ISOLATION AND STRUCTURE ELUCIDATION OF DITERPENES FROM HUNGARIAN EUPHORBIA SPECIES

Ph.D. Thesis

Andrea Vasas

Szeged
2006
Isolation and Structure Elucidation of Diterpenes from Hungarian Euphorbia Species

Ph.D. Thesis

Andrea Vasas

Szeged, Hungary
2006
List of publications related to the thesis


CONTENTS

ABBREVIATIONS AND SYMBOLS ........................................................................................................... 2

1. INTRODUCTION ................................................................................................................................. 3

1.1. Botany of the family Euphorbiaceae and the investigated *Euphorbia* species .................. 4

1.2. Chemical constituents of the family Euphorbiaceae ................................................................. 6

1.2.1. *Diterpenoids of the plant family Euphorbiaceae* ............................................................... 6

1.2.2. *Chemical constituents of Euphorbia esula* ........................................................................... 10

1.2.3. *Chemical constituents of Euphorbia lathyris* ................................................................. 10

1.2.4. *Chemical constituents of Euphorbia peplus* ................................................................. 11

1.3. Folk-medicinal use of the investigated *Euphorbia* species ................................................. 12

2. AIMS OF THE STUDY ...................................................................................................................... 13

3. MATERIALS AND METHODS ....................................................................................................... 14

3.1. Plant material ............................................................................................................................... 14

3.2. Screening of plant material for diterpene content ................................................................. 14

3.3. Extraction and isolation of diterpenes ....................................................................................... 14

3.3.1. *Extraction* .......................................................................................................................... 14

3.3.2. *Isolation and purification of compounds* ........................................................................... 15

3.4. Characterization and structure determination of the isolated compounds ......................... 16

4. RESULTS ........................................................................................................................................... 18

4.1. Screening of *E. esula*, *E. lathyris*, *E. peplus* and *E. villosa* for diterpene contents...... 18

4.2. Isolation of diterpenes ................................................................................................................. 18

4.2.1. *Isolation of diterpenes from E. esula* ............................................................................... 18

4.2.2. *Isolation of diterpene from E. lathyris* .............................................................................. 20

4.2.3. *Isolation of diterpenes from E. peplus* ............................................................................. 20

4.2.4. *Isolation of diterpenes from E. villosa* .............................................................................. 21

4.3. Characterization and structure determination of the isolated compounds ......................... 22

5. DISCUSSION ..................................................................................................................................... 36

6. SUMMARY ......................................................................................................................................... 42

7. REFERENCES ...................................................................................................................................... 44

ACKNOWLEDGEMENTS ..................................................................................................................... 52

APPENDIX ............................................................................................................................................... 53
### Abbreviations and Symbols

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D</td>
<td>one-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
<tr>
<td>COSY</td>
<td>correlated spectroscopy</td>
</tr>
<tr>
<td>cryst.</td>
<td>crystallization</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>DEPT</td>
<td>distortionless enhancement by polarization transfer</td>
</tr>
<tr>
<td>EIMS</td>
<td>electron-impact ionization mass spectroscopy</td>
</tr>
<tr>
<td>fr.</td>
<td>fraction</td>
</tr>
<tr>
<td>HMBC</td>
<td>heteronuclear multiple-bond correlation spectroscopy</td>
</tr>
<tr>
<td>HMQC</td>
<td>heteronuclear multiple-quantum coherence spectroscopy</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HREIMS</td>
<td>high-resolution electron ionization mass spectroscopy</td>
</tr>
<tr>
<td>HRFABMS</td>
<td>high-resolution fast atom bombardment mass spectroscopy</td>
</tr>
<tr>
<td>HSQC</td>
<td>heteronuclear single-quantum correlation spectroscopy</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>JMOD</td>
<td>$J$-modulated spin-echo experiment</td>
</tr>
<tr>
<td>MDR</td>
<td>multidrug resistance</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>nuclear Overhauser effect</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear Overhauser enhancement spectroscopy</td>
</tr>
<tr>
<td>NP</td>
<td>normal-phase</td>
</tr>
<tr>
<td>OCC</td>
<td>open-column chromatography</td>
</tr>
<tr>
<td>PLC</td>
<td>preparative-layer chromatography</td>
</tr>
<tr>
<td>RP</td>
<td>reversed-phase</td>
</tr>
<tr>
<td>TLC</td>
<td>thin-layer chromatography</td>
</tr>
<tr>
<td>$t_R$</td>
<td>retention time</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>VLC</td>
<td>vacuum-liquid chromatography</td>
</tr>
</tbody>
</table>

Ester groups: Ac = acetyl, Bz = benzoyl, Nic = nicotinoyl, iBu = isobutanoyl, $n$Bu = $n$-butanoyl
1. INTRODUCTION

One of the largest genera of flowering plants is *Euphorbia*, with approximately 2,000 species. This enormous genus belongs in the very diverse family Euphorbiaceae, with at least 8,000 species in 300 genera. The plants of the Euphorbiaceae are succulent or non-succulent, ranging from herbs and shrubs to trees and cacti, and occur in tropical and temperate regions throughout the world.\(^1,2\)

Many Euphorbiaceae species are characterized by the occurrence of highly irritant milky latex. These plants have been used to treat different cancers, tumours and warts from at least the time of Hippocrates.\(^3\) The folk-medicinal uses of *Euphorbia* species include the treatment of infections, gonorrhoea, migraine, intestinal parasites, rheumatism, snake-bites, asthma, obstipation, coughs, sores and skin diseases.\(^4\) In many African countries, these plants have often been used as sources of ingredients in arrow poisons, not only for their cohesive properties, but also to produce irritation at the site of the arrow wound, thereby promoting absorption of the poison. The constituents responsible for these irritant properties are well-known diterpene esters (mainly with a tigliane, ingenane or daphnane skeleton), which also exhibit mutagenic, cocarcinogenic and antileukaemic activities. Further important economic plants among the Euphorbiaceae are *Hevea brasiliensis* (pará rubber); *Euphorbia tetragonal* and *E. triangularis* (inferior rubber); *Manihot esculenta* (cassava, tapioca); *Croton tiglium* (croton oil); *Ricinus communis* (castor oil); and *Euphorbia resinifera* (‘euphorbium’).\(^5\)\(^-\)\(^7\)

Plants from the family Euphorbiaceae are sources of compounds with a variety of interesting biological activities. Especially the diterpenes are useful biochemical and pharmacological tools for elucidation of the biochemistry of diseased states in humans and animals and of the mechanisms of drug action. In recent years, the diterpene-type phorboids (ingenane, tigliane and daphnane esters) were amongst the most significant compounds isolated from higher plants in this respect. The extreme potency of phorboids, their specific biological actions and rigid chemical structure have made them invaluable assets in the study of cancer proliferation and inflammation. These compounds have played a large part in the elucidation of the protein kinase C (PKC) second messenger system of mammalian membranes. Moreover, antileukaemic ingenane diterpenes have been obtained from *Euphorbia esula*, *Croton tiglium* and *Cumuria spruceana*.\(^8\) Resiniferatoxin (RTX), isolated from the latex of *E. resinifera*, is another very important Euphorbiaceae diterpene, with an ultrapotent capsaicin-like effect. It is
a vanilloid receptor agonist, which interacts at a specific membrane recognition site (vanilloid receptor), expressed by primary sensory neurons involved in nociception and neurogenic inflammation. Further interesting diterpene esters with great structural variety and noteworthy biological activities have been isolated from Euphorbiaceae species.\textsuperscript{9-13}

In 1995, HOHMANN \textit{et al.} initiated a research programme in the Department of Pharmacognosy, University of Szeged, with the aim of the investigation of the secondary metabolites of Hungarian \textit{Euphorbia} species. In the course of these studies, many diterpene esters of different skeletal types have been isolated.\textsuperscript{14-21} One part of this programme involved the investigation of \textit{Euphorbia esula} L., \textit{E. lathyris} L., \textit{E. peplus} L. and \textit{E. villosa} Waldst. \& Kit. The present thesis summarizes the results of this phytochemical work.

\textbf{1.1. Botany of the family Euphorbiaceae and the investigated \textit{Euphorbia} species}

The Euphorbiaceae comprise one of the largest and most diversified families of angiosperms and, because of the range of morphological variation, may be polyphyletic in origin. There is great diversity in growth form, from tall rain forest trees to lianas, shrubs, perennial and annual herbs, geophytes, succulents and floating aquatics.\textsuperscript{2} The tribe Euphorbieae is characterized by the possession of a unique \textit{cyathium}, which consists of a central carpellate flower and four or five groups of basal male-flower clusters. \textit{Euphorbia} plants are monoecious.\textsuperscript{22,23}

The investigated plants, \textit{Euphorbia esula}, \textit{E. lathyris}, \textit{E. peplus}, and \textit{E. villosa} belong in the section \textit{Tithymalus} of the genus \textit{Euphorbia} in the family Euphorbiaceae, in the order Euphorbiales. \textit{Euphorbia esula} L. (leafy spurge; syn. \textit{Euphorbia pseudovirgata} (Schur) Soó, \textit{E. dalechampii} Haw., \textit{E. discolor} Led., \textit{E. intermedia} Brebis, \textit{E. racemosa} Tausch., \textit{E. tristis} Bess., \textit{E. triumfetti} Bert., \textit{Tithymalus esula} Moench.) is a glabrous or pubescent perennial herb, up to 120 cm in height. Stipules are absent, and the leaves are symmetrical at the base and stand opposite. The stems are usually unbranched at the base. It has up to 11 axillary non-flowering branches and 0-20 (-30) axillary rays. The leaves, measuring 15-85 × 0.5-15 mm, are linear to broadly ovate or obovate, entire and bluish-green in colour, although they turn yellowish or reddish-orange in late summer. The ray-leaves are shorter and often wider than cauline. The glands have a truncated or emarginated outer margin or 2 horns. The horns of the glands are
usually slender, or the outer margin of the glands is truncated or emarginated; the bracts between the male and female flowers are hirsute or plumose. The capsules measure 2.5-3 × 3.5 mm; they are deeply sulcated and granulated on the keels. The seeds measure 2 mm; they are ovoid, grey or brownish. This plant occurs in Europe, but only as an alien in the north, and in North America. It flowers from May to August.22-24

Euphorbia lathyris L. (caper spurge, mole plant; syn. Galarhoeus lathyris (L.) Haw., Euphorbia spongiosa Ledeb., Tithymalus lathyris Hill., T. lathyris Moench., T. lathyris Scop.) is a monoecious, always glabrous, glaucous biennial plant with a height of 150 cm. It has numerous axillary shoots; the leaves, measuring 30-150 × 5-25 mm, are linear to oblong-lanceolate, and entire. Stipules are absent; the leaves stand opposite and are decussated. The ray-leaves are ovate-lanceolate, but the raylet-leaves are triangular-ovate, acute, and paler-green than the cauline and ray-leaves. It has an umbel with 2-4 rays, up to 8 times dichotomous, the whole forming a cyathium. The flowers are green to greenish-yellow in colour. The seeds measure 5 mm; they are barrel-shaped, rugulose, brown or grey. This plant is a ruderal and weed of cultivated ground. It occurs throughout Europe, but is probably native only in the East and Central Mediterranean region.22-24

Euphorbia peplus L. (petty spurge; syn. Tithymalus peplus (L.) Hill, Galarhoeus peplus (L.) Rydb.) is a glabrous annual herb with 2 or more branches from the base and with 0-3 axillary rays. It grows up to 40 cm in height. The leaves measure 5-25 × 3-15 mm, with petioles up to 8 mm. They are ovate, suborbicular or obovate, and entire. The ray-leaves are like the cauline, but with shorter petioles. The raylet-leaves are smaller, and slightly obliquely ovate. The rays are 3-5 times dichotomous. The glands have 2 filiform horns. The capsule measure 2 × 2 mm; they are shallowly sulcated and smooth; each valve has two dorsal ridges. The seeds are 1.1-1.4 mm in size; they are ovoid-hexagonal, sulcated ventrally and pitted dorsally, pale-grey, but darker in the depressions. This species is a weed of cultivated ground. It occurs in most of Europe. The plant flowers from July to November.22-24

Euphorbia villosa W. et K. (syn. Tithymalus villosus (Waldst. & Kit.), E. austriaca Kerner, E. carpatica Woloszak, E. pilosa auct. Eur., non L., E. procera M.B., E. semivillosa Prokh., E. tauricola Prokh.) is a stout, glabrous or pubescent, rhizomatous perennial herb with a height of 30-120 cm. It is herb, or a slender, wiry dwarf shrub. The rhizome is without tubers.
The stems are stout, and usually numerous, often with non-flowering branches as well as axillary umbels, which are scaly below. The leaves are symmetrical at the base, and usually alternate; they are rarely opposite, but then are not decussated. The leaves are oblong, oblong-lanceolate to oblong-ovate or elliptical, 2-6 times as long as wide, obtuse to acute, and often mucronate, entire, or serrulate near the apex. The ray-leaves are ovate, obtuse and mucronate; the raylet-leaves are smaller, relatively wider and yellowish. The rays number (4)5 or more; they are trichotomous and then dichotomous. The capsule, measuring 3-6 mm, is smooth, minutely tuberculated or densely villous, and indurated. The seeds are 2.5-3.2 mm in size, smooth and brown. The plant occurs in damp meadows, open woods and river-banks throughout Europe. It flowers from May to June.22-24

1.2. Chemical constituents of the family Euphorbiaceae

This group of plants has been the subject of intense phytochemical examination. The isolated compounds include alkaloids (imidazoles, pyrimidines, pyrrolidines, pyridines, piperidines, quinolizinones, quinazolones, isoquinolines, morphinandienones, indoles, guanidines and diterpenes),24,25 diterpenoids, triterpenoids (tetra- and pentacyclic),25,26 flavonoids (particularly flavones, flavonols, and rarely flavanones),27 coumarins (in relatively few plants),2 lignans (in only two genera: Jatropha and Phyllanthus)28, tannins (hydrolysable),29 phenanthrenes and quinones,27 phenolic compounds,25 long-chain fatty alcohols (n-octacosanol and n-hexacosanol) and hydrocarbons, alkanes and amino acids. These compounds have been isolated from the latex and from different parts (bark, cortex, flowers, leaves, roots and stems) of the plants. The most important constituents of the latex of many Euphorbia species are triterpenes and diterpenes, mainly in ester form.30

1.2.1. Diterpenoids of the plant family Euphorbiaceae

The plants of the family Euphorbiaceae produce a great variety of diterpene esters, with various biological activities. Up to 2005, diterpenes were isolated from 187 Euphorbia species, or their presence was detected. One group of diterpenes occurs widely in the plant kingdom, but others appear to be restricted in distribution. In particular, the macrocyclic diterpenes and their cyclization products display only a very limited distribution in the families Euphorbiaceae and Thymeleaceae. These diterpenes can be classified as ‘lower terpenes’ (‘Euphorbiaceae diterpenes’), derived from a geranyl-geranyl-pyrophosphate
precursor through a ‘head-to-tail’ cyclization (Figure 1).\textsuperscript{31} The functionalization of diterpenes presumably proceeds after cyclization. The cembrene cation is a very reactive intermediate containing a 14-membered ring, which stabilizes through the formation of cembranoids.\textsuperscript{32} Cembrane diterpenes have been isolated from different plants and marine invertebrates; many have cytotoxic\textsuperscript{33}, antitumour\textsuperscript{34} and HIV-1-inhibitory\textsuperscript{35} activities.

![Figure 1. Hypothetical biogenic interconversions of diterpenes in the family Euphorbiaceae](image)

Large numbers of bicyclic diterpenes are to be found in the family Euphorbiaceae. The casbanes, formed from the cembrene cation by cyclization of the isopropyl group, have been considered to be precursors of a number of macrocyclic diterpenes.\textsuperscript{36} Casbanes with a trans or cis cyclopropane ring occur in the species of Euphorbia\textsuperscript{37,38} and other Euphorbiaceae genera.\textsuperscript{39,40} Some compounds have in vitro cytotoxic,\textsuperscript{37,40} antibacterial\textsuperscript{38} and antiproliferative\textsuperscript{38} activities. The bicyclic jatrophane diterpenes, with a bicyclo[10.3.0]pentadecane ring system, may be formed in the plant from a cembrene cation, or a casbane precursor by rearrangement. The first known member of this type was jatrophone, isolated from the roots of Jatropha gossypiifolia, which demonstrated significant activity against P-388 lymphocytic leukaemia. In 1975, two highly oxygenated jatrophane derivatives were obtained from E. kansui, kansuinine A and B, with analgesic and anti-writhing activities.\textsuperscript{41-43} Further antitumour jatrophane derivatives, euphornin from E. maddeni\textsuperscript{44} and euphoscopins A and B from E. helioscopia, were reported in 1981.\textsuperscript{45} The structure of euphornin was proved by X-ray analysis in 1984.\textsuperscript{46} In the 1980s, further jatrophane diterpenes were isolated from E. helioscopia,\textsuperscript{11,47,48} E. characias\textsuperscript{49} and E. lateriflora.\textsuperscript{50,51} YAMAMURA et al. reported 31 new jatrophane derivatives from the fresh plant material of E. helioscopia: euphornin A-K, euphoscopin A-L, epieuphoscopin B, D and F, euphohelioscopin A, B and euphohelionone.\textsuperscript{48} In the 1990s, jatrophane derivatives from further Euphorbia species were isolated:
From **E. terracina**, interesting bishomo-jatrophane derivatives, terracinolides and isoterracinolides were isolated by MARCO et al. Between 2000 and 2005, jatrophanes were isolated from **E. serrulata**, **E. salicifolia**, **E. paralias**, **E. turczaninowii**, **E. hyberna**, **E. dendroides**, **E. altotibetic**, **E. semiperfoliata**, **E. mongolica**, **E. obtusifolia**, **E. pubescens**, **E. characias**, **E. kansui**, **E. amygdaloides** and **E. helioscopia**. Many biological activities of jatrophanes have been reported, such as antifeeding activity against the larvae of *Spodoptera littoralis*, antiviral activity against HIV-1, microtubule-interacting activity, inhibitory activity on the mammalian mitochondrial respiratory chain, antitumour and multidrug resistance reversing (MDR) activities, and cell division-inhibitory effect on the *Xenopus laevis* embryo. Since the 1970s, more than 180 jatrophanes from natural sources have been identified. Their great structural variability stems from the number and positions of the double bonds, the nature and number of the oxygen functions, and the configuration of the diterpene core. The oxygen functions are hydroxy, keto, epoxy, ether and ester groups. Natural jatrophane diterpenes are mainly polyacylated derivatives. The number of ester moieties ranges between 2 and 8. The acyl residues are most frequently acetate, benzoate, isobutyrate, 2-methyl butyrate or nicotinate, and rarely propionate, butyrate, angelate, tiglate or cinnamate. The most heterogeneously esterified molecules have 4 different acyl groups, and there are only a few compounds with homogeneous ester groups. Hydroxy groups can usually be found on C-3, C-5, C-7, C-9 and C-15, but also on C-2, C-8 and C-14, and rarely on C-1, C-6 and C-13. Keto groups are usually located at C-9 or/and C-14, and occasionally at C-7 or C-12. Most of the compounds contain C-11/C-12 and C-6/C-17 double bonds. Rarely, C-5/C-6, C-14/C-15, C-3/C-4 and C-8/C-9 double bonds can be found.

Depending on their substitution, jatrophanes may have 5-10 chiral centres. The ringjunctions always prove to be *trans*, and the substituents at C-5, C-8 and C-9 are α and those at C-3, C-6 and C-7 are β, relative to the angular H-4, which is assumed to be α-oriented. Jatrophane diterpenes do not form a stereochemically homogeneous series, because the configurations of the remaining carbons (C-1, C-2, C-3, C-13 and C-14) are variable.

The absolute configurations of jatrophane derivatives have been investigated in only a few cases by X-ray analysis or by the circular dichroism exciton chirality method. The
absolute configurations of all natural jatrophane derivatives are identical as concerns the ring anellation: H-4 is \( \alpha \), and 15-OH or the 15-acyl group is \( \beta \)-oriented.

Irregular bicyclic compounds have been isolated from *Chrozophora obliqua* (Euphorbiaceae)\textsuperscript{81} and from *Jatropha integerrima* and *J. gossypifolia*\textsuperscript{82,83}

Euphorbiaceae species synthesize different types of tricyclic diterpenes. One of the largest groups of tricyclic diterpenes is the lathyrane group, which includes about 90 compounds. The hydrocarbon nucleus of casbene and its saturated analogue, casbane, may be considered to be the biogenetic precursor of these diterpenes (Figure 1).\textsuperscript{36} The first lathyrane diterpene was ‘euphorbiasteroid’, reported by Dublyansky in 1937 from the seed oil of *E. lathyris*.\textsuperscript{84} It was originally believed to be a steroid, but later studies revealed its diterpenoid nature. The configuration reported in 1970, as a consequence of X-ray analysis\textsuperscript{85,86} was revised by Appendino et al. in 2000.\textsuperscript{87,88} Lathyrane diterpenes have been reported from *Macaranga tanarius*,\textsuperscript{89} *Jatropha curcas*,\textsuperscript{90,91} *J. grossidenta*,\textsuperscript{92} *J. podagrica*,\textsuperscript{93} *J. weddelliana*,\textsuperscript{94} *Euphorbia jolkini*,\textsuperscript{95} *E. micractina*,\textsuperscript{96} *E. pithyusa* subsp. *cupani*\textsuperscript{97} and *E. hyberna*.\textsuperscript{63} An unusual lathyrane diterpene, bertyadionol, was isolated from *Bertya cupresseoides* in 1970.\textsuperscript{98} Pharmacological screening revealed that lathyrane-type compounds do not possess the pro-inflammatory activity characteristic of other Euphorbiaceae diterpenes.\textsuperscript{99} Ingols are 4,15-epoxy derivatives of lathyranes, which have given rise to considerable interest as cytotoxic,\textsuperscript{100,101} vasoactive,\textsuperscript{13,102} antineoplastic,\textsuperscript{44} and prostaglandin E\textsubscript{2}-inhibitory\textsuperscript{103} agents. Ingol was originally isolated from the latex of *Euphorbia ingens* in 1973. Its stereochemistry was elucidated by X-ray analysis.\textsuperscript{104} Further ingol esters have been isolated from *Euphorbia lactea*, *E. kamerunica*,\textsuperscript{100,101,105} *E. hermentiana*,\textsuperscript{106} *E. royleana*,\textsuperscript{107} *E. tirucalli*,\textsuperscript{108,109} *E. poisonii*,\textsuperscript{110,111} *E. antiquorum*,\textsuperscript{112} *E. portulacoides*,\textsuperscript{113} *E. canariensis*,\textsuperscript{13,114,115} *E. acrurensis*,\textsuperscript{116} *E. lactea*,\textsuperscript{117} *E. nivulia*,\textsuperscript{103,118} and *Synadenium compactum*.\textsuperscript{119}

Tigliane, ingenane, daphnane, jatropholane, crotopholane and rhamnopholane are further common types of ‘lower diterpenes’ with tri- or tetracyclic skeletons.\textsuperscript{31,120} Compounds with tigliane, ingenane or daphnane skeletons have attracted interest because of their skin-irritant activities.\textsuperscript{36} The most exhaustively investigated tiglianes are phorbols.
esterified at C-12 and C-13, which activate protein kinase C (PKC); they may therefore disturb the enzyme-regulated cellular activity. 12-\(\text{O}\)-Tetradecanoylphorbol-13-acetate (TPA) has become a classical activator of PKC, in many studies relating to signal transduction.\(^{121}\) Some tiglianes exert anti-HIV-1 activity.\(^{122,123}\)

Further tri- and tetracyclic diterpene types have been described from the family Euphorbiaceae. These minor diterpene classes are based on pepluane,\(^{55,80}\) segetane,\(^{124}\) jatrophatrione,\(^{125}\) euphoractine,\(^{126,127}\) myrsinane\(^{128,129}\) and cyclomyrsinane.\(^{128}\)

Among the diterpene constituents of the family Euphorbiaceae, non-specific ‘higher diterpenes’ may be mentioned. The skeletons of these compounds are formed by the classical ‘concertina-like’ cyclization typical of many diterpenoids, triterpenoids and steroids. Higher diterpenes, such as the bicyclic labdane\(^{130}\) and clerodane,\(^{131}\) the tricyclic abietane,\(^{113}\) and the tetracyclic bayerane,\(^{132}\) kaurane\(^{132}\) and atisane\(^{133}\) types, occur in many other plant families, too.

### 1.2.2. Chemical constitutents of Euphorbia esula

Earlier chemical investigations of \(E.\ esula\) revealed 3 ingenane diterpenoids: ingenol 3,20-dibenzoate, ingenol 3-dodecanoate, and ingenol 3-\(\Delta^{2,4,6,8,10}\)pentene tetradeconoate.\(^{8,152}\)

Macrocyclic diterpenes, viz. jatrophane esters, esulons A, B, and C, with moderately toxic and mildly inflammatory effects, have been isolated from leafy spurge roots collected in North Dakota, and lathyrane and jatrophane triesters from seeds collected in Canada.\(^{12,88,134}\) In 2002, Liu et al. isolated 2 jatrophane diterpenes with cytotoxic activity from the whole herb collected in China.\(^{135}\) From \(E.\ esula\), tertiary and quaternary alkaloids, triterpenoids (24-methylenecycloartenol, cycloartenol, lupeol, lupeol acetate, \(\alpha\)- and \(\beta\)-amyrin, \(\delta\)-amyrenone),\(^{33}\) steroids (\(\beta\)-sitosterol)\(^{33}\) flavonoid glycosides (kaempferol 3-glucuronide), hydrocarbons, long-chain alcohols (1-hexacosanol, 1-octacosanol),\(^{151}\) long-chain aldehydes (\(C_{26}\) and \(C_{28}\)),\(^{136}\) alkanes (\(C_{25}-C_{30}\), \(n\)-triacontane, \(n\)-hentriacontane, \(n\)-dotriacontane, \(n\)-tritriacontane), L-inositol, gallic acid, amino acids\(^{137}\) and rubber have also been identified.

### 1.2.3. Chemical constituents of Euphorbia lathyris

Chemical investigations of \(E.\ lathyris\) have revealed the presence of diterpenes. First, the diterpene ‘euphorbiasteroid’ was isolated from the seed oil. In 1989, Itokawa et al. isolated ingenol 3-hexadecanoate as active principle from the antitumour extract of the plant.\(^{138}\)
Besides ingenol esters, a series of diterpenes based on the lathyrane skeleton, *i.e.* ‘Euphorbia factors’ L₁-L₁₀, have also been isolated from the seeds₁³⁹-₁⁴⁴ and jolkinol A from a callus culture.¹⁴⁵ Among the ‘Euphorbia factors’, the ingenol-type compounds have proved to be potent PKC activators, and anticancer⁸ and anti-HIV agents, while the lathyrane-type factors have been demonstrated to be powerful P-glycoprotein inhibitors.¹³⁹,¹⁴⁶ Some ingenane diterpenes isolated from the latices of *E. lathyris* have been esterified with conjugated C₈–C₁₄ fatty acids.¹⁴⁴ The roots of *E. lathyris* have been less well investigated to date; only the isolation of a lathyrane diterpenoid, jolkinol A, was reported earlier.¹⁴⁵ In 2005, the first secolathyrane diterpenoid, and its biogenetic precursor, ‘Euphorbia factor’ L₁₁, were isolated from the seeds of the plant.¹³⁹ Besides diterpenes, triterpenoids (cycloartenol, euphol, lanosterol, isolanosterol, 24-methylene cycloartenol, 24-methylene-24-dihydro lanosterol, guimarenol, hopenol B, butyrosperrmol, taraxerol, taraxerone, betuline),³²,³³ steroids (campesterol, α- and β-euphorbiasterol, stigmasterol, Δ⁷-stigmasterol, β-sitosterol)³²,³³ flavonoids (kaempferol, quercetin, kaempferol 3-glucuronide, quercetin 3-glucuronide), coumarins (aesculetin, daphnetin, euphorbetin, isoeuphorbetin), p-coumaric acid, ferulic acid, lectins, hentriacontane, oleic acid, L-DOPA and rubber⁶ have been reported from this plant.

1.2.4. Chemical constituents of *Euphorbia peplus*

In 1984, 2 ingenane-type diterpene esters, ‘Euphorbia factors’ Pe₁ and Pe₂, were isolated by Gotta et al. from *E. peplus*.¹⁴⁷ One year later, the presence of the irritant toxin 20-deoxyingenol 3-angelate was reported from Egyptian *E. peplus*, together with the much less toxic ingenol 20-octanoate.¹⁵⁶ Jakupovic et al. investigated *E. peplus* samples collected in Chile and Germany. From the extract of the plant from Chile, 3 jatrophans, 1 tetracyclic diterpene with a pepluane skeleton and 2 ingenanes were isolated. The sample from Germany additionally afforded 3 other jatrophans.⁸⁰ In 1998, Zayed et al. tested the extracts of several herbaceous plants of the genus *Euphorbia* as regards irritancy on the mouse ear. As compared with croton oil, the extract of *E. peplus* was the most active. The results of these mouse ear assays led to ‘Euphorbia factors’ Pe₁-Pe₅ being identified as diterpene ester-type toxins of *E. peplus*.¹⁴⁸ Between 2000 and 2004, further pepluane-, jatropane- and ingenane-type diterpenes were isolated from the plant.¹⁴,¹⁴⁹ Besides diterpenes, *E. peplus* contains triterpenoids (β-amyrin acetate, nepheolin, alangidiol, simiarenone, cycloartenol, cycloartenone, 24-methylene cycloartenol, obtusifoliol,
citrostadeniol),\textsuperscript{150} steroids (lanosterol, campesterol, cholesterol, stigmasterol, \(\beta\)-sitosterol, 28-isofucosterol, \(\Delta^7\)-isofucosterol),\textsuperscript{150} flavonoids (quercetin, quercetin 3-glucoside, rhamnetin-rhamnoside, hiperoside, rhamnetin 3-galactoside, astragalin)\textsuperscript{150} and long-chain alcohols.\textsuperscript{150}

1.3. Folk-medicinal use of the investigated \textit{Euphorbia} species

\textit{E. esula} is toxic to livestock, and allelopathic to desirable forage plants.\textsuperscript{151} It is known to cause sheep mortality and to produce inflammation with the loss of hair from the feet of horses. The latex causes blistering with severe irritation if allowed to remain on the skin, and it can lead to partial blindness if dropped into the eyes.\textsuperscript{152} Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings and warts.\textsuperscript{3} Previous pharmacological studies demonstrated the pro-inflammatory, tumour-promoting and antitumour activities of the plant extracts. The extracts of \textit{E. esula} exhibited antileukaemic activity against P-388 lymphocytic leukaemia in mice.\textsuperscript{8}

Caper spurge (\textit{E. lathyris}) is often known as the 'mole plant' because animals nibbling the roots of the plant are killed because of its highly irritant gastric effect. The seeds have in the past been used for the production of oil; the oil content of the seeds is about 50 \%, of which 85 \% is in the form of oleic acid.\textsuperscript{153} The seeds of \textit{E. lathyris}, a common traditional Chinese medicine, have been used for the treatment of very diverse diseases, such as ascites, coprostatics, anuresis, amenorrhoea, venous stasis, terminal schistosomiasis and scabies.\textsuperscript{139} The seed oil is applied to burns. The root is equally purgative and emetic. According to HARTWELL, the latex is used in folk remedies for cancers and warts.\textsuperscript{3} Previous biological studies have demonstrated the pro-inflammatory property of the roots, seeds, aerial parts and milky latex, and an antitumour effect of the seeds of \textit{E. lathyris} against Sarcoma 180 ascites.\textsuperscript{138,145}

\textit{E. peplus} has been used in traditional medicine for the treatment of asthma and catarrh, but is known to induce painful vomiting and purgation, particularly in domestic animals.\textsuperscript{154} Its milky juice has also been used in the treatment of corns and warts. The plant has been utilized against cancer of the stomach, liver and uterus.\textsuperscript{155} Extracts of this plant have been recorded to exert C-mitotic action and have been reported to display proteolytic activities.\textsuperscript{3,156}

As concerns \textit{E. villosa}, neither any folk-medicinal use nor any pharmacological investigation has yet been published.
2. AIMS OF THE STUDY

The *Euphorbia* genus is the source of a large number of biologically active diterpenes. In 1995, HOHMANN *et al.* (Department of Pharmacognosy, University of Szeged) initiated a research programme with the aim of investigating the secondary metabolites of plants of the *Euphorbia* species. The aims of present work, as part of that programme, were the isolation and structural characterization of new diterpene polyesters, and investigation of their pharmacological effects.

In order to achieve these aims, the main tasks were:

- Screening of *E. esula*, *E. lathyris*, *E. peplus* and *E. villosa* for diterpene content.
- Extraction of the plant materials.
- Isolation and purification of the diterpene esters by the combination of various chromatographic methods (OCC, VLC, PLC and HPLC).
- Characterization and structure determination of the isolated compounds by different spectroscopic techniques (NMR, HR-MS, UV and IR spectroscopy).
- Evaluation of the pharmacological potential and chemotaxonomical relevance of the isolated diterpenes.
3. MATERIALS AND METHODS

3.1. Plant material

*E. esula*: Whole plants (11 kg) were collected in May 1994 on the banks of the Tisza River, in Szeged, Hungary, and identified by Károly Penszka*. *E. lathyris*: The roots (840 g) of the plant were collected from a 2-year-old stock in October 1996 in Székesfehérvár, Hungary. *E. peplus*: Whole plants were collected in June 1996 in Miskolc, Hungary. 200 g of fresh plant material was frozen and stored at -20 °C until preparation. *E. villosa*: The plants were collected in Vácrátót, Hungary, in June 2000, and identified by Vilmos Miklóssy V.†. The plant material was dried at room temperature, yielding 210 g of dried material.

3.2. Screening of plant material for diterpene content

20 g of fresh and crushed plant material of *E. esula*, 40 g of *E. lathyris*, 5 g of *E. peplus* and 5 g of dried and powdered plant material of *E. villosa* were percolated with methanol (300 ml, 400 ml, 100 ml and 100 ml) at room temperature. After concentration (to 30 ml, 40 ml, 10 ml, and 10 ml), water was added to the extract (30 ml, 40 ml, 10 ml and 10 ml) and the mixture was subjected to solvent-solvent partitioning with dichloromethane (3 × 50 ml, 3 × 40 ml, 3 × 20 ml and 3 × 20 ml). The dichloromethane-soluble phase was subjected to polyamide (1 g, 1 g, 0.5 g and 0.5 g) OCC, using a solvent system of methanol–water [1:4, 2:3, 3:2 and 4:1 (200 ml, 200 ml, 100 ml and 100 ml of each mixture)] as eluent. The fractions were concentrated and monitored by thin-layer chromatography (TLC), using mobile phases A and D (described in section 3.3.2).

3.3. Extraction and isolation of diterpenes

3.3.1. Extraction

The fresh *E. esula* and *E. lathyris*, and the frozen *E. peplus* plant material were crushed with a Waring CB–6 blender (model 33BL13), and extracted with methanol (75 l, 8 l and 1.8 l) at room temperature. The extracts were concentrated (to 2500 ml, 1500 ml and 100 ml) in vacuo, and liquid-liquid partition was then performed with dichloromethane (7 × 1500 ml, 3 × 200 ml and 4 × 100 ml).

* Department of Botany and Plant Physiology, Agricultural University of Gödöllő, Hungary
† Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary
\textit{E. villosa}: The dried plant material was crushed with a Bosch MKM 6000 grinder. The raw material was percolated with 3000 ml of methanol at room temperature. After the methanol extract had been concentrated to 200 ml, solvent-solvent partition was performed with 3 × 400 ml of chloroform.

3.3.2. Isolation and purification of compounds

\textit{Open column chromatography}: OCC was performed on polyamide (ICN) for column chromatography (420.0 g for the \textit{E. esula} extract, 25.0 g for the \textit{E. lathyris} extract, 20.0 g for the \textit{E. peplus} extract and 20.0 g for the \textit{E. villosa} extract). Mixtures of methanol–water [1:4, 2:3, 3:2 and 4:1 (\textit{E. esula} 2000 ml, \textit{E. lathyris} 500 ml, \textit{E. peplus} 400 ml, \textit{E. villosa} 300 ml of each)] were used as mobile phase for both plant extracts.

\textit{Vacuum-liquid chromatography}: For VLC, silica gel 60 G (15 \textmu m, Merck 11677) was used.

- **Column 1**
  - eluent: cyclohexane–acetone [19:1, 9:1, 4:1, 7:3, 1:1, 3:7 (1 000 ml each)]; volume of collected fractions: 100 ml; sorbent: 145 g
- **Column 2**
  - eluent: chloroform–methanol [100:0.3, 99.7:0.3, 99:1, 49:1, 24:1, 9:1, 4:1, 7:3 (500 ml each)]; volume of collected fractions: 20 ml; sorbent: 58 g
- **Column 3**
  - eluent: cyclohexane–ethyl acetate–ethanol [9:1:0, 8:2:0, 80:20:1, 30:20:1, 5:5:1 (150 ml each)]; volume of collected fractions: 15 ml; sorbent: 16 g
- **Column 4**
  - eluent: chloroform–acetone [49:1, 19:1, 9:1, 4:1, 7:3, 1:1 (500 ml each)]; volume of collected fractions: 50 ml; sorbent: 73 g
- **Column 5**
  - eluent: cyclohexane–ethyl acetate [19:1, 9:1, 4:1, 7:3, 3:2 (150 ml each)]; volume of collected fractions: 10 ml; sorbent: 10 g
- **Column 6**
  - eluent: cyclohexane–ethyl acetate [49:1, 19:1, 93:7, 9:1, 17:3, 4:1, 7:3 (200 ml each)]; volume of collected fractions: 10 ml; sorbent: 42 g

\textit{Layer chromatography}: PLC was performed on silica gel 60 F\textsubscript{254} (Merck 5715). Separation was monitored in UV light at 254 nm. Compounds were eluted from the scraped adsorbent with chloroform. The OCC, PLC and VLC fractions obtained, were monitored by TLC on silica gel 60 F\textsubscript{254} (Merck 5554).

**Mobile phases:**
- A cyclohexane–ethyl acetate–ethanol (30:10:1)
- B benzene–ethyl acetate (7:3)
- C \textit{n}-hexane–tetrahydrofuran–acetonitrile (20:5:1)
- D chloroform–acetone (19:1)
Visualization methods: UV light: at 254 nm, spraying with conc. H$_2$SO$_4$, and then heating at 110 °C for 5 min.

*Isocratic high-performance liquid chromatography*: HPLC was carried out on a pre-packed Hibar RT (250 mm, 4 mm) LiChrospher Si 100 (5 μm) column (Merck) and on a pre-packed Hibar RT (250 mm, 4 mm) LiChrospher RP–18 (5 μm) column (Merck), using Waters (1) and Waters Millipore (2) instruments:

2. Controller 600, Pump 600, Dual λ Absorbance Detector 2487, Injector Rheodyne 7725i.

Detection was carried out at 235 and 273 nm.

NP HPLC methods:
- Method 1 eluent: cyclohexane–ethyl acetate–ethanol (30:15:2); flow rate: 0.5 ml/min
- Method 2 eluent: cyclohexane–ethyl acetate–ethanol (30:10:1); flow rate: 0.5 ml/min
- Method 3 eluent: n-hexane–ethyl acetate (19:1); flow rate: 1 ml/min
- Method 4 eluent: cyclohexane–dichloromethane–methanol (70:50:1); flow rate: 1 ml/min

RP HPLC methods:
- Method 5 eluent: methanol–water (7:3); flow rate: 0.5 ml/min
- Method 6 eluent: methanol–water (7:3); flow rate: 0.7 ml/min
- Method 7 eluent: acetonitrile–water (7:3); flow rate: 0.5 ml/min
- Method 8 eluent: methanol–water (8:2); flow rate: 2 ml/min
- Method 9 eluent: methanol–water (8:2); flow rate: 1 ml/min

Mobile phases in all types of chromatography methods are given in terms of volume ratio v/v.

### 3.4. Characterization and structure determination of the isolated compounds

IR spectra were run as KBr discs on a Perkin-Elmer Paragon 1000 PC FTIR spectrometer. UV spectra were obtained in methanol on a Shimadzu UV-2101 PC spectrometer. Optical rotation $[\alpha]_D$ values were determined in chloroform at ambient temperature on a Perkin-Elmer 341 polarimeter. EIMS measurements were carried out on a Finnigan Mat 8430 spectrometer operating at 70 eV ionizing energy. HREIMS and HRFABMS were carried out on a VG ZAB SEQ instrument. The resolution of the instrument was 10000 (at 10% valley definition).

NMR spectra were recorded on a Bruker DRX 400 Avance spectrometer at 400 MHz ($^1$H) and 100 MHz ($^{13}$C), and on a Bruker Avance DRX 500 spectrometer at 500 MHz ($^1$H) and 125
MHz ($^{13}$C), with CDCl$_3$ or pyridine-$d_5$ as solvent and tetramethylsilane (TMS) as internal standard. Two-dimensional experiments were performed with standard Bruker software. In X-ray crystallography, a crystal was mounted on an Enraf-Nonius CAD4 diffractometer equipped with a graphite monochromator. Reflections were collected by using Cu Kα radiation and the ω-θ scan mode. The structure was determined by direct methods (Sheldrick, G.M. SHELXS-97 Program for Crystal Structure Solution, University of Göttingen) and refined by full-matrix least-squares analysis (Sheldrick, G.M. SHELXL-97 Program for Crystal Structure Refinement, University of Göttingen).
4. Results

4.1. Screening of *E. esula*, *E. lathyris*, *E. peplus* and *E. villosa* for diterpene contents

In the extracts of *E. esula*, *E. lathyris*, *E. peplus* and *E. villosa*, black, brown, blue and purple spots (R_f 0.08-0.75) were detected on the TLC chromatograms. The fractions obtained from the polyamide column with methanol–water 1:4, 2:3 and 3:2 were rich in diterpenes, which exhibit brown, black or blue spots on TLC chromatograms. The fractions eluted with methanol–water 4:1 contained mainly triterpenes and chlorophyll. These observations led to the conclusion that in the extracts of *E. esula*, *E. lathyris*, *E. peplus* and *E. villosa* a series of different diterpenes are present, which can be enriched in the 40 % and 60 % methanol fractions of the apolar extracts.

4.2. Isolation of diterpenes

4.2.1. Isolation of diterpenes from *E. esula*

The crushed, fresh plant material (11 kg) was percolated with methanol, an amphipolar solvent. The crude extract was concentrated, and then subjected to solvent-solvent partitioning to remove polar compounds (Figure 2). The methanol extract was exhaustively extracted with dichloromethane, and the organic phase was concentrated. The dark olive-drab oily residue (130 g) was chromatographed on a polyamide column with mixtures of methanol–water in order to remove chlorophyll and triterpenes from the extract. The diterpene-containing fractions were obtained with methanol–water 1:4. Diterpenes were detected in fractions 1-21. Fractions 5-21 afforded crystalline material, which was recrystallized from methanol to yield eup-1 (300 mg). After crystallization, fractions 1-15 (15 g) were combined and subjected to VLC column 1. In this step, a gradient system of cyclohexane-acetone, and silica gel with a mean particle size of 15 μm resulted in good selectivity. Fractions 23-27 from column 1 (2.24 g), eluted with cyclohexane-acetone 4:1, were transferred repeatedly to a silica gel VLC (column 2), and eluted with chloroform–methanol mixtures of increasing polarity. From fractions 24-27, obtained from column 2 with chloroform–methanol 99.7:0.3, 40 mg of eup-4 crystallized. Fractions 22-23, obtained from VLC column 2 with chloroform–methanol 100:0.3, were subjected to PLC on silica gel using mobile phase C and then further purified by NP-HPLC (method 2) to yield 5.1 mg of eup-10. Since these diterpenes do not exhibit UV
absorption, the HPLC purification was carried out with the use of an RI detector. Fractions 28-30, eluted from VLC column 2 with chloroform–methanol 100:0.3, were separated by RP-HPLC using method 5, to afford eup-11 (10 mg). VLC-fractions 31-32, obtained from column 2 with chloroform–methanol 100:0.3, were subjected to NP- and RP-HPLC by method 1 and method 7, respectively. These chromatographies allowed the isolation of 34.5 mg of eup-12 as white needles. Fractions 93-96 from column 2, obtained with chloroform–methanol 99:1, were further purified by PLC on silica gel using mobile phase B, and by NP-HPLC using method 1, to yield 1.2 mg of eup-6.

**Figure 2** Isolation of diterpenes from *Euphorbia esula*
4.2.2. Isolation of diterpene from E. lathyris

The fresh roots of *E. lathyris* (840 g) were extracted with methanol. The crude extract was concentrated *in vacuo* and extracted with dichloromethane. On evaporation, a dark-brown residue (4.27 g) of organic phase was obtained, which was chromatographed on a polyamide column with mixtures of methanol–water (1:4, 2:3, 3:2 and 4:1) as eluents. The fractions obtained with methanol–water 2:3 and 3:2 were combined (620 mg), and subjected to silica gel VLC, using column 3. Fractions 10-11 from column 3, eluted with cyclohexane–ethyl acetate 9:1, were further purified by PLC on silica gel, using mobile phase D, and then by NP-HPLC, using method 3, to yield 2.3 mg of eul-1 ($t_R$ 21.2 min).

4.2.3. Isolation of diterpenes from *E. peplus*

The fresh plant material was frozen and stored at -20 °C until preparation in order to stop the *post mortem* enzymatic processes.
The plant material was percolated with methanol and, after concentration, the crude extract was exhaustively extracted with dichloromethane (Figure 3). Evaporation of the organic phase gave a greenish-brown residue (4.7 g), which was chromatographed on a polyamide column with mixtures of methanol–water 1:4, 2:3, 3:2 and 4:1 as eluents. Fraction 3, obtained with methanol–water 3:2, was subjected to silica gel VLC, using column 4. Fractions 6-10 from column 4, eluted with chloroform, were further fractionated by RP-HPLC, using method 6. The compounds observed at retention times of 13.5, 16.2, 20.0, 20.4, 23.4 and 29.2 min were separated, and finally subjected to NP-HPLC, using method 2, to afford ep-1 (10.2 mg), ep-2 (2.3 mg), ep-3 (2.7 mg), ep-4 (4.7 mg), ep-5 (3.1 mg) and ep-8 (5.5 mg), respectively. Combined fractions 11-18, obtained from VLC column 4 with chloroform–acetone 49:1 as eluent, were further purified by NP-HPLC using method 2, to afford ep-7 (3.8 mg).

4.2.4. Isolation of diterpenes from E. villosa

The powdered raw plant material was percolated with methanol (Figure 4). After concentration under vacuo, the crude extract was subjected to solvent-solvent partitioning with chloroform. Evaporation of the chloroform-soluble phase resulted in a dark-brown, oily residue (6.12 g), which was fractionated on a polyamide column with mixtures of methanol–water 2:3, 3:2 and 4:1 as eluents. The fractions obtained with methanol–water 3:2 (fr. 2) and 4:1 (fr. 3) contained the diterpenes. Fraction 2 was chromatographed by VLC on column 5. TLC of the fractions showed that the main diterpene constituents of the E. villosa extract were present in fractions 5/48–57 (eluted with cyclohexane–ethyl acetate 9:1 and 8:2), 5/61–67 and 5/68–71 (eluted with cyclohexane–ethyl acetate 7:3). These fractions were further fractionated by RP-HPLC, using method 8. The UV absorption of the compounds allowed the final separation and purification by HPLC with UV detection at 235 and 273 nm. The compound from fractions 5/48-57, observed at a retention time of 21.1 min, crystallized from methanol as colourless prisms, and yielded ev-3 (13.8 mg). From fractions 5/61-67, a compound was crystallized, which was further purified by RP-HPLC, using method 8, to yield 9.4 mg of ev-1 ($t_R$ 27.6 min). Fractions 5/68–71, containing three main compounds, were separated by RP-HPLC, using method 8. The compound at $t_R$ 27.6 min was identical with ev-1 (1.6 mg). The compound at $t_R$ 24.7 min was isolated as ev-5 (3.0 mg). The fraction eluting at $t_R$ 16.6 min was subjected to NP-HPLC (method 4) to afford ev-7 (2.4 mg). Fraction 3, obtained with methanol–water 4:1 from the polyamide column, was repeatedly chromatographed on a
polyamide column with mixtures of methanol–water 3:2, 7:3 and 4:1 as eluents. Fraction 3/2, obtained with methanol–water 3:2, was separated by VLC on silica gel (column 6). Fractions 22-28, obtained from column 6 with cyclohexane–ethyl acetate 9:1 and 4:1, were further purified by RP-HPLC, using method 9, to afford 10.4 mg of ev-2 ($t_R$ 11.5 min.).

**Figure 4.** Isolation of diterpenes from *Euphorbia villosa*

### 4.3. Characterization and structure determination of the isolated compounds

The structure elucidation was performed by means of UV, NMR and mass spectroscopy and, in a few instances, IR spectroscopy and X-ray crystallography. Some physical data on the isolated compounds are listed in Table 1.

IR and UV spectroscopy provided only a little information on the molecules. The IR absorption bands at 3743–2983 cm$^{-1}$ and at 1749–1711 cm$^{-1}$ indicated the presence of hydroxy and C=O groups (ester and keto) in the molecules. The UV spectral data on the
compounds revealed the presence of aromatic ester groups (Table 1). UV maxima at 217-225 and 264 nm, characteristic of the pyridine ring, indicated a nicotinoyl group in ep-1, ep-3 and ep-7. In the case of benzoyl substitution (ep-2, ep-4, ep-5, ep-8, ev-1, ev-2 and ev-5), UV maxima were detected at 229-233 and 271-275 nm and a shoulder at 280-282 nm.

Table 1. Yields and physical data of the isolated compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield (mg)</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm) (log ε)</th>
<th>Optical rotation [α]&lt;sub&gt;D&lt;/sub&gt; (c)</th>
<th>Mp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>eup-1</td>
<td>300.0</td>
<td>202 (4.73), 219 sh (4.08)</td>
<td>-82 (0.1)</td>
<td>218-219</td>
</tr>
<tr>
<td>eup-4</td>
<td>40.0</td>
<td>210 (4.88), 284 (3.89)</td>
<td>-101 (0.1)</td>
<td>248-249</td>
</tr>
<tr>
<td>eup-6</td>
<td>1.2</td>
<td>209 (4.84), 273 (4.02)</td>
<td>+11 (0.06)</td>
<td>-</td>
</tr>
<tr>
<td>eup-10</td>
<td>5.1</td>
<td>204 (2.72), 267 (2.77)</td>
<td>-95 (0.1)</td>
<td>-</td>
</tr>
<tr>
<td>eup-11</td>
<td>10.0</td>
<td>216 (3.26), 296 (2.19)</td>
<td>-99 (0.6)</td>
<td>154-155</td>
</tr>
<tr>
<td>eup-12</td>
<td>34.5</td>
<td>230 (3.96), 275 (2.92)</td>
<td>-68 (1.1)</td>
<td>205-207</td>
</tr>
<tr>
<td>eul-1</td>
<td>2.3</td>
<td>218 (3.63), 280 (3.92)</td>
<td>+76 (0.1)</td>
<td>-</td>
</tr>
<tr>
<td>ep-1</td>
<td>10.2</td>
<td>225 (3.67), 264 (2.96)</td>
<td>+53 (0.51)</td>
<td>182-185</td>
</tr>
<tr>
<td>ep-2</td>
<td>2.3</td>
<td>229 (4.12), 274 (2.99), 282 sh (2.88)</td>
<td>+40 (0.2)</td>
<td>238-240</td>
</tr>
<tr>
<td>ep-3</td>
<td>2.7</td>
<td>217 (3.96), 264 (3.27)</td>
<td>-33 (0.1)</td>
<td>-</td>
</tr>
<tr>
<td>ep-4</td>
<td>4.7</td>
<td>230 (3.71), 272 (2.72), 280 sh (2.67)</td>
<td>-10 (0.19)</td>
<td>-</td>
</tr>
<tr>
<td>ep-5</td>
<td>3.1</td>
<td>230 (3.64), 271 (2.75), 281 sh (2.71)</td>
<td>+8 (0.3)</td>
<td>220-223</td>
</tr>
<tr>
<td>ep-7</td>
<td>3.8</td>
<td>223 (3.60), 264 (2.98)</td>
<td>+27 (0.04)</td>
<td>178-180</td>
</tr>
<tr>
<td>ep-8</td>
<td>13.9</td>
<td>230 (4.11), 272 (3.08), 280 sh (3.03)</td>
<td>+5 (0.06)</td>
<td>225</td>
</tr>
<tr>
<td>ev-1</td>
<td>11.0</td>
<td>230 (3.68), 275 (2.91), 282 sh (2.85)</td>
<td>-3° (0.1)</td>
<td>246-248</td>
</tr>
<tr>
<td>ev-2</td>
<td>10.4</td>
<td>233 (3.36), 271 (3.17)</td>
<td>-146° (0.1)</td>
<td>-</td>
</tr>
<tr>
<td>ev-3</td>
<td>13.8</td>
<td>214 (3.56), 277 (3.76)</td>
<td>+148° (0.2)</td>
<td>-</td>
</tr>
<tr>
<td>ev-5</td>
<td>3.0</td>
<td>232 (3.51), 275 (2.68), 282 sh (2.67)</td>
<td>-38° (0.1)</td>
<td>-</td>
</tr>
<tr>
<td>ev-7</td>
<td>2.4</td>
<td>231 (3.49), 259 (3.08), 285 sh (2.43)</td>
<td>+16° (0.033)</td>
<td>-</td>
</tr>
</tbody>
</table>

Optical rotation in chloroform at 25 °C (t = 28 °C); Mp. (melting point)

The HRMS measurements revealed the exact masses and molecular compositions of the compounds. The fragment ions observed in the mass spectra indicated the sequential loss of esterifying acids (acetic, benzoic, nicotinic and isobutyric acids) from the parent ions. The most useful data regarding the chemical structures of the compounds were obtained from advanced 1D and 2D NMR experiments, including HNMR, JMOD, 1H-1H COSY, NOESY, HMQC, HSQC and HMBC spectroscopy. For eup-1, eup-4, ep-5 and ep-8, X-ray diffraction analysis was performed, and from these experiments the absolute configurations of eup-1, ep-5 and ep-8 were determined.

Ep-3 (1)

The molecular formula of ep-3 was assigned via HREIMS and NMR investigations as C<sub>34</sub>H<sub>33</sub>NO<sub>11</sub>. The 1H and 13C NMR spectra of ep-3 revealed the presence of 1 nicotinoyl (Nic) and 4 acetyl groups (Table 1 in Appendix V). Additionally, the 1H and 13C NMR spectra contained resonances for skeletal carbons and protons, which were assigned on the basis of
the interpretation of the $^1$H-$^1$H COSY and HMQC spectra. The detected proton and carbon connectivities demonstrated that a diterpene core containing 20 carbons is present, consisting of 4 methyls, 1 trans-disubstituted olefin, 1 exocyclic and 2 alkyl-substituted methylenes, 7 methine groups, 1 ketone, and 3 quaternary carbons. The $^1$H-$^1$H COSY spectrum revealed 3 structural fragments with correlated protons: $\delta_H$ 3.01 d, 1.65 d, 2.22 m, 0.90 d (3H), 5.42 t, 2.71 d and 5.81 s [–CH$_2$–CH(CH$_3$)–CH(OR)–CH–CH(OR)–] (A); $\delta_H$ 4.84 dd, 2.18 m (2H) and 5.09 dd, [–CH(OR)–CH$_2$–CH(OR)–] (B); $\delta_H$ 5.94 d, 5.65 dd, 3.53 dq and 1.20 d (3H) [trans –CH=CH–CH(CH$_3$)–] (C) (R = acyl). Further, in the $^1$H-$^1$H COSY spectrum, $^4$J couplings were detected between the exomethylene [$\delta_H$ 5.07 s (2H) (H-17)] and 2 acyloxymethines [$\delta_H$ 5.81 s (H-5) and 4.84 dd (H-7)], indicating the linkage of sequences A and B. The remaining connectivities were established by inspection of the long-range C-H correlations observed in an HMBC spectrum (Table 1 in Appendix V). The correlation of the quaternary carbon signal at $\delta_C$ 92.9 (C-15) with the proton signals at $\delta_H$ 3.01, 1.65 (H-1$\alpha$, $\beta$), 2.22 (H-2), 2.71 (H-4) and 5.81 (H-5) showed that structural fragment A comprises a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes. Cross-peaks between the carbon signal at $\delta_C$ 40.7 (C-10) and the proton signals at $\delta_H$ 2.18 (H-8), 5.94 (H-11), 5.65 (H-12), 1.13 (H-18) and 1.11 (H-19) indicated that structural parts B and C comprised the twelve-membered ring of a jatrophane diterpene. The two and three-bond correlations between the carbon signal at $\delta_C$ 212.6 (C-14) and the proton signals at $\delta_H$ 3.01, 1.65 (H-1$\alpha$, $\beta$), 2.71 (H-4), 5.65 (H-12), 3.53 (H-13) and 1.20 (H-20) fixed the location of the keto group at C-14. The positions of the ester groups were also determined via the HMBC experiment by evaluation of the $J_{C-H}$ couplings between the oxymethine protons and the ester carbonyl carbons. Acetyl groups were placed at C-3, C-5 and C-7, and the nicotinoyl group at C-9. The remaining acetyl group ($\delta_H$ 2.11), which did not exhibit any long-range correlations, was of necessity situated on the quaternary carbon C-15. All of the above data were compatible with the structure of ep-3 being 3,5,7,15-tetraacetoxy-9-nicotinoyloxy-14-oxojatropa-6(17),11-diene.

The stereochemistry of ep-3 was assessed by analysing the coupling constants and the results of a NOESY experiment (Table 1 in Appendix V). Starting from the $\alpha$ position of the proton at the ring junction (H-4), it was found that a $\beta$-oriented methyl group is present on C-2 and a $\beta$-oriented ester group on C-3, with regard to the diagnostic NOE effects between H-4/H-1$\alpha$,.
H-1β/H-16, H-4/H-2 and H-4/H-3. The position of H-5 was concluded on the basis of the zero coupling constant between H-4/H-5 to be β, similarly as in the case of other jatrophanes.\textsuperscript{80,157,158} The NOE interaction between H-4/H-7 required a β-oriented acyl group on C-7. The NOESY cross-peak between H-5/H-13 dictated the β position of H-13. The coupling constants of H-9 (\(J = 2.7, 7.9\) Hz) were found to be similar to those of eup-11\textsuperscript{158} and 2α,3β,5α,7β,15β-pentaacetoxy-9α-nicotinoxyloxy-14-oxojatropha-6(17),11-diene,\textsuperscript{80} with a 9α-oriented ester group. Such a configuration of C-7 and C-9 resulted in an upfield-shifted acetyl group on C-7, when an aromatic substituent is present on C-9.\textsuperscript{80} In ep-3, the 7β-O-acetyl signal was observed at \(δ_H\) 1.52, because of the effect of the 9α-O-nicotinoxy group. With regard to the above data, the structure of this compound is formulated as 1.

![Chemical Structure](image)

1

Ep-1 (2), ep-4 (3), ep-5 (4) and ep-7 (5)

From the dichloromethane extract of \textit{E. peplus}, compounds ep-1 (2), ep-4 (3), ep-5 (4) and ep-7 (5) were also isolated and fully characterized by means of EIMS, HREIMS and 1D and 2D NMR spectroscopy, since these compounds had not been described earlier. In 1998, while our work was in progress, JAKUPOVIC \textit{et al.} reported jatropane diterpenes from \textit{E. peplus}.\textsuperscript{80} Among the diterpenes reported by JAKUPOVIC’s group, compounds 2, 3, 4 and 5 were found to be identical with ep-1, ep-4, ep-5 and ep-7, respectively. The stereochemistry of ep-5 (4) was analysed by X-ray crystallography and the absolute configuration was established for the first time as \((2R,3R,4S,5R,7S,8S,9S,13S,14S,15R)-2,5,9,14-tetraacetoxy-3-benzyloxy-8,15-dihydroxy-7-isobutanoyloxyjatropha-6(17),11E-diene.\) The determined solid-state conformation agreed well with the calculated conformation published by JAKUPOVIC \textit{et al.}\textsuperscript{80}
Ep-8 (6)

Ep-8 was obtained as white needles. Mass spectrometry and detailed NMR investigations indicated that ep-8 was identical with the pepluane diterpene isolated earlier by JAKUPOVIC et al. from the same plant.\textsuperscript{80} As a result of our work, some missing physical and spectral data were reported and the stereochemistry of the compound was revised by means of NOESY experiments and X-ray crystallography.

The NOESY spectrum of ep-8 demonstrated trans-fused A/B rings, as NOE effects were detected between 16-OH/H-1\textbeta, 16-OH/H-17, 16-OH and ortho-benzoyl protons, H-4/H-3, and H-3/H-2. These NOE interactions revealed a \beta-oriented methyl group on C-2 and a benzoyle group on C-3. The cross-peaks between H-4/H-18 and H-18/H-20 in the NOESY spectrum proved a cis B/C ring junction in the molecule. The NOESY correlations between 16-OH/H-15 and 16-OH/H-5 indicated the presence of ester groups in the \alpha position on C-5 and C-15. The 16-OH group also showed a NOE effect with H-13, from which the \beta orientation of H-13 was concluded. In the NOESY experiment, correlative signals were observed between 8-OAc/H-13 and between 8-OAc and ortho- and meta-benzoyle protons, pointing to cis-fused C/D rings, in contrast with the proposal of JAKUPOVIC et al.\textsuperscript{80} The stereochemistry of H-9 and C-11 could not be established on the basis of the NOESY spectrum, because of the missing diagnostical NOESY correlations.
In order to determine the complete relative and absolute configurations and solid-state conformation of ep-8, single-crystal X-ray analysis was performed (in collaboration with the Institute of Chemistry of the Chemical Research Centre of the Hungarian Academy of Sciences). The perspective view of ep-8 (Figure 2 in Appendix IV) depicts the absolute stereochemistry of the compound. In the crystal lattice, two conformers (I and II) were detected, which form dimers through O-H...O hydrogen-bonds. The two conformers, which differ only in the internal rotations of the 9-OAc and 11-OAc groups, unambiguously demonstrated an \( \alpha \)-oriented acetyl group on C-9 (in contrast with the structure published in ref. \(^{80}\)) and a \( \beta \)-oriented one on C-11. Additionally, it was found that ring B assumed a chair conformation and ring D a boat conformation, differing from the PCMODEL-calculated conformation in ref. \(^{80}\).

\[ \text{Ep-2 (7)} \]

Ep-2 gave a parent ion in the EIMS at \( m/z \) 658, appropriate for a molecular formula of \( \text{C}_{35}\text{H}_{46}\text{O}_{12} \). The \(^1\)H and \(^{13}\)C NMR spectra of ep-2 revealed the presence of 4 acetate and 1 benzoate groups (Table 1 in Appendix IV). Additionally, the spectra exhibited resonances closely related to that of ep-8 (6). After the \(^1\)H and \(^{13}\)C NMR data on ep-2 had been assigned by analysis of its \(^1\)H-\(^1\)H COSY, HMQC and HMBC spectra, it was obvious that compounds ep-8 and ep-2 were based on the same parent system and differed only in the esterification. The absence of 1 acetate signal and the appearance of 1 hydroxy signal (\( \delta_{\text{H}} \) 2.82 s) indicated the replacement of 1 acetyl residue by a hydroxy group. Comparison of the \(^1\)H and \(^{13}\)C NMR signals of ep-8 and ep-2 revealed a significant difference in the chemical shifts of C-11 (ep-8: \( \delta_{\text{C}} \) 79.8, ep-2: \( \delta_{\text{C}} \) 68.0), from which the position of the hydroxy group on C-11 was concluded. This was substantiated by the occurrence of long-range correlation between 11-OH/C-19 in the HMBC spectrum. A careful comparison of the NOESY spectra of
compounds ep-8 and ep-2 permitted determination of the same stereochemistry for ep-2 (7) as that of ep-8 (6).

**Eup-1 (8), eup-4 (9), eup-6 (10), eup-11 (11), eup-10 (12) and eup-12 (13)**

The NMR spectral analyses of eup-1 (8), eup-4 (9), eup-6 (10), eup-11 (11), eup-10 (12) and eup-12 (13) were reported in detail in the Ph.D. thesis of G. Günther.\(^{159}\)

**Eup-1** (esulatin A), which formed colourless crystals, was shown by elemental analysis and ESIMS to have the molecular formula C\(_{36}\)H\(_{50}\)O\(_{16}\). \(^1\)H and \(^{13}\)C NMR, \(^1\)H-\(^1\)H COSY, HMBC and NOESY spectral data are given in Table 1 in Appendix I. The absolute configuration of eup-1 (8) was determined by means of X-ray analysis to be (2\(R\),3\(R\),4\(S\),5\(R\),7\(S\),8\(R\),9\(S\),11\(R\),12\(S\),13\(R\),15\(R\))-2,3,5,8,9,15-hexaacetoxy-11,12-epoxy-7-(isobutanoyloxy)jatropha-6(17)-en-14-one.\(^{160}\)

![Diagram of Eup-1](image)

**Eup-4** (esulatin B) was isolated as colourless crystals, and had a molecular formula of C\(_{30}\)H\(_{40}\)O\(_{12}\), obtained from HREIMS (m/z [M]\(^+\) 592.2631; calcd. for 592.2520) and NMR analyses. The structure of eup-4 was formulated as 9 on the basis of the \(^1\)H and \(^{13}\)C NMR, \(^1\)H-\(^1\)H COSY, HMBC and NOESY spectral data (Table 2 in Appendix I). The stereochemistry of eup-4 was investigated by X-ray analysis.\(^{161}\) From the crystallographic data, the same configuration of C-2, C-3, C-4, C-5, C-7, C-13, C-15 was deduced for eup-4 (9) as that for eup-1 (8).

![Diagram of Eup-4](image)
Eup-6 (esulatin C), a minor diterpenoid of *E. esula*, was isolated as an amorphous white solid. HREIMS \(m/z\) [M–AcOH]⁺ 694.2839; calcd. for 694.2837) and NMR analyses revealed the molecular formula C_{36}H_{50}O_{17}. 1D and 2D NMR data are reported in Table 3 in Appendix I. The stereochemistry of C-7, C-8, C-9, C-11 and C-13 could not be determined on the basis of the *Overhauser* effects because of the high flexibility of this part of the molecule. The structure of eup-6 was elucidated as shown in formula 10.

![Formula 10](image.png)

Eup-11 (esulatin D) was obtained as colourless crystals from methanol. Its molecular formula \(\text{C}_{32}\text{H}_{44}\text{O}_{13}\) was derived from HREIMS and NMR analyses. \(^1\text{H}\) and \(^{13}\text{C}\) NMR, \(^1\text{H}-^1\text{H}\) COSY, HMBC and NOESY spectral data are to be found in Table 1 in Appendix II. With regard to these data, the structure of eup-11 is formulated as 11.

![Formula 11](image.png)

Eup-10 (esulatin E) was obtained as an amorphous white solid. Its molecular formula was assigned via HRMS and NMR analyses as C_{28}H_{36}O_{10} (EIIMS \(m/z\) [M]⁰ 532). The \(^1\text{H}\) and \(^{13}\text{C}\) NMR chemical shifts of eup-10 were assigned by means of \(^1\text{H}-^1\text{H}\) COSY, HMQC and HMBC spectral analysis. The relative configuration of eup-10 was investigated by means of NOESY measurements (Table 2 in Appendix II). The structure of eup-10 (12) was elucidated to be 2\(\alpha\),3\(\beta\),5\(\alpha\),15\(\beta\)-tetraacetoxyjatropha-6(17),7\(E\),11\(E\)-triene-9,14-dione.

![Formula 12](image.png)

Eup-12 (esulatin F) gave a parent ion at \(m/z\) 722.3200 on HREIMS, corresponding to the molecular formula C_{36}H_{50}O_{15}. \(^1\text{H}\) and \(^{13}\text{C}\) NMR, HMBC and NOESY spectral data are given in Table 1 in Appendix III. The relative configuration of eup-12 was the same as that found in
eup-1, eup-4 and eup-11. All of the spectral data led to the formulation of eup-12 as 13. The conformation of eup-12 was studied in three different solvents by means of NMR experiments. Dynamic NMR studies revealed conformational flexibility of the 12-membered ring. Two main conformers, a planar and a bent one, were identified (Figure 6 in Appendix III), and the energetic parameters of the dynamic process were determined.

**Eul-1 (14)**

Eul-1 was shown by HREIMS to have the molecular formula C_{26}H_{38}O_{7}. The $^1$H and $^{13}$C NMR spectra of eul-1 (Table 1 in Appendix VI) revealed the presence of 1 acetate group and 1 butanoate group. Characteristic signals at $\delta_H$ 1.10 dd (1H), 1.50 dd (1H), 1.06 s (3H) and 1.05 s (3H), and at $\delta_C$ 35.9, 28.3, 25.2, 28.3 and 15.7 suggested a gem-dimethyl-substituted cyclopropane ring, present in many types of diterpenes from plants in the Euphorbiaceae. The $^1$H NMR spectrum exhibited 15 further signals due to the parent skeleton, which were assigned with the aid of 2D experiments. The HMQC correlations indicated, besides ester groups, the presence of 14 protonated carbons: 6 methines, 4 methylenes and 4 methyls. The $^1$H-$^1$H COSY spectrum revealed the presence of the systems –CH\textsuperscript{2}–CH(CH\textsubscript{3})–CHR–CH–CHR– and –CH\textsuperscript{2}–CH\textsuperscript{2}–CH–CH– (R = acyl) in eul-1, which represented the structural moieties C-1–C-5 and C-7–C-11 of a lathyrane diterpene, respectively.

The HMBC spectrum of eul-1 showed correlative signals confirming the connection of the assignments of these structural parts. Further, the $^2$J\textsubscript{CH} and $^3$J\textsubscript{CH} correlations of H-4, H-5 and H-17a,b confirmed the presence of an exomethylene (C-17) group at C-6. The cross-peak between C-14/H-4 indicated that the keto group must be sited at C-14. Moreover, the HMBC correlations between C-14/H-20, C-12/H-20, C-13/H-20, C-13/H-11 and C-11/12-OH proved the C-12–C-14 structural part as depicted in formula 14.

The positions of the butanoyl and acetyl groups in eul-1 were also established via the HMBC experiment, which demonstrated the correlation of the butanoyl carbonyl signals with H-3, H-2’ and H-5, and the acetyl methyl protons. All of the above data are compatible with the structure of eul-1 being 5-O-acetyl-3-O-butanoyl-12-hydroxylathyrol.

The relative stereochemistry of eul-1 was studied by means of NOESY measurements. The trans-linked cyclopentane ring and the β-oriented OBu-3 and CH\textsubscript{3}-16 groups followed from the NOE interactions between H-1\textbeta, H-16 and OH-15 and between H-1β, H-2 and H-4. The cross-peak between H-4/H-20 in the NOESY spectrum required that the methyl group on
C-13 be oriented below the plane of the molecule. The NOE effect observed between H-20/H-11 revealed a *trans* configuration for the C-12/C-13 double bond. The correlative signals between H-11/H-9 dictated a *cis*-fused cyclopropane ring with α stereochemistry of H-9 and H-11, which is usual in lathyrane diterpenoids. The stereochemistry of H-5 was concluded to be β from its NOESY cross-peak with OH-12, both oriented above the plane of the molecule. Accordingly, the structure of this compound was elucidated to be as shown in formula **14**.

The demonstrated enolic form of eul-1 (14) is presumably stable because of hydrogen bonding, which probably exist between the 14-keto group and 12-OH. A similar enol structure was presented in the presumed biogenetic route of jatrophastrione by TORRANCE *et al.*

**Ev-3** (15)

Ev-3 (15) was found to be identical in all of its characteristics, including the $^1$H and $^{13}$C NMR spectral data, with the lathyrane diterpene isolated earlier from *Euphorbia hyberna*.63

**Ev-1** (16)

The molecular formula of ev-1 was C$_{30}$H$_{40}$O$_7$, established by HREIMS, which showed a molecular ion peak at $m/z$ 512.2806 [M]$^+$. The $^1$H NMR and JMOD spectra of ev-1 revealed the presence of 1 acetate, 1 benzoate and 1 methoxy group (Table 1 in Appendix VII). The JMOD and HMQC spectra suggested that the skeleton contained 20 carbons: 5 methyls, 3 methylenes, 7 methines and 5 quaternary carbons, with 1 ketone. The gradient $^1$H-$^1$H COSY spectra demonstrated 2 structural fragments with correlated protons: –CH$_2$–CH(CH$_3$)–CH(OR)–CH–CH(OR)– (A) and –CH$_2$–CH$_2$–CH–CH–CH(OR)– (B) (R = H, acyl or methyl).

The sequences A and B, tertiary methyls and quaternary carbons were connected by inspection of the long-range C-H correlations observed in a gradient HMBC spectrum. Figure 5 presents the main HMBC correlations, which allowed the construction of the planar
structure of ev-1 as a tetracyclic euphoractine-type diterpene with a 5-6-6-4 fused ring system. The positions of the acetyl, methoxy and hydroxy groups were also established via heteronuclear two and three-bond correlations. The benzoyl group, which did not show any HMBC correlations, was placed of necessity at the quaternary C-15. The relative configurations of the 10 stereogenic centres in ev-1 were investigated in a phase-sensitive NOESY experiment (Table 1 in Appendix VII), aided by consideration of the coupling constant values. Conventionally, as reference point, the position of H-4 was chosen to be \( \alpha \). The NOESY correlations observed between H-4/H-3, H-4/H-1\( \alpha \), H-4/5-OH, and H-4/H-17 indicated the \( \alpha \) stereochemistry of H-3, H-1\( \alpha \), 5-OH and H-17. The NOE interaction between H-1\( \alpha \)/H-16 suggested the \( \alpha \) position of H-16. A diagnostic NOE interaction was detected between H-5/H-2'.6' (benzoyl), proving the \( \beta \) position of the benzoyl group. The nuclear Overhauser effects observed between H-2',6'/H-5, H-2',6'/H-12, H-5/H-12, H-12/H-19 and H-1\( \beta \)/H-2 provided evidence of the \( \beta \) orientation, while the NOEs of H-11/H-9 and H-18/H-20 were indicative of the \( \alpha \) position of H-9, H-18 and H-20.

**Ev-7 (17)**

HRFABMS suggested that the molecular formula of ev-7 is \( \text{C}_{29}\text{H}_{39}\text{O}_7 \), with a molecular ion peak at \( m/z \ 499.2704 \ [\text{M+H}]^+ \). After the \(^1\text{H}\) and \(^{13}\text{C}\) chemical shift assignments (compound 2 in Table 2 in Appendix VII) of ev-7 had been achieved via the \(^1\text{H}-^1\text{H}\) COSY, HSQC and HMBC spectra, it was evident that compounds ev-1 and ev-7 differ in only one substituent: the methoxy group of ev-1 (16) is replaced by a hydroxy group in ev-7. The diamagnetically shifted C-11 signal (ev-7: \( \delta_{C-11} \ 73.6 \); ev-1: \( \delta_{C-11} \ 82.7 \)) demonstrated a hydroxy group instead of a methoxy group on C-11 in ev-7. The relative configuration of ev-7 was analysed in a NOESY experiment. The diagnostic nuclear Overhauser effects detected between H-2',6'/H-5, H-2',6'/H-12, H-2',6'/H-19, H-5/H-12, H-12/H-19 and H-1\( \beta \)/H-2 provided evidence of the \( \beta \) orientation, while the NOESY cross-peaks between H-1\( \alpha \)/H-16, H-1\( \alpha \)/H-4,
H-4/H-3, H-4/H-17, H-17/H-20, H-20/H-9, H-9/H-18, H-9/H-11 and H-11/H-20 pointed to the α position of these protons and groups. All of the above data indicate structure 17 for this compound.

**Ev-5 (18)**

The molecular formula (C₃₀H₄₀O₇), the substituents (1 benzoyl, 1 acetyl, 1 methoxy and 1 hydroxy) and structural fragments [–CH₂–CH(CH₃)–CH(OR)–CH–CH(OR)–] (A) and [–CH₂–CH₂–CH–CH–CH(OR)–] (B) (R = H, acyl or methyl)], 5 quaternary carbons and 4 methyls constructing the diterpene core were found to be the same as in the case of ev-1 (16) (compound 3 in Table 2 in Appendix VII). The difference between ev-1 and ev-5 was discovered by means of HMBC experiments, which showed long-range correlations between C-20/H-12, C-6/H-12 C-18/H-9 and C-18/H-11, proving a 5-6-7-3 condensed ring system (Figure 6).

![Figure 6. HMBC spectrum of ev-5 (18), with assignment of the main long-range correlations](image-url)
The long-range correlation between the hydroxy group and C-5 clearly indicated that the hydroxy group is located at C-5. The methoxy group was placed at C-12, as shown in the HMBC spectrum by the long-range correlation between the methoxy carbon and H-12. The position of the acetyl group was determined on the basis of the three-bond correlation between the ester carbonyl and H-3. Finally the benzoyl group, which did not display any long-range correlations, must be on C-15. The relative configuration of the molecule was investigated by a NOESY experiment. The overlapping of the $^1$H signals of H-2, H-4 and 3-OAc at $\delta_H 2.20$ did not allow a conclusion concerning the stereochemistry of H-4, but the coupling constants $J_{3,4}$ and $J_{4,5}$ in ev-5 were found to be very similar to those in ev-1 and ev-7, indicating the same $\alpha$ configuration of H-4. Further, the NOESY correlation of the overlapping signal at $\delta_H 2.20$ with that of H-17 can most probably be assigned to the H-4/H-17 correlation, suggesting the $\alpha$ orientation of H-17. Diagnostic Overhauser effects for the relative configuration of C-2, C-3, C-5, C-9, C-11, C-12, C-13 and C-15 are shown in Figure 6. All of the above data are compatible with structure 18 for this compound.

Ev-2 (19)

Ev-2, an amorphous solid, has the molecular formula C$_{29}$H$_{36}$O$_6$, determined via the molecular ion peak at $m/z$ 480.2526 [M]$^+$ in the HREIMS. Analysis of the $^1$H NMR and JMOD data revealed that ev-2 possesses 1 benzyol and 1 acetyl group in the molecule. The diterpene core consists of 5 methyls, 3 methylenes, 7 methines (1 vinylic, $\delta_C$ 144.5) and 5 quaternary carbons, with 1 ketone ($\delta_C$ 194.4) (compound 4 in Table 2 in Appendix VII). Interpretation of the HSQC and $^1$H–$^1$H COSY spectra led to the identification of 3 structural elements with correlated protons: $-\text{CH}_2-\text{CH}_(\text{CH}_3)-$, $-\text{CH}(\text{OR})-\text{CH}-\text{CH}-$ and $-\text{CH}_2-\text{CH}_2-\text{CH}-\text{CH}-\text{CH}=(\text{R = acyl})$. Their connectivities were determined on the basis of the HMBC spectrum. The long-range correlations of the quaternary carbons with the protons of the 3 fragments
(C-6/H-4, C-6/H-7, C-6/H-17, C-10/H-9, C-10/H-11, C-10/H-18, C-10/H-19, C-13/H-11, C-13/H-20, C-14/H-1, C-14/H-4, C-14/H-20, C-15/H-1, and C-15/H-3) indicated the bicyclic lathyrane ring system with oxygen functions at C-3, C-5, C-6, C-14 and C-15, and a double bond between C-12/C-13. The three-bond correlation between H-12 (δ_H 6.93 dd) and the keto signal placed the keto group at C-14. The chemical shifts of C-5 (δ_C 57.8), C-6 (δ_C 63.4) and H-5 (δ_H 3.53) were indicative of an epoxy group at C-5–C-6. The HMBC correlation between acetyl CO and H-3 pointed to the presence of the acetyl group at C-3, and the benzoyl group was of necessity located on C-15. The relative configuration of ev-2 was determined on the basis of a NOESY experiment. Because of the overlapping of the proton signals in CDCl_3 solution, the ^1H NMR, JMOD, HSQC and NOESY spectra were also run in C_6D_6, which resulted in better-resolved spectra and unambiguous assignments of all the ^1H and ^13C NMR signals. Starting from the α position of H-4, the nuclear Overhauser effects between H-4/H-1α, H-1α/H-16 and H-1α/H-3 indicated the α orientation of H-3 and H-16. The observed correlations of the ortho-benzoyl protons with H-5, 3-OAc and H-19, and between H-12/H-19 proved the β position of H-5 and H-19 and the acetyl group at C-3. The NOE interactions between H-5/H-7β, H-5/H-8β and H-5/H-12 allowed the steric differentiation of the C-7 and C-8 methylene protons and suggested that H-12 is oriented above the plane of the molecule. The NOE interactions between H-18/H-11 and H-18/H-9 suggested α-oriented H-11 and H-9. As regards the stereochemistry of C-17, the NOESY correlations between H-17/H-4, and H-17/H-7α were informative, proving the α orientation of the 17-methyl group. The NOE interaction between H-11/H-20 revealed that H-20 is oriented below the plane of the macrocycle, thereby confirming the E configuration for the C-12/C-13 double bond. The above NMR study demonstrated that the structure of ev-2 is 19.
5. **Discussion**

Phytochemical investigations of *E. esula*, *E. peplus*, *E. lathyris* and *E. villosa* led to the isolation of 19 diterpenes, including 13 new natural products. The structures were established by means of spectral analyses as esters of jatrophane, lathyrane, pepluane and euphoractine-type diterpenes. Biological investigations revealed that some of the isolated compounds possess noteworthy pharmacological activities (antiviral, antiproliferative and multidrug resistance reversing).

**Isolation of diterpenes**

- Screening of *E. esula*, *E. peplus*, *E. lathyris* and *E. villosa* led to the conclusion that the lipophilic extracts contain a number of diterpene esters, which can be enriched by polyamide OCC, mainly in the 60 % aqueous methanol fraction. The purification of the compounds generally requires the involvement of multistep separation methods because the plants produce complex mixtures of esters of the same diterpene nucleus (they may display very similar chromatographic characters), and the compounds occur merely in low quantities in the plants.

- The fresh or dried whole (except in the case of *E. lathyris*) plant material was extracted with methanol at room temperature by percolation. Methanol, an amphipolar solvent, suitable for the extraction of both lipophilic and polar compounds.

- In the initial step of separation, liquid-liquid extraction with dichloromethane or chloroform was applied in order to remove the polar constituents. The purification was continued with a classical column chromatographic technique. In accordance with the screening investigations, polyamide proved to be suitable as stationary phase for the preparative work, with the use of methanol-water solvent systems. The 20-60 % methanol fractions were rich in diterpenes, the 80 % methanol fractions contained mainly triterpenes, and the 100 % methanol fractions yielded chlorophyll.

- In the following steps, even more selective methods (VLC, PLC and HPLC) were applied. After polyamide OCC, adsorption chromatography on silica gel was used in all experiments. VLC separations of the diterpene fractions afforded crude fractionations of the main components. For final purification, NP- and RP-HPLC were applied since these were
the most effective and most selective separation methods. HPLC provided mild conditions for polyester-type (light- and heat-sensitive) diterpenes.

- The preparative work was completed with analytical thin layer chromatography on silica gel with various solvent systems. The aims of the TLC analysis were to model the separation methods, to combine fractions, and to check the purity of the isolated compounds. The detection was carried out in UV light at 254 nm, followed by spraying with cc. \( \text{H}_2\text{SO}_4 \).

- As a result of the isolation procedure, 19 compounds, occurring in low concentration, were obtained from the multicomponent samples. In some cases, compounds with very similar structures were separated: ep-1, ep-5 and ep-7, differing from each other in the ester groups, and ev-1 and ev-7, differing in only one substituent. After extensive chromatographic purification, 6 compounds were isolated from *E. esula* (eup-1, eup-4, eup-6 and eup-10–12), 1 from *E. lathyris* (eul-1), 7 from *E. peplus* (ep-1–5 and ep-7–8), and 5 from *E. villosa* (ev-1, ev-2, ev-3, ev-5 and ev-7).

**Structure elucidation**

- The isolated compounds are amorphous solids or crystals. They are optically active. The structures of the isolated compounds were elucidated by means of spectroscopic methods. IR and UV spectroscopy provided only a little information on the structures. From MS measurements, the molecular compositions were determined. The most useful data concerning the chemical structures were furnished by the 1D and 2D NMR spectroscopy. From the \(^1\)H and \(^{13}\)C NMR, \(^1\)H–\(^1\)H COSY, HMQC and HMBC experiments, the constitutions of the compounds were determined, and then, with the aid of the NOESY spectra, the relative configurations were elucidated. In the isolated compounds, the number of asymmetric carbons was 7-10, and, except for eup-6 (10) all of them were stereochemically characterized. As a result of the NMR studies (\(^1\)H NMR, JMOD, DEPT, \(^1\)H–\(^1\)H COSY, HSQC, HMQC and HMBC), complete \(^1\)H and \(^{13}\)C assignments were made for the characterization of the compounds. The absolute configurations of ep-5 (4), ep-8 (6) and eup-1 (8) were determined by X-ray crystallography, and proved to be in accordance with the diterpenes described to date from natural sources.

- Structurally, 11 of the compounds are jatrophanes, 3 are lathyranes, 3 are euphoractine-type compounds and 2 are pepluane derivatives. Ep-1 (2), ep-3 (1) and ep-7 (5) contain nicotinoyl group, and can be regarded as pseudoalkaloids. Eup-4 (9), eup-10 (12), eup-11 (11)
and ev-3 (15) are esterified only with acetyl groups. The diversity of the ester groups is characteristic of the members of ep series, e.g. in ep-1 (2) 4 (acetyl, isobutanoyl, nicotinoyl and benzooyl), and in ep-5 (4) and ep-7 (5) 3 different ester groups were found. Eup-1 and eup-6 are the most highly esterified jatrophane-type diterpenoids, with 7 ester groups. The parent diterpene alcohol found in 8–10 had not been described before our work. Besides ester groups, hydroxy, keto and epoxy functions and, in the cases of ev-1 (16) and ev-5 (18) methoxy groups are present. In eup-6 (10), an ether function can be found between C-11 and C-14, and it therefore possesses an unusual heterocyclic ring system. Such compounds have been isolated only from *E. kansui* and *E. esula*.\(^{41}\) Eup-10 (12) is the only jatrophane diterpene with a 7,8-ene structure known at present. It is highly unsaturated, having a triene-dione structure. The eup series is stereochemically homogeneous, characterized by 2β-methyl, 13α-methyl and 3β, 7β, 5α, 8α and 9α-acyl substitution. The ep series is heterogeneous: the configuration of C-20 can be α or β (α in ep-2, ep-3 and ep-8, and β in ep-1, ep-4, ep-5 and ep-7). Among the lathyranes, the 12-enol structure and the n-butanoyl esterification of eul-1 (14) are irregular. Ev-2 (19) is a lathyrane derivative containing the rare 5,6-epoxy function; its parent diterpene alcohol has not been described previously. The pepluane skeleton was first reported while our work was in progress. On the basis of our X-ray examinations, the structure of the first pepluane, ep-8 (6), was revised. The pepluane skeleton is a new Euphorbiaceae diterpene carbon framework, in which the geminal dimethyl group (originating from a cembrene-casbene source) is absent, because one of them is incorporated in a six-membered ring.

- The skeleton of ev-1 (16) and ev-7 (17) was previously found only in 3 natural compounds, euphoractine A, C and D, while the skeleton of ev-5 (18) was known only in euphoractine B and E. A biogenetic relationship can be presumed between the isolated compounds of *E. villosa*, since lathyrane diterpenes are regarded as the biosynthetic progenitors of the polycyclic derivatives. Ev-1 (16), ev-7 (17) and ev-5 (18) can be derived from a lathyrane precursor by transannular cyclization and, in the cases of 16 and 17, by expansion of the cyclopropane ring. A similar rearrangement was observed in synthetic studies, when the acid-catalysed conversion of an epoxylathyrane enone (Euphorbia factor L1) was achieved.\(^{85,162}\)
Chemotaxonomical significance

- Diterpenes are considered to be important taxonomic markers of the Euphorbiaceae family, because of its limited occurrence and structural diversity.

- On the basis of the diterpene composition, *E. esula* displays a close relationship with *E. salicifolia*, which belongs in the same section. They contain the same main diterpene component, eup-1 (8), and jatrophone diterpenes differing only in the esterification.

- The diterpenes isolated from *E. esula* in our experiment are not identical with those obtained by other workgroups. The samples of different origins (China, North America and Hungary) contain different jatrophone diterpenes. In the eup series obtained from the Hungarian collection, the aromatic acyl residues were missing and the alcohol core of the compounds was different. In conclusion, the morphological diversity (characteristic of this species) is manifested in the chemical features (the diterpene profile), too.

- As concerns *E. peplus*, four different investigations on jatrophone and pepluane diterpenes have been reported. The diterpenes of the samples originating from Chile, Germany and Hungary were found to be similar; they contain 4 identical compounds: ep-1, ep-4, ep-11 and ep-8. However, in the examinations on an Italian sample, different compounds were isolated (only one ep-11 is common). These facts indicated that chemovarietas must presumably exist for *E. peplus*.

- In the case of *E. lathyris*, we investigated for the first time the diterpenes of the roots and detected the presence of lathyranes. The isolated compound, eul-1 (14), is related to those lathyrane diterpenes isolated earlier from the aerial parts and the seeds.

- The chemical constituents of *E. villosa* have not been investigated previously.

- All of the isolated diterpenes were detected for the first time in the given plants; 5 compounds from *E. peplus* (ep-1, ep-4, ep-5, ep-7 and ep-8) were reported at the same time as our work.

Biological activities

The isolated compounds were tested for their phlogistic, antitumour, antiviral and anti-MDR activities.

- Phlogistic activity: The aim of our investigations was to determine whether jatrophone diterpenes possess pro-inflammatory activity similarly to that of the ingenane and tigliane diterpenes. Irritant doses (ID$_{50}$) were determined on the ears of mice according to a standard
procedure. The redness of the mouse ear was estimated 4 and 24 h after the application of solutions in acetone. As a reference, 12-O-tetradecanoylphorbol-13-acetate (TPA) was used (ID$_{50}^4$ = 0.00938 μg/ear, ID$_{50}^{24}$ = 0.00645 μg/ear). Compounds ep-4 (3), eup-1 (8), eup-4 (9), eup-11 (11) and eup-12 (13) were shown to be inactive up to a dose of 200 μg/ear, while ep-1 (2) exhibited a pro-inflammatory activity of ID$_{50}^4$ = ID$_{50}^{24}$ = 29 μg/ear. Consequently, for the highly irritant effect of E. esula and E. peplus, which cause many toxicological problems, primarily the phorboids are responsible.$^{148,156}$

- **Antitumour activity:** As a few experimental data have been published previously on the antitumour activity of jatrophone derivatives,$^{44,79}$ the cytotoxic activities of some jatrophone esters were evaluated against HeLa and L5178 mouse lymphoma cells, using the MTT test. The results presented in Table 2 revealed a cell growth-inhibitory effect of compounds eup-1 (8), eup-4 (9) and eup-12 (13) against the L5178 cell line. Cytotoxic assays on HeLa cells demonstrated only marginal activities for compounds ep-1 (2), ep-5 (4) and eup-1 (8).

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>9</th>
<th>11</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition (%)</td>
<td>13.4</td>
<td>20.1</td>
<td>4.3</td>
<td>16.3</td>
<td>4.3</td>
<td>17.5</td>
<td>6.5</td>
<td>9.6</td>
<td>11.8</td>
</tr>
<tr>
<td>IC$_{50}^*$ (μM)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>14.8</td>
<td>9.5</td>
<td>–</td>
<td>8.5</td>
</tr>
</tbody>
</table>

$^*$on HeLa cells ($c = 10 \mu M$), 'on L5178 mouse lymphoma cells

- **Antiviral activity:** The reported antiviral activities of some Euphorbiaceae diterpenes prompted us to investigate the virus replication-inhibitory activities of the isolated compounds. First, the cytotoxicities of the diterpenes were assayed by measuring their effects on the growth of Vero cells. The antiviral activities were investigated at concentrations which were lower than the CC$_{50}$ values. The antiviral effects on the multiplication of *Herpes simplex* virus type 2 (HSV-2) were studied by using the virus yield reduction method in cell cultures. Significant antiviral activity was recorded for ep-3 (1), ep-4 (3), ep-5 (4) ep-7 (5) and ep-2 (7) (Table 3).$^{163}$ The effects of the diterpenes on the virus infectivity were investigated under extracellular conditions. None of the tested compounds displayed virucidal activity against HSV-2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>7</th>
<th>8</th>
<th>acyclovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC$_{50}$ (μM)</td>
<td>31.2</td>
<td>4.3</td>
<td>36.5</td>
<td>37.2</td>
<td>35.6</td>
<td>21.0</td>
<td>30.1</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>IC$_{50}$ (μM)</td>
<td>3.9</td>
<td>–</td>
<td>3.8</td>
<td>5.5</td>
<td>6.0</td>
<td>6.4</td>
<td>11.2</td>
<td>0.89</td>
</tr>
<tr>
<td>SI CC$<em>{50}$/IC$</em>{50}^* $</td>
<td>8.0</td>
<td>–</td>
<td>9.6</td>
<td>6.8</td>
<td>5.9</td>
<td>3.3</td>
<td>2.7</td>
<td>&gt;1125</td>
</tr>
</tbody>
</table>

$^*$50 % cytotoxic conc. on Vero cells, 'inhibitory conc. for 50 % yield reduction, 'selectivity index
Multidrug resistance reversal activity: In the past few decades, there has been great clinical interest in the development of drugs that overcome multidrug resistance (MDR) to cytostatic chemotherapeutic agents in cancer cells. Extensive studies have been performed in the search for new, effective resistance modulators or chemosensitizers from natural sources. Investigation of the isolated diterpenes for their anti-MDR activity revealed that some jatrophane, pepluane and lathyrane diterpenes are able to enhance drug retention in the cells by inhibiting the efflux-pump activity mediated by the P-glycoprotein. Especially the members of the ep and ev series [ep-3 (1), ep-1 (2), ep-7 (5), ev-3 (15) and ev-2 (19)] displayed marked effects when tested on multidrug resistant mouse lymphoma cells, using rhodamine 123 exclusion test and verapamil as positive control (Table 4).\(^\text{16}\)

Table 4. Reversal of the MDR of mouse lymphoma cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>$c$ (μg/ml)</th>
<th>R</th>
<th>Compound</th>
<th>$c$ (μg/ml)</th>
<th>R</th>
<th>Compound</th>
<th>$c$ (μg/ml)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ep-3 (1)</td>
<td>4</td>
<td>34.74</td>
<td>ep-7 (5)</td>
<td>4</td>
<td>12.75</td>
<td>eup-11 (11)</td>
<td>4</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>74.27</td>
<td></td>
<td>40</td>
<td>71.98</td>
<td></td>
<td>8</td>
<td>4.03</td>
</tr>
<tr>
<td>ep-1 (2)</td>
<td>4</td>
<td>34.25</td>
<td>ep-2 (7)</td>
<td>4</td>
<td>4.71</td>
<td>eup-12 (13)</td>
<td>4</td>
<td>5.52</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>78.88</td>
<td></td>
<td>40</td>
<td>38.37</td>
<td></td>
<td>40</td>
<td>14.75</td>
</tr>
<tr>
<td>ep-4 (3)</td>
<td>4</td>
<td>16.77</td>
<td>eup-1 (8)</td>
<td>4</td>
<td>2.03</td>
<td>ev-3 (15)</td>
<td>1</td>
<td>8.90</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>29.48</td>
<td></td>
<td>40</td>
<td>1.93</td>
<td></td>
<td>10</td>
<td>44.16</td>
</tr>
<tr>
<td>ep-5 (4)</td>
<td>4</td>
<td>6.30</td>
<td>eup-4 (9)</td>
<td>4</td>
<td>2.58</td>
<td>ev-2 (19)</td>
<td>1</td>
<td>3.46</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>2.26</td>
<td></td>
<td>40</td>
<td>7.83</td>
<td></td>
<td>10</td>
<td>50.80</td>
</tr>
</tbody>
</table>

$R = \text{fluorescence activity ratio}$ [the fluorescence activity ratio of verapamil ($c = 10 \mu g/ml$) was 6.65] \n
From the above results, it could be concluded that jatrophane diterpenes do not play a significant role in the irritant effect of $Euphorbia$ species; they should rather be considered therapeutically relevant natural products.
6. SUMMARY

The aim of this work was the isolation and structure determination of diterpenes from *Euphorbia esula*, *E. peplus*, *E. lathyris* and *E. villosa*. First, the diterpene contents of the plant materials were investigated by using a CC, TLC-based screening method. The isolation was carried out by a multistep separation procedure, including OCC, VLC, PLC and NP- and RP-HPLC. The structures of the isolated compounds were elucidated by means of spectroscopic methods (IR, UV, HR-MS and NMR), and in a few instances X-ray crystallography. As a result of 2D NMR studies (COSY, HMBC, HSQC, HMQC), JMOD and DEPT, complete $^1$H and $^{13}$C assignments were made for the characterization of the compounds.

As a result of our work, 6 new (8-13) diterpene polyesters were isolated from *E. esula*, 3 new ones (1, 6-7) and 4 known ones (2-5) from *E. peplus*, 1 new one (14) from *E. lathyris*, and 4 new ones (16-19) and 1 known one (15) from *E. villosa*. 11 compounds are of jatrophane, 3 of lathyrane and 3 of euphoractine-type, and 2 are pepluane polyesters. Ep-3 (1), ep-1 (2) and ep-7 (5) contain nicotinoyl group, and they are pseudoalkaloids. Eup-4 (9), eup-11 (11), eup-10 (12) and ev-3 (15) are homogeneously esterified (only with acetyl groups), but others contain different acyl groups (benzoyl, nicotinoyl, isobutanoyl and n-butanoyl). Ep-1 (2) is the most diverse substituted diterpene, with 4 different ester groups (acetyl, benzoyl, i-butanoyl and nicotinoyl). Eup-1 (8) and eup-6 (10) are the most highly esterified jatrophane-type diterpenoids, with 7 ester groups. In eup-6 (10), an unusual ether function can be found between C-11/C-14. The parent diterpene alcohol present in 8-12 has not been described earlier. Eup-10 (12) is the only jatrophane diterpene known to date containing a double bond in position C-7/C-8. Eul-1 (14) is a lathyrane diterpene with an unprecedented 12-enol structure and n-butanoyl esterification. Ep-8 (6) and ep-2 (7) are based on a new carbon skeleton (named pepluane); they differ only in the esterification. Ev-2 (19) and ev-3 (15) are lathyrane derivatives; ev-2 (19) contains the rare 5,6-epoxy function. The parent diterpene alcohol of ev-2 (19) has not been described previously. Ev-1 (16) and ev-7 (17) have a tetracyclic 5-6-6-4, and ev-5 (18) a 5-6-7-3 fused ring system; they are euphoractine-type diterpenes.

On the basis of the diterpene composition, *E. esula* exhibits a relationship with *E. salicifolia*, as they contain the same main diterpene component, eup-1 (8), and related compounds.
differing only in the esterification. *E. esula* can be characterized as displaying high morphological and chemical diversity. The diterpenes of *E. peplus* samples of different origins were found to be similar, with the exception of an Italian sample, which contains compounds with different esterification pattern. *E. peplus* is most probably typified by chemovarietas.

All of the diterpenes were detected for the first time from the investigated plants; 5 compounds of *E. peplus* (ep-1, ep-4, ep-5, ep-7 and ep-8) were reported during our work. The chemical constituents of *E. villosa* have not been investigated previously.

The biological activities of some of the isolated compounds were investigated. In the phlogistic test, apart from an insignificant activity, the investigated jatrophane diterpenes were found to be inactive. Cytotoxic assays of some jatrophane esters demonstrated moderate or weak activity of ep-1 (2), ep-5 (4), eup-1 (8), eup-4 (9) and eup-12 (13) against human tumour cells. Significant antiviral activity was recorded for the compounds of the ep series: ep-3 (1), ep-4 (3) ep-5 (4), ep-7 (5) and ep-2 (7).

The isolated diterpenes were examined for their multidrug resistance reversing activity. The compounds in the ep and ev series were shown to enhance drug retention significantly in the tumour cells by inhibiting the efflux pump activity. Ep-3 (1), ep-1 (2) and ep-7 (5) exhibited much stronger effects than that of the positive control verapamil. The ability of jatrophane diterpenes to act as potent modulators of MDR has been evaluated here for the first time. Our results open up new opportunities in the design and development of drugs to overcome the MDR of human cancers.
7. REFERENCES


26 Warnaar F, Phytochemistry 1981; 20: 89-91
27 Rizk AM, Rimpler H, Ismail SI, Fitoterapia 1977; 48: 99-100
29 De Alvarenga MA, Gottlieb OR, Magalhaes MT, Phytochemistry 1976; 15: 844-845
30 Upadhyay RR, Hecker E, Phytochemistry 1974; 13: 752-753
31 Schmidt RJ, Bot. J. Linn. Soc. 1987; 94: 221-230
34 Rodríguez AD, Acosta AL, J. Nat. Prod. 1997; 60: 1134-1138
38 Xu ZH, Sun J, Xu RS, Qin GW, Phytochemistry 1998; 49: 149-151
49 Seip EH, Hecker E, *Phytochemistry* 1984; **23**: 1689-1694
50 Fakunle CO, Connolly JD, Rycroft DS, *J. Nat. Prod.* 1989; **52**: 279-283
51 Fakunle CO, Connolly JD, Rycroft DS, *Fitoterapia* 1992, **63**: 329-332
56 Marco JA, Sanz-Cervera JF, Checa J, Palomares E, Fraga BM, *Phytochemistry* 1999; **52**: 479-485
59 Marco JA, Sanz-Cervera JF, Yuste A, Jakupovic J, *Phytochemistry* 1997; **45**: 137-140
60 Abdelgaleil SAM, Kassem SMI, Doe M, Baba M, Nakatani M, *Phytochemistry* 2001; **58**: 1135-1139
Ferreira AVD, Carvalho LHM, Carvalho MJM, Sequeira MM, Silva AMS, *Phytochemistry*, 2002, **61**: 373-377


Valente C, Pedro M, Ascenso JR, Abreu PM, Nascimento MSJ, Ferreira MJU, *Planta Med.* 2004; **70**: 244-249


84 Hecker E, *Cancer Res.* 1968; **28**: 2338-2349
88 Onwukaeme ND, Rowan MG, *Phytochemistry* 1992; **31**: 3479-3482
89 Hui WH, Li MM, Ng KK, *Phytochemistry* 1975; **14**: 816-817
93 Aiyelaagbe OO, Gloer JB, Adesogan EK, Ekundayo O, *Fitoterapia* 1998; **69**: 381-382
100 Abo K, Evans FJ, *J. Pharm. Pharmacol.* 1981; **33**: 57
110 Fakunle CO, Connolly JD, Rycroft DS, *Fitoterapia* 1989; 60: 466-467
114 Marco JA, Sanz-Cervera JF, Yuste A *Phytochemistry* 1997; 45: 563-570

49
126 Shi JG, Jia ZJ, Yang L, Phytochemistry 1993; 32: 208-210
127 Shi JG, Jia ZJ, Phytochemistry 1995; 38: 1445-1447
133 Ng AS, Phytochemistry 1990; 29: 662-664
135 Liu LG, Meng JC, Wu SX, Li XY, Zhao XC, Tan RX, Planta Med. 2002; 68: 244-248
136 Starratt AN, Harris P, Phytochemistry 1971; 10: 1855-1856
139 Liao SG, Zhan ZJ, Yang SP, Yue JM, Org. Lett. 2005; 7: 1379-1382
142 Adolf W, Köhler I, Hecker E, Phytochemistry 1984; 23: 1461-1463


Rizk AM, Hammouda FM, Seif El-Nasr MM, Abou-Youssef AA, *Fitoterapia* 1980; **51**: 223-227

Manners GD, Davis DG, *Phytochemistry* 1984; **23**: 1059-1062


Calvin M, *Science* 1976; **194**: 46-47


ACKNOWLEDGEMENTS

This work was carried out at the Department of Pharmacognosy, University of Szeged, during the period 1995-2005. I am indebted to Professor Imre Máthé, Director of the Department of Pharmacognosy, for placing the facilities of the department at my disposal.

I express my sincere gratitude to my supervisor, Professor Judit Hohmann, for introducing me to the world of diterpenes. She has both always been there to answer questions and encourage me to continue the work. Her vast knowledge in different areas of pharmacognosy, her persistence and her diligence have been admirable.

I owe special thanks to Dr. Károly Penszka (Department of Botany and Plant Physiology, Agricultural University of Gödöllő) and Vilmos Miklóssy V. (Institute of Ecology and Botany of the Hungarian Academy of Sciences) for the identification and collection of the plant material.

In particular, I am deeply grateful to Dr. Gábor Günther, Dr. Ferenc Evanics (Institute of Pharmaceutical Analysis, University of Szeged) and Dr. Peter Forgo (Department of Organic Chemistry, University of Szeged) for the NMR measurements; to Dr. Gyula Jerkovich (Spectroscopic Department, Institute for Drug Research) and Dr. Pál Szabó (Institute of Chemistry, Chemical Research Centre of the Hungarian Academy of Sciences) for the mass spectra; to Professor Alajos Kálmán, Dr. Gyula Argay (Institute of Chemistry, Chemical Research Centre of the Hungarian Academy of Sciences) and Dr. Tanusree Kar (Department of Materials Science, Indian Association for the Cultivation of Science) for the X-ray measurements; to Dr. Gábor Blazsó (Department of Pharmacodynamics and Biopharmacy, University of Szeged) for the pro-inflammatory tests; and to Professor József Molnár and Dr. Ilona Mucsi (Department of Medical Microbiology, University of Szeged) for the anti-MDR, antiviral and cytotoxicity investigations.

My gratitude is due to all the staff at the Department, and especially Erzsébet Berta, for the help during my research and for the warm, down-to-earth atmosphere in the laboratory.

I am especially grateful to my family for providing me with a peaceful background.
APPENDIX

The thesis is based on the following publications referred to in the text:


I a
Macrocyclic Diterpene Polyesters of the Jatrophone Type from *Euphorbia esula*

Judith Hohmann,† Andrea Vasas,‡ Gábor Günther,‡ Imre Máthé,‡ Ferenc Evanics,‡ György Dombi,‡ and Gyula Jerkovich‡

Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Hungary, Department of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Hungary, and Spectroscopic Department, Institute for Drug Research Ltd., P.O. Box 82, H-1325 Budapest, Hungary

Received September 30, 1990.

Three new jatrophone diterpenes, esulatins A–C (1–3) were isolated and characterized from the whole, undried plant of *Euphorbia esula*. By means of spectral analysis, the structures were established as pentaeesters and heptaesters of hitherto unknown, polyfunctional diterpene parent alcohols. Esulatins A (1) and C (3) are the diterpenoids with the highest degree of esterification identified to date from the family Euphorbiaceae.

*Euphorbia esula* L., or leafy spurge (Euphorbiaceae) is distributed worldwide and contains a skin-irritant, toxic, milky latex. Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings, and warts. Previous phytochemical and pharmacological studies demonstrated the proinflammatory, tumor-promoting, and antitumor activity of the plant extracts, and three ingenane diterpenoids, ingenol 3,20-dibenzoate, ingenol 3-dodecanolate, and ingenol 3-A\(^2,4,5,8,10\)-pentene tetradeconolate, were found to be responsible for these activities.\(^{3-5}\)

Besides ingenol esters, macrocyclic diterpenes have also been isolated from *E. esula* namely, the jatrophone esters, esulons A, B, and C, with moderately toxic and mildly inflammatory effects from leafy spurge roots collected in North Dakota, and lathyrane and jatrophone triesters from seeds collected in Canada.\(^{6-8}\) All previous studies suggested a high variation of the diterpenoid compounds in *E. esula*, and significant differences were found between North American and European accessions.\(^{7}\)

As part of our studies on biologically active compounds from the family Euphorbiaceae, we have examined a Hungarian population of *E. esula* for its diterpene constituents. This paper deals with the isolation and structure elucidation of three new jatrophone esters, named esulatins A, B, and C (1–3).

Results and Discussion

The dichloromethane phase of a MeOH extract of the whole, undried plant of *E. esula* was fractionated by column chromatography on polyamide, then on Si gel, and further purified by preparative TLC and HPLC to afford esulatins A, B, and C (1–3).

Esulatin A (1) was shown by elemental analysis and ESIMS to have the molecular formula C\(_{39}\)H\(_{46}\)O\(_{4}\). The 1H- and 13C-NMR spectra of 1 revealed the presence of six acetate groups \(\delta_{H} 2.16 \text{ s}, 2.12 \text{ s}, 2.11 \text{ s}, 2.11 \text{ s}, 2.04 \text{ s}, 2.03 \text{ s}; \delta_{C} 170.0, 169.7, 169.5, 169.1, 168.6, 168.3 \text{ (CO)} \) and 22.2, 21.3, 21.2, 21.2, 20.8, 20.5 \text{ (CH\(_{3}\))} and one isobutanoate group \(\delta_{H} 2.60 \text{ sept (CH\(_{3}\))}, 1.21 \text{ d}, 1.18 \text{ d (CH\(_{3}\))}; \delta_{C} 175.0 \text{ (CO)}, 33.8 \text{ (CH\(_{3}\))}, 19.1, 18.4 \text{ (CH\(_{3}\))} \) (Table 1). The 13C-NMR and DEPT spectra suggested that the skeleton consisted of 20 carbons: four methyls, two methylenes, nine methines, and five quaternary carbons, including one ketone \(\delta_{C} 210.3\). The 1H-NMR spectrum contained 17 signals due to the parent skeleton, which were assigned with the aid of HMOC and H-H COSY experiments. The 1H-H COSY spectrum defined two structural fragments with correlated protons: \(-\text{CH}_2-\text{CR}_2-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-\text{CHR}-(\text{A})\) and \(-\text{CH(CH\(_3\))}-\text{CHR}-\text{CHR}-\text{CHR}-(\text{B})\). Their connectivities were determined from the long-range C-H correlations observed in an HMBC spectrum (Table 1). The long range correlations of the quaternary carbons \(\text{C} 6, \text{C} 10, \text{C} 2-2, \text{and C} 15\) with protons of the two fragments established the fragment A
Table 1. NMR Spectral Data of Esulatin A (8) [CDCl₃, TMS, δ (ppm) (J = Hz)]

<table>
<thead>
<tr>
<th>Atom</th>
<th>H</th>
<th>13C</th>
<th>H–H COSY</th>
<th>HMBC</th>
<th>NOEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.73 d (16.2)</td>
<td>46.2</td>
<td>H-1b, H-3</td>
<td>C-2, C-3, C-4, C-14</td>
<td>H-16</td>
</tr>
<tr>
<td>1b</td>
<td>2.04 d (16.2)</td>
<td>67.6</td>
<td>H-1a, H-3</td>
<td>C-15</td>
<td>H-16</td>
</tr>
<tr>
<td>2</td>
<td>86.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.58 br d (3.3)</td>
<td>78.0</td>
<td>H-1a, H-4</td>
<td>C-1, C-2, C-15, Ac-δ</td>
<td>H-7, H-16, H-17a, H-4</td>
</tr>
<tr>
<td>4</td>
<td>2.98 dd (3.3, 1.7)</td>
<td>49.8</td>
<td>H-3, H-5</td>
<td>C-3, C-14</td>
<td>H-3, H-7</td>
</tr>
<tr>
<td>5</td>
<td>6.06 br s</td>
<td>141.7</td>
<td>C-3, C-4, C-6, C-15, C-17, Ac-δ</td>
<td>168.3</td>
<td>H-7, H-8, H-12, H-13, H-17a</td>
</tr>
<tr>
<td>6</td>
<td>5.38 br s</td>
<td>86.5</td>
<td>H-8, H-7b</td>
<td>C-1’, C-6, C-9, C-17, i-Bu CO</td>
<td>H-3, H-4, H-5, H-8, H-9, H-11</td>
</tr>
<tr>
<td>7</td>
<td>5.51 d (4.1)</td>
<td>69.0</td>
<td>H-7, H-9</td>
<td>C-6, C-9, C-10, Ac-δ</td>
<td>169.5</td>
</tr>
<tr>
<td>8</td>
<td>4.95 d (4.1)</td>
<td>73.3</td>
<td>C-8, C-10, C-11, C-18, C-19, Ac-δ</td>
<td>169.1</td>
<td>H-7, H-11</td>
</tr>
<tr>
<td>10</td>
<td>39.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>3.00 d (2.1)</td>
<td>58.4</td>
<td>H-12</td>
<td>C-10, C-18, C-19</td>
<td>H-7, H-8, H-9, H-18, H-19, H-20</td>
</tr>
<tr>
<td>12</td>
<td>3.32 dd (4.7, 2.1)</td>
<td>57.1</td>
<td>H-11, H-13</td>
<td>C-13, C-14</td>
<td>H-5, H-8</td>
</tr>
<tr>
<td>13</td>
<td>3.67 dq (4.7, 6.9)</td>
<td>37.0</td>
<td>H-12, H-20</td>
<td>C-14, C-20</td>
<td>H-5</td>
</tr>
<tr>
<td>14</td>
<td>210.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>92.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.52 s</td>
<td>18.1</td>
<td>C-1, C-2, C-3</td>
<td>H-1a, H-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17a</td>
<td>5.09 s</td>
<td>111.9</td>
<td>H-5</td>
<td>C-7</td>
<td>H-3, H-5</td>
</tr>
<tr>
<td>17b</td>
<td>5.05 s</td>
<td>111.9</td>
<td>H-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>0.99 s</td>
<td>23.5</td>
<td>C-9, C-10, C-11, C-19</td>
<td>H-11</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>0.71 s</td>
<td>17.5</td>
<td>C-9, C-10, C-11, C-18</td>
<td>H-8, H-11</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.18 d (6.9)</td>
<td>15.3</td>
<td>H-13</td>
<td>C-12, C-13, C-14</td>
<td>H-11</td>
</tr>
</tbody>
</table>

Acetyl

| 2–CO | 169.7 |
| 2–CO Me | 2.11 s | 22.2 |
| 3–CO | 168.9 |
| 3–CO Me | 2.11 s | 22.2 |
| 5–CO | 168.3 |
| 5–CO Me | 2.18 s | 21.3 |
| 8–CO | 169.5 |
| 8–CO Me | 2.03 s | 20.5 |
| 9–CO | 169.1 |
| 9–CO Me | 2.04 s | 20.8 |
| 15–CO | 170.6 |
| 15–CO Me | 2.12 s | 21.2 |

α-β values are interchangeable.

and B to be C-1–C-9 (with an exocyclicene C-17 on C-6) and C-3–C-11 of a jatrophane diterpene, respectively. The 2JH and 3JH correlations between H-13, H-12, H-1β, and the carbon signal at δc 210.3 placed the keto group at C-14. The positions of ester groups were also established via an HMBC experiment. The correlation of the carbonyl signal at δc 173.0 (isobutanoyl CO) with the proton signals at δh 5.38 (H-7) and δh 1.21, 1.18 (methyl signals of isobutanoyl) indicated the presence of the isobutanoyl group at C-7. Similarly, the long-range couplings of the carbonyl carbon signals at δc 169.5, 169.1, 168.6, and 168.3 with the proton signals at δh 5.51 (H-8), 4.95 (H-9), 5.58 (H-3), and 6.06 (H-5) and the acetyl methyl signals at δh 2.03, 2.04, 2.11, and 2.16 demonstrated the presence of acetyl groups on C-8, C-9, C-3, and C-5, respectively. The two remaining acetyl groups did not show any long-range correlations, and it was therefore supposed that they were attached to quaternary carbons. When the 1H–NMR data of 1 were compared with those of analogous compounds having 2,3,5-triacyl substitution on the five-membered ring, similar δ values for H-1 and H-3 were found. Thus, the two acetyl groups (δh 2.11 and 2.12) were assigned to positions C-2 and C-15. With regard to the molecular formula and substituents mentioned above, the presence of a further epoxy group in the molecule was concluded. Chemical shift values of H-11, H-12 (δh 3.00 d and 3.32 dd), C-11, and C-12 (δc 58.4, 57.1) indicated that the epoxy group must be at position C-11, C-12.

The stereochemistry and absolute configuration of esulatin A were studied by NOESY measurements (Table 1) and X-ray analysis. On the basis of crystallographic investigations esulatin A was elucidated as (2R,3R,4S,5R,7S,8R,9S,11R,12S,13R,15R)-2,3,5,8,9,15-hexaacetoxy-11,12-epoxy-7-(isobutanoyloxy)jatrophiol (617) en-14-one (8); data are published elsewhere. The structure of 1 is very similar to that of kansuin B, isolated from *Euphorbia kansui*, which has been shown to possess analgesic and anti-writhing activities. Esulatin B had a molecular formula of C₃₆H₅₆O₁₂, obtained from HRMS and NMR analyses. Its EIMS spectrum exhibited fragment ion peaks due to the sequential loss of HOAc and ketene units, indicating the presence of five acetyl groups in the molecule (see Experimental Section). Accordingly, the 1H and 13C-NMR spectra of esulatin B contained signals corresponding to five acetyl groups (δH 2.16 s, 2.14 s, 2.13 s, 2.11 s, and 2.05 s, δC 170.4, 170.1, 169.7, 169.6, 169.0 (CO), and 22.1, 21.4, 21.2, 20.9, and 20.8 (CH₄)). Additionally, the 13C-NMR and DEPT spectra exhibited resonances for four methyls, three methylenes, seven methines, and six quaternary carbons, including two keto groups (δC 211.1 and 209.4) (Table 2). The 1H-NMR and 1H–1H COSY spectra revealed the presence of three tertiary methyls (δH 1.48 s, 1.17 s, 1.16 s) and the structural elements –CH₂CR₂CHRCHCHRCH– (CH₂CH=CHRCH₂ (A) and trans–CH(CH₂)CH=CH (B)). Fragment A was elucidated with the aid of the 4J couplings observed between H-1 and H-3, and between
Table 2. NMR Spectral Data of Esulatin B (2) [CDCl₃, TMS, δ (ppm) (J = Hz)]

<table>
<thead>
<tr>
<th>atom</th>
<th>H</th>
<th>¹³C</th>
<th>H-H COSY</th>
<th>HMBC</th>
<th>NOEY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.82 dd (16.0, 1.0)</td>
<td>4.66</td>
<td>H-1b, H-3</td>
<td>C-2, C-3, C-4, C-14, C-15</td>
<td>H-1b</td>
</tr>
<tr>
<td>1b</td>
<td>1.91 d (6.0)</td>
<td>86.2</td>
<td>H-1a</td>
<td>C-2, C-14, C-15, C-16</td>
<td>H-1a</td>
</tr>
<tr>
<td>2</td>
<td>5.51 (3.3)</td>
<td>78.3</td>
<td>H-1a, H-4</td>
<td>C-2, C-15, Ac δ 169.0</td>
<td>H-16, H-4, H-17</td>
</tr>
<tr>
<td>3</td>
<td>2.91 dd (3.5, 1.0)</td>
<td>49.7</td>
<td>H-3, H-5</td>
<td>C-3, C-4, C-14, C-15</td>
<td>H-3, H-5, H-7</td>
</tr>
<tr>
<td>4</td>
<td>5.80 s</td>
<td>68.2</td>
<td>H-4, H-17</td>
<td>C-3, C-4, C-6, C-7, C-15, C-17, Ac δ 169.7⁹</td>
<td>H-4, H-7, H-8b, H-11, H-13</td>
</tr>
<tr>
<td>5</td>
<td>4.94 d (9.6)</td>
<td>69.5</td>
<td>H-8b, H-8a, H-17</td>
<td>C-5, C-6, C-8, C-9, C-17, Ac δ 169.6⁹</td>
<td>H-4, H-5, H-19</td>
</tr>
<tr>
<td>6</td>
<td>3.26 dd (14.0, 1.0)</td>
<td>45.4</td>
<td>H-7, H-8a</td>
<td>C-6, C-7, C-9</td>
<td>H-8a, H-5, H-11</td>
</tr>
<tr>
<td>7</td>
<td>2.28 dd (14.0, 9.6)</td>
<td>5.8</td>
<td>H-7, H-8b</td>
<td>C-7, C-9</td>
<td>H-8b</td>
</tr>
<tr>
<td>8a</td>
<td>2.094</td>
<td>49.3</td>
<td>H-12</td>
<td>C-9, C-10, C-13, C-18</td>
<td>H-7, H-13, H-18</td>
</tr>
<tr>
<td>8b</td>
<td>6.07 d (15.9)</td>
<td>137.2</td>
<td>H-12</td>
<td>C-9, C-10, C-13, C-18</td>
<td>H-9, H-20</td>
</tr>
<tr>
<td>9</td>
<td>5.52 dd (15.9, 9.6)</td>
<td>133.2</td>
<td>H-11, H-13</td>
<td>C-10, C-14</td>
<td>H-11, H-5, H-20</td>
</tr>
<tr>
<td>10</td>
<td>3.35 q (9.6, 6.6)</td>
<td>44.7</td>
<td>H-12, H-20</td>
<td>C-11, C-12, C-14</td>
<td>H-12, H-13</td>
</tr>
<tr>
<td>11</td>
<td>1.48 s</td>
<td>17.8</td>
<td>H-12</td>
<td>C-1, C-2, C-3</td>
<td>H-3, H-1b</td>
</tr>
<tr>
<td>12</td>
<td>5.10 d (1.0)</td>
<td>110.1</td>
<td>H-5, H-7</td>
<td>C-5, C-6, C-7</td>
<td>H-3</td>
</tr>
<tr>
<td>13</td>
<td>5.11 d (1.0)</td>
<td>110.1</td>
<td>H-5, H-7</td>
<td>C-5, C-6, C-7</td>
<td>H-3</td>
</tr>
<tr>
<td>14</td>
<td>1.17 s³</td>
<td>26.7</td>
<td>H-9, C-10, C-11, C-19</td>
<td>H-3</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1.16 s⁴</td>
<td>23.0</td>
<td>H-9, C-10, C-11, C-18</td>
<td>H-3</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.12 d (6.6)</td>
<td>20.2</td>
<td>H-13</td>
<td>C-12, C-13, C-14</td>
<td>H-12, H-13</td>
</tr>
</tbody>
</table>

Acetyls
1. CO 2. COMe 3. CO 4. COMe 5. CO 6. COMe 7. CO

¹³C values are interchangeable.

Figure 1. HMBC correlations of quaternary carbons of esulatin B (2) (H–C).

H-5, H-7 and the exomethylene (H-17). The connection of the partial structures was clarified by means of an HMBC experiment. The correlations of the quaternary carbons (see Figure 1) to proximate protons supported the structure of esulatin B as 2,3,5,7,15-pentaacetoxynaphtho[6(17),11]dine, 9,14-dione.

The stereochemistry of esulatin B was investigated by X-ray analysis and NOESY measurements. From crystallographic data the same configuration of C-2, C-3, C-4, C-5, C-7, C-13, C-15 was deduced as that of esulatin A (1), and the structure of esulatin B was formulated as 2₁². NOESY correlations were in accordance with the stereochemistry elucidated from X-ray investigations. The full H- and ¹³C-NMR chemical shift assignments of 2 were carried out through H-1–H COSY, HMBC, HMBC, and NOESY spectral analysis, as listed in Table 2. The EIMS spectrum of esulatin B (2) revealed significant fragments, which seem to be characteristic of jatrophol-1-eine,9,11-dione derivatives. Peaks at m/z 123 (52%) C₇H₁₂O (found 123.0794, calcd 123.0810) and m/z 296 (70%) C₂H₁₂ (found 96.0934, calcd 96.0939) corresponding to the ions (CH₃)₂CcH=CH–C(CH=CH₂)=CH–CH==C(O)=O and (CH₃)₂CcH=CH–CH=CHCH₃⁺ were originated from the part of the molecule between the two keto groups.

Esulatin C, a minor diterpenoid of E. esula, was revealed by HRMS and NMR analyses to have the molecular formula C₂₀H₂₆O₇. The ¹H-NMR spectrum indicated the presence of seven ester groups: six acetate groups (δ₁H 2.16 s, 2.11 s, 2.06 s, 2.02 s, 2.00 s, and 1.99 s) and one isobutanolate group (δ₁H 2.61 sept (CH₃) 1.23 d, 1.16 d (CH₂)). Additionally, the ¹H-NMR spectrum exhibited signals attributed to four methyls (δ₁H 1.56 s, 1.24 d, 1.24 s, 1.12 s), one methylene (δ₁H 3.10 d, 2.80 d), one methyl-bearing methine (δ₁H 2.17 dq) and, in the range δ₁H 6.49–3.28, seven singlets and three doublets with coupling constants 3.5 and 1.9 Hz (Table 3). Correlations between these latter signals were detected in the H-1–H COSY spectrum. Relationships between the signals at δ₁H 6.20 (H-3), 3.28 (H-4), and 6.20 (H-5) and at δ₁H 6.49 (H-7), 5.65 (H-8), and 4.94 (H-9) were clearly defined. Further, in the H-1–H COSY spectrum 4J-couplings were detected between the signals at δ₁H 3.10 and 2.80 (H-1) and 6.02 (H-3); at δ₁H 6.20 (H-5), 6.49 (H-7), 5.04, and 4.95 (H-17a,b); at δ₁H 4.94 (H-9) and 4.13 (H-11); at δ₁H 4.13 (H-11) and 2.17 (H-13); and at δ₁H 2.17 (H-13) and 3.99 (H-14 OH). These data are compatible with a jatrophane diterpene substituted on C-2, C-3, C-5, C-7, C-8, C-9, C-11, C-12, C-14, and C-15.

The structure of esulatin C was further studied by means of HMQC and HMBC experiments. From the HMQC spectrum the chemical shifts of protonated carbons were assigned as listed in Table 3. On the basis of the HMBC experiment, the presence of five quaternary carbons attributed to the parent skeleton was detected. Cross peaks between H-1, H-3, H-16, and the signals at δ₁C 88.5 (C-2) and between H-1, H-4, H-5, and δ₁C 89.9...
Table 3. NMR Spectral Data of Esulatin C [CDCl₃, TMS, δ (ppm) (J = Hz)]

<table>
<thead>
<tr>
<th>Atom</th>
<th>JH</th>
<th>1³C</th>
<th>JH-H COSY</th>
<th>HMBC</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.10 d (16.5)</td>
<td>43.6</td>
<td>H-1b, H-3</td>
<td>C-2, C-3, C-4, C-14</td>
<td>H-1b, H-20</td>
</tr>
<tr>
<td>1b</td>
<td>3.80 d (16.5)</td>
<td>69.4</td>
<td>H-1a, H-3</td>
<td>C-14, C-15, C-16</td>
<td>H-1a, H-16</td>
</tr>
<tr>
<td>2</td>
<td>60.2 s</td>
<td>88.5</td>
<td></td>
<td>C-2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60.6 d (3.5)</td>
<td>74.2</td>
<td>H-1b, H-4</td>
<td>C-2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5.38 d (3.5)</td>
<td>49.7</td>
<td>H-3, H-5</td>
<td>C-3, C-5, C-6, C-14, C-15</td>
<td>H-3, H-5, H-7, 14-ΟΗ, H-16</td>
</tr>
<tr>
<td>5</td>
<td>6.20 s</td>
<td>69.4</td>
<td>H-4, H-7, H-17a</td>
<td>C-3, C-4, C-6, C-7, C-15</td>
<td>H-4, H-8</td>
</tr>
<tr>
<td>6</td>
<td>6.49 s</td>
<td>67.8</td>
<td>H-5, H-8, H-17a</td>
<td>C-6, C-8, C-9, C-17, C-1'</td>
<td>H-4, H-8, H-11</td>
</tr>
<tr>
<td>7</td>
<td>6.45 s</td>
<td>70.9</td>
<td>H-7, H-9</td>
<td>C-6, C-9, Ac δ 170.5</td>
<td>H-5, H-7, H-9, H-18</td>
</tr>
<tr>
<td>8</td>
<td>4.94 s</td>
<td>82.6</td>
<td>H-8, H-11</td>
<td>C-8, C-10, C-11, C-19, Ac δ 169.6</td>
<td>H-8, H-18, H-19</td>
</tr>
<tr>
<td>10</td>
<td>4.13 s</td>
<td>77.1</td>
<td>H-9, H-13</td>
<td>C-9, C-10, C-12, C-18</td>
<td>H-7, H-19</td>
</tr>
<tr>
<td>12</td>
<td>213.6</td>
<td>81.1</td>
<td>H-11</td>
<td>H-12</td>
<td>H-20</td>
</tr>
<tr>
<td>14</td>
<td>2.17 dq (7.0, 1.9)</td>
<td>21.6</td>
<td>H-11, 14-ΟΗ, H-20</td>
<td>C-12, C-14, C-20</td>
<td>H-4</td>
</tr>
<tr>
<td>15</td>
<td>15.2</td>
<td>89.9</td>
<td>H-13</td>
<td>H-14</td>
<td>H-4</td>
</tr>
<tr>
<td>16</td>
<td>1.56 s</td>
<td>19.3</td>
<td>H-15</td>
<td>C-1, C-2, C-3</td>
<td>H-1b, H-3</td>
</tr>
<tr>
<td>17a</td>
<td>5.04 s</td>
<td>107.9</td>
<td>H-5, H-7, H-17b</td>
<td>C-5, C-6, C-7</td>
<td>H-1b, H-3</td>
</tr>
<tr>
<td>17b</td>
<td>4.95 s</td>
<td>18.9</td>
<td>H-5, H-7, H-17a</td>
<td>C-5, C-6, C-7</td>
<td>H-1b, H-3</td>
</tr>
<tr>
<td>18</td>
<td>1.24 (d)</td>
<td>18.9</td>
<td>H-15</td>
<td>C-10, C-11, C-19</td>
<td>H-8, H-9</td>
</tr>
<tr>
<td>19</td>
<td>1.12 (d)</td>
<td>22.4</td>
<td>C-9, C-10</td>
<td>H-11</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.24 (d)</td>
<td>19.8</td>
<td>H-13</td>
<td>C-12, C-14</td>
<td>H-1a, H-5</td>
</tr>
<tr>
<td>iBu</td>
<td>176.6</td>
<td>176.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>2.61 s</td>
<td>34.2</td>
<td>H-13</td>
<td>C-1', C-4'</td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>1.16 d (7.0)</td>
<td>18.8</td>
<td>H-13</td>
<td>C-1', C-4'</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>1.23 d (7.0)</td>
<td>19.8</td>
<td>H-13</td>
<td>C-1', C-3'</td>
<td></td>
</tr>
</tbody>
</table>

Acetyls
8.CO  | 197.6 | 197.6 |          |      |       |
8.COMe | 206.6 | 21.2 | 8.CO |       |
9.CO  | 189.6 | 189.6 |          |      |       |
9.COMe | 212.2 | 22.2 | 9.CO |       |
2,3,5,15-CO | 168.6 | 168.6 |          |      |       |
2,3,5,15-COMe | 210.6 | 21.1 | Ac δ 169.6 |       |
2,3,5,15-COMe | 210.6 | 22.8 | Ac δ 169.6 |       |
2,3,5,15-COMe | 211.6 | 21.8 | Ac δ 168.6 |       |
2,3,5,15-COMe | 200.6 | 21.2 | Ac δ 167.5 |       |

δ values are interchangeable.

(C-15) suggested that a similarly substituted five-membered ring is present in the molecule as in esulatin A (1). Furthermore, in the HMBC spectrum a series of correlations was seen between H-5–H-11 and the surrounding carbons via two or three bonds, which confirmed an identical C-5–C-11 partial structure and esterification in positions C-5, C-7, C-8, and C-9 as in 1. A difference between the two compounds was found in the sequence C-12–C-14. Correlations between H-11, H-13, H-20, and the carbon signal at δC 213.6 indicated that a keto group must be located at C-12. On the basis of its long range couplings with H-1, H-4, H-13, and H-20, the quaternary carbon signal at δC 105.7 was assigned to C-14. A hydroxyl group was placed on C-14 because its doublet signal (J = 3.5 Hz) coupled to H-13 (J = 1.9 Hz), and the methine signal at δH 4.13 (H-11), which was observed at higher field than for esterified methines, is an ether functionality (deduced from the molecular formula and substituents discussed above) must be situated between C-11 and C-14.

The relative stereochemistry of esulatin C was studied by NOESY measurements. NOE interactions and coupling constants of H-1, H-3, and H-4 are very similar to that of esulatins A and B, suggesting the same configuration of C-2, C-3, C-4, and C-15. The stereochemistry of C-4 and C-15 was also followed from the fact that all jatrophane diterpenes subjected to X-ray analysis exhibit a transring junction and no NOE was observed between H-4 and 15-OAc. The zero coupling constant between H-4 and H-5 required that, similar to 1, 2, and kansuvin A, H-5 be β. The NOE effect observed between OH-14 and H-4 indicated the presence of an α hydroxyl group on C-14. The stereochemistry of C-7, C-8, C-9, C-11, and C-13 on the basis of NOESY correlations could not be determined because of the high flexibility of this part of the molecule. Thus, the structure of esulatin C was elucidated as shown in [formula 3].

Cesulatins A (1) and C (3) display a close relationship to a diterpenic constituent of E. kansui, kansuvin A, and Esulatins A (1) and C (3) are the most highly esterified Euphorbiaceae diterpenoids identified to date. Presumably the isolation of such highly esterified compounds is associated with the use of fresh plant material. The parent diterpene alcohol found in 1–3 has not been described earlier. The isolated compounds are additional members of the small group of jatrophane diterpenoids, which are considered to be the most important taxonomic members in this family. In vitro primary antitumor screening on esulatin A (1) by the National Cancer Institute (Bethesda, MD) showed it to be inactive.
Experimental Section

General Experimental Procedures. Melting points were determined on a Boetius apparatus and are uncorrected. IR spectra were run as KBr disks on a Perkin-Elmer Paragon 1000 FTIR spectrometer. UV spectra in MeOH were obtained on a Shimadzu UV-2101 spectrophotometer. Mass spectral measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. The NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 MHz (H) and 100 MHz (C), using CDCl3 as solvent and TMS as internal standard. Optical rotations were determined in CHCl3 at ambient temperature using a Perkin-Elmer 341 polarimeter. For column chromatography polyamide (ICN) and Si gel (Kieselgel GF254 15 μm, Merck) were used. HPLC was carried out on a Waters Millipore instrument with RI detection on a normal phase column (LiChrospher Si 100 5 μm, Merck). TLC was performed on Si gel 60 F254 plates using CHCl3–MeCO (19:1), CHCl3–C6H5–EtO (1:1:3) and cyclohexane–EtOAc–EtOH (20:10:1) as developing systems with visualization using 1% vanillin–H2SO4 spray reagent.

Plant Material. Euphorbia esula was collected in May 1994, in Szeged, Hungary, on the banks of the Tisza River and identified by Károly Penszka (Department of Botany and Plant Physiology, Agricultural University of Gödöllő, Hungary). A voucher specimen has been deposited at the Herbarium of the Museum of Natural Sciences in Budapest, Hungary.

Extraction and Isolation. The fresh and entire plants of E. esula (11 kg) were extracted with MeOH (75 L) at room temperature. The crude extract was concentrated in vacuo and partitioned between CHCl3 (7 × 1.5 L) and H2O. On evaporation, the organic phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixtures of H2O–MeOH (4:1, 3:2, 2:3, 1:4) as eluents. Fractions 5–21 obtained with H2O–MeOH (4:1) afforded a crystalline material upon standing, which was recrystallized from MeOH to yield esulatin A (1) (300 mg). The combined fractions 1–15 (15 g) were subjected to Si gel vacuum liquid chromatography (VLC) using a gradient system of cyclohexane–Me2CO (19:1, 9:1, 4:1, 3:1, 1:1, 3:7). Fractions from cyclohexane–Me2CO (4:1) were transferred repeatedly to a Si gel VLC and successively eluted with CHCl3–MeOH mixtures of increasing polarity. From fractions obtained with CHCl3–MeOH (99:7:0.3, 40 mg of esulatin B (2) was obtained as crystals. Fractions obtained with CHCl3–MeOH (99:1) were further purified by preparative TLC on Si gel using C6H6–EtOAc (7:3) as solvent and by HPLC using cyclohexane–EtOAc–EtOH (30:10:1) as eluent (flow 0.5 mL/min), to yield 1.2 mg of esulatin C (3).

Esulatin A (1): colorless crystals from MeOH: mp 218–219 °C; [α]23D −82° (c 0.1, CHCl3); UV (MeOH) λmax (log ε) 202 (4.73), 219 (sh, 4.08) nm; IR (KBr) νmax 2983, 2839, 1749, 1468, 1426, 1372, 1321, 1210, 1149, 1129, 1085, 1026, 994, 934, 911, 873, 833, 809, 746, 659, 634, 610 cm−1; H and 13C NMR, see Table 1; ESIMS m/z[M + K]+ 777 (36), [M + Na]+ 761 (40), [M + NH4]+ 756 (100); anal. C 58.08%, H 7.43%, calc. for C30H25O6C 58.51%, H 6.83%.

Esulatin B (2): colorless crystals from MeOH: mp 248–249 °C; [α]23D −101° (c 0.1, CHCl3); UV (MeOH) λmax (log ε) 210 (4.88), 284 (3.89) nm; IR (KBr) νmax 2984, 2939, 1741, 1659, 1431, 1374, 1251, 1229, 1133, 1099, 1042, 995, 926, 877, 805, 740, 629, 609 cm−1; H and 13C NMR, see Table 2; EIMS m/z[M]+ 592 (14), [M − AcOH]+ 532 (3), [M − AcOH − CH2CO]+ 490 (6), [M − 2 × AcOH]+ 472 (6), [M − 2 × AcOH − CH2CO]+ 430 (8), [M − 3 × AcOH]+ 412 (8), [M − 3 × AcOH − CH2CO]+ 370 (26), [M − 4 × AcOH]+ 352 (8), [M − 3 × AcOH − 2 × CH2CO]+ 328 (16), [M − 4 × AcOH − CH2CO]+ 310 (32), [C6H4]3O+ 123 (52), [C6H4]2OH+ 96 (70); HREIMS m/z 592.2631 (calcd for 592.2520 C30H25O6C)[M]+.

Esulatin C (3): white amorphous solid; [α]23D +11° (c 0.06, CHCl3); UV (MeOH) λmax (log ε) nm 209 (4.84), 273 (4.02); IR (KBr) νmax 3473, 2930, 1743, 1679, 1540, 1515, 1456, 1427, 1372, 1241, 1155, 1063, 1041 cm−1; H and 13C NMR, see Table 3; EIMS m/z[M − AcOH]+ 694 (22), [M − iBuOH]+ 666 (20), [M − iBuOH − OAc]+ 607 (53), [M − iBuOH − AcOH − CH2CO]+ 564 (17), [M − iBuOH − 2 × AcOH]+ 546 (38), [M − iBuOH − 2 × AcOH − CH2CO]+ 504 (43), [M − 3 × AcOH − CH2CO − CH4]+ 476 (71), [M − iBuOH − 3 × AcOH − CH2CO]+ 444 (100), [M − iBuOH − 4 × AcOH − CH2CO]+ 384 (31), [M − iBuOH − 5 × AcOH − CH2CO]+ 324 (20); HREIMS m/z 694.2839 (calcd for 694.2837 C30H25O6C)[M − AcOH]+, 666.2778 (calcd for 666.2524 C30H25O6C)[M − iBuOH]+.

Acknowledgment. This investigation was funded by Ministry of Culture and Education, Republic of Hungary, project no. MKM 495. We are greatly indebted to Dr. Károly Penszka, Department of Botany and Plant Physiology, Agricultural University of Gödöllő, Hungary, for identification of the plant species.

References and Notes


NP960669C
Ib
Jatrafánvázás makrociklusos diterpén-políészterek az *Euphorbia esula*-ból*

HOHMANN JUDIT¹, VASAS ANDREA¹, GÜNTHER GÁBOR², MÁTHÉ IMRE³, EVANICS FERENC², DOMBI GYÖRGY², JERKOVICH GYULA³

¹SZOTE Gyógynövény- és Drogismereti Intézet, 6701 Szeged, Pp. 121.
²SZOTE Gyógyszerkémiai Intézet, 6701 Szeged, Pp. 121.
³Gyógyszerkutató Intézet Rt., 1325 Budapest, Pp. 82.

Summary


Three new jatrophane diterpenes, esulatins A, B and C (1-3) were isolated and characterized from the whole, undried plant of *Euphorbia esula*. By means of spectral analysis, the structures were established as penta- and heptasteres of hitherto unknown, polyfunctional diterpene parent alcohols. Esulatin A (1) and C (3) are the diterpenoids with the highest degree of esterification identified to date from the family Euphorbiaceae.

Összefoglalás

Frissen gyűjtött *Euphorbia esula* növénymintából három új jatrafánvázás diterpén, az esulatin A-C (1-3) komponenseket izoláltak és szamosították. Spektroszkópiai vizsgálatok eredményeként megállapították, hogy a végyleletek korábban ismertlen, több feketeosz csoportot tartalmazó diterpénalkohóloktól penta- és heptaster esetében. Az esulatin A (1) és C (3) az Euphorbiaceae családból ezúttal legtöbb mértékben összetettebb diterpének.

Bevezetés

Az *Euphorbia esula* L., magyar néven sárkutya- tej (Euphorbiaceae) az egész világon elterjedt növényi faj, mely bőrirritáló, méregző tejedvet tartalmaz [1]. A növény kivonatait régóta széles körben használják a népgyógyszatban különféle dagana-tos megbetegedések, szemölcsök kezelésére [2]. A korábbi növénykémiai és farmakológiai vizsgál-tok igazolták a növényi kivonatok gyulladásként, tumorképzést elősegítő és antitumor aktivitását, valamint bizonyították, hogy ezen hatásért három ingenánvázás diterpén, az ingenol-3,20-dibenzoát, az ingenol-3-dodekanoát és az ingenol-3-Δ2,5,6,9,10-pentén-tetradekanoát a felelős [3-5].

Ingenol-észterek mellett makrociklusos diter-pénkeket is izoláltak az *E. esula*-ból: az Észak-Dakotában gyűjtött növény gyökeréből mérsékelt toxis-kus és enyhén gyulladásként hatást jatrafán-

észtereket, az esulaton A, B és C komponenseket különítették el, a Kanadából származó magminták-ból pedig latiráns- és jatrotán-trézsztereket nyertek [6-8]. A korábbi vizsgálatok egyértelműen arra utaltak, hogy a diterpénnek nagyfokú változatossá-

ga jellemző az *E. esula*-ra a származási helyettől füg-gően, azaz figyelemre méltó különbségek jelentkeznek észak-amerikai és európai eredetű növényminták között [7].

Az Euphorbiaceae család biológiaiágak aktiv ve-

gyületeinek megismerésére irányuló kutatási programunk keretében az *E. esula* magyarországi populációjának diterpénjeit vizsgáltuk. Ebben a dolgozatban három új jatrafánészter, az esulatin A, B és C (1-3) komponensei (1. ábra) izolálásáról és szerkezet-meghatározásáról számolunk be.
Kísérleti rész

1. Általános kísérleti eljárások

Az olvadáspont-meghatározás Boetius-készülékkel korrekció nélkül történt. Az IR spektrumok KB: pasztillában Perkin-Elmer Paragon 1000 PC FTIR spektrométerrel készültek. Az UV spektrumok felvétele metanolban Shimadzu UV 2101 spektrométerrel történt. A tömegspektroszkópia méréseket Finnigan MAT 8430 készüléken, 70 eV ionizáló energiával végeztük. Az NMR spektrumokat Bruker Avance DRX 400 spektrométerrel vettük fel 400 MHz-en (1H) és 100 MHz-en (13C), CDCl3-at, mint oldószt és TMS-t, mint belső standardot használva. Az optikai rotáció méresek klorofómban, termosztált hőmérsékleten Perkin-Elmer 341 polarímeterrrel történtek. Oszlopkromatográfához poliamidot (ICN) és szilikagélt (Kieselgel GF254 15 μm, Merck) használtunk. A HPLC elválasztás Waters Millipore készülékkel, RI detektálással, normál fázisú oszlopon (LiChrospher Si 100, 5 μm, Merck) folyt. A rétegkromatográfia vizsgálatokat szilikagélt (Kieselgel 60 F254) lemezekben, klorofórm-aceton benzetőben (1:1), benzetőben (1:1:3) és ciklohexán-aceton (20:10:1) kifejlesztő elegyek felhasználásával végeztük, a detektálás 1%-os vanillin-kénsav előhívó-reagenssel történt.

2. Növényi anyag

Az Euphorbia esula-t 1994 májusában Szegedben a Tisza partjáról gyűjtöttek, a faj azonosítását Dr. Pénzka Károly (Gödöllői Agrártudományi Egyetem, Növénytani és Növényfiziológiai Tarszék, Gödöllő) végezte. Egy herbárium mintapéldányt a Természettudományi Múzeum Herbáriumában, Budapesten helyeztünk el.

3. Extrakció és izolálás

A frissen gyűjtött, teljes növényt tartalmazó E. esula mintát (11 kg) 75 dm³ metanolból szobahőmérsékleten extraháltuk. A nyers kivonatot vákuumban betöményítettük és 7 x 1,5 dm³ döklőmetánnyal ráztuk ki. A szerves fázis beállását követően a maradéktartalmú (130 g) poliamid oszlopra (600 g) vittük és víz-metanol (4:1, 3:2, 2:3, 1:4) elegyekkel elültünk. A víz-metanol (4:1) elegygel kapott 5–21. frakcióból állás közben fehér kristályos anyag vált ki, melyet metanolból átkristályosítottunk, s így kaptuk az esulatin A jelzésű anyagot (1) (300 mg). Az 1–15. egyesített frakciót 15 g szilikagélt vákuum folyadékkromatográfiaival (VLC) tisztítottuk ciklohexán-aceton gradiens rendszert (19:1, 9:1, 4:1, 7:3, 1:1, 3:7) alkalmazva. A ciklohexán-aceton (4:1) elegygel elült frakciókat ismételten szilikagélt VLC-val fracionáltuk növekvő polaritású klorofórm-metanol elegyeket használva. A klorofórm-metanol (99:7:0.3) elegygel kapott frakciókból 40 mg kristályos anyag vált ki, jelzése esulatin B (2). A klorofórm-metanol (99:1) elegygel kapott frakció további tisztítása preparatív rétegkromatográfiaival történt szilikagélt benzol-etilacetát (7:3) elegygel. Ezt követően HPLC elválasztást végeztünk ciklohexán-etilacetát-etanol (30:10:1) összetételű eluenszel (tarnálósebesség 0.5 ml/perc), s folyón 1.2 mg tiszta anyagot kaptunk, jelzése esulatin C (3).
Esulatin A (1): szintén kristály MeOH-ból;
Op. 218-219 °C; [α]D² -82° (c 0,1, CHCl₃); UV (MeOH) λmax (log e) nm 207 (4,73), 219 (sh, 4,08);
IR (KBr) vmax 2983, 2939, 1749, 1648, 1426, 1372, 1321, 1210, 1149, 1085, 1026, 994, 934, 911,
873, 833, 809, 746, 659, 634, 610 cm⁻¹; ¹H és ¹³C NMR lásd I. táblázat.
HREIMS m/z [M⁺] 952 (14), [M – AcOH⁻] 532 (3), [M – AcOH - CH₂CO⁺] 490 (6), [M – 2xAcOH - CH₂CO⁺] 472 (6), [M – 3xAcOH - CH₂CO⁺] 430 (8), [M – 3xAcOH - CH₂CO⁻] 412 (8), [M – 3xAcOH - CH₂CO⁻] 370 (26), [M – 4xAcOH - CH₂CO⁺] 352 (8), [M – 3xAcOH - CH₂CO⁻] 328 (16), [M – 4xAcOH - CH₂CO⁻] 310 (32), [C₈H₁₄O⁺] 123 (52), [C₁₇H₂₄⁺] 96 (70), HREIMS m/z 592,2631
(számított 592,2520 C₃₀H₄₀O₁₂ [M⁺]).

Esulatin B (2): szintén kristály MeOH-ból;
Op. 248-249 °C; [α]D² -101° (c 0,1, CHCl₃); UV (MeOH) λmax (log e) nm 210 (4,88), 283 (3,89); IR
(KBr) vmax 2984, 2939, 1741, 1659, 1431, 1374, 1251, 1229, 1133, 1099, 1042, 955, 926, 877, 805, 740, 629,
609 cm⁻¹; ¹H és ¹³C NMR lásd III. táblázat.
HREIMS m/z [M⁺] 952 (14), [M – AcOH⁻] 532 (3), [M – AcOH - CH₂CO⁺] 490 (6), [M – 2xAcOH - CH₂CO⁺] 472 (6), [M – 2xAcOH - CH₂CO⁻] 430 (8), [M – 3xAcOH - CH₂CO⁻] 412 (8), [M – 3xAcOH - CH₂CO⁻] 370 (26), [M – 4xAcOH - CH₂CO⁺] 352 (8), [M – 3xAcOH - CH₂CO⁻] 328 (16), [M – 4xAcOH - CH₂CO⁻] 310 (32), [C₁₇H₂₄O⁺] 123 (52), [C₁₇H₂₄⁺] 96 (70), HREIMS m/z 592,2631
(számított 592,2520 C₃₀H₄₀O₁₂ [M⁺]).

Esulatin C (3): fehér amor fusum; [α]D² +11° (c 0,06, CHCl₃); UV (MeOH) λmax (log e) nm 207
(4,84), 273 (4,02); IR (KBr) vmax 3743, 2930, 1743, 1679, 1540, 1515, 1456, 1427, 1372, 1241, 1155, 1063,
1041 cm⁻¹; ¹H és ¹³C NMR lásd III. táblázat.
HREIMS m/z [M – AcOH⁻] 694 (22), [M – iBuOH⁻] 666 (20), [M – i-BuOH - AcOH - CH₂CO⁺] 564 (17), [M – i-BuOH - 2xAcOH⁻] 546 (38), [M – i-BuOH - 2xAcOH - CH₂CO⁺] 504 (43), [M – 3xAcOH - CH₂CO - C₃H₄O⁺] 476 (71), [M – i-BuOH - 3xAcOH - CH₂CO⁺] 444 (100), [M – i-BuOH - 4xAcOH - CH₂CO⁺] 438 (31), [M – i-BuOH - 5xAcOH - CH₂CO⁻] 324 (20), HREIMS m/z 694,2837 (számított 694,2837 C₂₉H₄₂O₁₆ [M – AcOH⁺] 666,2778
(számított 666,2524 C₃₂H₄₄O₁₈ [M – i-BuOH⁺]).

Az esulatin A (1) elemanalyzise és ESIMS vizsgálatával kapott összesköpe C₃₆H₅₀O₁₆. Az 1H-
és ¹³C-NMR spektruma hat acetilsopporten [H₂, 2,16 s, 2,12 s, 2,11 s, 2,11 s, 2,04 s, 2,03 s; C₁ₓ, 170,0, 169,7,
169,5, 169,1, 168,6, 168,3 (CO) és 22,2, 21,3, 21,2,
21,2, 20,8, 20,5 (CH₃)] és egy izobutaniovlsopporten δH, 2,60 sept (CH₂, 1,21 d, 1,18 d (CH₃); δC₁ₓ, 175,0 (CO),
33,8 (CH₃), 19,1, 18,4 (CH₃) jelenlétéit igazolta (I.
táblázat). A ¹³C-NMR és DEPT spektruma arra utalt, hogy a váz 20 szénatomból, azaz negy metil-, két metilén-, kilenc metincsopportból és öt kvaterner szénatomból – közülük egy ketossopport (δC, 210,3) – épül fel. Az ¹H-NMR spektrum 17 alap-
vázhoz tartozó jelet tartalmazott, melyek azonosításához ¹H- és HMQC kísérlet segítségével történt.
A ¹H-¹H COSY spektrum a protonkorrélációk alapján két részszereket bizonyított: CH₃-C₆H₅-CHR-CHR-CHR-CHR-C=CH₂-CHR-CHR-CHR-(A) és (CH₃)-CHR-CHR-CHR-(B). Kap-
szolódásainkat a HMBC spektrumban megfigyelhető távolításban (B) adja meg (I. táblázat). A kvaterner szénatomok (C-6, C-10, C-2 és C-15) és a két részszereket protonjainak két vagy három közös keresztüli korrélációi azt bizonyítják, hogy az A molekulározás megfelel egy játéfisházás díszperén C₈-C₁₉ szerkezeti nézé-
nek (a C-6-on egy exometilén-csoporttal), a B pedig
a C-13 és C-11 részre darabolható a H-13, H-12,
H-1b és a δC₁ₓ 210,3-nál jelentkező szénatom közötti
korrelációk igazolják, hogy a ketossop-
port a 14-helyzetben található. Az ésszercsopportok
poziciójára szintén HMBC mérésen volt meghatá-
rozható. A δC₁ₓ 175,0 karbonilból (izobutaniovls CO)
korrelációja a δC₁ₓ 5,38 (H-7) és δH₁ₓ 1,21, 1,18 (izobu-
tanol metilek) proton szignáljaihoz jeltekeje az
izobutaniovlsopport jelenlétéit a C-7-en. Használ-
képen a δC₁ₓ 169,5, 169,1, 168,6 és 168,3 ppm-nél je-
lentkező karbonilszén szignáljai és a δC₁ₓ 5,51 (H-8),
4,95 (H-9), 5,58 (H-3), 6,06 (H-5) protonjelek vala-
mint a δH₁ₓ 2,03, 2,04, 2,11 és 2,16 acetil metil-
szignáljai közötti jelentkező távolításokat igazoltak az acetilsopportok jelenlétéit a C-8, C-9,
C-3 és C-5 helyzetben. A fennmaradó két acetilso-
pport nem mutatott semmilyen távolító korrelá-
ciót, ezért feltételezhető volt, hogy ezek kvaterner
szénatomokhoz kapcsolódnak. Az 1 vegyület
¹H-NMR adatait az ottágú gyúrűn 2,3,15-triakionul-
szettált jatofán származékokéval összehasonlit-
va azt találtuk, hogy a H-11 és H-13 protonokra ha-
sóló 6 értékek jellemzőek [9]. Így a két acetilso-
pport (δH₁ₓ 2,11 és 2,12) a C-2 és C-15 helyzetben ta-
látható. A molekula összekapcsolással és először-
ben tárgyalt szubsztituenseket figyelembe véve
megállapíthatjuk, hogy ez a végyület egy epoxidsoportot is tartalmaz, mely szükségszerűen a C-11, C-12 helyzetben található. Ezt a H-11, H-12 (δH 3,00 d és 3,32 dd), C-11 és C-12 (δC 58,4, 57,1) kémiai eltolódásértékéi is megerősítik.


I. táblázat

<table>
<thead>
<tr>
<th>Atom</th>
<th>1H</th>
<th>13C</th>
<th>H-H COSY</th>
<th>HMBC</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3,73 d (16,2)</td>
<td>46,2</td>
<td>H-1b, H-3</td>
<td>C-2, C-3, C-4, C-14</td>
<td>H-16</td>
</tr>
<tr>
<td>1b</td>
<td>2,04 d (16,2)</td>
<td>65,7</td>
<td>H-1a, H-3</td>
<td>C-15</td>
<td>H-16</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>78,0</td>
<td>H-1a,b, H-4</td>
<td>C-1, C-2, C-15, Ac õ 186,5</td>
<td>H-7, H-16, H-17a, H-4</td>
</tr>
<tr>
<td>4</td>
<td>2,98 dd (3,3, 1,7)</td>
<td>49,9</td>
<td>H-3, H-5</td>
<td>C-3, C-14</td>
<td>H-3, H-7</td>
</tr>
<tr>
<td>5</td>
<td>6,06 brs</td>
<td>67,6</td>
<td>H-4, H-17a</td>
<td>C-3, C-4, C-6, C-15,</td>
<td>H-7, H-8, H-12,</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>141,7</td>
<td>-</td>
<td>C-17, Ac õ 186,5</td>
<td>H-13, H-17a</td>
</tr>
<tr>
<td>7</td>
<td>5,38 brs</td>
<td>68,5</td>
<td>H-8, H-17b</td>
<td>C-1, C-6, C-9, C-17, iBu-CO</td>
<td>H-3, H-4, H-5,</td>
</tr>
<tr>
<td>8</td>
<td>5,51 d (4,1)</td>
<td>69,0</td>
<td>H-7, H-9</td>
<td>C-6, C-9, C-10, Ac õ 186,5</td>
<td>H-4, H-5, H-11, H-12,</td>
</tr>
<tr>
<td>9</td>
<td>4,95 d (4,1)</td>
<td>77,3</td>
<td>H-8</td>
<td>C-8, C-10, C-11, C-18, C-19, Ac õ 186,1</td>
<td>H-7, H-11</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>39,6</td>
<td>-</td>
<td>C-10, C-18, C-19</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>3,00 d (2,1)</td>
<td>58,4</td>
<td>H-12</td>
<td>-</td>
<td>H-7, H-8, H-9,</td>
</tr>
<tr>
<td>12</td>
<td>3,32 dd (4,7, 2,1)</td>
<td>57,1</td>
<td>H-11, H-13</td>
<td>C-13, C-14</td>
<td>H-5, H-8</td>
</tr>
<tr>
<td>13</td>
<td>3,67 dq (4,7, 6, 9)</td>
<td>37,0</td>
<td>H-12, H-20</td>
<td>C-14, C-20</td>
<td>H-5</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>210,3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>92,6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>1,52 s</td>
<td>18,1</td>
<td>-</td>
<td>C-1, C-2, C-3</td>
<td>H-1a,b, H-3</td>
</tr>
<tr>
<td>17a</td>
<td>5,09 s</td>
<td>111,9</td>
<td>H-5</td>
<td>C-7</td>
<td>H-3, H-5</td>
</tr>
<tr>
<td>17b</td>
<td>5,05 s</td>
<td>58,4</td>
<td>H-7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>0,99 s ̈</td>
<td>23,5</td>
<td>-</td>
<td>C-9, C-10, C-11, C-19</td>
<td>H-11</td>
</tr>
<tr>
<td>19</td>
<td>0,71 s ̈</td>
<td>17,5</td>
<td>-</td>
<td>C-9, C-10, C-11, C-18</td>
<td>H-8, H-11</td>
</tr>
<tr>
<td>20</td>
<td>1,18 d (6,9)</td>
<td>15,3</td>
<td>H-13</td>
<td>C-12, C-13, C-14</td>
<td>H-11</td>
</tr>
<tr>
<td>iBu</td>
<td>-</td>
<td>175,0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>-</td>
<td>35,8</td>
<td>H-3', H-4'</td>
<td>C-1, C-3', C-4'</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>2,60 sept (7,0)</td>
<td>33,8</td>
<td>H-2'</td>
<td>C-1, C-2'n, C-4'</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>1,21 d (7,0)</td>
<td>19,1</td>
<td>H-2'</td>
<td>C-1, C-2', C-3'</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>1,18 d (7,0)</td>
<td>18,4</td>
<td>H-2'</td>
<td>C-1, C-2', C-3'</td>
<td>-</td>
</tr>
</tbody>
</table>

* k, c, d értékek felescérélhetők
Az esulatin B összegképlete a HRMS és NMR vizsgálatok szerint C_{19}H_{14}O_{7}. EIMS spekttrumában ececsav és keténegységek kilépésével keletkező fragmentek sorozata volt megfigyelhető (lásd a kísérleti részt) jelezve, hogy oly acetilcsoport van jelen a molekulában.

Ezzel összhangban áll az is, hogy az esulatin B ²H- és ¹³C-NMR spekttrumában öt acetilcsoportnak megfelelő szignálok jelenkeztek [δ_H 2,16 s, 2,14 s, 2,13 s, 2,11 s, és 2,05 s, δ_C 170,4, 170,1, 169,7, 169,6, 169,0 (CO), és 22,1, 21,4, 21,2, 20,9 és 20,8 (CH₃)]. Ezen felül a ¹³C-NMR és DEPT spektrum négy metil-, három metillén- és hét metinacsoportnak valamint hat kvaterner szénatomnak - beleértve két ketokcsoportot (δ_C 211,1 és 209,4) - megfelelő jelet tartalmazott (II. táblázat). Az ²H-NMR és ³H-²H COSY spektrum három tercier metilcsoport (δ_H 1,48 s, 1,17 s, 1,16 s) jelenlétéit bizonyította és azt mutatta, hogy a molekulában a következő szerkezeti elemek vannak jelen:

- CH₃-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-
- CH₃-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-C₆H₅-

Az esulatin B (2) kavarner szénatomjának HMBC körrelációi (HC)

tattott korrelációja az esulatin B-re a 2,3,5,7,13-pentaacetoxy-jatrofa-6(17),11-dién-9,14-dione szerkezetet valószínűsíti.


Az esulatin C relatív konfigurációját NOESY mérésekkel tanulmányoztuk. A H-1, H-3 és a H-4 közötti NOE interakciók és csatolási állandók nagon hasonlóak voltak az esulatin A és B-nél ta-
pasztaltakhoz, amely arra utalt, hogy a C-2, C-3, C-4 és C-15 konfigurációja azonos. A C-4 és C-15 konfigurációjára abban a tényből következtethetünk, hogy a H-4 és 15-OAc között nem jelentkezett NOE kölcsönhatás, valamint hogy minden eddig ismert jatrofán-diterpenoidban transz gyöngykapcsolódás fordul elő a röntgendiffракciós vizsgálatok szerint [6, 11, 14, 15]. A H-4 és H-5 közötti 0 csatolási állandó azt igazolta, hogy hasonlóan az 1, 2 vegyületekhez és a kansuinin A-hoz, a H-5-nek β térdírássúinak kell lennie [13]. A 14-OH és H-4 között észlelt NOE kölcsönhatás α-hidroxycsoportot jelzett a C-14-en. A C-7, C-8, C-9, C-11 és C-13 térszerkezetet a NOESY korrelációk alapján nem lehetett meghatározni, mert a molekulának ez a része nagy mértékben flexibilis. Az esulatin C tehát a 3 szerkezetet jellemzőnek. Ez a vegyület szoros szerkezeti rókságsort mutat az Euphorbia kansui-ból izolált egyik diterpénnek, a kansuinin A-val [13].

### Table: Az esulatin C (3) NMR adatok [CDCl₃, TMS, δ (ppm), (J = Hz)]

<table>
<thead>
<tr>
<th>Atom</th>
<th>H</th>
<th>C</th>
<th>H-1H COSY</th>
<th>HMBC</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.10 d (16.5)</td>
<td>43.6</td>
<td>H-1b, H-3</td>
<td>C-2, C-3, C-4, C-14</td>
<td>H-1b, H-20</td>
</tr>
<tr>
<td>1b</td>
<td>2.80 d (16.5)</td>
<td>43.6</td>
<td>H-1a, H-3</td>
<td>C-14, C-15, C-16</td>
<td>H-1a, H-16</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>88.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>6.02 d (3.5)</td>
<td>74.3</td>
<td>H-1b, H-4</td>
<td>C-2</td>
<td>H-4, H-16</td>
</tr>
<tr>
<td>4</td>
<td>3.26 d (3.5)</td>
<td>49.7</td>
<td>H-3, H-15</td>
<td>C-3, C-5, C-6, C-14, C-15</td>
<td>H-3, H-5, H-7, 14-OH, H-16</td>
</tr>
<tr>
<td>5</td>
<td>6.20 s</td>
<td>69.4</td>
<td>H-4, H-7, H-17a,b</td>
<td>C-3, C-4, C-6, C-7, C-15</td>
<td>H-4, H-8</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>147.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>6.49 s</td>
<td>67.8</td>
<td>H-5, H-8, H-17a,b</td>
<td>C-6, C-8, C-9, C-17, C-11</td>
<td>H-4, H-8, H-11</td>
</tr>
<tr>
<td>8</td>
<td>5.65 s</td>
<td>70.9</td>
<td>H-7, H-9</td>
<td>C-6, C-9, Ac 8 170.5</td>
<td>H-5, H-7, H-9, H-18</td>
</tr>
<tr>
<td>9</td>
<td>4.94 s</td>
<td>82.6</td>
<td>H-8, H-11</td>
<td>C-8, C-10, C-11, C-19, Ac 8 169.6</td>
<td>H-5, H-19</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>41.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>4.13 s</td>
<td>77.1</td>
<td>H-9, H-13</td>
<td>C-9, C-10, C-12, C-18</td>
<td>H-7, H-19</td>
</tr>
<tr>
<td>13</td>
<td>2.17 d (7.0, 1.9)</td>
<td>71.6</td>
<td>H-11, 14-OH, H-20</td>
<td>C-12, C-14, C-20</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>105.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14-OH</td>
<td>3.99 d (1.9)</td>
<td>-</td>
<td>H-13</td>
<td>-</td>
<td>H-4</td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>89.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>1.56 s</td>
<td>18.3</td>
<td>-</td>
<td>C-1, C-2, C-3</td>
<td>H-1b, H-3</td>
</tr>
<tr>
<td>17a</td>
<td>5.04 s</td>
<td>107.9</td>
<td>H-5, H-7, H-17b</td>
<td>C-5, C-6, C-7</td>
<td>-</td>
</tr>
<tr>
<td>17b</td>
<td>4.95 s</td>
<td>-</td>
<td>H-5, H-7, H-17a</td>
<td>C-5, C-6, C-7</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>1.24 s</td>
<td>18.9</td>
<td>-</td>
<td>C-10, C-11, C-19</td>
<td>H-8, H-9</td>
</tr>
<tr>
<td>19</td>
<td>1.12 s</td>
<td>22.4</td>
<td>-</td>
<td>C-9, C-10</td>
<td>H-11</td>
</tr>
<tr>
<td>20</td>
<td>1.24 d (7.0)</td>
<td>9.6</td>
<td>H-13</td>
<td>C-12, C-14</td>
<td>H-1a, H-5</td>
</tr>
<tr>
<td>1Bu</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>-</td>
<td>176.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2'</td>
<td>2.61 sept (7.0)</td>
<td>34.2</td>
<td>-</td>
<td>C-1', C-4'</td>
<td>-</td>
</tr>
<tr>
<td>3'</td>
<td>1.16 d (7.0)</td>
<td>18.5</td>
<td>-</td>
<td>C-1', C-4'</td>
<td>-</td>
</tr>
<tr>
<td>4'</td>
<td>1.23 d (7.0)</td>
<td>19.8</td>
<td>-</td>
<td>C-1', C-3'</td>
<td>-</td>
</tr>
<tr>
<td>Acetiléck</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8-CO</td>
<td>-</td>
<td>170.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8-COMe</td>
<td>2.06 s</td>
<td>21.2</td>
<td>-</td>
<td>8-CO</td>
<td>-</td>
</tr>
<tr>
<td>9-CO</td>
<td>-</td>
<td>169.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9-COMe</td>
<td>1.99 s</td>
<td>22.2</td>
<td>-</td>
<td>9-CO</td>
<td>-</td>
</tr>
<tr>
<td>2,3,5,15-CO</td>
<td>-</td>
<td>169.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>168.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>168.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>167.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2,3,5,15-</td>
<td>2.02 s</td>
<td>21.1</td>
<td>-</td>
<td>Ac 169.6</td>
<td>-</td>
</tr>
<tr>
<td>COMe</td>
<td>2.16 s</td>
<td>22.8</td>
<td>-</td>
<td>Ac 168.9</td>
<td>-</td>
</tr>
<tr>
<td>2,11 s</td>
<td>21.8</td>
<td>-</td>
<td>Ac 168.6</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2.00 s</td>
<td>21.2</td>
<td>-</td>
<td>Ac 167.5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*a A δ értékek felszerelhetők*
Következtetések

Az esulatin A (1) és C (3) az Euphorbiaceae családból ismert legmagasabban észterezett diterpennek, s ilyen nagy mértékben észterezett vegyületek ismertek a fajban. A 1-3 vegyületek olyan diterpen-políészterek, melyekben előforduló diterpenalkoholok korábban ismeretlenek voltak.

Az izolált vegyületek a jatrofánvázás diterpenoidok újabb képviselői, mely vegyületeket a család legfontosabb kemotaxonómiai bélyegeknek tekintenek [6–8]. Munkánkat megelőzően északkári és amerikai eredetű E. esula mintából közelítünk jatrofánvázás diterpenoidokat [6], európai mintából azonban ezt a vegyülettípus első alkalommal mutattuk ki. A két populációnban talált vegyületek különbözőségének kemotaxonómiai jelentősége lehet. Az esulatin A (1) a National Cancer Institute (Bethesda, MD, USA) előzetes in vitro antitumor szűrővizsgálatában hatástalannak bizonyult.

Köszönetnyilvánítás


Irodalom


[Érkezett: 1998. április 1.]
II
JATROPHONE DITERPENOIDS FROM EUPHORBIA ESULA

GÁBOR GÜNTHER*, JUDIT HOHIMANN†, ANDREA VASÁ†, IMRE MÁTHÉ†, GYÖRGY DOMB and
GYULA JERKOVICH‡‡

Department of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Hungary; † Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Hungary; ‡‡ Spectroscopic Department, Institute for Drug Research Ltd, P.O. Box 82, H 1325 Budapest, Hungary

(Received in revised form 18 June 1997)

Key Word Index—Euphorbia esula; Euphorbiaceae; jatrophone polyesters; macrocyclic diterpenes.

Abstract—Two new jatrophone diterpenoids, esulatin D and E, have been isolated from the dichloromethane extract of the whole, undried plant of Euphorbia esula. The structures have been assigned on the basis of spectral analysis, including 2D NMR experiments. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Plants of the Euphorbiaceae are known to produce highly irritant, carcinogenic, tumour-promoting diterpenoids with bi-, tri- or tetracyclic skeletons [1]. The jatrophone diterpenoids comprise a small group of the constituents of this family, they are known to occur only in a few species: Jatropha gossypifolia, J. macrorhiza, Euphorbia maddenii, E. kansui, E. helioscopia, E. characias, E. lathyriflora and E. esula L. [1–3]. These compounds have aroused interest because of their antitumour, antirhinthology, analgesic and phytotoxic activities [4–6].

As part of our studies on biologically active compounds from Hungarian Euphorbia species, E. esula was examined. Our earlier work yielded three new jatrophone polyesters, esulatin A–C, from the whole undried plant [7]. Further investigations, reported here, have led to the isolation of the related compounds esulatin D (1) and E (2), which have been established to be tetra- and hexaesters of hitherto unknown, polyfunctional diterpene parent alcohols.

RESULTS AND DISCUSSION

The dichloromethane-soluble fraction obtained from a methanol extract of the fresh, whole plant of E. esula was subjected to polyamide column chromatography and eluted with mixtures of water and methanol. The methanol-water (4:1) eluate was further purified by CC, TLC and HPLC to afford esulatin D and E.

Esulatin D (1) was obtained as colourless crystals. Its molecular formula (C39H64O13) was derived from the HR mass spectrum and NMR analyses. The Elmass spectrum displayed fragment peaks due to the sequential loss of HOAc and a ketene unit (see Experimental). The 1H and 13C NMR spectra of esulatin D showed signals corresponding to six acetyl groups [δH 2 × 2.14 s, 2.12 s, 2.11 s, 2 × 2.00 s; δC 170.4, 170.3, 169.9, 2 × 169.4 and 168.9 (CO) and 22.1, 2 × 21.3, 2 × 21.0 and 20.8 (CH3)]. Additionally, the 13C NMR and DEPT spectra exhibited resonances of one ketone, two oxygen-substituted and one alkyl-substituted quaternary carbons, one single unsaturated quaternary carbon, one unsaturated methylene carbon (exomethylene), two tertiary unsaturated carbons, two alkyl methylenes, four oxygen-substituted tertiary carbons, two alkyl-substituted tertiary carbons, one secondary and three tertiary methyl groups (Table 1). Thus, esulatin D was a derivative of the bicyclic diterpenoid jatrophone [1].

The 1H NMR spectrum of esulatin D contained 17 signals due to the parent skeleton. These signals were assigned on the basis of 1H–1H COSY and HMBC spectra, as listed in Table 1. The 1H NMR and 1H–1H COSY spectra revealed the presence of one secondary and three tertiary methyls (δH 1.14 d, 1.49 s, 1.06 s and 1.02 s) and four sequences of correlated protons: δH 3.80 br d and 1.93 d (—CH—) (A), δH 5.42 d, 2.85 d, 5.94 s, 5.21 s and 5.15 s [—CHR—CH—CHR—C—(CH2)—] (B); δH 4.96 t, 2.00 m and 4.74 dd [—CHR—CH—CHR—CH—CHR—] (C); δH 5.85 d, 5.54 dd, 3.48 dq and 1.14 d [trans—CHR—CH—CH2—] (D) (R = acetyl). The connection of these partial structures was determined from two- or three-bond long-range correlations observed in an HMBC spectrum.

* Author to whom correspondence should be addressed.
Structural elements were identified as C-1 (A), C-3-C-6 with an exomethylene on C-6 (B), C-7-C-9 (C) and C-11-C-13-C-20 (D) as follows. The quaternary carbon signal at δc 86.6 (C-2) showed correlation peaks with the proton signals at δh 3.80 and 1.93 (H-1) and 5.42 (H-3), which confirmed the linkage of structural elements A and B. Cross-peaks between the carbon signal at δc 146.0 (C-6) and the proton signals at δh 5.94 (H-5) and 4.96 (H-7) and between δc 111.7 (C-17) and H-5, H-7 revealed the connection of partial structure B to C. The 2JCH and 3JCH correlations between the carbon signal at δc 40.4 (C-10) and the proton signals at δh 2.00 (H-8), 4.74 (H-9), 5.85 (H-11) and 5.54 (H-12) suggested that molecular moiety C is adjacent to D. The quaternary carbon signal at δc 92.6 was assigned on the basis of its long-range couplings with H-1, H-3, H-4 and H-5 as C-15. Cross-peaks between the carbon signal at δc 210.9 and H-

<table>
<thead>
<tr>
<th>Atom</th>
<th>1H</th>
<th>13C</th>
<th>H-H COSY</th>
<th>HMBC</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.80 br d (16.1)</td>
<td>46.8</td>
<td>H-1a</td>
<td>C-2, C-14, C-15, C-16</td>
<td>H-1b C-2, C-3, C-4, C-14, C-15</td>
</tr>
<tr>
<td>1b</td>
<td>1.93, d (16.1)</td>
<td>46.8</td>
<td>H-1b</td>
<td>C-2, C-14, C-15, C-16</td>
<td>H-16</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>86.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>5.42 d (3.1)</td>
<td>78.2</td>
<td>H-4</td>
<td>C-1, C-2, C-15, 3-COMe</td>
<td>H-4, H-17b</td>
</tr>
<tr>
<td>4</td>
<td>2.85 d (3.1)</td>
<td>49.2</td>
<td>H-3, H-5</td>
<td>C-3, C-5, C-6, C-14, C-15</td>
<td>H-3, H-5, H-7</td>
</tr>
<tr>
<td>5</td>
<td>5.94</td>
<td>68.8</td>
<td>H-4, H-17a,b</td>
<td>C-3, C-4, C-6, C-15, 5-COMe</td>
<td>H-4, H-8, H-11, H-13</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>146.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>4.96 t (5.6)</td>
<td>68.0</td>
<td>H-8</td>
<td>C-5, C-6, C-8, C-9, C-17, 7-COMe</td>
<td>H-4, H-8</td>
</tr>
<tr>
<td>8</td>
<td>2.10 m</td>
<td>35.2</td>
<td>H-7, H-9</td>
<td>C-6, C-7, C-9, C-10</td>
<td>H-6, H-7, H-9, H-17a</td>
</tr>
<tr>
<td>9</td>
<td>4.74 dd (7.2, 4.4)</td>
<td>74.6</td>
<td>H-8</td>
<td>C-8, C-10, C-11, C-18, C-19, 9-COMe</td>
<td>H-8, H-9, H-11</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>40.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>5.15 d (16.0)</td>
<td>139.3</td>
<td>H-12</td>
<td>C-9, C-10, C-13, C-18, C-19</td>
<td>H-5, H-9, H-13, H-19</td>
</tr>
<tr>
<td>12</td>
<td>5.54 dd (10.0, 9.3)</td>
<td>130.0</td>
<td>H-11, H-13</td>
<td>C-10, C-13, C-14, C-20</td>
<td>H-18</td>
</tr>
<tr>
<td>13</td>
<td>3.48 d (9.3, 6.6)</td>
<td>43.7</td>
<td>H-12, H-20</td>
<td>C-11, C-12, C-14, C-20</td>
<td>H-5, H-11, H-20</td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>210.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>92.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>1.49 s</td>
<td>18.0</td>
<td>—</td>
<td>C-1, C-2, C-3</td>
<td>H-1b</td>
</tr>
<tr>
<td>17a</td>
<td>5.21 s</td>
<td>111.7</td>
<td>H-5</td>
<td>C-5, C-6, C-7</td>
<td>H-8</td>
</tr>
<tr>
<td>17b</td>
<td>5.15 s</td>
<td>111.7</td>
<td>H-5</td>
<td>C-5, C-6, C-7</td>
<td>H-5</td>
</tr>
<tr>
<td>18</td>
<td>1.06 s</td>
<td>21.8</td>
<td>—</td>
<td>C-9, C-10, C-11, C-19</td>
<td>H-12</td>
</tr>
<tr>
<td>19</td>
<td>1.02 s</td>
<td>26.8</td>
<td>—</td>
<td>C-9, C-10, C-11, C-18</td>
<td>H-9, H-11</td>
</tr>
<tr>
<td>20</td>
<td>1.14 d (6.6)</td>
<td>19.5</td>
<td>H-13</td>
<td>C-12, C-13, C-14</td>
<td>H-13</td>
</tr>
</tbody>
</table>

Acetils

- 2-CO
- 2-COMe
- 3-CO
- 3-COMe
- 4-CO
- 4-COMe
- 5-CO
- 5-COMe
- 6-CO
- 6-COMe
- 7-CO
- 7-COMe
- 8-CO
- 8-COMe
- 9-CO
- 9-COMe
- 10-CO
- 10-COMe
- 11-CO
- 11-COMe
- 12-CO
- 12-COMe
- 13-CO
- 13-COMe
- 14-CO
- 14-COMe

**Note:** δ values are interchangeable.
Jatrophone diterpenoids from *Euphorbia esula*

12. H-13 and H-20 indicated that the keto group must be sited at C-14. In addition, in the HMBC spectrum, correlations were observed between the carbonyl carbon signals of the acetyl groups and H-3, H-5, H-7 and H-9. Unfortunately, unambiguous assignment of the $^1$H and $^{13}$C NMR signals of the acetyl groups was not possible because the carbon signals of 5-COME and 7-COME and the proton signals of 3-COME and 9-COME were the same, and the acetyl groups attached to the quaternary carbons (2-OAc and 15-OAc) could not be differentiated in the absence of long-range correlations.

The relative stereochemistry of esulatin D was studied by means of the NOESY spectrum. A convenient point of reference was H-4, which was assumed to be axial. Cross-peaks between H-4 and H-3 proved the β-orientation of the acetyl group on C-3. The zero coupling constant between H-4 and H-5 required that H-5 was α as in esulatin A–C. In the NOESY spectrum, correlation signals between H-4 and H-7 revealed the presence of a β-oriented ester group at C-7. The proton signal at δH 5.94 (H-5) correlated with the proton signal at δC 5.85 (H-11), which indicated that H-11 is oriented above the plane of the macrocyclic ring. The cross-peak observed between H-11 and H-13 dictates the orientation of the methyl group on C-13. H-12, whose orientation is the opposite of that of H-11, showed an NOE interaction with one of the geminal methyl groups on C-10 (δH 1.06). Thus, this methyl group (C-18) is on the $\alpha$ side and the other (C-19) is in the β position. Correlation signals between H-19 and H-9 indicated the presence of an $\alpha$-oriented acetyl group on C-9. In the NOESY spectrum, no correlation was observed between H-4 and 15-OAc, which suggested the β orientation of the acetyl group on C-15.

With regard to the above data, the structure of esulatin D is formulated as I. The stereochemistry and absolute configuration of esulatin D were also studied by means of X-ray analysis [8]. The crystallographic data corroborated the structure elucidated above.

Esulatin E (2) was obtained as an amorphous white solid. Its molecular formula was assigned by HR mass spectrometry and NMR analyses as $C_{27}$H$_{40}$O$_6$. In the EI mass spectrum, a molecular ion was observed at $m/z$ 532, with prominent fragment ions at $m/z$ 490, 430, 370 and 310, representing losses of acetic acid and a ketene unit. Fragment peaks were also seen at $m/z$ 123 and 96, corresponding to the ions (CH$_3$)$_2$C=CH–CH=CH$_2$O$^-$ and (CH$_3$)$_2$C=CH–CH=CH$_2$, which were previously found to be characteristic of jatroaph-11-en-9,14-dione derivatives [7]. The $^1$H and $^{13}$C NMR spectra of esulatin E showed signals corresponding to four acetyl groups ($\delta$H 2.17 s, 2.15 s, 2.03 s and 2.02 s; $\delta$C 170.5 s, 170.1 s, 169.7 s and 168.8 s (CO) and 22.2, 21.4, 21.1 and 20.8 s (CH$_3$)). Additionally, the $^{13}$C NMR spectrum exhibited resonances for six quaternary carbons, including two ketones ($\delta$C 208.3 and 202.8), four olefinic carbons, two ester-bearing and two alkyl-substituted methines, two methylenes and four methyls. The $^1$H NMR and $^{13}$C NMR spectra revealed the presence of three tertiary methyls ($\delta$H 1.64 s, 1.77 s and 1.23 s) and structural elements $-C\equiv H\equiv(C\equiv H\equiv)$ ($\delta$H 3.46 d and 2.13 d) (A), $-C\equiv H\equiv(C\equiv H\equiv-C\equiv H\equiv-C\equiv H\equiv-C\equiv H\equiv$ ($\delta$H 5.33 d, 2.21 dd, 6.03 s, 5.73 s, 6.71 d and 6.58 d) (B) and $-C\equiv H\equiv(C\equiv H\equiv-C\equiv H\equiv-C\equiv H\equiv)$ ($\delta$H 5.70 d, 5.42 dd, 3.60 dq and 1.16 d) (C) (R = acetyl). Fragment B was elucidated with the aid of the $^1$J couplings observed between H-5, H-7 and the exomethylene (H-17). Connection of sequences A, B and C was performed by means of HMBC experiment (Table 2). The correlations of the quaternary carbons (Figure 1) supported the structure of esulatin E as 2,3,5,15-tetraacetoxysphatoph-6(17),7E,11E-triene-9,14-dione.

The relative configuration of esulatin E was investigated by means of NOESY measurements. The observation of an NOE effect between H-3 and H-4 revealed a β-oriented ester group on C-3. The relative small coupling constant between H-4 and H-5 indicated that, similarly as in esulatin A, B and D, H-5 is in the β position [7]. The coupling constants of H-7, H-8, H-11 and H-12 (16.4 and 15.9 Hz) proved that the C-7/C-8 and C-11/C-12 double bonds have the E configuration. The cross peaks between H-4 and H-8 and between H-8 and H-11 suggested that H-8 and H-11 are oriented below the plane of the molecule. NOESY correlations concerning the stereochemistry of C-2, C-13 and C-15 were not found, but on the basis of the close relationship with esulatin A, B and D, the β orientation of H-13 and the 15-OAc and 16-methyl groups seems likely. The above data led to the formulation of esulatin E as 2. The $^1$H and $^{13}$C NMR chemical shifts of 2 were assigned by means of $^1$H-$^1$H COSY, HMQC and HMBC spectral analysis, as listed in Table 2.

Previous phytochemical studies on *E. esula* resulted in the isolation of diterpenes with ingenane, lathyrate and jatrophone skeletons [5, 9–13]. In the collection of Hungarian plants which we investigated, ingenane and lathyrate diterpenoids were not found and the isolated jatrophones were different from those reported earlier from North American collections [5, 17, 18]. In the esulatin series obtained from the Hungarian collection, aromatic acyl residues were missing and the alcohol core of the compounds is new. This variation of jatrophone diterpenoids may be of chemotaxonomic significance.

**EXPERIMENTAL**

General experimental procedures. Mp are uncorr. UV: MeOH; EIMS: 70 eV, direct inlet; $^1$H and $^{13}$C NMR and DEPT: 400 MHz ($^1$H) and 100 MHz ($^{13}$C), CDCl$_3$, with TMS as int. standard. NOESY and 2D $^1$H-$^1$H and $^{13}$C-$^1$H correlation spectra were obtained using standard Bruker software. Optical rotations were determined in CHCl$_3$ at ambient temp; CC: polyamide (ICN). VLC: silica gel (Kieselgel GF$_254$, 15 μm, Merck). HPLC: LiChrospher RP-18 and LiChrospher
Table 2. NMR Spectral Data of 2 [CHCl₃, TMS, δ (ppm), (J = Hz)]

<table>
<thead>
<tr>
<th>Atom</th>
<th>δH</th>
<th>δC</th>
<th>δH-CH COSY</th>
<th>HMBC</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>3.46 s (15.9)</td>
<td>46.2</td>
<td>C-2, C-3, C-4, C-14</td>
<td>H-1b</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>2.13 d (15.9)</td>
<td>86.5</td>
<td>C-14, C-15, C-16</td>
<td>H-1a, H-16</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>86.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.33 d (6.9)</td>
<td>78.0</td>
<td>C-2, C-15, C-3-COMe</td>
<td>H-4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.21 d (6.9, 3.5)</td>
<td>48.1</td>
<td>C-3, H-5</td>
<td>H-3, H-5</td>
<td>H-3, H-5, H-8</td>
</tr>
<tr>
<td>5</td>
<td>6.03 d (3.5)</td>
<td>69.6</td>
<td>H-4, H-17</td>
<td>H-4, H-7, H-8, H-17</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>144.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.71 d (16.4)</td>
<td>136.5</td>
<td>C-5, C-8, C-9</td>
<td>H-5, H-7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>6.58 d (16.4)</td>
<td>129.6</td>
<td>C-6, C-7, C-9</td>
<td>H-4, H-5, H-11, H-18</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>209.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>49.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>5.70 d (15.9)</td>
<td>139.2</td>
<td>C-10, C-13, C-18, C-19</td>
<td>H-8, H-13, H-18, H-19, H-20</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.42 d (15.9, 9.0)</td>
<td>131.1</td>
<td>C-10, H-11, H-13</td>
<td>H-13, H-18, H-19, H-20</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5.69 d (9.0, 6.7)</td>
<td>45.5</td>
<td>H-12, H-20</td>
<td>H-11, H-12, H-20</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>208.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>89.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.64 s</td>
<td>19.8</td>
<td></td>
<td></td>
<td>H-1b</td>
</tr>
<tr>
<td>17</td>
<td>5.37 s (2 H)</td>
<td>116.6</td>
<td>C-5, C-6, C-7</td>
<td>H-5, H-7</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.27 s</td>
<td>24.4</td>
<td>C-9, C-10, C-11, C-19</td>
<td>H-11, H-12, H-8</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.23 s</td>
<td>23.0</td>
<td>C-9, C-10, C-11, C-18</td>
<td>H-11, H-12</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.16 s (6.7)</td>
<td>17.8</td>
<td>C-12, C-13, C-14</td>
<td>H-11, H-12, H-13</td>
<td></td>
</tr>
</tbody>
</table>

Acetyl
2-CO     |            | 170.1   |            |               |               |
2-COMe   | 2.17 s     | 21.4    | 2-COMe     |               |               |
3-CO     | 2.02 s     | 169.7   |            |               |               |
3-COMe   |            | 20.8    |            |               |               |
5-CO     |            | 170.5   |            |               |               |
5-COMe   | 2.03 s     | 22.2    | 5-COMe     |               |               |
15-CO    | 1.88 s     | 21.4    |            |               |               |
15-COMe  | 2.15 s     | 21.4    | 15-COMe    |               |               |

**Note:** δ values are interchangeable.

![Acetyl](image)

Fig. 1. HMBC correlations of quaternary carbons of esulatin E (2) (H → C).

Si 100 (5 µm), flow 0.5 ml/min⁻¹, with RI detection; TLC: Si gel 60 F₂₅₄.

Plant material. E. esula was collected in May 1994 in Szeged, Hungary, on the banks of the Tisza River and identified by Károly Penszka (Department of Botany and Plant Physiology, Agricultural University of Gödöllö, Hungary). A voucher specimen has been deposited at the Herbarium of the Museum of Natural Sciences in Budapest, Hungary.

Extraction and isolation. The fresh and entire plants of E. esula (11 kg) were extracted with MeOH (75 l) at room temp. The crude extract was concd in vacuo and partitioned between CH₂Cl₂ (7 × 1.5 l) and H₂O. On evapn, the organic phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixts of H₂O-MeOH (4:1, 3:2, 2:3, 1:4) as eluents. The combined frs 1–15 (15 g) were subjected to silica gel VLC (VLC I) using a gradient system of cyclohexane-Me₂CO (19:1, 9:1, 4:1, 3:1, 1:1, 1:3, 3:7). Frs eluted with cyclohexane-Me₂CO (4:1) were transferred repeatedly to a Silica gel VLC (VLC II) and successively eluted with CHCl₃-MeOH mixts of increasing polarity. Frs 22–23 obtained from VLC II with CHCl₃-MeOH (100:0.3) were further purified by prep. TLC on Si gel using n-hexane-THF-MeCN (20:5:1) as solvent and by HPLC using normal phase column and cyclohexane-CH₃OH (50:10:1) as eluent to yield 5.1 mg of esultan E (2). Frs 28–30, which were eluted from VLC II with CHCl₃-MeOH (100:0.3), after HPLC purification on reverse phase column using MeOH-H₂O (7:3) as eluent afforded esulatin D (1) (10 mg).


Esulatin E (2). Amorphous white solid. [Ω]D⁵⁺ -95
REFERENCES


Acknowledgements — This investigation was funded by Ministry of Culture and Education, Republic of Hungary, Project No. MKM 495 and by the National Scientific Research Fund, Project No. OTKA T022587.
III
Structural Characterization and Dynamic NMR Studies of a New Peracylated Macrocyclic Diterpene

Gábor Günther,1 Tamás Martinek,2 György Dombi,1 Judit Hohmann,1* and Andrea Vasas2

1 Institute of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University, 4 Somogyi Str, H-6720 Szeged, Hungary
2 Institute of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, P.O.B. 121, H-6701 Szeged, Hungary
3 Institute of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O.B. 121, H-6701 Szeged, Hungary

Received 21 August 1998; revised 2 December 1998; accepted 7 December 1998

ABSTRACT: A heptaster of a new diterpene alcohol was isolated from the dichloromethane extract of the whole plant of Euphorbia esula L. A detailed NMR and mass spectral analysis revealed that its structure is \((E)\cdot(2R, 3S, 4S, 5R, 7S, 8R, 9S, 13R, 15R)\cdot2,3,5,6,7,8,9\)-pentaacetoxy-1-tosbutanoxy-1\cdot4-oxojarophane-(11,14)-diene. Its conformation was studied in three different solutions. The temperature dependence of the spectral parameters revealed conformational changes through internal rotation of the macrocycle part of the molecule. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: NMR; conformational study; energetic calculation; diterpenoids

INTRODUCTION

Plants of the genus Euphorbia are well known for their acute and chronic toxic effects. Diterpenes containing a tigliane, ingenane or daphnane skeleton have been found to possess pro-inflammatory and tumour-promoting activities.1 The genus also produces macrocyclic diterpenes, which are regarded as progenitors of the polyacetylenic derivatives.2,3 The macrocyclic compounds to be found in Euphorbia species have received relatively little attention because their concentrations in plants are generally very low, and these substances do not exert a toxic effect. Some of the macrocyclic diterpenoids exhibit marked biological activity, e.g. jatrophone, jatrophatrine, euphospin A and B and euphorin display antitumour properties.4

In a systematic search for biologically active compounds from Hungarian Euphorbiaceae, we have previously reported esulatin A-E, a series of new macrocyclic diterpenes of the jatrophone type from Euphorbia esula L.4 The process of conformational exchange of different diterpenes has been investigated by means of NMR and force-field molecular mechanics calculations.5,6 The jatrophone diterpenes have been known for some years. The conformational flexibility of the skeleton has not been detected experimentally, and its energetic features have not yet been calculated. This is the first report dealing with a dynamic conformational study of a jatrophone diterpenoid, esulatin F (1), in solution. The isolation, structure elucidation and dynamic stereochemical analysis of 1 are reported.

RESULTS AND DISCUSSION

The dichloromethane-soluble portion of a methanolic extract of the fresh, whole plant of E. esula was fractionated by polyamide column chromatography, silica gel flash chromatography and high-performance liquid chromatography (HPLC) to afford a new crystalline (m.p. 205–207 °C, from methanol) and optically active ([\(\alpha\)]D)25

-67.7, c 1.1, chloroform) constituent, esulatin F (1).

Compound 1 gave a parent ion at m/z 722.3200 Da on high-resolution electron ionization mass spectrometry (HR-EI-MS), corresponding to the molecular formula C38H52O14 (calculated 722.3120). Its 1H NMR spectrum in CDCl3 at 300 K contained broad signals. Changing the solvent to pyridine-d5 resulted in a significantly better resolved spectrum. The 1H and 13C NMR spectra of 1 in pyridine-d5 showed the presence of one isobutyrate and six acetate groups. Additionally, the spectra exhibited resonances for 20 carbons comprising a diterpene core: three tertiary methyls and one secondary methyl, one trans-disubstituted olefin, one exocyclic methylene and one simple methylene, seven methine groups (including five acyl-substituted methines), one ketone and four quaternary carbons: two ester-bearing, a single unsaturated and an alkyl-substituted one (Table 1). To ascertain the proton and carbon connectivities, homo- and heteronuclear 2D-COSY experiments were performed. The 1H–1H COSY spectrum revealed the presence of three structural elements: \(-\text{CHR}\cdot\text{CHR}\cdot\text{CHR}\cdot\text{CHR}\) (A), \(-\text{CHR} \cdot \text{CHR} \cdot \text{CHR} \cdot \text{CHR}\) (B) and trans \(-\text{CH} = \text{CH} - \text{CH} = \text{CH}\) (C) (R = acyl). Their connectivities were established from the long range C–H correlations observed in an HMBC spectrum (Table 1).

The long-range correlations of the quaternary carbons with the methyl and methylene protons and with the protons of the structural elements A–C revealed that 1 is a jatrophone diterpene and that fragments A, B and C are is...
Table 1. NMR data for esulatin F (1)*

<table>
<thead>
<tr>
<th>Atom</th>
<th>$^1$H (Pyridine-$d_5$) (300 K)</th>
<th>$^1$H (CDCl$_3$) (300 K)</th>
<th>NOE: H No.</th>
<th>$^{13}$C (Pyridine-$d_5$) (350 K)</th>
<th>HMBC: H→C (350 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α</td>
<td>4.24 d (15.8)</td>
<td>3.79 d (16.0)</td>
<td>16</td>
<td>47.8</td>
<td>2, 3, 4, 14, 15</td>
</tr>
<tr>
<td>1β</td>
<td>2.28 d (15.8)</td>
<td>1.94 d (16.0)</td>
<td>16</td>
<td>87.1</td>
<td>14, 15</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>5.88 s</td>
<td>5.46 brs</td>
<td>4, 16</td>
<td>78.6</td>
<td>2, 15</td>
</tr>
<tr>
<td>4</td>
<td>3.37 brs</td>
<td>2.99 brs</td>
<td>3, 5, 7</td>
<td>50.2</td>
<td>3, 6, 14, 15</td>
</tr>
<tr>
<td>5</td>
<td>6.32 brs</td>
<td>5.93 brs</td>
<td>4, 8, 13, 17a</td>
<td>68.5</td>
<td>3, 4, 6, 7, 15, 17</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>145.9</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>5.51 brs</td>
<td>5.06 brs</td>
<td>4, 5</td>
<td>68.5</td>
<td>6, 8, 17</td>
</tr>
<tr>
<td>8</td>
<td>5.71 s</td>
<td>5.25 s</td>
<td>5, 9, 11, 18/19</td>
<td>72.7</td>
<td>6, 7, 10</td>
</tr>
<tr>
<td>9</td>
<td>5.36 d (1.9)</td>
<td>4.93 s</td>
<td>8, 11</td>
<td>78.2</td>
<td>7, 8, 10, 11, 18, 19</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>40.5</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>6.12 d (15.8)</td>
<td>5.90 d (15.8)</td>
<td>8, 9, 13, 18/19</td>
<td>138.5</td>
<td>10, 13, 18, 19</td>
</tr>
<tr>
<td>12</td>
<td>5.86 dd (15.8, 8.9)</td>
<td>5.63 dd (15.8, 9.0)</td>
<td>13, 20</td>
<td>132.0</td>
<td>10, 13, 14</td>
</tr>
<tr>
<td>13</td>
<td>5.90 dq (8.9, 6.7)</td>
<td>5.36 dq (9.0, 6.7)</td>
<td>5, 11, 12, 20, 21</td>
<td>44.1</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>211.2</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>93.2</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>1.61 s</td>
<td>1.49 s</td>
<td>1α, 1β, 3</td>
<td>18.4</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>17α</td>
<td>5.60 s</td>
<td>5.17 s</td>
<td>—</td>
<td>113.1</td>
<td>6, 7</td>
</tr>
<tr>
<td>17b</td>
<td>5.51 brs</td>
<td>—</td>
<td>8, 11</td>
<td>23.0</td>
<td>7</td>
</tr>
<tr>
<td>18</td>
<td>1.09 s</td>
<td>1.02 brs</td>
<td>8, 11</td>
<td>27.9</td>
<td>9, 10, 11</td>
</tr>
<tr>
<td>19</td>
<td>1.15 s</td>
<td>1.12 brs</td>
<td>8, 11</td>
<td>20.2</td>
<td>12, 13, 14</td>
</tr>
<tr>
<td>20</td>
<td>1.30 d (6.7)</td>
<td>1.14 d (6.7)</td>
<td>12, 13</td>
<td>20.2</td>
<td>12, 13, 14</td>
</tr>
</tbody>
</table>

* $^1$H NMR signals of the acyl groups: 2-OAc, 15-OAc, 2.20 s, 2.37 s; 3-OAc, 2.24 s; 5-OAc, 2.21 s; 8-OAc, 2.04 s; 9-OAc, 2.10 s; 7-OIFBu, 2.58 sept; (7.0) (→CH→), 1.18 d (7.0), 1.14 d (7.0) (→CH→). $^{13}$C NMR signals: 2-OAc, 15-OAc, 20.9, 22.3, 171.1, 170.4; 3-OAc, 21.2, 169.3; 5-OAc, 21.1, 169.4; 8-OAc, 20.4, 169.7; 9-OAc, 20.6, 169.8; 7-OIFBu, 175.6 (CO), 34.4 (→CH→), 17.7, 19.0 (→CH→).

Structural parts C-3—C-5, C-7—C-9 and C-11—C-13(C-20), respectively. The positions of the ester groups were determined via the HMBC experiment, with evaluation of the $^3$J(C, H) couplings between the oxy methane protons and the ester carbonyl carbons. The cross peaks of the carbon signals at $\delta_C$ 175.6 (isobutyroyl CO), 169.3, 169.4, 169.7 and 169.9 ppm (acyetyl CO) with the proton signals at $\delta_H$ 5.51 (H-7), 5.88 (H-3), 6.52 (H-5), 5.71 (H-8) and 5.36 ppm (H-9), respectively, unambiguously indicated the locations of five ester groups. The remaining two acetyl, which did not show any long-range correlations, were of necessity situated on quaternary carbons C-2 and C-15.

The relative configuration of 1 (Fig. 1) was assessed by analysing the coupling constants and the results of a NOESY experiment. The $\tau$ trans geometry of the C-11—C-12 double bond was indicated by the coupling constant of 15.8 Hz between H-11 and H-12. All protons geminal to the acyl groups in the macrocyclic ring gave singlets, suggesting their mutually orthogonal positions. A convenient point of reference was the proton at the ring junction (H-4), which was assumed to be $\alpha$. Diagnostic NOEs were observed between H-4 and H-7 and between H-4 and H-3, indicating $\beta$-oriented acyl groups at C-3 and C-7. The zero coupling constant between H-4 and H-5 required that H-5 was $\beta$, as in esulatin A—E.5 NOESY correlations between H-5 and H-8, between H-5 and H-13 and between H-8 and H-9 demonstrated the $\beta$-position of H-5, H-8, H-9 and H-13. The NOESY cross peaks between H-8 and H-11 dictated the position of H-11 as above the plane of the molecule. From the Overhauser effect between 20-CH$_3$ and H-12, the opposite position of H-12 could be concluded. All of the above data led to the formulation of esulatin F as 1. The relative configuration of 1 is the same as found in esulatin A, B and D, which was determined by x-ray crystallography.6 Esulatin F displayed a close structural similarity with terracinolide B, isolated from E. terracina.7 the esterification pattern and stereochemistry (except the configuration of C-13) are the same, and the two-carbon fragment at C-17 is missing in 1. The occurrence of such similar compounds is probably associated with their botanical relationship, since both species are members of the Section Esula.

Conformational study

The broadness of the $^1$H NMR signals and the missing $^{13}$C NMR signals from the spectra of esulatin F (1) recorded in CDCl$_3$ and CD$_2$Cl$_2$ at ambient temperature were taken to be indications of conformational flexibility of the 12-membered ring$^{10}$ or keto—enol tautomerism process due to the keto group at C-14.

![Figure 1. Structure and relative stereochemistry of 1.](image-url)
Figure 2. Signals of the acyloxymethine protons of the macrocyclic part of the esulatin F at 300 K in (1) pyridine-\(d_5\), (2) CD$_2$Cl$_2$ and (3) CDCl$_3$ as solvent.

Interpretation of the $^1$H NMR data relating to the macrocyclic part in CDCl$_3$ and CD$_2$Cl$_2$ was sometimes hampered by severe overlapped and broadened peaks of the diterpene core. In pyridine-$d_5$ as a weak basic solvent, sharp signals were detected (Fig. 2), suggesting the possibility of tautomerization.

To distinguish between the possibilities of tautomerization and a conformation change, $^1$H and $^{13}$C NMR spectra in CD$_2$Cl$_2$ and CDCl$_3$ were investigated in the temperature ranges 174–300 K [Fig. 3(a)] and 235–325 K, respectively. The complete signal assignments were proved by the HMOC and EXSY spectra. At about 240 K, the two components of the process have well separated signals in the $^1$H NMR spectra, and their ratio is 3:1. The intensity ratio changed only below 204 K in CD$_2$Cl$_2$ when signals corresponding to further components appeared. The hydrogen which should be involved in the tautomerization process (H-13, $\delta$H 3.57 ppm in CDCl$_3$) also splits into two signals (H-13 and H-13') with the above-mentioned intensity ratio, and the $^{13}$C NMR spectrum revealed two keto signals (a ratio of about 3:1), at $\delta$C 211.6 and 209.6 ppm. From the above facts it may be deduced that there is no tautomerization and a conformational exchange process$^{11-13}$ is present.

Figure 3. The conformational change of esulatin F (1) in (a) dichloromethane-$d_2$ and (b) pyridine-$d_5$. Peaks marked with sterohis correspond to the water in the solvent.
For analysis of the structure and conformational processes, esulatin F (1) was subjected to a series of $^1$H NMR measurements in the range 240-350 K in pyridine-$d_5$ [Fig. 3(b)], the signals being relatively sharp at ambient temperature.

Variation of the temperature causes a number of changes in the $^1$H NMR spectrum. The behaviour of the three resonances between $\delta_0$ 4.5 and 2.5 ppm (H-1a, H-4 and H-13) is particularly marked. The remaining part of the spectrum includes overlapping hydrogen resonances, and the broad signals lead to problems of interpretation of the low-temperature spectra. The signals sharpen above 320 K and develop into two well separated peaks at about 260 K. The peaks are unequal in intensity (again 2 : 1), showing that one structural form is favoured. The proportions of the two species did not change significantly with a decrease in temperature.

For investigation of the energetics of the ring system and mapping of the exchange pathways, a series of 2D EXSY spectra were recorded at 3 K intervals from 247 to 258 K in pyridine-$d_5$, from 174 to 310 K in CD$_2$Cl$_2$ and from 240 to 330 K in CDCl$_3$. The best indicator was the H-4 signal in the macrocycle at $\delta_0$ 3.32, 2.93 and 2.79 ppm, respectively; during cooling, this peak split into two signals (e.g. $\delta_0$ 3.42 and 2.92 ppm in pyridine-$d_5$). The cross peaks and the diagonal peaks of this proton in the two conformers were integrated and analysed by matrix formalism, where the rate constants of the exchange process at the given temperatures can be obtained.

The exchange was too slow in CD$_2$Cl$_2$ and CDCl$_3$ in this temperature range and peak integration was hampered by the high overlap of the signals. An initial value estimation was, therefore, used for the calculation of rate constants, but the measured values had too high a standard error. The rate constants in pyridine-$d_5$ were used with the Eyring equation $^{15}$ to calculate the enthalpy, $\Delta H^\ddagger$, entropy, $\Delta S^\ddagger$, free energy of activation, $\Delta G^\ddagger$ (Table 2) and activation parameters of the exchange process (Fig. 4).

These data might be used in comparisons of the energy profiles of analogous equilibria, and the results could also be used in calculations of reverse-field molecular mechanics.

The main conformational features of the jatropha-6(17), 11-diene skeleton are determined by the positions of the two double bonds and the keto group. The skeleton is strained by these three structural elements and by the five-membered ring. The coupling between H-4 and H-5 is a good indicator to determine the position of the exocyclic methylene as described Appendino et al.$^{17}$

Splitting of the signal of H-5 in to a doublet could not be observed, from which no change in the position of C-17 was concluded. The weak scalar coupling ($<$1 Hz) between H-4 and H-5 suggests their almost orthogonal position, and the exocyclic methylene is in the mean plane of the macrocyclic, i.e. it is in an exo position in both conformers. The diagnostic NOE pathways and the cross peak between H-4/H-5 and H-5/H-8 in the NOESY spectra prove these considerations. These cross peaks for the component present in a higher amount can also be detected at lower temperatures.

The spatial position of the keto group is relatively fixed, but the C-11—C-12 double bond can rotate around the C-13—C-10 axis. Moreover, the fragment between

\[ y = -3.3664x + 31.248 \]
\[ R^2 = 0.9973 \]
\[ \text{linear (k2)} \]
\[ y = -0.8562x + 22.08 \]
\[ R^2 = 0.9986 \]

Figure 4. Eyring plot of the exchange process in pyridine-$d_5$ calculated from the 2D EXSY build-up curves for H-4 and the energetic data for the exchange of 1 in pyridine-$d_5$.

Table 2. Energetic data on the equilibrium of 1 in pyridine$^a$

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$k_1$ (s$^{-1}$)</th>
<th>$k_2$ (s$^{-1}$)</th>
<th>$K$</th>
<th>$\Delta G^0$ (kJ mol$^{-1}$)</th>
<th>$\Delta G^\ddagger$ (kJ mol$^{-1}$)</th>
<th>$\Delta G^\ddagger$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>247</td>
<td>5.7(3)</td>
<td>16.1(3)</td>
<td>2.8(2)</td>
<td>-2.1(2)</td>
<td>56.5(1)</td>
<td>54.4(5)</td>
</tr>
<tr>
<td>250</td>
<td>9.0(7)</td>
<td>24.2(9)</td>
<td>2.6(8)</td>
<td>-2.1(4)</td>
<td>56.3(0)</td>
<td>54.2(4)</td>
</tr>
<tr>
<td>252</td>
<td>11.6(3)</td>
<td>30.3(3)</td>
<td>2.6(1)</td>
<td>-2.0(1)</td>
<td>56.1(6)</td>
<td>54.1(9)</td>
</tr>
<tr>
<td>255</td>
<td>18.7(4)</td>
<td>48.5(3)</td>
<td>2.5(9)</td>
<td>-2.0(2)</td>
<td>55.9(5)</td>
<td>53.9(4)</td>
</tr>
<tr>
<td>257</td>
<td>23.8(1)</td>
<td>62.4(5)</td>
<td>2.6(2)</td>
<td>-2.0(6)</td>
<td>55.8(2)</td>
<td>53.8(3)</td>
</tr>
</tbody>
</table>

*Standard errors of the calculations are given in parentheses.
C-10 and C-7 is extremely mobile. These motions can be characterized by the multiplicity of H-12 and by the difference in the chemical shifts of the conformers, suggesting that the protons are out of the plane of the macrocycle (Fig. 5).

The signal of H-12 in the planar arrangement of the macrocycle, which is the main conformer at higher temperature, displays dd splitting, whereas in the bent ring it exhibits only d splitting (Fig. 6). The 2D measurements show H-11/H-13, H-12/CH$_{2}$-20 NOESY cross peaks for the planar conformer and weak cross peaks between protons from the two ends of the molecule (e.g., CH$_{3}$-16/H-1) for the bent conformer and changes in the multiplicity of H-7, H-8 and H-9 in the EXSY spectra. The cross peaks relating to ester groups did not help in the elucidation of conformation because of overlapping signals and

Figure 5. NOESY cross peaks of esulatin F (1) for the conformational study. Thick arrows indicate the exo conformation of C-17 and thin arrows indicate the planar conformation.

Figure 6. The planar (I) and the bent (II) conformers of esulatin F (1).
their rotation around the \( \text{C} - \text{O} - \text{R} \) bonds, but their presence is indicative of considerable hindrance in the conformational motions. The results do not allow appreciable assumptions concerning the conformation of the component present in lower amount.

The x-ray structure provides information on the conformation of the 12-membered ring in solid state. Two conformers, one planar and the other bent, can be seen in the elemental cell of esulin A.18 These results are in accordance with those obtained from the NMR analysis.

**EXPERIMENTAL**

**General methods**

NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400.13 MHz (\(^1\)H) and 100.62 MHz (\(^13\)C). The signals of the deuterated solvents were taken as the reference. Two-dimensional experiments were performed with standard Bruker software. EXSY parameters are as follows: 1024 \( \times \) 512 data point, relaxation delay 2.5 s, mixing time 5 \( \mu \)s as the zero point and 5, 10, 20, 40, 80, 150, 300, 600 and 1000 ms at each temperature. The rate constants were calculated by using the matrix formalism implemented in MATLAB.17 Mass spectral measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. Optical rotations were determined in CHCl\(_3\) at ambient temperature using a Perkin-Elmer Model 341 polarimeter. Melting-points were not corrected. For column chromatography, polyamide (ICN) and silica gel (Kieselgel GF\(_254\), 15 \( \mu \)m, Merck) were used. HPLC was carried out on a Waters Millipore instrument, with refractive index detection on a normal-phase column (LiChropraph Si 100, 5 \( \mu \)m, Merck) and on a reversed-phase column (LiChropraph RP-18, 5 \( \mu \)m, Merck).

**Isolation of 1**

The extraction of *E. emula* and column chromatographic separations are described in Ref. 4. Fractions 21–35 eluted via tetrachloride liquid chromatography (VLC) with CHCl\(_3\)-MeOH (1:1 0.5) were subjected to normal- and reversed-phase HPLC, with elution with cyclohexane-EtOAc-EtOH (60:30:4) and acetonitrile-water (7:3), respectively. This allowed the isolation of 34.5 mg of esulin F (1) as white needles, m.p. 205–207°C (from MeOH); \([a]_D\)\(^{25}\) = –67.7 (c 1.1, CHCl\(_3\)); \(^1\)H and \(^13\)C NMR, see Table 1; EIMS (70 eV), \(m/z\) (relative intensity, %) 722 [M\(^+\)] (5), 662 [M – AcOH\(^+\)] (2), 634 [M – f-BuOH\(^+\)] (1), 602 [M – f-BuOH\(^+\)] (2), 532 [M – 2 \( \times \) AcOH\(^+\)] (3), 472 [M – 3 \( \times \) AcOH – (CH\(_2\))\(_2\)CO\(^+\)] (5), 412 [M – 4 \( \times \) AcOH – (CH\(_2\))\(_2\)CO\(^+\)] (3), 96 [CH\(_2\)\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)O\(^+\)] (29), 71 [C\(_6\)H\(_5\)O\(^+\)] (18), 43 [CH\(_2\)CO\(^+\)] (100); FABMS, observed \(m/z\) 722.3200 [M\(^+\)] (calculated for C\(_{45}\)H\(_{39}\)O\(_{12}\)), 722.3190.

**REFERENCES**

IV
Isolation and Structure Revision of Pepulane Diterpenoids from *Euphorbia peplus*

Judit Hohmann,*1 Gübar Günther,1 Andrea Vasas,1 Alajos Kálmán,6 and Gyula Argay6

Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szeged, Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University, 4 Somogyi u., H-6720 Szeged, Hungary, and Institute of Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary

Received July 23, 1998

A new pepulane diterpene polyester (2) was isolated from a CH2Cl2 extract of the whole, undried plant of *Euphorbia peplus*, together with the known compound 1. The structures were established by high-field spectroscopic methods, including 2D NMR techniques, and by X-ray crystallography, and the stereostructure of the first member of the pepulane diterpenoids (1) was revised.

Plants of the genus *Euphorbia* are known to produce a large variety of diterpenoids, some of which are highly irritant and have tumor-promoting activity, while others exhibit antileukemic, cytotoxic, and analgetic activity.1 Earlier investigations of this species have led to reports of the isolation of the skin irritant ingenane diterpenes, 2-deoxyingenol 3-O-angelate, ingenol 2-O-octanoate, and ingenol 2-O-acetate-3-O-angelate from the acetone extract of the latex and from a diethyl ether-soluble fraction of the plant.2,3 While the present work was in progress, jatropane diterpenoids and a tetracyclic diterpene (1) based on a new carbon framework, named pepulane, were reported from the dried, whole plant of *E. peplus*.6

In the course of our studies on biologically active compounds from Hungarian *Euphorbia* species, we have reinvestigated *E. peplus* for its diterpene constituents and have isolated two pepulane diterpenoids (1, 2) from a dichloromethane extract of the fresh plant. This paper deals with the isolation and structure elucidation of these compounds.

![Figure 1. NOE correlations for 1](image)

Results and Discussion

The dichloromethane-soluble fraction obtained from a methanolic extract of the fresh, whole plant of *Euphorbia peplus*, collected in Miskolc, Hungary, in 1996, was subjected to repeated column chromatography on polyamide (H2O—MeOH) and Si gel (CHCl3—Me2CO, gradient) fol-

* To whom correspondence should be addressed. Tel: (36)-62 455 558. Fax: (36)-62 426 146. E-mail: hohmann@pharma.szote.u-szeged.hu.
1 Department of Pharmacognosy, Albert Szent-Györgyi Medical University.
2 Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University.
3 Institute of Chemistry, Chemical Research Center, Hungarian Academy of Sciences.

10.1021/nr8031911 CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy Published on Web 12/05/1998

lowed by HPLC to afford, in crystalline form, compounds 1 (mp 225 °C) and 2 (mp 238—240 °C) in 0.0028% and 0.0012% yields, respectively.

Mass spectrometry and detailed NMR investigations indicated that compound 1 was identical with the pepulane diterpene 1 isolated earlier from the same plant.6 We now report the missing physical and spectral data (see Experimental Section) and a stereochemical study of 1 by means of NOESY experiments in solution, and X-ray crystallography.

The NOESY spectrum of 1, recorded in C6D6, demonstrated trans-fused A/B rings, as NOE effects were detected between 16-OH and H-1β, 16-OH and H-17, 16-OH and arthro-benzyol protons, H-4 and H-3, and H-3 and H-2 (Figure 1). These NOE interactions revealed a β-oriented methyl group on C-2 and a benzoyl group on C-3. The cross-peaks between H-4 and H-18 and between H-18 and H-20 in the NOESY spectrum proved a cis B/C ring junction in the molecule. The NOESY correlations between 16-OH and H-15 and between 16-OH and H-5 indicated the presence of ester groups in the α position on C-5 and C-15. The 16-OH group also participated in an NOE interaction with H-13, from which the β orientation of H-13 was concluded. In our NOESY experiment, correlation signals were observed between 8-OAc and H-13 and between 8-OAc and arthro- and meta-benzyol protons, pointing to cis-fused C/D rings, in contrast with the proposal of Jakupovic and co-workers.6 We could not establish the stereochemistry of H-9 on the basis of the NOESY spectrum, because this proton gave cross-peaks both with H-7α and H-7β and with H-10α and H-10β. Further, the configuration of C-11 could not be determined because of missing diagnostic NOESY correlations.

To determine the complete relative and absolute configurations and solid-state conformation of 1, single-crystal X-ray analysis was performed. Two conformers (I and II) (Figure 2) were detected in the crystal lattice, which form dimers through O–H···O hydrogen bonds. The geometry of these interactions [H...A (Å), D...A (Å), and DHA (°)] for

![Figure 2](image)
Figure 2. Perspective views of I conformers I and II. (In the latter case only the atoms in the molecular skeleton are labeled, using numbers increased by 30 relative to conformer I; bonds to the disordered atoms O33b, O41b, and O45b are drawn with dashed lines.)

O16–H16a..O41a and O46–H46..O15a contacts are 2.06, 2.87 (I), 170.4 and 2.27, 3.078 (3), 167.0. The two conformers, which differ only in the internal rotations of the 9-OAc and 11-OAc groups, unambiguously demonstrated an α-oriented acetyl group on C-9 and a β-oriented one on C-11. Additionally, it was found that ring B assumed a chair conformation and ring D a boat conformation, differing from the PCMODEL-calculated conformation. 6 The conformations of the symmetry-independent molecules exhibit a few visible differences. The five-membered ring A assumes a distorted envelope shape in molecule I, whereas it has a half-chair form in molecule II, with an almost perfect two-fold symmetry axis bisecting atom C32 and the opposite bond C34–C46. The conformation of the other three rings fused to each other with trans, cis, and cis junctions may be defined as follows: ring B is a slightly flattened (at C14/C44) chair, ring C is a distorted envelope, and ring D assumes a boat (1,4-diplanar) conformation.

Assuming the α position, the out-of-plane atoms are C9 (C39) and C12 (C42). This relatively rare conformation of ring D, with numerous substituents, may account for the misinterpretation of the NMR spectra by Jakupovic and co-workers 5a as concerns the C/D junction and the orientation of the acetoxymethyls on C8 and C9. Prior to the X-ray analysis of I, this feature of the molecular conformation presumably prevented the correct NMR elucidation of the substituent positions. On the whole, the four acetyl and one benzoyl functions exhibit differences only a few degrees (<10°) in rotation around the ester C–O–C bonds. Only the 11-OAc groups exhibit different rotations (ΔΨ ≈ 112°) about the C11–O11 bond. The second largest difference (ΔΨ ≈ 31°) in the substituent positions is displayed around the C3–O3 bond, accompanied by a second rotation of ΔΨ ≈ 30° about the C9b–C9c bond in the bulky benzoyl moiety.

Compound 2 gave a parent ion in the EIMS at m/z 658, appropriate for a molecular formula of C33H30O12. It exhibited IR absorptions bands at 3432, 1738, and 1711 cm−1 and UV maxima at 229, 274, and 282 nm, characteristic of hydroxy, ester, and phenyl groups. The 1H and 13C NMR spectra of 2 revealed four acetal and one benzozate groups (Table 1). Additionally, the spectra exhibited resonances closely related to that of 1. After the 1H and 13C NMR data on 2 had been assigned by analysis of its 1H–1H COSY, HMOC, and HMBC spectra, it was obvious that compounds 1 and 2 were based on the same parent system and differed only in esterification. The absence of one acetyl signal and the appearance of one hydroxyl signal (δH 2.82 s) indicated the replacement of one of the acetyl residues with a hydroxyl group. Comparison of the 1H and 13C signals of 1 and 2 revealed a significant difference in the chemical shift values of C-11 (δC-11: 79.8; δC-11: 68.0), from which the position of the hydroxyl group on C-11 was concluded. This was substantiated by the occurrence of 3J,1H–1H coupling between 11-OH and C-19 in the HMBC spectrum. A careful comparison of the NOESY spectra of compounds 1 and 2 (Table 1) enabled us to assume the same stereochemistry for 2 as that of 1. Thus, the structure of this compound was elucidated as shown in formula 2.

**Experimental Section**

General Experimental Methods. NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer at 400 MHz (1H) and 100 MHz (13C). The signals of the deuterated solvents were taken as the reference. Two-dimensional experiments were performed with standard Bruker software. MS measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. IR spectra of Kbr disks were run on a Perkin–Elmer Paragon 1000 PC FTIR instrument. Optical rotations were determined in CHCl3 at ambient temperature, using a Perkin–Elmer 341 polarimeter. Melting points were not corrected. For column chromatography, polyamide (ICN) and Si gel (Kieselgel GF254 15 µm, Merck) were used. HPLC was carried out on a Waters Millipore instrument with RI detection on a normal-phase (LiChrospher Si 100, 5 µm, 200 × 4 mm, Merck) and on a reversed-phase (LiChrospher RP-18, 5 µm, 200 × 4 mm, Merck) column.

**Plant Material.** *E. peplus* was collected in June 1996, in Miskolc, Hungary. A voucher sample has been deposited at the Department of Pharmacognosy, Albert Szent-Györgyi Medical University, Szeged, Hungary.

**Extraction and Isolation.** The fresh plant material (200 g) was percolated with MeOH (1800 mL) at room temperature. The crude extract was concentrated in vacuo to 100 mL, and

---

**Table 1. NMR Data on Compound 2 [CDCl3, δ (ppm) J = Hz]**

<table>
<thead>
<tr>
<th>Atom</th>
<th>Hα</th>
<th>δ (ppm)</th>
<th>NOESY H no.</th>
<th>HMBC C no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>2.17 dd (14.2, 11.6)</td>
<td>44.7 2</td>
<td>2, 3, 15, 16, 17</td>
<td></td>
</tr>
<tr>
<td>1β</td>
<td>1.51 dd (14.2, 5.1)</td>
<td>16-OH</td>
<td>2, 3, 4, 16, 17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.54 m</td>
<td>35.9 1α, 17</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.80 m</td>
<td>76.4 2, 4</td>
<td>1, 4, 16</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.40 dd (12.0, 4.3)</td>
<td>48.2 3, 18</td>
<td>5, 6, 18</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5.83 dd (12.0)</td>
<td>69.7 13, 16-OH</td>
<td>3, 4, 7, 16, 18</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>49.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7β</td>
<td>2.48 dd (16.0)</td>
<td>39.8 5</td>
<td>5, 6, 8, 13, 14</td>
<td></td>
</tr>
<tr>
<td>7α</td>
<td>1.58 dd (16.0)</td>
<td>18</td>
<td>5, 6, 8, 9, 18</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>88.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5.78 d (4.9)</td>
<td>68.3 8-OAc, 10α, 10β</td>
<td>7, 8, 11, 13</td>
<td></td>
</tr>
<tr>
<td>10α</td>
<td>1.97 dd (16.9, 5.7)</td>
<td>41.6 11-OH</td>
<td>11, 12</td>
<td></td>
</tr>
<tr>
<td>10β</td>
<td>1.85 d (16.9)</td>
<td>19</td>
<td>8, 9, 19</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>68.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12β</td>
<td>1.75 m</td>
<td>33.9</td>
<td>8, 10, 11, 13, 19</td>
<td></td>
</tr>
<tr>
<td>12α</td>
<td>1.69 t (12.9)</td>
<td>13, 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>4.30 dd (12.8, 6.6)</td>
<td>47.0 5, 11-OH,</td>
<td>9, 11, 12, 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15, 16-OH</td>
<td>15, 20</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>52.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>5.07 s</td>
<td>73.3 16-OH</td>
<td>1, 4, 6, 13, 14</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>84.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>1.05 d (7.3)</td>
<td>16.6 16-OH, 2</td>
<td>1, 2, 3</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>1.08 s</td>
<td>16.7 7α, 20</td>
<td>5, 6, 7, 14</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.29 s</td>
<td>31.5 10α</td>
<td>10, 11, 12</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.92 s</td>
<td>16.3 18</td>
<td>6, 13, 14, 15</td>
<td></td>
</tr>
<tr>
<td>11-OH</td>
<td>2.82 s</td>
<td>13, 10β</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>16-OH</td>
<td>3.17 s</td>
<td>15, 15, 17</td>
<td>4, 15, 16</td>
<td></td>
</tr>
</tbody>
</table>

1H NMR signals of the acyl groups: 5-OAc: 1.72 s, 8-OAc: 1.96 s, 9-OAc: 2.03 s, 15-OAc: 2.14 s, 3-OBz: 7.92 d (7.4 (H'-2', 6'), 7.54 ' (7.4) (H'-4'), 7.41 ' (7.7) (H'-3', 5'), 6 13C NMR signals: 5-OAc: 22.0, 170.4, 8-OAc: 20.9, 170.2, 9-OAc: 21.4, 169.3, 15-OAc: 20.9, 170.0, 3-OBz: 165.8 (CO2), 133.2 (C-4'), 129.3 (C-2', 6'), 128.5 (C-3', 5'), 130.0 (C-1').
exhaustively extracted with CH₂Cl₂. Evaporation of the organic phase gave a greenish-brown residue (4.7 g), which was chromatographed on a polyamide column (28 g) with mixtures of H₂O–MeOH (3.2 and 1.4) as eluents. Fractions obtained with H₂O–MeOH (2:3) were combined and subjected to Si gel flash chromatography, using a gradient system of CHCl₃ and Me₂CO. Fractions 6–10 eluted with CHCl₃ were further fractionated by reversed-phase HPLC with MeOH–H₂O (7:3) as eluent at a flow rate of 0.7 mL/min. Finally, compounds observed at retention times of 13.5 and 20.4 min were transferred to a normal-phase HPLC column eluted with cyclohexane–EtOAc–EtOH (30:10:1) to afford compounds 1 (5.5 mg) and 2 (2.3 mg).

**Compound 1**: white needles, mp 225 °C (from diethyl ether–n-hexane); [α]D²⁵+5° (c 0.06, CHCl₃); UV (MeOH) λmax 230 (ε 13.040), 272 (ε 1210), 280 (ε 1070) nm; IR (KBr) 3430, 1741, 1712, 1370, 1246, 1032, 717 cm⁻¹; ¹H NMR (CDCl₃ 400 MHz) δ 1.86 (1H, dd, J = 13.7, 11.4 Hz, H-1a), 1.12 (1H, dd, J = 13.7, 4.6 Hz, H-1b), 2.0 (1H, m, H-2), 5.76 (1H, dd, J = 5.7, 4.7 Hz, H-3), 2.18 (1H, dd, J = 11.9, 4.7 Hz, H-4), 6.09 (1H, d, J = 11.9 Hz, H-5), 1.66 (1H, d, J = 15.7 Hz, H-7a), 2.77 (1H, d, J = 15.7 Hz, H-7b), 6.29 (1H, d, J = 5.0 Hz, H-9), 1.80 (1H, d, J = 17.1 Hz, H-10a), 2.52 (1H, dd, J = 17.1, 5.0, 1.1 Hz, H-10b), 1.59 (1H, t, J = 13.3 Hz, H-12a), 2.59 (1H, ddd, J = 13.3, 5.8, 1.1 Hz, H-12b), 4.32 (1H, dd, J = 13.3, 5.8 Hz, H-13), 5.26 (1H, s, H-15), 0.84 (3H, d, J = 7.2 Hz, H-17), 1.07 (3H, s, H-18), 1.48 (3H, s, H-19), 0.89 (3H, s, H-20), 2.91 (1H, s, 16-OH), 1.76 (3H, s, 5-OAc), 1.80 (3H, s, 11-OAc), 1.98 (3H, s, 8-OAc), 1.58 (3H, s, 9-OAc), 1.71 (1H, s, 15-OAc), 8.15 (2H, d, J = 6.9 Hz, 3-OBz 2', 6'), 7.03–7.13 (3H, m, 3-OBz 3', 4', 5'); ¹³C NMR (CDCl₃ 100 MHz) δ 45.4 (C-1), 36.7 (C-2), 76.7 (C-3), 49.5 (C-4), 70.7 (C-5), 49.8 (C-6), 41.2 (C-7), 87.9 (C-8), 68.3 (C-9), 39.5 (C-10), 80.4 (C-11), 31.9 (C-12), 47.7 (C-13), 52.1 (C-14), 74.7 (C-15), 84.8 (C-16), 16.9 (C-17), 17.3 (C-18), 29.2 (C-19), 16.9 (C-20), 21.2, 170.4 (5-OAc), 23.1, 171.2 (11-OAc), 21.2, 170.4 (5-OAc), 21.4, 168.9 (8-OAc), 21.0, 169.6 (15-OAc), 165.4, 133.6, 131.8, 130.5, 129.3 (3-OBz); HREIMS obsd m/z 538.2619 [M - 2 × AcOH – CH₂CO]⁺, calcd for C₁₃H₁₉O₅ 538.2567, and obsd m/z 460.2392 [M - 4 × AcOH], calcd for C₁₂H₁₇O₅ 460.2250.

**Crystal Data on 1**: C₁₂H₁₇O₅, M = 700.75, orthorhombic, space group P₂₁₂₁, a = 10.178(5) Å, b = 18.387(2) Å, c = 39.621(5) Å, V = 7415(4) Å³, Z = 8, Dₐ = 1.255 gcm⁻³, F(000) = 2992, μ(Cu Kα) = 1.5418 Å = 0.789 mm⁻¹. Data were collected on an Enraf–Nonius CAD4 diffractometer in the range 2.23 < θ < 74.51°. The structure was determined by direct methods and refined by full-matrix least-squares analysis. All nonhydrogen atoms were refined anisotropically. There is disorder of some oxygen atoms, which have been modeled using two atomic sites and occupation factors of 0.5. The hydrogen atoms were introduced in idealized positions and added to the structure factor calculations. The final R values were R₁ = 0.062, wR₂ = 0.1617 for 10 738 reflections taken with Fo > 4σFo and R₁ = 0.083, wR₂ = 0.1749 for all 14 974 data. Crystallographic data on 1, including atomic coordinates, have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ UK [Fax: +44(0)1223-336303 or e-mail: deposit@ccdc.cam.ac.uk].

**Compound 2**: white needles, mp 238–240 °C (from diethyl ether); [α]D²⁵+40° (c 0.2, CHCl₃); UV (MeOH) λmax 229 (ε 13 200), 274 (+973), 282 (ε 776) nm; IR (KBr) 3432, 1738, 1711, 1369, 1241, 1027, 715 cm⁻¹; ¹H and ¹³C NMR, see Table 1; EIMS (70 eV) m/z (%) ref int 658 (0.1) [M⁺], 598 (1) [M - AcOH]⁺, 538 (55) [M - 2 × AcOH]⁺, 523 (53) [M - 2 × AcOH - CH₂CO]⁺, 478 (9) [M - 3 × AcOH]⁺, 460 (12) [M - 3 × AcOH - H₂O]⁺, 320 (49) [M - 3 × AcOH - BzOH - 2 × H₂O]⁺; HREIMS obsd m/z 538.2593, calcd for C₁₃H₁₉O₅ 538.2567 and obsd m/z 320.1786, calcd for C₁₀H₁₆O₄ 320.1776.

**Acknowledgment.** We are grateful to Dr. Gyula Jerkovich (Spectroscopic Department, Institute for Drug Research Ltd., Budapest, Hungary) for the mass spectroscopic measurements. This investigation was funded by the National Scientific Research Fund, project no. OTKA T022587.

**References and Notes**


NF980319L
Jatrophone diterpenoids from *Euphorbia peplus*

J. Hohmann\textsuperscript{a,*}, A. Vasas\textsuperscript{a}, G. Günther\textsuperscript{b}, Gy Dombi\textsuperscript{b}, G. Blazsó\textsuperscript{c}, Gy Falkay\textsuperscript{c}, I. Máthé\textsuperscript{a}, Gy Jerkovich\textsuperscript{d}

\textsuperscript{a}Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, 6701 Szeged, Hungary  
\textsuperscript{b}Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University, 4 Somogyi Street, 6720 Szeged, Hungary  
\textsuperscript{c}Department of Pharmacodynamics, Albert Szent-Györgyi Medical University, P.O. Box 121, 6701 Szeged, Hungary  
\textsuperscript{d}Spectroscopic Department, Institute for Drug Research Ltd., P.O. Box 82, 1325 Budapest, Hungary

Received 21 July 1998; received in revised form 26 January 1999; accepted 8 February 1999

**Abstract**

From the pro-inflammatory active extract of *Euphorbia peplus*, a new diterpene polyester (1) based on the jatrophone skeleton was isolated together with the known compounds 2–5. The irritant activities of some jatrophone diterpenes (2, 3 and 6–9) were also investigated: only compound 2 was found to exert a weak pro-inflammatory activity on mouse ear. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Euphorbia peplus*; Euphorbiaceae; Jatrophone diterpenes; Pro-inflammatory activity

1. Introduction

The widespread genus *Euphorbia* is the source of a large number of biologically active compounds. Besides the well-known skin irritant and tumour-promoting tuligane, ingenane and daphnane diterpenes, considerable attention has recently been paid to the macrocyclic diterpenes because of their high chemical diversity and therapeutically relevant bioactivity (Uemura; Katayama; Uno; Sasaki; & Hirata, 1975; Sahai; Rastogi; Jakupovic; & Bohliman; 1981; Evans & Taylor, 1983). Moreover, in recent years investigations on *Euphorbia* species have resulted in the discovery of several new classes of diterpenes (Ökşüz et al., 1996; Ahmad; Jassbi; & Parvez, 1998; Jakupovic et al., 1998a; Jakupovic; Morgenstern; Bittner; & Silva, 1998b; Jakupovic; Morgenstern; Marco; & Berendsohn, 1998c; Marco; Sanz-Cervera; Yuste; Jakupovic; & Jeske, 1998).

In continuation of our phytochemical studies on biologically active compounds from Hungarian *Euphorbia* species, we now describe the isolation and characterisation of a new jatrophone diterpene (1), together with the known jatrophone esters 2–5, which were obtained from the highly irritant extract of whole, fresh plants of *E. peplus* L. (Euphorbiaceae). As only a few experimental data have previously been published on the irritant activities of jatrophone derivatives (Seip & Hecker, 1984), some jatrophone esters (2, 3 and 6–9) isolated from *E. peplus* and *E. esula* (Hohmann et al., 1997; Günther et al., 1998) were evaluated in the mouse ear test, in order to establish whether they contribute to the pro-inflammatory activity of the plant extracts.

2. Results and discussion

The mouse ear irritant, dichloromethane extract (ID\textsubscript{50} = ID\textsubscript{50} = 25 \(\mu\)g/ear) of the fresh, whole plant of *E. peplus* was subjected to polyamide CC, silica gel flash chromatography and HPLC to afford a new optically active ([\(\alpha\)]\textsubscript{D} = –33, c, 0.1, CHCl\textsubscript{3}) constituent 1, together with the known compounds 2–5.

Compound 1 gave a parent ion in the HREIMS at \(m/z\) 641.2889, appropriate for a molecular formula of...
C_{24}H_{43}NO_{11} \text{ (calcd 641.2836). It exhibited UV maxima at 217 and 264 nm, characteristic of the nicotinoyl group. The } ^1\text{H and } ^{13}\text{C NMR spectra of 1 revealed the presence of one nicotinoyl and four acetyl groups (Table 1). Additionally, the } ^1\text{H and } ^{13}\text{C NMR spectra contained resonances for skeletal carbons and protons, which were assigned on the basis of the interpretation of the } ^1\text{H–}^1\text{H-COSY and HMOC spectra. The proton and carbon connectivities detected here demonstrated that a diterpene core containing 20 carbons is present, consisting of two tertiary and two secondary methyls, one } \textit{trans} \text{ disubstituted olefin, one exocyclic and two simple methylenes, seven methine groups, one ketone and three quaternary carbons. The } ^1\text{H–}^1\text{H COSY spectrum revealed three structural fragments with correlated protons: } \delta_\text{H} 3.01 \text{ d, 1.65 d, 2.22 m, 0.90 d, 5.42 t, 2.71 d and 5.81 s [–CH}_2–CH(CH}_2)–CH(OR)–CH–CH(OR)–] (A); } \delta_\text{H} 4.84 \text{ dd, 2.18 m and 5.09 dd, [–CH(OR)–CH}_2–CH(OR)–] (B); } \delta_\text{H} 5.94 \text{ d, 5.65 dd, 3.53 dq and 1.20 d [trans–CH=CH–CH(CH}_3)–] (C) (R = acyl). Further, in the } ^1\text{H–}^1\text{H COSY spectrum, } ^4\text{J couplings were also detected between the exomethylene (} \delta_\text{H} 5.07 \text{ s (2H) (H-17)) and two acyloxy groups (} \delta_\text{H} 5.81 \text{ s (H-5) and 4.84 dd (H-7)), indicating the linkage of sequences A and B. The remaining connectivities were established by inspection of long-range C–H cor-}
relations observed in an HMBC spectrum (Table 1). The correlation of the quaternary carbon signal at \( \delta_C \) 92.9 (C-15) with the proton signals at \( \delta_H \) 3.01, 1.65 (H-1α, β), 2.22 (H-2), 2.71 (H-4) and 5.81 (H-5) showed that structural fragment A constructs a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes (Evens & Taylor, 1983). Cross-peaks between the carbon signal at \( \delta_C \) 40.7 (C-10) and the proton signals at \( \delta_H \) 2.18 (H-8), 5.94 (H-11), 5.65 (H-12), 1.13 (H-18) and 1.11 (H-19) indicated that structural parts B and C comprised the twelve-membered ring of a jatropha diterpene. The \( J_{C-H} \) and \( J_{C-C} \) couplings between the oxymethine protons and the carbonyl carbons. The acetyl group at \( \delta_H \) 2.11, which did not exhibit any long-range correlations, was of necessity situated on a quaternary carbon (C-15). All of the above data are compatible with the structure of 1 being 3,5,7,15-tetraacetoxy-9-nicotinoyloxy-14-oxojatropha-6(17),11-diene.

The stereochemistry of 1 was assessed by analysing the coupling constants and the results of a NOESY experiment. Starting from the \( \alpha \) position of the proton at the ring junction (H-4), it was found that a \( \beta \)-oriented methyl group is present on C-2 and a \( \beta \)-oriented ester group on C-3, with regard to the diagnostic NOE effects between H-1α, H-2, H-3 and H-4 and between H-1β and H-16 (Table 1). The position of H-5 was concluded to be \( \beta \) on the basis of the zero coupling constant between H-4 and H-5, similarly as in case of co-occurring jatrophanes and esultains (Hohmann et
al., 1997; Günther et al., 1998; Jakupovic et al., 1998b). The NOE interaction between H-4 and H-7 required a β-oriented acyl group on C-7. The NOESY cross-peak between H-5 and H-13 dictated the β position of H-13. The α orientation of the nicotinoyl group could be concluded from the coupling constants of H-9, which were found to be similar to those of esulatin D (Günther et al., 1998) and 2x,3β,5α, 7β,15β-pentanoctoxy-9α-nicotinoyloxy-14-o xoatrophaphe-6(17),11-diene (Jakupovic et al., 1998b). Such a configuration of C-7 and C-9 signified a parallel position of the ester groups, as described by Jakupovic et al. (1998b), which is manifested in an upfield-shifted acetyl group on C-7 (δ 1H 1.52), influenced by the aromatic ring effect of the nicotinote on C-9. The stereochemistry of the 15-OAc group could not be determined on the basis of NOEY correlations. However, all known jatrophane diterpenes exhibit a trans ring junction (Hohmann et al., 1997; Jakupovic et al., 1998a, 1998b, 1998c; Günther et al., 1998; Marco et al., 1998) and, thus, the β orientation of the 15-OAc could be presumed. With regard to the above data, the structure of this compound is formulated as 1.

From the dichloromethane extract of *E. peplus*, compounds 2–5 were also isolated and fully characterised by means of EIMS, HREIMS and NMR spectroscopy, including 2D NMR techniques, since these compounds had not been described while this work was in progress. All the spectral data were identical with those reported previously (Jakupovic et al., 1998b). The stereochemistry of 4 was analysed by X-ray crystallography and the absolute configuration was established as (2R,3R,4S,5R,7S,8S,9S,13S,14S,15R)-2,5,9,14-tetraacetoxy-3-benzoxolyl-8,15-dihydroxy-7-isobutanolxyloxybatropha-6(17),11E-diene (4) (Kálman & Argay, in preparation). The solid-state conformation, determined here, agreed well with the calculated conformation published by Jakupovic et al. (1998b).

Compounds 2 and 3, together with jatrophanes 6–9, obtained from *E. esula* earlier (Hohmann et al., 1997; Günther et al., 1998), were tested for their skin irritant activity on mouse ear, using the standard assay (Hecker, Immich, Bresch, & Schairer, 1966). Compounds 3 and 6–9 were shown to be inactive up to a dose of 200 μg/ear, while 2 exhibited a pro-inflammatory activity of ID30 = ID30 = 29 μg/ear. These data, together with those published on jatrophanes of *E. characias* (Seip & Hecker, 1984), indicated that this type of diterpenes do not play a significant role in the skin irritant activity of *Euphorbia* species. For the highly irritant effect of *E. peplus*, which causes many toxicological problems, primarily ingenane diterpenes are responsible (Rizk, Hammouda, El-Missiry, Radwan, & Evans, 1985; Zayed, Farghaly, Taha, Gotta, & Hecker, 1998).

3. Experimental

3.1. General

M.p.'s are uncorrected. UV: MeOH. EIMS: 70 eV, direct inlet, Finnigan MAT 8430 spectrometer. 1H, 13C and 2D NMR (1H-1H COSY, NOESY, HMOC, HMBCC): 400 MHz (1H) and 100 MHz (13C), CDCl3, with TMS as internal standard, Bruker Avance DRX 400 spectrometer. Optical rotations were determined in CHCl3 at ambient temp.; CC: polyamide (ICN). VLC: silica gel (Kieselgel GF254 15 μm, Merck). HPLC: Li Chrsopher RP-18 (200x4 mm, 5 μm) and LiChrsopher Si 100 (200 × 4 mm, 5 μm) with RI detection.

3.2. Assay for irritant activity

Irritant doses 50 (ID50) were determined on the ears of mice according to a standard procedure (Hecker et al., 1966). The redness of the mouse ear was estimated 4 and 24 h after the application of solutions in Me2CO. As a reference, 12-O-tetradecanoylphorbol 13-acetate (TPA) was used: ID30 0.00938 μg/ear, ID30 0.00645 μg/ear.

3.3. Plant material

*E. peplus* was collected in June 1996 in Miskolc, Hungary. A voucher specimen has been deposited at the Department of Pharmacognosy, Albert Szent-Györgyi Medical University, Szeged, Hungary.

3.4. Extraction and isolation

Fresh and entire plants of *E. peplus* (200 g) were extracted with MeOH (1.8 l) at room temperature. The crude extract was concentrated in vacuo and partitioned between CH2Cl2 and H2O. Evaporation of the organic phase gave a residue (4.7 g), which was chromatographed over a polyamide column with mixtures of H2O/MeOH (3:2 and 1:4) as eluants. Fractions obtained with H2O/MeOH (2:3) were chromatographed by silica gel VLC, using CHCl3 and CHCl3/Me2CO mixtures of increasing polarity as eluants. Fractions 6–10 eluted with CHCl3 were transferred to a reverse-phase HPLC column and eluted with MeOH/H2O as mobile phase at a flow rate of 0.7 ml/min. Compounds observed at RT 20.0, 29.2, 16.2 and 23.4 min were further purified by HPLC on a normal-phase column with cyclohexane/EtOAc/EtOH (30:10:1) to yield compounds 1 (2.7 mg), 2 (10.2 mg), m.p. 182–185°C, [α]D25 + 53 (c 0.51, CHCl3), 3 (4.7 mg), [α]D25 –10 (c 0.19, CHCl3) and 4 (3.1 mg), m.p. 220–223°C, [α]D25 + 8 (c 0.3, CHCl3), respectively. Fractions 11–18 obtained from VLC with CHCl3/Me2CO (49:1) after HPLC purification on a normal-phase column with
cyclohexane/EtOAc/EtOH (30:10:1) as eluent afforded compound 5 (3.8 mg), \([\alpha]_{D}^{25} +27\) (c, 0.04, CHCl₃).

3.5. Compound I

Amorphous solid. \([\alpha]_{D}^{25} -33\) (c, 0.1, CHCl₃). UV \(\lambda_{max}\) nm (log \(\varepsilon\)): 217 (3.96), 264 (3.27). EIMS, \(m/z\) (rel. int.): 641 [M⁺] \((13)\), 571 [M? CH₂CO – CO]⁺ \((7)\), 391 \([571-3 \times \text{HOAc–C}_2\text{H}_4\text{NCOOH}⁺]⁺\) \((3)\), 328 \([571-2 \times \text{HOAc–C}_2\text{H}_4\text{NCOOH}⁺]⁺\) \((7)\), 124 \([\text{C}_6\text{H}_4\text{NCOOH}+\text{H}⁺]\) \((100)\), 123 \([\text{C}_8\text{H}_{11}\text{O}]⁺\) \((7)\), 96 \([\text{C}_7\text{H}_{12}]⁺\) \((27)\). HRMS: \(m/z\) 641.2889 \([\text{M}⁺] \text{C}_3\text{H}_{43}\text{NO}_{11}\) required 641.2836. \(^1\)H and \(^{13}\)C NMR: see Table 1.

Acknowledgements

This investigation was funded by the National Scientific Research Fund, Project No. OTKA T022587.

References


VI
A Novel Lathyran Diterpenoid from the Roots of *Euphorbia lathyris*

Judit Hohmann,∗† Ferenc Evani,† Andrea Vasas,† György Dombi,‡ Gyula Jerkovich,§ and Imre Máthé‡

Department of Pharmacognosy, Albert Szent-Györgyi Medical University, P.O. Box 121, H-6701 Szege, Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University, 4 Samogyi u., H-6720 Szege, Hungary, and Spectroscopic Department, Institute for Drug Research Ltd., P.O. Box 82, H-1325 Budapest, Hungary

Received July 9, 1998

A new lathyran diterpene (1) has been isolated and characterized from a CH₂Cl₂ extract of the roots of *Euphorbia lathyris*. Detailed spectral analysis revealed that the structure of 1, including relative stereochemistry, is that of a diester of a hitherto unknown, polyfunctional diterpene parent alcohol.

*Euphorbia lathyris* L., or caper spurge (Euphorbiaceae), is widely distributed throughout the Mediterranean region of Eastern and Middle Europe. It is sometimes planted in gardens in Hungary to deter moles. Animals nibbling the roots of the plant are killed because of its highly irritant gastric effect. Previous biological studies have demonstrated the proinflammatory property of the roots, seeds, aerial parts, and milky latex, and an antitumor effect of the seeds of *E. lathyris* against sarcoma 180 ascites. From the antitumor extract, a diterpenoid, ingenol 3-hexadecanoate was isolated as active principle; this compound displays paradoxical biological action, being also a known tumor-promoting agent. Besides ingenol esters, a series of diterpenes based on the lathyran skeleton have also been isolated (L₁–L₃, L₇, L₉) from the seeds. The roots of *E. lathyris* have been less well investigated to date; only the isolation of a lathyran diterpenoid, jolkinol B, was reported earlier.

As part of our studies on biologically active compounds from *Euphorbia* species found in Hungary, we have examined the roots of *E. lathyris* for its diterpenoid constituents. The dichloromethane phase of a methanolic extract of the undried roots of *E. lathyris* was fractionated by column chromatography on polyamide, then on Si gel, and further purified by preparative TLC and HPLC to afford compound 1.

![Compound 1](image)

Compound 1 was shown by HREIMS to have the molecular formula C₂₉H₄₈O₇. Its UV spectrum displayed a maximum at 280 nm, indicating an enone chromophore in the molecule. The ¹H and ¹³C NMR spectra of 1 (Table 1) revealed the presence of one acetate group [δH 2.08 s; δC 170.4 (CO) and 21.0 (CH₃)] and one butanoate group [δH

**Table 1. NMR Spectral Data of Compound 1**

<table>
<thead>
<tr>
<th>position</th>
<th>¹H</th>
<th>¹³C</th>
<th>HMBC (H-C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α</td>
<td>3.47 m</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>1β</td>
<td>2.04 t (12.3)</td>
<td>2, 15, 16</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.4 m</td>
<td>37.9</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5.83 t (3.6)</td>
<td>79.9</td>
<td>1', 15</td>
</tr>
<tr>
<td>4</td>
<td>3.01 dd (3.6, 10.5)</td>
<td>53.4</td>
<td>6, 14</td>
</tr>
<tr>
<td>5</td>
<td>6.56 d (10.5)</td>
<td>67.3</td>
<td>6, AcCO</td>
</tr>
<tr>
<td>6</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7α</td>
<td>2.54 dd (15.0, 5.4)</td>
<td>35.3</td>
<td></td>
</tr>
<tr>
<td>7β</td>
<td>2.13 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.94 m (2H)</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.10 dd (15.3, 8.3)</td>
<td>35.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>25.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.50 dd (11.8, 8.3)</td>
<td>28.3</td>
<td>13, 18</td>
</tr>
<tr>
<td>12</td>
<td>152.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>134.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>202.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>88.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>1.03 d (6.8)</td>
<td>14.1</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>17α</td>
<td>5.10 s</td>
<td>114.7</td>
<td>5</td>
</tr>
<tr>
<td>17β</td>
<td>5.07 s</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>18</td>
<td>1.06 s</td>
<td>28.3</td>
<td>9, 10, 11, 19</td>
</tr>
<tr>
<td>19</td>
<td>1.05 s</td>
<td>15.7</td>
<td>9, 10, 11, 18</td>
</tr>
<tr>
<td>20</td>
<td>1.91 s</td>
<td>12.2</td>
<td>12, 13, 14</td>
</tr>
<tr>
<td>12-OH</td>
<td>8.25 br s</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>15-OH</td>
<td>7.40 br s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Pyridine-δ, TMS, δ (ppm), J = Hz. † Additional assignments: 3-O-butanoyl: δH 2.36 m (H-2), 1.66 m (H-3), 0.81 t (7.4) (H-4); δC 173.8 (C-1), 35.9 (C-2), 18.1 (C-3), 13.2 (C-4), 5-O-acetyl: δH 2.08 s; δC 170.4 (CO), 21.0 (CH₃).

2.36 m (H-2), 1.66 m (H-3), and 0.81 t (C-4); δC 173.8 (C-1), 35.9 (C-2), 18.1 (C-3), and 13.2 (C-4). Characteristic signals at δH 1.10 dd, 1.50 dd, 1.06 s, and 1.05 s and at δC 35.9, 28.3, 25.2, 28.3, and 15.7 suggested a gem-dimethyl-substituted cyclopropane ring, present in many types of diterpenes from plants in the Euphorbiaceae. Additionally, the ¹H NMR spectrum exhibited 15 signals due to the parent skeleton, which were assigned with the aid of two-dimensional experiments. The ¹H–¹H COSY spectrum revealed the presence of the systems CH₂–CH(CH₃)–CH=CH–CH=CH– (A) and –CH₂–CH₂–CH=CH– (B) (R = acyl) in 1, which represented the structural moieties C-1–C-5 and C-7–C-11 of a lathyran diterpene, respectively. In position C-6, an exomethylene group was concluded on the basis of the singlet signals at δH 5.10 and 5.07, which did not show any correlations to other protons. In the ¹H NMR spectrum, a downfield-shifted tertiary methyl signal (δH 1.91 s) and two broad signals at δH 8.25 and 7.40 were also observed. The chemical shift assignments of the carbon atoms were established from the HMQC and HMBC spectra. The HMQC correlations

∗ To whom correspondence should be addressed. Tel.: (36)-62 456 456. Fax: (36)-62 426 146. E-mail: hohmann@pharm.pte.nste.u-szeged.hu.
† Department of Pharmacognosy, Albert Szent-Györgyi Medical University.
‡ Department of Pharmaceutical Analysis, Albert Szent-Györgyi Medical University.
§ Spectroscopic Department, Institute for Drug Research Ltd.

10.1021/jn980294q CCC: $18.00 © 1999 American Chemical Society and American Society of Pharmacognosy Published on Web 11/19/1998
indicated, besides ester groups, the presence of 14 protonated carbons: six methines, four methylenes, and four methyls. Moreover, it was considered that the two broad singlets at δH 8.25 and 7.40 corresponded to hydroxyl groups because of their missing HMBC cross peaks.

The HMBC spectrum of 1 revealed the presence of six quaternary carbons, whose correlative signals confirmed the connection of the partial structures proposed. Long-range correlations between the signals at δC 88.1 (C-15) and at δH 2.04 (H-1β) and 5.83 (H-3) proved that structural fragment A comprised the five-membered ring of 1. The 3JCH and 2JCH correlations of protons H-4, H-5, and H-17a,b (Table 1) confirmed the presence of an exomethylene group at C-6. The cross peak between the signals at δC 202.2 and δH 3.01 (H-4) indicated that a keto group must be situated at C-14. This keto group showed a correlation in the HMBC spectrum with the singlet methyl signal at δH 1.91, indicating that C-20 is attached to a quaternary carbon. Further, long-range 1H–13C couplings from H-20 to the quaternary carbons at δC 152.4 (C-12) and 134.2 (C-13) and from H-11 to δC 134.2 (C-13) were observed, and thus the assignments of C-12 and C-13 could be determined. A hydroxy group (δH 8.25) was situated at C-12, from the observation of its correlative signals with C-11 in the HMBC spectrum. The coupling of OH-12 and H-11 in the 1H NMR spectrum was detected in a proton decoupling experiment. On irradiation of the OH-12 signal (δH 8.25), simplification of the H-11 signal (δH 1.50 δd) to a doublet was observed.

The positions of ester groups in 1 were also established via the HMBC experiment. The correlation of the carbonyl signals at δC 173.8 (butanoyl CO) and 170.4 (acetyl CO) with the proton signals at δH 5.83 (H-3), 2.36 (H-2), and 6.56 (H-5), and at δH 2.08 (acetyl methyl), respectively, demonstrated the presence of the butanoyl group at C-3 and the acetyl group at C-5. All of the above data are compatible with the structure of 1 being 5-O-acetyl-3-O-butanoyl-12-hydroxylathyr.

The relative stereochemistry of 1 was studied by means of NOESY measurements. The trans-linked cyclopropene ring and the β-oriented OBU-3 and CH2-16 groups followed from the NOE interactions between H-1β, H-16, and OH-15 and between H-1α, H-2, and H-4, as shown in Figure 1. The cross peak between H-4 and H-20 in the NOESY spectrum required that the methyl group on C-13 be oriented below the plane of the molecule. The NOE effect observed between H-20 and H-11 revealed a trans configuration for the C-12/C-13 double bond. Correlative signals between H-11 and H-9 dictated a cis-fused cyclopropane ring with a stereochemistry of H-9 and H-11, which is usual in lathyrine diterpenoids.10 The stereochemistry of H-5 was concluded to be β from its NOESY cross peak with OH-12, both oriented above the plane of the molecule. On the above basis, the structure of this compound was elucidated as shown in formula 1. The identical configuration of C-5 was found in lathyrine ester Lα, jolkinoi B, and 15β-O-benzoyl-5α-hydroxyisovaleryl,8,11,12 and the opposite configuration in esters Lβ, Lα, and Lβ9,5,7 Our findings support the suggestion of Manners and co-workers that lathyrane and related jatrophane diterpenes should be stereochemically similar and that a stereochemical reexamination of some lathyrane diterpenes is necessary.13

The demonstrated enolic form of compound 1 is presumably stable because of hydrogen bonding, which can exist between the 14-keto group and OH-12 and OH-15. A similar enol structure was presented in the presumed biogenetic route of the antitumor-active jatrophastrone by Torrance et al.14

**Experimental Section**

**General Experimental Procedures.** The optical rotation was determined in CHCl₃ at ambient temperature, using a Perkin–Elmer 341 polarimeter. The UV spectrum in MeOH was obtained on a Shimadzu UV-2101 spectrophotometer. The NMR spectra were recorded on a Bruker DRX 400 Avance spectrometer at 400 MHz (1H) and 100 MHz (13C), using pyridine-d₅ as solvent and TMS as internal standard. MS measurements were carried out on a Finnigan MAT 8430 spectrometer operating at 70 eV ionizing energy. For column chromatography, polyamide (ICN) and Si gel (Kieselgel GF254 15 µm, Merck) were used. HPLC was carried out on a Waters Millipore instrument, with RI detection on a normal-phase column (LiChrospher Si 100 5 µm, Merck).

**Plant Material.** The roots of *E. lathyris* were collected from a two-year-old plant in October 1996, in Székesfehérvár, Hungary. A voucher specimen has been deposited at the Department of Pharmacognosy, Albert Szent-Györgyi Medical University, Szeged, Hungary.

**Extraction and Isolation.** The fresh roots of *E. lathyris* (840 g) were extracted with MeOH (8 L) at room temperature. The crude extract was concentrated in vacuo and partitioned between CHCl₃ and H₂O. On evaporation, the organic phase residue (4.27 g) was obtained, which was chromatographed on a polyamide column (30 g) with mixtures of H₂O–MeOH (4:1, 3:2, 2:3, and 1:4) as eluents. The fractions obtained with H₂O–MeOH (3:2 and 2:3) were combined and subjected to Si gel vacuum–liquid chromatography, using a gradient system of cyclohexane and cyclohexane–EtOAc (9:1, 4:1, 7:3, and 1:1). Fractions eluted with cyclohexane–EtOAc (9:1) were further purified by preparative TLC on Si gel, using CHCl₃–Me₂CO (19:1) as solvent, and by HPLC, using a normal-phase column and n-hexane–EtOAc (19:1) as eluent, to yield 2.3 mg of compound 1.

**Compound 1: amorphous solid; [α]D²⁰ +76º (c 0.1, CHCl₃); UV (MeOH) λmax (log ε) 218 (3.63), 280 (3.92) nm; 1H and 13C NMR, see Table 1; EIMS m/z [M – CH₄]⁺ 447 (16), [M – CH₃ – HOAc]⁺ 387 (36), [M – CH₃ – HOAc – CH₂COOH]⁺ 299 (23), [M – CH₃ – HOAc – CH₂COOH – CH₂]⁺ 298 (100), [C₇H₅CO]⁺ 71 (43), [CH₂CO]⁺ 43 (100); HREIMS m/z 447.2383 (calcld for 447.2383 C₂H₁₂O₃) [M – CH₄]⁺, 387.2165 (calcd for 387.2172 C₂H₁₀O₂ [M – CH₃ – HOAc]).

**Acknowledgment.** This investigation was funded by the Hungarian Ministry of Culture and Education, project no. MKE 495, and by the National Scientific Research Fund, project no. OTKA T022587.

**References and Notes**

3. Ikawa, H.; Ichihara, Y.; Watanabe, K.; Takaya, K. Planta Med. 1989, 55, 271–272. On the above basis, the structure of this compound was elucidated as shown in formula 1. The identical configuration of C-5 was found in lathyrine ester Lα, jolkinoi B, and 15β-O-benzoyl-5α-hydroxyisovaleryl,8,11,12 and the opposite configuration in esters Lβ, Lα, and Lβ9,5,7 Our findings support the suggestion of Manners and co-workers that lathyrane and related jatrophane diterpenes should be stereochemically similar and that a stereochemical reexamination of some lathyrane diterpenes is necessary.13
4. The demonstrated enolic form of compound 1 is presumably stable because of hydrogen bonding, which can exist between the 14-keto group and OH-12 and OH-15. A similar enol structure was presented in the presumed biogenetic route of the antitumor-active jatrophastrone by Torrance et al.14
5. 13C NMR, see Table 1; EIMS m/z [M – CH₄]⁺ 447 (16), [M – CH₃ – HOAc]⁺ 387 (36), [M – CH₃ – HOAc – CH₂COOH]⁺ 299 (23), [M – CH₃ – HOAc – CH₂COOH – CH₂]⁺ 298 (100), [C₇H₅CO]⁺ 71 (43), [CH₂CO]⁺ 43 (100); HREIMS m/z 447.2383 (calcd for 447.2383 C₂H₁₂O₃) [M – CH₄]⁺, 387.2165 (calcd for 387.2172 C₂H₁₀O₂ [M – CH₃ – HOAc]).
6. Acknowledgment. This investigation was funded by the Hungarian Ministry of Culture and Education, project no. MKE 495, and by the National Scientific Research Fund, project no. OTKA T022587.

NP980294Q
VII
New tri- and tetracyclic diterpenes from Euphorbia villosa

Andrea Vasas, a,1 Judit Hohmann,a,* Peter Forgo b and Pál Szabóc

a Department of Pharmacognosy, University of Szeged, PO Box 121, H-6720 Szeged, Hungary
b Department of Organic Chemistry, University of Szeged, H-6720 Szeged, Hungary

Institute of Chemistry, Chemical Research Centre, Hungarian Academy of Sciences, H-1525 Budapest, Hungary

Received 29 January 2004; revised 18 March 2004; accepted 8 April 2004

Abstract—Three new tetracyclic diterpenes were isolated from the chloroform-soluble extract of Euphorbia villosa, together with one new and one known lathyrane diterpene. The structures were elucidated by means of various spectroscopic methods, including HREI-MS, HRFAB-MS, UV, and 1D and 2D NMR techniques. Spectral analyses revealed that two of the tetracyclic compounds contain the rare 5-6-6-4 fused ring system, while the third has a 5-6-7-3 fused diterpene core. Such diterpene skeletons have previously been found only in euphoractines A-E isolated from Euphorbia microstyla. As a new structural feature, the diterpene framework described here has a C-2 epimer configuration. The new lathyrane diterpene is a diester of a hitherto unknown polyfunctional parent alcohol.

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The plants of the family Euphorbiaceae (spurges) produce a high diversity of diterpenoids based on various macrocyclic and polycyclic skeletons. Characteristic constituents of the family include the tigliane, ingenane and daphnomene diterpenes, referred to overall as phorbolides,1 which exhibit strong irritant and tumour-promoting activities as a consequence of their protein kinase C-activating and vanilloid receptor type 1 agonist effect.2,3 Diterpenes of other skeletal types, e.g. lathyrane, jatrophone, casbane and cembrane derivatives, have also attracted considerable interest because of their complex structures and therapeutically relevant bioactivities. Pharmacological studies have revealed their cytotoxic, antineoplastic, PGE2-inhibitory, multidrug-resistance-reversing, propyl endopeptidase-inhibitory and various vascular effects.4–6 As a continuation of our studies on the chemistry and pharmacology of the genus Euphorbia, we have now investigated Euphorbia villosa W. et K., a stout glabrous or pubescient, rhizomatous perennial plant widely distributed throughout Europe.7 No chemical and pharmacological investigation has been reported so far on this species. The present paper describes the isolation and structure determination of four new diterpenes (1–4) and one known diterpene (5) from the chloroform-soluble extract of E. villosa. We also discuss the conformational behaviour of the compounds, with an interpretation of the results of NOESY experiments and theoretical structure calculations.

Keywords: Euphorbia villosa; Euphorbiaceae; Terpenes and terpenoids; Lathyrane diterpenes; Tetracyclic diterpenes.

* Corresponding author. Tel.: +36-62-545-558; fax: +36-62-545-704; e-mail address: hohmann@pharma.szote.u-szeged.hu

2. Results and discussion

The chloroform phase of a methanol extract of the dried whole plant of E. villosa was fractionated by open column chromatography on polyamide, and then by vacuum liquid chromatography on NP silica gel, and it was further purified by RP HPLC to afford compounds 1–5. Compound 5 ([α]D ±148, c 0.2, CHCl3) was found to be identical in all of its characteristics, including the 1H and 13C NMR spectral data, with the lathyrane diterpene isolated earlier from Euphorbia hyberna.8
Table 1. NMR spectral data of 1 in CDCl₃ (δ in ppm, multiplicities, J in Hz)

<table>
<thead>
<tr>
<th>Atom</th>
<th>¹H</th>
<th>¹³C</th>
<th>HMBC (H No.)</th>
<th>NOESY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α</td>
<td>1.67dd (14.8, 9.6)</td>
<td>38.5</td>
<td>—</td>
<td>1β, 4, 16</td>
</tr>
<tr>
<td>1β</td>
<td>3.13dd (14.8, 8.0)</td>
<td>—</td>
<td>3, 16</td>
<td>1α, 2</td>
</tr>
<tr>
<td>2</td>
<td>2.25m</td>
<td>38.6</td>
<td>1α, 3, 16</td>
<td>1β, 3, 16</td>
</tr>
<tr>
<td>3</td>
<td>5.07dd (5.9, 1.7)</td>
<td>82.1</td>
<td>1β, 5, 16</td>
<td>2, 4, 16, OH</td>
</tr>
<tr>
<td>4</td>
<td>2.19m</td>
<td>54.1</td>
<td>1β</td>
<td>1α, 3, 17, OH</td>
</tr>
<tr>
<td>5</td>
<td>4.76dd (11.3, 3.1)</td>
<td>62.8</td>
<td>7α, 7β/4, 17</td>
<td>8β, 12, OH, 2', 6'</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>47.4</td>
<td>5, 4/7β, 7α, 17, 20, OH</td>
<td>—</td>
</tr>
<tr>
<td>7α</td>
<td>1.16m</td>
<td>32.4</td>
<td>8α, 8β, 17</td>
<td>7β, 8α, 17</td>
</tr>
<tr>
<td>7β</td>
<td>2.19m</td>
<td>—</td>
<td>8α, 8β, 17</td>
<td>7α, 8β</td>
</tr>
<tr>
<td>8α</td>
<td>1.42m</td>
<td>21.8</td>
<td>—</td>
<td>7α, 7β, 8β, 9, 18</td>
</tr>
<tr>
<td>8β</td>
<td>1.47dd (12.3, 3.2)</td>
<td>—</td>
<td>7α, 8α, 12, 19</td>
<td>9, 11, 20</td>
</tr>
<tr>
<td>9</td>
<td>1.01dt (3.8, 12.3)</td>
<td>38.6</td>
<td>8β, 12, 18, 19</td>
<td>7α, 8α, 11, 20</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>41.9</td>
<td>9, 12, 18, 19</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>2.83d (8.9)</td>
<td>82.7</td>
<td>12, 19, 19, OMe, 9, 11</td>
<td>9, 18, 20</td>
</tr>
<tr>
<td>12</td>
<td>2.65dd (12.3, 8.9)</td>
<td>46.9</td>
<td>—</td>
<td>5, 8β, 19, 2', 6'</td>
</tr>
<tr>
<td>13</td>
<td>—</td>
<td>56.1</td>
<td>11, 12, 17, 20</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>—</td>
<td>205.3</td>
<td>1α</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>91.2</td>
<td>1β, 3</td>
<td>—</td>
</tr>
<tr>
<td>16</td>
<td>1.16d (7.3)</td>
<td>19.8</td>
<td>1α, 3</td>
<td>1α, 2, 3</td>
</tr>
<tr>
<td>17</td>
<td>0.76a</td>
<td>17.8</td>
<td>5</td>
<td>4, 7α, 20</td>
</tr>
<tr>
<td>18</td>
<td>0.94a</td>
<td>28.9</td>
<td>9, 11, 19</td>
<td>8α, 11, 19</td>
</tr>
<tr>
<td>19</td>
<td>0.38s</td>
<td>14.6</td>
<td>9, 11, 18</td>
<td>8β, 12, 18, 2', 6'</td>
</tr>
<tr>
<td>20</td>
<td>1.14s</td>
<td>13.5</td>
<td>12</td>
<td>9, 11, 17</td>
</tr>
<tr>
<td>AcCO</td>
<td>—</td>
<td>172.3</td>
<td>3, AcMe</td>
<td>—</td>
</tr>
<tr>
<td>AcMe</td>
<td>—</td>
<td>22.9s</td>
<td>21.5</td>
<td>2', 6', 3', 5'</td>
</tr>
<tr>
<td>BrCO</td>
<td>—</td>
<td>163.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1'</td>
<td>—</td>
<td>131.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>2'</td>
<td>0.87d (7.3)</td>
<td>129.6</td>
<td>—</td>
<td>5, 12, 19, OMe, Ac</td>
</tr>
<tr>
<td>3', 5'</td>
<td>7.41t (7.7)</td>
<td>127.8</td>
<td>—</td>
<td>Ac</td>
</tr>
<tr>
<td>4'</td>
<td>7.53t (7.4)</td>
<td>132.6</td>
<td>—</td>
<td>3, 4, 5</td>
</tr>
<tr>
<td>OH</td>
<td>2.95dd (3.1)</td>
<td>—</td>
<td>—</td>
<td>2', 6'</td>
</tr>
<tr>
<td>OMe</td>
<td>2.86a</td>
<td>56.9</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

2.1. Structure of compound 1

Compound 1 was isolated as colourless prisms with [α]D20 = -3 (c 0.1, CHCl₃). The molecular formula C₃₀H₄₆O₂ was established by HR-EI-MS, which showed a molecular ion peak at m/z 512.2806 (M⁺) (Δ = 6.2 ppm). The ¹H NMR and JMOD spectra of 1 revealed the presence of one acetate group (δH 2.29s; δC 172.3 and 21.5) and one benzoate group (δH 7.587d, 7.35t and 7.41s; δC 163.8, 132.6, 131.6, 129.6, and 127.8) (Table 1). The JMOD and HMQC spectra suggested that the skeleton contained 20 carbons: five methyls, three methylenes, seven methines and five quaternary carbons, with one ketone (δC 205.3). The gradient ²H→¹H COSY and HMQC spectra demonstrated two structural fragments with correlated protons: —CH₂—CH(CH₂)—CH(OR)—CH—CH(OR)— (A) and —CH₂—CH—CH—CH—CH(OR)— (B) (R=H, acyl or methyl). The sequences A and B, tertiary methyls and quaternary carbons were connected by inspection of the long-range C–H correlations observed in a gradient HMBC spectrum, as presented in Figure 1. The correlations of quaternary C-15 with the H-3 and H-1β signals, and of C-4 with the H-1β signal revealed that structural fragment A together with quaternary C-15 forms a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes. HMBC cross-peaks between C-6 and H-5, C-6 and H-20, C-6 and H-17, and C-13 and H-17, C-13 and H-20, and C-14 and H-1 proved the presence of a six-membered ring B substituted with one keto and two methyl groups. The structure was further elucidated with the aid of two and three-bond correlations between C-6 and H-7, C-13 and H-11, and C-13 and H-12, indicating that structural fragments A and B are connected as depicted in Figure 1. The presence of six-membered ring C and four-membered ring D with geminal dimethyl groups was derived from the HMBC correlations between C-20 and H-12, C-10 and H-18, C-10 and H-19, C-10 and H-12, and C-10 and H-9. The JCH₂ and JCH correlations between H-1α and the carbon signal at δC 205.3 placed the keto group at C-14. The positions of the ester groups were also established via the HMBC experiment. The correlation of the carbonyl signal at δC 172.3 (acetyl CO) with the proton signal at δH 5.07 (H-3) indicated the presence of the acetyl group at C-3. The position of the hydroxy group at C-5 was determined via the coupling constants and the long-range correlations: in the ¹H NMR spectrum, the signal of the hydroxy group (δH 2.95d) was coupled to H-5 (δH 4.76dd), with J = 3.2 Hz, while in the HMBC spectrum, the hydroxyl proton showed a correlation to C-6. The HMBC correlation between H-11 and the methoxy group pointed to the position of the methoxy group at C-11, and of necessity the benzoate group was placed at the quaternary C-15. The relative

![Figure 1](https://example.com/figure1.png)
configurations of the ten stereogenic centres in 1 were investigated in a phase-sensitive NOESY experiment (Table 1), aided by consideration of the coupling constant values. Conventionally, as reference point, the position of H-4 was chosen to be α. The NOESY correlations observed between H-4 and H-3, H-4 and H-1α, H-4 and 5-OH, and H-4 and H-17 indicated the α stereochemistry of H-3, H-1α, 5-OH and H-17. The NOE interaction between H-1α and H-16 suggested the α position of H-16. A diagnostic NOE interaction was detected between H-5 and H-2', 6' (benzoyl), proving the β position of the benzoyl group. The nuclear Overhauser effects observed between H-5 and H-12, H-2', 6' and 11-OCH₃ and H-12 and H-19 demonstrated the β position of H-12, 11-OCH₃ and H-19, while the NOEs of H-11 with H-9, H-18 and H-20 were indicative of the α position of H-9, H-18 and H-20. The stereochemistry elucidated from the NOESY experiment was compared with the conformation generated by the semi-empirical PM3¹¹,¹² package of the HyperChem program¹² (Fig. 2). The calculated conformation is in good agreement with the detected nuclear Overhauser effects. Rings B and C are cis-fused, and in the model possess a chair—chair conformation. Thus, the distance between H-5 and H-12 is 1.9 Å, and consequently an intense NOESY cross-peak appeared between H-5 and H-12. The calculated conformation indicated the spatial proximity of the benzoyl and C-19 methyl groups in accordance with the observed NOESY correlation between H-2', 6' and H-19, and the upfield-shifted methyl signal (δH₂-19 0.38α) as a result of the anisotropic effect of the aromatic ring. From the above analysis, therefore, the structure of this compound was identified as 1, moreover complete and unambiguous ¹H and ¹³C chemical shift assignments were determined as listed in Table 1. Compound 1 has a tetracyclic 5-6-6-4 fused ring system. A similar diterpenoid core was previously found in euphoractines A, C and D (obtained from Euphorbia micractina) with a C-2 epimer configuration.¹⁴

2.2. Structure of compound 2

Compound 2 was isolated as an amorphous solid with [α]D²⁵ = +16 (c 0.033, CHCl₃). The HRFAB-MS suggested that the molecular formula is C₂₉H₃₉O₇, with a molecular ion peak at m/z 499.2704 (MH⁺) (Δ=1.6 ppm). The ¹H NMR and JMOD spectra of 2 contained signals corresponding to one acetyl (δH 2.32β; δC 172.1 and 21.5) and one benzoyl group (δH 8.12d, 7.58t and 7.44t; δC 166.3, 133.4, 130.6, 130.0 and 128.3). Additionally, the ¹H NMR and JMOD spectra exhibited resonances for five methyls, three methylenes, seven methines and five quaternary carbons, including one ketone (δC 205.3) (Table 2). The ¹H–¹H COSY spectrum indicated two sequences of correlated protons: –CH₂–CH(CH₃)–CH(OR)–CH(OR)– and –CH₂–CH₃–CH–CH(OR)– (R=H or acyl). After the ¹H and ¹³C chemical shift assignment of 2 had been achieved via the ¹H–¹H COSY, HSQC and HMBC spectra, it was evident that compounds 1 and 2 differ only in one substituent: the methoxy group of 1 is replaced by a hydroxy group in 2. The diamagnetically shifted C-11 signal (2: δC₁₁ 73.6; 1: δC₁₁ 82.7) demonstrated a hydroxy group instead of a methoxy group on C-11 in 2.

The relative configuration of 2 was analysed by a NOESY experiment. Diagnostic nuclear Overhauser effects detected between H-2', 6'/H-5, H-2', 6'/H-12, H-2', 6'/H-19, H-5/H-12, H-12/H-19 and H-1/β/H-2 provided evidence of the β orientation, while NOESY cross-peaks between H-1α/H-16, H-1α/H-4, H-4/H-3, H-4/H-17, H-17/H-20, H-20/H-9, H-9/H-18, H-9/H-11 and H-11/H-20 pointed to the α position of these protons and groups. All of the above data indicate structure 2 for this compound.

2.3. Structure of compound 3

Compound 3 was isolated as an amorphous solid with [α]D²⁵ = 38 (c 0.1, CHCl₃). The molecular formula C₂₉H₃₉O₇ was established by HREI-MS, with a molecular ion peak at m/z 512.2801 (M⁺) (Δ=5.2 ppm). In the ¹H NMR and JMOD spectra, signals of one benzoyl, one acetyl and one methoxy group were observed (Table 2). Further analysis of the ¹H NMR and JMOD resonances revealed that the remaining moiety consisted of 20 carbons: five methyls, three methylenes, seven methines and five quaternary carbons (Table 2). A quaternary carbon at δC 20.4 and
two methines at $\delta_{\text{H}}$ 0.76 and 0.32, and $\delta_{\text{C}}$ 26.3 and 26.8 indicated a *gem*-dimethyl-substituted cyclopropane ring.\textsuperscript{10} The gradient $^1\text{H}$$-$$^1\text{H}$ COSY and HSQC spectra identified two isolated fragments of correlated protons: -CH$_2$-CH(CH$_3$)$_2$-CH(OR)-CH(CH$_3$)(OR)- and -CH$_2$-CH$_2$-CH-CH(OR)-(R=H, acyl or methyl). One signal at $\delta_{\text{H}}$ 3.01, which did not provide any correlation in the HSQC spectrum, pointed to the presence of one hydroxy group in the molecule. The HMBC correlations between C-15 and H-1, C-15 and H-3 and C-4 and H-1 suggested that structural fragment A, together with a quaternary carbon (C-15), is joined to a methyl-substituted five-membered ring in 3. The long-range correlations between C-5 and H-7, C-5 and H-17, C-17 and H-7, C-6 and OH, C-6 and H-7, C-14 and H-20, C-13 and H-20, C-20 and H-12, and C-6 and H-12 led to the assembly of units A and B together with two methyl groups (C-17 and C-20) through quaternary carbons (C-6, C-13 and C-14), resulting in a 5-6-7-3 condensed ring system. The long-range correlation between the hydroxy group and C-5 clearly indicated that the hydroxy group is located at C-5. The methoxy group was placed at C-12, as shown in the HMBC spectrum by the long-range correlation between the methoxy carbon and H-12. The position of the acetyl group was determined on the basis of the three-bond correlation between the ester carbonyl and H-3. Finally, the benzoxy group, which did not display any long-range correlations, must be on C-15. The relative configuration of the molecule was investigated by a NOESY experiment. Starting from the $\alpha$ position of H-3, the nuclear Overhauser effects between H-3 and 5-OH and H-3 and H-16 were indicative of the $\alpha$ position of the hydroxy group at C-5 and the methyl group at C-2. The NOESY correlations between H-16 and H-1$\alpha$ allowed the conclusion that this H-1 is $\alpha$-oriented. The NOE effects between H-5 and H-8$\beta$, H-5 and H-12 suggested the $\beta$ position, and that between H-8$\alpha$ and H-7$\alpha$ the $\alpha$ configuration of these protons. The $\beta$ orientation of the 19-methyl group followed from the nuclear Overhauser effects between H-12 and H-19. The cross-peaks of the benzyloxy protons (H-$\gamma$, $\delta$) with H-5, H-12 and H-19 suggested the $\beta$ position of the benzyloxy group. The $\alpha$ orientation of H-11, H-9 and H-20 was deduced from the NOE correlations between H-18 and H-11, H-11 and H-20, and H-11 and H-9. The overlapping of the $^1$H signals of H-2, H-4 and 3-OAc at $\delta_{\text{H}}$ 2.20 did not allow a conclusion concerning the stereochemistry of H-4, but the coupling constants of H-3 and H-5 in 3 were found to be very similar to those in 1 and 2, indicating the same $\alpha$ configuration of H-4. Further, the NOESY correlation of the overlapping signal at $\delta_{\text{H}}$ 2.20 with that of H-17 can most probably be assigned to the H-4/H-17 correlation, suggesting the $\alpha$ orientation of H-17. All of the above data are compatible with structure 3 for this compound.

2.4. Structure of compound 4

Compound 4, an amorphous solid with $[\alpha]_{\text{D}}^{25} = 146$ (c 0.1, CHCl$_3$), has the molecular formula C$_{20}$H$_{22}$O$_4$, determined via the molecular ion peak at $m/z$ 480.2526 (M$^+$).
(Δ=3.0 ppm) in the HREI-MS. Analysis of the ¹H NMR and JMOD data revealed that 4 possesses one benzoyl and one acetyl group in the molecule. The diterpene core consists of five methyls, three methylenes, seven methines (one vinylic, δC 144.5) and five quaternary carbons, with one ketone (δC 194.4) (Table 2). Interpretation of the HSQC and ¹H-¹H COSY spectra led to the identification of three structural elements with correlated protons: –CHO–CH(CH₃)₂ = (A), –CH(OR)–CH–CH = (B) and –CH₂–CH₂–CH–CH = (C), (R=acyl). Their connectivities were determined on the basis of the HMBC spectrum. The long-range correlations of the quaternary carbons with the protons of the three fragments (C-6 and H-4, C-6 and H-7, C-6 and H-17, C-10 and H-9, C-10 and H-11, C-10 and H-18, C-10 and H-19, C-13 and H-11, C-13 and H-20, C-14 and H-1, C-14 and H-4, C-14 and H-20, C-15 and H-1, and C-15 and H-3) indicated the bicyclic lathyrane ring system with oxygen functions at C-3, C-5, C-6, C-14 and C-15, and a double bond between C-12 and C-13. The three-bond correlation between H-12 (δH 6.93(dd)) and the carbon signal at δC 194.4 placed the keto group at C-14. The chemical shifts of C-5 (δC 57.8), C-6 (δC 63.4) and H-5 (δH 3.53) were indicative of an epoxy group at C-5-C-6.¹² The HMBC correlation between acetyl CO and H-3 pointed to the presence of the acetyl group at C-3. The benzoyl group, which did not exhibit any long-range correlations, is located on quaternary C-15. The relative configuration of 4 was determined on the basis of a NOE experiment. Because of the overlapping of the proton signals in CDCl₃ solution, the ¹H NMR, JMOD, HSQC and NOESY spectra were also run in C₂D₆, which resulted in better-resolved spectra and unambiguous assignments of all the ¹H and ¹³C NMR signals. Starting from the α position of H-4, the nuclear Overhauser effects between H-4 and H-1α, H-1α and H-16 and H-1α and H-3 indicated the α orientation of H-3 and H-16. The observed correlations of the ortho-benzoyl protons with H-5, 3-OAc and H-19, and between H-12 and H-19 proved the β position of H-5 and H-19 and the acetyl group at C-3. The NOE interactions between H-5 and H-7α, H-5 and H-8β and H-5 and H-12 allowed the steric differentiation of the C-7α and C-8 methyl protons and suggested that H-12 is oriented above the plane of the molecule. The NOE interactions between H-18 and H-11 and between H-18 and H-9 suggested the α-oriented H-11 and H-9. As regards the stereochemistry of C-17, the NOE interactions between H-17 and H-14, and H-17 and H-7α were informative, proving the α orientation of the 17-methyl group. The NOE interaction between H-11 and H-20 revealed that H-20 is oriented below the plane of the macrocycle, thereby confirming the E configuration for the C-12/C-13 double bond. As a result of the above NMR study, the structure of this compound was demonstrated to be 4, and the complete ¹H and ¹³C NMR data were assigned as listed in Table 2. Compound 4 is a lathyrane derivative containing the rare 5,6-epoxy function. The parent diterpene alcohol of 4 has not been described previously.

Biogenetic relationship can be supposed between the isolated compounds, since lathyrane diterpenes are regarded as the biosynthetic progenitors of the naturally occurring polycyclic derivatives. Compounds 1-3 can be derived from a lathyrane precursor by transannular cyclization, and in case of 1 and 2, by expansion of the cyclopropane ring.

Similar rearrangement was observed in synthetic studies when acid-catalysed conversion of an epoxythyrane enone (Euphorbia factor L₁) was performed.¹⁶,¹⁷

3. Experimental

3.1. General

Melting points were uncorrected. HRMS measurements were carried out on a VG ZAB SEQ instrument in EI and FAB ionization mode. The resolution of the instrument was 10,000 (at 10% valley definition). NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (¹H) and 125 MHz (¹³C). The signals of the deuterated solvents were taken as reference. Two-dimensional experiments were performed with standard Bruker software. Optical rotations were determined in CHCl₃ by using a Perkin-Elmer 341 polarimeter. The UV spectra were recorded on a Shimadzu UV-2101 PC spectrometer. For column chromatography, polyamide (ICN) and silica gel (Kieselgel GF₂₅₄, 15 μm, Merck) were used. HPLC was carried out on a Waters Millipore instrument, with detection at 254 nm on LiChrospher Sí 100 and LiChrospher RP-18 (5 μm, 200×4 mm) columns.

3.2. Plant material

E. villosa W. et K. was collected in Vácártó, Hungary, in June 2000, and identified by Vilmos Miklós Szy V. (Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácártó). A voucher specimen (No. 550) has been deposited in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged.

3.3. Extraction and isolation

The dried plant material (210 g) was percolated with methanol (3:1) at room temperature. The crude extract was concentrated in vacuo to 300 ml and exhaustively extracted with chloroform (1200 ml). On evaporation, the organic phase gave a residue (6.12 g), which was chromatographed on a polyamide column (20 g) with mixtures of MeOH–H₂O (2:3, 3:2 and 4:1) as eluents. The fractions obtained with MeOH–H₂O (3:2) were subjected to silica gel (40 g) flash chromatography, using a gradient system of cyclohexane–EtOAc (19:1, 9:1, 8:2, 7:3 and 6:4). Fractions from cyclohexane–EtOAc (9:1 and 8:2) were further fractionated by reverse-phase HPLC with MeOH–H₂O (8:2 and 9:1) eluent at a flow rate of 2.0 ml/min. Purification of the peaks observed at retention times of 27.6, 21.1 and 24.7 min yielded compounds 1 (11.0 mg), 5 (13.8 mg), and 3 (3.0 mg), respectively. The fraction eluting at tR 16.6 min was subjected to NP-HPLC with cyclohexane–CH₂Cl₂–MeOH (70:50:1) as eluent to afford 2 (2.4 mg). The fractions obtained with MeOH–H₂O (4:1) were repeatedly chromatographed on a polyamide column with mixtures of MeOH–H₂O (3:2, 7:3 and 4:1) as eluents. The fractions obtained with MeOH–H₂O (3:2) were separated by flash chromatography on silica gel, using a gradient system of cyclohexane–EtOAc (49:1, 9:1, 4:1 and 7:3). The fractions obtained were further purified by RP-HPLC with
MeOH–H₂O (8:2) as eluent at a flow rate of 1.0 ml/min to afford compound 4 (10.4 mg, t₉₀=11.5 min).

3.3.1. Compound 1. Colourless prisms; mp 246–248 °C; [α]D²⁰ = –3 (c 0.1, CHCl₃); UV (MeOH): 230 (3.68), 275 (2.91); HREI-MS m/z 512.2806 (M⁺) (Δ=6.2 ppm). Calcd for C₃₀H₃₀O₁₂: 512.2774. ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 1.

3.3.2. Compound 2. An amorphous solid; [α]D²⁰ = +16 (c 0.033, CHCl₃); UV (MeOH): 231 (3.49), 259 (3.08), 285 (2.43); HRFAB-MS m/z 499.2704 (MH⁺) (Δ=1.6 ppm). Calcd for C₂₉H₂₀O₁₀: 498.2617. ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 2.

3.3.3. Compound 3. An amorphous solid; [α]D²⁰ = –38 (c 0.1, CHCl₃); UV (MeOH): 232 (3.51), 275 (2.68), 282 (2.67); HREI-MS m/z 512.2801 (M⁺) (Δ=5.2 ppm). Calcd for C₃₀H₃₀O₁₂: 512.2774. ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 2.

3.3.4. Compound 4. An amorphous solid; [α]D²⁰ = –146 (c 0.1, CHCl₃); UV (MeOH): 233 (336), 271 (3.17); HREI-MS m/z 480.2526 (M⁺) (Δ=3.0 ppm). Calcd for C₂₉H₂₀O₁₀: 480.2512. ¹H NMR (500 MHz, CDCl₃) and ¹³C NMR (125 MHz, CDCl₃) data, see Table 2.

3.4. PC model

Molecular conformation of 1 was generated by the semi-empirical PM3¹¹,¹² package of the HyperChem program.¹³ The Polak–Ribiere algorithm was used with the termination condition of 0.1 kcal/(A mol) RMS gradient. The calculation was performed on a single molecule placed in vacuo.

Acknowledgements

The authors thank Vilmos Miklóssy V. (Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátóti) for the identification and collection of the plant material. Financial support from the Hungarian Research Fund Agency (OTKA-T038390) and the Hungarian Ministry of Education (FKFP-0024/2001) are gratefully acknowledged.

References and notes

1. Evans, F.; Taylor, S. E. Progress in the Chemistry of Organic Natural Products; Springer: Vienna, 1983; Vol. 44. p 1.