ISOLATION AND STRUCTURE ELUCIDATION OF DITERPENES FROM HUNGARIAN *EUPHORBIA* SPECIES

Ph.D. Thesis

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List of publications related to the thesis

I a) Hohmann J, **Vasas A**, Günther G, Máthé I, Evanics F, Dombi G, Jerkovich G: Macrocyclic diterpene polyesters of the jatrophane type from *Euphorbia esula*, *J. Nat. Prod*. 1997; **60**: 331-335

b) Hohmann J, **Vasas A**, Günther G, Máthé I, Evanics F, Dombi Gy, Jerkovich Gy: Jatorfánvázas makrociklusos diterpén-poliészterek az *Euphorbia esula*-ból, *Acta Pharm. Hung*. 1998; **68**: 175-182

II Günther G, Hohmann J, **Vasas A**, Máthé I, Dombi G, Jerkovich G: Jatrophane diterpenoids from *Euphorbia esula*, *Phytochemistry* 1998; **47**: 1309-1313

III Günther G, Martinek T, Dombi G, Hohmann J, **Vasas A**: Structural characterization and dynamic NMR studies of a new peracylated macrocyclic diterpene, *Magn. Reson. Chem*. 1999; **37**: 365-370

IV Hohmann J, Günther G, **Vasas A**, Kálmán A, Argay G: Isolation and structure revision of pepluane diterpenoids from *Euphorbia peplus*, *J. Nat. Prod*. 1999; **62**: 107-109

V Hohmann J, **Vasas A**, Günther G, Dombi G, Blazsó G, Falkay G, Máthé I, Jerkovich G: Jatrophane diterpenoids from *Euphorbia peplus*, *Phytochemistry* 1999; **51**: 673-677

VI Hohmann J, Evanics F, **Vasas A**, Dombi G, Jerkovich G, Máthé I: A novel lathyrane diterpenoid from the roots of *Euphorbia lathyris*, *J. Nat. Prod*. 1999; **62**: 176-178

VII **Vasas A**, Hohmann J, Forgo P, Szabó P: New tri- and tetracyclic diterpenes from *Euphorbia villosa*, *Tetrahedron* 2004; **60**: 5025-5030

CONTENTS

ABBREVIATIONS AND SYMBOLS

1D	one-dimensional
2D	two-dimensional
COSY	correlated spectroscopy
cryst.	crystallization
δ	chemical shift
DEPT	distortionless enhancement by polarization transfer
EIMS	electron-impact ionization mass spectroscopy
fr.	fraction
HMBC	heteronuclear multiple-bond correlation spectroscopy
HMQC	heteronuclear multiple-quantum coherence spectroscopy
HPLC	high-performance liquid chromatography
HREIMS	high-resolution electron ionization mass spectroscopy
HRFABMS	high-resolution fast atom bombardment mass spectroscopy
HSQC	heteronuclear single-quantum correlation spectroscopy
IR	infrared
JMOD	*J*-modulated spin-echo experiment
MDR	multidrug resistance
NMR	nuclear magnetic resonance
NOE	nuclear *Overhauser* effect
NOESY	nuclear *Overhauser* enhancement spectroscopy
NP	normal-phase
OCC	open-column chromatography
PLC	preparative-layer chromatography
RP	reversed-phase
TLC	thin-layer chromatography
t_R	retention time
UV	ultraviolet
VLC	vacuum-liquid chromatography
Ester groups:	Ac = acetyl, Bz = benzoyl, Nic = nicotinoyl, *i*Bu = 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 *Cunuria 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.[9-13]

In 1995, HOHMANN *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 *Euphorbia* species. In the course of these studies, many diterpene esters of different skeletal types have been isolated.[14-21] One part of this programme involved the investigation of *Euphorbia esula* L., *E. lathyris* L., *E. peplus* L. and *E. villosa* Waldst. & Kit. The present thesis summarizes the results of this phytochemical work.

1.1. Botany of the family Euphorbiaceae and the investigated *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.[2] The tribe Euphorbieae is characterized by the possession of a unique *cyathium*, which consists of a central carpellate flower and four or five groups of basal male-flower clusters. *Euphorbia* plants are monoecious.[22,23]

The investigated plants, *Euphorbia esula*, *E. lathyris*, *E. peplus*, and *E. villosa* belong in the section Tithymalus of the genus *Euphorbia* in the family Euphorbiaceae, in the order Euphorbiales.

Euphorbia esula L. (leafy spurge; syn. *Euphorbia pseudovirgata* (Schur) Soó, *E. dalechampii* Haw., *E. discolor* Led., *E. intermedia* Brebis, *E. racemosa* Tausch., *E. tristis* Bess., *E. triumfetti* Bert., *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, quinolizidines, 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).[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.[32] Cembrane diterpenes have been isolated from different plants and marine invertebrates; many have cytotoxic[33], antitumour[34] and HIV-1-inhibitory[35] activities.

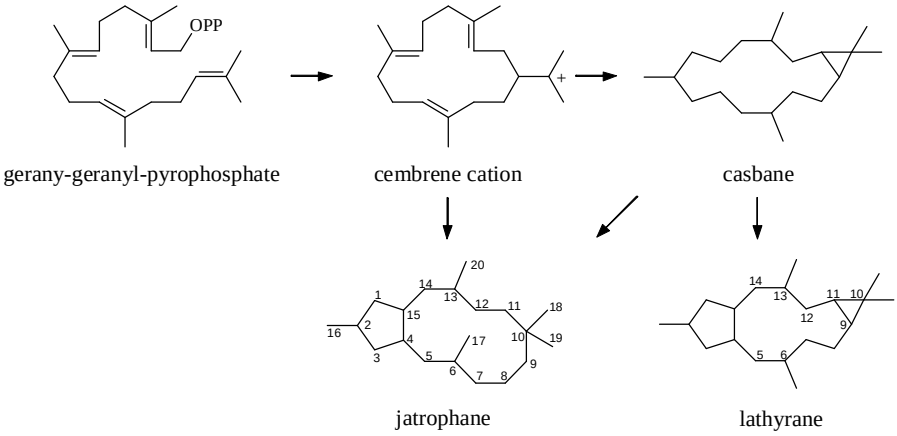


Figure 1. Hypothetical biogenic interconversions of diterpenes in the family Euphorbiaceae

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.[36] Casbanes with a *trans* or *cis* cyclopropane ring occur in the species of *Euphorbia*[37,38] and other Euphorbiaceae genera.[39,40] Some compounds have *in vitro* cytotoxic,[37,40] antibacterial[38] and antiproliferative[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.[41-43] Further antitumour jatrophane derivatives, euphornin from *E. maddeni*[44] and euphoscopins A and B from *E. helioscopia*, were reported in 1981.[45] The structure of euphornin was proved by X-ray analysis in 1984.[46] In the 1980s, further jatrophane diterpenes were isolated from *E. helioscopia*,[11,47,48] *E. characias*[49] and *E. lateriflora*.[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.[48] In the 1990s, jatrophane derivatives from further *Euphorbia* species were isolated:

E. terracina,[52] *E. paralias*,[53] *E. semiperfoliata*,[54] *E. segetalis*[55] and *E. obtusifolia*.[56] From *E. terracina*, interesting bishomo-jatrophane derivatives, terracinolides and isoterracinolides were isolated by MARCO *et al*.[57-59] Between 2000 and 2005, jatrophanes were isolated from *E. serrulata*,[15-17] *E. salicifolia*,[18] *E. paralias*,[60] *E. turczaninowii*,[61] *E. hyberna*,[62,63] *E. dendroides*,[64] *E. altotibetic*,[65] *E. semiperfoliata*,[66] *E. mongolica*,[20] *E. obtusifolia*,[67] *E. pubescens*,[68-71] *E. characias*,[72] *E. kansui*,[73-75] *E. amygdaloides*[76] and *E. helioscopia*.[77] Many biological activities of jatrophanes have been reported, such as antifeeding activity against the larvae of *Spodoptera littoralis*, antiviral activity against HIV-1,[60] microtubule-interacting activity,[66] inhibitory activity on the mammalian mitochondrial respiratory chain,[67] antitumour and multidrug resistance reversing (MDR) activities,[64,68-72,76] and cell division-inhibitory effect on the *Xenopus laevis* embryo.[73,74] 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[48,49] and 8.[55] The acyl residues are most frequently acetate, benzoate, isobutyrate, 2-methyl butyrate or nicotinate, and rarely propionate, butyrate, angelate, tigliate or cinnamate. The most heterogeneously esterified molecules have 4 different acyl groups,[58] and there are only a few compounds with homogeneous ester groups.[52,53,61] Hydroxy groups can usually be found on C-3, C-5, C-7, C-9 and C-15, but also on C-2,[53,65] C-8[51] and C-14,[48] and rarely on C-1,[42] C-6,[55,78] and C-13.[57,64] Keto groups are usually located at C-9 or/and C-14, and occasionally at C-7[48] or C-12.[41] Most of the compounds contain C-11/C-12 and C-6/C-17 double bonds. Rarely, C-5/C-6,[44,46,49,68] C-14/C-15,[48] C-3/C-4 and C-8/C-9[79] 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,[73] C-2,[48,63] C-13[48,80] and C-14[48]) are variable.

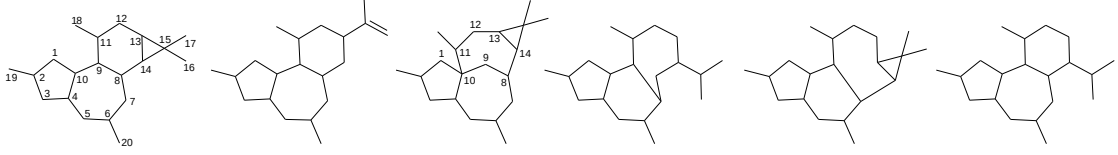
The absolute configurations of jatrophane derivatives have been investigated in only a few cases by X-ray analysis or by the circular dichroism exciton chirality method.[42,45,46,65] The

absolute configurations of all natural jatrophane derivatives are identical as concerns the ring anellation: H-4 is α, and 15-OH or the 15-acyl group is β-oriented.

Irregular bicyclic compounds have been isolated from *Chrozophora obliqua* (Euphorbiaceae)[81] and from *Jatropha integerrima* and *J. gossypifolia*.[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).[36] The first lathyrane diterpene was 'euphorbiasteroid', reported by DUBLYANSKAYA in 1937 from the seed oil of *E. lathyris*.[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[85,86] was revised by APPENDINO *et al*. in 2000.[87,88] Lathyrane diterpenes have been reported from *Macaranga tanarius*,[89] *Jatropha curcas*,[90,91] *J. grossidenta*,[92] *J. podagrica*,[93] *J. weddelliana*,[94] *Euphorbia jolkini*,[95] *E. micractina*,[96] *E. pithyusa* subsp. *cupani*[97] and *E. hyberna*.[63] An unusual lathyrane diterpene, bertyadionol, was isolated from *Bertya cuppressoidea* in 1970.[98] Pharmacological screening revealed that lathyrane-type compounds do not possess the pro-inflammatory activity characteristic of other Euphorbiaceae diterpenes.[99] Ingols are 4,15-epoxy derivatives of lathyranes, which have given rise to considerable interest as cytotoxic,[100,101] vasoactive,[13,102] antineoplastic[44] and prostaglandin E_2-inhibitory[103] agents. Ingol was originally isolated from the latex of *Euphorbia ingens* in 1973. Its stereochemistry was elucidated by X-ray analysis.[104] Further ingol esters have been isolated from *Euphorbia lactea*, *E. kamerunica*,[100,101,105] *E. hermentiana*,[106] *E. royleana*,[107] *E. tirucalli*,[108,109] *E. poisonii*,[110,111] *E. antiquorum*,[112] *E. portulacoides*,[113] *E. canariensis*,[13,114,115] *E. acrurensis*,[116] *E. lactea*,[117] *E. nivulia*,[103,118] and *Synadenium compactum*[119].

Tigliane, ingenane, daphnane, jatropholane, crotopholane and rhamnopholane are further common types of 'lower diterpenes' with tri- or tetracyclic skeletons.[31,120]



tigliane daphnane ingenane crotopholane jatropholane rhamnopholane

Compounds with tigliane, ingenane or daphnane skeletons have attracted interest because of their skin-irritant activities.[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-*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 constituents 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 tetradecanoate.[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, α- and β-amyrin, δ-amyrenone),[33] steroids (β-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' L_1-L_{10}, have also been isolated from the seeds[139-144] and jolkinol A from a callus culture.[145] Among the 'Euphorbia factors', the ingenol-type compounds have proved to be potent PKC activators, and anticancer[8] 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 C_8–C_{14} 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' L_{11}, were isolated from the seeds of the plant.[139] Besides diterpenes, triterpenoids (cycloartenol, euphol, lanosterol, isolanosterol, 24-methylenecycloartenol, 24-methylene-24-dihydrolanosterol, guimarenol, hopenol B, butyrospermol, taraxerol, taraxerone, betuline),[32,33] steroids (campesterol, α- and β-euphorbiasterol, stigmasterol, Δ^7-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 rubber[6] have been reported from this plant.

1.2.4. *Chemical constituents of Euphorbia peplus*

In 1984, 2 ingenane-type diterpene esters, 'Euphorbia factors' Pe_1 and Pe_2, 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 jatrophanes, 1 tetracyclic diterpene with a pepluane skeleton and 2 ingenanes were isolated. The sample from Germany additionally afforded 3 other jatrophanes.[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' Pe_1-Pe_5 being identified as diterpene ester-type toxins of *E. peplus*.[148] Between 2000 and 2004, further pepluane-, jatrophane- and ingenane-type diterpenes were isolated from the plant.[14,149]

Besides diterpenes, *E. peplus* contains triterpenoids (β-amyrin acetate, nepehinol, alangidiol, simiarenone, cycloartenol, cycloartenone, 24-methylenecycloartenol, obtusifoliol,

citrostadeniol),[150] steroids (lanosterol, campesterol, cholesterol, stigmasterol, β-sitosterol, 28-isofucosterol, Δ^7-isofucosterol),[150] flavonoids (quercetin, quercetin 3-glucoside, rhamnetin-rhamnoside, hiperoside, rhamnetin 3-galactoside, astragalin)[150] and long-chain alcohols.[150]

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

E. esula is toxic to livestock, and allelopathic to desirable forage plants.[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.[152] Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings and warts.[3] Previous pharmacological studies demonstrated the pro-inflammatory, tumour-promoting and antitumour activities of the plant extracts. The extracts of *E. esula* exhibited antileukaemic activity against P-388 lymphocytic leukaemia in mice.[8]

Caper spurge (*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.[153] The seeds of *E. lathyris*, a common traditional Chinese medicine, have been used for the treatment of very diverse diseases, such as ascites, coprostasis, anuresis, amenorrhoea, venous stasis, terminal schistosomiasis and scabies.[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.[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 *E. lathyris* against Sarcoma 180 ascites.[138,145]

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.[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.[155] Extracts of this plant have been recorded to exert C-mitotic action and have been reported to display proteolytic activities.[3,156]

As concerns *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).

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[†] Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary

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*

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

Vacuum-liquid chromatography: For VLC, silica gel 60 G (15 μm, 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

Layer chromatography: PLC was performed on silica gel 60 F_{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_{254} (Merck 5554).

Mobile phases:
- A cyclohexane–ethyl acetate–ethanol (30:10:1)
- B benzene–ethyl acetate (7:3)
- C *n*-hexane–tetrahydrofuran–acetonitrile (20:5:1)
- D chloroform–acetone (19:1)

Visualization methods: UV light: at 254 nm, spraying with conc. H_2SO_4, and then heating at 110 ºC 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:

1. Gradient Controller 680, Solvent Delivery System 6000A, Differential Refractometer R-400, Data Module 730, Injector Rheodyne 7725i.
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.

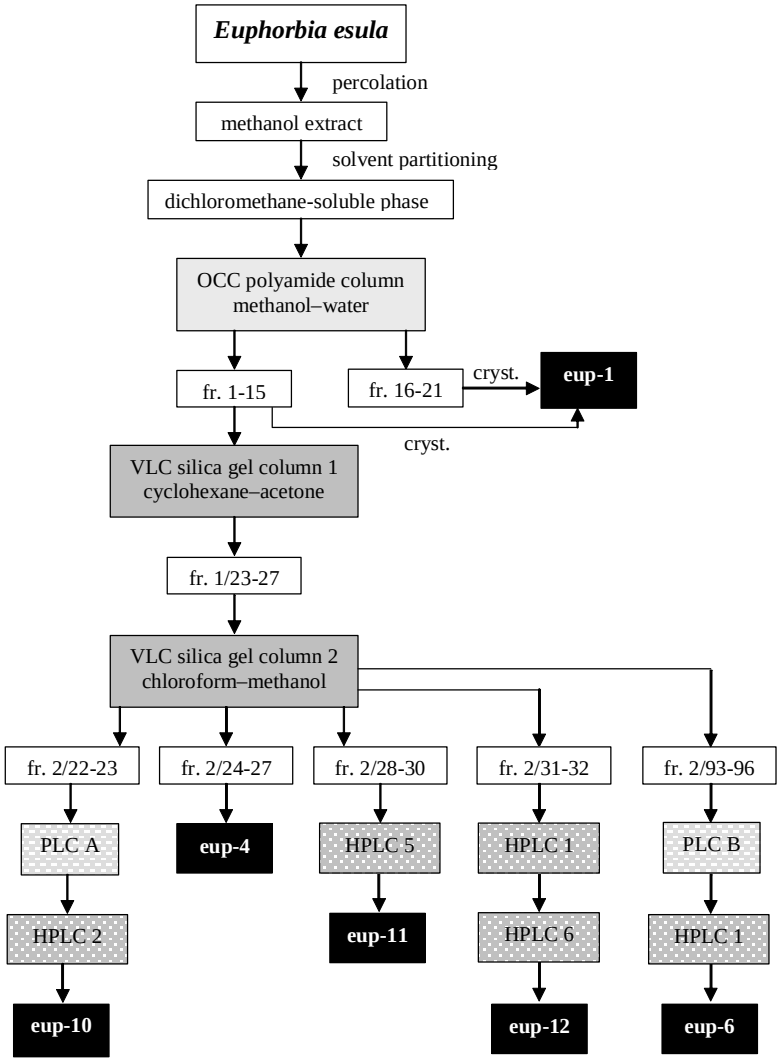


Figure 2 Isolation of diterpenes from *Euphorbia esula*

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 ºC until preparation in order to stop the *post mortem* enzymatic processes.

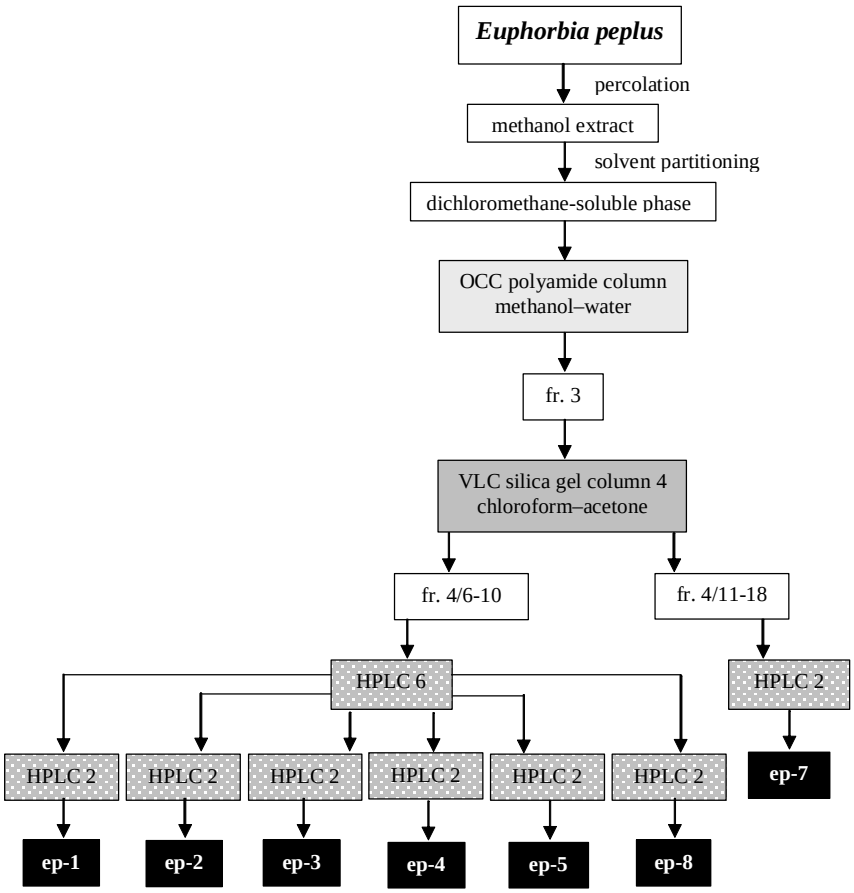


Figure 3. Isolation of diterpenes from *Euphorbia peplus*

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.).

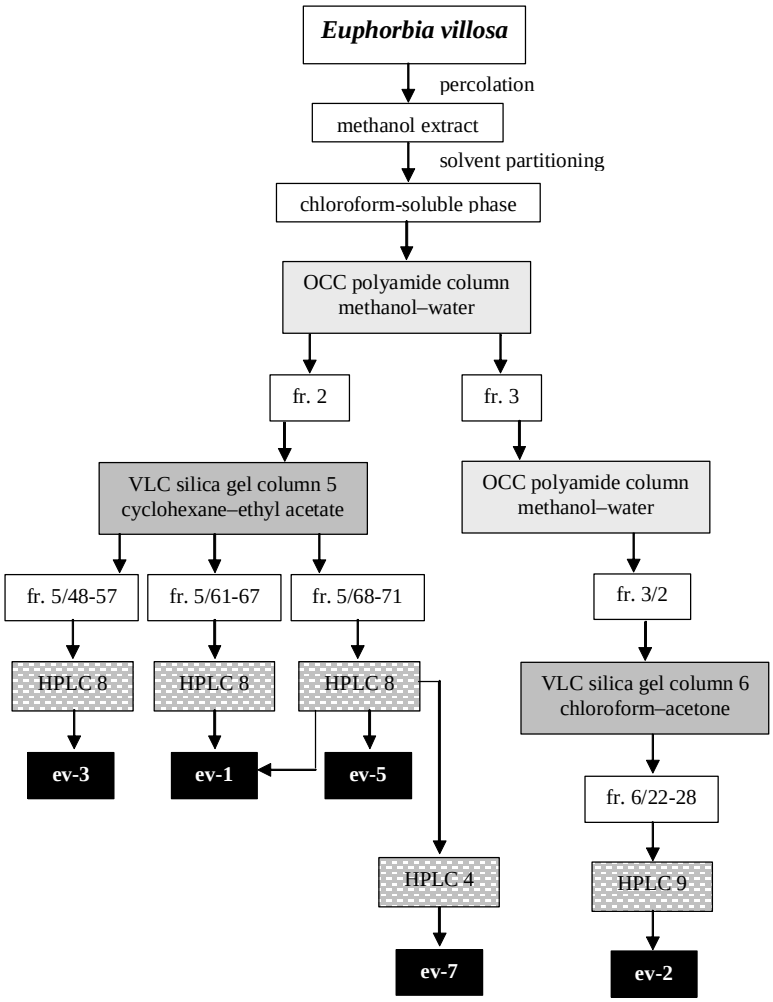


Figure 4. Isolation of diterpenes from *Euphorbia villosa*

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

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

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 ^{1}H NMR, JMOD, ^{1}H-^{1}H 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.

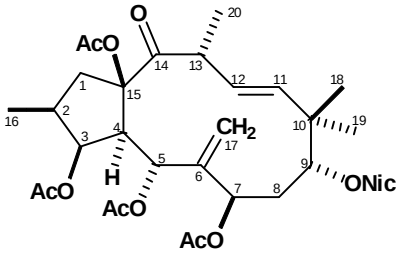
Ep-3 (**1**)

The molecular formula of ep-3 was assigned via HREIMS and NMR investigations as $C_{34}H_{43}NO_{11}$. The ^{1}H and ^{13}C NMR spectra of ep-3 revealed the presence of 1 nicotinoyl (Nic) and 4 acetyl groups (Table 1 in Appendix V). Additionally, the ^{1}H and ^{13}C 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: d_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); d_H 4.84 dd, 2.18 m (2H) and 5.09 dd, [–CH(OR)–CH_2–CH(OR)–] (B); d_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, ^4J couplings were detected between the exomethylene [d_H 5.07 s (2H) (H-17)] and 2 acyloxymethines [d_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 d_C 92.9 (C-15) with the proton signals at d_H 3.01, 1.65 (H-1*a*, *b*), 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 d_C 40.7 (C-10) and the proton signals at d_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 d_C 212.6 (C-14) and the proton signals at d_H 3.01, 1.65 (H-1*a*, β), 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 (d_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-oxojatropha-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 *a* position of the proton at the ring junction (H-4), it was found that a *b*-oriented methyl group is present on C-2 and a *b*-oriented ester group on C-3, with regard to the diagnostic NOE effects between H-4/H-1*a*,

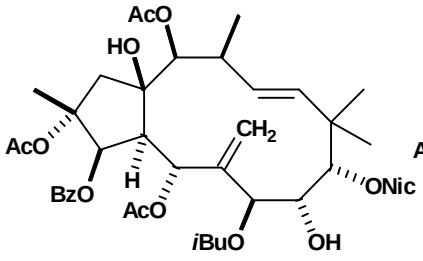
H-1*b*/H-16, H-4/H-2 and H-4/H-3. The position of H-5 was concluded on the basis of the zero coupling constant between H-4/H-5 to be *b*, similarly as in the case of other jatrophanes.[80,157,158] The NOE interaction between H-4/H-7 required a *b*-oriented acyl group on C-7. The NOESY cross-peak between H-5/H-13 dictated the *b* 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[158] and 2*a*,3*b*,5*a*,7*b*,15*b*-pentaacetoxy-9*a*-nicotinoyloxy-14-oxojatropha-6(17),11-diene,[80] with a 9*a*-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.[80] In ep-3, the 7*b*-*O*-acetyl signal was observed at δ_H 1.52, because of the effect of the 9*a*-*O*-nicotinoyl group. With regard to the above data, the structure of this compound is formulated as **1**.



1

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

From the dichloromethane extract of *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 *et al*. reported jatrophane diterpenes from *E. peplus*.[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 (2*R*,3*R*,4*S*,5*R*,7*S*,8*S*,9*S*,13*S*,14*S*,15*R*)-2,5,9,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutanoyloxyjatropha-6(17),11*E*-diene. The determined solid-state conformation agreed well with the calculated conformation published by JAKUPOVIC *et al*.[80]



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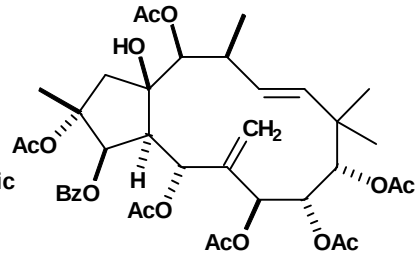

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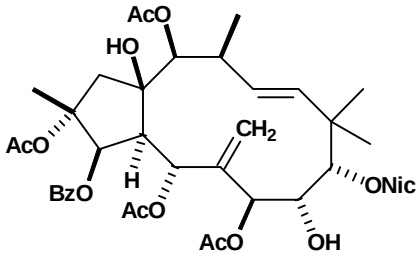

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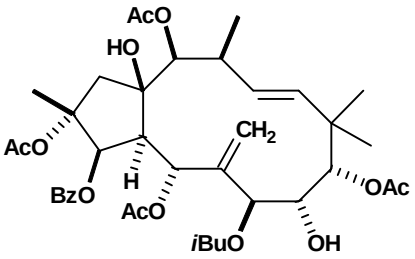
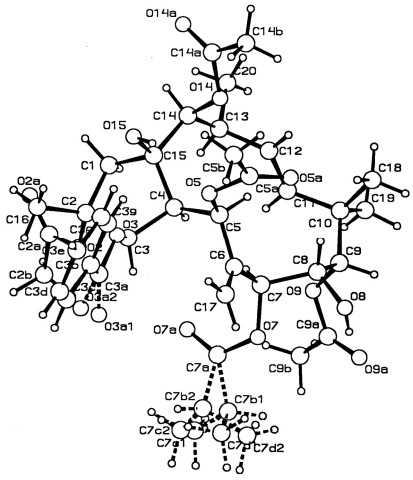




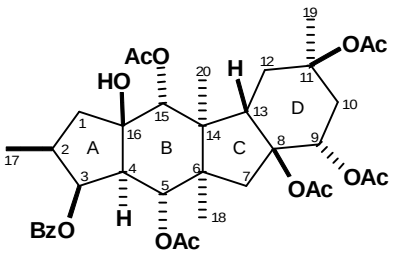
4

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.[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*b*, 16-OH/H-17, 16-OH and *ortho*-benzoyl protons, H-4/H-3, and H-3/H-2. These NOE interactions revealed a *b*-oriented methyl group on C-2 and a benzoyl 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 *a* position on C-5 and C-15. The 16-OH group also showed a NOE effect with H-13, from which the *b* 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*-benzoyl protons, pointing to *cis*-fused C/D rings, in contrast with the proposal of JAKUPOVIC *et al.*[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 α-oriented acetyl group on C-9 (in contrast with the structure published in ref. [80]) 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 in ref. [80].



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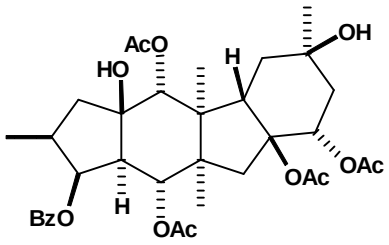


7

Ep-2 (**7**)

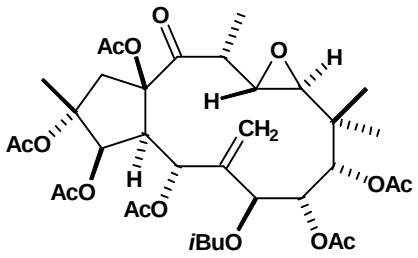
Ep-2 gave a parent ion in the EIMS at *m/z* 658, appropriate for a molecular formula of $C_{35}H_{46}O_{12}$. The ^{1}H and ^{13}C NMR spectra of ep-2 revealed the presence of 4 acetate and 1 benzoate groups (Table 1 in Appendix IV). Additionally, the spectra exhibited resonances closely related to that 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 (d_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: $d_{C\text{-}11}$ 79.8, ep-2: $d_{C\text{-}11}$ 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

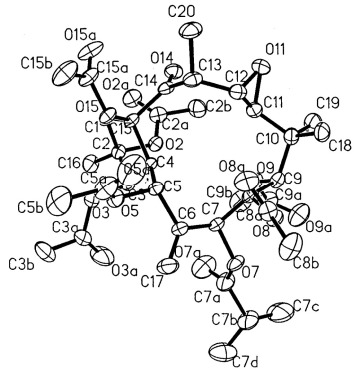
compounds ep-8 and ep-2 permitted determination of the same stereochemistry for ep-2 (**7**) as that of ep-8 (**6**).

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]

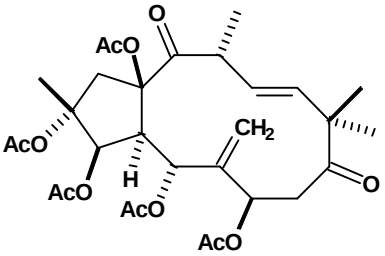
Eup-1 (esulatin A), which formed colourless crystals, was shown by elemental analysis and ESIMS to have the molecular formula $C_{36}H_{50}O_{16}$. ^1H and ^{13}C NMR, ^1H-^1H COSY, HMBC and NOESY spectral data are given in Table 1 in Appendix I. The absolute configuration of eup-1 (**8**) was determined 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]

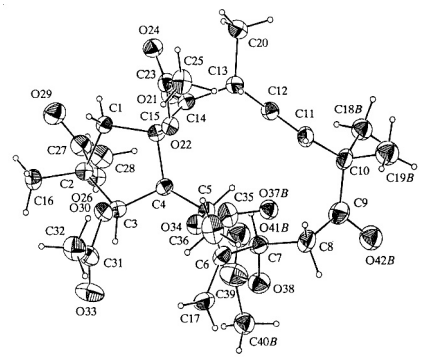




8

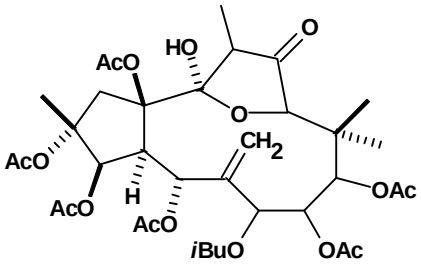
Eup-4 (esulatin B) was isolated as colourless crystals, and had a molecular formula of $C_{30}H_{40}O_{12}$, obtained from HREIMS (*m/z* $[M]^+$ 592.2631; calcd. for 592.2520) and NMR analyses. The structure of eup-4 was formulated as **9** on the basis of the ^1H and ^{13}C NMR, ^1H-^1H COSY, HMBC and 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**).





9

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**.



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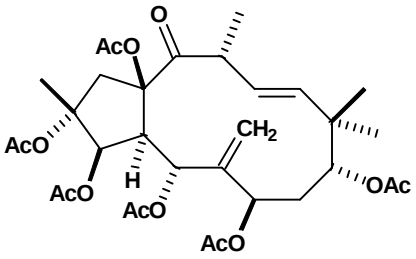
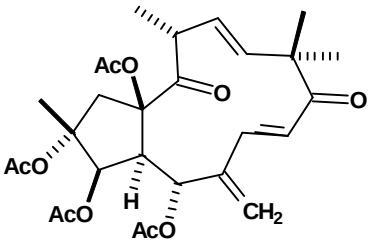


11

Eup-11 (esulatin D) was obtained as colourless crystals from methanol. Its molecular formula ($C_{32}H_{44}O_{13}$) was derived from HREIMS and NMR analyses. ^{1}H and ^{13}C NMR, ^{1}H-^{1}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**.

Eup-10 (esulatin E) was obtained as an amorphous white solid. Its molecular formula was assigned via HRMS and NMR analyses as $C_{28}H_{36}H_{10}$ (EIMS *m/z* [M]$^+$ 532). The ^{1}H and ^{13}C NMR chemical shifts of eup-10 were assigned by means of ^{1}H-^{1}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*α*,3*β*,5*α*,15*β*-tetraacetoxyjatropha-6(17),7*E*,11*E*-triene-9,14-dione.



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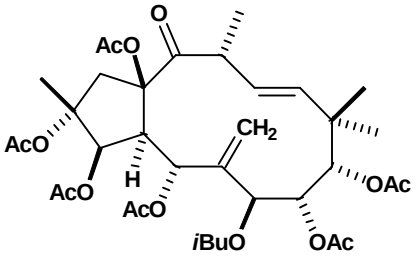


13

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}H and ^{13}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 d_H 1.10 dd (1H), 1.50 dd (1H), 1.06 s (3H) and 1.05 s (3H), and at d_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.[36] 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_2–CH(CH_3)–CHR–CH–CHR– and –CH_2–CH_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.

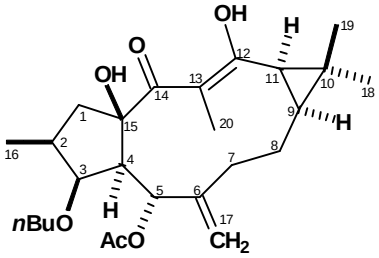
The HMBC spectrum of eul-1 showed correlative signals confirming the connection of the assignments of these structural parts. Further, the $^2J_{CH}$ and $^3J_{CH}$ correlations of H-4, H-5 and H-17a,b confirmed the presence of an exomethylene (C-17) group at C-6. The cross-peak between C-14/H-4 indicated 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 *b*-oriented OBu-3 and CH_3-16 groups followed from the NOE interactions between H-1*b*, H-16 and OH-15 and between H-1*a*, 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 *a* stereochemistry of H-9 and H-11, which is usual in lathyrane diterpenoids.[36] The stereochemistry of H-5 was concluded to be *b* 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 jatrophatrione by TORRANCE *et al.*[125]



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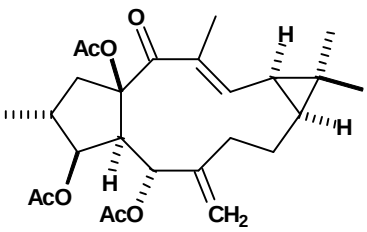


15

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 *α*. The NOESY correlations observed between H-4/H-3, H-4/H-1*α*, H-4/5-OH, and H-4/H-17 indicated the *α* stereochemistry of H-3, H-1α, 5-OH and H-17. The NOE interaction between H-1*α*/H-16 suggested the *α* position of H-16. A diagnostic NOE interaction was detected between H-5/H-2',6' (benzoyl), proving the *β* position of the benzoyl group. The nuclear *Overhauser* effects observed between H-5/H-12, H-2',6'/11-OCH_3 and H-12/H-19 demonstrated the *β* position of H-12, 11-OCH_3 and H-19, while the NOEs of H-11/H-9 and H-18/H-20 were indicative of the *α* position of H-9, H-18 and H-20.

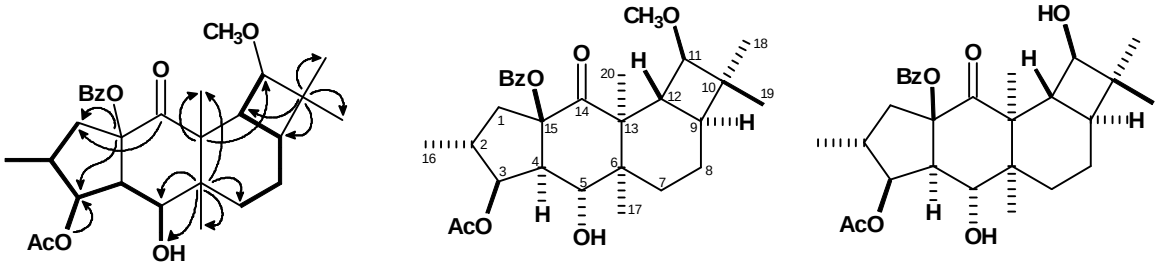


Figure 5. Selected ^{1}H-^{1}H COSY (**—**) and HMBC (C→H) correlations for ev-1 (**16**)

16 **17**

Ev-7 (**17**)

HRFABMS suggested that the molecular formula of ev-7 is $C_{29}H_{39}O_7$, with a molecular ion peak at ***m/z*** 499.2704 $[M+H]^+$. After the ^{1}H and ^{13}C chemical shift assignments (compound **2** in Table 2 in Appendix VII) of ev-7 had been achieved via the ^{1}H-^{1}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: $\delta_{C\text{-}11}$ 73.6; ev-1: $\delta_{C\text{-}11}$ 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*β*/H-2 provided evidence of the *β* orientation, while the NOESY cross-peaks between H-1*α*/H-16, H-1*α*/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 ($C_{30}H_{40}O_7$), the substituents (1 benzoyl, 1 acetyl, 1 methoxy and 1 hydroxy) and structural fragments [–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)], 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).

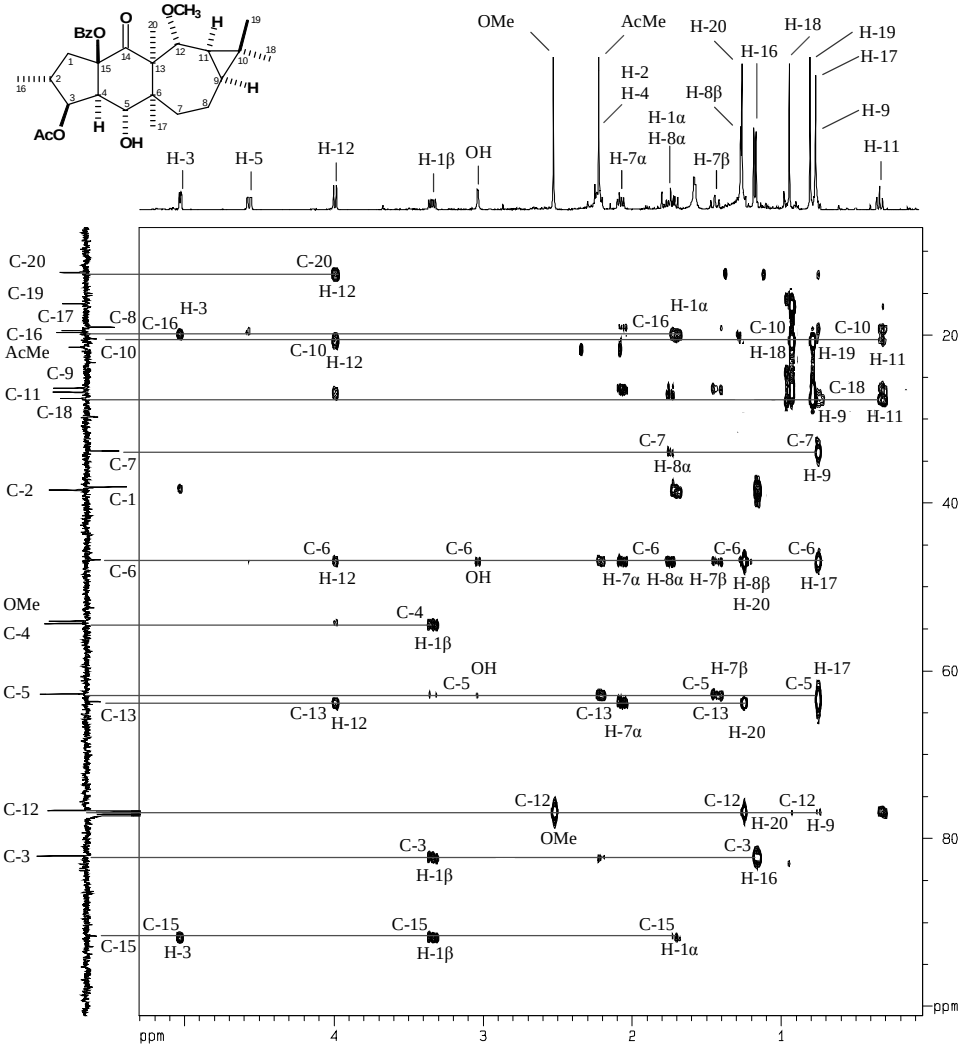
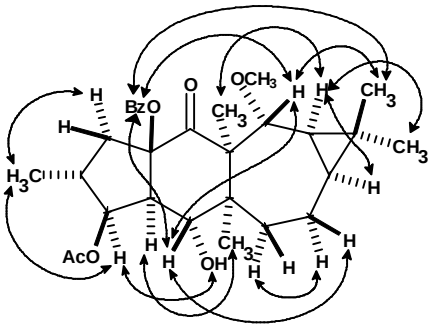
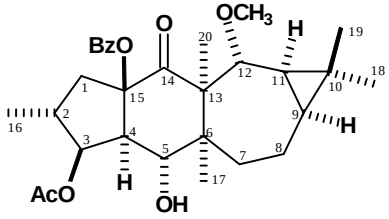


Figure 6. HMBC spectrum of ev-5 (**18**), with assignment of the main long-range correlations

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 δ_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 α configuration of H-4. Further, the NOESY correlation of the overlapping signal at δ_H 2.20 with that of H-17 can most probably be assigned to the H-4/H-17 correlation, suggesting the α 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.





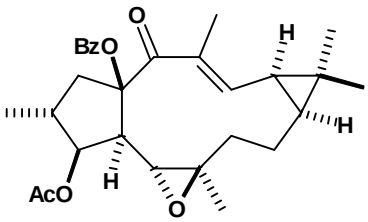
18

Figure 7. Diagnostic NOESY correlations of ev-5 (**18**)

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 benzoyl and 1 acetyl group in the molecule. The diterpene core consists of 5 methyls, 3 methylenes, 7 methines (1 vinylic, δ_C 144.5) and 5 quaternary carbons, with 1 ketone (δ_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: –CH_2–$CH(CH_3)$–, –CH(OR)–CH–CH– and –CH_2–CH_2–CH–CH–CH= (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.[92] The HMBC correlation between acetyl *C*O 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 ^{13}C 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**.



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. H_2SO_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 benzoyl), 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-casbane 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 L_1) 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 jatrophane diterpenes differing only in the esterification.
- The diterpenes isolated from *E. esula* in our experiment are not identical with those obtained by other workgroups.[12,88,134] The samples of different origins (China, North America and Hungary) contain different jatrophane 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 jatrophane 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.[80] However, in the examinations on an Italian sample, different compounds were isolated (only one ep-11 is common).[149] 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 jatrophane 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 jatrophane derivatives,[44,79] the cytotoxic activities of some jatrophane 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 2. Antiproliferative activities of diterpenes on tumour cells

Compound	**1**	**2**	**3**	**4**	**6**	**8**	**9**	**11**	**13**
Inhibition (%)[*]	13.4	20.1	4.3	16.3	4.3	17.5	6.5	9.6	11.8
IC_{50}[†] **(μM)**	–	–	–	–	–	14.8	9.5	–	8.5

[*]on HeLa cells (c = 10 μM), [†]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 3. Cytotoxic and anti-herpes virus activities of some diterpenes

Compound	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**acyclovir**
CC_{50}[*] **(μM)**	31.2	4.3	36.5	37.2	35.6	21.0	30.1	>1000
IC_{50}[†] **(μM)**	3.9	–	3.8	5.5	6.0	6.4	11.2	0.89
SI CC_{50}/IC_{50}[‡]	8.0	–	9.6	6.8	5.9	3.3	2.7	>1125

[*]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).[16]

Table 4. Reversal of the MDR of mouse lymphoma cells

Compound	*c* (*μ*g/ml)	R	Compound	*c* (*μ*g/ml)	R	Compound	*c* (*μ*g/ml)	R
ep-3 (**1**)	4	34.74	ep-7 (**5**)	4	12.75	eup-11 (**11**)	4	2.47
	40	74.27		40	71.98		8	4.03
ep-1 (**2**)	4	34.25	ep-2 (**7**)	4	4.71	eup-12 (**13**)	4	5.52
	40	78.88		40	38.37		40	14.75
ep-4 (**3**)	4	16.77	eup-1 (**8**)	4	2.03	ev-3 (**15**)	1	8.90
	40	29.48		40	1.93		10	44.16
ep-5 (**4**)	4	6.30	eup-4 (**9**)	4	2.58	ev-2 (**19**)	1	3.46
	8	2.26		40	7.83		10	50.80

R = fluorescence activity ratio [the fluorescence activity ratio of verapamil (*c* = 10 *μ*g/ml) was 6.65]

- 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.[16] Our results open up new opportunities in the design and development of drugs to overcome the MDR of human cancers.

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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) Hohmann J, **Vasas A**, Günther G, Máthé I, Evanics F, Dombi G, Jerkovich G: Macrocyclic diterpene polyesters of the jatrophane type from *Euphorbia esula*, *J. Nat. Prod*. 1997; **60**: 331-335

b) Hohmann J, **Vasas A**, Günther G, Máthé I, Evanics F, Dombi Gy, Jerkovich Gy: Jatrofánvázas makrociklusos diterpén-poliészterek az *Euphorbia esula*-ból, *Acta Pharm. Hung*. 1998; **68**: 175-182

II Günther G, Hohmann J, **Vasas A**, Máthé I, Dombi G, Jerkovich G: Jatrophane diterpenoids from *Euphorbia esula*, *Phytochemistry* 1998; **47**: 1309-1313

III Günther G, Martinek T, Dombi G, Hohmann J, **Vasas A**: Structural characterization and dynamic NMR studies of a new peracylated macrocyclic diterpene, *Magn. Reson. Chem*. 1999; **37**: 365-370

IV Hohmann J, Günther G, **Vasas A**, Kálmán A, Argay G: Isolation and structure revision of pepluane diterpenoids from *Euphorbia peplus*, *J. Nat. Prod*. 1999; **62**: 107-109

V Hohmann J, **Vasas A**, Günther G, Dombi G, Blazsó G, Falkay G, Máthé I, Jerkovich G: Jatrophane diterpenoids from *Euphorbia peplus*, *Phytochemistry* 1999; **51**: 673-677

VI Hohmann J, Evanics F, **Vasas A**, Dombi G, Jerkovich G, Máthé I: A novel lathyrane diterpenoid from the roots of *Euphorbia lathyris*, *J. Nat. Prod*. 1999; **62**: 176-178

VII **Vasas A**, Hohmann J, Forgo P, Szabó P: New tri- and tetracyclic diterpenes from *Euphorbia villosa*, *Tetrahedron* 2004; **60**: 5025-5030

I a

Macrocyclic Diterpene Polyesters of the Jatrophane Type from *Euphorbia esula*

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Three new jatrophane 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 pentaesters 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.[1] Extracts of the plant have been widely used in folk medicine to treat various cancers, swellings, and warts.[2] 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-dodecanoate, and ingenol 3-$\Delta^{2,4,6,8,10}$-pentene tetradecanoate, were found to be responsible for these activities.[3–5]

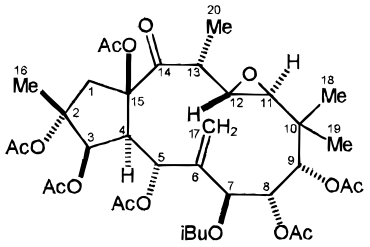
Besides ingenol esters, macrocyclic diterpenes have also been isolated from *E. esula*, namely, the jatrophane esters, esulons A, B, and C, with moderately toxic and mildly inflammatory effects from leafy spurge roots collected in North Dakota, and lathyrane and jatrophane triesters from seeds collected in Canada.[6–8] All previous studies suggested a high variation of the diterpene 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 jatrophane 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 $C_{36}H_{50}O_{16}$. The ^{1}H- and ^{13}C-NMR spectra of **1** revealed the presence of six acetate groups [δ_H 2.16 s, 2.12 s, 2.11 s, 2.11 s, 2.04 s, 2.03 s; δ_C 170.0, 169.7, 169.5, 169.1, 168.6, 168.3 (*C*O) and 22.2, 21.3, 21.2, 21.2, 20.8, 20.5 (*C*H$_3$)] and one isobutanoate group [δ_H 2.60 sept (C*H*), 1.21 d, 1.18 d (C*H*$_3$); δ_C 175.0 (*C*O), 33.8 (*C*H), 19.1, 18.4 (*C*H$_3$)] (Table 1). The ^{13}C-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 (δ_C 210.3). The ^{1}H-NMR spectrum contained 17 signals due to the parent skeleton, which were assigned with the aid of HMQC and ^{1}H–^{1}H COSY experiments. The ^{1}H–^{1}H COSY spectrum defined two structural fragments with correlated protons: –CH$_2$–CR$_2$–CHR–CHR–CHR–C(=CH$_2$)–CHR–CHR–CHR– (A) and –CH(CH$_3$)–CHR–CHR– (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 (C-6, C-10, C-2, and C-15) with protons of the two fragments established fragment A



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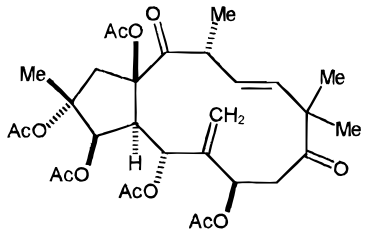

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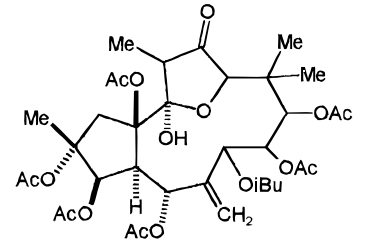

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Table 1. NMR Spectral Data of Esulatin A (**1**) [$CDCl_3$, TMS, δ (ppm) (J = Hz)]

atom	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC	NOESY
1a	3.73 d (16.2)	46.2	H-1b, H-3	C-2, C-3, C-4, C-14	H-16
1b	2.04 d (16.2)		H-1a, H-3	C-15	H-16
2		86.7			
3	5.58 br d (3.3)	78.0	H-1a,b, H-4	C-1, C-2, C-15, Ac δ 168.6	H-7, H-16, H-17a, H-4
4	2.98 dd (3.3, 1.7)	49.8	H-3, H-5	C-3, C-14	H-3, H-7
5	6.06 br s	67.6	H-4, H-17a	C-3, C-4, C-6, C-15, C-17, Ac δ 168.3	H-7, H-8, H-12, H-13, H-17a
6		141.7			
7	5.38 br s	68.5	H-8, H-17b	C-1′, C-6, C-9, C-17, i-Bu-CO	H-3, H-4, H-5, H-8, H-9, H-11
8	5.51 d (4.1)	69.0	H-7, H-9	C-6, C-9, C-10, Ac δ 169.5	H-4, H-5, H-11, H-12, H-19
9	4.95 d (4.1)	77.3	H-8	C-8, C-10, C-11, C-18, C-19, Ac δ 169.1	H-7, H-11
10		39.6			
11	3.00 d (2.1)	58.4	H-12	C-10, C-18, C-19	H-7, H-8, H-9, H-18, H-19, H-20
12	3.32 dd (4.7, 2.1)	57.1	H-11, H-13	C-13, C-14	H-5, H-8
13	3.67 dq (4.7, 6.9)	37.0	H-12, H-20	C-14, C-20	H-5
14		210.3			
15		92.6			
16	1.52 s	18.1		C-1, C-2, C-3	H-1a,b, H-3
17a	5.09 s	111.9	H-5	C-7	H-3, H-5
17b	5.05 s		H-7		
18	0.99 s[d]	23.5		C-9, C-10, C-11, C-19	H-11
19	0.71 s[d]	17.5		C-9, C-10, C-11, C-18	H-8, H-11
20	1.18 d (6.9)	15.3	H-13	C-12, C-13, C-14	H-11
i-Bu					
1′		175.0			
2′	2.60 sept (7.0)	33.8	H-3′,4′	C-1′, C-3′, C-4′	
3′	1.21 d (7.0)	19.1	H-2′	C-1′, C-2′, C-4′	
4′	1.18 d (7.0)	18.4	H-2′	C-1′, C-2′, C-3′	
Acetyls					
2-CO		169.7[b]			
2-COMe	2.11 s[a]	22.2[c]		2-CO	
3-CO		168.6			
3-COMe	2.11 s[a]	21.2[c]		3-CO	
5-CO		168.3			
5-COMe	2.16 s	21.3		5-CO	
8-CO		169.5			
8-COMe	2.03 s	20.5		8-CO	
9-CO		169.1			
9-COMe	2.04 s	20.8		9-CO	
15-CO		170.0[b]			
15-COMe	2.12 s[a]	21.2		15-CO	

[a–d] δ values are interchangeable.

and B to be C-1–C-9 (with an exomethylene C-17 on C-6) and C-13–C-11 of a jatrophane diterpene, respectively. The $^2J_{CH}$ and $^3J_{CH}$ correlations between H-13, H-12, H-1β, and the carbon signal at δ_C 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 δ_C 175.0 (isobutanoyl *C*O) with the proton signals at δ_H 5.38 (H-7) and δ_H 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 δ_C 169.5, 169.1, 168.6, and 168.3 with the proton signals at δ_H 5.51 (H-8), 4.95 (H-9), 5.58 (H-3), and 6.06 (H-5) and the acetyl methyl signals at δ_H 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 ^{1}H-NMR data of **1** were compared with those of analogous compounds having 2,3,15-triacyl substitution on the five-membered ring, similar δ values for H-1 and H-3 were found.[9] Thus, the two acetyl groups (δ_H 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 (δ_H 3.00 d and 3.32 dd), C-11, and C-12 (δ_C 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 (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)jatroph-6(17)-en-14-one (**1**); data are published elsewhere.[10] The structure of **1** is very similar to that of kansuinin B, isolated from *Euphorbia kansui*, which has been shown to possess analgesic and anti-writhing activities.[11]

Esulatin B had a molecular formula of $C_{30}H_{40}O_{12}$, 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 ^{1}H- and ^{13}C-NMR spectra of esulatin B contained signals corresponding to five acetyl groups [δ_H 2.16 s, 2.14 s, 2.13 s, 2.11 s, and 2.05 s, δ_C 170.4, 170.1, 169.7, 169.6, 169.0 (*C*O), and 22.1, 21.4, 21.2, 20.9, and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C-NMR and DEPT spectra exhibited resonances for four methyls, three methylenes, seven methines, and six quaternary carbons, including two keto groups (δ_C 211.1 and 209.4) (Table 2). The ^{1}H-NMR and ^{1}H–^{1}H COSY spectra revealed the presence of three tertiary methyls (δ_H 1.48 s, 1.17 s, 1.16 s) and the structural elements –$CH_2CR_2CHRCHRCHRC$(=CH_2)$CHRCH_2$– (A) and *trans*–$CH(CH_3)CH$=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**) [$CDCl_3$, TMS, δ (ppm) (J = Hz)]

atom	^1H	^{13}C	^1H–^1H COSY	HMBC	NOESY
1a	3.82 dd (16.0, 1.0)	46.6	H-1b, H-3	C-2, C-3, C-4, C-14, C-15	H-1b
1b	1.91 d (16.0)		H-1a	C-2, C-14, C-15, C-16	H-1a
2		86.2			
3	5.51 d (3.3)	78.3	H-1a, H-4	C-2, C-15, Ac δ 169.0	H-16, H-4, H-17
4	2.91 dd (3.5, 1.0)	49.7	H-3, H-5	C-3, C-6, C-14, C-15	H-3, H-5, H-7
5	5.80 s	68.2	H-4, H-17	C-3, C-4, C-6, C-7, C-15, C-17, Ac δ 169.7[b]	H-4, H-7, H-8b, H-11, H-13
6		147.0			
7	4.94 d (9.6)	69.5	H-8b, H-8a, H-17	C-5, C-6, C-8, C-9, C-17, Ac δ 169.6[b]	H-4, H-5, H-19
8b	3.21 dd (14.0, 1.0)	45.4	H-7, H-8a	C-6, C-7, C-9	H-8a, H-5, H-11
8a	2.28 dd (14.0, 9.6)		H-7, H-8b	C-7, C-9	H-8b
9		209.4			
10		49.3			
11	6.07 d (15.9)	137.2	H-12	C-9, C-10, C-13, C-18, C-19	H-5, H-8b, H-13, H-18
12	5.52 dd (15.9, 9.6)	133.2	H-11, H-13	C-10, C-14	H-19, H-20
13	3.56 dq (9.6, 6.6)	44.7	H-12, H-20	C-11, C-12, C-14	H-11, H-5, H-20
14		211.1			
15		92.1			
16	1.48 s	17.8		C-1, C-2, C-3	H-3, H-1b
17	5.10 d (1.0)	110.1	H-5, H-7	C-5, C-6, C-7	H-3
	5.11 d (1.0)			C-5, C-6, C-7	H-3
18	1.17 s[d]	26.7		C-9, C-10, C-11, C-19	
19	1.16 s[d]	23.0		C-9, C-10, C-11, C-18	
20	1.12 d (6.6)	20.2	H-13	C-12, C-13, C-14	H-12, H-13
Acetyls					
2-CO		170.1			
2-COMe	2.13 s	20.9			
3-CO		169.0			
3-COMe	2.16 s	21.4		3-CO	
5-CO		169.7[b]			
5-COMe	2.05 s[a]	20.8[c]		5-CO	
7-CO		169.6[b]			
7-COMe	2.14 s[a]	21.2[c]		7-CO	
15-CO		170.4			
15-COMe	2.11 s	22.1			

[a–d] δ values are interchangeable.

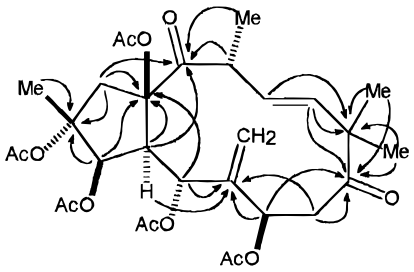

Figure 1. HMBC correlations of quaternary carbons of esulatin B (**2**) (H→C).

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-pentaacetoxyjatropha-6(17),11-diene-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 **2**.[12] NOESY correlations were in accordance with the stereochemistry elucidated from X-ray investigations. The full ^1H- and ^{13}C-NMR chemical shift assignments of **2** were carried out through ^1H–^1H COSY, HMQC, 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 jatroph-11-ene-9,11-dione derivatives. Peaks at m/z 123 (52%) $C_8H_{11}O$ (found 123.0794, calcd 123.0810) and m/z96 (70%) C_7H_{12} (found 96.0934, calcd 96.0939) corresponding to the ions $(CH_3)_2C{=}CH{-}CH{=}CCH_3C{=}O^+$ and $(CH_3)_2C{=}CH{-}CH{=}CHCH_3^+$, 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 $C_{36}H_{50}O_{17}$. The ^1H-NMR spectrum indicated the presence of seven ester groups: six acetate groups (δ_H 2.16 s, 2.11 s, 2.06 s, 2.02 s, 2.00 s, and 1.99 s) and one isobutanoate group [(δ_H 2.61 sept (C*H*), 1.23 d, 1.16 d (CH_3)]. Additionally, the ^1H-NMR spectrum exhibited signals attributed to four methyls (δ_H 1.56 s, 1.24 d, 1.24 s, 1.12 s), one methylene (δ_H 3.10 d, 2.80 d), one methyl-bearing methine (δ_H 2.17 dq) and, in the range δ_H 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 ^1H–^1H COSY spectrum. Relationships between the signals at δ_H 6.02 (H-3), 3.28 (H-4), and 6.20 (H-5) and at δ_H 6.49 (H-7), 5.65 (H-8), and 4.94 (H-9) were clearly defined. Further, in the ^1H–^1H COSY spectrum ^4J couplings were detected between the signals at δ_H 3.10 and 2.80 (H-1) and 6.02 (H-3); at δ_H 6.20 (H-5), 6.49 (H-7), 5.04, and 4.95 (H-17a,b); at δ_H 4.94 (H-9) and 4.13 (H-11); at δ_H 4.13 (H-11) and 2.17 (H-13); and at δ_H 2.17 (H-13) and 3.99 (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 six quaternary carbons attributed to the parent skeleton was detected. Cross-peaks between H-1, H-3, H-16, and the signals at δ_C 88.5 (C-2) and between H-1, H-4, H-5, and δ_C 89.9

Table 3. NMR Spectral Data of Esulatin C (**3**) [$CDCl_3$, TMS, δ (ppm) (J = Hz)]

atom	^1H	^{13}C	$^1H-^1H$ COSY	HMBC	NOESY
1a	3.10 d (16.5)	43.6	H-1b, H-3	C-2, C-3, C-4, C-14	H-1b, H-20
1b	2.80 d (16.5)		H-1a, H-3	C-14, C-15, C-16	H-1a, H-16
2		88.5			
3	6.02 d (3.5)	74.2	H-1b, H-4	C-2	H-4, H-16
4	3.28 d (3.5)	49.7	H-3, H-5	C-3, C-5, C-6, C-14, C-15	H-3, H-5, H-7, 14-OH, H-16
5	6.20 s	69.4	H-4, H-7, H-17a,b	C-3, C-4, C-6, C-7, C-15	H-4, H-8
6		147.0			
7	6.49 s	67.8	H-5, H-8, H-17a,b	C-6, C-8, C-9, C-17, C-1′	H-4, H-8, H-11
8	5.65 s	70.9	H-7, H-9	C-6, C-9, Ac δ 170.5	H-5, H-7, H-9, H-18
9	4.94 s	82.6	H-8, H-11	C-8, C-10, C-11, C-19, Ac δ 169.6	H-8, H-18, H-19
10		41.4			
11	4.13 s	77.1	H-9, H-13	C-9, C-10, C-12, C-18	H-7, H-19
12		213.6			
13	2.17 dq (7.0, 1.9)	21.6	H-11, 14-OH, H-20	C-12, C-14, C-20	
14		105.7			
14-OH	3.99 d (1.9)		H-13		H-4
15		89.9			
16	1.56 s	18.3		C-1, C-2, C-3	H-1b, H-3
17a	5.04 s	107.9	H-5, H-7, H-17b	C-5, C-6, C-7	
17b	4.95 s		H-5, H-7, H-17a	C-5, C-6, C-7	
18	1.24 s[a]	18.9		C-10, C-11, C-19	H-8, H-9
19	1.12 s[a]	22.4		C-9, C-10	H-11
20	1.24 d (7.0)	9.6	H-13	C-12, C-14	H-1a, H-5
i-Bu					
1′		176.6			
2′	2.61 sept (7.0)	34.2		C-1′, C-4′	
3′	1.16 d (7.0)	18.8		C-1′, C-4′	
4′	1.23 d (7.0)	19.8		C-1′, C-3′	
Acetyls					
8-CO		170.5			
8-COMe	2.06 s	21.2		8-CO	
9-CO		169.6			
9-COMe	1.99 s	22.2		9-CO	
2,3,5,15-CO		169.6			
		168.9			
		168.6			
		167.5			
2,3,5,15-COMe	2.02 s	21.1		Ac δ 169.6	
	2.16 s	22.8		Ac δ 168.9	
	2.11 s	21.8		Ac δ 168.6	
	2.00 s	21.2		Ac δ 167.5	

[a] 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 δ_C 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 δ_C 105.7 was assigned to C-14. A hydroxyl group was placed on C-14 because its doublet signal (δ_H 3.99) coupled to H-13 (J = 1.9 Hz). Judging from the quaternary carbon signal at δ_C 105.7 (C-14) and the methine signal at δ_H 4.13 (H-11), which was observed at higher field than for esterified methines,[13] an ether functionality (deduced from the molecular formula and substituents discussed above) must be sited 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 *trans* ring junction[6,11,14,15] 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 kansuinin A, H-5 be β.[13] 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**. Esulatin C (**3**) displayed a close relationship to a diterpene constituent of *E. kansui*, kansuinin A.[13]

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.[6–8] Jatrophane diterpenoids from North American accession of *E. esula* were reported earlier,[6] but this type of compound was isolated for the first time from a European accession. It may be of chemotaxonomic significance that compounds found in these two collections of leafy spurge are different.

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 PC 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 (^{1}H) and 100 MHz (^{13}C), using $CDCl_3$ as solvent and TMS as internal standard. Optical rotations were determined in $CHCl_3$ at ambient temperature using a Perkin-Elmer 341 polarimeter. For column chromatography polyamide (ICN) and Si gel (Kieselgel GF_{254} 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 F_{254} plates using $CHCl_3$–Me_2CO (19:1), C_6H_6–$CHCl_3$–Et_2O (1:1:3) and cyclohexane–EtOAc–EtOH (20:10:1) as developing systems with visualization using 1% vanillin–H_2SO_4 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 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 temperature. The crude extract was concentrated *in vacuo* and partitioned between CH_2Cl_2 (7 × 1.5 L) and H_2O. On evaporation, the organic-phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixtures of H_2O–MeOH (4:1, 3:2, 2:3, 1:4) as eluents. Fractions 5–21 obtained with H_2O–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–Me_2CO (19:1, 9:1. 4:1, 7:3, 1:1, 3:7). Fractions from cyclohexane–Me_2CO (4:1) were transferred repeatedly to a Si gel VLC and successively eluted with $CHCl_3$–MeOH mixtures of increasing polarity. From fractions obtained with $CHCl_3$–MeOH (99.7:0.3), 40 mg of esulatin B (**2**) was obtained as crystals. Fractions obtained with $CHCl_3$–MeOH (99:1) were further purified by preparative TLC on Si gel using C_6H_6–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; $[\alpha]^{25}$D −82° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 202 (4.73), 219 (sh, 4.08) nm; IR (KBr) ν_{max} 2983, 2939, 1749, 1468, 1426, 1372, 1321, 1210, 1149, 1129, 1085, 1026, 994, 934, 911, 873, 833, 809, 746, 659, 634, 610 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m/z* $[M + K]^+$ 777 (36), $[M + Na]^+$ 761 (40), $[M + NH_4]^+$ 756 (100); *anal.* C 58.08%, H 7.43%, calcd for $C_{36}H_{50}O_{16}$ C 58.51%, H 6.83%.

Esulatin B (2): colorless crystals from MeOH; mp 248–249 °C; $[\alpha]^{25}$D −101° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.88), 284 (3.89) nm; IR (KBr) ν_{max} 2984, 2939, 1741, 1659, 1431, 1374, 1251, 1229, 1133, 1099, 1042, 995, 926, 877, 805, 740, 629, 609 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* $[M]^+$ 592 (14), $[M - AcOH]^+$ 532 (3), $[M - AcOH - CH_2CO]^+$ 490 (6), $[M - 2 \times AcOH]^+$ 472 (6), $[M - 2 \times AcOH - CH_2CO]^+$ 430 (8), $[M - 3 \times AcOH]^+$ 412 (8), $[M - 3 \times AcOH - CH_2CO]^+$ 370 (26), $[M - 4 \times AcOH]^+$ 352 (8), $[M - 3 \times AcOH - 2 \times CH_2CO]^+$ 328 (16), $[M - 4 \times AcOH - CH_2CO]^+$ 310 (32), $[C_8H_{11}O]^+$ 123 (52), $[C_7H_{12}]^+$ 96 (70); HREIMS *m/z* 592.2631 (calcd for 592.2520 $C_{30}H_{40}O_{12}$) $[M]^+$.

Esulatin C (3): white amorphous solid; $[\alpha]^{25}$D +11° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) nm 209 (4.84), 273 (4.02); IR (KBr) ν_{max} 3743, 2930, 1743, 1679, 1540, 1515, 1456, 1427, 1372, 1241, 1155, 1063, 1041 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 3; EIMS *m/z* $[M - AcOH]^+$ 694 (22), $[M - i\text{-}BuOH]^+$ 666 (20), $[M - i\text{-}BuOH - OAc]^+$ 607 (53), $[M - i\text{-}BuOH - AcOH - CH_2CO]^+$ 564 (17), $[M - i\text{-}BuOH - 2 \times AcOH]^+$ 546 (38), $[M - i\text{-}BuOH - 2 \times AcOH - CH_2CO]^+$ 504 (43), $[M - 3 \times AcOH - CH_2CO - C_4H_4O]^+$ 476 (71), $[M - i\text{-}BuOH - 3 \times AcOH - CH_2CO]^+$ 444 (100), $[M - i\text{-}BuOH - 4 \times AcOH - CH_2CO]^+$ 384 (31), $[M - i\text{-}BuOH - 5 \times AcOH - CH_2CO]^+$ 324 (20); HREIMS *m/z* 694.2839 (calcd for 694.2837 $C_{34}H_{46}O_{15}$) $[M - AcOH]^+$, 666.2778 (calcd for 666.2524 $C_{32}H_{42}O_{15}$) $[M - i\text{-}BuOH]^+$.

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Jatrofánvázas makrociklusos diterpén-poliészterek az *Euphorbia esula*-ból*

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Summary

Hohmann, J., Vasas, A., Günther, G., Máthé, I., Evanics,. F., Dombi, Gy., Jerkovich, Gy.: ***Macrocyclic Diterpene Polyesters of the Jatrophane Type from Euphorbia esula.***

Three new jatrophane diterpenes, esulatin 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 heptaesters 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 jatrofánvázas diterpént, az esulatin A-C (1-3) komponenseket izoláltuk és azonosítottuk. Spektroszkópiai vizsgálataink eredményeként megállapítottuk, hogy a vegyületek korábban ismeretlen, több funkciós csoportot tartalmazó diterpénalkoholok penta- illetve heptaészterei. Az esulatin A (1) és C (3) az Euphorbiaceae családból ezidáig izolált legnagyobb mértékben észterezett diterpének.

Bevezetés

Az *Euphorbia esula* L., magyar néven sárkutyatej (Euphorbiaceae) az egész világon elterjedt növényfaj, mely bőrirritáló, mérgező tejnedvet tartalmaz [1.] A növény kivonatait régóta széles körben használják a népgyógyászatban különféle daganatos megbetegedések, szemölcsök kezelésére [2]. A korábbi növénykémiai és farmakológiai vizsgálatok igazolták a növényi kivonatok gyulladáskeltő, tumorképződést elősegítő és antitumor aktivitását, valamint bizonyították, hogy ezen hatásért három ingenánvázas diterpén, az ingenol-3,20-dibenzoát, az ingenol-3-dodekanoát és az ingenol-3-$\Delta^{2,4,6,8,10}$-pentén-tetradekanoát a felelős [3–5].

Ingenol-észterek mellett makrociklusos diterpéneket is izoláltak az *E. esula*-ból: az Észak-Dakotában gyűjtött növény gyökeréből mérsékelten toxikus és enyhén gyulladáskeltő hatású jatrofánésztereket, az esulon A, B és C komponenseket különítették el, a Kanadából származó magmintákból pedig latirán- és jatrofán-triésztereket nyertek [6–8]. A korábbi vizsgálatok egyértelműen arra utaltak, hogy a diterpének nagyfokú változatossága jellemző az *E. esula*-ra a származási helytől függően, azaz figyelemre méltó különbségek jelentkeznek észak-amerikai és európai eredetű növényminták között [7].

Az Euphorbiaceae család biológiailag aktív vegyületeinek megismerésére irányuló kutatási programunk keretében az *E. esula* magyarországi populációjának diterpénjeit vizsgáltuk. Ebben a dolgozatban három új jatrofánészter, az esulatin A, B és C (1–3) komponensek *(1. ábra)* izolálásáról és szerkezet-meghatározásáról számolunk be.

*A közleményt a szerzők Petri Gizella professzor asszony 70. születésnapjára ajánlják

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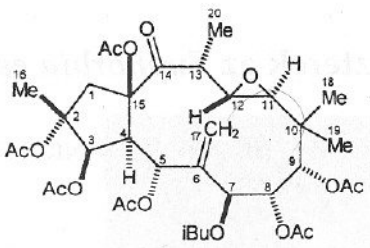

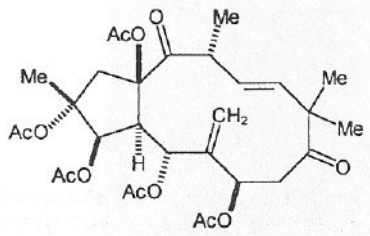

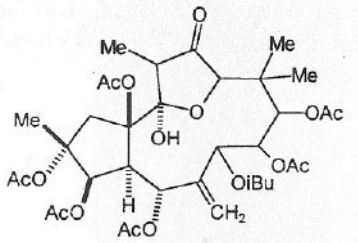

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1. ábra

Kísérleti rész

1. Általános kísérleti eljárások

Az olvadáspont-meghatározás Boetius-készülékkel korrekció nélkül történt. Az IR spektrumok KBr pasztillában Perkin-Elmer Paragon 1000 PC FTIR spektrométerrel készültek. Az UV spektrumok felvétele metanolban Shimadzu UV 2101 spektrométerrel történt. A tömegspektroszkópiás méréseket Finnigan MAT 8430 készüléken, 70 eV ionizáló energiával végeztük. Az NMR spektrumokat Bruker Avance DRX 400 spektrométerrel vettük fel 400 MHz-en (^{1}H) és 100 MHz-en (^{13}C), $CDCl_3$-at, mint oldószert és TMS-t, mint belső standardot használva. Az optikai rotációs mérések kloroformban, termosztált hőmérsékleten Perkin-Elmer 341 polariméterrel történtek. Oszlopkromatográfiához poliamidot (ICN) és szilikagélt (Kieselgel GF_{254} 15 μm, Merck) használtunk. A HPLC elválasztás Waters Millipore készülékkel, RI detektálással, normál fázisú oszlopon (LiChrospher Si 100, 5 μm, Merck) folyt. A rétegkromatográfiás vizsgálatokat szilikagél (Kieselgel 60 F_{254}) lemezeken, kloroform aceton (19:1), benzol-kloroform-éter (1:1:3) és ciklohexán-etil-acetát-etanol (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 Szegeden a Tisza partjáról gyűjtöttük, a faj azonosítását *Dr. Penszka Károly* (Gödöllői Agrártudományi Egyetem, Növénytani és Növényfiziológiai Tanszék, Gödöllő) végezte. Egy herbáriumi mintapéldányt a Természettudományi Múzeum Herbáriumában, Budapesten helyeztünk el.

3. Extrakció és izolálás

A frissen gyűjtött, teljes növényt tartalmazó *E. esula* mintát (11 kg) 75 dm^3 metanollal szobahőmérsékleten extraháltuk. A nyers kivonatot vákuumban betöményítettük és 7 x 1,5 dm^3 diklórmetánnal ráztuk ki. A szerves fázis bepárlását követően a maradékot (130 g) poliamid oszlopra (600 g) vittük és víz-metanol (4:1, 3:2, 2:3, 1:4) elegyekkel eluáltunk. A víz-metanol (4:1) eleggyel 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 szilikagélen vákuum folyadékkromatográfiával (VLC) tisztítottuk ciklohexán-aceton grádiens rendszert (19:1, 9:1, 4:1, 7:3, 1:1, 3:7) alkalmazva. A ciklohexán-aceton (4:1) eleggyel eluált frakciókat ismételten szilikagél VLC-val frakcionáltuk növekvő polaritású kloroform-metanol elegyeket használva. A kloroform-metanol (99,7:0,3) eleggyel kapott frakciókból 40 mg kristályos anyag vált ki, jelzése esulatin B (**2**). A kloroform-metanol (99:1) eleggyel kapott frakció további tisztítása preparatív rétegkromatográfiával történt szilikagélen benzol-etilacetát (7:3) eleggyel. Ezt követően HPLC elválasztást végeztünk ciklohexán-etilacetát-etanol (30:10:1) összetételű eluenssel (áramlási sebesség 0,5 ml/perc), s ílymódon 1,2 mg tiszta anyagot kaptunk, jelzése esulatin C (**3**).

Esulatin A (1): színtelen kristály MeOH-ból; Op. 218-219 °C; $[\alpha]^{25}_D$ –82° (*c* 0,1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm 202 (4,73), 219 (sh, 4,08); IR (KBr) υ_{max} 2983, 2939, 1749, 1468, 1426, 1372, 1321, 1210, 1149, 1129, 1085, 1026, 994, 934, 911, 873, 833, 809, 746, 659, 634, 610 cm^{-1}; ^1H és ^{13}C NMR lásd *I. táblázat;* ESIMS *m/z* $[M + K]^+$ 777 (36), $[M + Na]^+$ 761 (40), $[M + NH_4]^+$ 756 (100); anal. C 58,08%, H 7,43%, számított $C_{36}H_{50}O_{16}$ C 58,51%, H 6,83%.

Esulatin B (2): színtelen kristály MeOH-ból; Op. 248-249 °C; $[\alpha]^{25}_D$ –101° (*c* 0,1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm 210 (4,88), 284 (3,89); IR (KBr) υ_{max} 2984, 2939, 1741, 1659, 1431, 1374, 1251, 1229, 1133, 1099, 1042, 995, 926, 877, 805, 740, 629, 609 cm^{-1}; ^1H és ^{13}C NMR lásd *II. táblázat;* EIMS *m/z* $[M]^+$ 592 (14), $[M - AcOH]^+$ 532 (3), $[M - AcOH - CH_2CO]^+$ 490 (6), $[M - 2xAcOH]^+$ 472 (6), $[M - 2xAcOH - CH_2CO]^+$ 430 (8), $[M - 3xAcOH]^+$ 412 (8), $[M - 3xAcOH - CH_2CO]^+$ 370 (26), $[M - 4xAcOH]^+$ 352 (8), $[M - 3xAcOH - 2xCH_2CO]^+$ 328 (16), $[M - 4xAcOH - CH_2CO]^+$ 310 (32), $[C_8H_{11}O]^+$ 123 (52), $[C_7H_{12}]^+$ 96 (70); HREIMS *m/z* 592,2631 (számított 592,2520 $C_{30}H_{40}O_{12}$) $[M]^+$.

Esulatin C (3): fehér amorf anyag; $[\alpha]^{25}_D$ +11° (*c* 0,06, $CHCl_3$); UV (MeOH) λ_{max} (log ε) nm 209 (4,84), 273 (4,02); IR (KBr) υ_{max} 3743, 2930, 1743, 1679, 1540, 1515, 1456, 1427, 1372, 1241, 1155, 1063, 1041 cm^{-1}; ^1H és ^{13}C NMR lásd *III. táblázat;* EIMS *m/z* $[M - AcOH]^+$ 694 (22), $[M - i\text{-}BuOH]^+$ 666 (20), $[M - i\text{-}BuOH - OAc]^+$ 607 (53), $[M - i\text{-}BuOH - AcOH - CH_2CO]^+$ 564 (17), $[M - i\text{-}BuOH - 2xAcOH]^+$ 546 (38), $[M - i\text{-}BuOH - 2xAcOH - CH_2CO]^+$ 504 (43), $[M - 3xAcOH - CH_2CO - C_4H_4O]^+$ 476 (71), $[M - i\text{-}BuOH - 3xAcOH - CH_2CO]^+$ 444 (100), $[M - i\text{-}BuOH - 4xAcOH - CH_2CO]^+$ 384 (31), $[M - i\text{-}BuOH - 5xAcOH - CH_2CO]^+$ 324 (20); HREIMS *m/z* 694,2839 (számított 694,2837 $C_{34}H_{46}O_{15}$) $[M - AcOH]^+$, 666, 2778 (számított 666,2524 $C_{32}H_{42}O_{15}$) $[M - i\text{-}BuOH]^+$.

Eredmények és értékelésük

A frissen gyűjtött *E. esula* metanolos kivonatából készített diklór-metános fázist poliamid és szilikagél oszlopon kromatografáltuk. Ezt követően további elválasztást végeztünk preparatív rétegkromatográfiával és nagyhatékonyságú folyadékkromatográfiával, s így három anyagot nyertünk, jelzésük esulatin A, B és C (**1–3**).

Az esulatin A (**1**) elemanalízise és ESIMS vizsgálattal kapott összegképlete $C_{36}H_{50}O_{16}$. Az **1** ^1H- és ^{13}C-NMR spektruma hat acetilcsoport [δ_H 2,16 s, 2,12 s, 2,11 s, 2,11 s, 2,04 s, 2,03 s; δ_C 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 (CH_3)] és egy izobutanoilcsoport δ_H 2,60 sept (C*H*), 1,21 d, 1,18 d (CH_3); δ_C 175,0 (CO), 33,8 (CH), 19,1, 18,4 (CH_3)] jelenlétét igazolta (*I. táblázat).* A ^{13}C-NMR és DEPT spektruma arra utalt, hogy a váz 20 szénatomból, azaz négy metil-, két metilén-, kilenc metincsoportból és öt kvaterner szénatomból – közülük egy ketocsoport (δ_C 210,3) – épül fel. Az ^1H-NMR spektrum 17 alapvázhoz tartozó jelet tartalmazott, melyek asszignálása ^1H-^1H COSY és HMQC kísérlet segítségével történt. A ^1H-^1H COSY spektrum a protonkorrelációk alapján két részszerkezetet bizonyított: -CH_2-CR_2-CHR-CHR-CHR-C(=CH_2)-CHR-CHR-CHR- (A) és -CH(CH_3)-CHR-CHR- (B). Kapcsolódásaikat a HMBC spektrumban megfigyelhető távolható C-H korrelációk alapján határoztuk meg (*I. táblázat).* A kvaterner szénatomok (C-6, C-10, C-2 és C-15) és a két részszerkezet protonjainak két vagy három kötésen keresztüli korrelációi azt bizonyítják, hogy az A molekularész megfelel egy jatrofánvázas diterpén C-1–C-9 szerkezeti részének (a C-6-on egy exometilén-csoporttal), a B pedig a C-13–C-11 részszerkezetnek. A H-13, H-12, H-1b és a δ_C 210,3-nál jelentkező szénatom közötti $^2J_{CH}$ és $^3J_{CH}$ korrelációk igazolták, hogy a ketocsoport a 14-helyzetben található. Az észtercsoportok pozíciója szintén HMBC méréssel volt meghatározható. A δ_C 175,0 karboniljel (izobutanoil CO) korrelációja a δ_H 5,38 (H-7) és δ_H 1,21, 1,18 (izobutanoil metilek) proton szignálokkal jelezte az izobutanoilcsoport jelenlétét a C-7-en. Hasonlóképpen a δ_C 169,5, 169,1, 168,6 és 168,3 ppm-nél jelentkező karbonilszén szignálok és a δ_H 5,51 (H-8), 4,95 (H-9), 5,58 (H-3), 6,06 (H-5) protonjelek valamint a δ_H 2,03, 2,04, 2,11 és 2,16 acetil metilszignálok között jelentkező távolható csatolások igazolták az acetilcsoportok jelenlétét a C-8, C-9, C-3 és C-5 helyzetben. A fennmaradó két acetilcsoport nem mutatott semmilyen távolható korrelációt, ezért feltételezhető volt, hogy ezek kvaterner szénatomokhoz kapcsolódnak. Az **1** vegyület ^1H-NMR adatait az öttagú gyűrűn 2,3,15-triacilszubsztituált jatrofán származékokéval összehasonlítva azt találtuk, hogy a H-1 és H-3 protonokra hasonló δ értékek jellemzőek [9]. Így a két acetilcsoport (δ_H 2,11 és 2,12) a C-2 és C-15 helyzetben található. A molekula összegképletét és az előzőekben tárgyalt szubsztituenseket figyelembe véve

megállapíthatjuk, hogy ez a vegyület egy epoxicsoportot is tartalmaz, mely szükségszerűen a C-11, C-12 helyzetben található. Ezt a H-11, H-12 (δ_H 3,00 d és 3,32 dd), C-11 és C-12 (δ_C 58,4, 57,1) kémiai eltolódásértékei is megerősítik.

Az esulatin A (**1**) térkémiájának és abszolút konfigurációjának tanulmányozása NOESY mérésekkel (*I. táblázat*) és röntgendiffrakciós analízissel történt. A röntgenkrisztallográfiai vizsgálatok bizonyították, hogy az esulatin A (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-hexaacetoxi-11,12-epoxi-7-(izobutanoiloxi)jatrofa-6(17)-en-14-on (**1**) szerkezettel jellemezhető [10]. Az **1** szerkezete igen hasonló az *Euphorbia kansui*-ból izolált kansuinin B-éhez, mely analgetikus és anti-writhing hatással rendelkezik [11].

I. táblázat

Az esulatin A (1) NMR adatai [$CDCl_3$, TMS, (ppm), (J = Hz)]

Atom	^1H	^{13}C	^1H-^1H COSY	HMBC	NOESY
1a	3,73 d (16,2)	46,2	H-1b, H-3	C-2, C-3, C-4, C-14	H-16
1b	2,04 d (16,2)		H-1a, H-3	C-15	H-16
2	-	86,7	-	-	-
3	5,58 brd (3,3)	78,0	H-1a,b, H-4	C-1, C-2, C-15, Ac δ 168,6	H-7, H-16, H-17a, H-4
4	2,98 dd (3,3, 1,7)	49,8	H-3, H-5	C-3, C-14	H-3, H-7
5	6,06 brs	67,6	H-4, H-17a	C-3, C-4, C-6, C-15, C-17, Ac δ 168,3	H-7, H-8, H-12, H-13, H-17a
6	-	141,7	-	-	-
7	5,38 brs	68,5	H-8, H-17b	C-1', C-6, C-9, C-17, iBu-CO	H-3, H-4, H-5, H-8, H-9, H-11
8	5,51 d (4,1)	69,0	H-7, H-9	C-6, C-9, C-10, Ac δ 169,5	H-4, H-5, H-11, H-12, H-19
9	4,95 d (4,1)	77,3	H-8	C-8, C-10, C-11, C-18, C-19, Ac δ 169,1	H-7, H-11
10	-	39,6	-	-	-
11	3,00 d (2,1)	58,4	H-12	C-10, C-18, C-19	H-7, H-8, H-9, H-18, H-19, H-20
12	3,32 dd (4,7, 2,1)	57,1	H-11, H-13	C-13, C-14	H-5, H-8
13	3,67 dq (4,7, 6,9)	37,0	H-12, H-20	C-14, C-20	H-5
14	-	210,3	-	-	-
15	-	92,6	-	-	-
16	1,52 s	18,1	-	C-1, C-2, C-3	H-1a,b, H-3
17a	5,09 s	111,9	H-5	C-7	H-3, H-5
17b	5,05 s	-	H-7	-	-
18	0,99 s[d]	23,5	-	C-9, C-10, C-11, C-19	H-11
19	0,71 s[d]	17,5	-	C-9, C-10, C-11, C-18	H-8, H-11
20	1,18 d (6,9)	15,3	H-13	C-12, C-13, C-14	H-11
iBu					
1'	-	175,0	-	-	-
2'	2,60 sept (7,0)	33,8	H-3',4'	C-1', C-3', C-4'	-
3'	1,21 d (7,0)	19,1	H-2'	C-1', C-2'n, C-4'	-
4'	1,18 d (7,0)	18,4	H-2'	C-1', C-2', C-3'	-
Acetilek					
2-CO	-	169,7[b]	-	-	-
2-COMe	2,11 s[a]	22,2[c]	-	2-CO	-
3-CO	-	168,6	-	-	-
3-COMe	2,11 s[a]	21,2[c]	-	3-CO	-
5-CO	-	168,3	-	-	-
5-COMe	2,16 s	21,3	-	5-CO	-
8-CO	-	169,5	-	-	-
8-COMe	2,03 s	20,5	-	8-CO	-
9-CO	-	169,1	-	-	-
9-COMe	2,04 s	20,8	-	9-CO	-
15-CO	-	170,0[b]	-	-	-
15-COMe	2,12 s[a]	21,2	-	15-CO	-

[a, b, c, d] δ értékek felcserélhetők

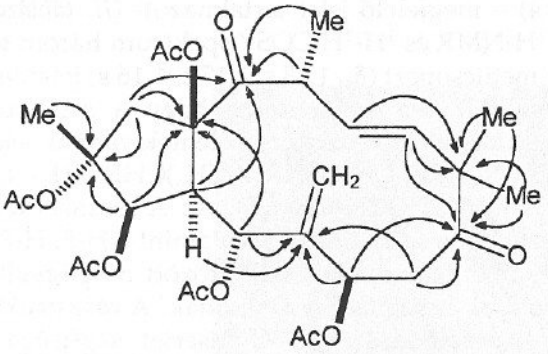
Az esulatin B összegképlete a HRMS és NMR vizsgálatok szerint $C_{30}H_{40}O_{12}$. EIMS spektrumában ecetsav és keténegységek kilépésével keletkező fragmensek sorozata volt megfigyelhető (lásd a kísérleti részt) jelezve, hogy öt acetilcsoport van jelen a molekulában. Ezzel összhangban áll az is, hogy az esulatin B ^{1}H- és ^{13}C-NMR spektrumában öt acetilcsoportnak megfelelő szignálok jelentkeztek [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 (CH_3)]. Ezen felül a ^{13}C-NMR és DEPT spektrum négy metil-, három metilén- és hét metincsoportnak valamint hat kvaterner szénatomnak – beleértve két ketocsoportot (δ_C 211,1 és 209,4) – megfelelő jelet tartalmazott (*II. táblázat*). Az ^{1}H-NMR és ^{1}H-^{1}H COSY spektrum három tercier metilcsoport (δ_H 1,48 s, 1,17 s, 1,16 s) jelenlétét bizonyította és azt mutatta, hogy a molekulában a következő szerkezeti elemek vannak jelen: -CH_2-CR_2-CHR-CHR-CHRC(-CH_2)CHR-CH_2- (A), *transz* -CH(CH_3)CH-CH- (B). Az A részszerkezet levezetéséhez a H-1 és H-3, valamint a H-5, H-7 és az exometiléncsoport (H-17) között megfigyelt ^4J csatolások is segítséget nyújtottak. A részstruktúrák kapcsolódását HMBC kísérlet segítségével tisztáztuk. A kvaterner szénatomok 2. *ábrán* bemu-

II. táblázat

Az esulatin B (2) NMR adatai [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^{1}H	^{13}C	^{1}H-^{1}H COSY	HMBC	NOESY
1a	3,82 dd (16,0, 1,0)	46,6	H-1b, H-3	C-2, C-3, C-4, C-14, C-15	H-1b
1b	1,91 d (16,0)		H-1a	C-2, C-14, C-15, C-16	H-1a
2	-	86,2	-	-	-
3	5,51 d (3,3)	78,3	H-1a, H-4	C-2, C-15, Ac δ 169,0	H-16, H-4, H-17
4	2,91 dd (3,5, 1,0)	49,7	H-3, H-5	C-3, C-6, C-14, C-15	H-3, H-5, H-7,
5	5,80 s	68,2	H-4, H-17	C-3, C-4, C-6, C-7, C-15, C-17, Ac δ 169,7	H-4, H-7, H-8b, H-11, H-13
6	-	147,0	-	-	-
7	4,94 d (9,6)	69,5	H-8b, H-8a, H-17	C-5, C-6, C-8, C-9, C-17, Ac δ 169,6[b]	H-4, H-5, H-19
8a	3,21 dd (14,0, 1,0)	45,4	H-7, H-8a	C-6, C-7, C-9	H-8a, H-5, H-11
8b	2,28 dd (14,0, 9,6)		H-7, H-8b	C-7, C-9	H-8b
9	-	209,4	-	-	-
10	-	49,3	-	-	-
11	6,07 d (15,9)	137,2	H-12	C-9, C-10, C-13, C-18, C-19	H-5, H-8b, H-13, H-18
12	5,52 dd (15,9, 9,6)	133,2	H-11, H-13	C-10, C-14	H-19, H-20
13	3,56 dq (9,6, 6,6)	44,7	H-12, H-20	C-11, C-12, C-14	H-11, H-5, H-20
14	-	211,1	-	-	-
15	-	92,1	-	-	-
16	1,48 s	17,8	-	C-1, C-2, C-3	H-3, H-1b
17	5,10 d (1,0)	110,1	H-5, H-7	C-5, C-6, C-7	H-3
	5,11 d (1,0)			C-5, C-6, C-7	H-3
18	1,17 s[d]	26,7	-	C-9, C-10, C-11, C-19	-
19	1,16 s[d]	23,0	-	C-9, C-10, C-11, C-18,	-
20	1,12 d (6,6)	20,2	H-13	C-12, C-13, C-14	H-12, H-13
Acetilek					
2-CO	-	170,1	-	-	-
2-COMe	2,13 s	20,9	-	-	-
3-CO	-	169,0	-	-	-
3-COMe	2,16 s	21,4	-	3-CO	-
5-CO	-	169,7[b]	-	-	-
5-COMe	2,05 s[a]	20,8[c]	-	5-CO	-
7-CO	-	169,6[b]	-	-	-
7-COMe	2,14 s[a]	21,2[c]	-	7-CO	-
15-CO	-	170,4	-	-	-
15-COMe	2,11 s	22,1	-	-	-

[a, b, c, d] δ értékek felcserélhetők



2. ábra. Az esulatin B (2) kvaterner szénatomjainak HMBC korrelációi (HC)

tatott korrelációja az esulatin B-re a 2,3,5,7,15-pentaacetoxi-jatrofa-6(17),11-dién-9,14-dion szerkezetet valószínűsíti.

Az esulatin B sztereokémiáját az esulatin A-hoz hasonlóan röntgendiffrakciós és NOESY mérésekkel vizsgáltuk. A röntgenkrisztallográfiai adatokból a C-2, C-3, C-4, C-7, C-13, C-15 ugyanolyan konfigurációja volt levezethető, mint az esulatin A (**1**) esetén, így tehát az esulatin B a **2** szerkezettel jellemezhető [12]. A NOESY korrelációk összhangban álltak a röntgendiffrakciós vizsgálatok alapján feltárt térszerkezettel. A **2** vegyület valamennyi hidrogén- és szénatomjának kémiai eltolódásértékét ^{1}H-^{1}H COSY, HMQC, HMBC és NOESY vizsgálatok alapján határoztuk meg, amint a *II. táblázatban* látható. Az esulatin B (**2**) EIMS vizsgálata során megállapítottuk, hogy a spektrum olyan fragmenseket is tartalmaz, melyek a jatrofa-11-én-9,11-dion-származékokra jellemzőnek tekinthetők. Az *m/z* 123 (52%) $C_8H_{11}O$ (mért tömeg *m/z* 123,0794, számított 123,0810) és *m/z* 96 (70%) C_7H_{12} (mért tömeg *m/z* 96,0934, számított 96,0939) fragmensek a $(CH_3)_2C$-CH-CH-CCH_3CO^+ és $(CH_3)_2$C-CH-CH-$CHCH_3^+$ ionoknak felelnek meg, melyek a molekula két ketocsoportja közötti részből származnak.

Az *E. esula* minor diterpenoidja, az esulatin C a HRMS és NMR vizsgálatok szerint $C_{36}H_{50}O_{17}$ összegképlettel jellemezhető. Az ^{1}H-NMR spektrum hét észtercsoport, hat acetil- (δ_H 2,16 s, 2,11, 2,06 s, 2,02 s, 2,00 s és 1,99 s) és egy izobutanoilcsoport [δ_H 2,61 sept (C*H*), 1,23 d, 1,16 d (CH_3)] jelenlétét mutatta. Az ^{1}H-NMR spektrum további szignáljai négy metil- (δ_H 1,56 s, 1,24 d, 1,24 s, 1,12 s), egy metilén- (δ_H 3,10 d, 2,80 d) és egy metilhez kapcsolódó metincsoportra (δ_H 2,17 dq) engedtek következtetni, valamint a δ_H 6,49-3,28 tartományban hét szinglet és három dublett szignál (3,5 és 1,9 Hz csatolási állandókkal) jelent meg (*III. táblázat*). Ez utóbbi jelek egyértelmű asszignálása érdekében ^{1}H-^{1}H COSY mérést végeztünk. A δ_H 6,02 (H-3), 3,28 (H-4) és 6,20 (H-5), és a δ_H 6,49 (H-7), 5,65 (H-8) és 4,94 (H-9) rezonancia jelek közötti korrelációk egyértelműen meghatározhatók voltak. Az ^{1}H-^{1}H COSY spektrumban ezen felül ^4J csatolásokat is észleltünk a δ_H 3,10 és 2,80 (H-1a,b) és a 6,02 (H-3); a δ_H 6,20 (H-5), 6,49 (H-7) és 5,04, 4,95 (H-17a,b); a δ_H 4,94 (H-9) és 4,13 (H-11); δ_H 4,13 (H-11) és 2,17 (H-13); a δ_H 2,17 (H-13) és 3,99 (14-OH) között. Ezen adatok 2,3,5,7,8,9,11,12,14,15-helyzetben szubsztituált jatrofánszármazékot jeleztek.

Az esulatin C szerkezetének további tanulmányozása HMQC és HMBC kísérlettel történt. A HMQC spektrumból a protonált szénatomok kémiai eltolódásértékeit határoztuk meg, melyeket a *III. táblázatban* tüntettünk fel. A HMBC kísérlet alapján hat alapvázhoz tartozó kvaterner szénatom jelenlétét mutattuk ki. A H-1, H-3, H-16 és a δ_C 88,5-nél jelentkező szénatom (C-2) valamint a H-1, H-4, H-5 és a δ_C 89,9-nél lévő szén (C-15) közötti kölcsönhatások arra utaltak, hogy az esulatin A (**1**)-hoz hasonlóan szubsztituált öttagú gyűrű van jelen ebben a molekulában is. A HMBC spektrumban a H-5–H-11 és a környező szénatomok két illetve három kötésen keresztüli kölcsönhatásai megerősítették, hogy az esulatin C az **1** vegyületben is meglévő a C-5–C-11 részszerkezetet tartalmazza és mindkét vegyületben ugyanolyan észtercsoportok találhatók az 5,7,8,9-helyzetben. A két vegyület tehát mindössze a C-12–C-14 molekularészen különbözik. A H-11, H-13, H-20 és a δ_C 213,6-nál lévő szénszignál korrelációja azt igazolta, hogy a C-12-n egy ketocsoportnak kell lennie. A δ_C 105,7-nél jelentkező kvaterner szénatom a H-1, H-4, H-13 és H-20 protonokkal mutatott keresztcsúcsa alapján a C-14-hez volt rendelhető. A 14-helyzetben valószínűleg egy hidroxilcsoport kapcsolódik, tekintve, hogy annak dublett jele (δ_H 3,99) a H-13-al mutatott csatolást (J = 1,9 Hz). A C-14 (δ_C 105,7) és a H-11 kémiai eltolódásértékéből (δ_H 4,13), mely észterezett metinekhez viszonyítva a magasabb térerőnél volt megfigyelhető [13], egy éterkötésre következtethettünk. Az éterkötés, mely az összegképletből és a fentiekben tárgyalt szubsztituensekből is következik, szükségszerűen a C-11 és C-14 között helyezkedik el.

Az esulatin C relatív konfigurációját NOESY mérésekkel tanulmányoztuk. A H-1, H-3 és a H-4 közötti NOE interakciók és csatolási állandók nagyon hasonlóak voltak az esulatin A és B-nél ta-

III. táblázat

Az esulatin C (3) NMR adatai [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^1H	^{13}C	^1H-^1H COSY	HMBC	NOESY
1a	3,10 d (16,5)	43,6	H-1b, H-3	C-2, C-3, C-4, C-14	H-1b, H-20
1b	2,80 d (16,5)		H-1a, H-3	C-14, C-15, C-16	H-1a, H-16
2	-	88,5	-	-	-
3	6,02 d (3,5)	74,2	H-1b, H-4	C-2	H-4, H-16
4	3,28 d (3,5)	49,7	H-3, H-5	C-3, C-5, C-6, C-14, C-15	H-3, H-5, H-7, 14-OH, H-16
5	6,20 s	69,4	H-4, H-7, H-17a,b	C-3, C-4, C-6, C-7, C-15	H-4, H-8
6	-	147,0	-	-	-
7	6,49 s	67,8	H-5, H-8, H-17a,b	C-6, C-8, C-9, C-17, C-1'	H-4, H-8, H-11
8	5,65 s	70,9	H-7, H-9	C-6, C-9, Ac δ 170,5	H-5, H-7, H-9, H-18
9	4,94 s	82,6	H-8, H-11	C-8, C-10, C-11, C-19, Ac δ 169,6	H-8, H-18, H-19
10	-	41,4	-	-	-
11	4,13 s	77,1	H-9, H-13	C-9, C-10, C-12, C-18	H-7, H-19
12	-	213,6	-	-	-
13	2,17 dq (7,0, 1,9)	21,6	H-11, 14-OH, H-20	C-12, C-14, C-20	-
14	-	105,7	-	-	-
14-OH	3,99 d (1,9)	-	H-13	-	H-4
15	-	89,9	-	-	-
16	1,56 s	18,3	-	C-1, C-2, C-3	H-1b, H-3
17a	5,04 s	107,9	H-5, H-7, H-17b	C-5, C-6, C-7	-
17b	4,95 s	-	H-5, H-7, H-17a	C-5, C-6, C-7	-
18	1,24 s[a]	18,9	-	C-10, C-11, C-19	H-8, H-9
19	1,12 s[a]	22,4	-	C-9, C-10	H-11
20	1,24 d (7,0)	9,6	H-13	C-12, C-14	H-1a, H-5
iBu					
1'	-	176,6	-	-	-
2'	2,61 sept (7,0)	34,2	-	C-1', C-4'	-
3'	1,16 d (7,0)	18,8	-	C-1', C-4'	-
4'	1,23 d (7,0)	19,8	-	C-1', C-3'	-
Acetilek					
8-CO	-	170,5	-	-	-
8-COMe	2,06 s	21,2	-	8-CO	-
9-CO	-	169,6	-	-	-
9-COMe	1,99 s	22,2	-	9-CO	-
2,3,5,15-CO	-	169,6	-	-	-
	-	168,9	-	-	-
	-	168,6	-	-	-
	-	167,5	-	-	-
2,3,5,15-	2,02 s	21,1	-	Ac 169,6	-
COMe	2,16 s	22,8	-	Ac 168,9	-
	2,11 s	21,8	-	Ac 168,6	-
	2,00 s	21,2	-	Ac 167,5	-

[a] A δ értékek felcserélhetők

pasztaltakhoz, amely arra utalt, hogy a C-2, C-3, C-4 és C-15 konfigurációja azonos. A C-4 és C-15 konfigurációjára abból a tényből következtethettünk, hogy a H-4 és 15-OAc között nem jelentkezett NOE kölcsönhatás, valamint hogy minden eddig ismert jatrofán-diterpenoidban *transz* gyűrűkapcsolódás fordul elő a röntgendiffrakciós 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 térállásúnak kell lennie [13]. A 14-OH és H-4 között észlelt NOE kölcsönhatás α-hidroxicsoportot jelzett a C-14-en. A C-7, C-8, C-9, C-11 és C-13 térszerkezetét a NOESY korrelációk alapján nem lehetett meghatározni, mert a molekulának ez a része nagy mértékben flexibilis. Az esulatin C tehát a 3 szerkezettel jellemezhető. Ez a vegyület szoros szerkezeti rokonságot mutat az *Euphorbia kansui*-ból izolált egyik diterpénnel, a kansuinin A-val [13].

Következtetések

Az esulatin A (**1**) és C (**3**) az Euphorbiaceae családból izolált legmagasabban észterezett diterpének, s ilyen nagy mértékben észterezett vegyületek izolálása feltehetően összefüggésben áll azzal, hogy friss növényi nyersanyag került felhasználásra. Az **1-3** vegyületek olyan diterpén-poliészterek, melyekben előforduló diterpénalkoholok korábban ismeretlenek voltak.

Az izolált vegyületek a jatrofánvázas diterpenoidok újabb képviselői, mely vegyületeket a család legfontosabb kemotaxonómiai bélyegének tekintenek [6–8]. Munkánkat megelőzően északamerikai eredetű *E. esula* mintából közöltek jatrofánvázas diterpenoidokat [6], európai mintából azonban ezt a vegyülettípust első alkalommal mutattuk ki. A két populációban talált vegyületek különbözőségének kemotaxonómiai jelentősége lehet. Az esulatin A (**1**) a National Cancer Institute (Bethesda, MD, USA) előzetes *in vitro* antitumor szűrővizsgálataiban hatástalannak bizonyult.

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JATROPHANE DITERPENOIDS FROM *EUPHORBIA ESULA*

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

Abstract—Two new jatrophane 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 jatrophane diterpenoids comprise a small group of the constituents of this family; they are known to occur only in a few species: *Jatropha gossypiifolia*, *J. macrorhiza*, *Euphorbia maddeni*, *E. kansui*, *E. helioscopia*, *E. characias*, *E. latheriflora* and *E. esula* L. [1–3]. These compounds have aroused interest because of their antitumour, antiwrithing, 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 jatrophane 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 methanolic 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 ($C_{32}H_{44}O_{13}$) was derived from the HR mass spectrum and NMR analyses. The EI-mass spectrum displayed fragment peaks due to the sequential loss of HOAc and a ketene unit (see Experimental). The ^1H and ^{13}C NMR spectra of esulatin D showed signals corresponding to six acetyl groups [δ_H 2×2.14 *s*, 2.12 *s*, 2.11 *s*, 2×2.00 *s*; δ_C 170.4, 170.3, 169.9, 2×169.4 and 168.9 (*C*O) and 22.1, 2×21.3, 2×21.0 and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C 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 jatrophane [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 HMQC spectra, as listed in Table 1. The ^1H NMR and ^1H-^1H COSY spectra revealed the presence of one secondary and three tertiary methyls (δ_H 1.14 *d*, 1.49 *s*, 1.06 *s* and 1.02 *s*) and four sequences of correlated protons: δ_H 3.80 *br d* and 1.93 *d* (—C*H*$_2$—) (A); δ_H 5.42 *d*, 2.85 *d*, 5.94 *s*, 5.21 *s* and 5.15 *s* [—C*H*R—C*H*—C*H*R—C(=C*H*$_2$)—] (B); δ_H 4.96 *t*, 2.00 *m* and 4.74 *dd* [—C*H*R—C*H*$_2$—C*H*R—] (C); δ_H 5.85 *d*, 5.54 *dd*, 3.48 *dq* and 1.14 *d* [*trans*-C*H*=C*H*—C*H*(C*H*$_3$)—] (D) (R = acetyl). The connection of these partial structures was determined from two- or three-bond long-range correlations observed in an HMBC spectrum.

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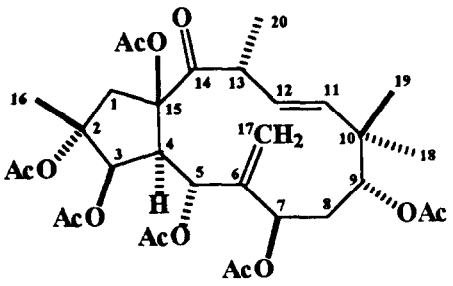

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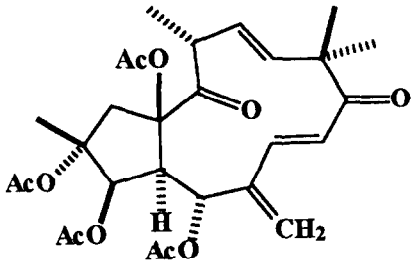

2

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 δ_C 86.6 (C-2) showed correlation peaks with the proton signals at δ_H 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 δ_C 146.0 (C-6) and the proton signals at δ_H 5.94 (H-5) and 4.96 (H-7) and between δ_C 111.7 (C-17) and H-5, H-7 revealed the connection of partial structure B to C. The $^2J_{CH}$ and $^3J_{CH}$ correlations between the carbon signal at δ_C 40.4 (C-10) and the proton signals at δ_H 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 δ_C 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 δ_C 210.9 and H-

Table 1. NMR Spectral Data of **1** [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^{1}H	^{13}C	^{1}H-^{1}H COSY	HMBC	NOESY
1a	3.80 *br d* (16.1)		46.8	H-1b	C-2, C-3, C-4, C-14, C-15
1b	1.93 *d* (16.1)	H-1a	C-2, C-14, C-15, C-16	H-16	
2	—	86.6		—	—
3	5.42 *d* (3.1)	78.2	H-4	C-1, C-2, C-15, 3-*C*OMe	H-4, H-17b
4	2.85 *d* (3.1)	49.2	H-3, H-5	C-3, C-5, C-6, C-14, C-15	H-3, H-5, H-7
5	5.94 *s*	68.8	H-4, H-17a,b	C-3, C-4, C-6, C-15, C-17, 5-*C*OMe	H-4, H-8, H-11, H-13
6	—	146.0	—	—	—
7	4.96 *t* (5.6)	68.0	H-8	C-5, C-6, C-8, C-9, C-17, 7-*C*OMe	H-4, H-8
8	2.00 *m*	35.2	H-7, H-9	C-6, C-7, C-9, C-10	H-5, H-7, H-9, H-17a
9	4.74 *dd* (7.2, 4.4)	74.6	H-8	C-8, C-10, C-11, C-18, C-19, 9-*C*OMe	H-8, H-19, H-11
10	—	40.4	—	—	—
11	5.85 *d* (16.0)	139.3	H-12	C-9, C-10, C-13, C-18, C-19	H-5, H-9, H-13, H-19
12	5.54 *dd* (16.0, 9.3)	130.0	H-11, H-13	C-10, C-13, C-14, C-20	H-18
13	3.48 *dq* (9.3, 6.6)	43.7	H-12, H-20	C-11, C-12, C-14, C-20	H-5, H-11, H-20
14	—	210.9	—		—
15	—	92.6	—		—
16	1.49 *s*	18.0		C-1, C-2, C-3	H-1b
17a	5.21 *s*	111.7	H-5	C-5, C-6, C-7	H-8
17b	5.15 *s*	—	H-5	C-5, C-6, C-7	H-3
18	1.06 *s*	21.8	—	C-9, C-10, C-11, C-19	H-12
19	1.02 *s*	26.8	—	C-9, C-10, C-11, C-18	H-9, H-11
20	1.14 *d* (6.6)	19.5	H-13	C-12, C-13, C-14	H-13
Acetyls					
2-CO	—	170.4[c]			—
2-CO*Me*	2.11 *s*[a]	21.0		2-*C*OMe	—
3-CO		168.9	—	—	—
3-CO*Me*	2.14 *s*	21.3[c]		3-*C*OMe	—
5-CO	—	169.4	—	—	—
5-CO*Me*	2.00 *s*[b]	20.8[d]	—	5-*C*OMe	—
7-CO	—	169.4	—	—	—
7-CO*Me*	2.12 *s*[b]	22.1[d]	—	7-*C*OMe	—
9-CO	—	169.9		—	—
9-CO*Me*	2.14 *s*	21.3[c]	—	9-*C*OMe	
15-CO		170.3[c]	—		—
15-CO*Me*	2.00 *s*[a]	21.0		15-*C*OMe	—

[a–c] δ values are interchangeable.

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 ^1H and ^{13}C NMR signals of the acetyl groups was not possible because the carbon signals of 5-*C*OMe and 7-*C*OMe and the proton signals of 3-CO*Me* and 9-CO*Me* 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 α. 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 β 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 δ_H 5.94 (H-5) correlated with the proton signal at δ_H 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 α 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 (δ_H 1.06). Thus, this methyl group (C-18) is in the α position and the other (C-19) is in the β position. Correlative signals between H-19 and H-9 indicated the presence of an α-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 **1**. 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 $C_{28}H_{36}O_{10}$. 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 $(CH_3)_2C{=}CH{-}CH{=}CCH_3C{=}O^+$ and $(CH_3)_2C{=}CH{-}CH{=}CHCH_3^+$, which were previously found to be characteristic of jatroph-11-ene-9,14-dione derivatives [7]. The ^1H and ^{13}C NMR spectra of esulatin E showed signals corresponding to four acetyl groups [δ_H 2.17 *s*, 2.15 *s*, 2.03 *s* and 2.02 *s*; δ_C 170.5, 170.1, 169.7 and 168.8 (*C*O) and 22.2, 21.4, 21.1 and 20.8 (*C*H$_3$)]. Additionally, the ^{13}C NMR spectrum exhibited resonances for six quaternary carbons, including two ketones (δ_C 208.3 and 202.8), four olefinic carbons, two ester-bearing and two alkyl-substituted methines, two methylenes and four methyls. The ^1H NMR and ^1H-^1H COSY spectra revealed the presence of three tertiary methyls (δ_H 1.64 *s*, 1.27 *s* and 1.23 *s*) and structural elements —C$^1H_2$— (δ_H 3.46 *d* and 2.13 *d*) (A), —C^3HR—C^4H—C^5HR—C^6(C$^{17}H_2$)—C$^7H{=}$C^8H— (δ_H 5.33 *d*, 2.21 *dd*, 6.03 *d*, 5.37 *s*, 6.71 *d* and 6.58 *d*) (B) and —C^{11}H=C^{12}H—C^{13}H(C^{20}H$_3$)— (δ_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 ^4J 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-tetraacetoxyjatropha-6(17),7*E*,11*E*-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 ^1H and ^{13}C NMR chemical shifts of **2** were assigned by means of ^1H-^1H 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, lathyrane and jatrophane skeletons [5, 9–13]. In the collection of Hungarian plants which we investigated, ingenane and lathyrane diterpenoids were not found and the isolated jatrophanes were different from those reported earlier from North American collections [5, 12, 13]. 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 jatrophane diterpenoids may be of chemotaxonomic significance.

EXPERIMENTAL

General experimental procedures. Mp are uncorr. UV: MeOH; EIMS: 70 eV, direct inlet; ^1H and ^{13}C NMR and DEPT: 400 MHz (^1H) and 100 MHz (^{13}C), $CDCl_3$, with TMS as int. standard. NOESY and 2D ^1H-^1H and ^{13}C-^1H 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** [$CDCl_3$, TMS, δ (ppm), (J = Hz)]

Atom	^1H	^{13}C	^1H-^1H COSY	HMBC	NOESY
1a	3.46 *d* (15.9)	46.2	H-1b	C-2, C-3, C-4, C-14	H-1b
1b	2.13 *d* (15.9)		H-1a	C-14, C-15, C-16	H-1a, H-16
2	—	86.5	—	—	—
3	5.33 *d* (6.9)	78.6	H-4	C-2, C-15, 3-COMe	H-4
4	2.21 *dd* (6.9, 3.5)	48.1	H-3, H-5	—	H-3, H-5, H-8
5	6.03 *d* (3.5)	69.6	H-4, H-17	—	H-4, H-7, H-8, H-17
6	—	144.0	—	—	—
7	6.71 *d* (16.4)	136.5	H-8, H-17	C-5, C-8, C-9	H-5, H-17
8	6.58 *d* (16.4)	129.6	H-7	C-6, C-7, C-9	H-4, H-5, H-11, H-18
9	—	202.8	—	—	—
10	—	49.4	—	—	—
11	5.70 *d* (15.9)	139.2	H-12	C-10, C-13, C-18, C-19	H-8, H-13, H-18, H-19, H-20
12	5.42 *dd* (15.9, 9.0)	131.1	H-11, H-13	C-10	H-13, H-18, H-19, H-20
13	3.60 *dq* (9.0, 6.7)	45.5	H-12, H-20	—	H-11, H-12, H-20
14	—	208.3	—	—	—
15	—	89.9	—	—	—
16	1.64 *s*	19.8	—	C-1, C-2, C-3	H-1b
17	5.37 *s* (2 H)	118.6	H-5, H-7	C-5, C-6, C-7	H-5, H-7
18	1.27 *s*	24.4	—	C-9, C-10, C-11, C-19	H-11, H-12, H-8
19	1.23 *s*	23.0	—	C-9, C-10, C-11, C-18	H-11, H-12
20	1.16 *d* (6.7)	17.8	H-13	C-12, C-13, C-14	H-11, H-12, H-13
Acetyls	—	—			
2-CO	—	170.1[c]	—		—
2-CO*Me*	2.17 *s*[a]	21.4[b]	—	2-COMe	—
3-CO	—	169.7	—	—	—
3-CO*Me*	2.02 *s*	20.8	—	3-COMe	—
5-CO	—	170.5[c]	—	—	—
5-CO*Me*	2.03 *s*[a]	22.2[b]	—	5-COMe	—
15-CO	—	168.8[c]	—	—	—
15-CO*Me*	2.15 *s*[a]	21.1[b]	—	15-COMe	—

[a,b,c] δ values are interchangeable.

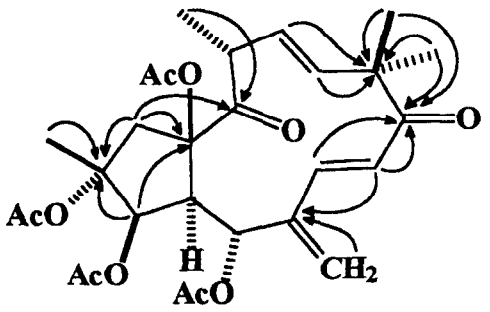

Fig. 1. HMBC correlations of quaternary carbons of esulatin E (**2**) (H → C).

Si 100 (5 μm), flow 0.5 ml/min^{-1}, with RI detection; TLC: Si gel 60 F_{254}.

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 CH_2Cl_2 (7 × 1.5 l) and H_2O. On evapn, the organic phase residue (130 g) was obtained, which was chromatographed over a polyamide column (600 g) with mixts of H_2O–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–Me_2CO (19:1, 9:1, 4:1, 7:3, 1:1, 3:7). Frs eluted with cyclohexane–Me_2CO (4:1) were transferred repeatedly to a Silica gel VLC (VLC II) and successively eluted with $CHCl_3$–MeOH mixts of increasing polarity. Frs 22–23 obtained from VLC II with $CHCl_3$–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–EtOAc–EtOH (30:10:1) as eluent to yield 5.1 mg of esulatin E (**2**). Frs 28–30, which were eluted from VLC II with $CHCl_3$–MeOH (100:0.3), after HPLC purification on reverse phase column using MeOH–H_2O (7:3) as eluent afforded esulatin D (**1**) (10 mg).

Esulatin D (**1**). Colourless crystals from MeOH; mp 154 5°. $[\alpha]_D^{25}$ −99 (*c*, 0.6, $CHCl_3$). UV λ_{max} nm (log ε): 216 (3.26), 296 (2.19). EIMS, *m/z* (rel. int.): 636 $[M]^+$ (6), 576 $[M–HOAc]^+$ (3), 516 $[M–2 \times HOAc]^+$ (3), 386 $[M–3 \times HOAc–CH_2CO–CO]^+$ (13), 43 $[MeCO]^+$ (100). HRMS: *m/z*: 516.2406 $[M–2 \times HOAc]^+$ $C_{28}H_{36}O_9$ required 516.2359. ^1H and ^{13}C NMR: see Table 1.

Esulatin E (**2**). Amorphous white solid. $[\alpha]_D^{25}$ −95

(c, 0.1, $CHCl_3$). UV λ_{max} nm (log ε): 204 (2.72), 267 (2.77). EIMS, m/z (rel. int.): 532 $[M]^+$ (0.3), 490 $[M-CH_2CO]^+$ (3), 430 $[M-HOAc-CH_2CO]^+$ (3), 370 $[M-2\times HOAc-CH_2CO]^+$ (6), 310 $[M-3\times HOAc-CH_2CO]^+$ (15), 123 $[C_8H_{11}O]^+$ (23), 96 $[C_7H_{12}]^+$ (27), 43 $[MeCO]^+$ (100). HRMS, m/z: 430.1972 $[M-HOAc-CH_2CO]^+$ $C_{24}H_{30}O_7$ required 430.1992. ^1H and ^{13}C NMR: see Table 2.

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III

MAGNETIC RESONANCE IN CHEMISTRY
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Structural Characterization and Dynamic NMR Studies of a New Peracylated Macrocyclic Diterpene

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ABSTRACT: A heptaester 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*)-(2*R**, 3*R**, 4*S**, 5*R**, 7*S**, 8*R**, 9*S**, 13*R**, 15*R**)-2,3,5,8,9-pentaacetoxy-7-isobutanoyloxy-14-oxojatropha-6(17),11-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 polycyclic derivatives.[1,2] 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, jatrophatrione, euphoscopin A and B and euphornin display antitumour properties.[3]

In a systematic search for biologically active compounds from Hungarian Euphorbiaceae, we have previously reported esulatin A–E, a series of new macrocyclic diterpenoids of the jatrophane 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.[5–7] The jatrophane 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 jatrophane diterpenoid, esulatin F (**1**), in solution. The isolation, structure elucidation and dynamic stereochemical analysis of **1** are reported.

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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-EIMS), corresponding to the molecular formula $C_{36}H_{50}O_{15}$ (calculated 722.3150). Its ^{1}H NMR spectrum in $CDCl_3$ at 300 K contained broad signals. Changing the solvent to pyridine-d_5 resulted in a significantly better resolved spectrum. The ^{1}H and ^{13}C NMR spectra of **1** in pyridine-d_5 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 ^{1}H–^{1}H COSY spectrum revealed the presence of three structural elements: —CHR—CH—CHR—(A), —CHR—CHR—CHR— (B) and *trans* —CH=CH—CH(CH_3)—(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 jatrophane diterpene and that fragments A, B and C are its

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Table 1. NMR data for esulatin F (**1**)[a]

Atom	^{1}H (Pyridine-d_5) (300 K)	^{1}H ($CDCl_3$) (300 K)	NOE: H No	^{13}C (Pyridine-d_5) (350 K)	HMBC: H→C (350 K)
1α	4.24 d (15.8)	3.79 d (16.0)	16	47.8	2, 3, 4, 14, 15
1β	2.28 d (15.8)	1.94 d (16.0)	16		14, 15
2	—	—	—	87.1	—
3	5.88 s	5.46 brs	4, 16	78.6	2, 15
4	3.37 brs	2.99 brs	3, 5, 7	50.2	3, 6, 14, 15
5	6.32 brs	5.93 brs	4, 8, 13, 17a	68.5	3, 4, 6, 7, 15, 17
6	—	—	—	145.9	—
7	5.51 brs	5.06 brs	4, 5	68.5	6, 8, 17
8	5.71 s	5.25 s	5, 9, 11, 18/19	72.7	6, 7, 10
9	5.36 d (1.9)	4.93 s	8, 11	78.2	7, 8, 10, 11, 18, 19
10	—	—	—	40.5	—
11	6.12 d (15.8)	5.90 d (15.8)	8, 9, 13, 18/19	138.5	10, 13, 18, 19
12	5.86 dd (15.8, 8.9)	5.63 dd (15.8, 9.0)	13, 20	132.0	10, 13, 14
13	3.90 dq (8.9, 6.7)	3.56 dq (9.0, 6.7)	5, 11, 12, 20	44.1	—
14	—	—	—	211.2	—
15	—	—	—	93.2	—
16	1.61 s	1.49 s	1α, 1β, 3	18.4	1, 2, 3
17a	5.60 s	5.17 brs	—	113.1	6, 7
17b	5.51 brs		—		7
18	1.09 s	1.02 brs	8, 11	23.0	9, 10, 11, 19
19	1.15 s	1.12 brs	8, 11	27.9	9, 10, 11
20	1.30 d (6.7)	1.14 d (6.7)	12, 13	20.2	12, 13, 14

[a] ^{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-O*i*Bu, 2.58 sept (7.0) (=CH—), 1.16 d (7.0), 1.14 d (7.0) (—CH_3). ^{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, 169.9; 7-O*i*Bu, 175.6 (CO), 34.4 (=CH—), 18.7, 19.0 (—CH_3).

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 ^3J(C, H) couplings between the oxymethine protons and the ester carbonyl carbons. The cross peaks of the carbon signals at δ_C 175.6 (isobutyroyl CO), 169.3, 169.4, 169.7 and 169.9 ppm (acetyl CO) with the proton signals at δ_H 5.51 (H-7), 5.88 (H-3), 6.32 (H-5), 5.71 (H-8) and 5.36 ppm (H-9), respectively, unambiguously indicated the locations of five ester groups. The remaining two acetyls, 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 *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 α. Diagnostic NOEs were observed between H-4 and H-7 and between H-4 and H-3, indicating β-oriented acyl groups at C-3 and C-7. The zero coupling constant between H-4 and H-5 required that H-5 was β, as in esulatin A–E.[4] NOESY correlations between H-5 and H-8, between H-5 and H-13 and between H-8 and H-9 demonstrated the β-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.[8] Esulatin F displayed a close structural similarity with terracinolide B, isolated from *E. terracina*:[9] 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*.

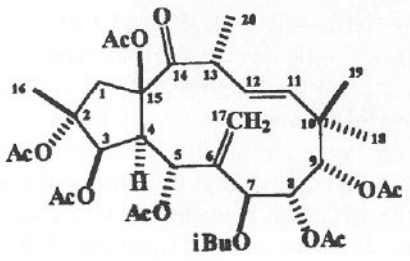


Figure 1. Structure and relative stereochemistry of **1**.

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_2Cl_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.

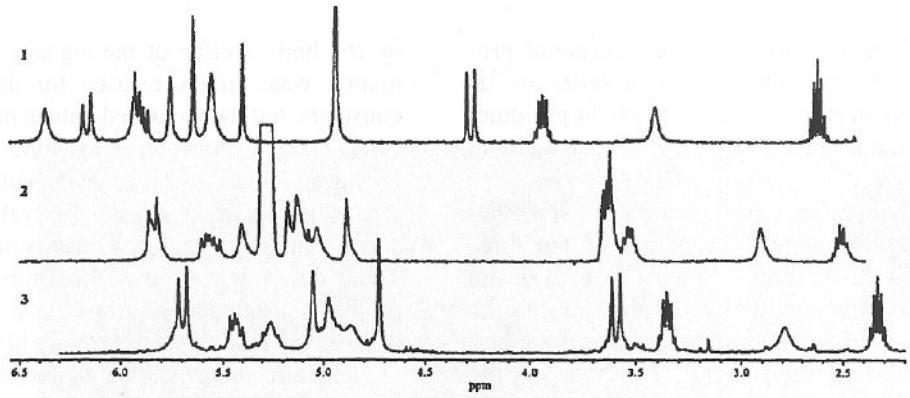


Figure 2. Signals of the acyloxymethine protons of the macrocyclic part of the esulatin F at 300 K in (1) pyridine-d_5, (2) CD_2Cl_2 and (3) $CDCl_3$ as solvent.

Interpretation of the ^1H NMR data relating to the macrocyclic part in $CDCl_3$ and CD_2Cl_2 was sometimes hampered by severe overlapped and broadened peaks of the diterpene core. In pyridine-d_6 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, ^1H and ^{13}C NMR spectra in CD_2Cl_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 HMQC and EXSY spectra. At about 240 K, the two components of the process have well separated signals in the ^1H NMR spectra, and their ratio is 3 : 1. The intensity ratio changed only below 204 K in CD_2Cl_2 when signals corresponding to further components appeared. The hydrogen which should be involved in the tautomerization process (H-13, δ_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 δ_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.

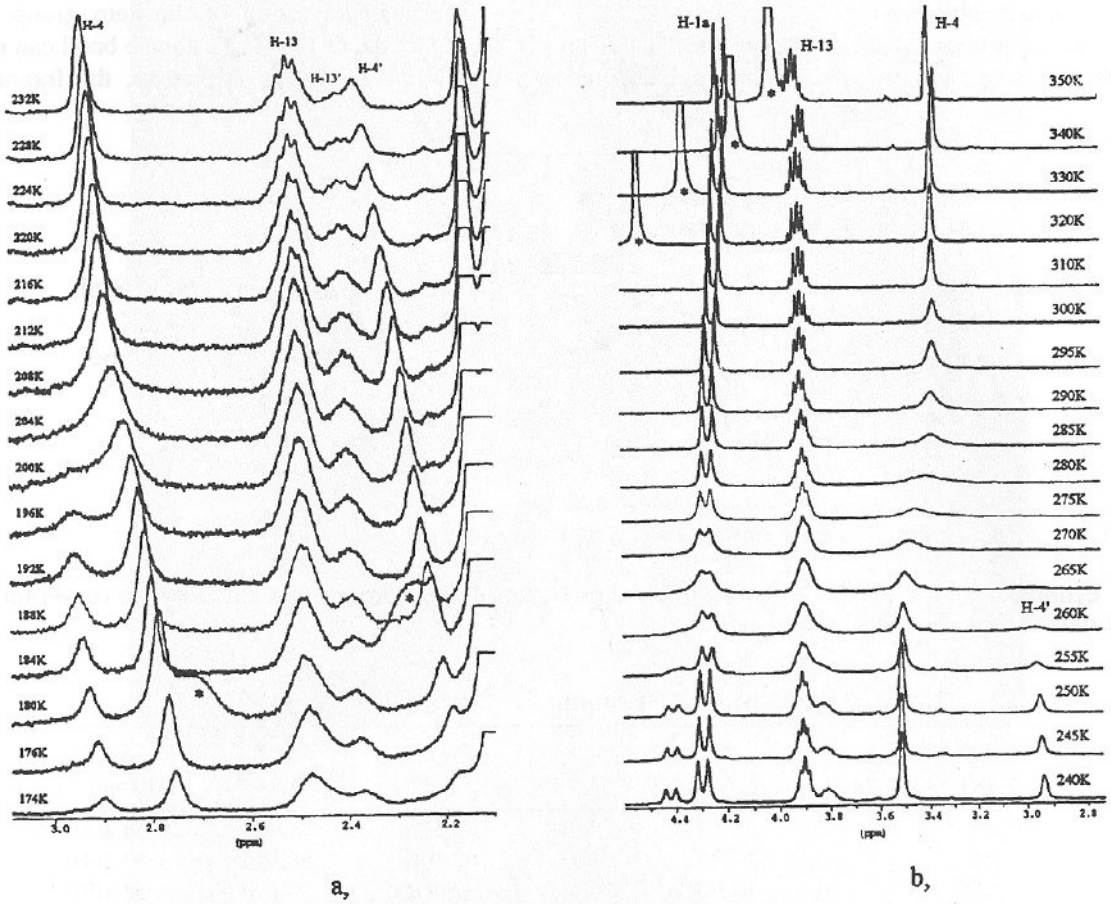


Figure 3. The conformational change of esulatin F (1) in (a) dichloromethane-d_2 and (b) pyridine-d_5. Peaks marked with sterishs correspond to the water in the solvent.

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.

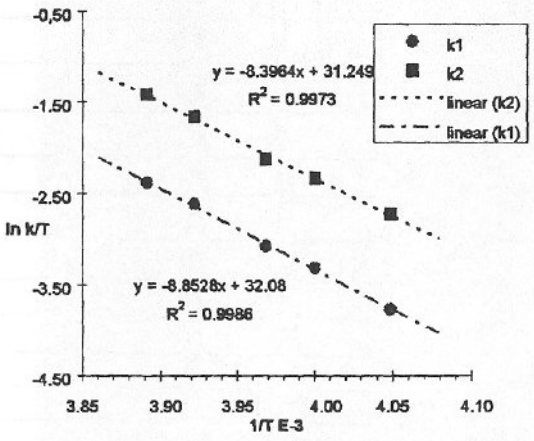
Variation of the temperature causes a number of changes in the ^{1}H NMR spectrum. The behaviour of the three resonances between δ_H 4.5 and 2.5 ppm (H-1a, H-4 and H-13) is particularly marked. The remaining part of the spectrum includes overlapping hydrogen resonances, and the broad signals lead to 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 3 : 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_2Cl_2 and from 240 to 330 K in $CDCl_3$. The best indicator was the H-4 signal in the macrocycle at δ_H 3.32, 2.93 and 2.79 ppm, respectively; during cooling, this peak split into two signals (e.g. δ_H 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,[14] where the rate constants of the exchange process at the given temperatures can be obtained.

The exchange was too slow in CD_2Cl_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, $\Delta H^{\neq}$, entropy, $\Delta S^{\neq}$ (Fig. 4), free energy of activation, $\Delta G^{\neq}$ (Table 2)[16] 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 force-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 macrocycle, 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 proved 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



$\Delta H^{\#}_1$ 73.60 kJ/mol
$\Delta S^{\#}_1$ 69 J/K
$\Delta H^{\#}_2$ 69.81 kJ/mol
$\Delta S^{\#}_2$ 62 J/K

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]

T (K)	k_1 (s^{-1})	k_2 (s^{-1})	K	ΔG^0 (kJ mol^{-1})	$\Delta G_1^{\neq}$ (kJ mol^{-1})	$\Delta G_2^{\neq}$ (kJ mol^{-1})
247	5.7(3)	16.1(3)	2.8(2)	−2.1(2)	56.5(1)	54.4(5)
250	9.0(7)	24.2(9)	2.6(8)	−2.1(4)	56.3(0)	54.2(4)
252	11.6(3)	30.3(3)	2.6(1)	−2.0(1)	56.1(6)	54.1(9)
255	18.7(4)	48.5(3)	2.5(9)	−2.0(2)	55.9(5)	53.9(4)
257	23.8(1)	62.4(5)	2.6(2)	−2.0(6)	55.8(2)	53.8(3)

[a] Standard errors of the calculations are given in parentheses.

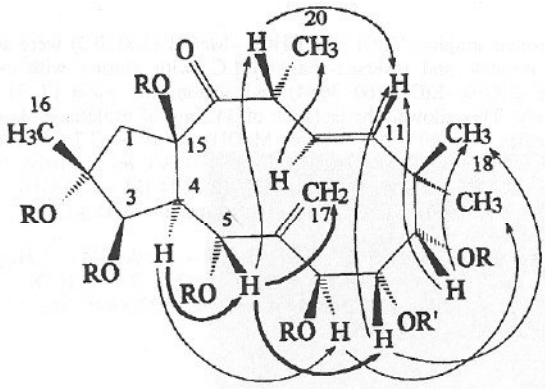
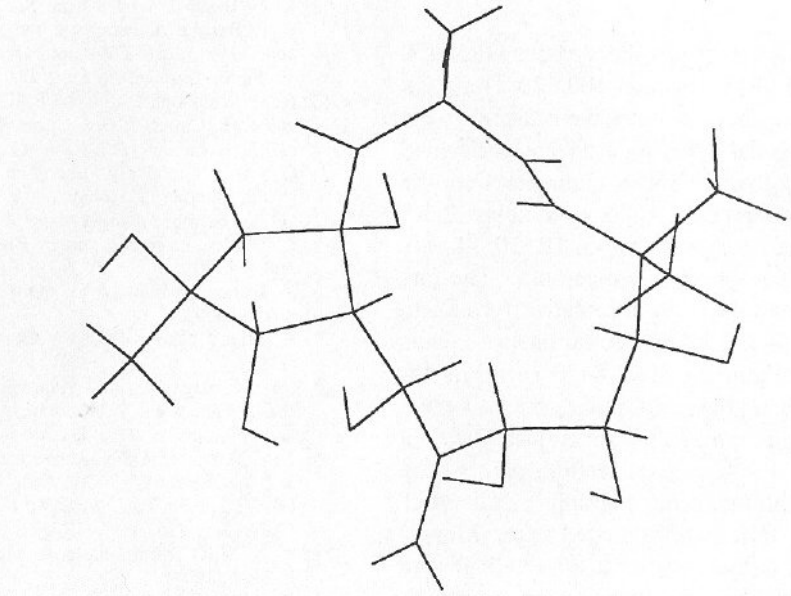


Figure 5. NOESY cross peaks of esulatin F (**1**) for the conformational study. Thick arrows indicate the *exo* conformation of C-17 and thin arrows indicate the planar conformation.

C-10 and C-7 is extremly 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_3-20 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



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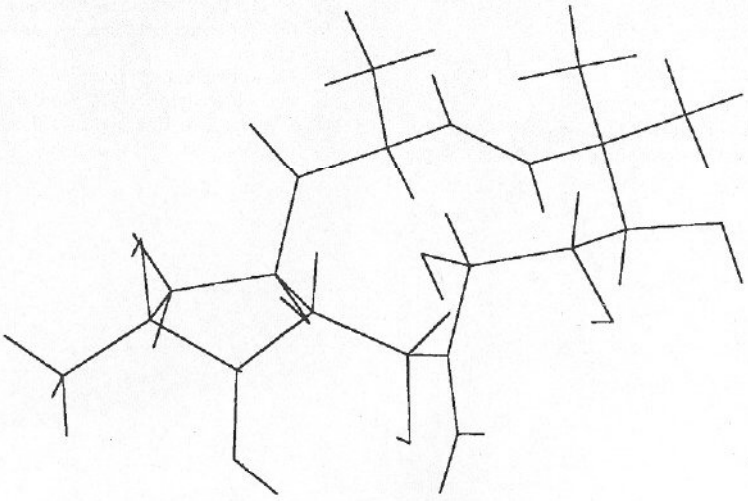

II

Figure 6. The planar (I) and the bent (II) conformers of esulatin F (**1**).

their rotation around the >C—O—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 esulatin D.[8c] 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 the follows: 1024 × 512 data point, relaxation delay 2.5 s, mixing time 5 μs 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.[18] 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 μm, Merck) were used. HPLC was carried out on a Waters-Millipore instrument, with refractive index detection on a normal-phase column (LiChrospher Si 100, 5 μm, Merck) and on a reversed-phase column (LiChrospher RP-18, 5 μm, Merck).

Isolation of 1

The extraction of *E. esula* and column chromatographic separations are described in Ref. 4. Fractions 31–32 obtained on vacuum liquid chromatography (VLC) with $CHCl_3$–MeOH (100:0.3) 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 esulatin F (**1**) as white needles, m.p. 205–207 °C (from MeOH); $[\alpha]_D^{25}$ −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 $[M - i\text{-}BuOH]^+$ (1), 602 $[M - 2 \times AcOH]^+$ (2), 532 $[M - 2 \times AcOH - (CH_3)_2CCO]^+$ (5), 472 $[M - 3 \times AcOH - (CH_3)_2CCO]^+$ (5), 412 $[M - 4 \times AcOH - (CH_3)_2CCO]^+$ (4), 96 $[(CH_3)_2C{=}CHCH{=}CHCH_3]^+$ (22), 71 $[C_4H_7O]^+$ (18), 43 $[CH_3CO]^+$ (100); HREIMS, observed *m/z* 722.3200 $[M]^+$ (calculated for $C_{36}H_{50}O_{15}$, 722.3150).

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IV

Isolation and Structure Revision of Pepluane Diterpenoids from *Euphorbia peplus*

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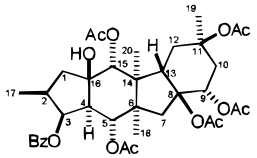
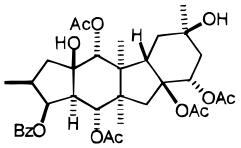
A new pepluane diterpene polyester (**2**) was isolated from a CH_2Cl_2 extract of the whole, undried plant of *Euphorbia peplus*, together with the known compound **1**. The structures were established by high-field spectroscopic methods, including 2D NMR techniques, and by X-ray crystallography, and the stereostructure of the first member of the pepluane 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] *Euphorbia peplus* L. (Euphorbiaceae) is a small, annual, herbaceous plant with milky latex, which occurs all over the world. The plant has been used as an antiasthmatic and anticatarrhal agent and to treat cancerous conditions in traditional medicine in many areas of the world.[2,3] 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.[4,5] While the present work was in progress, jatrophane diterpenoids and a tetracyclic diterpene (**1**) based on a new carbon framework, named pepluane, 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 pepluane diterpenoids (**1**, **2**) from a dichloromethane extract of the fresh plant. This paper deals with the isolation and structure elucidation of these compounds.



1



2

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 (H_2O→MeOH) and Si gel ($CHCl_3$−Me_2CO, gradient) followed 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.

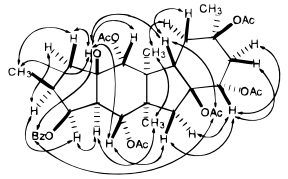


Figure 1. NOESY correlations for **1**.

Mass spectrometry and detailed NMR investigations indicated that compound **1** was identical with the pepluane 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 C_6D_6, 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 *ortho*-benzoyl 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, correlative signals were observed between 8-OAc and H-13 and between 8-OAc and *ortho*- and *meta*-benzoyl protons, pointing to cis-fused C/D rings, in contrast with the proposal of Jakupovic and coworkers.[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

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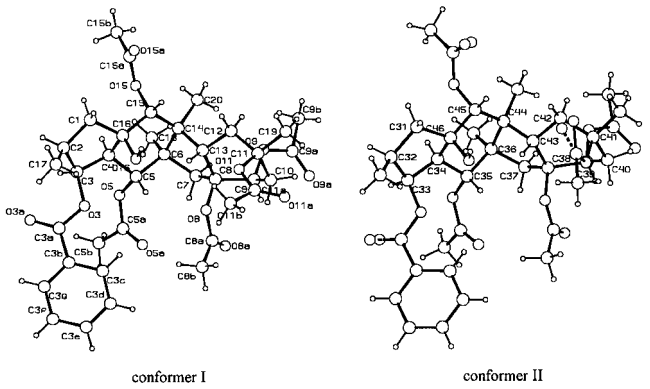

Figure 2. Perspective views of **1** conformers I and II. (In the latter case only the atoms in the molecular skeleton are labeled, using numbers increased by 30 relative to conformer I; bonds to the disordered atoms O33b, O41b, and O45b are drawn with dashed lines.)

O16−H16...O41a and O46−H46...O15a contacts are 2.06, 2.87 (1), 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[6] as concerns the C/D junction and the orientation of the acetoxy moieties on C8 and C9. Prior to the X-ray analysis of **1**, 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 ($\Delta\rho \approx 112°$) about the C11−O11 bond. The second largest difference ($\Delta\rho \approx 31°$) in the substituent positions is displayed around the C3−O3 bond, accompanied by a second rotation of $\Delta\varphi \approx 30°$ about the C_{ph}−C_{acyl} 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 $C_{35}H_{46}O_{12}$. It exhibited IR absorption 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 ^{13}C NMR spectra of **2** revealed four acetate and one benzoate groups (Table 1). Additionally, the spectra exhibited resonances closely related to that of **1**. After the ^1H and ^{13}C NMR data on **2** had been assigned by analysis of its ^1H−^1H COSY, HMQC, 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 acetate signal and the appearance of one hydroxy signal (δ_H 2.82 s) indicated the replacment of one of the acetyl residues with a hydroxy group. Comparison of the ^1H and ^{13}C signals of **1** and **2** revealed a significant difference in the chemical shift values

Table 1. NMR Data on Compound 2 [$CDCl_3$, δ (ppm) J = Hz)]

atom	^1H[a]	^{13}C[b]	NOESY H no.	HMBC C no.
1α	2.17 dd (14.2, 11.6)	44.7	2	2, 3, 15, 16, 17
1β	1.51 dd (14.2, 5.1)		16-OH	2, 3, 4, 16, 17
2	2.54 m	35.9	1α, 3, 17	17
3	5.80 m	76.4	2, 4	1, 4, 16
4	2.40 dd (12.0, 4.3)	48.3	3, 18	5, 6, 16
5	5.83 d (12.0)	69.7	7β, 13, 16-OH	3, 4, 7, 16, 18
6		49.0		
7β	2.48 d (16.0)	39.8	5	5, 6, 8, 13, 14
7α	1.58 d (16.0)		18	5, 6, 8, 9, 18
8		88.6		
9	5.78 d (4.9)	68.3	8-OAc, 10α,β	7, 8, 11, 13
10β	1.97 dd (16.9, 5.7)	41.6	11-OH	11, 12
10α	1.85 d (16.9)		19	8, 9, 19
11		68.0		
12β	1.75 m	33.9		8, 10, 11, 13, 19
12α	1.69 t (12.9)			13, 14
13	4.30 dd (12.8, 6.6)	47.0	5, 11-OH, 15, 16-OH	9, 11, 12, 14, 15, 20
14		52.0		
15	5.07 s	73.3	13, 16-OH	1, 4, 6, 13, 14, 16
16		84.2		
17	1.05 d (7.3)	16.6	16-OH, 2	1, 2, 3
18	1.08 s	16.7	4, 7α, 20	5, 6, 7, 14
19	1.29 s	31.5	10α	10, 11, 12
20	0.92 s	16.3	18	6, 13, 14, 15
11-OH	2.82 s		13, 10β	19
16-OH	3.17 s		1β, 5, 13, 15, 17	4, 15, 16

[a] ^1H NMR signals of the acyl groups: 5-OAc: 1.72 s, 8-OAc: 1.96 s, 9-OAc: 2.03 s, 15-OAc: 2.13 s, 3-OBz: 7.92 d (7.4) (H-2′, 6′), 7.54 't' (7.4) (H-4′), 7.41 't' (7.7) (H-3′, 5′). [b] ^{13}C 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 (CO), 133.2 (C-4′), 129.3 (C-2′, 6′), 128.5 (C-3′, 5′), 130.0 (C-1′).

of C-11 (**1**, δ_{C-11} 79.8; **2**, δ_{C-11} 68.0), from which the position of the hydroxy group on C-11 was concluded. This was substantiated by the occurrence of $^3J_{C-H}$ 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 (^{13}C). 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 $CHCl_3$ at ambient temperature, using a Perkin−Elmer 341 polarimeter. Melting points were not corrected. For column chromatography, polyamide (ICN) and Si gel (Kieselgel GF_{254} 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

exhaustively extracted with CH_2Cl_2. 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 H_2O–MeOH (3:2 and 1:4) as eluents. Fractions obtained with H_2O–MeOH (2:3) were combined and subjected to Si gel flash chromatography, using a gradient system of $CHCl_3$ and Me_2CO. Fractions 6–10 eluted with $CHCl_3$ were further fractionated by reversed-phase HPLC with MeOH–H_2O (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); $[\alpha]^{25}_D$ +5° (*c* 0.06, $CHCl_3$); UV (MeOH) λ_{max} 230 (ϵ 13 040), 272 (ϵ 1210), 280 (ϵ 1070) nm; IR (KBr) 3430, 1741, 1712, 1370, 1246, 1032, 717 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) δ 1.86 (1H, dd, J = 13.7, 11.4 Hz, H-1α), 1.12 (1H, dd, J = 13.7, 4.6 Hz, H-1β), 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-7α), 2.77 (1H, d, J = 15.7 Hz, H-7β), 6.29 (1H, d, J = 5.0 Hz, H-9), 1.80 (1H, d, J = 17.1 Hz, H-10α), 2.52 (1H, ddd, J = 17.1, 5.0, 1.1 Hz, H-10β), 1.59 (1H, t, J = 13.3 Hz, H-12α), 2.59 (1H, ddd, J = 13.3, 5.8, 1.1 Hz, H-12β), 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 (3H, 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′); ^{13}C NMR (C_6D_6, 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 (9-OAc), 21.0, 169.6 (15-OAc), 166.4, 133.6, 131.8, 130.5, 129.1 (3-OBz); HREIMS obsd *m/z* 538.2619 [M − 2 × AcOH − CH_2CO]$^+$, calcd for $C_{31}H_{38}O_8$ 538.2567, and obsd *m/z* 460.2392 [M − 4 × AcOH], calcd for $C_{29}H_{32}O_5$ 460.2250.

Crystal Data on 1: $C_{37}H_{48}O_{13}$, M = 700.75, orthorhombic, space group $P2_12_12_1$, a = 10.178(5), b = 18.387(2), c = 39.621-(5) Å, V = 7415(4) Å^3, Z = 8, D_c = 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 R_1 = 0.062, wR_2 = 0.1617 for 10 738 reflections taken with Fo > 4σFo and R_1 = 0.083, wR_2 = 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)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Compound 2: white needles, mp 238–240 °C (from diethyl ether); $[\alpha]^{25}_D$ +40° (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} 229 (ϵ 13 200), 274 (ϵ 975), 282 (ϵ 766) nm; IR (KBr) 3432, 1738, 1711, 1369, 1241, 1027, 715 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS (70 eV) *m/z* (% rel int) 658 (0.1) [M]$^+$, 598 (1) [M − AcOH]$^+$, 538 (55) [M − 2 × AcOH]$^+$, 523 (53) [M − 2 × AcOH − CH_3]$^+$, 478 (9) [M − 3 × AcOH]$^+$, 460 (12) [M − 3 × AcOH − H_2O]$^+$, 320 (49) [M − 3 × AcOH − BzOH − 2 × H_2O]$^+$; HREIMS obsd *m/z* 538.2593, calcd for $C_{31}H_{38}O_8$ 538.2567 and obsd *m/z* 320.1786, calcd for $C_{22}H_{24}O_2$, 320.1776.

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PHYTOCHEMISTRY

Jatrophane diterpenoids from *Euphorbia peplus*

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Abstract

From the pro-inflammatory active extract of *Euphorbia peplus*, a new diterpene polyester (**1**) based on the jatrophane skeleton was isolated together with the known compounds **2–5**. The irritant activities of some jatrophane 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; Jatrophane 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 tigliane, 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, & Bohlmann, 1981; Evans & Taylor, 1983). Moreover, in recent years investigations on *Euphorbia* species have resulted in the discovery of several new classes of diterpenes (Öksüz 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 jatrophane diterpene (**1**), together with the known jatrophane 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 jatrophane derivatives (Seip & Hecker, 1984), some jatrophane 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 ($ID_{50}^{4} = ID_{50}^{24} = 25$ μg/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 ($[\alpha]_D^{25}$ −33, *c*, 0.1, $CHCl_3$) 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

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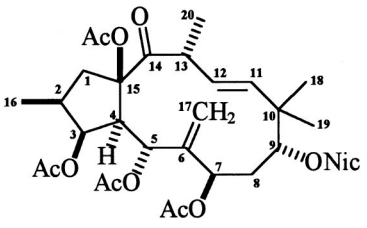

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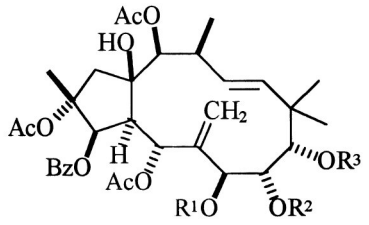
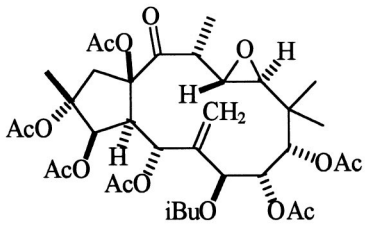


	R^1	R^2	R^3
2	iBu	H	Nic
3	Ac	Ac	Ac
4	iBu	H	Ac
5	Ac	H	Nic



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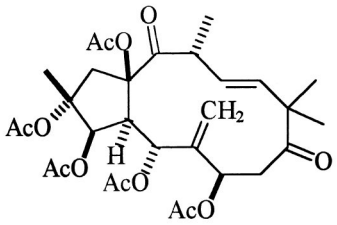

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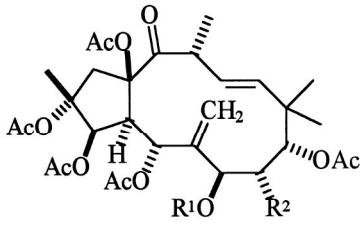

	R^1	R^2
8	Ac	H
9	iBu	OAc

$C_{34}H_{43}NO_{11}$ (calcd 641.2836). It exhibited UV maxima at 217 and 264 nm, characteristic of the nicotinoyl group. The ^1H and ^{13}C NMR spectra of **1** revealed the presence of one nicotinoyl and four acetyl groups (Table 1). Additionally, the ^1H and ^{13}C NMR spectra contained resonances for skeletal carbons and protons, which were assigned on the basis of the interpretation of the ^1H–^1H-COSY and HMQC 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 *trans* disubstituted olefin, one exocyclic and two simple methylenes, seven methine groups, one ketone and three quaternary carbons. The ^1H–^1H COSY spectrum revealed three structural fragments with correlated protons: δ_H 3.01 d, 1.65 d, 2.22 m, 0.90 d, 5.42 t, 2.71 d and 5.81 s [–C*H*$_2$–C*H*(C*H*$_3$)–C*H*(OR)–C*H*–C*H*(OR)–] (A); δ_H 4.84 dd, 2.18 m and 5.09 dd, [–C*H*(OR)–C*H*$_2$–C*H*(OR)–] (B); δ_H 5.94 d, 5.65 dd, 3.53 dq and 1.20 d [*trans*–C*H*=C*H*–C*H*(C*H*$_3$)–] (C) (R = acyl). Further, in the ^1H–^1H COSY spectrum, ^4J couplings were also detected between the exomethylene (δ_H 5.07 s (2H) (H-17)) and two acyloxymethines (δ_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 long-range C–H cor-

Table 1
NMR spectral data on **1** ($CDCl_3$, TMS, δ (ppm), (J=Hz))

Atom	^1H	^{13}C	HMBC (H→C) C No.	NOESY
1α	3.01 dd (14.0, 7.7)	46.3	2, 3, 4, 14, 15	1β, 2
1β	1.65 dd (14.0, 12.7)		2, 14, 15, 16	1α, 16
2	2.22 m	38.3	–	1α, 3, 4, 16
3	5.42 t (3.0)	76.7	15, 3-COMe	2, 4, 16
4	2.71 d (3.0)	52.9	3, 14, 15	2, 3, 5, 7
5	5.81 s	68.5	3, 4, 6, 7, 15, 17, 5-COMe	4, 8, 11, 13, 17
6	–	146.9	–	–
7	4.84 dd (7.1, 2.3)	69.1	5, 8, 7-COMe	4, 8, 9, 11, 17, 18/19
8	2.18 m (2H)	34.0	7, 9, 10	5, 7, 9, 17
9	5.09 dd (2.7, 7.9)	75.9	7, NicCO	7, 8, 18/19
10	–	40.7	–	–
11	5.94 d (16.0)	137.9	10, 13, 18, 19	5, 7, 13, 18/19
12	5.65 dd (16.0, 9.0)	130.3	10, 13, 14, 20	13, 17, 18/19, 20
13	3.53 dq (9.0, 6.7)	43.3	11, 12, 14, 20	5, 11, 12, 20
14	–	212.6	–	–
15	–	92.9	–	–
16	0.90 d (6.6)	13.3	1, 2, 3	1β, 2, 3
17	5.07 s (2H)	110.2	5	5, 7, 8, 12
18	1.13 s	26.5	9, 10, 11, 19	7, 8, 9, 11, 12,
19	1.11 s	23.3	9, 10, 11, 18	7, 8, 9, 11, 12,
20	1.20 d (6.7)	19.4	12, 13, 14	12, 13
Nic CO	–	164.1	–	
2′	9.23 d (1.5)	151.1	3′, 4′, 6′	
3′	–	125.7	–	
4′	8.21 dt (7.9, 1.5)	136.8	2′, 6′, NicCO	
5′	7.43 dd (7.9, 4.8)	123.5	3′	
6′	8.80 dd (4.8, 1.5)	153.8	4′, 5′	
Acetyls				
3-CO		169.9	–	
3-COMe	2.13 s	21.2	3-COMe	
5-CO		169.6	–	
5-COMe	2.15 s	21.2	5-COMe	
7-CO		169.7	–	
7-COMe	1.52 s	20.5	7-COMe	
15-CO		169.9	–	
15-COMe	2.11 s	21.2	15-COMe	

relations observed in an HMBC spectrum (Table 1). The correlation of the quaternary carbon signal at δ_C 92.9 (C-15) with the proton signals at δ_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 construct a methyl-substituted five-membered ring, present in many types of Euphorbiaceae diterpenes (Evans & Taylor, 1983). Cross-peaks between the carbon signal at δ_C 40.7 (C-10) and the proton signals at δ_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 $^2J_{C-H}$ and $^3J_{C-H}$ correlations between the carbon signal at δ_C 212.6 (C-14) and the proton signals at δ_H 3.01, 1.65 (H-1α, β), 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 ester groups were also determined via the HMBC experiment, from an evaluation of the J_{C-H} couplings between the oxymethine protons and the carbonyl carbons. The acetyl group at δ_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 α position of the proton at the ring junction (H-4), it was found that a β-oriented methyl group is present on C-2 and a β-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 β on the basis of the zero coupling constant between H-4 and H-5, similarly as in case of co-occurring jatrophanes and esulatins (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 2α,3β,5α, 7β,15β-pentaacetoxy-9α-nicotinoyloxy-14-oxojatropha-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_H 1.52), influenced by the aromatic ring effect of the nicotinoate on C-9. The stereochemistry of the 15-OAc group could not be determined on the basis of NOESY 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 (2*R*,3*R*,4*S*,5*R*,7*S*,8*S*,9*S*,13*S*,14*S*,15*R*)-2,5,9,14-tetraacetoxy-3-benzoyloxy-8,15-dihydroxy-7-isobutanoyloxyjatropha-6(17),11*E*-diene (**4**) (Kálmán & 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 $ID_{50}^{4} = ID_{50}^{24} = 29$ 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, ^{13}C and 2D NMR (^1H–^1H COSY, NOESY, HMQC, HMBC): 400 MHz (^1H) and 100 MHz (^{13}C), $CDCl_3$, with TMS as internal standard, Bruker Avance DRX 400 spectrometer. Optical rotations were determined in $CHCl_3$ at ambient temp.; CC: polyamide (ICN). VLC: silica gel (Kieselgel GF_{254} 15 μm, Merck). HPLC: Li Chrospher RP-18 (200x4 mm, 5 μm) and LiChrospher Si 100 (200 × 4 mm, 5 μm) with RI detection.

3.2. Assay for irritant activity

Irritant doses 50 (ID_{50}) 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 Me_2CO. 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.

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 CH_2Cl_2 and H_2O. Evaporation of the organic phase gave a residue (4.7 g), which was chromatographed over a polyamide column with mixtures of H_2O/MeOH (3:2 and 1:4) as eluents. Fractions obtained with H_2O/MeOH (2:3) were chromatographed by silica gel VLC, using $CHCl_3$ and $CHCl_3$/Me_2CO mixtures of increasing polarity as eluents. Fractions 6–10 eluted with $CHCl_3$ were transferred to a reverse-phase HPLC column and eluted with MeOH/H_2O 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, $[\alpha]_D^{25}$ +53 (*c*, 0.51, $CHCl_3$), **3** (4.7 mg), $[\alpha]_D^{25}$ −10 (*c*, 0.19, $CHCl_3$) and **4** (3.1 mg), m.p. 220–223°C, $[\alpha]_D^{25}$ +8 (*c*, 0.3, $CHCl_3$), respectively. Fractions 11–18 obtained from VLC with $CHCl_3$/Me_2CO (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, $CHCl_3$).

3.5. Compound 1

Amorphous solid. $[\alpha]_D^{25}$ −33 (*c*, 0.1, $CHCl_3$). UV λ_{max} nm (log ε): 217 (3.96), 264 (3.27). EIMS, *m/z* (rel. int.): 641 $[M]^+$ (13), 571 [M? $CH_2CO - CO]^+$ (7), 391 [571-3 × HOAc]$^+$ (3), 328 [571–2 × HOAc–$C_5H_4NCOOH]^+$ (7), 124 $[C_6H_4NCOOH+H]^+$ (100), 123 $[C_8H_{11}O]^+$ (7), 96 $[C_7H_{12}]^+$ (27). HRMS: *m/z* 641.2889 $[M]^+$ $C_{34}H_{43}NO_{11}$ required 641.2836. ^1H and ^{13}C NMR: see Table 1.

Acknowledgements

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VI

A Novel Lathyrane Diterpenoid from the Roots of *Euphorbia lathyris*

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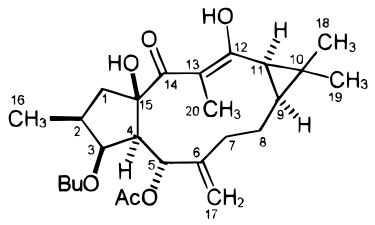
A new lathyrane diterpene (**1**) has been isolated and characterized from a CH_2Cl_2 extract of the roots of *Euphorbia lathyris*. Detailed spectral analysis revealed that the structure of **1**, including relative stereochemistry, is that of a diester of a hitherto unknown, polyfunctional diterpene parent alcohol.

Euphorbia lathyris L., or caper spurge (Euphorbiaceae), is widely distributed throughout the Mediterranean region of Eastern and Middle Europe.[1] 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.[2−4] 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.[3] Besides ingenol esters, a series of diterpenes based on the lathyrane skeleton have also been isolated (L_1−L_3, L_7, L_8) from the seeds.[2,5−9] The roots of *E. lathyris* have been less well investigated to date; only the isolation of a lathyrane diterpenoid, jolkinol B, was reported earlier.[4]

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 diterpene 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**.



1

Compound **1** was shown by HREIMS to have the molecular formula $C_{26}H_{38}O_7$. Its UV spectrum displayed a maximum at 280 nm, indicating an enone chromophore in the molecule. The ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed the presence of one acetate group [δ_H 2.08 s; δ_C 170.4 (*C*O) and 21.0 (*C*H$_3$)] and one butanoate group [δ_H 2.36 m (H-2′), 1.66 m (H-3′), and 0.81 t (C-4′); δ_C 173.8 (C-1′), 35.9 (C-2′), 18.1 (C-3′), and 13.2 (C-4′)]. Characteristic signals at δ_H 1.10 dd, 1.50 dd, 1.06 s, and 1.05 s and at δ_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.[10] 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 CH_2−CH(CH_3)−CHR−CH−CHR− (A) and −CH_2−CH_2−CH−CH− (B) (R = acyl) in **1**, which represented the structural moieties C-1−C-5 and C-7−C-11 of a lathyrane diterpene, respectively. In position C-6, an exomethylene group was concluded on the basis of the singlet signals at δ_H 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 (δ_H 1.91 s) and two broad signals at δ_H 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

Table 1. NMR Spectral Data of Compound **1**[a]

position	^1H[b]	^{13}C	HMBC (H→C)
1α	3.47 m	50.3	
1β	2.04 t (12.3)		2, 15, 16
2	2.4 m	37.9	
3	5. 83 t (3.6)	79.9	1′, 15
4	3.01 dd (3.6, 10.5)	53.4	6, 14
5	6.56 d (10.5)	67.5	6, AcCO
6		149.8	
7a	2.54 dd (15.0, 5.4)	35.3	
7b	2.13 m		
8	1.94 m (2H)	21.6	
9	1.10 dd (15.3, 8.3)	35.9	
10		25.2	
11	1.50 dd (11.8, 8.3)	28.3	13, 18
12		152.4	
13		134.2	
14		202.2	
15		88.1	
16	1.03 d (6.8)	14.1	1, 2, 3
17a	5.10 s	114.7	5
17b	5.07 s		5
18	1.06 s	28.3	9, 10, 11, 19
19	1.05 s	15.7	9, 10, 11, 18
20	1.91 s	12.2	12, 13, 14
12-OH	8.25 br s		11
15-OH	7.40 br s		

[a] Pyridine-d_5, TMS, δ (ppm), J = Hz. [b] Additional assignments: 3-*O*-butanoyl: δ_H 2.36 m (H-2′), 1.66 m (H-3′), 0.81 t (7.4) (H-4′); δ_C 173.8 (C-1′), 35.9 (C-2′), 18.1 (C-3′), 13.2 (C-4′); 5-*O*-acetyl: δ_H 2.08 s; δ_C 170.4 (*C*O), 21.0 (*C*H$_3$).

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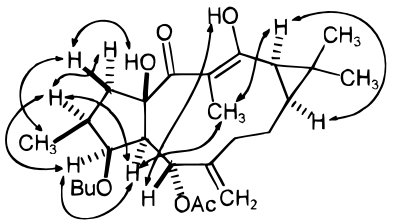

Figure 1. Major NOESY correlations of **1**.

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 δ_H 8.25 and 7.40 corresponded to hydroxyl groups because of their missing HMQC 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 δ_C 88.1 (C-15) and at δ_H 2.04 (H-1β) and 5.83 (H-3) proved that structural fragment A comprised the five-membered ring of **1**. The $^2J_{CH}$ and $^3J_{CH}$ 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 δ_C 202.2 and δ_H 3.01 (H-4) indicated that a keto group must be sited at C-14. This keto group showed a correlation in the HMBC spectrum with the singlet methyl signal at δ_H 1.91, indicating that C-20 is attached to a quaternary carbon. Further, long-range $^1H-^{13}C$ couplings from H-20 to the quaternary carbons at δ_C 152.4 (C-12) and 134.2 (C-13) and from H-11 to δ_C 134.2 (C-13) were observed, and thus the assignments of C-12 and C-13 could be determined. A hydroxy group (δ 8.25) was sited 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 (δ_H 8.25), simplification of the H-11 signal (δ_H 1.50 dd) 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 δ_C 173.8 (butanoyl *C*O) and 170.4 (acetyl *C*O) with the proton signals at δ_H 5.83 (H-3), 2.36 (H-2′), and 6.56 (H-5), and at δ_H 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-hydroxylathyrol.

The relative stereochemistry of **1** was studied by means of NOESY measurements. The trans-linked cyclopentane ring and the β-oriented OBu-3 and CH_3-16 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 α stereochemistry of H-9 and H-11, which is usual in lathyrane 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 lathyrane ester L_9, jolkinol B, and 15β-*O*-benzoyl-5α-hydroxyisolathyrol,[8,11,12] and the opposite configuration in esters L_1, L_2, and L_{7b}.[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 jatrophatrione by Torrance et al.[14]

Experimental Section

General Experimental Procedures. The optical rotation was determined in $CHCl_3$ 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 (^{13}C), using pyridine-d_5 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 GF_{254} 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 CH_2Cl_2 (3 × 0.2 L) and H_2O. On evaporation, the organic phase residue (4.27 g) was obtained, which was chromatographed on a polyamide column (30 g) with mixtures of H_2O–MeOH (4:1, 3:2, 2:3, and 1:4) as eluents. The fractions obtained with H_2O–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 $CHCl_3$–Me_2CO (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; $[\alpha]^{25}_D$ +76° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 218 (3.63), 280 (3.92) nm; ^1H and ^{13}C NMR, see Table 1; EIMS *m/z* $[M - CH_3]^+$ 447 (16), $[M - CH_3 - HOAc]^+$ 387 (36), $[M - CH_3 - HOAc - C_3H_7COOH]^+$ 299 (23), $[M - CH_3 - HOAc - C_3H_7COOH - H]^+$ 298 (100), $[C_3H_7CO]^+$ 71 (43), $[CH_3CO]^+$ 43 (100); HREIMS *m/z* 447.2299 (calcd for 447.2383 $C_{25}H_{35}O_7$) $[M - CH_3]^+$, 387.2165 (calcd for 387.2172 $C_{23}H_{31}O_5$) $[M - CH_3 - HOAc]^+$.

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VII

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Tetrahedron

New tri- and tetracyclic diterpenes from *Euphorbia villosa*

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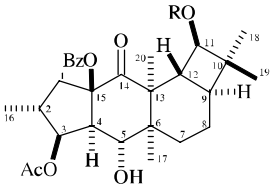
Abstract—Three new tetracyclic diterpenes were isolated from the chloroform-soluble extract of *Euphorbia villosa*, together with one new and one known lathyrane diterpene. The structures were elucidated by means of various spectroscopic methods, including HREI-MS, HRFAB-MS, UV, and 1D and 2D NMR techniques. 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 micractina*. 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.

1. Introduction

The plants of the family Euphorbiaceae (spurges) produce a high diversity of diterpenoids based on various macrocyclic and polycylic skeletons. Characteristic constituents of the family include the tigliane, ingenane and daphnane diterpenes, referred to overall as phorboids,[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, jatrophane, 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, PGE_2-inhibitory, multidrug-resistance-reversing, propyl endopeptidase-inhibitory and various vascular effects.[4–8] 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 pubescent, rhizomatous perennial plant widely distributed throughout Europe.[9] 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.



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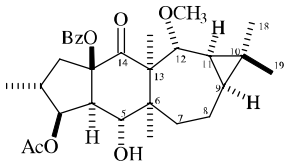

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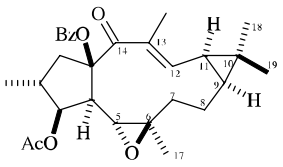

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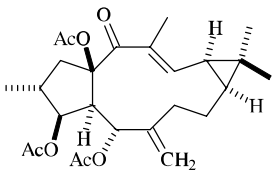

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2. Results and discussion

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** ($[\alpha]_D^{28}$=+148, *c* 0.2, $CHCl_3$) was found to be identical in all of its characteristics, including the ^1H and ^{13}C NMR spectral data, with the lathyrane diterpene isolated earlier from *Euphorbia hyberna*.[10]

Keywords: *Euphorbia villosa*; Euphorbiaceae; Terpenes and terpenoids; Lathyrane diterpenes; Tetracyclic diterpenes.
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Table 1. NMR spectral data of **1** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)

Atom	^1H	^{13}C	HMBC (H No.)	NOESY
1α	1.67dd (14.8, 9.6)	38.5	—	1β, 4, 16
1β	3.13dd (14.8, 8.0)	—	3, 16	1α, 2
2	2.25m	38.6	1α, 3, 16	1β, 3, 16
3	5.07dd (5.9, 1.7)	82.1	1β, 5, 16	2, 4, 16, OH
4	2.19m	54.1	1β	1α, 3, 17, OH
5	4.76dd (11.3, 3.1)	62.8	7α, 7β/4, 17	8β, 12, OH, 2′, 6′
6	—	47.4	5, 4/7β, 7α, 17, 20, OH	—
7α	1.16m	32.4	—	7β, 8α, 9, 17
7β	2.19m	—	8α, 8β, 17	7α, 8β
8α	1.42m	21.8	—	7α, 7β, 8β, 9, 18
8β	1.47dd (12.3, 3.2)	—	7α, 12	5, 7β, 8α, 12, 19
9	1.01dt (3.8, 12.3)	38.6	8β, 12, 18, 19	7α, 8α, 11, 20
10	—	41.9	9, 12, 18, 19	—
11	2.83d (8.9)	82.7	12, 18, 19, OMe,	9, 18, 20
12	2.65dd (12.3, 8.9)	46.9	9, 11	5, 8β, 19, 2′, 6′
13	—	56.1	11, 12, 17, 20	—
14	—	205.3	1α	—
15	—	91.2	1β, 3	—
16	1.16d (7.3)	19.8	1α, 3	1α, 2, 3
17	0.76s	17.8	5	4, 7α, 20,
18	0.94s	28.9	9, 11, 19	8α, 11, 19
19	0.38s	14.6	9, 11, 18	8β, 12, 18, 2′, 6′
20	1.14s	13.5	12	9, 11, 17
AcCO	—	172.3	3, AcMe	—
AcMe	2.29s	21.5	—	2′, 6′, 3′, 5′
BzCO	—	163.8	—	—
1′	—	131.6	—	—
2′, 6′	8.07d (7.3)	129.6	—	5, 12, 19, OMe, Ac
3′, 5′	7.41t (7.7)	127.8	—	Ac
4′	7.53t (7.4)	132.6	—	—
OH	2.95d (3.1)	—	—	3, 4, 5,
OMe	2.86s	56.9	12	2′, 6′

2.1. Structure of compound 1

Compound **1** was isolated as colourless prisms with $[\alpha]_D^{28}=-3$ (c 0.1, $CHCl_3$). The molecular formula $C_{30}H_{40}O_7$ was established by HREI-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 (δ_H 2.29s; δ_C 172.3 and 21.5) and one benzoate group (δ_H 8.07d, 7.53t and 7.41t; δ_C 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 (δ_C 205.3). The gradient ^1H–^1H COSY and HMQC spectra demonstrated two 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, 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 $^2J_{CH}$ and $^3J_{CH}$ correlations between H-1α and the carbon signal at δ_C 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 δ_C 172.3 (acetyl *C*O) with the proton signal at δ_H 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 (δ_H 2.95d) was coupled to H-5 (δ_H 4.76dd), with J=3.2 Hz, while in the HMBC spectrum, the hydroxy 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 benzoyl group was placed at the quaternary C-15. The relative

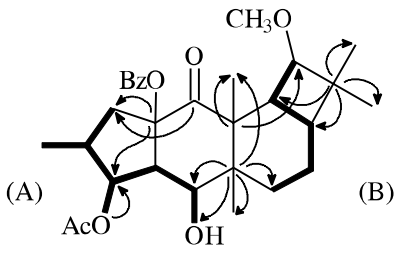

Figure 1. Selected ^1H–^1H-COSY (▬) and HMBC (C→H) correlations for **1**.

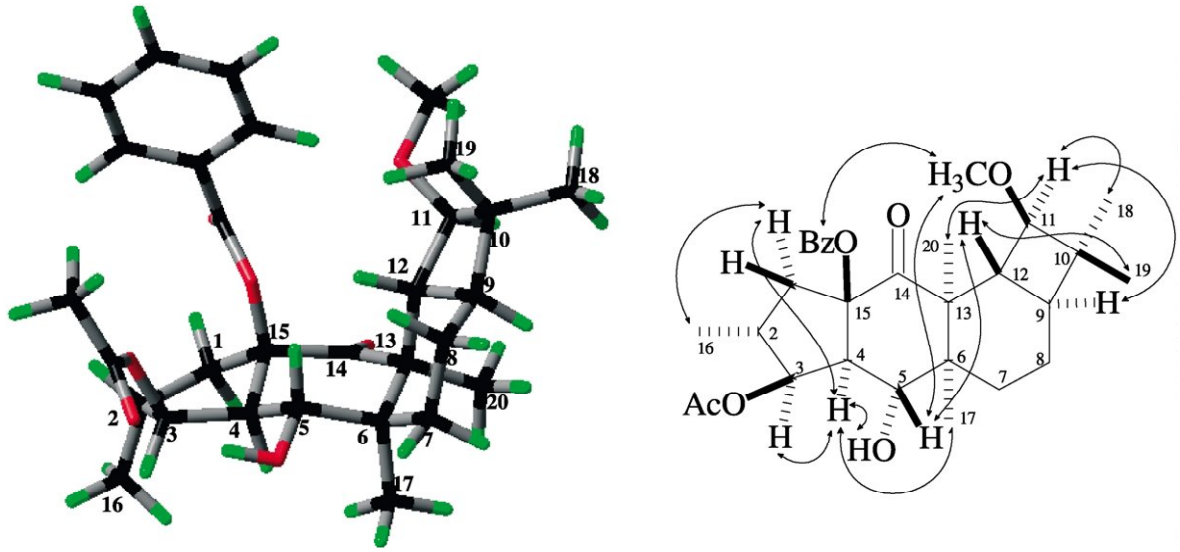

Figure 2. Calculated conformation and significant NOESY correlations (↔) of compound **1**.

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-OCH_3 and H-12 and H-19 demonstrated the β position of H-12, 11-OCH_3 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 PM3[11,12] package of the HyperChem program[13] (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 ($\delta_{H\text{-}19}$ 0.38s) 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 ^{13}C chemical shift assignments were determined as listed in Table 1. Compound **1** has a tetracyclic 5-6-6-4 fused ring system. A similar diterpene 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 $[\alpha]_D^{28}$=+16 (*c* 0.033, $CHCl_3$). The HRFAB-MS suggested that the molecular formula is $C_{29}H_{39}O_7$, 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 (δ_H 2.32s; δ_C 172.1 and 21.5) and one benzoyl group (δ_H 8.12d, 7.58t and 7.44t; δ_C 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 (δ_C 205.3) (Table 2). The ^1H–^1H COSY spectrum indicated two sequences of correlated protons: $-CH_2-CH(CH_3)-CH(OR)-CH-CH(OR)-$ and $-CH_2-CH_2-CH-CH-CH(OR)-$ (R=H or acyl). After the ^1H and ^{13}C 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**: $\delta_{C\text{-}11}$ 73.6; **1**: $\delta_{C\text{-}11}$ 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-1β/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 $[\alpha]_D^{28}$= 38 (*c* 0.1, $CHCl_3$). The molecular formula $C_{30}H_{40}O_7$ 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 δ_C 20.4 and

Table 2. NMR spectral data of **2–4** in $CDCl_3$ (δ in ppm, multiplicities, J in Hz)

Atom	2		3		4			
	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H[a]	^{13}C[a]
1α	1.76dd (14.9, 9.4)	39.3	1.70m	38.1	3.09dd (14.4, 4.4)	43.2	2.44dd (14.7, 7.8)	43.8
1β	2.81dd (14.9, 8.1)		3.34dd (14.8, 8.0)		2.31dd (14.4, 7.9)		3.34dd (14.7, 5.5)	
2	2.3m	39.0	2.20m	38.5	2.37m	38.3	2.33m	39.2
3	5.11dd (6.0, 1.7)	82.2	5.03d (5.7)	82.1	5.16dd (5.6, 2.4)	83.4	5.45dd (5.9, 3.0)	83.9
4	2.22m	53.8	2.20m	54.4	1.94m	48.6	2.13dd (9.3, 6.0)	49.5
5	4.73d (11.2)	63.0	4.57brd (10.8)	62.7	3.53d (9.2)	57.8	3.78d (9.3)	58.4
6	—	47.7	—	46.8	—	63.4	—	63.4
7α	1.16m	32.5	2.07dd (14.7, 7.2)	33.8	1.99m	38.7	1.75brt (12.3)	39.7
7β	2.21m		1.43t (13.3)		1.61m		1.58m	
8α	1.44m	22.0	1.69dd (15.0, 9.6)	19.0	1.99m	23.1	1.58m	23.7
8β	1.44m		1.25m		1.34m		1.34m	
9	1.13m	39.1	0.76m	26.3	1.08m	34.3	0.59m	34.3
10	—	41.6	—	20.4	—	26.2	—	25.9
11	3.35d (8.6)	73.6	0.32t (9.5)	26.8	1.45dd (11.2, 8.0)	29.6	1.16dd (11.1, 8.0)	29.9
12	2.47dd (12.1, 8.6)	48.5	3.99d (9.5)	76.7	6.93dd (11.2, 0.6)	144.5	7.03m	143.4
13	—	56.4	—	63.7	—	134.6	—	135.9
14	—	205.3	—	203.5	—	194.4	—	194.4
15	—	92.1	—	91.6	—	93.3	—	94.3
16	1.18d (7.3)	19.8	1.16d (7.3)	19.7	1.06d (7.0)	17.7	0.99d (7.1)	18.0
17	0.77s	17.8	0.76s	19.4	1.15s	20.1	1.05s	20.7
18	0.91s	27.8	0.96s	27.5	1.03s	29.0	0.79s	29.1
19	0.37s	14.2	0.79s	16.2	0.29s	14.8	0.29s	15.4
20	1.16s	13.6	1.25s	12.5	1.90s	12.5	1.96s	13.2
AcCO	—	172.1	—	172.5	—	169.9	—	169.6
AcMe	2.32s	21.5	2.21s	21.4	2.16s	21.3	1.84s	21.2
BzCO	—	166.3	—	163.2	—	164.9	—	165.2
1′	—	130.6	—	131.2	—	130.2	—	131.6
2′, 6′	8.12d (7.2)	130.0	8.11d (7.7)	129.8	8.04d (7.1)	129.6	8.12d (7.2)	130.2
3′, 5′	7.44t (7.7)	128.3	7.43t (7.7)	128.3	7.47t (7.7)	128.7	7.11t (7.4)	129.1
4′	7.58t (7.4)	133.4	7.57t (7.5)	133.1	7.60t (7.5)	133.6	7.03m	133.8
OH	2.84s	—	3.01s	—	—	—	—	—
OMe	—	—	2.52s	54.1	—	—	—	—

[a] In C_6D_6.

two methines at δ_H 0.76 and 0.32, and δ_C 26.3 and 26.8 indicated a *gem*-dimethyl-substituted cyclopropane ring.[10] The gradient ^1H–^1H COSY and HSQC spectra identified two isolated fragments of correlated protons: $-CH_2-CH(CH_3)-CH(OR)-CH-CH(OR)-$ and $-CH_2-CH_2-CH-CH-CH(OR)-$ (R=H, acyl or methyl). One signal at δ_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 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. Starting from the α position of H-3, the nuclear Overhauser effects between H-3 and 5-OH and H-3 and H-16 were indicative of the α position of the hydroxy group at C-5 and the methyl group at C-2. The NOESY correlations between H-16 and H-1α allowed the conclusion that this H-1 is α-oriented. The NOE effects between H-5 and H-8β, and H-5 and H-12 suggested the β position, and that between H-8α and H-7α the α configuration of these protons. The β orientation of the 19-methyl group followed from the nuclear Overhauser effects between H-12 and H-19. The cross-peaks of the benzoyl protons (H-2′, 6′) with H-5, H-12 and H-19 suggested the β position of the benzoyl group. The α 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 ^1H signals of H-2, H-4 and 3-OAc at δ_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 α configuration of H-4. Further, the NOESY correlation of the overlapping signal at δ_H 2.20 with that of H-17 can most probably be assigned to the H-4/H-17 correlation, suggesting the α 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 $[\alpha]_D^{28}=$ 146 (*c* 0.1, $CHCl_3$), has the molecular formula $C_{29}H_{36}O_6$, determined via the molecular ion peak at *m/z* 480.2526 (M^+)

(Δ=3.0 ppm) in the HREI-MS. Analysis of the ^{1}H 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, δ_C 144.5) and five quaternary carbons, with one ketone (δ_C 194.4) (Table 2). Interpretation of the HSQC and ^{1}H–^{1}H COSY spectra led to the identification of three structural elements with correlated protons: $-CH_2-CH(CH_3)-$ (A), $-CH(OR)-CH-CH-$ (B) and $-CH_2-CH_2-CH-CH-CH=$ (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 (δ_H 6.93dd) and the carbon signal at δ_C 194.4 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.[15] The HMBC correlation between acetyl *C*O 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 NOESY experiment. Because of the overlapping of the proton signals in $CDCl_3$ solution, the ^{1}H 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 ^{1}H and ^{13}C 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-7β, H-5 and H-8β and H-5 and 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 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 NOESY correlations between H-17 and H-4, 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 ^{1}H and ^{13}C 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 epoxylathyrane enone (Euphorbia factor L_1) was performed.[16,17]

3. Experimental

3.1. General

Melting points are 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 (^{1}H) and 125 MHz (^{13}C). The signals of the deuterated solvents were taken as reference. Two-dimensional experiments were performed with standard Bruker software. Optical rotations were determined in $CHCl_3$ 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 GF_{254} 15 μm, Merck) were used. HPLC was carried out on a Waters Millipore instrument, with detection at 254 nm on LiChrospher Si 100 and LiChrospher RP-18 (5 μm, 200×4 mm) columns.

3.2. Plant material

E. villosa W. et K. was collected in Vácrátót, Hungary, in June 2000, and identified by Vilmos Miklóssy V. (Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót). 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 l) 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–H_2O (2:3, 3:2 and 4:1) as eluents. The fractions obtained with MeOH–H_2O (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–H_2O (8:2 and 9:1) as 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 t_R 16.6 min was subjected to NP-HPLC with cyclohexane–CH_2Cl_2–MeOH (70:50:1) as eluent to afford **2** (2.4 mg). The fractions obtained with MeOH–H_2O (4:1) were repeatedly chromatographed on a polyamide column with mixtures of MeOH–H_2O (3:2, 7:3 and 4:1) as eluents. The fractions obtained with MeOH–H_2O (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–H_2O (8:2) as eluent at a flow rate of 1.0 ml/min to afford compound **4** (10.4 mg, t_R=11.5 min).

3.3.1. Compound 1. Colourless prisms; mp 246–248 °C; $[\alpha]_D^{28}$=−3 (*c* 0.1, $CHCl_3$); UV (MeOH): 230 (3.68), 275 (2.91); HREI-MS *m/z* 512.2806 (M^+) (Δ=6.2 ppm). Calcd for $C_{30}H_{40}O_7$: 512.2774. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1.

3.3.2. Compound 2. An amorphous solid; $[\alpha]_D^{28}$=+16 (*c* 0.033, $CHCl_3$); UV (MeOH): 231 (3.49), 259 (3.08), 285 (2.43); HRFAB-MS *m/z* 499.2704 (MH^+) (Δ=1.6 ppm). Calcd for $C_{29}H_{38}O_7$: 498.2617. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$, $^aC_6D_6$) data, see Table 2.

3.3.3. Compound 3. An amorphous solid; $[\alpha]_D^{28}$=−38 (*c* 0.1, $CHCl_3$); UV (MeOH): 232 (3.51), 275 (2.68), 282 (2.67); HREI-MS *m/z* 512.2801 (M^+) (Δ=5.2 ppm). Calcd for $C_{30}H_{40}O_7$: 512.2774. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2.

3.3.4. Compound 4. An amorphous solid; $[\alpha]_D^{28}$=−146 (*c* 0.1, $CHCl_3$); UV (MeOH): 233 (336), 271 (3.17); HREI-MS *m/z* 480.2526 (M^+) (Δ=3.0 ppm). Calcd for $C_{29}H_{36}O_6$: 480.2512. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 2.

3.4. PC model

Molecule conformation of **1** was generated by the semi-empirical PM3[11,12] 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.

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