Department of Pharmacognosy
University of Szeged

Isolation and structure elucidation of compounds with antitumor activity from *Tamus communis* and *Xanthium italicum*

Ph.D. Thesis

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List of publications related to the thesis

1. Réthy B, **Kovács A**, Zupkó I, Forgo P, Vasas A, Falkay Gy, Hohmann J
   Cytotoxic phenanthrenes from the rhizomes of *Tamus communis*
   Planta Med. **72**, 767-770 (2006)

2. **Kovács A**, Forgo P, Zupkó I, Réthy B, Falkay Gy, Szabó P, Hohmann J
   Phenanthrenes and a dihydrophenanthrene from *Tamus communis* and their cytotoxic activity
   Phytochemistry **68**, 687-691 (2007)

3. **Kovács A**, Vasas A, Hohmann J
   Natural phenanthrenes and their biological activity
   Phytochemistry **69**, 1084-1110 (2008)

4. **Kovács A**, Vasas A, Forgo P, Réthy B, Zupkó I, Hohmann J
   Xanthanolides with antitumour activity from *Xanthium italicum*
   Z. Naturforsch,. C (in press)

CONTENTS

ABBREVIATIONS AND SYMBOLS

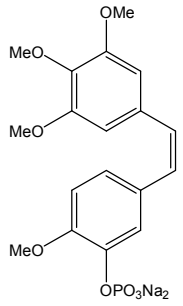
1D	one-dimensional
δ	chemical shift
2D	two-dimensional
A431	skin epidermoid carcinoma
COSY	correlated spectroscopy
CPC	centrifugal planar chromatography
cryst.	crystallization
EIMS	electron-impact ionization mass spectroscopy
fr	fraction
HeLa	cervix adenocarcinoma
HMBC	heteronuclear multiple-bond correlation
HPLC	high-performance liquid chromatography
HREIMS	high resolution electron impact mass spectrometry
HSQC	heteronuclear single quantum correlation
JMOD	*J*-modulated spin-echo experiment
MCF7	breast epithelial adenocarcinoma
MTT	[3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide]
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

1. INTRODUCTION

Natural products play a highly significant role in the drug discovery and development process. They not only serve as drugs or templates for drugs directly, but in many instances lead to the discovery of novel mechanisms of action that provide a better understanding of the targets and pathways involved in the disease process. Natural products have additionally been used as starting templates in the synthesis of combinatorial libraries.

Recent progress towards the discovery of drugs from natural product sources has resulted in compounds that are being developed to treat different diseases, especially cancer, resistant bacteria and viruses and immunosuppressive disorders. In the area of cancer chemotherapy, some 67% of the effective drugs may be traced to a natural origin, such as the vinca alkaloids vinblastine and vincristine, isolated from *Catharanthus roseus*; paclitaxel (Taxol), originally isolated from the bark of *Taxus brevifolia*, and the analogue, docetaxel; etoposide and teniposide, derived semisynthetically from epipodophyllotoxin, an epimer of podophyllotoxin, isolated from roots of *Podophyllum* species; camptothecin, isolated from the bark of *Camptotheca acuminata*, a precursor to the semisynthetic drugs topotecan and irinotecan.[1]

Currently one of the most interesting areas of antitumor drug research is the group of *cis*-stilbene combretastatins because of their potential use in cancer chemotherapy. The most potent member, combretastatin A-4, originally isolated from the southern African plant *Combretum caffrum*, in disodium phosphate (**1**) prodrug form is undergoing testing in clinical trials, and has been found to be effective against different solid tumors, including multidrug-resistant cancers.[2]



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The alkoxy-substituted phenanthrenes, which are conformationally restricted congeners of the antitumor *cis*-stilbenes, have not been investigated in detail, but on the basis of their structural similarity they can be regarded as promising anticancer agents.

Sesquiterpenes are another group of secondary plant metabolites which have been investigated extensively for their antitumor effects. These compounds are characteristic primarily for Asteraceae family. Some species of the genus *Xanthium* (Asteraceae) were used in traditional medicine for the treatment of basal cell carcinoma and different cancers and cold tumors, their extracts and sesquiterpene compounds exhibiting high activity against several human cell lines.[3-5]

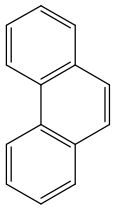
In recent years, HOHMANN *et al*. initiated a research programme at the Department of Pharmacognosy, in collaboration with the Department of Pharmacodynamics and Biopharmacy, University of Szeged, with the aim of the isolation and identification of antitumor compounds from medicinal plants. In the course of this program, a comprehensive antitumor screening of Hungarian Asteraceae species were carried out, and the active extracts were separated using bioassay-guided fractionation, yielding the compounds with tumor cell growth inhibitory effect. These studies also involved some promising species from other plant families. As part of these studies, my research activities have involved investigations of *Tamus communis* L. and *Xanthium italicum* Moretti. The present thesis summarizes the results of this phytochemical work.

2. OVERVIEW OF THE LITERATURE DATA

2.1. Structural characteristics of natural phenanthrenes and xanthanolides

2.1.1. Phenanthrenes

Compounds based on phenanthrene skeleton (**2**) are a rather uncommon class of aromatic metabolites which are presumably formed by oxidative coupling of the aromatic rings of stilbene precursors. A fairly large number of phenanthrenes have been reported from higher plants, mainly in the Orchidaceae family, in 49 species: in particular *Dendrobium*, *Bulbophyllum*, *Eria*, *Maxillaria, Bletilla*, *Coelogyna*, *Cymbidium*, *Ephemerantha* and *Epidendrum*. A few phenanthrenes have been found in the Marchantiacea, Hepaticae, Dioscoreaceae, Combretaceae and Betulaceae families. The phenanthrenes were mainly isolated from the whole plants, but in some cases the cortex, tubers or stems were studied and found to contain such compounds.



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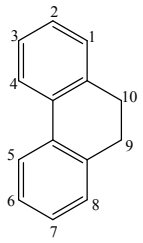

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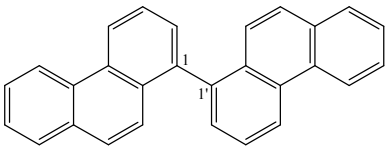
The phenanthrenes isolated so far may be classified into three major groups: monophenanthrenes, diphenanthrenes and triphenanthrenes. Monophenanthrenes are subdivided according to the number and type of the structural moieties, while diphenanthrenes can be classified by the type of connection of the phenanthrene units. Most natural phenanthrenes occur in monomeric form and almost the half of this group are only hydroxy- and/or methoxy-substituted, equally 9,10-dehydroderivatives (**2**) or 9,10-dihydrophenantres (**3**). Besides hydroxy and methoxy groups, further substituents can be found in monomeric phenanthrenes, such as methyl, hydroxymethyl, carboxy, formyl, prenyl, and vinyl, mainly in *Juncus* and *Stemona* species.



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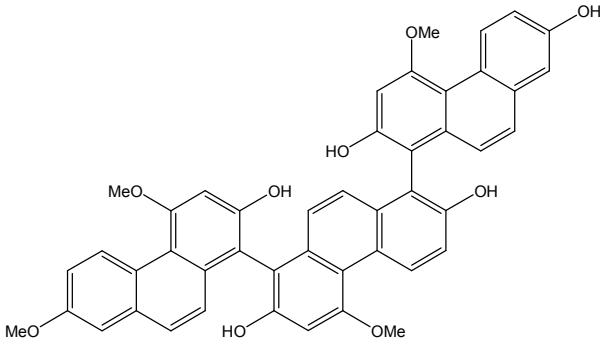

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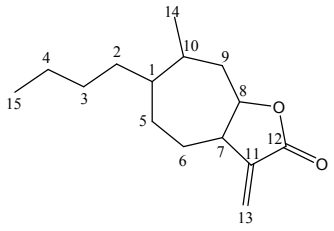
The dimeric phenanthrenes include 9,10-dihydro- and dehydroderivatives. The monomers are mostly 1-1'-linked (**4**), but 1-3', 1-8' and 3-3' linkages also occur in the natural compounds. They are usually hydroxy and methoxy-substituted. The only triphenanthrene (**5**) described so far was isolated from the tubers of an orchidaceous plant, *Cremastra appendiculata.*[6]

2.1.2. Xanthanolides

Xanthanolides (**6**), a bicyclic subtype of sesquiterpene lactones can be characterized by a 5,7-fused system containing a γ-lactone moiety and bearing a C_4 side-chain at C-1 and a C_1 unit at C-10. They can be formed from xanthane (**7**), which is an irregular sesquiterpene. These compounds have been reported from higher plants, mainly in genus *Xanthium*, in which sesquiterpenes having xanthanolide skeleton are widespread.



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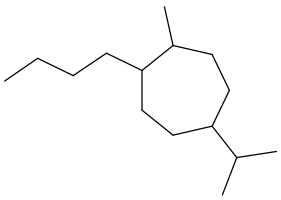

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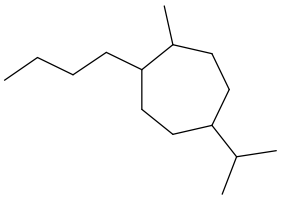
Xanthanolides usually have an unsaturated four-carbon side-chain attached to the cycloheptane ring. Sometimes 1,5-epoxy group is present, but mainly a double bond can be found in this position in the seven-membered ring. The xanthanolide skeleton has a lactone ring, conjugated with exomethylene group, but rarely at C-11 a methyl group can be found. In all isolated xanthanolides at C-10 is a methyl group. In the side-chain keto, hydroxy and acetyl groups may be present in positions C-2 and C-4, but α-β unsaturated carbonyl group also occurs.

2.2. Biological activity of natural phenanthrenes and xanthanolides

2.2.1. Phenanthrenes

Many phenanthrene-containing plants have been used in traditional medicine throughout the world, but mainly in China, and phytochemical-pharmacological investigations which have resulted in the identification of phenanthrenes as their active principles have provided support for the use of these plants in the ethnomedical practice.

In the 1970's at first the cell growth inhibitory activities of phenanthrenes from *Combretum caffrum* were tested on murine P388 lymphocytic leukaemia cell lines.[7] Later numerous plants and their constituents were investigated. Lusianthridin (**8**) and denbinobin (**9**) isolated from *Dendrobium nobile* were found to exert cytotoxic effects both *in vitro* and *in vivo* against A549 (human alveolar basal epithelial carcinoma); SK-OV-3 (human ovary adenocarcinoma) and HL-60 (human promyelocytic leukemia) cell lines.[8]



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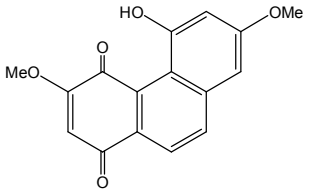

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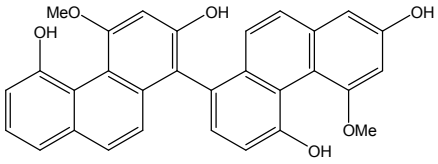

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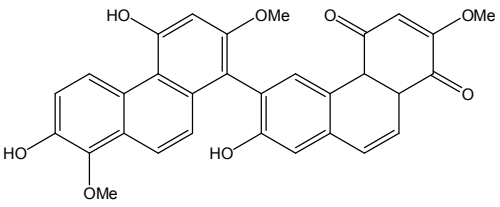

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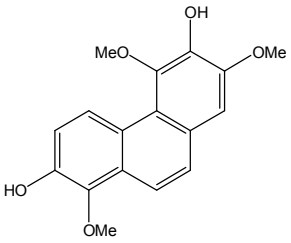
Five phenanthrenes, isolated from the stems of *Dendrobium thyrsiflorum,* were assayed against several tumor cell lines. Two dimeric phenanthrenes [denthyrsinol (**10**), denthyrsinone (**11**)] and denthirsinin (**12**) displayed significant cytotoxicity against HeLa, K-562 (human immortalised myelogenous leukaemia) and MCF-7 cells.[9] Moscatin (**13**) and hircinol (**14**) were less effective in killing HeLa and MCF-7 cells than denthyrsinol (**10**). Structure-activity relationship analyses revealed that the dimerization of phenanthrenes is a very important factor for the inhibition of cancer cell growth. This was supported by the investigation of mono-, bi- and

triphenanthrenes of *Cremastra appendiculata,* too. The isolated monophenanthrenes were inactive against all the tested cell lines (A549, A2780, Bel7402, BGC-823, HCT-8, MCF-7 and WISH). Biphenanthrenes and the unusual triphenanthrene (**5**) proved to be active compounds in this investigation.[6]



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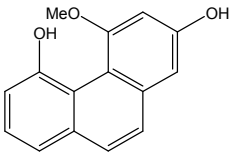

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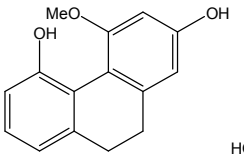

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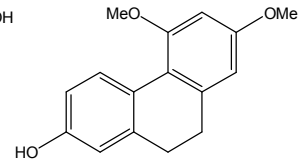

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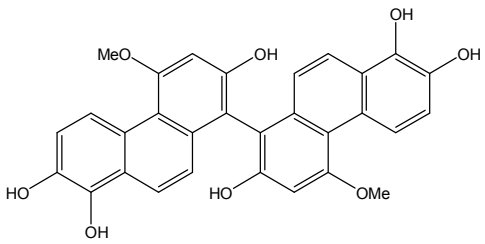


16

Compounds from *Domohinea perrieri* and hydroxybenzyl-phenanthrenes from *Spiranthes sinensis* were proved to be cytotoxic *in vitro* against a number of cancer cell lines.[10,11]

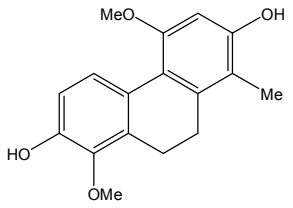
The accumulation of phytoalexins in plants is a response to infection by pathogenic fungi. Phytoalexins are utilized by plants to stop the growth of the attacking fungus. Orchid phytoalexins are phenanthrenes and dihydrophenanthrenes, as exemplified by orchinol (**15**) from *Orchis militaris* and hircinol (**14**) from *Loroglossum hircinum*.[12]

The tubers of *Bletilla striata* were investigated, because this has been used in traditional medicine in China to treat pneumonorrhagia and pneumonophthisis. While the methanol extracts of *B. striata* and lusianthridin (**8**) were mainly active against Gram-positive bacteria, but weakly active against certain fungi, the biphenanthrenes were active against the Gram-positive bacteria. Blestriarene B (**16**) exhibited the most potent activity against both test organisms.[13]

The dihydrophenanthrene stemanthrene D (**17**) from *Stemona collinsae* demonstrated a weak activity against the fungi. The antifungal properties of stemanthrenes A-D from *Stemona peirrei* were also investigated. In this study dihydrophenanthrenes exerted a weak activtity, similarly as in previous studies.[14,15] The virus replication inhibitory effects of phenanthrenes of *T. communis* have been tested on RNA enveloped virus:

vesicular stomatitis virus (VSV) and human rhinovirus serotype 1B (HRV 1B). The results of the screening revealed marked inhibitory action on plaque formation against VSV. On HRV, these compounds produced a low decrease in viral multiplication.[16]

Spasmolytic effects have been investigated in case of phenanthrenes isolated from *Scaphyglottis livida,*[17] *Maxillaria densa*[18] and *Epidendrum boothii.*[19] *S. livida* and *M. densa* are used by indigenous people in the tropical forests of Mexico. The ground herb of *S. livida* is applied to eliminate ectoparasites. The decoction is used for the treatment of stomach aches and to avoid abortion. *M. densa* is utilized for the same purposes. In all cases spasmolitic effect were confirmed.



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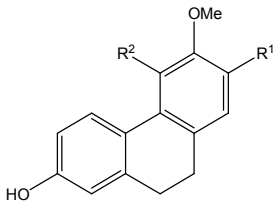
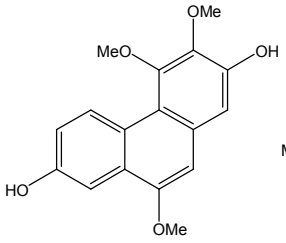


18 R^1=OH R^2=OMe
19 R^1=OMe R^2=OH



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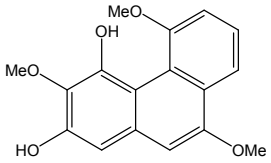


21

Denbinobin (**9**), a phenanthraquinone from *Dendrobium moniliforme,* showed *in vitro* anti-inflammatory activity. This compound significantly inhibited the formation of tumor necrosis factor α and prostaglandin E_2 in RAW 264.7 and N9 cells.[20] The tubers of *Gymnadenia conopsea* have been used also in traditional Chinese medicine for the treatment of asthma, neurasthenia and chronic hepatitis. The methanol extract of the tubers was found to shown an antiallergic effect on the ear passive cutaneous anaphylaxis reaction in mice.[21] Phenanthrenes of the *Stemona* species were tested in an *ex vivo* leukotriene biosynthesis inhibition assay. The potency of phenanthrenes in this test system suggests that these substances might be the anti-inflammatory and antiasthmatic principles of the *Stemona* species.[22] In 2006, four compounds were isolated from the rhizomes of *T. communis* by BORDAT *et al.* and their anti-inflammatory activities were analysed. The activity was evaluated from the production of prostaglandin PG6KF1-α.[23] An ethanolic extract of the stems of *E. lonchophylla* exhibited antiplatelet aggregation activity. CHEN *et al*. isolated erianthridin (**18**), and denbinobin (**9**) from the stems of this plant. Dose-response analyses of compounds indicated that inhibition against AA-induced aggregation was effective, and erianthridin (**18**) proved to be the most potent component.[24] *Dendrobium loddigesii* is

used with the same aims as *E. lonchophylla* in China. The active principle, moscatin (**13**), strongly inhibited both AA and collagen-induced platelet aggregation.[25]

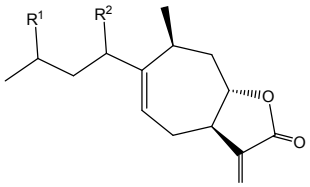
Gymnopusin (**20**) and erianthridin (**18**) from *Maxillaria densa* inhibited the radical elongation of *Amaranthus hypochondriacus* seedlings. Both phenanthrene derivatives exhibited moderate cytotoxicity towards all mammalian cells tested.[26] Compounds ephemeranthol-A (**19**) and fibrinol A (**21**) isolated from *E. rigidum* also demonstrated substantial phytotoxicity against *A. hypochondriacus*.[27]

The antialgal activities of 41 monomeric and 5 dimeric phenanthrenes were investigated against *Selenastrum capricornutum* by DELLAGRECA *et al*. in numerous studies.[28-32] It was observed that antialgal activities of the dimeric phenanthrenes were higher than those of the 9,10-dihydrophenanthrenes.

2.2.2. Xanthanolides

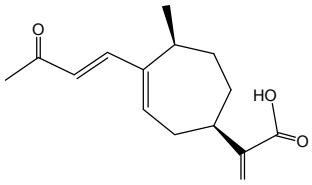
Xanthanolide sesquiterpenes have shown several biological activities such as antimicrobial toward methicillin-resistant *Staphylococcus aureus* (MRSA), chloroqiune-resistant *Plasmodium falciparum* or *Candida albicans*, cytotoxic activity toward human cancer cell lines or anti-ulcerogenic activity.

TSANKOVA *et al.* demonstrated that, xanthinin (**22**) and xanthatin (**23**) has significant activity against Gram-positive bacteria, and showed antiviral activity against influenza virus A, Newcastle diseases virus and pseudorabies virus A-2 strain.[33] LAVAULT *et al.* reported the antifungal screening of *Xanthium macrocarpum* and the isolation of xanthinosin (**24**), xanthatin (**23**), 4-hydroxyxanthinosin (**25**), xanthinin (**22**), 4-epixanthanol (**26**), 4-epiisoxanthanol (**27**), 2-hydroxyxanthinosin (**28**) and 4-oxobedfordia acid (**29**) against *Candida albicans*, *C. glabrata* and *Aspergillus fumigatus*. Xanthatin (**23**) and xanthinin (**22**) exhibited significant fungistatic activities with MIC 80 and 32 μg/ml, respectively. No significant fungicidal activities were revealed for these two compounds. The antileishmanial activity of the compounds was also tested on *Leishmania infantum* and *L. mexicana*. Five of the xanthanolides [xanthinosin (**24**), xanthatin (**23**), xanthinin (**22**), 4-epixanthanol (**26**) and 4-epiisoxanthanol (**27**)] were found to be leishmanicidal, and xanthinin (**22**) was the most active.[34]



	R^1	R^2
22	=O	OAc
23	=O	2,3-dehydro-
24	=O	H
25	OH	=O
26	β-OH	α-OAc
27	α-OAc	β-OH
28	=O	OH

In another study the antifungal activity of xanthatin derivatives were also investigated. Tomentosin (**30**), xanthumin (**31**) and 8-epi-xanthatin (**32**), isolated from aerial parts of *Xanthium strumarium*, showed antimalarial activity against chloroqiune-resistant *Plasmodium falciparum* strain.[35] SATO *et al.* isolated xanthatin (**23**) from the fruits of *X. strumarium*, as MRSA inhibitors. MIC of xanthatin (**23**) against twenty strains of MRSA and seven strains of MSSA (methicillin-susceptible *S. aureus*) was between 7.8-15.6 μg/ml.[36]



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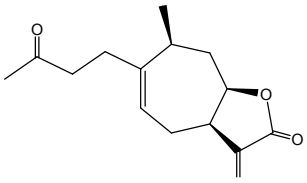

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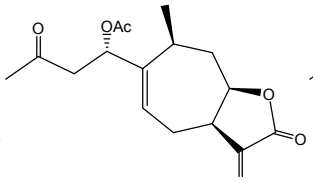

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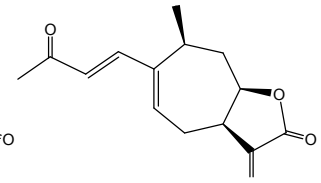

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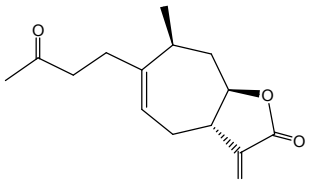
From *X. strumarium* not only antimicrobial compounds were isolated. In three studies sesquiterpene lactones were investigated against human tumor cell lines. Cytotoxicity guided fractionation of the plant led to xanthatin (**23**), 8-epi-xanthatin (**32**) and 8-epi-tomentosin (**33**) by AHN *et al*.[37] 8-Epi-xanthatin (**32**) was found to be more active than 8-epi-tomentosin (**33**), which lacks the conjugated enone moiety present in 8-epi-xanthatin (**32**).



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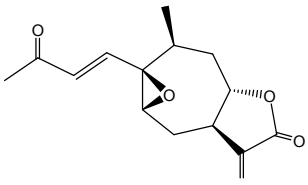


34

Generally, the exomethylene substituted lactone moiety regarded as an active group for cytotoxic properties. By KIM *et al*. 8-epi-xanthatin (**32**) and 8-epi-xanthatin-epoxide (**34**) were isolated and demonstrated a significant inhibition on A549, SK-OV-3, SK-MEL-2 (melanoma), HCT-15 (colon) and XF498 (CNS) cell lines *in vitro*.

In this study the inhibitory effect of the compounds on farnesyltransferase (FTase) were also examined and consequently both sesquiterpenes were found to inhibit remarkably FTase in a dose-dependent manner *in vitro*.[38] SATO *et al.* described xanthatin as an active compound with IC_{50} of 3.0, 2.2 and 1.5 μg/ml against MCF-7, A431 and HepG2 cell lines.[39]

Finally, the preventive effect of natural xanthanolides on ulcer formation induced by absolute ethanol in rats was examined and xanthatin (**23**) demonstrated the strongest protective activity.[40]

2.3. Botany of Tamus communis *and* Xanthium italicum

The black bryony*, Tamus communis* L. (Dioscoreaceae) belongs to a family of twining and climbing plants, which generally spring from large tubers. It is a very common plant in woods and hedges, with weak stems twining. The stem is up to 4 m, longitudinally striate, sometimes branched, glabrous. The leaves are heart-shaped pointed, smooth, dark shining green, with 3-9 curved and branched veins and long petiolate. Late in autumn they turn dark purple or bright yellow. In winter, the stems die down, though the root is perennial. The flowers are small, greenish-white, 3-6 mm in diameter. Flowers are in axillary racemes. Perianth of male flowers is urceolate-campanulate, with 6 subequal lobes; the numbers of stamens are 6, inserted at base of perianth. The perianth of female flowers is with 6 minute, narrow segments. The berry is 10-12 mm in diameter. The large, fleshy root is black on the outside and exceedingly acrid, nearly cylindrical, 2.5-4 cm in diameter, the length 7.5-10 cm or more. It occurs in oaken and gallery forest from North England to Iran.[41] The plant is covert in Hungary.[42]

X. italicum Moretti (nom. illegit. *Xanthium strumarium* subsp. *italicum* Moretti, *X. californicum* E.L. Greene, *X. echinatum* Murray, *X. strumarium* subsp. *cavanillesii* D. Löve&Dansereau) is an annual herb. Stems and branches are often with violet or brownish lines or dots. Capitula are solitary or in axillary clusters, unisexual, the males are above the females. Male capitula is subglobose; involucral bracts are in 1 row; the receptacle is cylindrical, with scales; florets are numerous; stamens are 5; anthers are free and hooked at apex, filaments are connate, style andovary rudimentary. Female capitula is ovoid, involucral bracts are in 2 rows, the outer is small, free, the inner is connacte, coriaceous, prickly, ending in 2 beaks and forming a

2-locular structure containing 2 florets. Involucre is 15-35 × 6-25 mm in fruit, yellow or brown when ripe, vovered with stout spines. It distributed from America over the whole world, mainly in meadow, flood plain, building operations. It is native to Hungary.[41,43]

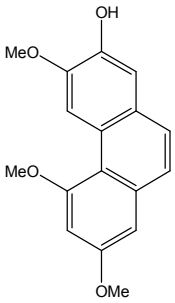
2.4. Chemical constituents of **Tamus communis** *and* **Xanthium italicum**

2.4.1. *Tamus communis*

Phytochemical investigation of *T. communis* started in the middle of the 1960's. At the Department of Pharmacognosy, Medical University of Szeged, BÁTHORI *et al.* isolated nine *N*-free phenanthrenes from the rhizome of *T. communis*, named Ta I – Ta IX (**35-42**).[44-49] These compounds were tetra- and pentasubstituted phenanthrenes with hydroxy- and methoxy substitution. Later LETCHER *et al*. revised the structures of some phenanthrenes after these compounds were synthetised.[50-52]

In the middle of the 1980's Italian researchers identified further dihydrophenanthrenes (**43-44**) from the petroleum ether extract of the rhizome.[16,53,54]

T. communis also contains spirostane and furostane glycosides as characteristic for the Dioscoreaceae family (**45a, 45b**, **46a-46d**).[16]



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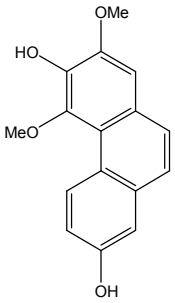

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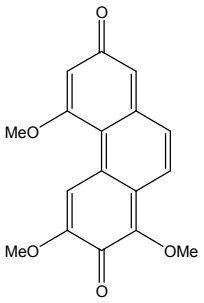

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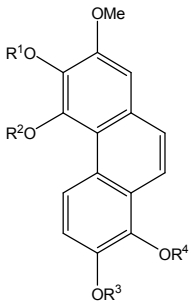
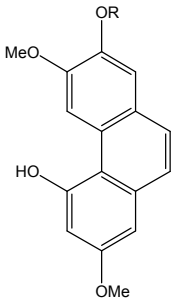
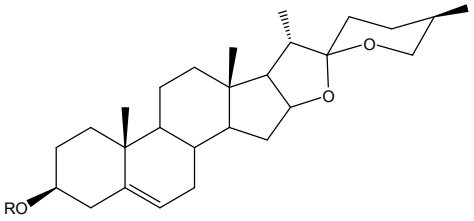


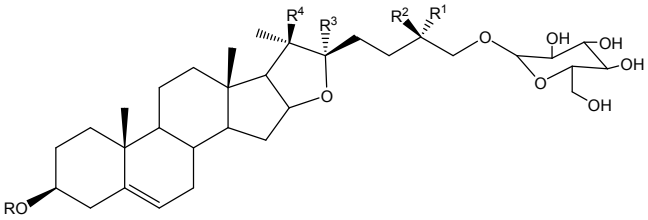
	R^1	R^2	R^3	R^4
38	H	CH_3	-CH_2-	
39	CH_3	CH_3	CH_3	H
40	CH_3	CH_3	-CH_2-	
41	CH_3	CH_3	CH_3	CH_3
42	CH_3	H	CH_3	H



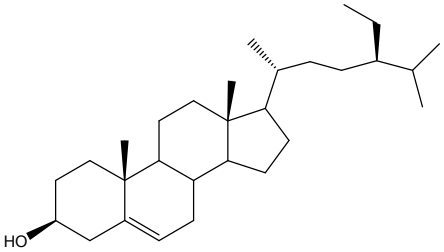


43 R=H
44 R=OH

45 a R=glu-rha$_2$
45 b R=glu$_2$-rha



	R	R^1	R^2	R^3
46 a	-glu-rha$_2$	H	CH_3	OCH_3
46 b	-glu-rha$_2$	CH_3	H	OCH_3
46 c	-glu-rha$_2$	H	CH_3	$\Delta^{20,22}$
46 d	-glu-rha$_2$	H	CH_3	OCH_3



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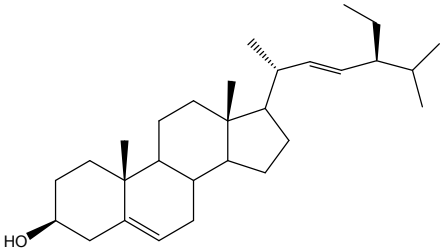

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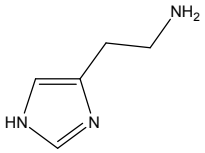
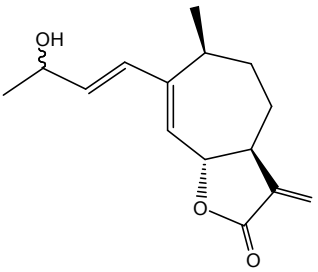


49

Other phytochemical investigations revealed the presence of sterols (**47**-**48**) and histamine (**49**) in the plant.[55,56]

2.4.2. *Xanthium italicum*

The isolation, synthesis and structure determination of xanthanolides started in 1957, but the phytochemical investigation of *Xanthium italicum* were first described only in 1993 by MARCO *et al.* and TSANKOVA *et al*. in the same time.[57,58] 4-*O*-Dihydroinusoniolide (**50**), deacetylxanthanol (**51**), xanthinosin (**24**), 4-epixanthanol (**26**) and 4-epiisoxanthanol (**27**) were isolated from the aerial part of the plant by MARCO *et al.*



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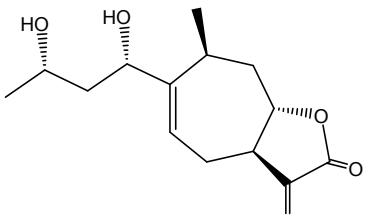

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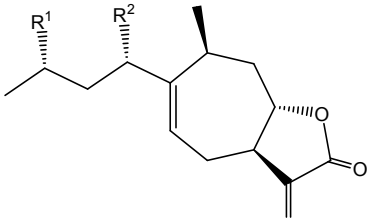


	R^1	R^2
52	OH	OAc
53	OAc	OH

TSANKOVA *et al.* reported the isolation of xanthinin (**22**) and five related compounds xanthatin (**23**), xanthinosin (**24**), xanthanol (**52**), isoxanthanol (**53**) and 2-hydroxyxanthinosin (**28**) from the leaves of *X. italicum,* while from the roots of this plant carbohydrates (glucose, sucrose), phytosterols (*β*-sitosterol, *β*-sitosterol-D-glucoside, stigmasterol, campesterol), triterpenoids (taraxerol, taraxerylacetate) and an eremophylane-type lactone, xanthanodiene were described.[33,58]

2.5. Folk-medicinal use of Tamus communis *and* Xanthium italicum

Both the rhizomes and the berries of *Tamus communis* have a reputation in folk medicine as effective rubefacients, and they have therefore traditionally been used in several countries for the treatment of rheumatism, arthrosis, lumbago and dermatosis.[59] Moreover, different parts of the plant have been applied in traditional medicine for the treatment of polyps and tumors.[60] Rhizomes of the plant cause irritation when rubbed on the skin.[61] The rash is apparently caused by mechanical irritation, being the result of penetration of the skin by acicular calcium oxalate crystals.

The genus *Xanthium* (Asteraceae) is represented by limited number of species (n=5), which are distributed in all parts of the world. Some species were used in the traditional medicine for the treatment of basal cell carcinoma and different cancers and cold tumors. *X. strumarium* is a species found abundantly throughout China and used in traditional Chinese medicine to treat nasal sinusitis, headache, urticaria, and arthritis.[62] In other countries, plants of the genus *Xanthium* are also used as diuretic and emetic agents, and are reported to be effective against prostata disease.[63]

3. AIMS OF THE STUDY

T. communis and *X. italicum* were used in the traditional medicine for the treatment of different types of cancers. Previous phytochemical studies revealed the presence of phenanthrenes in *T. communis*, and xanthanolides in *X. italicum*, but the chemistry and pharmacology of these plants were not investigated thoroughly.

The aims of the present work related to the bioassay-guided isolation, identification and antitumor evaluation of compounds derived from *T. communis* and *X. italicum*. In order to achieve these aims, the main tasks were:

- The collection of the plant materials
- The screening of *T. communis* and *X. italicum* for tumor cell proliferation-inhibitory activity
- The extraction of the plant materials
- The bioassay-guided fractionation of the active extracts and purification of the compounds responsible for the activity by means of combinations of various chromatographic methods (OCC, VLC, CPC, preparative TLC, HPLC and gel chromatography)
- The characterization and structure determination of the isolated compounds via spectroscopic techniques (UV, NMR and HREIMS)
- The evaluation of the pharmacological potential of the isolated compounds.

4. MATERIALS AND METHODS

4.1. Plant material

Tamus communis

The rhizomes and the herbs of the plant were collected in June 2003 in mountain Mecsek near Pécs, Hungary and identified by Prof. László Gy. Szabó (Department of Botany, Institute of Biology, University of Pécs). The 1200 g of fresh rhizomes was frozen and stored at -20°C until preparation. A voucher specimen (No. 619) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged.

Xanthium italicum

Plant materials were collected in August 2006 in Budaörs, and in September 2006 on the banks of the River Tisza in Szentes, Hungary, and identified by Dr. Anikó Böszörményi (Department of Plant Biology, University of Szeged). Voucher specimens (765 and 766) are deposited in the Herbarium of the Department of Pharmacognosy. The plant materials were dried at room temperature, yielding 1070 g of aerial part and 310 g of root from the first gathering, and 1020 g of leaf, 680 g of root, 150 g of flower and 870 g of stem from the second gathering.

4.2. Preparation of the extracts for antiproliferative screening

10 g of fresh rhizomes of *T. communis* were percolated with 100 ml methanol at room temperature. After concentration, 10 ml of water was added to the extracts (10 ml), and the mixture was subjected to solvent-solvent partitioning with 5 × 20 ml petroleum ether (40–60°C) and 5 × 20 ml chloroform. The fractions were concentrated, and the compounds were investigated by TLC using mobil phase A.

5 g of different parts of the dried and powered plant of *X. italicum* (root, flower, stem and leaf) were extracted with 50 ml methanol. The extracts were concentrated *in vacuo* to 5 ml, 5 ml water was then added, and subjected to liquid-liquid partition with *n*-hexane (3 × 10 ml) and chloroform (3 × 10 ml). The fractions were concentrated and monitored by TLC using mobil phase K.

The fractions obtained were tested for tumor cell growth-inhibitory effect against HeLa cell lines.

4.3. Extraction and isolation of phenanthrenes

4.3.1. Extraction

The frozen *T. communis* rhizomes (1200 g) were crushed with a Warning CB-6 blender (model 33BL13), and extracted with methanol (10 l) by percolation at room temperature. The extract were concentrated (to 500 ml) *in vacuo*, and extracted with 5 × 400 ml petroleum ether, and then with 5 × 400 ml chloroform.

X. italicum: The dried leaves (1020 g) of plant were crushed with Retsh rinder. The raw material was percolated with 10 l methanol at room temperature. After the methanol extract had been concentrated to 500 ml and 500 ml of water was added to the extract, solvent-solvent partition was performed with 5 × 1000 ml *n*-hexane and 5 × 1000 ml chloroform.

4.3.2. Isolation and purification of the compounds

The following chromatographic methods were used for the isolation of the compounds:

Vacuum-liquid chromatography: For VLC, silica gel 60 G (15 μm, Merck 11677) and the following experimental parameters were used.

VLC method-1: eluent: cyclohexane–ethyl acetate–ethanol gradient [9:1:0, 4:1:0, 7:3:0, 70:30:1, 70:30:2, 70:30:5, 5:5:1 (2100 ml each)]; volume of the fractions: 20 ml; sorbent: 60 g

VLC method-2: eluent: cyclohexane–ethyl acetate gradient [49:1, 19:1, 9:1, 85:15, 4:1, 1:1 (1800 ml each)]; volume of the fractions: 20 ml; sorbent: 60 g

VLC method-3: eluent: *n*-hexane–chloroform–methanol gradient [60:30:0.5, 60:40:0.7, 60:50:1, 60:50:1.5, 30:30:1, 6:6:1 (300 ml each)]; volume of the fractions: 10 ml; sorbent: 5 g

VLC method-4: eluent: chloroform–methanol gradient [100:0, 99:1, 49:1, 93:5 (120 ml each)]; volume of the fractions: 3 ml; sorbent: 5 g

VLC method-5: eluent: *n*-hexane–acetone gradient [9:1, 4:1, 7:3, 1:1 (500 ml each)]; and chloroform–methanol [9:1, 7:3, 1:1 (500 ml each)]; volume of the fractions: 100 ml; sorbent: 150 g

VLC method-6: eluent: toluene–ethyl acetate gradient [4:1, 7:3, 65:35, 3:2, 1:1, 2:3, (50 ml each)]; volume of the fractions: 5 ml; sorbent: 30 g

Open column chromatography: For OCC, ICN Polyamide (Lot23) was used. Eluent: methanol–water [4:1, 3:2, 2:3, 1:4 (1000 ml each)]; volume of collected fractions: 100 ml; stationary phase: 160 g

Centrifugal chromatography: CPC was performed on Chromatotron (Harrison Research) apparatus, using manually coated silica gel (60 GF_{254}, Merck) plates.

CPC method-1: eluent: cyclohexane–ethyl acetate gradient [9:1, 85:15, 4:1, 7:3, 1:1 (250 ml each)]; volume of collected fractions: 5 ml; thickness: 1.0 mm

CPC method-2: eluent: toluene–ethyl acetate gradient [9:1, 4:1, 7:3, 3:2, 1:1 (100 ml each)]; volume of collected fractions: 5 ml; thickness: 2.0 mm

Gel chromatography: Gel chromatography was performed on Sephadex LH-20 (Pharmacia Fine Chemicals) and methanol was used as mobil phase.

Thin layer chromatography: For preparative TLC silica gel 60 F_{254} (Merck 5715) was used as sorbent. Preparative separations were monitored in UV light at 254 nm. The compounds were eluted from the scraped adsorbent with chloroform. The OCC, preparative TLC, CPC and VLC fractions obtained, were monitored by analytical TLC on silica gel 60 F_{254} (Merck 5554).

Mobil phases of TLCs:

A cyclohexane–ethyl acetate–ethanol (20:10:1)

B benzene–diethylether–petroleum ether (2:1:1)

C chloroform–acetone (19:1)

D cyclohexane–ethyl acetate–ethanol (60:15:1)

E *n*-hexane–chloroform–acetone (70:40:3)

F *n*-hexane–chloroform–acetone (70:30:3)

G benzene–ethyl acetate (9:1)

H *n*-hexane–chloroform–acetone (50:49:1)

Visualization: UV light at 254 nm, spraying with conc. H_2SO_4, and then heating at 110 °C for 5 min.

High performance liquid chromatography: Instrument: Waters Pump 600E, Dual λ Absorbance Detector 2487, Injector Rheodyne 7725i. Detection was carried out at 254 and 280 nm. RP-HPLC was carried out on a LiChrospher 100 RP-18 (10 μm, 250×4 mm) reversed-phase (RP) column (Merck) using the following elutions:

HPLC method-1 eluent: methanol–water (7:3); flow rate: 1 ml/min

HPLC method-2 eluent: methanol–water (3:2); flow rate: 1 ml/min

NP-HPLC was carried out on a LiChrospher Si 60 (10 μm, 250×4 mm) normal-phase (NP) column (Merck) using the following elution:

HPLC method-3 eluent: cyclohexane–ethyl acetate–ethanol (20:10:1); flow rate: 1 ml/min

Mobil phases in all type of chromatography are given in terms of volume ratio *v*/*v*. Extracts and fractions were concentrated under vacuum with a Rotavapor RE (Büchi) rotary evaporation system, dipped in a water bath not warmer than 45°C. Compounds isolated from *T. communis* were marked in the experiment with TC-1–TC-11, and constituents of *X. italicum* as Xan-1–Xan-4.

UV spectroscopy: UV spectra were measured in methanol on a Shimadzu UV-201 PC spectrometer.

NMR spectroscopy: NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). The signals of the deuterated solvents were taken as reference (7.26 ppm in ^1H NMR and 77.0 ppm in JMOD). Two-dimensional experiments (^1H,^1H-COSY, NOESY, HSQC, HMBC) were set up, performed and processed with the standard Bruker protocol.

Mass spectrometry: HREIMS spectra were obtained on a Finnigan MAT 95S spectrometer.

4.4. Bioassays

Cytotoxic effects were measured *in vitro* on HeLa, MCF-7 and A431 cell lines, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. A limited amount of human cancer cells (5000/well) were seeded onto a 96-well microplate, which were allowed to adhere overnight before the drugs were introduced. The original medium was then removed, 200 μl culture medium containing the compounds of interest was added and the cells were incubated for 72 h. The tested extracts and compounds were dissolved in DMSO. The final concentration of DMSO never exceeded 0.3%, and therefore had no essential effect on the cell growth. Next the living cells were assayed: aliquots (20 μl at 5 mg/ml) of the MTT stock solution were pipetted into each well and reduced by viable cells to an insoluble formazan product during a further 4 h. After this contact period, the medium was removed and the formazan crystals were dissolved in 100 μl DMSO by gentle shaking for 60 min. Finally, the absorbance was measured at 545 nm with a microplate reader.[64] In this way the cell growth or drug toxicity was determined. The 50%

inhibitory concentration (IC_{50}) was derived from the dose–response curves fitted to the measured points by GraphPad Prism 2.01. All *in vitro* experiments were carried out on two microplates with at least five parallel wells. Cisplatin and doxorubicine were used as positive controls.

5. RESULTS

5.1. Screening of Tamus communis *and* Xanthium italicum *for antiproliferative activity*

The fresh rhizomes of *T. communis* were extracted with MeOH at room temperature. The MeOH extract was concentrated and extracted with petroleum ether and $CHCl_3$. The organic fractions were tested for their cytotoxic activity on the HeLa cell line, using the MTT assay. Both the petroleum ether and the $CHCl_3$ fractions exhibited high, concentration-dependent cytotoxic activity on HeLa cells at 0.2 μg/ml and 0.01 μg/ml concentration (Figure 1), and therefore both fractions were subjected to detailed phytochemical studies.

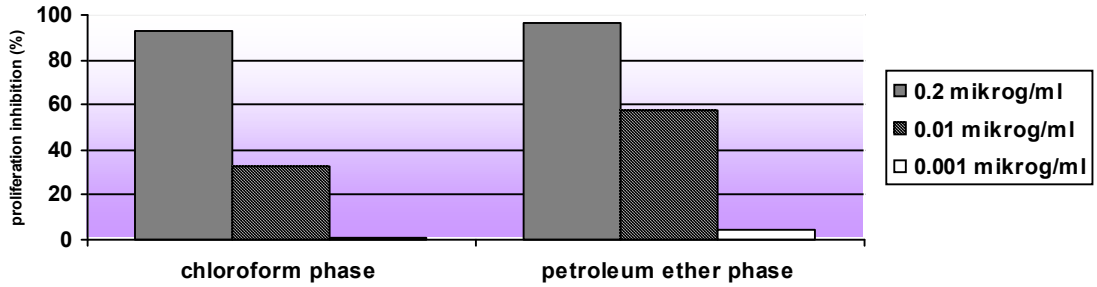
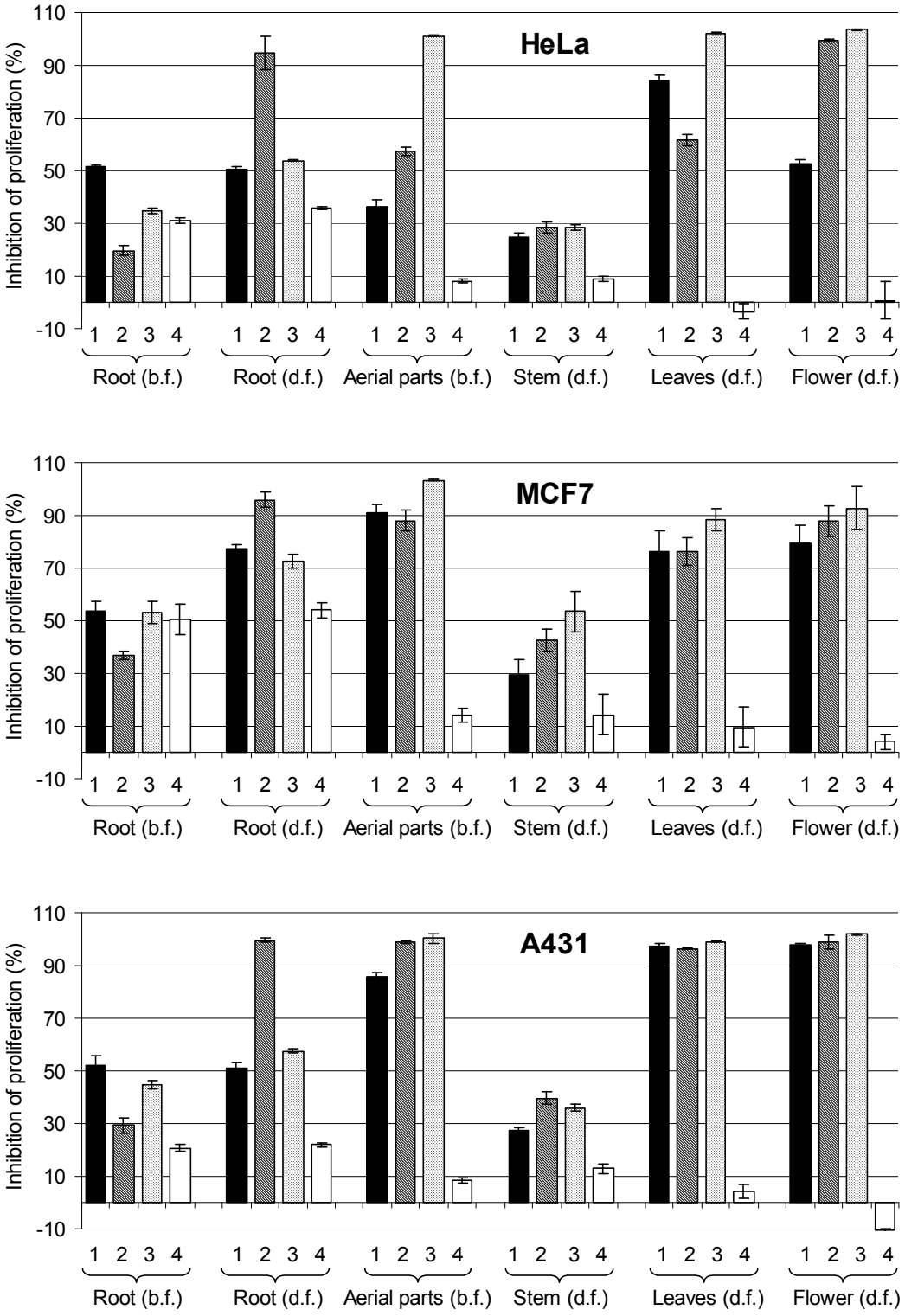


Figure 1. Antiproliferative activity of the $CHCl_3$ and petroleum ether fractions of the rhizome of *T. communis* on HeLa cells

Dried and powdered plant parts (root, flower, stem and leaf) of *X. italicum*, collected before and during the flowering period extracted with MeOH and after evaporation, the residues from the extracts were subjected to solvent-solvent partitioning. Extraction with lipophilic solvents yielded *n*-hexane-, $CHCl_3$- and H_2O-soluble phases. These fractions, together with the original MeOH extract, were tested for their tumor cell proliferation-inhibitory activities on the HeLa, A431 and MCF7 cell lines (Figure 2). It was observed that the active components predominantly accumulated in the leaves and flowers, especially in the *n*-hexane and $CHCl_3$ extracts. The extracts from the stems exerted only low effects (< 53%) on all three cell lines. The extracts from the roots in the flowering stage were more active than those from before the flowering period.



1: MeOH extract; 2: *n*-hexane fraction; 3: chloroform fraction; 4: water phase; b.f.: before flowering; d.f.: during flowering)

Figure 2. Antitumor effects of the extracts from different parts of *X. italicum* on human cell lines

The fractions with different polarities revealed that the $CHCl_3$ extracts exerted the highest activity, with exception of the root during flowering period, where the active compounds accumulated in the *n*-hexane extracts. The $CHCl_3$ extract of the leaves exhibited marked cell growth-inhibitory activities of 102.0, 88.5 and 99.1% on the HeLa, MCF7 and A431 cells, respectively, and this extract was therefore selected for detailed study.

5.2. Bioassay-guided isolation of phenanthrenes and xanthanolides

5.2.1. Isolation of phenanthrenes from *T. communis*

The frozen rhizomes of the plant were exhaustively percolated with MeOH at room temperature. Similarly as in the preliminary screen, the MeOH extract was concentrated and extracted with petroleum ether and $CHCl_3$. The extraction and isolation procedure, involving a combination of various chromatographic methods, is outlined in Figure 3. The chloroform fraction (2.03 g) was chromatographed by vacuum liquid chromatography (VLC) on silica gel, using VLC method-1, in order to separate the compounds according to their polarity. The fractions were monitored by TLC (mobil phase A) and, appropriate separation after combinations 12 main subfractions (I-XII) were obtained, and tested for their cytotoxic activity on the HeLa cell line (Figure 4). Subfractions IV, V, VIII and XII of the $CHCl_3$ phase were found to exert significant activity.

Subfraction IV was separated by gel chromatography on Sephadex LH-20 with MeOH, and then further chromatographed by HPLC (HPLC Method-1) to yield TC-3 (t_R=10.65, 6.0 mg) and TC-4 (t_R=25.52, 7.4 mg). Subfraction V (66.2 mg) was separated by VLC on silica gel (VLC method-3), using mixtures of *n*-hexane–$CHCl_3$–MeOH with increasing polarity. The fractions containing the main component were subjected to preparative TLC (PLC 1, mobil phase B) with benzene–diethyl ether–petroleum ether (2:1:1) as solvent system (R_f=0.5) and finally purified by RP-HPLC (HPLC method-2), affording TC-2 (t_R=25.97 min, 1.5 mg). Subfractions VII and VIII were separated in the same manner, first by VLC (VLC method-4) on silica gel, and then by preparative TLC (PLC 2, mobil phase C) on silica gel, with the use of $CHCl_3$–acetone (19:1) as mobile phase. These separations yielded TC-5 (R_f=0.53, 5.2 mg) and TC-6 (R_f=0.37, 20.6 mg). Upon standing, β-sitosterine (TC-1) crystallized from

subfraction III. Subfraction XII, which showed high cytotoxic activity was not investigated, because its small quantity and complexity.

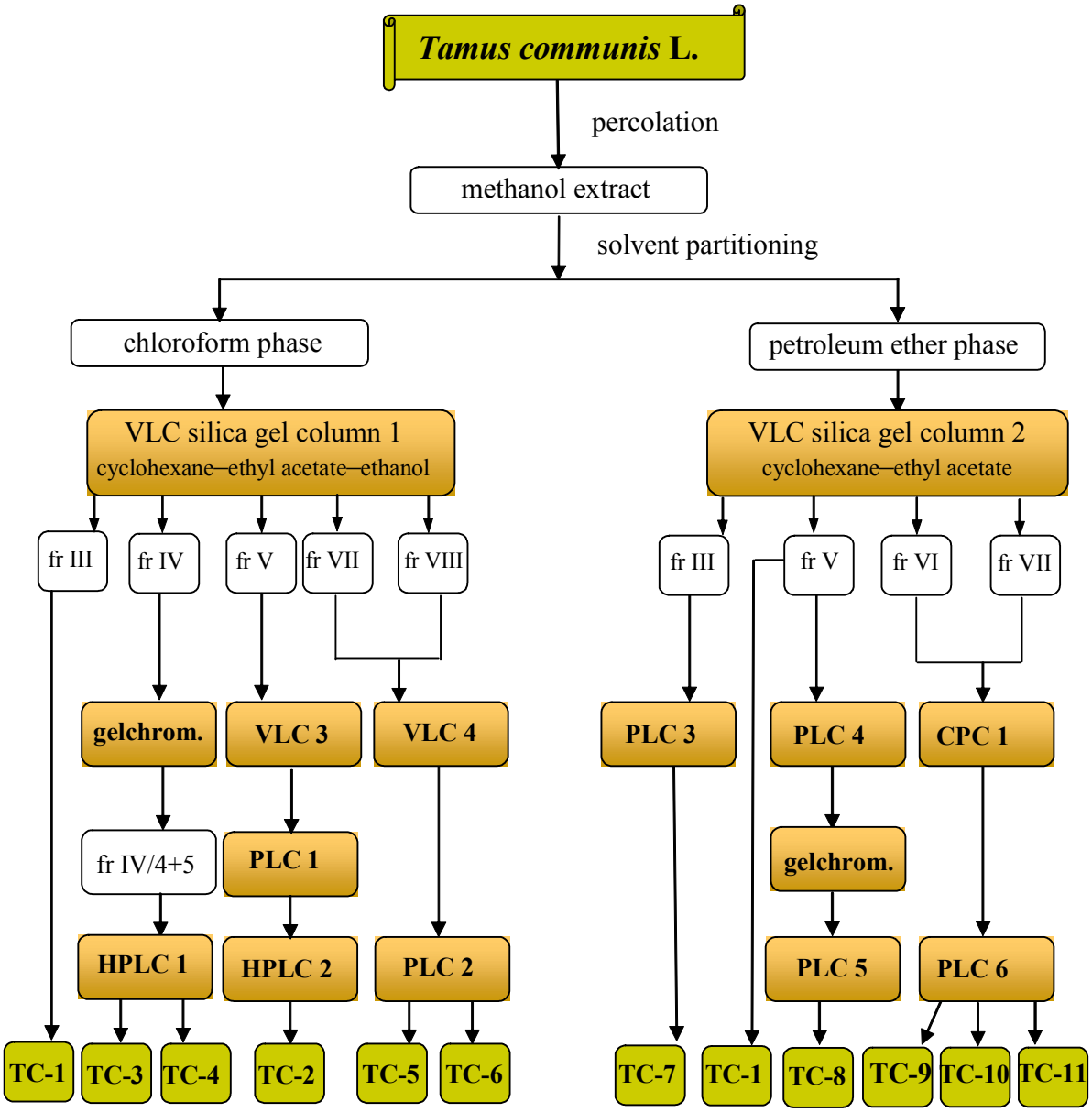


Figure 3. Isolation of phenanthrenes from *Tamus communis*

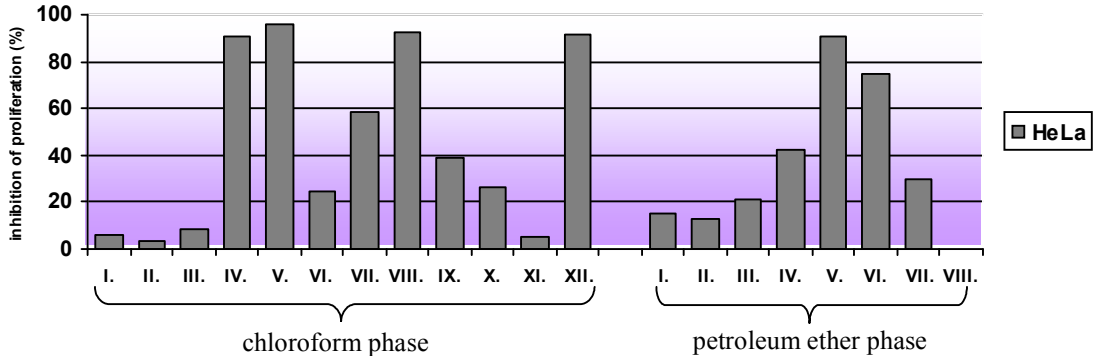


Figure 4. Antiproliferative activity of the VLC fractions on HeLa cell line

The petroleum ether phase (1.32 g) was chromatographed by vacuum liquid chromatography. The VLC chromatography using VLC method-2 was carried out with a gradient system of cyclohexane–EtOAc of increasing polarity as eluent. The collected fractions were combined on the basis of TLC (mobil phase D) monitoring, affording eight main subfractions (I–VIII). From the petroleum ether phase subfractions V and VI demonstrated high antiproliferative activity against HeLa cells (Figure 4.), but another subfraction (III) was also processed, because it contains chemically similar compounds, as detected by analytical TLC (Figure 3).

Subfraction III (53.8 mg) was separated by preparative TLC (PLC 3, mobil phase E) on silica gel with the use of *n*-hexane–$CHCl_3$–acetone (70:40:3) as mobile phase, yielding TC-7 (9.3 mg). Subfraction V (76.9 mg) afforded a considerable amount of a crystalline material, which was identified as *β*-sitosterine (TC-1). The remaining part of this subfraction was subjected to preparative TLC (PLC 4), using mobil phase F, and then further purified by gel chromatography on Sephadex LH-20, eluted with MeOH. Finally, the main compound was purified by preparative TLC (PLC 5) on silica gel with mobil phase G, to furnish TC-8 (2.6 mg). Subfractions VI (52.4 mg) and VII (138.5 mg) were processed in the same manner, first by centrifugal chromatography (CPC method-1), and then by preparative TLC (PLC 6, mobil phase H) on silica gel in two steps, to afford TC-9 (2.6 mg), TC-10 (16.3 mg) and TC-11 (10.5 mg).

5.2.2. Bioassay-guided fractionation and purification of xanthanolides

In order to identify the active components of *X. italicum* leaves, a large-scale isolation procedure was achieved. After extraction of the dried leaves (1020 g) solvent-solvent partitioning was performed with *n*-hexane and chloroform (see in *4.3.1*). The chloroform fraction (31.32 g) was chromatographed on a polyamide column with gradient system of MeOH–H_2O in order to remove chlorophyll from the extract (Figure 5).

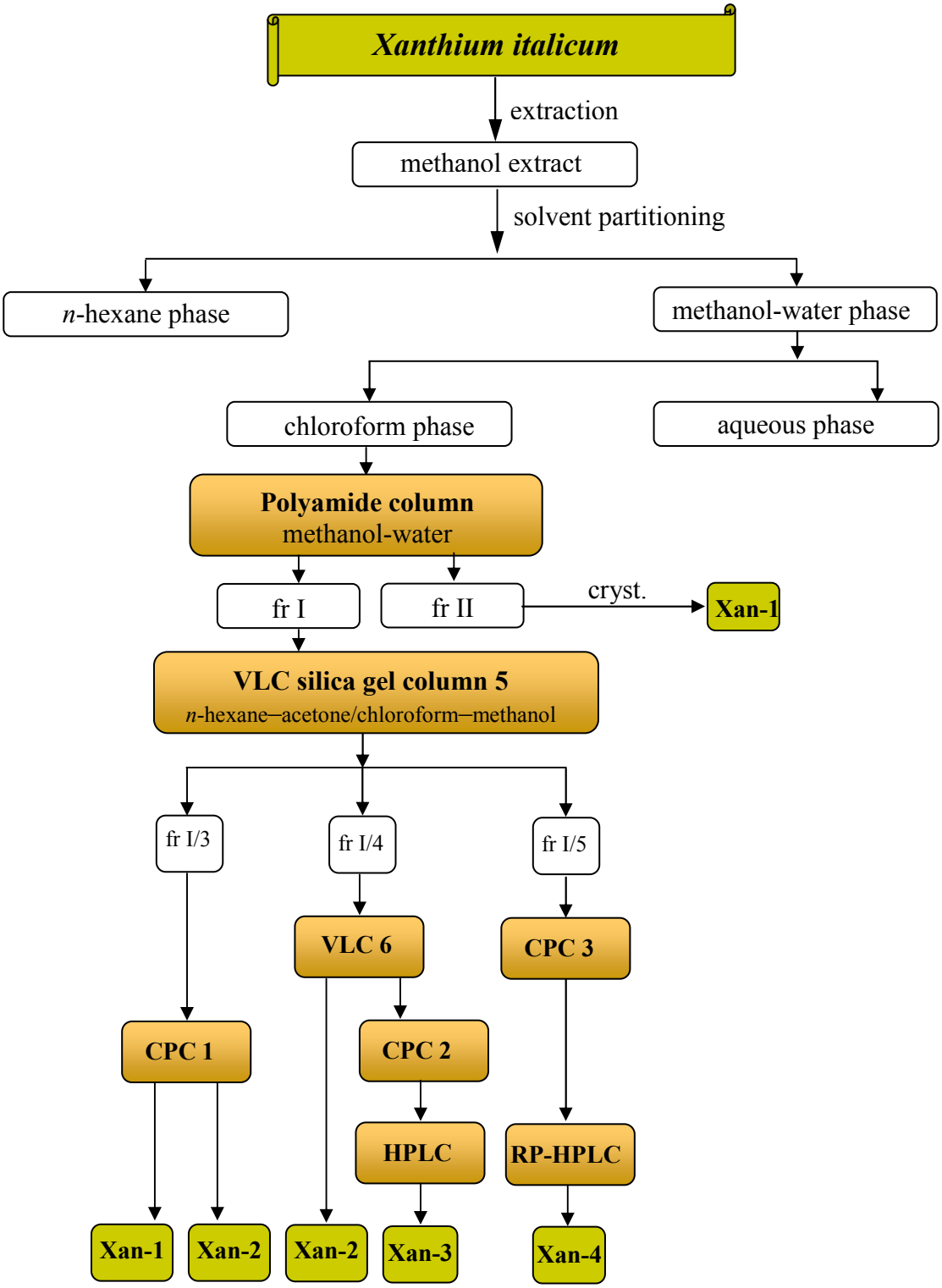


Figure 5. Isolation of xanthanolides from *Xanthium italicum*

The fractions were combined according to their composition, yielding subfractions I-V, among which subfractions I, II and IV exhibited cell growth-inhibitory activities between 85.0–97.2% on HeLa, 88.2–101.2% on A431 and 86.3–93.2% on MCF cell lines (Figure 6).

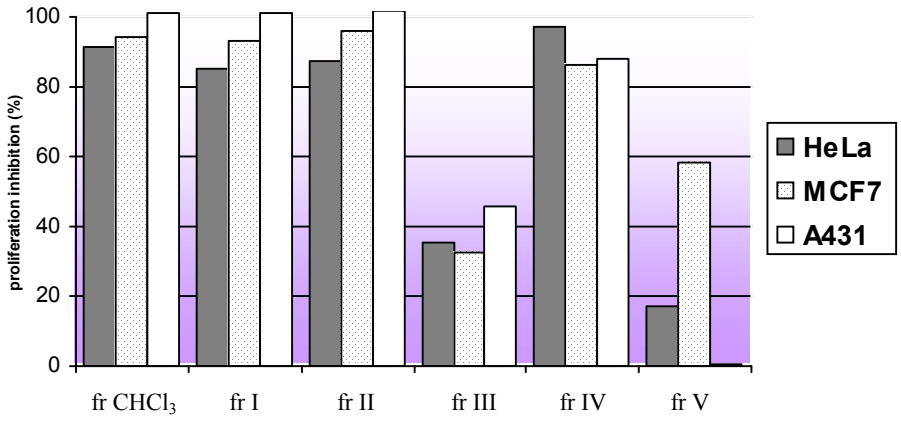


Figure 6. Antiproliferative activity of the $CHCl_3$ fraction of leaves on HeLa, MCF7 and A431 cell lines

In subfraction I sesquiterpene-type compounds were detected. Subfraction II afforded on standing crystalline material, which was recrystallized from methanol to yield Xan-1 (1.12 g). Subfractions I (10.22 g) was subjected to VLC (VLC method-5). The gradient elution with *n*-hexane–acetone and $CHCl_3$–MeOH on silica gel resulted after combinations ten subfractions I/1-10, according to their composition, as indicated by TLC (mobil phase A). The subfractions were tested again on HeLa, MCF-7 and A431 cell lines. Subfractions I/2, I/3, I/4 and I/5 showed significant activity (Figure 7).

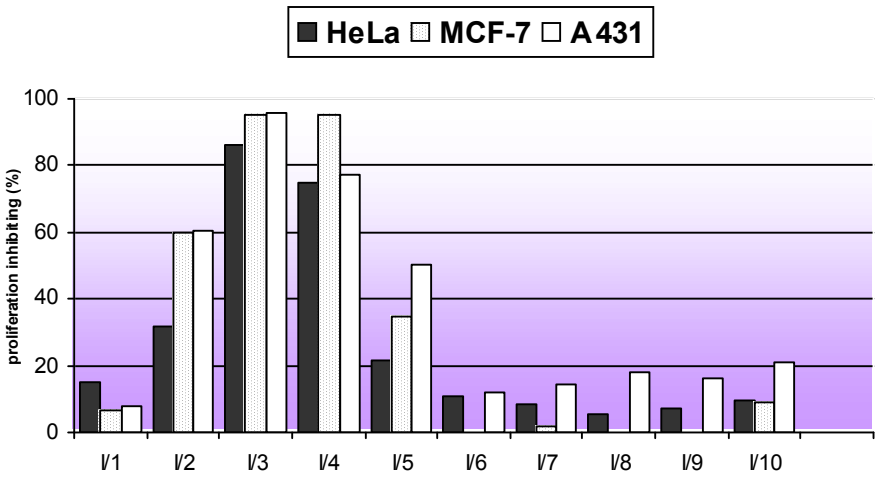


Figure 7. Antiproliferative activity of the VLC subfractions (I/1–I/10) on HeLa, MCF7 and A431 cell lines

Subfraction I/3 were separated by CPC using method-1, to yield Xan-1 (20.1 mg) and Xan-2 (5.4 mg).

Subfraction I/4 (3.39 g), obtained with *n*-hexane–acetone 7:3, were transferred repeatedly to a silica gel VLC (method-6) and eluted with toluene–EtOAc mixtures of increasing polarity. From subfractions I/4.1, 60.3 mg of Xan-2 was crystallized. The remaining part of this fraction were separated by centrifugal chromatography (CPC method-2) at first, using a gradient system of toluene–EtOAc and finally purified by NP-HPLC (method-3), to afford Xan-3 (42 mg). Subfraction I/5 (1.7 g) obtained with *n*-hexane–acetone 1:1, were further purified by CPC method-2 on silica gel using the gradient system of toluene–EtOAc and by RP-HPLC using HPLC method-2. The compound eluted at retention time of 9.56, yielded Xan-4 (10.8 mg).
Subfractions I/2 and IV, which showed pronounced cytotoxic activity was not investigated, because of its small quantity and complexity.

5.3. Characterization and structure determination of the isolated compounds

The structure elucidation was performed by means of HREIMS measurements, UV spectroscopy and detailed NMR studies.
The HREIMS spectra revealed the exact mass and molecular composition of the compounds. The most useful data on the chemical structures were obtained from advanced 1D and 2D NMR experiments, including ^{1}H NMR, JMOD, ^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC measurements.

5.3.1 Phenanthrenes and dihydrophenanthrene

Compounds isolated from *T. communis* proved to be phenanthrene derivatives. The UV spectral data of the compounds were very similar. The UV spectra of TC-2–TC-11 exhibited one definite absorption maximum at 259-268 nm and four-six maxima at 280–365 nm with lower intensity, characteristic of phenanthrene (TC-2–TC-7, TC-9–TC-11) and dihydrophenanthrene (TC-8). Some physical properties of the isolated compounds are listed in Table 1.

Table 1. Yields and physical data of the compounds isolated from *T. communis*

Experimental code	Structure	Yield (mg)	UV maxima λ (nm) (log ε)	Mp. (°C)
TC-2	**54**	1.5	259 (3.89), 283 (3.28), 292 (3.19), 303 (2.96), 347 (2.48), 364 (2.48)	amorphous solid
TC-3	**55**	6.0	259 (3.94), 283 (3.24), 292 (3,13), 303 (2,93)	185-187
TC-4	**56**	7.4		amorphous solid
TC-5	**57**	5.2	263 (3,96), 312 (3,08), 328 (2,40), 345 (2,54), 353 (2,34), 363 (2,58)	amorphous solid
TC-6	**58**	20.6		amorphous solid
TC-7	**40**	9.3	233 (4.54), 268 (4.81), 277 (4.69), 293 (4.18), 305 (4.08), 315 (3.94), 360 (3.57), 375 (3.64)	149-151
TC-8	**59**	2.6	218 (4.17), 268 (3.86), 277 (3.91), 303 (3.81), 313 (3.75)	amorphous solid
TC-9	**35**	2.6	234 (4.34), 251 (4.73), 261 (4.94), 284 (4.19), 320 (3.34), 330 (3.46), 345 (3.73), 362 (3.84)	176-177
TC-10	**38**	16.3	232 (4.52), 268 (4.81), 276 (4.70), 294 (4.10), 307 (4.06), 320 (3.97), 340 (3.55), 355 (3.77), 373 (3.81)	159-161
TC-11	**60**	10.5	233 (4.26), 263 (4.80), 287 (4.11), 294 (3.99), 308 (3.97), 331 (3.26), 352 (3.31), 365 (3.38)	183-186

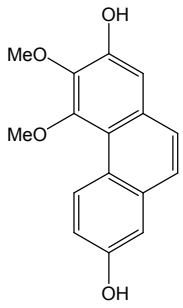
TC-2

TC-2 was obtained as yellowish-white amorphous solid and it was found to be identical in all of its characteristics, including the ^{1}H (Table 2) and ^{13}C NMR spectral data with nudol (**54**) (2,7-dihydroxy-3,4-dimethoxyphenanthrene), isolated earlier from *Bulbophyllum vaginatum,*[65] *Dendrobium rotundatum,*[66] *Eria carinata,*[67] *Eria stricta,*[67] *Eulophia nuda*[67,68] and *Maxillaria densa.*[18,69]

TC-3

TC-3 obtained as yellowish-white crystals, possessed the molecular formula $C_{17}H_{16}O_4$, as confirmed by HREIMS. The ^{1}H-NMR spectrum showed resonances for three methoxy groups [δ_H 4.01 (6H, s), 4.03 (3H, s)] and six aromatic protons (Table 2). One set of aromatic protons comprised an ABX spin system [δ_H 9.39 (1H, d, J=9.2 Hz), 7.19 (1H, dd, J=9.2, 2.6 Hz), 7.22 (1H, d, J=2.6 Hz)]. The most deshielded aromatic proton signal (δ_H 9.39) of this system was typical for H-5 of a phenanthrene, and therefore a 7-substituted ring C was concluded. The signals of a pair of *ortho*-coupled aromatic protons [δ_H 7.56, 7.52 (each 1H, each d, J=8.8 Hz)] were attributed on the basis of literature values to H-9 and H-10. Moreover, an isolated aromatic proton at δ_H 7.07 (1H, s) indicated a trisubstituted ring A. The substitution pattern of

TC-3 was further studied in a NOESY experiment. Nuclear *Overhauser* effects were detected between the signals of H-1 and H-10; H-10 and H-9; H-9 and H-8; and H-5 and H-6, demonstrating oxygen functionalities at C-2, C-3, C-4 and C-7. Besides the three methoxy groups, the presence of one hydroxyl group was deduced from the molecular composition. The location of two methoxy groups at C-2 and C-4 was evident from the NOESY cross-peaks obsrevered between the methoxy signals and the signals of H-1 and H-5. The third methoxy group was placed at C-3, and the hydroxy group at C-7, with regard to the absence of diagnostic NOEs between the methoxy group and H-6 and H-8. Furthermore, the chemical shifts of the aromatic protons were in good agreement with those published for phenanthrenes having the same functionalities on ring A or C. On the basis of these spectral data, the structure of TC-3 was elucidated as 7-hydroxy-2,3,4-trimethoxyphenanthrene (**55**). This compound is a new natural product.



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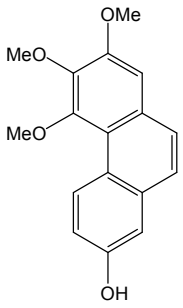

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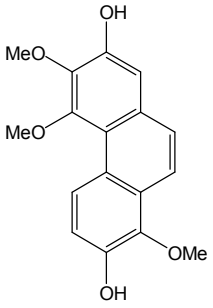

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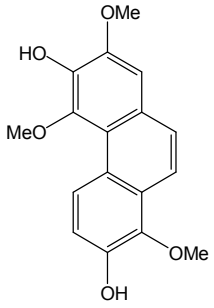

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TC-4

TC-4 was obtained as an amorphous solid. On comparison of its physical and spectral data (MS, NMR and NOESY) (Table 2) it was found to be identical with confusarin (**56**). This compound was obtained for the first time from *Tamus communis*, but previously described from different Orchideaceae species. Confusarine was isolated from *Bulbophyllum gymnopus,*[70,71] *B. reptans*[72] and *Eria confusa*.[73]

TC-5

TC-5 was isolated as yellowish white crystals. It was found to be identical in all of its characteristics [UV (Table 1), MS, NMR (Figure 8, 9) and NOESY] with those reported in the literature, for 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene (denthyrsinin) (**57**). This compound were obtained for the first time from *Tamus*

communis, but previously described from *Cymbidium pendulum*,[74] *Dendrobium densiflorum*,[75] *D. thyrsiflorum*,[9] *Eulophia nuda*,[68] *Nidema boothii*,[19] *Scaphyglottis livida*[17] and *Thunia alba*.[76] In 2007 RADIX *et al*. reported on the synthesis of this compound by radical cyclization from the corresponding stilbenes intermediates.[77]

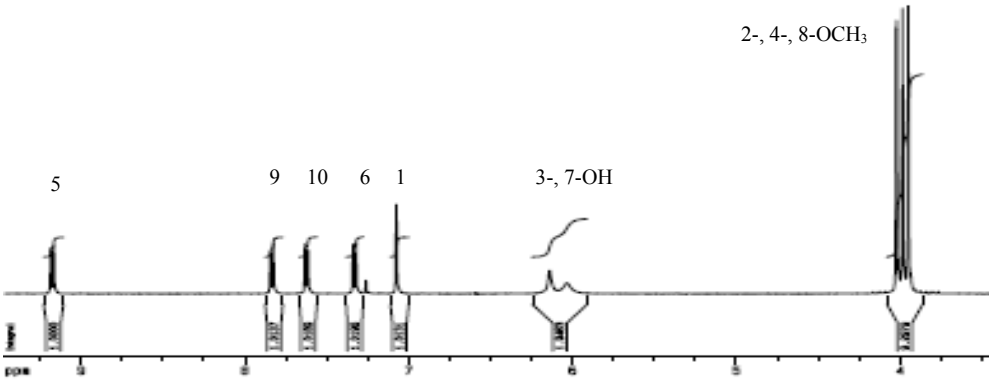


Figure 8. ^{1}H NMR spectrum of TC-5 (**57**) (500 MHz, $CDCl_3$)

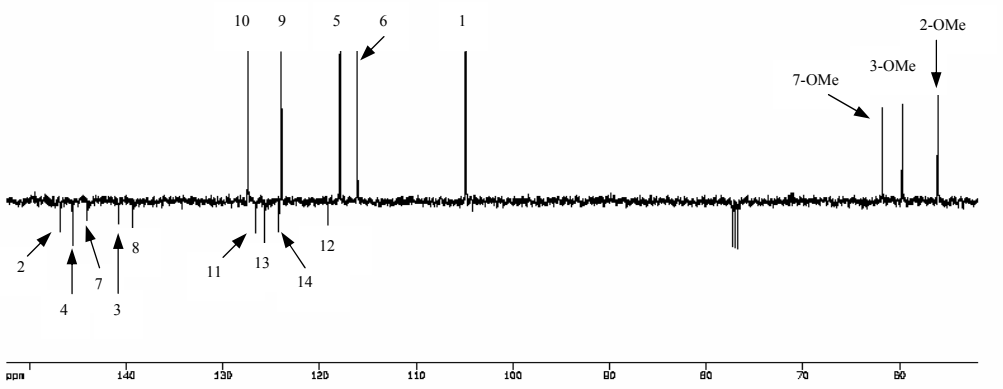
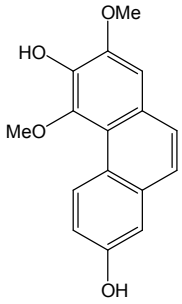


Figure 9. JMOD spectrum of TC-5 (**57**) (125 MHz, $CDCl_3$)

TC-6

TC-6 proved to be identical with 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**58**), on comparison of its NMR data (Table 2) with those published by MAJUMDER *et al*.[78] This compound was isolated earlier from *Bulbophyllum vaginatum*,[65] *Dendrobium plicatile*,[79] *Eulophia nuda*,[68] *Ephemerantha lonchophylla*,[80] *Eria flava*,[78] *Nidema boothii*,[19] *Scaphyglottis livida*[17,81] and *Thunia alba.*[76]



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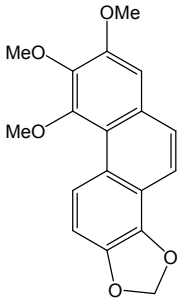

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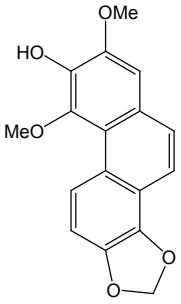

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TC-7 and TC-10

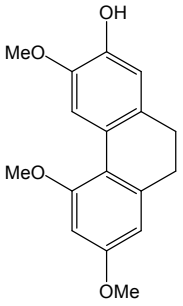
TC-7 and TC-10 are yellowish-white crystals. Their structures, 2,3,4-trimethoxy-7,8-methylenedioxyphenanthrene (**40**) and 3-hydroxy-2,4-dimethoxy-7,8-methylene-dioxy-phenanthrene (**38**) were elucidated, respectively. The compounds are identical with "TaI" and "TaIV", isolated earlier by BÁTHORI *et al*.,[46] from the rhizomes of this plant. After the first description of these compounds, the structures were revised in the 1970s by LETCHER *et al*.[51,52] In our experiments, the ^{13}C NMR signals of **40** and **38** were assigned for the first time (Table 3), and the position of the substituents was checked with the aid of NOESY spectra.

TC-8

TC-8 was obtained as a white amorphous solid with the molecular formula $C_{17}H_{18}O_4$, as determined via the molecular ion peak at *m*/*z* 286.1217 (calcd. for 286.1205) in the HREIMS. Its UV spectrum exhibited absorption bands characteristic of a dihydrophenanthrene derivative. In the ^1H-NMR spectrum of TC-8, the chemical shift of H-9 (δ_H 2.74 m) and H-10 (δ_H 2.67 m) were indicative of a dihydrophenanthrene derivative (Table 2). The ^1H NMR spectrum of TC-8 disclosed the presence of an isolated methylene, one *para*- and one *meta*-coupled proton pair, three methoxy groups and one hydroxy group.

The substitution pattern was determined with the aid of a NOESY spectrum. Nuclear *Overhauser* effects were detected between H-1/H-10, H-10/H-9, H-9/H-8, H-8/7-OCH_3, 7-OCH_3/H-6, H-6/5-OCH_3, 5-OCH_3/H-4 and H-4/3-OCH_3 (Figure 10). These NOESY correlations led to the formulation of TC-8 as 2-hydroxy-3,5,7-trimethoxy-9,10-dihydrophenanthrene (**59**). This is the second reported isolation of this compound from a natural source: it was first obtained from the heartwood of *Combretum psidioides*.[82] Interestingly, a similar compound, 5-hydroxy-2,3,7-

trimethoxy-9,10-dihydrophenanthrene, differing only in the positions of two substituents, was identified earlier from the chloroform extract of the rhizomes of *T. communis*.[53]



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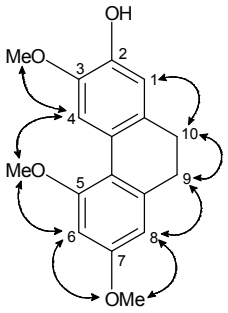
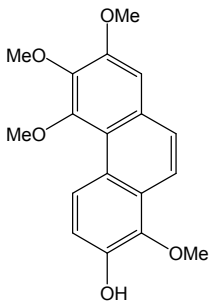


Figure 10. Diagnostic NOESY correlations of **59**

TC-9

TC-9 was found to be identical in all of its characteristics, including the ^{1}H NMR spectral data (Table 2), with 2-hydroxy-3,5,7-trimethoxyphenanthrene (**35**) isolated earlier from *Combretum psidioides*,[82] *Dendrobium rotundata*[83] and *T. communis*.[45,49,50,53,54]



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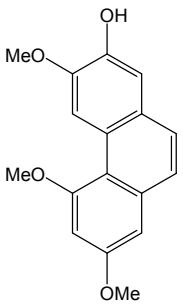

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Table 2. ^{1}H NMR spectral data of the isolated phenanthrenes 1-10 [500 MHz, $CDCl_3$, δ ppm (J=Hz)]

Atom	TC-2 (54)	TC-3 (55)	TC-4 (56)	TC-5 (57)	TC-6 (58)	TC-7 (40)	TC-8 (59)	TC-9 (35)	TC-10 (38)	TC-11 (60)
1	7.17 s	7.07 s	7.21 s	7.07 s	7.08 s	7.06 s	6.78 s	7.31 s	7.06 s	7.09 s
4	-	-	-	-	-	-	7.88 s	9.09 s	-	-
5	9.36 d (9.2)	9.39 d (9.2)	9.22 d (9.4)	9.18 d (9.3)	9.33 d (9.2)	9.10 d (9.1)	-	-	9.03 d (9.0)	9.24 d (9.3)
6	7.17 dd (2.8, 9.1)	7.19 dd (9.2, 2.6)	7.31 d (9.4)	7.33 d (9.3)	7.18 dd (9.2, 2.8)	7.22 d (9.1)	6.46 d (2.5)	6.75 d (2.0)	7.23 d (9.0)	7.31 d (9.3)
8	7.21 d (2.8)	7.22 d (2.6)	-	-	7.21 d (2.7)	-	6.44 d (2.5)	6.88 d (2.0)	-	-
9	7.50 d (8.7)	7.52 d (8.8)	7.88 d (9.1)	7.84 d (8.9)	7.48 d (8.8)	7.68 d (8.9)	2.74 m (2H)	7.52 d (8.7)	7.63 d (8.9)	7.88 d (9.0)
10	7.53 d (8.7)	7.56 d (8.8)	7.62 d (9.1)	7.62 d (8.9)	7.57 d (8.8)	7.55 d (8.9)	2.67 m (2H)	7.59 d (8.7)	7.55 d (8.9)	7.64 d (9.0)
2-OCH_3	-	4.03 s	4.00 s	4.02 s	4.05 s	4.00 s	-	-	4.03 s	4.01 s
3-OCH_3	4.11 s	4.01 s	4.14 s	-	-	4.03 s	3.92 s	4.09 s	-	4.03 s
4-OCH_3	3.98 s	4.01 s	-	3.98 s	3.96 s	4.00 s	-	-	3.94 s	4.00 s
5-OCH_3	-	-	-	-	-	-	3.88 s	4.10 s	-	-
7-OCH_3	-	-	3.99 s	-	-	-	3.84 s	3.95 s	-	-
8-OCH_3	-	-	-	3.95 s	-	-	-	-	-	3.98 s
OH	-	-	-	6.00 s 6.14 s	6.00 s 6.00 s	-	5.57 s	5.84 s	6.03 s	5.78 s
-OCH_2O-	-	-	-	-	-	6.16 s (2H)	-	-	6.16 s (2H)	-

TC-11

TC-11 was obtained as colourless crystals. Its HREIMS exhibited a molecular ion $[M]^+$ at m/z 314.1161 (calcd. for $C_{18}H_{18}O_5$, 314.1154), suggesting the molecular formula $C_{18}H_{18}O_5$. In the ^{1}H NMR spectrum of TC-11, two *ortho*-coupled doublet 1H pairs [δ_H 9.24 d (9.3), 7.31 d (9.3), 7.88 d (9.0), 7.64 d (9.0)], an aromatic singlet signal (δ_H 7.09 s) and four methoxy signals (δ_H 4.03 s, 4.01 s, 4.00 s, 3.98 s) were observed (Table 2). The JMOD spectrum revealed the presence of five *O*-substituted, four *C*-substituted aromatic quaternary carbons and five aromatic methines, in addition to four methoxy groups, indicating a pentasubstituted phenanthrene derivative (Table 3). The positions of the substituents (one hydroxy and four methoxy groups) were determined by a NOESY experiment and the coupling constants. In the NOESY spectrum a series of correlative signals were observed between 2-OCH_3/H-1, H-1/H-10, H-10/H-9, H-9/8-OCH_3, 8-OCH_3/7-OH, H-6/H-5 and H-5/4-OCH_3, which

provided evidence for the 7-hydroxy-2,3,4,8-tetramethoxyphenanthrene (**60**) structure of TC-11.

Table 3. ^{13}C NMR data of TC-5, TC-7, TC-10 and TC-11 [125 MHz, $CDCl_3$, δ_C (ppm)]

atom	TC-5 (57)	TC-7 (40)	TC-10 (38)	TC-11 (60)
1	104.9	105.8	105.3	105.3
2	146.8	152.0	144.1	148.0
3	140.8	143.1	139.4	143.1
4	145.5	151.9	146.7	151.9
4a	119.2	117.6	117.8	119.4
4b	125.7	119.4	119.0	124.8
5	117.9	120.5	120.3	124.2
6	116.1	108.9	108.7	116.2
7	144.0	143.4	143.4	145.5
8	139.3	142.3	142.2	140.8
8a	124.2	125.0	124.5	126.4
9	123.9	118.6	117.1	119.3
10	127.4	127.1	127.3	127.3
10a	126.6	129.1	125.9	128.4
OCH_3	56.1	55.8	56.1	55.9
OCH_3	59.7	60.1	59.7	60.2
OCH_3	61.8	61.2	-	61.2
OCH_3	-	-	-	61.9
$-OCH_2O-$	-	101.4	101.4	-

5.3.2. Xanthanolides

Xan-1

Xan-1 was obtained as white crystals, and identified as xanthatin (**23**) by comparison of its ^{13}C-NMR spectral data with those published by MARCO *et al*.[57] Our detailed NMR studies, including ^{1}H-NMR, JMOD, $^{1}H,^{1}H$-COSY, NOESY, HSQC and HMBC (Figure 11) experiments, resulted in complete ^{1}H chemical shift assignments for xanthatin for the first time (Table 4).

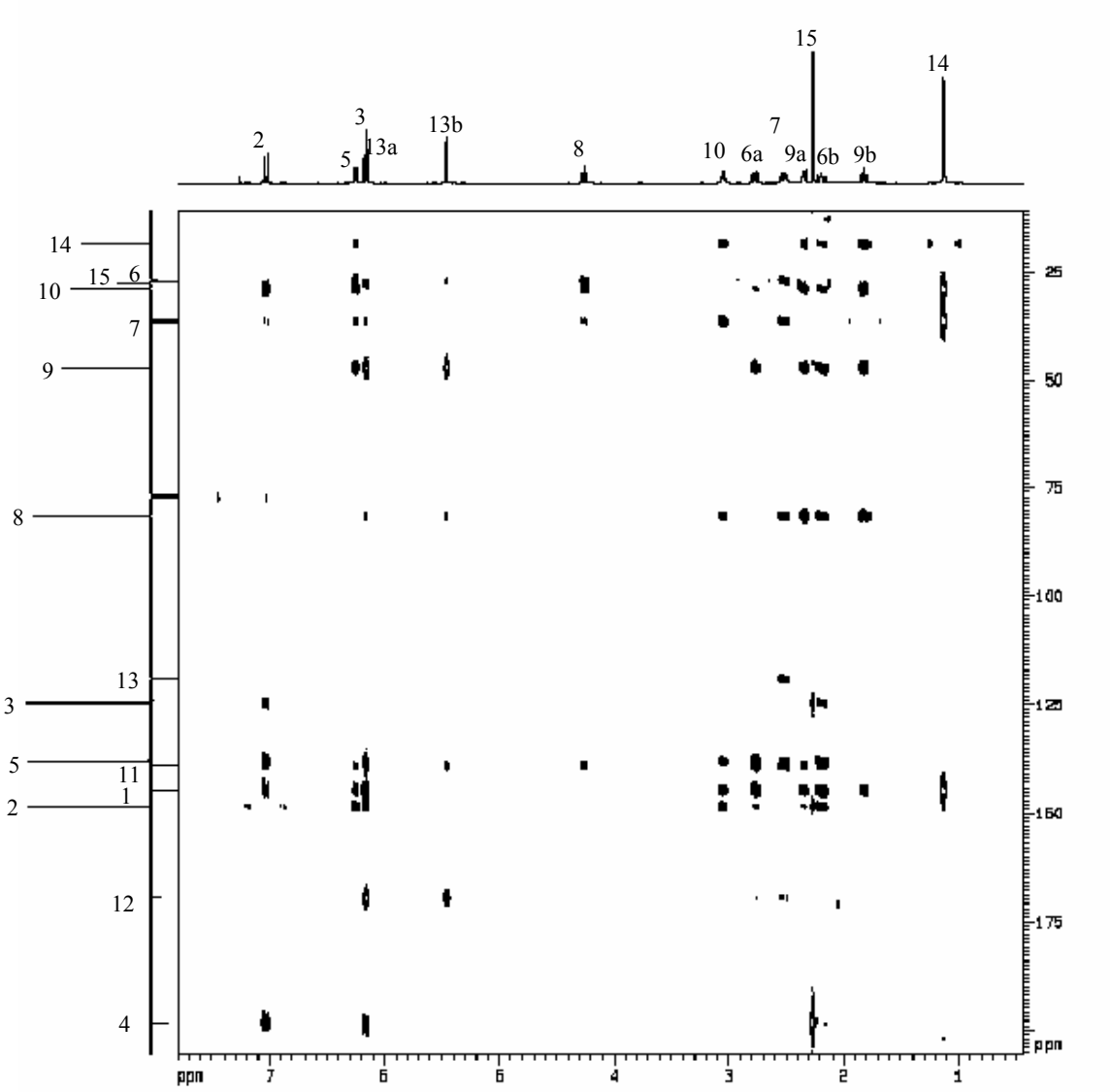
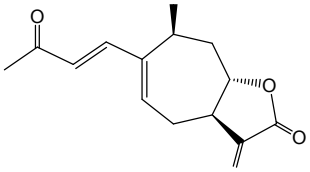


Figure 11. HMBC spectrum of Xan-1 (xanthatin **23**)

The NOESY experiment provided information on the stereochemistry of the chiral centres (C-7, C-8 and C-10). The nuclear *Overhauser* effects detected between H-8/H-14, H-8/H-6β and H-9β/H-8, and between H-6α/H-7 and H-7/H-9α, proved the *trans* lactone ring junction and the *β*-oriented 14-methyl group (Figure 12). Xanthatin (**23**), which was obtained in the highest yield, is one of the main xanthanolides: it has been isolated previously from *X. italicum*,[84] *X. macrocarpum*,[85] *X. spinosum*,[86] *X. sibiricum*[87] and *X. strumarium*.[88] This compound has attracted considerable attention because of its antileishmanial, antifungal,[85] and nitric oxide synthesis and COX-2 inhibitory activities.[89]

Table 4. NMR spectral data of Xan-1 (xanthatin **23**) [$CDCl_3$, TMS, δ(ppm) (J= Hz)]

atom	^{1}H	^{13}C	HMBC	NOESY
1	-	144.6	C-2, C-3, C-5, C-6a, C-6b, C-9a, C-9b, C-10, C-14-Me	
2	7.03 d (15.2)	148.4	C-3, C-5, (C-6b), C-10	
3	6.17 d (15.2)	124.6	C-2	H-5
4	-	198.4	C-2, C-3, C-15-Me	
5	6.26 dd (9.1, 3.3)	138.1	C-2, C-6a, C-6b, C-7, C-10	H-2, H-6a
6a	2.77 ddd (16.7, 9.1, 2.6)	27.1	C-5, C-7, C-8, C-13b	H-7, H-13b
6b	2.20 ddd (16.7, 12.2, 3.3)			H-8
7	2.52 dt (12.2, 2.6)	47.3	C-5, C-8, C-10, C-13a	H-6a, H-9b
8	4.26 dt (12.2, 2.6)	81.4	C-6b, C-7, C-9a, C-9b, 10, 13a, 13b	H-6b, H-14Me
9a	2.34 ddd (12.8, 3.8, 2.6)	36.5	C-5, C-6a, C-6b, C-13a, C-13b	H-8, H-14Me
9b	1.82 dt (12.8, 3.8)			H-7, H-10
10	3.04 m	29.0	C-2, C-5, C-8, C-9a, C-9b, 14-Me,	H-9b
11	-	139.1	C-7, C-8, C-9a, C-13a, C-13b	
12	-	169.6	C-7, C-13a, C-13b	
13a	6.15 d (3.3)	118.8	C-7	
13b	5.46 d (3.3)			H-5, H-6a
14	1.12 d (7.5)	18.7	C-5, C-9a, C-9b, C-10	H-8, H-9a
15	2.26 s	27.8	C-3	



23

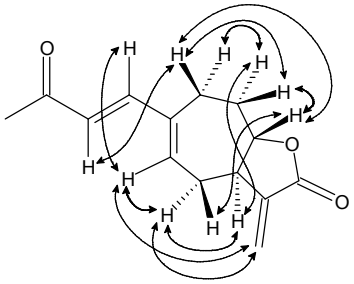
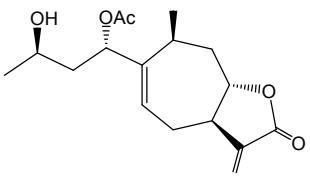


Figure 12. Diagnostic NOESY correlations of xanthatin (**23**)

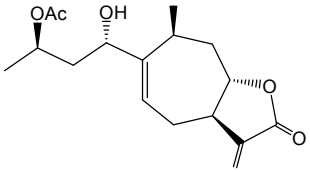
Xan-2 and Xan-3

Xan-2 and Xan-3 were obtained as colourless crystals. The ^{1}H NMR spectrum of these compounds showed that in this case the keto group was reduced, as an additional methyl doublet at δ_H 1.20 (Xan-2) and 1.24 (Xan-3) was observed due to H-15. The ^{1}H and ^{13}C NMR spectra of Xan-2 and Xan-3 revealed the presence of one

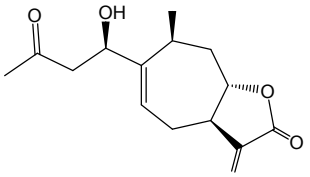
acetyl [δ_H 2.08 (Xan-2) and 2.04 (Xan-3)] and one hydroxy groups. The only relevant difference between their ^{1}H NMR spectra is the chemical shifts of H-2 [5.38 dd (Xan-2) and 3.95 dd (Xan-3)] and H-4 [3.71 m (Xan-2) and 5.06 m (Xan-3)]. These compounds, therefore, differ only in the stereochemistry of the side chains. On the basis of their ^{1}H and ^{13}C NMR data, they were identified as 4-epixanthanol (**26**) and 4-epi-isoxanthanol (**27**). These compounds were isolated earlier from *X. strumarium* and *X. italicum*.[57]



26



27



61

Xan-4

Xan-4 was isolated as a colourless gum. Its ^{1}H-NMR and JMOD spectra (Table 5) revealed the presence of a methylene-substituted lactone ring (δ_H 6.16 d, 5.45 d, 4.30 dt, 2.44 m; δ_C 169.6, 139.4, 118.5, 82.2, 48.2 ppm), and contained signals for a lactone ring condensed methyl-substituted seven-membered ring [δ_H 5.86 dd, 2.55 ddd, 2.11 ddd, 2.44 m, 4.30 dt, 2.32 dt, 1.69 dt, 2.81 m], characteristic of a xanthanolide. Additionally, a four-carbon containing side-chain was suggested by the signals at δ_H 4.50 dd, 2.73 dd, 2.60 dd and 2.21 s, and δ_C 209.1, 73.9, 48.7 and 30.9 ppm. The HSQC and ^{1}H-^{1}H COSY spectra led to the identification of two methyl, five methine, and four methylene groups and four quaternary carbons in the molecule. The HMBC correlations of C-1 with H-2, H-3 and H-5, of C-4 with H-2, H-3 and H-15, and of C-2 with H-3, H-5 and H-10 proved the 2-hydroxy-4-oxo-1(5)-ene substituted xanthanolide structure. The stereochemistry of Xan-4 was investigated by NOESY experiments (Figure 13).

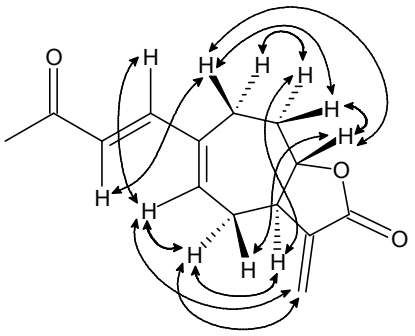


Figure 13. Diagnostic NOESY correlations of Xan-4 (2-hydroxyxanthinosin **61**)

The nuclear *Overhauser* effects between H-7/H-9α, H-10/H-2, H-5/H-2 and H-5/H-6α indicated protons below the plane of the molecule, while the NOE interactions between H-8/H-9β, H-8/H-6β and H-8/H-14 demonstrated *β*-oriented H-8 and H-14 (Figure 13). All of the above data supported the structure of 2-hydroxyxanthinosin (**61**) for Xan-4, with the first stereochemical characterization of C-2, and determination of the complete ^{13}C-NMR assignments as listed in Table 5. This compound was obtained previously from *Telekia speciosa*.[90]

Table 5. NMR spectral data of Xan-4 (2-hydroxyxanthinosin **61**) [500 MHz (^{1}H), 125 MHz (^{13}C) $CDCl_3$, TMS, δ (ppm) (J = Hz)]

Atom	^{1}H	^{13}C	HMBC (C→H)	NOESY
1	-	148.3	H-2, H-3a, H-3b, H-5, H-6α, H-6β, H-9α, H-9β, H-10, H-14	-
2	4.50 dd (9.3, 1.4)	73.9	H-3a, H-3b, H-5, H-10	H-3b, H-5, H-10
3a	2.73 dd (17.2, 9.6)	48.7	H-15	H-2, H-3b, H-10, H-14
3b	2.60 dd (17.2, 2.7)			H-3a
4	-	209.1	H-2, H-3a, H-3b, H-15	-
5	5.86 dd (9.2, 3.2)	123.7	H-2, H-6α, H-6β, H-10	H-2, H-6α
6α	2.55 ddd (15.1, 9.2, 2.2)	25.2	H-5, H-8	H-5, H-6β, H-7, H-13b
6β	2.11 ddd (15.1, 12.0, 3.2)			H-6α, H-7, H-8, H-14
7	2.44 m	48.2	H-6α, H-6β, H-9α, H-9β, H-13a, H-13b	H-6α, H-6β, H-9α
8	4.30 dt (12.5, 2.8)	82.2	H-6β, H-9α, H-9β	H-6β, H-9β, H-14
9β	2.32 dt (13.1, 3.7)	36.9	H-10, H-14	H-8, H-9α, H-10, H-14
9α	1.69 dt (12.4, 3.4)			H-7, H-9β, H-10
10	2.81 m	29.7	H-2, H-5, H-8, H-9α, H-9β, H-14	H-2, H-3a, H-9β, H-9α, H-14
11	-	139.4	H-6α, H-13a, H-13b	-
12	-	169.5	H-13a, H-13b	-
13a	6.16 d (3.2)	118.5		H-13b
13b	5.45 d (3.2)			H-6α, H-13a
14	1.17 d (7.4)	19.3	H-5, H-9α, H-9β, H-10	H-6β, H-8, H-9β, H-10
15	2.21 s	30.9		H-3a, H-3b

5.4. Biological activity of the isolated compounds

5.4.1. Compounds from *Tamus communis*

Multistep separation procedure of the chloroform and petroleum ether fraction of *T. communis* resulted the isolation of nine phenanthrenes (**35**, **38**, **40**, **54-58**, **60**) and one 9,10-dihydrophenanthrene (**59**).

The isolated compounds were tested for their cytotoxic activity on a HeLa cell line using the MTT assay, compared with cisplatin and doxorubicin as positive controls. The cell growth-inhibitory potencies of phenanthrenes, expressed as IC_{50} values in μM, are shown in Table 6.

Table 6. Cytotoxic activities of phenanthrenes on HeLa cell lines

Experimental code	Structure	IC_{50}±SEM (μM)
TC-2	**54**	20.18 ± 0.54
TC-3	**55**	13.85 ± 0.56
TC-4	**56**	0.97 ± 0.009
TC-5	**57**	>30
TC-6	**58**	6.66 ± 0.25
TC-7	**40**	>30
TC-8	**59**	14.21 ± 1.64
TC-9	**35**	11.49 ± 0.68
TC-10	**38**	3.64 ± 0.12
TC-11	**60**	8.52 ± 0.70
Doxorubicin		0.15 ± 0.028
Cisplatin		12.43 ± 1.05

Confusarin (**56**) exhibited the most potent cytotoxic activity (IC_{50} = 0.97 ± 0.009 μM), followed by 3-hydroxy-2,4-dimethoxy-7,8-methylenedioxyphenanthrene (TC-10) (**38**) (IC_{50} = 3.64 ± 0.12 μM) and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (TC-6) (**58**) (IC_{50} = 6.66 ± 0.25 μM). TC-2 (**55**), TC-3 (**54**), TC-8 (**59**), TC-9 (**35**) and TC-11 (**60**), displayed moderate cell growth inhibitory effect (IC_{50} = 8.52–20.18 μM), whereas TC-7 (**40**) and TC-5 (**57**) were found to be inactive.

5.4.2. Compounds from *Xanthium italicum*

Chloroform extract of the leaves of *X. italicum* was subjected to multiple chromatographic purifications yielding xanthanolide constituents: xanthatin (**23**), 4-epixanthanol (**26**), 4-epi-isoxanthanol (**27**) and 2-hydroxyxanthinosin, (**61**). The

compounds were tested on HeLa, MCF-7 and A431 cell lines, and found that xanthatin (**23**) demonstrated the highest activity with IC_{50} values of 8.00, 3.44 and 5.19 μM on the HeLa, A431 and MCF-7 cell lines, respectively; its potency was close to that of the positive control, cisplatin. 2-Hydroxyxanthinosin (**61**) displayed significant activity (IC_{50} = 7.78 ± 1.21 μM) against HeLa cells, and 4-epixanthanol (**26**) and 4-epi-isoxanthanol (**27**) exerted moderate effects (IC_{50} 15.53 – 37.62 μM) against all three tumour cell lines (Table 7).

Table 7. Cytotoxic activities of xanthanolides on HeLa, A431 and MCF-7 cell lines (IC_{50}±SEM μM)

Experimental code	Compound	HeLa	A431	MCF7
Xan-1	xanthatin (**23**)	8.00 ± 0.27	3.44 ± 0.27	5.19 ± 2.16
Xan-2	4-epixanthanol (**26**)	15.53 ± 1.46	20.85 ± 0.66	26.81 ± 3.42
Xan-3	4-epi-isoxanthanol (**27**)	29.83 ± 2.21	37.62 ± 2.09	17.65 ± 1.37
Xan-4	2-hydroxyxanthinosin (**61**)	7.78 ± 1.21	97.84 ± 4.12	27.94 ± 1.44
	Doxorubicin	0.15 ± 0.03	0.15 ± 0.04	0.28 ± 0.01
	Cisplatin	12.43 ± 1.05	2.84 ± 0.61	9.63 ± 0.75

6. DISCUSSION

The aim of this work was investigation of the cytotoxic activity of the extracts from *T. communis* and *X. italicum*, and identification of the compounds responsible for the cytotoxicity. The present thesis reports the isolation, structure elucidation and tumor cell growth-inhibitory activities of phenanthrenes and xanthanolides obtained from the petroleum ether and/or chloroform extracts through cytotoxic assay guidance.

Cytotoxic screening of *T. communis* led to the conclusion that the lipophilic extracts of the rhizome contain the active compounds, while in case of *X. italicum* the tumor cell growth inhibitory constituents are present in the chloroform fractions of the leaves and flowers.

The plant materials were extracted with methanol, which is an amphipolar solvent suitable for the extraction of lipophilic and polar compounds from plant material. In the initial step of separations, liquid-liquid extractions were applied to separate the constituents of different polarity. These fractions were tested for their cytotoxic activity on human cell lines using the MTT assay. Thereafter, in case of *X. italicum*, a specific purification method was used (OCC on polyamide), which resulted in a xanthanolide-rich fraction free from chlorophyll. Repeated VLC separations of the xanthanolide fraction afforded subfractions containing only a few main components. The next purification methods were chosen with regard to the degree of purity and complexity of the fractions. The relatively complex subfractions were subjected to NP- and RP-HPLC purification. This on-line preparation technique resulted in the isolation of numerous constituents under mild conditions. The preparative TLC and gel chromatographic methods were applied for the purification of large fractions containing 1-2 main components. Successful separations were achieved when different stationary and mobile phases were used in the subsequent steps of purification.

After extensive chromatographic purification 11 compounds (TC-1–TC-11), including 10 phenanthrenes (**35**, **38**, **40**, **54**–**60**), were isolated from *Tamus communis*, and 4 sesquiterpenes (Xan-1–Xan-4) (**23**, **26**, **27** and **61**) containing a xanthanolide skeleton from *Xanthium italicum*. The isolated phenanthrenes were amorphous solids or crystals, while xanthanolides were crystals, oils or gums.

The structures of the isolated compounds were elucidated by means of spectroscopic methods. The UV spectra of the compounds from *T. communis* revealed the presence of the phenanthrene nucleus. Through the utilization of HREIMS spectrometry, the molecular compositions were determined. The most useful data concerning the chemical structures were furnished by 1D and 2D NMR spectroscopy. As a result of the detailed NMR studies, complete ^{1}H and ^{13}C chemical-shift assignments of compounds TC-5 (**57**), TC-7 (**40**), TC-10 (**38**), TC-11 (**60**) and Xan 1–4 (**23**, **26**, **27**, **61**) proved possible. For compounds TC-2 (**54**), TC-3 (**55**), TC-4 (**56**), TC-6 (**58**), TC-8 (**59**) and TC-9 (**35**) only ^{1}H NMR assignments were determined. The positions of the substituents in the phenanthrenes, and relative configurations of the chiral centres in the xanthanolides were identified on the basis of NOESY measurements.

Nine phenanthrenes (**35**, **38**, **40**, **54-58**, **60**) and one 9,10-dihydrophenanthrene (**59**) were identified from the lipophilic phase of the rhizome of *T. communis*. All the isolated phenanthrenes have monomeric structure, and they are hydroxy- and methoxy-substituted. TC-7 (**40**) and TC-10 (**38**) contain methylenedioxy group at C(7)–C(8). TC-3 (**55**) and TC-11 (**60**) are new natural products, and further five compounds (TC-2 **54**, TC-4 **56**, TC-5 **57**, TC-6 **58**, TC-8 **59**) were described for the first time from *T. communis*.

The isolated phenanthrenes were tested for their cytotoxic activity on the HeLa cells. Confusarin (TC-4) (**56**) exhibited the highest cytotoxic activity ($IC_{50} = 0.97 \pm 0.009$ μM), but other compounds [TC-3 (**55**), TC-9 (**35**), TC-10 (**38**), TC-8 (**59**) and TC-11 (**60**)] also displayed remarkable effect ($IC_{50} = 3.64–14.21$ μM). Although the low numbers of the compounds do not allow a thorough evaluation of structure-activity relationships, some conclusions can be drawn concerning the importance of various structural elements, as concerns tumor cell proliferation-inhibitory activity. The cytotoxic potencies indicate that the C(9)–C(10) double bond is not essential for the cytotoxic effect, as the compounds TC-9 (**35**) and TC-8 (**59**), which differ only in this structural feature, are almost equally effective. A similar observation was reported earlier on structurally related alkoxy-substituted phenanthrenes and dihydrophenanthrenes.[7] The number and positions of the methoxy groups on the phenanthrene skeleton seem to be crucial factors as regards the efficacy, similarly as for the conformationally less restricted analogues, the *cis*-stilbene combretastatins. Although the highly active combretastatins require a trimethoxy-substituted benzene ring in the molecule,[91] our results reveal that this is not essential

for the phenanthrenes, since compound TC-7 (**40**), containing 3 methoxy substituents, proved to be the least active, and compounds TC-10 (**38**) and TC-4 (**56**), with only 2 methoxy groups, were the most effective in the MTT assay. Comparison of the effects of compounds TC-10 (**38**)**,** TC-7 (**40**), TC-4 (**56**) and TC-11 (**60**) suggests that the presence of a hydroxy group on either ring A or ring B is favourable relative to full alkoxy substitution. All of these findings demonstrate that the structure–activity relationships established for combretastatins cannot be directly applied to the congeners, *e.g.* to the conformationally constrained phenanthrenes.

The compounds (**23**, **26**, **27** and **61**) isolated from *X. italicum* are members of the group of sesquiterpene lactones, one of them 2-hydroxyxanthinosine (**61**) was first identified from the plant.

The cytotoxic effects of the obtained xanthanolides were screened on three human cell lines (HeLa, A431 and MCF7), and it was found that xanthatin (**23**) demonstrated high activity against all of these cell lines, and 2-hydroxyxanthinosine (**61**) displayed significant activity against HeLa cells. 4-Epixanthanol (**26**) and 4-epi-isoxanthanol (**27**) had moderate or weak activity against A431 cells. All these xanthanolides (**23**, **26**, **27**, **61**) contain an α-methylene-γ-lactone ring, which is generally regarded as a structural requirement of sesquiterpenes for cytotoxic activity. The most potent xanthatin (**23**) has an additional α,β-unsaturated carbonyl group in the side-chain; this structural feature presumably enhances the antitumor activity. In previous pharmacological studies, this side-chain was similarly found to be responsible for pronounced biological activities, *e.g.* iNOS and COX-2 expression suppressive effects.[89]

In conclusion, the strong inhibitory effect of the leaves of *X. italicum* on the proliferation of cultured human tumor cell lines (HeLa, MCF7 and A431) may be attributed to xanthanolides. Predominantly the content of the most active main compound, xanthatin (**23**), determines the antitumor activity of the extracts, with the minor xanthanolides (**26**, **27**, **61**) playing additional roles in this effect.

7. SUMMARY

The aim of the present study was the bioassay-guided isolation, structure determination and pharmacological evaluation of cytotoxic compounds of the rhizome of *Tamus communis* and leaves of *Xanthium italicum*.

The preliminary antiproliferative screening of these species showed that the lipophilic extracts have significant cell growth-inhibitory potency against human tumor cells (HeLa, MCF-7, A431). The most active fractions, prepared with petroleum ether and chloroform from the rhizomes of *T. communis*, and chloroform phase from the leaves of *X. italicum*, were selected for detailed phytochemical-pharmacological analysis in order to identify the compounds responsible for the cytotoxicity. The isolation was carried out by a multistep separation procedure, including extraction with methanol, solvent-solvent partitioning, VLC, CPC, preparative TLC, NP- and RP-HPLC and gel chromatography.

Ten phenanthrene-type compounds (TC-2–TC-11) (**35**, **38**, **40**, **54**–**60**) were isolated from the extracts of *T. communis* besides *β*-sitosterine (TC-1), and four compounds (Xan-1–Xan-4) (**23**, **26**, **27** and **61**) based on xanthanolide skeleton were obtained from *X. italicum*. The structures of the compounds were elucidated by means of UV spectroscopy, HREIMS measurements and advanced 1D and 2D NMR experiments. As a result of ^{1}H NMR, JMOD and 2D NMR studies (^{1}H,^{1}H-COSY, NOESY, HSQC, HMBC), complete ^{1}H and ^{13}C assignments were made for four phenanthrenes [TC-5 (**57**), TC-7 (**40**), TC-10 (**38**), TC-11 (**60**)] and all xanthanolides [Xan-1 (**23**), Xan-2 (**26**), Xan-3 (**27**) and Xan-4 (**61**)].

Seven phenanthrenes, including two new natural products (TC-3 **55**, TC-11 **60**) and five known phenanthrenes (TC-2 **54**, TC-4 **56**, TC-5 **57**, TC-6 **58**, TC-8 **59**) were detected for the first time from *T. communis*. Nine compounds are 9,10-dehydrophenantrenes (**35**, **38**, **40**, **54-58**, **60**) and one is 9,10-dihydrophenanthrene (**59**). From *X. italicum* four known xanthanolide-type sesquiterpene lactones (**23**, **26**, **27**, **61**) were isolated. This was the first identification of one of them, 2-hydroxyxanthinosine (**61**), from this plant.

The biological activities of the isolated compounds were investigated against HeLa, MCF-7 and A431 cells through use of the MTT assay. Some compounds, especially confusarin (TC-4 **56**), 3-hydroxy-2,4-dimethoxy-7,8-methylenedioxyphenanthrene

(TC-10 **38**) and xanthatin (Xan-1 **23**) demonstrated high cell growth inhibitory activity with IC_{50} values between 0.97–3.64 μM. Other compounds, such as TC-2 (**54**), TC-3 (**55**), TC-6 (**58**), TC-8 (**59**), TC-9 (**35**), TC-11 (**60**), 4-epixanthanol (**26**) and 4-epi-isoxanthanol (**27**) also revealed substantial or moderate antiproliferative effect; these results allowed some statements as regards the structure-activity relationships. In the literature the mechanisms of action of phenanthrenes and xanthanolides have not been reported earlier, therefore these compounds are worthy of further pharmacological studies, including investigations of their molecular mechanism of action, and studies on *in vivo* animal models.

In conclusion, our results indicate that naturally occurring phenanthrenes, dihydrophenanthrenes and xanthanolides may be promising starting structures for antitumor drug development.

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I

Cytotoxic Phenanthrenes from the Rhizomes of *Tamus communis*

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Abstract

From the fresh rhizomes of *Tamus communis* five phenanthrenes (**1 – 5**) were isolated under the guidance of cytotoxic assays in HeLa cells. The compounds were obtained from the highly active $CHCl_3$ fraction of the MeOH extract by using multistep chromatographic purifications, including VLC, preparative TLC, HPLC and gel filtration. The compounds were identified by means of EI-mass, UV and NMR spectroscopy as 7-hydroxy-2,3,4-trimethoxyphenanthrene (**1**), 2,7-dihydroxy-3,4-dimethoxyphenanthrene (nudol) (**2**), 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene (**3**), 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene (confusarin) (**4**), and 3,7-dihydroxy-2,4-dimethoxyphenanthrene (**5**). Compound **1** is a new natural product, and **2 – 4** were isolated for the first time from *T. communis*. In the cytotoxic assays, compounds **1 – 3** and **5** significantly inhibited the growth of HeLa cells (IC_{50} = 0.97 – 20.18 μM). Compound **3**, with an IC_{50} value of 0.97 μM, is of special interest because of its high activity.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

In recent years, the *cis*-stilbene combretastatins have attracted great interest because of their potential use in cancer chemotherapy. The most potent member, combretastatin A-4 in sodium phosphate prodrug (CA4P) form, is currently undergoing testing in Phase II clinical trials, and has been found to be effective against different solid tumours, including multidrug-resistant cancers [1]. The aim of the present work was to perform an antitumour evaluation of structurally related compounds: phenanthrenes, which are the conformationally restricted congeners of *cis*-stilbenes. Previous phytochemical examinations of *Tamus communis* L. (Dioscoreaceae) have demonstrated the presence of alkoxy-substituted phenanthrenes [2], [3], [4], [5], [6], but no data have been reported on their antitumour potency. The present paper deals with the bioguided isolation, structure elucida-

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Dedication
This work is dedicated to Professor Kálmán Szendrei on the occasion of his 70th birthday

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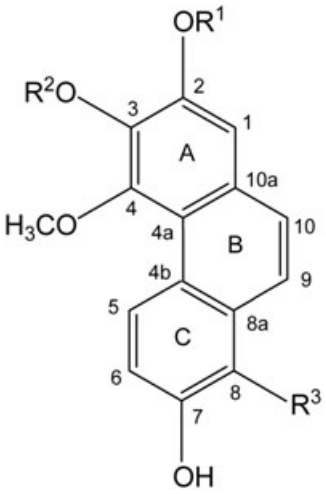

	R^1	R^2	R^3
1	CH_3	CH_3	H
2	H	CH_3	H
3	H	CH_3	OCH_3
4	CH_3	H	OCH_3
5	CH_3	H	H

tion and cytotoxic activity of phenanthrenes **1 – 5** from the fresh rhizomes of *T. communis*.

Compound **1**, obtained as yellowish-white crystals, possessed the molecular formula $C_{17}H_{16}O_4$, as confirmed by the HR-EI-MS. Its UV spectrum [λ_{max} (log ε) = 259 (3.94), 283 (3.24), 292 (3.13), 303 (2.92) nm] was characteristic of a phenanthrene derivative [7], [8]. The ^{1}H-NMR spectrum showed resonances for three methoxy groups [δ = 4.01 s (6H, s), 4.03 (3H, s)] and six aromatic protons. One set of aromatic protons comprised an ABX spin system [δ = 9.39 (1H, d, J = 9.2 Hz), 7.19 (1H, dd, J = 9.2, 2.6 Hz), 7.22 (1H, d, J = 2.6 Hz)]. The most deshielded aromatic proton signal (δ = 9.39) of this system was typical for H-5 of a phenanthrene [7], [8], and therefore a 7-substituted ring C was concluded. The signals of a pair of *ortho*-coupled aromatic protons [δ = 7.56, 7.52 (each 1H, each d, J = 8.8 Hz)] were attributed on the basis of literature values to H-9 and H-10 [5], [7], [8]. Moreover, an isolated aromatic proton at δ = 7.07 (1H, s) indicated a trisubstituted ring A. The substitution pattern of **1** was further studied in a NOESY experiment. Overhauser effects were detected between the signals of H-1 and H-10; H-10 and H-9; H-9 and H-8; and H-5 and H-6, demonstrating oxygen functionalities at C-2, C-3, C-4 and C-7. Besides the three methoxy groups, the presence of one hydroxy group was deduced from the molecular composition. The location of two methoxy groups at C-2 and C-4 was evident from the NOESY cross-peaks observed between the methoxy signals and the signals of H-1 and H-5. The third methoxy group was placed at C-3, and the hydroxy group at C-7, with regard to the absence of diagnostic NOEs between the methoxy group and H-6 and H-8. Furthermore, the chemical shifts of the aromatic protons were in good agreement with those published for phenanthrenes having the same functionalities on ring A or C [8], [9]. On the basis of these spectral data, the structure of **1** was elucidated as 7-hydroxy-2,3,4-trimethoxyphenanthrene. This compound is a new natural product.

By comparison of their physical and spectral data (UV, MS, NMR and NOESY) with those reported in the literature, compounds **2 – 4** were identified as nudol (**2**) [7], [9], [10], confusarin (**3**) [11], [12], and 3,7-dihydroxy-2,4,8-trimethoxyphenanthrene (**4**) [7], [13]. These compounds were previously described from different Orchidaceae species, but have been obtained for the first time from *T. communis*. Compound **5** proved to be identical with 3,7-dihydroxy-2,4-dimethoxyphenanthrene (= "TaVIII"), isolated earlier from the rhizomes of this plant [2], [14].

The cytotoxic assays of the isolated phenanthrenes in HeLa cells, using the MTT test, revealed that compounds **1 – 3** and **5** possess marked cell growth inhibitory activity if compared with those of the positive controls cisplatin and doxorubicin (Table **1**). Primarily 2,7-dihydroxy-3,4,8-trimethoxyphenanthrene (**3**) is worthy of interest because of its high activity (IC_{50} = 0.97 μM). Similarly, as in the case of the chemically closely related combretastatins [15], [16], the different cytotoxicities of compounds **1 – 5** demonstrated that the positions of the hydroxy and methoxy groups on the carbon skeleton are crucial for inhibition of cancer cell growth.

A large variety of combretastatin analogues have been investigated in order to elucidate the structure-activity relationship. It is generally accepted that a diaryl system linked by a carbon-carbon double bond is essential for the cytostatic effect together with the three methoxy groups on one of the rings [17]. However, the two most active members of the currently tested phenanthrenes, compounds **3** and **5**, have only two methoxy groups on one of the rings. Moreover, the substance closest to combretastatin regarding the three methoxy groups, compound **1**, has only a limited cytostatic effect. These findings do not fit into the previously published structure-activity relationship as concerns combretastatin analogues [17]. On the other hand, the olefinic part can be considered as an additional target for modification of the chemical structure. Many conformationally restricted analogues, including sulfonate and azetidinone derivatives of combretastatin, have clearly revealed that inhibition of the rotation favours antiproliferative action [18], [19]. Accordingly, it is conceivable that the structure-activity relationship observed for the substituted stilbenes is not directly applicable to the conformationally constrained phenanthrene skeleton.

Materials and Methods

The rhizomes of *Tamus communis* L. were collected in the Mecsek Hills (Hungary) in June 2003. A voucher specimen (No. 619) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

Chemicals used in the isolation protocol were purchased from Molar Chemicals Kft (Budapest, Hungary) except for petroleum ether and diethyl ether (Merck; Darmstadt, Germany). Resins for chromatography were from Merck (Darmstadt, Germany) (TLC Silica gel and LiChrospher) and from Pharmacia (Upsala, Sweden) (Sephadex LH 20).

The fresh rhizomes of the plant material (1.2 kg) were crushed and percolated with MeOH (10 L). The MeOH extract was concentrated to 400 mL and extracted with petroleum ether (5 × 400 mL) and $CHCl_3$ (5 × 400 mL). The $CHCl_3$ fraction (2.03 g) was chromatographed by vacuum liquid chromatography (VLC) on silica gel (GF_{254} 15 μm), using a gradient system of cyclohexane-EtOAc-EtOH (9 : 1 : 0, 8 : 2 : 0, 7 : 3 : 0, 70 : 30 : 1, 70 : 30 : 2, 70 : 30 : 5, 50 : 50 : 10, 15 × 20 mL of each), yielding thirteen main fractions (I - XIII) after combinations. Fraction IV was fractiona-

Table 1 Cytotoxic activity of the isolated phenanthrenes **1 – 5** in HeLa cells

Compound	*IC_{50} ± SEM (μM)*
1	13.85 ± 0.56
2	20.18 ± 0.54
3	0.97 ± 0.009
4	> 30
5	6.66 ± 0.25
Doxorubicin	0.15 ± 0.028
Cisplatin	12.43 ± 1.05

ted by gel chromatography on Sephadex LH-20 with MeOH, and then further chomatographed by HPLC on a LiChrospher RP-18 (5 μm, 250 × 4 mm) column, using MeOH-H_2O (7 : 3), with detection at 250 nm, flow rate 2 mL/min. In this way, compounds **1** (t_R = 10.65 min, 6.0 mg) and **3** (t_R = 25.52 min, 7.4 mg) were isolated. Fraction V was separated by VLC on silica gel, using mixtures of *n*-hexane-$CHCl_3$-MeOH with increasing polarity. The subfractions containing the main component were subjected to TLC with benzene-diethyl ether-petroleum ether (2 : 1 : 1) as solvent system (R_f = 0.50), and finally purified by RP-HPLC under the above conditions, affording compound **2** (t_R = 25.97 min, 0.0015 g). Fractions VII and VIII were processed in the same manner, first by VLC on silica gel, using $CHCl_3$, $CHCl_3$-MeOH (99 : 1 and 98 : 2) and MeOH as eluents (30 mL of each), and then by TLC on silica gel, with the use of $CHCl_3$-Me_2CO (19 : 1) as mobile phase. These separations yielded compounds **4** (R_f = 0.53, 5.2 mg) and **5** (R_f = 0.37, 20.6 mg). Upon standing, β-sitosterine crystallized from fraction III.

7-Hydroxy-2,3,4-trimethoxyphenanthrene (**1**): Yellowish-white crystals; m. p. 185 – 187 °C; UV (MeOH): see text; HR-EI-MS: *m/z* 284.10505 $[M]^+$ (calcd. for $C_{17}H_{16}O_4$: 284.10486); ^{1}H-NMR (500 MHz, $CDCl_3$): see text.

2,7-Dihydroxy-3,4-dimethoxyphenanthrene (= nudol) (**2**): Yellowish-white amorphous solid; UV (MeOH): λ_{max} (log ε) = 259 (3.89), 283 (3.28), 292 (3.19), 303 (2.96), 347 (2.48), 364 nm (2.48); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.17 (1H, s, H-1), 9.36 (1H, d, *J* = 9.2 Hz, H-5), 7.17 (1H, dd, *J* = 9.1, 2.8 Hz, H-6), 7.21 (1H, d, *J* = 2.8 Hz, H-8), 7.50 (1H, d, *J* = 8.7 Hz, H-9), 7.53 (1H, d, *J* = 8.7 Hz, H-10), 4.11 (3H, s, OCH_3), 3.98 (3H, s, OCH_3).

3,7-Dihydroxy-2,4,8-trimethoxyphenanthrene (**4**): Yellowish white crystals; UV (MeOH): λ_{max} (log ε) = 263 (3.96), 282 (3.30), 299 (3.41), 312 (3.08), 347 (2.54), 363 nm (2.58); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 7.07 (1H, s, H-1), 9.18 (1H, d, *J* = 9.3 Hz, H-5), 7.33 (1H, d, *J* = 9.3 Hz, H-6), 7.84 (1H, d, *J* = 8.9 Hz, H-9), 7.62 (1H, d, *J* = 8.9 Hz, H-10), 6.00, 6.14 (each 2H, each brs, 2 × OH), 3.95, 3.98, 4.02, (each 3H, each s, 3 × OCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 104.9 (C-1), 146.8, 145.5 (C-2, C-4), 140.8, 139.3 (C-3, C-8), 119.2 (C-4a), 125.7 (C-4b), 117.9 (C-5), 116.1 (C-6), 144.0 (C-7), 124.2 (C-8a), 123.9 (C-9), 127.4 (C-10), 126.6 (C-10a), 56.1, 59.7, 61.8 (3 × OCH_3); assignments were made by comparison with previously assigned spectra [5], [10], [20].

Cytotoxic assay: Cytotoxic effects were measured *in vitro* in a HeLa (cervix adenocarcinoma) cell line (ECACC; Salisbury, UK), using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Human cancer cells (5000/well) were seeded into a 96-well microplate. During an overnight preincubation, the cells attached to the bottom of the well. On the second day of the procedure, the original medium was removed and 200 μL new medium containing the test substances were added. The tested extracts and compounds were dissolved in DMSO. After an incubation period of 72 hours, living cells were assayed by the addition of 20 μL 5 mg/mL MTT solution (Sigma-Aldrich; Budapest, Hungary). MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a 4-hour contact period. The medium was then removed and the precipitated crystals were dissolved in 100 μL DMSO during a 60-minute period of shaking. Finally, the reduced MTT was assayed at 545 nm by using a microplate reader. All *in vitro* experiments were carried out on two microplates with at least 5 parallel wells.

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Phenanthrenes and a dihydrophenanthrene from *Tamus communis* and their cytotoxic activity

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Abstract

From the petroleum ether extract of the rhizomes of *Tamus communis*, the 7-hydroxy-2,3,4,8-tetramethoxyphenanthrene (**1**) was isolated, together with the known 2,3,4-trimethoxy-7,8-methylenedioxyphenanthrene (**2**), 3-hydroxy-2,4,-dimethoxy-7,8-methylenedioxyphenanthrene (**3**), 2-hydroxy-3,5,7-trimethoxyphenanthrene (**4**) and 2-hydroxy-3,5,7-trimethoxy-9,10-dihydrophenanthrene (**5**), through cytotoxic assay guidance. The structures were determined by means of HREIMS, ^{1}H NMR, JMOD and NOESY experiments. The cytotoxic effects of the isolated compounds were tested on cervix adenocarcinoma (HeLa) cells, with the MTT assay. The results demonstrated that, with the exception of **2**, all these compounds displayed pronounced cytotoxic activity; especially **1** and **3** exhibited significant cell growth inhibitory effects, with $IC_{50} = 8.52 \pm 0.70$ and 3.64 ± 0.12 μM, respectively.

Keywords: *Tamus communis*; Dioscoreaceae; Structure elucidation; Cytotoxicity; Phenanthrenes; Dihydrophenanthrene

1. Introduction

Only four species of the family Dioscoreaceae are native in Europe: *Dioscorea balcanica* Košanin, *Borderea pyrenaica* Miégeville, *Borderea chouardii* (Gausen) Heslot and *Tamus communis* L. *Dioscorea* and *Borderea* species are endemic in the Balkan Peninsula and the Pyrenees, whereas *T. communis* displays a broader distribution in South, South-Central and West Europe (Tutin et al., 1972).

T. communis (black bryony) is a climbing plant with large tubers which causes irritation when rubbed on the skin (Schmidt and Moult, 1983). Both the rhizomes and the berries have a reputation in folk medicine as effective rubefacients, and they have therefore traditionally been used in several countries for the treatment of rheumatism, arthrosis, lumbago and dermatosis (Duke, 2002). Moreover, different parts of the plant have been applied in traditional medicine for the treatment of polyps and tumours (Hartwell, 1969). Previous phytochemical investigations revealed the presence of spirostane and furostane glycosides (Aquino et al., 1991), sterols (Capasso et al., 1983), histamine (Schmidt and Moult, 1983) and hydroxy/alkoxy-substituted phenanthrenes and dihydrophenanthrenes (Reisch et al., 1969, 1972, 1973; Aquino et al., 1985a,b). The aim of the present work was an investigation of the cytotoxic activity of the petroleum ether extract from the fresh rhizomes of *T. communis*, and identification of the compounds responsible for the cytotoxicity. The present paper reports the isolation, structure elucidation and tumour cell growth-inhibitory activities of one new **1** and three known phenanthrenes **2**–**4**, together with a dihydrophenanthrene **5**, all obtained from the petroleum ether extract through cytotoxic assay guidance.

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2. Results and discussion

The MeOH extract of the fresh rhizomes of *T. communis* was subjected to solvent–solvent partitioning, to yield petroleum ether-, $CHCl_3$- and H_2O-soluble phases. The petroleum ether extract exhibited high cytotoxic activity on cervix adenocarcinoma (HeLa) cells, with IC_{50} 8.02 ± 0.20 (mean ± SEM) μg/ml. Fractionation of the petroleum ether extract by vacuum liquid chromatography resulted in nine main fractions, among which fractions VI and VII exhibited cell growth-inhibitory activities of $90.8 \pm 1.01\%$ and $74.6 \pm 1.3\%$ (mean ± SEM), respectively, at a final concentration of 10 μg/ml. The active fractions (VI and VII) were subjected to multiple chromatographic purifications, which afforded compounds **1**, **3** and **4**. From the less active fractions III and V, chemically similar constituents **2** and **5** were isolated with a view to obtaining further compounds for structure–activity relationship studies.

Compound **1** was obtained as colorless crystals. Its HREIMS exhibited a molecular ion $[M]^+$ at *m/z* 314.1161 (calcd. for $C_{18}H_{18}O_5$, Δ 2 ppm), suggesting the molecular formula $C_{18}H_{18}O_5$. The UV absorption bands were indicative of a phenanthrene derivative (Reisch et al., 1969). In the ^{1}H NMR spectrum of **1**, two *ortho*-coupled doublet 1H pairs, an aromatic singlet signal and four methoxy signals were observed (Table 1). The JMOD spectrum revealed the presence of five *O*-substituted, four *C*-substituted aromatic quaternary carbons and five aromatic methines, in addition to four methoxy groups, indicating a pentasubstituted phenanthrene derivative. The positions of the substituents (one hydroxy and four methoxy groups) were determined by a NOESY experiment and the coupling constants. In the NOESY spectrum a series of correlation signals were observed between 2-OCH_3/H-1, H-1/H-10, H-10/H-9, H-9/8-OCH_3, 8-OCH_3/7-OH, H-6/H-5 and H-5/4-OCH_3 (Fig. 1), which provided evidence for the 2,3,4,8-tetramethoxy-7-hydroxyphenanthrene structure of compound **1**.

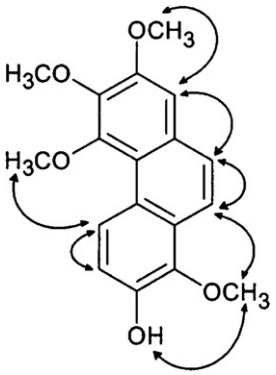

Fig. 1. Diagnostic Overhauser effects (↔) detected in the NOESY spectrum of **1**.

Compounds **2**–**4** proved to be 2,3,4-trimethoxy-7,8-methylenedioxyphenanthrene (**2**), 3-hydroxy-2,4-dimethoxy-7,8-methylenedioxyphenanthrene (**3**), and 2-hydroxy-3,5,7-trimethoxyphenanthrene (**4**), isolated earlier as compounds "TaI", "TaIV" and "TaVI", respectively, from the rhizomes of this plant (Reisch et al., 1969). After the first description of these compounds, the structures were revised in the 1970s (Letcher and Nhamo, 1972a; Letcher and Wong, 1978, 1979). In our experiments, the ^{13}C NMR signals of **2** and **3** were assigned for the first time, and in the cases of **2**–**4** the position of the substituents on the molecules were checked with the aid of the NOESY spectra.

Compound **5** was obtained as a white amorphous solid with the molecular formula $C_{17}H_{18}O_4$, as determined via the molecular ion peak at *m/z* 286.1217 in the HREIMS. Its UV spectrum exhibited absorption bands characteristic of a dihydrophenanthrene derivative (Letcher and Nhamo, 1972b). The ^{1}H NMR spectrum of **5** (Table 1) disclosed the presence of an isolated methylene, one *para*- and one *meta*-coupled proton pair, three methoxy groups and one hydroxy group (Table 1). The

Table 1
^{1}H NMR spectral data of the isolated phenanthrenes **1**–**5** [500 MHz, $CDCl_3$, δ ppm (J = Hz)]

H	1	2	3	4	5
1	7.09 *s*	7.06 *s*	7.06 *s*	7.31 *s*	6.78 *s*
4		–	–	9.09 *s*	7.88 *s*
5	9.24 *d* (9.3)	9.10 *d* (9.1)	9.03 *d* (9.0)	–	–
6	7.31 *d* (9.3)	7.22 *d* (9.1)	7.23 *d* (9.0)	6.75 *d* (2.0)	6.46 *d* (2.5)
8	–	–	–	6.88 *d* (2.0)	6.44 *d* (2.5)
9	7.88 *d* (9.0)	7.68 *d* (8.9)	7.63 *d* (8.9)	7.52 *d* (8.7)	2.74 *m* (2H)
10	7.64 *d* (9.0)	7.55 *d* (8.9)	7.55 *d* (8.9)	7.59 *d* (8.7)	2.67 *m* (2H)
2-OCH_3	4.01 *s*	4.00 *s*	4.03 *s*	–	–
3-OCH_3	4.03 *s*	4.03 *s*	–	4.09 *s*	3.92 *s*
4-OCH_3	4.00 *s*	4.00 *s*	3.94 *s*	–	–
5-OCH_3	–	–	–	4.10 *s*	3.88 *s*
7-OCH_3	–	–	–	3.95 *s*	3.84 *s*
8-OCH_3	3.98 *s*	–	–	–	–
OH	5.78 *s*	–	6.03 *s*	5.84 *s*	5.57 *s*
–OCH_2O–	–	6.16 *s* (2H)	6.16 *s* (2H)	–	–

Table 2
Cytotoxic activity of the isolated phenanthrenes **1**–**5** on HeLa cells

Compound	$IC_{50} \pm SEM$ (μM)
1	8.52 ± 0.70
2	>30
3	3.64 ± 0.12
4	11.49 ± 0.68
5	14.21 ± 1.64
Doxorubicin	0.15 ± 0.028
Cisplatin	12.43 ± 1.05

substitution pattern was determined with the aid of a NOESY spectrum. Overhauser effects were detected between H-1/H-10, H-10/H-9, H-9/H-8, H-8/7-OCH_3, 7-OCH_3/H-6, H-6/5-OCH_3, 5-OCH_3/H-4 and H-4/3-OCH_3. These NOESY correlations led to the formulation of compound **5** as 2-hydroxy-3,5,7-trimethoxy-9,10-dihydrophenanthrene. This is the second reported isolation of this compound from a natural source: it was first obtained from the heartwood of *Combretum psidioides* (Letcher and Nhamo, 1972b). Interestingly, a similar compound, 5-hydroxy-2,3,7-trimethoxy-9,10-dihydrophenanthrene, differing only in the positions of two substituents, was identified earlier from the $CHCl_3$ extract of the rhizomes of *T. communis* (Aquino et al., 1985b).

The isolated compounds were tested for their cytotoxic activity on a HeLa cell line using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, compared with cisplatin and doxorubicin as positive controls. The cell growth-inhibitory potencies of compounds **1**–**5**, expressed as IC_{50} values, are shown in Table 2. 3-Hydroxy-2,4-dimethoxy-7,8-methylenedioxyphenanthrene (**3**) exhibited the most potent cytotoxic activity ($IC_{50} = 3.64 \pm 0.12$ μM), while compounds **1**, **4** and **5** displayed moderate cytotoxicities ($IC_{50} = 8.52$–14.21 μM), whereas **2** was found to be inactive.

3. Conclusions

The distribution of phenanthrene and dihydrophenanthrene in the plant kingdom appears to be limited; their occurrence has been reported to date in only a few plant families: Orchidaceae, Dioscoreaceae, Combretaceae, etc. These compounds are known to be endogenous plant growth regulators and potent phytoalexins. As concerns their pharmacological profile, a number of phenanthrenes have been reported to exert antiviral activity against vesicular stomatitis virus and human rhinovirus serotype 1B (Aquino et al., 1991), and smooth muscle-relaxing activity (Estrada et al., 1999). Some natural phenanthrenes and dihydrophenanthrenes have also been reported to display an antitumour effect (Pettit et al., 1988; Lee et al., 1995; Long et al., 1997; Shagufta et al., 2006). Our results provide further evidence that hydroxy/alkoxy-substituted phenanthrenes are promising antitumour agents. However, the low number of compounds **1**–**5** are not sufficient for a thorough structure-activity relationships, the cytotoxic potencies clearly indicate that the C(9)–C(10) double bond is not essential for the cytostatic effect, as compounds **4** and **5** are almost equally effective. A similar observation was reported earlier on structurally related alkoxy-substituted phenanthrenes and dihydrophenanthrenes (Pettit et al., 1988). The number and positions of the methoxy groups on the phenanthrene skeleton seem to be crucial factors as regards the efficacy, similarly as for the conformationally less restricted analogues, the *cis*-stilbene combretastatins. Although the highly active combretastatins require a trimethoxy-substituted benzene ring in the molecule (Srivastava et al., 2005), our results reveal that this is not essential for the phenanthrenes, since compound **2**, containing three methoxy substituents, proved to be the least active, and compound **3**, with only two methoxy groups, was the most effective in the MTT assay. Comparison of the effects of compounds **1**–**3** suggests that the presence of a hydroxy group on either ring A (**3**) or ring B (**1**) is favourable relative to full alkoxysubstitution (**2**). All of these findings demonstrate that the structure–activity relationships established for combretastatins cannot be applied to the congeners, e.g. to phenanthrenes. The present results can contribute to the design of further synthetic phenanthrenes, in which limited methoxylation is expected to be a favourable feature.

4. Experimental

4.1. General experimental procedures

Melting points are uncorrected. UV spectra were recorded in MeOH on a Shimadzu UV-2101 PC UV–VIS spectrophotometer. NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 11.7 T (500 MHz for ^1H and 125 MHz for ^{13}C); the signals of the deuterated solvent were taken as the reference (7.26 ppm in ^1H NMR and 77.0 ppm in JMOD). Two-dimensional experiments (NOESY) were set up, performed and processed with the standard Bruker protocol. HREIMS spectra were obtained on a Finnigan MAT 95 S spectrometer. For vacuum liquid chromatography, silica gel (Kieselgel GF_{254} 15 μm, Merck) was used. Preparative TLC was carried out on silica gel (Kieselgel $60F_{254}$, Merck). The chromatograms were visualized under UV light at 254 and 365 nm, and by spraying with concentrated H_2SO_4, followed by heating at 110 °C for 10 min. Sephadex LH-20 (Pharmacia Fine Chemicals) was used for gel chromatography. Centrifugal chromatography was carried out on Chromatotron (Harrison Research) apparatus, using manually coated silica gel (60 GF_{254}, Merck) plates with 1.0 mm thickness.

4.2. Plant material

The rhizomes of *Tamus communis* L. were collected in the Mecsek Hills (Hungary) in June 2003. A voucher specimen

(no. 619) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

4.3. Extraction and isolation of compounds

The fresh rhizomes of the plant (1.2 kg) were crushed and then percolated with MeOH (10 l) at room temperature. The MeOH extract was concentrated and extracted with petroleum ether 40–60 °C (5 × 400 ml) and $CHCl_3$ (5 × 400 ml). The petroleum ether fraction (1.32 g) was chromatographed by vacuum liquid chromatography, using a gradient system of cyclohexane–EtOAc (98:2, 95:5, 9:1, 85:15, 8:2 and 1:1, 15 × 20 ml of each). A total of 90 fractions were collected and combined on the basis of TLC monitoring, affording nine main fractions (I–IX). Fraction III was separated by preparative TLC on silica gel with the use of *n*-hexane–$CHCl_3$–Me_2CO (70:40:3) as mobile phase, yielding compound **2** (9.3 mg). Fraction V afforded a considerable amount of a crystalline material upon standing, which was identified as β-sitosterine. The mother liquor of this fraction was subjected to preparative TLC, using *n*-hexane–$CHCl_3$–Me_2CO (70:30:3) as developing system, and then further purified by gel chromatography on Sephadex LH-20, with elution with MeOH. Finally, the main compound from this separation was chromatographed by preparative TLC on silica gel with benzene–EtOAc (9:1), to furnish compound **5** (2.6 mg). Fractions VI and VII were processed in the same manner, first by centrifugal chromatography, using a gradient solvent system of cyclohexane–EtOAc (9:1, 85:15, 8:2, 7:3 and 1:1), and then by preparative TLC on silica gel in two steps. First, benzene–EtOAc (9:1) was used as solvent system, while in the second step *n*-hexane–$CHCl_3$–Me_2CO (50:49:1) was applied, which afforded compounds **1** (10.5 mg), **3** (16.3 mg) and **4** (2.6 mg).

4.3.1. 6-Hydroxy-2,3,4,8-tetramethoxyphenanthrene (*1*)

Colorless crystals; m.p. 183–186 °C; UV (MeOH) λ_{max} nm (log ε): 233 (4.26), 263 (4.80), 287 (4.11), 294 (3.99), 308 (3.97), 331 (3.26), 352 (3.31), 365 (3.38); ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 105.3$ (C-1), 148.0 (C-2), 143.1 (C-3), 151.9 (C-4), 119.4 (C-4a), 124.8 (C-4b), 124.2 (C-5), 116.2 (C-6), 145.5 (C-7), 140.8 (C-8), 126.4 (C-8a), 119.3 (C-9), 127.3 (C-10), 128.4 (10a), 55.9, 60.2, 61.2, 61.9 (4×OCH_3), the signals were tentatively assigned; HREIMS *m*/*z* 314.1161 $[M]^+$ (calcd. for $C_{18}H_{18}O_5$, 314.1154).

4.3.2. 2,3,4-Trimethoxy-7,8-methylenedioxyphenanthrene (*2*)

Yellowish-white crystals; 149–151 °C; UV (MeOH) λ_{max} nm (log ε): 233 (4.54), 268 (4.81), 277 (4.69), 293 (4.18), 305 (4.08), 315 (3.94), 360 (3.57), 375 (3.64); ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$): δ 105.8 (C-1), 152.0, 151.9 (C-2, C-4), 143.1 (C-3), 117.6, 119.4 (C-4a, C-4b), 120.5 (C-5), 108.9 (C-6), 143.4 (C-7), 142.3 (C-8), 125.0 (C-8a), 118.6 (C-9), 127.1 (C-10), 129.1 (C-10a), 101.4 (–OCH_2O–), 55.8, 60.1, 61.2 (3 × OCH_3).

4.3.3. 3-Hydroxy-2,4-dimethoxy-7,8-methylenedioxyphenanthrene (*3*)

Yellowish-white crystals; m.p. 159–161 °C; UV (MeOH) λ_{max} nm (log ε): 232 (4.52), 268 (4.81), 276 (4.70), 294 (4.10), 307 (4.06), 320 (3.97), 340 (3.55), 355 (3.77), 373 (3.81); ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1; ^{13}C NMR (125 MHz, $CDCl_3$): δ 105.3 (C-1), 144.1, 146.7 (C-2, C-4), 139.4 (C-3), 117.8, 119.0 (C-4a, C-4b), 120.3 (C-5), 108.7 (C-6), 143.4 (C-7), 142.2 (C-8), 124.5 (C-8a), 117.1 (C-9), 127.3 (C-10), 125.9 (C-10a), 101.4 (–OCH_2O–), 56.1, 59.7 (2×OCH_3).

4.3.4. 2-Hydroxy-3,5,7-trimethoxyphenanthrene (*4*)

Yellowish-white crystals; m.p. 176–177 °C; UV (MeOH) λ_{max} nm (log ε): 234 (4.34), 251 (4.73), 261 (4.94), 284 (4.19), 320 (3.34), 330 (3.46), 345 (3.73), 362 (3.84); ^{1}H NMR (500 MHz, $CDCl_3$), see Table 1.

4.3.5. 2-Hydroxy-3,5,7-trimethoxy-9,10-dihydrophenanthrene (*5*)

White amorphous solid; UV (MeOH) λ_{max} nm (log ε): 218 (4.17), 268 (3.86), 277 (3.91), 303 (3.81), 313 (3.75); ^{1}H NMR (500 MHz, $CDCl_3$), see in Table 1; HREIMS: *m*/*z* 286.1217 $[M]^+$ (calcd. for $C_{17}H_{18}O_4$, 286.1205).

4.4. Bioassays

Cytotoxic effects were measured *in vitro* on a HeLa (cervix adenocarcinoma) cell line, using the MTT colorimetric assay. The cytotoxicity tests were carried out in 96-well microtitre plates, using 5000 cells per well in all cases, which were allowed to adhere overnight before the drugs were introduced. The original medium was then removed, 200 μl culture medium containing the compounds of interest was added and the cells were incubated for 72 h. The tested extracts and compounds were dissolved in DMSO. The final concentration of DMSO never exceeded 0.3%, and therefore had no essential effect on the cell growth. Next the living cells were assayed: aliquots (20 μl at 5 mg/ml) of the MTT stock solution were pipetted into each well and reduced by viable cells to an insoluble formazan product during a further 4 h. After this contact period, the medium was removed and the formazan crystals were dissolved in 100 μl DMSO by gentle shaking for 60 min. Finally, the absorbance was measured at 545 nm with a microplate reader (Mosmann, 1983). In this way the cell growth or drug toxicity was determined. The 50% inhibitory concentration (IC_{50}) was derived from the dose–response curves fitted to the measured points by GraphPad Prism 2.01. All *in vitro* experiments were carried out on two microplates with at least five parallel wells.

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Review

Natural phenanthrenes and their biological activity

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Abstract

The aim of this review is to survey the various naturally occurring phenanthrene compounds that have been isolated from different plants. Only one review has previously been published on this topic. Gorham [Gorham, J., 1989. Stilbenes and phenanthrenes. Meth. Plant Biochem. 1, 159–196] reviewed the structures, biosynthesis, separations and spectroscopy of stilbenes and phenanthrenes.

The present study furnishes an overview of the hydroxy or/and methoxy-substituted 9,10-dihydro/phenanthrenes, methylated, prenylated and other monomeric derivatives, dimeric and trimeric phenanthrenes and their biological activities.

A fairly large number of phenanthrenes have been reported from higher plants, mainly in the Orchidaceae family, in the species *Dendrobium*, *Bulbophyllum*, *Eria*, *Maxillaria*, *Bletilla*, *Coelogyna*, *Cymbidium*, *Ephemerantha* and *Epidendrum*. A few phenanthrenes have been found in the Hepaticae class and Dioscoreaceae, Combretaceae and Betulaceae families. Their distribution correlates strongly with the taxonomic divisions.

These plants have often been used in traditional medicine, and phenanthrenes have therefore been studied for their cytotoxicity, antimicrobial, spasmolytic, anti-inflammatory, antiplatelet aggregation, antiallergic activities and phytotoxicity.

On the basis of 120 references, this review covers the phytochemistry and pharmacology of phenanthrenes, describing 252 compounds. This contribution stems from our work on the medicinal plant *Tamus communis*.

Keywords: Phenanthrenes; Dihydrophenanthrenes; Biological activity; Dimeric phenanthrenes; Phenanthraquinones

Contents

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1. Introduction

The phenanthrenes are a rather uncommon class of aromatic metabolites which are presumably formed by oxidative coupling of the aromatic rings of stilbene precursors. Besides these stilbene derived compounds, phenanthrenes most likely originated from diterpenoid precursors are also included in the present review. A large number of differently substituted phenanthrenes have been reported to occur in plants and have been demonstrated to possess various biological activities.

The phenanthrenes isolated so far may be classified into three major groups: monophenanthrenes, diphenanthrenes and triphenanthrenes. Monophenanthrenes are subdivided according to the number and type of the structural moieties, while diphenanthrenes can be classified by the type of connection of the phenanthrene units. Up to present only one compound of the triphenanthrene group was described.

2. Occurrence of phenanthrenes

A fairly large number of phenanthrenes have been reported from higher plants, mainly in the Orchidaceae family, in 49 species: in particular *Dendrobium*, *Bulbophyllum*, *Eria*, *Maxillaria*, *Bletilla*, *Coelogyna*, *Cymbidium*, *Ephemerantha* and *Epidendrum*. A few phenanthrenes have been found in the Hepaticae class and Dioscoreaceae, Combretaceae and Betulaceae families (Table 1). The phenanthrenes were mainly isolated from the whole plants, but in some cases the cortex, tubers or stems were studied and found to contain such compounds. The greatest number of phenanthrenes has been described from the *Juncus* species, but notable numbers have been isolated from *Bletilla striata* and *Bulbophyllum vaginatum*.

3. Structural characteristics of phenanthrenes

3.1. Monomeric phenanthrenes

Most natural phenanthrenes occur in monomeric form; this group consists of about 210 compounds (Tables 2–9). Of these, almost 100 are only hydroxy- and/or methoxy-substituted, and equally 9,10-dihydro- or dehydro derivatives (Tables 2–5). Their great structural diversity stems from the number and position of their oxygen functions. The hydroxy and methoxy moieties number are between 3 and 6, and can usually be found on C-2, C-3, C-5, C-6 or C-7. Homogeneously substituted phenanthrenes are rare; there are only four such compounds among the trisubstituted phenanthrenes bearing only methoxy functionalities (**2**, **10**, **12** and **20**). Only two of the tetrasubstituted compounds are all-hydroxy-substituted (**29**, **58**) and three are substituted only with methoxy groups [callosumin (**26**), **46**, callosuminin (**57**)]. The structures of two compounds [rotundatin (**40**) and **61**] are unusual as they contain a hydroxy or methoxy group on C-9 or C-10. None of the pentasubstituted compounds bears only hydroxy or only methoxy groups. Interestingly, one compound [plicatol-A (**97**)] contains methoxy functions on both C-9 and C-10. There are only two hexasubstituted monophenanthrenes (**98**, **99**) (Table 5).

Besides hydroxy and methoxy groups, further substituents can be found in monomeric phenanthrenes, such as methyl [stemanthrene A (**104**)], hydroxymethyl (**120**), carboxy (**123**), formyl [dehydroeffusal (**153**)], prenyl [gancaonin U (**154**), sinensol G (**156**)] and vinyl (**123**–**128**) (Table 6). Compounds, substituted with methyl and vinyl groups can be found in *Micrandropsis*, *Sagotia*, *Stemona*, *Juncus* and *Domohinea* species. Euphorbiaceae family can be characterized by the occurrence of a wide range of diterpenes, thus it can be assumed that methyl and oxymethyl-substituted phenanthrenes in *Micrandropsis*, *Sagotia* and *Domohinea* have diterpenoid origin (de Alvarenga et al., 1976). *Juncus* phenanthrenes obviously also derived from a specific biosynthetic pathway. Glycosides are relatively rare: they have been reported only in *Juncus effusus* [effusides I–V. (**157**–**161**)], *Epimedium koreanum* [epimedoicariside A (**162**)], *Dendrobium chrysanthum* [denchryside A (**163**)] and *B. striata* (**164**–**167**). In *Coelogyne ochracea* a carboxy group [ochrolic acid (**168**)], in *Micrandropsis scleroxylon* the uncommon *S*-methyl group [micrandrol C (**169**)], and in *Polysyphonia ferulacea* two bromide groups [polysiphenol (**170**)] are attached to the phenanthrene skeleton. Yamaki et al. (1993a) isolated a novel type of phenanthrene with a spirolactone ring, blespirol (**171**), from *B. striata* (Table 7). The structure was proved by X-ray analysis. In general, unusual substitution is somewhat more likely in 9,10-dihydrophenanthrenes.

Another type of monomeric phenanthrenes is the group of phenanthraquinones; altogether 19 compounds belong in this group (Table 8). They are usually hydroxy, methoxy or methyl-substituted, but in *Cremastra appendiculata* a 2-oxopropyl group (**184**, **185**), in *Spiranthes sinensis* a prenyl group [spiranthoquinone (**186**)], and in *Plectranthus* species a 2-propenyl [plectranthon A, C, D (**187**, **188**, **190**)] or 2-acetoxypropyl group (**189**) can be found in the molecule. Plectanthrones A–D (**187**–**190**) are 1,4-phenanthraquinones, which biogenetically are derived from the diterpenoid type abietanoic precursors. (Alder et al., 1984). In the 1990s, a new bisbibenzyl derivative, shancilin (**246**) was isolated from the tubers of the orchid *Pleione bulbocodioides*, and were two **244** and **245** from *Frullania convoluta*. Guo et al. (2006) isolated an unusual molecule, a bibenzyl-dihydrophenanthrene ether, phoyunnanin D (**247**), from the whole plant of *Pholidota yunnanensis*.

Isolation of monomeric phenanthrenes was carried out in general similarly. Usually, the plant materials are extracted with MeOH, EtOH, $CHCl_3$, Me_2CO or MeOH–$CHCl_3$ (1:1). After concentration *in vacuo*, the residue is extracted successively with Et_2O, EtOAc, $CHCl_3$ or *n*-hexane. Extracts are chromatographed on a silica gel

Table 1
Occurrence of phenanthrenes in plant families

	Latin name	Drug	Compounds	Activity
Berberidaceae	*Epimedium koreanum*		**162**	
Betulaceae	*Alnus maximowiczii*	Herba	**20**	
Combretaceae	*Combretum apiculatum*	Cortex	**27**, **31**, **62**, **65–70**, **76**, **78–80**, **83**, **84**	
	Combretum caffrum	Cortex	**62**, **66**, **67**, **69**, **82**	Antitumour
	Combretum molle	Cortex	**65**, **68–70**, **80**, **84**	
	Combretum psidioides	Cortex	**25**, **56**, **62**, **66**, **71**, **75**, **81**, **82**	
Dioscoreaceae	*Dioscorea batatas*		**53–55**	Antifungal
	Dioscorea bulbifera		**29**, **58**	
	Dioscorea decipiens	Radix	**74**	
	Dioscorea prazeri	Tuber, yam	**69**	
	Dioscorea rotundata	Tuber, yam	**6**, **53**, **56**	Antifungal
	Tamus communis	Radix	**25**, **27**, **28**, **42**, **43**, **47**, **48**, **56**, **85–93**, **182**	Antitumour, antiviral
Euphorbiaceae	*Domohinea perrieri*	Stem	**108–111**, **179**	Cytotoxic
	Micrandropsis scleroxylon	Trunk wood	**100**, **102**, **169**	
	Sagotia racemosa	Trunk wood	**100–103**	
Fabaceae	*Glycyrrhiza uralensis*	Aerial part	**154**, **155**	
Hepaticae	*Frulania convoluta*		**244**, **245**	
	Marchantia polymorpha	Herba	**21**, **22**, **226–228**	
	Plagiochila killarniensis	Aerial part	**8–14**, **23**, **26**	
	Plagiochila oresitropha			
Juncaceae	*Juncus acutus*	Aerial part	**112–128**, **131**, **133–138**, **142**, **146–148**, **152**, **236–241**	Antialgal
	Juncus effusus	Aerial part	**112**, **115–118**, **121–124**, **126–128**, **130**, **132–135**, **139–141**, **143–146**, **149–151**, **153**, **157–161**	
	Juncus roemerianus	Root	**115**, **116**, **129**, **146**	Antimicrobial
Lamiaceae	*Plectranthus* species		**187–190**	
Orchidaceae	*Agrostophyllum callosum*	Whole plant	**3**, **24**, **26**, **31**, **57**, **221**, **222**	
	Agrostophyllum khasiyanum	Whole plant	**221**, **222**	
	Arundina graminifolia	Rhizoma	**202**	
	Bletilla formosana	Whole plant	**41**, **192–201**, **218**	
	Bletilla striata	Tuber	**1**, **2**, **4**, **17**, **46**, **164–167**, **171**, **191–195**, **197**, **207**, **214–216**, **218**, **219**, **229**, **249–252**	Antitumour/antimicrobial
	Bulbophyllum gymnopus	Whole plant	**62**, **75**, **85**, **96**	
	Bulbophyllum leopardium	Whole plant	**51**	
	Bulbophyllum reptans	Whole plant	**1**, **19**, **62**, **75**, **85**, **96**, **214**, **223–225**	
	Bulbophyllum vaginatum	Whole plant	**1**, **19**, **24**, **30**, **36**, **42–44**, **50**, **52**, **61–64**, **75–77**, **233**	
	Cirrhopetalum andersonii	Whole plant	**34**, **45**	
	Cirrhopetalum maculosum		**223**	
	Coelogyne cristata	Whole plant	**73**, **95**	
	Coelogyne elata	Whole plant	**1**	
	Coelogyne flaccida	Whole plant	**31**	
	Coelogyne ochracea	Whole plant	**1**, **168**, **176**, **177**	
	Cremastra appendiculata	Tuber	**18**, **19**, **184**, **185**, **223**, **230**, **231**, **243**	Cytotoxic
	Cymbidium aloifolium	Root	**1**, **24**, **180**, **181**	
	Cymbidium pendulum	Whole plant	**86**, **99**	
	Cypripedium tibeticum		**173**, **178**	
	Dendrobium chrysanthum	Herba	**163**	
	Dendrobium densiflorum	Stems	**4**, **15**, **86**, **173**, **175**	
	Dendrobium loddigesii	Stems	**15**	Antiplatelet aggregation inhibitory
	Dendrobium moniliforme	Stem	**172**, **183**	Anti-inflammatory
	Dendrobium moscatum		**15**	
	Dendrobium nobile	Aerial part	**4**, **172**	Antitumour
	Dendrobium plicatile	Stems	**4**, **15**, **36**, **40**, **43**, **97**, **174**, **213**	
	Dendrobium rotundatum	Whole plant	**40**, **42**, **62**, **75**	
	Dendrobium thyrsiflorum	Stems	**86**, **220**, **242**	Cytotoxic
	Ephemerantha fimbriata		**38**, **50**, **94**	
	Ephemerantha lonchophylla	Stems	**35–37**, **43**, **48**, **172**, **174**	Antiplatelet aggregation inhibitory

Table 1 (*continued*)

	Latin name	Drug	Compounds	Activity
	Epidendrum rigidum	Whole plant	**35**, **94**	Phytotoxic
	Eria carinata	Whole plant	**42**	
	Eria confusa	Whole plant	**85**, **98**	
	Eria flava	Whole plant	**1**, **19**, **37**, **43**, **214**	
	Eria stricta	Whole plant	**42**	
	Eulophia nuda	Tuber	**1**, **32**, **33**, **42**, **43**, **59**, **60**, **86**, **232**	
	Eulophia petersii	Root	**1**, **4**, **16**, **32**, **59**	
	Gymnadenia conopsea	Tuber	**1**, **7**, **194**, **195**, **210**, **211**, **214**, **217**	Antiallergic
	Loroglossum hircinum		**5**, **6**	Antifungal
	Lusia indivisa	Whole plant	**4**, **16**	
	Lusia volucris		**234**	
	Maxillaria densa	Whole plant	**36**, **39**, **42**, **49**, **94**, **96**	Phytotoxic, spasmolytic
	Nidema boothii	Whole plant	**4**, **37**, **43**, **72**, **86**, **174**	Spasmolytic
	Orchis militaris		**3**	Antifungal
	Pholidota yunnanensis	Whole plant	**247**, **248**	NO production inhibitory
	Pleione bulbocodioides		**1**, **4**, **195**, **207**, **246**	
	Scaphyglottis livida	Whole plant	**1**, **43**, **86**	Spasmolytic/aorta dilatatory
	Spiranthes sinensis	Aerial part	**156**, **186**, **202–206**, **208**, **209**, **235**	Cytotoxic
	Thunia alba	Whole plant	**4**, **43**, **86**, **214**, **223**	
Rhodomelaceae	*Polysyphonia ferulacea*		**170**	
Stemonaceae	*Stemona collinsae*		**104–107**	
	Stemona pierrei		**104–107**	
	Stemona tuberosa		**104–107**	

column or on Sephadex LH-20 or subjected to preparative TLC and HPLC. Majumder et al. (1982) developed a special method for the isolation of phenanthrenes. The plant materials were soaked in MeOH for three weeks. The MeOH extract was then drained off, concentrated under reduced pressure, and diluted with H_2O, and the liberated solids were exhaustively extracted with Et_2O. The Et_2O extract was fractionated into acidic and non-acidic fractions with 2 M aqueous NaOH. The aqueous alkaline solution was acidified in the cold with conc. HCl, and the liberated solids were extracted with Et_2O, the extract was washed with H_2O and dried, and the solvent was removed.

3.2. Dimeric phenanthrenes and a triphenanthrene

The nearly 40 dimeric phenanthrenes include 9,10-dihydro- and dehydro derivatives (Tables 10 and 11). The monomers are mostly 1-1′-linked, but 1-3′, 1-8′ and 3-3′ linkages also occur in the natural compounds. The compounds are usually hydroxy and methoxy-substituted. In 2003, DellaGreca et al. isolated five dimeric 9,10-dihydrophenanthrenoids with interesting hepta- or octacyclic structures (**237**–**241**) from the rhizome of *Juncus acutus*. From the stems of *Dendrobium thyrsiflorum*, a derivative of phenanthrene–phenanthraquinone, denthyrsinone (**242**), was obtained (Zhang et al., 2005). Investigation of the tubers of *B. striata* led to the isolation of four unusual *bis*(dihydrophenanthrene)ethers (**249**–**252**), in which phenanthrene monomers are coupled through ether bridge. The structures of blestrin C (**251**) and D (**252**) were confirmed by X-ray analysis (Yamaki et al., 1992) (see Table 11).

The only triphenanthrene (**243**) described so far was isolated from the tubers of an orchidaceous plant, *C. appendiculata* (Xue et al., 2006).

4. Biological activities

4.1. Anticancer effects of phenanthrenes

In 1979, Pettit et al. began a study of cancer cell growth inhibitors present in the African willow tree *Combretum caffrum* which resulted in the isolation and structural determination of a series of active phenanthrenes, dihydrophenanthrenes and stilbenes. The cell growth inhibitory activities of phenanthrenes from *C. caffrum* (**62**, **66**, **69**, **82**) were tested on murine P388 lymphocytic leukaemia cell lines; the IC_{50} values were 2.2, 2.8, 2.6 and 2.0 μg/ml, respectively. A series of publications by other researchers reported similar effects of phenanthrenes (Pettit et al., 1988, and references cited therein).

Lusianthridin (**4**) and denbinobin (**172**) isolated from *Dendrobium nobile* were found to exert cytotoxic effects both *in vitro* and *in vivo*. The significant activities of these compounds on A549 human lung carcinoma [ED_{50}: 7.7 μg/ml (**4**); 1.3 μg/ml (**172**)]; SK-OV-3 human ovary adenocarcinoma [ED_{50}: 9.4 μg/ml (**4**); 3.5 μg/ml (**172**)] and HL-60 human promyelocytic leukaemia [ED_{50}: 9.8 μg/ml (**4**); 0.11 μg/ml (**172**)] cell lines were also demonstrated. Lusianthridin (**4**) appears to be less effective than denbinobin (**172**). Their methylated derivatives did not exhibit activities, suggesting that a free phenolic hydroxy group

Table 2
Trisubstituted dihydro/phenanthrenes with hydroxy- or/and methoxy-substitution

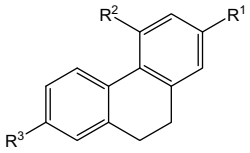
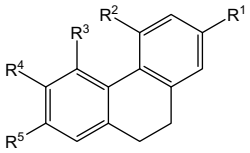
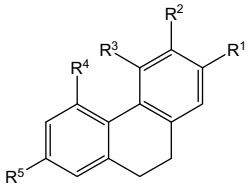
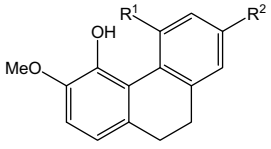
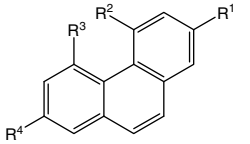
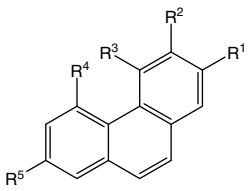
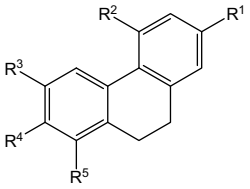
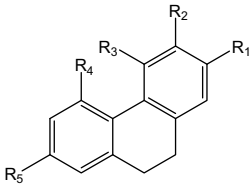
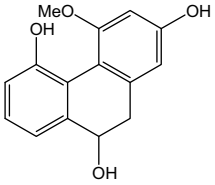
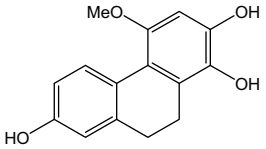
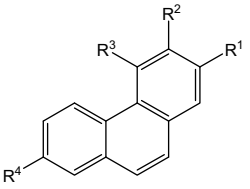
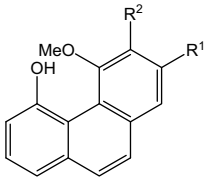
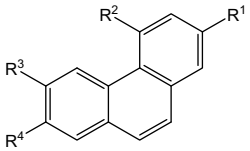
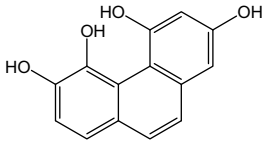
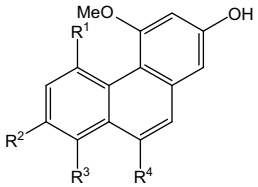
	**1** $R^1 = OH$, $R^2 = OMe$, $R^3 = OH$; coelonin	*B. reptans* (Majumder et al., 1999) *B. striata* (Yamaki et al., 1991) *B. vaginatum* (Leong et al., 1997) *C. aloifolium* (Juneja et al., 1987) *C. elata* (Majumder et al., 1982) *C. ochracea* (Majumder et al., 1982) *E. flava* (Majumder and Banerjee, 1990a) *E. nuda* (Tuchinda et al., 1988) *E. petersii* (Blitzke et al., 2000) *G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006) *P. bulbocodioides* (Bai et al., 1996) *S. livida* (Estrada et al., 1999a)
	2 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$	*B. striata* (Yamaki et al., 1991)
	3 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$; orchinol	*A. callosum* (Majumder et al., 1995, 1996) *O. militaris* (Baller et al., 1957; Fisch et al., 1973)
	4 $R^1 = OMe$, $R^2 = OH$, $R^3 = OH$; lusianthridin	*B. striata* (Takagi et al., 1983) *D. densiflorum* (Fan et al., 2001) *D. nobile* (Lee et al., 1995) *D. plicatile* (Yamaki and Honda, 1996) *E. petersii* (Blitzke et al., 2000) *L. indivisa* (Majumder and Lahiri, 1990d) *N. boothii* (Hernandez-Romero et al., 2004) *P. bulbocodioides* (Bai et al., 1996) *T. alba* (Majumder et al., 1998b)
	**5** $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = H$, $R^5 = H$; loroglossol	*L. hircinum* (Fisch et al., 1973)
	6 $R^1 = OH$, $R^2 = OMe$, $R^3 = OH$, $R^4 = H$, $R^5 = H$; hircinol	*L. hircinum* (Fisch et al., 1973) *D. rotundata* (Coxon et al., 1982)
	**7** $R^1 = OMe$, $R^2 = H$, $R^3 = OH$, $R^4 = OH$, $R^5 = H$	*G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006)
	8 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$, $R^5 = H$	*Plagiochila* sp. (Anton et al., 1997) *P. killarniensis* (Rycroft et al., 1999)
	9 $R^1 = OMe$, $R^2 = OMe$, $R^3 = H$, $R^4 = OH$, $R^5 = H$	*Plagiochila* sp. (Anton et al., 1997)
	10 $R^1 = OMe$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$, $R^5 = H$	*Plagiochila* sp. (Anton et al., 1997)
	11 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = H$, $R^5 = OMe$	*Plagiochila* sp. (Anton et al., 1997)
	12 $R^1 = OMe$, $R^2 = OMe$, $R^3 = H$, $R^4 = H$, $R^5 = OMe$	*Plagiochila* sp. (Anton et al., 1997)
	**13** $R^1 = OH$, $R^2 = H$	*Plagiochila* sp. (Anton et al., 1997) *P. killarniensis* (Rycroft et al., 1999)
	14 $R^1 = H$, $R^2 = OMe$	*Plagiochila* sp. (Anton et al., 1997)
	**15** $R^1 = OH$, $R^2 = OMe$, $R^3 = OH$, $R^4 = H$; plicatol-B, moscatin	*D. plicatile* (Honda and Yamaki, 2000) *D. rotundatum* (Majumder and Sen, 1987b; Majumder and Pal, 1992) *D. densiflorum* (Fan et al., 2001) *D. moscatum* (Majumder and Sen, 1987b) *D. loddigesii* (Chen et al., 1994)
	16 $R^1 = OMe$, $R^2 = OH$, $R^3 = H$, $R^4 = OH$; lusianthrin	*E. petersii* (Blitzke et al., 2000) *L. indivisa* (Majumder and Lahiri, 1990d)
	17 $R^1 = OMe$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$	*B. striata* (Yamaki et al., 1991)
	18 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$	*C. appendiculata* (Xue et al., 2006)
	19 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = OH$; flavanthrinin	*B. reptans* (Majumder et al., 1999) *B. vaginatum* (Leong et al., 1997) *E. flava* (Majumder and Banerjee, 1990a) *C. appendiculata* (Xue et al., 2006)
	**20** $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = H$, $R^5 = H$	*A. maximowiczii* (Tori et al., 1995)
	21 $R^1 = OMe$, $R^2 = OMe$, $R^3 = H$, $R^4 = H$, $R^5 = OH$	*M. polymorpha* (Adam and Becker, 1994)
	22 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = H$, $R^5 = OH$	*M. polymorpha* (Adam and Becker, 1994)
	23 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$, $R^5 = H$	*Plagiochila* sp. (Anton et al., 1997)

Table 3
Tetrasubstituted dihydro/phenanthrenes with hydroxy- or/and methoxy-substitution

	**24** $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$; 6-methoxy-coelonin	*A. callosum* (Majumder et al., 1995, 1996) *B. vaginatum* (Leong et al., 1997) *C. aloifolium* (Juneja et al., 1987)
	25 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$;	*C. psidioides* (Letcher and Nhamo, 1972b) *T. communis* (Kovács et al., 2007)
	26 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = H$; callosumin	*A. callosum* (Majumder et al., 1996) *Plagiochila* sp. (Anton et al., 1997)
	27 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$	*T. communis* (Aquino et al., 1985a, 1991; Letcher and Nhamo, 1972a) *C. apiculatum* (Letcher and Nhamo, 1971)
	28 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = H$	*T. communis* (Aquino et al., 1985a, 1991)
	29 $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$, $R^5 = H$	*D. bulbifera* (Wij and Rangaswami, 1978)
	30 $R^1 = OH$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OH$, $R^5 = H$	*B. vaginatum* (Leong et al., 1999)
	31 $R^1 = OH$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OMe$, $R^5 = H$; callosin	*A. callosum* (Majumder et al., 1995, 1996) *C. flaccida* (Majumder et al., 1995) *C. apiculatum* (Letcher and Nhamo, 1971)
	32 $R^1 = OMe$, $R^2 = OH$, $R^3 = H$, $R^4 = OMe$, $R^5 = OH$; eulophiol	*E. nuda* (Tuchinda et al., 1988) *E. petersii* (Blitzke et al., 2000)
	33 $R^1 = OH$, $R^2 = OMe$, $R^3 = H$, $R^4 = OMe$, $R^5 = OH$	*E. nuda* (Tuchinda et al., 1988)
	**34** $R^1 = R^2 = –O–CH_2–O–$, $R^3 = OH$, $R^4 = H$, $R^5 = OH$; cirrhopetalanthridin	*C. andersonii* (Majumder and Basak, 1991a)
	35 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = H$, $R^5 = OH$; ephemeranthol-A	*E. lonchophylla* (Tezuka et al., 1991) *E. rigidum* (Hernandez-Romero et al., 2005)
	36 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = H$, $R^5 = OH$; erianthridin	*B. vaginatum* (Leong et al., 1997) *D. plicatile* (Yamaki and Honda, 1996) *E. lonchophylla* (Chen et al., 2000; Tezuka et al., 1991) *M. densa* (Estrada et al., 1999b, 2004; Valencia-Islas et al., 2002)
	37 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = H$, $R^5 = OH$; ephemeranthol-B, flavanthridin	*E. lonchophylla* (Tezuka et al., 1991) *E. flava* (Majumder and Banerjee, 1990a) *N. boothii* (Hernandez-Romero et al., 2004)
	38 $R^1 = OH$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$; ephemeranthol-C	*E. fimbriata* (Tezuka et al., 1993)
	39 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = H$	*M. densa* (Estrada et al., 1999b)
	**40** plicatol-C, rotundatin	*D. plicatile* (Honda and Yamaki, 2000) *D. rotundatum* (Majumder and Pal, 1992)
	**41**	*B. formosana* (Lin et al., 2005)
	**42** $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$; nudol	*B. vaginatum* (Leong et al., 1997) *D. rotundatum* (Majumder and Pal, 1992) *E. carinata* (Bhandari et al., 1985) *E. stricta* (Bhandari et al., 1985) *E. nuda* (Bhandari et al., 1985; Tuchinda et al., 1988) *M. densa* (Estrada et al., 1999b, 2004) *T. communis* (Réthy et al., 2006)

(*continued on next page*)

Table 3 (*continued*)

	43 R^1 = OMe, R^2 = OH, R^3 = OMe, R^4 = OH;	*B. vaginatum* (Leong et al., 1997) *D. plicatile* (Yamaki and Honda, 1996) *E. flava* (Majumder and Banerjee, 1990a) *E. lonchophylla* (Chen et al., 2000) *E. nuda* (Tuchinda et al., 1988) *N. boothii* (Hernandez-Romero et al., 2004) *S. livida* (Estrada et al., 1999a; Estrada-Soto et al., 2006) *T. alba* (Majumder et al., 1998b) *T. communis* (Réthy et al., 2006)
	44 R^1 = OH, R^2 = OH, R^3 = OMe, R^4 = OH	*B. vaginatum* (Leong et al., 1997, 1999)
	45 $R^1 = R^2$ = –O–CH_2–O–, R^3 = OMe, R^4 = OH, cirrhopetalin	*C. andersonii* (Majumder and Basak, 1990b, 1991a)
	46 R^1 = OMe, R^2 = OMe, R^3 = OMe, R^4 = OMe	*B. striata* (Yamaki et al., 1991)
	47 R^1 = OMe, R^2 = OMe, R^3 = OMe, R^4 = OH;	*T. communis* (Bordat et al., 2006; Réthy et al., 2006)
	48 R^1 = OMe, R^2 = OMe, R^3 = OH, R^4 = OH; TaVIII.	*E. lonchophylla* (Tezuka et al., 1991) *T. communis* (Aquino et al., 1985a,b; Letcher and Wong, 1979; Reisch et al. 1973)
	**49** R^1 = OH, R^2 = OMe	*M. densa* (Estrada et al., 1999b, 2004)
	50 R^1 = OH, R^2 = OH; fimbriol-B	*B. vaginatum* (Leong et al., 1997) *E. fimbriata* (Tezuka et al., 1993)
	51 R^1 = OMe, R^2 = OH; bulbophyllanthrin	*B. leopardium* (Majumder et al., 1985)
	**52** R^1 = OH, R^2 = OMe, R^3 = OMe, R^4 = OH	*B. vaginatum* (Leong et al., 1997)
	53 R^1 = OMe, R^2 = OMe, R^3 = OH, R^4 = OMe; batatasin I	*D. batatas* (Takasugi et al., 1987) *D. rotundata* (de Alvarenga and Gottlieb, 1974)
	54 R^1 = OMe, R^2 = OMe, R^3 = OH, R^4 = OH	*D. batatas* (Takasugi et al., 1987)
	55 R^1 = OH, R^2 = OMe, R^3 = OMe, R^4 = OH	*D. batatas* (Takasugi et al., 1987)
	56 R^1 = OMe, R^2 = OMe, R^3 = OMe, R^4 = OH; TaVI	*C. psidioides* (Letcher and Nhamo, 1972b) *D. rotundata* (Coxon et al., 1982) *T. communis* (Aquino et al., 1985a,b; Kovács et al., 2007; Letcher and Nhamo, 1972a; Reisch et al., 1969, 1973)
	57 R^1 = OMe, R^2 = OMe, R^3 = OMe, R^4 = OMe; callosuminin	*A. callosum* (Majumder et al., 1996)
	**58**	*D. bulbifera* (Wij and Rangaswami, 1978)
	**59** R^1 = H, R^2 = OH, R^3 = OMe, R^4 = H	*E. nuda* (Tuchinda et al., 1988) *E. petersii* (Blitzke et al., 2000)
	60 R^1 = H, R^2 = OMe, R^3 = OH, R^4 = H	*E. nuda* (Tuchinda et al., 1988)
	61 R^1 = OH, R^2 = H, R^3 = H, R^4 = OMe	*B. vaginatum* (Leong et al., 1997)

is essential for inhibitory activity (Lee et al., 1995). Lusianthridin (**4**) isolated from *B. striata* did not exert activity against the leukaemic P388 cell line *in vitro* (Takagi et al., 1983). In contrast, at a dose of 20 μg/kg, lusianthridin (**4**) displayed an antitumour effect, while denbinobin (**172**) was inactive in ICR mice implanted intraperitoneally with 10^6 cells of sarcoma 180 (Takagi et al., 1983).

D. thyrsiflorum has been used in Chinese ethnomedicine. Five phenanthrenes isolated from the stems were assayed against several tumour cell lines by use of the MTT test. Two dimeric phenanthrenes (**220**, **242**) and denthirsinin (**86**) displayed significant cytotoxicity against HeLa, K-562 and MCF-7 cells. The IC_{50} values (μM) on these cell lines were: denthyrsinol (**220**) 9.3, 1.6 and -, denthyrsinone (**242**) 9.9, 6.0 and 3.5, and denthirsinin (**86**) 2.7, 2.3 and 4.8, respectively. Hircinol (**6**) ($IC_{50} > 100$, 6.3 and >100 μM) and moscatin (**15**) ($IC_{50} > 100$, 7.1 and >100 μM) were less effective in killing HeLa and MCF-7 cells than denthyrsinol (**220**). Structure–activity relationship analyses revealed that the dimerization of phenanthrenes is a very important factor for the inhibition of cancer cell growth (Zhang et al., 2005).

Table 4
Pentasubstituted dihydro/phenanthrenes with hydroxy- or/and methoxy-substitution

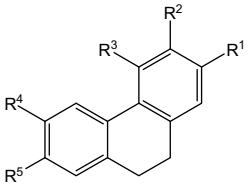
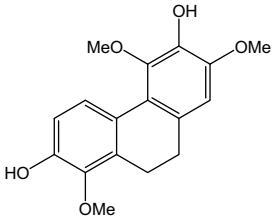
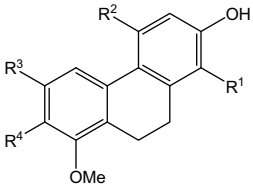
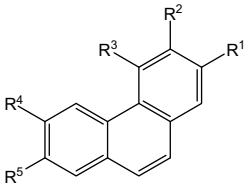
	**62** $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*B. gymnopus* (de Alvarenga et al., 1976; Majumder and Banerjee, 1988b) *B. reptans* (Majumder et al., 1999) *B. vaginatum* (Leong et al., 1997) *C. apiculatum* (Malan and Swinny, 1993) *C. caffrum* (Pettit et al., 1988) *C. psidioides* (Letcher and Nhamo, 1972b) *D. rotundatum* (Majumder and Pal, 1992)
	63 $R^1 = OH$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*B. vaginatum* (Leong et al., 1999)
	64 $R^1 = OH$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$	*B. vaginatum* (Leong et al., 1999)
	65 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OH$, $R^5 = OH$	*C. apiculatum* (Malan and Swinny, 1993) *C. molle* (Letcher et al., 1972)
	66 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*C. apiculatum* (Malan and Swinny, 1993) *C. caffrum* (Pettit et al., 1988) *C. psidioides* (Letcher and Nhamo, 1972b)
	67 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OMe$	*C. apiculatum* (Pettit et al., 1988) *C. caffrum* (Pettit et al., 1988)
	68 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OMe$, $R^5 = OH$	*C. apiculatum*, (Letcher and Nhamo, 1971; Malan and Swinny, 1993) *C. molle* (Letcher et al., 1972)
	69 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$; prazerol	*C. apiculatum* (Malan and Swinny, 1993) *C. caffrum* (Pettit et al., 1988) *C. molle* (Letcher et al., 1972; Malan and Swinny, 1993) *D. prazeri* (Biswas et al., 1988)
	70 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OMe$	*C. apiculatum* (Letcher and Nhamo, 1971) *C. molle* (Letcher and Nhamo, 1971; Letcher et al., 1972)
	71 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$	*C. psidioides* (Letcher and Nhamo, 1972b)
	**72**	*N. boothii* (Hernandez-Romero et al., 2004)
	**73** $R^1 = H$, $R^2 = OH$, $R^3 = OH$, $R^4 = OMe$, coeloginanthridin	*C. cristata* (Majumder et al., 2001)
	74 $R^1 = H$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$	*D. decipiens* (Sunder et al., 1978)
	**75** $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*B. gymnopus* (DellaGreca et al., 1993; Majumder and Banerjee, 1988b) *B. reptans* (Majumder et al., 1999) *B. vaginatum* (Leong et al., 1997) *C. psidioides* (Letcher and Nhamo, 1972b) *D. rotundatum* (Honda and Yamaki, 2000; Majumder and Pal, 1992)
	76 $R^1 = OH$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*B. vaginatum* (Leong et al., 1997, 1999) *C. apiculatum* (Malan and Swinny, 1993)
	77 $R^1 = OH$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$	*B. vaginatum* (Leong et al., 1999)
	78 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$	*C. apiculatum* (Malan and Swinny, 1993)
	79 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*C. apiculatum* (Malan and Swinny, 1993)
	80 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R_4 = OMe$, $R^5 = OH$	*C. apiculatum* (Malan and Swinny, 1993) *C. molle* (Letcher et al., 1972)
	81 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OH$	*C. psidioides* (Letcher and Nhamo, 1972b)
	82 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$	*C. caffrum* (Pettit et al., 1988) *C. psidioides* (Letcher and Nhamo, 1972b)
	83 $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OMe$	*C. apiculatum* (Letcher and Nhamo, 1971)
	84 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OH$, $R^5 = OH$	*C. apiculatum* (Letcher and Nhamo, 1971) *C. molle* (Letcher et al., 1972)

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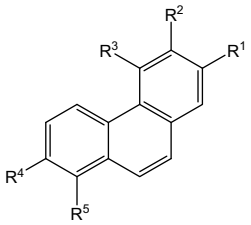
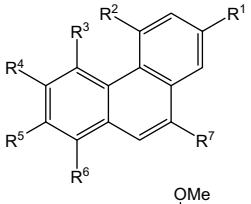
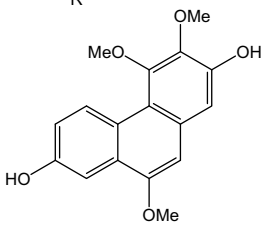
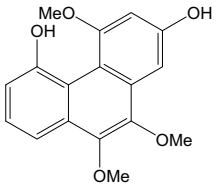
Table 4 (*continued*)

	**85** $R^1 = OH$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OMe$; confusarin	*B. gymnopus* (de Alvarenga et al., 1976; Majumder and Banerjee, 1988b) *B. reptans* (Majumder et al., 1999) *E. confusa* (Majumder and Kar, 1987a) *T. communis* (Réthy et al., 2006; Bordat et al., 2006)
	86 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OMe$; denthyrsinin	*C. pendulum* (Majumder and Sen, 1991b) *D. densiflorum* (Fan et al., 2001) *D. thyrsiflorum* (Zhang et al., 2005) *E. nuda* (Tuchinda et al., 1988) *N. boothii* (Hernandez-Romero et al., 2004) *S. livida* (Estrada et al., 1999a) *T. alba* (Majumder et al., 1998b) *T. communis* (Réthy et al., 2006; Bordat et al., 2006)
	87 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = OH$, $R^5 = OMe$;	*T. communis* (Kovács et al., 2007)
	88 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OMe$, $R^4 = R^5 = –O–CH_2–O–$; TaI	*T. communis* (Majumder and Basak, 1991a; Letcher and Wong, 1978)
	89 $R^1 = OMe$, $R^2 = OH$, $R^3 = OMe$, $R^4 = R^5 = –O–CH_2–O–$; TaIV	*T. communis* (Majumder and Basak, 1991a; Letcher and Wong (1979))
	90 $R^1 = OMe$, $R^2 = R^3 = –O–CH_2–O–$, $R^4 = OMe$, $R^5 = OMe$ TaI	*T. communis* (Aquino et al., 1985b, 1991; Reisch et al., 1969, 1970)
	91 $R^1 = OMe$, $R^2 = R^3 = –O–CH_2–O–$, $R^4 = OH$, $R^5 = OMe$; TaIV	*T. communis* (Aquino et al., 1985b, 1991; Letcher and Wong, 1979; Reisch et al., 1969, 1970)
	92 $R_1 = OMe$, $R_2 = OMe$, $R_3 = OMe$, $R_4 = OMe$, $R_5 = OH$; TaV	*T. communis* (Aquino et al., 1985a,b, 1991; Letcher and Wong, 1978; Reisch et al., 1970, 1973)
	93 $R^1 = OMe$, $R^2 = OMe$, $R^3 = OH$, $R^4 = OMe$, $R^5 = OH$; TaIX	*T. communis* (Aquino et al., 1985a,b, 1991; Reisch et al., 1973; Takasugi et al., 1987)
	**94** $R^1 = H$, $R^2 = OH$, $R^3 = OMe$, $R^4 = OMe$, $R^5 = OH$, $R^6 = H$, $R^7 = OMe$; fimbriol-A	*E. fimbriata* (Tezuka et al., 1993) *E. rigidum* (Hernandez-Romero et al., 2005) *M. densa* (Estrada et al., 1999b, 2004)
	95 $R^1 = OH$, $R^2 = OH$, $R^3 = H$, $R^4 = OH$, $R^5 = OMe$, $R^6 = OMe$, $R^7 = H$; coeloginanthrin	*C. cristata* (Majumder et al., 2001)
	**96** gymnopusin	*B. gymnopus* (Majumder and Banerjee, 1988b) *B. reptans* (Majumder et al., 1999) *M. densa* (Estrada et al., 1999b, 2004; Fisch et al., 1973; Valencia-Islas et al., 2002)
	**97** plicatol-A	*D. plicatile* (Honda and Yamaki, 2000)

This was supported by the investigation of mono-, bi-, and triphenanthrenes of *C. appendiculata*. The tuber of this plant has been used in traditional Chinese medicine for the treatment of various cancers. The isolated phenanthrenes (**18**, **19**) were inactive ($IC_{50} > 5$ μg/ml) against all the tested cell lines (A549, A2780, Bel7402, BGC-823, HCT-8, MCF-7 and WISH). Biphenanthrenes (**223**, **231**) and the unusual triphenanthrene (**243**) proved to be active compounds in this investigation (Xue et al., 2006).

Many phenanthrenes have been isolated from petroleum ether and chloroform extracts of the rhizomes of *Tamus communis* through cytotoxic assay guidance. The antitumour effects of eight phenanthrenes and one dihydrophenanthrene were tested on the HeLa cell line by Hohmann et al.; the compounds showed high cytotoxic activities with IC_{50}s of 20.18 (**42**), 6.66 (**43**), 13.85 (**47**), 0.97 (**85**), >20 (**86**), 15.86 (**25**), 12.18 (**56**), 8.52 (**87**) and 3.64 μM (**89**) (Réthy et al., 2006; Kovács et al., 2007).

Aquino et al. studied the cytotoxicities of compounds **27**, **28**, **90**, **92** and **93**, isolated from *T. communis* on CER (chicken embryo-related) and HeLa cells. The HeLa cells were found to be generally more susceptible than the

Table 5
Hexasubstituted dihydro/phenanthrenes with hydroxy- or/and methoxy-substitution

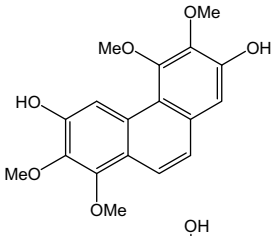
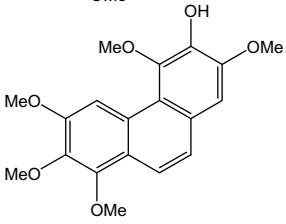
	**98** confusaridin	*E. confusa* (Majumder and Kar, 1987a)
	**99** pendulin	*C. pendulum* (Majumder and Sen, 1991b)

Table 6
Methyl, oxymethyl, vinyl and prenylsubstituted monomeric dihydro/phenanthrenes

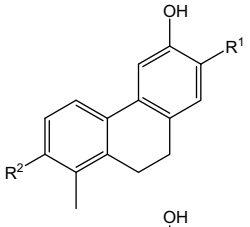
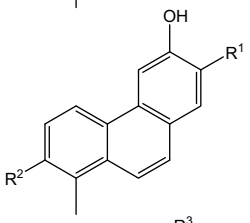
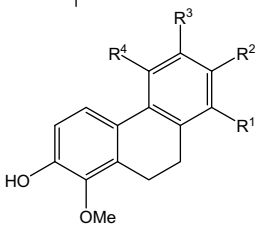
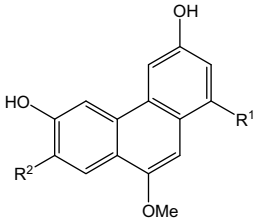
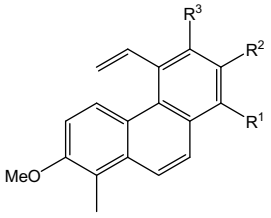
	**100** R^1 = Me, R^2 = OH; micrandrol-B	*M. scleroxylon* (de Alvarenga and Gottlieb, 1974) *S. racemosa* (de Alvarenga et al., 1976)
	101 R^1 = OMe, R^2 = Me; micrandrol-F	*S. racemosa* (de Alvarenga et al., 1976)
	**102** R^1 = Me, R^2 = OH; micrandrol-A	*M. scleroxylon* (de Alvarenga and Gottlieb, 1974) *S. racemosa* (de Alvarenga et al., 1976)
	103 R^1 = OMe, R^2 = Me; micrandrol-E	*S. racemosa* (de Alvarenga et al., 1976)
	**104** R^1 = H, R^2 = OH, R^3 = Me, R^4 = OMe; stemanthrene A	*S. collinsae* (Adams et al., 2005) *S. pierrei* (Adams et al., 2005; Kostecki et al., 2004) *S. tuberosa* (Adams et al., 2005)
	105 R^1 = H, R^2 = OMe, R^3 = Me, R^4 = OH; stemanthrene B	*S. collinsae*, (Adams et al., 2005) *S. pierrei* (Adams et al., 2005; Kostecki et al., 2004) *S. tuberosa* (Adams et al., 2005)
	106 R^1 = Me, R^2 = OH, R^3 = Me, R^4 = OMe; stemanthrene C	*S. collinsae* (Adams et al., 2005) *S. pierrei* (Adams et al., 2005; Kostecki et al., 2004) *S. tuberosa* (Adams et al., 2005)
	107 R^1 = Me, R^2 = OH, R^3 = H, R^4 = OMe; stemanthrene D, racemosol	*S. collinsae* (Adams et al., 2005; Pacher et al., 2002) *S. pierrei* (Adams et al., 2005) *S. tuberosa* (Adams et al., 2005)
	**108** R^1 = Me, R^2 = Me **109** R^1 = CH_2OH, R^2 = Me **110** R^1 = Me, R^2 = CH_2OH **111** R^1 = CH_2OH, R^2 = CH_2OH	*D. perrieri* (Long et al., 1997) *D. perrieri* (Long et al., 1997) *D. perrieri* (Long et al., 1997) *D. perrieri* (Long et al., 1997)
	**112** R^1 = OH, R^2 = H, R^3 = Me	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993)
	113 R^1 = H, R^2 = OH, R^3 = H	*J. acutus* (DellaGreca et al., 2004)
	114 R^1 = H, R^2 = Me, R^3 = H	*J. acutus* (DellaGreca et al., 2004)

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Table 6 (*continued*)

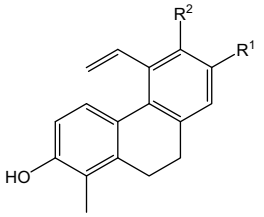
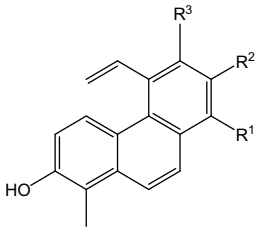
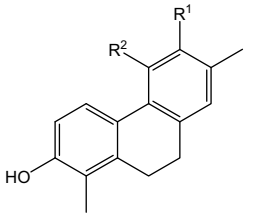
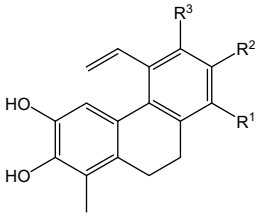
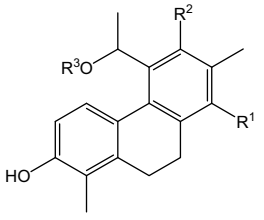
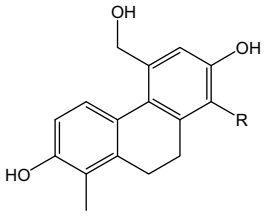
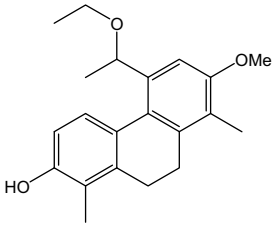
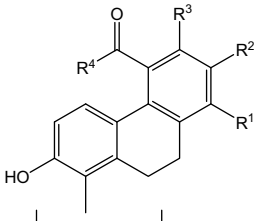
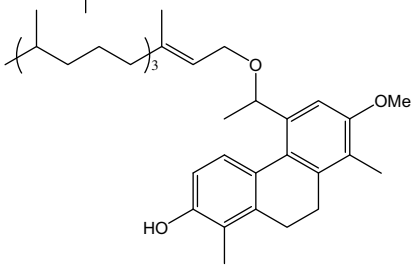
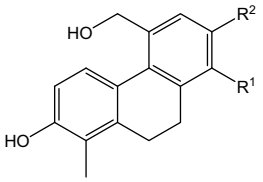
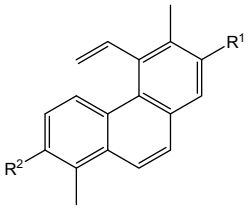
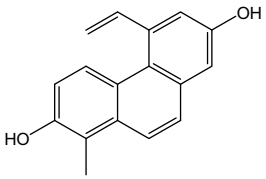
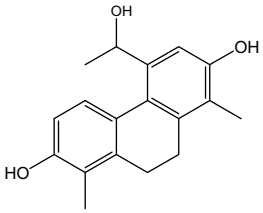
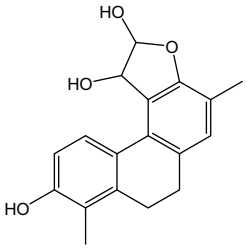
	**115** R^1 = Me, R^2 = H, juncunol	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993) *J. roemerianus* (Sarkar et al., 1988)
	116 R^1 = OH, R^2 = Me, juncusol	*J. acutus* (DellaGreca et al., 2002a,b, 2004) *J. effusus* (DellaGreca et al., 1993; Shima et al., 1991) *J. roemerianus* (Chapatwala et al., 1981; Sarkar et al., 1988)
	117 R^1 = Me, R^2 = OH	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1993)
	118 R^1 = OH, R^2 = H, effusol	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1993; Shima et al., 1991)
	119 R^1 = H, R^2 = Me	*J. acutus* (DellaGreca et al., 2004)
	120 R^1 = OH, R^2 = CH_2OH	*J. acutus* (DellaGreca et al., 2004)
	121 R^1 = CH_2OH, R^2 = H	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1997)
	122 R^1 = H, R^2 = CH_2OH	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1997)
	**123** R^1 = COOH, R^2 = H, R^3 = H	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1993)
	124 R^1 = H, R^2 = COOH, R^3 = H	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1993)
	125 R^1 = H, R^2 = H, R^3 = COOH	*J. acutus* (DellaGreca et al., 2004)
	126 R^1 = Me, R^2 = OH, R^3 = H	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993)
	127 R^1 = OH, R^2 = H, R^3 = Me	*J. acutus* (DellaGreca et al., 2004) *J. effusus* (DellaGreca et al., 1993)
	128 R^1 = Me, R^2 = OMe, R^3 = H	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993)
	**129** R^1 = OH, R^2 = Ac; juncunone	*J. roemerianus* (Sarkar et al., 1988)
	130 R^1 = OH, R_2 = H	*J. effusus* (DellaGreca et al., 1993)
	**131** R^1 = OH, R^2 = H, R^3 = Me	*J. acutus* (DellaGreca et al., 2004)
	132 R^1 = H, R^2 = Me, R^3 = H	*J. effusus* (DellaGreca et al., 1993)
	**133** R^1 = H, R^2 = OH, R^3 = H	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993, 1997)
	134 R^1 = H, R^2 = OH, R^3 = Me	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993)
	135 R^1 = OH, R^2 = H, R^3 = H	*J. acutus* (DellaGreca et al., 2002a, 2004) *J. effusus* (DellaGreca et al., 1993)
	**136** R = H	*J. acutus* (DellaGreca et al., 2004)
	137 R = Me	*J. acutus* (DellaGreca et al., 2004)

Table 6 (*continued*)

	**138** *J. acutus* (DellaGreca et al., 2002a, 2004)
	**139** $R^1 = H$, $R^2 = Me$, $R^3 = OH$, $R^4 = Me$ *J. effusus* (DellaGreca et al., 1993) **140** $R^1 = Me$, $R^2 = OMe$, $R^3 = H$, $R^4 = H$ *J. effusus* (DellaGreca et al., 1993) **141** $R^1 = H$, $R^2 = Me$, $R^3 = OH$, $R^4 = H$ *J. effusus* (DellaGreca et al., 1993)
	**142** *J. acutus* (DellaGreca et al., 2002a, 2004)
	**143** $R^1 = Me$, $R^2 = OH$ *J. effusus* (DellaGreca et al., 1997) **144** $R^1 = Me$, $R^2 = OMe$ *J. effusus* (DellaGreca et al., 1997) **145** $R^1 = H$, $R^2 = Me$ *J. effusus* (DellaGreca et al., 1997)
	**146** $R^1 = OH$, $R^2 = OH$, dehydrojuncusol *J. effusus* (Shima et al., 1991) *J. roemerianus* (Sarkar et al., 1988) *J. acutus* (DellaGreca et al., 2002a, 2004) **147** $R^1 = H$, $R^2 = OH$ *J. acutus* (DellaGreca et al., 2002a, 2004) **148** $R^1 = OMe$, $R^2 = OMe$ *J. acutus* (DellaGreca et al., 2002a, 2004)
	**149** dehydroeffusol *J. effusus* (Shima et al., 1991)
	**150** *J. effusus* (DellaGreca et al., 1997)
	**151** *J. effusus* (DellaGreca et al., 1997)

(*continued on next page*)

Table 6 (*continued*)

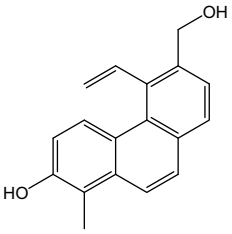
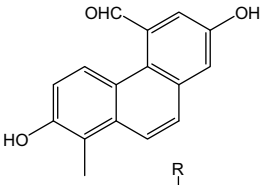
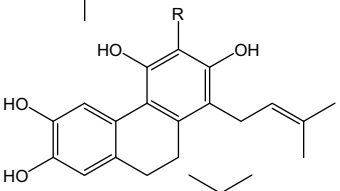
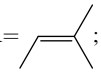
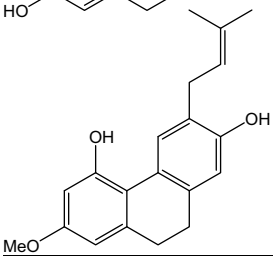
	**152**	*J. acutus* (DellaGreca et al., 2004)
	**153** dehydroeffusal	*J. effusus* (Shima et al., 1991)
	**154** R=  ; gancaonin U	*G. uralensis* (Fukai et al., 1991)
	155 R = H; gancaonin V	*G. uralensis* (Fukai et al., 1991)
	**156** sinensol G	*S. sinensis* (Lin et al., 2001)

Table 7
Miscellaneous monomeric phenanthrene derivatives

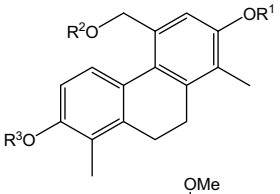
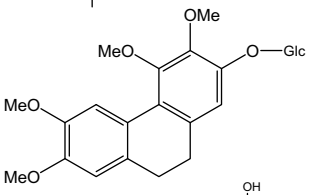
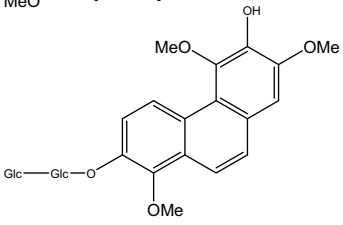
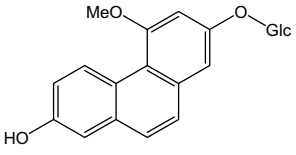
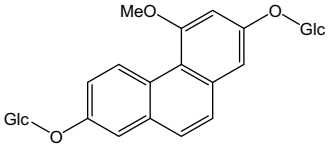
	**157** R^1 = Me, R^2 = Glc, R^3 = H, effuside I	*J. effusus* (DellaGreca et al., 1995)
	158 R^1 = H, R^2 = Glc, R^3 = H, effuside II	*J. effusus* (DellaGreca et al., 1995)
	159 R^1 = Glc, R^2 = H, R^3 = H, effuside III	*J. effusus* (DellaGreca et al., 1995)
	160 R^1 = H, R^2 = H, R^3 = Glc, effuside IV	*J. effusus* (DellaGreca et al., 1995)
	161 R^1 = Me, R^2 = Glc, R^3 = Glc, effuside V	*J. effusus* (DellaGreca et al., 1995)
	**162** epimedoicarisoside A	*E. koreanum* (Li et al., 1995)
	**163** denchryside A	*D. chrysanthum* (Ye et al., 2003)
	**164**	*B. striata* (Yamaki et al., 1993b)
	**165**	*B. striata* (Yamaki et al., 1993b)

Table 7 (*continued*)

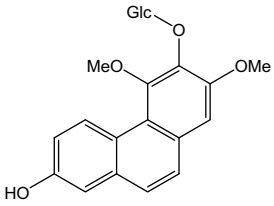
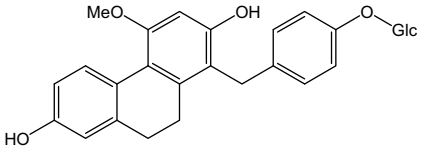
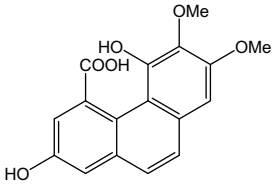
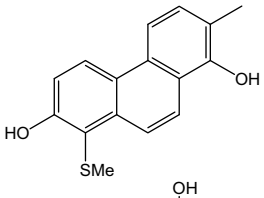
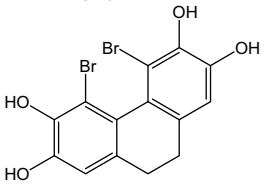
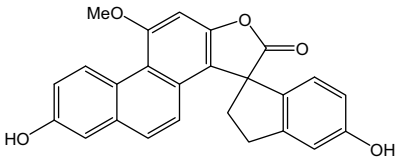
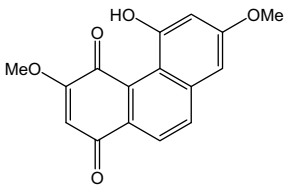
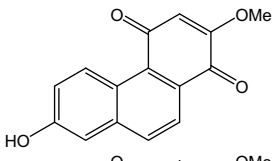
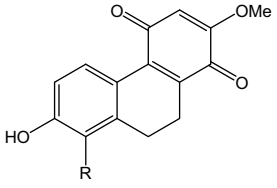
	**166**	*B. striata* (Yamaki et al., 1993b)
	**167**	*B. striata* (Yamaki et al., 1993b)
	**168** ochrolic acid	*C. ochracea* (Anuradha et al., 1994)
	**169** micrandrol C	*M. scleroxylon* (de Alvarenga and Gottlieb, 1974)
	**170** polysiphenol	*P. ferulacea* (Aknin et al., 1992)
	**171** blespirol	*B. striata* (Yamaki et al., 1993a)

Table 8
Phenanthraquinones

	**172** denbinobin	*D. moniliforme* (Lin et al., 2001) *D. nobile* (Lee et al., 1995) *E. lonchophylla* (Chen et al., 2000; Tezuka et al., 1991)
	**173** densiflorol B, cypritibetquinone A	*C. tibeticum* (Liu et al., 2005a) *D. densiflorum* (Fan et al., 2001)
	**174** R = H; ephemeranthoquionone **175** R = OMe; cypripedin	*D. plicatile* (Yamaki and Honda, 1996) *E. lonchophylla* (Tezuka et al., 1991) *N. boothii* (Hernandez-Romero et al., 2004) *D. densiflorum* (Fan et al., 2001)

(*continued on next page*)

Table 8 (*continued*)

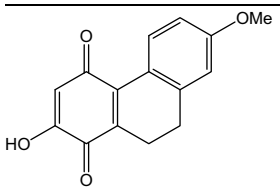
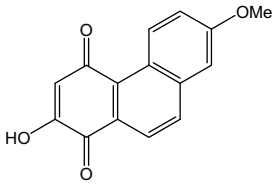
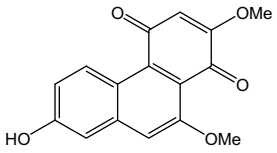
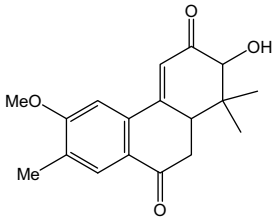
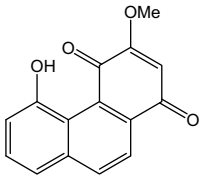
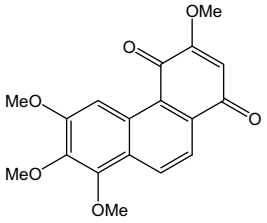
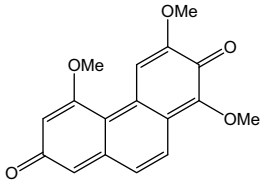
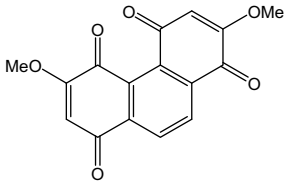
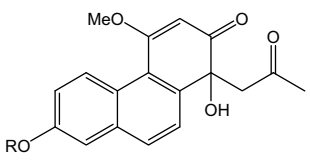
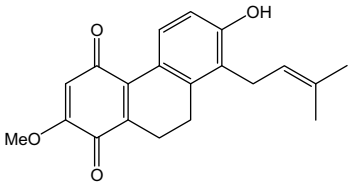
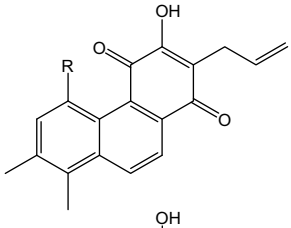
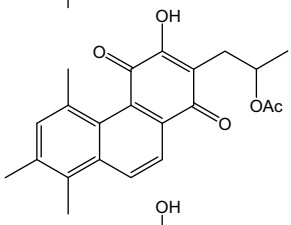
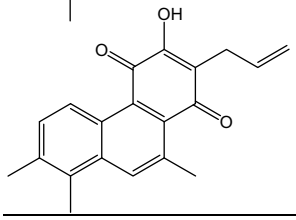
	**176** ochrone A	*C. ochracea* (Bhaskar et al., 1991)
	**177** ochrone B	*C. ochracea* (Bhaskar et al., 1991)
	**178** cypritibetquinone B	*C. tibeticum* (Liu et al., 2005a)
	**179** domohinone	*D. perrieri* (Long et al., 1997)
	**180** cymbinodin A	*C. aloifolium* (Barua et al., 1990)
	**181** cymbinodin B	*C. aloifolium* (Ghosh et al., 1992)
	**182** TaII	*T. communis* (Reisch et al., 1970)
	**183** moniliformin	*D. moniliforme* (Lin et al., 2001)
	**184** R = H **185** R = Me	*C. appendiculata* (Xue et al., 2006) *C. appendiculata* (Xue et al., 2006)

Table 8 (*continued*)

	**186** spiranthoquinone	*S. sinensis* (Tezuka et al., 1990)
	**187** R = Me, plectranthon A **188** R = H, plectranthon C	*Plectranthus* sp. (Alder et al., 1984) *Plectranthus* sp. (Alder et al., 1984)
	**189** plectranthon B	*Plectranthus* sp. (Alder et al., 1984)
	**190** plectranthon D	*Plectranthus* sp. (Alder et al., 1984)

CER cells to the toxic action of these compounds, which were toxic to the cells at concentrations above 4–20 μg/ml, with the exception of **90** (100 μg/ml), the only compound in which hydroxy groups are missing. This is a further indication that free phenolic hydroxy groups are essential for the inhibitory activity (Aquino et al., 1991).

Compounds from *Domohinea perrieri* were screened for *in vitro* cytotoxicity against a number of cancer cell lines. Compounds **108** and **109** were found to demonstrate significant cytotoxic responses against several lines, with some cell-type selectivity. Compound **108** was more active against drug-resistant KB cells, while **109** was active against HT (fibrosarcoma) and U373 (glioma) cell lines. **110**, **111** and **179** were not significantly active against any of the cell lines tested. Compounds with a hydroxymethyl group on C-7 were not active, suggesting that an unsubstituted methyl group on C-7 is important for the activity (Long et al., 1997).

Hydroxybenzyl-phenanthrenes [sinensol A–F (**202**–**206** and **208**)] from *S. sinensis* proved to be active against the MS-G2 cell line and were cytotoxic at 20 μg/ml, but showed no anti-hepatitis B virus e antigen (HBeAg) effect at non-cytotoxic (5 or 10 μg/ml) doses (Lin et al., 2000).

4.2. Antimicrobial effects

The accumulation of phytoalexins in plants is a response to infection by pathogenic fungi. Phytoalexins are utilized by plants to stop the growth of the attacking fungus. Orchid phytoalexins are phenanthrenes and dihydrophenanthrenes, as exemplified by orchinol (**3**) from *Orchis militaris* and hircinol (**6**) from *Loroglossum hircinum*. Their antifungal activities, together with those of loroglossol (**5**), were investigated on *Candida lipolitica* in 1973. Loroglossol was inactive, while orchinol (**3**) (at either 50 or 100 ppm) was considerably more active than hircinol (**6**). Orchinol (**3**) at 100 ppm inhibited the growth of the cells completely for the first 6 days, whereas hircinol (**6**) or the control did so for only 3 days (Fisch et al., 1973).

The tubers of *B. striata* were investigated, because this has been used in traditional medicine in China to treat pneumonorrhagia and pneumonophthisis. The methanol extracts of *B. striata* and lusianthridin (**4**) were mainly active against Gram-positive bacteria, but weakly active against certain fungi. Biphenanthrenes [blestriareneA-C (**214**, **218**, **229**)] were active against the Gram-positive bacteria *Staphylococcus aureus* and *Streptococcus nutans*. Blestriarene B (**218**) exhibited the most potent activity against both test organisms (Yamaki et al., 1989).

The dihydrophenanthrene stemanthrene D (**107**) from *Stemona collinsae* demonstrated a weak activity against the fungi: *Fusarium avenaceum*, *Cladosporium herbarium* and *Pyricularia grisea*, with EC_{50} values >200 μg/ml. The antifungal properties of stemanthrenes A–D (**104**–**107**) from *Stemona pierrei* were also investigated. In this study

Table 9
Monomeric phenanthrenes with hydroxybenzyl substitution

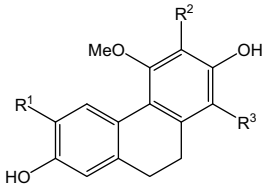
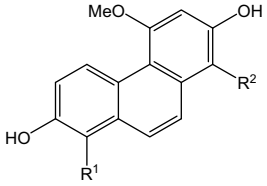
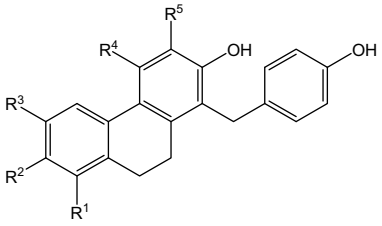
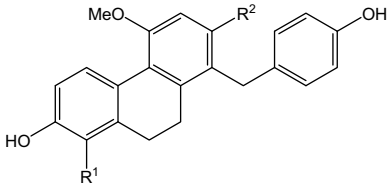
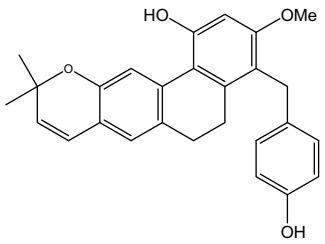
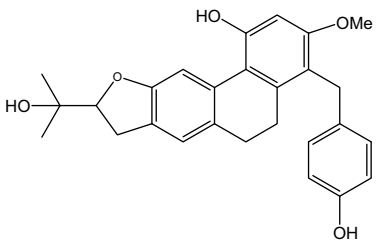
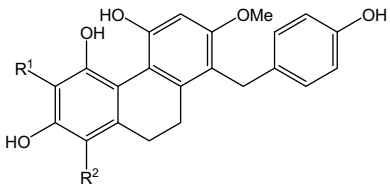
	**191** R^1 = H, R^2 = hydroxybenzyl, R^3 = H	*B. striata* (Yamaki et al., 1990)
	192 R^1 = hydroxybenzyl, R^2 = H, R^3 = hydroxybenzyl	*B. formosana* (Lin et al., 2005) *B. striata* (Yamaki et al., 1990)
	193 R^1 = H, R^2 = hydroxybenzyl, R^3 = hydroxybenzyl	*B. formosana* (Lin et al., 2005) *B. striata* (Bai et al., 1993)
	194 R^1 = H, R^2 = H, R^3 = hydroxybenzyl	*B. formosana* (Lin et al., 2005) *B. striata* (Bai et al., 1993; Yamaki et al., 1990) *G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006)
	**195** R^1 = H, R^2 = hydroxybenzyl	*B. formosana* (Lin et al., 2005) *B. striata* (Yamaki et al., 1990) *G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006) *P. bulbocodioides* (Bai et al., 1996)
	196 R^1 = OMe, R^2 = hydroxybenzyl	*B. formosana* (Lin et al., 2005)
	197 R^1 = hydroxybenzyl, R^2 = hydroxybenzyl	*B. formosana* (Lin et al., 2005) *B. striata* (Bai et al., 1991)
	**198** R^1 = H, R^2 = OH, R^3 = hydroxybenzyl, R^4 = OMe, R^5 = hydroxybenzyl	*B. formosana* (Lin et al., 2005)
	199 R^1 = H, R^2 = OMe, R^3 = H, R^4 = OMe, R^5 = H	*B. formosana* (Lin et al., 2005)
	200 R^1 = OMe, R^2 = OH, R^3 = H, R^4 = OMe, R^5 = H	*B. formosana* (Lin et al., 2005)
	201 R^1 = hydroxybenzyl, R^2 = OH, R^3 = H, R^4 = OMe, R^5 = H	*B. formosana* (Lin et al., 2005)
	**202** R_1 = H, R^2 = OMe; arundinaol, sinensol A	*A. graminifolia* (Liu et al., 2005b) *S. sinensis* (Lin et al., 2000)
	203 R^1 =  ; R^2 = OMe; sinensol B	*S. sinensis* (Lin et al., 2000)
	204 R^1 =  ; R^2 = H; sinensol C	*S. sinensis* (Lin et al., 2000)
	**205** sinensol D	*S. sinensis* (Lin et al., 2000)
	**206** sinensol E	*S. sinensis* (Lin et al., 2000)
	**207** R^1 = H, R^2 = H; shancidin	*B. striata* (Takagi et al., 1983) *P. bulbocodioides* (Bai et al., 1996) *S. sinensis* (Lin et al., 2000)
	208 R^1 =  ; R^2 = hydroxybenzyl; sinensol F	
	209 R^1 = hydroxybenzyl, R^2 = hydroxybenzyl; sinensol H	*S. sinensis* (Lin et al., 2001)

Table 9 (*continued*)

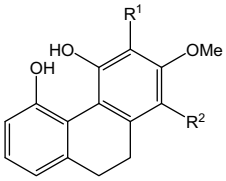
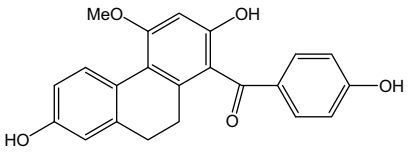
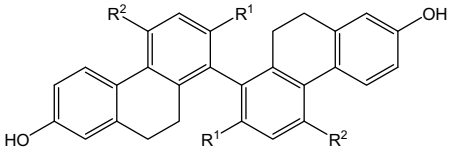
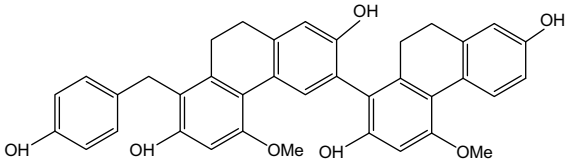
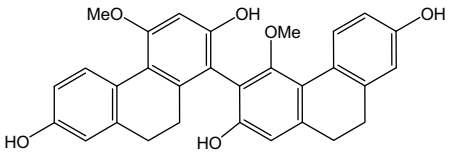
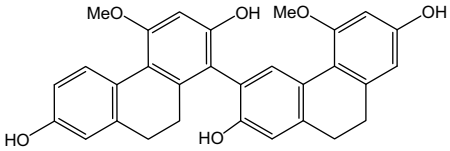
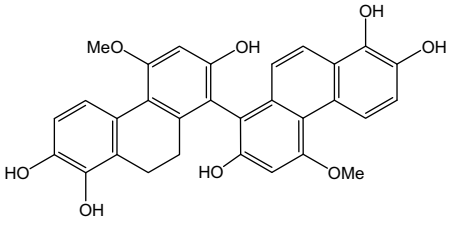
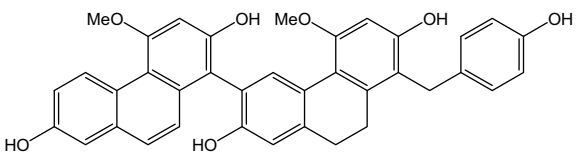
	**210** R^1 = H, R^2 = hydroxybenzyl; gymconopin A	*G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006)
	211 R^1 = hydroxybenzyl, R^2 = H; gymconopin B	*G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006)
	**212**	*B. striata* (Bai et al., 1993)

Table 10
Dihydro/phenanthrene dimers and a triphenanthrene

	**213** R^1 = OMe, R^2 = OH **214** R^1 = OH, R^2 = OMe; blestriarene A, flavanthrin	*D. plicatile* (Yamaki and Honda, 1996) *B. reptans* (Majumder et al., 1999) *B. striata* (Yamaki et al., 1989) *E. flava* (Majumder and Banerjee, 1988a, 1990a) *G. conopsea* (Matsuda et al., 2004; Morikawa et al., 2006) *T. alba* (Majumder et al., 1998b)
	**215** blestrianol B	*B. striata* (Bai et al., 1991)
	**216** blestrianol A	*B. striata* (Bai et al., 1991)
	**217** gymconopin C	*G. conopsea* (Matsuda et al., 2004)
	**218** blestriarene B	*B. striata* (Yamaki et al., 1989) *B. formosana* (Lin et al., 2005)
	**219** blestrianol C	*B. striata* (Bai et al., 1991)

(*continued on next page*)

Table 10 (*continued*)

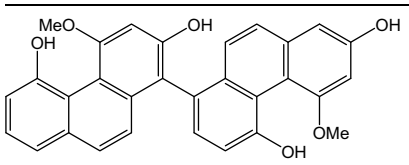
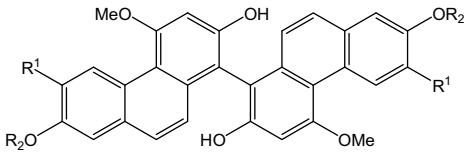
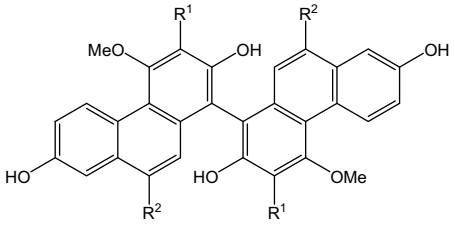
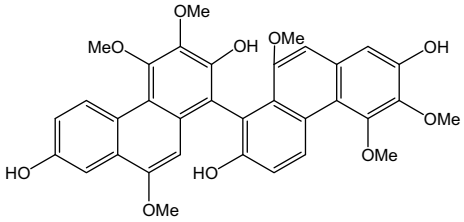
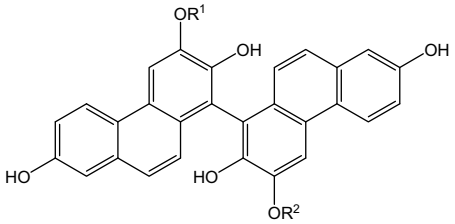
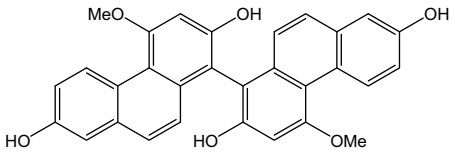
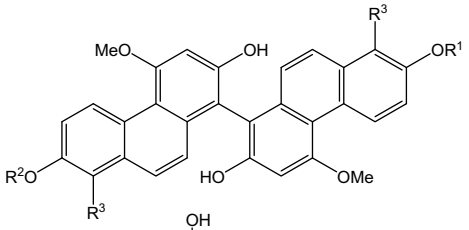
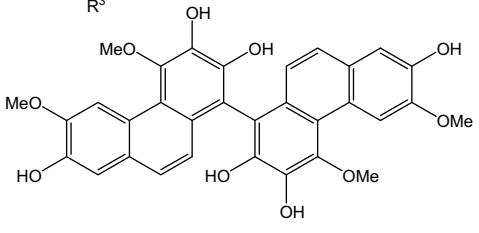
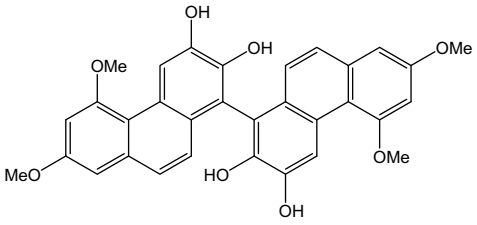
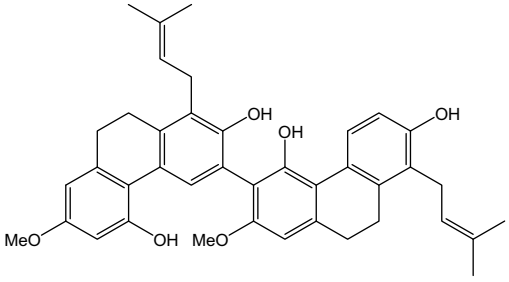
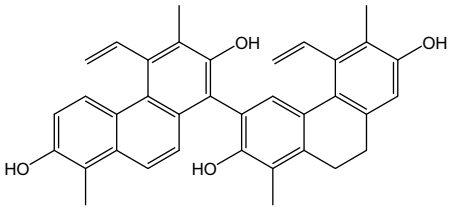
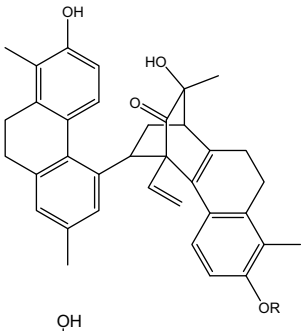
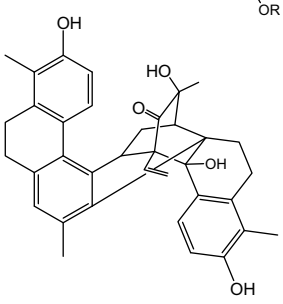
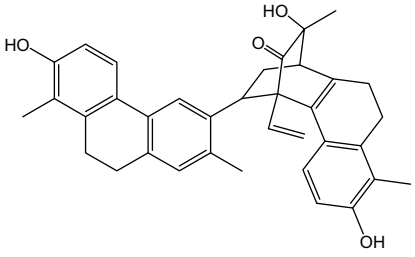
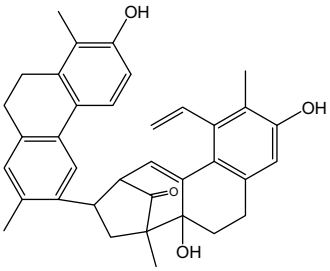
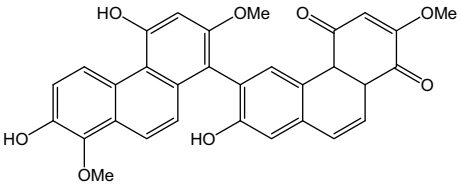
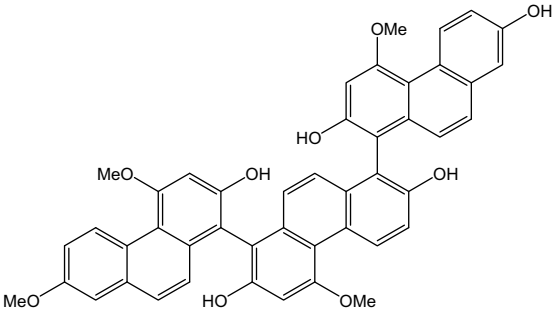
	**220** denthyrsinol	*D. thyrsiflorum* (Zhang et al., 2005)
	**221** R^1 = OMe, R^2 = H; agrostonin **222** R^1 = OH, R^2 = Me; agrostonidin	*A. khasiyanum* (Majumder et al., 1998a) *A. callosum* (Majumder et al., 1998a) *A. khasiyanum* (Majumder et al., 1998a) *A. callosum* (Majumder et al., 1998a)
	**223** R^1 = H, R^2 = H; cirrhopetalanthrin **224** R^1 = OMe, R^2 = OMe; reptanthrin	*C. maculosum* (Majumder et al., 1990e) *B. reptans* (Majumder et al., 1999) *T. alba* (Majumder et al., 1998b) *C. appendiculata* (Xue et al., 2006) *B. reptans* (Majumder et al., 1999)
	**225** isoreptanthrin	*B. reptans* (Majumder et al., 1999)
	**226** R^1 = Me, R^2 = Me **227** R^1 = Me, R^2 = H **228** R^1 = H, R^2 = H	*M. polymorpha* (Adam and Becker, 1994) *M. polymorpha* (Adam and Becker, 1994) *M. polymorpha* (Adam and Becker, 1994)
	**229** blestriarene C	*B. striata* (Yamaki et al., 1989)
	**230** R^1 = Me, R^2 = H, R^3 = H **231** R^1 = Me, R^2 = Me, R^3 = H **232** R^1 = H, R^2 = H, R^3 = OMe	*C. appendiculata* (Xue et al., 2006) *C. appendiculata* (Xue et al., 2006) *E. nuda* (Tuchinda et al., 1988)
	**233**	*B. vaginatum* (Leong and Harrison, 2004)

Table 10 (*continued*)

	**234** volucrin	*L. volucris* (Majumder and Lahiri, 1990c)
	**235** spiranthesol	*S. sinensis* (Tezuka et al., 1990)
	**236**	*J. acutus* (DellaGreca et al., 2003)
	**237** R = H **238** R = Me	*J. acutus* (DellaGreca et al., 2003)
	**239**	*J. acutus* (DellaGreca et al., 2003)
	**240**	*J. acutus* (DellaGreca et al., 2003)

(*continued on next page*)

Table 10 (*continued*)

	**241**	*J. acutus* (DellaGreca et al., 2003)
	**242** denthyrsinone	*D. thyrsiflorum* (Zhang et al., 2005)
	**243**	*C. appendiculata* (Xue et al., 2006)

dihydrophenanthrenes exerted a weak activity, similarly as in previous studies (Kostecki et al., 2004; Pacher et al., 2002).

The microbiological effects of juncusol (**116**) have been tested on several species. *Bacillus* species were inhibited at all concentrations, while *Plannococcus* species were inhibited only at the highest concentration. *Pseudomonas* species, *Mycobacterium smegmatis*, *Enterobacter aerogenes* and *Escherichia coli* were not inhibited at any of the concentrations used (Chapatwala et al., 1981).

The virus replication inhibitory effects of phenanthrenes of *T. communis* (**27**, **28**, **90**, **91**, **92**, **93**) have been tested on RNA enveloped virus: vesicular stomatitis virus (VSV) and human rhinovirus serotype 1B (HRV 1B). The results of the screening revealed marked inhibitory action on plaque formation against VSV (**27**), with an IC_{50} of 9 μg/ml. On HRV, these compounds produced a low decrease in viral multiplication (Aquino et al., 1991).

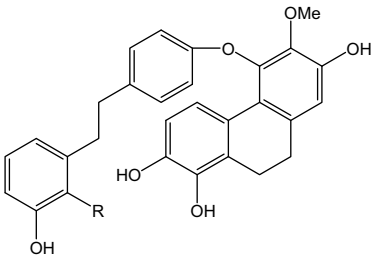
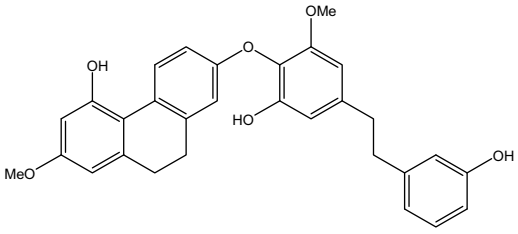
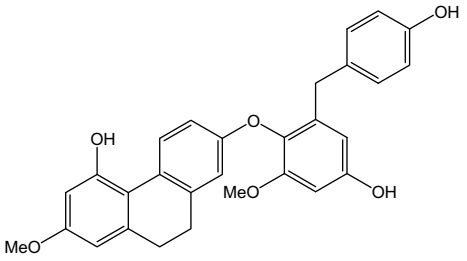
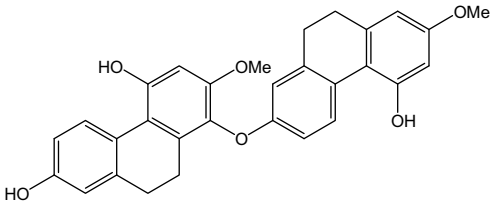
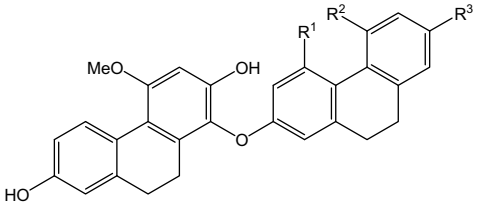
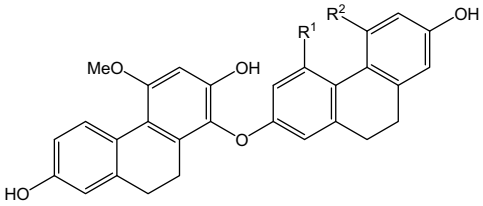
4.3. Spasmolytic effects

Spasmolytic effects have been investigated in case of phenanthrenes isolated from *Scaphyglottis livida*, *Maxillaria densa* and *Nidema boothii*. *S. livida* and *M. densa* are used by indigenous people in the tropical forests of Mexico. The ground herb of *S. livida* is applied to eliminate ectoparasites. The decoction is used for the treatment of stomach aches and to avoid abortion. *M. densa* is utilized for the same purposes.

Estrada et al. investigated the spasmolytic action of compounds of *S. livida* (Estrada et al., 1999a). Bioactivity-guided fractionation of the active extract resulted in the isolation of lusianthridin (**4**), **43** and denthirsinin (**86**). Compound **43** significantly antagonized the histamine-induced contractions of the rat ileum, in contrast with lusianthridin (**4**), and denthirsinin (**86**), which were inactive. The contractions evoked by $BaCl_2$ were inhibited slightly by **43** and denthirsinin (**86**), but lusianthridin (**4**) enhanced the contractions elicited by $BaCl_2$. The relaxatory response elicited by natural products is probably mediated by the neuronal release of NO. Compounds of *S. livida* induced the production of NO in ileal tissue, which in turn provoked relaxation of the ileal muscles by elevating the cyclic GMP content. Later, the vasorelaxing effect of **43** was studied on intact and denuded rat aorta rings. Compound **43** and denthirsinin (**86**) provoked the inhibition of NA-evoked contractions in the endothelium and appeared to induce a dose–response vasorelaxation by more than one mechanism (Estrada et al., 1999a, 2006). Phenanthrenes [erianthridin (**36**), **39**, nudol (**42**), **49** and gymnopusin (**96**)] isolated from *M. densa* provoked a concentration-dependent inhibition of the spontaneous contractions of the rat ileum induced by histamine, $BaCl_2$ and L-NAME. All these compounds antagonized the contractions, but they exerted a weaker smooth muscle relaxant activity than the extract; this could be due to a synergistic effect of the isolated compounds (Estrada et al., 2004).

Bioassay-guided fractionation of the active extract of *E. boothii* led to the identification of a new dihydrophenanthrene (**72**) and numerous known stilbenoids

Table 11
Dihydro/phenanthrenes with unusual structure

	**244** R = H **245** R = OH	*F. convoluta* (Flegel et al., 1999) *F. convoluta* (Flegel et al., 1999)
	**246** shancilin	*P. bulbocodioides* (Bai et al., 1996)
	**247** phoyunnanin D	*P. yunnanensis* (Guo et al., 2006)
	**248** phoyunnanin E	*P. yunnanensis* (Guo et al., 2006)
	**249** R^1 = OMe, R^2 = H, R^3 = OH; blestrin A **250** R^1 = H, R^2 = OH, R^3 = OMe; blestrin B	*B. striata* (Bai et al., 1990) *B. striata* (Bai et al., 1990)
	**251** R^1 = OMe, R^2 = H; blestrin C **252** R^1 = H, R^2 = OMe; blestrin D	*B. striata* (Yamaki et al., 1992) *B. striata* (Yamaki et al., 1992)

[lusianthridin (**4**), flavanthridin (**37**), **43**, denthirsinin (**86**) and **174**]. Denthyrsinin (**86**) and **72** induced noteworthy concentration-dependent inhibition of the spontaneous contractions of the guinea-pig ileum (Hernandez-Romero et al., 2004).

4.4. Antiallergic and anti-inflammatory activities

The tubers of *Gymnadenia conopsea* have been used in traditional Chinese medicine for the treatment of asthma, neurasthenia and chronic hepatitis. The methanolic

extract of the tubers was found to shown an antiallergic effect on the ear passive cutaneous anaphylaxis reaction in mice. Column chromatography of the methanolic extract afforded a fraction which inhibited the antigen-induced release of β-hexosaminidase, a marker of degranulation, in RBL-2H3 cells sensitized with anti-DNP IgE. From the active fraction, numerous phenanthrenes were isolated, but these compounds were not investigated (Matsuda et al., 2004).

Denbinobin (**172**), a phenanthraquinone from *Dendrobium moniliforme*, showed *in vitro* anti-inflammatory activity. This compound, at 1 μM, stimulated with 1 μg/ml of lipopolysaccharide, significantly inhibited the formation of tumour necrosis factor α and prostaglandin E2 in RAW 264.7 and N9 cells (Lin et al., 2001).

Various species from the genus *Stemona* have long been used in traditional Asian medical practices for the treatment of inflammation-related diseases, such as asthma. Phenanthrenes of the *Stemona* species were tested in an *ex vivo* leukotriene biosynthesis inhibition assay, using human neutrophile granulocytes. Stemanthrene A (**104**) and D (**107**) displayed clear activity in a dose-dependent manner, with IC_{50} values of 8.5 μM and 4.8 μM, respectively. Stemanthrene B (**105**) and C (**106**) initially caused very high inhibition (100%) at 25 μM, but because of the degradation of the compounds during storage the activity was lost. The potency of phenanthrenes in this test system suggests that these substances might be the anti-inflammatory and antiasthmathic principles of the *Stemona* species (Adams et al., 2005).

In 2006, four compounds were isolated from the rhizomes of *T. communis* (**47**, **85**, **86** and **92**) and their anti-inflammatory activities were analysed. The activity of **86** was evaluated from the production of prostaglandin PG6KF1-α, induced in keratinocytes by the stimulation of arachidonic acid and calcium ionophore A23187 (Bordat et al., 2006). The compounds showed a significant anti-inflammatory activity at 1 μg/ml, 3 μg/ml and 10 μg/ml with the inhibition percent of the release of PG6KF1-α of 25%, 59% and 90%, respectively.

4.5. Antiplatelet aggregation, phytotoxicity and antialgal activity

Ephemerantha lonchophylla was used in traditional Chinese medicine as a health tonic, to regulate the body fluid balance, and as an antipyretic. An ethanolic extract of the stems of *E. lonchophylla* exhibited antiplatelet aggregation activity. Chen et al. isolated erianthridin (**36**), **43** and denbinobin (**172**) from the stems of this plant. The *in vitro* antiplatelet effects of these compounds were evaluated on washed rabbit platelets against aggregation induced by either thrombin, arachidonic acid (AA), collagen or PAF. At high concentration (100 μg/ml) the isolated compounds displayed significant inhibitory effects against the aggregation caused by AA, collagen and PAF. Further dose–response analyses indicated that inhibition against AA-induced aggregation was effective, erianthridin (**36**) proving to be the most potent compound, with an IC_{50} of about 9 μM, while for **43** IC_{50} was 24 μM. The antiplatelet effects may be due to the inhibition of thromboxane A_2 formation because the platelet aggregation induced by AA or collagen was most readily inhibited (Chen et al., 2000).

Dendrobium loddigesii is used with the same aims as *E. lonchophylla* in China. The active principle, moscatin (**15**), was isolated by using bioassay-guided fractionation by Chen et al. This compound strongly inhibited both AA and collagen-induced platelet aggregation. The antiplatelet effects of this compound may be due to the inhibition of thromboxane A_2 formation (Chen et al., 1994).

The phytotoxicities of compounds obtained from *M. densa* and *Epidendrum rigidum* were examined on *Amaranthus hypochondriacus*. Gymnopusin (**96**) and erianthridin (**36**) from *M. densa* inhibited the radical elongation of *A. hypochondriacus* seedlings, with IC_{50} values of 330 and 58.2 μM, respectively. Both phenanthrene derivatives exhibited moderate cytotoxicity towards all mammalian cells tested (Valencia-Islas et al., 2002). Compounds ephemeranthol-A (**35**) and fimbriol A (**94**) isolated from *E. rigidum* also demonstrated substantial phytotoxicty against *A. hypochondriacus*, with IC_{50} values of 0.12 and 5.9 μM, respectively (Hernandez-Romero et al., 2005).

The antialgal activities of 41 monomeric and 5 dimeric phenanthrenes were investigated against *Selenastrum capricornutum* by DellaGreca et al. in numerous studies. (DellaGreca et al., 1993, 1995, 1997, 2002a,b, 2003). The antialgal activities of the dimeric phenanthrenes were higher than those of the 9,10-dihydrophenanthrenes. As already observed in monomeric 9,10-dihydrophenanthrenes, a reduction of the polarity in related compounds causes a decrease in activity.

5. Conclusions

Numerous phenanthrenes and their derivatives have been found, mainly in higher plants, and their biological activities have been studied. The phenanthrenes are a promising and expanding group of biologically active natural compounds whose potential has not yet been investigated sufficiently throughly, and which have not been exploited by the pharmaceutical industry.

Many phenanthrene-containing plants have been used in traditional medicine throughout the world, but mainly in China, and phytochemical–pharmacological investigations which have resulted in the identification of phenanthrenes as their active principles have provided support for the use of these plants in ethnomedical practice. On the other hand, the mechanisms of action and the structure–activity relationships of these compounds have been reported only rarely and are worthy of future investigations.

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