Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 825–833
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Lignans from *Phyllanthus urinaria*

Chia-Chuan Chang, Yu-Chin Lien, Karin C.S. Chen Liu, Shoei-Sheng Lee*

School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan, ROC

Received 21 January 2003; received in revised form 2 April 2003

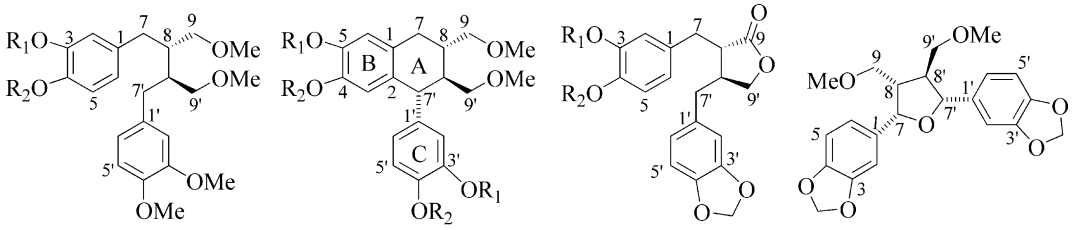
Abstract

Chemical investigation on the aerial and the root parts of *Phyllanthus urinaria* L. culminated in the isolation of four lignans, namely 5-demethoxyniranthin, urinatetralin, dextrobursehernin, urinaligran, together with nine known lignans. Their structures, including the absolute stereochemistry, were elucidated by spectral analysis (NMR and CD) and chemical correlation.

Keywords: *Phyllanthus urinaria*; Euphorbiaceae; Lignans; Aerial and root parts; CD; Chemical correlation

1. Introduction

Phyllanthus urinaria L. (Euphorbiaceae), widely distributed in tropical and subtropical regions in Asian countries, has long been used in folk medicine for liver protection, diabetes, hepatitis, jaundice and dropsy (Satyan et al., 1995). A number of lignans isolated from *Phyllanthus* plants have been shown to possess cytotoxic and biological activities (Prakash et al., 1995; Zhou et al., 1997; Meixia et al., 1995). Chemical components in the aerial and underground parts often demonstrate different bioactivities in many species (Carrier et al., 1998; Mizuno et al., 1988; Afsharypuor et al., 1995). Hence comparison of chemical constituents of both parts was undertaken. This effort led to the isolation and identification of 13 lignans (**1**–**13**) from the aerial

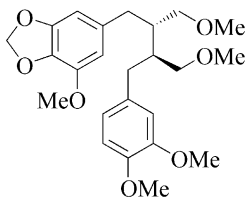


1 $R_1+R_2=CH_2$
5 $R_1=R_2=Me$

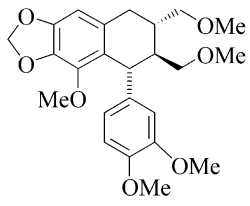
2 $R_1+R_2=CH_2$
7 $R_1=R_2=Me$

3 $R_1=R_2=Me$
12 $R_1+R_2=CH_2$

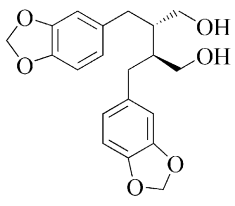
4



6



9



17

* Corresponding author. Tel.:/Fax: +886-2-23916127.
E-mail address: shoeilee@ha.mc.ntu.edu.tw (S.S. Lee).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00371-6

parts and 12 lignans (**2**–**13**) from the roots. Of the lignans, isolated, compounds **1**–**4** are new. The following describes the isolation and structural determination of these secondary metabolites.

2. Results and discussion

The water-insoluble part of the EtOH extract of the aerial portion of *P. urianria* was divided into *n*-hexane- and chloroform-soluble fractions and a residue by trituration with the corresponding solvent. Repeated chromatography over Sephadex LH-20, aluminum oxide and silica gel of these two soluble fractions yielded 13 lignans (**1**–**13**). Of these compounds **5**–**13** were found to be phyllanthin (**5**) (Somanabandhu et al., 1993), niranthin (**6**) (Anjaneyulu et al., 1973), phyltetralin (**7**) (Stevenson and Williams, 1977), hypophyllanthin (**8**) (Somanabandhu et al., 1993), nirtetralin (**9**) (Anjaneyulu et al., 1973), lintetralin (**10**) (Ward et al., 1979), isolintetralin (**11**) (Huang et al., 1992), heliobuphthalmin lactone (**12**) (Rao and Bramley, 1971) and virgatusin (**13**) (Huang et al., 1996) by comparison of their physical data ($[\alpha]_D$, NMR, MS) with those reported in the literatures.

Compound **1** had a molecular formula $C_{23}H_{30}O_6$, established by HR-EI-MS data. The spectral data indicated **1** contained a methylenedioxy function [IR ν_{max} 940 cm^{-1}, δ_H 5.89 (2H, *s*), δ_C 101.1 (*t*)], two aryl methoxyl groups (δ_H 3.80, 3.84), two methoxymethyl groups [δ_H 3.265 (3H, *s*), 3.273 (3H, *s*), δ 3.270 (4H, *m*)], six aryl protons displayed as two ABX systems, two aliphatic methine (δ 2.02, *m*, 2H) and four benzylic protons (δ 2.56, *m*, 2H; δ 2.61, *m*, 2H). These structural moieties would constitute a 3,4,3′,4′-tetrasubstituted diarylbutane skeleton for **1**, similar to that of niranthin (**6**) except for the substitution in the aryl groups. Comparison of these data with those of **6** (Anjaneyulu et al., 1973) suggested **1** as likely to be 5-demethoxyniranthin. This structure for **1** was further supported by analysis of COSY-90, HMQC and HMBC NMR spectra, from which the chemical shifts of ^1H and ^{13}C NMR signals of **1** (Table 1) were assigned unambiguously. The (8*S*,8′*S*)-absolute stereochemistry was confirmed by the similar specific optical rotation and circular dichroic curve as those of **5** (*see Experimental*), whose (8*S*,8′*S*)- stereochemistry was assigned by a synthetic approach (Row and Satyanarayana, 1967) and X-ray crystallography of the iodo derivatives (Rao and Murthy, 1968). Therefore, structure **1** was established to be (8*S*,8′*S*)-3,4-methylenedioxy-3′,4′,9,9′-tetramethoxylignan.

Compound **2** had a molecular formula $C_{22}H_{24}O_6$, as deduced from HR-EI-MS. It contained an aryltetralin

Table 1
^1H NMR, ^{13}C NMR, and HMBC data for 5-demethoxyniranthin (**1**) and urinatetralin (**2**) in $CDCl_3$ (δ in ppm, *J* in Hz)

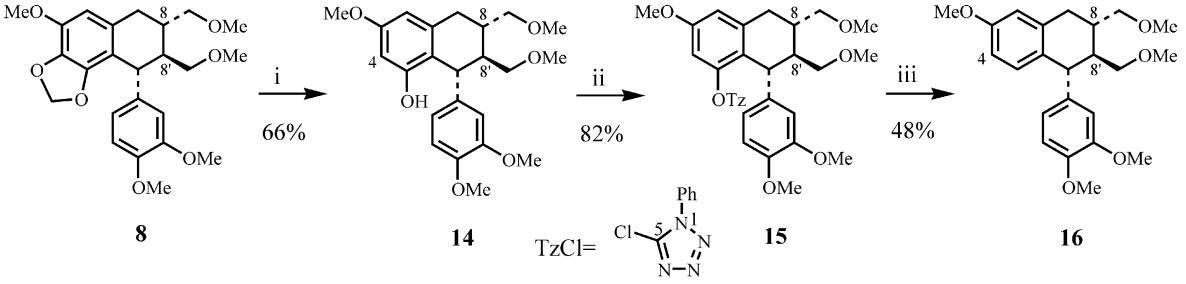
Position	**1**			**2**		
	^1H	^{13}C	HMBC (*J* = 8 Hz) (H→C)	^1H	^{13}C	HMBC (*J* = 8 Hz) (H→C)
1		135.3 *s*			129.8 *s*	
2	6.56 *br s*	109.8 *d*	4, 5, 6, 7		133.1 *s*	
3		147.8 *s*		6.19 *s*	109.5 *s*	1, 5, 6, 7′
4		145.9 *s*			145.6 *s*	
5	6.67 *d* (7.8)	108.3 *d*	1, 3, 4		145.5 *s*	
6	6.54 *br d* (7.8)	122.3 *d*	4, 5, 7	6.55 *s*	108.0 *d*	2, 4, 7
7	2.61 *m*	35.3 *t*	1, 2, 6, 9, 8′	2.77 *m*	33.5 *t*	1, 2, 6, 8, 9, 8′
8	2.02 *m*	41.3 *d*		2.12 *m*	36.2 *d*	1, 7, 9, 7′, 8′, 9′
9	3.270 *m*	72.9 *t*	7, 8, 7′, 8′, 9-OMe	3.38 *dd* (9.3, 6.4)	75.2 *t*	7, 8′, 9-OMe
				3.44 *dd* (9.3, 3.9)		7, 8, 8′, 9-OMe
1′		134.0 *s*			139.4 *s*	
2′	6.60 *br s*	112.5 *d*	4′, 6′, 7′	6.54 *d* (1.6)	109.2 *d*	4′, 6′, 7′
3′		149.1 *s*			147.8 *s*	
4′		147.5 *s*			146.0 *s*	
5′	6.74 *d* (8.1)	111.4 *d*	3′, 4′, 7′	6.72 *d* (7.9)	107.8 *d*	2′, 3′, 7′
6′	6.64 *br d* (8.1)	121.5 *d*	4′, 5′, 7′	6.62 *dd* (7.9, 1.6)	122.7 *d*	2′, 4′, 7′
7′	2.56 *m*	35.2 *t*	8, 1′, 2′, 6′, 9′	3.91 *d* (10.4)	47.5 *d*	1, 6, 8, 1′, 2′, 6′, 8′, 9′
8′	2.02 *m*	41.1 *d*		1.76 *m*	44.8 *d*	6, 7, 8, 9, 1′, 7′, 9′
9′	3.270 *m*	73.0 *t*	7, 8, 7′, 8′, 9-OMe	3.08 *dd* (9.6, 3.3)	71.2 *t*	8, 7′, 9′-OMe
				3.35 *m*		7, 8, 7′, 8′, 9′-OMe
9-OMe	3.265 *s*	59.0 *q*	9	3.265 *s*	59.0 *q*	9
3′-OMe	3.80 *s*	56.1 *q*	3′			
4′-OMe	3.84 *s*	56.3 *q*	4′			
9′-OMe	3.273 *s*	59.1 *q*	9′	3.273 *s*	59.1 *q*	9′
3,4-OCH_2O-	5.89 *s*	101.1 *t*	3, 4			
4,5-OCH_2O-				5.80 *s*	100.5 *t*	5
3′,4′-OCH_2O-				5.90 *s*	100.8 *t*	4′

skeleton similar to the lignan phyltetralin (Stevenson and Williams, 1977), as established by the characteristic ^{1}H NMR spectral signals (Table 1), of which five aryl protons appear as two singlets (H-3 and H-6) and an ABX system (H-2′, H-5′ and H-6′), and two methylenedioxy groups appear at δ 5.80 (2H, *s*) and 5.90 (2H, *s*), suggesting their location at C-4/C-5 and C-3′/C-4′ in the aromatic rings. An HMBC spectrum revealed three-bond couplings of both C-4 and C-5 to one methylenedioxy singlet (δ_H 5.80), and both C-3′ and C-4′ to the other (δ_H 5.90), distinguishing their assignments. Further analysis of this 2D NMR spectrum also allowed the complete assignment of its ^{13}C NMR spectroscopic data (Table 1). The (7′*R*,8*S*,8′*S*)- stereochemistry for **2** was assigned based on the similarity of CD curves between **2** and the known isolintetralin (**11**) (*see Experimental*) and was also deduced from optical activity tests (Hulbert et al., 1981). Compound **2** was named urinatetralin [(7′*R*,8*S*,8′*S*)-9,9′-dimethoxy-4,5:3′,4′-*bis*(methylenedioxy)-2,7′-cyclolignan] after its plant origin and structural property.

Some aryltetralins possessing the stereochemistry might show different sign of specific optical rotation such as lintetralin (right-handed) and isolintetralin (left-handed). In addition, the steric effect arisen by the B-ring substituents and the bottom rings will influence the CD curves. Thus, the CD curves of those 3,4,5-trisubstituted compounds such as hypophyllanthin (**8**) and nirtetralin (**9**) are usually not clear enough for the determination of their stereochemistry. In such cases, the relative stereochemistry was determined by analysis of NOESY spectra, such as for **8** and **9**. Their absolute configuration is determined by comparing the CD curve of a chemically modified product, void of above-mentioned steric effect, to that of a well-characterized related lignan such as phyltetralin (**7**). A typical example is illustrated in Scheme 1. Reductive cleavage of the methylenedioxy function in **8** by sodium in liquid ammonia (Lee et al., 1996) yielded a phenolic compound **14**, $\delta_{H\text{-}4}$ 6.30 (*d*, J = 2.4 Hz) and $\delta_{H\text{-}6}$ 6.18 (*d*, J = 2.4 Hz,), $\delta_{\underline{OH}}$ 4.88 (s, D_2O exchangeable). Treatment of the phenol with 5-chloro-1-phenyltetrazole (Ram and Neumeyer, 1981) yielded compound **15**, δ 7.44–7.38 (5H, *m*, Tz-*Ph*), 6.65 (1H, *d*, J = 2.4 Hz) and 6.69 (1H, *d*, J = 2.4 Hz) (H-4 and H-6). Hydrogenolysis of **15** yielded 4-demethoxyphyltetralin (**16**), $[\alpha]_D^{27}$ −22 (c = 1.0, $CHCl_3$); $\delta_{H\text{-}3}$ 6.62 (*d*, J = 8.6 Hz), $\delta_{H\text{-}4}$ 6.54 (*dd*, J = 8.6, 2.5 Hz) and $\delta_{H\text{-}6}$ 6.64 (*d*, J = 2.5 Hz). Although the optical rotation of **16** is left-handed, different from the right-handed **7**, the CD curve of **16** is similar in shape to that of **7**, suggesting the same (7′*R*,8*S*,8′*S*)- configuration and consequently the same stereochemistry for hypophyllanthin (**8**).

Compound **3** had a molecular formula $C_{21}H_{22}O_6$, established by EI-MS and NMR spectroscopic data. Its NMR data were almost identical to those of bursehernin, a diaryl butyrolactone isolated from *Bursera schlech* (Mcdoniel and Cole, 1972) and *Hernandia guianensis* (Richomne et al., 1984), except for the opposite sign of specific optical rotation. Thus, **3** could be the enantiomer of bursehernin. Elaborate analysis of the COSY and HMBC data (Table 2) also supported this suggestion for the structure. Two NOE experiments upon irradiation on the methoxy signals at δ 3.81 and 3.84, enhancing the signals of H-2 (δ 6.64, *d*, J = 1.8 Hz) and H-5 (δ 6.77, *d*, J = 8.0 Hz), respectively, are also supportive. The 8*S*,8′*S*-configuration was elucidated by comparison of its CD data and optical rotation (Jakupovic et al., 1986) with heliobuphthalmin lactone (**12**). To confirm this stereochemistry, the related and more abundant **12** from this studied plant was reduced by $LiAlH_4$ to give a phyllanthin derivative (**17**), whose optical property ($[\alpha]_D$ and CD) is similar to that of **5**, supporting the assignment of stereochemistry. Following its right-handed optical property, compound **3**, (8*S*,8′*S*)-3,4-dimethoxy-3′,4′-methylenedioxylignan-9,9′-olide, was named dextrobursehernin.

Urinaligran (**4**) had a molecular formula $C_{22}H_{24}O_7$, established by its HR-EI-MS spectrum. It contained two 3,4-methylenedioxyphenyl groups as established by the presence of two sets of ABX system for six aryl protons (one for H-2, H-5 and H-6, and the other for H-2′, H-5′, and H-6′) and two methylenedioxy singlets (δ 5.940 and 5.936) in its ^{1}H NMR spectrum (Table 2). Other ^{1}H NMR signals included two doublets for two



Scheme 1. Preparation of 4-demethoxyphyltetralin (**16**) from hypophyllanthin (**8**). (i) Na/liq. NH_3, THF, −78°; (ii) TzCl. K_2CO_3, THF, Δ; (iii) H_2 (75 psi)-Pd/C, HOAc, 50°, 4 d.

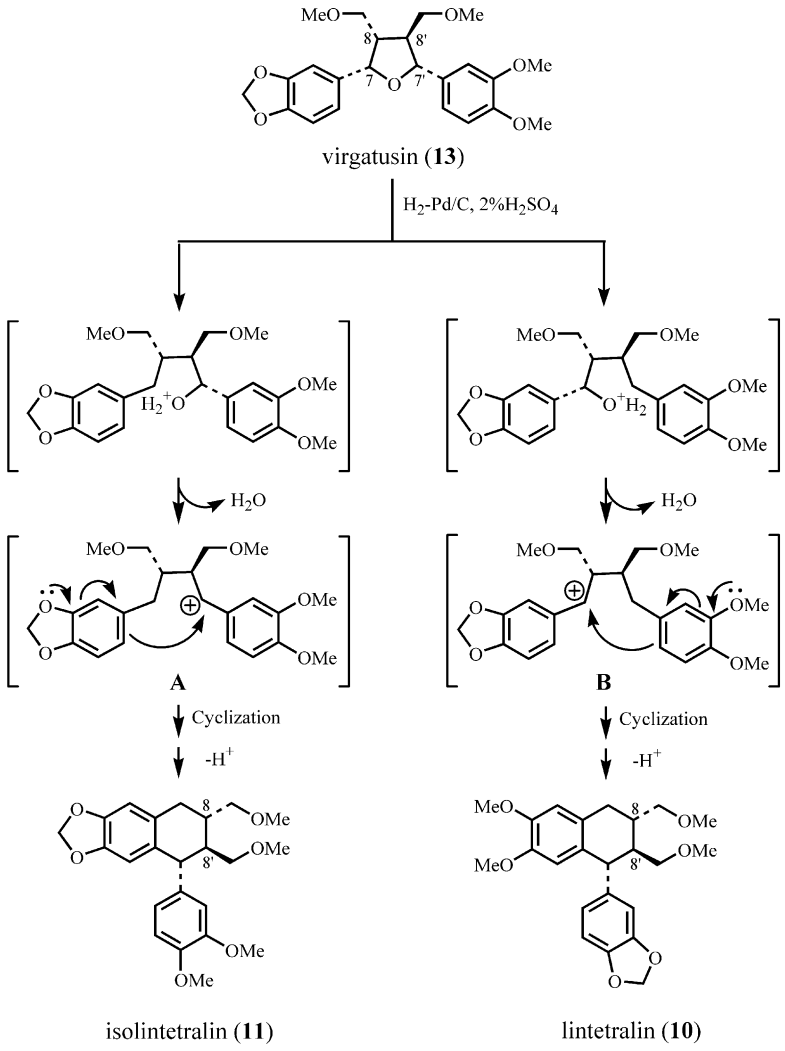
relatively deshielded benzylic protons ($\delta_{H\text{-}7}$ 4.67, 1H and $\delta_{H\text{-}7'}$ 5.02, 1H), two multiplets for two aliphatic methines (H-8 and H-8′) and signals for two methoxymethyl groups [δ 2.95 (*dd*, $J=9.2$, 5.7 Hz), δ 3.03 (*t*, $J=9.2$ Hz); δ 3.45 (*dd*, $J=9.5$, 4.9 Hz), δ 3.51 (*dd*, $J=9.5$, 5.4 Hz)], verified by a COSY spectrum (Table 2). These data constituted a structure for **4**, 9,9′-dimethoxy-3,4:3′,4′-*bis*(methylenedioxy)-7,7′-epoxylignan. This assigned structure was supported by comparison of its spectral data with those reported for virgatusin (**13**) (Huang et al., 1996), which was also isolated from this study. Complete ^{1}H and ^{13}C NMR spectral assignment of **4** was made by analysis of Homo-COSY, HSQC and HMBC data.

Relative stereochemistry of **4** was determined by NOE techniques. The *cis* relationship of H-7 to H-8 and H-7′ was established by the observation of the enhanced signals for H-7′ (δ 4.67, 8.7%) and H-8 (δ 2.95, 11.6%) upon irradiating at the frequency of H-7 (δ 5.02). While the *cis* H-8/H-7′ and *trans* H-8′/H-7′ relationships were deduced based on the extent of enhancement of H-7 (8.7%), H-8 (11.6%) and H-8′ (δ 2.26) (2.7%) upon irradiating at the frequency of H-7′. The (7*S*,8*S*,7′*R*,8′*S*)- absolute configuration in **4** was established by the following chemical correlation. Catalytic hydrogenolysis of the related virgatusin (**13**), under strongly acidic conditions (H_2-10% Pd/C, 2% H_2SO_4) yielded two products, identical to the aryltetralin lintetralin (**10**) and isolintetralin (**11**), both possessing (8*S*,8′*S*)- stereochemistry. Since the stereochemistry of C-8 and C-8′ in **13** was intact during the reaction process, the (8*S*,8′*S*)- configuration in compound **4** and virgatusin (**13**) were thus ascertained. Following the *cis* relationship of H-7 to H-8 and H-7′, as indicated above, designated the (7*S*,7′*R*)- stereochemistry. Compound **4** is thus determined to be (7*S*,8*S*,7′*R*,8′*S*)-9,9′-dimethoxy-3,4:3′,4′-*bis*(methylenedioxy)-7,7′-epoxylignan, and was named urinaligran after the plant origin and structural property.

Possible mechanism for the formation of compound **10** and **11** is suggested as shown in Scheme 2. Hydrogenolysis at the benzylic position, C_7 or $C_{7'}$, led to the cleavage of C_7-O or $C_{7'}$-O bond to give a benzylic alcohol, which then reacted with 2% sulfuric acid to produce the cationic intermediates (**A** and **B**). Subsequent SN'_1 cyclization reaction from the direction of the less sterically hindered site yielded product **10** (from **B**) and **11** (from **A**).

Table 2
^{1}H NMR, ^{13}C NMR, and HMBC data for dextrobursehernin (**3**) and urinaligran (**4**) in $CDCl_3$ (δ in ppm, J in Hz)

Position	**3**			**4**		
	^{1}H	^{13}C	HMBC ($J=8$ Hz) (H→C)	^{1}H	^{13}C	HMBC ($J=8$ Hz) (H→C)
1		130.1 *s*			132.7 *s*	
2	6.64 *d* (1.8)	112.2 *d*	4, 6, 7	6.90 *d* (1.5)	107.0 *d*	1, 4, 6, 7
3		149.1 *s*			147.4 *s*	
4		147.9 *s*			146.6 *s*	
5	6.77 *d* (8.0)	111.1 *d*	1, 3	6.76 *d* (8.0)	107.9 *d*	1, 3
6	6.66 *dd* (8.0, 1.8)	121.4 *d*	2, 4, 7	6.83 *dd* (8.0, 1.5)	119.6 *d*	1, 3, 4, 7
7	2.87 *dd* (14.1, 6.9) 2.94 *dd* (14.1, 5.1)	34.6 *t*	1, 2, 6, 8, 9, 8′	5.02 *d* (7.3)	81.4 *d*	1, 2, 6, 8, 9
8	2.53 *dd* (13.1, 7.9)	46.5 *d*	7, 9, 7′, 8′	2.56 *m*	46.4 *d*	8′, 9′
9		178.6 *s*		2.95 *dd* (9.2, 5.7) 3.03 *t* (9.2)	73.2 *t*	7, 8, 8′, 9-OMe
1′		131.6 *s*			135.5 *s*	
2′	6.41 *d* (1.5)	108.3 *d*	4′, 6′, 7′	7.00 *d* (1.6)	106.95 *d*	1′, 3′, 4′, 6′, 7′
3′		148.0 *s*			147.8 *s*	
4′		146.4 *s*			147.0 *s*	
5′	6.66 *d* (7.8)	108.8 *d*	1′, 3′	6.78 *d* (8.1)	108.0 *d*	1′, 3′
6′	6.43 *dd* (7.8, 1.5)	121.5 *d*	2′, 4′, 7′	6.91 *dd* (8.1, 1.6)	120.0 *d*	2′, 4′, 7′
7′	2.44 *m* 2.57 *dd* (17.0, 9.8)	38.3 *t*	8, 1′, 2′, 6′, 8′, 9′	4.67 *d* (8.0)	82.6 *d*	1′, 2′, 6′, 8′, 9′
8′	2.46 *m*	41.1 *d*	7, 8, 7′, 9′	2.26 *m*	51.4 *d*	8, 9, 1′, 7′
9′	3.83 *m* 4.09 *dd* (9.2, 7.0)	71.2 *t*	7′, 8′ 9, 7′, 8′	3.45 *dd* (9.5, 4.9) 3.51 *dd* (9.5, 5.4)	72.9 *t*	8, 7′, 8′, 9′-OMe
3-OMe	3.81 *s*	55.8 *q*	3			
4-OMe	3.84 *s*	55.9 *q*	4			
9-OMe				3.07 *s*	58.6 *q*	9
9′-OMe				3.34 *s*	59.0 *q*	9′
3,4-OCH_2O-				5.940 *s*	101.0 *t*	3, 4
3′,4′-OCH_2O-	5.90 *br s* 5.91 *br s*	101.1 *t*	3′, 4′	5.936 *s*	100.9 *t*	3′, 4′



Scheme 2. Proposed mechanism for formation of aryltetralins **10** and **11** from diaryltetrahydrofuran **13**.

A modified partitioning procedure was applied to the isolation of lignans in the root of the same plant. The ethanol extract of this part was partitioned by the solvent system $CHCl_3$–MeOH–H_2O (2:2:1) to give fractions soluble in aqueous (upper) and organic (lower) layers, the latter fraction yielding 12 lignans (**2**–**13**). They were identified efficiently by characteristic ^1H NMR signals, including mixture fractions. Analysis on intensity of aromatic and methoxy signals allowed the distinction of various lignans and determination of their abundance in the aerial and root parts. This study found that phyllanthin (6.63%), phyltetralin (3.06%) and hypophyllanthin (0.76%) are major in the aerial parts while phyllanthin (5.36%), niranthin (2.27%) and hypophyllanthin (1.41%) are most abundant in the roots. In addition, a thorough survey of ^1H NMR spectra resulted in the exclusion of 5-demethoxyniranthin (**1**) in the extract of the root.

3. Experimental

3.1. General

The physical data of the compounds were obtained using the following instruments: $[\alpha]_D$ ($CHCl_3$): JASCO DIP-370 Digital Polarimeter; IR (KBr disc): JASCO IR Report-100 Infrared Spectrometer; UV (MeCN): Hitachi 150-20 Double Beam Spectrophotometer; CD (MeCN): JASCO J-710 Spectropolarimeter. NMR: Bruker AMX400 spectrometer; EIMS: JEOL JMS D300 Mass Spectrometer (70 eV). TLC (silica gel): EtOAc–hexane (3:7).

3.2. Plant material

The plant material, collected in 1994 in Xishuangbanna, Yunnan Province, Mainland China, was

authenticated by colleagues in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing, China. A voucher specimen (PHNTU 9410) has been deposited in the School of Pharmacy. College of Medicine, National Taiwan University.

3.3. Extraction and isolation

The dry powders of the aerial and underground parts were percolated with 95% EtOH to give EtOH extracts (2.26 and 0.59 kg, respectively) upon concentration under reduced pressure. The EtOH extract of the aerial part was triturated with H_2O vigorously to give H_2O insoluble fraction (832 g). Part of this fraction (189 g) was triturated with *n*-hexane and then $CHCl_3$ to give *n*-hexane soluble fraction (68 g) and $CHCl_3$ soluble fraction (82 g). The $CHCl_3$-soluble fraction was applied on to a Sephadex LH-20 [750 g, MeOH–$CHCl_3$ (1:1)] and a silica gel column [finer than 230 mesh, 1.5 kg, MeOH–$CHCl_3$ (1:19)] to yield two fractions (B-1, B-2). Fraction B-1 (53 g) was reapplied to a silica gel column (230–400 mesh, 4 kg, 10–60% EtOAc–hexane) to give 19 subfractions. Subfraction 12 (315.8 mg) yielded compound **1** (7.0 mg) upon further separation [silica gel, 230–400 mesh, acetone–$CHCl_3$–hexane (2:49:49); Sephadex LH-20, MeOH–$CHCl_3$ (7:3)]. Subfraction 15 (22.97 g) yielded compounds **12** (80.4 mg), **6** (2.32 g) and **8** (902.1 mg) by repeated silica gel cc (EtOAc–hexane, acetone–toluene, EtOAc–toluene) (compounds **12** and **6**) and recrystallization from Me_2CO–hexane solvent pairs (compound **8**). Subfraction 16 (14.14 g) yielded compound **5** (1.74 g) by silica gel chromatography (230–400 mesh, 1% acetone–toluene). The hexane-soluble fraction (68 g, 17 g × 4) was fractionated on a Sephadex LH-20 column (750 g) with MeOH–$CHCl_3$ (7:3) to give four fractions (I–IV). Fraction II (59 g) was applied on to a silica gel column (230–400 mesh, 2.0 kg, 10–60% EtOAc–hexane) to yield 27 subfractions. Subfraction 12 (3.53 g) yielded **2** (188 mg) via a Sephadex LH-20 column (MeOH–$CHCl_3$ 7:3), subfraction 17 (1.54 g) yielded **4** (103 mg) after separation on a Sephadex LH-20 (MeOH–$CHCl_3$ 7:3) and a silica gel column (<230 mesh, 1% Me_2CO-toulene), subfraction 19 (2.53 g) yielded **10** (21 mg) after separation on Sephadex LH-20 columns [MeOH–$CHCl_3$ (7:3) and $CHCl_3$–hexane (1:1)] and a preparative silica gel TLC [EtOAc–petroleum ether (1:4)], subfraction 20 (2.32 g) yielded **11** (39 mg) after separation on a Sephadex LH-20 column [MeOH–$CHCl_3$ (7:3)] and an aluminum oxide column [Me_2CO–$CHCl_3$–hexane (0.1:30:70)], subfraction 21 (1.04 g) yielded **9** (433 g) after separation on Sephadex LH-20 columns [MeOH–$CHCl_3$ (7:3 and 3:7)], subfraction 24 yielded **13** (724 mg) after separation on a silica gel [230–400 mesh, Me_2CO–$CHCl_3$–hexane (2:49:49–5: 47.5: 47.5)] and an aluminum oxide column [Me_2CO–$CHCl_3$–hexane (0.1: 30: 70)], subfraction 25 (504 mg) yielded **7** (150 mg) and **3** (22 mg) after separation on Sephadex LH-20 columns [MeOH–$CHCl_3$ (7:3 and 1:1)].

Part of the EtOH extract (437 g) of the root was triturated with the aqueous layer of $CHCl_3$–MeOH–H_2O (2:2:1) vigorously to give soluble and insoluble parts. The latter was further triturated with the organic layer for five times, which afforded 216 g of residue after evaporation. This soluble fraction was triturated with *n*-hexane and then $CHCl_3$ to give fractions soluble in *n*-hexane (69 g) and $CHCl_3$ (68 g). The *n*-hexane soluble fraction, divided into two portions (34 g and 35 g), was fractionated on a Sephadex LH-20 column [1.5 kg, MeOH–$CHCl_3$ (3:7)] to give 4 fractions (A–D). Part of fraction C (27 g) was further subjected to a silica gel cc [230–400 mesh, 1.0 kg, EtOAc–hexane (1:9)] to yield 21 fractions, of which fractions 9 and 10 yielded a mixture of stigmasterol and β-sitosterol (1.93 g). Subfraction 7 (981 mg) yielded **2** (19.3 mg) after separation on Sephadex LH-20 columns [50 and 1.5 g, MeOH–$CHCl_3$ (7:3)]. Subfractions 12–14 (7.94 g) yielded compounds **6** (4.66 g) and **9** (37.1 mg) after separation through silica gel [230–400 mesh, 320 g, EtOAc–hexane (2:8)] and Sephadex LH-20 columns. The $CHCl_3$ soluble fraction (68 g) was fractionated on a Sephadex LH-20 column [1.4 kg, MeOH–$CHCl_3$ (7:3)] to give three fractions (I–III). Fraction II was applied on to a silica gel column [230–400 mesh, 1.1 kg, EtOAc–hexane (1:9 to 3:2)] to give 21 subfractions. Recrystallization of subfraction 4 from Me_2CO–hexane solvent pairs yielded friedelin (147.5 mg). Subfraction 6 (0.93 g) was reapplied on to silica gel [Me_2CO–toluene (2:98) and (3: 97)] and Sephadex LH-20 columns [MeOH–$CHCl_3$ (7:3) and EtOAc–hexane (3:7)] to give **12** (4.8 mg), **4** (3.4 mg), **10** (10.3 mg) and **11** (3.1 mg). Subfraction 10 (4.23 g) yielded **8** (107.4 mg), **13** (38.9 mg), and **7** (84.4 mg) after separation through a silica gel column [230–400 mesh, 160 g, EtOAc–toluene (1:19)], followed by a Sephadex LH-20 column [MeOH–$CHCl_3$ (7:3)]. Subfraction **11** (6.07 g) yielded **5** (4.23 g), after silica gel cc [230–400 mesh, Me_2CO–toluene (1:19)], and **3** (25.6 mg), and subsequent separation on a Sephadex LH-20 column [MeOH–$CHCl_3$ (7:3)].

3.4. 5-Demethoxyniranthin (*1*)

White amorphous solid; R_f 0.31; $[\alpha]_D^{25}$ +15.4° (c = 0.19); IR ν_{max} 2925, 2850, 1610, 1590, 1510, 1490, 1450, 1240, 1190, 1160, 1140, 1115, 1040, 940, 810, 770 cm^{-1}; UV λ_{max} (log ε) 226.6 (4.26, *sh*), 282.8 (3.90) nm; CD ($c = 2.49 \times 10^{-5}$ M) ($\Delta\varepsilon$) 331 (−2.20), 293 (+1.39), 280 (−0.85), 272 (−1.07), 259 (+0.88), 248 (−0.74), 234 (+1.93), 226 (+1.91), 211 (−0.83); for ^1H and ^{13}C NMR spectroscopic data, see

Table 1; EIMS *m/z* 402 (18, M^+), 386 (35), 371 (80), 315 (38), 293 (21), 152 (16), 151 (45), 135 (21), 85 (79), 83 (100); HREIMS: *m/z* 402.2030 (calc. for $C_{23}H_{30}O_6$ 402.2018).

3.5. Urinatetralin (2)

Liquid; R_f 0.49; $[\alpha]_D^{25}$ +7.0° (*c* = 1.0); IR ν_{max} 2900, 1610, 1500, 1485, 1440, 1380, 1300, 1240, 1190, 1120, 1040, 940, 900, 870, 800, 760 cm^{-1}; UV λ_{max} (log ε) 231.8 (4.11, *sh*), 287.0 (3.91) nm; CD (*c* = 2.60 × 10^{-5} M) ($\Delta\varepsilon$) 312 (0), 299 (+6.83), 282 (−1.96), 258 (+0.92), 246 (−1.27), 225 (+6.34), 213 (−4.59), 203 (+36.03); for ^1H and ^{13}C NMR spectroscopic data, see Table 1; EIMS *m/z* 384(12, M^+), 352 (12), 307 (26), 185 (12), 135 (58), 85 (48), 83 (78), 45(100); HREIMS: *m/z* 384.1559 (calc. for $C_{22}H_{24}O_6$ 384.1545).

3.6. Dextrobursehernin (3)

Colorless liquid; R_f 0.16; $[\alpha]_D^{25}$ +36.0° (*c* = 1.0); IR ν_{max} 3020, 2930, 2850, 1740, 1540, 1520, 1450, 1250, 1220, 1040, 1030, 935, 760 cm^{-1}; UV λ_{max} (log ε) 225.2 (4.22, *sh*), 283.0 (3.90) nm; CD (*c* = 2.7 × 10^{-5} M) ($\Delta\varepsilon$) 297 (+0.80), 291 (+1.66), 282 (−0.69), 274 (+0.42), 265 (+0.15), 259 (+1.13), 254 (+0.78), 236 (+3.34), 227 (+3.77), 219 (+2.51), 213 (+2.18), 204 (+13.29); for ^1H and ^{13}C NMR spectroscopic data, see Table 2; EIMS *m/z* 370 (26, M^+), 234 (7), 208 (4), 151 (100), 135 (20).

3.7. Urinaligran (4)

Colorless liquid; R_f 0.36; $[\alpha]_D^{25}$ +19.0° (*c* = 1.0); IR ν_{max} 2925, 2880, 1615, 1505, 1490, 1445, 1400, 1250, 1200, 1120, 1100, 1040, 940, 870, 810 cm^{-1}; UV λ_{max} (log ε) 234.8 (4.01), 285.8 (3.89) nm; CD (*c* = 2.40 × 10^{-5} M) ($\Delta\varepsilon$) 299 (+0.02), 293 (+1.03), 288 (+0.21), 278 (+0.21), 273 (−0.48), 268 (−0.18), 260 (+0.63), 255 (+0.20), 248 (+0.39), 233 (+0.28), 227 (+1.80), 213 (−0.11), 205 (1.81), 200 (−3.41); for ^1H and ^{13}C NMR spectroscopic data, see Table 2; EIMS *m/z* 400 (89, M^+), 255 (44), 250 (22), 208 (87), 192 (70), 173 (100), 149 (50), 135 (20) 115 (37); HREIMS: *m/z* 400.1508 (calc. for $C_{22}H_{24}O_7$ 400.1494).

3.8. Preparation of 4-demethoxyphyltetralin (16) from hypophyllanthin (8) (Scheme 1)

3.8.1. Reaction of 8 with sodium in liquid ammonia

The solution of compound **8** (130.4 mg) dissolved in dry THF (6.5 ml) was added to a dark blue solution of sodium (350 mg)/ liquid ammonia (70 ml) dropwise with vigorous stirring under nitrogen in a dry ice-acetone bath (−70 °C) (Ram and Neumeyer, 1981). After 2 h, ammonia was evaporated in a well-ventilated hood at room temp. To the residue was slowly added MeOH (16 ml) to destroy excessive sodium, and the organic solvent was removed by condensation. The aqueous solution of the residue (100 ml) was then adjusted to pH 7 by 1 N HCl and partitioned against Et_2O (100 ml × 3). The combined Et_2O layers after drying over Na_2SO_4 and subsequent condensation gave a residue (137 mg), which was applied onto a silica gel column [230–400 mesh, 6 g, Me_2CO–$CHCl_3$–hexane (5: 47.5: 47.5)] to give **14** (86.7 mg, 66% yield). **14**: R_f 0.32 [Me_2CO-$CHCl_3$ (1:19)], ^1H NMR ($CDCl_3$) δ 6.78 (2H, *s*, H-5′ and H-6′), 6.68 (1H, *brs*, H-2′), 6.30 (1H, *d*, *J* = 2.4 Hz, H-6), 6.18 (1H, *d*, *J* = 2.4 Hz, H-4), 4.88 (*s*, −O<u>H</u>, D_2O exchangeable), 4.06 (1H, *d*, *J* = 9.0 Hz, H-7′), 3.83 (3H, *s*, 5-OMe), 3.77 (3H, *s*) and 3.71 (3H, *s*) (3′- and 4′-OMe), 3.30 (3H, *s*) and 3.29 (3H, *s*) (9- and 9′-OMe); EI-MS (rel. int.%) 402 (38, $[M]^+$), 339 (21), 325 (26), 85 (64), 83 (100).

3.8.2. Preparation of tetrazolyl derivative 15

The mixture of **14** (53.2 mg, 0.132 mM), 5-chloro-1-phenyltetrazole (48 mg, 0.267 mM), dry THF (5.5 ml) and K_2CO_3 (39 mg, 0.283 mM) in a 10-ml round bottom flask was stirred and refluxed under nitrogen overnight (Mcdoniel and Cole, 1972). After cooling, the solution was washed with H_2O, dried over anhyd. $MgSO_4$ and condensed to give a residue (48 mg), which was purified through a silica gel column [230–400 mesh, 5 g, Me_2CO–$CHCl_3$–hexane (1:18:2)] to afford **15** (43.7 mg, 82.1% yield). **15**: ^1H NMR ($CDCl_3$) δ 7.44-7.38 (5H, *m*, Tz-*Ph*), 6.69 (1H, *d*, *J* = 2.4 Hz) and 6.65 (1H, *d*, *J* = 2.4 Hz) (H-4 and H-6), 6.44 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.36 (1H, d, *J* = 1.9 Hz, H-2′), 6.32 (1H, *dd*, *J* = 8.2, 1.9 Hz, H-6′), 4.11 (1H, *d*, *J* = 8.0 Hz, H-7′), 3.50 (3H, *s*, 5-OMe), 3.76 (3H, *s*) and 3.72 (3H, *s*) (3′- and 4′-OMe), 3.26 (3H, *s*) and 3.21 (3H, *s*) (9′- and 9-OMe); EI-MS (rel. int.%) 546 (50, $[M]^+$), 402 (37), 401 (32), 339 (25), 325 (27), 307 (42), 292 (30), 151 (22), 118 (100), 91 (56), 77 (36).

3.8.3. Preparation of 4-demethoxyphyltetralin (16) by hydrogenolysis of 15

The mixture of **15** (30 mg), HOAc (10 ml) and 10% Pd/C (30 mg) in a high-pressure reactor after degassing was introduced H_2 (75 psi) at 50 °C. After 4 days' reaction, the reaction mixture was filtered through a Celite cake. The Celite pad was exhaustively washed with $CHCl_3$. The combined $CHCl_3$ washings and the filtrate after drying over $MgSO_4$ and subsequent condensation gave a residue (26 mg), which was purified by silica gel cc [230–400 mesh, 6 g, Me_2CO–$CHCl_3$–hexane (1:18:2)] to afford **16** (14.4 mg, 48% yield). **16**: R_f 0.58 [Me_2CO–$CHCl_3$ (1:19)]; $[\alpha]_D^{27}$ −22 (*c* = 1.0); IR ν_{max} 2900, 2730, 1610, 1590, 1460, 1425, 1385, 1320, 1260, 1235, 1190, 1160, 1140, 1120, 1030, 960, 895, 860, 810, 760 cm^{-1}; UV λ_{max} (log ε) 228.2 (4.23, *sh*), 280.8 (3.74); CD (*c* = 2.59 × 10^{-5} M) ($\Delta\varepsilon$) 298 (0), 288 (+4.18), 273 (−3.09), 247 (+2.24), 233 (−5.02), 221 (−2.88), 211

(−15.01), 200 (+20.83); ^{1}H NMR ($CDCl_3$) δ 6.79 (1H, *d*, *J* = 8.2 Hz, H-5′), 6.68 (1H, *dd*, *J* = 8.2, 1.8 Hz, H-6′) 6.61 (1H, *d*, *J* = 1.8 Hz, H-2′), 6.64 (1H, *d*, *J* = 2.5 Hz, H-6), 6.62 (1H, *d*, *J* = 8.6 Hz, H-3), 6.54 (1H, *dd*, *J* = 8.6, 2.5 Hz, H-4), 3.95 (1H, *d*, *J* = 10.6 Hz, H-7′), 3.86 (3H, *s*, 5-OMe), 3.79 (3H, *s*) and 3.73 (3H, *s*) (3′- and 4′-OMe), 3.34 (3H, *s*) and 3.24 (3H, *s*) (9′- and 9-OMe); EI-MS *m*/*z* (rel. int.%) 386 (68, $[M]^+$), 354 (38), 323 (100), 309 (92), 294 (28), 291 (53), 282 (22), 239 (20), 151 (31); HR-EI-MS *m*/*z* 386.2108 (calc. for $C_{23}H_{30}O_5$, 386.2122).

3.9. Optical rotation and CD data for phyllanthin (5)

$[\alpha]_D^{25}$ +15.0° (*c* = 1.00); UV λ_{max} (log ε) 225.8 (4.18, *sh*), 278.8 (3.73) nm; CD (*c* = 2.39 × 10^{-5} M) ($\Delta\varepsilon$) 334 (−0.84), 292 (+1.81), 283 (0), 258 (+1.46), 248 (−0.14), 236 (+1.62), 227 (+3.54), 208 (−5.57).

3.10. Optical rotation and CD data for niranthin (6)

$[\alpha]_D^{25}$ +12.0 (*c* = 1.00); UV λ_{max} (log ε) 200.8 (4.83), 227.2 (4.16, *sh*), 278.4 (3.65) nm; CD (*c* = 2.31 × 10^{-5} M) ($\Delta\varepsilon$) 331 (−1.32), 302 (+0.83), 293 (+1.31), 278 (−0.25), 259 (+0.99), 249 (−0.23), 231 (+3.51), 210 (−1.55), 203 (+2.33).

3.11. Optical rotation and CD data for phyltetralin (7)

$[\alpha]_D^{25}$ +40.0 (*c* = 0.50); UV λ_{max} (log ε) 232.2 (4.23, *sh*), 282.2 (3.85) nm; CD (*c* = 2.40 × 10^{-5} M) ($\Delta\varepsilon$) 306 (0), 291 (+5.96), 274 (−1.28), 265 (−1.06), 258 (+0.71), 240 (−7.29), 225 (+2.72), 218 (+1.31), 206 (+23.05).

3.12. Optical rotation and CD data for hypophyllanthin (8)

$[\alpha]_D^{25}$ +6.0 (*c* = 1.00); UV λ_{max} (log ε) 199 (4.86), 231.0 (4.30, *sh*), 278.8 (3.72) nm; CD (*c* = 2.33 × 10^{-5} M) ($\Delta\varepsilon$) 315 (0), 300 (+1.43), 286 (0), 277 (+1.06), 272 (0), 259 (+1.32), 246 (−4.25), 225 (+10.10), 218 (+16.29), 203 (−17.36).

3.13. Optical rotation and CD data for nirtetralin (9)

$[\alpha]_D^{25}$ +7.0 (*c* = 1.00); UV λ_{max} (log ε) 201.0 (4.91), 228.8 (4.34, *sh*), 281.2 (3.85) nm; CD (*c* = 2.33 × 10^{-5} M) ($\Delta\varepsilon$) 310 (0), 292 (+1.42), 282 (−1.95), 275 (−2.05), 255 (+0.88), 249 (0), 239 (+10.16), 228 (+6.46), 212 (+30.11).

3.14. Optical rotation and CD data for lintetralin (10)

$[\alpha]_D^{25}$ −7.0 (*c* = 1.00); UV λ_{max} (log ε) 224.8 (4.40, *sh*), 274.8 (3.92) nm; CD (*c* = 2.50 × 10^{-5} M) ($\Delta\varepsilon$) 308 (−1.39), 293 (+2.08), 280 (−2.03), 274 (−0.92), 269 (−1.40), 262 (0), 246 (−3.35), 226 (+1.43), 215 (−0.43), 205 (+10.51).

3.15. Optical rotation and CD data for isolintetralin (11)

$[\alpha]_D^{25}$ +10.0 (*c* = 1.00); UV λ_{max} (log ε) 226.4 (4.25, *sh*), 283.2 (3.85) nm; CD (*c* = 2.50 × 10^{-5} M) ($\Delta\varepsilon$) 313 (−0.62), 293 (+4.48), 280 (−3.03), 270 (−1.34), 254 (0), 248 (−2.51), 228 (+3.23), 213 (−4.60), 204 (+19.61).

3.16. Hydrogenolysis of virgatusin (13) under highly acidic conditions

The suspension of virgatusin (15.2 mg), EtOAc (3 ml), 7.2 N H_2SO_4 (0.3 ml) and 10%Pd/C (10.6 mg) after degassing was hydrogenated under a hydrogen balloon at room temp for 21 h. After a general work-up procedure, the crude products obtained (10.5 mg) were separated on a silica gel column, eluted by 20% EtOAc in petroleum ether to give lintetralin (**10**, 4.4 mg), R_f 0.38 [Me_2CO–toluene (1:19)] and isolintetralin (**11**, 2.0 mg), R_f 0.34 [Me_2CO–toluene (1:19)].

3.17. Optical rotation and CD data for heliobuphthalmin lactone (12)

$[\alpha]_D^{25}$ +38.0 (*c* = 1.00); UV λ_{max} (log ε) 226.4 (4.25, *sh*), 284.6 (3.95) nm; CD (*c* = 2.82 × 10^{-5} M) ($\Delta\varepsilon$) 298 (+0.79), 292 (+1.84), 287 (+0.95), 274 (+0.20), 268 (+0.93), 246 (+0.37), 236 (+2.43), 227 (+2.88), 220 (+1.75), 211 (+0.62), 204 (+9.46).

Acknowledgements

This work was supported by the National Science Council, R.O.C., under grant NSC-89-2320-B-002-271.

References

Afsharypuor, S., Mostajeran, A., Mokhtary, R., 1995. Variation of scopolamine and atropine in different parts of *Datura metel* during development. Planta Med. 61, 383–384.

Anjaneyulu, A.S.R., Rao, K.J., Row, L.R., Subrahmanyam, C., 1973. Crystalline constituents of *Euphorbiaceae*—XII. Isolation and structure elucidation of three new lignans from the leaves of *Phyllanthus niruri* Linn.. Tetrahedron 29, 1291–1298.

Carrier, D.J., Beek, T.A., Van Heijden, R., Van der Verpoorte, R., 1998. Distribution of gingkolides and terpenoid biosynthetic activity in *Gingko biloba*. Phytochemistry 48, 89–92.

Huang, Y.L., Chen, C.C., Hsu, F.L., Chen, C.F., 1996. A new lignan from *Phyllanthus virgatus*. J. Nat. Prod. 59, 520–521.

Huang, Y.L., Chen, C.C., Ou, J.C., 1992. Isolintetralin: a new lignan from *Phyllanthus niruri*. Planta Med. 58, 473–474.

Hulbert, P.B., Klyne, W., Scopes, P.M., 1981. Chiroptical studies. Part 100. Lignans. J. Chem. Res. 401–451 (Miniprint).

Jakupovic, J., Schuster, A., Bohlmann, F., King, R.M., Robinson, H., 1986. New lignan and isobutylamides from *Heliopsis buphthalmoides*. Planta Med. 18–20.

Lee, S.S., Chen, C.K., Chen, I.S., Chen, C.H., 1996. Chemical constituents from *Dehaasia triandra*. 1. Three new alkaloids, iso-

corydione, norisocorydione, and dehatriphine, from the leaves. J. Nat. Prod. 59, 55–58.

Mcdoniel, P.B., Cole, J.R., 1972. Antitumor activity of *Bursera schlech* Tendali: isolation and structure determination of two new lignans. J. Pharm. Sci. 61, 1992–1994.

Meixia, W., Haowie, C., Yanjun, L., Linmin, M., Goulong, Z., Kai, M., 1995. Herbs of the genus *Phyllanthus* in the treatment of chronic hepatitis B: observations with three preparations from different geographic sites. J. Lab. Chin. Med. 126, 350–352.

Mizuno, M., Iinuma, M., Tanaka, T., Sakakibara, N., Fujikawa, T., 1988. Flavonol glycosides in the roots of *Epimedium diphyllum*. Phytochemistry 27, 3645–3648.

Prakash, K.S., Satyan, K.S., Wahi, P., Singh, R.P., 1995. Comparative hepatoprotective activity of three *Phyllanthus* species, *P. urinaria*, *P. niruri* and *P. simplex*, on carbon tetrachloride induced liver injury in the rat. Phytother. Res. 9, 594–596.

Ram, V.J., Neumeyer, J.L., 1981. Aporphines. 35. Synthesis of (*R*)-(−)- and (*S*)-(+)-apomorphine from thebaine and (+)-bulbocapnine. J. Org. Chem. 46, 2830–2831.

Rao, G.S., Bramley, R., 1971. Hypophyllanthin. Tetrahedron Lett. 34, 3175–3178.

Rao, K.V.K., Murthy, K.S., 1968. Crystal structure of iodophyllanthin. Tetrahedron Lett. 4859–4860.

Richomne, P., Lavault, M., Jacquemin, H., Bruneton, J., 1984. Studies on *Hernandiaeae* VI. Lignans and alkaloids of *Hernandia guianensis*. Planta Med. 50, 20–22.

Row, L.R., Satyanarayana, P., 1967. Crystalline constituents of *Euphorbiaceae* VI. The synthesis and absolute configuration of phyllanthin. Tetrahedron 23, 1915–1918.

Satyan, K.S., Prakash, A., Singh, R.P., Srivastava, R.S., 1995. Phthalic acid bis-ester and other phyto-constituents of *Phyllanthus urinaria*. Planta Med. 61, 293–294.

Somanabandhu, A., Nitayangkura, S., Mahidol, C., Ruchirawat, S., Likhitwitayawuid, K., Shieh, H.L., Chai, H., Pezzuto, J.M., Cordell, G.A., 1993. ^{1}H- and ^{13}C- NMR assignments of phyllanthin and hypophyllanthin: lignans that enhance cytotoxic responses with cultured multi-drug resistant cells. J. Nat. Prod. 56, 233–239.

Stevenson, R., Williams, J.R., 1977. Concerning phyltetralin. Synthesis of lignan aryltetralin isomers. Tetrahedron 33, 2913–2917.

Ward, R.S., Satyanarayana, P., Row, L.R., Rao, B.V.G., 1979. The case for a revised structure for hypophyllanthin—an analysis of the ^{13}C N.M.R. spectra of aryltetralins. Tetrahedron Lett. 32, 3043–3046.

Zhou, S.W., Xu, C.F., Zhou, N., Huang, Y.P., Huang, L.Q., Chen, X.H., Hu, Y.M., Liao, Y.Q., 1997. Mechanism of protective action of *Phyllanthus urinaria* L. against injuries of liver cells. Zhong-guo Zhongyao Zazhi 22, 109–111.