



PHYTOCHEMISTRY OF *ILlicium dunnianum* AND THE SYSTEMATIC POSITION OF THE ILLICACEAE

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Key Word Index—*Illicium dunnianum*; Illiciaceae; chemotaxonomy; cladistics; evolution; phenylpropanoid; neolignan; cycloartane; *seco*-cycloartane.

Abstract—Detailed chemical investigation of a dichloromethane extract of *Illicium dunnianum* yielded six phenylpropanoids (two of which, 1-[(3-methylbut-2-enyl)oxy]-2-methoxy-4-(prop-1-en-3-ol)benzene and 1,2-(methylenedioxy)-4-(propan-1,2-diol)benzene, were novel), five known neolignans, three known cycloartane triterpenes and a novel ring-A cleaved cycloartane, 3,4-*seco*-(24*Z*)-cycloart-4(28),24-diene-3,26-dioic acid 3-methyl ester. The significance of these chemical findings is assessed in the context of the historical debate concerning the systematic position and phylogenetic relationships of the genus. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Illicium dunnianum Tutch. are shrubs or small trees, indigenous to southeast China [1]. The genus is comparatively primitive, and is generally classified in the Magnoliidae (*sensu* Takhtajan [2] and Cronquist [3]) or equivalent taxon. The evolutionary relationships of the genus have been the source of considerable discussion, although its affinities with the Magnoliales have long been recognized.

There are three previous reports concerning the chemistry of *I. dunnianum* [4–6] which have described the isolation of a number of unusual sesquiterpenes and neolignans. Several other members of the genus *Illicium* have also been the subject of chemical investigation, including *I. anisatum* L. (syn. *I. religiosum* Sieb. & Zucc.) [7–15], *I. arborescens* Hayata [16, 17], *I. floridanum* Ellis, *I. macranthum* A. C. Sm., *I. majus* Hook. f. & Thoms. [18–21], *I. manipurens* Watt ex King, *I. tashiroi* Maxim. [16, 17, 22–24] and *I. verum* Hook. f. [25]. Both the primary literature and reviews [26–28] indicate that *Illicium* is characterized by prenylated phenols, neolignans and sesquiterpenes (several common flavanoids and monoterpenes are also reported).

The systematic position of *Illicium* is elucidated in the present publication by comparing the distribution

of 30 phytochemical characters [26–28] among various plant genera that have been suggested as putative relatives (see Table 1). Thirteen plant genera are included in the study, viz.: *Drimys* J. R. Forst. & G. Forst. (Winteraceae), *Liriodendron* L. (Magnoliaceae), *Magnolia* L. (Magnoliaceae), *Michelia* L. (Magnoliaceae), *Nelumbo* Adans. (Nelumbonaceae), *Nuphar* Sm. (Nymphaeaceae), *Illicium* L. (Illiciaceae), *Kadsura* Kaempf. ex Juss. (Schisandraceae), *Schisandra* Michx. (Schisandraceae), *Canella* P. Browne (Canellaceae), *Warburgia* Engl. (Canellaceae), *Austrobaileya* C.T. White (Austrobaileaceae) and *Euptelea* Siebold & Zucc. (Eupteleaceae). Cladistic methods of data analysis [29] are used for phylogenetic reconstruction.

RESULTS

Extraction of the fresh leaves and twigs of *I. dunnianum* with methylene chloride followed by detailed chemical analysis, involving exhaustive column chromatography and HPLC separations, yielded 15 compounds of which three (**1**, **5** and **12**) were novel.

Compound **1** showed a molecular ion in the HREI mass spectrum at m/z 248.1416 corresponding to the molecular formula $C_{15}H_{20}O_3$. IR spectroscopy showed absorption in the hydroxyl region of the spectrum (3614 cm^{-1}) whilst $^{13}\text{C}/\text{DEPT}$ spectra confirmed the presence of 15 distinct carbon resonances with only 19 attached protons, indicating that **1** contained a

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Table 1. Binary data set* used in cladistic analysis

	Chemical class†																													
Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
<i>Calycanthus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Drimys</i>	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Liriodendron</i>	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
<i>Magnolia</i>	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1	1	0	0	0	1	1
<i>Michelia</i>	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1
<i>Nelumbo</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>Nuphar</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0
<i>Illicium</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Kadsura</i>	0	0	1	0	0	0	1	0	0	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
<i>Schisandra</i>	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Canella</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Warburgia</i>	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Austrobaileya</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Euptelea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* 0 = absence of a chemical class; 1 = presence, from literature reviewed.

† Chemical classes reviewed: (1) drimanes; (2) colorenones; (3) germacranes; (4) eudesmanes; (5) guaianes; (6) eremophilanes; (7) elemenes; (8) cadinanes; (9) chamigranes; (10) sesquicaranes; (11) prezaanenes; (12) lanostanes; (13) *seco*-lanostanes; (14) cycloartanes; (15) *seco*-cycloartanes; (16) oleananes; (17) phenylpropanoids; (18) 3,3'-neolignans; (19) 8'8'-lignans; (20) 8,3'-neolignans; (21) 8,8',2,2'-cyclooctane lignans; (22) 4-O-3'-neolignans; (23) triphenylneolignans; (24) phenolic amines; (25) taspines; (26) piperizidine alkaloids; (27) quinolizidine alkaloids; (28) thiospirane alkaloids; (29) benzyloquinoline precursors; (30) benzyloquinolines.

single hydroxyl group. The broad structure of **1**, consisting of a 1,2,4-substituted benzene ring with isoprenyloxy, methoxyl and prop-1-en-3-ol substituents was determined by consideration of PFG-HSQC spectra (which indicate ^{13}C and ^1H connected by a single bond), PFG-HMBC spectra (^{13}C and ^1H connected by two or three bonds) and ^1H - ^1H COSY spectra (^1H sharing J -coupling) (Table 2), although the order of these three substituents on the benzene ring was not completely defined. However, it was possible to unambiguously define the substitution pattern through application of a NOESY experiment (which defines ^1H atoms close in space) and consequently to arrive at full NMR assignments for **1** (Table 2).

The extract also contained related compounds 1-[(3-methylbut-2-enyl)oxy]-2-methoxy-4-(2-propenyl)benzene (**2**), dictagymnin (**3**) and methoxyeugenol (**4**) previously isolated from *I. anisatum* and *I. tashiroi* [11, 16], which were identified by comparison with spectroscopic data in the literature. Full NMR assignments for **3** were also made by the above method for the first time (Table 3), and previous assignments for quaternary carbons in **2** and **4** [16] are revised. The NMR data for **2-4** provided further evidence in confirming the structure of the novel compound **1**.

Compound **5** gave a molecular ion in the HREI mass spectrum corresponding to the molecular formula $\text{C}_{10}\text{H}_{12}\text{O}_4$. IR spectroscopy showed absorption in the hydroxyl region of the spectrum whilst $^{13}\text{C}/\text{DEPT}$ spectra confirmed the presence of 10 carbons with 10 directly attached protons, indicating that **5** contained

two hydroxyl groups. The full structure of **5** was determined from 2D NMR analysis as previously. HMBC correlations were sufficient to assign the structure unambiguously and were confirmed by the results of ^1H - ^1H COSY (in which δ 4.29 showed a correlation with δ 3.82, which in turn showed a correlation with δ 1.06). A comparison of the complete NMR assignments for 1,2-(methylenedioxy)-4-(2-propenyl)benzene (**6**) (Table 3), also present as a major component of the extract, provided further confirmation for the structure of the novel compound **5**.

The biphenyl neolignans magnolol (**7**), 2,2'-dihydroxy-3-methoxy-5,5'-di-(2-propenyl)biphenyl (**8**) and dehydrodieugenol (**9**) have all been reported previously as natural products [30-32]. The full NMR data for **8**, assigned by 2D NMR techniques as previously (Table 4) show that some previous assignments [30] were in error, and chemical shift values for **7** and **9** (also rigorously assigned) supported this conclusion. Triphenyl neolignans **10** and **11** were also isolated and have been reported previously as constituents of *I. dunnianum* [5, 6].

In addition to phenylpropanoids and neolignans, *I. dunnianum* also yielded a number of cycloartane triterpenoids. The novel *seco*-cycloartane methyl ester **12** gave a parent ion corresponding to the molecular formula $\text{C}_{31}\text{H}_{48}\text{O}_4$ in the HREI mass spectrum, and was completely identified by 2D NMR, as previously (see Fig. 1 for HMBC correlations). *seco*-Cycloartanes are comparatively rare, although several closely related structures are known from the genus *Kadsura*

Table 2. NMR data for compound **1** (CDCl₃)

Assigned	δ (¹³ C)	Mult. *	δ (¹ H)	PFG-HMBC correlation from ¹ H to ¹³ C	¹ H- ¹ H COSY correlation	NOESY correlation from ¹ H to ¹ H
1	148.5	C				
2	149.5	C				
3	109.0	CH	6.95	148.5, 119.6	6.90	6.55, 6.24, 3.89
4	137.7	C				
5	119.6	CH	6.90	148.5, 109.0	6.95, 6.82	6.55, 6.82, 6.24
6	112.9	CH	6.82	149.5, 129.7	6.90	6.90, 4.58
7	131.3	CH	6.55		6.24	6.95, 6.90, 4.31
8	126.48	CH	6.24	129.7, 63.9	6.55, 4.31	6.95, 6.90, 4.31
9	63.9	CH ₂	4.31		6.24, 1.40	6.55, 6.24, 3.89
1'	65.8	CH ₂	4.58	137.7, 119.9	5.52, 1.77, 1.73	6.82, 5.52, 3.89, 1.73
2'	119.9	CH	5.52		4.58, 1.77, 1.73	4.58, 1.77
3'	137.7	C				
4'	25.8	Me	1.77	137.7, 119.9, 18.3	5.52, 4.58	5.52
5'	18.3	Me	1.73	137.7, 119.9, 25.8	5.52, 4.58	4.58
2-OMe	55.8	Me	3.89	149.5		6.95, 4.58, 4.31
9-OH			1.40		4.31	

* Multiplicity determined from DEPT spectra

Table 3. ¹³C and ¹H NMR data for compounds **2–6** (CDCl₃)

Assignment	δ (¹³ C)					δ (¹ H)				
	2	3	4	5	6	2	3	4	5	6
1	146.6	157.3	133.0	145.8	145.8					
2	149.4	114.6	147.0	147.4	147.6		6.84			
3	112.1	129.5	105.1	107.1	109.1	6.70	7.09	6.41	6.86	6.67
4	132.7	132.0	131.1	135.0	133.9					
5	120.3	129.5	105.1	120.4	121.3	6.69	7.09	6.41	6.81	6.63
6	113.3	114.6	147.0	108.2	108.2	6.81	6.84		6.77	6.73
7	39.8	39.4	40.2	79.4	39.2	3.32	3.32	3.30	4.29	3.33
8	137.3	137.9	137.6	72.3	137.6	5.95	5.95	5.95	3.82	5.92
9	115.6	115.4	115.6	18.8	115.7	5.05	5.07	5.03	1.06	5.05
						5.07	5.08	5.05		5.07
1'	65.8	64.8				4.55	4.48			
2'	120.2	119.8				5.51	5.49			
3'	137.3	138.0								
4'	18.2	18.2				1.72	1.74			
5'	25.8	25.8				1.76	1.79			
2-OMe	55.8		56.3			3.85		3.87		
O-CH ₂ -O				101.1	100.8				5.96	5.92

[33–36]. Three known cycloartanes, schizandronic acid (**13**), schizandrolid acid (**14**) and magniferolic acid (**15**), were also present in the extract and were identified by comparison with the literature data [37–39]. Full assignments of ¹H and ¹³C data for these compounds were made for the first time by 2D NMR and provided support for the proposed structure of **12** (Table 5). The stereochemistry of **12** has been drawn as

for **13–15**, on the assumption that **12** is biogenetically derived from the cycloartane skeleton.

DISCUSSION

Table 1 details the presence or absence of the 30 phytochemical groups [26–28] among the selected plant genera. Cladistic analysis of these data resulted

Table 4. ^{13}C and ^1H NMR data for compounds 7–9 (CDCl_3)

Assignment	δ (^{13}C)			δ (1H)		
	7	8	9	7	8	9
1	151.3	151.9	140.9			
1'		139.9				
2	123.8	125.2	124.4			
2'		124.0				
3	131.2	130.9	123.1	7.08	7.10	6.75
3'		123.6			6.76	
4	133.1	132.5	131.9			
4'		132.9				
5	129.9	129.5	110.7	7.12	7.11	6.72
5'		110.4			6.75	
6	116.7	117.8	147.2	6.95	6.97	
6'		146.5				
7	39.4	39.5	40.0	3.36	3.37	3.36
7'		40.0			3.37	
8	137.5	137.8	137.7	5.95	6.00	6.00
8'		137.4			5.90	
9	115.8	115.5	115.7	5.06, 5.06	5.05, 5.05	5.00, 5.10
9'		116.0			5.00, 5.10	
6-OMe		—	56.1	—		3.92
6'OMe		56.2			3.95	

in the construction of 11 different but equally parsimonious cladograms. An example of one of these cladograms is presented in Fig. 2(a); this has a consistency index (CI) of 75 (indicating the level of homoplasy in the data) and a retention index (RI) of 71 (indicating the level of synapomorphy). Homoplasies are represented in this figure as evolutionary parallelisms for ease of presentation; each of the phytochemical characters used, however, represents the presence or absence of a biochemical pathway resulting from the interaction of numerous genes, and evolutionary reversal is therefore more probable than parallelism. The various tree topologies contain three consistent clades (*Illicium*–*Schisandra*–*Kadsura*, *Drimys*–*Warburgia*–*Canella* and *Liriodendron*–*Magnolia*–*Michelia*); topological variation among the 11 cladograms is therefore restricted to the relationships between the remaining in-group taxa. The strict consensus tree derived from these cladograms is presented in Fig. 2(b).

Illicium was classified in the family Magnoliaceae *sensu lato* in much of the early taxonomic literature [e.g. 40, 41]; this approach is rarely adopted today, although Law [42] is a significant recent exception. There is a limited degree of phytochemical similarity between *Illicium* and the Magnoliaceae, with the common possession of phenylpropanoids in *Illicium*, *Magnolia* and *Michelia* (character 17 in Fig. 2(a)), and neolignans (character 18 in Fig. 2(a)) in *Illicium* and *Magnolia*. The former character is of comparatively little chemotaxonomic significance, since phenylpropanoids are quite widespread in higher plants.

3,3'-Neolignans, however, are of much more restricted distribution and have only been consistently recorded from *Magnolia*.

Illicium was subsequently excluded from the Magnoliaceae by Smith [1] and Bailey and Nast [43] on the basis of floral morphology and vegetative anatomy, and assigned familial rank as the Illiciaceae. These authors furthermore suggested a close relationship with the family Schisandraceae (comprising two genera of woody lianes, *Schisandra* and *Kadsura*); this is commonly reflected today in the isolation of these two families as the order Illiciales *sensu* Takhtajan [2], Dahlgren [44] and Cronquist [3] or as Annonales suborder Illiciinae *sensu* Thorne [45]. The relationship with the Schisandraceae has received wide support, with taxonomic data derived from a broad range of sources including general morphology [46], palynology [47–49], embryology [50] and cytology [51]. To date, no phytochemical evidence has been published which corroborates this classification. The common possession of the cycloartanes (character 14) and *seco*-cycloartanes (character 15) in both *Illicium dunnianum* and the Schisandraceae (*Schisandra* and *Kadsura*) is therefore very significant (Fig. 2(a)). The newly described *seco*-cycloartane 12 is furthermore very closely related to kadsuric acid (described from *Kadsura coccinea* (Lem.) A. C. Sm. [52]) and changnanic acid (described from *K. longipedunculata* Finet & Gagnep. [33]). The suggestion by Gottlieb *et al.* [53] that the Illiciaceae and Schisandraceae both possess esters of angelic and tiglic acids is incorrect (we are unaware of any reports of such esters from *Illicium*) and there-

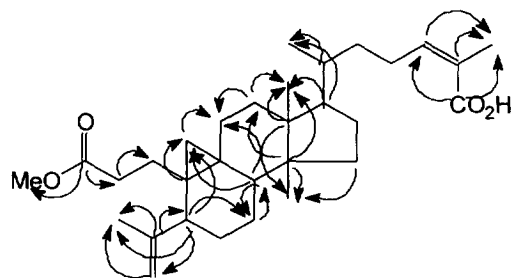
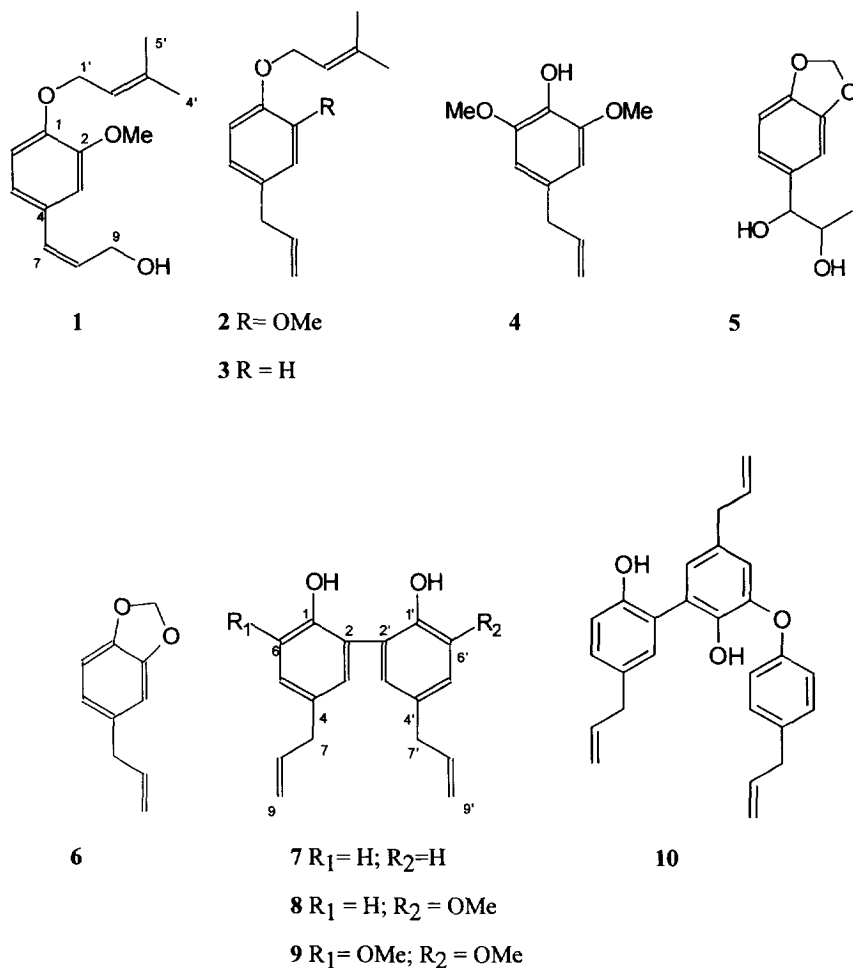


Fig. 1. HMBC correlations for compound **12** (arrows represent correlation from ^{13}C to ^1H).

fore does not provide any indication of a close affinity between the two families. The definition of lignan and neolignans adopted by Gottlieb *et al.* [53] was also not the currently accepted one. In this review, lignans and neolignans have been defined according to Whiting [54].

The Winteraceae have often been cited as putative relatives of the Illiciaceae, most recently in studies based on palynology [47–49] and general morphology [46]. There are significant anatomical differences

between the two families [55, 56], however, and this is reflected in the absence of the common possession of any of the 30 phytochemical groups studied (Table 1).

Wilson [57] suggested a relationship between the Illiciaceae and the families Canellaceae and Eupteleaceae on the basis of wood anatomy. This interpretation is not supported by the phytochemical data, with none of the chemical groups studied occurring in both *Illicium* and *Euptelea*, and only one (phenylpropanoids) co-occurring in *Illicium* and the Canellaceae (Table 1; Fig. 2(a)).

More recently, molecular data has been used to interpret phylogenetic relationships in the Magnoliidae. Qiu *et al.* [58] used nucleotide sequences of the plastic gene *rbcL* to conclude a close relationship with *Austrobaileya* (Austrobaileyaaceae) and a lesser relationship with the Nymphaeales (which includes the genera *Nuphar* and *Nelumbo*). Neither of these relationships is supported by the phytochemical data, with none of the investigated compounds held in common with *Illicium* (Table 1; Fig. 2(a)); *Nuphar* is shown to possess a very distinctive phytochemistry, with piperizidine, quiniolizidine and thiospirane alkaloids

Table 5. ^{13}C and ^1H NMR data for compounds 12–15 (CDCl_3)

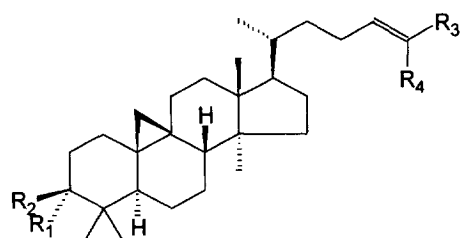
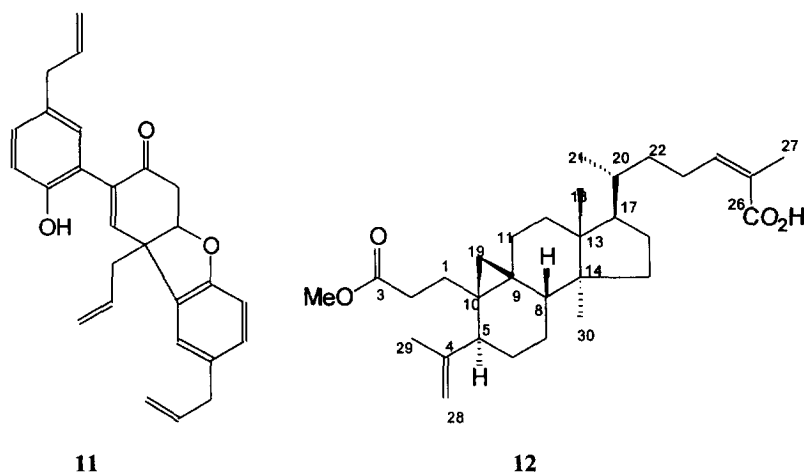
Assignment	δ (^{13}C)				δ (^1H)			
	12	13	14	15	12	13	14	15
1	29.0	33.4	27.5	27.5	2.05	1.90	1.88	1.88
					1.37	1.55	1.02	1.02
2	31.5	37.4	28.6	28.6	2.50	2.70	1.95	1.95
					2.25	2.30	1.65	1.65
3	174.5	216.7	77.1	77.1			3.48	3.48
4	149.5	50.3	39.6	39.6				
5	45.9	48.4	44.1	41.1	2.42	1.72	1.83	1.83
6	28.1	28.1	21.1	21.2	1.92	1.93	1.50	1.50
					1.29	1.33	0.79	0.77
7	25.0	21.5	25.7	25.7	1.30	1.13	1.30	1.60
					1.10	1.13	1.15	1.30
8	47.7	47.9	48.1	48.1	1.55	1.60	1.53	1.55
9	21.4	21.1	19.8	19.8				
10	27.1	26.0	26.5	26.5				
11	27.0	26.8	26.3	26.3	2.08	2.04	2.02	2.02
					1.25	1.45	1.15	1.15
12	33.0	32.8	32.9	32.9	1.65	1.68	1.62	1.62
					1.65	1.68	1.62	1.62
13	45.2	45.4	45.3	45.3				
14	49.0	48.8	48.9	49.0				
15	35.6	35.6	35.0	35.5	1.29	1.33	1.30	1.30
					1.29	1.33	1.30	1.30
16	27.8	25.9	28.2	28.2	1.52	1.40	1.29	1.29
					1.08	1.15	1.10	1.10
17	52.2	52.2	52.2	52.2	1.60	1.62	1.60	1.60
18	18.1	18.2	18.0	18.1	0.96	0.90	0.96	0.97
19	30.0	29.6	29.9	29.9	0.72	0.78	0.50	0.52
					0.40	0.58	0.34	0.35
20	36.1	36.0	36.1	36.0	1.41	1.43	1.42	1.43
21	18.1	18.1	18.1	18.0	0.89	0.90	0.89	0.91
22	35.9	35.8	35.9	34.8	1.52	1.55	1.55	1.58
					1.15	1.16	1.15	1.18
23	27.0	26.7	26.9	26.0	2.55	2.55	2.56	2.25
					2.45	2.45	2.45	2.12
24	147.4	147.2	146.9	145.8	6.09	6.09	6.06	6.89
25	125.7	125.7	125.9	126.6				
26	172.9	172.8	172.7	172.6				
27	20.6	20.6	19.3	12.0	1.92	1.92	1.92	1.87
28	111.5	20.8	25.9	25.9	4.80	1.05	0.95	0.95
					4.73			
29	19.8	22.2	21.3	21.3	1.68	1.10	0.88	0.89
30	19.3	19.3	19.3	19.3	0.93	0.91	0.90	0.90
3-OMe	51.6				3.65			

(characters 26–28) that are unique among the taxa studied. Hamby and Zimmer [59] used sequences from the 18S and 26S regions of ribosomal RNA, and suggested a close relationship with *Trochodendron* (Trochodendronaceae). *Trochodendron* has not received detailed phytochemical study, however, and consequently could not be incorporated in this study.

EXPERIMENTAL

General. Chemical shifts are expressed in ppm (δ) relative to TMS as int. standard. All NMR experi-

ments were run on a Bruker DRX 500 instrument with CDCl_3 as solvent. PFG-HSQC and PFG-HMBC experiments were normally recorded with 2048 data points in F_2 and 128 data points in F_1 . EIMS: (70 eV) (Finnigan-MAT 95 mass spectrometer); FTIR spectra were recorded in CCl_4 on a Shimadzu FTIR-8201 PC instrument. TLC plates were developed using *p*-anisaldehyde; HPLC: PREP-SIL 20 mm $\times$ 25 cm column, flow rate 8 ml min^{-1} . *Illicium dunnianum* (1 kg) was collected in November, whilst fruiting from Plover Cove Country Park, New Territories, Hong Kong. The sample was ground to a fine powder under



13 $R_1, R_2 = \text{=O}$; $R_3 = \text{Me}$; $R_4 = \text{CO}_2\text{H}$

14 $R_1 = \text{H}$; $R_2 = \text{OH}$; $R_3 = \text{Me}$; $R_4 = \text{CO}_2\text{H}$

15 $R_1 = \text{H}$; $R_2 = \text{OH}$; $R_3 = \text{CO}_2\text{H}$; $R_4 = \text{Me}$

liquid N_2 and immediately extracted with CH_2Cl_2 in a Soxhlet apparatus (8 hr). The organic extract was then dried and evapd under red. pres. to yield a dark green oil (21.96 g; 2.2% w/w) which was sepd chromatographically to yield **1** (4.9 mg), **2** (49.6 mg), **3** (15.6 mg), **4** (15.7 mg), **5** (7.2 mg), **6** (1 056 mg), **7** (19.5 mg), **8** (13.2 mg), **9** (13.4 mg), **10** (704 mg), **11** (47.3 mg), **12** (59.4 mg), **13** (9.8 mg), **14** (27.5 mg) and **15** (10.2 mg). A voucher specimen of *I. dunnianum* (GDBROWN 96/3) is deposited at the University of Hong Kong Herbarium (HKU).

Compound 1 (1-[(3-methylbut-2-enyl)oxy]-2-methoxy-4-(prop-1-en-3-yl)benzene). Oil. HREIMS m/z (rel. int.): 248.1416 ($[\text{M}]^+$ $\Delta -0.3$ mmu for $\text{C}_{15}\text{H}_{20}\text{O}_3$) (3), 180 (100), 137 (66), 124 (52); IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 3614, 2924, 2853, 1506, 1458, 1265, 1229; ^1H NMR: δ 6.95 (1H, *d*, $J = 2.0$ Hz), 6.90 (1H, *dd*, $J = 8.2, 2.0$ Hz), 6.82 (1H, *d*, $J = 8.2$ Hz), 6.55 (1H, *d*, $J = 15.8$ Hz), 6.24 (1H, *dt*, $J = 15.8, 6.0$ Hz), 5.52 (1H, *t*, $J = 6.4$ Hz), 4.58 (2H, *d*, $J = 6.4$ Hz), 4.31 (2H, *br d*, $J = 6.0$ Hz), 3.89 (3H, *s*), 1.77 (3H, *s*), 1.73 (3H, *s*), 1.40 (1H, *br s*).

Compound 5 (1,2-(methylenedioxy)-4-(propan-1,2-

diol)benzene). Oil, $[\alpha]_{\text{D}} = +12.9^\circ$ (*c*, 0.61, CHCl_3). HREIMS m/z (rel. int.): 196.0730 ($[\text{M}]^+$ $\Delta 0.5$ mmu for $\text{C}_{10}\text{H}_{12}\text{O}_4$) (25), 151 (100), 123 (9), 93 (20); IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 3410, 2930, 2856, 1734, 1504, 1487, 1442, 1248; ^1H NMR: δ 6.86 (1H, *d*, $J = 1.2$ Hz), 6.81 (1H, *dd*, $J = 8.0, 1.2$ Hz), 6.77 (1H, *d*, $J = 8.0$ Hz), 5.96 (2H, *s*), 4.29 (1H, *d*, $J = 7.6$ Hz), 3.82 (1H, *dq*, $J = 7.6, 6.3$ Hz), 2.56 (1H, *br s*), 2.44 (1H, *br s*), 1.06 (3H, *d*, $J = 6.3$ Hz).

Compound 12 (3,4-seco(24Z)-cycloart-4(28),24-diene-3,26-dioic acid 3-methyl ester). Oil, $[\alpha]_{\text{D}} = +43.3^\circ$ (*c* 2.56, CHCl_3). HREIMS m/z (rel. int.): 484.3554 ($[\text{M}]^+$ $\Delta -0.1$ mmu for $\text{C}_{31}\text{H}_{48}\text{O}_4$) (25), 469 (100), 451 (13), 385 (16), 343 (20), 316 (9), 249 (16); IR $\nu_{\text{max}}^{\text{CCl}_4}$ cm^{-1} : 2932, 2874, 1740, 1690, 1639, 1458, 1437, 1375, 1259, 1217, 1167; ^1H NMR: δ 6.09 (1H, *t*, $J = 7.0$ Hz), 4.80 (1H, *d*, $J = 1.4$ Hz), 4.73 (1H, *d*, $J = 1.4$ Hz), 3.65 (3H, *s*), 1.92 (3H, *d*, $J = 0.9$ Hz), 1.68 (3H, *s*), 0.96 (3H, *s*), 0.93 (3H, *s*), 0.89 (3H, *d*, $J = 6.4$ Hz), 0.72 (1H, *d*, $J = 4.4$ Hz), 0.40 (1H, *d*, $J = 4.4$ Hz).

Phylogenetic reconstruction. Parsimony cladistic methods [29] were employed for the phylogenetic

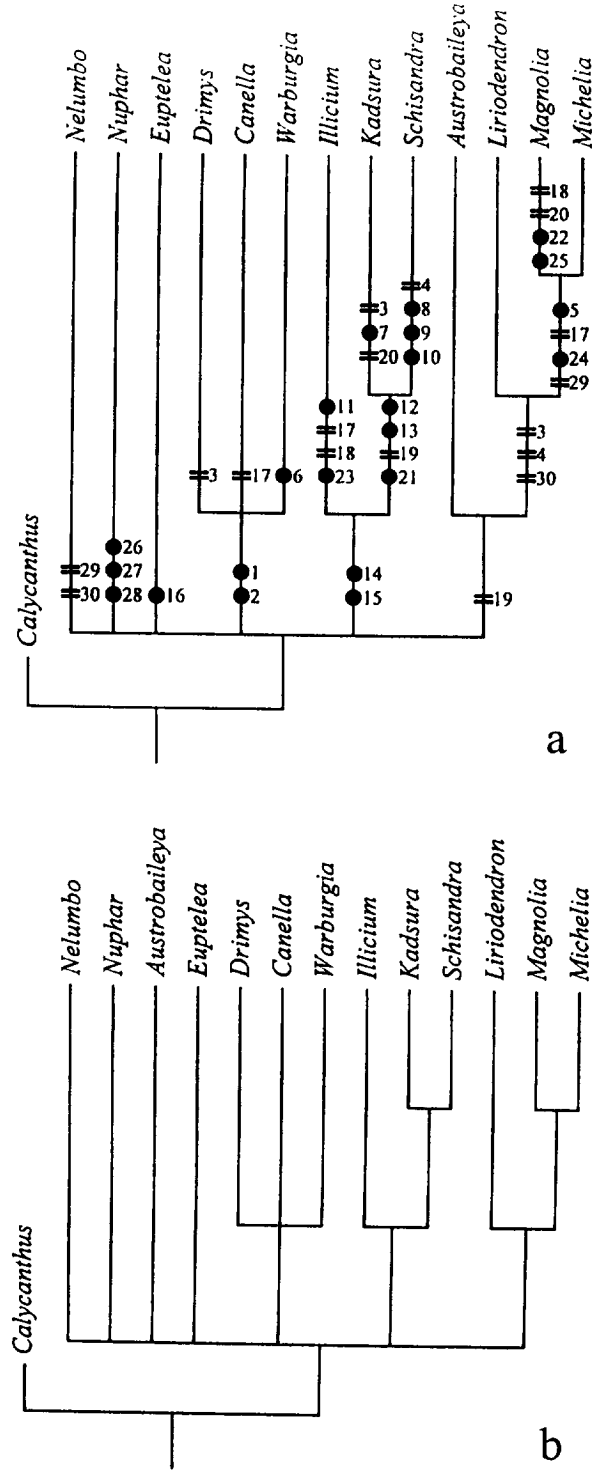


Fig. 2. (a) One of 11 equally parsimonious cladograms, illustrating character distribution (indicated by numbers, cf. Table 1); (b) strict consensus tree, derived from 11 equally parsimonious cladograms. (●) Apomorphy; (=) evolutionary parallelism.

analysis of data, using *Hennig86* software [60]. The genus *Calycanthus* L. (Calycanthaceae) was included as an out-group taxon for comparative purposes, based on its relative position in recent cladograms [46]. Cladograms were constructed using the implicit enumeration (IE) option in *Hennig86*, with the strict consensus tree computed by the *Nelsen* option.

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