Phytochemistry, Vol. 21, No. 11, 2719–2723, 1982.
Printed in Great Britain.

0031-9422/82/112719-05$03.00/0

HORSFIELDIN, A LIGNAN AND OTHER CONSTITUENTS FROM *HORSFIELDIA IRYAGHEDHI**

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(*Received* 11 *March* 1982)

Key Word Index—*Horsfieldia iryaghedhi*; Myristicaceae; seeds; horsfieldin; *d*-asarinin; (−)-dihydrocubebin; dodecanoylphloroglucinol; myristic acid; trimyristin; sitosterol; structure elucidation; absolute configuration; chemotaxonomy.

Abstract—A new lignan, horsfieldin [2-(3-hydroxy-4-methoxyphenyl)-6-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3,3,0)octane], *d*-asarinin, (−)-dihydrocubebin, dodecanoylphloroglucinol, myristic acid, trimyristin and sitosterol have been isolated and characterized from the hot methanol extract of *Horsfieldia iryaghedhi* seeds. The absolute configuration of the lignans and the chemotaxonomic significance of their occurrence is discussed.

INTRODUCTION

Horsfieldia iryaghedhi Warb. (= *Myristica horsfieldia*, *M. Iryaghedhi*; *Sinhala*, Iryaghedhi, Ruk), which is indigenous to Sri Lanka is a large tree with a tall straight trunk and numerous long drooping branches; the seeds are *ca* 3.5 cm long and of an oblong shape[1]. In a previous chemical investigation of the seeds of *H. iryaghedhi*, the occurrence of *d*-asarinin (**1**), dodecanoylphloroglucinol (**5**) and several unidentified constituents have been described [2]. A recent report from our laboratories dealt with the characterization of **1**, **5** and an antimicrobial agent, (−)-dihydrocubebin (**4**) from the bark, leaf and timber extractives of this plant[3]. As a consequence of our interest in the medicinal and related plants of Sri Lanka we studied the hot methanol extract of *H. iryaghedhi* seeds and herein we report the isolation of a new lignan, horsfieldin and the elucidation of its structure as **2**. In addition, we have also isolated *d*-asarinin (**1**), (−)-dihydrocubebin (**4**), dodecanoylphloroglucinol (**5**), sitosterol, myristic acid and trimyristin. Although the biological activity of horsfieldin has not been evaluated in the present study it is noteworthy that tetrahydrofuran lignans have been shown to possess insecticidal [4] and some medicinal [5, 6] properties.

RESULTS AND DISCUSSION

The hot methanol extract of the seeds of *H. iryaghedhi* on concentration precipitated trimyristin, whose identity was confirmed by comparison with a synthetic sample [4]. The mother liquor after concentration was extracted successively and exhaustively with hot petrol, hot benzene, hot chloroform, hot ethyl acetate and hot methanol. From the hot petrol extract myristic acid was isolated. The hot benzene extract on CC and prep. TLC separation afforded *d*-asarinin (**1**), sitosterol and (−)-dihydrocubebin (**4**). Purification of the hot chloroform extract by CC and prep. TLC gave dodecanoylphloroglucinol (**5**) and a new lignan named horsfieldin which was identified as 2-(3-hydroxy-4-methoxyphenyl)-6-(3, 4-methylenedioxyphenyl)-3,7-dioxabicyclo(3,3,0)octane (**2**) by the evidence presented below.

The ^{1}H NMR spectrum of horsfieldin indicated it to be a lignan and the IR spectrum showed the presence of a hydroxyl group. The phenolic nature was confirmed by a positive ferric chloride test (green colouration); a positive Gibbs test[5] indicated that the position *para* to the hydroxyl in the aromatic ring was free. The ^{1}H NMR spectrum (see Table 1) also showed the presence of –OMe and $-OCH_2O-$ substituents in the aromatic rings. In the mass spectrum the M^+ was found at *m/z* 356 and this gave the molecular formula $C_{20}H_{20}O_6$ for the lignan. The spectrum also had fragments typical of a lignan with nonidentical aromatic rings, one with a $-OCH_2O-$ substituent and the other with hydroxyl and methoxyl substituents (Table 2). Comparison of the ^{13}C NMR spectrum of horsfieldin with that of *d*-asarinin (**1**) (Table 3) confirmed that they have similar oxygenation patterns. The hydroxyl group in the aromatic ring was located at C-3″, since horsfieldin gave a positive Gibbs test (see above). Methylation of

*Part 8 in the series "Studies on Medicinal and Related Plants of Sri Lanka". For Part 7 see, Gunatilaka, A. A. L., Sultanbawa, M. U. S., Surendrakumar, S. and Somanathan, R. *J. Chem. Soc. Perkin Trans.* 1 (submitted).

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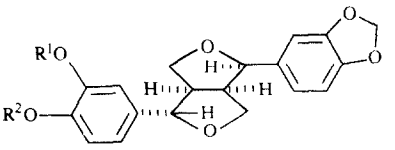

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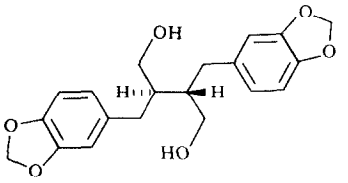

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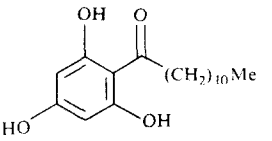

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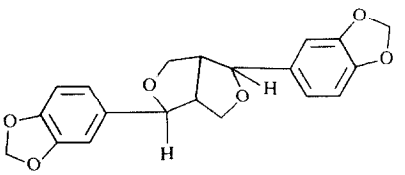

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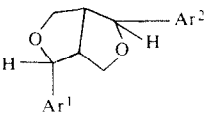
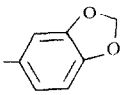
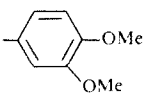
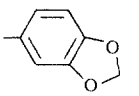


7 $Ar^1 = Ar^2 =$ 

8 $Ar^1 =$  , $Ar^2 =$ 

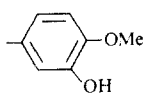
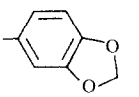
9 $Ar^1 =$  , $Ar^2 =$ 

Table 1. ^{1}H NMR data of lignans **1–3** [δ/$CDCl_3$ (TMS)]

Lignan	C-1, C-4	C-3a, C-6a	C-3, C-6	OCH_2O	OMe	ArH
1	4.80(1H, *d*)	3.35(1H, *m*)	4.03(2H, *m*)	5.90(4H, *s*)	—	
	4.40(1H, *d*)	2.85 (1H, *m*)	3.85 (1H, *m*)			6.80, 6.75 (6H, *m*)
			3.35(1H, *m*)			
2	4.93(1H, *d*)	3.35(1H, *m*)	4.03(2H, *m*)	5.96(2H, *s*)	3.93(3H, *s*)	
	4.53(1H, *d*)	2.87(1H, *m*)	3.93(1H, *m*)			7.00, 6.83 (6H, *m*)
			3.35(1H, *m*)			
3	4.88(1H, *d*)	3.35(1H, *m*)	4.17(2H, *m*)	5.95(2H, *s*)	3.90(6H, *s*)	
	4.45(1H, *d*)	2.90(1H, *m*)	3.80(1H, *m*)			6.96, 6.85 (6H, *m*)
			3.35(1H, *m*)			

Table 2. Typical fragments (*m/z*) in the mass spectra of lignans **1–3**

Lignan	Ar	M^+	$ArCH_2^+$	$ArCHO^{+\cdot}$	$ArC{\equiv}O^+$	$ArCH{=}\overset{+}{C}HCH_2$	$ArCH{=}\overset{+}{O}H$	ArH^+	Ar^+
1	Ar^1	354	135	150	149	161	—	122	121
2	Ar^1		135	150	149	161	151	—	121
		356							
	Ar^2		137	152	151	163	153	124	123
3	Ar^1		135	150	149	161	151	122	121
		370							
	Ar^3		151	166	165	177	167	138	137

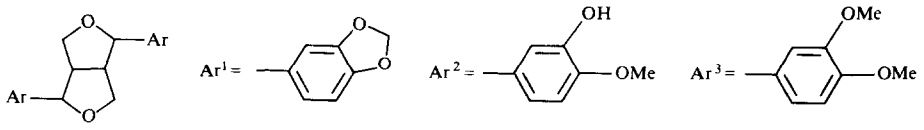


Table 3. ^{13}C NMR chemical shifts in *d*-asarinin (**1**) and horsfieldin (**2**) [δ/(multiplicity)$CDCl_3$ (TMS)]

Carbon(s)	1	2
1, 4	82.0(*d*), 87.6(*d*)	82.1(*d*), 87.7(*d*)
3, 6	50.1(*t*), 54.6(*t*)	50.2(*t*), 54.6(*t*)
3a, 6a	69.6(*d*), 70.9(*d*)	69.7(*d*), 71.1(*d*)
1′, 1″	132.3(*s*), 135.3(*s*)	130.4(*s*), 135.3(*s*)
2′, 2″	108.1(*d*)	108.5(*d*), 114.4(*d*)
3′, 3″	146.5(*s*), 147.2(*s*)	144.8(*s*), 147.2(*s*)
4′, 4″	147.6(*s*), 147.9(*s*)	146.5(*s*), 148.0(*s*)
5′, 5″	118.7(*d*), 119.5(*d*)	118.5(*d*), 119.5(*d*)
6′, 6″	106.3(*d*), 106.5(*d*)	106.5(*d*), 108.2(*d*)
OCH_2O	101.0(*t*)	101.0(*t*)
OMe	—	56.0(*q*)

horsfieldin yielded the monomethyl derivative which was shown to be identical with fargesin (**3**) reported previously from the flower buds of *Magnolia fargesii* [7].

The absolute configuration of asarinin has been a matter of controversy [8–10]. According to Freudenberg and Sidhu [8] all (+)-sesamin type lignans belong to the same series with the absolute configuration *R* at the bridge carbons C-3a and C-6a. The absolute configuration of (+)-sesamin has been determined as in **6** by ^1H NMR studies [10], and this was confirmed recently by application of ^1H NMR lanthanide-induced shift (LIS) techniques [11]. Since acid isomerization of (+)-sesamin **6** gives *d*-asarinin (**1**) [12] the absolute configuration of the latter can be represented as in **7**. Absolute configuration of fargesin (**3**) has been determined to be as in **8** by application of the ^1H NMR LIS technique [11]. Thus the absolute configuration of horsfieldin (**2**) can be represented as in **9**.

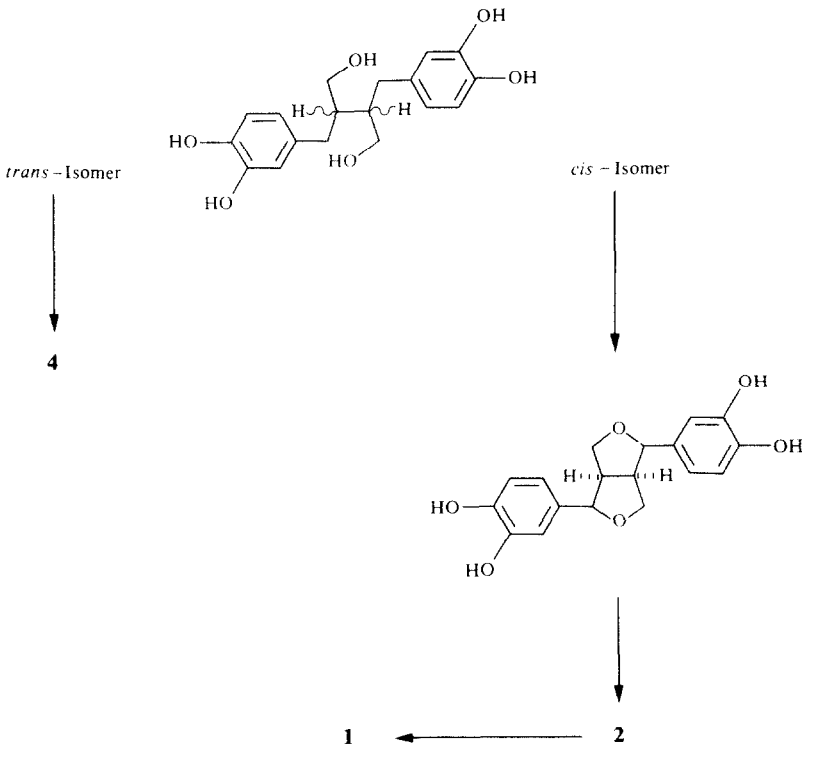
The occurrence of myristic acid and trimyristin in a plant belonging to the Myristicaceae is not surprising and they may serve as chemotaxonomic markers at the family level. The presence of *l*-asarinin has previously been reported from two *Asarum* species (Aristolochiaceae) [13, 14] and two *Xanthoxylum* species (Rutaceae) [15, 16] whereas *d*-asarinin has been previously found to occur only in *Acronychia muelleri* (Rutaceae) [12]. (−)-Dihydrocubebin has been encountered twice before in nature in *Piper guineense* (Piperaceae) [17, 18] and in *Cleistanthus collinus* (Euphorbiaceae) [19]. The co-occurrence of horsfieldin and *d*-asarinin in *H. iryaghedhi* is significant as the former could be considered to be the biosynthetic precursor of the latter lignan. A possible biosynthetic relationship between (−)-dihydrocubebin (**4**), horsfieldin (**2**) and *d*-asarinin (**1**) is presented in Scheme 1.

EXPERIMENTAL

General procedures. Mps are uncorr. UV spectra were recorded in EtOH, IR spectra in KBr discs and ^1H NMR spectra in $CDCl_3$ at 60 MHz. ^{13}C NMR spectra were measured in $CDCl_3$ at 50.11 MHz. Petrol refers to the fraction of bp 60–80°. Si gel (Merck) plates (0.25 mm) were used for TLC; for prep. TLC these were 1 mm thick.

Extraction. Air-dried seeds of *H. iryaghedhi* (9 kg) collected at Kanneliya were exhaustively extracted with hot MeOH. This extract on concn yielded an off-white solid (9 g, 0.1%). The mother liquor was evaporated *in vacuo* to afford a dark-brown solid (2.7 kg, 30%) which was re-extracted with hot petrol, hot C_6H_6, hot $CHCl_3$ and hot EtOAc. 100 g of the methanolic extract yielded the following extracts; hot petrol (7 g, 2.1%), hot C_6H_6 (10.5 g, 3.05%), hot $CHCl_3$ (20 g, 6.0%), hot EtOAc (30 g, 9.0%) and insoluble (20.7 g, 6.21%).

Isolation of trimyristin. TLC of the off-white solid obtained on concn of the MeOH extract indicated it to consist essentially of a single compound. This solid was recrystallized several times from hot MeOH to yield trimyristin as a white crystalline solid (4.71 g, 0.06%), mp 54–55°, lit. 56.5–57° [20]. IR ν_{max}^{KBr} cm^{-1}: 2900, 1740 (ester C=O) and 1460; ^1H NMR: δ 5.36–5.07 (1H, *m*, $\underset{\text{C–O}}{\overset{\text{C–O}}{\underset{|}{\overset{|}{\text{H–C}}}}}$–O), 4.33–4.13 (4H, *q*, $\underset{\text{CHO}}{\overset{\text{CH}_2\text{O}}{\underset{|}{\overset{|}{\text{CHO}}}}}$), 2.40–2.17 (6H, *t*, 3 × $COCH_2$), 1.27 (30H, *m*, CH_2 envelope) and 0.87 (9H, *t*, 3 × Me).



Scheme 1. Possible biosynthetic relationship between (−)-dihydrocubebin (**4**), horsfieldin (**2**), and *d*-asarinin, (**1**).

Synthesis of trimyristin [21]. Myristic acid (3 g) was dissolved in dry C_6H_5N (25 ml), benzenesulphonyl chloride (0.80 g) was added and the mixture heated under reflux. After 3 hr glycerol (10 g) in C_6H_5N (15 ml) was added and heating continued for a further 1 hr (TLC control). Work-up by usual procedures afforded trimyristin (3.6 g, 90%) as a colourless crystalline solid, mp 54–56° (from MeOH) which was found to be identical (mmp, co-TLC and IR) with the natural sample.

Isolation of myristic acid. The hot petrol extract (3 g) was subjected to CC on Si gel. Elution with petrol gave a further small quantity (0.8 g, 0.00003%) of trimyristin. The major compound (0.35 g, 0.00012%) eluted with 25% C_6H_6 in petrol was identified as myristic acid, mp and mmp 45–46°.

Isolation of d-*asarinin* (**1**). The hot C_6H_6 extract (4 g) was chromatographed over Si gel and eluted with 75% C_6H_6 in petrol. The first few fractions on evaporation gave *d*-asarinin (0.5 g, 0.00014%), mp 120–122°, $(\alpha)_D$ + 120°, lit. [22] mp 122–123°, $(\alpha)_D$ + 118.6°. For MS, ^1H and ^{13}C NMR data, see Tables 1–3.

Isolation of sitosterol. Elution of the above column with C_6H_6 afforded the next polar compound contaminated with *d*-asarinin. Purification by prep. TLC gave sitosterol as white prisms (0.037 g, 0.00001%), mp 137–139°, $(\alpha)_D$ − 36° ($CHCl_3$), lit. [23] mp 138–139°, $(\alpha)_D$ − 35.0° whose identity was confirmed by comparison with an authentic sample (mmp and co-TLC).

Isolation of (−)-dihydrocubebin (**4**). Elution of the column with $CHCl_3$ afforded crude **4** which was further purified by prep. TLC (eluent: $CHCl_3$–MeOH, 95:5) to give off-white crystals (0.015 g, 0.000004%), mp and mmp 100–101° lit. [3] mp 101–102°, $(\alpha)_D$ − 37°.

Isolation of horsfieldin (**2**). The $CHCl_3$ extract (13 g) was chromatographed over 250 g of Si gel. Elution with 50% $CHCl_3$ in petrol gave further quantities of *d*-asarinin (**1**) (1.59 g, 0.0003%) and (−)-dihydrocubebin (**4**) (0.04 g, 0.000007%). Elution with 75% $CHCl_3$ in petrol gave crude **2** which was purified by prep. TLC ($CHCl_3$–MeOH, 49:1) to afford colourless crystals (0.11 g, 0.00002%) mp 164–165°, $(\alpha)_D$ + 38.0° ($CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 3454 (OH), 2830, 1600, 1450, 1440, 1235, 1225, 1215, 1070, 1030, 960, 930, 855, 820, 775, 760, and 735; for MS, ^1H and ^{13}C NMR data, see Tables 1–3.

Methylation of **2** *to give fargesin* (**3**). Methylation of **2** (65 mg) with excess CH_2N_2–Et_2O gave the monomethyl derivative **3** (56 mg, 86.0%) as a colourless crystalline solid, mp 137–139°, $(\alpha)_D$ + 119° ($CHCl_3$) lit. [7] mp 133–134°, $(\alpha)_D$ + 121°; IR ν_{max}^{KBr} cm^{-1}: 1605, 1590, 1505, 1240, 1020, 920, 860 and 810; for MS and ^1H NMR data, see Tables 1 and 2. The methylated product was shown to be identical (mp, mmp, co-TLC, and IR) with an authentic sample of fargesin [7].

Isolation of dodecanoylphloroglucinol (**5**). Further elution of the column with $CHCl_3$ afforded a mixture of compounds. The major compound was purified by prep. TLC ($CHCl_3$–MeOH, 9:1) to give dodecanoylphloroglucinol (**5**) (1.95 g, 0.0003%) as a colourless crystalline solid, mp 128–130°, lit. [2] 125–126°. Its identity was further confirmed by comparison (mmp, co-TLC and co-IR) with an authentic sample [3].

Acknowledgements—We thank Professor S. Balasubramaniam (Department of Botany, University of Peradeniya) for identification of plant material; Professor Carl Djerassi (Stanford University) and Professor R. H. Thomson (University of Aberdeen) for mass spectral data and Dr. R. Somanathan (University of California, Davis) for ^{13}C NMR data; Prof. H. Kakisawa (Tokyo Kyoiku University) for an authentic sample of fargesin; Messrs. Z. A. M.

Faizal, V. S. B. Imbuldeniya, D. B. Egodawela and P. Liyanage for technical assistance; Mrs. S. C. Weerasekera for typing the manuscript. Financial assistance from the National Science Council (Sri Lanka) and International Foundation for Science (Sweden) is gratefully acknowledged.

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