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CHEMICAL CONSTITUENTS OF THREE RUTACEAE SPECIES FROM SRI LANKA

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ABSTRACT.—The stem bark of *Luvunga angustifolia* yielded a new acridone alkaloid, 5-methoxyarborinine [**1**], and several known compounds. Several known compounds were also isolated from *Limonia acidissima*. *Pleiospermium alatum* afforded a rare coumarin glycoside, apiosylskimmin [**5**], and two known limonoids **6** and **7**. Nmr studies of **6** demonstrated strong nOe effects due to "through-space" coupling and led to revision of some previous carbon signal assignments. A probable biosynthetic relationship between some limonoids of the Rutaceae is suggested.

The family Rutaceae comprises about 150 genera and over 1600 plant species (1). In Sri Lanka, the Rutaceae is represented by 44 species distributed in 20 genera (2); the genera *Luvunga, Limonia (Feronia)*, and *Pleiospermium* each contain a single species, viz. *Luvunga angustifolia* (Oliv.) Tan.(=*Luvunga eleutheranthera*), *Limonia acidissima* (L.) Swingle (=*Feronia limonia; Feronia elephantum*), and *Pleiospermium alatum* (Wight & Arn.) Swingle (=*Hesperethusa alata*). *Lu. angustifolia* is endemic to Sri Lanka, and *Li. acidissima* and *P. alatum* are used in the indigenous system of medicine in Sri Lanka (3,4). In this paper we report the isolation and structure elucidation of several metabolites new to these three species. There is no reported work on *Lu. angustifolia*, whereas previous studies on *Li. acidissima* have resulted in the isolation of coumarins (5–12), alkaloids (12), a benzoquinone (5), flavone glycosides (10,13), sterols (9,11,12), and triterpenoids (11,13); *P. alatum* has yielded alkamides (14,15), coumarins (16,17), acridone alkaloids (17,18), stigmasterol (17), and lupeol (16,17).

The hot petroleum ether extract of the stem of *Lu. angustifolia* on chromatographic fractionation afforded (in the order of increasing polarity) lupeol, stigmasterol, suberosin, a new acridone alkaloid identified as 5-methoxyarborinine [**1**], 5-hydroxyarborinine [**2**], and ostruthin. The uv and ir spectra of the new natural product were typical of a 9-acridone. It remained unchanged on treatment with CH_2N_2, and hence the OH group discernible in the ^{1}H-nmr spectrum (D_2O exchangeable 1H singlet at δ 14.03) was placed at C-1. The ^{1}H-nmr spectrum also indicated the presence of three OMe and one NMe groups. A singlet at δ 6.37 was assignable to H-4. Remaining signals appearing in the aromatic region showed an ABX pattern. Methylation of 5-hydroxyarborinine [**2**] occurring in the same extract (see later) with CH_2N_2 gave 5-methoxyarborinine [**1**], identical with the new alkaloid. The physical data of the more polar alkaloid compared well with those reported (17,18) for 5-hydroxyarborinine [**2**]. This is the first report of the natural occurrence of **1**; **2** has previously been isolated from three other Sri Lankan Rutaceae species, *Glycosmis bilocularis* (19), *P. alatum* (17,18), and *Atalantia monophylla* (20).

Occurrence of the coumarins bergapten, psoralen, xanthotoxin, and osthenol (5) and a new limonoid, acidissimin [**8**] (21) [=jangomolide (22)] in the root bark, and bergapten, psoralen, and the limonoid obacunone [**11**] (21) in the stem bark of *Li.*

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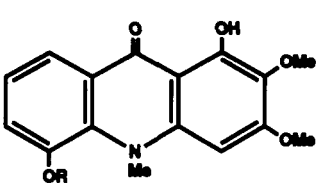

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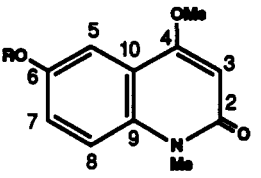

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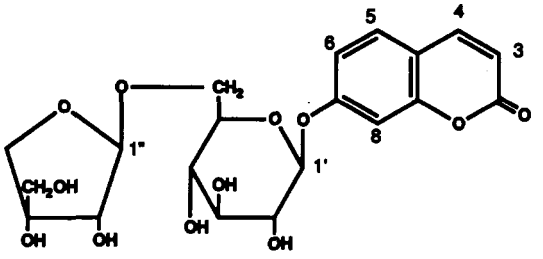

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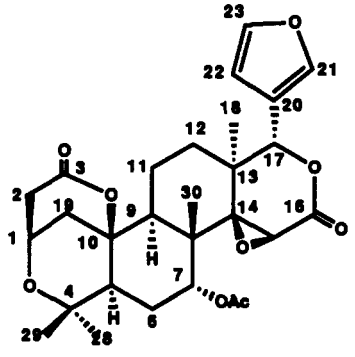

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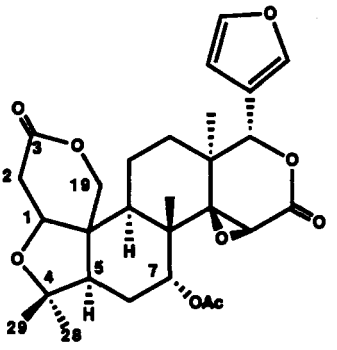

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acidissima were reported earlier. The current study of the root bark afforded osthol, aurapten, stigmasterol, isopimpinellin, and integriquinolone [**3**], while the stem bark yielded lupeol, (+)-marmesin, bergapten, and psoralen.

Chromatographic fractionation of the petroleum ether extract of the root bark of *Li. acidissima* gave (in the order of increasing polarity) osthol, aurepten, stigmasterol, and isopimpinellin. The CH_2Cl_2 extract of the root bark of *Li. acidissima* when subjected to cc fractionation afforded further quantities of aurepten and isopimpinellin. Elution of the column with 1% MeOH in $CHCl_3$ followed by preparative tlc (5% MeOH in $CHCl_3$) afforded an off-white crystalline solid, mp 255–258°, spectral data of which indicated it to be an *N*-methylquinolone with an OH and an MeO substitutent. Acetylation gave the monoacetate, mp 170–172°. Detailed ^1H- and ^{13}C-nmr spectral analysis of the parent alkaloid along with nOe studies suggested it to be integriquinolone [**3**]. In the nOe difference spectra, irradiation of the proton signals at δ 3.51 (NMe) and 3.91 (OMe) enhanced the intensities of the signals at δ 7.33 (H-8) and at δ 5.97 (H-3), respectively, while irradiation at δ 9.52 (OH) increased the intensities of the signals at

δ 7.10 (H-7) and 7.23 (H-5). Comparison with an authentic sample (23) confirmed its identity. Integriquinolone has previously been encountered in another Rutaceous plant, *Xanthoxylum integrifolium* (23). The petroleum ether extract of the stem bark of *Li. acidissima* afforded lupeol and (+)-marmesin, whereas the CH_2Cl_2 extract yielded psoralen, bergapten, and (+)-marmesin.

Our previous work on the root bark of *P. alatum* revealed the presence of 5 acridone alkaloids, 5 coumarins, lupeol, and stigmasterol (17). Present study has resulted in the isolation of two limonoids, 7α-limonyl acetate [**7**] and 1-(10↛19)*abeo*-7α-acetoxy-10β-hydroxyiso-obacunoic acid-3,10-lactone [**6**] from the hexane extract and a rare coumarin glycoside, apiosylskimmin [**5**], from the EtOAc extract of the root bark of *P. alatum*.

Chromatographic fractionation of the hexane extract yielded two crystalline solids with mp 292–294° (less polar solid) and 267–269° (more polar solid). Hrms analysis suggested the molecular formula $C_{28}H_{34}O_9$ for both compounds. ^1H- and ^{13}C-nmr spectra of the two compounds were completely analyzed with the aid of their ^1H-^1H, ^1H-^{13}C, and long-range ^1H-^{13}C COSY spectra. These data along with their uv and ir spectra suggested them to be limonoids, and they were identified as the lactone **6** and 7α-limonyl acetate [**7**] by comparison of our spectral data with those reported (24). ^1H- and ^{13}C-nmr spectral assignments for the two limonoids **6** and **7** are given in Tables 1 and 2. It is noteworthy that the ^1H-^1H COSY spectrum of **6** showed significant cross

TABLE 1. High-field ^1H-nmr Chemical Shifts for the Limonoids **6** and **7**.[a]

Proton	Limonoid					
	6			**7**		
H-1	4.41	td	(4.5, 1)	4.04	br d	(4)
H-2	2.65[b]	dd	(19, 5.5)	2.72	dd	(13, 6.5)
	2.84[e,f]	br d	(19)	2.93	dd	(16.5, 4)
H-5	1.88	m		2.25	dd	(13.5, 3)
Hα-6	1.89	m		1.90	dt	(15, 3)
Hβ-6	1.78	br d	(2.5)	1.80	ddd	(15, 13.5, 3)
H-7	4.55	d	(2.5)	4.56	t	(3)
H-9	2.47[c,d]	dd	(12, 7)	2.72	dd	(13, 6.5)
Hα-11	1.93	dtd	(12, 7, 1.5)	1.795	dddd	(13, 10.5, 7.5, 6.5)
Hβ-11	1.83	tt	(12, 9.5)	1.95	tdd	(13, 10, 2.5)
Hα-12	1.56	ddd	(13, 9.5, 1.5)	1.60	ddd	(13.5, 10.5, 2.5)
Hβ-12	1.74[c]	ddd	(13, 9.5, 7)	1.70	ddd	(13.5, 10, 7.5)
H-15	3.54	s		3.50	s	
H-17	5.65	s		5.59[d]	s	
H-19	1.75[e]	ddd	(13, 4.5, 2.5)	4.41	d	(13)
H-19	1.97[f]	br d	(13)	4.48	d	(13)
H-21	7.423	d	(1)	7.41	d	(1.2)
H-22	6.34	dd	(1.5, 1)	6.33	t	(1.2)
H-23	7.420	d	(1.5)	7.41	d	(1.2)
H_3-18	1.20	s		1.29	s	
H_3-28	1.19[g]	s		1.12	s	
H_3-29	1.16[g]	s		1.24	s	
H_3-30	1.40	s		0.95	s	

[a]Spectra were recorded in $CDCl_3$ at 400 MHz; chemical shifts are reported in ppm relative to TMS; figures in parentheses are coupling constants in Hz.

[b,c,d]Long-range coupling was observed with H-29, H-18, and H-30, respectively, in the ^1H-^1H COSY.

[e,f,g]Long-range coupling was observed between each other in the ^1H-^1H COSY.

TABLE 2. ^{13}C-nmr Data for the Limonoids **6** and **7**.[a]

Carbon	Compound							
	6				**7**			
	H/C long-range correlation				H/C long-range correlation			
	δ		^3JCH	^2JCH	δ		^3JCH	^2JCH
C-1 . . .	64.9	d		2,19α,β	80.0	d		2,19
C-2 . . .	38.2	t	19		35.7	t		
C-3 . . .	169.6	s	1	2α,β	169.4	s	1,19	2α,β
C-4 . . .	73.0[b]	s	1,6α	28,29	80.7	s		5,28,29
C-5 . . .	45.2	d	7,19,28,29		53.6	d	7,19α,β,28,29	6α,β
C-6 . . .	24.0[b]	t			23.5	t		5
C-7 . . .	72.5	d	30		73.2	d	30	6α
C-8 . . .	41.3	s	6β,7,11β	9,30	42.9	s	6α	30
C-9 . . .	39.6	d	7,12α,30	11α	43.3	d	5,7,12α,19,20	11β
C-10 . . .	80.7[b]	s	6α,β	1,19α,β	45.5	s	2,6α	5
C-11 . . .	14.4	t			17.5	t		
C-12 . . .	24.8[b]	t	18	11α	25.7	t	18	
C-13 . . .	39.0	s	11α	12α,β,17,18	38.8	s	11β	12α,17,18
C-14 . . .	69.8	s	12α,17,18,30		68.9	s	12α,18,30	
C-15 . . .	57.2	d			56.6	d		
C-16 . . .	167.5	s		15	167.0	s		15
C-17 . . .	78.0	d	18		78.1	d	18	
C-18 . . .	16.8	q	12β,17		17.5	q		
C-19 . . .	33.8	t			65.6	t	5	
C-20 . . .	120.3	s	23	17,21,22	120.2	s	23	17,21,22
C-21 . . .	141.2	d	17,22,23		141.3	d	17	22
C-22 . . .	109.8	d	21	23	109.8	d	21	23
C-23 . . .	143.1	d	21	22	143.2	d		22
C-28 . . .	32.6	q	29		21.3	q	5,29	
C-29 . . .	24.5	q	5,28		30.3	q	5,28	
C-30 . . .	18.7	q			18.4	q		
Ac	21.0	q			21.1	q		
Ac	169.9	s	7	$COCH_3$	169.6	s	7	$COCH_3$

[a]Spectra were recorded in $CDCl_3$ at 100 MHz; chemical shifts are reported in ppm relative to TMS; multiplicities of carbon signals were determined by DEPT method.

[b]Previous assignments by Bennett and Hasegawa (24) were revised.

peaks between the H_3-29 (δ 1.16) and H-2 (δ 2.65). In the nOe difference spectrum, irradiation at H_3-29 caused a strong nOe enhancement of the signal at δ 2.65 (H-2) suggesting a "through-space" coupling due to their close proximity. A cross peak observed between H-9 and H_3-18 may also be due to similar coupling. "Through-space" coupling has previously been observed for aromatic systems (25) and fluorinated steroids (27). For a theoretical discussion, see Barfield and Karplus (26). The observed nOe effects of **6** can be rationalized by considering its conformation, which compares well with the solid state conformation of 7α-acetoxydihydronomilin which has been analyzed by X-ray crystallography (28). Detailed analysis of the long-range ^{1}H-^{13}C COSY spectrum enabled us to assign all the carbon signals in **6**, leading to revision of some previous assignments (24) (Table 2).

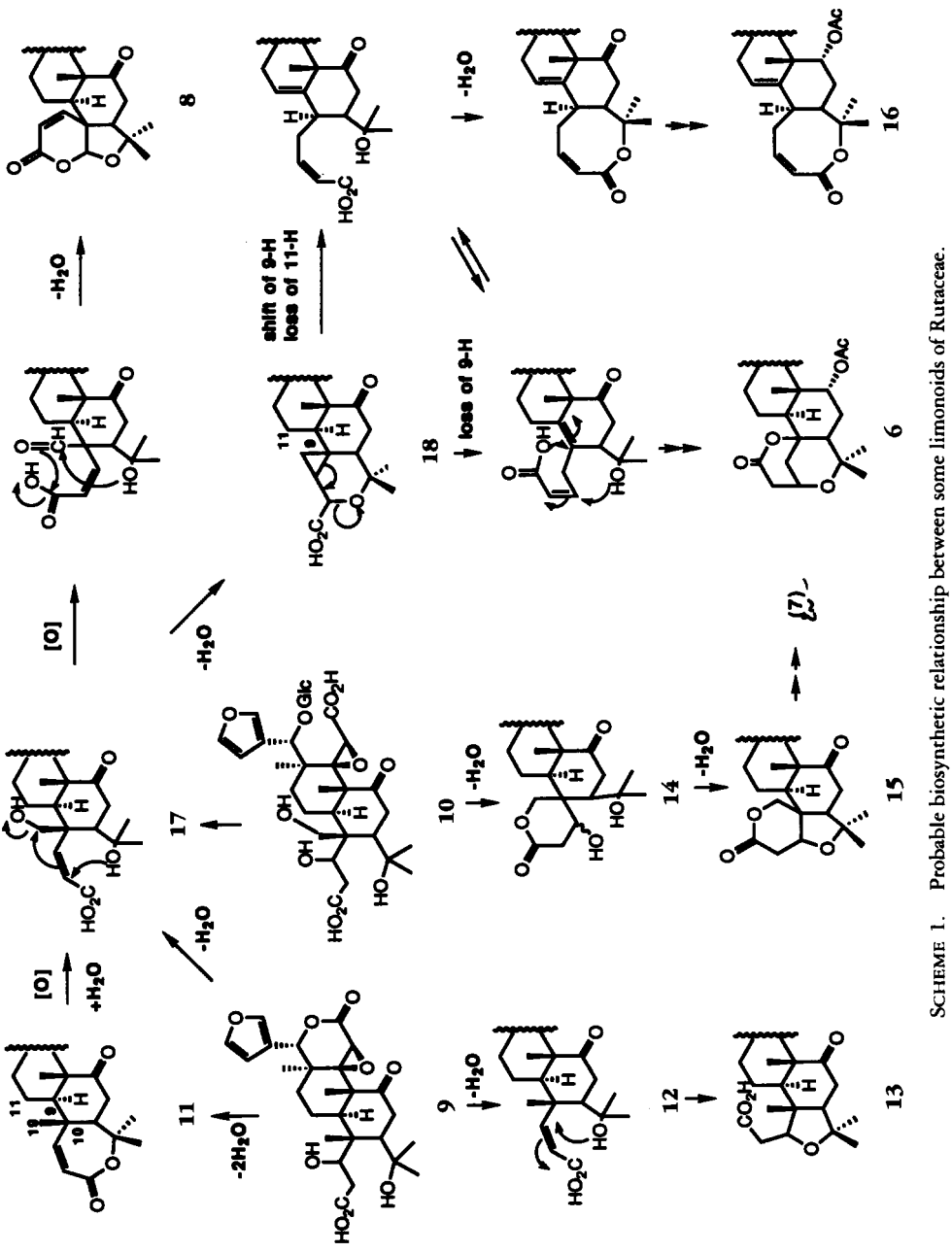
Chromatographic fractionation of the EtOAc extract over Si gel followed by gel filtration using Sephadex afforded a white powder, mp 128–130°. The fabms data were consistent with the molecular formula $C_{20}H_{24}O_{12}$. Uv and ir data along with its

molecular formula suggested it to be a coumarin glycoside, and this was confirmed by its ^{1}H-nmr spectrum which was fully analyzed by the use of ^{1}H-^{1}H shift correlation techniques. Acetylation (Ac_2O/C_5H_5N) gave the hexaacetate, mp 116°–118°, whereas hydrolysis with 2 M H_2SO_4 furnished umbelliferone and a mixture of two sugars. One of the sugars was identified as glucose by paper chromatography with an authentic sample. Partial hydrolysis with Amberlite resin provided a glycoside that was identified as skimmin from its physical data and conversion into its tetraacetate. The ^{13}C-nmr spectrum of the parent compound, analyzed with the aid of ^{1}H-^{13} COSY, while confirming the compound to be a coumarin glycoside, revealed the presence of signals characteristic of two anomeric carbons at δ 102.1 (C-1′, glucose moiety) and δ 111.2 (C-1″, apiose moiety). In the ^{1}H-nmr spectrum, the irradiation of the anomeric proton at δ 5.61 (glucose) caused an nOe enhancement of the signals at δ 7.21 and 7.29 of the coumarin, while the irradiation of the anomeric proton at δ 5.71 (apiose) caused enhancement of the signal at δ 4.15 (H-6′ of glucose). The foregoing evidence suggested the compound to be apiosylskimmin [**5**], and our ^{1}H- and ^{13}C-nmr spectral data showed a very close resemblance to those reported for **5** (29). The minor differences observed may be attributable to the solvents used. We found that by the use of pyridine-d_5 as the solvent in the ^{13}C-nmr spectrum, the glycosidic carbons were better resolved (C-2′ from C-4″ and C-5′ from C-2″) than those reported for $CDCl_3$ (29). Apiosylskimmin [**5**] has previously been isolated from *Gmelina arborea* of the family Verbenaceae (29). However, some physical constants (mp and [α]D) of our apiosylskimmin and its hexaacetate were found to be significantly different from those reported for these two compounds (29).

The plants of the Rutaceae contain closely related A- and D-ring seco-limonoids believed to be biosynthetically derived from obacunone [**11**] (30). Extensive radioactive tracer work by Hasegawa and coworkers on *Citrus* species have suggested that deacetylnomilinic acid [**9**] is the initial limonoid to be biosynthesized, and it may be the biosynthetic precursor of obacunone [**11**], obacunoic acid [**12**], and isoobacunoic acid [**13**] (31–33). The majority of limonoids in the Rutaceae appear to be C-19 oxidized derivatives, and thus far no experimental evidence has been provided for their biosynthetic origin although the aglycone of **10** has been postulated as an intermediate in the biosynthesis of limonin [**15**] (32). Recent isolation of 19-hydroxydeacetylnomilinic acid 17-β-D-glucopyranoside [**10**] from *Citrus aurantium* further supports this hypothesis (34). Co-occurrence of 1-(10↛19)*abeo*-obacun-9(11)en-7α-yl acetate [**16**] and the lactone **6** in *Citrus paradisii* has prompted Bennett and Hasegawa (24) to suggest the biosynthetic relationship between these (10↛19)*abeo* derivatives; subsequently Dreyer (30) postulated a plausible biosynthetic route to **6** and **16** from the co-occurring 7α-limonyl acetate [**7**]. However, the origin of limonin [**15**] and its derivatives in the Rutaceae still remains obscure, although ischangin [**14**] has been suspected to be a possible precursor (35). Co-occurrence of the limonoids, jangomolide (=acidissimin) [**8**], and obacunone [**11**] in *Li. acidissima* (17) and 7α-limonyl acetate [**7**] and the lactone **6** in *P. alatum* (this work) prompts us to suggest their biosynthetic interrelationship to a common C-19 oxidized intermediate **17** as depicted in Scheme 1. The origin of **6** and **16** from **17** may involve a common cyclopropyl intermediate **18**. A cyclopropyl intermediate analogous to **18** has been recently invoked as a possible biosynthetic precursor of the spongian diterpene, luterosin (36).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Unless otherwise stated, collection of plant material, extraction, general isolation procedures, and instrumentation were the same as those described in our previous publications (5,17,21). Voucher specimens of *Lu. angustifolia, Li. acidissima,* and *P. alatum* are preserved at the Department of Botany, University of Peradeniya, Sri Lanka. Sephadex LH-20 (Pharmacia) was employed for gel filtration. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL JNM-GX 400 instru-



SCHEME 1. Probable biosynthetic relationship between some limonoids of Rutaceae.

ment at 400 and 100 MHz, respectively; chemical shifts are reported in ppm (δ) downfield from internal TMS. Multiplicities of carbon signals were determined by the DEPT method. ^{1}H-^{1}H, ^{1}H-^{13}C, and long-range ^{1}H-^{13}C COSY spectra were measured by the use of the JEOL standard pulse sequences, and collected data were treated with the JEOL standard software, NOe difference spectra were measured with the JEOL standard pulse sequence with 5 sec irradiation. The ms were recorded on JEOL JMS-D300 with a direct inlet system; for fabms glycerol was used as the matrix. All known compounds were identified by comparison of their physical and spectral properties (mp, ir, ^{1}H-nmr, ms) directly or with those reported.

EXTRACTIVES OF *LU. ANGUSTIFOLIA* STEM.—Air-dried and powdered stem (1.25 kg) of *Lu. angustifolia* collected at the Sinharaja forest of Sri Lanka was exhaustively extracted with hot petroleum ether. Evaporation afforded a dark brown semi-solid (37.0 g). A portion (35.0 g) of this was chromatographed over a column of Si gel (300 g) made up in hexane and eluted with hexane containing increasing amounts of EtOAc. The fraction eluted with 10% EtOAc in hexane followed by flash chromatography over Si gel yielded lupeol (48 mg) and stigmasterol (34 mg). The middle fraction eluted with the same solvent system on further purification by preparative tlc (30% EtOAc in hexanes) afforded suberosin (27 mg), mp 87–88°, having spectral data (ir, ^{1}H nmr, ms) identical with those reported (37). The final column fraction eluted with 10% EtOAc, in hexane, followed by flash chromatography (10% $CHCl_3$ in hexane) and preparative tlc (1% MeOH in CH_2Cl_2) gave 5-methoxyarborinin [**1**] as a yellow crystalline solid (34 mg), mp 130–132° (from CH_2Cl_2/hexanes) [lit. (18) 134–135°]; uv λ max (EtOH) 264, 277, 284, 316, 338, 412 nm (log ε 4.53, 4.33, 4.50, 4.06, 3.94, 3.78); ir ν max (KBr) 1620, 1590, 1560, 1490 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 3.75 (3H, s, OMe), 3.78 (3H, s, NMe), 3.97 (3H, s, OMe), 4.00 (3H, s, OMe), 6.37 (1H, s, H-4), 7.13–7.37 (2H, m, H-6 and -7), 7.95 (1H, dd, J = 7, 3 Hz, H-8), 14.03 (1H, s, 1-OH).

The column fraction eluted with 20% EtOAc in hexane contained two compounds and these were separated by flash chromatography over Si gel (25% $CHCl_3$ in hexane). The less polar of the two compounds on further purification by preparative tlc (80% Et_2O in petroleum ether) yielded 5-hydroxyarborinine [**2**] as a yellow crystalline solid (47 mg), mp 205–207° [lit. (19) 206–207°], spectral data of which were found to be identical with those reported (17, 19). Methylation of **2** with CH_2N_2 in Et_2O afforded a monomethyl ether that was identical with 5-methoxyarborinine [**1**] isolated above. The more polar of the two compounds obtained from the above flash chromatographic separation gave ostruthin (31 mg) on further purification by preparative tlc ($CHCl_3$).

EXTRACTIVES OF *LI. ACIDISSIMA* ROOT BARK.—Cc fractionation of the hot petroleum ether extract (30 g) representing 2.0 kg of the root bark of *Li. acidissima* afforded (in the order of increasing polarity) osthol (90 mg), aurapten (800 mg), stigmasterol, and isopimpinellin (500 mg). Isolation of psoralen and bergapten from this column has been reported previously (5). The hot CH_2Cl_2 extract (35 g) on cc fractionation gave aurapten, isopimpinellin, and integriquinolone [**3**] (200 mg), mp 255–258° (dec) [lit. (23) 257–260°]. Acetylation of **3** (20 mg) with Ac_2O (0.5 ml) and pyridine (0.5 ml) at 25° for 24 h gave the acetate **4** (21 mg), mp 170–172°; ir ν max ($CHCl_3$) 1760, 1680, 1610, 1470, 1380, 1330, 1250, 1130 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 7.68 (1H, m, H-5), 7.32 (2H, m, H-7, -8), 6.03 (1H, s, H-3), 3.92 (3H, s, OMe), 3.63 (3H, s, NMe), 2.32 (3H, s, OAc).

EXTRACTIVES OF *LI. ACIDISSIMA* STEM BARK.—Air-dried and powdered stem bark (4 kg) was successively and exhaustively extracted with petroleum ether and CH_2Cl_2 under reflux conditions, and removal of solvents in vacuo gave 22 g and 30 g of the respective extracts. A part (16 g) of the petroleum ether extract on cc fractionation yielded (in the order of increasing polarity) lupeol, psoralen (80 mg), bergapten (95 mg), stigmasterol (145 mg), and (+)-marmesin.

EXTRACTIVES OF *P. ALATUM* ROOT BARK.—Air-dried and powdered root bark (1.65 kg) of *P. alatum* was successively and exhaustively extracted with hot hexane, CH_2Cl_2, and EtOAc under reflux conditions. Evaporation of solvent in vacuo yielded hexane (90 g), CH_2Cl_2 (34 g), and EtOAc (35 g) extracts. A portion (30 g) of the hexane extract was separated by cc affording seselin, xanthyletin, xanthoxyletin, lupeol, stigmasterol, 1-hydroxy-2,3,5,6-tetramethoxy-10-methyl-9-acridone, 1,5-dihydroxy-2,3-dimethoxy-10-methyl-9-acridone, 5-hydroxyacronycine, suberenol, and umbelliferone (17), two limonoids, 1-(10↦19)-*abeo*-7α-acetoxy-10β-hydroxyiso-obacunoic acid-3,10-lactone [**6**] (50 mg), mp 292–294° [lit. (24) 271–274°]; $[\alpha]^{25}D$ −15° (c = 1.0 in $CHCl_3$), and 7α-limonyl acetate [**7**] (7 mg), mp 267–269° [lit. (38) 255–260°]; $[\alpha]^{25}D$ −58° (c = 1.0 in $CHCl_3$). For ^{1}H- and ^{13}C-nmr data of **6** and **7**, see Tables 1 and 2.

A portion (30 g) of the above EtOAc extract was chromatographed over a column of Si gel (120 g) made up in $CHCl_3$ and eluted with $CHCl_3$ containing increasing amounts of MeOH. The fraction eluted with 10% MeOH in $CHCl_3$ was further purified by gel filtration on Sephadex. Elution with 30% $CHCl_3$ in MeOH gave apiosylskimmin [**5**] as a colorless crystalline solid (3.1 g): mp 138–140° [lit. (29) 141–142°]; $[\alpha]^{25}D$ −127° (c = 1.0 in pyridine); ^{1}H nmr (pyridine-d_5) δ 7.56 (1H, br d, J = 9.5 Hz, H-4), 7.41 (1H, br d, J = 8.5 Hz, H-5), 7.29 (1H, d, J = 2 Hz, H-8), 7.21 (1H, dd, J = 8.5, 2 Hz, H-6), 6.26 (1H, br d,

J = 9.5 Hz, H-3), 5.71 (1H, d, J = 2 Hz, H-1″), 5.61 (1H, d, J = 8 Hz, H-1′), 4.81 (1H, d, J = 2 Hz, H-2″), 4.74 (1H, dd, J = 11, 1.5 Hz, H-6′), 4.66 (1H, d, J = 9.5 Hz, H-4″), 4.37 (1H, d, J = 9.5 Hz, H-4″), 4.35 (1H, t, J = 8 Hz, H-3′), 4.34 (1H, m, H-5′), 4.31 (1H, t, J = 8 Hz, 2-H′), 4.24 (2H, s, H-5″), 4.15 (1H, dd, J = 11, 7 Hz, H-6′), 4.12 (1H, t, J = 8 Hz, H-4′); ^{13}C nmr (pyridine-d_5) δ 161.3 (s, C-2), 160.9 (s, C-7), 155.8 (s, C-8a), 143.7 (d, C-4), 129.4 (d, C-5), 114.0 (d, C-6), 113.9 (s, C-4a), 113.6 (d, C-3), 111.2 (d, C-1″), 104.5 (d, C-8), 102.1 (d, C-1′), 80.3 (s, C-3″), 78.4 (d, C-3′), 77.8 (d, C-2″), 77.3 (d, C-5′), 75.0 (t, C-4″), 74.6 (d, C-2′), 71.5 (d, C-4′), 69.0 (t, C-6′), 65.5 (t, C-5″). Hexaacetate: mp 116–118° [lit. (29) 80°]; [α]D −110° (c = 1.0 in $CHCl_3$); ir ν max (KBr) 1750, 1620, 1350, 1225, 1070, 1040; ^{1}H nmr ($CDCl_3$) δ 7.66 (1H, br d, J = 9.8 Hz, H-4), 7.42 (1H, d, J = 7.4 Hz, H-5), 6.94 (1H, d, J = 2.1 Hz, H-8), 6.93 (1H, dd, J = 7.4, 2.1 Hz, H-6), 6.31 (1H, d, J = 9.8 Hz, H-3), 5.32 (1H, dd, J = 9.2 Hz, H-3′), 5.32 (1H, d, J = 0.6 Hz, H-2″), 5.27 (1H, dd, J = 9.2, 7.3 Hz, H-2′), 5.19 (1H, d, J = 7.3 Hz, H-1′), 4.55 (1H, d, J = 12.2 Hz, H-5″), 4.21 (1H, d, J = 10.4 Hz, H-4″), 4.13 (1H, d, J = 10.4 Hz, H-4″), 3.62 (1H, dd, J = 11.3, 6.4 Hz, H-6′), 1.12, 2.07, 2.06, 2.04 (each 3H, s, OAc), 2.02 (6H, s, 2 × OAc); ^{13}C nmr ($CDCl_3$) δ 160.6 (s, C-2), 159.3 (s, C-7), 155.3 (s, C-8a), 142.9 (d, C-4), 129.1 (d, C-5), 114.6 (d, C-6), 114.5 (s, C-4a), 113.7 (d, C-3), 106.0 (d, C-1″), 104.5 (d, C-8), 98.3 (d, C-1′), 83.8 (s, C-12), 76.1 (d, C-3′), 73.5 (d, C-2″), 72.6 (d, C-5′), 72.5 (t, C-4″), 71.0 (d, C-2′), 68.6 (d, C-4′), 66.3 (t, C-6′), 62.9 (t, C-5″), 170.6, 170.1, 169.6, 169.3, 169.2, 168.9 (each s, OCOMe), 21.1, 20.7, 20.6, 20.5 (each q, $OCOCH_3$).

The glycoside **5** (200 mg) was hydroxyzed with 2 M H_2SO_4 (5 ml) under reflux for 30 min. The reaction mixture was cooled, diluted with H_2O, and extracted with Et_2O. The organic extract was washed with H_2O, dried ($MgSO_4$), and evaporated yielding umbelliferone (47 mg), mp 222–224° [lit. (39) 223–224°]. The aqueous solution was basified with excess $BaCO_3$, the resulting precipitate was removed by filtration, and the filtrate was freeze-dried. Paper chromatographic analysis [EtOAc-C_5H_5N-H_2O-HOAc (5:5:2:1)] of the resulting residue showed the presence of 2 sugars, one of which had the same R_f as D-glucose. Partial hydrolysis of **5** (100 mg) with Amberlite IR-120 (H^+ form, 1 g) in H_2O (3 ml) at 65° for 1 h, filtration, and freeze-drying gave a residue (87 mg) which ws purified by Si gel cc (EtOAc) to give white needles of skimmin (67 mg), mp 218–220°, [α]D −80° (c = 0.6 in C_5H_5N) [lit. (40) 219–221°, −79.8°]. Acetylation (Ac_2O/C_5H_5N) gave its tetraacetate, mp 181–183°, [α]D −63° (c = 1 in $CHCl_3$) [lit. (40) 183–184°, −63.3°]; ir ν max (KBr) 1740, 1620, 1380, 1250, 1220, 1080, 1050, 1030; ^{1}H nmr ($CDCl_3$) δ 7.66 (1H, d, J = 9.8 Hz, H-4), 7.42 (1H, d, J = 8.9 Hz, H-5), 6.96 (1H, d, J = 2.6 Hz, H-8), 6.91 (1H, dd, J = 8.9, 2.6 Hz, H-6), 6.32 (1H, d, J = 9.8 Hz, H-3), 5.32 (1H, dd, J = 7.7, 7.3 Hz, H-2′), 5.30 (1H, dd, J = 8.9, 7.7 Hz, H-3′), 5.18 (1H, d, J = 7.3 Hz, H-1′), 5.17 (1H, dd, J = 10.3, 8.9 Hz, H-4′), 4.29 (1H, dd, J = 12.4, 2.6 Hz, H-6), 4.19 (1H, dd, J = 12.4, 5.6 Hz, H-6′), 3.93 (1H, ddd, J = 10.3, 5.62, 2.6 Hz, H-5′), 2.12, 2.07, 2.06, 2.04 (each 3H, OAc).

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