Phytochemistry, Vol. 23, No. 2, pp. 323–328, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00

TERPENOID AND BIFLAVONOID CONSTITUENTS OF *CALOPHYLLUM CALABA* AND *GARCINIA SPICATA* FROM SRI LANKA*

A. A. LESLIE GUNATILAKA,† A. M. Y. JASMIN DE SILVA, SUBRAMANIAM SOTHEESWARAN, SINNATHAMBY BALASUBRAMANIAM‡ and MOHAMED I. M. WAZEER§

Department of Chemistry, University of Peradeniya, Peradeniya, Sri Lanka; ‡Department of Botany, University of Peradeniya, Sri Lanka; §Department of Chemistry, University of Petroleum & Minerals, Dhahran, Saudi Arabia

(*Revised received* 25 *June* 1983)

Key Word Index—*Calophyllum calaba*; *Garcinia spicata*; Guttiferae; foliar constituents; bark acids; chapelieric acid; isochapelieric acid; biflavanones; sitosterol; friedelin; friedelan-3β-ol; canophyllol; canophyllal; canophyllic acid; friedelan-3β,28-diol; chemotaxonomy.

Abstract—A new bark acid, isochapelieric acid (*cis*-chapelieric acid), chapelieric acid, friedelin, friedelan-3β-ol, canophyllal, canophyllol, friedelan-3β,28-diol, canophyllic acid and amentoflavone have been isolated and characterized from leaf extractives of *Calophyllum calaba*. ^{13}C NMR spectra of methyl chapelierate and methyl isochapelierate have been recorded and interpreted. Leaf extractives of *Garcinia spicata* afforded an unidentified long chain carboxylic acid, friedelin, friedelan-3β-ol, sitosterol and the biflavanones GB-1, GB-1a, GB-2a and morelloflavone. Chemotaxonomic significance of the occurrence of some of the above foliar constituents in *Calophyllum* and *Garcinia* species is discussed.

INTRODUCTION

The Guttiferae (Clusiaceae), a pantropical family of plants, is represented in Sri Lanka by 24 species distributed in four genera of which 17 species are endemic to the country [2]. Of these, 11 species have been recorded for the genus *Calophyllum* and 10 for the genus *Garcinia*, containing 10 and five endemic species, respectively. Reported medicinal uses of some *Calophyllum* [3] and *Garcinia* [4, 5] species prompted us to undertake this study and in this paper we present our results on the leaf extractives of the endemic species, *Calophyllum calaba* L. and the non-endemic species *Garcinia spicata* (W. & A.) Hook. f.

The bark and timber extractives of *C. calaba* have been investigated previously and the occurrence of calabaxanthone, jacareubin, 6-deoxyjacareubin, 1,5,6-trihydroxyxanthone, 1,6-dihydroxy-5-methoxyxanthone, 2,8-dihydroxy-1-methoxyxanthone, 2-hydroxy-1,8-dimethoxyxanthone, scriblitifolic acid, guanandin, taraxerol and taraxerone have been reported [6]. Previous investigations of the bark of *G. spicata* have resulted in the isolation of three biflavonoids, *viz.* (+)-fukugetin, (±)-fukugetin(morelloflavone) and (±)-3′-*O*-methylfukugetin [7–10].

RESULTS AND DISCUSSION

Calophyllum calaba

The dried and powdered leaves of *C. calaba* were exhaustively and successively extracted with hot petrol and hot ethyl acetate. TLC examination of the former extract revealed it to be a complex mixture. A partial separation of this was achieved by a re-extraction with hot methanol whereby the polar compounds went into solution. These two fractions, methanol soluble and insoluble, were further separated into acidic (sodium carbonate soluble) and non-acidic fractions. The non-acidic fraction of the methanol soluble part of the petrol extract had mainly chlorophyll and its breakdown products and was not investigated further.

The acidic fraction of the methanol soluble part on TLC showed it to be an inseparable mixture of two components. Thus, they were methylated (CH_2N_2) and separated on silver nitrate impregnated TLC into two isomeric esters (see later). UV spectra of these indicated the presence of chromanone–chromene type chromophore and comparable with those of some bark acids, previously isolated from several other *Calophyllum* species [3]. The phenolic nature of these compounds was indicated by the positive ferric chloride test (green colour). The ^1H NMR spectrum (Table 1) of one of the isomers was found to be identical with that reported [11] for chapelieric acid methyl ester (**2**), previously isolated from *Calophyllum chapelieri*. Comparison of UV, IR and MS data with those reported and the ^{13}C NMR spectrum (Table 2) further confirmed its identity.

The UV, IR and MS data of the other methyl ester were identical to those reported [11] for **2**, whereas ^1H and ^{13}C NMR spectra exhibited certain differences (see Tables 1 and 2, respectively) indicating this to be a stereoisomer of chapelieric acid methyl ester. The ^1H NMR coupling constants (4.5 Hz) observed for the protons at C-1 and C-2 of this isomer (**5**) compared with the *J* value of 12 Hz for the corresponding protons in **2** suggested the relative configuration of the methyl groups at C-1 and C-2 to be *cis* in this isomer (**5**) in contrast to the

*Part 11 in the series "Studies on Medicinal and Related Plants of Sri Lanka." For Part 10 see ref. [1].

†To whom correspondence should be addressed.

Table 1. ^{1}H NMR data for chapelieric and isochapelieric acid derivatives [δ/$CDCl_3$(TMS)]

Proton(s)	2	3	5	6
C1-H	4.04 (*m*, *J* = 12 Hz)	4.31 (*m*)	4.53 (*dm*, *J* = 4.5 Hz)	4.38 (*m*)
C2-H	2.56 (*m*, *J* = 8 Hz)	2.42 (*m*)	2.51 (*dm*, *J* = 4.5 Hz)	2.48 (*m*)
C5-OH	12.94 (*s*)	—	12.76 (*s*)	—
C5-OAc	—	2.31 (*s*)	—	2.32 (*s*)
C7-H	6.51 (*d*, *J* = 10 Hz)	6.48 (*d*, *J* = 10 Hz)	6.47 (*d*, *J* = 10 Hz)	6.46 (*d*, *J* = 10 Hz)
C8-H	5.37 (*d*, *J* = 10 Hz)	5.45 (*d*, *J* = 10 Hz)	5.33 (*d*, *J* = 10 Hz)	5.45 (*d*, *J* = 10 Hz)
C13-H	5.03 (*t*, *J* = 8 Hz)	4.65 (*t*, *J* = 8 Hz)	5.02 (*t*, *J* = 8 Hz)	4.65 (*t*, *J* = 8 Hz)
C14-H	3.16 (*d*, *J* = 8 Hz)	3.02 (*d*, *J* = 8 Hz)	3.13 (*d*, *J* = 8 Hz)	3.09 (*d*, *J* = 8 Hz)
C1-Me	1.56 (*d*, *J* = 8 Hz)	1.48 (*d*, *J* = 6 Hz)	1.28 (*d*, *J* = 8 Hz)	1.35 (*d*, *J* = 6 Hz)
C2-Me	1.22 (*d*, *J* = 6 Hz)	1.15 (*d*, *J* = 6 Hz)	1.50 (*d*, *J* = 8 Hz)	1.09 (*d*, *J* = 8 Hz)
C9-Me_2	1.13 (*s*)	0.82 (*s*)	1.11 (*s*)	0.78 (*s*)
Ph	7.20 (*m*)	7.18 (*m*)	7.15 (*m*)	7.15 (*m*)
CO_2Me	3.56 (*s*)	3.62 (*s*)	3.53 (*s*)	3.65 (*s*)

Table 2. ^{13}C NMR chemical shifts in methyl chapelierate (2) and methyl isochapelierate (5) [δ/(multiplicity) $CDCl_3$ (TMS)]

Carbon(s)	2	5	Carbon(s)	2	5
1	78.8 (*d*)	76.0 (*d*)	12	155.4 (*s*)	155.1 (*s*)
2	45.6 (*d*)	44.3 (*d*)	13	34.7 (*d*)	35.0 (*d*)
3	173.2 (*s*)	173.3 (*s*)	14	36.6 (*d*)	36.7 (*d*)
4	111.3 (*s*)	111.2 (*s*)	15	161.3 (*s*)	161.8 (*d*)
5	143.5 (*s*)	143.3 (*s*)	1-Me	19.6 (*q*)	16.3 (*q*)
6, 11	101.7 (*s*)	101.4 (*s*)	2-Me	10.1 (*q*)	9.4 (*q*)
	101.4 (*s*)	101.0 (*s*)	9-Me_2	27.3 (q)	27.8 (q)
7	125.9 (d)	125.8 (d)		28.3 (q)	28.1 (q)
8	115.7 (d)	115.7 (d)	Ph, *o*	127.8 (*d*)	127.7 (*d*)
9	78.0 (*s*)	78.1 (*s*)	*m*	127.7 (*d*)	127.6 (*d*)
10	159.4 (*s*)	159.5 (*s*)	*p*	125.8 (*d*)	125.8 (*d*)
			OMe	51.5 (*q*)	51.4 (*q*)

trans arrangement in chapelieric acid methyl ester (**2**). Thus the two bark acids present in the original extract should be chapelieric acid (**1**) and its isomer, isochapelieric acid (**4**). This constitutes the first report of the occurrence of isochapelieric acid in nature.

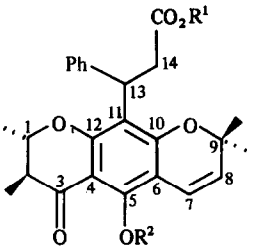
The acidic fraction of the methanol insoluble part consisted of mainly a single compound as indicated by TLC, which on purification by prep. TLC gave an unidentified fatty acid, mp 45–46°. The non-acidic fraction of the methanol insoluble part on prep. TLC afforded (in the order of increasing polarity) friedelin (**7**), friedelan-3β-ol (**8**), canophyllal (**9**), canophyllol (**10**), friedelan-3β, 28-diol (**11**) and canophyllic acid (**12**). The identities of compounds **7**–**10** and **12** were confirmed by comparison with authentic samples and the identity of **11** was confirmed by oxidation with CrO_3–pyridine to obtain **9** which was compared with authentic canophyllal.

The hot ethyl acetate extract was washed with 10% aqueous borax solution and the borax soluble fraction on usual work-up yielded a yellow solid. Purification of this by prep. TLC afforded a pale yellow crystalline solid, mp 245–246°, giving the ferric chloride test for phenols (green) and magnesium–hydrochloric acid test for flavonoids (red). IR and UV spectra supported the flavonoid structure whereas the MS fragmentation [12] indicated it to be the biflavonoid, amentoflavone (**13**). Acetylation with acetic anhydride–pyridine gave the hexa-acetate (**14**), mp 234–236°, $[M]^+$ at *m/z* 538, whereas methylation with dimethyl sulphate–potassium carbonate–acetone afforded the tetramethyl ether (**15**), mp 228–229°.

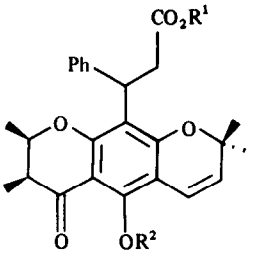
Garcinia spicata

The dried and powdered leaves of *G. spicata* were exhaustively and successively extracted with hot petrol and hot ethyl acetate. The petrol extract on combined CC and prep. TLC afforded an unidentified long chain carboxylic acid, friedelin (**7**), friedelan-3β-ol (**8**), and sitosterol.

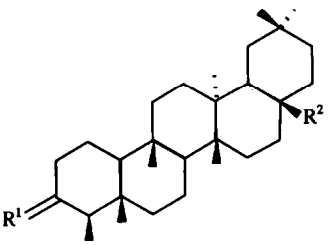
The ethyl acetate extract was separated into borax soluble and borax insoluble fractions. TLC examination indicated the former fraction to consist of one major compound and the borax insoluble fraction to be a complex mixture of compounds. Purification of the borax soluble fraction by TLC afforded an off-white crystalline solid, mp 216–218°, giving positive response to phenol (ferric chloride—green) and flavonoid (magnesium–hydrochloric acid—red) tests. The characteristic UV maxima and shifts with sodium acetate and aluminium chloride indicated it to be a flavonoid dimer and this was



1 $R^1 = R^2 = H$
2 $R^1 = Me$, $R^2 = H$
3 $R^1 = Me$, $R^2 = Ac$



4 $R^1 = R^2 = H$
5 $R^1 = Me$, $R^2 = H$
6 $R^1 = Me$, $R^2 = Ac$



7 $R^1 = O$, $R^2 = Me$
8 $R^1 = \alpha\text{-H}, \beta\text{-OH}$, $R^2 = Me$
9 $R^1 = O$, $R^2 = CHO$
10 $R^1 = O$, $R^2 = CH_2OH$
11 $R^1 = \alpha\text{-H}, \beta\text{-OH}$, $R^2 = CH_2OH$
12 $R^1 = \alpha\text{-H}, \beta\text{-OH}$, $R^2 = CO_2H$

confirmed by negative ion MS (M^+, *m/z* 558). The MS fragmentation and the ^1H NMR spectrum were identical to those reported [13–15] for the biflavonoid GB-2a [I-4′,II-4′,II-5′,1-5,II-5,I-7,II-7-heptahydroxy-(I-3,II-8)-biflavone] [**16**].

The borax insoluble fraction of the ethyl acetate extract on separation by combined polyamide CC and silica gel prep. TLC afforded three further pigments which were identified as GB-1a [1-4,II-4′,I-5,II-5,I-7,II-7-hexahydroxy-(I-3, II-8)-biflavone] (**17**) [13–15], GB-1 [II-3, I-4′,II-4′,I-5,II-5,I-7,II-7-heptahydroxy-(I-3,II-8)-biflavone] (**18**) [13–15] and morelloflavone (**19**) [16].

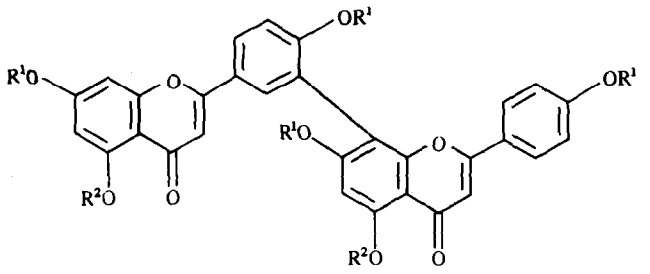
Chemotaxonomic aspects

This constitutes the first report of the occurrence of bark acids in leaves of a *Calophyllum* species. Thus far about 27 *Calophyllum* species have been chemically investigated and about 20 different bark acids have been encountered [17]. Chapelieric acid (**1**) has been isolated previously from *C. chapelieri* [11]. This is the first report of the occurrence of isochapelieric acid (**4**) in nature. These two acids differ in the stereochemistry of the two methyl groups of the chromanone ring. Papuanic and isopapuanic acids in *C. papuanum* [18] represent a pair of related stereoisomers. The biflavanones have been more commonly encountered in genera *Garcinia* [13] and *Allanblackia* [16], both belonging to the tribe Garcineae of the subfamily Clusioideae [3]. All the biflavonoids encountered so far in *Garcinia* species contain (I-3,II-8) interflavonoid linkage except for amentoflavone (**13**) and cupressuflavone isolated from *G. multiflora* [19, 20] and *G. livingstonii* [20], where the linkage is (I-8,II-8). Thus, the occurrence of amentoflavone in *Calophyllum calaba* is of chemotaxonomic significance. It is interesting that *G. spicata* leaves contained both GB type biflavonoids, e.g. GB-1 (**18**), GB-1a (**17**), GB-2a (**16**) and morelloflavone (**19**). A variety of triterpenes have been isolated from several species of the Guttiferae [3]. These include members of the friedelane, oleanane, taraxerane and adianane groups. However, in both species investigated in the present study triterpenes belonging only to the friedelane group have been encountered.

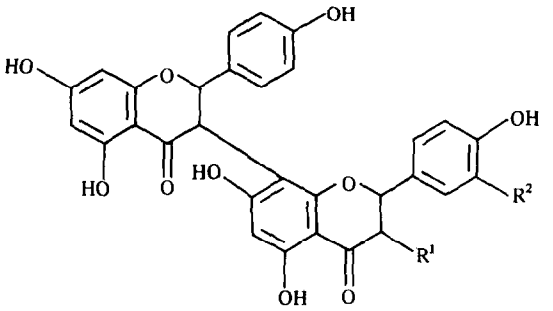
EXPERIMENTAL

General procedures. Mps are uncorr. UV spectra were recorded in EtOH. Unless otherwise stated, IR spectra were recorded in KBr discs and ^1H NMR spectra in $CDCl_3$ at 60 MHz and 220 MHz. ^{13}C NMR spectra were measured in $CDCl_3$ at 50.11 MHz. Petrol refers to the fraction of bp 60–80°. Silica gel (Merck) plates (0.25 mm) were used for TLC; for prep. TLC these were 1 mm thick.

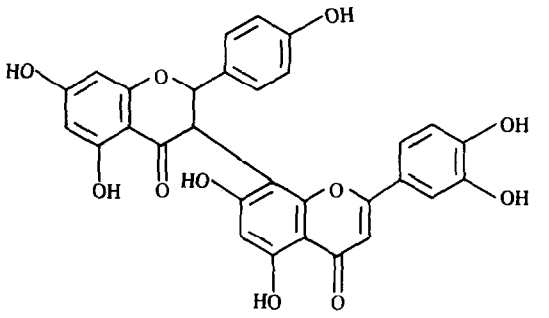
Calophyllum calaba—*extraction.* Dried and powdered leaves of *C. calaba* (3.0 kg) collected at Kanneliya, Sri Lanka were successively and exhaustively extracted with hot petrol and hot EtOAc. Evaporation gave 149 g of petrol extract and 159 g of



13 $R^1 = R^2 = H$
14 $R^1 = R^2 = Ac$
15 $R^1 = Me$, $R^2 = H$



16 $R^1 = H$, $R^2 = OH$
17 $R^1 = R^2 = H$
18 $R^1 = OH$, $R^2 = H$



19

EtOAc extract. The former extract (30 g) was re-extracted with hot MeOH to obtain MeOH soluble (24.5 g) and MeOH insoluble (5 g) fractions. These two fractions in ether were separately washed with 10% Na_2CO_3 to yield Na_2CO_3 soluble fractions of the MeOH soluble (fraction A; 18 g) and MeOH insoluble (B; 0.82 g); Na_2CO_3 insoluble fractions of MeOH soluble (C; 6.2 g) and MeOH insoluble (D, 4.0 g). Of these only fractions A and D were subjected to detailed investigations.

Methyl chapelierate (**2**) *and methyl isochapelierate* (**5**). The fraction A (1.5 g) from above on prep. TLC yielded a mixture of inseparable acids (1.3 g) which was methylated (CH_2N_2) affording a green gum (1.03 g). This methylated mixture (0.12 g) was separated by TLC (SiO_2–5% $AgNO_3$) to afford two isomeric esters as pale yellow gums. The less polar compound (0.077 g, 1.2 $\times 10^{-6}$%) was found to have physical data compatible with those reported [11] for methyl chapelierate (**2**), except for rotation; $[\alpha]_D$ −40.5° (*c* 2.0; $CHCl_3$), *lit.* −165° [11]; UV λ_{max}^{EtOH} nm (log ε): 266 (4.40), 274 (4.60), 298 (4.00), 312 (4.10) and 370 (3.65); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–3200 (*OH*), 1745 (*CO*), 1720 (ester CO), 1620 (chromanone CO), 1345, 1100, 760, 685; MS *m/z* (rel. int.): 436$[M]^+$ (36), 421 (100), 389 (14), 363 (29), 361 (14), 333 (11), 291 (22), 259 (11), 203 (11), 121 (22), and 28 (36); for ^1H and ^{13}C NMR data, see Tables 1 and 2. Acetylation of **2** with Ac_2O in pyridine afforded the acetyl derivative (**3**),

mp 64–65° (from C_6H_6–petrol); $[\alpha]_D$ −60.1° (*c* 1.9; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}; 1780 (OAc), 1745 (ester CO), 1650 and 1640 (chromanone), 750, 685; for ^1H NMR data, see Table 1.

The more polar ester (0.30 g; 0.7×10^{-6}%), methyl isochapelierate (**5**), $[\alpha]_D$ −113.5° (*c* 2.0; $CHCl_3$) had the following physical properties; UV λ_{max}^{EtOH} nm (log ε): 265 (4.20), 270 (4.50), 292 (4.05), 310 (4.10) and 370 (3.60); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300–3200(OH), 1740 (CO), 1720 (ester CO), 1620 (chromanone CO), 1330, 1100, 760, 680; MS was found to be identical with that of methyl chapelierate (**2**); for ^1H and ^{13}C NMR data see Tables 1 and 2. With Ac_2O in pyridine it gave the acetyl derivative (**6**), mp 60–61° (from C_6H_6–petrol); $[\alpha]_D$ −120.1° (*c* 2.0; $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (OAc), 1745 (ester CO), 1650 and 1640 (chromanone CO), 750, 685; for ^1H NMR data, see Table 1.

Friedelin (**7**), *friedelan-3β-ol* (**8**), *canophyllal* (**9**) *and canophyllol* (**10**). The fraction D (0.4 g) on repeated prep. TLC (eluant: petrol–C_6H_6–$CHCl_3$, 6:1:3) gave friedelin (**7**) (0.061 g, 6.6×10^{-3}%), friedelan-3β-ol (**8**) (0.041 g, 4.3×10^{-3}%), canophyllal (**9**) (0.068 g, 7.4×10^{-3}%), and canophyllol (**10**) (0.024 g, 2.6×10^{-3}%) which were identified by their mp, $[\alpha]_D$ data and mmp and co-TLC with authentic samples. The lowest R_f band from above prep. TLC separation afforded a mixture of polar triterpenes as a colourless solid (0.15 g). This mixture was further separated by prep. TLC (eluant: $CHCl_3$–MeOH, 99:1) into **11** and **12**.

Friedelan-3β,28-diol (**11**). The less polar of the above triterpene mixture gave **11** as a colourless crystalline solid (0.068 g, 7.4×10^{-3}%); mp 308–310° (from $CHCl_3$–MeOH). $[\alpha]_D$ −20.1° (*c* 1.5; $CHCl_3$), lit. 270–272°, $[\alpha]_D$ −21° [21]; IR ν_{max}^{KBr} cm^{-1}: 3555, 2592, 1380, 1120; ^1H NMR ($CDCl_3$): δ 3.60 (2H, *br, s*, CH_2OH), 2.58 (1 H, *m*, CHOH), 1.10 (3 H, *s*, Me), 1.06 (6H, *s*, Me), 0.96 (6H, *s*, Me) and 0.85 (3H, *s*, Me); MS *m/z* (rel. int.): 427 $[M]^+$ (100%), 410 (4), 383 (36), 289 (64), 272 (18), 245 (86), 227 (22). CrO_3–pyridine oxidation [22] of **11** gave canophyllal (**9**) whose identity was confirmed by comparison (mp, mmp, co-TLC, co-IR) with an authentic sample.

Canophyllic acid (**12**). The more polar of the above triterpene mixture gave **12** as a colourless crystalline solid (0.018 g, 0.2×10^{-3}%), mp 310–312° (from $CHCl_3$–MeOH), $[\alpha]_D$ +19.8° (*c* 1.2; $CHCl_3$), lit. 312°, $[\alpha]_D$ +20.89° (pyridine) [23]; IR ν_{max}^{KBr} cm^{-1}: 3510 (OH), 1690 (CO_2H), 1210. Comparison (mmp, co-TLC and co-IR) with an authentic sample further confirmed its identity.

Amentoflavone (**13**). The hot EtOAc extract (0.80 g) of the leaves of *C. calaba* was separated into borax soluble (0.30 g) and insoluble (0.50 g) fractions. The major pigment in the borax soluble fraction (0.3 g) was separated by prep. TLC to yield amentoflavone (**13**) as a pale yellow crystalline solid (0.137 g, 1.7×10^{-3}%), mp 246–248°, lit. 249–250° [23, 24]; IR ν_{max}^{KBr} cm^{-1}: 3500–3250 *br* (OH), 1650 (flavone CO), 1600, 1490, 1420, 1350, 1240, 1160, 1105, 1030, 900, 840, 630; UV and ^1H NMR data were found to be identical to those reported [23, 24].

Amentoflavone hexa-acetate (**14**). Acetylation of **13** (0.1 g) with Ac_2O (3 ml) in pyridine (5 ml) at room temp. overnight and usual work-up afforded the hexa-acetate (**14**) as a pale yellow crystalline solid (0.068 g, 67%), mp 232–234° (from petrol–$CHCl_3$), lit. 234–236° [23]; IR and ^1H NMR spectra were almost identical with those reported for amentoflavone hexa-acetate [23].

Amentoflavone tetramethyl ether (**15**). Methylation of **13** with Me_2SO_4–K_2CO_3–Me_2CO and purification of the product by prep. TLC afforded the tetramethyl ether (**15**) as pale yellow crystals, mp 228–229° (from petrol–$CHCl_3$); IR ν_{max}^{KBr} cm^{-1}: 1750, 1650, 1600, 1430, 1365, 1320, 1300, 1200, 1100, 960, 840, 760; ^1H NMR ($CDCl_3$): δ 12.00 (1H, chelated OH), 11.30 (1H, chelated OH), 7.86 (1H, *s*), 7.82 (2H, *d*, *J* = 8 Hz), 7.52 (1H, *d*, *J* = 9 Hz), 7.31 (1H, *d*, *J* = 9 Hz), 7.20 (2H, *d*, *J* = 9 Hz), 7.00 (1H, *s*), 6.60 (1H, *s*), 6.56 (1H, *s*), 6.50 (1H, *s*).

Garcinia spicata—*extraction.* Dried and powdered leaves of *G. spicata* (1.75 kg) collected at Tanamalwila, Sri Lanka were subjected to separate extractions successively and exhaustively with hot petrol and hot EtOAc yielding 82.2 g (4.7%) of petrol extract and 118.0 g (6.7%) of EtOAc extract.

Separation of the petrol extract—isolation of friedelin (**7**), *friedelan-3β-ol* (**8**) *and sitosterol.* The hot petrol extract (30 g) was chromatographed on a column of silica gel (300 g) made up in petrol. Elution with petrol gave an unidentified long chain fatty acid (60 mg, 0.008%), mp 49–50°; IR ν_{max}^{KBr} cm^{-1}: 1690. Fractions eluted with petrol–$CHCl_3$ (9:1) on evaporation afforded friedelin (**7**) as a colourless crystalline solid (1.6 g, 5.0%), mp 264–265°, $[\alpha]_D$ −21.6° (*c* 2.0; $CHCl_3$), lit. mp 264°, $[\alpha]_D$ −22.1° [25] identical (mmp, co-TLC and co-IR) with an authentic sample. Elution with petrol–$CHCl_3$ (85:15) gave friedelan-3β-ol (**8**) (0.3 g, 1.0%) mp 283–285° (from $CHCl_3$–MeOH), $[\alpha]_D$ +24° (*c* 1.8; $CHCl_3$), lit. mp 284°, $[\alpha]_D$ +24° [26], identical (mmp, co-TLC and co-IR) with an authentic sample. Further elution of the column with petrol–$CHCl_3$ (1:1) gave sitosterol (0.046 g, 0.15%), mp 137–138°, $[\alpha]_D$ −37° (*c* 1.8; $CHCl_3$), lit. mp 138–139°, $[\alpha]_D$ −38° [27], identical (mmp, co-TLC and co-IR) with an authentic sample.

Separation of the EtOAc *extract—isolation of biflavanone GB-2a* (**16**). The hot EtOAc extract of *G. spicata* (50 g) was separated into borax soluble (17 g) and borax insoluble (32 g) fractions. The major compound of the former fraction (0.5 g) on purification by prep. TLC afforded the biflavanone GB-2a (**16**) as an off-white solid (0.265 g, 0.05%), mp 216–218°, $[\alpha]_D$ +28.2° (*c* 2.1; Me_2CO), lit. mp 210°, $[\alpha]_D$ +28° [13]; IR ν_{max}^{KBr} cm^{-1}: 3350, 3200, 1650, 1610, 1200, 1000, 865, 780; MS *m/z* (rel. int.) 558 $[M]^+$ (72%), 540 (28), 538 (5), 432 (30), 430 (70), 298 (28), 288 (21), 270 (17), 269 (5), 242 (37); UV and ^1H NMR data agreed well with those reported [13] for the biflavonoid GB-2a.

Isolation of biflavanone GB-1a (**17**). The borax insoluble fraction (2 g) was subjected to CC over polyamide (40 g). Elution with $CHCl_3$–Me_2CO (1:1) gave crude **17** which on purification by prep. TLC afforded pure GB-1a as a pale yellow solid (0.064 g, 0.013%), mp 220–229°, lit. 200° [13]; IR ν_{max}^{KBr} cm^{-1}: 3300, 3200, 1650, 1615, 1200, 850, 780, 710; UV and ^1H NMR data were identical to those reported [13, 14]. Comparison (mmp, co-TLC, and Co-IR) with an authentic sample further confirmed its identity.

Isolation of biflavanone GB-1 (**18**). Elution with $CHCl_3$–Me_2CO (4:6) gave crude GB-1 which after prep. TLC afforded pure biflavanone (**18**) as a pale yellow solid (0.039 g, 0.008%) mp 204–205°, lit. 200° [28]; IR ν_{max}^{KBr} 3350, 1650; the UV spectrum was found to be identical to that reported previously [14]. Comparison (mmp, co-TLC and co-IR) with an authentic sample confirmed its identity.

Isolation of morelloflavone (**19**). Elution of the above polyamide column with $CHCl_3$–Me_2CO (3:7) yielded impure morelloflavone which was purified by prep. TLC to afford pure **19** (0.072 g, 0.014%), mp 300–302°, lit. 304° [29]; IR ν_{max}^{KBr} cm^{-1}: 3300, 1650, 1610, 1200, 860, 785; UV and ^1H NMR data were identical to those reported [14]. Comparison (mmp, co-TLC and co-IR) with an authentic sample further confirmed its identity.

Acknowledgements—The authors thank Prof. R. H. Thomson (University of Aberdeen) and Dr. A. G. M. Barrett (Imperial College) for MS data; Prof. M. U. S. Sultanbawa and Dr. V. Kumar (University of Peradeniya) for authentic samples of biflavanones and triterpenoids; Ms. V. S. B. Imbuldeniya and Z. A. M. Faizal for technical assistance; Mrs. S. C. Weerasekera for typing the manuscript. Financial assistance from the Natural Resources, Energy and Science Authority (Sri Lanka) and

International Foundation for Science (Sweden) is gratefully acknowledged.

REFERENCES

1. Gunatilaka, A. A. L., Sultanbawa, M. U. S., Wimalasena, K. and Balasubramaniam, S. (1983) *Planta Med.* **48**, 61.
2. Kostermans, A. J. G. H. (1980) in *A Revised Handbook to the Flora of Ceylon* (Dassanayake, M. D. and Fosberg, F. R., eds) Vol. 1. Amerind, New Delhi.
3. Sultanbawa, M. U. S. (1973) *J. Nat. Sci. Council, Sri Lanka* **1**, 123.
4. Crichton, E. G. and Waterman, P. G. (1979) *Phytochemistry* **18**, 1553.
5. Cotterill, P. J., Scheinmann, F. and Stenhouse, I. A. (1978) *J. Chem. Soc. Perkin Trans.* 1, 532.
6. Somanathan, R. and Sultanbawa, M. U. S. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1935.
7. Perkin, A. G. and Phipps, S. (1904), *J. Chem. Soc.* 58.
8. Murakami, M. (1936) *Bull. Chem. Soc.* (Japan) **11**, 288.
9. Kubota, T. and Arai, I. (1955) *Nippon Kagaku Zasshi* **76**, 1069.
10. Konoshima, M., Ikeshiro, Y., Nishinaga, A., Matsuura, T., Kubota, T. and Sakamoto, H. (1969) *Tetrahedron Letters* 121.
11. Guerreiro, E., Kunesh, G. and Polonsky, J. (1971) *Phytochemistry* **10**, 2139.
12. Locksley, H. D. (1973) *Fortschritte d. Chem. Org. Naturst.* **30**, 208.
13. Jackson, B., Locksley, H. D., Scheinmann, F. and Wolstenholme, W. A. (1971) *J. Chem. Soc.* (C), 3791.
14. Bandaranayake, W. M., Selliah, S., Sultanbawa, M. U. S. and Ollis, W. D. (1975) *Phytochemistry* **14**, 1878.
15. Herbin, G. A., Jackson, B., Locksley, H. D., Scheinmann, F. and Wolstenholme, W. A. (1970) *Phytochemistry* **9**, 221.
16. Locksley, H. D. and Murray, I. G. (1971) *J. Chem. Soc.* (C) 1332.
17. Silva, A. M. Y. J. de (1983) M. Phil. Thesis, University of Peradeniya, Sri Lanka.
18. Stout, G. H., Hickernell, G. K. and Sears, K. D. (1968) *J. Org. Chem.* **33**, 4191.
19. Meei, L. Y., Ching, C. F. and Chin, H. J. (1975) *Phytochemistry* **14**, 818.
20. Pelter, A., Warren, P. A. R., Chexal, K. K., Handa, B. K. and Raman, W. R. (1971) *Tetrahedron* **27**, 1625.
21. Dominguez, X. A. and Meneses, A. Y. (1974) *Phytochemistry* **13**, 1292.
22. Govindachari, T. R., Viswanathan, N., Pai, B. R., Ramadas Rao, U. and Srinivasan, M. (1967) *Tetrahedron* **23**, 1901.
23. Pelter, A., Warren, R., Hameed, N., Ilyas, I. and Rahman, W. (1971) *J. Indian Chem. Soc.* **48**, 204.
24. Ilyas, M., Usmani, J. N., Bhatnagar, S. P., Rahman, W. and Pelter, A. (1966) *Bull. Chem. Soc.* (*Japan*) **39**, 538.
25. Arthur, H. R., Lee, C. M. and Ma, C. N. (1956) *J. Chem. Soc.* 1461.
26. Choppe, C. W., Howden, M. E. and Jonsten, G. A. R. (1962) *J. Chem. Soc.* 498.
27. Majumdar, G. S. and Thakur, S. (1968) *J. Indian Chem. Soc.* **45**, 785.
28. Cotterill, P. J., Scheinmann, F. and Stenhouse, I. A. (1978) *J. Chem. Soc. Perkin Trans.* 1, 532.
29. Karangaskar, C. G., Radhakrishnan, P. V. and Wolstenholme, W. A. (1967) *Tetrahedron Letters* 787.