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XANTHONES AND NEOFLAVONOIDS FROM TWO ASIAN SPECIES OF *CALOPHYLLUM*

STEPHEN A. AMPOFO* and PETER G. WATERMAN

Phytochemistry Research Laboratories, Department of Pharmacy (Pharm. Chem.), University of Strathclyde, Glasgow G1 1XW, Scotland, U.K.

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Key Word Index—*Calophyllum macrocarpum*; *C. walkeri*; Guttiferae; stem bark; neoflavonoids; 1,3,7-oxygenated xanthones; chemical systematics.

Abstract—The stem barks of both *Calophyllum macrocarpum* and *C. walkeri* have yielded the known neoflavonoid apetalic acid. In addition, *C. walkeri* bark gave four 1,3,7-oxygenated xanthones with prenyl substituents at C-2 and C-8. One of the xanthones has been identified as thwaitesixanthone, and the other three as the related novel derivatives thwaitesixanthonol, demethylcalabaxanthone and 2″-isopropenyl-3″-hydroxydihydrofuranodemethylcalabaxanthone.

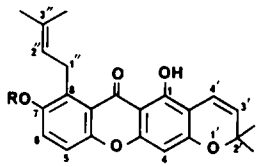
INTRODUCTION

The pan-tropical genus *Calophyllum* L. is a well-known source of prenylated xanthones and neoflavonoids [1]. In this paper we report the results of a study of the chemistry of the stem bark of two species. *C. macrocarpum* is a large rain-forest tree distributed throughout the Malayan peninsula, where it is found particularly on the banks of rivers [2]. As far as we have been able to ascertain, it has not previously been chemically investigated. *C. walkeri* is also a large forest tree and is distributed throughout southern India and Sri Lanka [3]. Previous investigation of Sri Lankan material [4] of *C. walkeri* yielded a number of triterpenes and xanthones, one of which was calabaxanthone (**1**). More recently, the neoflavonoid calozeylanic acid (**2**) has been reported [5].

RESULTS AND DISCUSSION

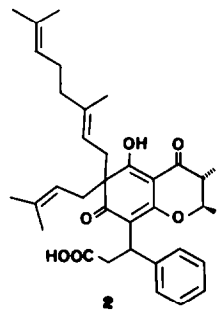
The stem bark of *C. macrocarpum* was extracted with petrol and then ethyl acetate. Column chromatography of the concentrated petrol extract yielded five compounds, three of which were identified as sitosterol, stigmasterol and betulinic acid.

The major compound analysed for $C_{22}H_{28}O_6$ and gave the spectral characteristics of the chromanone acid neoflavonoids which have been isolated from many *Calophyllum* species [5]. It was identified as apetalic acid (**3**) by comparison with previous data [5–8], salient features being (a) a J of 4 Hz between H-2 and H-3 indicative of their *cis* relationship, (b) a large paramagnetic shift of H-6 in the 5-acetate, requiring the pyran ring to be linear, and (c) an OR of + 23°, in agreement with previous reports for apetalic acid [6] but not with the large negative OR recorded for enantiomers [5]. The ^{13}C NMR spectrum of **3** was recorded for the first time (see Experimental) and resonances were assigned by comparison with published data for isoapetalic acid [8].



1 R = CH_3

7 R = H



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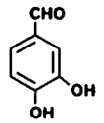

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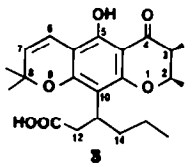

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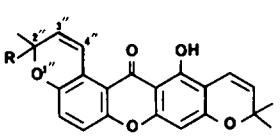
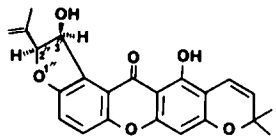


5 R = CH_3

6 R = CH_2OH



8

The most polar compound to be obtained from *C. macrocarpum* was identified as protocatechualdehyde (**4**) by direct comparison with an authentic sample. No xanthones could be detected in this species and this could explain the presence of **4**. Xanthones are thought to be formed from combination of 2C units with a C_6C_1 moiety

*Present address: Department of Chemistry, University of Iowa, Iowa City, IA 52242, U.S.A.

[9], and accumulation of **4** in this species may simply reflect the loss of the enzyme capable of catalysing the coupling of C_6C_1 and 2C units.

Similar treatment of a petrol extract of *C. walkeri* stem bark yielded **3**, the triterpene friedelin and four xanthones. The latter, identified as A–D in order of their elution from a silica gel column, analysed as follows: A ($C_{23}H_{20}O_5$), B ($C_{23}H_{20}O_6$), C ($C_{23}H_{22}O_5$) and D ($C_{23}H_{20}O_6$). All four shared a number of features in common.

(a) The UV spectra were characteristic of 1-hydroxyxanthones oxygenated at C-1, C-3 and C-7 [10].

(b) The ^{1}H NMR spectra showed signals typical of a 2,2-dimethylpyran moiety situated at C-2 and C-3 of the xanthone. For xanthones A and B, this was confirmed by preparation of the 1-acetoxy derivative with the anticipated shift of the *peri* H-4′ proton (Table 1).

(c) All four xanthones gave a positive Gibbs test suggesting the presence of a free position at C-4.

(d) The presence of deshielded resonances in all four compounds assignable to the proton(s) of the α-carbon of the second prenyl unit indicated that it was situated at C-8 within the deshielding cone of the *peri*-carbonyl.

The ^{1}H NMR spectrum (Table 1) of the major xanthone (A) revealed two 2,2-dimethylpyran units identifying it as thwaitesixanthone (**5**), which has previously been reported as a constituent of *C. thwaitesii* [4]. The ^{1}H NMR spectrum of xanthone D (Table 1) was very similar to that of **5**, again showing AB quartets for the olefinic protons of two pyran ring systems. However, the spectrum of D showed the presence of only three methyl resonances, gave an additional AB quartet (δ3.76, 3.66, J = 11.7 Hz) indicative of a hydroxymethyl substituent, and had non-equivalent chemical shifts for the H-5 and H-6 protons. From these data xanthone D was obviously identical to **5** except that one of the methyls had been oxidized to give a primary alcohol. Gentle acetylation yielded a monoacetate, the hydrogen-bonded C-1 phenolic group still being present. The ^{1}H NMR spectrum of the acetate showed the anticipated deshielding of the oxymethylene and one methyl resonance. In addition, a small shielding of the H-3″ proton was observed but there was no effect on H-3′. The structure of xanthone D must therefore be **6**, to which we have assigned the trivial name of thwaitesixanthonol.

Xanthone C gave spectral data very similar to those for calabaxanthone (**1**) but lacked the resonance for a methoxyl substituent. Cyclization using DDQ gave a product identical by TLC to **5**, confirming that xanthone C was demethylcalabaxanthone (**7**).

The ^{1}H NMR spectrum of the final xanthone

Table 1. ^{1}H NMR chemical shifts for **5–8**, the monoacetates of **5** and **6** and the diacetate of **8**

Proton No.	A **5**	**5-Ac**	D **6**	**6-Ac**	C **7**	B **8**	**8-Ac**
1-OH	13.47	—	13.46	13.45	13.50	12.86	—
H-4	6.24	6.66	6.28	6.28	6.24	6.35	6.69
H-5	7.13	7.12	7.18	7.18	7.18	7.26	7.31
H-6	7.13	7.14	7.23	7.21	7.18	7.39	7.40
2′-Me_2	1.45	1.41	1.48	1.47	1.46	1.49	1.49
H-3′	5.59	5.71	5.59	5.59	5.55	5.63	5.75
H-4′	6.72	6.47	6.73	6.73	6.72	6.74	6.48
7,8-Pyran							
2″-Me_2	1.45	1.46	1.40*	1.47*	—	—	—
2″-OCH_2	—	—	3.67	4.11	—	—	—
	—	—	3.76	4.21	—	—	—
H-3″	5.78	5.84	5.81	5.74	—	—	—
H-4″	8.00	7.95	8.19	8.19	—	—	—
7,8-Furan							
H-2″	—	—	—	—	—	5.11	5.02
H-3″	—	—	—	—	—	5.69	6.44
=Me	—	—	—	—	—	1.84	1.84
$=CH_2$	—	—	—	—	—	4.99	4.89
	—	—	—	—	—	5.22	5.01
3″-OH	—	—	—	—	—	4.86	—
8-Prenyl							
1″-CH_2	—	—	—	—	4.27	—	—
2″=CH	—	—	—	—	5.32	—	—
3″-Me	—	—	—	—	1.76	—	—
	—	—	—	—	1.87	—	—
OAc	—	2.49	—	2.03	—	—	2.09/2.46

*Signal for one Me only.

J values: in all xanthones $J_{3'-4'}$ = 10; J_{5-6} = 9 Hz; in **5** and **6**: $J_{3''-4''}$ = 10 Hz; in **6**: J_{gem} for 2″-OCH_2 = 11.7 Hz; in **7**: $J_{1''-2''}$ = 6 Hz; in **8**: $J_{4-4'}$ = 0.8; $J_{2''-3''}$ = 6; $J_{6-3''}$ = 0.8 Hz.

(xanthone B) gave data for ring A and its linear pyran substituent comparable to that for the other three compounds (Table 1). For ring B, the resonances of H-5 and H-6 showed marked non-equivalence (0.13 ppm) whilst the signals for the second prenyl group revealed a single vinylic methyl (δ1.83), an exocyclic methylene (δ4.98, 5.21), two oxymethine protons (δ5.11, 5.69) and a non-phenolic hydroxyl (δ4.86). These resonances are indicative of a 2-isopropenyl-3-hydroxydihydrofuran system, similar to that found in the coumarin xanthoarnol [11] and, without the hydroxyl substituent, in rotenone [12]. The strongly deshielded resonance of the H-3″ oxymethine proton must be attributed to it lying *peri* to the xanthone carbonyl and to it approaching planarity with the xanthone nucleus, a hypothesis supported by the appearance of long-range (*Z*-bond) coupling between this proton and H-6. The coupling constant of 6 Hz between H-3″ and H-2″ indicated a *cis* relationship. This was supported by acetylation of the 3″-hydroxyl group which caused further deshielding of H-3″ to 6.44 but shielding of the protons of the exocyclic methylene by 0.10 and 0.21 ppm, suggesting that they lie on the same side of the furan ring as the acetyl group. On this basis, xanthone D has been assigned structure **8**.

This is the first report on the phytochemistry of Indian material of *C. walkeri* and it is interesting to compare its chemistry with that of Sri Lankan material of this and the closely allied species *C. trapezifolium* Thw. and *C. thwaitesii* Planch. *et* Triana. Xanthones with 1,3,7-substitution are known from all three taxa: **1** from the first two and **5** from *C. thwaitesii* (which also yielded friedelin [4]). Although xanthones with different oxygenation patterns are known from the heartwood of all three Sri Lankan taxa, only *C. trapezifolium* has yielded another pattern (1,3,5-oxygenation) from the bark. The stem barks of all three Sri Lankan taxa have also yielded chromone acids: *C. walkeri* and *C. thwaitesii* yield **2** whilst *C. trapezifolium* yields the 2,3-*trans* isomer of **3** [5]. Thus the bark chemistry of Indian *C. walkeri* shares common features with all three Sri Lankan taxa.

EXPERIMENTAL

Mps are uncorr. UV: EtOH. IR: KCl discs. ^{1}H and ^{13}C NMR: run in $CDCl_3$ with TMS as internal standard (field strength in text). EIMS were run at 70 eV at elevated temp. Petrol refers to bp 40–60° fraction.

Plant material. Stem bark of *C. macrocarpum* Hook. f. was collected from the Kuala Lompat study area in the Krau Game Reserve, Malaysia [13]. A voucher specimen has been deposited at the National Herbarium at Kepong. *C. walkeri* Wight was collected in the Kakachi study area in the primary tropical evergreen forest of the Agastya Malai range of the western Ghat mountains [14].

Isolation of compounds from C. macrocarpum. Ground stem bark (700 g) was extracted with petrol and then with EtOAc. TLC analysis revealed several compounds in the petrol extract but only polyphenolics in the EtOAc extract, which was discarded. CC of the petrol extract over silica gel gave, on elution with toluene, large amounts of fats. Further elution with toluene, acidified with HOAc and containing increasing amounts of EtOAc, gave (a) with 10% EtOAc, **3** (43 mg) followed by a mixture of sitosterol and stigmasterol: (b) with 20% EtOAc, betulinic acid (14 mg); and (c) with 50% EtOAc, **4** (19 mg).

Apetalic acid (**3**). Amorphous solid. Found: $[M]^+$ 388.1859; $C_{22}H_{28}O_6$ requires: 388.1886. UV λ_{max} nm: 264, 273, 312, 364; (+NaOH) 273, 290, 380. IR ν_{max} cm^{-1}: 3500, 1710, 1640. ^{1}H NMR (90 MHz): δ0.86 (3H, *t*, *J* = 7 Hz, Me-16), 1.16 (3H, *d*, *J* = 7 Hz, 3-Me), 1.30 (3H, *d*, *J* = 7 Hz, 2-Me), 1.41, 1.46 (2 × 3H, 2 × *s*, 8-Me$_2$), 2.53 (1H, *dq*, *J* = 7, 4 Hz, H-3), 2.79 (2H, *d*, *J* = 7 Hz, 12-CH$_2$), 3.72 (1H, *m*, H-11), 4.54 (1H, *dq*, *J* = 7, 4 Hz, H-2), 5.54, 6.55 (2H, AB*q*, *J* = 10 Hz, H-7, H-6), 9.80 (1H, *br s*, COOH), 12.60 (1H, *br s*, 5-OH). ^{13}C NMR (62.5 Hz): singlets at 78.7 (C-8), 100.8, 100.9 (C-4a, C-10), 110.0 (C-5a), 154.0 (C-5), 159.9 (C-10a), 162.3 (C-9a), 178.7 (C-13), 200.6 (C-4); doublets at 30.2 (C-11), 44.4 (C-3), 76.0 (C-2), 115.8 (C-7), 125.6 (C-6); triplets at 20.8 (C-15), 35.2 (C-14), 38.1 (C-12); quartets at 9.40 (3-Me), 13.9 (C-16), 16.4 (2-Me), 28.4 (8-Me$_2$). EIMS *m/z* (rel. int.): 388 $[M]^+$ (16), 373 (100). *Apetalic acid methyl ester.* **3** (15 mg) was methylated using excess CH_2N_2, the product being purified by centrifugal prep. TLC to give the Me ester (12 mg) as a yellow gum, $[\alpha]_D^{25}$ + 23° (*c* 0.1; $CHCl_3$) (lit. [6] + 28.4°). ^{1}H NMR (90 MHz): δ3.60 (3H, *s*, OMe). *Acetylation of apetalic acid methyl ester.* The Me ester of **3** (9 mg) was acetylated using normal procedures to yield the corresponding 5-acetate (5 mg). ^{1}H NMR (90 MHz): δ2.35 (3H, *s*, OAc), 5.55, 6.32 (2H, AB*q*, *J* = 10 Hz, H-7, H-6).

Betulinic acid. Needles from MeOH, mp > 300°. Found: $[M]^+$ 456.3590; $C_{30}H_{48}O_3$ requires: 456.3603. Identical in all respects (TLC, IR, ^{1}H NMR, EIMS) with authentic material.

Protocatechualdehyde (**4**). Cluster crystals from MeOH, mp 156°. Found: $[M]^+$ 138.0272; $C_7H_6O_3$ requires: 138.1317. Identical in all respects (mmp, TLC, UV, IR, ^{1}H NMR) with authentic material.

Extraction of compounds from C. walkeri. Ground stem bark (400 g) was extracted with petrol and then with EtOAc. TLC examination of the two extracts revealed the presence of several compounds in the petrol extract but nothing of note in the EtOAc extract. CC of the petrol extract over silica gel eluting with petrol and then with petrol–EtOAc mixtures gave the following: (a) with petrol, **5** (110 mg); (b) with 1% EtOAc, friedelin (52 mg); (c) with 2% EtOAc, impure **8** which was then subjected to prep. TLC (silica gel; petrol–EtOAc, 9:1) to give **8** (5 mg), 3% EtOAc, **7** (7 mg) and 5% EtOAc, **6** (15 mg). Further elution with EtOAc gave a mixture of acidic compounds from which **3** (56 mg, identified by direct comparison with **3** above) was separated by centrifugal prep. TLC (silica gel, toluene–EtOAc–HOAc, 90:10:1).

Thwaitesixanthone (**5**). Yellow needles from petrol–EtOAc, mp 231° (lit. [4] 221–223°). Found: $[M]^+$ 376.1298; $C_{23}H_{20}O_5$ requires: 376.1311. UV λ_{max} nm: 225, 245, 283, 290, 327, 404. ^{1}H NMR (90 MHz): see Table 1. IR, EIMS, ^{13}C NMR in close agreement with lit. [4, 8]. Treatment of **5** (10 mg) with Ac_2O in C_5H_5N yielded the corresponding acetate. ^{1}H NMR (250 MHz): see Table 1.

Friedelin. Needles from petrol–EtOAc, mp 260°. Identical (TLC, IR, ^{1}H NMR, mmp) with an authentic sample.

Thwaitesixanthonol (**6**). Yellow prisms from petrol–EtOAc, mp 176°. Found: $[M]^+$ 392.1268; $C_{23}H_{20}O_6$ requires: 392.1260. UV λ_{max} nm: 245, 283, 290, 327, 404; (+NaOH) 258, 282, 313, 360. IR ν_{max} cm^{-1}: 3400, 1655, 1610, 1460, 1300, 1250, 820, 720. ^{1}H NMR (250 MHz): see Table 1. EIMS *m/z* (rel. int.): 392 $[M]^+$ (4), 377 $[M - Me]^+$ (3), 361 $[M - CH_2OH]^+$ (100), 173 (77)—doubly charged ion. *Thwaitesixanthonol monoacetate.* **6** (12 mg) was acetylated by normal procedures to give a monoacetate (4 mg); ^{1}H NMR (250 MHz): see Table 1.

Demethylcalabaxanthone (**7**). Yellow needles, mp 85°. Found: $[M]^+$ 378.1440; $C_{23}H_{20}O_5$ requires: 378.1467. UV λ_{max} nm: 235, 286, 315, 385; (+NaOH) 240, 300, 415. IR ν_{max} cm^{-1}: 3400, 1645, 1600, 1460, 1140, 820. ^{1}H NMR (90 MHz): see Table 1. EIMS *m/z* (rel. int.): 378 $[M]^+$ (45), 363 $[M - Me]^+$ (100), 335 $[M - C_3H_7]^+$ (34), 319 $[M - C_5H_9]^+$ (39). *Cyclization of* **7**.

7 (3 mg), DDQ (20 mg) and C_6H_6 were refluxed for 2 hr, cooled and filtered. TLC of the reaction mixture revealed the presence of **5**.

2"-Isopropenyl-3"-hydroxydihydrofuranodemethylcalabaxanthone (**8**). Yellow amorphous solid from petrol–EtOAc. Found: $[M]^+$ 392.1257; $C_{23}H_{20}O_6$ requires: 392.1260. UV λ_{max} nm: 225, 287, 315, 345, 390; (+ NaOH) 230, 315, 420. ^{1}H NMR (250 MHz): see Table 1. EIMS *m/z* (rel. int.): 392 $[M]^+$ (43), 377 $[M - Me]^+$ (32), 359 $[M - Me - H_2O]^+$ (100). *Acetylation* of **8** (4 mg) for 24 hr at 60° following normal procedures gave the corresponding diacetate; ^{1}H NMR spectrum: see Table 1.

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