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Xanthones from *Calophyllum teysmannii* var. *inophylloide*

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Abstract

Further study of the wood of *Calophyllum teysmannii* Miq. var. *inophylloide* from Thailand yielded the xanthones 1,2,8-trimethoxyxanthone, 6-hydroxy-1,2,5,7-tetramethoxyxanthone and 3-(3′-carboxybutyl)-4-methoxyxanthone (teysmannic acid) in addition to scriblitifolic acid, 1,7-dihydroxyxanthone and the benzoic acid derivative leiocarpic acid. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Calophyllum teysmannii var. *inophylloide*; Guttiferae; Xanthones; 1,2,8-Trimethoxyxanthone; 6-Hydroxy-1,3,5,7-tetramethoxyxanthone; 6-(3′-carboxybutyl)-5-methoxyxanthone; Scriblitifolic acid; Leiocarpic acid

1. Introduction

Xanthones are prominent constituents of the wood of *Calophyllum* species (Guttiferae), but recent attention has centered on a group of dipyranocoumarins from several Southeast Asian *Calophyllum* species which act as potent inhibitors of HIV-1 reverse transcriptase. Among the sources are leaves and twigs of an unspecified variety of *C. teysmannii* Miq. from Malaysia (Patil et al., 1993), latex, leaves and twigs of collections of *C. teysmannii* Miq. var. *inophylloide* (King) P. Stephen from Sarawak (Gustafson et al., 1994; Fuller et al., 1994; McKee et al., 1996, 1998) and stem bark of the same variety from Sabah (Cao, Sim, Pereira & Goh, 1996a, 1996b; Cao, Chang, Vittal, Sim & Goh, 1998, Cao et al., 1998). However, there are no reports on other constituents of *C. teysmannii* var. *inophylloide*.

We recently described isolation of nine known xanthones from the wood of a collection of *C. teysmannii* var. *inophylloide* from Narathiwat Province, Southern Thailand, and their immunomodulatory activity (Gonzalez et al., 1999). Further work on the extract has now resulted in isolation of euxanthone (**1**; Locksley, Moore & Scheinmann, 1966), the new xanthones 1,2,8-trimethoxyxanthone (**2**) and 1,3,5,7-tetramethoxyxanthone (**3**), scriblitifolic acid (**4a**; Jackson, Locksley & Scheinmann, 1967) and a new analog **4c** of the latter, which we have named teysmannic acid. An additional component of the extract was the benzoic acid derivative leiocarpic acid (**5**; Foetsch, Gründemann, Pfeifer, Hiller & Salzwedel, 1982).

2. Results and discussion

The ^{1}H-NMR spectrum ($CDCl_3$) of xanthone **2**, $C_{16}H_{14}O_5$, exhibited three–OMe signals at δ 3.88, 3.97 and 4.00, two mutually coupled doublets of vicinal aromatic protons at δ 7.25 and δ 7.11 in one of the rings and a system of three mutually coupled aromatic protons at δ 7.50 (*t*, J = 8.4 Hz), 6.92 (*dd*, J = 8.4, 1 Hz) and 6.72 (*dd*, J = 8.4, 1 Hz) in the other. The

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chemical shifts of the vicinal system in the first ring compared with those of H-3 and H-4 in 1,2-dimethoxy-8-hydroxyxanthone, while those in the second ring compared with the shifts of H-5, H-6 and H-7 in 1,8-dimethoxy-2-hydroxyxanthone (Gonzalez et al., 1999). The ^{13}C-NMR spectrum (Table 1) exhibited three–OMe signals, one of which (δ 61.6) was flanked by the xanthone carbonyl carbon and another–OMe. The C=O signal was found at δ 176.3, while C-9a appeared at δ 118.5. The remaining signals coincided with those in ring A of 1,2-dimethoxy-8-hydroxyxanthone and in ring B of 1,8-dimethoxy-2-hydroxyxanthone (Gonzalez et al., 1999). Consequently, the new substance was 1,2,8-trimethoxyxanthone (**2**).

The ^{1}H-NMR spectrum of xanthone **3**, $C_{17}H_{16}O_7$, exhibited four–OMe signals, the singlet of an aromatic proton at δ 7.45 whose chemical shift indicated that it was located between the xanthone carbonyl and a hydroxyl or—OMe group, i.e. at C-1 or C-8, and signals of two *meta*-coupled protons at δ 6.56 and 6.32 whose chemical shifts were essentially identical with H-2 and H-4 of 1,3-dimethoxyxanthones isolated previously (Gonzalez et al., 1999). In the ^{13}C-NMR spectrum (Table 1), the chemical shift of the C=O signal (δ 174.7) indicated that the one hydroxyl group required by the empirical formula was not located on either C-1 or C-8; furthermore, only one of the four—OMe signals had a chemical shift higher than 60 ppm which indicated that only one of the four—OMe groups, that on C-5, was flanked by two oxygenated substituents.

Table 1
^{13}C-NMR spectral data of **2**, **3**, **4a**, **4b** and **4c** (50 MHz, CDCl$_3$)[a]

C	**2**	**3**	**4a**	**4b**	**4c**
1	149.2	161.8	161.8	150.0	126.7
2	148.6	95.4	110.6	118.3	124.1
3	119.0	164.5	137.7	134.5	134.8
4	112.2	92.8	107.1	116.1	118.1
4a	150.1	159.6	155.6	156.9	155.8
5	105.1	134.1	146.0	145.9	146.2
6	134.2	143.9	142.4	141.6	141.6
7	109.3	144.6[b]	125.2	125.2	125.0
8	160.5	100.8	120.4	118.3	121.2
8a	113.0	115.7	120.3	122.0	121.6
9	176.3	174.7	182.1	175.6	177.0
9a	118.5	106.8	108.7	114.8	121.6
10a	157.3	144.6[b]	149.8	149.0	149.8
1-OMe	61.6	56.5[c]			
2-OMe	56.9				
3-OMe		56.4[c]			
5-OMe		61.7	61.7	61.7	61.7
7-OMe		55.8[c]			
8-OMe	56.3				
1′			28.0	27.9	28.0
2′			33.7	33.9	34.0
3′			39.0	40.0	39.0
4′			182.3	181.8	181.1
5′			17.0	17.9	17.0
Ac				169.9, 21.2	

[a] Assignment of multiplets by DEPT and HETCOR.

[b] Double intensity.

[c] Interchangeable assignments.

The HMBC spectrum of **3** illustrated in Fig. 1, which shows connectivities between hydrogens and carbons in the range of two or three bonds, demonstrated that the proton appearing at δ 6.32 (H-2) was correlated with carbons at δ 92.8 (C-4), 106.8 (C-9a), 161.8 (C-1) and 164.5 (C-3) and that the proton appearing at δ 6.56 (H-4) was correlated with carbons at δ 95.4 (C-2), 106.8 (C-9a), 159.6 (C-4a) and 164.5 (C-3), thus establishing the nature of ring A. Likewise in ring B, the proton appearing at δ 7.45 (C-8) was correlated with carbons at δ 115.7 (C-8a), 144.6 (C-7, 10a) and 174.7 (C-9), while the protons of the methoxy groups were correlated with the carbons to which they were attached in the manner shown and listed in Table 1, thus completing the structure assignment. Hence, **3** is the 1,3-dimethyl ether of 1,3,6-trihydroxy-5,7-dimethoxyxanthone recently reported from *C. austroindicum* (Iinuma, Tosa, Toriyama, Tanaka, Ito & Chelladurai, 1996).

^{13}C-NMR spectra of scriblitifolic acid (**4a**) and its acetate **4b** have not been recorded previously and are included in Table 1. The structure of its new 1-deoxy derivative teysmannic acid (**4c**; in the absence of a substituent on C-1, the numbering of ring carbons is chosen parallel to that of **4a**) was obvious from the ^{1}H-NMR spectrum (see Section 3) which showed that ring A was unsubstituted and from the ^{13}C-NMR spectrum (Table 1) when the chemical shifts of ring B were compared with those of **4a**.

A minor constituent of the extract was leiocarpic acid (**5**). This substance has been obtained earlier by hydrolysis of the main glycoside constituent of *Solidago virgaurea* L. var. *leiocarpa* (Benth.) A. Gray (Hiller, Gil-Rjong, Franke & Gründemann, 1979; Foetsch et al., 1982) but has never, to our knowledge, been reported either as a natural product or by synthesis. It may perhaps be viewed as a precursor of a shikimate-acetate derived benzophenone implicated in the biosynthesis of xanthones found in higher plants (Locksley, Moore & Scheinmann, 1969; Carpenter, Locksley & Scheinmann, 1969).

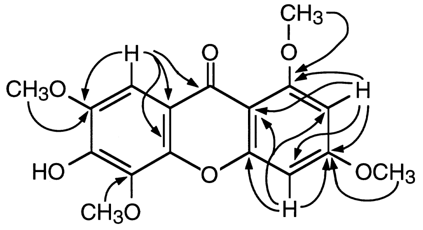
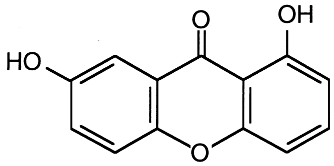
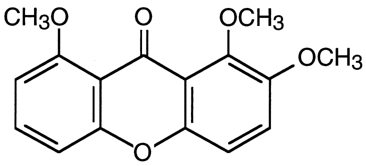


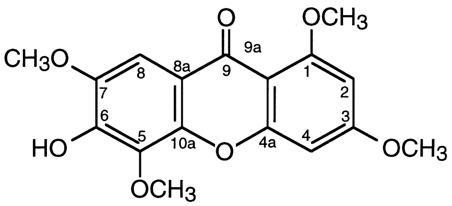
Fig. 1. HMBC correlations of **3**.



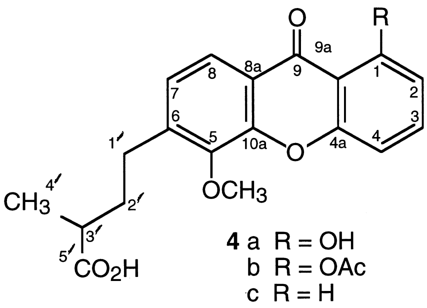
1



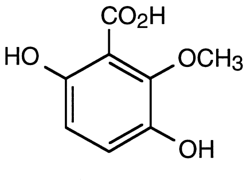
2



3



4 a R = OH
b R = OAc
c R = H



5

3. Experimental

Extraction of the wood of *C. teysmannii* var. *inophylloide* from Narathiwat Province, Southern Thailand, was described earlier (Gonzalez et al., 1999). The crude $CHCl_3$ extract (20 g) from 600 g of dry powdered wood was chromatographed over silica gel (200 g) and eluted with petrol–$CHCl_3$ and $CHCl_3$–Me_2O, 500 ml frs. being collected as follows: frs. 1–60 (petrol–$CHCl_3$, 3 : 2), 61–89 (petrol–$CHCl_3$, 1 : 1) 90–100 (petrol–$CHCl_3$, 2 : 3), 101–110 (petrol–$CHCl_3$, 1 : 4), 111–120 ($CHCl_3$), 121–177 ($CHCl_3$–Me_2O, 9 : 1), 178–225 ($CHCl_3$–Me_2O, 7 : 3), 245–284 ($CHCl_3$–Me_2O, 1 : 1). Frs. 4–27 and frs. 74–86 were studied earlier (Gonzalez et al., 1999) and furnished 1-methoxy-2-hydroxyxanthone, 1-methoxy-2,-dihydroxyxanthone, 1,8-dimethoxy-2-hydroxyxanthone, 1,2-dimethoxy-8-hydroxyxanthone, 1,2-dimethoxy-3,8-dihydroxyxanthone, 1,6-dihydroxy-5methoxyxanthone, 1,3-dimethoxy-5,8-dihydroxyxanthone, 1,3-dihydroxy-4,6,8-trimethoxyxanthone and 1,5-dihydroxy-6-(4-hydroxy-3-methylbutyl)-xanthone. Frs. 28–30 (620 mg) were rechromatographed (silica gel 60), 50 ml subfrs. being collected as follows: subfrs. 1–49 (petrol–$CHCl_3$, 4 : 1) and subfrs. 50–116 (petrol–$CHCl_3$, 7 : 3). Purification of subfrs. 24–40 by TLC (silica gel, petrol–EtOAc–HCO_2H, 70 : 30 : 1) gave more 1-methoxy-2,8-dihydroxyxanthone (20 mg), while similar purification of subfrs. 53–67 by TLC gave more 1,2-dimethoxy-3,8-dihydroxyxanthone (12 mg) and more 2-hydroxy-1-methoxyxanthone (21 mg). Combination of subfrs. 68–87 followed by TLC (silica gel, petrol–EtOAc–HCO_2H, 70 : 30 : 1) gave, in order of elution, xanthone **3** (21 mg), more 2-hydroxy-1-methoxyxanthone (10 mg), more 1,2-dimethoxy-3,8-dihydroxyxanthone (12 mg), and euxanthone (**1**, 17 mg).

Frs. 87–159 (0.415 mg) were also rechromatographed (silica gel 60), 50 ml subfrs. being collected as follows: subfrs. 1–118 (petrol–$CHCl_3$, 7 : 3), 119–158 (petrol–$CHCl_3$, 1 : 1) and 159–200 (petrol–$CHCl_3$, 1 : 9). Purification of subfrs. 65–157 (107 mg) by TLC (silica gel, $CHCl_3$–Me_2O–HCO_2H, 90 : 10 : 0.5) gave leiocarpic acid (**5**, 10 mg). Frs. 177–225 (1.2 g) were combined and rechromatographed (silica gel 60), 100 ml subfrs. being collected as follows: subfrs. 1–20 (petrol–$CHCl_3$, 1 : 9), 21–37 (petrol–$CHCl_3$, 1 : 1), 38–99 (silica gel, $CHCl_3$–Me_2O–HCO_2H, 70 : 30 : 0.5), 100–130 (petrol–$CHCl_3$, 1 : 9). Subfrs. 21–27 were combined and recrystallized from petrol–$CHCl_3$ to give scriblitifolic acid (**4a**) (30 mg). Subfrs. 39–67 on combination and purification by TLC (silica gel, $CHCl_3$–Me_2O–HCO_2H, 70 : 30 : 0.5) furnished more **4a** (15 mg) and xanthone **3** (27 mg), while TLC of subfrs. 68–78 (silica gel, $CHCl_3$–Me_2O–HCO_2H, 70 : 30 : 0.5) furnished additional **4a** (17 mg).

Frs. 226–275 (1.6 g) were combined and rechromatographed (silica gel 60), 100 ml subfrs. being collected as follows: 1–22 (petrol–$CHCl_3$, 7 : 3), 23–40 (petrol–$CHCl_3$, 1 : 1), 41–59 (petrol–$CHCl_3$, 3 : 7), 76–100 ($CHCl_3$), 101–142 ($CHCl_3$–Me_2O, 9 : 1), 143–160, ($CHCl_3$–Me_2O, 7 : 3), and 161–180 ($CHCl_3$–Me_2O, 1 : 1). Subfrs. 95–156 were combined and purified by TLC ($CHCl_3$–Me_2O–HCO_2H, 90 : 10 : 0.5) to give **5** (30 mg).

3.1. 1,2,8-Trimethoxyxanthone (**2**)

Mp 139–141°C (MeOH); EI MS *m/z* (rel. int): 286 [M^+] (65), 271 (100), 235 (15), 237 (40), 228 (45), 211 (15), 200 (15); FAB HRMS + H^+, *m/z* 287.0919; calcd. for $C_{16}H_{14}O_5 + H^+$, 287.0919; UV (MeOH) λ_{max} nm (log ε): 365 (3.7), 241 (4.5), 203 (4.3); IR λ_{max}^{KBr} cm^{-1}: 3461, 3388, 2923, 2854, 1668, 1600, 1473, 1267, 1091, 1035; ^{1}H-NMR spectral data (200 MHz, $CDCl_3$): δ 7.50 (*dd*, *J* = 8.4, 8.4 Hz, H-6), 7.25 (*d*, *J* = 9.2 Hz, H-3), 7.11 (*d*, *J* = 9.2 Hz, H-4), 6.92 (*dd*, *J* = 8.4, 1.0 Hz, H-5), 6.72 (*dd*, *J* = 8.3, 1.0 Hz, H-7), 4.00 (*s*, 3p, 1-OMe), 3.97 (*s*, 3p, 2-OMe), 3.88 (*s*, 3p, 8-OMe).

3.2. 6-Hydroxy-1,3,5,7-tetramethoxyxanthone (**3**)

Mp 284–286°C (MeOH); EI MS *m/z* (rel. int): 332 [M^+] (100), 317 (20), 286 (20), 264 (25), 149 (50); FAB HRMS + H^+, *m/z* 333.0974; calcd. for $C_{17}H_{16}O_7 + H^+$, 333.0974; UV (MeOH) λ_{max} nm (log ε): 338 (3.4), 306 (3.6), 250 (4.0) 202 (4.0); UV (MeOH + NaOH) λ_{max} nm (log ε): 369 (3.7), 239 (4.0), 211 (4.4); ^{1}H-NMR spectral data (300 MHz, $CDCl_3$): δ 7.45 (*s*, H-8), 6.56 (*d*, *J* = 2.3 Hz, H-4), 6.32 (*d*, *J* = 2.3, Hz, H-4), 4.08 (*s*, 3p, 5-OMe), 3.96 (*s*, 3p, 7-OMe), 3.94 (*s*, 3p, 1-OMe), (*s*, 3-OMe).

3.3. Scriblitifolic acid (**4a**)

Mp 163–164°C (petrol–$CHCl_3$); lit. 164–167°C; EI MS *m/z* (rel. int.): 342 (100), 268 (65), 253 (25), 241 (15), 225 (30), 197 (15); ^{1}H-NMR spectral data (300 MHz, $CDCl_3$): δ 12.63 (–OH), 7.91 (*d*, *J* = 8.6 Hz, H-8), 7.58 (*dd*, 8.4, 8.4 Hz, H-4), 7.19 (*d*, *J* = 8.3 Hz, H-7), 6.98 (*dd*, *J* = 8.4, 0.9 Hz, H-4), 6.78 (*dd*, *J* = 8.3, 0.9 Hz, H-2), 4.02 (*s*, 3p, 5-OMe), 2.82 (*t*, *J* = 8 Hz, 2p, H-1′a,b), 2.54 (*m*, H-3′) 2.04 and 1.76 (both *m*; H-2′a,b), 1.26 (*d*, *J* = 7 Hz, 3p, H-4′); ^{13}C-NMR spectral data in Table 1. The acetate **4b** was prepared in the usual manner; mp 139–141°C; ^{1}H-NMR spectral data (300 MHz, $CDCl_3$): δ 7.89 (*d*, *J* = 8.3 Hz, H-8), 7.68 (*dd*, *J* = 8.0, 8.1 Hz, H-3), 7.46 (*dd*, *J* = 8.0, 1.1 Hz, H-2), 7.16 (*d*, *J* = 8.3 Hz, H-7), 6.99 (*d*, *J* = 8.0, 1.1 Hz, H-4), 4.01 (*s*, 3p, 5-OMe), 2.80 (*t*, *J* = 8.1 Hz, 2p, H-1′a,b), 2.52 (*m*, H-3′), 2.47 (*s*, 3p, Ac), 2.03 and 1.7 (both *m*, H-2′a,b), 1.25 (*d*, *J* = 7 Hz, 3p, H-4′); ^{13}C-NMR spectral data in Table 1.

3.4. Teysmannic acid (**4c**)

Viscous liquid; EI MS *m/z* (rel. int.): 326 [M^+] (80), 253 (100), 238 (30), 237 (40), 209 (35), 181 (25), 149 (20); FAB HRMS + H^+, *m/z* 327.1232; calcd. for $C_{19}H_{18}O_5 + H^+$, 327.1235; ^{1}H-NMR spectral data (200 MHz, $CDCl_3$): δ 8.31 (*ddd*, *J* = 8.0, 1.5, 0.4 Hz, H-1), 7.99 (*d*, *J* = 8.3 Hz, H-8), 7.72 (*ddd*, *J* = 8.5, 7.0, 1.7 Hz, H-2), 7.54 *ddd* (*J* = 8.5, 1.0, 0.4 Hz, H-4), 7.37 (*ddd*, *J* = 8.0, 7.0, 1.1 Hz, H-3), 7.18 (*d*, *J* = 8.3 Hz, H-7), 4.04 (*s*, 3p, –OMe), 2.82 (*t*, 7.8 Hz, 2p, H-1′a,b), 2.55 (*m*, H-3′), 2.07 and 1.79 (both *m*, H-2′a,b), 1.26 (*d*, *J* = 7, 3p, H-4′); ^{13}C-NMR spectral data in Table 1.

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