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TWO XANTHONES FROM ROOTS OF *CALOPHYLLUM INOPHYLLUM*

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Key Word Index—*Calophyllum inophyllum*; Clusiaceae; roots; heartwood; xanthone; caloxanthones D and E.

Abstract—From the root bark of *Calophyllum inophyllum*, a new xanthone named caloxanthone D and from the root heartwood, another new xanthone, caloxanthone E, in addition to four known xanthones [1,3,8-trihydroxy-7-methoxy-, 1,3-dihydroxy-7,8-methoxy-, 1,3,5-trihydroxy-2-methoxy- and 6-hydroxy-1,5-dimethoxy-] were isolated. The structures were determined by analysis of NMR spectral data including 2D techniques.

INTRODUCTION

In our previous papers, the structures of three new xanthones, caloxanthones A, B [1] and C [2] in the root bark of *Calophyllum inophyllum* were described as well as the structure of the simple oxygenated xanthones in the root heart. The chemical composition between the root bark and the root heartwood is markedly different. To characterize the localization of the xanthones based on the presence or absence of isoprenyl group(s), xanthone derivatives in the underground parts were further isolated. In the present paper, further structures of a new prenylated xanthone, caloxanthone D, in the root bark and a new simple oxygenated xanthone, caloxanthone E, in the root heartwood are described.

RESULTS AND DISCUSSION

Underground parts of *C. inophyllum* collected in Okinawa, Japan were divided into root bark and root heartwood. Each part was extracted with *n*-hexane, acetone, methanol and 70% methanol, successively, after being dried and ground. The acetone extract of the root bark was repeatedly chromatographed on silica gel and Sephadex LH-20 to give compound **1**. The acetone extract of the root heartwood suspended in water was partitioned between benzene, ethyl acetate and *n*-butanol, successively. The combined benzene and ethyl acetate layers were subjected to vacuum liquid chromatography (VLC) on silica gel to give compounds **2**–**6**.

Compound **1**, caloxanthone D, obtained as a pale yellow amorphous, reacted positively to the Gibbs and $FeCl_3$ tests. The high resolution EI mass spectrum showed the $[M]^+$ at *m/z* 426.1343 which corresponds to $C_{23}H_{22}O_8$. UV and IR spectra indicated that **1** was a xanthone derivative. The ^1H NMR spectrum showed the presence of two aromatic protons [δ6.35 and 7.42 (1H each, *s*)], two phenolic hydroxyl groups [δ9.96 (1H, *br s*) and 13.56 (1H, *s*, chelated)] and a dimethylchromene ring [δ1.43, 1.44 (3H each, *s*, Me × 2) and 5.76, 6.62 (1H, each, *d*, J = 10 Hz, *cis*-olefinic protons)]. In the HMBC spectrum (Fig. 1), the chelated hydroxyl group caused three cross-peaks with three quaternary carbons at δ102.4, 103.7 and 156.6. One of the quaternary carbons (δ156.6) was correlated to the *cis*-olefinic proton of the chromene ring (δ6.62), the quaternary ones (δ102.4 and 103.7) to the aromatic proton (δ6.35). These results indicated that the chelated hydroxyl group, the chromene ring and the aromatic proton were located on the same ring and that the chromene ring was fused in a linear form with the xanthone nucleus. On the other hand, an NOE was observed with the other aromatic proton (δ7.42) which caused a long-range correlation with a carbonyl group through ^3J in the HMBC spectrum, when the phenolic group (δ9.96) was irradiated (Fig. 2). Consequently, the aromatic proton was assigned to H-8 and the hydroxyl group (δ9.96) was substituted at C-7 position. These results indicated that **1** was a 1,2,3,5,6,7-hexasubstituted xanthone. The ^1H NMR spectrum further showed the presence of two methyls adjacent to an oxygen function (δ1.14 and 1.23) and two oxygenated methine protons (δ4.39 and 5.57) coupled by J = 3 Hz, in addition to the other protons [δ4.68 (1H, *br s*) and 5.87 (1H, *br d*, J = 7 Hz)] which caused no cross-peak in the C–H COSY spectrum. The latter two protons (δ4.68 and 5.87) were then assigned to hydroxyl groups. In the H–H COSY spectrum, the hydroxyl signal at δ5.87 was correlated with the methine signal at δ5.57. Therefore, the partial structure could be expanded to **1a** or **1b** (Fig. 3). Upon

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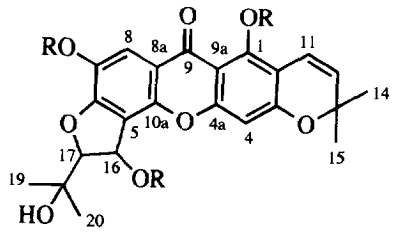
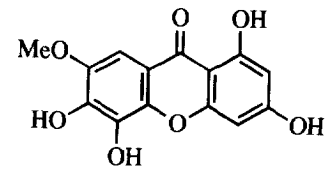


1: R = H
1a: R = Ac



6

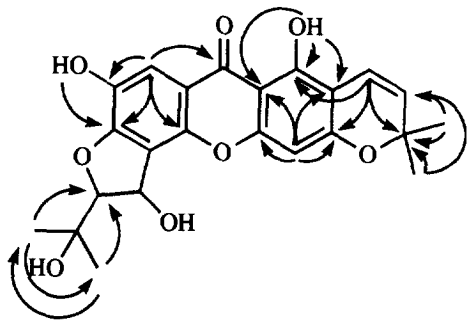
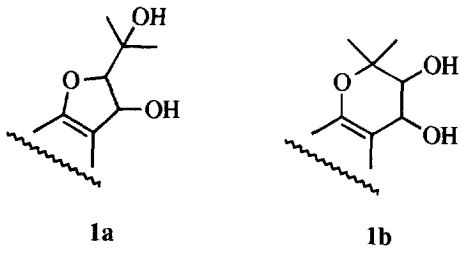


Fig. 1. HMBC spectrum (J = 10 Hz) of **1**.



1a **1b**

Fig. 3. Partial structures of **1**.

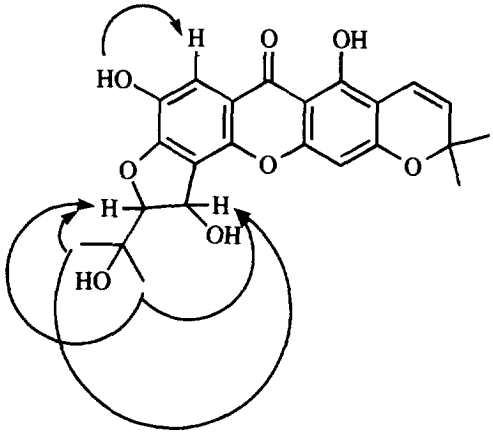


Fig. 2. NOEs observed in **1**.

acetylation, **1** gave a triacetate, indicating that one of the alcoholic hydroxyls was tertiary and NOEs (Fig. 2) were observed between the two methines and respective methyls, the results of which supported that **1a** is preferable to **1b** as the partial structure of **1**. The side-chain was determined to be a 4-hydroxy-5-hydroxyisopropyldihydrofuran, the hydroxyl group of which was in the *trans*-form supported by the NOE experiments. There remained to be determined the orientation of the dihydrofuran ring. In the HMBC spectrum, the hydroxyl group (δ9.96) and the aromatic proton (δ7.42) were correlated with an aromatic carbon at δ156.1 through ^3J, which implied that the furan ring oxygen belongs to the hydroxyl group at C-7. Finally, the structure of caloxanthone D was characterized as **1**.

Compound **6**, caloxanthone E, obtained as a pale red amorphous, reacted positively with the Gibb's and $FeCl_3$ tests. The high resolution EI mass spectrum showed the $[M]^+$ at m/z 290.0451 which corresponds to $C_{14}H_{10}O_7$. Its UV and IR spectra were suggestive of a xanthone derivative. The ^1H NMR spectrum displayed the presence of four phenolic hydroxyl groups including a chelated one [δ9.56, 9.99 and 10.85 (1H each, *br s*) and δ13.15 (1H, *s*, chelated OH)] and a methoxyl group [δ3.88 (3H, *s*)] in addition to an aromatic [δ7.06 (1H, *s*)] and *meta*-coupled protons [δ6.14 and 6.38 (1H each, *d*, J = 2 Hz)]. All protonated carbons were assigned by C–H COSY (Table 1). In the HMBC spectrum of **6** (Fig. 4), the aromatic proton at δ7.06 was correlated to a carbonyl carbon (δ179.1), indicating that one of the *peri*-positions of the carbonyl group was unsubstituted. An NOE was observed at the aromatic proton (δ7.06) when the methoxyl group (δ3.88) was irradiated. These results suggested that the partial structure of **6** was a 1-hydroxy-7-methoxyxanthone. On the other hand, in the HMBC spectrum, a long-range correlation was observed between the chelated hydroxyl group (δ13.15) and the aromatic carbon at δ97.7 which was also correlated to one of the *meta*-coupled protons (δ6.14) in the C–H COSY spectrum. This result showed that **6** was a 1,3-dihydroxyxanthone derivative. The structure of caloxanthone E was thus determined to be 1,3,5,6-tetrahydroxy-7-methoxyxanthone.

Compounds **2**–**5** were identified as 1,3,8-trihydroxy-7-methoxy- [3], 1,3-dihydroxy-7,8-dimethoxy- [4], 1,3,5-trihydroxy-2-methoxy- [5] and 6-hydroxy-1,2-dimethoxyxanthone, respectively, by spectral analysis.

Table 1. ^{13}C NMR spectral data of compounds **1** and **6**

C	**1** (Acetone-d_6)	**6** (DMSO-d_6)
1	156.6	162.5
2	103.7	97.7
3	159.2	164.7
4	95.3	93.6
5	117.6	133.1
6	156.1[a]	141.9[a]
7	140.0	145.8
8	109.8	95.3
9	179.0	179.1
4a	156.1[a]	157.1
8a	113.2	111.1
9a	102.4	101.5
10a	147.4	141.9[a]
11	114.5	—
12	128.2	—
13	78.0	—
14, 15	27.7	—
16	69.6	—
17	99.3	—
18	94.5	—
19, 20	24.9, 25.7	—
OMe	—	55.8

All carbons were assigned with the aid of C–H COSY and HMBC.
[a]Overlapping signal.

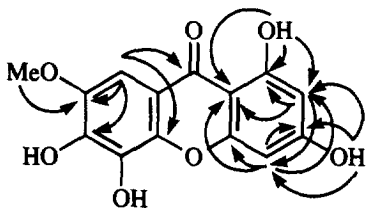


Fig. 4. Long-range correlations in the HMBC spectrum (J = 10 Hz) of **6**.

EXPERIMENTAL

Plant material. Underground parts of *C. inophyllum* L. cultivated in the Botanical Garden of the University of Ryukyu were collected in November, 1992. Voucher specimens are deposited in the Herbarium of Gifu Pharmaceutical University.

Extraction and isolation. Dried and ground root bark (1.15 kg) and root heartwood (2.2 kg) were extracted with *n*-hexane, Me_2CO, MeOH and 70% MeOH, successively, under reflux. The Me_2CO extract (100 g) of the root bark after evapn was chromatographed on silica gel CC eluted with *n*-hexane–EtOAc. The *n*-hexane–EtOAc (3:1) eluate was further chromatographed on silica gel CC and eluted with *n*-hexane–EtOAc (3:1) to give 4 frs. A second fr. was purified by VLC on silica gel with *n*-hexane–EtOAc. The eluate with *n*-hexane–EtOAc (1:1) was further chromatographed on Sephadex LH-20 and eluted with MeOH to give compound **1** (1.5 mg).

The Me_2CO extract of root heartwood (45 g) was suspended in H_2O and partitioned with benzene, EtOAc and *n*-BuOH, successively. The benzene-sol. extract (4 g) was purified by TLC on silica gel using *n*-hexane–EtOAc. The *n*-hexane–EtOAc (3:1) eluate was further purified by VLC eluting with benzene–Me_2CO (10:1) to give **2** (2 mg), **3** (4 mg) and **4** (8 mg). The eluate with *n*-hexane–EtOAc (1:1) was further purified by VLC with the same solvent system to give **5** (5 mg). Compound **6** (6 mg) was obtained from the benzene–Me_2CO (5:1) eluate of the EtOAc sol. extract (6 g) using VLC eluted with benzene–Me_2CO.

Compound **1** (*caloxanthone D*). Pale yellow amorphous. $[\alpha]_D^{24}$ + 96° ($CHCl_3$; *c* 0.017). HREIMS *m/z* 426.1343 for $C_{23}H_{22}O_8$ (calcd 426.1314). EIMS *m/z* (rel. int.): 426 $[M]^+$, 31), 411 (100), 393 (8), 351 (9), 339 (11), 335 (23), 284 (4), 283 (4). IR ν (cm^{-1}, KBr): 3605, 3230, 2960, 1650, 1620, 1585. UV λ (nm, MeOH): 221, 239sh, 249, 280sh, 290, 337, 365sh; + NaOMe: 285, 302, 350, 403. ^1H NMR (400 MHz, DMSO-d_6) δ: 1.14 and 1.23 (3H each *s*, Me × 2, H-19 and 20), 1.43 and 1.44 (3H each, *s*, Me × 2, H-14 and H-15), 4.39 (1H, *d*, J = 3 Hz, H-16), 4.68 (1H, *br s*, C-18-OH), 5.57 (1H, *br dd*, J = 7, 3 Hz, H-16), 5.76 (1H, *d*, J = 10 Hz, H-12), 5.87 (1H, *br d*, J = 7 Hz, C-16-OH), 6.35 (1H, *s*, H-4), 6,62 (1H, *d*, J = 10 Hz, H-11), 7.42 (1H, *s*, H-8), 9.96 (1H, *br s*, C-7-OH), 13.56 (1H, *s*, C-1-OH).

Acetylation of **1**. To a soln of pyridine (1 ml), **1** (1 mg) was dissolved and Ac_2O (0.5 mg) was added. The soln was left at room temp. for 24 hr. The reaction mixt. was poured into ice-H_2O and then extracted with EtOAc. The organic layer was evapd and the residue purified by prep. TLC (*n*-hexane–EtOAc–MeOH = 8:2:1) to give the triacetate (**1a**, 0.5 mg). Compound **1a** pale yellow amorphous. ^1H NMR (270 MHz) δ:1.26 and 1.30 (3H each, *s*, Me × 2, H-19, H-20), 1.46 (6H, *s*, Me × 2, H-14 and H-15), 2.12 (3H, *s*, OAc-C-16), 2.33 and 2.40 (3H each, *s*, OAc-C-1 and C-7), 4.91 and 6.90 (1H each, *d*, J = 3 Hz, H-16 and H-17), 5.98 and 6.58 (1H each, *d*, J = 10 Hz, H-11 and H-12), 6.66 (1H, *s*, H-4), 7.84 (1H, *s*, H-8).

Compound **6** (*1,3,5,6-tetrahydroxy-7-methoxyxanthone, caloxanthone E*). Pale red amorphous. HREIMS *m/z* 290.0451 for $C_{14}H_{10}O_7$ (calcd 290.0426). EIMS *m/z* (rel. int.): 290 (100), $[M]^+$), 275 (21), 260 (28), 247 (27), 219 (11), 145 (7), 69 (14). IR ν (cm^{-1}, KBr): 3430, 3300, 1645, 1605, 1595. UV λ (nm, MeOH): 215, 254, 284, 325, 360sh; + NaOMe: 218, 242, 263, 293, 385; + $AlCl_3$: 223, 235sh, 267, 290sh, 399; + $AlCl_3$/HCl: 229, 267, 289, 349, 395sh; + NaOAc: 217, 240, 254, 335sh, 378; + NaOAc/H_3BO_3: 218, 235sh, 256, 285sh, 353. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.88 (3H, *s*, OMe-C-7), 6.14 (1H, *d*, J = 2 Hz, H-2), 6.38 (1H, *d*, J = 2 Hz, H-4), 7.06 (1H, *s*, H-8), 9.56 and 9.99 (1H each, *br s*, OH × 2), 10.85 (1H, *br s*, C-3-OH), 13.15 (1H, *s*, C-1-OH).

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