TWO NEW XANTHONES IN THE UNDERGROUND PART OF *CALOPHYLLUM INOPHYLLUM*

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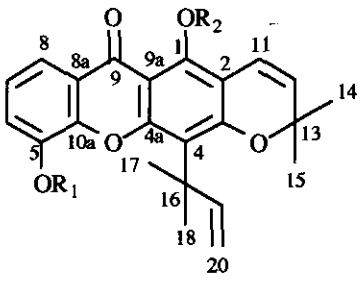
Abstract — From the root bark of *Calophyllum inophyllum* L. (Guttiferae), a new xanthone named caloxanthone C (**1**) and 4-hydroxyxanthone (**2**), and from the heartwood of root, a new xanthone 1-hydroxy-2-methoxyxanthone (**3**) in addition to three known xanthones [1,2-dimethoxy- (**4**), 2-hydroxy-1-methoxy- (**5**)], and 6-deoxyjacareubin (**6**) were isolated. The structures were characterized by means of a spectroscopic analysis.

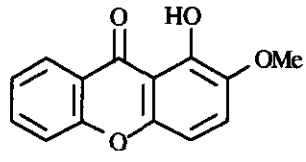
In our previous paper,[1] the structures of new xanthones named caloxanthones A and B in the root bark of *Calophyllum inophyllum* were described. In continuation of our study oriented to reach for xanthone derivatives with bioactive potency in Guttiferaeous plants, xanthone derivatives, in particular, from the their underground parts were further investigated, and two new xanthones were isolated.

The underground part of *C. inophyllum* collected in Okinawa, Japan was separated into its root bark and root heartwood. Each part was successively extracted with *n*-hexane, acetone, MeOH and 70% MeOH, after dried and ground, respectively. The acetone extract of the root bark and the *n*-hexane extract of the heartwood were

repeatedly subjected to silica gel column chromatography. From the former extract, compounds (**1**) and (**2**), from the latter, compounds (**3-6**) were isolated.



1 : R1 = R2 = H
1a: R1 = R2 = Me
1b: R1 = H, R2 = Me



3

Compound (**1**), named caloxanthone C, was obtained as yellow needles and positively reacted to Gibbs and $FeCl_3$ reactions. The $[M^+]$ at *m/z* 378.1487 in the hrms corresponds to $C_{23}H_{22}O_5$ (Calcd 378.1467). The uv and ir spectrum were suggestive of a xanthone derivative. In the ^{1}H-nmr spectrum, two one-proton double doublets (δ 7.36 and 7.65, each *J*= 2, 8 Hz) and a one-proton triplet like double doublet (δ 7.26, *J*= 8, 8 Hz) in an ABC system were observed in addition to a hydroxyl signal (δ 8.69) and a chelated hydroxyl signal (δ 13.70). The spectrum further showed the presence of two methyl groups in a singlet (δ 1.51) and two *cis*-olefinic protons in doublets (δ 5.74 and 6.71, each *J*= 10 Hz), implying the presence of a dimethylchromene ring, and two methyl groups in a singlet (δ 1.75) and three one-proton signals (δ 4.89, dd, *J*= 1, 11 Hz; 5.05,dd, *J*= 1, 17 Hz; and 6.52, dd, *J*= 11, 17 Hz), suggesting the presence of a 1,1-dimethylallyl chain. All protonated carbons of **1** were assigned by C-H COSY experiment (See Experimental). In the HMBC spectrum (Figure 1), the chelated hydroxyl group was correlated to three quaternary carbons (δ 104.6, 105.9 and 157.2), and one of which (δ 157.2) was also correlated to the *cis*-olefinic proton of the chromene ring (δ 6.71). Another *cis*-olefinic proton (δ 5.74) caused a cross peak with one of the quaternary carbons (δ 105.9). These results permitted that the dimethylchromene ring was fused in a linear form located near the chelated hydroxyl group. The positions of the 1,1-dimethylallyl chain and another non-chelated hydroxyl group were decided as follows. In the ^{13}C-nmr spectrum of the dimethyl ether of **1** (**1a**), one of methoxyl carbons was observed in a lower field (δ 62.5) and assigned to a methoxyl group at C-1, while another (δ 56.7) was a methoxyl group located at one of *ortho*-positions not to be substituted. The chemical shifts of the quaternary carbons with *O*-function found at δ 146.0 and 147.1 in **1** and 146.0 and 149.6 in **1a** suggested that the ring was a 1,2-dioxygenative substitution.

In the ^{1}H-nmr spectrum of **1a**, an nOe was observed between a methoxyl group (δ 4.03) and one of aromatic protons (δ 7.37, dd, *J*= 2, 8 Hz), which indicated that the methoxyl group was located at C-5 and the 1,1-dimethylallyl chain was substituted at C-4. The structure of caloxanthone C was thus characterized as **1**, the structure of which is a dehydroxy-derivative of macluraxanthone isolated from *Maclura* (Moraceae),[2,3] *Rheedia* [4-6] and *Garcinia* (Guttiferae).[7] We have also obtained macluraxanthone in this plant, and all assignment of the ^{13}C-nmr spectrum was accomplished[1]. The spectral data based on their partial structures were superimposed each other. Trapezifolixanthone which has a γ,γ-dimethylallyl chain at C-4 instead of a 1,1-dimethylallyl in **1** was isolated from *Calophyllum calaba* var. *calaba*.[8]

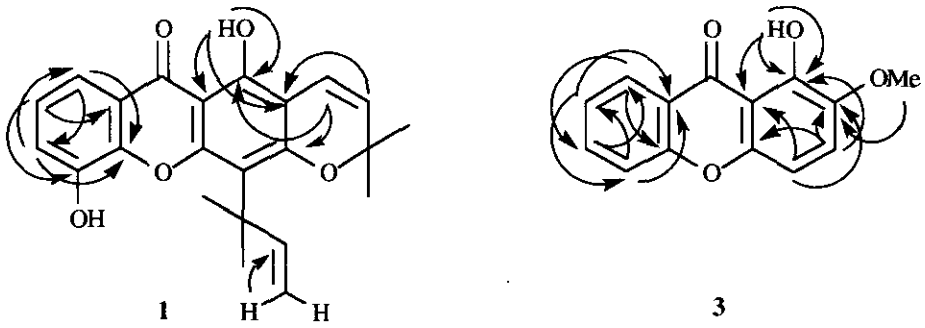


Figure 1 Correlations in the HMBC spectra of **1** and **3**

Compound (**3**) obtained as yellow needles showed the [M$^+$] at *m/z* 242.0593 in the hrms, which corresponds to $C_{14}H_{10}O_4$ (Calcd 242.0620). The uv and ir spectrum were also suggestive to a xanthone derivative. In the ^{1}H-nmr spectrum, signals for a chelated hydroxyl (δ 12.77), a methoxyl group (δ 3.91), two *ortho*-coupled protons (δ 7.00 and 7.52, each d, *J*= 9 Hz) and a 1,2-disubstituted benzene ring (δ 7.48, 1H, t like m; 7.58, 1H, br d, *J*= 8 Hz; 7.90, 1H, t like m; and 8.25, 1H, dd, *J*= 1, 8 Hz) were shown. An nOe was observed between the methoxyl group and one of the *ortho*-coupled protons (δ 7.52), which indicated that the structure of **3** is 1-hydroxy-2-methoxyxanthone. The complete assignment of ^{13}C-nmr spectrum was achieved by the aids of C-H COSY and HMBC spectra (Figure 1). The HMBC spectral data gained the definitive information on the structure of **3**. However **3** itself has been already synthesized,[9] it is the first isolation of **3** from a natural source. The structures of known xanthones (**2**, **4**[10], **5**[11] and **6**[12]) were identified with their spectral data.

EXPERIMENTAL

Plant material. Root bark and root heartwood of *C. inophyllum* which was cultivated in Botanical Garden of the University of Ryukyu were collected in November, 1992. The voucher specimens are deposited in the Herbarium of Gifu Pharmaceutical University.

Extract and isolation. The dried and ground root bark (1.15 kg) and the such root heartwood (2.2 kg) were successively extracted with *n*-hexane, acetone, MeOH, and 70% MeOH under reflux with respect. After each solvent was removed, the resulting acetone extract (100 g) of the root bark was chromatographed on Si CC eluted with a *n*-hexane-EtOAc system. A *n*-hexane-EtOAc (10 : 1) eluent was further chromatographed on Sephadex LH-20 eluted with MeOH to give **1** (30 mg). An eluent with *n*-hexane-EtOAc (3 : 1) was further chromatographed on Si CC eluted with a same system to give **2** (10 mg). Compound (**5**) (15 mg) was obtained from the *n*-hexane extract of the root heartwood (1.5 g) by recrystallization, and the remaining extract was purified by vacuum liquid chromatography on silica gel with a *n*-hexane-EtOAc system. Compound (**3**) (8 mg), (**4**) (5 mg) and (**6**) (10 mg) were purified by recrystallization.

Compound 1 (caloxanthone C): mp 210.5°C (*n*-hexane-C6H6); yellow needles, hrms *m/z* 378.1487 (Calcd 378.1467 for C23H22O5); eims *m/z* (rel. int.): 378 (M^+, 33), 363 (100), 337 (7), 335 (7), 174 (5), 154 (9); uv λ (nm, MeOH) (log ε): 236 (4.34), 249sh, 280sh, 292 (5.65), 310sh, 340sh, 375 (3.60), +NaOMe: 281, 301, 330sh, 400, +AlCl3: 235, 250sh, 272sh, 292, 308sh, 338sh, 375sh, +AlCl3/HCl: 235, 249sh, 272sh, 293, 307sh, 340sh, 375sh, +NaOAc: 285, 300, 330sh, 386, +NaOAc/H3BO3: 274sh, 292, 320sh, 379; ir υ (cm^{-1}, KBr): 3460, 3420, 2960, 2920, 1645, 1625; ^{1}H-nmr (270 MHz, acetone-*d6*) δ: 1.51 (6H, s, C14, 15-Me), 1.75 (6H, s, C17, 18-Me), 4.89 (1H, dd, *J*= 1, 11 Hz, H-20*Z*), 5.05 (1H, dd, *J*= 1, 18 Hz, H-20*E*), 5.74 (1H, d, *J*= 10 Hz, H-12), 6.52 (1H, dd, *J*= 11, 18 Hz, H-19), 6.71 (1H, d, *J*= 10 Hz, H-11), 7.26 (1H, t like dd, *J*= 8, 8 Hz, H-7), 7.36 (1H, dd, *J*= 2, 8 Hz, H-6), 7.65 (1H, dd, *J*= 2, 8 Hz, H-8), 8.69 (1H, br s, C5-OH), 13.70 (1H, s, C1-OH); ^{13}C-nmr (67.5 MHz, acetone-*d6*) δ: 29.0 (C-14, C-15), 29.9 (C-17, C-18), 41.8 (C-16), 79.3 (C-13), 104.6 (C-9a), 105.9 (C-2), 107.4 (C-20), 114.5 (C-4), 116.0 (C-8), 116.3 (C-11), 120.9 (C-6), 121.7 (C-8a), 124.9 (C-7), 128.6 (C-12), 146.0 (C-10a), 147.1 (C-5), 152.7 (C-19), 155.9 (C-4a), 157.2 (C-1), 160.1 (C-3), 182.3 (C-9).

Methylation of 1. To an acetone solution (5 ml) containing **1** (10 mg, 0.026 mmol)), CH3I (15 mg, 0.11 mmol) and K2CO3 (1 g, 7.2 mmol) were added and the solution was heated for 6 h under reflux. The reaction mixture was poured into water, and then extracted with EtOAc. After evaporation of the EtOAc layer, the residue was chromatographed on silica gel with *n*-hexane-EtOAc-MeOH (8 : 2 : 1) to give the dimethyl ether (**1a**) (4 mg) as a colorless oil and the monomethyl ether (**1b**) (3 mg) as a yellow amorphous powder. **1a**: hrms: 406.1806 (Calcd

406.1780 for $C_{25}H_{26}O_5$); eims *m/z* (rel. int.): 406 (M^+, 38), 391 (100), 361 (8), 188 (7), 168 (7); uv λ (MeOH, nm) (log ε): 219 (4.37), 280 (4.78), 332 (3.93), 356 (3.95); ^{1}H-nmr (270 MHz, acetone-*d*6) δ: 1.50 (6H, s, C14, 15-Me), 1.76 (6H, s, C17, 18-Me), 3.87 (3H, s, C1-OMe), 4.03 (3H, s, C5-OMe), 4.83 (1H, dd, *J*= 1, 11 Hz, H-20*Z*), 4.95 (1H, dd, *J*= 1, 18 Hz, H-20*E*), 5.86 (1H, d, *J*= 10 Hz, H-12), 6.46 (1H, dd, *J*= 11, 18 Hz, H-19), 6.76 (1H, d, *J*= 10 Hz, H-11), 7.30 (1H, t like dd, *J*= 8 Hz, H-7), 7.37 (1H, dd, *J*= 2, 8 Hz, H-6), 7.70 (1H, dd, *J*= 2, 8 Hz, H-8); ^{13}C-nmr (67.5 Mz, acetone-*d*6) δ: 29.0 (C-14, C-15), 30.1 (C-17, C-18), 56.7 (C5-OMe), 62.5 (C1-OMe), 78.6 (C-13), 108.2 (C-20), 113.0 (C-9a), 115.7 (C-6), 117.1 (C-11), 117.2 (C-8), 117.3 (C-4), 120.0 (C-2), 124.0 (C-8a), 124.3 (C-7), 131.2 (C-12), 146.0 (C-10a), 149.6 (C-5), 151.6 (C-19), 155.7 (C-4a), 158.0 (C-1), 158.2 (C-3), 175.5 (C-9). **1b**: hrms *m/z* 392.1600 (Calcd 392.1623 for $C_{24}H_{24}O_5$); eims *m/z* (rel. int.): 392 (M^+, 38), 377 (100), 351 (7), 349 (8), 337 (7), 319 (4), 181 (11), 160 (13), 149 (9); uv λ (MeOH, nm) (log ε): 237 (4.36), 270sh, 291 (4.61), 308sh, 350sh, 370sh; ^{1}H-nmr (400 MHz, acetone-*d*6) δ: 1.50 (6H, s, C14, 15-Me), 1.74 (6H, s, C17, 18-Me), 4.04 (3H, s, C5-OMe), 4.85 (1H, dd, *J*= 1, 11 Hz, H-20*Z*), 4.98 (dd, *J*= 1, 18 Hz, H-20*E*), 5.74 (1H, d, *J*= 10 Hz, H-12), 6.39 (1H, dd, *J*= 11, 18 Hz, H-19), 6.71 (1H, d, *J*= 10 Hz, H-11), 7.37 (1H, t like dd, *J*= 8 Hz, H-7), 7.47 (1H, dd, *J*= 2, 8 Hz, H-6), 7.72 (1H, dd, *J*= 2, 8 Hz, H-8), 13.68 (1H, s, C1-OH); ^{13}C-nmr (100 MHz, acetone-*d*6) δ: 28.1 (C-14, C-15), 30.4 (C-17, C-18), 41.8 (C-16), 56.8 (C5-OMe), 79.3 (C-13), 104.4 (C-9a), 105.9 (C-2), 108.3 (C-20), 114.5 (C-4), 116.2 (C-6), 116.5 (C-8), 117.0 (C-11), 121.4 (C-8a), 124.8 (C-7), 128.7 (C-12), 146.9 (C-10a), 150.0 (C-5), 151.4 (C-19), 156.0 (C-4a), 157.1 (C-1), 160.2 (C-3), 182.3 (C-9).

Compound 3 (1-hydroxy-2-methoxyxanthone): mp 135-136°C (*n*-hexane-EtOAc), yellow needles; hrms *m/z* 242.0593 (Calcd 242.0620 for $C_{14}H_{10}O_4$); eims *m/z* (rel. int.): 242 (M^+, 100), 227 (52), 213 (13), 199 (77), 115 (8), 77 (27); uv λ (MeOH, nm) (log ε): 203 (3.90), 232 (4.02), 245sh, 259 (4.07), 281sh, 383 (3.18), +NaOMe: 211, 230, 250, 270, 297, 308, 430, +AlCl3: 204, 233, 250sh, 270, 275sh, 310sh, 318, 364, 463, +AlCl3/HCl: 203, 233, 250sh, 255, 269, 277, 307, 316, 364, 457, +NaOAc: 216, 258, 295sh, 307sh, 385, +NaOAc/H3BO3: 216, 259, 280sh, 379; ir υ (cm^{-1}, KBr): 3450, 1655, 1610, 1585; ^{1}H-nmr (400 MHz, acetone-*d*6) δ: 3.91 (3H, s, C2-OMe), 7.00 (1H, d, *J*= 9 Hz, H-4), 7.48 (1H, t like m, H-7), 7.52 (1H, d, *J*= 9 Hz, H-3), 7.58 (1H, br d, *J*= 8 Hz, H-5), 7.90 (1H, t like m, H-6), 8.25 (1H, dd, *J*= 1, 8 Hz, H-8), 12.77 (1H, s, C1-OH). ^{13}C-nmr (100 MHz, acetone-*d*6) δ: 57.5 (OMe), 106.5 (C-4), 109.8 (C-9a), 118.8 (C-5), 120.6 (C-8a), 123.0 (C-3), 124.9 (C-7), 126.5 (C-8), 137.0 (C-6), 143.8 (C-2), 150.8 (C-4a), 152.0 (C-1), 157.4 (C-10a), 183.8 (C-9).

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Received, 22nd September, 1993