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TWO XANTHONES FROM ROOTS OF *CRATOXYLUM FORMOSANUM*

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Key Word Index—*Cratoxylum formosanum*; Guttiferae; 2,7-dihydroxy-1,8-dimethoxyxanthone; 1,4,7-trihydroxy-8-methoxyxanthone; 1,4,7-trihydroxyxanthone.

Abstract—From the roots of *Cratoxylum formosanum*, two new xanthones, 2,7-dihydroxy-1,8-dimethoxyxanthone and 1,4,7-trihydroxy-8-methoxyxanthone, were isolated, in addition to seven known xanthones and two flavonoids. Among the xanthones, 1,4,7-trihydroxyxanthone was the first isolation from the natural sources. Structures were determined by spectral analyses.

INTRODUCTION

The genus *Cratoxylum* has about six species [1] which are distributed mainly in Southeast Asia. Some species have been used as traditional medicines [2] and the occurrence of flavonoids [3], triterpenoids [4] and xanthones [5] has been reported. In our search for biologically active compounds in Guttiferous plants [6–8], we now report the isolation and structural determination of two new xanthones with a simple oxygenated pattern in the roots of *C. formosanum*.

RESULTS AND DISCUSSION

Roots collected in the Philippines were dried, ground and extracted with benzene, acetone and 70% MeOH, successively. The benzene and acetone extracts were repeatedly chromatographed on silica gel and Sephadex LH-20 to give **1**–**7**, **10** and **11** (from the acetone extract) and **8** and **9** (from the benzene extract).

Compound **1** was obtained as pale yellow needles; $FeCl_3$ and Gibb's tests were negative. The high resolution EI-mass spectrum showed the $[M]^+$ at *m/z* 288.0646, corresponding to $C_{15}H_{12}O_6$. The ^1H NMR spectrum showed signals for *ortho*-coupled protons [δ 7.15 and 7.33 (*d*, $J = 8.8$ Hz)], a methoxyl group [δ 3.94 (*s*)] and a hydroxyl group [δ 8.00 (*br s*)]. The IR (1660 cm^{-1}) and ^{13}C NMR spectra (δ 175.9) suggested the presence of a carbonyl group. In the ^{13}C NMR spectrum, signals due to quaternary carbon were significantly attenuated, indicating that **1** was a xanthone derivative with a symmetrical axis in its structure; thus, the number of hydroxyl and methoxyl groups and of hydrogens was duplicated. In the ^{13}C NMR spectrum, the methoxyl carbons appeared at δ 62.1, indicating that both *ortho*-positions of the methoxyl were substituted [9]. Furthermore, the quaternary carbons with an *O*-function were observed at δ 146.1, 147.1 and 150.8, respectively, which suggested that **1** had a 1,3,4-trioxygenated benzene ring. Thus, the structure of **1** was determined to be 2,7-dihydroxy-1,8-dimethoxyxanthone. This deduced structure was clearly supported by the HMBC spectrum (Fig. 1). To the best of our knowledge, this is the first isolation of a symmetrically substituted xanthone.

Compound **2**, a yellow amorphous solid, gave a positive $FeCl_3$ test. The $[M]^+$ at *m/z* 274.0470 in the HREI-mass spectrum corresponded to the molecular formula $C_{14}H_{10}O_6$. The UV and IR spectra suggested that **2** was also a xanthone derivative. In the ^1H NMR spectrum the presence of three hydroxyls [δ 8.17 (2H, *br s*) and 12.08 (1H, *s*, chelated)] and a methoxyl group [δ 3.82 (3H, *s*)] was suggested, in addition to two sets of *ortho*-coupled protons [δ 6.46 and 7.12 (1H each, *d*, $J = 8.8$ Hz) and δ 7.15 and 7.33 (1H each, *d*, $J = 9.2$ Hz)]. In the ^{13}C NMR spectrum, the methoxyl group appeared at δ 62.4, indicating that both *ortho*-positions of the methoxyl group were occupied by substituents. All protonated carbons were assigned from the CH COSY spectrum (Table 1). In the HMBC spectrum of **2** (Fig. 1), the chelated hydroxyl group was correlated to an aromatic carbon at δ 109.5, which gave a cross-peak to the *ortho*-coupled proton at δ 6.46 in the CH COSY spectrum. These results indicated the partial structure of **2** to be a 1,4-dihydroxyxanthone derivative. The positions of other substituents were determined as follows. In the ^{13}C NMR spectrum, aromatic carbons with an *O*-function were observed at δ 146.3, 147.8 and 151.5, respectively, which suggested the presence of another 1,3,4-trioxygenated

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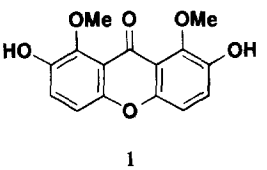

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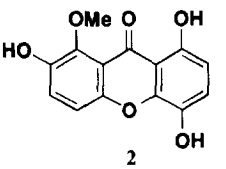

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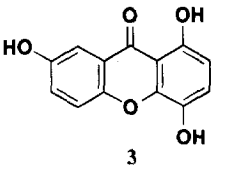

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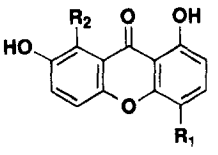
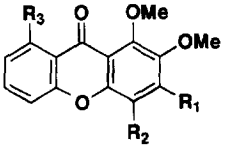
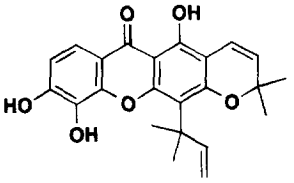


4 : R_1 = OMe, R_2 = H
5 : R_1 = R_2 = H
9 : R_1 = H, R_2 = OMe



6 : R_1 = R_2 = R_3 = OMe
7 : R_1 = R_3 = OH, R_2 = H
7a : R_1 = R_3 = OMe, R_2 = H



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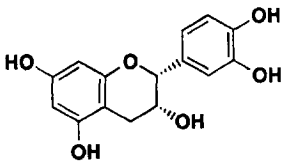

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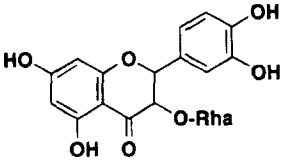

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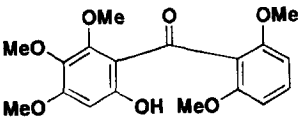

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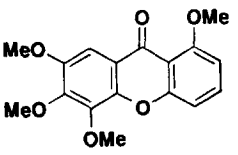

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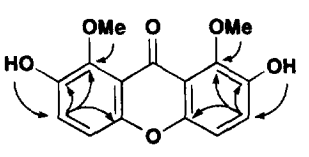
Table 1. ^{13}C NMR spectral data of compounds **1**–**3**

CNo.	1	2	3
1	146.1	155.0	154.7
2	147.1	109.5	109.3
3	123.4	123.8	124.0
4	113.8	137.7	138.1
5	113.8	114.6	120.3
6	123.4	125.3	126.1
7	147.1	147.8	155.0
8	146.1	146.3	109.3
9	175.9	183.1	183.0
4a	150.8	144.6	145.2
8a	117.7	116.2	121.9
9a	117.7	110.1	109.5
10a	150.8	151.5	151.0
OMe	62.1	62.4	

All carbons assigned with the aid of HMQC and HMBC spectra.

benzene ring. When the chemical shift of the methoxyl group in the ^{13}C NMR spectrum was taken into account, the structure of **2** was elucidated to be 1,4,7-trihydroxy-8-methoxyxanthone. This structure was supported by the correlations observed in the HMBC spectrum (Fig. 1).

Compound **3**, a yellow amorphous solid, also reacted positively with $FeCl_3$. The HREI-mass spectrum showed the $[M]^+$ at *m/z* 244.0358, which corresponds to $C_{13}H_8O_5$. UV and IR absorptions showed that **3** was a xanthone. In the ^{1}H NMR spectrum, the presence of three hydroxyl groups [δ 8.35, 8.92 (1H each, *br s*) and 11.93 (1H, *s*, chelated)] and *ortho*-coupled protons [δ 6.62 and 7.29 (1H each, *d*, J = 8.8 Hz)], in addition to a 1,3,4-trisubstituted benzene ring [δ 7.42 (1H, *dd*, J = 8.7, 3.0 Hz), 7.52 (1H, *d*, J = 8.7 Hz) and 7.61 (1H, *d*, J = 3.0 Hz)] were detected. In the HMBC spectrum (Fig. 1), the chelated hydroxyl group gave a cross-peak



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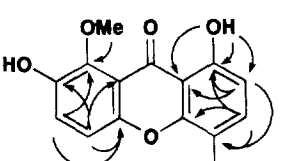

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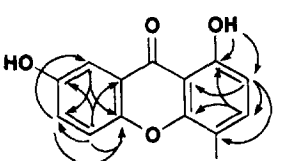

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Fig. 1. HMBC spectra (J = 10 Hz) of compounds **1**–**3**.

with an aromatic carbon at δ 109.3. In the CH COSY spectrum, this aromtic carbon was correlated to one of the *ortho*-coupled protons (δ 6.62). These results indicated that **3** was a 1,4-dihydroxyxanthone derivative. The position of remaining hydroxyl group was determined as follows. Comparison of the ^{1}H and ^{13}C NMR spectral data with those of **5** (1,7-dihydroxyxanthone) isolated from *Harungana madagascariensis* [6]; both spectral data based on a 7-hydroxyxanthone moiety were superimposable on each other. Thus, the structure of **3** was concluded to be 1,4,7-trihydroxyxanthone, which was further supported by the HMBC spectrum (Fig. 1). Although this xanthone was previously synthesized [10], this is the first report of its occurrence as a natural compound.

Compound **7** was a xanthone with two hydroxyls and two methoxyl groups. The structure was considered to be either 3,8-dihydroxy-1,2-dimethoxy- or 1,7-dihydroxy-5,6-dimethoxyxanthone. To clarify the substitution, **7** was derivatised to form a permethyl ether (**7a**), because 1,2,3,8-tetramethoxy- and 1,5,6,7-tetramethoxyxanthone (**13**) are available. The latter xanthone (**13**) was derived from 1,5,6-trihydroxy-7-methoxyxanthone isolated from *H. madagascariensis* [6], the former prepared synthetically by condensation of 2,6-dimethoxybenzoic acid and 3,4,5-trimethoxyphenol. The ^{1}H NMR spectral data of **7a** was identical to the former. Therefore, the structure of **7** was 3,8-dihydroxy-1,2-dimethoxyxanthone which has previously been isolated from roots of *Polygala nyikensis* (Polygalaceae) [11].

Compounds **4**–**6** and **8**–**11** were identified as 1,7-dihydroxy-4-methoxy- (**4**), 1,7-dihydroxy- (**5**), 1,2,3,4,8-pentamethoxyxanthone (**6**), macluraxanthone (**8**), 1,7-dihydroxy-8-methoxyxanthone (**9**), (−)-epicatechin (**10**) and astilbin (**11**), respectively, by spectroscopic analysis.

EXPERIMENTAL

Plant material. Roots of *C. formosanum* (Jack) Dyer were collected in the Philippines, in August, 1993. Voucher specimens are deposited in the Philippine National Herbarium and the herbarium of Gifu Pharmaceutical University.

Extraction and isolation. Dried and ground roots (915 g) were extracted under reflux with benzene (2 l × 12 hr × 3) (wt of extractive after removal of solvent, 15 g), acetone (2 l × 12 hr × 3) (16 g) and 70% MeOH (2 l × 12 hr × 3) (48 g), successively. The benzene extract (7 g) was chromatographed on silica gel eluted with a benzene–Me_2CO system. The benzene eluent was recrystallized from *n*-hexane to give **8** (11 mg). The benzene–Me_2CO (10:1) eluent was further chromatographed on Sephadex LH-20 eluting with $CHCl_3$–Me_2CO (1:1) to give 4 frs. The fourth fr. was subjected to prep. TLC with *n*-hexane–EtOH (20:1) to give **9** (2 mg). The Me_2CO extract (14 g) was subjected to silica gel CC eluting with a benzene–Me_2CO system to give 9 frs (frs 1–9). Fr. 3 was further chromatographed on Sephadex LH-20 eluting with MeOH to give 4 frs. The fourth fr. was recrystallized from benzene–Me_2CO to give **4** (10 mg). After the amorphous crystals were filtered off, the filtrate was purified using prep. TLC with benzene–Me_2CO (10:1) to give **5** (10 mg). Fr. 4 was further chromatographed on Sephadex LH-20 eluting with $CHCl_3$–Me_2CO (1:1) and prep. TLC with *n*-hexane–EtOAc–MeOH (8:2:1) to give **7** (5 mg). Fr. 5 was purified by Sephadex LH-20 using $CHCl_3$–Me_2CO (1:1) to give 5 frs. The first and fifth frs were subjected to prep. TLC usng *n*-hexane–EtOAc (5:1) and $CHCl_3$–MeOH (20:1), respectively, to give **6** (8 mg) (from the first fr.) and **2** (8 mg), **3** (5 mg) (from the fifth fr.). Fr. 7 was recrystallized from benzene–Me_2CO to give **9** (30 mg). After the amorphous precipitations were filtered off, the filtrate was chromatographed on Sephadex LH-20 with $CHCl_3$–Me_2CO (1:1) to give **1** (10 mg). Fr. 8 was subjected to vacuum liquid chromatography on silica gel using a $CHCl_3$–MeOH system. From the $CHCl_3$–MeOH (10:1) eluent, **10** (220 mg) was obtained.

Compound **1** (*2,7-dihydroxy-1,8-dimethoxyxanthone*). Mp 243–244° (*n*-hexane–EtOAc), pale yellow needles. HREI-MS *m/z* 288.0646 for $C_{15}H_{12}O_6$ (calcd 288.0634); EI-MS *m/z* (rel. int.): 288 $[M]^+$ (100), 273 (22), 245 (25), 213 (20), 202 (14), 149 (9), 123 (5), 79 (4). UV λ (nm, MeOH): 207, 235 sh, 250 sh, 282, 305 sh, 350. IR ν (cm^{-1}, KBr): 3380, 1660, 1623. ^{1}H NMR (400 MHz, acetone-d_6): δ 3.94 (6H, *s*, OMe-C-1, 8), 7.15 (2H, *d*, J = 8.8 Hz, H-4, 5), 7.33 (2H, *d*, J = 8.8 Hz, H-3, 6), 8.00 (2H, *br s*, OH-C-2, 7).

Compound **2** (*1,4,7-trihydroxy-8-methoxyxanthone*). Yellow amorphous. HREI-MS *m/z* 274.0470 for $C_{14}H_{10}O_6$ (calcd 274.0477); EI-MS *m/z* (rel. int.): 274 $[M]^+$ (100), 256 (81), 244 (9), 231 (53), 228 (17), 200 (8), 149 (5), 123 (5), 102 (5), 79 (4). UV λ (nm, MeOH): 205, 238, 269, 333, 410. IR ν (cm^{-1}, KBr): 3390, 1650, 1615, 1600. ^{1}H NMR (400 MHz, acetone-d_6): δ 3.82 (3H, *s*, OMe-C-8), 6.46 (1H, *d*, J = 8.8 Hz, H-2), 7.12 (1H, *d*, J = 8.8 Hz, H-3), 7.15 (1H, *d*, J = 9.2 Hz, H-5), 7.33 (1H, *d*, J = 9.2 Hz, H-6), 8.17 (2H, *br s*, OH-C-4, 7), 12.08 (1H, *s*, OH-C-1).

Compound **3** (*1,4,7-trihydroxyxanthone*). Yellow amorphous. HREI-MS *m/z* 244.0358 for $C_{13}H_8O_5$ (calcd 244.0372); EI-MS *m/z* (rel. int.): 244 $[M]^+$ (100), 243 (14), 187 (5), 131 (5). UV λ (nm, MeOH): 235, 268. IR ν (cm^{-1}, KBr): 3390, 1655, 1615, 1600. ^{1}H NMR (400 MHz, acetone-d_6): δ 6.62 (1*H*, *d*, J = 8.8 Hz, H-2), 7.29 (1H, *d*, J = 8.8 Hz, H-3), 7.42 (1H, *dd*, J = 8.7, 3.0 Hz, H-6), 7.52 (1H, *d*, J = 8.7 Hz, H-5), 7.61 (1H, *d*, J = 3.0 Hz, H-8), 8.35, 8.92 (1H, *br s*, OH-C-4, 7), 11.93 (1H, *s*, OH-C-1).

Methylation of **7**. Compound **7** (2 mg) was methylated with MeI (0.5 ml) and K_2CO_3 (1 g) in Me_2CO (5 ml) under reflux for 5 hr. The reaction mixt. was poured into dil. HCl (200 ml) and extracted with EtOAc (20 ml). After evapn of EtOAc, the residue was purified by prep. TLC (*n*-hexane–EtOAc–MeOH, 8:2:1) to give 1,2,3,8-tetramethoxyxanthone (**7a**) (1 mg).

Synthesis of **7a**. 2,6-Dimethoxybenzoic acid (500 mg) was dissolved in $SOCl_2$ (1.5 g) and heated under reflux for 2 hr. After evapn of $SOCl_2$, the reaction mixt. was dissolved in dry Et_2O (200 ml) and 3,4,5-trimethoxyphenol (500 mg) and $AlCl_3$ (1 g) added. After the mixt. was stirred at room temp. for 48 hr, the solvent was evapd under red. pres. and the residue was poured into dil. HCl. The acidified suspension was extracted with EtOAc and solvent evapd. The resulting mixt. was subjected to silica gel CC eluting with benzene–Me_2CO (50:1) to give 2-hydroxy-4,5,6,2′,6′-pentamethoxybenzophenone (**12**) (680 mg). Pale yellow amorphous. EI-MS *m/z* (rel. int.): 348 $[M]^+$ (51), 317 (98), 287 (11), 210 (100), 195 (81), 167 (40), 165 (14). ^{1}H NMR (400 MHz, acetone-d_6): δ 3.31 (3H, *s*, OMe-C-5), 3.64 (3H, *s*, OMe-C-6), 3.73 (6H, *s*, OMe-C-2′,6′), 3.93 (3H, *s*, OMe-C-4), 6.32 (1H, *s*, H-3), 6.70 (2H, *d*, J = 8.3 Hz, H-3′,5′), 7.30 (1H, *t*, J = 8.3 Hz, H-4′), 13.39 (1H, *s*, OH-C-2). A part of **12** (100 mg) was treated with 20% NaOH (10 ml) in MeOH (5 ml) under reflux for 10 hr. Usual work-up of the reaction mixt. gave **7a** (70 mg). Colourless oil. EI-MS *m/z* (rel. int.): 316 $[M]^+$ (28), 301 (100), 258 (22), 243 (10), 151 (7). UV λ (nm, MeOH): 220, 247, 253 sh, 293, 344. IR ν (cm^{-1}, KBr): 2950, 1655, 1605, 1595. ^{1}H NMR (400 MHz, acetone-d_6): δ 3.81 (3H, *s*, OMe-C-2), 3.92 (3H, *s*, OMe-C-8), 3.93 (3H, *s*, OMe-C-1), 3.99 (3H, *s*, OMe-C-3), 6.78 (1H, *s*, H-4), 6.89 (1H, *br d*, J = 8.3 Hz, H-7), 6.94 (1H, *br d*, J = 8.3 Hz, H-5), 7.58 (1H, *t*, J = 8.3 Hz, H-6).

Methylation of 1,5,6-*trihydroxy*-7-*methoxyxanthone*. 1,5,6-Trihydroxy-7-methoxyxanthone (5 mg), isolated from the roots of *H. madagascariensis* [6], was methylated with MeI (1 ml) and K_2CO_3 (1 g) in Me_2CO (10 ml) under reflux for 5 hr. The reaction mixt. was purified by prep. TLC (*n*-hexane–EtOAc–MeOH, 8:2:1) to give 1,5,6,7-tetramethoxyxanthone (**13**) (4 mg). Colourless amorphous. ^{1}H NMR (400 MHz, acetone-d_6): δ 3.94, 3.95, 3.98 and 4.04 (3H each, *s*, OMe-C-1,5,6,7), 6.95 (1H, *br d*, J = 8.3 Hz, H-2), 7.14 (1H, *br d*, J = 8.3 Hz, H-4), 7.52 (1H, *d*, J = 8.7 Hz, H-5), 7.39 (1H, *s*, H-8), 7.68 (1H, *t*, J = 8.3 Hz, H-3).

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