Cytotoxic Xanthones from *Cudrania tricuspidata*

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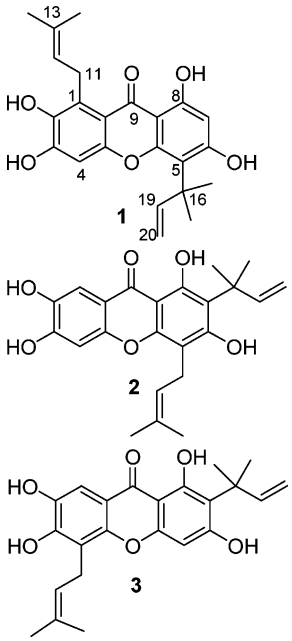
The new isoprenylated tetrahydroxyxanthone, 2,3,6,8-tetrahydroxy-1-(3-methylbut-2-enyl)-5-(2-methylbut-3-en-2-yl)-9*H*-xanthen-9-one (**1**), was isolated from the root bark of *Cudrania tricuspidata* together with macluraxanthone B (**2**) and cudraxanthone L (**3**), which were fully characterized by NMR spectroscopic and X-ray crystallographic analyses.

The whole plant of *Cudrania tricuspidata* has become one of most important folk remedies for cancer in Korea during the last few decades and has also been used as a traditional medicine for curing neuritis and inflammation in Asia.[1] Previous studies have demonstrated that it contains biologically active xanthones,[2−6] flavonoids,[7−11] and benzenoids.[12] In the course of our studies searching for cytotoxic substances from higher plants, the chloroform extract of the root bark of *C. tricuspidata* was found to show significant cytotoxicity against human cancer cell lines. Activity-guided fractionation yielded three isoprenylated xanthones showing strong cytotoxic activity. Determination of the position of substituents in isoprenylated xanthones by NMR is difficult due to the number of quaternary carbon atoms present in these skeletons, with similar chemical shift values being observed between structural isomers. For instance, macluraxanthone B (**2**) was initially reported as a yellow amorphous solid.[13] Herein, we report the isolation and identification of a new isoprenylated tetrahydroxyxanthone (**1**) and full characterization of known macluraxanthone B (**2**) and cudraxanthone L (**3**)[6] with the aid of the X-ray crystal structures.

The ^{1}H NMR, ^{13}C NMR, IR, UV, and EIMS data obtained for compounds **2** and **3** agreed with the previous report for macluraxanthone B (**2**) and cudraxanthone L (**3**).

The isolated compounds **2** and **3** were recrystallized in a $CHCl_3$/acetone solution to give yellow prisms of **2** (mp 152 °C) and **3**, respectively. Thus, the positions of the substituents in **2** and **3** were assigned on the basis of X-ray crystallographic analysis and HMBC experiments (Figure 1).

Compound **1** was obtained as yellowish solid having the molecular formula $C_{23}H_{24}O_6$ and 12 degrees of unsaturation, as deduced from its HREIMS data. The IR spectrum of **1** showed absorptions at 3418, 1636, 1593, and 1514 cm^{-1}, suggesting the presence of hydroxyl, conjugated carbonyl, and aromatic ring moieties. The UV spectrum of **1** resembled the spectra of 1,3,6,7-tetrahydroxyxanthone derivatives.[14−16] The structure of **1** was inferred from a detailed analysis of ^{1}H and ^{13}C NMR data, together with 2D-NMR experiments. The ^{1}H and ^{13}C NMR data with DEPT experiments showed the presence of 23 carbon atoms as one carbonyl, one sp^2 methylene, one sp^3 methylene, four methines, four methyls, and 13 quaternary carbons. The ^{13}C NMR data enabled one carbonyl and eight double bonds to be characterized, and these accounted for nine of the 12 degrees of unsaturation. The assignment of the 3,3-dimethylallyl group was determined on the basis of successive connectivities from C-11 to C-15 in the ^{1}H−^{1}H COSY spectrum. The HMBC correlation of C-2 with H-4 and H-11, and C-9a with H-4 and H-11, placed the 3,3-dimethylallyl group at C-1 (Figure 2). The presence of the 1,1-dimethylallyl group was deduced from the connectivity between H-19 (δ_H 6.44) and the vinylic protons H-20$_{\alpha/\beta}$ (δ_H 5.34 and 5.44) in the ^{1}H−^{1}H COSY spectrum and the correlation between C-17, 18 (δ_C 28.1) and H-19 in the HMBC experiment. This 1,1-dimethylallyl group was placed at C-5 due to the correlations of C-5 with H-7 and H-17, 18 in the HMBC experiment (Figure 2). Furthermore, the HMBC results of **1** were compared with that of **3** to solve an ambiguity in the position of the 1,1-dimethylallyl group. A correlation between H-7 and C-8 was observed in compound **1** but not in compound **3**, which further established that the 1,1-dimethylallyl group was located at C-5. The NMR data of **1** were also compared with the previously reported monomethyl ether of **1**.[3] Thus, compound **1** was identified as 2,3,6,8-tetrahydroxy-1-(3-methylbut-2-enyl)-5-(2-methylbut-3-en-2-yl)-9*H*-xanthen-9-one.



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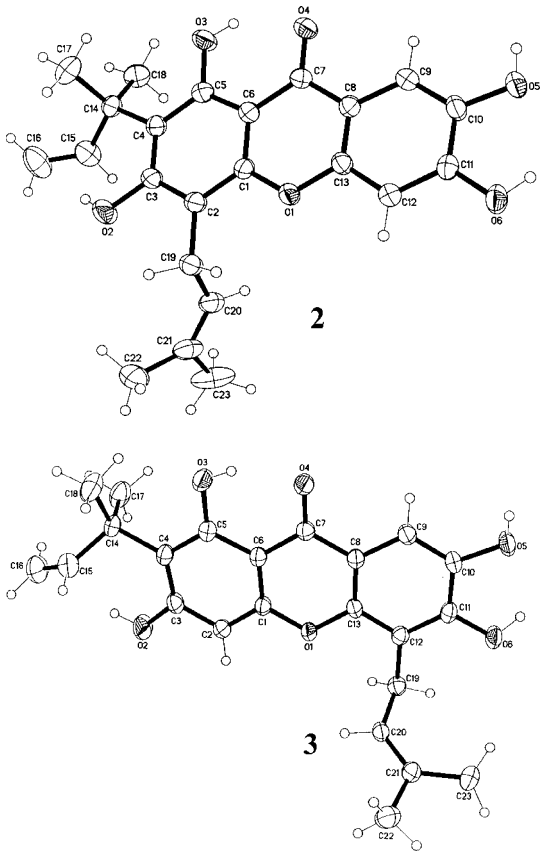

Figure 1. ORTEP views of **2** and **3**.

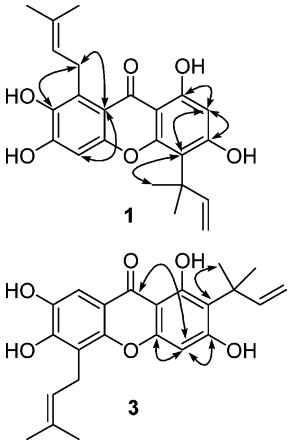

Figure 2. HMBC correlations of **1** and **3**.

The three xanthone derivatives (**1**, **2**, and **3**) and Taxol (positive control) were examined for their in vitro cytotoxic activity against A549 (human lung cancer cell) and SK-OV3 (human ovarian cancer cell) cell lines. The IC_{50} values of compounds (**1**−**3**) and Taxol are presented Table 2.

Experimental Section

General Experimental Procedures. Melting points were measured on a Thomas Scientific capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Bruker IFS66 infrared Fourier transform spectrophotometer (KBr), and UV spectra were measured on a Beckman DU650 spectrophotometer. ^1H and ^{13}C NMR along with 2D-NMR data were obtained on a Bruker AM 500 (^1H NMR at 500 MHz, ^{13}C NMR at 125 MHz) spectrometer in $CDCl_3$ and CD_3OD; EIMS and HREIMS data were collected on a JEOL JMS-700 spectrometer.

Plant Materials. *Cudrania tricuspidata* (Carr.) Bureau was collected in Hyoupchun (Korea) and identified by Prof. Jae-Hong Pak. A voucher specimen (Park, K. H. 110) of this raw material is deposited at Herbarium of Kyungpook National University (KNU).

Table 1. ^{13}C NMR Data of Compounds **1**−**3** at 125 MHz (ppm, m)[a]

position	1	2	3
1	127.4 (s)	107.0 (d)	106.7 (d)
2	140.0 (s)	141.9 (s)	144.2 (s)
3	150.9 (s)	152.8 (s)	152.9 (s)
4	100.6 (d)	102.1 (d)	117.1 (s)
4a	152.9 (s)	151.8 (s)	151.3 (s)
4b	155.0 (s)	153.3 (s)	157.6 (s)
5	108.9 (s)	107.2 (s)	95.5 (d)
6	161.8 (s)	161.1 (s)	165.7 (s)
7	100.0 (d)	113.2 (s)	116.3 (s)
8	161.7 (s)	160.4 (s)	163.7 (s)
8a	104.6 (s)	102.6 (s)	103.7 (s)
9	183.0 (s)	180.3 (s)	182.1 (s)
9a	111.1 (s)	112.2 (s)	114.0 (s)
11	26.0 (t)	22.1 (t)	23.7 (t)
12	121.6 (d)	122.3 (d)	123.3 (d)
13	135.0 (s)	131.8 (s)	133.1 (s)
14	18.1 (q)	17.9 (q)	18.6 (q)
15	25.9 (q)	25.8 (q)	26.4 (q)
16	40.8 (s)	41.5 (s)	42.5 (s)
17, 18	28.1 (q)	27.4 (q)	29.9 (q)
19	149.4 (d)	149.9 (d)	152.0 (d)
20	113.1 (t)	113.4 (t)	108.6 (t)

[a] The chemical shifts of compounds **1** and **2** were determined in $CDCl_3$. Compound **3** was measured in CD_3OD.

Table 2. In Vitro Cytotoxicity of Compounds **1**−**3** and Taxol on Human Cell Lines[a]

	cell lines	
compound	A549	SK-OV3
1	5.93 ± 0.71	7.09 ± 0.61
2	2.88 ± 0.53	4.24 ± 0.40
3	3.15 ± 0.45	4.72 ± 0.58
Taxol	1.25 ± 0.19	1.35 ± 0.29

[a] Results are expressed as IC_{50} values (μM).

Extraction and Isolation. The dried root barks (3 kg) were chopped and extracted with $CHCl_3$ (2 × 15 L) at room temperature. The combined extracts were concentrated, and the dark residue (86 g) was partitioned between H_2O and $CHCl_3$ (300 mL:400 mL). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and then concentrated to give a dark brown residue (41 g). Chromatography was with silica gel column (7 × 60 cm, 230−400 mesh, 1 kg) eluting with $CHCl_3$ (2 L), then with a gradient of $CHCl_3$/MeOH [30:1 (1 L), 20:1 (1 L), 10:1 (1 L), 5:1 (1 L)]. The fraction (1 L) of $CHCl_3$/MeOH (10:1) was concentrated to give a brown residue (8.6 g). Chromatography of this residue was over a silica gel column (5 × 50 cm 230−400 mesh, 250 g) eluting with a gradient of hexane/EtOAc [15:1 (600 mL), 10:1 (500 mL), 5:1 (500 mL), and then 1:1 (500 mL); 10 mL each]. Fractions 18−21 were combined and evaporated to give 120 mg of compound **2** [R_f 0.53 ($CHCl_3$/acetone, 4:1)]. Fractions 23−25 and 30−34 were evaporated to give compound **1** (60 mg) [R_f 0.47 ($CHCl_3$/acetone, 4:1)] and **3** (70 mg) [R_f 0.41 ($CHCl_3$/acetone, 4:1)], respectively. Spectroscopic data of **2** and **3** were in agreement with previously published data.

2,3,6,8-Tetrahydroxy-1-(3-methylbut-2-enyl)-5-(2-methylbut-3-en-2-yl)-9*H*-xanthen-9-one (1): yellowish solid from $CHCl_3$; mp 187 °C; UV (MeOH) λ_{max} (log ϵ) 242 (4.60), 257 (4.66), 317 (4.36), 370 (4.24) nm; IR (KBr) ν_{max} 3478, 1636, 1593, 1514, 1426 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.68 (6H, s, H-17, 18), 1.88 (3H, s, H-14), 2.19 (3H, s, H-15), 4.30 (2H, d, J = 6.7 Hz, H-11), 5.30 (1H, m, H-12), 5.35 (1H, d, J = 10.5 Hz, H-20a), 5.44 (1H, d, J = 17.8 Hz, H-20b), 6.22 (1H, s, H-7), 6.45 (1H, dd, J = 17.8, 10.5 Hz, H-19), 6.83 (1H, s, H-4); ^{13}C NMR data, see Table 1; EIMS *m/z* 396 $[M]^+$ (81), 381 (100), 353, (74), 337 (32), 325 (56), 311 (22), 297 (17), 285 (13), 269

(9), 253 (5), 205 (6), 182 (7), 161 (6), 149 (6), 135 (3), 115 (6), 91 (6), 69 (10), 59 (7); HREIMS *m/z* 396.1528 [M$^+$] (calcd for $C_{23}H_{24}O_6$, 396.1573).

2,3,6,8-Tetrahydroxy-5-(3-methylbut-2-enyl)-7-(2-methylbut-3-en-2-yl)-9*H*-xanthen-9-one (2): yellowish prisms from $CHCl_3$/MeOH; mp 152 °C; UV (CH_3Cl) λ_{max} (log ϵ) 234 (4.51), 260 (4.60), 319 (4.32), 375 (4.25) nm; IR (KBr) ν_{max} 3517, 1632, 1619, 1588, 1482 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.68 (6H, s, H-17, 18), 1.70 (3H, s, H-14), 1.83 (3H, s, H-15), 3.44 (2H, d, J = 7.0 Hz, H-11), 5.20 (1H, m, H-12), 5.40 (1H, d, J = 10.5 Hz, H-20a), 5.50 (1H, d, J = 17.8 Hz, H-20b), 6.41 (1H, s, H-4), 6.53 (1H, dd, J = 17.8, 10.5 Hz, H-19), 7.51 (1H, s, H-1); ^{13}C NMR data, see Table 1; EIMS *m/z* 396 [M]$^+$ (67), 381 (100), 355 (29), 341 (19), 325 (49), 297 (18), 285 (12), 272 (7), 257 (4), 241 (2), 203 (2), 176 (3), 165 (5), 143 (3), 121 (4), 115 (3), 77 (4), 55 (4); HERIMS *m/z* 396.1568 [M$^+$] (calcd for $C_{23}H_{24}O_6$, 396.1573).

X-ray Crystal Structure Analysis of 2. $C_{23}H_{24}O_6$, M = 396.42, monoclinic, space group, $P2_1/c$, a = 9.7949(9) Å, b = 6.4418(6) Å, c = 30.252(3) Å, β = 93.258(2)°, V = 1905.7(3) Å^3, Z = 4, T = 173(2) K, D_c = 1.382 g cm^{-3}, $F(000)$ = 840, μ = 0.100 mm^{-1}, R_1 = 0.0640, R_w = 0.1720 for 4494 independent reflections.[18]

X-ray Crystal Structure Analysis of 3. $C_{23}H_{24}O_6$, M = 396.42, triclinic, space group, $P\bar{1}$, a = 8.0223(14) Å, b = 8.6013(15) Å, c = 14.450(3) Å, α = 96.767(3)°, β = 99.053(3)°, γ = 98.177(3)°, V = 964.6(3) Å^3, Z = 2, T = 173(2) K, D_c = 1.365 g cm^{-3}, $F(000)$ = 420, μ = 0.098 mm^{-1}, R_1 = 0.0627, R_w = 0.1775 for 4219 independent reflections.[18]

Cytotoxic Activity. The compounds (**1**−**3**) were examined for their in vitro cytotoxic activity against human cell lines such as A549 and SK-OV3. Cancer cells were incubated for 48 h at 37 °C in the presence of various concentrations of compounds from DMSO-diluted stock. The growth inhibitory property was determined by in vitro treatment of the respective cell lines using the sulforhodamine B assay (SRB).[17]

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Supporting Information Available: ^{1}H, ^{13}C NMR, DEPT 90, 135, COSY, HMQC, and HMBC for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as deposition Nos. CCDC-222303 (2) and CCDC-222304 (3). Copies of data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: + 44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

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