New Isoprenylated Xanthones from *Cudrania tricuspidata*

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A phytochemical investigation of the roots of *Cudrania tricuspidata* afforded three new isoprenylated xanthones, cudratricusxanthones N–P (**1**–**3**, resp.), together with five known compounds, **4**–**8**. The structures of the new compounds were elucidated by extensive spectroscopic analyses.

Introduction. – *Cudrania tricuspidata* (CARR.) BUR. belongs to the family Moraceae and is a deciduous shrub distributed in China, Korea, and Japan. Its roots have been used as Chinese folk medicine for the treatment of gonorrhea, rheumatism, jaundice, hepatitis, boils, scabies, bruising, and dysmenorrhea [1]. Isoprenylated xanthones and flavonoids are the main chemical constituents of *C. tricuspidata* [2]. Our previous phytochemical research on the roots of *C. tricuspidata* afforded a series of isoprenylated xanthones and flavonoids, some of which showed significant inhibitory effects on some human digestive apparatus tumor cell lines [2b][2e]. Cudratricusxanthone G, the main active compound from this plant, showed potent inhibition activity on colon cancer cell proliferation, migration, and invasion by suppressing the activities of Rac1 and its downstream transcriptional factor AP-1 [3]. Continuing our studies on *C. tricuspidata*, three new isoprenylated xanthones, cudratricusxanthones N–P (**1**–**3**, resp.), and five known compounds, cudratricusxanthones K [2a] and F (**4** and **5**, resp.) [2e], macluraxanthone B (**6**) [4], toxyloxanthone C (**7**) [5], and wighteone (**8**) [6], were isolated and characterized. Herein, we describe the structure elucidation of the new compounds **1**–**3**.

Results and Discussion. – *Structure Elucidation*. The EtOH extract of the roots of *C. tricuspidata* was purified by repeated column chromatography on *Diaion HP-20*, silica gel, and *Sephadex LH-20*, as well as preparative HPLC, to yield three new isoprenylated xanthones, **1**–**3**, and five known compounds, **4**–**8** (*Fig. 1*). Their structures were determined by spectroscopic methods.

Cudratricusxanthone N (**1**), yellow amorphous powder, had the molecular formula of $C_{23}H_{22}O_6$ as deduced from HR-EI-MS (*m/z* 394.1417 (M^+, $C_{23}H_{22}O_6^+$; calc. 394.1416). The IR spectrum evidenced the presence of OH (3224 cm^{-1}), a conjugated C=O (1633 cm^{-1}), and aromatic (1582 and 1483 cm^{-1}) moieties. The UV data resembled those of 1,3,6,7-tetraoxygenated xanthone derivatives [2f]. The ^{1}H- and ^{13}C-NMR spectra (*Table*) exhibited signals of a H-bonded OH group (δ(H) 14.41 (*s*, 1 H)), two aromatic H-atoms (δ(H) 7.56 and 7.02 (*s*, each 1 H)), a 1,1-dimethylallyl group (δ(H) 6.32 (*dd*, J = 17.5, 10.5, 1 H), 4.91 (*dd*, J = 17.5, 1.0, 1 H), 4.81 (*dd*, J = 10.5,

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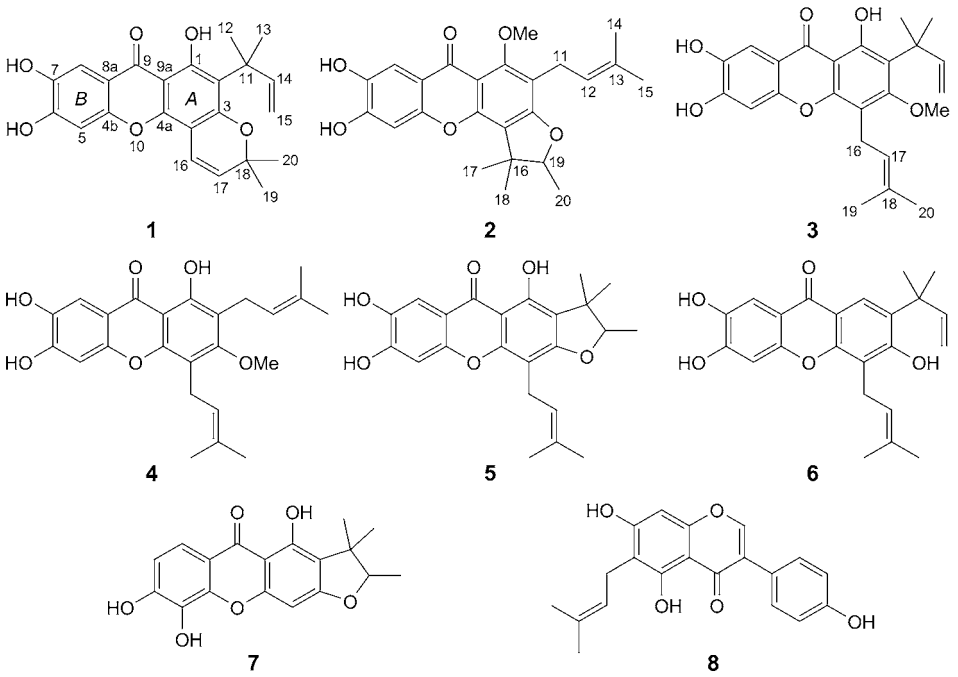

Fig. 1. *Structures of compounds* **1**–**8**

1.0, 1 H), and 1.61 (*s*, 6 H); δ(C) 151.5, 107.6, 41.6, and 29.5 for two Me groups), and a 2,2-dimethylpyran ring (δ(H) 6.84 (*d*, $J = 10.0$, 1 H), 5.70 (*d*, $J = 10.0$, 1 H), and 1.48 (*s*, 6 H); δ(C) 127.6, 116.1, 79.0, and 27.9 for two Me groups). Moreover, the ^{13}C-NMR spectrum displayed signals of a conjugated C=O group (δ(C) 181.1) and six O-bearing C-atoms (δ(C) 163.1, 159.6, 154.3, 152.3, 151.3, and 144.1) (*Table*). The aforementioned data indicated that **1** was a xanthone with a 1,1-dimethylallyl group, a 2,2-dimethylpyran ring, and three OH groups. The position of these substituents on the xanthone skeleton was determined on the basis of HMBC spectrum. In the HMBC spectrum (*Fig. 2*), the correlations of the OH group (δ(H) 14.41 (HO–C(1))) with C(1) (δ(C) 163.1), C(9a) (δ(C) 103.4), and C(2) (δ(C) 116.9) suggested its location at C(1). The HMBC cross-peaks of Me(12) and Me(13) (δ(H) 1.61) with C(2) indicated that the 1,1-dimethylallyl group was at C(2). The 2,2-dimethylpyran ring was located at C(3) and C(4), as deduced from the HMBC correlations of H–C(16) (δ(H) 6.84) with C(3) (δ(C) 159.6), and of the H–C(17) (δ(H) 5.70) with C(4) (δ(C) 101.8). These data assigned the structure of the *A* ring moiety. The HMBC correlations of H–C(8) (δ(H) 7.56) with C(6) (δ(C) 154.3), C(7) (δ(C) 144.1), C(8a) (δ(C) 113.7), and C(9) (δ(C) 181.1), and of H–C(5) (δ(H) 7.02) with C(6) and C(4b) (δ(C) 152.3) established a 6,7-dihydroxylated *B* ring. Thus, the structure of **1** was determined as 6,9,10-trihydroxy-3,3-dimethyl-5-(2-methylbut-3-en-2-yl)-3*H*,7*H*-pyrano[2,3-*c*]xanthen-7-one and named cudratricusxanthone N (*Fig. 1*).

Cudratricusxanthone O (**2**), yellow amorphous powder, was assigned the molecular formula of $C_{24}H_{26}O_6$ by HR-EI-MS (*m*/*z* 410.1731 (M^+, $C_{24}H_{26}O_6^+$; calc. 410.1729).

Table. *^1H- and ^{13}C-NMR Data* (in (D_6)acetone) *of Compounds* **1**–**3**[a]). δ in ppm, J in Hz.

Position	**1**[b])		**2**[b])		**3**[c])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		163.1		160.5		161.1
2		116.9		115.6		122.8
3		159.6		156.3		164.4
4		101.8		125.0		114.2
4a		151.3		158.7		154.2
4b		152.3		151.9		152.7
5	7.02 (*s*)	103.5	6.91 (*s*)	103.4	6.96 (*s*)	103.3
6		154.3		153.1		155.0
7		144.1		143.6		144.2
8	7.56 (*s*)	109.3	7.55 (*s*)	110.1	7.55 (*s*)	108.9
8a		113.7		116.0		113.3
9		181.1		174.5		181.6
9a		103.4		106.8		106.0
11		41.6	3.52 (br. *d*, J = 6.5)	23.5		41.6
12	1.61 (*s*)	29.5	5.28 (br. *t*, J = 6.5)	123.9	1.59 (*s*)	27.5
13	1.61 (*s*)	29.5		132.1	1.59 (*s*)	27.5
14	6.32 (*dd*, J = 17.5, 10.5)	151.5	1.89 (br. *s*)	18.2	6.43 (*dd*, J = 17.4, 10.6)	150.6
15	4.91 (*dd*, J = 17.5, 1.0), 4.81 (*dd*, J = 10.5, 1.0)	107.6	1.69 (*d*, J = 0.5)	25.8	4.93 (br. *d*, J = 17.4), 4.75 (br. *d*, J = 10.6)	104.8
16	6.84 (*d*, J = 10.0)	116.1		44.5	3.49 (br. *d*, J = 7.0)	23.4
17	5.70 (*d*, J = 10.0)	127.6	1.47 (*s*)[d])	26.2[d])	5.24 (br. *t*, J = 7.0)	123.8
18		79.0	1.19 (*s*)[d])	22.3[d])		131.9
19	1.48 (*s*)	27.9	4.47 (*q*, J = 6.5)	90.9	1.85 (br. *s*)	18.1
20	1.48 (*s*)	27.9	1.39 (*d*, J = 6.5)	14.3	1.65 (br. *s*)	25.8
OH	14.41 (*s*)				14.29 (*s*)	
MeO			3.94 (*s*)	63.3	3.57 (*s*)	62.9

[a]) C-Atom numbering as indicated in *Fig. 1*. [b]) ^{1}H-NMR at 500 Hz, ^{13}C-NMR at 125 Hz. [c]) ^{1}H-NMR at 400 Hz, ^{13}C-NMR at 100 Hz. [d]) Signals may be exchanged.

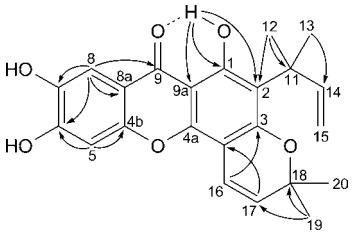

Fig. 2. *Selected HMBCs* (H → C) *of compound* **1**

The ^{1}H- and ^{13}C-NMR spectra (*Table*) exhibited signals of a C=O group (δ(C) 174.5), six O-bearing C-atoms (δ(C) 160.5, 158.7, 156.3, 153.1, 151.9, and 143.6), two aromatic H-atoms (δ(H) 7.55 and 6.91 (*s*, each 1 H)), a MeO group (δ(H) 3.94 (*s*); δ(C) 63.3), a 3-methylbut-2-enyl (prenyl) group (δ(H) 5.28 (br. *t*, $J = 6.5$, 1 H), 3.52 (br. *d*, $J = 6.5$, 2 H), 1.89 (br. *s*, 3 H), and 1.69 (*d*, $J = 0.5$, 3 H); δ(C) 132.1, 123.9, 25.8, 23.5, and 18.2), and a 2,3,3-trimethylfuran ring (δ(H) 4.47 (*q*, $J = 6.5$, 1 H), 1.47 (*s*, 3 H), 1.39 (*d*, $J = 6.5$, 3 H), and 1.19 (*s*, 3 H); δ(C) 90.9, 44.5, 26.2, 22.3, and 14.3). These data suggested that **2** was a tetraoxygenated xanthone with two isoprenoid, a MeO, and two OH groups. Comparison of the NMR data of **1** and **2** revealed that the OH group (δ(H) 14.41) in **1** disappeared in **2**, and the signal of the C(9)=O group at δ(C) 174.5 in **2** was shifted upfield by 6.6 ppm. These observations indicated that there was no OH group at C(1) in **2**. In the HMBC spectrum (*Fig. 3*), the correlations of the MeO group (δ(H) 3.94) with C(1) (δ(C) 160.5), and of CH_2(11) (δ(H) 3.52) with C(1), C(2) (δ(C) 115.6), and C(3) (δ(C) 156.3) indicated that the MeO and the prenyl groups were located at C(1) and C(2), respectively. The 2,3,3-trimethylfuran ring was connected to C(3) and C(4) based on the HMBC cross-peak Me(17) (δ(H) 1.47)/C(4) (δ(C) 125.0). The *B* ring moiety was identical to that of **1** as determined by the HMBC correlations in *Fig. 3*. The absolute configuration at C(19) was not assigned by the available data. Thus, the structure of **2** was elucidated as 1,2-dihydro-8,9-dihydroxy-5-methoxy-1,1,2-trimethyl-4-(3-methylbut-2-en-1-yl)-6*H*-furo[2,3-*c*]xanthen-6-one and named cudratricusxanthone O (*Fig. 1*).

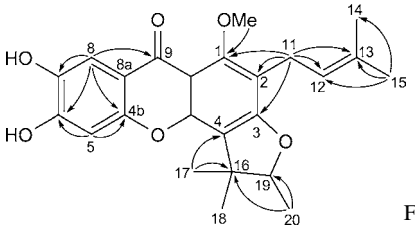

Fig. 3. *Selected HMBCs* (H → C) *of compound* **2**

Cudratricusxanthone P (**3**), yellow amorphous powder, had the molecular formula of $C_{24}H_{26}O_6$ as deduced from HR-EI-MS (*m*/*z* 410.1733 (M^+, $C_{24}H_{26}O_6^+$; calc. 410.1729). The ^{1}H- and ^{13}C-NMR spectra (*Table*) evidenced the presence of a OH group (δ(H) 14.29 (*s*, 1 H)), two aromatic H-atoms (δ(H) 7.55 and 6.96 (*s*, each 1 H)), a MeO group (δ(H) 3.57 (*s*, 3 H); δ(C) 62.9), and two isoprenoid groups including a 1,1-

dimethylallyl group (δ(H) 6.43 (*dd*, $J = 17.4, 10.6$, 1 H), 4.93 (br. *d*, $J = 17.4$, 1 H), 4.75 (br. *d*, $J = 10.6$, 1 H), and 1.59 (*s*, 6 H); δ(C) 150.6, 104.8, 41.6, and 27.5 for two Me groups), and a prenyl group (δ(H) 5.24 (br. *t*, $J = 7.0$, 1 H), 3.49 (*d*, $J = 7.0$, 1 H), 1.85 (br. *s*, 3 H), and 1.65 (br. *s*, 3 H); δ(C) 131.9, 123.8, 25.8, 23.4, and 18.1) (*Table*). The HMBC spectrum established ring *A* of **3** by the following key cross-peaks: Me(12) and Me(13) (δ(H) 1.59)/C(2) (δ(C) 122.8); MeO (δ(H) 3.57)/C(3) (δ(C) 164.4); and CH_2(16) (δ(H) 3.49)/C(3), C(4) (δ(C) 114.2), and C(4a) (δ(C) 154.2). Compound **3** possessed the same 6,7-dihydroxylated *B* ring as that of **1** and **2**, as confirmed by comparison their NMR data (*Table*) and the HMBC spectrum of **3**. Finally, the structure of **3** was identified as 1,6,7-trihydroxy-3-methoxy-2-(2-methylbut-3-en-2-yl)-4-(3-methylbut-2-en-1-yl)-9*H*-xanthen-9-one, named cudratricusxanthone P (*Fig. 1*).

The five known compounds were identified as cudratricusxanthone K (**4**) [2a], cudratricusxanthone F (**5**) [2e], macluraxanthone B (**6**) [4], toxyloxanthone C (**7**) [5], and wighteone (**8**) [6], by comparing their spectroscopic data with those reported.

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Experimental Part

General. TLC: Precoated silica gel GF_{254} plates (10–40 μm; *Yantai Institute of Chemical Technology*, P. R. China). Column chromatography (CC): *Diaion HP-20* (*Mitsubishi Chemical Corporation*, Japan), silica gel *H* (200–300 mesh; *Yantai Institute of Chemical Technology*, P. R. China), and *Sephadex LH-20* (*GE Healthcare Amersham Biosciences*, Sweden). HPLC: *Agilent 1200* (*Agilent Technologies*, USA), *Sepax Amethyst* C_{18} column (10 × 150 mm, 5 μm; *Sepax Techologies, Inc.*, USA). Optical rotations: *Jasco P1030* polarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Avatar-360* spectrometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-400* and *DRX-500* instruments; δ in ppm rel. to residual solvent peaks of (D_6)acetone (δ(H) 2.05; δ(C) 206.0), *J* in Hz. EI-MS: *Agilent 5973N* mass spectrometer; in *m*/*z* (rel. %). HR-EI-MS: *Waters Micromass GCT* mass spectrometer; in *m*/*z*.

Plant Material. The roots of *C. tricuspidata* (CARR.) BUR. were collected in Dali, Yunnan, P. R. China, in July 2007, and air-dried. The plant material was identified by Dr. *Yun Kang*, Fudan University, and a voucher specimen (TCM 2007–7 Hou) was deposited with the Herbarium of Department of Pharmacognosy, School of Pharmacy, Fudan University.

Extraction and Isolation. The air-dried and powdered roots (30 kg) were extracted with 95% EtOH (150 l) at r.t. The filtrate was evaporated *in vacuo* to provide a residue (1 kg). A portion of the residue (160 g) was dissolved in 30% EtOH and subjected to CC (*Diaion HP-20*; 30, 50, and 90% EtOH). The 90% EtOH fraction (50 g) was seperated by CC (SiO_2; petroleum ether/AcOEt 16:1, 12:1, 9:1, 4:1, and 3:2) to give six fractions: *Frs. A–F. Fr. B* (8.0 g) was subjected to CC (*Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to yield three fractions: *Frs.* B_1–B_3. *Fr.* B_3 (5.2 g) was further separated by CC (SiO_2; $CHCl_3$/MeOH 50:1, 25:1, 10:1, 5:1, and 1:1) to afford **1** (700 mg) and further six fractions: *Frs.* $B_{3\text{-}1}$–$B_{3\text{-}6}$. *Fr.* $B_{3\text{-}5}$ (2.1 g) was purified by prep. HPLC (MeOH/H_2O 8.5:1.5, 1 ml/min, 210 nm) to give **2** (14 mg), **3** (125 mg), **4** (9 mg), and **5** (107 mg). Further separation of *Fr.* $B_{3\text{-}6}$ (1.3 g) by prep. HPLC (MeOH/H_2O 8:2, 1ml/min, 210 nm) gave **6** (90 mg), **7** (7 mg), and **8** (25 mg).

Cudratricusxanthone N (*= 5-(1,1-Dimethylprop-2-en-1-yl)-6,9,10-trihydroxy-3,3-dimethyl-3*H,*7*H-*pyrano[2,3-*c*]*xanthen-7-one*; **1**). Yellow amorphous powder. UV (MeOH): 349 (3.50), 314 (3.61), 270 (3.62), 239 (3.28), 224 (3.07). IR (KBr): 3224, 2972, 2929, 1633, 1582, 1483, 1442, 1367, 1295, 1244, 1203, 1160, 1127. ^{1}H- and ^{13}C-NMR: *Table.* EI-MS: 394 (30), 379 (100), 365 (5), 351 (13), 339 (9), 311 (36). HR-EI-MS: 394.1417 (M^+, $C_{23}H_{22}O_6^+$, calc. 394.1416).

Cudratricusxanthone O (*=1,2-Dihydro-8,9-dihydroxy-5-methoxy-1,1,2-trimethyl-4-(3-methylbut-2-en-1-yl)-6*H-*furo[2,3-*c*]xanthen-6-one*; **2**). Yellow amorphous powder. $[\alpha]_D^{20} = -16.8^\circ$ ($c = 0.2$, acetone). UV (MeOH): 370 (3.05), 290 (3.33), 273 (3.49), 255 (3.67). IR (KBr): 3443, 2959, 2921, 1614, 1516, 1466, 1423, 1384, 1284, 1246, 1216, 1161, 1111, 1075. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 410 (65), 395 (100), 327 (57), 312 (12), 180 (22), 163 (17). HR-EI-MS: 410.1731 (M^+, $C_{24}H_{26}O_6^+$, calc. 410.1729).

Cudratricusxanthone P (*=2-(1,1-Dimethylprop-2-en-1-yl)-1,6,7-trihydroxy-3-methoxy-4-(3-methylbut-2-en-1-yl)-9*H-*xanthen-9-one*; **3**). Yellow amorphous powder. UV (MeOH): 381 (4.10), 307 (4.15), 259 (4.52). IR (KBr): 3384, 2915, 1630, 1582, 1477, 1294, 1205, 1171, 1113. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS: 410 (58), 395 (100), 381 (49), 367 (36), 355 (33), 341 (46), 327 (71), 311 (29), 299 (36), 285 (37), 153 (25). HR-EI-MS: 410.1733 (M^+, $C_{24}H_{26}O_6^+$, calc. 410.1729).

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