A Novel Xanthone from *Garcinia oligantha*

by **Xue-Mei Gao***[a]), **Ming-Zhu Cui**[a]), **Ting Yu**[b]), **Qiu-Fen Hu**[a]), **Jian-Xin Pu**[c]), **Xue Du**[c]), **Tian-Cheng Liu**[a]), and **Kathy Qian Luo***[b])

[a]) Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan University of Nationalities, Kunming 650031, P. R. China (phone: +86-13388808396; fax: +86-871-5910017; e-mail: gao_xuemei@hotmail.com)

[b]) Division of Bioengineering, School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore 637457

[c]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China

One novel xanthone, oliganthone A (**1**), was isolated from the stems of the plant *Garcinia oligantha*. It features the O-bearing C(3)-atom and absence of C(4) compared with the structures of related known xanthones, which have never been reported before. The structure of this compound was elucidated by spectroscopic analysis. Compound **1** showed strong HeLa cell growth-inhibiting effects with IC_{50} values below 10 μM.

Introduction. – *Garcinia oligantha* (Guttiferae family), a 1–3-m-tall shrub, is distributed, at 200–1200 m altitude, in dense forests in Hainan and Guangdong Provinces [1]. We have already reported the isolation and characterization of various bioactive compounds including terpenes, xanthones, benzophenones, and depsidones from *Garcinia paucinervis* [2][3], *G. multiflora* [4], and *G. oligantha* [5]. In our continuing search for biologically active and structurally unique compounds from the genus *Garcinia*, one novel polyisoprenylated xanthone, oliganthone A (**1**), together with known flavonoids, were isolated from the acetone extract of the dried stems *Garcinia oligantha*. Herein, we describe the isolation and structure elucidation of **1**. The compound was also assayed for cytotoxic activity against Hela C3 cells.

Results and Discussion. – Oliganthone A (**1**), obtained as a yellow amorphous powder from an acetone extract of the stems of *G. oligantha*, exhibited a molecular-ion peak at *m/z* 507.2384 ($[M+H]^+$) in the HR-ESI-MS spectrum, corresponding to the molecular formula $C_{30}H_{34}O_7$, which indicated 14 degrees of unsaturation. The inspection of its ^{1}H- and ^{13}C-NMR data (*Table*) indicated the presence of seven Me, three CH_2, and six CH (five olefinic) groups, and fourteen quaternary C-atoms (nine olefinic, one O-bearing, and three C=O). The absorption bands in its IR spectrum suggested the presence of OH groups (3447 cm^{-1}) and of a xanthone C=O group (1656 cm^{-1}). With the aid of 1D- and 2D-NMR experiments, all ^{1}H- and ^{13}C-NMR signals of **1** were assigned by comparison of its NMR data with those of reference compounds. The deduced structure of **1** is shown in *Fig. 1*, and the ^{1}H- and ^{13}C-NMR data of **1** are compiled in the *Table*.

Table. *^{1}H- and ^{13}C-NMR Data of Compound* **1** *in $CDCl_3$*[a]). Arbitrary atom numbering as indicated in *Fig. 1*; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
1		58.5 (*s*)	15	1.81 (*s*)	28.2 (*q*)
2		212.2 (*s*)	16	2.60–2.66 (*m*)	33.6 (*t*)
3		75.9 (*s*)	17	4.89–4.91 (*m*)	119.7 (*d*)
5	6.30 (*s*)	100.7 (*d*)	18		135.9 (*s*)
6		162.0 (*s*)	19	1.56 (*s*)	17.7 (*q*)
7		101.7 (*s*)	20	1.64 (*s*)	25.9 (*q*)
8		159.7 (*s*)	21	2.70–2.75 (*m*)	34.6 (*t*)
9		180.1 (*s*)	22	4.85–4.89 (*m*)	118.9 (*d*)
4a		164.0 (*s*)	23		135.6 (*s*)
8a		106.0 (*s*)	24	1.53 (*s*)	17.7 (*q*)
9a		119.8 (*s*)	25	1.58 (*s*)	25.8 (*q*)
10a		152.2 (*s*)	26	2.77–2.82 (*m*)	42.8 (*t*)
11	6.63 (*d*, $J = 10.0$)	114.4 (*d*)	27		208.4 (*s*)
12	5.61 (*d*, $J = 10.0$)	127.8 (*d*)	28	2.26 (*s*)	31.9 (*q*)
13		78.3 (*s*)	HO–C(3)	5.15 (br.)	
14	1.49 (*s*)	28.4 (*q*)	HO–C(8)	12.66 (*s*)	

[a]) Spectra were recorded with a *Bruker DRX-400* MHz spectrometer, chemical shifts (δ) were expressed in ppm, J in Hz; assignments were confirmed by $^{1}H,^{1}H$-COSY, HMQC, and HMBC, experiments.

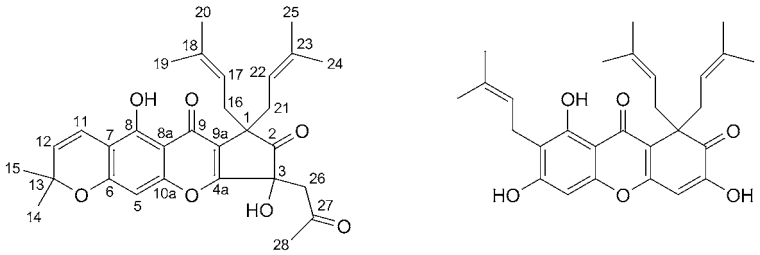

Fig. 1. *Structures of compounds* **1** *and* **2**

The ^{1}H-NMR spectrum of **1** displayed a set of signals at δ(H) 4.89–4.91 and 4.85–4.89 (*m*, H–C(17) and H–C(22), resp.), 2.60–2.66 and 2.70–2.75 (2*m*, CH_2(16) and C(21), resp.), 1.56, 1.64, 1.53, and 1.58 (4*s*, Me(19), Me(20), Me(24), and Me(25), resp.). These resonances are typical of a geminal bis(3-methylbut-2-en-1-yl) group, linked to a sp^3-C-atom (δ(C) 58.5 (C(1))) [6][7]. Except the signal of the xanthone C=O group at δ(C) 180.1 (C(9)), an additional C=O signal at δ(C) 212.2 (C(2)) suggested a xanthenedione skeleton. The ^{1}H- and ^{13}C-NMR spectra of **1** displayed signals for a xanthenedione skeleton that contains three isoprene units [6]. Further comparison with the isolated compounds confirmed that **1** was an analog of a known xanthenedione named allanxanthone C [6].

Compared with the NMR data of allanxanthone C (**2**) [6], this new compound did not exhibit signals for a third prenyl group. One of the C_5 group was established to be attached to C(6), forming a dimethyl-2*H*-pyrano-xanthone skeleton, by the HMBCs of δ(C) 162.0 (C(6)) with the chelated HO–C(8), and the *cis*-coupled H–C(11) and H–C(12), and those of H–C(12) with the O-bearing quaternary C(13) and the Me(14) and Me(15). The other two 3-methylbut-2-enyl groups are located at C(1), according to the HMBCs of H–C(16) with C(1), and of H–C(21) with C(2). The remaining aromatic H-atom was indicated to be H–C(5) by its HMBCs with C(6), C(7), C(8a), and C(10a) (*Fig. 2*).

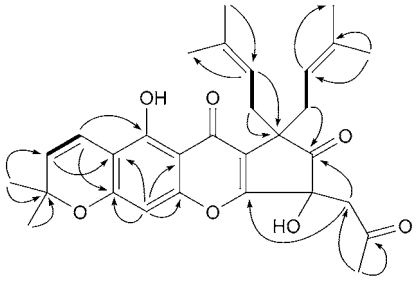

Fig. 2. *Selected HMB* (H → C) *and* $^1H,^1H$*-COSY* (—) *correlations of* **1**

However, different C- and H-atom chemical shifts for C(3), C(26), C(27), and C(28) indicated that the structure of **1** differed from that of allanxanthone C (**2**) [6] with respect to the side chain at C(3). In the HMBC spectrum of **1**, the correlations of the H-atom signals at δ(H) 5.15 (br., HO–C(3)) with the C-atom signals at δ(C) 212.2 (C(2)), 164.0 (C(4a)), and 42.8 (C(26)) suggested that C(4a) and C(3) linked to each other directly, which is without precedent. The O-bearing C-atom signal at δ(C) 75.9 (C(3)) and the absence of the C(4) signal further confirmed the above deduction. The O-bearing C(3)-atom and absent C(4) suggested compound **1** as a new xanthone, and shed a new insight into structural diversity of xanthone analogs. The HMBCs of the signals at δ(H) 2.77 – 2.82 (*m*, CH_2(26)) with those at δ(C) 212.2 (C(2)), 75.9 (C(3)), 208.4 (C(27)), and 31.9 (C(28)), and of the signals at δ(H) 2.26 (*s*, Me(28)) with those at δ(C) 42.8 (C(26)) and 208.4 (C(27)) indicated an acetonyl moiety at C(3). Since we used acetone as solvent in the extraction procedure, we tested the acetone, MeOH, and CH_2Cl_2 extracts of the dried plant material by LC/MS, respectively. As a result, we detected the peak corresponding to compound **1** in all of the three extracts, which indicated that compound **1** was not an artefact of the extraction procedure. From these spectroscopic data, the structure of compound **1** was determined as shown, and it was named oliganthone A.

To determine the cytotoxicity of the compound, we measured its the IC_{50} value on HeLa cells. Oliganthone A (**1**) showed strong HeLa cell growth-inhibiting effects with the IC_{50} value of 7.27 ± 0.23 μM, which is below 10 μM.

Experimental Part

General. Column chromatography (CC): silica gel *GF_{254}* (SiO_2; 200–300 mesh, *Qingdao Marine Chemical, Inc.*); *Sephadex LH-20* (*Pharmacia*); reversed-phase (RP) C_{18} SiO_2 (250 mesh; *Merck*). TLC: Precoated SiO_2 *60 GF_{254}* (*Qingdao Marine Chemical, Inc.*). HPLC: *Agilent 1100* series, with an *Alltima C_{18}* column (4.6 × 250 mm) for HPLC analysis, and semi-prep. *Alltima C_{18}* columns (9.4 × 250 mm) for sample preparation. Optical rotation: *JASCO DIP-1000* polarimeter. UV Spectra: *Perkin-Elmer Lambda L14* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer Spectrum 100* FT-IR spectrometer in KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR spectra ($CDCl_3$): *Bruker AV-400* spectrometer at 400 (^1H) and 100 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: nanoLC-MS/MS system, with a *nanoAcquity UPLC* module and a *Q-TOF* spectrometer equipped with a nanoelectrospray ion source (*Waters*, Milford, MA; in *m/z*).

Plant Material. The stems of *G. oligantha* were collected in October 2008 in Hainan Province, P. R. China. The plant was identified by *Rong-Jing Zhang*. A voucher specimen (YNGXM-0001) has been deposited with the Key Laboratory of Ethnic Medicine Resource Chemistry (Yunnan University of Nationalities), State Ethnic Affairs Commission & Ministry of Education.

Extraction and Isolation. The air-dried and powdered stems of *G. oligantha* (5.5 kg) were extracted with acetone (5.0 l × 3, 24 h each) at r.t., and the extract was concentrated under vacuum condition. Then, the acetone extract was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble portion (242 g) was decolorized by *MCI GEL®* and eluted with 90% MeOH. The decolorized portion (130 g) was subjected to column chromatography (CC; SiO_2; hexane/acetone 1:0, 4:1, 2:1, 1:1, and 0:1) to afford five fractions, *Frs. I – V. Fr. I-1* (5.05 g) was then separated by CC (*RP-18*; MeOH/H_2O 80–100%) to give 14 fractions, *Frs. I-1.1 – I-1.14*. *Fr. I-1.4* was separated by CC (*Sephadex LH-20*; MeOH) and then subjected to semi-prep. HPLC (MeOH/H_2O 80:20) to yield *oliganthone A* (**1**; 5.2 mg).

Bioassay. We determined the IC_{50} values of **1** by using MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. MTT Powder was dissolved in PBS (phosphate-buffered saline) at a concentration of 5 mg/ml. First, 2,500 HeLa cells suspended in 100 µl of MEM (minumum essential *Eagle* medium) were seeded in a 96-well plate. After 24 h incubation, fresh medium containing various concentrations of each compound were added into the 96-well plate to replace the old medium. The concentrations applied were ranged from 100 to 1.56 µM, which was achieved by twofold dilutions for 6 times. After 72-h treatment, 10 µl of MTT soln. was added into each well of a 96-well plate. After 2 h incubation at 37°, 100 µl of 10% SDS (sodium dodecyl sulfate) soln. with 0.01M HCl was added to dissolve the purple crystals. After 24 h incubation, the optical density (*OD*) values at 595 nm of the control groups at 0 and 72 h together with the compound-treated groups at 72 h from the MTT assay were measured using a plate reader. Paclitaxel at 500 nM was used as the positive control. IC_{50} is the concentration of a compound inhibiting 50% of the cell growth.

Oliganthone A (= *7,9-Dihydro-5,9-dihydroxy-2,2-dimethyl-7,7-bis(3-methylbut-2-en-1-yl)-9-(2-oxopropyl)-2*H*-cyclopenta[*e*]benzo[1,2-*b*:5,4-*b*′]dipyran-6,8-dione*; **1**). Yellow amorphous powder. $[\alpha]_D^{25.3} = -3.6$ ($c = 0.03$, MeOH). UV (MeOH): 269 (4.08), 228 (2.68), 205 (2.34). IR (KBr): 3447, 3377, 3133 2286, 1656, 1597, 1439, 1266, 1122, 1064, 861, 817, 621, 569. ^1H- and ^{13}C-NMR: *Table.* HR-ESI-MS (pos.): 507.2384 ($[M+H]^+$, $C_{30}H_{35}O_7^+$; calc. 507.2384).

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