Anticancer Activity of Diterpenoids from *Amoora ouangliensis* and *Amoora stellato-squamosa*

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A new *ent*-halimane-type diterpene, named 5(10),14-*ent*-halimadien-3*β*,13*S*-diol (**1**), was isolated from the bark of *Amoora ouangliensis* and its chemical structure determined on the basis of spectroscopic analysis. Additionally, ten other diterpenoids were obtained from *A. ouangliensis* and *A. stellato-squamosa*. The bioactive experiments of all compounds against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) cells were documented.

Key words: 5(10),14-*ent*-Halimadien-3*β*,13*S*-diol, *Amoora ouangliensis*, *Amoora stellato-squamosa*

Introduction

During the course of our phytochemical investigations on the genus *Amoora* (Meliaceae), tirucallane and dammarane triterpenes, and neoclerodane diterpenes were isolated successively (Yang *et al*., 2004a, b, c, 2005). This paper presents a new *ent*-halimane-type diterpene, 5(10),14-*ent*-halimadien-3*β*,13*S*-diol (**1**), obtained during our continuing study on the bark of *Amoora ouangliensis*. Ten other diterpenoids, namely neoclerod-14-en-3*α*,4*β*,13*S*-triol (**2**), 6-*O*-acetyl-austroinulin (**3**), (3*α*,4*β*)-neoclerod-13(*E*)-en-3,4,15-triol (**4**), 3,13(*E*)-2-oxo-neoclerodadien-15-ol (**5**), methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate (**7**), (13*S*)-2-oxoneocleroda-3,14-dien-13-ol (**8**), (13*E*)-neocleroda-3,13-dien-15,18-diol (**9**), 15-hydroxy-8(17),13(*E*)-labdadien-19-oic acid (**10**), 8(17),12(*E*),14-labdatrien-19-oic acid (**11**), (3*α*,4*β*,14*RS*)-neoclerod-13(16)-en-3,4,14,15-tetrol (**12**), were isolated from the barks of *A. ouangliensis* and the twigs of *A. stellato-squamosa*. The bioactive experiments on **1**–**13** (**6** and **13** were the acetylized products of **4** and **12**, respectively) against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) were also assayed. Among them, **1** and **6** exhibited significant activities against these cells with IC_{50} values of 21.52 and 28.47 μM, and 25.73 and 23.34 μM, respectively.

Results and Discussion

Compound **1**, obtained as colourless oily solid, showed a molecular ion peak at *m/z* 306 in the EI mass spectrum. Its molecular formula was determined to be $C_{20}H_{34}O_2$ by HR-ESI-MS (*m/z* 329.2457 $[M+Na]^+$; calcd. 329.2456), corresponding to four degrees of unsaturation. The IR spectrum revealed an absorption band for the hydroxy group (3418 cm^{-1}). The ^{13}C NMR (DEPT) spectrum of **1** (see Table I) indicated the presence of five CH_3 (δ_C 27.4, 15.9, 25.1, 20.1, 21.2), seven CH_2 (δ_C 23.9, 27.1, 25.5, 27.3, 29.8, 36.4, 111.6), three CH groups (δ_C 33.2, 76.1, 145.1), and five quaternary carbon atoms (δ_C 39.8, 40.1, 73.2, 136.1, 131.6). The ^1H NMR spectrum showed the signals for one terminal vinyl group at δ_H 5.84 (1H, dd, J = 17.4, 10.7 Hz), 5.15 (1H, dd, J = 17.4, 1.0 Hz), and 5.01 (1H, dd, J = 10.7, 1.0 Hz), one oxymethine [δ_H 3.39 (1H, dd, J = 11.2, 3.2 Hz)], and five CH_3 groups: one double-peak signal at δ_H 0.78 (3H, d, J = 6.8 Hz) and four single-peak signals at δ_H 1.22, 0.99, 0.90, 0.77. These spectral data were quite similar to those of 3-hydroxy-5(10),13(*E*)-halimadien-15-al, except for the resonance attributable to the side chain (Nagashima *et al.*, 1995). Hence,

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Table I. ^{1}H NMR, ^{13}C NMR and HMBC spectral data of **1** in $CDCl_3$ (500 and 125 MHz, respectively).

Position	δ_H	δ_C	HMBC
1	2.07, 1.85 (m)	23.9 (t)	C-2, 10
2	1.38–1.45, 1.04–1.11 (m)	27.1 (t)	C-1, 3, 4
3	3.39 (dd, 11.2, 3.2)	76.1 (d)	C-1, 2, 4, 5, 18, 19
4	–	39.8 (s)	–
5	–	136.1 (s)	–
6	1.88–1.95 (m)	25.5 (t)	C-5, 7, 10
7	1.67–1.74, 1.57–1.61 (m)	27.3 (t)	C-5, 6, 8, 9
8	1.52 (m)	33.2 (d)	C-9
9	–	40.1 (s)	–
10	–	131.6 (s)	–
11	1.28–1.34 (m)	29.8 (t)	C-8, 9, 10, 12, 13
12	1.38–1.45, 1.04–1.11 (m)	36.4 (t)	C-11, 13, 14, 16
13	–	73.2 (s)	–
14	5.84 (dd, 17.4, 10.7)	145.1 (d)	C-12, 13, 16
15	5.15 (dd, 17.4, 1.0), 5.01 (dd, 10.7, 1.0)	111.6 (t)	C-13, 14
16	1.22 (s)	27.4 (q)	C-12, 13, 14
17	0.78 (d, 6.8)	15.9 (q)	C-7, 8, 9
18	0.90 (s)	20.1 (q)	C-3, 4, 5, 18
19	0.99 (s)	25.1 (q)	C-3, 4, 5, 19
20	0.77 (s)	21.2 (q)	C-8, 9, 10, 11

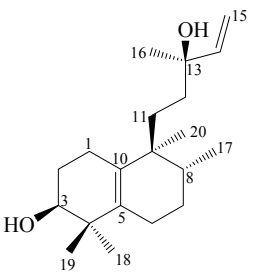
compound **1** was expected to contain a halimane skeleton with a $\Delta^{5,10}$ C=C bond. This assumption was confirmed by the HMBC spectrum; δ_H 2.07, 1.85 (each 1H, m, H-1) showed cross-peaks with δ_C 27.1 (t, C-2) and 131.6 (s, C-10), and δ_H 0.99 (3H, s, Me-19) and 0.90 (3H, s, Me-18) with δ_C 136.1 (s, C-5) (see Table I).

The structure of the side chain was determined by ^{1}H, ^{13}C NMR, HMQC and HMBC spectra, as well as by comparison with analogues. The HMBC spectrum showed the following long-range correlations: δ_H 1.28–1.34 (2H, m, H-11) to δ_C 33.2 (d, C-8), 40.1 (s, C-9), 131.6 (s, C-10), 36.4 (t, C-12), 73.2 (s, C-13); δ_H 1.22 (3H, s, Me-16) to δ_C 145.1 (d, C-14), 36.4 (t, C-12), 73.2 (s, C-13); and δ_H 5.84 (1H, dd, H-14) to δ_C 27.4 (q, C-16), 36.4 (t, C-12), 73.2 (s, C-13) (see Table I). Therefore, it was deduced that the side chain was a 3-methylpent-3 -hydroxy-1-ene moiety. Furthermore, due to the extremely similar chemical shifts of C-13 and those of its connected carbon atoms, it was concluded that **1** had the same side chain as **2**, which means they had the same stereochemistry at C-13 (Meragelman *et al.*, 1996).

In the ^{1}H NMR spectrum, H-3 showed a dd signal; the coupling constant J = 11.2, 3.2 Hz between H-3 and $H_{\alpha,\beta}$-2 indicated that 3-OH was in β-equatorial position.

A ROESY experiment was deduced in order to determine the relative configuration of **1**, but except for NOES between H-3 and Me-19, no other useful information could be obtained. Me-17 and Me-20 were assumed to be α-orientated on the basis of comparison with other halimane-type diterpenes and on biogenetic groups (co-occurrence of labdane- and neoclerodane-type diterpenes in the same plant) (Nagashima *et al.*, 1995). So compound **1** was established as 5(10),14-*ent*-halimadien-3β,13*S*-diol (Fig. 1).

The occurrence of halimane-type diterpenes in Nature is not so common (Kernan and Faulkner, 1988; Nagashima *et al.*, 1995; Calderón *et al.*,



1

Fig. 1. Chemical structure of compound **1**.

1983); this is the first report on a halimane-type diterpene from the family Meliaceae.

Compounds **1**–**13** were assayed for their cytotoxic activity toward AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) cells; the results are shown in Table II. **1** and **6** exhibited significant activities against these cells with IC_{50} values of 21.52 and 28.47 μM, and 25.73 and 23.34 μM, respectively. **3** and **9** showed moderate activities, others showed no remarkable activity towards the two cells.

Table II. Cytotoxicity of compounds **1**–**13**.

Compound	IC_{50} [μM][a]	
	AGZY 83-a	SMMC-7721
cis-Platin[b]	5.67	3.95
1	21.52	28.47
2	n.a.[c]	n.a.
3	69.61	56.38
4	n.a.	n.a.
5	n.a.	n.a.
6	25.73	23.34
7	n.a.	n.a.
8	n.a.	n.a.
9	50.10	40.67
10	n.a.	n.a.
11	86.59	n.a.
12	n.a.	n.a.
13	n.a.	n.a.

[a] AGZY 83-a, human lung cancer cells; SMMC-7721, human liver cancer cells.
[b] *cis*-Platin as positive control.
[c] n.a., no activity.

Experimental

General

Silica gel (200–300 mesh) for column chromatography (CC) and silica gel GF_{254} for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, P.R. China. Melting points: XRC-1 apparatus (Sichuan University, Sichuan, P. R. China). Optical rotations: Horiba SEAP-300 polarimeter (Kyoto, Japan). IR spectra: Bio-Rad FTS-135 spectrophotometer (Richmond, CA, USA). NMR spectra: Bruker AM-400 or DRX-500 spectrometers (Karlsruhe, Germany). MS data: VG Autospec-3000 spectrometer (Manchester, England).

Plant material

The barks of *A. ouangliensis* and the twigs of *A. stellato-squamosa* were collected in Xishuangbanna County of Yunnan Province, P. R. China, in January 2002. The plants were identified by Prof. Jing-Yun Cui, Xishuangbanna Tropical Botanical Garden, Academia Sinica, Mengla County, China.

Extraction and isolation

The air-dried barks of *A. ouangliensis* (7.0 kg) were extracted three times with EtOH/H_2O (9:1) at reflux temperature for 4 h each). After evaporation, the residue was suspended in H_2O and extracted with $CHCl_3$. The extract (110 g) was subjected to CC [SiO_2; petroleum ether/EtOAc (1:0) → (2:8)] to afford eight fractions (Frs. 1–8), as judged by TLC. Fr. 4 (20 g) and Fr. 5 (9.5 g) were repeatedly chromatographed [1. SiO_2, $CHCl_3$/Me_2CO (9:1) → (1:1); 2. RP-18 gel, MeOH/H_2O (1:1) → 1:0)]. **1** (37 mg), **3** (57 mg), and **5** (336 mg) were obtained from Fr. 4, **2** (1.01 g) and **4** (1.28 g) from Fr. 5.

The isolation of **4**, **5**, **7**–**9**, **12** from the twigs of *A. stellato-squamosa* were as described previously (Yang *et al.*, 2004a), **10** (854 mg) was obtained from Fr. 3, and **11** (188 mg) from Fr. 2.

Bioassays

An improved MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colourimetric assay was performed in 96-well plates; the experimental details have been reported previously (Niu *et al.*, 2002).

Compounds **1**–**13** were assayed for their cytotoxic activity toward AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) cells; the results are shown in Table II.

5(10),14-ent-Halimadien-3β,13S-diol (**1**): Colourless oily solid. – $[\alpha]_D^{25.2}$ +78.71° (*c* 0.303, $CHCl_3$). – IR (KBr): ν = 3418, 2968, 2938, 2876, 1640, 1462, 1379, 1369, 1282, 1184, 1122, 1105, 1049, 996, 920 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see Table I. – EI-MS: *m/z* = 306 (2, $[M]^+$), 288 (3), 270 (28), 255 (30), 227 (10), 207 (100), 189 (77), 121 (60), 163 (25), 147 (30), 135 (43), 119 (37), 107 (31), 95 (21), 83 (26), 71 (18), 55 (22). – HR-ESI-MS: *m/z* = 329.2457 $[M+Na]^+$ (calcd. for $C_{20}H_{34}O_2Na$, 329.2456).

Acetylation of **12**

The synthetic method for **13** from **12** was the same as for **6** from **4** (Yang *et al.*, 2004a).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.38 (1H, tt, J = 7.9, 2.7 Hz, H-14), 5.07, 4.98 (each 1H, brs, H-16), 4.74 (1H, t, J = 2.5 Hz, H-3), 4.11 (4.08) (1H, dd, J = 12.5, 7.9 Hz, H-15a), 4.26 (1H, dd, J = 11.8, 3.7 Hz, H-15b), 2.06, 2.07, 2.09/2.10 (each 3H, q, $COOC\underline{H}_3$), 1.12 (3H, s, Me-18), 1.08 (3H, s, Me-19), 0.79/0.80 (3H, d, J = 7.9 Hz, Me-17), 0.75 (3H, s, Me-20). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 16.82/16.79 (t, C-1), 27.4 (t, C-2), 76.9 (d, C-3), 75.6 (s, C-4), 41.4 (s, C-5), 32.0 (t, C-6), 26.6 (t, C-7), 36.1 (d, C-8), 38.7 (s, C-9), 40.3 (d, C-10), 37.16/37.12 (t, C-11), 26.31/26.42 (t, C-12), 145.4 (s, C-13), 73.75/73.83 (d, C-14), 64.60/64.66 (t, C-15), 112.50/112.56 (t, C-16), 15.96/16.01 (q, C-17), 18.3 (q, C-18), 20.8 (q, C-19), 16.9 (q, C-20), 21.06, 21.48, 21.14/21.18 (q, 3 × $\underline{C}H_3COO$), 170.1, 170.2, 170.8 (s, 3 × $CH_3\underline{C}OO$).

The spectral data of **4**–**9** and **12** were just like those we have reported previously (Yang *et al.*, 2004a). The structures of the other diterpenoids, **2** (Meragelman *et al.*, 1996), **3** (Darise *et al.*, 1983), **10** (Fang *et al.*, 1989), **11** (Barrero and Altarejos, 1993), were elucidated on the basis of spectral data and comparison with published data.

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