Anticancer Activity of Tirucallane Triterpenoids from *Amoora dasyclada*

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A new tetranortriterpene 3α-acetoxy-24,25,26,27-tetranortirucalla-7-ene-23(21)-lactone (**3**), and eleven other compounds were isolated from the twigs of *Amoora dasyclada*. The structure of compound **3** was identified on the basis of spectroscopic data, and the bioactive experiments of **1** and **3**–**5** against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) are documented. Among them, compound **5** exhibited a strong activity against SMMC-7721.

Key words: Amoora dasyclada, Tirucallane Triterpenoid, Anticancer Activity

Introduction

In previous papers, we reported four new tirucallane-type triterpenoids (**1**, **2**, **4**, **5**) from the twigs of *Amoora dasyclada* (How et T. Chen) C. Y. Wu (Yang *et al*., 2004a, b); here we present another tirucallane-type triterpenoid obtained during our continuing study on the same plant: 3α-acetoxy-24,25,26,27-tetranortirucalla-7-ene-23(21)-lactone (**3**). Seven other compounds, taraxerone (**6**), taraxerol (**7**) and taraxerol acetate (**8**), scopoletin (**9**), stigmast-5-en-3β,7α-diol (**10**), β-sitosterol (**11**), β-sitosterol-D-glucoside (**12**), were isolated from the same source. The bioactive experiments of **1** and **3**–**5** against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) were also assayed. Among them, compound **5** exhibited a strong activity against SMMC-7721 with the IC_{50} value of 8.41×10^{-3} μM/ml.

Results and Discussion

Compound **3** (white needles) has a molecular formula of $C_{28}H_{42}O_4$ established by HR-ESI-MS (*m/z* 465.2989 $[M + Na]^+$). The ^1H and ^{13}C NMR spectra of it were in good agreement with those of **2** indicating that **3** was also a tirucallane derivative (Yang *et al*., 2004a). The IR spectrum showed the presence of a γ-lactone group (1784 cm^{-1}); it was further confirmed by the long correlations between δ_H 4.37 (1H, t, J = 8.7 Hz, H-21α); 3.90 (1H, t, J = 9.3 Hz, H-21β) with δ_C 176.9 (s, C-23), 39.2 (d, C-20) and 34.2 (t, C-22); δ_H 2.52 (1H, dd, J = 19.0, 6.5 Hz, H-22α); 2.17 (1H, dd, J = 18.3, 13.8 Hz, H-22β) with δ_C 72.4 (t, C-21), C-20 and C-23. The signals at δ_H 2.52, 2.17 showed a large coupling constant of 13.8 Hz and a small coupling constant of 6.5 Hz, respectively, which revealed axial orientations for H-20 and H-22α. Comparing the ^1H and ^{13}C NMR spectra of **3** with those of **2**, except the γ-lactone group [δ_C 176.9 (s, C-23)], there was an acetoxy group in **3** [δ_C 170.7(s), 21.3 (q)], and the signals at δ_H 2.04 (3H, s) and 4.66 (bs) also indicated that an α-acetoxy group was located at C-3 instead of a hydroxy group in **2**. The cross peaks between δ_H 2.04 (3H, s) and δ_C 170.7 (s, CH_3**C**OO), δ_H 4.66 (bs) and δ_C 170.7, 33.6 (s, C-4), 45.6 (d, C-5), 22.9 (t, C-2), 31.8 (t, C-1) in the HMBC spectrum supported this assumption.

The strongly negative optical rotation of **3** (–42.13°) suggested that it belongs to the tirucallanes (C-20α) (Sherman *et al*., 1980; Jolad *et al*., 1981). In the ROESY spectrum the strong cross peaks between δ_H 4.37 (H-21α) and 3.90 (H-21β) with 1.35 (H-12a), δ_H 4.37 (H-21α) with 0.86 (Me-18), δ_H 3.90 (H-21β) with 1.73 (1H, m, H-17) and 1.71 (H-12b) also indicated that **3** preferred H-20α (C-20*S*) configuration to C-20*R* configuration proving the tirucallane-type triterpene (Mohamad *et al*., 1999; Wang *et al*., 2003). So compound **3** was a biodegraded product of tirucallane

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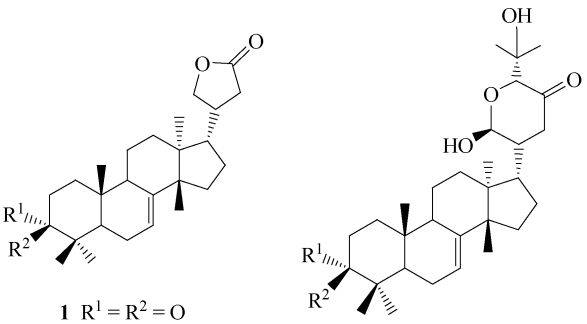
with the loss of four carbon atoms at the side-chain and it was determined to be 3α-acetoxy-24,25,26,27-tetranortirucalla-7-ene-23(21)-lactone (Fig. 1).



1 $R^1 = R^2 = O$
2 $R^1 = OH, R^2 = H$
3 $R^1 = OAc, R^2 = H$
4 $R^1 = R^2 = O$
5 $R^1 = OH, R^2 = H$

Fig. 1. The structures of compounds **1**–**5**.

Experimental

General

Melting points: XRC-1 apparatus (Sichuan University, Sichuan, P.R. China), uncorrected. Optical rotations: Horiba SEAP-300 polarimeter (Kyoto, Japan). IR spectra: Bio-Rad (Richmond, CA, USA) FTS-135 infrared spectrophotometer. One- and two-dimensional NMR spectra: Bruker AM-400 or DRX-500 spectrometers (Karlsruhe, Germany). MS data: VG Autospec-3000 spectrometer (Manchester, England).

Plant material

The twigs of *A. dasyclada* were collected in Xishuangbanna County of Yunnan Province, P.R. China, in January 2002. The plant was identified by Mr. Jingyun Cui, Xishuangbanna Tropical Botanical Garden, CAS, P.R. China.

Extraction and isolation

The first step of the isolation was the same as previously described (Yang *et al.*, 2004b). Then fraction 2 was repeatedly chromatographed by CC over silica gel eluted with petroleum ether/EtOAc (from 1:0 to 8:2, v/v) to give compounds **6** (191 mg) and **8** (7 mg); fraction 4 was subject to repeated CC on silica gel eluted with petroleum ether/Me_2CO (from 98:2 to 7:3) to obtain compounds **1** (440 mg), **3** (19 mg), **7** (140 mg) and **11** (2.1 g); fraction 5 was submitted to repeated CC on silica gel eluted with $CHCl_3$/EtOAc (from 95:5 to 3:1) and then purified on a RP-18 column eluted with MeOH/H_2O (from 1:1 to 1:0) to yield compounds **2** (20 mg) and **10** (17 mg); fraction 8 was repeatedly chromatographed by CC over silica gel eluted with $CHCl_3$/Me_2CO (from 95:5 to 3:1) and then purified on a RP-18 and Sephadex LH-20 column successively to afford compound **9** (21 mg); fraction 9 was repeatedly chromatographed over silica gel eluted with $CHCl_3$/MeOH (from 95:5 to 1:1) to produce compound **12** (610 mg).

Bioassays

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

The results of anticancer activity tests of the four tirucallane triterpenoids **1** and **3**–**5** against AGZY 83-a (human lung cancer cells) and SMMC-7721 (human liver cancer cells) are given in Table I. In the test, **1** and **3** showed inactivity to these two cell lines, **4** and **5** exerted weak activity against AGZY 83-a, **5** exhibited strong activity against SMMC-7721.

*3α-Acetoxy-24,25,26,27-tetranortirucalla-7-ene-23(21)-lactone (**3**):* White needles. – M.p. 214–

Table I. Cytotoxicity[a] of compounds **1** and **3**–**5**.

	IC_{50} [μM/ml][b]				
	cis-Platin[c]	**1**	**3**	**4**	**5**
AGZY 83-a	5.673×10^{-3}	no activity	no activity	0.065 ± 0.013	0.050 ± 0.005
SMMC-7721	3.947×10^{-3}	no activity	0.171 ± 0.044	no activity	0.018 ± 0.005

[a] AGZY 83-a, human lung cancer cells; SMMC-7721, human liver cancer cells.
[b] The IC_{50} values are presented as means ± SE.
[c] *cis*-Platin as positive control.

216 °C. – $[\alpha]_D^{16}$ –42.13° (*c* 0.178, $CHCl_3$). – IR (KBr): ν = 2922, 1781, 1728, 1631, 1462, 1373, 1248, 1174, 1101, 1032, 1019 cm^{-1}. – ^1H NMR (500 MHz, $CDCl_3$): δ = 5.26 (1H, d, *J* = 2.9 Hz, H-7), 4.37 (1H, t, *J* = 8.7 Hz, H-21α), 3.90 (1H, t, *J* = 9.3 Hz, H-21β), 2.55 (1H, m, H-20), 2.52 (1H, dd, *J* = 19.0, 6.5 Hz, H-22α), 2.30 (1H, m, H-9), 2.17 (1H, dd, *J* = 18.3, 13.8 Hz, H-22β), 2.05, 1.95 (each 1H, m, H-6), 2.04 (3H, s, **C**H_3COO), 1.91, 1.32 (1H, m, H-16), 1.85, 1.67 (each 1H, m, H-2), 1.75 (1H, m, H-5), 1.73 (1H, m, H-17), 1.72, 1.38 (2H, m, H-1), 1.71, 1.35 (each, 1H, m, H-12), 1.64–1.48 (4H, m, H-11 and H-15), 0.97 (3H, s, Me-30), 0.94 (3H, s, Me-29), 0.86 (3H, s, Me-18), 0.82 (3H, s, Me-28), 0.76 (3H, s, Me-19). – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 31.8 (t, C-1), 22.9 (t, C-2), 78.2 (d, C-3), 36.6 (s, C-4), 45.6 (d, C-5), 23.8 (t, C-6), 118.6 (d, C-7), 144.9 (s, C-8), 48.4 (d, C-9), 34.9 (s, C-10), 17.3 (t, C-11), 31.9 (t, C-12), 43.7 (s, C-13), 50.6 (s, C-14), 34.1 (t, C-15), 27.3 (t, C-16), 51.0 (d, C-17), 22.6 (q, C-18), 12.9 (q, C-19), 39.2 (d, C-20), 72.4 (t, C-21), 34.6 (t, C-22), 176.9 (s, C-23), 27.4 (q, C-28), 21.4 (q, C-29), 27.0 (q, C-30), 170.7 (s, CH_3**C**OO), 21.3 (q, **C**H_3COO). – EI-MS: *m/z* = 442 (17, $[M]^+$), 426 (13), 382 (9), 367 (100), 324 (3), 297 (4), 259 (13), 245 (6), 213 (5), 187 (13), 159 (11), 147 (9), 119 (12), 105 (9), 81 (5). – HR-ESI-MS: *m/z* = 465.2989 $[M + Na]^+$ (calcd. for $C_{28}H_{42}O_4Na$, 465.2980).

Three known compounds **6**–**8** were identified by spectral analysis results and by comparison with the published data (Sakurai *et al*., 1987). The R_f values of **9**–**12** were coincident with the standard samples in different developing solvents.

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