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-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\textsubscript{50} value of \(8.41 \times 10^{-3} \mu M/ml\).

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 \(^13C\) 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 \(\delta_\text{H} 4.37 (1H, t, J = 8.7 Hz, H-21\alpha)\); 3.90 (1H, t, \(J = 9.3\) Hz, H-21\(β\)) with \(\delta_\text{C} 176.9 (s, C-23)\), 39.2 (d, C-20) and 34.2 (t, C-22); \(\delta_\text{H} 2.52 (1H, dd, J = 19.0, 6.5\) Hz, H-22\(α\)); 2.17 (1H, dd, \(J = 18.3, 13.8\) Hz, H-22\(β\)) with \(\delta_\text{C} 72.4 (t, C-21)\), 20-C and C-23. The signals at \(\delta_\text{C} 2.52, 2.17\) 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-22\(α\). Comparing the \(^1H\) and \(^13C\) NMR spectra of 3 with those of 2, except the γ-lactone group [\(\delta_\text{C} 176.9 (s, C-23)\)], there was an acetoxy group in 3 [\(\delta_\text{C} 170.7 (s)\)], 21.3 (q)], and the signals at 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 \(\delta_\text{H} 2.04 (3H, s)\) and \(\delta_\text{C} 170.7 (s, CH_3COO)\), \(\delta_\text{H} 4.66 (bs)\) and \(\delta_\text{C} 170.7, 33.6 (s, C-4)\), 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-20\(α\)) (Sherman et al., 1980; Jolad et al., 1981). In the ROESY spectrum the strong cross peaks between \(\delta_\text{H} 4.37 (H-21\alpha)\) and 3.90 (H-21\(β\)) with 1.35 (H-12\(a\)), \(\delta_\text{H} 4.37 (H-21\alpha)\) with 0.86 (Me-18), \(\delta_\text{H} 3.90 (H-21\β)\) with 1.73 (1H, m, H-17) and 1.71 (H-12\(b\)) also indicated that 3 preferred H-20\(α\) (C-20\(α\)) 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
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).

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/Me2CO (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 CHCl3/EtOAc (from 95:5 to 3:1) and then purified on a RP-18 column eluted with MeOH/H2O (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 CHCl3/Me2CO (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 CHCl3/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>
<thead>
<tr>
<th>IC50 [µm/ml]b</th>
<th>1</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGZY 83-a</td>
<td>5.673 × 10⁻³</td>
<td>no activity</td>
<td>no activity</td>
<td>0.065 ± 0.013</td>
</tr>
<tr>
<td>SMMC-7721</td>
<td>3.947 × 10⁻³</td>
<td>no activity</td>
<td>0.171 ± 0.044</td>
<td>0.050 ± 0.005</td>
</tr>
</tbody>
</table>

a AGZY 83-a, human lung cancer cells; SMMC-7721, human liver cancer cells.
b The IC50 values are presented as means ± SE.
c cis-Platin as positive control.
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216 °C. – $[\alpha]_D^{20} = -42.13^\circ$ (c 0.178, CHCl₃). – IR (KBr): $\nu = 2922, 1781, 1728, 1631, 1462, 1373, 1248,$ 1174, 1101, 1032, 1019 cm⁻¹. – ¹H NMR (500 MHz, CDCl₃): $\delta = 5.26$ (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), 2.20, 2.05 (each 1H, m, H-6), 2.04 (3H, s, CH₃COO), 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). – ¹³C NMR (125 MHz, CDCl₃): $\delta = 31.8$ (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, CH₃COO), 21.3 (q, CH₃COO). – 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 C₂₈H₄₂O₄Na, 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 Rf values of 9–12 were coincident with the standard samples in different developing solvents.

Acknowledgements

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