Triterpenoids from *Bignonia unguiscati* roots

Biswa Nath Dinda*, Utpal Chandra De
Shiho Arima*, Nariko Sato* & Yoshihiro Harigaya*
Department of Chemistry, Tripura University, Suryamaninagar,
Agartala 799 130, India
E.mail: dindabtu@rediffmail.com

*School of Pharmaceutical Sciences, Kitasato University,
Minato-ku, Tokyo 108 8641, Japan

Received 16 October 2003; accepted (revised) 20 June 2005

One new triterpenoid named acetylbignonic acid 1 along with 2α-hydroxyursolic acid 2 have been isolated from the roots of *Bignonia unguiscati*. On the basis of chemical and spectral evidence, the structure of 1 has been established as 3β-acetoxy-19α-hydroxyurs-12-en-29β-oic acid.

**Keywords:** Triterpenoid, acetylbignonic 2α-hydroxyursolic acid, *Bignonia unguiscati*

**IPC:** Int.Cl.7 A 61 K 35/00

*Bignonia unguiscati* L. syn. *B. gracilis* Lodd syn. *Doxantha unguis* Miers (Bignoniaceae) is an Indian folk medicine, the roots of which are used for treatment of ulcers1. Earlier investigations reported the isolation of lapachol, ceryl alcohol, β-amyrin, β-sitosterol, octacosanol, tectol and ellagic acid from its aerial parts2,3 and β-sitosterol cerotate and 2-(4-hydroxy-3-nitrophenyl)ethyl stearate from its roots4. As a part of our continuing chemical study on this plant, two more compounds were isolated and identified on the basis of chemical and spectral methods. Among them, one compound 1 is a new triterpenoid and the other compound 2 is a known triterpenoid, previously reported from the tissue culture of *Isodon japonicus* Hara5. In this note, we report the isolation and structure elucidation of these compounds.

**Results and Discussion**

The methanol extract of the roots of *B. unguiscati* was fractioned by the usual procedure (see Experimental) and the chloroform soluble fraction on column chromatography afforded compounds 1 and 2.

Acetylbignonic acid 1, white needles, mp 250°C, showed a positive reaction to the Liebermann Burchard test, which indicated its triterpenoid nature. The compound had the molecular formula C32H50O5, as determined by HRFABMS (positive): m/z 537.3573[M+Na]+ (100%). Calcd for C32H50O5Na: m/z 537.7338 as well as by elemental analysis. The IR spectrum of 1 exhibited hydroxyl (3400 cm⁻¹), ester (1728 cm⁻¹), carboxyl (1700 cm⁻¹) and olefinic (1640 cm⁻¹) absorptions. The 400 MHz 1H NMR spectrum of 1 in CDCl3 (Table I) revealed the signals for six tertiary methyl groups (δ 0.72, 0.85, 0.87, 1.21, 1.24 and 1.25, each 3H, s) and one secondary methyl group (δ 0.95, 3H, d, J = 6.5 Hz), one acetate methyl group (δ 2.05, 3H, s), one methine proton (δ 4.50, dd, J = 9.5 and 5.0 Hz), one olefinic proton (δ 5.34, t, J = 3.5 Hz)
and one carboxyl group (δ 10.8 - 11.6, brs, disappeared on D$_2$O exchange), all of which suggested an acetyl hydroxy ursane skeletal structure. The 100 MHz $^{13}$C NMR spectrum (Table I) revealed 32 carbon signals which were assigned by DEPT experiments as eight methyl, nine methylene, six methine and nine quaternary (including one acetoxyl and one carboxyl) carbons. Both $^1$H and $^{13}$C NMR chemical shifts were in good agreement with the 3β-acetoxy-19α-hydroxyurs-12-en-29β-oic acid structure 1 for the compound $^6$$^7$. The position the carboxyl and hydroxyl groups at C-19 was assigned on the basis of HMBC correlation of C-18 proton with C-19 and C-29 carbons and of C-20 proton with C-19 and C-29 carbons (Table I). Moreover, upfield chemical shifts of H-18 and H$_3$-30 at δ 2.53 (s) and 0.95 (3H, d) respectively compared to that of pomolic acid $^3$, where the values are at δ 3.05 and 1.13, respectively, also supported this assignment. The assignment of $^1$H and $^{13}$C NMR chemical shift values was made with the help of $^1$H-$^1$H COSY, HMQC and HMBC experiments. The EIMS of 1 showed molecular ion

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_c$</th>
<th>$\delta_H$</th>
<th>HMBC(H→C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.1(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>28.2(t)</td>
<td>1.63(m), 1.97(m)</td>
<td>C-1, C-3</td>
</tr>
<tr>
<td>3</td>
<td>81.0(d)</td>
<td>4.50(dd, $J = 9.5$, 5.0 Hz)</td>
<td>C-2, C-4, C-23, C-24, C-31</td>
</tr>
<tr>
<td>4</td>
<td>39.5(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>55.2(d)</td>
<td>1.40(m)</td>
<td>C-6</td>
</tr>
<tr>
<td>6</td>
<td>18.3(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>32.6(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>40.0(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>47.1(d)</td>
<td>1.78(dd, $J = 9.0$, 9.5 Hz)</td>
<td>C-8, C-10</td>
</tr>
<tr>
<td>10</td>
<td>37.7(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>23.5(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>129.3(d)</td>
<td>5.34(t, $J = 3.5$ Hz)</td>
<td>C-9, C-11, C-14, C-18</td>
</tr>
<tr>
<td>13</td>
<td>138.0(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>41.9(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>28.2(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>25.3(t)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>36.9(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>52.8(d)</td>
<td>2.53(s)</td>
<td>C-12, C-13, C-17, C-19, C-20, C-29</td>
</tr>
<tr>
<td>19</td>
<td>73.1(s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>41.1(d)</td>
<td>1.54(m)</td>
<td>C-19, C-21, C-29</td>
</tr>
<tr>
<td>21</td>
<td>29.7(t)</td>
<td>1.40(m)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>37.5(t)</td>
<td>2.18(m)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>28.1(q)</td>
<td>1.21(s)</td>
<td>C-4, C-5, C-24</td>
</tr>
<tr>
<td>24</td>
<td>15.3 (q)</td>
<td>0.85(s)</td>
<td>C-4, C-5, C-23</td>
</tr>
<tr>
<td>25</td>
<td>16.1(q)</td>
<td>0.72(s)</td>
<td>C-1, C-9, C-10</td>
</tr>
<tr>
<td>26</td>
<td>16.9(q)</td>
<td>0.87(s)</td>
<td>C-8, C-9, C-14</td>
</tr>
<tr>
<td>27</td>
<td>24.5(q)</td>
<td>1.25(s)</td>
<td>C-8, C-13, C-14</td>
</tr>
<tr>
<td>28</td>
<td>27.4(q)</td>
<td>1.24(s)</td>
<td>C-18, C-21, C-22</td>
</tr>
<tr>
<td>29</td>
<td>183.9(s)</td>
<td>10.8-11.6(brs)</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>16.6(q)</td>
<td>0.95(d, $J = 6.5$ Hz)</td>
<td>C-18, C-19, C-21</td>
</tr>
<tr>
<td>31 (32)</td>
<td>-OCOCH$_3$</td>
<td>171.0(s)</td>
<td></td>
</tr>
<tr>
<td>31 (32)</td>
<td>21.3 (q)</td>
<td>2.05(s)</td>
<td>C-3, C-31</td>
</tr>
</tbody>
</table>

Table I — $^1$H and $^{13}$C NMR spectral data of compound 1 (400 MHz for $^1$H and 100 MHz for $^{13}$C).
peak at m/z 514 and some intense mass peaks at m/z 496, 468, 454, 439, 396, 264, 249, 248, 246, 218, 203, 190, 189, 175, 146 (base peak) and 133, which could be rationalized by considering the said structure (Scheme I)\(^8\). The base peak at m/z 146 was obtained by retro-Diels-Alder cleavage of mass peak at m/z 248. Similar base peak at m/z 146 was also reported in the EIMS of barbinervic acid \(4\) isolated from the stem of *Coussarea brevicaulis*\(^9\). The mass peak at m/z 249 supported the presence of acetoxyl group in ring-A of the compound. The compound on hydrolysis with 1\(M\) methanolic HCl gave bignonic acid \(5\), \(C_{30}H_{48}O_4\), \([M + 472]\) as determined from HRFABMS (positive): \(m/z\) 495.3450 \([M+Na]^+\). Calcd for \(C_{30}H_{48}O_4Na\): \(m/z\) 495.6966. Its IR spectrum showed absorptions for hydroxyl (3400 cm\(^{-1}\)), carboxyl (1700 cm\(^{-1}\)) and olefinic acid (1640 cm\(^{-1}\)) functions. The 400 MHz \(^1\)H NMR spectrum in CDCl\(_3\) revealed five singlet methyl signals (\(\delta\) 0.77, 0.85, 0.98, 1.04, 1.09, each 3H) and two doublet methyl signals (\(\delta\) 0.83 and 0.90, \(J = 6.5\) Hz in each case), three methine proton signals (\(\delta\) 2.15, 1H, \(d\), \(J = 10.0\) Hz, H-18; 2.92, 1H, \(d\), \(J = 10.0\) Hz, H-3; 3.62, 1H, m, H-2), one olefinic proton signal (\(\delta\) 5.22, \(t\), \(J = 3.4\) Hz) and one carboxyl proton signal (\(\delta\) 10.95-11.20, brs, disappeared on D\(_2\)O exchange) suggesting \(2\alpha,3\beta\)-dihydroxyurs-12-en-28-oic structure \(2\) for the

![Scheme I — Mass fragmentation of compound 1](image)

(Labiatae)\(^{11}\). It is the first report that a carboxyl and a hydroxyl group are attached at the same carbon atom in a triterpenoid.

Compound \(2\), granular crystals, mp 242°C, had the molecular formula \(C_{30}H_{48}O_4\) as determined from HRFABMS (positive): \(m/z\) 495.3450 \([M+Na]^+\). Calcd for \(C_{30}H_{48}O_4Na\): \(m/z\) 495.6966. Its IR spectrum showed absorptions for hydroxyl (3400 cm\(^{-1}\)), carboxyl (1700 cm\(^{-1}\)) and olefinic acid (1640 cm\(^{-1}\)) functions. The 400 MHz \(^1\)H NMR spectrum in CDCl\(_3\) revealed five singlet methyl signals (\(\delta\) 0.77, 0.85, 0.98, 1.04, 1.09, each 3H) and two doublet methyl signals (\(\delta\) 0.83 and 0.90, \(J = 6.5\) Hz in each case), three methine proton signals (\(\delta\) 2.15, 1H, \(d\), \(J = 10.0\) Hz, H-18; 2.92, 1H, \(d\), \(J = 10.0\) Hz, H-3; 3.62, 1H, m, H-2), one olefinic proton signal (\(\delta\) 5.22, \(t\), \(J = 3.4\) Hz) and one carboxyl proton signal (\(\delta\) 10.95-11.20, brs, disappeared on D\(_2\)O exchange) suggesting \(2\alpha,3\beta\)-dihydroxyurs-12-en-28-oic structure \(2\) for the
compound. The $^{13}$C NMR spectral data in CDCl$_3$ with DEPT, in combination with HMQC and HMBC experiments also supported the said structure (see Experimental). The $^{13}$C NMR chemical shifts are almost similar to those reported for methyl 2α-hydroxyursolate$^5$ 6. The EIMS of the compound recorded molecular ion peak at m/z 472, which also agreed with the molecular formula. The base peak at m/z 248 was obtained from retro-Diels-Alder fragmentation of ring-C having a Δ12-double bond$^8, 9$. Another intense mass peak at m/z 203 was derived by loss of carboxyl radical from mass peak at m/z 248. Acetylation of the compound with Ac$_2$O in pyridine at room temperature afforded a diacetate 7 (C$_{34}$H$_{52}$O$_6$) (M$^+$ 556). Therefore, the structure of the compound was assigned as 2α,3β-dihydroxyurs-12-en-28-oic acid or 2α-hydroxyursolic acid 2. It may be noted that only $^{13}$C NMR data of this compound was reported earlier$^5$.

**Experimental Section**

**General.** Melting points are uncorrected. All solvents were distilled before use. Silica gel GF 254 (Merck) and silica gel 60-120 mesh (Merck) were used for TLC and CC, respectively and spots on TLC were visualized by spraying with 10% ethanolic H$_2$SO$_4$ and heating at 110°C. IR, MS and NMR spectra were recorded on Perkin-Elmer 577, Jeol JMS-AX 505 HA and Varian XL-400 spectrometers, respectively. HRFABMS were recorded on a Jeol JMS – 700 Mstation mass spectrometer.

**Plant material.** The roots of Bignonia unguis-cati were supplied by M/s United Chemical and Allied Products, Kolkata in October, 2001. A voucher specimen has been preserved in the herbarium of Shibpur Botanical Garden, Howrah, West Bengal, India.

**Extraction and isolation of compounds 1 and 2**

Air-dried milled roots (1.0 kg) of B. unguis-cati were extracted with MeOH by percolation. The MeOH extract was concentrated under reduced pressure to a gummy residue (4.2 g). The residue was dissolved in minimum quantity of H$_2$O and partitioned with C$_6$H$_6$, CHCl$_3$ and n-BuOH, respectively. The CHCl$_3$ soluble fraction was concentrated and subjected to CC. Elution of the column with C$_6$H$_6$-CHCl$_3$ (1:4) eluate gave a residue which on repeated CC afforded 1 in colourless needles (0.05 g, yield 5×10$^{-3}$ %) [R$_f$, 0.75 in CHCl$_3$-MeOH (95:5)]; Anal. Found: C, 74.21; H, 9.35. Caled for C$_{32}$H$_{50}$O$_6$: C, 74.69; H, 9.79 %.

Elution of the column with CHCl$_3$-MeOH (9:1) afforded a residue which on repeated CC gave 2 in granular crystals (0.04 g, yield 4×10$^{-3}$ %) [R$_f$, 0.85 in CHCl$_3$-MeOH (8:1)]. Anal. Found: C, 75.73; H, 9.81. Caled for C$_{30}$H$_{48}$O$_4$: C, 76.23; H, 10.23%.

**Compound 1:** EIMS m/z(%): 514 (M$^+$, 4), 496 (M$_{-}$H$_2$O, 2), 454 (26), 468 (26), 439 (12), 396 (20), 264 (13), 249 (27), 248 (75), 246 (29), 218 (24), 203 (62), 190 (90), 189 (56), 175 (29), 146 (100), 133 (45).

**Compound 2:** EIMS m/z(%): 472 (M$^+$, 2), 454 (M$_{-}$H$_2$O, 2), 426 (M-COOH-H, 4), 408 (M$_{-}$H$_2$O-COOH-H$_2$O, 2), 454 (26), 468 (26), 439 (12), 396 (20), 264 (13), 249 (27), 248 (75), 246 (29), 218 (24), 203 (62), 190 (90), 189 (56), 175 (29), 146 (100), 133 (45).

**Acid hydrolysis of compound 1.** Compound 1 (0.015 g) was dissolved in 1M methanolic HCl (10 mL) and refluxed for 2 hr on a water-bath. The reaction mixture was concentrated , diluted with water and extracted with chloroform. The CHCl$_3$ extract was concentrated and purified through column to get bignonic acid 5 as colourless needles (0.008g), mp 286°C. The aqueous layer was concentrated. The concentrated solution responded positive cacodyl oxide test for acetic acid.

**Compound 5:** EIMS m/z 472[M$^+$]; IR (KBr): 3400(OH), 1700(COOH), 1635 cm$^{-1}$(>C=C<); $^1$H NMR (CDCl$_3$): δ 7.07, 0.86, 0.88, 1.18, 1.22, 1.24 (each 3H, s, H3-23, H3-24, H3-25, H3-26, H3-27 and H3-28), 0.96(3H, d, J = 6.5 Hz, H-18), 2.55(1H, s, H-19), 3.38(1H, m, H-20), 5.30(1H, m, H-19), 3.06(1H, m, H-21), 3.67(1H, m, H-22), 28.5(q, C-23), 16.4(q, C-24), 16.6(q, C-25), 16.9(q, C-26), 23.4(q, C-27), 180.6(s, C-28), 18.2(q, C-29), 21.1(q, C-30).

**Acetylation of compound 2.** Compound 2 (0.01 g) was dissolved in 3 drops of pyridine and to it 1 mL of Ac$_2$O was added and the reaction mixture was kept at room temperature overnight. Work-up as usual gave a diacetate 7 (0.005 g) as white amorphous powder, mp 224°C ; EIMS: m/z 556[M$^+$]; $^1$H NMR (CDCl$_3$): δ 0.76, 0.84, 1.00, 1.06, 1.11 (each 3H, s), 0.86(3H, d, J = 6.5 Hz), 1.02(3H, d, J = 6.5 Hz), 2.02, 2.04 (each 3H, s, 2× -OAc), 2.18(1H, d, d = 10.5 Hz, H-18), 4.56(1H, m, H-3), 4.78(1H, m, H-2), 5.24(1H, t, J = 3.4 Hz, H-12), 10.95-11.20(1H, brs, HOOC-28).
Acknowledgement

The authors thank RSIC, Lucknow, India for microanalysis. One of the authors (UCD) is grateful to the CSIR, New Delhi for the grant of SRF.

References