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3β,6β-Dihydroxyolean-12-en-27-oic acid: a cytotoxic and apoptosis-inducing oleanane triterpenoid from the rhizome of *Astilbe chinensis*

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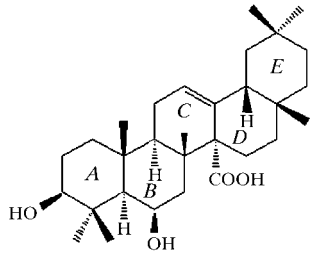
3β,6β-Dihydroxyolean-12-en-27-oic acid, $C_{30}H_{48}O_4$, a cytotoxic and apoptosis-inducing oleanane triterpenoid, which was isolated from the rhizome of *Astilbe chinensis*, consists of a linear array of five fused six-membered rings. The central ring has a slightly distorted half-chair conformation, while the four outer rings adopt chair conformations. Two hydroxy groups and one carboxy group serve simultaneously as hydrogen-bond donors and acceptors, forming molecular chains.

Comment

Astilbe chinensis (Maxim.) Franch. et Savat. (Saxiffagaceae) is a perennial herbaceous plant growing at an altitude of 390–3600 m in China, Russia, Japan and Korea. Its rhizome, known as 'Luo Xinfu' (Chinese name), has been used to treat headaches, arthralgia, chronic bronchitis and stomachalgia in traditional Chinese medicine (Pan, 1985, 1995). Pharmacological experiments indicate that extracts from *A. chinensis* have antineoplastic and immunopotent activities (Chen *et al.*, 1996). Previously, we have isolated β-sitosterol palmitate, daucosterol, β-sitosterol and bergenin from the rhizome of *A. chinensis* (Sun *et al.*, 2002). To investigate the bioactive natural products from *A. chinensis*, chemical studies of the rhizome of *A. chinensis* were undertaken by screening using antineoplastic tests *in vitro*, and we have obtained a cytotoxic and apoptotic-inducing triterpenoid, *viz.* 3β,6β-dihydroxyolean-12-en-27-oic acid, (I), from the petroleum ether extract. Compound (I) exhibits significant cytotoxic activity against the human ovarian carcinoma cell (HO-8910), the human cervical squamous carcinoma cell (Hela), the human leukemia cell (HL60), the human colorectal carcinoma cell line (COLO 205), human androgen-independent prostate adenocarcinoma (PC_3), human breast cancer (Bcap37) and human chronic myelogenous leukemia (K562) *in vitro*. The 50% inhibitory concentration (IC_{50}) values are 22.24 (0.57), 11.91 (1.02), 24.24 (3.82), 29.08 (0.16), 30.59 (2.56), 28.91 (0.76) and 31.08 (0.92) μg ml^{-1}, respectively. Moreover, (I) induced apoptosis of COLO 205 by disturbing DNA replication, down-regulating bcl-2 expression and up-regulating bax expression, lowering relative mitochondrial transmembrane potential and activating the caspase-3 pathway (Sun *et al.*, 2004). The structure of (I) was elucidated by extensive spectroscopic analysis, including two-dimensional NMR spectroscopy, and confirmed by single-crystal X-ray diffraction analysis.



(I)

Compound (I) was obtained as colorless prisms in the orthorhombic space group $P2_12_12_1$. Our X-ray data allowed determination of the relative stereochemistry of the molecules in the crystal studied; a view of the molecule of (I) with our numbering scheme is shown in Fig. 1 and selected dimensions are given in Table 1. The molecule is composed of five six-membered rings, *viz*. *A* (C1–C5/C10), *B* (C5–C10), C (C8/C9/C11–C14), *D* (C13–C18) and *E* (C17–C22). Rings *A*, *B*, *D* and *E* adopt chair conformations, while ring *C* adopts a slightly distorted half-chair conformation as a result of the double bond between atoms C12 and C13. All rings are *trans* fused, except for the *D*/*E* junction, which is *cis* fused. The configurations at the other chiral centers are as follows: C5—H, C6—OH, C18—H, C8—Me, C17—Me and the carboxy group at C14 are axial, and C3—OH is equatorial.

The hydroxy groups located at atoms C3 and C6, and the carboxy group located at atom C14 participate in hydrogen bonding. The three groups serve simultaneously as hydrogen-bond donors and acceptors (Table 2 and Fig. 2), resulting in

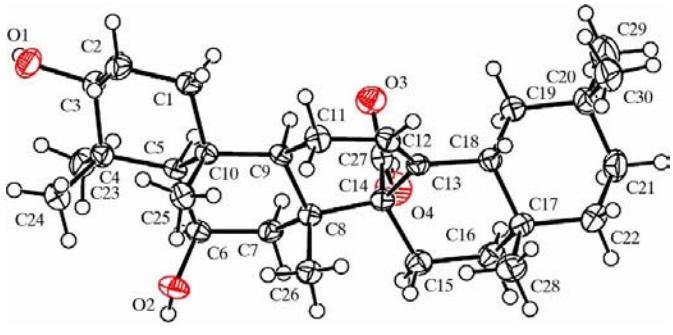


Figure 1
A view of (I), with the atomic numbering scheme. Displacement ellipsoids are drawn at the 50% probability level. H atoms have been omitted for clarity.

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three intermolecular O—H···O hydrogen bonds. All of the hydrogen bonds are between molecules of different asymmetric units. The significant inhibitory effect of (I) on the HO-8910, Hela, HL60, COLO 205, PC_3, Bcap37 and K562 cell lines *in vitro*, and the elevated 50% inhibitory concentration (IC_{50}) values of its disodium phosphate, imply that the hydroxy groups help the molecule to bind to enzymes in the tumor cell and that these groups are implicated in inhibitory activity.

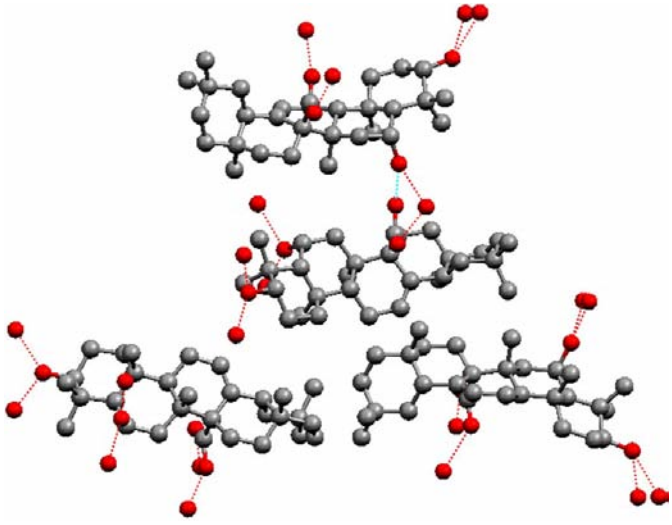

Figure 2
The intermolecular hydrogen bonding in (I), viewed normal to the (001) plane. H atoms have been omitted for clarity, except for those involved in hydrogen bonds. Hydrogen bonds are shown as dashed lines.

Experimental

The rhizome of *A. chinensis* was collected in Anji county, Zhejiang province, People's Republic of China, in August 2001. The plants were identified as *A. chinensis* (Maxim.) Franch. et Savat. by Professor Xiang-Ji Xue, College of Pharmaceutical Science, Zhejiang University. A voucher specimen (No. 200120) was deposited in the Laboratory of Nature and Biochemistry, Zhejiang University. The rhizomes of *A. chinensis* were dried in the dark, in a ventilated hood, and ground. The material (5.0 kg) was extracted three times with MeOH (each 25 l) at room temperature. The MeOH extract (366 g) was partitioned between petroleum ether (20 l) and water (2 l), and the petroleum ether solution was concentrated *in vacuo*, yielding a gelatinous material (41.2 g). Part of the extract (40.0 g) was absorbed onto silica gel (60 g) and chromatographed on a silica-gel (600 g) column, eluting with petroleum ether–EtOAc gradients (50:1, 30:1, 15:1, 5:1, 3:1, 2:1). The eluted fractions were evaluated by thin-layer chromatography and combined to give fractions 1–19, respectively. Fraction 13 was recrystallized from EtOAc–CH_3OH (1:1) to afford the pure title compound, (I) (4.2 g, m.p. 512–514 K). ^{13}C NMR (125 MHz, C_5ND_5, p.p.m.): 179.8 (C27), 133.2 (C13), 128.5(C12), 79.1 (C3), 60.2 (C18), 55.9 (C14), 55.1 (C5), 46.8 (C9), 40.9 (C22), 39.8 (C19), 39.8 (C8), 38.7 (C1), 38.6 (C4), 37.5 (C20), 36.9 (C10), 36.6 (C7), 33.7 (C17), 30.4 (C21), 29.0 (C28), 28.9 (C16), 28.1 (C23), 27.0 (C2), 22.7 (C11), 22.4 (C15), 21.3 (C30), 18.2 (C6), 18.2 (C26), 17.8 (C29), 16.5 (C25), 15.7 (C24). Crystals suitable for X-ray structure analysis were obtained by slow evaporation from methanol at room temperature.

Crystal data

$C_{30}H_{48}O_4$
M_r = 472.68
Orthorhombic, $P2_12_12_1$
a = 12.016 (2) Å
b = 14.141 (2) Å
c = 15.406 (2) Å
V = 2617.6 (4) Å^3
Z = 4
D_x = 1.199 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 36 reflections
θ = 5.8–14.2°
μ = 0.08 mm^{-1}
T = 288 (2) K
Prism, colorless
0.58 × 0.58 × 0.38 mm

Data collection

Siemens $P4$ diffractometer
ω scans
3481 measured reflections
3209 independent reflections
2549 reflections with $I > 2\sigma(I)$
R_{int} = 0.007
θ_{max} = 27.0°
$h = 0 \rightarrow 15$
$k = 0 \rightarrow 18$
$l = -1 \rightarrow 19$
3 standard reflections every 97 reflections
intensity decay: 2.1%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.037
$wR(F^2)$ = 0.086
S = 0.94
3209 reflections
327 parameters
H atoms treated by a mixture of independent and constrained refinement
$w = 1/[\sigma^2(F_o^2) + (0.0509P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.22 e Å^{-3}
$\Delta\rho_{min}$ = −0.16 e Å^{-3}
Extinction correction: *SHELXL*97
Extinction coefficient: 0.0030 (5)

Table 1
Selected geometric parameters (Å, °).

O1—C3	1.449 (3)	C8—C14	1.616 (3)
O2—C6	1.443 (3)	C11—C12	1.489 (3)
O3—C27	1.219 (3)	C12—C13	1.323 (3)
O4—C27	1.320 (3)		
O1—C3—C2	108.56 (18)	C12—C13—C18	119.8 (2)
O1—C3—C4	111.71 (18)	C12—C13—C14	122.1 (2)
C2—C3—C4	112.83 (19)	C18—C13—C14	118.09 (18)
O2—C6—C7	113.27 (18)	O3—C27—O4	123.0 (2)
O2—C6—C5	113.40 (17)	O3—C27—C14	123.6 (2)
C7—C6—C5	110.36 (17)	O4—C27—C14	113.4 (2)
C1—C2—C3—O1	−176.49 (19)	C13—C14—C27—O3	−43.0 (3)
O1—C3—C4—C23	63.7 (2)	C15—C14—C27—O3	−161.4 (2)
O1—C3—C4—C24	−53.5 (3)	C8—C14—C27—O3	77.9 (3)
O1—C3—C4—C5	179.73 (18)	C13—C14—C27—O4	137.59 (19)
C10—C5—C6—O2	72.7 (2)	C15—C14—C27—O4	19.2 (3)
C4—C5—C6—O2	−67.3 (2)	C8—C14—C27—O4	−101.5 (2)
O2—C6—C7—C8	−76.6 (2)		

Table 2
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
O1—H1O···O3^{i}	0.82 (2)	2.255 (19)	2.999 (2)	150 (3)
O2—H2O···O1^{ii}	0.817 (18)	1.975 (14)	2.758 (2)	160 (3)
O4—H4O···O2^{iii}	0.821 (13)	1.940 (14)	2.738 (2)	164 (3)

Symmetry codes: (i) $\frac{1}{2}-x, 1-y, \frac{1}{2}+z$; (ii) $1-x, y-\frac{1}{2}, \frac{3}{2}-z$; (iii) $x-\frac{1}{2}, \frac{1}{2}-y, 1-z$.

Hydroxy atoms H10, H20 and H40 were located from diffraction measurements and refined with distance restraints on their bonds to atoms O1, O2 and O4, respectively. Other H atoms were placed at

calculated positions and allowed to ride on their parent atoms using *SHELXL*97 defaults. The absolute structure could not be refined because of the absence of significant anomalous effects; Friedel pairs were merged before the final cycles of the refinement.

Data collection: *XSCANS* (Siemens, 1994); cell refinement: *XSCANS*; data reduction: *SHELXTL*/*PC* (Siemens, 1991); program(s) used to solve structure: *SHELXS*97 (Sheldrick, 1997); program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *SHELXTL*/*PC*; software used to prepare material for publication: *SHELXTL*/*PC*.

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Supplementary data for this paper are available from the IUCr electronic archives (Reference: FA1051). Services for accessing these data are described at the back of the journal.

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