Acta Crystallographica Section E
Structure Reports Online
ISSN 1600-5368

3β-Hydroxyolean-12-en-27-oic acid methanol solvate: a cytotoxic and apoptosis-inducing triterpenoid from the rhizome of *Astilbe chinensis*

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Key indicators

Single-crystal X-ray study
T = 296 K
Mean σ(C–C) = 0.007 Å
R factor = 0.057
wR factor = 0.158
Data-to-parameter ratio = 10.1

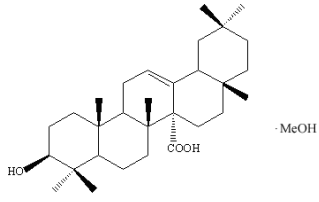
For details of how these key indicators were automatically derived from the article, see http://journals.iucr.org/e.

Received 6 May 2003
Accepted 6 June 2003
Online 24 June 2003

The title compound, $C_{30}H_{48}O_3\cdot CH_4O$, is a cytotoxic and apoptosis-inducing triterpenoid which was isolated from the rhizome of *Astilbe chinensis*. The molecule is composed of five six-membered rings. The cyclohexene ring adopts a slightly distorted half-chair conformation and the cyclohexane rings adopt chair conformations. The hydroxy and carboxy groups both 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 for headache, arthralgia, chronic bronchitis and stomachalgia, in traditional Chinese medicine (Pan, 1985, 1995). Pharmacological experiments have indicated that the extracts from *A. chinensis* have antineoplastic and immunopotent activities (Chen *et al.*, 1996). Previously, we 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 on the rhizome of *A. chinensis* were undertaken by screening using antineoplastic tests *in vitro*, and we have obtained cytotoxic and apoptosis-inducing triterpenoid 3β-hydroxyolean-12-en-27-oic acid (I), from its petroleum ether extract. It exhibits significant cytotoxic activity against human ovarian carcinoma cell line (HO-8910), human cervical squamous carcinoma cell line (HeLa) and human leukemia cell (HL60) *in vitro*; its 50% inhibitory concentration ($IC_{20}50$) values were 8.0 (9), 3.94 (13) and 3.67 (19) μg ml^{-1}, respectively. Moreover, it induces apoptotic cell death of the HO-8910 in a dose-dependent manner, ranging from 2.5 μg ml^{-1} to 40.0 μg ml^{-1}. The structure of the title compound, (I), was elucidated by spectroscopic analysis including two-dimensional NMR spectroscopy and confirmed by single-crystal X-ray diffraction analysis.



(I)

The structure of (I) with the atom-numbering scheme is shown in Fig. 1. The molecule is composed of five six-

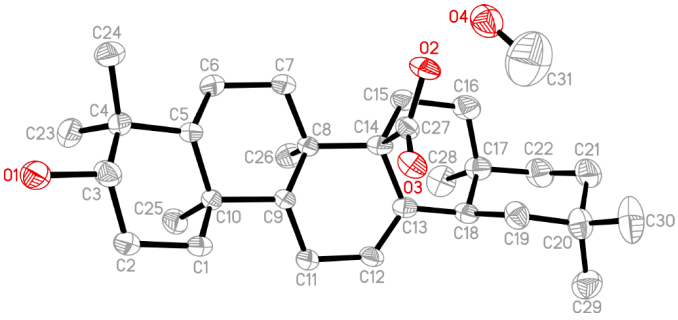


Figure 1
A view of (I), with the atomic numbering scheme. Displacement ellipsoids are drawn at the 35% probability level. H atoms have been omitted for clarity.

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 owing to the C12═C13 double bond. All rings are *trans* fused except for the *D*/*E* junction, which is *cis* fused. The orientation of the carboxy group is axial and that of the hydroxy group is equatorial.

The hydroxy and carboxy groups both serve simultaneously as hydrogen-bond donors and acceptors (Table 2). In the asymmetric unit, the two molecules are linked by O2—H2···O4 hydrogen bonds. In the solid state, the screw-related molecules are linked by O1—H1···O3^{i} hydrogen bonds (Table 2), to form molecular chains along the *a* axis. The chain formation is further stabilized by the methanol solvent through O4—H4O···O1^{i} hydrogen bonds. The crystal structure is further stabilized by C—H···O interactions.

Experimental

The rhizomes of *Astilbe chinensis* were collected in Anji county, Zhejiang province, 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 *Astilbe Chinensis* were dried at 313 K in the dark, in a ventilated hood, and ground. The material (5.0 kg) was extracted three times with CH_3OH (each 25 l) at room temperature. The CH_3OH extract (366 g) was partitioned between petroleum ether (20 l) and water (2 l). The petroleum ether solution was concentrated in vacuum to give 41.2 g of a gelatinous material. A part of the extract (40.0 g) was absorbed on to silica gel (60 g) and chromatographed on a silica gel (600 g) column eluted with petroleum ether–EtOAc (50:1, 30:1, 15:1, 5:1, 3:1, 2:1) gradients. The eluted fractions were evaluated by TLC and combined to give fractions 1 to 19, respectively. Fraction 12 was recrystallized from EtOAc—CH_3OH (1:1) to afford 2.5 g of the pure title compound, (I). m.p.: 513.5–515.5 K. ^{13}C NMR (125 MHz, C_5ND_5, p.p.m): 179.4 (C27), 138.2 (C13), 126.2 (C12), 79.4 (C3), 55.8 (C14), 55.2 (C5), 49.0 (C18), 47.2 (C9), 43.9 (C19), 39.7 (C8), 7 (C4), 38.6 (C1), 37.1 (C10), 36.5 (C21), 36.3 (C7), 34.3 (C22), 33.5 (C29), 38 32.8 (C17), 31.1 (C20), 28.2 (C28), 28.1 (C23), 27.5(C2), 26.9 (C16), 23.6 (C30), 22.8 (C11), 22.2 (C15), 18.2 (C6), 18.0 (C26), 16.4 (C25), 16.0 (C24). Crystals suitable for X-ray structure analysis were obtained by slow evaporation from methanol at room temperature.

Crystal data

$C_{30}H_{48}O_3{\cdot}CH_4O$
M_r = 488.73
Orthorhombic, $P2_12_12_1$
a = 7.538 (2) Å
b = 13.710 (3) Å
c = 27.629 (8) Å
V = 2855.3 (13) Å^3
Z = 4
D_x = 1.137 Mg m^{-3}
Mo $K\alpha$ radiation
Cell parameters from 38 reflections
θ = 2.9–13.8°
μ = 0.07 mm^{-1}
T = 296 (2) K
Prism, colorless
0.58 × 0.40 × 0.24 mm

Data collection

Siemens *P*4 diffractometer
ω scans
Absorption correction: none
3492 measured reflections
3203 independent reflections
1876 reflections with $I > 2\sigma(I)$
R_{int} = 0.021
θ_{max} = 26.0°
$h = 0 \rightarrow 9$
$k = 0 \rightarrow 16$
$l = -1 \rightarrow 34$
3 standard reflections
every 97 reflections
intensity decay: 1.5%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.057
$wR(F^2)$ = 0.158
S = 0.89
3203 reflections
316 parameters
H-atom parameters constrained
$w = 1/[\sigma^2(F_o^2) + (0.0951P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max} < 0.001$
$\Delta\rho_{max}$ = 0.21 e Å^{-3}
$\Delta\rho_{min}$ = −0.19 e Å^{-3}
Extinction correction: *SHELXTL*
Extinction coefficient: 0.0116 (17)

Table 1
Selected geometric parameters (Å, °).

O1—C3	1.449 (5)	C12—C13	1.336 (6)
O2—C27	1.323 (5)	O4—C31	1.369 (8)
O3—C27	1.213 (5)		
O1—C3—C2	107.5 (4)	C12—C13—C14	119.9 (4)
O1—C3—C4	112.4 (4)	C18—C13—C14	120.2 (4)
C6—C5—C4	115.8 (4)	C13—C14—C27	105.2 (4)
C4—C5—C10	116.9 (3)	C16—C15—C14	115.1 (4)
C8—C9—C10	116.3 (3)	O3—C27—O2	121.4 (4)
C13—C12—C11	126.7 (4)	O3—C27—C14	124.6 (4)
C12—C13—C18	119.9 (4)	O2—C27—C14	114.0 (4)
C1—C2—C3—O1	−178.2 (4)	C15—C14—C27—O3	−159.3 (4)
O1—C3—C4—C24	67.2 (5)	C8—C14—C27—O3	78.1 (5)
O1—C3—C4—C23	−49.5 (5)	C13—C14—C27—O2	139.9 (4)
O1—C3—C4—C5	−175.7 (3)	C15—C14—C27—O2	22.5 (5)
C13—C14—C27—O3	−42.0 (6)	C8—C14—C27—O2	−100.0 (4)

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

D—H···*A*	*D*—H	H···*A*	*D*···*A*	*D*—H···*A*
O2—H2···O4	0.82	1.82	2.619 (5)	164
O1—H1···O3^{i}	0.82	2.17	2.991 (5)	178
O4—H4O···O1^{i}	0.82	1.96	2.755 (5)	164

Symmetry code: (i) $x-\frac{1}{2}, \frac{1}{2}-y, 1-z$.

After their location in a difference map, all H atoms were geometrically fixed and allowed to ride on their attached atoms using *SHELXL*97 (Sheldrick, 1997) defaults. The absolute configuration could not be established because of the absence of significant anomalous effects. Friedel pairs were merged for the final cycles of refinement.

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

This work was supported by a Grant-in-Aid from the Zhejiang Provincial Natural Science Foundation of China (No. 300456) and Zhejiang Provincial Science and Technology Council (2002C3306).

References

Chen, P. F., Lai, P. F., Zhang, P. & Chen, L. L. (1996). *China J. Chin. Mater. Med.* **21**, 302–303.
Pan, J. T. (1985). *Acta Phytotaxonom. Sin.* **23**, 432–443.
Pan, J. T. (1995). *Acta Phytotaxonom. Sin.* **33**, 390–402.
Sheldrick, G. M. (1997). *SHELXS*97 and *SHELXL*97. University of Göttingen, Germany.
Siemens (1991). *SHELXTL/PC*. Version 4.2. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA.
Siemens (1994). *XSCANS*. Version 2.10b. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, USA.
Sun, H. X., Ye, Y. P. & Yang, K. (2002). *China J. Chin. Mater. Med.* **27**, 751–754.