Crystal Structure of 3-*O*-Acetyloleanolic Acid

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The X-ray crystal structure of the antileishmanial 3-*O*-acetyloleanolic acid was established. It crystallizes in the orthorhombic space group $P2_12_12_1$. The absolute configuration was determined by calculating the $F(+)/F(-)$ ratios of Bijvoet pairs. Solid-state data could be used to clarify the mechanism of action implicating this antileishmanial hemisynthetic compound.

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Leishmania are protozoan parasites responsible for a spectrum of diseases collectively known as leishmaniasis, which are regarded as a major public health problem, causing significant morbidity and mortality in Africa, Asia and Latin America. The World Health Organization (WHO) estimates that there are 1.5

to 2 million new cases of leishmaniasis worldwide annually. Overall, the disease affects 12 million individuals in 88 countries where Leishmania is present, with 350 million individuals at risk of infection. Infection by various strains of Leishmania causes a wide spectrum of disease in humans, with many

Fig. 1 Chemical structure of 3-*O*-acetyloleanolic acid.

Fig. 2 View of the molecular structure of 3-*O*-acetyloleanolic acid with our numbering scheme. Displacement ellipsoids are drawn at the 30% probability level.

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Table 1 Crystal and experimental data

Chemical formula: $C_{32}H_{50}O_4 \cdot 2CH_3OH$
Formula weight: 562.80
$T = 213(2)K$
Crystal system: orthorhombic
Space group: $P2_12_12_1$
$a = 7.4588(8)\text{\AA}$ $\beta = 90^\circ$
$b = 19.2001(18)\text{\AA}$
$c = 23.167(2)\text{\AA}$
$V = 3317.8(6)\text{\AA}^3$
$Z = 4$
$D_x = 1.127\text{ Mg/m}^3$
Radiation: Cu K_α ($\lambda = 1.54180\text{\AA}$)
$F(000) = 1240$
Crystal size = $0.12 \times 0.07 \times 0.05\text{ mm}^3$
No. of reflections collected = 45548
No. of independent reflections = 5990
No. of reflections used [$I > 2\sigma(I)$] = 3493
θ range for data collection: 6.65 to 68.14°
Data/Restraints/Parameters = 5990/0/375
Goodness-of-fit = 1.007
R indices [$I > 2\sigma(I)$]: $R1 = 0.0712$, $wR2 = 0.1257$
R indices (all data): $R1 = 0.1239$, $wR2 = 0.1407$
$(\Delta\rho)_{\max} = 0.036$
$(\Delta\rho)_{\max} = 0.275\text{ \AA}^{-3}$
$(\Delta\rho)_{\min} = -0.165\text{ \AA}^{-3}$
Measurement: R-Axis Rapid ⁶
Structure determination: SHELXS-97 ⁷
Refinement: SHELXL-97 ⁸
CCDC deposition number: 683211

Table 2 Hydrogen-bonding geometry (Å, °)

D-H...A	H-A	D-A	D-H...A
O(26)-H(26)...O(37, II)	1.79	2.603(5)	164
O(37)-H(37)...O(39)	1.81	2.640(5)	171
O(39)-H(39)...O(27)	2.06	2.713(4)	134
C(1)-H(1)...O(27)	2.45	2.806(4)	100
C(36)-H(36B)...O(31)	2.53	2.929(5)	104

Symmetry codes: II (1+x, y, z).

different clinical manifestations, that is, cutaneous, mucocutaneous and visceral.¹ The current treatment of the disease is based on a limited number of chemotherapeutic agents that are rapidly becoming ineffective, and are characterized by high toxicity and cost. Novel approaches are now currently used to identify leishmanicidal compounds as diverse as antimicrobial peptides and natural plant extracts. The plant kingdom is undoubtedly valuable as a source of new medicinal agents.^{1,2}

Very recently, we reported on the synthesis of a series of new ursolic and oleanolic acid derivatives, previously isolated from the South American species *Ilex paraguariensis* and *Ilex dumosa*, and its *in vitro* antiparasitic activity on *Leishmania amazonensis* and *Leishmania infantum* strains.³ We report herein on the structural characterization of one of them, *i.e.* the 3-*O*-acetyloleanolic acid (Fig. 1), which shows the most promising antileishmanial activity *in vitro* against the promastigote forms of *Leishmania amazonensis* (IC₅₀ = 5 μM).

The present crystal structure determination will not only help us to understand the detailed three-dimensional arrangement of the compound, which could be useful for designing new derivatives, but will also contribute to a structural database in which there are very few structures containing the oleanolic acid moiety.⁴

3-Acetyloleanolic acid was synthesized by stirring oleanolic acid, previously extracted from the south American species *Ilex dumosa*, with acetic anhydride in dry pyridine at room temperature.³ Colorless crystals suitable for X-ray analysis were obtained by slow crystallization from a chloroform-methanol solution at 4°C.

The molecular structure of 3-*O*-acetyloleanolic acid is depicted in Fig. 2. 3-Acetyloleanolic acid crystallizes in the system orthorhombic space group *P*2₁2₁2₁ with two molecules of methanol in the asymmetric unit. Crystal and experimental data are given in Table 1. The absolute configuration of this hemisynthetic compound was determined by measuring Bijvoet pairs intensities differences, leading to a Flack parameter value of −0.2(3). Based on these results, the absolute configurations at C-1, C-6, C-9, and C-21 were determined to be *S*, and were found to be *R* at C-13, C-14, C-17, and C-18.

The bond lengths and angles are very similar to those given in the literature for other triterpenes, such as ursolic and oleanolic acids derivatives.^{4,5} The C(10)–C(11) double bond was noticed at 1.325(4) Å, as typically observed for C=C double bonds, and the C_{sp3}–C_{sp3} bond lengths range from 1.502(5) to 1.593(4) Å. The three C_{sp3}–C_{sp2} bond distances are 1.482(4), 1.515(4) and 1.538(4) Å. Three of the five six-membered rings in the molecule [C17–C22 (A); C13–C18 (B); C1–C6 (E)] are in *chair* conformations. The other two rings [C9–C14 (C); C6–C10, C1 (D)] exhibit twisted-chair conformations due to a flattening of the C10–C11 double bond. The carboxyl group at C6 is equatorial with respect to ring E and axial with respect to ring D. Since one of the common atoms between rings C and D is on the double bond, the arrangement between these two rings indicates that the C28 methyl group is *trans* to the C29 one.

Table 3 Final atomic coordinates and equivalent isotropic thermal parameters ($U_{eq} \times 10^4$ (Å²))

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{eq}
C(1)	644(5)	-323(2)	647(1)	40(1)
C(2)	-582(5)	-131(2)	1167(1)	47(1)
C(3)	-494(6)	-652(2)	1667(1)	48(1)
C(4)	1439(6)	-733(2)	1848(1)	52(1)
C(5)	2669(5)	-943(2)	1362(1)	51(1)
C(6)	2585(5)	-451(2)	831(1)	40(1)
C(7)	3496(5)	243(2)	954(1)	42(1)
C(8)	3521(5)	713(2)	415(1)	43(1)
C(9)	1684(5)	849(2)	137(1)	35(1)
C(10)	475(5)	201(2)	161(1)	35(1)
C(11)	-833(5)	123(2)	-221(1)	42(1)
C(12)	-1247(5)	589(2)	-713(1)	49(1)
C(13)	15(5)	1226(2)	-767(1)	41(1)
C(14)	1911(5)	1060(2)	-525(1)	39(1)
C(15)	3127(5)	1710(2)	-578(1)	40(1)
C(16)	3051(5)	2057(2)	-1179(1)	43(1)
C(17)	1118(5)	2246(2)	-1346(1)	36(1)
C(18)	-54(5)	1578(2)	-1380(1)	37(1)
C(19)	-1986(5)	1808(2)	-1484(1)	43(1)
C(20)	-2200(5)	2290(2)	-2008(1)	46(1)
C(21)	-1025(6)	2923(2)	-1942(1)	44(1)
C(22)	979(5)	2775(2)	-1861(1)	39(1)
C(23)	-1317(6)	-1354(2)	1491(2)	62(1)
C(24)	-1610(6)	-366(2)	2174(1)	67(1)
C(25)	3554(7)	-840(2)	362(2)	46(1)
O(26)	5337(4)	-823(1)	407(1)	65(1)
O(27)	2865(4)	-1186(1)	-19(1)	64(1)
C(28)	755(5)	1422(2)	496(1)	45(1)
C(29)	2811(5)	450(2)	-857(1)	44(1)
C(30)	485(5)	1086(2)	-1876(1)	44(1)
O(31)	-1190(4)	3355(1)	-2459(1)	49(1)
C(32)	-2627(7)	3767(2)	-2494(2)	50(1)
O(33)	-3757(4)	3819(1)	-2127(1)	57(1)
C(34)	-2597(6)	4167(2)	-3051(2)	70(1)
C(35)	1877(5)	2523(2)	-2424(1)	52(1)
C(36)	1909(6)	3462(2)	-1688(1)	54(1)
O(37)	-3127(5)	-1582(2)	-381(2)	113(1)
C(38)	-3983(9)	-2030(5)	-729(4)	244(5)
O(39)	220(5)	-1585(2)	-751(1)	94(1)
C(40)	775(7)	-2262(3)	-924(2)	113(2)

$$U_{eq} = (1/3) \sum_i \sum_j U_{ij} (a_i^* a_j^*) (a_i a_j).$$

There are solvent accessible voids in the crystal lattice, *i.e.* two methanol molecules are contained in the asymmetric crystallographic unit. Crystal cohesion is ensured by a three-dimensional network of van der Waals interactions and by hydrogen bonds between the carboxylic acid function of acetyloleanolic acid at C6 and methanol molecules (Table 2).

The final atomic coordinates and the equivalent isotropic thermal parameters are listed in Table 3.

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