Crystal Structure of 5 β ,6 β -Epoxy-4 β ,27-dihydroxy-1-oxo-22*R*-witha-2,24-dienolide Isolated from *Withania somnifera* Leaves

Pankaj BANDHORIA,* Vivek K. GUPTA,*† Pardeep KUMAR,** Naresh K. SATTI,**
Prabhu DUTT,** and Krishan A. SURI**

*Post-Graduate Department of Physics, University of Jammu, Jammu Tawi-180 006, India

**Natural Products Chemistry Division, Regional Research Laboratory, Jammu Tawi-180 001, India

5 β ,6 β -Epoxy-4 β ,27-dihydroxy-1-oxo-22*R*-witha-2,24-dienolide (C₂₈H₃₈O₆) was isolated from the leaves of *Withania somnifera*. The structure of the withanolide was established by spectral analysis and X-ray diffraction studies as withaferin A. The compound crystallizes in the orthorhombic space group *P*2₁2₁2₁ with unit-cell parameters: *a* = 10.697(1), *b* = 12.344(2), *c* = 18.714(2) Å, *Z* = 4. The crystal structure was solved by direct methods and refined to *R* = 0.0382 for 2471 observed reflections. The ring conformations are: A *twist-boat*, B *half-chair*, C *chair*, D *envelope*, E *distorted sofa*. The twist along the length of the steroid nucleus is negligible [C19–C10...C13–C18 = 1.9°]. Both hydroxy groups are involved in hydrogen bonding.

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In continuation to our investigation on *Withania somnifera*,¹ we report here on the crystal structure of a withanolide, 5 β ,6 β -epoxy-4 β ,27-dihydroxy-1-oxo-22*R*-witha-2,24-dienolide (withaferin A), isolated from the 95% alcohol extract of the leaves of *Withania somnifera*. The structure of the isolate was established by spectral analysis, followed by X-ray diffraction studies. The crystal structure of the isomer of withaferin A has already been reported by Modawi *et al.*^{2*}

Withania somnifera (Aswagandha, Indian ginseng) is widely used in Ayurvedic medicine (traditional system of medicine in India). It is an ingredient of many formulations prescribed for a variety of musculoskeletal conditions, and as a general health tonic for elderly persons and lactating mothers. To authenticate its use as a multipurpose medicinal plant, a battery of

Fig. 1 Chemical structure of withaferin A.

† To whom correspondence should be addressed.

E-mail: vivek_gupta2k5@yahoo.co.in

*Modawi *et al.* have published the crystal and molecular structures of an isomer of withaferin A (5 β ,6 β -epoxy-4 α ,27-dihydroxy-1-oxo-22*R*-witha-2,24-dienolide, m.p. 228 – 230°C).² Upon comparing the X-ray data of withaferin A with its isomer as reported by Modawi *et al.*, we came to the conclusion that both structures are the same. The name and configuration assigned to the compound by Modawi *et al.* (i.e. isomer of withaferin A and 4 α -hydroxy...) is incorrect.

Table 1 Crystal and experimental data

Crystal description	Colorless rectangular
Empirical formula	C ₂₈ H ₃₈ O ₆
Formula weight	470.58
Wavelength	1.5418 Å
Crystal system	orthorhombic
Space group, <i>Z</i>	<i>P</i> 2 ₁ 2 ₁ 2 ₁ , 4
Unit cell dimensions	
<i>a</i>	10.697(1) Å
<i>b</i>	12.344(2) Å
<i>c</i>	18.714(2) Å
Volume (<i>V</i>)	2471.1(5) Å ³
<i>D_x</i>	1.265 Mg m ⁻³
Absorption coefficient	0.707 mm ⁻¹
Absorption correction	ψ -scan (<i>T</i> _{min} = 0.9373 and <i>T</i> _{max} = 0.9807)
Extinction coefficient	0.0029(5)
Reflections collected/unique	2639/2552 [<i>R</i> _{int} = 0.0096]
Reflections observed (<i>I</i> > 2 σ (<i>I</i>))	2471
θ range for entire data collection	4.29 < θ < 67.90°
No. of parameters refined	460
Final <i>R</i>	0.0382
<i>wR</i> (<i>F</i> ²)	0.1156
Goodness-of-fit	1.003
($\Delta\sigma$) _{max}	−0.028 (for X H112)
($\Delta\rho$) _{max}	0.265 e Å ⁻³
($\Delta\rho$) _{min}	−0.150 e Å ⁻³
Measurement	Enraf-Nonius CAD4
Program system	SHELX
Structure determination	Direct methods
Refinement	Full-matrix least-squares on <i>F</i> ²
Structure drawing	ORTEP

CCDC-285229 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Fig. 2 ORTEP view of the molecule with displacement ellipsoids drawn at the 40% probability level.

pharmacological investigations have been reported. The plant is also used in the traditional medicine systems of several countries as a narcotic, anti-epileptic, against female sterility, hypotonic, for stomachache, ulcers, colds, rashes, gonorrhoea, sedative and for its antiseptic properties. The fruit of the plant has been used in folk medicine as a febrifuge, diuretic and antirheumatic under the name "Morgan" in Egypt. Genuine interest arose in withanolides when it was found that they showed antitumour activity in a number of animal studies.³ In addition, cytotoxicity, immunosuppressive, antimicrobial, hepatoprotective, insect antifeedant and anti-inflammatory properties were also observed.

The leaves of *W. somnifera* (L) Dunal were collected in Aug. 2004 from RRL fields. A voucher specimen (No. RRLWS002L04) has been deposited in the Herbarium. Air-dried and powdered leaves (500 g) of *W. somnifera* were extracted with 95% ethanol at room temperature while stirring for two hours. The extract was filtered through muslin cloth, followed by centrifugation. The marc was again extracted twice as mentioned above. All of the three extracts were pooled and concentrated to 1/8th of its volume. A syrupy solution was diluted with water, and the resulting suspension was extracted sequentially with CHCl_3 , EtOAc and 1-BuOH. The CHCl_3 extract was subjected to chromatography over silica gel (60–120 mesh) and the elution was carried in increasing polarity with CHCl_3 , 2% MeOH in CHCl_3 , 5% MeOH in CHCl_3 and MeOH. Fractions that were eluted in 2% MeOH in CHCl_3 were pooled and concentrated; the residue after crystallization from EtOAc yielded withaferin A (200 mg) (Fig. 1), mp 252–253°C. The other physical and spectroscopy data are in good agreement with the literature.⁴ Because of the absence of any significant anomalous scatterers in the compound, the absolute configuration of the molecule has not been determined from the X-ray diffraction experiment, and the assumed chirality of the molecule was determined from the synthesis route.⁵

The crystal and experimental data are listed in Table 1. All of the hydrogen atoms were located from a difference Fourier map; their positions and isotropic temperature factors were refined along with all of the parameters of the non-hydrogen atoms. An ORTEP-III drawing of the molecule is shown in Fig. 2.

The bond distances and bond angles of the title compound are in good agreement with the corresponding values obtained in the case of related withanolides. The mean bond lengths are: $\text{C}(\text{sp}^3)\text{--C}(\text{sp}^3) = 1.532(3)$; $\text{C}(\text{sp}^3)\text{--C}(\text{sp}^2) = 1.501(3)$; $\text{C}(\text{sp}^2)\text{--C}(\text{sp}^2) = 1.476(4)$; $\text{C}(\text{sp}^2)\text{=C}(\text{sp}^2) = 1.329(4)$; $\text{C}(\text{sp}^3)\text{--O} =$

Table 2 Hydrogen bonds

D-H...A	D-H(Å)	D...A(Å)	H...A(Å)	D-H...A(°)
O4-H4O...O27 ⁱ	0.93(4)	2.942(3)	2.07(4)	157(4)
O27-H27O...O26 ⁱⁱ	1.03(4)	2.990(4)	1.97(4)	171(3)
Symmetry code : (i) x, y, z+1		(ii) x+1/2, -y-1/2, -z-1		

1.445(3); $\text{C}(\text{sp}^2)\text{--O} = 1.336(3)$; $\text{C=O} = 1.214(3)$ Å. The bond angles ($\text{C12--C13--C17} = 117.2(2)$, $\text{C14--C13--C17} = 99.8(2)$, $\text{C8--C14--C15} = 117.9(2)$, $\text{C8--C14--C13} = 115.2(2)$, $\text{C13--C14--C15} = 104.4(2)^\circ$) show significant deviations from the ideal tetrahedral value of 109.4° . Deviations of this kind are the consequence of a strain caused by fusion of the five- and six-membered rings.

Ring A has a $1\alpha,4\alpha$ -twist boat conformation with C1 and C4 $-0.346(3)$ and $-0.576(2)$ Å, respectively, from the C2, C3, C5, C10 plane. Ring B is *cis* fused to ring A and has a *half chair* conformation with $8\beta,9\alpha$ orientation [$\Delta\text{C}_2(\text{C8--C9}) = 7.9$]. The overall shape of ring C is close to the *chair* conformation. Distortion from the ideal form could be expressed as being due to the loss of rotational symmetry [$\Delta\text{C}_2(\text{C11--C12}) = 12.14$] with a retention of the perpendicular mirror symmetry through atoms C9 and C13 [$\Delta\text{C}_s(\text{C9}) = 2.06$]. The conformation of the D ring is *C13-envelope* [$\Delta\text{C}_s(\text{C13}) = 4.02$] with the phase angle of pseudorotation being $\Delta = 27.96^\circ$ and maximum angle of torsion $\phi_m = 48.02^\circ$. The δ -lactone ring E adopts a 22α -distorted sofa conformation [$\Delta\text{C}_s(\text{C22}) = 9.07$] with atom C22 disposed $-0.654(2)$ Å below the plane defined by other five ring atoms. Ring E makes a dihedral angle of $59.0(1)^\circ$ with the plane of the steroid nucleus.

The distance between the terminal atoms, O4 and O27, is 16.468 Å and the C3...C16 distance, which is a measure of the length of the steroid nucleus, is 8.471 Å. The pseudo-torsion angle, $\text{C19--C10...C13--C18}$, is 1.9° , indicating that the steroid nucleus is untwisted. This is attributed to the short intramolecular C-H...O contacts [$\text{C11--H11...O1} = 3.056(3)$ and $\text{C19--H191...O1} = 2.808(4)$ Å] present in the molecule. The crystal structure of withaferin A is dictated by two intermolecular hydrogen bonds. Atom O4 of the hydroxy group in the molecule at (x, y, z) acts as a hydrogen-bond donor to hydroxy atom O27 in the molecule at (x, y, z + 1), producing a chain. Chains of molecules are packed together to form well-defined layers. Molecules within the layers are arranged in an antiparallel manner and are stabilized by the second hydrogen bond (O27--H27O...O26) (see Table 2 for details).

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