

ISOLATION AND CHARACTERIZATION OF 3-OXOATISANE-16 α , 17-DIOL FROM *EUPHORBIA ACAULIS*

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ABSTRACT.—The new *ent*-atis-16-ene diterpene isolated from the rhizomes of *Euphorbia acaulis* has been shown to be 3-oxoatisane-16 α , 17-diol [**1**] by a combination of spectroscopic data and X-ray crystallography. This is believed to be the first diterpene with the atisane skeleton isolated from a Euphorbiaceae species.

In our search for the active principle in the *n*-hexane extract of *Euphorbia acaulis* Roxb. (Euphorbiaceae) rhizomes, which showed anti-inflammatory activity comparable to phenylbutazone in experimental animals (1), we have isolated a minor constituent (0.002%) that was shown to be 3-oxoatisane-16 α , 17-diol [**1**]. Earlier we reported (2) the high resolution nmr and X-ray crystallographic data for caudicifolin isolated from the plant. The atisane skeleton is unusual for oxygenated, nitrogen free compounds obtained from plant sources.

RESULTS AND DISCUSSION

Compound [**1**] showed the following physical properties: mp 138°, [α]_D²⁷ -30.8 (c 1%, CHCl₃); eims (Probe) 70 ev *m/z* (rel. int) 320 (M⁺) (0.5), 289 (M-CH₂OH)⁺ (100), 271 (M-CH₂OH-H₂O)⁺ (93); ir ν max (KBr) 3300-3400 (primary & tertiary-OH groups), 1708 (C=O); ¹H nmr (CDCl₃) δ 0.96, 0.97, and 1.02 (three tertiary methyl groups), 2.50 (*m*, 4H, 2H attached to carbon α to carbonyl and 2H due to 2-OH groups as indicated by exchange with D₂O), 3.70 (2H, ABq, *J*=11 Hz, -CH₂OH); ¹³C nmr (CDCl₃) revealed the presence of 20 carbon atoms in the molecule, and their assignments are summarized in Table 1. Its monoacetate and acetonide derivatives had a mp 138° and 172°, respectively.

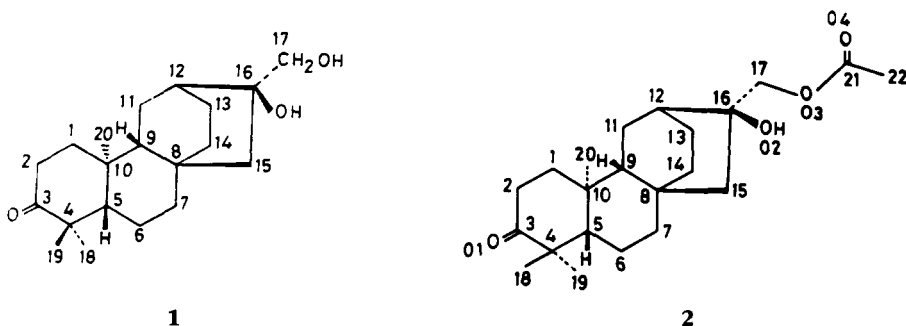
These structural data could be rationalized in terms of a tetracyclic *ent*-kaurane or *ent*-atisane diterpene skeleton with a keto group at C-3 and hydroxyl groups at C-16 and C-17. The position of the keto group at C3 was fixed on the basis of the chemical shifts of the C-18 and C-19 methyl groups in the ¹³C-nmr spectra which are normally in the vicinity of 33.1 and 21.5, respectively. However, the presence of a keto group at C-3 shields the neighboring geminal methyl carbon atoms at C-18 by 6.8 ppm and at C-19

TABLE 1. ¹³C-nmr (CDCl₃) Spectral Data of Compound 1

Catom	Multiplicity	Catom	Multiplicity
1	43.0, t	11	23.3, t
2	33.9, t	12	32.0, d
3	217.5, s	13	23.5, t
4	47.5, s	14	26.0, t
5	55.5, d	15	52.0, t
6	19.5, t	16	73.5, s
7	43.5, t	17	68.5, t
8	42.0, s	18	27.0, q
9	51.5, d	19	21.0, q
10	33.0, s	20	13.5, q

by 0.5 ppm (3). The resonances for the two methyl groups in the compound under investigation were recorded at 27.0 (-6.1) and 21.0 (-0.5), respectively. Placement of the hydroxyl group at C-17 was mainly due to the presence of a base peak at m/z 289 ($M-CH_2OH$)⁺ in the ms of **1**. The second hydroxyl [¹³C nmr=73.5 (s)] was located at C-16 because **1** readily yielded an acetoneide.

An insufficient quantity of **1** excluded chemical degradation; thus, to determine its carbon skeleton, we resorted to X-ray crystallography. The natural product did not yield crystals suitable for the desired studies, but the acetylation product **2** proved satisfactory.



CRYSTAL STRUCTURE ANALYSIS OF 2.—The molecular structure of **2** is illustrated in Figure 1, and the atomic coordinates are listed in Table 2. In general, bond lengths and angles do not deviate significantly from the expected values (4). The conformation of ring A is that of a slightly distorted chair, while ring B possesses a very complex conformation. The torsional angles indicate that the conformation of the bicyclo [2.2.2] octyl moiety is neither fully eclipsed as observed in bicyclo [2.2.2] octane-1,4-dicarboxylic acid (5) nor staggered as found in 1-*p*-(bromophenyl sulfoxyl) methyl] bicyclo [2.2.2] octane (6).

The cd curve¹ of **1** showed a negative Cotton effect at 285 nm ($[\theta] = -6.3 \times 10^2$) due to the isolated carbonyl chromophore at C-3 and a positive Cotton effect at 313 nm ($[\theta] = 2.9 \times 10^2$). In comparison to the reported value ($[\theta] = -589$ at 285 nm) of atisane-3 β ,16 α -diol (7), **2** is regarded as having an absolute configuration of non-steroid type as indicated in **2**.

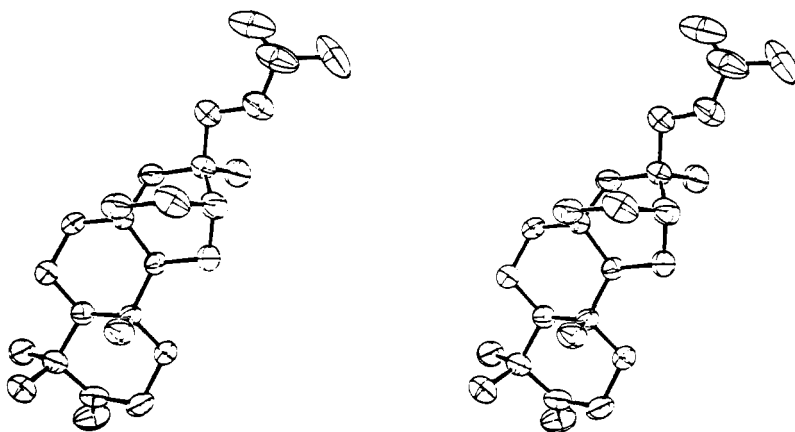


FIGURE 1. An ORTEP drawing of compound **2**

¹The cd curve was recorded in MeOH ($c=4.68$ mmol/liter at 27°) on a JASCO J-500C spectropolarimeter.

TABLE 2. Fractional Atomic Coordinates ($\times 10^4$) of Compound 2

Atom	x	y	z
C1	4713 (5) ^a	987 (11)	-1787 (6)
C2	5191 (6)	1029 (11)	-2901 (6)
C3	5105 (6)	2969 (11)	-3406 (6)
C4	3955 (5)	3896 (11)	-3663 (5)
C5	3462 (5)	3677 (10)	-2546 (5)
C6	2302 (6)	4533 (11)	-2663 (6)
C7	2046 (5)	4844 (11)	-1462 (6)
C8	2131 (5)	3061 (10)	-730 (5)
C9	3233 (5)	2015 (10)	-752 (5)
C10	3508 (5)	1720 (10)	-1988 (5)
C11	3312 (6)	220 (11)	5 (6)
C12	2314 (6)	122 (12)	612 (6)
C13	1241 (6)	0 (0)	-355 (6)
C14	1105 (5)	1816 (12)	-1088 (6)
C15	2189 (5)	3587 (11)	553 (5)
C16	2297 (5)	1857 (11)	1353 (5)
C17	1343 (6)	1896 (13)	2002 (6)
C18	4120 (6)	5974 (12)	-3872 (6)
C19	3248 (6)	3045 (13)	-4785 (6)
C20	2723 (6)	284 (11)	-2728 (6)
C21	900 (6)	160 (19)	3590 (6)
C22	1084 (8)	-1689 (19)	4217 (7)
O1	5938 (4)	3707 (9)	-3565 (5)
O2	3315 (4)	2019 (8)	2212 (4)
O3	1420 (4)	218 (10)	2685 (4)
O4	356 (5)	1440 (12)	3784 (5)

^aThe standard deviation for the last digit is given in parentheses.

EXPERIMENTAL

Mps are uncorrected; mass spectra were recorded on a JEOL JMS D-300 and the nmr spectra on a JEOL FX-90Q 90MHz for ¹H nmr and 22.49 MHz for ¹³C nmr with TMS as internal reference. The ir spectra are for KBr pellets on a Perkin-Elmer 377 spectrophotometer.

E. acaulis rhizomes were collected in Tharu village of the Kheri District, in the vicinity of Dudhwa National Park, Madhya Pradesh, India and adjoining the territory of Nepal in the State of Uttar Pradesh between 27° 41' and 28° 42' N and 80° 20' and 81° 19' E. A voucher specimen of the plant material has been deposited in the Herbarium Section of the National Botanical Research Institute, Lucknow, India. The air-dried plant material was finely powdered and extracted with *n*-hexane at room temperature (28 ± 2°). The extract was concentrated under reduced pressure at 40 ± 1°, charged on a column of Si gel in petroleum ether (40-60°), and eluted with increasing proportions of EtOAc in petroleum ether. Elution with petroleum ether-EtOAc (9:1) yielded a constituent, compound 1, which was observed to be homogeneous on tlc. Compound 1 was crystallized from EtOAc. The pure constituent yielded 2 on acetylation.

X-RAY DATA.²—Data collection was performed on a Rigaku AFC-5 FOS four circle diffractometer using graphite, monochromated Cu-K α ($\lambda \pm 1.5418 \text{ \AA}$) radiation. The 1562 unique intensities were collected by 2 θ - ω scan method within a limit of $2\theta \leq 120$. The crystal data are as follows: Formula C₂₂H₃₄O₄; size ca. 0.15 × 0.30 × 0.45 mm, monoclinic; space group P2₁ (Z=2); cell dimensions *a* = 12.3004 (3), *b* = 7.2052 (1), *c* = 11.723 (2) \AA , β = 102.45 (1)°, *V* = 1014.6 (3) \AA^3 . All the non-hydrogen atomic positions were revealed by direct methods (MULTAN) (8). The positions of hydrogen atoms, except for those of hydroxyl and acetoxymethyl groups, were calculated on the basis of stereochemical and geometrical considerations. The block-diagonal least-squares refinement for the 1162 observed reflections ($F_o \geq 2 F_c$) with

²Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK.

anisotropic thermal parameters for non-hydrogen atoms and isotropic thermal parameters for hydrogen atoms converged to a final *R* value of 0.049 [UNICS III (9)].³

ACKNOWLEDGMENTS

The authors are grateful to Assoc. Prof. Shigeaki Kawano of the College of General Education, Kyushi University, for the Program UNICS III and to Prof. Tsunehiro Takano of the Faculty of Pharmaceutical Sciences, Setsunan University, for the preparation of ORTEP drawing. Our thanks are also due to Dr. J.K. Maheshwari, Deputy Director, NBRI (CSIR), Lucknow.

LITERATURE CITED

1. G.B. Singh, S. Kaur, N.K. Satti, C.K. Atal, and J.K. Maheshwari, *J. Ethnopharmacol.*, **10**, 225 (1984).
2. N.K. Satti, O.P. Suri, K.L. Dhar, C.K. Atal, T. Kawasaki, K. Miyahara, and S. Kawano, *Phytochemistry*, **25**, 1411 (1986).
3. S.O. Almqvist and C.R. Enzell, *Acta Chem. Scand*, **B29**, 659 (1975).
4. L.E. Sutton, "Tables for Interatomic Distances and Configuration in Molecules and Ions," (Spec. Publ. No. 18.) The Chemical Society, London, 1965.
5. V.O. Ermer and J.D. Dunitz, *Helv. Chim. Acta*, **52**, 1861 (1969).
6. A. Cameron, G. Ferguson, and D. Morris, *J. Chem. Soc. B*, 1249 (1968).
7. F.J. Schmitz, D.J. Vanderah, K.H. Hollenbeak, C.E.L. Enwall, Y. Gopichand, P.K. SenGupta, M.B. Hossain, and D. Helm, *J. Org. Chem.*, **48**, 3941 (1983).
8. P. Main, M.M. Woolfson, and G. Germain, "A Computer Programme for the Automatic Solution of Crystal Structures," Univ. of York, York, England and Univ. de Louvain, Louvain, Belgium, 1971.
9. T. Sakurai and K. Kobayasi, *Rikagaku Kenkyusbo Hokoku*, **55**, 69 (1979).

Received 27 October 1986

³All the calculations were performed on a TOSBAC DS-600 computer.