Journal of Natural Products
Vol. 58, No. 7, pp. 1056-1058, July 1995

A NOVEL PENTACYCLIC TRITERPENE ACID FROM *ADENOCALYMMA ALLIACEUM* LEAVES

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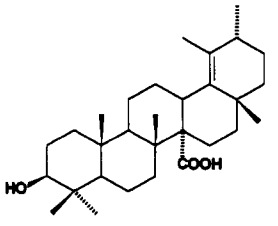
ABSTRACT.—A novel pentacyclic triterpene acid has been isolated from the leaves of *Adenocalymma alliaceum*. It has been characterized as 3β-hydroxyurs-18-en-27-oic acid [**1**] by spectral data and chemical studies.

The leaves of *Adenocalymma alliaceum* Miers (Bignoniaceae), a South American dicotyledonous plant, have been reported to possess biological activity (1,2). In continuation of our earlier phytochemical studies on the leaves of this plant (3–5), we have now isolated a new triterpene acid [**1**] based on the ursane skeleton.

Chemical tests (Liebermann-Burchard, Noller, tetranitromethane, $NaHCO_3$, and acetylation) suggested **1** to be an unsaturated triterpenoid containing -OH and -COOH functions. The ir spectrum demonstrated the presence of hydroxy (3450), monomeric carboxylic acid (1700 and 1100), double bond (1640), and *gem*-dimethyl (1390 and 1380 cm^{-1}) groups. The unsaturation was inferred to be a tetrasubstituted olefinic bond due to absence of bands at 960 or 840 cm^{-1} (6–9). The ir spectrum of the acetate derivative [**2**] showed the presence of -COOH (3440 and 1700) and ester (1730 and 1250 cm^{-1}) functionalities in the molecule. A high-field ^{1}H-nmr (400 MHz) spectrum of **1** displayed signals for seven Me groups at δ 0.70 (3H, d, J=6.0 Hz), 0.80 (3H, s), 0.82 (3H, s), 0.89 (3H, s), 0.97 (3H, s), 1.16 (3H, s), and 1.68 (3H, s), thereby suggesting the pentacyclic nature of the triterpenoid. Observation of the recorded spectral data revealed that one Me out of the seven appeared as a doublet at δ 0.70, while another appeared as a singlet at comparatively low field (δ 1.68). In view of this, **1** was concluded to be a member of the ursane series of triterpenoids. As one of the two secondary methyls of the ursane skeleton appeared downfield as a singlet, it could be attached with an olefinic carbon. Thus, the above-mentioned diagnostic features of these methyls indicated the possibility of the double bond being located either at C-18 or C-20 in ring E. As the ir of **1** had already suggested the double bond to be tetra-substituted, it was assigned at C-18. In view of this, the isolate was concluded to possess a Δ^{18}-ursene skeleton (10). A one-proton triplet resonating at δ 3.08 (J=8.0 Hz) in the ^{1}H-nmr spectrum suggested the presence of a proton on the carbon attached to the lone -OH group which could be assigned as 3β-OH on the basis of the ir (1040, 1030, 997 cm^{-1}) data. Further, the appearance of a one-proton singlet centered at δ 5.05 was attributed to an -OH proton since the -COOH proton resonated in the off-set region (6). The ^{1}H-nmr spectrum (400 MHz) of the acetate **2** revealed the appearance of a



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three-proton singlet at δ 2.05 (acetyl), a one-proton multiplet at δ 4.50 (-C*H*-$OCOCH_3$), and the disappearance of the signal in **1** for the hydroxyl proton.

The ms data proved to be very useful in determining the structure of **1**. The eims molecular ion peak appeared at *m/z* 456. The appearance of fragment ions at *m/z* 249, 248 (base peak), 208, 207, 203, 190, and 189 could only be explained by placing the lone -OH group at C-3 in ring A as suggested earlier and the -COOH group on any of the rings C, D, or E, with the possible positions being C-27, C-28, C-29, or C-30. Assignment of the -COOH to C-29 or C-30 could be ruled out by the presence of Me groups in these positions. Assignment to C-28 would give Δ^{18}-ursolic acid. This possibility was ruled out by mmp and tlc comparison with an authentic sample of Δ^{18}-ursolic acid (10). On this basis, the carboxyl was assigned to C-27 and the unknown **1** was assigned the structure 3β-hydroxyurs-18-en-27-oic acid.

Fission of the C-8–C-14 and C-9–C-11 σ bonds of ring C followed by the transfer of a hydrogen from C-26 to C-11 gave a fragment ion at *m/z* 207 from which a molecule of H_2O was eliminated to produce an ion peak at *m/z* 189. The formation of the base peak at *m/z* 248 was possibly due to the cleavage of the C-8–C-14 and C-9–C-11 bonds without involving hydrogen transfer. This radical ion probably further isomerized into the more stable allylic species accompanied by the shift of one hydrogen. Expulsion of the -COOH group from this allylic moiety accounted for another resonance-stabilized fragment ion at *m/z* 203. A peak appearing at *m/z* 208 was formed as a counterpart of the base peak. The formation of all these fragments from the parent molecule [**1**] is as previously proposed (11,12). The ms of **2** showed the molecular ion peak at *m/z* 498 (monoacetate) and other important fragments at *m/z* 483, 468, 453, 438, 392, 250, 249, 248 (base peak), 219, 203, 190, 189, and 43.

Compound **1** is a triterpenoid being reported for the first time. Triterpenes of the Δ^{18}-ursene skeleton are very rare, and this is the second report of a triterpene of this series. However, triterpenes with two double bonds in the ursane skeleton, vanguerolic acid (Δ^{12} and Δ^{18}); ilexolic acid (Δ^{12} and Δ^{18}); kanerocin (Δ^{18} and Δ^{20}); 28-hydroxy-20α-urs-12,18(19)-dien-3β-yl acetate; 3-oxo-urs-20α-12,18(19)-dien-28-oic acid; and randialic acid B_1 (Δ^{12} and Δ^{18}) have already been reported (13–18).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All the reported mps are uncorrected. Ir spectra were recorded on a Beckman Acculab 10 ir spectrophotometer. ^{1}H-Nmr spectra were measured in $CDCl_3$ with TMS as internal standard at 400 MHz on a Bruker WM-400 NMR spectrometer. Eims were recorded on a JEOL JMS D-300 mass spectrometer. Si gel (Qualigens) was used for tlc and cc. Tlc visualization was conducted by uv light, I_2 vapor, or by heating the plates after spraying with 10% H_2SO_4.

PLANT MATERIAL.—Plants of *Adenocalymma alliaceum*, identified by Dr. S.K. Singh, Department of Botany, University of Gorakhpur, were collected locally in Gorakhpur, in December 1985. A voucher specimen (No. 5) has been placed in the herbarium of the Natural Products Research Laboratory, Department of Chemistry, University of Gorakhpur, Gorakhpur, India. The leaves were separated, air-dried, and ground to a coarse powder.

EXTRACTION AND ISOLATION.—Air-dried and powdered leaves (5 kg) were thoroughly extracted with petroleum ether. The extract was filtered and then concentrated under reduced pressure to yield a semi-solid mass (105 g). The petroleum ether extract (105 g) was chromatographed over a column of Si gel (1.5 kg) eluted with *n*-hexane, *n*-hexane-C_6H_6 (3:1, 1:1, 1:3) mixtures, C_6H_6, and C_6H_6-EtOAc (3:1, 1:1) mixtures. Fractions showing similar chromatographic patterns on tlc plates were pooled, and the solvent was removed under reduced pressure.

3β-Hydroxyurs-18-en-27-oic acid [**1**].—Fractions 6–17 of the C_6H_6-EtOAc (1:1) eluate yielded a solid residue which was recrystallized from MeOH as white crystals (95 mg), mp 222–224°; $[\alpha]^{23}_D$ +17° (pyridine); ir ν max (KBr) 3450, 2925, 2900, 1700, 1640, 1465, 1455, 1390, 1380, 1100, 1040, 1030, 997 cm^{-1}; ^{1}H nmr (400 MHz, $CDCl_3$) δ 0.70 (3H, d, J=6.0 Hz, Me), 0.80 (3H,

s, Me), 0.82 (3H, s, Me), 0.89 (3H, s, Me), 0.97 (3H, s, Me), 1.16 (3H, s, Me), 1.68 (3H, s, Me-19), 3.08 (1H, t, J=8.0 Hz, H-3α), 5.05 (1H, s, OH-3β); eims M^+ *m/z* 456 (6) for $C_{30}H_{48}O_3$, 441 (0.5), 439 (0.5), 438 (2), 423 (1), 411 (1), 395 (1), 394 (1), 249 (15), 248 (100), 208 (4), 207 (20), 203 (38), 190 (5), 189 (10), 133 (5); tlc, R_f 0.45 (C_6H_6-EtOAc, 1:3), 0.34 ($CHCl_3$-MeOH, 19:1).

ACETYLATION OF **1**.—An aliquot (40 mg) of **1** and 3 ml each of pyridine and Ac_2O were taken in a stoppered flask and left overnight at room temperature. After the usual workup, the mixture afforded colorless crystals of the acetate derivative [**2**], 25 mg, mp 211–213°; ir ν max (KBr) 3440, 2930, 1730, 1700, 1620, 1460, 1370, 1360, 1250, 1100, 1020 cm^{-1}; ^1H nmr (400 MHz, $CDCl_3$) δ 0.65 (3H, d, J=5.50 Hz, Me), 0.87 (3H, s, Me), 0.89 (3H, s, Me), 0.92 (3H, s, Me), 1.05 (3H, s, Me), 1.20 (3H, s, Me), 1.65 (3H, s, Me-19), 2.05 (3H, s, -$OCOCH_3$), 4.50 (1H, m, H-3α); eims M^+ *m/z* 498 (1) for $C_{32}H_{50}O_4$, 483 (0.5), 468 (0.5), 453 (0.5), 438 (4), 392 (2), 300 (3), 268 (3), 266 (3), 250 (5), 249 (25), 248 (100), 219 (10), 203 (85), 190 (35), 189 (38), 133 (15), 43 (80); tlc R_f 0.62 (C_6H_6-EtOAc, 1:3), 0.42 ($CHCl_3$-MeOH, 19:1).

ACKNOWLEDGMENTS

The authors are thankful to Prof. S. Giri, Head, Department of Chemistry, University of Gorakhpur, Gorakhpur, and RSIC, CDRI, Lucknow, for providing laboratory facilities and spectral analyses, respectively. Thanks are due to Dr. J.S. Tandon, CDRI, Lucknow, for providing an authentic sample of Δ^{18}-ursolic acid. H.S.P. is grateful to CSIR, New Delhi, for financial assistance.

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Received 15 February 1993