STRUCTURE OF A NEW TERPENE, URS-3-O-ACETYL-20(30)-ENE-28-OIC ACID FROM STEM BARK OF *SAPIUM EUGNIFOLIUM*

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ABSTRACT

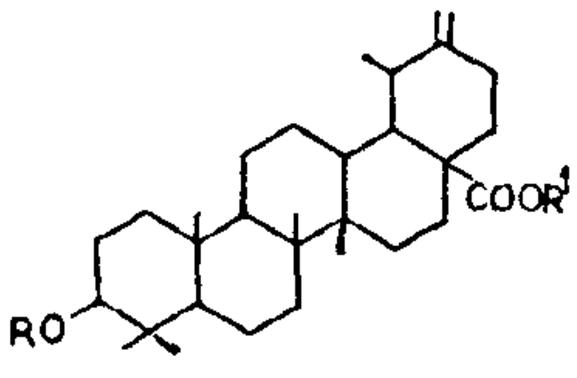
Evidence is presented for the structure of a new triterpenic acid, urs-3-O-acetyl-20(30)-ene-28-oic acid, which occurs together with moretenone in the stem bark of *Sapium eugnifolium*.

INTRODUCTION

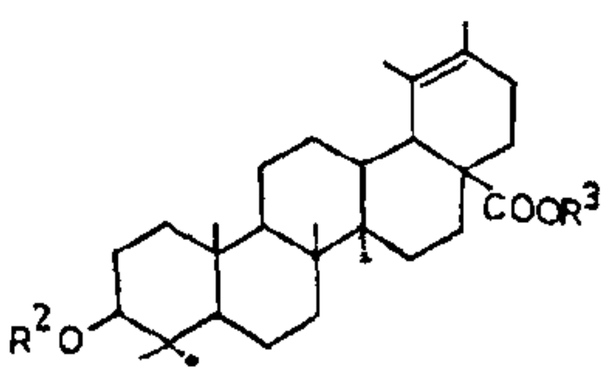
Previous study on *Sapium eugnifolium* has disclosed the presence of taraxenone, taraxerol, β-sitosterol[1,2] and a new ester triterpene[3]. Examination for pharmacological activity[4] revealed that the EtOAc extract of *S. eugnifolium* had antibacterial and antifungal activity and affected perfused frog heart. We therefore became interested in studying the constituents responsible for such activity. A new triterpenic acid (1) has been isolated along with moretenone from the stem bark of *S. eugnifolium*. The structure of the new compound was established as urs-3-O-acetyl-20(30)-ene-28-oic acid on the basis of chemical and spectral data.

RESULTS AND DISCUSSION

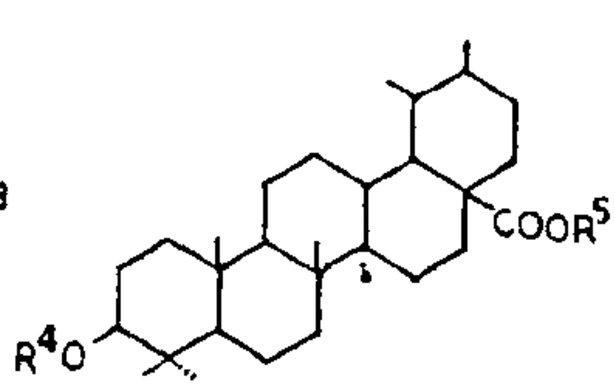
Compound (1), m.p. 220–22°C, $C_{32}H_{50}O_4$(M^+ 498) responded positively to the reactions characteristic for triterpenoids. The principal peaks in the IR spectrum of (1) indicated the presence of methyl, acetate, carboxylic acid and exomethylene double bond. The ^{1}H NMR spectrum of (1) displayed signals for the presence of five tertiary methyls (δ 1.05–1.20), one secondary methyl (δ 0.85, *d*, J = 4 Hz), polymethylene and methine protons, and an acetate group. Further the ^{1}H NMR spectrum exhibited a signal at δ 4.50 (*t*, 1H, J = 7 Hz) for H-3 proton as observed earlier in triterpenoid series. The chemical shift and coupling constant as well as biosynthetic considerations lead to the assumption that an acetate group is present at C-3 position. The exocyclic methylene group showed a fused *dq* in the ^{1}H NMR of (1) (δ 5.5). From the above data it is clear that one of the methyl groups in the triterpene skeleton must be present as a COOH group and most probably occupies position C-28. The presence of acetate and COOH groups at positions C-3 and C-28 respectively has been confirmed by the following set of reactions. Compound (1) on attempted hydrogenation (10% Pd-C)[5] isomerised to (2), the IR spectrum of which showed the band characteristic for a tetrasubstituted double bond (ν_{max} 850 cm^{-1}). The compound (2) on deacetylation (1% MeOH–KOH) yielded (3). LAH reduction of the methyl ester of (3) afforded a known diol, heterobetulin, m.p. 244–45°C (lit. m.p. 246–47°C)[6]. Compound (1) on treatment with CH_2N_2 gave (4), the IR spectrum of which showed the absorption for ester group (IR: 1735 cm^{-1}) and disappearance of the peak of COOH group, confirming the presence of COOH in (1). Compound (4) on hydrogenation with PtO_2–AcOH yielded (5), followed by deacetylation (1% MeOH–KOH) to afford (6). Compound (6) on treatment with C_6H_5COCl gave a known product, m.p. 212–13°C (lit. m.p. 213–14°C)[7].



1: R = Ac; R^1 = H
4: R = Ac; R^1 = Me



2: R^2 = Ac; R^3 = H
3: R^2 = R^3 = H



5: R^4 = Ac; R^5 = Me
6: R^4 = H; R^5 = Me

EXPERIMENTAL

General experimental procedures

Melting points were determined using a Toshniwal melting point apparatus and are uncorrected. IR spectra in KBr were recorded on a Perkin-Elmer-577 spectrophotometer. ^{1}H NMR spectrum was obtained in $CDCl_3$ at 90 MHz on an FT-instrument using TMS internal standard. Chemical shifts are given in δ ppm. The mass spectrum was recorded on a Jeol JMS-D300 spectrometer.

Isolation and purification of the compounds

Air-dried and powdered stem bark of *S. eugnifolium* (3 kg), procured from the United Chemical and Allied products, Calcutta, was exhaustively extracted with ethanol under reflux for 180 h on a water bath. The ethanol (20 l) from the percolates was removed under reduced pressure to get a solid mass which was extracted with petroleum ether. The petroleum ether extract was concentrated and examined by TLC which showed the presence of two compounds. It was then passed through a column of neutral alumina and successively eluted with petroleum ether-hexane (8:2) and petroleum ether; yield 500 mg, moretenone, m.p. 202–4°C (mmp and Co-TLC). The petroleum ether fraction (800 ml) was concentrated and the product was crystallized as colourless needles from C_6H_6: $CHCl_3$ to give compound (1) (yield 950 mg).

Characterization of compound (1)

IR: ν_{max}(in cm^{-1}) 2920 and 2840(Me), 1725(OAc), 1680 (COOH), 1460, 1360, 1235, 1020, 990 and 890 (exocyclic double bond). ^{1}H NMR: 0.90(*d*, J = 4 Hz; 3H, 1×Me), 1.05(*s*, 6H, 2×Me), 1.10(*s*, 3H, 1×Me), 1.20(*s*, 6H, 2×Me), 1.40–1.84(CH_2 and CH), 4.50(*t*, J = 7 Hz, 1H, H-3) and 5.60(fused *dq*, 2H, =CH_2). MS at *m/z*: 498(M^+, 30), 483(5), 480(10), 465(12), 453(35), 439(34), 416(5), 249(10), 219(56), 189(100), 150(80), 136(15), 135(28), 109(24), 95(32), 81(30%).
Found: C, 76.89; H, 10; $C_{32}H_{50}O_4$ required C, 77.10; H, 10.04%.

Attempted catalytic hydrogenation of compound (1)

A solution of (1) (400 mg) in *n*-heptane (240 ml) was exposed to H_2 at a little above atmospheric pressure in presence of 10% Pd–C (0.10 g) for 4 h. The catalyst was removed and the filtrate after concentration of the solvent gave a residue which was crystallized from ethyl acetate as fine needles, (2), m.p. 180–82°C (yield 350 mg), ν_{max} 850 cm^{-1} (tetrasubstituted double bond). Found: C, 76.80; H, 10; $C_{32}H_{50}O_4$ required C, 77.10; H, 10.04%.

Deacetylation of compound (2)

Compound (2) (300 mg) was hydrolysed with 1% methanolic potassium hydroxide (30 ml) under reflux for 1 h as usual. The product, (3), was crystallized as colourless rhombs from $CHCl_3$: MeOH mixture, m.p. 204–5°C (yield 250 mg). Found: C, 78.64; H, 10.50; $C_{30}H_{48}O_3$ required C, 78.94; H, 10.52%.

LAH reduction of the methyl ester of the compound (3)

The methyl ester of compound (3) (200 mg) in THF (20 ml) was added slowly to LAH (200 mg) in anhydrous ether (20 ml). The mixture was refluxed on a water bath for 3 h. It was then cooled and excess of LAH decomposed by the addition of a saturated aqueous solution of sodium sulphate. The solution was filtered, concentrated and evaporated to dryness to yield an amorphous solid which on crystallization from $CHCl_3$: MeOH mixture yielded heterobetulin. Found: C, 80.59; H, 10.82; $C_{30}H_{50}O_2$ required C, 81.44; H, 11.31%.

Methylation of compound (1)

The compound (1) (450 mg) was methylated with CH_2N_2 (prepared by the method described by Amstutz and Myers[8]) as usual. The product, (4), was crystallized from ether-benzene mixture as colourless needles, m.p. 140–42°C (yield 400 mg), ν_{max} 1735 (ester carbonyl). Found C, 77.28; H, 10.10; $C_{33}H_{52}O_4$ required C, 77.34; H, 10.15%.

Hydrogenation of compound (4)

The compound (4) (300 mg) in acetic acid (40 ml) was hydrogenated over platinum oxide for 20 h. The product, (5), was crystallized from ether: $CHCl_3$ mixture as colourless needles, m.p. 160–65°C (yield 250 mg). Found: C, 77; H, 10.48; $C_{33}H_{54}O_4$ required C, 77.04; H, 10.50%.

Deacetylation of compound (5)

The compund (5) (150 mg) was hydrolysed with 1% methanolic potassium hydroxide (15 ml) under reflux for 1 h as usual. The product, (6), was crystallized as colourless rhombs from $CHCl_3$: MeOH mixture, m.p. 128–30°C (yield 120 mg). Found: C, 78.68; H, 10.50; $C_{31}H_{52}O_3$ required C, 78.81; H, 11.01%.

Benzoylation of compound (6)

The compound (6) (100 mg) was benzoylated with benzoyl chloride (5 ml) and pyridine (5 ml) on a water bath for 6 h and worked up as usual. The product was crystallized from ether as prismatic crystals, λ_{max} 230 (lit. λ_{max} 239)[7], $[\alpha]_D^{25}+30°$ ($CHCl_3$), (lit. $[\alpha]_D+32°$)[7]. Found: C, 79.10; H, 9.68; $C_{38}H_{36}O_4$ required C, 79.16; H, 9.72%.

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ANNOUNCEMENT

DR A. K. GANGULY FELICITATION PRIZE

Nominations are invited by the 'A. K. Ganguly Felicitation Prize Committee for Indian Association for Radiation Protection' from Head of Institutions/ guiding teachers/immediate superiors/colleagues of any Indian scientist below 40 years of age as on 1-1-1989 who had in their opinion done meritorious work in the field of radiation protection/radiation in the environment. The work should have been carried out in India during the period January 1983 to December 1987.

The Prize consists of a citation and a cash award of Rs. 1000/-. The Prize will be awarded during the XVI Conference of IARP to be held at Bombay during January 9–12, 1989. The last date for submission of the nominations is October 31, 1988, to Dr. B. L. Gupta, Member-Secretary, Dr. A. K. Ganguly Felicitation Prize Committee, Division of Radiological Protection, Bhabha Atomic Research Centre, Bombay 400 085.