

## A NATURAL TRITERPENE ACID FROM *HYPTIS SUAVEOLENS*

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(Received 15 March 1983)

**Key Word Index**—*Hyptis suaveolens*; Labiatae; triterpenoids; heptacosanone; 3 $\beta$ -hydroxylup-20(29)-en-27-oic acid.

**Abstract**—A new natural triterpenoid, characterized as 3 $\beta$ -hydroxylup-20(29)-en-27-oic acid, has been isolated from the roots of *Hyptis suaveolens* in addition to heptacosanone, sitosterol- $\beta$ -D-glucoside, ursolic acid and betulinic acid. This is first example of a naturally occurring triterpene acid where the carboxyl group is situated as C-27 of the lup-20(29)-ene series.

### INTRODUCTION

In continuation of our earlier work [1–3], five more compounds (four of which are known) have been isolated from the roots of *Hyptis suaveolens*. The present paper deals with the isolation and characterization of a new natural triterpene acid, 3 $\beta$ -hydroxylup-20(29)-en-27-oic acid.

### RESULTS AND DISCUSSION

A benzene extract of the powdered roots was chromatographed over silica gel and the column was eluted with solvents of increasing polarity. Five compounds were isolated, four of which were identified (by colour reactions, TLC, IR and preparation of acetates where required [4–9]) as heptacosanone, sitosterol- $\beta$ -D-glucoside, ursolic acid and betulinic acid.

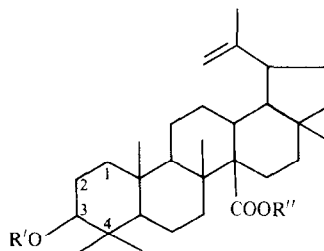
The benzene-ethyl acetate (3:1) eluate gave a solid which afforded colourless needles after repeated crystallization from aqueous methanol, 1, mp 307–308°,  $[\alpha]_D^{25} + 31^\circ$  (pyridine). It was soluble in ethyl acetate, methanol, acetone and chloroform. It gave colour reactions [4, 5, 7, 8] indicating that it was an unsaturated triterpenoid. Its molecular weight by mass spectrometry was 456 and elemental analyses gave the formula as C<sub>30</sub>H<sub>48</sub>O<sub>3</sub>. The IR spectrum showed hydroxyl (3400 cm<sup>-1</sup>), carboxyl (1690 cm<sup>-1</sup>) and unsaturation (1638 cm<sup>-1</sup>) functions; bands at 1370 and 1360 cm<sup>-1</sup> were indicative of the presence of a gem-dimethyl group, and vibrations at 1217 and 1178 cm<sup>-1</sup> indicated the quaternary nature of the gem-dimethyl group [10]. Bands at 3070, 1638 and 880 cm<sup>-1</sup> could be attributed to a terminal methylene group [11]. The C–O–H stretching vibrations at 1035 and 1025 cm<sup>-1</sup> suggested the presence of an equatorial hydroxyl group provided that it was present at C-3 of an A/B-*trans*-triterpenoid [12].

The NMR spectrum of the compound displayed signals due to five tertiary methyl groups [13] at  $\delta$ 0.68 (6H, s), 0.76 (3H, s) and 0.90 (6H, s), one vinylic methyl group at  $\delta$ 1.61 (3H, s) and two vinylic protons of the terminal methylene group at  $\delta$ 4.63 (1H, d,  $J = 3.0$  Hz) and 4.48 (1H, d,  $J = 3.0$  Hz). The above observations can be accounted by assuming the presence of a terminal isopropenyl group in the molecule [14]. Further, the charac-

teristic signals for the olefinic protons present can be obtained only when it has the lup-20(29)-ene skeleton (those of hop-22(29)-ene give only a broadened singlet [15]). The carbinolic proton resonated as a triplet centred at  $\delta$ 3.65, suggesting that it was adjacent to a single methylene group [16]. All these data lead to the conclusion that the compound is a monohydroxy-monocarboxy-triterpene acid of lup-20(29)-ene series [17].

Acetylation yielded the acetate 2, mp 301–302°, having the expected IR and NMR spectral data. Treatment of 1 with ethereal diazomethane afforded a monomethyl ester 3, mp 189–190° (lit. 190–191°) whose NMR spectrum showed carbomethoxy protons as a singlet at  $\delta$ 3.65.

The mass spectrum of 1 gave prominent peaks at  $m/z$  456, 248, 234, 220, 216, 207 and 189 (cf. [18]). The formation of ion 248 can be accounted by assuming that the lone carboxyl group is at either of the two junctions of rings C and D, or D and E. Since the compound is not betulinic acid the carboxyl group must be at C-27. The compound is therefore 3 $\beta$ -hydroxylup-20(29)-en-27-oic acid. This compound has been prepared synthetically [19] and the identity of the new natural acid was confirmed by comparison of mp and optical rotation with those of the synthetic product.



- 1 R' = R'' = H
- 2 R' = Ac, R'' = H
- 3 R' = H, R'' = Me

## EXPERIMENTAL

Mps are uncorr. NMR were recorded using  $\text{CDCl}_3$  or  $\text{DMSO}-d_6$  as solvent and TMS as internal standard. MS were on a JEOL high resolution mass spectrometer JMS-D 300 with data acquisition system. Optical rotations were measured in pyridine or  $\text{CHCl}_3$ . TLC was on silica gel G. CC was with silica gel and neutral  $\text{Al}_2\text{O}_3$ .

**Plant material.** Roots of *H. suaveolens* Poit (Labiateae) were collected from the campus of Gorakhpur University, Gorakhpur. A specimen has been preserved at the Herbarium of the Natural Products Research Laboratory, Department of Chemistry, University of Gorakhpur. The roots were air-dried and ground to a coarse powder.

**Extraction and isolation of compounds.** Powdered roots (5 kg) were exhaustively extracted with hot  $\text{C}_6\text{H}_6$  and worked up in the usual manner. Chromatography over a silica gel column and elution with hexane, hexane- $\text{C}_6\text{H}_6$  (3:1, 1:1, 1:3),  $\text{C}_6\text{H}_6$ ,  $\text{C}_6\text{H}_6$ -EtOAc (3:1, 1:1, 1:3) and EtOAc were monitored with co-TLC.

**Heptacosanone** was obtained with hexane- $\text{C}_6\text{H}_6$  (1:1) and identified by mp, IR and comparison with an authentic sample.

**Sitosterol- $\beta$ -D-glucoside** was eluted with hexane- $\text{C}_6\text{H}_6$  (1:3) and its identity verified by colour tests, IR and TLC of the compound and its acetate.

**Ursolic acid** was obtained with  $\text{C}_6\text{H}_6$  and its identity confirmed as above.

**Betulinic acid** was eluted with  $\text{C}_6\text{H}_6$ -EtOAc (1:1) and its identity confirmed as above.

**3 $\beta$ -Hydroxyilup-20(29)-en-27-oic acid (1).** Elution with  $\text{C}_6\text{H}_6$ -EtOAc (3:1) gave a solid which yielded colourless needles (300 mg) from MeOH, mp 307–308°,  $[\alpha]_D^{25} + 31^\circ$  (pyridine); IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3400, 3070, 2907, 2825, 1690, 1638, 1451, 1370, 1360, 1217, 1178, 1035, 1025 and 880;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  0.68 (6H, s), 0.76 (3H, s), 0.90 (6H, s), 1.61 (3H, s), 3.65 (1H, t,  $J = 7.5$  Hz), 4.63 (1H, d,  $J = 3.0$  Hz) and 4.48 (1H, d,  $J = 3.0$  Hz); MS  $m/z$  (rel. int.): 456  $[\text{M}]^+$  (14.95), 439 (4.56), 438 (3.69), 415 (6.56), 411 (3.56), 248 (39.22), 234 (20.36), 220 (19.32), 216 (4.69), 207 (45.39), 203 (16.50), 202 (8.95), 189 (100), 176 (10.56) and 175 (6.95).

**3 $\beta$ -Acetoxylup-20(29)-en-27-oic acid (2).** The acetate ( $\text{Ac}_2\text{O}$ -pyridine) was obtained as needles from aq. MeOH, mp 301–302°; IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3030, 2850, 1737, 1690, 1640, 1450, 1420, 1370, 1360, 1250, 1240, 1075, 1040 and 880;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.70 (6H, s), 0.82 (3H, s), 0.92 (3H, s), 0.96 (3H, s), 1.68 (3H, s), 2.05 (3H, s), 4.90 (1H, m) and 4.56 (2H, d,  $J = 3.0$  Hz).

**Methyl-3 $\beta$ -hydroxylup-20(29)-en-27-oate (3).** The methyl ester (ethereal  $\text{CH}_2\text{N}_2$ ), mp 189–190°, had IR  $\nu_{\text{max}}^{\text{KBr}}$   $\text{cm}^{-1}$ : 3390, 3020, 2920, 2890, 1740, 1640, 1470, 1420, 1375, 1365, 1250, 1220, 1150, 1050, 920 and 890;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  0.70 (6H, s), 0.82 (3H, s),

0.92 (3H, s), 0.96 (3H, s), 1.65 (3H, s), 3.65 (3H, s) and 4.56 (2H, d,  $J = 3.0$  Hz).

**Acknowledgements**—We are grateful to the Head, Department of Chemistry, Gorakhpur University, for laboratory facilities. We are also grateful to Dr. Nitya Nand, Director, and Dr. R. S. Kapil, Medicinal Chemistry Division, Central Drug Research Institute, Lucknow, for spectral analyses, authentic samples and useful discussions during the structure elucidation of the compounds. Financial assistance provided by the C.S.I.R., New Delhi, is also gratefully acknowledged.

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