CHEMICAL CONSTITUENTS OF *HYPTIS SUAVEOLENS*. PART I. SPECTRAL AND BIOLOGICAL STUDIES ON A TRITERPENE ACID

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Abstract.—β-Sitosterol, oleanolic acid, urs-12-en-3β-ol-27-oic acid (α-peltoboykinolic acid) and an unidentified triterpene acid have been isolated from the roots of *Hyptis suaveolens*. The nmr and ms of α-peltoboykinolic acid were studied. Some biological activity of the acid is also reported.

From the aerial parts of many species of the genus *Hyptis* (Labiatae), several essential oils (1–4), di- and triterpenoids (5–7), a steroid (7), a flavonoidal glycoside (8), certain agglutinins (9), fatty compounds (5,10), and a lactone (11) were reported earlier. Two cytotoxic (antimitotic) principles, β-pellatin and 4'-demethyldesoxypodophyllotoxin (12), were isolated from the leaves of *H. verticillata*. The roots of *H. suaveolens* are said to act as a stomachic, and a decoction thereof is valued as an appetizer (13,14). It was noticed that the benzene extract of the roots of *H. suaveolens* prevented the growth of the pathogenic fungus *Helminthosporium oryzae*. Since no chemical investigation has been reported on the roots of this species, it was thought worthwhile to pursue a systematic chemical examination of suitable root extracts.

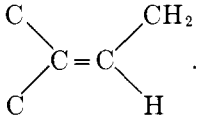
A benzene extract (70 g) of air-dried and powdered roots was chromatographed over silica gel. The column was eluted with hexane, hexane-benzene mixtures (3:1, 1:1, 1:3), benzene, benzene-ethyl acetate (3:1, 1:1, 1:3) and ethyl acetate successively.

The benzene eluate yielded a white solid which, on repeated crystallization from ethanol, gave colorless needles mp 136–138°, $[\alpha]^{24}D-29°$ ($CHCl_3$). It was identified as β-sitosterol by mmp determination, co-tlc and super-imposable ir with an authentic sample. On acetylation, it gave an acetate mp 130–131°.

From the benzene-ethyl acetate (3:1) eluate, three compounds oleanolic acid, IV and V were obtained. Oleanolic acid was recrystallized from aqueous methanol as colorless needles, mp 300–302°, $[\alpha]^{24}D+78°$ ($CHCl_3$). It was identified as oleanolic acid by comparison of its ir, co-tlc and mmp determination with an authentic sample.

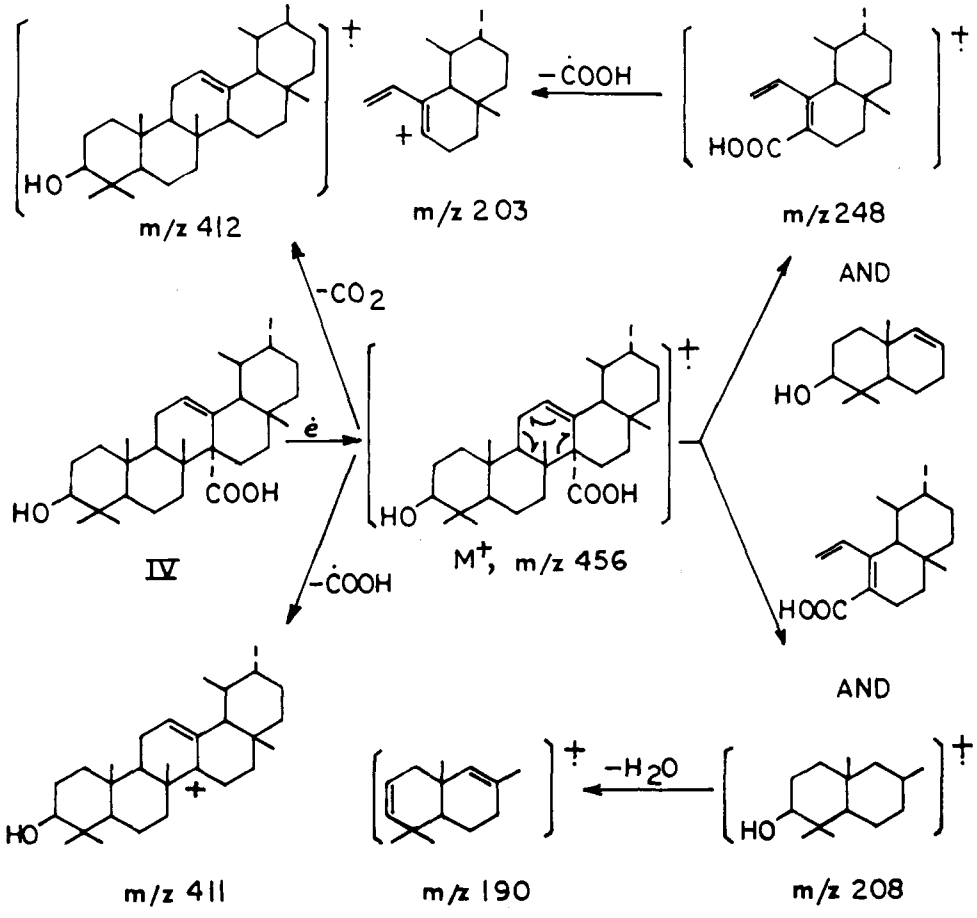
Compound IV was recrystallized from aqueous methanol as white needles, mp 235–236° (decomp.), $[\alpha]^{24}D+121°$ ($CHCl_3$). From elemental analyses and molecular weight determination (456, from ms), it analyzed for $C_{30}H_{48}O_3$. It gave a deep violet color with Liebermann-Burchard reagent (15,16) and a yellow (turning pink) color with Noller's reagent (17) suggesting that the compound is a triterpenoid. The compound responded positively with tetranitromethane (18) indicating the presence of C=C in the molecule. The ir spectrum of the compound showed the presence of hydroxyl (3400), carboxyl (1690) and unsaturation (1640 Cm^{-1}) in the molecule. Bands at 1100 and 1035 Cm^{-1} indicated the presence of C–O linkage in the molecule.

A high resolution nmr ($CDCl_3$) spectrum of the compound displayed signals at δ 0.53 (3H, S), 0.64 (3H, d, $J=5$ cps), 0.77 (3H, d, $J=2.5$ cps), 0.87 (6H, S) and 1.18 (6H, S). These signals located in the high field region of the spectrum (integrated to a total of 21 protons) show the presence of seven C-methyl groups. These observations are suggestive of the pentacyclic nature of the isolated triterpenoid (19). The bands at δ 5.02 and 5.20 indicated the presence of a hydroxyl and an olefinic proton in the molecule. Further, the nmr spectrum of this compound showed a triplet centered at δ 4.10 (1H, $J=7.5$ cps) which is due to the

presence of a proton on the hydroxyl-bearing carbon adjacent to a single methylene group. A single olefinic proton resonating as a triplet centered at δ 5.20 (1H, $J=2.5$ cps) indicated the presence of a C=C residue present between a bridgehead carbon and an adjacent ring carbon as  . The proton of the carboxyl group resonated in the offset region at δ 11.0 (1H, S).

The ms of compound IV proved to be very helpful in arriving at the structure. The parent ion M^+ appeared at m/z 456. The fragment ions at m/z 441, 439, 438, 412 and 411 are attributed to the ions formed by the loss of .CH_3, .OH, H_2O, CO_2 and .COOH, respectively, from the parent ion. The base peak appeared at even mass number 248. Another peak at m/z 208 is presumably due to the counterpart ion of the neutral fragment corresponding to the base peak. These ions are characteristics of the ms of Δ^{12}-pentacyclic triterpenoids of ursane and oleanane series (20, 21) containing a carboxyl group at any position in rings C, D and E and a hydroxyl group in ring A or B. These are formed in a retro-Diels Alder reaction as shown in the scheme. In the nmr spectrum of the compound two doublets for methyl groups (C_{29}, C_{30}) appeared at δ 0.64 and 0.77 suggesting the ursane skeleton of the triterpene because in the case of oleanane triterpene all

SCHEME



the methyl groups would have appeared as a singlet. Thus the compound belongs to the Δ^{12}-ursane series of triterpenoids. The lone hydroxyl group is placed as 3β-OH because in the ir spectrum of the triterpene a sharp band appeared at 1035 Cm^{-1}, which is a characteristic feature of a 3β-hydroxyl grouping in A/B trans triterpenoids (22).

The assignment of the carboxyl group as C_{28} would give ursolic acid, a well known compound whose available data are quite different from those reported here. The allocation of this group either as C_{29} or C_{30} of an ursane skeleton can possibly be ruled out on the basis of the following observations. Such an assignment should show a single proton signal for *H*–C–COOH in the down field region, which is not observed in the recorded nmr spectrum. Also the appearance of two methyl groups as two doublets in the nmr spectrum rules out the presence of a carboxyl group in any position in ring E (C_{29} and C_{30}) because, in that case, only one doublet would have appeared corresponding to a single methyl group. The urs-12-en-3β-ol-29-oic acid and urs-12-en-3β-ol-30-oic acid are well known compounds, namely, bryonolic and katonic acids, respectively. These acids have distinctly different mps and optical rotations as compared to the isolated compound. The molecular weight, mp, optical rotation, and ir data of this compound closely resemble those of urs-12-en-3β-ol-27-oic acid (α-peltoboykinolic acid), originally derived from quinovic acid by Ruzicka *et al.* (23) as early as 1946 and later isolated from the rhizomes of *Peltoboykinia tellimoides* (maxim) Hara by Nagai *et al.* (24). The identity of this acid was confirmed by direct comparison (mmp and co-tlc) with an authentic sample. Compound IV gave an acetate, mp 242–243° [243°, (24)], and methyl ester, mp 193–194° (193–195°, ibid), $[\alpha]^{24}D$+130° ($CHCl_3$) [+132°, (24)]. Thus compound IV was identified as α-peltoboykinolic acid.

Compound V, when recrystallized from aqueous methanol, gave a white solid, mp 305–307°. It gave a molecular ion peak at *m/z* 456 and base peak at *m/z* 189. It responded positively to Liebermann-Burchard and Noller's tests for triterpenoids. Detailed characterization of this compound leading to its final identification is in progress.

In view of the antifungal nature of the original benzene extract of the roots, the fungitoxicity of α-peltoboykinolic acid was tested by the method envisaged by Spencer *et al.* (25) at 10, 100 and 1000 ppm against *H. oryzae*. The recorded inhibition of the mycelial growth was not significant (below 10% at 1000 ppm); hence, it can not be recognized as a useful antifungal component.

EXPERIMENTAL[1]

PLANT MATERIAL.—Roots of *H. suaveolens* POIT were collected from the campus of Gorakhpur University, Gorakhpur. A repository stock can be had from India, Ceylon, and the U.S.A. A herbarium specimen has been placed in the herbarium of the Natural Products Research Laboratory, Department of Chemistry, University of Gorakhpur, Gorakhpur, India. The roots were air dried and ground to a coarse powder.

EXTRACTION PROCEDURE.—Powdered roots (5 kg) were exhaustively extracted with benzene. The extract was filtered and the solvent was removed by distillation under reduced pressure to give a dark brownish mass (80 g).

ISOLATION OF COMPOUNDS.—The benzene extract (70 g) was chromatographed on a silica gel column. The column was eluted with hexane, hexane-benzene mixtures (3:1, 1:1, 1:3), benzene, benzene-ethyl acetate (3:1, 1:1, 1:3) and ethyl acetate in succession. The progress of elution was monitored by intermittent co-tlc examinations of the 200 ml effluent fractions. Chromatographically identical fractions were mixed, and the solvent was removed under reduced pressure.

ISOLATION OF β-SITOSTEROL.—The benzene eluate yielded a colorless compound after recrystallization from ethanol (200 mg), mp 136–138°, $[\alpha]^{24}D$ −29° ($CHCl_3$); ir ν max (KBr) 3239, 2907, 2825, 1655, 1451, 1372, 1059, 972, 845 and 804 cm^{-1}.

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[1]Melting points are uncorrected. The ir spectra were recorded on a Perkin-Elmer-177 spectrophotometer; nmr spectra were recorded on a Perkin-Elmer R-32 instrument (90 MC) with $CDCl_3$ as solvent and TMS as an internal reference, ms analyses were carried out with a JEOL High Resolution Mass Spectrometer JMS-D 300 with data acquisition system and optical rotations were taken in $CDCl_3$ on a Carl Zeiss-370265 Spectropolarimeter. Silica gel G was used for tlc, and the spots were visuallized by spraying the plates with 10% H_2SO_4 followed by heating in an oven.

ACETYLATION OF β-SITOSTEROL.—β-Sitosterol (25 mg) treated with acetic anhydride/pyridine (1:1) at room temperature overnight afforded β-sitosterol acetate, mp 130–131°, ir ν max (KBr) 2907, 2786, 1727, 1451, 1362, 1292, 1043, 962 and 807 cm^{-1}.

ISOLATION OF OLEANOLIC ACID.—The benzene-ethyl acetate (3:1) eluate yielded a solid mass which, on repeated crystallization from aqueous methanol, gave colorless needles (135 mg) of oleanolic acid, mp 300–302°, $[\alpha]_D$+78° ($CHCl_3$); ir ν max (KBr) 3400, 3185, 2874, 2793, 1695, 1640, 1462, 1391, 1362, 1037 and 996 cm^{-1}.

ISOLATION OF α-PELTOBOYKINOLIC ACID (*IV*).—The benzene-ethyl acetate (3:1) eluate yielded a second compound which, on recrystallization from aqueous methanol, afforded colorless needles (120 mg) of IV, mp 235–236° (decomp.), $[\alpha]^{24}_D$+121° ($CHCl_3$); ir ν max (KBr) 3400, 1690, 1640, 1100 and 1035 cm^{-1}; nmr ($CDCl_3$) δ 0.53 (3H, S), 0.64 (3H, d, J=5. cps), 0.77 (3H, d, J=2.5 cps), 0.87 (6H, S), 1.18 (6H, S), 4.1 (1H, *H*–C–O, t, J=7.5 cps), 5.02 (1H, H–C–O*H*, s), 5.20 (1H, C=C–*H*, t, J=2.5 cps), 11.0 (1H, –C–O–*H*, s); ms M^+ m/z 456 (2.26%) for $C_{30}H_{48}O_3$, 454 (.32), 441 (.41), 439 (.67), 438 (1.99), 437 (1.70), 412 (.17), 411 (1.08), 248 (100), 247 (2.55), 208 (6.38), 207 (29.75), 204 (12.29), 203 (48.42), 191 (8.90), 190 (13.37) and 189 (19.17).

ACETYLATION OF *IV*.—A mixture of *IV* (20 mg), pyridine (1 ml) and acetic anhydride (1 ml) was allowed to stand overnight at room temperature. After the completion of the reaction, the solvent was removed under reduced pressure. The residue when washed with water and recrystallized from methanol gave a colorless solid, mp 242–243°.

METHYLATION OF IV.—Compound IV (30 mg) was treated with ethereal diazomethane at room temperature for 10 minutes. Evaporation afforded a residue which, when recrystallized from methanol, gave needles of methyl ester, mp 193–195°, $[\alpha]^{24}_D$+138° ($CHCl_3$).

ISOLATION OF COMPOUND V.—Benzene-ethyl acetate (3:1) eluate gave a third compound which, on recrystallization from aqueous methanol, afforded colorless needles (150 mg), mp 305–307°; ms M^+ m/z 456, base peak at m/z 189. It gave positive tests for triterpenoids. Further study of this compound is in progress.

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