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CHEMICAL CONSTITUENTS OF *VERNONIA CINEREA*, PART I. ISOLATION AND SPECTRAL STUDIES OF TRITERPENES

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The leaves, roots, and seeds of some species of *Vernonia* (Compositae) have been ascribed medicinal properties (1,2). *Vernonia cinerea* Less is an annual herb that grows in India. It is used as a tonic, stomachic, and astringent and is also a known cure for tridosa, consumption, asthma, and bronchitis (3). An aqueous ethanolic (50%) extract of the plant showed activity against Ranikhetvirus disease (4). It also showed anticancer activity against Sarcoma 180 in mice (4). The root is bitter and is used as an anthelmintic and diuretic (5). Fresh juice of leaves is given to treat dysentery and is locally applied for the extraction of guinea-worms (5). The seeds are also used as an anthelmintic and alexipharmic, and they are quite effective against round-worms and thread-worms. They are also given for coughs, flatulence, intestinal colic, and chronic skin diseases (5). A paste of seeds with lime-juice is used to destroy pediculi (3,5). The flowers are used to treat conjunctivitis, fever, and rheumatism (3). β-Amyrin, lupeol, and their acetates; β-sitosterol; stigmasterol; α-spinasterol; phenolic resin; and KCl have been isolated from the whole plant (6). Because no detailed chemical investigation of this plant has been reported, the present study deals with a systematic chemical examination of its roots.

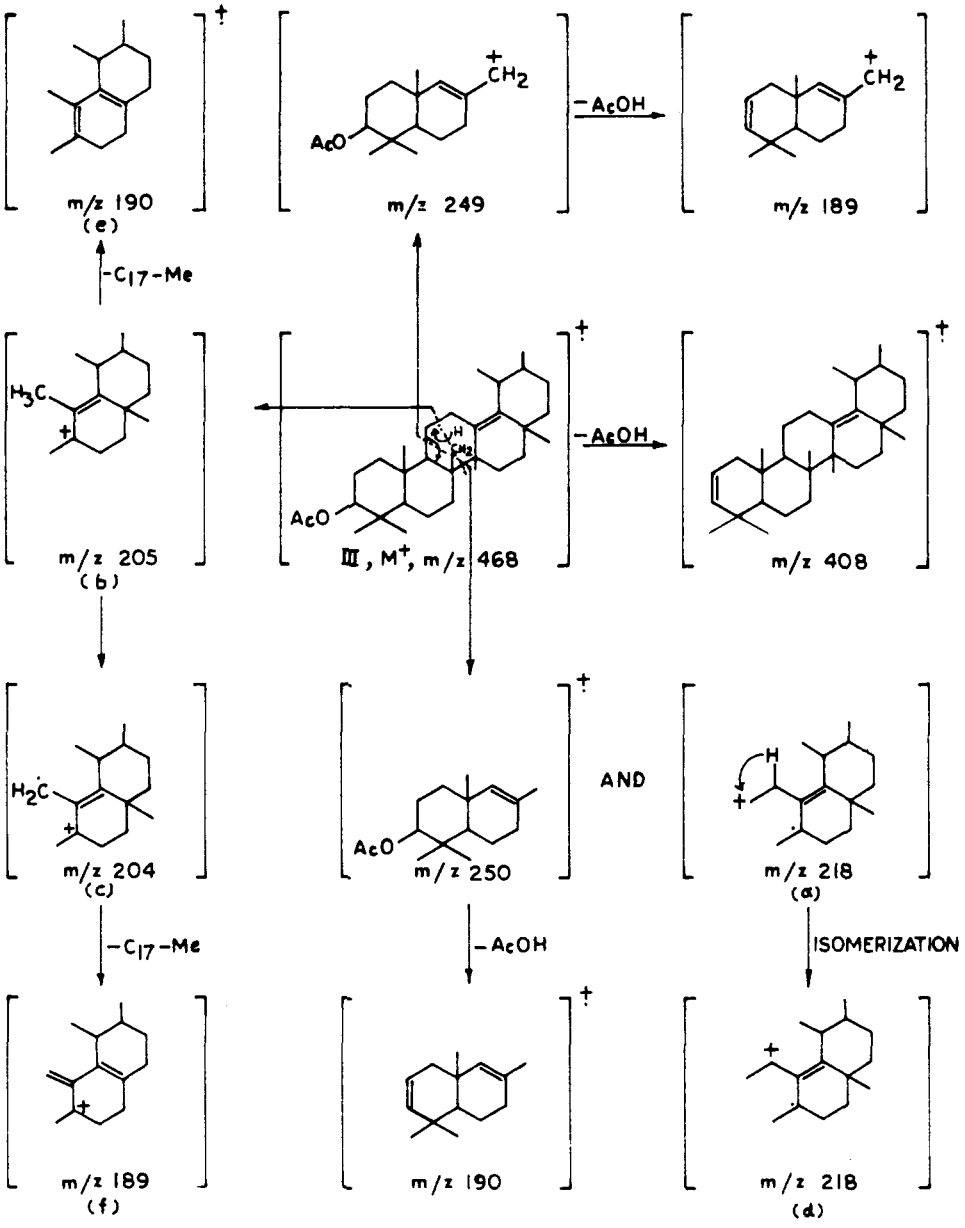
The roots of *V. cinerea* have yielded six triterpenes, δ-amyrin acetate, α-amyrin acetate, 3β-acetoxyurs-13(18)-ene (**1**), β-amyrin acetate, β-amyrin, and α-amyrin. Compound **1** has been synthesized by earlier workers, but its isolation from natural sources and spectral studies have been done for the first time. Identity of all the six compounds has been established with the help of mp, optical rotation, ir, ^{1}H-nmr, ms, and chemical reactions.

From elemental analyses and molecular weight determination (468, from ms), compound **1** analyzed for $C_{32}H_{52}O_2$. It gave a deep red color with Liebermann-Burchard reagent (7,8) and a yellow (turning to violet) color with Noller's reagent (9), suggesting that **1** is a triterpenoid. The compound responded positively with tetranitromethane (10), indicating the presence of C=C in the molecule. The ir spectrum had peaks corresponding to ester carbonyl (1730 cm^{-1}), unsaturation (1640 cm^{-1}), and *gem*-dimethyl (1390 and 1380 cm^{-1}) functions in the molecule. Absence of typical bands for di- and trisubstituted double bonds at 960 and 840 cm^{-1} (11,12), respectively, leads to the conclusion that the triterpene must contain a tetrasubstituted double bond.

A high resolution nmr ($CDCl_3$) spectrum of the compound displayed signals for eight C-methyl groups at δ 0.80 (6H, s), 0.87 (3H, s). 0.93 (3H, s), 0.96 (3H, s), 1.00 (3H, s), 1.32 (3H, d, J=4.5 cps), and 1.56 (3H, d, J=3.6 cps). This observation indicates the pentacyclic nature of the isolated triterpenoid (13,14). A singlet at δ 1.95 (3H, s) is attributed to acetoxyl methyl group, and a multiplet centered at δ 4.50 (1H, m) is assigned to the proton on the acetoxyl bearing carbon adjacent to a single methylene group. Absence of a peak for olefinic proton could be due either to the absence of unsaturation or to the presence of a tetrasubstituted olefinic bond (15).

A clearer picture of the newly isolated molecule emerged with the help of ms

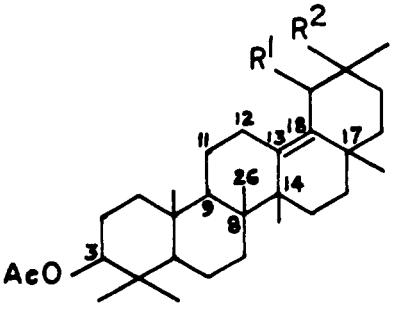
studies. The parent ion M^+ appeared at *m/z* 468. Appearance of fragment ions (see Scheme 1) at *m/z* 250,218(a), 205(b), and 204(c) is accountable on the basis of a $\Delta^{13(18)}$-oleanene or ursene skeleton (16,17). Also, the formation of these fragment ions required the placement of a lone acetoxyl group at C-3 and no group to either rings C, D, or E (17). Satellite a, which is an important fragment of appreciable intensity, is formed by the cleavage of 8-14 and 9-11 bonds. It further isomerizes into more stable allylic cation (d) by the shift of one hydrogen. Satellites e (*m/z* 190) and f (*m/z* 189) are formed by the loss of C_{17}-methyl from species b and c, respectively. A fragment ion at *m/z* 249 with rings A



SCHEME 1

and B intact is attributable to the species formed by the cleavage of 9-11 and 8-14 bonds with the transfer of one hydrogen from C-26 to C-11; it is also a characteristic peak of $\Delta^{13(18)}$-ene (16). Peak at *m/z* 408 is formed by the loss of AcOH from the molecular ion M^+.

The fact that the peak corresponding to *m/z* 189 is of higher intensity than that at *m/z* 203 is indicative of its ursane skeleton (18). As discussed earlier, the appearance of two doublets for two methyl groups (C-29, C-30) at δ 1.32 and 1.56 also suggests the ursane skeleton because, in the case of oleanane triterpene, all the methyl groups would appear as singlets (19). Thus, compound **1** can be assigned to the $\Delta^{13(18)}$-ursene series of triterpenoids. The lone acetoxyl group is placed as 3β-OAc because, in the ir spectrum, a sharp band appeared at 1260 cm^{-1}, which is a characteristic feature of a 3β-acetoxyl grouping in A/B *trans*-triterpenoids (20-22). Observations and assignments as made above on the basis of ir, nmr, and ms data lead to the structure of compound **1** as 3β-acetoxyurs-13(18)-ene.



1; R^1=Me, R^2=H

Hydrolysis with 3% ethanolic KOH gave 3β-hydroxyurs-13(18)-ene, mp 200-202° [201-202° (23)]. Its ir spectrum showed complete disappearance of ester peaks (1730 and 1260 cm^{-1}) and appearance of a peak for hydroxyl group at 3320 cm^{-1}. J.M. Beaton *et al.* (23), while studying the constitution and stereochemistry of the ursane group of triterpenoids, synthesized urs-13(18)-en-3β-ol and its acetate derivative. These compounds have also been synthesized by other workers (24-26). The mp, optical rotation, and ir data (26) of compound **1** and its hydrolyzed product closely resemble those of the synthesized products, namely, urs-13(18)-en-3β-acetate and urs-13(18)-en-3β-ol, respectively. Therefore, the newly isolated compound was identified as 3β-acetoxyurs-13(18)-ene. Because an authentic sample was not available, a direct comparison could not be made.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All the reported mps are uncorrected. The ir spectra were determined on a Perkin-Elmer-177 spectrometer ranging from 4000-650 cm^{-1}. Pmr spectra were recorded on a Perkin-Elmer-R-32 instrument (90 MHz) in $CDCl_3$ with TMS as internal standard. Mass spectra were recorded with a JEOL High Resolution Mass spectrometer JMS-D-300 with data acquisition system. Optical rotations were measured in chloroform on Carl Zeiss-370265 Spectropolarimeter. Silica gel G (BDH) was used for tlc. The detections of spots were made by uv lamp, iodine chamber, and heating the plates in an oven after spraying with 10% H_2SO_4.

PLANT MATERIAL.—Plant of *Vernonia cinerea* Less was collected from the campus of Gorakhpur University, Gorakhpur, in December, 1981. A repository stock can be had from India and Pakistan. 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 separated, washed, air dried, and ground to a coarse powder.

EXTRACTION PROCEDURE.—Powdered roots (10 kg) were exhaustively extracted with petroleum ether. The extract was filtered, and the solvent was removed by distillation under reduced pressure to give a dark brownish mass (450 g).

ISOLATION OF COMPOUNDS.—The solid mass (260 g) was chromatographed over a column of silica gel (2.5 kg). The column was eluted with petrol and petrol-C_6H_6 (9:1, 1:1, 1:3). The progress of elution was monitored by intermittent Co-tlc examination of the 200-ml effluent fractions. Chromatographically identical fractions were mixed, and the solvent was removed under reduced pressure.

ISOLATION OF δ-AMYRIN ACETATE.—The pure petrol eluate yielded a white compound after recrystallization from $CHCl_3$-MeOH (250 mg),

mp 197-198°, $[\alpha]_D$ −22° ($CHCl_3$), and was identified as δ-amyrin acetate using ir, Pmr, and ms. δ-Amyrin acetate (60 mg) was refluxed (6 h) with 3% ethanolic KOH to obtain δ-amyrin, mp 212-213°, $[\alpha]^{24}_D$ −44° ($CHCl_3$).

ISOLATION OF α-AMYRIN ACETATE.—Fractions 1-3 of petrol-C_6H_6 (9:1) eluate yielded a crude solid that, on fractional crystallization from $CHCl_3$-MeOH, yielded white needles (150 mg), mp 220-222°, $[\alpha]^{20}_D$ +76° ($CHCl_3$), and was identified as α-amyrin acetate using ir, Pmr, and ms. Hydrolysis of α-amyrin acetate (25 mg) with 3% ethanolic KOH gave α-amyrin, mp 180-182°.

ISOLATION OF 3β-ACETOXYURS-13 (18)-ENE (**1**).—A solid was obtained from fractions 4-7 of petrol-C_6H_6 (9:1) eluate which, on repeated crystallization from $CHCl_3$-MeOH, gave white crystals (150 mg), mp 208-210°, $[\alpha]^{19}_D$ −22° ($CHCl_3$); ir ν max (KBr) 1730, 1640, 1470, 1390, 1380, 1260, 1210, 1160, 1020, 990, 890 and 730 cm^{-1}; nmr ($CDCl_3$) δ 0.80 (6H, s), 0.87 (3H, s), 0.93 (3H, s), 0.96 (3H, s), 1.00 (3H, s), 1.32 (3H, d, J=4.5 cps), 1.56 (3H, d, J=3.6 cps), 1.95 (3H, O-$\overset{\overset{O}{\|}}{C}$-$CH_3$, s) and 4.50 (1H, -$CH$-OAc, m); ms M^+ *m/z* 468 (27%) for $C_{32}H_{52}O_2$, 453 (2.2), 408 (10), 393 (15.1), 250 (10.1), 249 (13.1), 219 (5.4), 218 (45.1), 205 (100), 204 (40.4), 203 (25.3), 191 (32.4), 190 (30.6), and 189 (40).

HYDROLYSIS OF **1**.—A 30-mg sample was refluxed for 6 h with 3% ethanolic KOH, and the reaction mixture was poured into cold water. It was filtered and washed to yield white crystals (20 mg), mp 200-202°, ir ν max (KBr) 3320, 1640, 1470, 1390, 1200, 1160, 1040, 990, 890, and 730 cm^{-1}.

ISOLATION OF β-AMYRIN ACETATE.—Fractions 8-10 of petrol-C_6H_6 (9:1) eluate gave a compound that was recrystallized from $CHCl_3$-MeOH into white crystals (200 mg), mp 236-237°, $[\alpha]_D$ +81° ($CHCl_3$), and was identified as β-amyrin acetate using ir, Pmr, and ms. On hydrolysis, β-amyrin acetate (40 mg) gave β-amyrin, mp 191-192°.

ISOLATION OF β-AMYRIN.—Petrol-C_6H_6 (1:1) eluate yielded a dirty white solid which, on repeated crystallization from $CHCl_3$-MeOH, gave white needles (200 mg), mp 193-195°, $[\alpha]_D$ +88° ($CHCl_3$), and was identified as β-amyrin using ir, pmr, and ms. β-Amyrin (25 mg) was treated with acetic anhydride and pyridine (1 ml each) at room temperature and, on keeping overnight, it afforded β-amyrin acetate, mp 235-236°.

ISOLATION OF α-AMYRIN.—Petrol-C_6H_6 (1:3) eluate yielded a solid which, on repeated crystallization from MeOH, gave white needles (150 mg), mp 180-181°, and was identified as α-amyrin using ir, pmr, and ms. α-Amyrin (30 mg) was acetylated with acetic anhydride and pyridine (1 ml each) at room temperature into α-amyrin acetate, mp 220-222°.

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