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CHEMICAL CONSTITUENTS OF *VERNONIA CINEREA*. ISOLATION AND STRUCTURE ELUCIDATION OF A NEW PENTACYCLIC TRITERPENOID

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Vernonia cinerea Less (Compositae) has been reputed to have medicinal value (1,2). As a continuation of our earlier phytochemical studies (3-5), we have now isolated a new triterpenoid having the taraxerane skeleton along with campesterol and α-spinasterol.

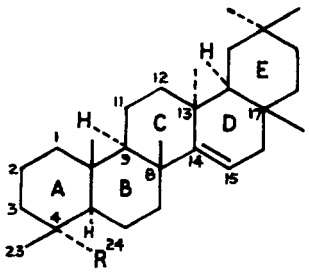
Positive tests with Liebermann-Burchard (6,7), Noller (8), and tetranitromethane (9) reagents indicated the new compound to be an unsaturated triterpenoid. Its ir spectrum demonstrated the presence of primary hydroxyl (10) (3350, 1050 cm^{-1}), *gem*-dimethyl (1380, 1370 cm^{-1}), and trisubstituted (10,11) unsaturation (1640, 840 cm^{-1}) functions. On acetylation it gave an acetate (**2**). The ir spectrum of the acetate, **2**, showed complete disappearance of the primary hydroxyl peaks (3350, 1050 cm^{-1}) and appearance of an ester peak at 1730 cm^{-1}. It also showed a broad band at 1160 cm^{-1} and weak bands at 1180 and 1140 cm^{-1}, instead of a strong peak at 1245 cm^{-1}, which suggests that the primary hydroxyl group is axial.

Further insight into the structure of **1** was gained by the study of the pmr spectrum, which showed singlets for seven tertiary methyl groups at δ 0.70 (3H, s), 0.80 (6H, s), 0.92 (6H, s), and 0.96 (6H, s). Signals were also exhibited for -CH_2OH (2H, m, centered at δ 3.10) and -CH_2OH (1H, m, centered at δ4.50). A well-split double doublet for an olefinic proton at δ 5.10 was observed, which is a characteristic of the taraxerane series (13). A perusal of the spectral data on hand suggested that the isolate had the taraxerane skeleton with one of the pendant axial methyls as -CH_2OH and a trisubstituted double bond.

The ms of **1** was characteristic of a pentacyclic triterpenoid having the Δ^{14}-taraxerene skeleton (14). The molecular ion peak appeared at *m/z* 426 and other principal fragment ions were observed at *m/z* 395 (M^+-CH_2OH), 302 (base peak), 287 (302-Me), 271 (302-CH_2OH), 256 (287-CH_2OH), 222, 219, 207, 204, 189 (204-Me), and 124. The characteristic mass fragments at *m/z* 302 and 124 were formed by retro-Diels-Alder decomposition of ring D of a taraxer-14-ene skeleton. The base peak, by the loss of a C_8-Me group, gave a fragment at *m/z* 287.

The formation of the above fragments confirmed the position of a trisubstituted double bond between C-14 and C-15 and also suggested that the hydroxyl group is present at any of the methyl groups in rings A, B, and C. Further, the spectrum showed an ion radical at *m/z* 204 derived from rings D and E. During its formation, the missing electron of the molecular ion is first removed from the carbon-carbon double bond, C-13 methyl group migrates to C-14, and finally the fissions of 11-12 and 8-14 bonds take place. Formation of the above ion radical and its counterpart at *m/z* 222 indicated that the primary hydroxyl group is located in either of the rings A or B and ruled out the possibility of its location in either rings D or E. Since no axial methyl group is present in ring B, the hydroxyl group as such could be assigned only to ring A. As taraxerene contains eight tertiary methyl groups and compound **1** contains an axial primary

hydroxyl group in ring A along with seven tertiary methyl groups, the hydroxyl group is ultimately assigned at C-24. In view of the above discussion, compound **1** was assigned as 24-hydroxytaraxer-14-ene.



1 R=CH_2OH
2 R=CH_2OAc
3 R=CH_2OTs
4 R=CH_3

This structural assignment of **1** was substantiated by formation of taraxer-14-ene itself, from the isolated compound **1**. Compound **1** on tosylation with *p*-toluene sulphonyl chloride gave a tosyl derivative **3**. Its ir spectrum showed intense bands due to the tosyl group (1603, 1500, 1186, 1170, and 1095 cm^{-1}) instead of bands characteristic of the hydroxyl group (3350, 1050 cm^{-1}). The tosyl derivative on selective reduction with $LiAlH_4$ in dry Et_2O yielded **4**, whose mp (15) and optical rotation values resemble closely those reported for taraxer-14-ene.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps are uncorrected. Pmr were measured in $CDCl_3$ at 90 MHz with TMS as internal standard. Mass spectra were recorded with a JEOL High resolution Mass Spectrometer JMS-D-300 with a data acquisition system. Silica gel was used for tlc and column chromatography. Spots on the tlc plates were detected by uv and iodine.

PLANT MATERIAL.—Plants of *V. cinerea* were collected from the campus of Goprakhpur University, Gorakhpur. A voucher specimen representing the collection is deposited in the herbarium of the Natural Products Research Laboratory, Department of Chemistry, University of Gorakhpur, Gorakhpur, India.

EXTRACTION AND SOLVENT FRACTIONATION.—The air-dried and powdered roots (10 kg) of *V. cinerea* were extracted with petroleum and then with EtOH. Evaporation of the alcoholic extract *in vacuo* afforded a solid that was partitioned betweren EtOH and C_6H_6. A solid residue (50 g) was obtained after processing the C_6H_6 soluble fraction.

CHROMATOGRAPHIC SEPARATION.—The C_6H_6 soluble residue (50 g) was chromatographed over a column packed with silica gel G (1.5 kg). In continuation of our earlier work (5), further elution was performed with the solvents of increasing polarity [C_6H_6 (3000 ml), C_6H_6-EtOAc (9:1, 1000 ml)] and monitored by intermittent co-tlc examinations of 200 ml eluates (20 fractions) emerging from the column.

ISOLATION OF CAMPESTEROL.—Fractions 1-5 of the C_6H_6 eluate on crystallization from MeOH gave white crystals (250 mg), mp 157-159°, which were identified as campesterol by optical rotation $[\alpha]^{23}D$ −35° ($CHCl_3$) and ir data (16). Campesterol (50 mg) on acetylation with Ac_2O and pyridine (2 ml each) gave campesterol acetate, which was identified by mp (137-140°) and ir (16).

ISOLATION OF α-SPINASTEROL.—Fractions 10-15 of the C_6H_6 eluate gave a crude solid which on recrystallization from Me_2CO afforded colorless crystals (200 mg), mp 172-173°, $[\alpha]^{17}D$ −3° ($CHCl_3$), which were identified as α-spinasterol using ir, pmr, ms (17) and positive responses towards color tests (6,7,9). Acetylation of α-spinasterol (50 mg) was carried out with Ac_2O and pyridine (2 ml each). The mixture on usual work-up afforded α-spinasterol acetate derivative as white needles from Me_2CO, mp 185-186° (17).

ISOLATION OF 24-HYDROXYTARAXER-14-ENE (**1**).—A solid was obtained from the C_6H_6-EtOAc (9:1) eluate, which on repeated crystallization from Me_2CO gave white needles of **1** (300 mg) identified as 24-hydroxytaraxer-14-ene, mp 264-265°, $[\alpha]^{25}D$ +5° ($CHCl_3$); ir ν max (KBr) 3350, 2900, 1640, 1440, 1380, 1370, 1240, 1050, 930, and 840 cm^{-1}; pmr ($CDCl_3$) δ 0.70 (3H, s), 0.80 (6H, s), 0.92 (6H, s), 0.96 (6H, s), 3.10 (2H, m, $-CH_2OH$), 4.50 (1H, m, $-CH_2OH$), and 5.10 (1H, dd, J=5 Hz); ms M^+ *m/z* 426 (33.8%) for $C_{30}H_{50}O$, 395 (20.3), 302 (100.0), 287 (30.4), 284 (15.2), 271 (35.5), 256 (40.2), 222 (50.5), 219 (25.0), 207 (55.0), 204 (62.5), 203 (42.5), 191 (45.2), 189 (67.5), and 124 (23.4). A mixture of **1** (45 mg) and Ac_2O and pyridine (2 ml each) was allowed to stand overnight at room temperature in a stoppered flask. The mixture on usual work-up afforded **2** as white needles from Me_2CO, mp 250-251°; ir ν max (KBr) 2900, 1730, 1645, 1480, 1380, 1370, 1280, 1180, 1160, 1140, 1040, 1000, 900, and 840 cm^{-1}.

REDUCTION OF **1** TO **4**.—A mixture of **1** (100 mg) in dry pyridine (20 ml) and freshly crystallized *p*-toluene sulphonyl chloride (100 mg) in dry pyridine (20 ml) was kept at room temperature for 80 h and then poured into crushed ice and taken up in Et_2O. After evaporating the solvent, a crude solid was obtained, which on recrystallization from Me_2CO gave the tosylate derivative **3** (85 mg), mp 245-246°; ir ν max (KBr) 2900, 1645, 1603, 1500, 1380, 1370, 1186, 1170, 1095, 900, and 840 cm^{-1}. Derivative **3** (60 mg) in dry Et_2O was added dropwise to a slurry of lithium aluminium hydride (25 mg in dry Et_2O) at 0° with stirring, which was continued for 30 min. The contents were then refluxed for 15 h. The excess of lithium aluminum hydride was decomposed cautiously by the addition of moist Et_2O and H_2O. The Et_2O layer was separated, washed with H_2O, and then crystallized with MeOH to yield white crystals (40 mg) of taraxer-14-ene, **4**, mp 235-236°, $[\alpha]^{25}D$ +1° ($CHCl_3$).

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LITERATURE CITED

1. K.R. Kirtikar and B.D. Basu, "Indian Medicinal Plants," II. Dehradun, India: New Connaught Place, 1975, p. 1322.
2. "The Wealth of India, Raw Materials," X. New Delhi: CSIR, 1976, p. 448.
3. T.N. Misra, R.S. Singh, J. Upadhyay, and R. Srivastava, *J. Nat. Prod.*, **47**, 368 (1984).
4. T.N. Misra, R.S. Singh, J. Upadhyay, and R. Srivastava, *Phytochemistry*, **73**, 415 (1984).
5. T.N. Misra, R.S. Singh, J. Upadhyay, and R. Srivastava, *Experientia*, (Communicated), 1983.
6. C. Liebermann, *Ber. Dtsch. Chem. Res.*, **18**, 1803 (1885).
7. Burchard, *Chem. Zeutr.*, **61**, 25 (1890).
8. C.R. Noller, R.A. Smith, G.H. Harris, and J.W. Walker, *J. Am. Chem. Soc.*, **64**, 3047 (1942).
9. R.D. Haworth, *Ann. Repts. Prog. Chem., Chem. Soc. London*, **34**, 328 (1937).
10. K. Nakanishi, "Infrared Absorption Spectroscopy," San Francisco: Holden-Day, Inc., 1962, p. 24, 31.
11. R.M. Silverstein and G.C. Bassler, "Spectrometric Identification of Organic Compounds," Stanford Research Institute. New York: John Wiley and Sons, Inc., 1967, p. 108.
12. P.S. Bory and M. Fetzon, *Bull. Soc. Chim. Fr.*, 570 (1964).
13. S.B. Katti and J.S. Tondon, *Indian J. Chem.*, **18**, 189 (1979).
14. H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 3688 (1963).
15. I. Heilbron and H.M. Bunbury, "Dictionary of Organic Compounds," V. London: Eyre and Spottiswoode, 1965, p. 2942.
16. I. Heilbron and H.M. Bunbury, "Dictionary of Organic Compounds," I. London: Eyre and Spottiswoode, 1953, p. 411.
17. I. Heilbron and H.M. Bunbury, "Dictionary of Organic Compounds," IV. London: Eyre and Spottiswoode, 1953, p. 369.

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