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Chemical Constituents of *Vernonia Cinerea* Less

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β-Amyrin acetate, lupeol acetate, β-amyrin, lupeol, β-sitosterol, stigmasterol, α-spinasterol, and potassium chloride have been isolated from the whole plant of *V. cinerea*.

Vernonia cinerea Less (*Compositae*) is an annual shrub growing wildly in India. The leaves, roots, and seeds of some species of *Vernonia* have been ascribed medicinal properties[1] and some have yielded physiologically active compounds. The anthelmintic principle[2] of the seeds of *V. anthelmintica* was shown to be a bitter acid and from the leaves of *V. altissima* three inositols were isolated[3]. A literature survey revealed no therapeutic application of the *cinerea* species nor any chemical study relative to isolation of its constituents.

A detailed chemical examination of the petroleum ether extract of the whole plant by the method of chromatographic resolution over Brockmann's alumina has now shown the occurrence in the plant of four triterpene compounds, viz., β-amyrin acetate, lupeol acetate, β-amyrin, and lupeol, and three sterols, viz., β-sitosterol, stigmasterol, and α-spinasterol. All the isolated constituents have been characterised by the preparation of suitable derivatives. The ethanol extract of the petroleum ether-exhausted plant material gave, besides a phenolic resin, a large amount of crystalline potassium chloride.

EXPERIMENTAL

All samples for analysis were dried at 110° *in vacuo* over P_2O_5 for 12 hrs. Petroleum ether refers to fraction of b.p. 60-80°. Optical rotations were determined in chloroform solution at room temperature.

Air-dried, powdered whole plant (2 kg.) of *V. cinerea* was soxhleted in turn with petroleum ether and 95% ethanol. The residue from the petroleum ether extract was adsorbed in benzene solution on a column of Brockmann's alumina (500 g.). The column was then eluted successively with petroleum ether (2 litres), petroleum ether-benzene (1:1, 500 ml), benzene (1 litre), and ether-benzene (1:4, 200 ml). The petroleum ether eluate, on removal of the solvent and crystallisation of the residue from chloroform-methanol, gave a colorless solid (5.5 g., Fraction I), m.p. 165-90°, which developed a pink colour in

*M.p.s. are uncorrected. Analyses were done by Drs. Weiler and Strauss, Oxford (U.K.).

1. Chopra, Nayar, and Chopra, "Glossary of Indian Medicinal Plants", p. 253, C. S. I. R., New 1956.
2. Chopra *et al.*, *Indian J. Med. Res.*, 1934, 22, 183.
3. Rowe *et al.*, *J. Amer. Pharm. Assoc., Sci. Ed.*, 1955, 44, 308.

the Liebermann-Burchard test. The residue from the petroleum ether-benzene eluate crystallised from methanol, yielding a crystalline substance (0.3 g., Fraction II), m.p. 158-62° (pink colour in the Liebermann-Burchard test). The benzene and ether-benzene eluates left residues crystallising from methanol as colorless solids of m.p. 137-52° and 145-54° respectively, both of which developed in the Liebermann-Burchard reaction a transient pink colour, changing rapidly to blue, and finally green and were therefore combined (0.5 g., Fraction III).

Fraction I was taken up in petroleum ether (50 ml) and rechromatographed over alumina (300 g.) using benzene-petroleum ether (1:19) as eluent, collecting 50 ml portions. The first eight fractions yielded solids, m.p. 187-226°, which on two crystallisations from ether-ethanol melted at 234-36° (Fraction I_A). The next five fractions left solids, m.p. 164-225°, which on further crystallisations gave a product identical with Fraction I_A. From the subsequent forty fractions solids, m.p. 180-200°, were obtained which showed no improvement in m.p. on fractional crystallisations from mixed solvents. On rechromatography, however, using a long column and crystallisation from chloroform-methanol, a colorless solid, m. p. 204-206° (Fraction I_B), was obtained from it. The last ten fractions gave traces of solids, m.p. 195-232°, which on repeated crystallisation from benzene-ethanol and ethyl acetate furnished colorless shining flakes (Fraction I_C) with an extended m. p. 225-46°. Fraction I_C was found to be insufficient for further investigation.

β-Amyrin Acetate.—Fraction I_A after several crystallisations from ether-ethanol was obtained as colorless needles, m. p. 236-37°; $[\alpha]^{26}_D$+83.1° (*c*, 1.16). (Found: C, 81.86; H, 11.4. Calc. for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11%).

Hydrolysis of the above product with ethanolic KOH (5%) on the steam bath for 3 hrs. gave *β-amyrin*, crystallising from ethanol as needles; m.p. and mixed m.p. with an authentic sample, 196-97°; $[\alpha]^{31}_D$+89.4° (*c*, 0.78). (Found: C, 84.22; H, 11.96. Calc. for $C_{30}H_{50}O$: C, 84.50; H, 11.73%).

It formed a *benzoate* on heating with benzoyl chloride and pyridine at 100° for 2 hrs., which crystallised from acetone as shining flakes, m.p. 231-32°; $[\alpha]^{27}_D$+97.9° (*c*, 0.73). (Found: C, 83.58; H, 10.26. Calc. for $C_{37}H_{54}O_2$: C, 83.77; H, 10.19%). Thus Fraction I_A is shown to be β-amyrin acetate.

Lupeol Acetate.—Fraction I_B after several crystallisations from ether-ethanol yielded lupeol acetate; m. p. and mixed m.p. with an authentic sample, 215-16°; $[\alpha]^{30}_D$ +46.2° (*c*, 1.45). (Found: C, 81.78; H, 11.32. Calc. for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11%).

The above product on hydrolysis furnished microcrystalline needles from aqueous ethanol, m.p. 212-13°; undepressed in m. p. with an authentic sample of lupeol; $[\alpha]^{30}_D$ +26.5° (*c*, 1.05). (Found: C, 84.34; H, 11.89. Calc. for $C_{30}H_{50}O$: C, 84.50 ; H, 11.73%).

It formed a benzoate crystallising from benzene-ethanol as flakes; m.p. and mixed m.p. with an authentic sample of lupeol benzoate, 259-61°; $[\alpha]^{31}_D$+60.05° (*c*, 1.04). (Found: C, 83.68; H, 10.45. Calc. for $C_{37}H_{54}O_2$: C, 83.77; H, 10.19%).

Fraction II was directly benzoylated and the benzoate in petroleum ether (10 ml) was chromatographed over alumina (50 g.). Elution with benzene-petroleum ether (1:19)

gave in the earlier fractions solids, m.p. 210-23° (Fraction II_A) and the latter fractions melted at 246-55° (Fraction II_B), the intermediate fractions being mixtures of the two.

β-Amyrin.—Fraction II_A on repeated crystallisation from chloroform-methanol yielded colorless flakes of β-amyrin benzoate, m.p. 230-31° (undepressed mixed m.p. and same optical rotation). This on hydrolysis gave β-amyrin. Thus Fraction II contains β-amyrin in the free state.

Lupeol.—Fraction II_B after several crystallisations from chloroform-methanol was obtained in shining flakes, m.p. 259-62°, identical with lupeol benzoate. Hydrolysis of the benzoate regenerated lupeol. Thus lupeol also is present in Fraction II in the free state.

Fraction III was converted into the benzoate which was dissolved in petroleum ether (20 ml) and chromatographed over alumina (60 g.), using benzene-petroleum ether (1:19) as eluent. From the earlier fractions, solids, m.p. 137-40° (Fraction IIIA), were obtained, but the subsequent fractions yielded solids, m.p. 142-57° (Fraction III_B). The strongly adsorbed benzoate, which could only be eluted with benzene-petroleum ether (1:9), melted at 170-95° (Fraction III_C).

β-Sitosterol.—Fraction III_A was crystallised from acetone when β-sitosteryl benzoate was obtained in colorless flakes; m.p. and mixed m. p. with an authentic specimen, 146-47°; $[\alpha]^{29}_D$ −13.5° (*c*, 0.85). (Found: C, 83.35; H, 10.28. Calc. for $C_{36}H_{54}O_2$: C, 83.39; H, 10.42%).

Hydrolysis of the benzoate furnished β-sitosterol as shining leaflets; m.p. and mixed m.p. 136-37°; $[\alpha]^{31}_D$ −35.2° (*c*, 0.55). (Found: C, 83.63; H, 11.93. Calc. for $C_{29}H_{50}O$: C, 83.05; H, 12.07%).

The sterol formed an acetate crystallising from methanol as plates, m.p. 126-27°, $[\alpha]^{31}_D$ −39.5° (*c*, 0.42), identical with β-sitosteryl acetate.

Stigmasterol.—Further crystallisations of Fraction III_B from benzene-acetone yielded stigmasteryl benzoate as flakes; m.p. and mixed m.p. with an authentic sample, 160-61°; $[\alpha]^{32}_D$ −28.5° (*c*, 1.2). (Found: C, 83.69; H, 10.02. Calc. for $C_{36}H_{52}O_2$: C, 83.72; H, 10.07%).

The benzoate on hydrolysis gave stigmasterol, crystallising from ethanol as plates, m.p. and mixed m.p. 168-61°; $[\alpha]^{31}_D$ −50.4° (*c*, 0.85). (Found: C, 84.16; H, 12.03. Calc. for $C_{29}H_{48}O$: C, 84.46; H, 11.65%).

It furnished an acetate, crystallising from ethanol as flakes, m.p. 143-44°; $[\alpha]^{32}_D$ −54.8° (*c*, 0.67). It is identical with stigmasteryl acetate.

α-Spinasterol.—Fraction III_C on repeated crystallisation from benzene-acetone yielded α-spinasteryl benzoate as shining flakes; m.p. and mixed m.p. with an authentic sample, 199-200°; $[\alpha]^{29}_D$ +2.5° (*c*, 0.75). (Found: C, 83.56; H, 10.13. Calc. for $C_{36}H_{52}O_2$: C, 83.72; H, 10.07%).

On hydrolysis of the benzoate, α-spinasterol was obtained as plates from chloroform-methanol, m.p. 169-70°; $[\alpha]^{32}_D$ -4.2° (*c*, 0.62). (Found: C, 84.28; H, 11.73. Calc. for $C_{29}H_{48}O$: C, 84.46; H, 11.65%).

Acetylation of the sterol yielded ∝-spinasteryl acetate crystallising from chloroform-methanol as flakes; m.p. and mixed m.p. with an authentic sample, 185-86°; $[\alpha]^{31}_{D}$ +5.5° (*c*, 0.45). (Found: C, 81.61; H, 11.30. Calc. for $C_{31}H_{50}O_2$: C, 81.93; H, 11.01%).

Thus Fraction III contains the three sterols in the free state.

The ethanol extract of the plant after distillation of the solvent *in vacuo* left a dark green residue which was washed well with ether. The ether washings gave negative tests for alkaloids and on extraction with 2% NaOH yielded a phenolic resin which could be neither induced to crystallise nor converted into a crystalline derivative. The ether-insoluble material on trituration with ethanol left a solid residue from which potassium chloride was obtained after crystallisation from water, using Norit for decolorisation. No further crystalline constituent could be located in the ethanol mother liquors either before or after acid hydrolysis.

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