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STUDIES ON NEPETA RUDERALIS HAMILT. EXAMINATION OF THE PETROLEUM ETHER EXTRACTIVE OF THE FLOWERS AND STEMS

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Nepetol, a new triterpene, m.p. 180-181°C, hentriacontane and β -sitosterol have been isolated from the flowers and stems of *Nepeta ruderalis* Hamilt.

Introduction

Nepeta ruderalis Hamilt, syn, *Nepeta hindostana* (Roth) (Haines) (local name, Badranjboya) N.O. Labiatae, is found in the hilly parts of Pakistan, both in the East and West wings and also in similar regions of India. It is used in the indigenous systems of medicine for fevers and as a cardiac tonic.¹

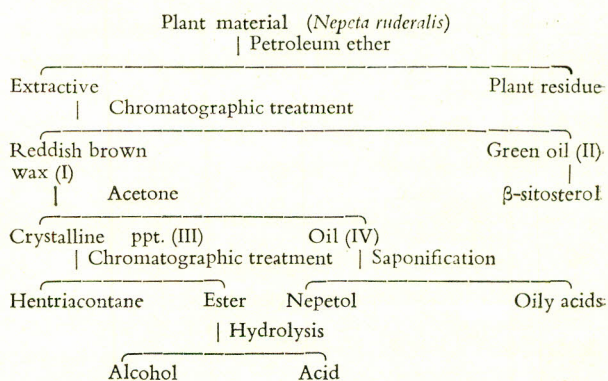
As reference to literature showed that no chemical examination had been carried out it was considered of interest to undertake a study of its constituents. During the course of our investigations a report of the isolation of oleanolic acid was made² but no product of medicinal interest was reported. The present paper deals with the petroleum ether extractive obtained from mixed flowers and stems. Through chromatography of this extractive on an alumina column it could be divided into two main fractions: (I) brown, waxy and (II) green, oily (see chart). The procedure employed for the further handling of these two fractions is shown in the following chart. On treating the wax (I) with acetone a precipitate (III) was obtained which could not be separated into its constituents by crystallization and was therefore chromatographed on alumina to yield a hydrocarbon, hentriacontane and an ester. Hentriacontane has been identified, *inter alia*, by its refractive index above its melting point. The ester has been examined only to the extent of its confirmation of being a wax.

The acetone containing mother liquor, on removal of the solvent, gave an oil (IV), which was saponified. The unsaponifiable matter was

crystallized from acetone to give a new compound $C_{30}H_{50}O$, m.p. 180-181°C, $[\alpha]_D^{20} +92$ (in chloroform). The purity of the analytical sample was checked by several experiments in thin layer chromatography. Two derivatives, the acetate, m.p. 230°C, and the benzoate, m.p. 230°C, were prepared and also checked for uniformity by thin layer chromatography. It has been provisionally named 'nepetol'. It may either be a triterpene or a sterol. However, its ultraviolet spectrum gives a peak at 278 $m\mu$ with a $\log \epsilon$ of 1.88, which corresponds to that of certain triterpenes (e.g. cf. spectrum of escigenin, λ_{max} 275, ($\log \epsilon$ 1.58)³. The same can be said about its infrared spectrum.⁴

The oil (II) on the addition of acetone gave crystals which were identified as β -sitosterol through its acetyl and benzoyl derivatives.

CHART



Experimental

All the melting points are uncorrected. The microanalyses have been carried out by Messrs. Alfred Bernhardt, Muelheim, West Germany.

Nepeta ruderalis flowers and stems (930 g) were extracted exhaustively by steeping in petroleum ether (b.p. 60-80°C) at room temperature. The combined extracts were freed of the solvent on the water bath *in vacuo* below 50°C when a dark green waxy residue (18.4 g) was obtained.

A portion of this residue (15.4 g) was dissolved in benzene (40 ml) and chromatographed on a column (2.25 × 53 cm) of alumina Brockmann (160 g). The following main fractions were obtained at first (I), benzene (240 ml), a reddish brown wax (4.6 g) (II), 98:2 benzene-ethanol (400 ml), a dark green oily substance (2.6 g). On subsequent elution with increasing amounts of ethanol in benzene, different substances were obtained, but each of these was too small in quantity to merit any further examination.

TREATMENT OF FRACTION I

10 g of the substance was treated with acetone (1 l.); this immediately produced a white flocculent precipitate. After cooling in the ice-chest the precipitate was centrifuged out and washed twice with acetone (1.51 g). The decanted golden-yellow solution and the washings of the precipitate were combined and concentrated *in vacuo* to 500 ml. A second crop of the precipitate (483 mg) was obtained, which was separated in the same way. A third crop (152 mg) was similarly obtained on concentration to 250 ml. Further treatment did not yield any more material.

The precipitate, m.p. 64-68°C (2.15 g), was dissolved in 15 ml of petroleum ether (b.p. 60-80°C), and passed through a column (2.5 × 85 cm) of silica gel (107 g). The following fractions were eluted:

- (a) petroleum ether (b.p. 60-80°C), (800 ml), hydrocarbon (1.38 g).
- (b) petroleum ether (b.p. 60-80°C), (300 ml), ester (179 mg).

Further elution with a mixture of 95:5 petroleum ether-chloroform gave a colourless greasy substance, the infrared spectrum of which indicated that it was mainly a fat. It was not examined further.

HENTRIACONTANE

The hydrocarbon, (1.38 g), was recrystallized thrice from benzene-alcohol to yield colourless leaflets, m.p. 69°C (Found: C, 85.53; H, 14.30%; mol. wt. (osmometric) 433, 433. Calc. for C₃₁H₆₄: C, 85.23; H, 14.77%; mol. wt. 436.82). The substance was readily soluble in chloroform and benzene, insoluble in acetone, ethanol and methanol. Its infrared spectrum was similar to that of nonacosane and dotriacontane.⁵ It had n_D^{20} 1.4359 (Lit.⁶ n_D^{20} 1.4354). It showed no optical rotation.

INVESTIGATION OF THE ESTER

The ester, m.p. 75-76°C, (179 mg.) was crystallized twice from benzene-ethanol, m.p. 76°C. (Found: C, 81.97; H, 13.66; O, 4.63%; mol. wt. 678).

Its infrared spectrum exhibited a strong peak at 1750 cm⁻¹ indicating that it was an ester, other peaks at 2945, 2880, 1475, 1450, 1195, 1180, 960, 925, 732, 722 cm⁻¹ suggesting that it was a wax.

It was saponified by refluxing with alcoholic potassium hydroxide (7.5%) on a water bath for 17 hours. The unsaponifiable matter was obtained in the usual way and on recrystallization from benzene-ethanol gave the *alcohol*, m.p. 82°C, ν_{\max} 3450 cm⁻¹ (OH).

The acid portion was treated with hydrochloric acid till acidic to Congo red paper and extracted with ether. The extract was washed with water, dried and freed of the solvent. The acid was recrystallized from ethyl acetate, m.p. 88°C, ν_{\max} 1705 cm⁻¹ (COOH).

ISOLATION OF NEPETOL

100 ml of N/2 alcoholic potassium hydroxide were added to 6 g of the oil (IV; see Chart) obtained on the removal of the solvent from the acetone-containing mother liquor and the solution refluxed on the water bath for 9 hours. The solvent was then removed *in vacuo* till only 30 ml remained. Water (200 ml) was added and the mixture extracted with ether four times. The organic layer was separated, washed with water, dried over calcium sulphate and the solvent removed *in vacuo* to give an oily residue (3.3 g). This oil was dissolved in acetone (20 ml) and kept in the refrigerator. After one week colourless needle-shaped crystals appeared, which were sucked off and washed with acetone (3 ml), m.p.

178.5°C (199 mg.). Two further crops m.p. 178°C and 167-169°C (total 232 mg) were similarly obtained after standing for long periods in the ice-chest.

The above crops of crystals were combined in (431 mg) and recrystallized from methanol (50 ml) to yield fine needles (250 mg) m.p. 180-181°C $[\alpha]_D^{25} +92$ (chloroform). (Found: C, 84.29; H, 12.06; O, 3.91%; mol. wt. 423. $C_{30}H_{50}O$ requires C, 84.44; H, 11.81; O, 3.75%; mol. wt. 426.7).

The homogeneity of the product was checked by thin layer chromatography on silica gel G, using three different developing solvents (a) hexane-ether (9:1), (b) chloroform-ethyl acetate (80:20) (c) benzene-ethyl acetate (80:20). In the first case the spot was detected on spraying with H_2SO_4 (50%) and heating in the oven at 100°C, and in the second and third iodine vapour was used for the purpose. The $R_f \times 100$ value in the latter two cases was found to be 56 and 55 respectively. A similar chromatography in the reversed phase, with 10% dodecane as the stationary phase, was done, the plate being developed with chloroform, methanol, water (5:15:1) saturated with dodecane and the spot detected with iodine vapour. In each of the above four chromatograms a single spot was obtained.

The substance was readily soluble in the cold in chloroform, benzene, ether and ethyl acetate, moderately soluble in petroleum ether and acetone, sparingly soluble in ethanol and insoluble in methanol. It gave a positive Liebermann-Burchard reaction and the reddish-violet coloration

persisted for hours. In the Salkowski test it gave a violet coloration in both layers. The substance was dissolved in a mixture of glacial acetic acid, zinc chloride, and acetyl chloride, and boiled when a red colour appeared.⁷ It gave a negative digitonin test. Its infrared spectrum (KBr disc) indicated the presence of hydroxyl group or groups and furnished peaks at 3310, 2950, 2355, 1648, 1460, 1395, 1365, 1315, 1200, 1142, 1100, 1039, 1002, 955, 815 and 750 cm^{-1} (Fig. 1). Its ultraviolet spectrum (n-hexane) gave a peak at 278 $m\mu$, $\log \epsilon$ 1.88.

NEPETYL ACETATE

The substance (50 mg), acetic anhydride (2 ml), and dry pyridine (2 ml) were refluxed for three hours. On cooling, the mixture was poured into 100 ml of ice-cold 2N hydrochloric acid solution, when the impure acetate was obtained as a granular white precipitate. However, after exhaustively extracting with ether, the organic layer was washed with 2N sodium carbonate solution, then with water and dried over calcium sulphate. On the removal of the solvent a crystalline residue was obtained (52 mg), m.p. 181-190°C. Recrystallization from benzene-ethanol mixture gave colourless long rod-shaped crystals, m.p. 230°C. The substance sublimed at its melting point. (Found: C, 82.42; H, 11.29%. $C_{32}H_{52}O_2$ required C, 81.99; H, 11.18%).

The purity of the acetate was checked by thin-layer chromatography on silica gel G, the developing agent being chloroform-ethyl acetate

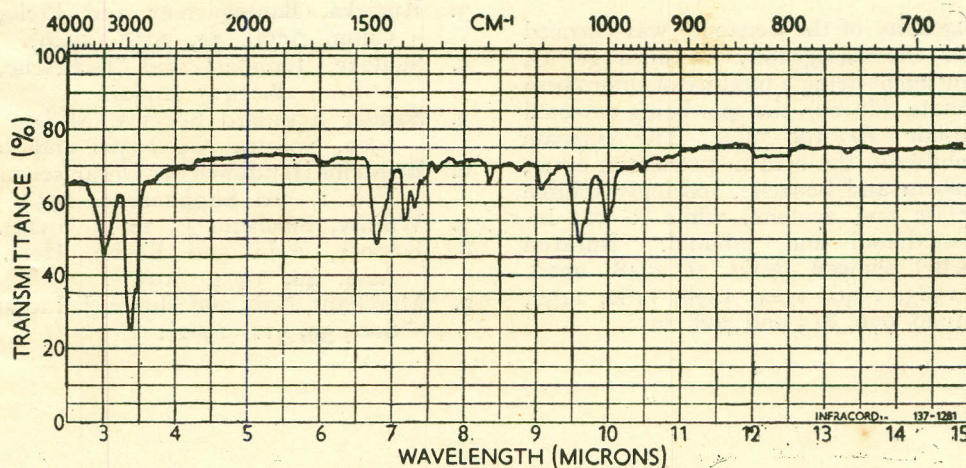


Fig. 1.—I R spectrum of nepetal.

(80:20) mixture. On exposure of the chromatogram to iodine vapour a single spot was obtained. ($R_f \times 100 = 75$).

In the cold the acetate was found readily soluble in benzene, ether, chloroform, and petroleum ether, moderately so in acetone and ethyl acetate and sparingly in ethanol and methanol. The infrared spectrum (KBr) exhibited peaks at 2957, 2880, 1737, 1458, 1362, 1245, 1149, 1095, 1022, 1000, 966, 958, 901 and 810 cm^{-1} .

NEPETHYL BENZOATE

The substance (95 mg) was dissolved in a mixture of dry benzene (10 ml) and dry pyridine (5 ml). To the well-cooled homogeneous mixture benzoyl chloride (4 ml) was added. It was refluxed on the water bath for 3 hours and then on the oil bath for 6 hours at a bath temperature of $130\text{--}140^\circ\text{C}$. The mixture which had turned somewhat red in colour was poured into ice-cold 2N hydrochloric acid solution, and exhaustively extracted with diethyl ether. The ether layer was washed with 2N sodium carbonate solution, then with water and dried over calcium sulphate. On the removal of the solvent a reddish-brown oil was obtained. This oil was poured on to a column (11 cm. \times 1.5 cm dia) containing 25 g of alumina Brockmann (standard). On elution with petroleum ether (b.p. $60\text{--}80^\circ\text{C}$) the first two fractions (4 ml each) gave a crystalline substance impregnated with oil. The residue from these two fractions were mixed and dissolved in acetone. On cooling overnight in the ice-chest colourless crystals appeared, m.p. 222°C . Recrystallization from acetone gave colourless rectangular plates, m.p. 230°C . (Found: C, 84.06; H, 10.13%. $\text{C}_{37}\text{H}_{54}\text{O}_2$ requires C 83.72, H 10.25%).

The homogeneity of the benzoate was checked by thin layer chromatography on silica gel G, the developing agent being a mixture of chloroform and ethyl acetate (80:20), the spot being detected by iodine vapour ($R_f \times 100 = 75$). The benzoate was found soluble in the cold in petroleum ether, ether, chloroform and benzene, sparingly soluble in ethyl acetate and acetone, while it was insoluble in methanol and ethanol. Infrared spectrum (KBr) showed peaks at 2948, 2920, 1718, 1451, 1383, 1346, 1314, 1278, 1180, 1152, 1110, 1066, 1029, 970, 815 and 708 cm^{-1} .

β -SITOSTEROL

The dark green oily substance (II) was dissolved in acetone (10 ml) and kept in the refrigerator overnight. Colourless crystals appeared which were sucked off, washed with acetone, and dried. The yield was 30 mg m.p. $137\text{--}138^\circ\text{C}$. On recrystallization from ethanol, plates were obtained (20 mg) $143\text{--}144^\circ\text{C}$. (lit.⁸ 142°C). The substance gave positive Liebermann-Burchard and Salkowski tests.

The acetate was prepared with the sterol (4.5 mg), dry pyridine, and acetic anhydride. The crude acetate (5 mg) was recrystallized from ethanol, m.p. 125°C (lit.⁸ 127°C).

The benzoate was made with the sterol (8 mg), benzoyl chloride and dry pyridine and crystallized from acetone, m.p. 143°C (lit.⁹ $144\text{--}145^\circ\text{C}$).

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