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A phytochemical investigation of *Trigonella corniculata* Linn.

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The extraction of ground seeds of *Trigonella corniculata* with light petroleum has yielded triacontane and 22,23-dihydrostigmasterol. Further extraction of the seeds with ethanol yielded choline and betaine. Trigonelline, the major alkaloid of *Trigonella foenum-graecum* was not found.

THE seeds and leaves of *Trigonella corniculata* Linn. commonly known in Punjab and Kashmir as "Kasuri Methi" are an important article of commerce. The bitter fruit is astringent and styptic. It is applied to swellings and bruises (Kirtikar & Basu, 1933; Chopra, Nayar & Handa, 1956). The seeds are hot and dry and are used as a household remedy for backaches and in diseases of puerperal women. The plant is cultivated extensively in many parts of India, particularly in Northern India and is used as a green vegetable. The seeds and leaves have a characteristic pleasant odour and are used as flavouring agents and spice.

Experimental

The powdered drug (2·5 kg) was extracted with light petroleum (b.p. 60–80°) in a soxhlet apparatus for 60–70 hr. Removal of the solvent furnished a dark green lipid residue (147·5 g). 50 g of this was saponified (2 hr) with 0·5N ethanolic potassium hydroxide (500 ml) on a water-bath. The yellowish orange unsaponifiable matter (6·4 g) was extracted with cold light petroleum to remove the carotenoid material. The residual amorphous mass (4 g) was applied to a column of alumina (125 g, activity grade I) in light petroleum (b.p. 60–80°) (100 ml). Elution proceeded as follows: (i) Light petroleum (b.p. 60–80°) (250 ml). (ii) Benzene (500 ml). (iii) Benzene: ether (95:5 progressively increasing to 50:50) (300 ml). (iv) Ether (550 ml). (v) Ether: absolute ethanol (80:20) (150 ml).

Fraction (i) yielded triacontane m.p. 64–65°, crystallised twice from acetone (Heilbron & Banbury 1953 report m.p. 66°).

Fraction (iv) crystallised twice from ethanol gave 2·04 g of colourless crystals m.p. 134°. Chromatography of this on alumina (50 g) yielded 22,23-dihydrostigmasterol m.p. 137° $[\alpha]_D$ 36·60° ($CHCl_3$) giving no depression on admixture with an authentic specimen. Its identity was further confirmed by preparing its acetate m.p. 126–127° and benzoate m.p. 145–146°. (Merck Index, 1952, reports 22,23-dihydrostigmasterol acetate m.p. 127–128°, benzoate m.p. 146–147° and $[\alpha]^{25}_D$–37·0 ($CHCl_3$).

EXTRACTION OF ALKALOIDS

The powdered drug (2·5 kg) previously exhausted with light petroleum, ether and chloroform was extracted further with ethanol (95% v/v) in a

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soxhlet apparatus for 140 hr. There resulted 250 g of reddish-brown semi-solid mass which on paper chromatography showed the presence of two alkaloids.

The residue was treated with cold ethanol (95% v/v) (500 ml) and the solution filtered from insoluble gummy matter. The ethanolic solution was then treated with a concentrated solution of lead acetate until no further precipitation occurred. The precipitate was filtered off and filtrate and washings were freed from lead salts with hydrogen sulphide. The residual solution was concentrated under reduced pressure at 60° to 200 ml.

The above concentrated solution (200 ml) was acidified to congo red with 3% sulphuric acid. Overnight at 0° a gelatinous precipitate was deposited: this was rejected. To the clear pale solution, a 4% aqueous solution of ammonium reineckate was slowly added with constant stirring, until a sample of the supernatant solution no longer gave any turbidity. A 10% excess was then added to ensure completeness of precipitation. The rose-coloured precipitate was washed with water, sucked dry and finally dried in a desiccator to a pink chalky powder (21 g). This was decomposed by the method of Kapfhammer as modified by Wieland (Dutcher, 1946), and the syrupy solution of base hydrochlorides thus obtained, dried in a vacuum desiccator (6·2 g).

PURIFICATION OF CRUDE ALKALOIDAL MIXTURE

Part of this mixture was soluble in ethanol (Fraction A), part was apparently insoluble (Fraction B). Paper chromatography indicated that Fraction A was a mixture of two alkaloids while Fraction B was a single alkaloid.

ISOLATION AND CHARACTERISATION OF ALKALOID A

Fraction A was purified further by column chromatography over Merck alumina (50 g, activity grade I) in absolute ethanol. Elution proceeded as follows: (i) Absolute ethanol (120 ml). (ii) Absolute ethanol: methanol (120 ml). (iii) Methanol (180 ml). (iv) Distilled water (60 ml).

Fraction (i) was light yellow in colour. Because of the hygroscopic nature of the residue, the melting-point could not be determined. The residue was redissolved in absolute ethanol and decolourised with charcoal. The residue from the colourless filtrate, disolved in a small quantity of absolute ethanol, on addition of dry ether deposited colourless needle-shaped crystals m.p. 288–290°.

Found for Alkaloid 'A': C, 42·4; H, 10·5; Cl, 24·2; N, 10·5. Calculated for $C_5H_{14}ClNO$(choline chloride): C, 43·0; H, 10·2; Cl, 25·4; N, 10·0%. Alkaloid A gave an Rf value of 0·41 on a paper buffered to pH 7·4 using n-butanol: hydrochloric acid: water (5:2:1, upper phase) as the solvent system. Mixed application of alkaloid A and authentic choline chloride gave only a single spot on paper chromatograms. Using n-butanol; acetic acid: water (4:1:5, upper phase) and unbuffered papers, alkaloid A gave Rf value 0·67 and authentic choline chloride 0·68.

Picrate, picrolonate and reineckate of alkaloid A prepared by the usual methods gave m.p. 239–240°, 178–179° and 250–252° respectively. It appears that the melting-points of picrate and picrolonate of choline are not recorded in literature, these salts, prepared from authentic choline chloride gave melting-points identical with those of the isolated sample.

ISOLATION AND CHARACTERISATION OF ALKALOID B

Fraction (iii) obtained from column chromatography of Fraction A. with methanol as eluant, yielded almost colourless residues m.p. 224–225°, This was combined with the ethanol-insoluble Fraction B (m.p. 226°). The combined alkaloid, on recrystallisation from methanol gave colourless, shining crystals m.p. 227° (Heilbron & Banbury, 1953 report m.p. 227–228°).

Found for Alkaloid B: C, 39·2; H, 7·7; Cl, 22·9; N, 9·1%. Calculated for $C_5H_{11}NO_2$.HCl(betaine hydrochloride) C, 39·1; H, 7·8; Cl, 23·1; N, 9·1%.

The base betaine was prepared from the hydrochloride by rubbing it with freshly prepared moist silver oxide, taking up the liberated base with methanol (Merck) and repeatedly crystallizing it from absolute ethanol, when colourless crystals were obtained, m.p. 264–265° with decomposition (Mulliken, 1916 reported, for betaine 273° ± 3°, with decomposition).

The Rf value of alkaloid B using the solvent system butanol: hydrochloric acid: water (5:2:1, upper phase), and *n*-butanol; acetic acid; water (25:1:6, upper phase) was 0·26 and 0·14 respectively. These values correspond with those obtained for authentic betaine hydrochloride.

Picrate, picrolonate and reineckate of alkaloid B prepared by usual methods gave m.p. 181–182°, 200°, and 148–150° (with decomposition) respectively. These melting-points are in close agreement with the values reported for these derivatives.

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