PHYTOCHEMICAL STUDIES ON THE GENUS *CROTALARIA*

PART I. A PHYTOCHEMICAL INVESTIGATION OF THE SEEDS OF *Crotalaria sericea* RETZ

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By chromatography of an unsaponifiable fraction of a petroleum extract, a saturated hydrocarbon triacontane, 22,23-dihydrostigmasterol and α-carotene were isolated. The identity of triacontane was confirmed by its melting point and elemental analysis. 22,23-Dihydrostigmasterol was identified by its melting point, mixed melting point, melting points of its acetate and benzoate and by its specific rotation in chloroform. α-Carotene was identified by its absorption maxima at 460 and 480 mμ. From the ethanolic extracts a mixture of alkaloids was separated which on chromatography over alumina gave a crystalline alkaloid, monocrotaline, $C_{16}H_{23}NO_6$, m.p. 196–197° and a new crystalline base m.p. 180–181°, named sericine which has not been completely identified. It has the tentative empiric formula $C_{17}H_{24}NO_7$ and may be a new alkaloid. Presence of two more alkaloids was shown by paper chromatography.

Crotalaria sericea Retz. (Syn: *C. spectabilis*) of the family *Leguminosae* has been used in the indigenous system of medicine since very early times. This plant as well as other species of *crotalaria*, which are found all over the world, are toxic to livestock and have evoked much interest in the scientific field. Some of the toxic effects produced (Chopra, Badhwar and Gosh, 1949) are termed "crotalism" and "Missourie bottom" disease. The animals suffering from "crotalism" show inflammation and outgrowth of hoofs, and a general decline of bodily vigour, culminating in stupor, coma and death. A large number of animals are reported to die annually by consuming these plants.

Neal, Rusoff and Ahmann (1935) isolated an alkaloid, monocrotaline, from the Florida grown seeds of *C. spectabilis* and showed that this alkaloid had hypotensive properties. It lowers the blood pressure in dogs and decreases the rate and amplitude of terrapin heart. These authors isolated monocrotaline by extraction of the seeds with aqueous ammonia and gave it the tentative formula $C_{16}H_{23}NO_6$ by careful analysis of monocrotaline, its hydrochloride and methiodide. Structure and synthesis of monocrotaline and monocrotalic acid have also been established.

A study of literature revealed that no alkaloid other than monocrotaline had been isolated from *C. sericea*. As work on the non alkaloidal constituents of this plant has never been reported, a detailed phytochemical investigation was undertaken.

EXPERIMENTAL

The material used in this study was an authentic sample consisting of the seeds of *Crotalaria sericea* collected from Panjab University campus in

December, 1959. The dried seeds were coarsely powdered in a flour mill. By using suitable sieves the hard testa and horny endosperm could be effectively separated from the coarsely powdered embryo. The coarse powder representing the embryos was used in all subsequent studies.

Preliminary Chemical Examination

To obtain a general idea about the type of constituents of the seeds, tests for the presence of alkaloids, glycosides, tannins, phenols, steroids, flavonoids, reducing sugars, gums, resins and proteinous matter were made. Alkaloids, sterols, gums and proteins were detected. It was further observed by histochemical tests that the alkaloids were present chiefly in the embryo whereas the gums were present exclusively in the endosperm.

Preparation of Unsaponifiable Fraction

The powdered seeds (2·5 kg.) were extracted with light petroleum (b.p. 60–80°) in a soxhlet apparatus for 18 hr. After the removal of the solvent a yellowish brown oily substance (100 g.) was obtained. This, (50 g.) was processed for the preparation of unsaponifiable matter according to the B.P. (1958) method. The unsaponifiable fraction was obtained as a yellowish orange amorphous mass (7·6 g.) which gave positive tests for phytosterols and carotenoids. It was further fractionated by treating with cold light petroleum. In this way an orange coloured fraction, (2·6 g.), freely soluble in cold light petroleum and a yellowish white solid fraction (5 g.) sparingly soluble in cold light petroleum, were separated.

Isolation of Triacontane and 22, 23-Dihydrostigmasterol

The yellowish white solid residue (5 g.) obtained from the unsaponifiable matter was dissolved in light petroleum (b.p. 60–80°) (100 ml.). The solution, after cooling, was passed through a column containing Merck chromatographic alumina (125 g.). The column was eluted with 32 fractions each of 50 ml.; those numbered 1 and 2 with light petroleum; those 3 to 8 with benzene; those 9 to 10 with benzene: ethyl ether (19 : 1); those 11 to 13 with benzene: ethyl ether (4 : 1); those 14 to 29 with ethyl ether; and those numbered 30 to 32 with ethanol.

Triacontane. The product obtained from fractions 1 and 2 was white, waxy, and had a m.p. 63°. After two crystallizations from acetone and drying in a desiccator, colourless needles m.p 64–65° were obtained. The elemental analysis was found to be C, 84·42: H, 14·08 per cent, (calculated for $C_{30}H_{62}$, C, 85·21 : H, 14·69 per cent). Triacontane, $C_{30}H_{62}$ is reported to have m.p. 66°. No authentic sample was available.

22,23-*Dihydrostigmasterol.* The residues obtained from fractions 14–29 gave positive tests for sterols and had m.p. 134–137°. The combined residue on crystallisation from ethanol gave plate like crystals (4·2 g.) m.p. 136–137° (Shriner, Fuson and Curtin, 1956, reported 22,23-dihydrostigmasterol to have m.p. 137°). Its melting point with an authentic sample of 22,23-dihydrostigmasterol (Atal and Lamba, 1960) was undepressed. Its identity was further confirmed by determining its specific

rotation in chloroform (found −36·65°: reported, Merk Index, 1952, −37°). Yield 0·17 per cent of the air dried seeds.

22,23-*Dihydrostigmasterol acetate.* 22,23-*Dihydrostigmasterol* (50 mg.) was treated with acetic anhydride (1 ml.) and pyridine (1·5 ml.) The solution was refluxed in a water bath for 2 hr. It was evaporated to a small volume and poured in water. The precipitate was washed and dried. Needle-like crystals were obtained m.p. 126–127° (Shriner, Fuson and Curtin, 1956, give 126–127°).

Isolation of α-Carotene

The orange coloured fraction (2·69 g.), derived from the unsaponifiable matter, was redissolved in light petroleum (50 ml.) and passed through a column of Merck aluminium oxide (150 g.). The column was eluted with 30 fractions each of 50 ml.; those numbered 1 to 8 with light petroleum; those 9 to 12 with benzene; those 13 to 22 with benzene: ethanol (200 : 1). The fractions numbered 23 to 26 were eluted with benzene: ethanol (100 : 1) and those numbered 27 to 30 with ethanol.

The few mg. orange coloured residues from fractions 9–12, 16–18, 20–21 and 24 gave tests for carotenoids (Strain, 1935). The residue from fraction 23 was dissolved in benzene and its absorption measured spectrophotometrically (Hilger). It gave absorption maxima at 460 and 488 mμ identical with that reported (Strain, 1935) for α-carotene. The other three carotenoid residues did not give clear maxima and may represent decomposition products of carotenes. Such decomposition is known to occur on alumina columns (Strain, 1945).

Preparation of Crude Alkaloidal Mixture

The powdered drug (2·5 kg.), after being successively extracted with light petroleum, benzene and chloroform, was extracted in a soxhlet apparatus for 24 hr. with ethanol (95 per cent). The ethanol was evaporated when a semisolid dark brown residue (160 g.) was obtained. It gave positive tests for alkaloids. The residue was dissolved in 0·5 N hydrochloric acid (500 ml.), the acid solution filtered and made alkaline with a dilute solution of ammonia and the alkaloids extracted by shaking with chloroform. On evaporation of the solvent a dirty-white crude alkaloid (16 g.) was obtained. Paper chromatography of the crude alkaloid, using butanol saturated with 5 per cent acetic acid as the mobile phase and modified Dragendorff's reagent, as the spray (Munier and Macheboeuf, 1957) showed the presence of four alkaloids (R_F 0·157, 0·224, 0·521 and 0·620). An attempt to separate the individual alkaloids from the mixture by fractional crystallisations did not succeed.

Isolation of Sericine and Monocrotaline

The alkaloidal mixture (8 g.) was dissolved in benzene (100 ml.) and added to a column containing chromatographic aluminium oxide (225 g.). The column was eluted with 42 fractions each of 50 ml.; those numbered 1 to 23 with benzene; those 24 to 38 with chloroform and those 39 to 42 with ethanol. Fractions 1–3 gave a light yellow waxy residue (0·060 g.)

which could not be crystallised. Fractions 4–6 gave a residue of large prismatic colourless crystals (1·833 g.) which gave positive tests for alkaloids m.p. 180–181°. The residue (4·723 g.) from fractions 7–42 was in the form of long fine needles, alkaloid positive, m.p. 195–197°, which was probably identical with monocrotaline.

Sericine. The crystalline residue from fraction 4–6 is regarded tentatively as a new alkaloid as evidenced by its crystal structure and melting point. To confirm this view and to prove that it was not an impure form of monocrotaline (fractions 7–42), the technique of mixed spotting on paper chromatograms was used. On a sheet of Whatman No. 1 filter paper, impregnated with pH 7·4 phosphate buffer, four spots were applied. Spot I was of residue from fraction 4–6, Spot 2 of residue from fraction 7–42, Spot 3 of a mixture of residues from fractions 4–6 and 7–42, and Spot 4 of an authentic sample of monocrotaline. Butanol saturated with 5 per cent acetic acid was used as the mobile phase. A comparison of these alkaloids on the chromatogram shows that the residue from fractions 7–42 is identical with monocrotaline (R_F 0·54) while residue from fraction 4–6 represents the new substance (R_F 0·63). The name sericine has been given to this crystalline base. The elemental analysis gave C, 58·8; H, 6·77; N, 3·96 per cent which corresponds to the tentative empirical formula $C_{17}H_{24}NO_7$. Yield of sericine was 0·07 per cent of the air dried seeds.

Sericine hydrochloride. The base (10 mg.) was dissolved in absolute ethanol (3 ml.) and neutralized to congo red by dilute hydrochloric acid. The solution was evaporated to dryness. The residue was taken up in 2 ml. hot methanol and ether added to turbidity. On cooling, the product was filtered and recrystallized from methanol-ether, m.p. 232–233°.

Sericine picrate. The base (20 mg.) was dissolved in absolute ethanol (2 ml.) and to it a saturated solution of picric acid in ethanol was added. The heavy crystalline precipitate so obtained was recrystallized from ethanol, m.p. 233–235°.

Sericine methiodide. The base (30 mg.) was dissolved in chloroform (1 ml.) and ethanol (0·1 ml.) and 0·2 ml. of methyl iodide added. On cooling an oily product separated which on crystallization from methanol-chloroform gave a crystalline addition compound, m.p. 242–245° (decomp.).

Monocrotaline. As shown above, the residues from fractions 7–42 were probably identical with monocrotaline, based on the melting point and R_F values (found m.p. 196–197°) (Rogers and Rogers, 1939, gave monocrotaline, 196–197°). The elemental analysis gave C, 59·16: H, 6·83 per cent (calculated for monocrotaline, $C_{16}H_{23}NO_6$, C, 59·07: H, 7·09 per cent). A mixed melting point with an authentic sample of monocrotaline was undepressed. The yield of monocrotaline was 0·5 per cent on the basis of air dried seeds.

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