MATERIALS AND METHODS
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Research material consisting of seeds was procured from cultivated as well as wild plants. The seeds were powdered by flour mill or Raymond's mill depending upon nature of seeds. They were sifted through suitable sieve.

*C. sericea* Retz.

The plants of *C. sericea* grow wild in abundance in Chandigarh. The seeds were collected from Panjab University campus in November, 1959, air dried and powdered in flour mill. The hard testa and gummy endosperm were effectively removed by using suitable sieve. The coarse powder consisting mainly of embryo was used in the present investigation.

*C. medicaginea* Lam.

Seeds of *C. medicaginea* were also obtained from wild source. The collection of seeds was made in November, 1961 from the naturally growing plants around Panjab University campus, Chandigarh. The air dried seeds were powdered in flour mill. The testa was removed by sifting through coarse sieve. The sifted material, consisting mainly of embryo, was utilised for the extraction purpose.
C. striata DC.

The collection of seeds was arranged from Kurseong near Darjeeling in November, 1960. The dried seeds were powdered in Raymond's mill and were used in the present investigation.

C. grahamiana W. & A.

The seeds of C. grahamiana were obtained from Nilgiri Seed Depot, Coimbatore. They were powdered in Raymond's mill. The testa and gummy endosperm were removed by using suitable sieve. The sifted material consisting mainly of embryo was used in the present investigation.

C. ferruginea Grah.

The plants of C. ferruginea were grown in Pharmacy drug garden, Panjab University, Chandigarh. The collection of seeds was made in December, 1962. The air dried seeds were powdered in Raymond's mill and used for the isolation of alkaloids.

Assay

The method of assay of pyrrolizidine bases in the form of tertiary bases and their N-oxides has been reported by Culvenor. This method has been modified to give a rapid method of analysis and has been applied in assay of
Crotalaria seeds reported in subsequent work. The modified method is given below:

Crotalaria seeds (100 g) were extracted completely with alcohol in Soxhlet apparatus. The alcohol was evaporated off and resulting residue treated with sulphuric acid (5%). The mixture was shaken and filtered. The residue on the filter paper was washed with more of acid till it gave negative tests for alkaloid. The combined acid extract was divided into two parts. One part was made strongly alkaline with dilute solution of ammonia and extracted with chloroform. On evaporation of chloroform solid or liquid base resulted which represented 'unreduced base'. To the other part was added zinc dust and sufficient sulphuric acid to make the solution (2 N). The mixture was kept for 6 hours with occasional shaking. It was filtered, made strongly alkaline with dilute solution of ammonia and extracted with chloroform. Evaporation of chloroform gave 'reduced base'. Both 'reduced' and 'unreduced bases' were dissolved in appropriate volume of anhydrous chloroform. Anhydrous chloroform was prepared by distilling chloroform BDH, rejecting first and last fractions, the middle fraction being passed through column of alumina and stored in air tight container.

The aliquot portion of chloroform was titrated with 0.01 N p-toluenesulphonic acid in anhydrous chloroform using
dimethyl yellow as indicator prepared in absolute alcohol. The results were expressed on the basis of monocrotaline Eq. wt. 325. The titre value of unreduced base gave alkaloid present as tertiary bases and that of reduced base present as tertiary bases as well as its N-oxides.

**Paper Chromatography**

Throughout the investigation Rf value was determined by ascending method of paper chromatography. Solvent system consisting of equal volume of n-Butanol Merck and acetic acid (5%) was prepared by thorough shaking and using upper layer as mobile phase. The lower layer was used for saturating chromatographic paper. The paper was allowed to saturate for 8-10 hours. The mobile solvent was allowed to run for 10-12 hours at 17±5°C. The paper was air dried and spots developed by suspending the paper in a chamber containing iodine vapour.

**Equivalent Weight**

The equivalent weight of base was determined by preparing known percentage of solution of base in anhydrous chloroform. Method of preparation of anhydrous chloroform was same as that used in assay of alkaloid. The aliquot portion of base solution was titrated against standard solution of p-toluenesulphonic acid in anhydrous chloroform using dimethyl yellow as indicator. The titre value was found
for 100 ml. of alkaloid solution. The result was calculated by the following formula:

$$\text{Eq. wt.} = \frac{w \times 1000}{v \times n}$$

Where

- $w$ = grams of alkaloid dissolved in 100 ml. of anhydrous chloroform.
- $v$ = titre value for 100 ml. of solution.
- $n$ = normality of p-toluenesulphonic acid solution.

### Specific Rotation

Specific rotation was determined under identical conditions as reported for specific alkaloid. The optical rotation was observed by Zeiss polarimeter using filter Winkel number one in chloroform or ethanol. Known percentage of alkaloid solution was prepared in ethanol or chloroform and circular scale reading noted adjusting the reading for blank. The result was calculated by the following formula:

$$\left(\frac{\alpha}{D}\right)_D = \frac{a \times 100}{l \times c}$$

Where

- $\alpha$ = specific rotation.
- $a$ = circular scale reading (optical rotation).
- $l$ = length of the column of liquid in decimeters.
- $c$ = number of grams of substance in 100 ml. of solution.