377. *Sophora Alkaloids. Part V. The Alkaloids of the Seeds of a Possibly New Species from Anawhata, New Zealand.*

By LINDSAY H. BRIGGS and J. L. MANGAN.

From the seeds of a possibly new species, cytisine, methylcytisine, and matrine have been isolated. The bearing of these results on the botanical classification is discussed.

DR. H. H. ALLAN, Director of the Botany Division, Department of Scientific and Industrial Research, New Zealand, has suggested to the authors that the tree of which the examination is now reported and which occurs in a restricted area round Anawhata, near Auckland, is either a variety of *S. microphylla*, already examined (Part I, *J.*, 1937, 1795), or a distinct species. Baker and Smith ("A Research on the Eucalypts and their Essential Oils", 1920, 2nd Edtn.) and Manske (*Canadian J. Res.*, 1939, **17**, *B*, 1) have shown that species of *Eucalyptus* and *Senecio* respectively, taxonomically indistinguishable, can be separated by differences in the essential oils and the alkaloids they contain, and it was considered, therefore, that an examination would be of assistance in its botanical classification as attempted in Part IV (*J.*, 1942, 555).

Following the methods outlined in earlier papers of this series, extraction of the seeds has yielded a crude alkaloid mixture from which cytisine, methylcytisine, and matrine have been identified. Only 55% of the alkaloids present in the crude mixture distilled in a high vacuum, of which 81% was accounted for by the isolation, as either the free base or the picrate, of methylcytisine (71%), cytisine (7%), and matrine (3%), corresponding to 40, 4, and 1·5% respectively of the total alkaloids, and no crystalline material could be obtained from the distillation residues. In an attempt to obtain a better quantitative yield of the alkaloids present, aliquot portions of the crude alkaloid mixture were converted into their picrates and picrolonates severally and fractionally crystallised. No sharp separation could be achieved with the picrates, from which only those of cytisine and methylcytisine were obtained. Crystallisation of the picrolonates, however, afforded a better separation in amounts corresponding to 23 and 29% of cytisine and methylcytisine, respectively, but from neither method could derivatives of matrine be isolated. No other bases, *e.g.*, sophochrysine, could be detected by any of the above methods.

In this series of experiments on the alkaloids of the seeds of *S. tetraptera*, *S. microphylla*, and this tree, although the total yield of alkaloids actually isolated in a pure state is far from quantitative, the methods of isolation have been similar in all cases and may therefore be considered roughly comparable. The composition (in %) of the alkaloids is given in the following table :

Species.	Matrine.	Methylcytisine.	Cytisine.	Sophochrysine.
S. tetraptera	45	3	—	1
S. microphylla	27	20	0·05	trace
Unknown *Senecio* spp.	1·5	40	4 *	—

* Isolated by solvent extraction; 23% through separation of the picrolonates.

There is thus a marked difference between the composition of the alkaloids of *S. microphylla* and this tree, as much as the difference between that of *S. microphylla* and *S. tetraptera*. If phytochemical evidence is to be taken into account in the differentiation of species, then the above results would support the classification of this plant as a new species.

Burger (*J. Amer. Chem. Soc.*, 1945, **67**, 1615) has recommended the use of lithium hydroxide for regenerating bases from their picrates owing to the greater solubility of lithium picrate than of sodium or potassium picrate. A similar solubility relationship holds for the lithium, sodium, and potassium picrolonates and we have shown that the free bases are also conveniently regenerated from their picrolonates by using lithium hydroxide.

EXPERIMENTAL.

The obvious differences of this species or variety are that the leaves consist of about 40 pairs of pinnæ, *ca.* 4 mm. long, much smaller than those of *S. microphylla*, and the characteristic dull greyish-green foliage in contrast to the normal bright green of *S. microphylla*.

1·9 Kg. of the crushed seeds of this species gathered from an area at Anawhata less than 50 yards across were worked up as described in Part II (*J.*, 1938, 1206). Chloroform is twice as efficient as trichloroethylene or ether in removing the alkaloids from the aqueous solution, but even then continuous extraction for 150 hours was required. The crude alkaloid mixture was obtained as a brown viscous oil (40 g.). An aliquot sample was dissolved in 1% hydrochloric acid and filtered. After the addition of excess of silicotungstic acid the granular precipitate was filtered off, dried at 110°, and ignited to red heat. Assuming the composition of the precipitate to be $SiO_2,12WoO_3,2H_2O,x$(alkaloid), converted on ignition into $SiO_2,12WoO_3$, the percentage of alkaloid in the crude mixture was found to be 69·5% and the total yield of alkaloids from the seeds *ca.* 1·5%.

Separation of Bases.—(a) *By distillation and solvent extraction.* No apparent separation occurred during the distillation of the crude bases, a single fraction being obtained, b. p. 165—190°/0·01—0·02 mm., with the final bath temperature 240°. Of 19·5 g. distilled, 5·8 g. were lost as solvent, corresponding almost exactly to the figure indicated by the silicotungstate method (it is extremely difficult to remove solvent from this mixture except by distillation). Of the residue, 7·6 g. (55% of the total alkaloids) distilled, leaving a residue of 6·1 g. (45% of the total alkaloids). The distilled bases rapidly crystallised in contact with light petroleum (b. p. below 40°) but separation was best achieved by extraction in a Soxhlet extractor. The material was first exhaustively extracted with this solvent, and repeated crystallisation of the material separating on cooling gave 4·38 g. of base A, m. p. 128—131°. From the mother-liquors 0·21 g. of base B was obtained in crystalline condition, m. p. 66—72°, also from the same solvent. The intermediate fractions consisted of 1·29 g. of oily base A and 0·66 g. of oily base B, but only from the former could a further quantity of base A be obtained as the picrate (1·24 g.).

The residue in the Soxhlet readily and completely dissolved in chloroform (cf. sophochrysine, which is only sparingly soluble in chloroform). The semisolid material remaining after removal of the chloroform by crystallisation from benzene–light petroleum (b. p. 100—120°) and again from benzene yielded base C, m. p. 155·5—157° (0·24 g.). The mother-liquors afforded a further quantity (0·64 g.) as the picrate. From these quantities the yields of bases A, B, and C were calculated to be 71, 2·8, and 7%, respectively, of the distilled bases, or 40, 1·5, and 4%, respectively, of the total alkaloids.

No other bases could be isolated from the mother-liquors, nor could any crystalline material be obtained from the residue of the distillation.

Base A. The m. p. of this base, identified as methylcytisine, was raised to a constant value of 135—136° by recrystallisation from light petroleum (b. p. under 40°), and from this material the picrate, m. p. 224°, and picrolonate, m. p. 223°, were prepared (for the literature on all these bases and derivatives, see Parts I—IV). No depressions of m. p. were observed on admixture with methylcytisine and its appropriate derivatives respectively; $[\alpha]_D^{15^\circ}$ −194·1° (l = 0·25; c, 2·025 in water).

Base B. Recrystallisation of this base, identified as α-matrine, from light petroleum (b. p. below 40°) afforded long colourless needles, m. p. 76—77°, from which the picrate, m. p. 60—135°, and aurichloride, m. p. 199—200° (decomp.), were prepared. The m. p.s of the free base and its aurichloride were not depressed by authentic specimens, and the readily prepared picrate shows the indefinite m. p. characteristic also of matrine picrate; $[\alpha]_D^{15^\circ}$ +42·3° (l = 0·25; c, 1·700 in water).

Base C. This has been identified as cytisine, the purified base separating from absolute alcohol–acetone or amyl alcohol in colourless needles, m. p. 156—157°; $[\alpha]_D^{15^\circ}$ $-126{\cdot}2^\circ$ ($l = 0{\cdot}25$; c, 1·965 in water). The free base, its aurichloride, m. p. 221°, picrolonate, m. p. 266° (decomp.), and its benzenesulphonyl derivative, m. p. 259° (Ing, *J.*, 1935, 1053, records m. p. 263—264°), were not depressed in m. p. by cytisine and its appropriate derivatives respectively.

(b) *By fractional crystallisation of the picrolonates.* 0·5510 G. of the crude bases was dissolved in a little water, filtered, and to the hot solution picrolonic acid (0·6 g.) in alcohol was added. Systematic crystallisation of the crude picrolonates formed and working up of the mother-liquors therefrom afforded only two products: (*a*) golden needles, m. p. 269° (decomp.), from 50% alcohol, and (*b*) short yellow needles, m. p. 224°, from 50% methyl alcohol and absolute alcohol, undepressed in m. p. by cytisine and methylcytisine picrolonates, respectively. The yields of these derivatives corresponded to 23% and 29% of the total alkaloids, respectively.

Cytisine picrolonate (75 mg.) was decomposed with lithium hydroxide solution, and the free base extracted with chloroform and converted into the perchlorate. After recrystallisation from methyl alcohol it formed colourless needles, m. p. and mixed m. p. 296° (decomp.).

(c) *By fractional crystallisation of the picrates.* 0·5016 G. of the crude bases was dissolved in water, and picric acid (1 g.) in hot water added. Careful fractionation of the product and working up of the mother-liquors again afforded only two products: (*a*) yellow needles from water, m. p. 228·5—229°, and (*b*) yellow needles from aqueous alcohol, m. p. above 300°. The former was not depressed in m. p. by methylcytisine picrate. The latter corresponds to the m. p. of 302° recorded by Manske (*Canadian J. Res.*, 1943, **21**, *B*, 144) for cytisine picrate, which is considerably higher than the values 270° and 278° recorded by Clemo and Raper (*J.*, 1935, 10) and by Briggs and Ricketts (Part I, *loc. cit.*), respectively. The identity with cytisine, however, was confirmed by conversion of the picrate into the free base by Burger's method and conversion into the picrolonate. After recrystallisation from aqueous alcohol this formed yellow needles, m. p. and mixed m. p. 269° (decomp.) (Found: C, 55·4; H, 4·7. Calc. for $C_{11}H_{14}ON_2,C_{10}H_8O_5N_4$: C, 55·5; H, 4·85%).

Despite the indefinite m. p. of matrine picrate, it crystallises readily and would have been observed in the above separation if present in quantity.

The analysis is by Mr. J. Mills, University of Adelaide.

We are indebted to the Chemical Society and the Australian and New Zealand Association for the Advancement of Science for continued grants.

AUCKLAND UNIVERSITY COLLEGE, AUCKLAND, NEW ZEALAND. [*Received, December* 31*st*, 1947.]