THE PARTITION CHROMATOGRAPHY OF ALKALOIDS

PART II.—THE ALKALOIDS OF AUSTRALIAN *DATURA FEROX* AND OF INDIAN HENBANE

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IN Part I[1] attention was directed to the possibility that *Datura ferox* may yield different alkaloids when grown in different localities. Australian-grown *D. ferox* was found by Barnard and Finnemore[2] to contain hyoscyamine as the major alkaloid and material grown in the Crimea afforded, according to Libizov[3], hyoscyamine and hyoscine. We found that plants grown in this country produced hyoscine as the chief alkaloidal constituent and meteloidine as a relatively minor alkaloid. In view of the well authenticated cases of variation in the nature of the alkaloids within certain species of the Solanaceæ, viz., *Duboisia species* and *Datura metel*[4] it appeared of interest to accumulate more evidence on this point for *D. ferox*. Moreover, it would appear from the results reported in Part I and to be described in this communication that *D. ferox* may provide a convenient commercial source of hyoscine.

Samples of Indian henbane are from time to time offered for sale in this country and presumably are used for the manufacture of galenical preparations. As far as we are aware, no information is available either on the plant source of these materials[5] or on the nature of the alkaloids contained in them. Accordingly a comparison has been made of the alkaloids of Indian henbane with those of authentic *Hyoscyamus niger*.

EXPERIMENTAL

The Australian *D. ferox* available consisted of about 120 g. of whole plant grown at Canberra and seeds from plants grown at Shepparton, Victoria. The Indian henbane was a commercial sample purchased in this country. The botanical source of this material is doubtful but we are informed by Mr. J. L. Forsdike that it differs from *H. niger*.

Quantities of 100 g. of both the whole plant and the seeds of *D. ferox* were extracted by a modification of the Pharmacopœial assay process[6]. An ethereal solution of the total alkaloids was in each case chromatographically fractionated first with ether and then with chloroform on a column of 20 g. of kieselguhr on which was distributed 10 ml. of M/2 phosphate buffer of *p*H 7·3. Details of this technique and the method of collecting fractions of the eluate are described in Part I. The alkaloids in the fractions of eluting solvent were identified as their picrates.

For comparison of the alkaloids of Indian henbane with those of *H. niger*, 500 g. of the Indian drug and 100 g. of *H. niger* were extracted and chromatographically fractionated by the same procedure, the M/2 phosphate buffer having *p*H 6·8 in this instance. Because of the low proportion of alkaloids in the Indian henbane, a total of 10 kg. was extracted in batches of 2 kg. for the identification of the alkaloids after

chromatographic fractionation. The titration liquors corresponding to a given peak were combined; the aqueous layer, containing the alkaloidal sulphate was separated from the organic solvent and indicator was removed from it by shaking with chloroform; after concentrating under reduced pressure, the solution was available for the preparation of solid derivatives by double decomposition with the appropriate reagent.

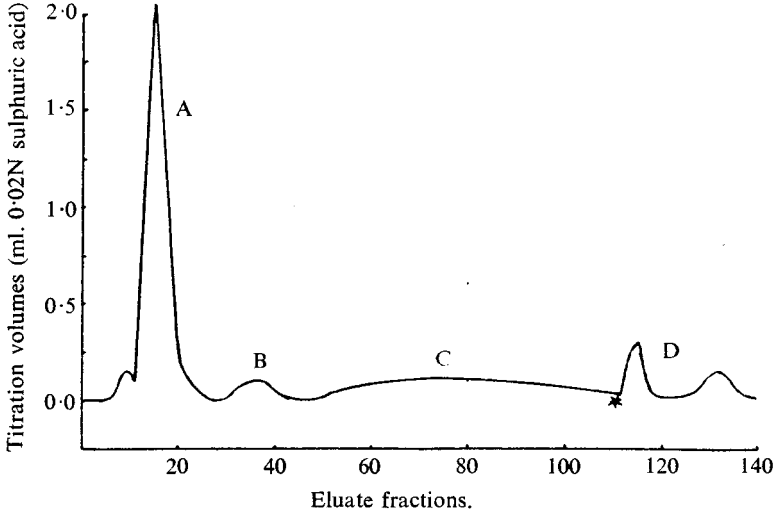

FIG. 1.—Separation of alkaloids from 100g. of Australian *Datura ferox* (whole plant)

Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 7·3.

* Chloroform B.P. used as eluant.

Figure 1 shows the separation of the alkaloids of the whole plant of *D. ferox*. The eluate fractions represented by the large peak A were shown to contain hyoscine by the preparation of the picrate, m.pt. 187°C., not depressed on admixture with authentic hyoscine picrate. The proportion of hyoscine found by titration was 0·06 per cent. and by isolation of the picrate, 0·05 per cent. From the eluate fractions corresponding to peak B, 5 mg. of a picrate, m.pt. 230°C., was isolated. Fractions 52 to 112 of the eluate (peak C) afforded meteloidine picrate, m.pt. 174° to 175°C., undepressed on admixture with authentic material. The melting-point of this picrate was considerably depressed when mixed with hyos-

TABLE I

Alkaloid	*H. niger*	Indian henbane
	per cent.	per cent.
Total alkaloids, calculated as hyoscyamine ...	0·073	0·031
Hyoscine	0·028	0·016
Hyoscyamine	0·041	0·010
Tropine	0·0025	0·0015

cyamine picrate or with atropine picrate. The weight of meteloidine picrate isolated was 3 mg.; no reasonably accurate estimation of the proportion of this alkaloid was possible by a summation of the very small,

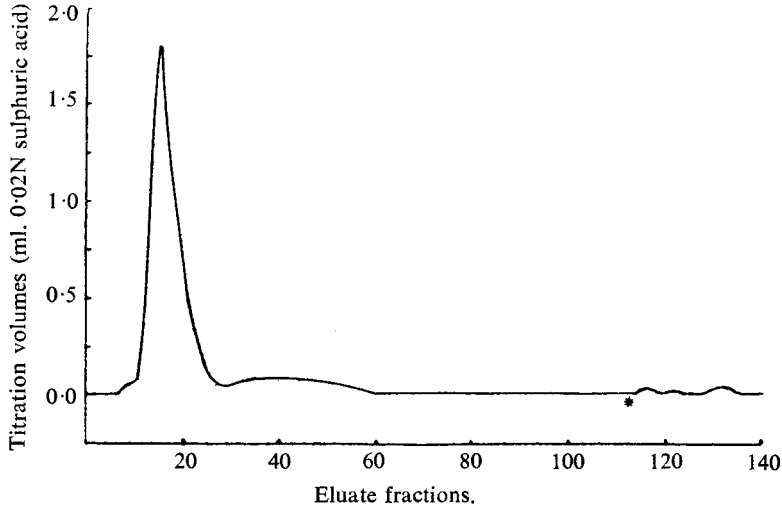

FIG. 2.—Separation of alkaloids from 100g. of Australian *Datura ferox* seeds. Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 7·3. * Chloroform B.P. used as eluant.

individual eluate-fraction titres. The partition chromatogram of *D. ferox* seeds is represented in Figure 2. The only significant peak was shown to refer to hyoscine, 0·093 per cent., which afforded hyoscine

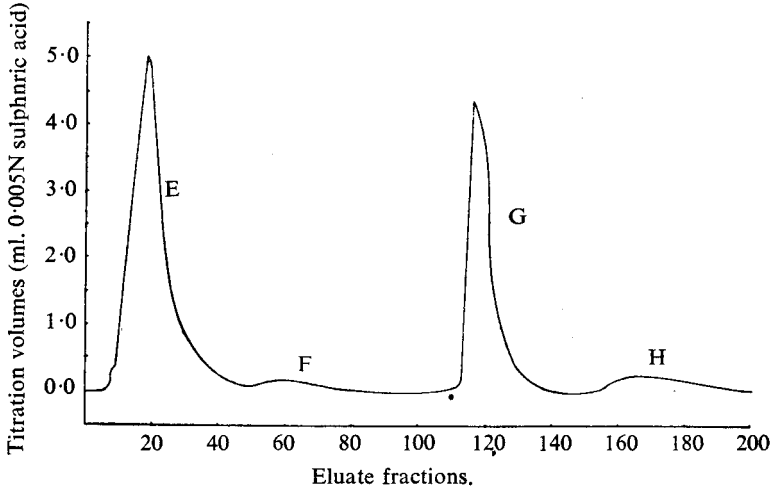

FIG. 3.—Separation of alkaloids from 100g. of Indian henbane. Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 6·8. * Chloroform B.P. used as eluant.

picrate, m.pt. 187°C., undepressed by authentic hyoscine picrate. The proportion of total alkaloids, calculated as hyoscine, was 0·095 per cent.

For Indian henbane and *H. niger*, the partition chromatograms were as shown in Figures 3 and 4. Quantitative data obtained from the respective 500 g. and 100 g. quantities of these two drugs are summarised in Table I. The shape of the chromatogram curve from the larger-scale extraction of Indian henbane was similar to Figure 3.

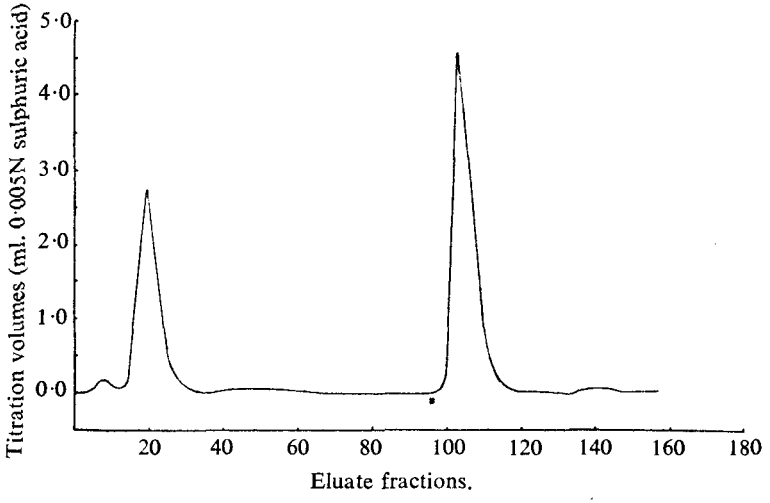

FIG. 4.—Separation of alkaloids from 100g. of *Hyoscyamus niger*.
Column, 20g. kieselguhr with 10 ml. 0·5 M phosphate buffer, *p*H 6·8.
* Chloroform B.P. used as eluant.

Peak E was demonstrated to correspond to hyoscine by the preparation of the picrate, m.pt. and mixed m.pt. 186°C. and the aurichloride, m.pt. and mixed m.pt. 204°C., with decomposition. From the fractions represented by peak F, a picrate, m.pt. 230°C., was isolated; this material was identical with the picrate of the same melting-point isolated from *D. ferox*. The alkaloid corresponding to peak G was found to be hyoscyamine, since it afforded an aurichloride, m.pt. 163°C., not depressed on admixture with authentic hyoscyamine aurichloride, and also a picrate, m.pt 164° to 165°C., not depressed by mixing with authentic material. For the identification of the alkaloid corresponding to peak H, the auribromide and Reineckate were found to be satisfactory. The auribromide crystallised from water or dilute hydrobromic acid as dark brown needles, m.pt. 196°C., with decomposition. Found: C, 14·9; H, 2·4; N. 2·4 per cent. $C_8H_{15}ON,HAuBr_4$ requires C, 14·6; H, 2·4; N, 2·1 per cent. The melting-point of this substance was undepressed on admixture with the auribromide prepared from tropine made by the hydrolysis of hyoscyamine according to Ladenburg[7]. The Reineckate, m.pt. 239° to 240°C., with decomposition, did not depress the melting-point of authentic tropine Reineckate[1]. Found: N, 20·3 per cent. $C_8H_{15}ON,H[Cr(SCN)_4(NH_3)_2]$, H_2O requires N,20·5 per cent.

Discussion of Results

Contrary to previous findings, the experiments described in this communication and in Part I[1] show that samples of *D. ferox* grown in Australia and in this country contain hyoscine as the principal alkaloidal constituent. Its proportion is very variable since material grown in Nottingham contained 0·4 per cent, whereas the Australian material contained only 0·06 per cent. This plant is common in Australia and in view of the low proportion of other alkaloidal constituents, may, if the constancy of alkaloidal type is confirmed for other Australian samples, provide a convenient commercial source of hyoscine. Since the partition chromatogram indicated the complete absence of meteloidine from the seeds, it would appear that this alkaloid is not a product of metabolism of the reproductive organs of the plant. It is hoped that further information on the nature of the alkaloid which afforded a picrate, m.pt. 230° C., and on the alkaloids of *D. ferox* which are eluted with chloroform (peak D) will be presented in a further communication.

The major alkaloids of Indian henbane are hyoscine and hyoscyamine with tropine as a minor alkaloid, but the total alkaloidal content is very low. Notwithstanding anatomical differences, the shape of the partition chromatogram curve, which is a function of the R_F values characteristic of the alkaloids, demonstrates that the only significant difference between Indian henbane and *H. niger* is in the relative proportions of the alkaloids. The small final peak in the curve for *H. niger* is likely to correspond to tropine since a comparison of the curves for *H. niger* and Indian henbane shows that the R_F value must be very close to that of the tropine independently identified in the Indian henbane. This sample of Indian henbane bore no resemblance in its constituents to *H. reticulatus,* which according to Konovalova and Magidson[8], contains hyoscyamine and 1 : 4-*bis*dimethylaminobutane. The characterisation of the small quantity of tropine presented some difficulty. The picrate and aurichloride were too soluble to afford convenient derivatives and the auribromide decomposed slightly on repeated recrystallisation. We now find that the melting-point of the Reineckate varies appreciably with the rate of heating; in Part I the value recorded was 251° to 252°C., with decomposition, after sintering at 246° to 248°C., whereas the best reproducible value is probably 245° to 246°C., with decomposition.

We are greatly indebted to Dr. C. Barnard of the Council for Scientific and Industrial Research, Australia for providing the samples of *D. ferox* and to Mr. J. L. Forsdike for procuring the sample of Indian henbane.

Summary

1. Australian-grown *Datura ferox* has been found to contain as principal alkaloids, hyoscine and meteloidine.

2. The chief alkaloidal constituents of Indian henbane are hyoscine, hyoscyamine and tropine; in this respect, Indian henbane differs from *Hyoscyamus niger* only in the absolute and relative proportions of the alkaloids.

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