VARIATION IN THE ALKALOIDS OF CLONES OF NORTHERN *DUBOISIA MYOPOROIDES* R. BR.

By K. LOFTUS HILLS* and CYNTHIA N. RODWELL*

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Summary

Samples of leaf were obtained from seven clones of the northern or hyoscine type of *Duboisia myoporoides* for three successive summers at Canberra, Australian Capital Territory, and from the same clones for a winter and a summer at Nambour, south-eastern Queensland. The alkaloids were separated by partition chromatography and the hyoscine determined quantitatively. Some of the other alkaloids present were identified.

Consistent differences in hyoscine content were evident between certain of the clones and it is presumed that they differ genetically in their capacity to produce this alkaloid. The average percentage of hyoscine per clone ranged from 0.6 to 1.5 per cent. of the dry weight of the leaf.

The yield of both total alkaloids and of hyoscine were of the same order of magnitude at Canberra and Nambour but there was considerable variation between samplings at each location. Interaction was evident between the yield of hyoscine from individual clones and the year or season of sampling.

Alkaloids other than hyoscine accounted for from 30 to 90 per cent. of the alkaloids found in individual samples. They comprised in various combinations hyoscyamine, valeroidine, an unidentified alkaloid with a high melting point picrate, and one or more unidentified alkaloidal substances.

I. INTRODUCTION

Preliminary investigations (Loftus Hills, Trautner, and Rodwell 1945) have shown that both heredity and environment are important in determining the proportion of hyoscine in leaves of *Duboisia* species. Certain major geographical races have been described (Loftus Hills and Kelenyi 1947) but an evaluation of the variation within these groups was not possible until suitable quantitative analytical methods were developed. The successful application of the partition chromatography of Evans and Partridge (1948) to *Duboisia* leaf extracts, and the use of a modification of the method of Trautner, Neufeld, and Rodwell (1948) for the quantitative estimation of hyoscine have enabled further progress to be made.

This paper describes the variation in total alkaloids and in the hyoscine content of seven clones† from the northern or hyoscine-dominant group of *Duboisia myoporoides* for three successive summers at Canberra, A.C.T., and a winter and a summer at Nambour, south-eastern Queensland (see representative meteorological data in Table 1). An exhaustive investigation of the

* Division of Plant Industry, C.S.I.R.O., Canberra, A.C.T.

† Four of the leaf samples of the seventh clone were too small for assay, and were replaced by composite samples from siblings of the original selection.

genotypes available within any particular zone was clearly impracticable. The project was therefore confined to a study of the environmental interaction of a limited number of clones, selected at random in regard to alkaloid propensities, but with some regard to geographical origin and to the more obvious variations in vegetative form observed in a number of seedling progenies being grown for selection. The authors are unaware of any comparable investigations with other alkaloid-bearing species.

TABLE 1
METEOROLOGICAL DATA FOR CANBERRA AND NAMBOUR

	Normal Mean Minimum Temperature (°F.)		Normal Mean Maximum Temperature (°F.)		Normal Mean Relative Humidity (%)		Mean Annual Rainfall (in.)	Latitude
	July	Jan.	July	Jan.	July	Jan.		
Canberra	33	55	52	82	83	53	23	35° 20'S.
Nambour (Gympie)	42	67	71	88	75	74	47	26° 11'S.

II. MATERIALS AND METHODS

(*a*) *Botanical*

From 10 to 30 vegetative propagants were struck from each of a number of individuals selected from seedling progenies of *D. myoporoides* growing at Canberra, A.C.T. One-half of each of these clonal populations were transplanted into the field at Canberra and the remainder were established similarly at Nambour. Seven of them were selected for further study on the basis of maximum vigour in both environments, diversity of geographical origin of the parental seed, and diversity of leaf shape and plant form.

The location of the parent trees from which the selected seedlings were derived, together with the northern and southern limits of distribution of the hyoscine-dominant type of *D. myoporoides* are shown in Figure 1. Clones 437-2, 455-1, 468-1, and to a lesser extent 355-1 and 428-1, may be regarded as vegetatively normal for the group, whereas 327-1, with small, narrow, olive-green leaves, and 370-1 with glossy, prominently veined, dark green leaves, are atypical. The vegetative characters vary throughout the region of occurrence but not apparently in any regular manner.

Fertility was maintained during the course of the experiment by regular dressings of NPK fertilizer. After the initial watering in of the transplants growth was maintained by natural rainfall. Leaf samples were taken during the first, second, and third summers after establishment in the field at Canberra, and in July and November 1947 at Nambour. The methods used for sampling and drying were the same as those described previously (Loftus Hills, Trautner, and Rodwell 1945).

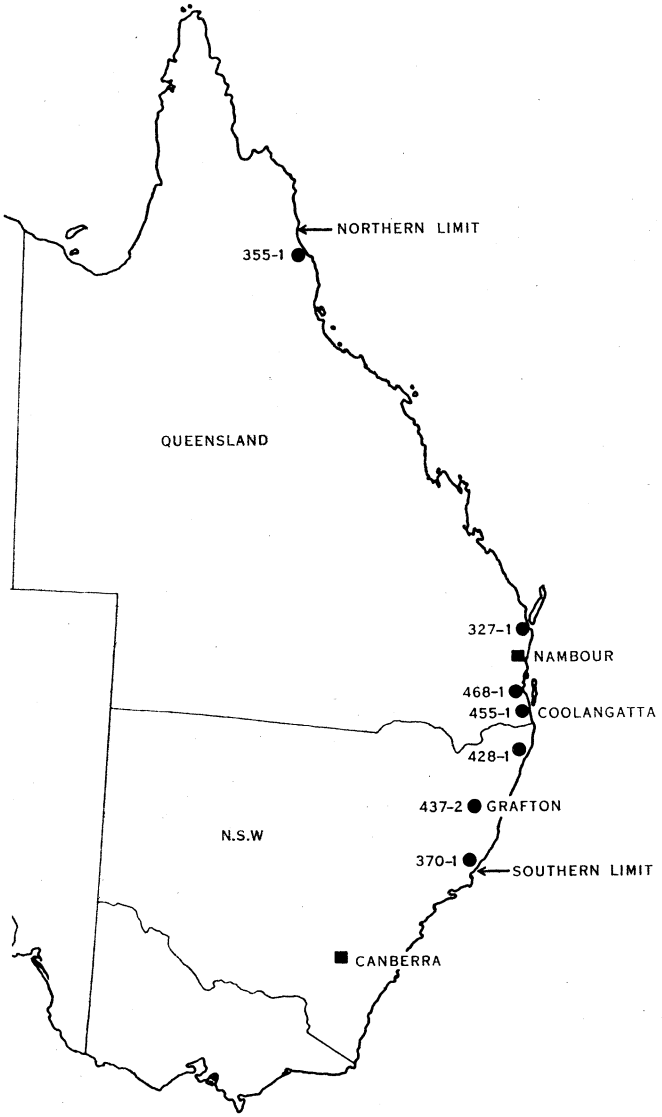

Fig. 1.—The geographical origin of the seed from which the clones of northern *D. myoporoides* were established.

(*b*) *Chemical*

The method of Loftus Hills, Trautner, and Rodwell (1945) was designed to give a rapid overall picture of a large number of small samples. It gave an approximate (possibly too high) figure for total alkaloids, and a general assessment of the nature of the main alkaloids present, based on the identification of the picrates. The methods used in the present investigation gave a more exact figure for the total alkaloids, a quantitative evaluation of the hyoscine, and allowed of the identification of some of the other alkaloids.

The total alkaloids obtained by the earlier method were estimated by titration of an organic extract without passage through water or other purification. The methods used in the present investigation entailed repeated cycles through water and organic solvent, until a constant titration figure was reached. This procedure led, in every case, to a substantial decrease in the titration of bases. The decrease was greatest between the first and second titrations and became less with each succeeding cycle. In some cases the second and third titrations were sufficiently close for the third one to be taken as final but more often four or five cycles were necessary. The nature of the titratable material discarded by this procedure is not known — a few chromatograms of the first (i.e. least pure) ether extracts gave no indication of the presence of additional bases in any significant amount. The final titration of bases was occasionally as little as 30 per cent. of the first one, but was usually about 70 per cent. The figures were similar whether the chloroform extract was titrated with *p*-toluenesulphonic acid, or the aqueous extract titrated with sulphuric acid.

Two main methods were used. The first was based on the estimation of hyoscine as the picrate, and the second involved the separation of the bases by partition chromatography and the subsequent estimation of hyoscine by titration.

(i) *The Picrate Method.*—Ten g. of dry, powdered leaf was extracted by shaking with successive 150 ml. portions of cold, purified methanol until a small portion, on evaporation with dilute acid, showed no reaction with Mayer's reagent (K_2HgI_4). The methanol was evaporated under reduced pressure and the residue acidified to congo red with 1 per cent. sulphuric acid. The precipitate was broken up, stirred thoroughly, filtered under reduced pressure, and the filter cake washed with very dilute acid until free from alkaloids.

The filtrate was transferred to a separating funnel, washed once with chloroform which was extracted with dilute acid, and then discarded; the acid wash being added to the main extract. The main extract was made alkaline to phenolphthalein with solid sodium carbonate and exhausted with chloroform. The chloroform was run off carefully through a dry filter paper, concentrated to half its volume and titrated with N/20 *p*-toluenesulphonic acid as in the method described by Loftus Hills, Trautner, and Rodwell (1945), to give the first titration of bases.

After titration the chloroform extract was exhausted with dilute acid, the acid extract made alkaline with sodium carbonate, and exhausted with chloroform. The chloroform was run off through a dry filter paper, concentrated, and then titrated with N/20 *p*-toluenesulphonic acid to give the second titration of bases.

This purification cycle was repeated until two successive titrations did not differ by more than 0.75 ml. N/20 acid, equivalent to 0.1 per cent. of bases calculated on a molecular weight of 300.

The final chloroform solution was exhausted with dilute acid and, after being made alkaline, shaken back into chloroform. The chloroform solution

was dried and titrated with standard N/20 picric acid in chloroform with dimethylaminoazobenzene as indicator. One-third volume of benzene was added, the solution left standing for 24 hr., and then the precipitated hyoscine picrate was filtered off, dried, and weighed. The mother liquors, after removal of hyoscine picrate, were examined by the addition of further small quantities of benzene and light petroleum and by the removal of all organic solvents and recrystallization of the oily residue from water, in an attempt to separate the other alkaloid picrates present.

(ii) *The Chromatographic Method.*—A continuous extraction cell of stainless steel was designed to facilitate several of the laborious operations involved in this method (Fig. 2). The most suitable dimensions depend on the characteristics of the shaker to be used but the sizes shown proved satisfactory with a shaker consisting of a box swinging on a 3 in. arc of a circle of 12 in. radius at 50 cycles per min.

Five g. of ground leaf was introduced into a compartment of the cell and carefully moistened with 40 ml. of 10 per cent. sodium carbonate. Standard N/10 sulphuric acid (15 ml.) was introduced into the other compartment and ether added until the partition was well covered. The cell was set lengthwise in the shaking machine for 24 hr., during which time the ether became green or yellow-green, but the acid remained colourless. The acid was pipetted out, the compartment rinsed twice with water, and the excess acid titrated with standard N/10 sodium carbonate, using methyl orange as indicator. A further 2 ml. of standard acid was introduced, shaken for several hours, pipetted out and the excess titrated as before. Extraction was continued until no more than 0.5 ml. of N/10 acid was used in a 12-hr. run. Complete extraction from the leaf was slow, often taking 6-7 days, but the time required for manipulation was short and the extraction proceeded steadily without the use of heat. No evidence of hydrolysis was observed.

The titrated solution was made alkaline by the addition of solid sodium carbonate and replaced in the cell. Acid was placed in the other half as before and the second stage completed in a similar manner. This process was repeated until the total titration values at the completion of successive cycles did not differ by more than 0.4 ml. of N/10 acid, equivalent to 0.1 per cent. of bases calculated on a molecular weight of 300. The first cycle was usually complete in 48 hr., and later ones within 24 hr.

The final aqueous solution was made alkaline and exhausted, either by repeated extraction with chloroform in a separating funnel or in a continuous liquid-liquid extractor. In the latter case the chloroform slowly darkened to orange as extraction proceeded. In each case the resulting chloroform solution was evaporated to 10 ml., and an aliquot of the solution equivalent to 1 g. of the original plant material was titrated against N/100 *p*-toluenesulphonic acid in chloroform, with dimethylaminoazobenzene as indicator. In spite of several lengthy extractions, a further loss of bases occurred during this transfer. The residual aqueous extract was negative to Mayer's reagent and no further basic material could be extracted with chloroform. Complete transfer of known

mixtures of hyoscine and hyoscyamine was effected under similar conditions and without difficulty. It is assumed that this final titration of bases in chloroform gave the best estimation of the total alkaloids present.

The alkaloids were separated on a chromatographic column of the type described by Evans and Partridge (1948), and the various bases estimated by titration. The support used for the stationary phase was "Hyflo-Supercel,"

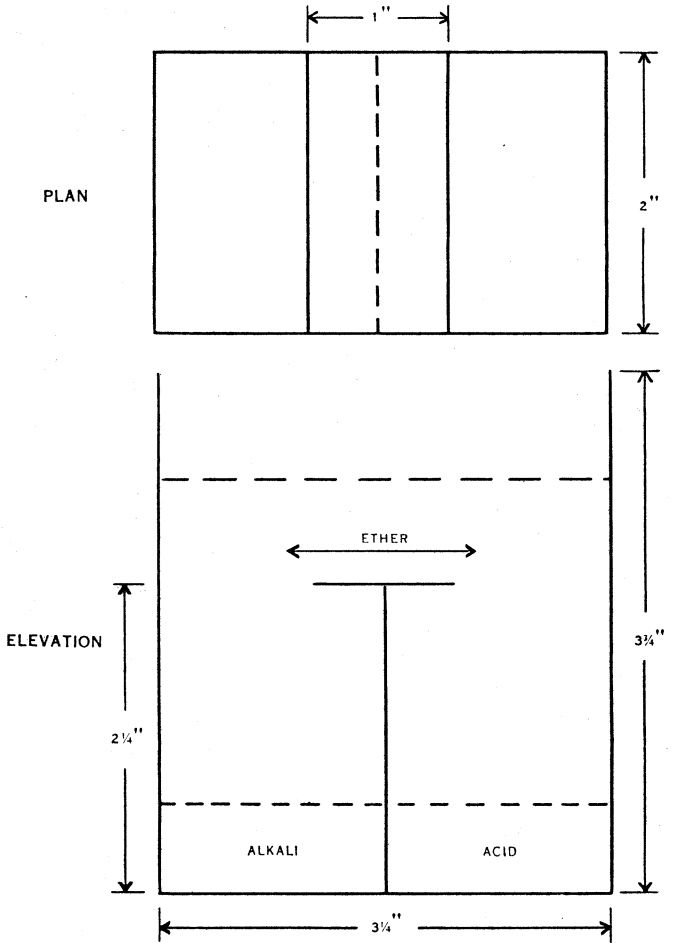

Fig. 2.—The cell for continuous extraction of the alkaloids.

a standardized kieselguhr prepared by the John's-Manville Co., U.S.A. Twenty g. of the material were moistened evenly with 4 ml. of M/2 phosphate buffer at pH 7.0, and packed into a Pyrex glass tube 40 cm. long by 1 cm. in diameter, with a stopcock sealed on the lower end. Packing was done carefully by first partly filling the tube with ether saturated with the buffer and then introducing the supercel in small lots, packing each lot firmly with a perforated disc

plunger. After the packing was completed 100 ml. of buffered ether were run through under gravity. The column was then mounted on a mechanically operated fraction collector arranged so that 5 ml. aliquots were delivered to individual test tubes set on the periphery of an intermittently rotating circular platform.

An aliquot of the chloroform extract equivalent to 1 or 2 g. of dried leaf was used for the separation of the alkaloids. After evaporation of the chloroform the residue was taken up in ether, introduced into the top of the column, and allowed to soak in. A reservoir containing ether that had previously been saturated with the buffer was attached to the top of the column and a constant air pressure of 15-20 mm. of mercury applied.

The ether was evaporated from each 5 ml. aliquot and the residue dissolved in chloroform and titrated with N/100 *p*-toluenesulphonic acid. Development with ether was continued for 60 aliquots (300 ml.), or more if titratable bases were still appearing in the eluate. Development was then continued with chloroform saturated with the buffer until no further bases appeared. If the total titration value of the eluate was still appreciably lower than that determined just before chromatography, development was continued with 1:1 butanol-chloroform.

Hyoscine first appeared at about the fifth aliquot and reached its maximum concentration very rapidly. It was usually recovered *in toto* by the fifteenth aliquot although, particularly with plant extracts, the titration did not always immediately fall to zero.

Hyoscyamine generally appeared at about the thirtieth aliquot, rising and falling very slowly, so that often it was not completely recovered until the sixtieth aliquot. However, its exact position varied from time to time, even when dealing with replicate samples from the same solution of pure alkaloids.

Three of the minor alkaloids of *Duboisia* described by Barger, Martin, and Mitchell (1937) were also investigated. Tigloidine appeared in the eluate fractions together with hyoscine but it has not yet been possible to devise a means of separating them. Valeroidine was eluted with ether and appeared between hyoscine and hyoscyamine, although some variation in its position has been observed, and overlapping, particularly with the latter, may occur. Base Z, which is a mixture of poroidine and *iso*poroidine only appeared after development with chloroform.

In certain cases the alkaloids appearing in the region of particular peaks were identified by examination of their picrates. The titrated solutions were exhausted with dilute acid, the acid extract made alkaline with ammonia, and the resulting solution exhausted with ether. The ether was then layered over saturated aqueous picric acid in a test tube, and allowed to stand while crystallization proceeded at the interface. Hyoscine was particularly easy to identify by this means, and the melting point of the crystals was seldom more than a few degrees below that of the pure salt. The peak representing a mixture of hyoscine and tigloidine yielded a picrate with a melting point close to that of hyoscine picrate, so that tigloidine could not be detected by the method.

If hyoscyamine was present in reasonable quantity it could usually be identified, although overlapping impurities such as valeroidine and other minor alkaloids were apparently often also present in the region in which hyoscyamine occurs. This general problem, together with the characterization of other peaks, is still under investigation.

The recovery of total alkaloids from the column ranged from 100 to 65 per cent. Results with known mixtures showed that when the recovery was less than 100 per cent. a higher proportion of hyoscine was recovered than of alkaloids of greater basicity. Duplicate aliquots of each extract were fractionated separately and the amount of hyoscine calculated from the duplicate showing the greater recovery.

In the first of the five groups of samples assayed by this method (Canberra 1946-1947) the ground leaf was extracted with methanol in the manner described for the picrate assay, instead of with ether in the extraction cell. The former procedure generally led to somewhat higher titrations for total bases than did the latter.

TABLE 2

TOTAL ALKALOID CONTENT OF CLONES OF NORTHERN *D. MYOPOROIDES* AT DIFFERENT TIMES AND PLACES

	Total Alkaloids* as Percentage of Dry Matter of Leaf					
Clone No.	Canberra, 1946-47 Summer	Canberra, 1947-48 Summer	Canberra, 1948-49 Summer	Nambour, 1947 Winter	Nambour, 1947-48 Summer	Mean† per Clone
327-1	2.0	5.0	3.3	3.3	4.3	3.6
355-1	2.0	1.2	2.0	3.7	2.5	2.7
370-1	2.0	3.0	1.5	1.1	2.7	2.1
428-1	1.7	3.3	1.5	2.6	2.7	2.4
437-2	2.0	2.7	3.0	2.1	3.3	2.6
455-1	1.6	3.6	2.6	2.3	3.8	2.8
468-1	1.3	3.6	2.5	3.3	2.6	2.9
Mean ‡ per sampling	1.8	3.2	2.3	2.6	3.1	

* Calculated from the final titration of bases immediately prior to the separation of the alkaloids in the chromatographic method.

† Minimum difference for significance at the 5% level = 0.92.

‡ Minimum difference for significance at the 5% level = 0.79.

(*c*) *Results and Discussion*

(i) *Total Alkaloids.*—The percentages of total alkaloids calculated from the final titration of bases in chloroform are given in Table 2. The individual values range from 1.1 to 5.0 per cent. and the means per clone from 2.1 to 3.6 per cent. The differences between clones are of marginal significance ($P = 0.07$). There is no significant difference between locations but the differences between times within locations are highly significant ($P < 0.01$).

Differences between clones and between times of harvest are not unexpected but it is of interest that a subtropical coastal species may produce as great a quantity of alkaloids when grown in an inland environment having a continental climate as when cultivated in its original habitat.

(ii) *Hyoscine.*—The hyoscine content of all five sets of samples was determined by the chromatographic method, and also, in three of them, by the picrate method. The percentages of hyoscine found by both methods are shown in Table 3. The results are in reasonably good agreement, with the exception of clone 437-2, which often failed to yield picrate precipitates, but gave a positive identifiable hyoscine component when fractionated in the column.

TABLE 3

HYOSCINE CONTENT OF CLONES OF NORTHERN *D. MYOPOROIDES* AT DIFFERENT TIMES AND PLACES

	Hyoscine as Percentage of Dry Matter of Leaf								
	Canberra, 1946-47 Summer	Canberra, 1947-48 Summer		Canberra, 1948-49 Summer		Nambour, 1947 Winter		Nambour, 1947-48 Summer	
Clone No.	Chrom. Method	Picrate Method	Chrom. Method	Picrate Method	Chrom. Method	Picrate Method	Chrom. Method	Chrom. Method	Mean† per Clone
327-1	0.9	1.5	1.6	1.5	1.5	0.6	0.7	2.6	1.5
255-1	0.9	—	1.0	—	—	—	1.7	1.1*	1.2
370-1	0.3	1.0	1.3	0.8	0.6	0.6	0.4	1.7	0.9
428-1	0.5	0.8	1.1	0.8	0.5	0.6	0.6	1.5	0.8
437-2	0.2	0.0	0.3	0.2	0.3	0.0	0.3	1.7	0.6
455-1	0.5	2.0	1.9	1.9	1.8	0.6	0.5	2.1	1.4
468-1	0.5*	1.7*	1.8*	1.7*	1.6*	0.9	1.2	1.1*	1.2
Mean per sampling‡ excluding clone 355-1	0.48		1.33		1.05		0.62	1.78	

* A composite sample from a number of siblings of the clone.

† Minimum difference for significance at the 5% level = 0.50.

‡ Minimum difference for significance at the 5% level = 0.46.

Although there is considerable variation in the relative yields of the clones due to environmental factors and possibly also to errors of assay, it is clear that some clones produce hyoscine in greater quantity on the average than others; as the overall differences between them are statistically significant ($P < 0.01$). The interaction between clones and seasons within places is greater than that between clones and places, which suggests that clones may rank similarly in different environments. However, only two locations are involved in the present data and it is possible that interactions may occur with other geographical locations.

During the course of preliminary experiments, over 200 samples were obtained at various times from many seedling lines and clones of *D. myoporoides*

growing in a number of plots. These were all assayed by a semi-quantitative method based on the characterization of the alkaloid picrates (Loftus Hills, Trautner, and Rodwell 1945), the amounts of hyoscine picrate being rated visually on a scale giving an observed total maximum score of 8. On this basis 15 samples of 437-2 and related seedlings gave an average rating of 0.5 and 20 samples of family 327 an average of 4.0.

It is clear that a range of genotypes exists within the northern section of *D. myoporoides*. Clone 437-2 is typical of those yielding appreciable quantities of hyoscine only under exceptional circumstances, and 327-1 of those yielding substantial amounts of the alkaloid under most circumstances. A selection programme, particularly in relation to a specific geographical environment, should be effective in establishing high hyoscine-yielding clones.

The geographical origin of the seed from which the clones were derived does not appear to have influenced the hyoscine content in any regular manner, the maximum and minimum values being observed in clones having their origin about the middle of the zone of distribution, and intermediate values being recorded from both the northern and southern geographical extremities. It should be noted that we have been dealing only with a few individual seedling isolates and it is impossible to generalize regarding regional population trends from the results presented. It does not follow, for example, that trees grown from bulk seed collected at Grafton (437-2) will prove inferior in hyoscine content to material from the Coolangatta area (455-1, 468-1).

Visual estimates indicated that the yield of leaf per tree was of the same order for each of the seven clones. For example there was little if any difference in the weight of leaf produced by clones 437-2 and 455-1, and hence the considerable differences between them in percentage of hyoscine may be regarded as indicative of differences in yield of hyoscine per tree and per unit area.

The differences in average hyoscine content between samplings are statistically highly significant ($P < 0.01$); the concentration of hyoscine being least for the first sampling after establishment at both centres and greatest for the summer harvest at Nambour. It is noteworthy that the hyoscine content of the clones was not substantially different in the two contrasting environments.

Hyoscine seldom comprised more than two-thirds of the total alkaloids found and occasionally represented as little as one-tenth of the whole. In Table 4 the hyoscine figures are given as percentages of the total alkaloids. There was considerable variation, and little can be deduced beyond the fact that the alkaloids of clone 437-2 were frequently characterized by an unusually low proportion of hyoscine.

The relationship of the amount of hyoscine to the other alkaloids present is of interest, as it may give some indication whether production is limited by competition for common precursor materials or whether hyoscine synthesis proceeds independently of that of other alkaloids.

No correlation was found within clones at different times between percentage of hyoscine and percentage of alkaloids not hyoscine ($r = -0.0094$).

This result is in conformity with an hypothesis of independent production but other interpretations are possible* and the final solution must await further experimental evidence.

TABLE 4

PROPORTION OF HYOSCINE IN ALKALOIDS FROM CLONES OF NORTHERN *D. MYOPOROIDES* AT DIFFERENT TIMES AND PLACES

Clone No.	Hyoscine Content as Percentage of Total Alkaloids Found					
	Canberra, 1946-47 Summer	Canberra, 1947-48 Summer	Canberra, 1948-49 Summer	Nambour, 1947 Winter	Nambour, 1947-48 Summer	Mean per Clone
327-1	43	42	47	27	60	44.5
355-1	61	93	---	65	42	65.3
370-1	16	63	37	34	59	41.6
428-1	28	38	38	30	57	38.1
437-2	11	13	13	15	56	21.7
455-1	36	68	78	20	59	51.2
468-1	39*	61*	72*	46	42*	51.2
Mean per sampling	33.4	54.0	47.5	33.9	53.6	

* A composite sample from a number of siblings of the clone.

(iii) *Alkaloids other than Hyoscine.*—Alkaloids other than hyoscine accounted for from about 30 to 90 per cent. of the total alkaloids found. Even in samples yielding maximum amounts of hyoscine, nearly half the alkaloids remain unaccounted for. Efforts are now being made to characterize these substances so only preliminary data are presented. Some information has been obtained from the chromatograms, some by the examination of picrates prepared from the filtrates after the removal of hyoscine picrate, and from bulk extracts from four of the clones at Canberra in 1948. Positive identification of one alkaloid does not exclude the possible presence of additional alkaloids in major amounts, and in fact in certain cases where hyoscyamine has been identified other alkaloids are indicated by the presence of further major peaks in the chromatograms. Similarly, failure to establish the presence of identifiable alkaloids does not necessarily exclude their presence, although in such cases they are unlikely to occur in major amounts.

Typical chromatograms are shown in Figure 3. Figure 3*A* is typical of samples high in hyoscine and relatively free from other alkaloidal substances. Two-thirds of the alkaloids recovered from this column are accounted for by the clearly defined hyoscine peak. The remaining third is made up by two unidentified peaks, one lying between hyoscine and the expected position for hyoscyamine, and the other in the chloroform zone. Figure 3*B* represents a

* An alternative explanation is that the ratio of hyoscine to other alkaloids changes as the total amount of alkaloids changes, the change in the ratio with total amount to be such as to give no correlation in the absolute amounts of each produced. Such an hypothesis cannot be excluded even though the correlation within clones between the ratio of hyoscine to remaining alkaloids and the total amounts is not significant ($r = +0.1049$).

sample yielding appreciable amounts of both hyoscine and hyoscyamine. In contrast to the previous chromatogram the successive titration values rarely fall to the base level and it would appear that at least four substances of different basicities are present. A still greater contrast is provided by the sample of 437-2 illustrated in Fig. 3*C*. In this case only a small amount of hyoscine appeared, the major alkaloids being hyoscyamine (m.p. of picrate = 167°C.) and an unknown represented by a prominent peak in the chloroform zone (m.p. of picrate = 206°C.).

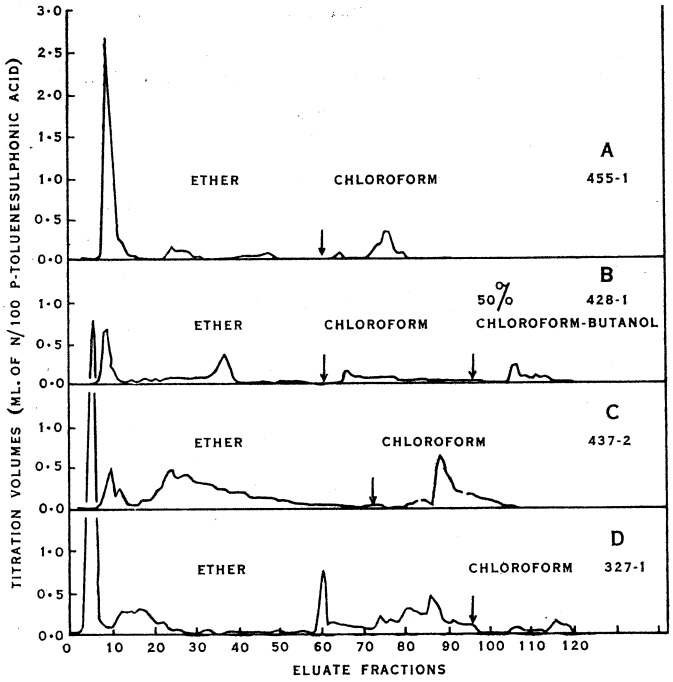

Fig. 3.—Typical chromatograms showing the bases eluted through a buffered kieselguhr column by successive 5 ml. aliquots of solvent, for four contrasting leaf samples from clones of northern *D. myoporoides*.

An approximate estimate of the amount of hyoscyamine was made from some of the chromatograms where the alkaloid appeared as a well-isolated peak and could be identified. The values ranged from 0.3 to 1.2 per cent. It only appeared in quantity at the first sampling after transplanting at both locations when it was positively identified in nine of the 14 samples. Seasonal conditions at the two places are unrelated and it seems possible that the effect may be associated with establishment factors rather than environmental conditions in general. The data do not establish that the presence or absence of hyoscyamine is an inherent character of any particular clone, although unpublished data concerning further material of northern *D. myoporoides* suggests that the tendency to produce hyoscyamine is in fact a genetic variable within the group.

Valeroidine was found in the bulk extract of clone 327-1 to the extent of 0.2 per cent. of the dry weight of the original leaf, but was not found in the other three clones extracted in bulk. The chromatogram is shown in Figure 3*D*. A substantial peak is apparent in the expected position for valeroidine. Although valeroidine could not be positively identified in the other four samples of 327-1, characteristic valeroidine peaks were apparent in three of them. Occasional samples in certain of the other clones showed minor peaks in the expected position but they could not be positively identified. It does appear that clone 327-1 has a greater capacity for the production of valeroidine than the other six, and in fact it may be that some of them are incapable of synthesizing that alkaloid.

Barger, Martin, and Mitchell (1937) found valeroidine in small amounts in leaf of *D. myoporoides* of unstated origin. Its occurrence in larger quantities in a particular clone suggests the interesting possibility that some at least of the so-called minor alkaloids of *Duboisia* may occur in major amount in certain genotypes of infrequent occurrence. The character of such individual trees would be lost in bulk collections. Support to the idea is given by the earlier observation (Loftus Hills, Trautner, and Rodwell 1945) that certain individual trees yield an as yet unidentified alkaloid with a high melting point picrate in major amounts.

Of the 10 samples for which additional information regarding the alkaloids other than hyoscine was obtained by examination of the filtrates after the removal of hyoscine picrate, six yielded appreciable quantities of picrate melting between 200° and 220°C. The data are compatible with the suggestion that clone 428-1 yields this unknown alkaloid in greater quantity than do some other clones, such as 370-1.

In general therefore it seems that the alkaloids other than hyoscine include singly or in various combinations hyoscyamine, valeroidine, an unknown alkaloid with a picrate of high melting point, and one or more other alkaloids of unknown character and composition. The pattern of these alkaloids remains indefinite and it has not yet been possible to relate their appearance to either season or place.

III. Conclusions

(1) Consistent differences in hyoscine content occur among clones derived from seed originating within the zone of distribution of the northern or hyoscine type of *Duboisia myoporoides.* The most probable explanation is that the clones differ genetically in their capacity to produce hyoscine. The variation does not appear to be related in any regular manner to the geographical origin of the seed from which the clones were derived, or to their vegetative characters.

(2) The average percentage of hyoscine in seven clones sampled for three summers at Canberra, A.C.T., and a summer and winter at Nambour, Queensland, ranged from 0.6 to 1.5 per cent. with values for individual samples ranging from 0.2 to 2.6 per cent.

(3) There was considerable variation between samplings in each of the two locations, but the yield of hyoscine was of the same order of magnitude

at both. Some interaction occurred between location and time of sampling, and the yield of hyoscine from individual clones.

(4) Alkaloids other than hyoscine comprised from 30 to 90 per cent. of the alkaloids found in individual samples. The amount of hyoscine present was not related to the amount of other alkaloids. The other alkaloids included singly or in various combinations hyoscyamine, valeroidine, an unidentified alkaloid having a picrate of high melting point, and one or more additional unidentified alkaloids.

(5) The presence of hyoscyamine in quantity was characteristic of the first sampling in both environments, but genetic differences in the tendency to produce hyoscyamine were not established.

(6) Valeroidine appeared in quantity in one clone. The tendency to produce it is probably influenced by both environmental and genetic factors.

(7) An unidentified alkaloid with a high melting point picrate appeared frequently. The tendency to produce it is probably influenced by both environmental and genetic factors.

(8) The total alkaloid content varied from year to year at Canberra, and from winter to summer at Nambour, but was of the same order of magnitude at both places. There was some indication that the total alkaloids were influenced by the genotype, but the data were not conclusive.

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V. References

Barger, G., Martin, W. F., and Mitchell, W. (1937).—The minor alkaloids of *Duboisia myoporoides*. I. *J. Chem. Soc.* **1937**: 1820-23.

Evans, W. C., and Partridge, M. W. (1948).—The partition chromatography of alkaloids. I. Solanaceous alkaloids. *Quart. J. Pharm.* **21**: 126-37.

Loftus Hills, K.. and Kelenyi, G. P. (1946).—A preliminary report upon the cultivation of *Duboisia* spp. *J. Coun. Sci. Industr. Res. Aust.* **19**: 359-75.

Loftus Hills, K., Trautner, E. M., and Rodwell, Cynthia N. (1945).—A preliminary report upon variation in the nature and quantity of the main alkaloids in *Duboisia myoporoides* and *Duboisia leichhardtii*. *J. Coun. Sci. Industr. Res. Aust.* **18**: 234-53.

Trautner, E. M., Neufeld, O. E., and Rodwell, Cynthia N. (1948).—The titration of hyoscine, hyoscyamine and atropine with picric acid in chloroform. Separation and fractionation of the picrates. *Aust. Chem. Inst. J. and Proc.* **15**: 55-61.