ALKALOID BIOGENESIS

PART II. CHANGES IN THE ONTOGENETIC PRODUCTION OF ALKALOIDS IN *Atropa* AND *Datura*

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IN the course of a programme of experimental work on the biogenesis of the alkaloids of the tropane group, information was required on changes in the proportions of the individual alkaloids typical of the species during the life cycle of the plant. This work has afforded evidence from which may be made certain speculations on the problem of alkaloid biogenesis. Moreover, the results have some relevance to the medicinal use of these plants. Fluctuations in the proportions of the individual alkaloids, although not detected by the usual methods for the standardisation of these drugs, could be expected to lead to fluctuations in the physiological effects of galenical preparations of the drugs.

EXPERIMENTAL

The plants studied were *Atropa belladonna* and *Datura tatula* var. *inermis*. The results of a limited number of experiments on *Datura ferox* are also discussed. Plant material from specimens, cultivated in the usual manner and dried at 60° to 65° C., was analysed by the procedure we have described previously[1]. The identity of the alkaloids was checked in a number of cases by the preparation of crystalline derivatives. Collection of the aerial parts of *A. belladonna* was continued each year until damage due to frost occurred. The roots and rootstock of third-year plants were so large as to render sampling difficult; the material analysed was a representative sample from 4 plants.

Analytical results are recorded in Tables I and II and, for clarity, certain of the results are presented graphically in Figures 1 and 2.

DISCUSSION

In the interpretation of the results, it is recognised that information on the percentage of alkaloid in a dried sample of plant material may in many instances be of little use in making deductions relating to the process of alkaloid synthesis in the plant. Difficulties in expressing the results in a significant manner are illustrated by the values recorded in Table III. In 2 sets of samples selected for approximate constancy of the average weight of their roots, the percentage of alkaloids and the absolute weight per root are roughly equivalent. The average weights of the shoots growing on these roots differed widely and whereas the percentage of alkaloid decreased during the month's growth, the absolute weight of alkaloid increased. However, certain useful deductions may be made when a given alkaloid is found not to be formed or when changes occur in the relative proportions of the individual alkaloids. The re-location of the alkaloids said to occur

TABLE I

HYOSCINE AND HYOSCYAMINE IN *A. belladonna*

Sample	Date of collection	Hyoscine		Hyoscyamine	
		In dried sample per cent.	Proportion of total alkaloids per cent.	In dried sample per cent.	Proportion of total alkaloids per cent.
First year plants—					
Small seedlings	1.6.51	0·040	89	0·005	11
Young plants beginning to flower	8.8.51	0·062	15	0·34	85
Flowering plants with young fruits:—					
Aerial parts	27.8.51	0·030	10	0·27	90
Old leaves from base of plants	27.8.51	0·004	5	0·072	95
Tops of flowering branches	27.8.51	0·070	15	0·40	85
Young shoots from decapitated plants	11.9.51	0·15	26	0·42	74
Fruiting plants:—					
Aerial parts	26.9.51	0·00	0	0·21	100
Tops of branches bearing leaves	26.9.51	0·031	6	0·51	94
Roots and rootstock	26.9.51	0·022	6	0·32	94
Shoots from decapitated plants	3.11.51	0·00	0	0·11	100
Second year plants—					
Plants with unexpanded shoots, 3 in. tall:—					
Aerial parts	8.3.52	0·052	8	0·60	92
Roots and rootstock	8.3.52	0·020	4	0·49	96
Plants 6 in. to 9 in. tall:—					
Aerial parts	15.4.52	0·028	6	0·44	94
Roots and rootstock	15.4.52	0·015	2	0·61	98
Plants 3 ft. tall beginning to flower:—					
Aerial parts	12.5.52	0·002	1	0·28	99
Roots and rootstock	12.5.52	0·001	0·5	0·33	99·5
Flowering plants:—					
Aerial parts	13.6.52	0·006	3	0·19	97
Tops of flowering branches	13.6.52	0·018	4	0·39	96
Roots and rootstock	13.6.52	0·001	0·5	0·27	99·5
Flowering plants with young fruits:—					
Aerial parts	15.7.52	0·001	0·5	0·18	99·5
Tops of flowering branches	15.7.52	0·022	4	0·59	96
Unripe fruits	15.7.52	0·005	2	0·31	98
Roots and rootstock	15.7.52	0·003	1	0·32	99
Fruiting plants with immature fruits:—					
Aerial parts	14.8.52	0·00	0	0·21	100
Tops of flowering and fruiting branches	14.8.52	0·00	0	0·36	100
Roots and rootstock	14.8.52	0·007	2	0·27	98
Fruiting plants with mature fruits:—					
Aerial parts	19.9.52	0·00	0	0·095	100
Tops of fruiting branches	19.9.52	0·003	1	0·21	99
Fruits	19.9.52	0·00	0	0·12	100
Roots and rootstock	19.9.52	0.00	0	0·31	100
Shoots from decapitated plants	19.9.52	0·00	0	0·67	100
Roots and rootstock	20.10.52	0·002	0·5	0·32	99·5
Roots and rootstock	15.12.52	0·005	1	0·50	99
Underground buds	15.12.52	0·00	0	0·64	100
Third year plants—					
Roots and rootstock	12.1.53	0·00	0	0·40	100
Underground shoots	12.1.53	0·005	1	0·70	99
Seeds (viable)	23.1.53*	0·003	1	0·29	99
Emerging shoots	17.2.53	0·013	4	0·32	96
Roots and rootstock	17.2.53	0·004	1	0·36	99
Plants 2 in. to 3 in. tall:—					
Aerial parts	16.3.53	0·008	1	0·70	99
Roots and rootstock	16.3.53	0·00	0	0·49	100
Plants 3 in. to 4 in. tall:—					
Aerial parts	27.3.53	0·00	0	0·59	100
Roots and rootstock	27.3.53	0·00	0	0·53	110

* Date of analysis.

during drying[2] can have no effect upon the results since each part was dried separately before analysis.

Changes in the total alkaloid content of the aerial parts of *A. belladonna* shown in Figure 1 are in agreement with the observations of other investigators[3,4,5,6] and demonstrate that during the first year, the concentration rises to a maximum at the beginning of flowering and then declines during fruiting. Young shoots arising during the period of the late stages of flowering on decapitated plants are considerably richer in alkaloids than similar shoots which develop later in the season.

TABLE II

HYOSCINE AND HYOSCYAMINE IN *D. tatula*

Sample	Date of collection	Hyoscine		Hyoscyamine	
		In dried sample per cent.	Proportion of total alkaloids per cent.	In dried sample per cent.	Proportion of total alkaloids per cent.
Seeds (viable)	20.9.51	0·045	24	0·14	76
Young plants, 2 in. to 3 in. tall ..	27.6.52	0·17	82	0·038	18
Plants beginning to flower ..	15.7.52	0·15	65	0·080	35
Flowering plants with immature fruits	27.7.52	0·14	52	0·13	48
Flowering plants with immature fruits	15.8.52	0·075	45	0·093	55
Fruiting plants:—					
Aerial parts	10.9.52	0·053	32	0·11	68
Leaves and tops	10.9.52	0·065	32	0·14	68
Fruiting plants	7.10.52	0·045	29	0·11	71
Seeds (dried)	7.10.52	0·058	29	0·14	71
Seeds (viable)	7.10.52*	0·090	33	0·18	67

* Date of analysis 16.2.53.

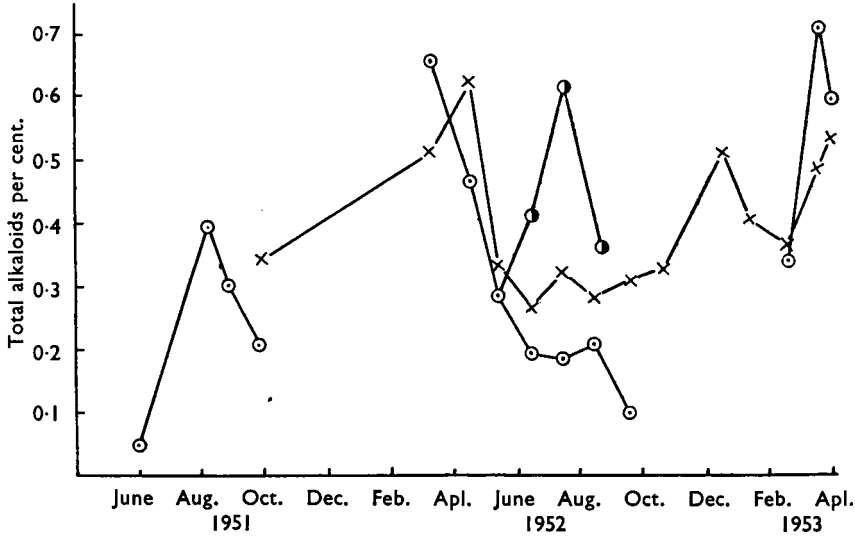

FIG. 1. Variations in total alkaloids of the aerial parts, roots and rootstock and tops of flowering branches of *Atropa belladonna*.

⊙——⊙ Aerial parts.
×——× Root and rootstock.
◑——◑ Tops of flowering branches.

Alkaloid accumulation in the aerial parts during the second year follows a somewhat different course. The concentration decreases rapidly during growth until flowering begins, remains steady during flowering and declines during fruiting. The changes in concentration of total alkaloids in the young tops resemble those in the aerial parts of the first-year plants; a

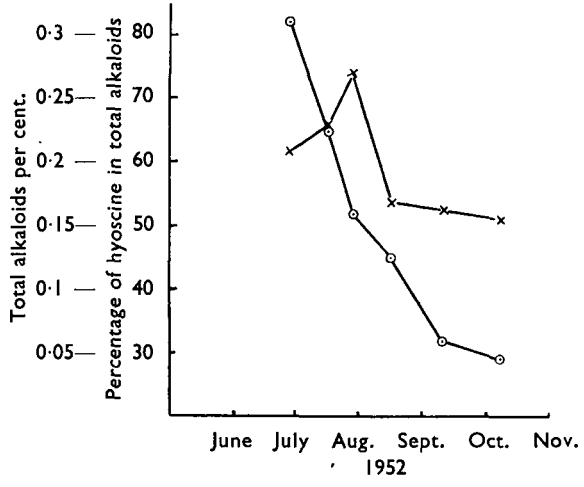

FIG. 2. Variation in the total alkaloids and the percentage of hyoscine in the total alkaloids of *Datura tatula*.

⊙——⊙ Percentage of hyoscine in total alkaloids.

×——× Total alkaloids.

maximum concentration is attained at the period of flowering. It would therefore appear that by excluding the young tops from the aerial parts of the second-year plants, a steady decline in alkaloid concentration would be observed. This implies that there is some parallelism between total

TABLE III

TOTAL ALKALOID PRODUCTION DURING ONE MONTH'S GROWTH OF *A. belladonna*

Date of collection	Average weight of dried part studied		Total alkaloids in dried material		Weight of total alkaloids per individual part	
	Root	Shoot	Root	Shoot	Root	Shoot
	g.	g.	per cent.	per cent.	g.	g.
15.4.52	18	10	0·63	0·47	0·113	0·048
12.5.52	22	67	0·33	0·28	0·072	0·187

alkaloid production and overall growth of the young parts of the plant. In agreement with this, shoots developing on decapitated plants during the period of decline in alkaloid concentration in the normal plant contain roughly 3 times the concentration of alkaloid found in the tops of fruiting branches of the intact plant. The early stages of the third-year plants

resemble those observed during the second year; the concentration falls markedly during about 1 inch growth in 10 days in March.

Changes in alkaloid concentration in the roots were followed during about 18 months from the end of the first season and were found to follow roughly the same pattern as those recorded for the aerial parts.

The ratio of hyoscine to hyoscyamine in *A. belladonna* is not simply related to the quantity of total alkaloids. Seeds in which hyoscine forms only 1 per cent. of the total alkaloids produce seedlings in which hyoscine is the predominant alkaloid. This condition is reversed by the time the young plants have begun to flower and thereafter the proportion of hyoscine in the total alkaloids declines until little or none is detectable in the second- and third-year plants. It is probable that the capacity to produce hyoscine does not disappear completely at any time, since, under the stimulation of new growth produced during the normal growing season, significant quantities are usually produced; this is particularly well demonstrated by the quantity found in the young shoots formed on decapitated plants. These findings provide corroboration of the histochemical evidence for the production of alkaloids in meristematic tissues[4]. The production of hyoscine in the root apparently declines with age.

The changes in the total alkaloids of *D. tatula* var. *inermis* summarised in Table II and Figure 2 show that the concentration rises to a maximum at the time of flowering. In a short series of experiments, similar changes were observed in *D. ferox*.

Changes in the relative proportions of hyoscine and hyoscyamine in *D. tatula* appear to have no simple relationship to the concentration of total alkaloids. From seeds in which hyoscine forms 24 per cent. of the total alkaloids, there are produced seedlings in which this proportion has risen to 82 per cent. From Figure 2, it can be seen that in the latter stages of growth up to fruiting the proportion of hyoscine gradually drops to about 30 per cent. of the total alkaloids and then remains almost steady. Hegnauer[6] has recorded qualitative evidence which agrees with these results.

In *D. ferox* the total alkaloids in the seeds contain 95 per cent. of hyoscine and no meteloidine. On germination, the proportion of hyoscine falls to 50 per cent. and that of meteloidine rises to 26 per cent. of the total alkaloids; in the later stages of growth up to flowering the proportion of hyoscine rises gradually to 85 per cent., whereas meteloidine then forms only 7 per cent. of the total alkaloids. Unidentified bases, other than hyoscine and meteloidine, make up the proportions of total alkaloids not accounted for.

In these 3 solanaceous plants, each of which produces hyoscine, the pattern of changes in its ratio to the other alkaloids differs in each case, although in *A. belladonna* and *D. tatula* there exists the quantitative similiarity of a decrease in its proportion during growth. However, in all cases, changes in the rate of production of alkaloids oxygenated at the 6 and 7 positions of the tropane ring appear to be related to the processes occurring in the plant at times of great metabolic activity.

In *A. belladonna*, where the production of an oxygenated tropane

alkaloid is very small, the site of alkaloid biogenesis is the roots[7]. The aerial parts of *D. tatula* and *D. ferox* are capable of synthesising hyoscyamine and meteloidine respectively and it is probable that hyoscine is formed in the aerial parts of *D. tatula* and in both the roots and aerial parts of *D. ferox*[8].

In the absence of further biological evidence, an interpretation of these results in terms of the various speculations on chemical mechanisms for the biosynthesis of tropane alkaloids[9,10,11,12] is not possible. The results recorded here appear to provide some indication that the product of synthesis is related to the site of synthesis. When synthesis occurs in the aerial parts significant quantities of the oxygenated alkaloids, hyoscine and meteloidine, are produced, whereas in the roots the tendency is towards the production of hyoscyamine with the reduced tropane ring. Our earlier findings[8] on the site of biogenesis of the alkaloids of *D. innoxia* are, however, in direct contradiction to these deductions, since in this plant hyoscine is formed in the roots and hyoscyamine in the aerial parts. Unfortunately no information is available on changes in the ontogenetic production of the alkaloids in this plant. As biological evidence accumulates, it is becoming increasingly apparent that alkaloid synthesis is unlikely to be simply related to the general metabolic processes of the plant.

Summary

1. Changes in the ontogenetic production of the alkaloids of *A. belladonna* and *D. tatula* have been studied.

2. The greatest changes in the ratio of hyoscine to hyoscyamine usually occur at times of greatest meristematic activity. Similar features are to be observed in changes in the ratio of hyoscine and meteloidine in *D. ferox*.

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