RESEARCH PAPERS

ALKALOID BIOGENESIS

PART I. THE SITE OF SYNTHESIS OF ALKALOIDS IN *DATURA*

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From the University, Nottingham

Received February 3, 1953

IN a general investigation of the biogenesis of the alkaloids of a given plant the first step would appear to be the identification of the organ in which the synthetic process occurs. An abundance of experimental results connected with this problem is available[1,2]. Much of the information is not definitive because of the difficulties of the design and interpretation of experiments on this topic. Accordingly, it is not surprising that relatively little is known with certainty of the tissues involved, of the chemical mechanisms, of the enzymes associated with the process and of the correlation of alkaloid biogenesis with the general metabolism of the plant and with environmental factors.

THE PROBLEM OF THE LOCATION OF THE SITE OF ALKALOID BIOGENESIS

Sources of Evidence. Evidence on the site of alkaloid biosynthesis has been derived mainly from three sources. The selective accumulation of alkaloids throughout the plant tissues during the life-cycle has been studied by histochemical and macrochemical methods. Artificially cultured, isolated plant organs have been examined for the augmentation of alkaloids already present and for the appearance of alkaloids in organs initially devoid of them. Grafts, usually involving members of different genera in which there are normally significantly different capacities, both qualitative and quantitative, for alkaloid production, have been studied for the presence or absence of alkaloids foreign to one or both members of the graft combination.

Interpretation of Results. Probably the greatest hindrance to the making of valid deductions from experimental observations is related to the somewhat low degree of biochemical specialisation of plant cells and tissues. The growth of a plant organ in an abnormal environment may cause the adventitious development of degradative or synthetic biochemical processes which are normally latent. Experiments involving grafts and more especially the culture of isolated organs may afford misleading results for this reason.

Contrariwise, consideration must be given to the possibility of a certain degree of tissue or organ specialisation both for the whole process and for individual steps in the process. Tropine has been detected in a tobacco scion growing on a stock of *Duboisia myoporoides* and from this it has been suggested that, in the intact *Duboisia*, tropine formed in the root is converted into hyoscine in the aerial parts[3]. Dawson[4] has recently shown that demethylation of nicotine to nornicotine occurs in the green cells of the leaf-blade and petiole of *Nicotiana glutinosa*. This observation serves

also to direct attention to the possibility of secondary degradations of alkaloids as part of the overall metabolic process.

Since solanaceous alkaloids have been detected in the bleeding sap of *Datura* and of *Atropa belladonna*[5,6,7] and nicotine occurs in the fluid exuding from the cut xylem of tobacco[8], it can hardly be doubted that alkaloids can be translocated from the root to the aerial parts in the living plant. No evidence has been adduced of downward translocation in the phloem. Furthermore, if translocation across the graft union did not occur in grafted plants, the interpretation of numerous results would involve the improbable hypothesis that the act of grafting induces the development of totally new synthetic capabilities in the scion. It is therefore not possible to make unequivocal deductions from studies of the selective accumulation of alkaloids in plant tissues and organs.

Stock-scion Relationship in Grafts. The degree of abnormality of the conditions of growth for the members of a graft combination would seem to depend on the nature of the scion and the stock. A small alteration in metabolism caused by grafting may be sufficient to have an important effect on one or more of the stages of alkaloid biosynthesis; it is unnecessary to postulate an effect so fundamental as the total loss of synthetic powers or as the development of new synthetic powers.

Evidence is available to show that the stock and scion exert mutual influences upon one another[9]. Mothes and Romeike[10] have found that for the synthesis of alkaloids in belladonna scions on tomato roots, it is by no means immaterial what variety of tomato is used as the stock; similarly, the concentration of alkaloid in tomato scions on *Datura* stocks is influenced by the variety of tomato[11].

In the most favourable case, where the graft involves union of species of the same genus which produce alkaloids of similar structure, differences in the materials translocated between the stock and the scion are likely to be unimportant. With generically distinct plants, exhibiting both qualitative and quantitative differences in alkaloid production, the metabolic dissimilarities may be of considerable importance from the point of view of alkaloid biogenesis. The scion and stock of a graft of two such plants develop under conditions which may be decidedly abnormal and reliable information on the site of alkaloid biosynthesis is not necessarily obtainable from such experimental material.

Analytical Procedures. Experimental work on this topic has involved qualitative and quantitative analyses. Much work carried out hitherto has not attained a high degree of precision because of the use of non-specific procedures. This is particularly true of histochemical work and of tests based on the physiological activity of crude plant extracts. Only in a few instances have the alkaloids been isolated and authenticated as crystalline derivatives. The importance of complete authentification is illustrated by the observations on tobacco-*Duboisia* grafts previously mentioned[3].

Site of Biogenesis of Tropane Alkaloids

By histochemical tests, it has been shown that there is significant correlation between the occurrence of alkaloids and metabolically active

tissues, such as meristems and wound cambia. For example, in belladonna seedlings, strong reactions for alkaloids are obtained in both apical meristems but not in the hypocotyl. Alkaloids are detectable in wound cambia as soon as growth begins and then they spread to surrounding tissues[1]. These features appear to be more consistent with the local formation of the alkaloids than of translocation of alkaloids to the tissues.

Results derived from examinations of artificially cultured, isolated organs may be inconclusive when extrapolated to the problem of the site of synthesis of tropane alkaloids in the intact plant. That excised roots of *D. Stramonium* elaborate hyoscyamine when grown in sterile culture was shown by Peacock, Leyerle and Dawson[6]; parallel experiments on excised stem tips have not been carried out. James[12], in an extensive series of experiments on the culture of isolated leaves of belladonna, detected the production of a small but statistically significant quantity of alkaloids responding to the Vitali-Morin reaction, and concluded that some capacity for alkaloid synthesis resides in the leaves of this plant. Dawson[2], however, found this evidence unconvincing.

Amongst plants producing tropane alkaloids, grafts have been prepared which have involved the union of *Atropa belladonna*, *Datura stramonium* and *Duboisia myoporoides* with tomato, tobacco and potato[3,5,6,11,12,13,14,15,16,17,18,19]. From this work, it was concluded that the nature of the stock controlled the alkaloids in the scion. With a stock of a plant which normally produces tropane alkaloids, these were detectable in at least some parts of the scion; no tropane alkaloids were detected when such a plant was the scion. Similar conclusions have been reached from experiments with grafts involving *Hyoscyamus muticus*, *H. niger*, tomato, and *Physalis alkekengi*[20]. Strasburger[21] recorded the isolation of a small quantity of atropine from tubers formed on a potato stock on which a *D. Stramonium* scion had been grafted, but later workers[22,23] were unable fully to confirm this observation; mydriatic alkaloids were detected just below the graft union[24].

For reasons already given, the simple conclusion that the root is the sole site of alkaloidal biosynthesis may not be completely valid. Indeed, conflicting evidence is available. Traces of alkaloid localised in the meristems have been detected in belladonna leaves grafted on to tomato[17] and when tomato of the variety "Lukullus" is employed as the stock, a strong reaction for mydriatic alkaloids is obtained in the belladonna scion[10]. Subnormal quantities of alkaloids containing a small proportion of atropine appeared to be present in the young leaves, fruits, and seeds of *Datura* grafted on to tomato[25]. In grafts of *D. tatula* var. *inermis* on tomato stocks, small quantities of mydriatic alkaloids were found to be present in all parts of the plant, including the tomato stock; the tomato scion of the reciprocal graft contained up to nearly 0·3 per cent. of hyoscyamine, together with a small quantity of hyoscine, distributed in a manner consistent with its passive accumulation by translocation from the stock[7].

In order to avoid the possibility of factitious results obtained with grafts involving plants differing as widely as those employed hitherto, James and Thewlis[26] have studied grafts prepared from belladonna and *Datura*

innoxia. The differing ratios of the quantities of hyoscine and hyoscyamine normally produced in these plants were employed as the means of locating the site of synthesis. They concluded that the root was the site of synthesis of both alkaloids in these plants. Consideration was not given to the possibility that in *D. innoxia* hyoscine is formed in the roots and at least part of the hyoscyamine is formed in the aerial parts. We believe this to be the simplest conclusion from our experiments described below. The small quantity of hyoscyamine produced in the aerial parts of a *D. innoxia* scion on a belladonna stock when added to the relatively large quantity of hyoscyamine translocated to the scion from the stock would have a very small effect on the hyoscine:hyoscyamine ratio. Moreover, they assumed that the tropane alkaloid eluted from a partition column at *p*H 6·8 by chloroform was hyoscyamine. We find that *D. innoxia* contains, in addition to hyoscine and hyoscyamine, meteloidine which under the conditions they employed is eluted from the column with hyoscyamine[27].

It is therefore clear that it is possible to prepare grafts in which tropane alkaloids are formed mainly in the roots of the tropane-alkaloid-producing member of the combination. Some capacity for alkaloid synthesis appears to be retained with this member as the scion, but, even with the additional information provided by histochemical tests and isolated organ culture, the evidence is not conclusive.

Experimental

Cultivation of Grafts. The species studied were *Datura ferox*, *D. tatula* var. *inermis*, and *D. innoxia*. Seeds were sown in sterile, John Innes seed compost and kept for germination in a greenhouse at 25° C. Grafting was carried out when the seedlings had developed the first two foliage leaves. To prepare one type of graft, the plant destined to become the stock was decapitated near the seed leaves by a long, obliquely transverse cut. The stem of the plant destined to become the scion was sliced tangentially with a cut penetrating to the centre of the stem at its deepest part and equal in length to the transverse cut of the stock. The two cut stems were bound moderately firmly with stout thread and the plants were kept in the greenhouse for 5 days for the formation of the union. The scion root was then cut transversely at about 1 cm. below the graft union and the cut surface was sealed with soft paraffin. The binding was retained in position until the expanding stem showed the first signs of constriction and was then gradually slackened during about 7 days. A support for the plant was provided at this stage to prevent undue strain on the union.

For the preparation of combinations in which two aerial shoots, one normal to the species and the other of a different species, were allowed to grow on one root, the true approach method of grafting was employed. After the union had been allowed to develop for 5 days, the stem of the specifically different member was cut 0·5 cm. below the union and sealed with soft paraffin.

The plants were potted on in John Innes potting compost No. 2 and

planted out in the open at the end of May. The control plants were raised alongside the graft combinations.

In both seasons of 1951 and 1952 most of the graft combinations flourished and produced plants sufficiently large for analyses of individual plants to be carried out. The graft of *D. tatula* scion on *D. ferox* stock in the two seasons remained somewhat stunted, matured early, and developed little foliage; this necessitated the use of several plants for a single complete analysis. This combination appeared to be particularly susceptible to attack by insects and was badly attacked in the 1952 season.

The plants were collected at the flowering and fruiting stage, and the aerial parts, graft union, and roots were dried separately at 50 to 60° C.

Method of Analysis. Samples of the aerial parts of the grafts were analysed by the procedure we have described previously[28]; for a 10-g. sample, a column consisting of 5 ml. of phosphate buffer (0·5M) of *p*H 7·3 distributed on 10 g. of kieselguhr was used for the fractionation of the alkaloids. Eluate fractions corresponding to the separate peaks on the elution curve were combined for the preparation of crystalline derivatives.

From previous experience, it appeared probable that hyoscyamine and meteloidine, which could be expected to occur together in some of these graft combinations, would not be separated on a partition column of the type used. The behaviour of artificial mixtures was therefore examined. It was found that they were indeed eluted together from the column with

TABLE I

ALKALOIDS IN NORMAL AND GRAFTED *D. tatula*, *D. ferox* AND *D. innoxia*

Sample	Date of collection	Dry weight of scion g.	Hyoscine per cent.	Hyoscyamine per cent.	Meteloidine per cent.	Other bases as hyoscyamine per cent.
1. *D. tatula* var. *inermis*, flowering and fruiting tops (control)	18.8.51	—	0·12	0·24	—	0·01
	15.8.52	—	0·075	0·09	—	—
2. *D. ferox*, flowering and fruiting tops (control)	10.8.51	—	0·31	—	0·10	0·11
3. *D. ferox* scion grown on *D. tatula* stock:						
(*a*) Flowering tops	13.9.51	30	0·39	—	0·050	0·04
(*b*) Flowering and fruiting tops	20.8.52	110	0·25	—	0·034	0·03
4. *D. tatula* scion grown on *D. ferox* stock:						
(*a*) Aerial parts, excluding fruits	13.8.51	26	0·065	0·11	—	0·05
(*b*) Aerial parts, excluding fruits	28.7.52	14·7	0·043	0·025	—	—
5. *D. ferox* scion grown with *D. tatula* top on *D. tatula* stock:						
(*a*) *D. ferox* scion	11.8.52	75	0·175	—	0·037	0·03
(*b*) *D. tatula* tops	11.8.52	46	0·036	0·095	—	0·04
6. *D. tatula* scion grown with *D. ferox* top on *D. ferox* stock:						
(*a*) *D. tatula* scion	4.9.52	13	0·082	0·061	—	0·01
(*b*) *D. ferox* tops	4.9.52	71	0·105	—	0·044	0·03
7. *D. innoxia* tops (control)	26.9.52	—	0·235	0·033*		—
8. *D. innoxia* scion grown on *D. ferox* stock	6.9.52	73	0·095	0·103†		—
	26.9.52	25	0·043	0·025†		0·06
9. *D. ferox* scion grown on *D. innoxia* stock	19.9.52	15	0·43	—	0·095	—

* Meteloidine with a small quantity of hyoscyamine, calculated as meteloidine.
† Hyoscyamine and meteloidine, calculated as hyoscyamine.

ether or chloroform. Their separation by fractional crystallisation of their picrates was accordingly investigated.

RESULTS

Analytical results obtained from the grafts and control plants are given in Tables I and II. Certain of these results have already been reported in a preliminary communication[29].

TABLE II

CHARACTERS OF ALKALOIDAL PICRATES

Sample yielding picrate	M.pt. ° C.	Mixed m.pt. with appropriate authentic specimen. ° C.	Nature of picrate
1	163 to 164	163 to 164*	Hyoscyamine
2	175 to 176	175 to 176*	Meteloidine
3(a)	175 to 177	176 to 177	Meteloidine
3(b)	176 to 177	176 to 177	Meteloidine
4(a)	162 to 163	162 to 163	Hyoscyamine
4(b)	162 to 163	162 to 163	Hyoscyamine
5(a)	175 to 176	176 to 177	Meteloidine
5(b)	162 to 163·5	162 to 163	Hyoscyamine
6(a)	163 to 164	163 to 164	Hyoscyamine
6(b)	175 to 176	175 to 176	Meteloidine
7	176 to 177 (first fraction)	175 to 177	Meteloidine
	about 150 (crude second fraction)	—	—
	162 to 164 (after fractionation of second fraction)	162 to 164	Hyoscyamine†
	175 to 177 (after fractionation of second fraction)	175 to 177	Meteloidine
8	158 to 159 (crude)	—	—
	163 to 164 (after fractionation)	163 to 164	Hyoscyamine
	174 to 175 (after fractionation)	174 to 176	Meteloidine†
9	174 to 175	174 to 176	Meteloidine

* A mixture of equal quantities of hyoscyamine and meteloidine picrates melts at 148° to 158° C.
† Isolated in very small amount.

The titration liquors corresponding to the hyoscine peak on the elution curves afforded a picrate, m.pt. 188° to 190° C., and an aurichloride, m.pt. 202° C., undepressed on admixture with authentic specimens of hyoscine picrate and aurichloride respectively. The characters of the picrates prepared from the liquors corresponding to the second major peak on the elution curves are summarised in Table II. The crystals of hyoscyamine and meteloidine picrates can usually be distinguished by their habits; meteloidine picrate crystallises from water in nodules, whereas hyoscyamine picrate crystallises as flat plates. Unfortunately, these habits are not changed when either of the picrates is a major component of a mixture of the two, and the presence of the minor component is not revealed by the habit of the mixture.

DISCUSSION

It is reasonable to suppose that by employing grafts involving species of the same genus, deductions relating to the site of at least the final stages of alkaloid biogenesis can be made with greater certainty than with the types of combination studied hitherto. Grafts represented by examples 5 and 6 (Table I) have not previously been investigated. In these, the

simultaneous growth on the same root of two tops, one grafted and the other normal, provides a still more reliable control.

The quantitative results obtained with the controls and the reciprocal grafts of *D. tatula* and *D. ferox* (Table I, 1, 2, 3, 4) have most significance in showing that the process of grafting has no great influence on the overall production of alkaloids. Complete proof of the absence of hyoscyamine or meteloidine was not achieved in instances where they are reported as not having been found. From our experience of the fractional crystallisation of artificial mixtures of these picrates, it is unlikely that the alkaloid reported as absent is present in more than traces.

Unequivocal conclusions on the site of synthesis of hyoscine in these two plants cannot be made. The aerial parts of *D. tatula*, both as the whole plant (Table, I, 1) and as a scion (Table I, 4) appear to be characterised by a lower proportion of hyoscine than the aerial parts of *D. ferox*, as the whole plant (Table I, 2) and as a scion (Table I, 3). These facts are not in disagreement with the suggestion that the aerial parts of both plants have some faculty for synthesis of this alkaloid.

In both types of graft, the occurrence of hyoscyamine in *D. tatula* scions (Table I, 4, 6) and of meteloidine in *D. ferox* scions (Table I, 3, 5) in significant quantities shows that the aerial parts exhibit a well-marked capacity for the synthesis of hyoscyamine and of meteloidine respectively.

We failed to detect meteloidine in the *D. tatula* scion grown together with the normal *D. ferox* top on *D. ferox* roots (Table I, 6) and hyoscyamine in the *D. ferox* scion grown analogously on *D. tatula* (Table I, 5). Secondary transformations of alkaloids translocated from the stock to the scion are possible, but for these species, no information on this point is available. If this possibility is excluded, the evidence indicates that either their roots have little capacity, if any, for the synthesis of these alkaloids or that their translocation to the scion does not occur.

From the relatively high concentration of hyoscine found in ungrafted *D. innoxia* (Table I, 7) and in the *D. ferox* scion grown on a *D. innoxia* stock (Table I, 9), and from its low concentration in a *D. innoxia* scion grown on a *D. ferox* stock (Table I, 8), it appears that in *D. innoxia*, hyoscine is synthesised mainly in the roots and in *D. ferox* some synthetic capacity is present in the roots. This alkaloid apparently accumulates in the scion mainly as a result of translocation from the stock.

In agreement with the conclusions derived above, the distribution of hyoscyamine and meteloidine in these reciprocal grafts (Table I, 8, 9) indicates that *D. ferox* is capable of synthesising meteloidine in its aerial parts.

Information on the site of synthesis of hyoscyamine in *D. innoxia* is provided by the results obtained with graft combinations 8 and 9 (Table I). Since hyoscyamine is not produced by *D. ferox*[28], its occurrence in the *D. innoxia* scion grown on a *D. ferox* stock (Table I, 8) shows that it is formed in the aerial parts of *D. innoxia*. This conclusion is corroborated by the observation that hyoscyamine is apparently not present in the *D. ferox* scion grown on the *D. innoxia* stock (Table I, 9). No conclusions can be drawn with regard to the site of synthesis of meteloidine in *D. innoxia*.

D. innoxia therefore shows some degree of organ specialisation for synthesis. Dawson[30] has reported on an almost analogous case in *Nicotiana glauca*, in which nicotine is produced in the roots and anabasine is formed in both the roots and the aerial parts.

Summary

1. The problem of the location of the site of alkaloid biogenesis is discussed and work related to the tropane alkaloids is reviewed.

2. From a study of the distribution of alkaloids in grafts involving *Datura tatula*, *D. ferox* and *D. innoxia* the following conclusions are drawn:

(*a*) The aerial parts of *D. tatula* and *D. ferox* are capable of synthesising hyoscyamine and meteloidine respectively.

(*b*) In *D. innoxia*, hyoscine is formed mainly in the roots and hyoscyamine in the aerial parts.

3. The evidence obtained is consistent with the following hypotheses:

(*a*) In *D. ferox*, hyoscine is synthesised in both the roots and the aerial parts.

(*b*) In *D. tatula*, the aerial parts exhibit some faculty for the synthesis of hyoscine.

(*c*) In *D. ferox* and *D. tatula*, either the roots are incapable of synthesising meteloidine and hyoscyamine respectively or translocation of the alkaloid from the stock to the scion does not occur.

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