ALKALOID BIOGENESIS

PART III. THE PRODUCTION OF BIOSYNTHETIC RADIOACTIVE HYOSCINE AND METELOIDINE

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THE use of ^{14}C in experimental alkaloid chemistry has hitherto involved studies of two types. By growing plants in $^{14}CO_2$, radioactive nicotine[1,2,3], morphine[4], colchicine[5], cinchona alkaloids[6] and veratrum alkaloids[7], usually of high specific activity, have been produced. In the second series of experiments, specific labelled precursors of a fragment of the alkaloid molecule, especially the methyl group, have been employed in studies of the mechanisms of synthesis occurring in plants[8–17]. From this work, important deductions have been made relating to the origin of gramine, nicotine and hordenine and to methylation processes occurring in plants. In plants containing alkaloids of the tropane group, the observation[18] that *Atropa belladonna* does not furnish radioactive alkaloids from ^{14}C-labelled putrescine has provided powerful evidence that this compound is not a precursor of the alkaloids[19].

This communication describes the production of radioactive hyoscine and meteloidine in *Datura ferox* after injection of glycine-2-^{14}C.

EXPERIMENTAL

Vigorously growing plants of *Datura ferox* which had been raised under glass and planted out in the open in June were selected for study.

Method of Injection. Injection was carried out on a fine day. The main stem, beneath one of the dichasial branches, was slit longitudinally about 5 cm. and transversely to a depth of one-third to one-half the diameter of the stem in order to produce a flap joined to the stem at its upper end. About 3 mm. of the lower end of the flap was cut away and a glass tube was fixed in position with the tip of the flap touching the bottom of the tube. Immediately the tube was in position it was partly filled with water, plugged with cotton-wool and provided with a dust cap. At no stage was the cut tissue of the flap allowed to become dry. Capillary creep of the liquid from the tube down the cut stem did not occur provided that the tube was not too full and that the flap was directed into the tube at a wide angle. The plants were firmly supported with stakes. All plants to be injected were prepared in this manner.

For injection, the water was pipetted from the tube and replaced by the solution to be injected. Uptake was complete by evening when the injection solution was placed in the tube in the middle of the afternoon. As the tubes emptied, two successive quantities of water were added to complete the uptake of the injection solution. During the first day, the plants were covered with cloches when rain threatened. The doses administered varied from 15 to 50 μc. of glycine-2-^{14}C in the form of a

0·12 per cent. solution in water; control plants received the same quantities of glycine.

Autoradiographs. After intervals of 1 to 44 days, 3 discs, each of 1·5 cm. diameter, were cut from the leaves on the side of the plant above the site of injection. The fresh and air-dry weight of the 3 discs were 0·1 g. and 0·02 g. respectively.

For extraction, the discs were macerated with 1 drop of dilute solution of ammonia and 3 to 4 ml. of ethanol; after 3 hours the menstruum was pipetted off and the maceration was repeated with a further quantity of ethanol. The leaf tissue rapidly became brittle and was powdered under the solvent with a glass rod. After evaporation of the solvent, the residue was disolved in 2 drops of ethanol and the solution was placed on a paper strip. The components of the mixture were separated chromatographically by capillary rise, the organic solvent layer from a mixture of light petroleum (b.pt. 60° to 80° C.) 1 volume, amyl alcohol, glacial acetic acid and water, of each, 3 volumes, being used for the development.

Organic solvents on the developed paper strip were allowed to evaporate spontaneously and autoradiographs were obtained using Ilford, Industrial G, X-ray film. The time of exposure was 2 to 4 weeks. In order to test for the absence of chemical fogging, the control paper chromatograms were submitted to the same treatment. After marking coloured and fluorescent zones, the paper strip was cut longitudinally in order to locate amino-acids by means of ninhydrin reagent and alkaloids by means of modified Dragendorff's reagent[20].

Chromatograms and autoradiographs were prepared from the dried plant material after harvesting. In order to concentrate the alkaloids in this material, 0·2 g. was macerated as described above and the extract was evaporated to dryness. A solution of the residue in 0·2N sulphuric acid was washed with successive quantities of chloroform until colouring matter had been removed; traces of alkaloid were recovered from the chloroform with 0·2N acid and then the alkaloids were liberated by ammonia and collected in chloroform. All the solutions were evaporated to a small volume and placed on paper strips for chromatography.

Isolation of Radioactive Hyoscine and Meteloidine. The plant material was powdered in a ball mill and mixed with one-tenth its weight of calcium hydroxide. Sufficient water was added to moisten the mixture and, after an hour, the alkaloids were extracted by repeated maceration with ether. The ether was evaporated and the alkaloids were fractionated chromatographically by the method we have previously described[21].

The solutions remaining after titration of the two alkaloids were faintly acidified, indicator was removed by washing with chloroform, and the alkaloids were precipitated as their picrates; these salts were recrystallised from water and characterised by their melting-points and mixed melting-points.

Hydrolysis of the Alkaloids. Hyoscine picrate (3 mg.) was heated with 2 ml. of solution of barium hydroxide (5 per cent.) in a sealed tube in a boiling water bath for 30 minutes. Oscine was collected in chloroform,

converted into its hydrochloride by the addition of ethanolic hydrogen chloride and the solution was evaporated to dryness on a counting planchet. For the recovery of tropic acid, the aqueous liquid was made acid with hydrochloric acid and extracted with chloroform. Triethylamine was added to convert the acid in the chloroform into its salt and the solution was evaporated on a planchet. Meteloidine was hydrolysed and tiglic acid recovered in a similar manner; the remaining mother liquor was evaporated to dryness and the residue was extracted with ethanol to recover teloidine hydrochloride.

In *ad hoc* experiments, it was shown that hyoscine and meteloidine were completely hydrolysed under the conditions described above.

Isolation of Radioactive Calcium Oxalate. A portion of the marc of the leaves remaining after the extraction of the alkaloids was macerated with 3 successive quantities of dilute hydrochloric acid. After treatment of the extract with charcoal, calcium oxalate was precipitated by the addition of solution of ammonia, collected and washed thoroughly. The oxalate was decomposed by acidified potassium permanganate and the liberated carbon dioxide was swept into solution of barium hydroxide and recovered as barium carbonate.

Results

Not more than a trace of radioactive glycine was detected on the autoradiographs within one day of its injection but 6 different radioactive areas coincident with those responding to ninhydrin reagent were observed. Up to 9 days after injection, the number of active zones diminished and thereafter no significant change was detected; the greatest activity was associated with the green pigments and with a zone exhibiting a blue fluorescence. Radioactive amino-acids had disappeared. Active zones on the autoradiographs prepared from the seeds of a plant receiving 50 μc. of radioactivity occupied similar positions to some of those present in the leaf autoradiographs. No radioactive alkaloid was detected by autoradiography in these samples.

In spite of changes which occurred on drying the plants as demonstrated by changes in their ultra-violet fluorescence chromatograms, the dried plant appeared to contain the same radioactive compounds as the fresh plant. Radioactive amino-acids were not detected in the dried plant.

The autoradiographs of the chromatograms prepared from 0·2 g. of dried material from the plant receiving 50 μc. of radioactivity, exhibited radioactive zones coincident with the alkaloid zones on the chromatogram. Appreciably more radioactive material was present in the fraction containing the colouring matter and in the alkaline aqueous liquid remaining after removing the alkaloids. The latter solution contained amino-acids but none was radioactive.

In Table I values are given for the activities of the samples listed as determined by counting. The products of hydrolysis had the following activities in disintegrations per minute per milliatom of carbon: hyoscine, $1{\cdot}25 \times 10^5$; oscine, $1{\cdot}5 \times 10^5$; tropic acid, $2{\cdot}1 \times 10^4$; meteloidine,

$1{\cdot}15 \times 10^5$; teloidine, $1{\cdot}7 \times 10^5$; tiglic acid, $2{\cdot}6 \times 10^4$. The dried leaves of the plant receiving 50 μc. of radioactivity contained about 4 per cent. of calcium oxalate which had an activity of 9×10^4 disintegrations per minute per millatom of carbon.

TABLE I

Sample	Dose μc.	Period between injection and harvest days	Radioactivity† Disintegrations per minute per milligram				
			Powdered material	Exhausted material	Hyoscine picrate	Meteloidine picrate	Colouring matter
Plant A*	50	44					
(i) Leaves from above site of injection ..			6×10^3	$3{\cdot}5 \times 10^3$	4×10^3	3×10^3	6×10^3
(ii) Leaves from side opposite to site of injection			5×10^2	—	3×10^3	3×10^3	—
(iii) Root			2×10^3	—	4×10^3**		—
Plant B, leaves from whole plant	15	32	$1{\cdot}5 \times 10^3$	—	$3{\cdot}5 \times 10^2$	$3{\cdot}5 \times 10^2$	—

* Dried material contained hyoscine, 0·11 per cent. and meteloidine 0·03 per cent.
** Mixed picrates of total bases.
† Corrected for background count and self-absorption.

Discussion

The evidence of the autoradiographs showed that glycine was rapidly metabolised to other amino-acids or simple ethanol-soluble peptides. This was followed by a slower disappearance of all the radioactive materials giving a colour with ninhydrin. The appearance of radioactivity in fractions associated with the colouring matter and in calcium oxalate implies that a considerable proportion of the glycine entered the general metabolic processes of the plant.

The general distribution of radioactivity throughout the plant was by no means uniform. Of that injected, the greatest quantity remained in the organs immediately above the site of injection; some was translocated to the root and a still smaller proportion to the side of the plant opposite the site of injection.

Glycine did not appear to be a very efficient starting material for the biosynthesis of these alkaloids. Marked differences in the activity of the alkaloids formed in the more mature plant receiving 50 μc. and in the less mature plant receiving 15 μc. indicated that the state of maturity of the plant was of some significance. The distribution of radioactive alkaloids was fairly uniform although hyoscine, but not meteloidine, recovered from near the site of injection appeared to be more active than that occurring on the opposite side of the plant.

Radioactive carbon was not uniformly distributed throughout the alkaloid molecules. Glycine provided a considerably more efficient source of carbon for the tropane ring than for the tropic acid or tiglic acid.

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SUMMARY

1. The production of biosynthetic radioactive hyoscine and meteloidine in *Datura ferox* after injection of glycine-2-^{14}C is described.

2. The major part of the radioactive carbon is concentrated in the tropane residues.

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