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BIOGENESIS OF THE ALKALOIDS OF DATURA INNOXIA

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Datura innoxia Mill. has been found to contain 11 bases belonging to two groups of alkaloids, the pyrrolidine group and, mainly, the tropane group [1].

The literature has information on the use in the biosynthesis of the tropanols of ornithine [3, 5, 6, 8–11, 13], phenylalanine [2, 7, 10], methylamine [11], acetate [3, 4, 13], and acetone [11]. Ornithine, methylamine, acetate, and acetone are used in the biosynthesis of the amino alcohol moiety of the tropane alkaloids and phenylalanine in the biosynthesis of tropic and atropic acids.

We have studied a number of organic substances assumed to be precursors of the alkaloids of Datura innoxia, in particular [2-C^{14}] acetate, [1, 3-C^{14}] acetone, [2-C^{14}] acetone, and [C^{14}] methylamine. The acetate and methylamine were used as precursors of the methyl group of the alkaloids and the acetone as a precursor of the 3-carbon fragment of the tropane alkaloids consisting of the atoms $\overset{2}{C}-\overset{3}{C}-\overset{4}{C}$. In the experiment with acetate we assumed that decarboxylation and utilization of methyl took place. Methylamine must be included wholly in the formation of the N-methyl group of the alkaloids.

As our experiments have shown, all the precursors used were incorporated in the plant alkaloids.

Table 1

Specific Radioactivity of the Alkaloids of Datura innoxia

Plant organ	Alkaloids	Specific Radioactivity, μC/g			
		[2-C^{14}]-acetate	[1,3-C^{14}]-acetone	[2-C^{14}]-acetone	methylamine
Roots	hyoscyamine	0.23	0.09	0.05	0.99
	scopolamine	0.08	0.08	0.02	0.88
Stems	hyoscyamine	0.19	0.19	0.06	0.55
	scopolamine	0.13	0.05	0.06	0.79
Leaves	hyoscyamine	0.21	0.24	0.19	0.26
	scopolamine	0.07	0.25	0.06	0.21
Generative organs	hyoscyamine	0.12	0.06	0.08	0.29
	scopolamine	0.03	0.01	0.00	0.06
Plant as a whole	hyoscyamine	0.14	0.14	0.07	0.72
	scopolamine	0.07	0.08	0.02	0.39

In almost all cases, the specific radioactivity was higher in hyoscyamine than in scopolamine (Table 1). The higher radioactivity of hyoscamine is a proof of the priority of the synthesis of hyoscyamine, from which scopolamine is formed subsequently. The conversion of the hyoscyamine formed into scopolamine must take place more rapidly than the decomposition of the scopolamine. In this case, the plants will contain more newly formed hyoscyamine than scopolamine. The whole of the scopolamine present before the experiment, like the other alkaloids, is nonradioactive, but during the experiment radioactive alkaloids are formed. The large amount of the scopolamine previously existing and the low rate of its decomposition and formation (less than the rate of formation of hyoscamine) leads to a decrease in the specific radioactivity of the scopolamine together with a relatively high percentage inclusion of C^{14} in comparison with hyoscyamine.

The greatest formation of hyoscyamine from acetate and methylamine takes place in the roots. The highest specific radioactivity is there. Scopolamine is formed from it in the stems. Part of the acetate is probably converted into some 3-carbon compound which, like acetone, takes part in the synthesis of the alkaloids in the leaves. In this case, the specific radioactivity of the alkaloids is greatest in the leaves, as can be seen from Table 1.

Data showing the high specificity of acetone as a precursor of the tropane alkaloids are given in Tables 2 and 3.

The carbon of the precursors is incorporated into scopolamine more intensively because scopolamine is the end-product of the biogenesis chain of the alkaloids and is therefore less reactive. The inclusion differs in the various organs of the plant. The conversion of the alkaloids into other substances affects their radioactivity less than their interconversion.

In a study of the distribution of radioactivity in the molecule of the alkaloids, the inclusion of C^{14} in the amino alcohol and in the esterifying acid was found (Table 4).

Table 2

Incorporation of C^{14} in the Alkaloids of Datura innoxia

Plant organs	Alkaloids	Inclusion of C^{14}, % of the C^{14} assimilated			
		[2-C^{14}]-acetate	[1,3-C^{14}]-acetone	[2-C^{14}]-acetone	methyl-amine
Roots	hyoscyamine	0.02	0.72	0.38	0.15
	scopolamine	0.03	1.37	0.35	0.22
Stems	hyoscyamine	0.01	0.20	0.49	0.07
	scopolamine	0.04	0.59	1.39	0.16
Leaves	hyoscyamine	0.02	0.28	0.47	0.12
	scopolamine	0.07	1.39	0.79	0.55
Generative organs	hyoscyamine	0.02	0.43	14.83	0.10
	scopolamine	0.02	1.06	6.10	0.43
Plant as a whole	hyoscyamine	0.02	0.27	0.48	0.12
	scopolamine	0.04	0.95	0.58	0.22

All the radioactivity indices of the alkaloids depend on exposure. In the majority of cases there are two-peaked curves. This is probably due to the different rates of formation of the various fragments of the alkaloid molecules. Hyoscyamine accumulates intensively in the roots and scopolamine in the generative organs (Table 5). The total content of scopolamine in the plant exceeds that of hyoscyamine by a factor of 3.5–5.75.

Table 3

Relative Radioactivity of the Alkaloids of Datura innoxia

Plant organ	Alkaloids	Relative radioactivity (specific radioactivity of the alkaloid / specific radioactivity of raw material)			
		[2-C^{14}]-acetate	[1,3-C^{14}]-acetone	[2-C^{14}]-acetone	methyl-amine
Roots	hyoscyamine	0.33	7.90	4.72	1.86
	scopolamine	0 21	6.75	2.15	1.71
Stems	hyoscyamine	0.70	14.49	15.62	2.18
	scopolamine	0.46	3.77	14.38	3.27
Leaves	hyoscyamine	1.05	9.56	16.26	4.41
	scopolamine	0.37	9.39	10.17	3.72
Generative organs	hyoscyamine	0.39	8.97	597.62	3.14
	scopolamine	0.10	1.16	0.67	0.76
Plant as a whole	hyoscyamine	0.45	6.58	10.82	2.81
	scopolamine	0.24	4.06	3.44	1.54

The precursor had little effect on the accumulation of hyoscyamine. Methylamine has a somewhat suppressive effect on the accumulation of scopolamine and acetone proved to have some accumulating effect.

Experimental

The plants for the experiment were grown in vegetation vessels in a clay soil and were fertilized with potassium chloride, sodium dihydrogen phosphate, and ammonium nitrate.

In the period of full flowering, the plants were removed from the soil and placed individually in jars each of which contained a 0.1% solution of an assumed precursor. After 1–2 hr, pure water was added. The exposure was 1–5 days. The activity per variant was 200 μC.

The plants, separated into their organs, were fixed at 105° C for 15 min, dried, and ground. The alkaloids were extracted quantitatively from the plant material steeped in ammonia by a mixture of ether and chloroform (1:1) and then with 0.5 N hydrochloric acid. The hydrochloric acid extract was washed with ether, made alkaline with ammonia, and extracted with chloroform.

The chloroform solution of the total alkaloids was transferred into a citrate buffer with pH 6.5. After eight extractions from the buffer, practically all the scopolamine passed into the chloroform and the hyoscyamine remained in the buffer. Then these alkaloids were freed of impurities by chromatography on thin fixed layers of alumina containing gypsum (19:1). The mobile phase was chloroform. The alkaloids were dissolved in methanol and were hydrolyzed with caustic soda for 2 hr. After the methanol had been distilled off, the amino alcohol was extracted with ether. The remaining mother liquor was acidified and the esterifying acid was extracted with ether.

Table 4

Distribution of the Radioactivity in Alkaloid Molecules, %

Precursor	Hyoscyamine		Scopolamine	
	tropane	tropic acid	scopine	tropic acid
[1,3-C^{14}]acetone	51	49	33	67
[2-C^{14}]acetone	40	60	73	27
[C^{14}]methylamine	58	42	62	33
[2-C^{14}]acetate	60	40	82	18

Table 5

Relative Contents of Alkaloids in the Organs of Datura innoxia

Plant organs	Alkaloids	Content of alkaloids, %			
		acetate	acetone	methylamine	control
Roots	hyoscyamine	0.08	0.09	0.08	0.09
	scopolamine	0.18	0.18	0.13	0.17
Stems	hyoscyamine	0.01	0.02	0.03	0.02
	scopolamine	0.08	0.12	0.05	0.09
Leaves	hyoscyamine	0.02	0.03	0.03	0.03
	scopolamine	0.17	0.12	0.15	0.14
Generative organs	hyoscyamine	0.04	0.04	0.04	0.04
	scopolamine	0.27	0.60	0.52	0.50
Plant as a whole	hyoscyamine	0.04	0.04	0.04	0.04
	scopolamine	0.16	0.19	0.14	0.17

Summary

1. It has been shown that acetone, acetate, and methylamine are utilized in the biosynthesis of the tropane alkaloids. The most specific precursor of the alkaloids was acetone.

2. Acetate and methylamine are implicated in alkaloid biosynthesis in the roots, and acetone in the leaves. The biosynthetic process does not have a smooth nature but one with a number of maxima.

3. The accumulation of hyoscyamine takes place predominantly in the roots and that of scopolamine in the generative organs. The content of scopolamine is higher.

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