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THE BIOSYNTHESIS OF THE ALKALOIDS OF *Goebelia pachycarpa*

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We have previously [1-4] shown a possible route for the interconversion of the matrine alkaloids in Goebelia pachycarpa C. A. Mey. In the present paper we give concrete information on this question obtained as the result of a detailed study of the alkaloids isolated after feeding the plant with various labelled precursors. In order to determine the optimum time of feeding we studied the dynamics of the accumulation of alkaloids in the plant. The qualitative composition of the combined alkaloids was studied by thin-layer chromatography on alumina and by paper chromatography in various systems.

The combined alkaloids, obtained by a known method, [1] were separated by ascending thin-layer chromatography on plates coated with alumina by a method described previously [3]. The amounts of the individual alkaloids were determined by titration with silicotungstic acid in parallel with standard solutions of the corresponding alkaloids (Table 1).

Analysis of Table 1 shows that the main alkaloids in the initial growth period are present in the epigeal and hypogeal parts of the plant in approximately equal amounts; in the period of vigorous flowering and fruit-bearing, the maximum synthesis and accumulation take place in the epigeal parts of the plants.

Beginning with the fruit-bearing period, the alkaloid content gradually falls, and therefore to feed the plant with assumed precursors of the matrine alkaloids we selected the period of flowering and budding (Table 2).

The results of a study of the percentage inclusion of labelled precursors in the matrine alkaloids (see Table 2) show that in the periods considered an intensive biosynthesis of the alkaloids takes place.

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TABLE 1. Amounts of Alkaloids in the Plant During the Vegetation Period, %

Alkaloid	Phase of development, time of collection, and part of the plant							
	initial growth period		budding, May 5		incipient flowering, May 15		flowering, May 25	fruit-bearing, June 5
	hypo	epi	hypo	epi	hypo	epi	epigeal	
Total alkaloids	1,03	1,61	1,12	1,80	0,70	1,40	2,41	1,03
Start	+	+	+	+	+	+	+	+
Matrine	18,6	15,1	12,3	18,9	11,2	9,8	11,4	8,7
Matrine N-oxide	12,0	19,2	6,8	8,2	5,7	11,3	13,1	11,6
Sophocarpine	5,7	8,1	4,2	12,3	8,8	13,5	12,3	8,7
Sophocarpine N-oxide	4,2	6,7	3,8	11,2	4,1	12,2	13,7	10,1
Sophoramine	tr.	tr.	3,1	8,4	2,3	12,7	9,4	6,2
Pachycarpine	—	2,8	—	10,3	—	15,8	20,7	17,2
Cytisine	11,2	7,2	5,8	8,1	2,4	6,3	3,6	8,1
Sophorbenzamine	—	—	—	—	—	tr.	3,2	—
Alkaloid X_2	—	—	—	—	—	tr.	2,5	—
Total amount of identified alkaloids	61,7	59,1	35,0	77,4	34,5	81,6	90,0	70,6

TABLE 2. Specific Radioactivities of the Alkaloids in the Plant in Various Vegetation Periods on Feeding with Labelled Precursors

Compound	Budding		Incipient flowering		Incipient ripening on the seeds	
	counts/min/mmole	percentage inclusion	counts/min/mmole	percentage inclusion	counts/min/mmole	percentage inclusion
[2-^{14}C]Lysine	$1,5\cdot10^7$	—	$3,24\cdot10^9$	—	$1,6\cdot10^7$	—
Matrine	$7,4\cdot10^4$	0,49	$1,65\cdot10^7$	0,51	$2,5\cdot10^4$	0,17
Matrine N-oxide	$3,3\cdot10^4$	0,26	$2,54\cdot10^7$	0.78	$1,65\cdot10^4$	0,10
Sophocarpine	$2,4\cdot10^4$	0,16	$1,55\cdot10^7$	0,47	$1,7\cdot10^4$	0,10
Sophocarpine N-oxide	$2,6\cdot10^4$	0,17	$1,68\cdot10^7$	0,26	$7,8\cdot10^3$	0,05
Sophoramine	$3,6\cdot10^4$	0,24	$2,85\cdot10^6$	0,08	$1,2\cdot10^4$	0,07
[1,5-^{14}C]Cadaverine	—	—	—	—	$1,5\cdot10^7$	—
Matrine	$2,5\cdot10^5$	1,8	—	—	$8,7\cdot10^4$	0,59
Matrine N-oxide	$1,6\cdot10^5$	1,1	—	—	$5,3\cdot10^4$	0,35
Sophocarpine	$1,1\cdot10^5$	0,80	—	—	$6,10\cdot10^4$	0,41
Sophocarpine N-oxide	$1,0\cdot10^5$	0,71	—	—	$2,6\cdot10^4$	0,16
Sophoramine	$1,2\cdot10^5$	0,86	—	—	$3,7\cdot10^4$	0,24

In a study of the biosynthetic connections of some matrine alkaloids, plants were fed with biosynthesized matrine and sophocarpine, and the alkaloids isolated were studied. The specific radioactivities of the alkaloids isolated in the flowering period from a mature plant fed with labelled matrine and sophocarpine after exposure for 3 days are given below:

Compound	Activity, counts/min/mole	% inclusion
[2,6,10,11,15,17-^{14}C]Matrine	$1.65\cdot10^7$	—
Matrine N-oxide	$1.18\cdot10^6$	7.15
Sophocarpine	$8.35\cdot10^5$	5.10
[2,6,10,11,15,17-^{14}C]Sophocarpine	$1.55\cdot10^7$	—
Sophocarpine N-oxide	$7.45\cdot10^5$	4.8
Sophoramine	$4.85\cdot10^5$	3.1

Consequently, in the plant matrine is first oxidized to matrine N-oxide and this is then converted into sophocarpine, and the sophocarpine is converted via the N-oxide form into sophoramine.

Results obtained by the oxidation of the alkaloids isolate show that their mutual conversion takes place without intramolecular rearrangement:

Fragment	Isolated mg	Counts/min/mg	Counts/min/mmole	% inclusion
[2,6,10,11,15,17-^{14}C] Sophocarpine	0.370	2123	$5.46 \cdot 10^5$	–
Succinic acid	0.003	797	$9.50 \cdot 10^4$	17.2
γ-Aminobutyric acid	0.0041	748	$7.70 \cdot 10^5$	14.1
β-Alanine	0.0021	1170	$1.04 \cdot 10^5$	18.08
Matrine obtained by reduction from [2,6,10,11,15,17-^{14}C]-sophocarpine	0.100	2097	$5.15 \cdot 10^5$	–
Glutaric acid	0.0032	1638	$2.13 \cdot 10^5$	40.1
Succinic acid	0.002	840	$9.91 \cdot 10^4$	19.2
γ-Aminobutyric acid	0.0039	790	$8.13 \cdot 10^4$	15.8
β-Alanine	0.0012	1020	$9.07 \cdot 10^4$	17.56
Glycine	0.002	987	$8.38 \cdot 10^4$	16.2
[2,6,10,11,15,17-^{14}C] Sophoramine	0.051	–	$4.85 \cdot 10^5$	–
Succinic acid	0.001	680	$8.02 \cdot 10^4$	16.5
γ-Aminobutyric acid	0.0021	710	$7.34 \cdot 10^4$	15.2

EXPERIMENTAL

Production of [^{14}C] Matrine and [^{14}C] Sophocarpine. The lateral roots of plants growing under natural conditions were dug up and were cut, and test-tubes containing labelled [2-^{14}C] lysine and [1,5-^{14}C] cadaverine were placed in position. After exposure for 3 days, the plant was worked up and the alkaloids were isolated in the usual way.

Feeding was carried out with matrine and sophocarpine in a similar way, and after the appropriate working up matrine N-oxide and sophocarpine were isolated from the plant fed with matrine, and sophocarpine N-oxide and sophoramine from a plant fed with sophocarpine.

Oxidation of the Alkaloids Matrine and Sophocarpine. The oxidative degradation of the alkaloids to aliphatic acids and amino acids, and their separation were performed by a known procedure [5]. The radioactivities of the alkaloids and the products of oxidative cleavage were measured in a gas-flow counter; the counting efficiency was 30-33%. In the calculation of the distribution of radioactivity between the alkaloids and their cleavage products, the total radioactivity of the initial alkaloid was taken as 100%.

SUMMARY

1. Qualitative and quantitative compositions of the main alkaloids in five development phases of the Goebelia pachycarpa C. A. Mey. have been determined.

2. The radioactivities of the alkaloids isolated after feeding the plant with assumed precursors – [2-^{14}C] lysine and [1,5-^{14}C] cadaverine – show that vigorous synthesis of alkaloids takes place in the flowering and budding periods.

3. The results of an investigation of the oxidation products and of the alkaloids introduced and isolated after the feeding of plants with labelled matrine and labelled sophocarpine show that the alkaloids undergo interconversion without intramolecular changes.

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