BIOSYNTHESIS AND INTERCONVERSIONS OF ALKALOIDS IN *Ammothamnus lehmannii*

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UDC 547.944/945

We have previously [1] studied the alkaloid composition of *Ammothamnus lehmannii* Bge. according to the vegetation periods and have shown that the alkaloid spectrum changes during ontogenesis in the individual organs. The present paper gives the results of a study of the biosynthesis of the alkaloids of *A. lehmannii* by feeding the plant with assumed precursors of the alkaloids.

When the plant *A. lehmannii* was supplied with [1,5-$^{14}C_2$] cadaverine, we isolated labelled sophocarpidine, sophocarpine, and pachycarpine, which shows the participation of the [1,5-$^{14}C_2$] cadaverine in their formation.

Below we give information on the amounts and specific radioactivities of the alkaloids isolated from shoots of *A. lehmannii* after their feeding with [1,5-$^{14}C_2$] cadaverine:

Alkaloid	Alkaloid isolated, mg	mp, °C	Specific radioactivity, counts/mm/mmole	Fraction of inclusion, %
[1,5-^{14}C]Cadaverine	25	—	$5.5 \cdot 10^9$	—
Sophocarpidine	95	204	$3.95 \cdot 10^7$	0.71
Sophocarpine	68	52	$1.84 \cdot 10^7$	0.33
Pachycarpine	16	208*	$1.75 \cdot 10^7$	0.31

*Picrate.

The specific radioactivity of the sophocarpidine was almost twice that of the sophocarpine and, therefore, the label is more actively included in the N-oxide forms of the alkaloids, which agrees with a statement concerning the active role of the N-oxide forms of basis as oxygen carriers [2].

The possible routes of the biosynthesis of pachycarpine, sophocarpine, and sophocarpinine in *A. lehmannii* are given in the scheme. A similar route for the formation of these alkaloids in other plants has been shown previously [3-9]. It is most likely that these bases are formed similarly in *A. lehmannii*.

In order to study possible intertransitions of the alkaloids in the plants, we supplied young shoots of *A. lehmannii* with the labelled alkaloids [^{3}H] matrine, [^{3}H] sophocarpine and [^{3}H] pachycarpine. The results obtained in this experiment are:

Alkaloid	Alkaloid isolated, mg	mp, °C	Specific radioactivity, counts/min/mmole	Fraction of inclusion, %
			[^{3}H]Matrine	
[^{3}H]Matrine	20.1	74	$2.5 \cdot 10^{10}$	—
Sophocarpidine	105	204	$1.4 \cdot 10^8$	0.56
Sophocarpine	96	52	$9.5 \cdot 10^7$	0.38

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		[^{3}H]Sophocarpine		
[^{3}H]Sophocarpine	42.7	52	$4.9 \cdot 10^{10}$	—
Sophocarpidine	84	203	$1.49 \cdot 10^{9}$	3.02
Sophocarpine	76	152*	$5.2 \cdot 10^{8}$	1.06
Pachycarpine	11	208*	$1.6 \cdot 10^{8}$	0.32
		[^{3}H]Pachycarpine		
[^{3}H]Pachycarpine	30.1	235	$6.55 \cdot 10^{10}$	—
Sophocarpine	5.8	152*	$5.1 \cdot 10^{8}$	0.78
Sophocarpidine	126	203	$3.85 \cdot 10^{9}$	0.58
Pachycarpine	13.1	208*	$2.55 \cdot 10^{8}$	0.38
Pachycarpine N-oxide	8.2	153	$7.6 \cdot 10^{7}$	0.1

*Picrate.

Judging from the experimental results, in the plant organism matrine undergoes dehydrogenation and then oxidation of the N_1 tertiary nitrogen atom, giving sophocarpine N-oxide. By supplying the plant with labelled [^{3}H] pachycarpine we obtained labelled sophocarpine, sophocarpidine, and pachycarpine N-oxide. Consequently, in the plant there are intertransitions between these alkaloids which apparently take place according to the scheme given above.

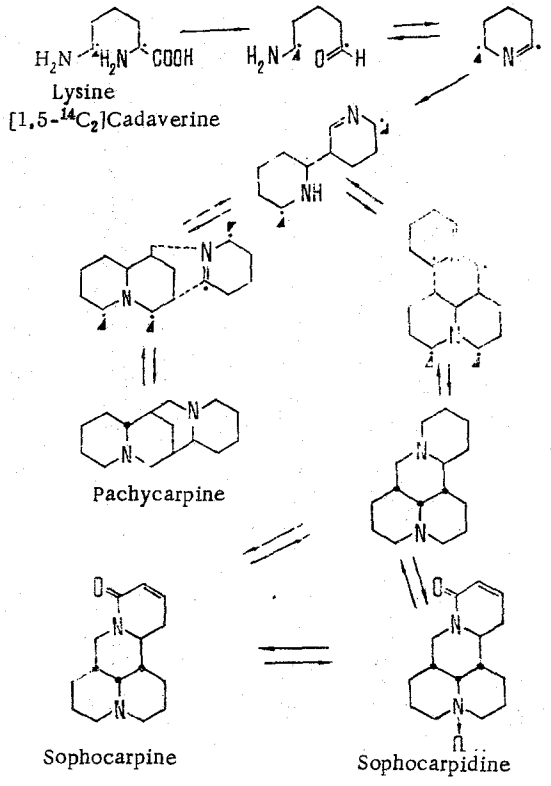
Thus, as a result of the investigations performed it has been shown that cadaverine is a possible precursor of the alkaloids sophocarpine, sophocarpidine, and pachycarpine in *A. lehmannii* and they are transformed into one another in the development process of the plants.

EXPERIMENTAL

For thin-layer chromatography we used Merck type HF_{252} silica gel layer thickness 0.9 mm and the following solvent systems: 1) chloroform–benzene–methanol (20:5:3); 2) ethyl acetate–isopropanol–25% ammonia (50:35:25); and 3) cyclohexane–diethylamine (7:3).

Chromogenic reagents: Dragendorff's reagent, iodine vapor, UV rays, and a 3% ethereal solution of ferric chloride.

The labelling of matrine, sophocarpine, and pachycarpine with tritium was done in a Wilzbach-Tritierung instrument on a $BaCO_3$ support with the aid of uranium tritide. The labelled alkaloids were purified in the following way. The tritium-labelled alkaloids were separated by decantation from the $BaCO_3$ support with methanol. The methanolic solution was boiled for 30 min and the methanol was distilled off, and the residue was again dissolved in



methanol. This operation was repeated 5 times. The dry alkaloid residue was recrystallized from petroleum ether. The labelled alkaloids were rechromatographed three times by the preparative method on silica gel and an autoradiogram (scannogram) was taken on the Dünschicht-Scanner-II instrument.

The melting points of the individual bases and the derivatives were determined on a Mikroheiztisch Boëtius.

The alkaloids isolated from the plants by the TLC method were identified by comparison with authentic samples. The radioactivities (^{14}C and ^{3}H) of the bases were measured in Packard Automatic Tri-Carb Liquid Scintillation Spectrophotometer Model 3365 scintillation counter.

Supplying the Plant with [1,5-$^{14}C_2$] Cadaverine. Fresh sprouts were placed in Knopp's solution (1/2 standard) containing 24 mg of cadaverine dihydrochloride with a specific radioactivity of $1.8 \cdot 10^9$ counts/min/mmole which we synthesized previously by a known method [10]. After exposure for 2 days, the sprouts were carefully washed with water and dried to constant weight. The weight of the air-dry plant was 51.2 g.

The plant material was comminuted and after it had been moistened with a solution of ammonia (15%) the alkaloids were extracted with methanol in a Soxhlet apparatus.

The combined alkaloids (0.9 g) were separated into the individual bases by thin-layer chromatography on plates with dimensions of 200 × 200 mm bearing a fixed layer of silica gel.

Sophocarpine and sophocarpidine were isolated by chromatography of the alkaloids in system 1, and pachycarpine in system 2.

The chromatograms were revealed with the Dragendorff's reagent. Spots with the same R_f value were collected, made alkaline with ammonia, and eluted with methanol. The methanolic extracts were evaporated to dryness, made alkaline, and extracted with chloroform. The amounts of alkaloids isolated were determined quantitatively by the gravimetric method. After the elimination of the solvent, the individual bases sophocarpine, sophocarpidine, and pachycarpine were obtained. The yields and physicochemical constants of these alkaloids are given above.

Supplying Sprouts of *A. lehmannii* with [^{3}H]Matrine. Fresh sprouts were placed in Knopp's solution (1/2 standard) containing 28.1 mg of [^{3}H] matrine with a specific radioactivity of $2.5 \cdot 10^{10}$ counts/min/mmole. The time of exposure and the method of isolating the combined alkaloids and separating them into their component parts were similar to those of the first experiment.

The individual bases sophocarpidine and sophocarpine were obtained. Information on their specific radioactivities and the fraction of inclusion by the alkaloids is given above.

Supplying Plants with [^{3}H] Sophocarpine. Freshly cut sprouts were placed in Knopp's solution (1/2 standard) containing 42.7 mg of [^{3}H] sophocarpine with a specific radioactivity of $4.9 \cdot 10^{10}$ counts/min/mmole. The experiment was performed in the same way as the preceding one.

The amounts, the physicochemical constants, the specific radioactivities and the fractions of inclusion of the label of the alkaloids isolated are given above.

Supplying Sprouts of *A. lehmannii* with [^{3}H] Pachycarpine. Sprouts of the plant were fertilized in Knopp's solution (1/2 standard) containing 30.1 g of [^{3}H] pachycarpine with a specific radioactivity of $6.55 \cdot 10^{10}$ counts/min/mmole.

The experiment was performed similarly to the preceding one. The amounts, physicochemical constants, the specific radioactivities, and fractions of the inclusion of the tritium label of the alkaloids isolated are given above.

SUMMARY

The biosynthesis of the alkaloids of *Ammothamnus lehmannii* Bge. have been studied by supplying the plants with [1,5-$^{14}C_2$] cadaverine. It has been shown that cadaverine is a precursor of sophocarpidine, sophocarpine, and pachycarpine. Possible routes of intercon-

versions have been studied by supplying the plants with the tritium-labelled alkaloids [^{3}H] sophocarpine, [^{3}H] matrine, and [^{3}H] pachycarpine.

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ALKALOIDS OF *Korolkovia severtzovii*

STRUCTURE OF KORSIDINE

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UDC 547.944/945

We have continued our investigation of the alkaloids of *Korolkovia severtsovii* Rgl. [1] growing in Khamzaabade, Fergana oblast. From the epigeal part collected in the flowering stage, by chloroform extraction we isolated 0.93% of combined alkaloids, and from the hypogeal part 1.64%. Separation of the combined alkaloids from the epigeal part gave sevkorine, korseveriline, and severtzidine [2-4], and the new alkaloid korsidine with mp 316-318°C $[\alpha]_D$ ±0°, $C_{27}H_{43}NO_2$ (I).

The IR spectrum of the base showed absorption bands at (cm^{-1}) 3400-3200 (OH), 2975-2830 (—CH_3, —CH_2—), and 2776 (trans-quinolizidine). The mass spectrum of (I) showed the peaks of ions with m/e 97, 98, 111, 112, 122, 149, 165, 179, 183, 201, 202, 244, (M—29), (M—18), (M—15), 413 (100%) M^+, which are characteristic for C-nor-D-homosteroid alkaloids of the cevine group [5, 6]. The NMR spectrum of korsidine was not recorded because of its poor solubility in organic solvents.

In korsidine, the oxygen atoms are present in the form of secondary hydroxy groups, as was confirmed by the preparation of diacetylkorsidine (II). The IR spectrum of (II) has absorption frequencies at 1738, 1250, and 1232 cm^{-1} (ester C=O).

No signal from an olefinic proton was observed in the NMR spectrum, but in a weak sulfuric acid solution korsidine instantaneously decolorizes potassium permanganate solution, which shows the presence of a double bond.

The oxidation of korsidine with chromium trioxide gave a diketone — korsidinedione (III). In the UV spectrum the latter has λ_{max} 300 nm (log ε 2.39), which is characteristic for unconjugated carbonyl groups. The IR spectrum of (III) had absorption bands at 1710 cm^{-1} (carbonyl in a six-membered ring) and lacked absorption bands of hydroxy groups.

The catalytic hydrogenation of korsidine according to Adams formed dihydrokorsidine (IV). In the IR spectrum of (IV), the fingerprint region was similar to that of petilinine

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Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 780-783, November-December, 1976. Original article submitted May 18, 1976.