LITERATURE CITED

1. Flora of the USSR [in Russian], Vol. XIV, Moscow–Leningrad (1949), p. 637.
2. L. T. Sin'ko, Rast Res., 8, 323 (1976).
3. R. Tschesche, C. Spilles and G. Eckhardt, Chem. Ber., 107, 1329 (1974).
4. H. Otsuka, Y. Ogihara, and S. Shibata, Phytochem, 13, 2016 (1974).
5. R. Tschesche, M. Elgamal, G. Eckhardt, and M. Radloff, Phytochem., 13, 2328 (1974).
6. E. W. Warnhoff, Forschr. Chem. Org. Naturstoffe, 28, 162 (1971).
7. R. Tschesche and E. U. Kaufmann, in: The Alkaloids (ed. R. H. F. Manske), Vol. 15, Academic Press, New York (1975), p. 165.
8. R. Tschesche, I. Khokhar, H. Wilhelm, and G. Eckhardt, Phytochem., 15, 541 (1976).
9. I. A. Israilov, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 811 (1975).
10. Kh. G. Kiryakov, I. A. Israilov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 411 (1975).
11. S. R. Johns, J. A. Lamberton, A. A. Sioumis, and H. J. Tweeddale, Aust. J. Chem., 22, 1277 (1969).
12. H. Guinaudeau, M. Leboeuf, and A. Cave, Lloydia, 38, 275 (1975).
13. E. Gellert and R. Rudzats, Aust. J. Chem., 25, 2477 (1972).
14. N. Obashi, J. M. Wilson, H. Budzikiewicz, M. Shamma, W. A. Slusarchyk, and C. Djerassi, J. Am. Chem. Soc., 85, 2807 (1963).
15. A. W. Sangster and K. L. Stuart, Chem. Rev, 65, 69 (1965).
16. N. C. Franca, A. M. Giesbrecht, O. R. Gottlieb, A. F. Magalhaes, and E. G. Magalhaes, Phytochem., 14, 1671 (1975).
17. M. U. Ibragimova, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 438 (1970).
18. T. Irgashev, I. A. Israilov, N. D. Abdullaev, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 127 (1977).
19. G. Grethe, M. Uskokovic, and A. Brossi, J. Org. Chem., 33, 2500 (1968).

BIOSYNTHESIS OF ALKALOIDS IN *Sophora alopecuroides*

Yu. K. Kushmuradov, Kh. A. Aslanov, Kh. R. Sohütte, and S. Kuchkarov

UDC 547.944/945

The alkaloid composition of Sophora alopecuroides L. has been studied by various workers [1-4]. This plant is characterized by a high content of quinolizidine alkaloids of the matrine, sparteine, cytisine, and aloperine groups.

We [5] have established that in S. alopecuroides sophoridine, matrine and sophocarpine, and sophocarpidine are formed from lysine or cadaverine. It was shown that when shoots were fed with [1,5-^{14}C]cadaverine the proportion of it included in the sophoridine was considerably higher than in the matrine. It is obvious that this plant species contains specific enzymes ensuring the formation of a A/B, A/C, C/D-trans, and B/C-cis conformations and of rings B/C in the boat form (sophoridine) that are considerably more effective than in the case of the synthesis of matrine (A/B-trans, A/B-cis, B/C-cis, C/D-trans linkage).

The schemes for the biosynthesis of alkaloids known in the literature [6], determined with the aid of labelled atoms, do not explain fine details of the alkaloid spectra connected with the stereochemical features of biosynthesis. In view of this we shall consider how the conformational state of the precursor of the quinolizidine alkaloids can affect the isomeric composition of the alkaloids formed. In the first place, we may note that, depending on the species of plant within a given family and its vegetation phase, a *l*-(amino acid) – lysine – leads to the formation of both the *l*- and d-forms of the quinolidizine bases (for example, pachycarpine,

V. I. Lenin Tashkent State University. Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 244-247, March-April, 1977. Original article submitted December 2, 1976.

sparteine). This indirectly shows a loss of asymmetry in the process of forming the alkaloids, which is in with the idea that a common procursor of these alkaloids, as well, is cadaverine → amino aldehyde → Δ^1-piperideine [7-9], which have no asymmetric centers [10].

The cyclic system of Δ^1-piperideine exists in two half-chair forms and is characterized by the absence of elements of symmetry. The two forms undergo rapid interconversion in solutions. The substitution of the α-carbon or the dimerization of two molecules of Δ^1-piperideine may retard this process, and the products will be distinguished by their conformations and configurations according to which forms react.

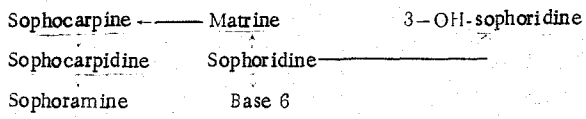
Apparently, the interaction of the two forms of Δ^1-piperideine is necessary for the appearance of the trans-quinolizidine system of the alkaloids matrine and pachycarpine. On the appearance of an asymmetric center at C_6 in one molecule of Δ^1-piperideine, one of the forms most frequently proves to be predominant. In the formation of tetracyclic systems, interactions of the *l*- and d-forms of the products of their transformation are possible with the formation of *l*-sparteine, d-sparteine, or matrine isomers (sophoridine, albertidine, darvasamine, leontine). In order to show in what stereochemical ratios the alkaloids of the quinolizidine series exist in the process of biogenesis we have studied the biogenesis and interconversions of the bases of S. alopecuroides with the aid of labelled precursors and the introduction of the corresponding labelled alkaloids into shoots.

Shoots of the plant were fed with [^{3}H] sophoridine, and with the conformationally stable isomers [^{3}H]isosophoridine (A/B-cis, A/C-cis, B/C-trans, C/D-trans) and [^{3}H]allomatrine (A/B-trans, A/C-trans, B/C-trans, C/D-trans), and after a four-day exposure the combined alkaloids were obtained from the roots in the usual way; from them by preparative thin-layer chromatography we isolated and identified the following labelled alkaloids (+, picrate; ++, hydriodide):

Alkaloid	Amount, mg	mp, °C	Specific radioactivity, counts/min/mole	Proportion of inclusion, %
		[^{3}H]sophoridine		
[^{3}H]sophoridine	10.38	107	$1.74 \cdot 10^{10}$	—
Sophoridine	2	107	$8.06 \cdot 10^{9}$	47
3-OH-sophoridine	3.3	162	$1.93 \cdot 10^{9}$	6.6
Base 6	1.7	165	$1.43 \cdot 10^{8}$	0.83
Sophocarpidine	1.5	204	$7.4 \cdot 10^{7}$	0.43
Sophocarpine	5.2	52	$5.6 \cdot 10^{7}$	0.32
Sophoramine	2	164	$4.4 \cdot 10^{7}$	0.25
Matrine	3.1	74	$3.81 \cdot 10^{7}$	0.21
		[^{3}H]isosophoridine		
[^{3}H]isosophoridine	14.7	109	$4.82 \cdot 10^{10}$	—
Isosophoridine	9.5	109	$4.92 \cdot 10^{9}$	20.6
Isosophoridine N-oxide	3.5	158	$1.04 \cdot 10^{9}$	2.1
Base 6	57	165	$1.5 \cdot 10^{6}$	0.031
Sophoridine	4	107	$1.2 \cdot 10^{6}$	0.025
		[^{3}H]allomatrine		
[^{3}H]allomatrine	14	104	$2.11 \cdot 10^{10}$	—
Allomatrine	4	104	$1.35 \cdot 10^{10}$	64.7
Base 6	41	165	$4.68 \cdot 10^{6}$	0.02
		[^{3}H]pachycarpine		
[^{3}H]pachycarpine	15	235++	$6.55 \cdot 10^{10}$	—
Pachycarpine	6	208+	$2.8 \cdot 10^{9}$	4.3
Pachycarpine N-oxide	18.1	153	$8 \cdot 10^{8}$	1.2
Sophocarpine	1.5	52	$5.1 \cdot 10^{8}$	0.78
Neosophoramine	8.5	129	$3.16 \cdot 10^{8}$	0.49
		[^{3}H]cytisine		
[^{3}H]cytisine	13.5	155	$1.08 \cdot 10^{10}$	—
Cytisine	9.7	155	$0.84 \cdot 10^{9}$	8.4
Methylcytisine	19	136	$8.4 \cdot 10^{7}$	0.84
		[^{3}H]methylcytisine		
[^{3}H]methylcytisine	20.1	136	$2.04 \cdot 10^{10}$	—
Methylcytisine	23.8	136	$5.3 \cdot 10^{8}$	2.56
Neosophoramine	15.5	129	$1.45 \cdot 10^{8}$	0.72
Cytisine	4.5	155	$5.4 \cdot 10^{7}$	0.30

It can be seen from the figures given that in the plant organism [^{3}H]sophoridine, at the same time as it is converted into 3-OH-sophoridine and base 6, partially isomerizes into the matrine alkaloids.

Below we give a scheme of the interconversion of sophoridine in S. alopecuroides:



It may be assumed that the conformationally stable isomers do not take part in the metabolic process: since isosophoridine has a cis-quinolizidine ring, it should readily be oxidized at the N_1 nitrogen atom. In actual fact, when shoots were supplied with labelled [^{3}H]isosophoridine we isolated isosophoridine N-oxide from the plant, from which it is normally absent.

When S. alopecuroides was fed with [^{3}H]pachycarpine, the resulting pachycarpine N-oxide, sophocarpine, and neosophoramine proved to be radioactive. A large part of the radioactivity was concentrated in the pachycarpine N-oxide, which shows the high activity of oxidative processes in the root parts of the plant. This experiment showed the possibility of the transformation of the sparteine alkaloids into matrine alkaloids in the ontogenesis of plants. The experiments on feeding shoots of S. alopecuroides showed that processes of methylation and demethylation take place in the plant simultaneously, and it was found that the rate of the methylation of cytisine in the plant is approximately three times higher than the demethylation reaction.

Thus, on the basis of the experimental facts it may be concluded that in the metabolism of the quinolizidine alkaloids the main role is played by the conformational state of the precursors and of the alkaloids themselves.

EXPERIMENTAL

For thin-layer chromatography we used Merck HF_{252} silica gel in a layer thickness of from 0.5 to 0.9 mm. Solvent systems: chloroform–benzene–methanol (20 : 5 : 3); 2) ethyl acetate–isopropanol–25% ammonia (50 : 35 : 25); and 3) toluene–acetone–ethanol–25% ammonia (40 : 40 : 8 : 3). Revealing agents: iodine vapor, Dragendorff's reagent, UV light, and a 3% solution of ferric chloride in ether.

The alkaloids sophoridine, allomatrine, isosophoridine, pachycarpine, cytisine, and N-methylcytisine were labelled with tritium in a special Wilzbach-Trittierung apparatus on $BaCO_3$ as support with the aid of uranium tritide. The purification of the labelled alkaloids has been described previously [5]. The labelled alkaloids were twice rechromatographed preparatively on silica gel, and their autoradiograms (scannograms) were recorded on a Dünnschicht-Scanner-II instrument. The melting points of the bases and their derivatives were determined on a "Mikroheiztisch Boëtius" block.

Alkaloids isolated from the plants were identified by comparison with authentic samples. The radioactivities (^{14}C and ^{3}H) of the bases were measured on a Packard Automatic Tri-Carb Liquid Scintillation Spectrophotometer Model 3363 counter.

The Feeding of the Shoots of S. alopecuroides with Labelled [^{3}H]Sophoridine. Four-week shoots were placed in an aqueous solution of 10.38 mg of [^{3}H]sophoridine with a specific radioactivity of $1.74 \cdot 10^{10}$ counts/min/mmole. After 2 days' exposure, the shoots were carefully washed with water. The fresh plant material was ground to particles with a size of 0.5–1 cm, covered with methanol, and comminuted with the aid of a homogenizer. The methanolic extract was filtered from residues of plant tissue and these were washed with methanol, and then the solvent was distilled off to dryness on a rotary evaporator. The residue was treated in the usual way, and the combined bases were obtained.

The combined alkaloids were separated into their individual components by comparative chromatography on 200 × 200 mm plates with a fixed layer of silica gel. The chromatogram was revealed with the Dragendorff reagent. The zones with the same R_f value were combined, made alkaline with ammonia, and extracted with methanol. The methanolic extracts were distilled to dryness and the residues were made alkaline and were again extracted, with chloroform.

After the chloroform had been distilled off the alkaloids were recrystallized from appropriate solvents. This gave the labelled individual bases.

Feeding of the Shoots of the Plant S. alopecuroides with the Labelled Alkaloids [^{3}H]isosophoridine, [^{3}H]-allomatrine: [^{3}H]pachycarpine, [^{3}H]cytisine, and [^{3}H]N-methylcytisine. Experiments were performed as in the preceding case. For feeding the plant shoots the labelled alkaloids were taken in the following amounts (mg): [^{3}H]isosophoridine 14.7; [^{3}H]allomatrine 14; [^{3}H]pachycarpine 15; [^{3}H]cytisine 14.5; and [^{3}H]N-methyl-cytisine 20.1.

SUMMARY

The interconversions of the alkaloids of Sophora alopecuroides L. have been studied by feeding the plants with tritium-labelled alkaloids ([^{3}H]sophoridine, [^{3}H]pachycarpine, [^{3}H]cytisine, [^{3}H]isosophoridine, [^{3}H]allomatrine, and [^{3}H]N-methylcytisine). It has been established that the conformationally stable isomers of the alkaloids – isosophoridine and allomatrine – do not take part actively in the metabolism of the alkaloids. It has also been shown that the alkaloids of the sparteine group are converted into matrine alkaloids in the development of the plant. The methylation of cytisine to N-methylcytisine and the reverse transition have been shown experimentally in the plant organism.

LITERATURE CITED

1. A. Orechoff, Ber., 66, 948 (1933).
2. E. Orechoff, N. Proskurnina, and R. Konovalova, Ber., 68, 431 (1935).
3. T. E. Monakhova, N. F. Proskurnina, O. N. Tolkachev, V. S. Kabanov, and M. E. Perel'son, Khim. Prirodn. Soedin., 59 (1973).
4. T. E. Monakhova, O. N. Tolkachev, V. S. Kabanov, M. E. Perel'son, and N. F. Proskurnina, Khim. Prirodn. Soedin., 472 (1974).
5. J. K. Kuschmuradov, D. Gross, and H. R. Schütte, Phytochem., 11, 3441 (1972).
6. K. Mothes and H. R. Schütte, Biosynthese der Alkaloide, Akademie Verlag, Berlin (1968).
7. H. T. Schütte, H. Aslanov, and C. Schäfer, Arch. Pharm., 295, 34 (1962).
8. S. Shibata and U. Sankawa, Chem. Ind. (London), 1161 (1963).
9. K. Hasse, Abh. dtsch. Akad. Wiss. Berlin. Kä. Chem. Geol. Biol., No. 3, 187 (1966).
10. G. E. Saxton, Alkaloids, 3, 25 (1973).

BIOSYNTHEIS OF THE ALKALOIDS OF *Ammodendron karelinii*

Yu. K. Kushmuradov, H. R. Schütte
Kh. A. Aslanov, and S. Kuchkarov

UDC 547.944 / 945

There is information in the literature [1] according to which transitions of certain types of alkaloids into others take part in the plant organism. In the present paper we give information obtained in a study of the biosynthesis and interconversions of the alkaloids in the plant A. karelinii Fisch et Mey.

When young shoots of the plant were fed with [1,5-^{14}C]cadaverine, labelled anagyrine, pachycarpine, ammodendrine, N-methylcytisine, and cytisine were isolated.

Judging from the experimental results, a probable precursor of the quinolizidine [2, 3] and piperidine [4] alkaloids of this plant is [1,5-^{14}C]cadaverine (+, picrate, ++, hydriodide):

Alkaloid	Amount, mg	mp, °C	Specific radioactivity, counts/min/mmole	Proportion of inclusion, %
[1,5-^{14}C]cadaverine	20	—	$5.5 \cdot 10^9$	—
Anagyrine	4.5	253+	$7.42 \cdot 10^7$	1.34
Pachycarpine	154	235++	$4.27 \cdot 10^7$	0.77
Ammodendrine	20.14	73	$4.16 \cdot 10^7$	0.75
N-methylcytisine	7.5	136	$2.4 \cdot 10^6$	0.43
Cytisine	11.1	135	$2.98 \cdot 10^6$	0.05

V. I. Lenin Tashkent State University. Institute of Plant Biochemistry, Academy of Sciences of the GDR, Halle.

Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 247-250, March-April, 1977. Original article submitted December 2, 1976.