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COMPARATIVE BIOSYNTHESIS OF QUINOLIZIDINE ALKALOIDS FROM DL-LYSINE IN FIVE SPECIES OF *LEGUMINOSAE**

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Abstract—Five species of the leguminosae produced radioactive lupine alkaloids after feeding with DL-lysine-[2-^{14}C]. Saturated alkaloids and compounds with a pyridone ring were radioactive. The specific radioactivity of the isolated compounds provides evidence that conversion of lysine into the saturated alkaloids, and by further oxidation to compounds both with a pyridone ring and without a D ring.

INTRODUCTION

Previous studies [1–7] have established that lysine is probably the only substrate necessary for biosynthesis of lupine alkaloids in species of *Lupinus*, *Laburnum* and *Sarothamnus*. All experiments with ^{14}C labeled lysine and cadaverine resulted in the biosynthesis of radioactive lupanine, lupinine, sparteine and other saturated lupine alkaloids in *Lupinus* and cytisine in *Laburnum* [8]. Other compounds such as acetate, succinate and aspartate proved to be less effective.

In the present study, our aim was to demonstrate that the lysine conversion pathway is the main route for biosynthesis of all lupine alkaloids with the quinolizidine ring system. A further aim was to determine the isotope dilution factor for the individual alkaloids. The plants chosen were species with different alkaloid composition so that it would be possible to establish divergent pathways for the conversion of the alkaloids in the species investigated.

RESULTS AND DISCUSSION

The incorporation of radioactivity from DL-lysine-[2-^{14}C] into lupine alkaloids in three species of *Lupinus*, one species of *Thermopsis* and one species of *Baptisia* are shown in Tables 1–3. *Lupinus luteus* contains the simplest alkaloid structurally speaking; *L. angustifolius* accumulates

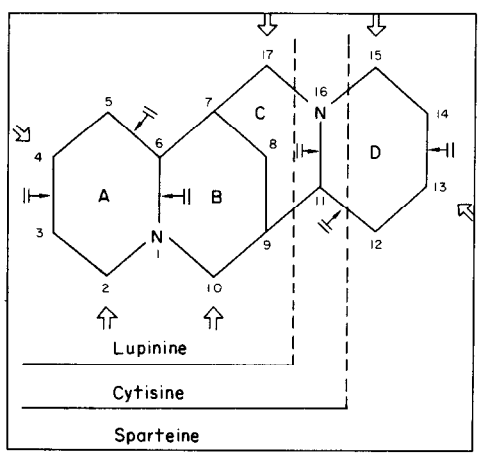

Fig. 1. The Structure of Lupine alkaloids; four ring—sparteine type; three ring—cytisine type; two ring—lupinine type; ⇨ most common oxidation and hydroxylation; ||→ most common dehydrogenation.

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Table 1. Biosynthesis of *Baptisia*, *Thermopsis* and *Lupinus* alkaloids from DL-lysine-[2-^{14}C]

Plant	Hydroxy-spartiene	Spart-eine	Lupanine*	Therm-opsine	Cytisine	Methyl-cytisine	Bapti-foline	Not identified
Baptisia leucophea								
Distribution of isolated alkaloids (%) (654 mg total)	1·6	2·0	2·6	4·5	49·3	20·3	2·0	17·7
Distribution of radioactivity (6657 dpm total × 10^{-2})	5·1	22·7	6·1	12·2	30·1	5·4	5·5	12·3
Specific activity (dpm/mmol × 10^{-2})	537	1768	387	438	79	36	445	—
Thermopsis macrophylla								
Distribution of isolated alkaloids (%) (350 mg total)		11·6	26·9	13·9	9·1	23·7	—	12·1
Distribution of radioactivity (%) (13 500 dpm total) × 10^{-2}	—	8·9	9·4	41·9	5·3	8·8	—	26·1
Specific activity (dpm/mmol × 10^{-2})	—	2440	1060	9830	1490	1030	—	—
Lupinus luteus								
Distribution of isolated alkaloids (%) (932 mg total)	—	42	5	—	—	—	—	53
Distribution of radioactivity (%) (242 dpm total × 10^{-2})	—	39·2	6·2	—	—	—	—	54·6
Specific activity (dpm/mmol × 10^{-2})	—	530	504	—	—	—	—	—

* In *L. Luteus—lupinine*.

Table 2. Biosynthesis of *Lupinus angustifolius* alkaloids from DL-lysine-[2-^{14}C]

	Lupanine	Hydroxylupanine	Angustifoline	Not identified
Distribution of isolated alkaloids (%) (1235 mg total)	46	32	20	2
Distribution of radioactivity (%) (145 dpm total × 10^{-2})	20·7	10·3	6·2	62·3
Specific activity (dpm/mmol × 10^{-2})	163	127	103	—

Table 3. Biosynthesis of *Lupinus nanus* alkaloids from DL-lysine-[2-^{14}C]

	L_1	L_2	pl^{-4}	Thermopsine	Not identified
Distribution of isolated alkaloids (%) (590 mg total)	5	10	2	70	13
Distribution of radioactivity (%) (5449 dpm total × 10^{-2})	36·5	24·2	14·9	13·9	8·4
Specific activity (dpm/mmol × 10^{-2})	931	327	—	26	—

L_1 was an unidentified lupine alkaloid with a molecular weight of 234; L_2 was an unidentified alkaloid with a molecular weight of 248; pl^{-4} were a series of unidentified alkaloids present in trace amounts.

alkaloids which are further conversion products of sparteine; and *L. nanus* contains thermopsine, an alkaloid with a pyridone ring, and two unknown saturated alkaloids with molecular weights of 234 and 248. *Thermopsis* and *Baptisia* contain considerable quantities of cytisine and *N*-methylcytisine; neither of these alkaloids contain a fourth ring (Fig. 1).

Biosynthesis of quinolizidine alkaloids from DL-*lysine*

The biosynthesis experiments with DL-lysine-[2-^{14}C] confirmed the previous data [9] that all quinolizidine alkaloids are derived from this amino acid, a fact that was true for both the saturated as well as the dehydrogenated ring systems (Tables 1–4). The highest efficiency of conversion of lysine into these alkaloids was achieved in plants which produced the pyridone alkaloids, i.e. *L. nanus* (Table 3), *Thermopsis macrophyla* (Table 1) and *Baptisia leucophea* (Table 1); the next was *L. angustifolius* (Table 2) and the lowest was *L. luteus* (Table 1). These results may be explained based on the requirement that more steps are necessary to convert lysine to thermopsine (*L. nanus*) than to sparteine (*L. luteus*) and that the main alkaloid is the last compound on the biosynthetic pathway for that particular plant. It may exhibit an inhibitory feedback effect on biosynthesis, hence in plants with a longer alkaloid pathway more of the precursor can be converted into alkaloids before the conversion is effectively inhibited. In *L. luteus* 50% of all the radioactivity extracted with methylene chloride was found to occur as amines. A considerable portion, up to 30%, of this amine fraction was cadaverine. In *L. angustifolius* only traces of radioactivity were found in the amine fraction. Amines other than cadaverine were not identified in any of the samples.

Following the feeding of lysine to *L. nanus* (Table 3) the specific activity of the unsaturated alkaloids (L_1 and L_2) was 13–37 times higher than that of thermopsine. After lysine administration to

Table 4. Biosynthesis of quinolizidine alkaloids from DL-lysine-[2-^{14}C] in *Leguminosae*

Alkaloid		*L. luteus*	*L. angustifolius*	*L. nanus*	*T. macrophylla*	*B. leucophaea*
Crude alkaloids isolated						
Amount, mg		932	1235	590	350	654
Radioactivity,	dpm	24200	14500	544900	1350000	665700
Incorporation,	%	0·13	0·07	1·1	3·6	1·8
Purified alkaloids						
Lupinine	a	50·36	—	—	—	—
	b	100	—	—	—	—
Sparteine*	a	35·36	—	62·09	162·6	117·8
	b	70	—	100	25	100
Hydroxy-	a	—	—	—	—	36·8
sparteine	b	—	—	—	—	30
Lupanine†	a	—	10·93	21·82	70·1	26·8
	b	—	100	35	11	22
Hydroxy-	a	—	8·74	—	—	—
lupanine	b	—	77	—	—	—
Thermopsine	a	—	—	1·75	655·3	29·3
	b	—	—	3	100	25
Baptifoline	a	—	—	—	—	29·7
	b	—	—	—	—	25
Angustifoline	a	—	7·39	—	—	—
	b	—	68	—	—	—
Cytisine	a	—	—	—	135·0	7·2
	b	—	—	—	21	6
Methylcytisine	a	—	—	—	86·0	3·0
	b	—	—	—	13	3

(a)—dpm/mmol C.
(b)—Relative activity compared to alkaloids with highest radioactivity.
* In *L. nanus* sparteine is replaced by an alkaloid with MW 234; chromatographically it is not a sparteine.
† Lupanine in *L. nanus* is replaced by an alkaloid with MW 248 again not identical with lupanine on TLC.

Table 5. Inhibition of thermopsine biosynthesis in *Lupinus nanus* from DL-lysine-[2-^{14}C]

Compound added	Young plants (%)	Flowering plants (%)
None (dpm = 100%)	(2296)	(1475)
Putrescine	96·1	58·1
Hexamethylene diamine	90·6	57·9
Sparteine	72·9	—
Cadaverine	54·6	50·8

L. luteus both identified alkaloids had similar specific activity yet due to the different proportion of C:N:O in lupinine and sparteine the specific activity calculated per mmole of carbon was higher for lupinine (Table 4).

In *L. angustifolius* (Table 2) lupanine had the highest specific activity with both hydroxylupanine and angustifoline being less labeled. In plants with both saturated and dehydrogenated alkaloids the former were more radioactive in *Baptisia* (Table 1) and *L. nanus* (Table 1), less radioactive in *Thermopsis* (Table 1).

Inhibition of alkaloid biosynthesis

When *L. nanus* was fed simultaneously with DL-lysine-[2-^{14}C] and inactive cadaverine (Table 5) incorporation of label into thermopsine was significantly lower than in the control experiments. Putrescine and hexamethylene diamine inhibited the incorporation only slightly; however, sparteine proved more effective than cadaverine. Cadaverine, putrescine, hexamethylene diamine were effective in flowering plants as inhibitors.

CONCLUSION

As conversion of lysine into all quinolizidine alkaloids was confirmed. The specific radioactivity of the isolated alkaloids suggest a conversion pathway leading from the saturated alkaloids to the compounds with the pyridone ring. Alkaloids without ring D, such as cytisine and methylcytisine had the lowest specific activity. Such results may be caused by the relatively high pool of these compounds but it can also be explained by the longer pathway leading to their formation. Although no sparteine was detected in the steam distillate of *L. nanus* alkaloid extracts, an intermediate on the oxidation level of sparteine with a MW of 234 was found. It is postulated that this unknown compound is probably the precursor of thermopsine since sparteine is effective in reducing the incorporation of lysine into thermopsine.

Inactive diamines when added to flowering plants inhibit the conversion of lysine into thermopsine. This may be a further indication that cadaverine is the first intermediate to be formed from lysine in the biosynthesis of the quinolizidine ring system.

EXPERIMENTAL

Plant material. Lupinus luteus, the sparteine-rich strain "CS bred at Przebedowo", *L. angustifolius*, the alkaloid-rich strain "Wielkopolski gorzki" and *L. nanus*, selected strain "nasz", were grown in the greenhouse and in the plant growth chamber; non-flowering plants 4–8-weeks-old were used. *Thermopsis macrophylla* plants growing in the plant growth chamber and *Baptisia leucophea* plants growing along U.S. Highway 177 south of Stillwater were used.

Origin of seeds. All lupine seeds were from the collection of cultivated plants at the Laboratory for Phytochemistry, Przebedowo, Poland, where the plants had been propagated and selected for chemical uniformity. Seeds of *T. macrophylla* were obtained from the Department of Environmental Horticulture, University of California at Davis.

Alkaloids. L-Sparteine was obtained from Cefarm, Warsaw; α-iso-sparteine, α-isolupanine, epi-lupinine and angustifoline were gifts from Professor Dr. M. Wierwiórowski. Lupanine, hydroxylupanine, lupinine, cytisine and thermopsine were isolated and *N*-methyl cytisine was synthesized from cytisine by refluxing the latter compound with methyl iodide. DL-lysine hydrochloride-[2-^{14}C] was purchased from New England Nuclear Corporation; it was assayed and subjected to TLC before use and only one spot was observed.

Feeding experiments. Rootless plants were placed in 1·5 ml of the solution to be fed. After all of the solution was taken up the plants were transferred into H_2O. The remaining radioactivity in the vessel was assayed and the actual amount taken up by the plant calculated. The plants were then allowed to metabolize for 24 hr in all *Lupinus* experiments for 48 hr in the *Thermopsis* and *Baptisia* experiments. The plants were ground to a powder and moistened with 20% $NaHCO_3$. Excess moisture was absorbed by adding Sigmacell cellulose. The mixture was extracted overnight (soxhlet) with CH_2Cl_2. The extract was evaporated and the alkaloids transferred into 2% HCl. Aliquots (20 μl) were taken for the determination of total radioactivity and for chromatographic analysis.

Chromatography. Alkaloids as free bases were applied with standards to 5 × 20 cm TLC plates (Silica gel F_{254}). The plates were developed in a $CHCl_3$–MeOH (5:1), scanned with a 2π strip counter to locate the radioactivity and sprayed with a solution of Dragendorff's reagent to locate the alkaloids. The radioactive spots were extracted with MeOH and assayed in a liquid scintillation spectrometer [10]. Purified alkaloids as HCl salts were decomposed with sat. $NaHCO_3$, dried (Na_2SO_4) and extracted successively with hexane and CH_2Cl_2. Each fraction was separately chromatographed on preparative (2 mm) silica gel TLC. The final isolation steps used for pure alkaloids were: sparteine by steam distillation, lupanine by recrystallization as its perchlorate from *iso*-PrOH, isolupanine and thermopsine by crystallization as free bases from hexane. In experiments where the amount of alkaloid was insufficient for crystallization the

amount was determined after chromatography by UV analysis at 305nm. This method was applicable only for thermopsine, cytisine and *N*-methylcytisine. Cadaverine was isolated by steam distillation, then converted to its benzoylderivative. In some cases, such as when the plant extract contained both saturated tetracyclic alkaloids and cytisine, minute amounts of cytisine were isolated as its benzoylderivative as the *N*-benzoylcytisine had a very different R_f value and thus was more easily isolatable.

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