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METABOLISM OF L-SPARTEINE, DL-LUPANINE AND L-THERMOPSINE IN SPECIES OF *LEGUMINOSAE**

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Key Word Index—Leguminosae; quinolizidine alkaloids; biosynthesis; interconversions.

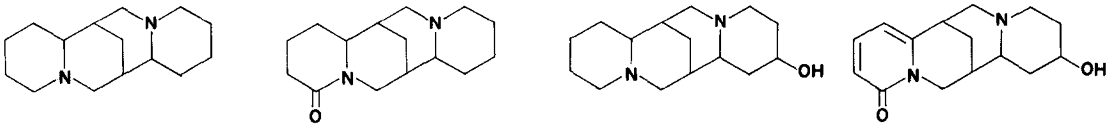
Abstract—L-Sparteine-[^{14}C], DL-lupanine-[^{14}C] and L-thermopsine-[^{14}C] fed to plants served as precursors to the more highly oxidized alkaloids. Radioactivity was also found in amino acids.

Lysine has previously been shown to be a precursor of all known lupine alkaloids [1–5]. The similarity in the chemical structure of various lupine alkaloids suggested the probability of their interconversion. Attempts have been previously made [6,7] to study the transformation of lupine alkaloids by administering sparteine to lupine plants; however, these experiments did not use radioactive substrates.

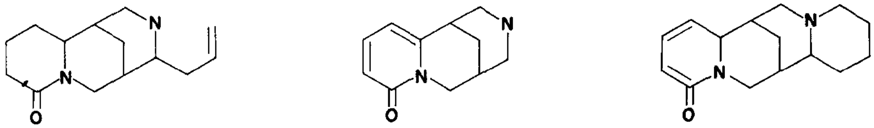
Certain species of *Leguminosae* produce, beside the common lupine alkaloids, alkaloids with a dehydrogenated ring. Such as thermopsine, anagyrine, baptifoline, cytisine and methylcytisine which are found in species of *Baptisia*, *Thermopsis* and in *Lupinus nanus*.

Some information on the biogenesis of the quinolizidine alkaloids are now known[8]. Certain *in vivo* conversions have been established, such as lupine into sparteine, and sparteine into lupanine and hydroxylupanine [9–11]. Other possible conversions, such as that of the tetracyclic sparteine type of alkaloids into the tricyclic cytisine alkaloids, have been suggested [5]. Some data on the metabolism of lupine alkaloids were obtained by means of genetic experiments [12–14]. All these observations confirmed the suggestions presented by Robinson [15] and Leete [16].

In spite of the fact that many gaps remain in these biosynthetic pathways, we decided to investi-



Sparteine Lupanine Hydroxy sparteine Baptifoline



Angustifoline Cytisine Thermopsine

Structural formulae of alkaloids investigated.

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Table 1. Metabolism of DL-sparteine-[^{14}C] and DL-lupanine-[^{14}C] in *Lupinus nanus*

	L_1	L_2	p^{1-4}	Thermopsine	Not identified
Sparteine					
Distribution of isolated alkaloids (%) (640 mg total)	14	7	1	48	30
Distribution of radioactivity (%) (10133 dpm total)	15·5	22·3	9·5	14·5	38·2
Specific activity (dpm/mmol)	2592	7904	—	738	—
Lupanine					
Distribution of isolated alkaloids (%) (670 mg total)	18	11	4	48	19
Distribution of radioactivity (%) (7217 dpm total)	26·7	26·0	10·6	13·3	23·4
Specific activity (dpm/mmol)	2508	4241	—	487	—

L_1 is an unidentified lupine alkaloid with a MW of 234; L_2 an unidentified alkaloid with a MW of 248; p^{1-4} were a series of unidentified alkaloids present in trace amounts.

gate the conversion of saturated tetracyclic lupine alkaloids into the pyridone tetracyclic alkaloids and the latter group into the three tricyclic alkaloids.

RESULTS

The incorporation of L-sparteine-[^{14}C] and DL-lupanine-[^{14}C] into the alkaloids of *L. nanus* is shown in Table 1. The incorporation of DL-lupanine-[^{14}C] into the alkaloids of *Thermopsis* and *Baptisia* plants is shown in Table 2. It can be seen that the introduction of sparteine-[^{14}C] or lupanine-[^{14}C] gave radioactivity in alkaloids with a dehydrogenated ring A (pyridone ring) such as thermopsine and cytisine, and the specific activity of the isolated compounds suggests that there is direct pathway between the two. When an alkaloid with a dehydrogenated ring A, e.g., thermopsine was introduced, only 12% of radioactivity was found in alkaloids with hydrogenated rings (Table

Table 2. Metabolism of DL-lupanine-[^{14}C] in *Thermopsis* and *Baptisia*

	Hydroxy-sparteine	Sparteine	Lupanine	Thermo-psine	Cytisine	Methyl-cytisine	Bapti-foline	Not identified
Thermopsis macrophylla								
Distribution of isolated alkaloids (%) (402 mg total)	—	10·6	31·5	14·2	10·1	22·7	—	11·0
Distribution of radioactivity (%) (3705 dpm total)	—	5·2	37·7	18·4	8·3	16·9	—	13·4
Specific activity (dpm/mmol)	—	424	1137	1094	584	567	—	—
Baptisia leucopheya								
Distribution of isolated alkaloids (%) (701 mg total)	1	2·5	10·6	5·2	24·2	25·2	2·0	29·3
Distribution of radioactivity (%) (3607 dpm total)	1·3	1·3	28·5	10·3	8·8	7·8	4·7	37·3
Specific activity (dpm/mmol)	115	45	240	174	25	23	206	—

Table 3. Metabolism of DL-thermopsine-[^{14}C] in *Thermopsis* and *Baptisia*

	Hydroxy-sparteine	Sparteine	Lupanine	Thermo-psine	Cytisine	Methyl-cytisine	Bapti-foline	Not identified
Thermopsis macrophylla								
Distribution of isolated alkaloids (%) (410 mg total)	—	10·1	26·4	27·2	8·3	18·7	—	9·3
Distribution of radioactivity (%) (1157 dpm total)	—	—	11·8	51·1	10·3	12·6	—	14·2
Specific activity (dpm/mmol)	—	—	129	530	274	160	—	—
Baptisia leucopheya								
Distribution of isolated alkaloids (%) (702 mg total)	1·5	2·0	2·6	10·5	35	31	3·7	14·3
Distribution of radioactivity (%) (1375 dpm total)	1·0	1·1	1·7	30·7	12·7	7·9	9·9	34·9
Specific activity (dpm/mmol)	23	18	23	981	95	72	897	—

3). After sparteine, lupanine and thermopsine were administered, regardless of the pool size of the investigated alkaloids, more radioactivity was usually detected in the higher oxidized compounds. The distribution of radioactivity presents more information about the conversion than the specific radioactivity of isolated alkaloids. The specific radioactivity is highly affected by the pool size, while the total radioactivity is less.

The experiments involving the feeding of lupine seeds with radioactive alkaloids seemed to indicate that the alkaloids were utilized during germination and then converted into other compounds, e.g. amino acids (Table 4). Experiments with pyridone-^{14}C feeding confirmed the previous experiments with nicotinic acid feeding [17] that the pyridone ring is synthesized, like all other rings of lupine alkaloids, from lysine and not from glyceraldehyde-3-phosphate and aspartate which serve as precursors of nicotinic acid in plants, since only 0·8% of the administered label entered the alkaloid fraction.

According to these results we suggest that the biosynthetic pathway from lysine to the lupin alkaloids is via sparteine and lupanine and that these compounds are further metabolized to alkaloids with a dehydrogenated ring A. Such alkaloids may be further oxidized to give components which give no reaction with alkaloid detecting reagents. The decomposition products are amino acid which are metabolized via the respective amino acid pools. These results are similar to the finding on nicotine degradation in plants [18].

EXPERIMENTAL

The origin of plant material, the reagents and the procedures applied in feeding experiments, as well as the isolation procedures, were identical with those described previously.

Radioactive alkaloids. DL-Lysine HCl-[2^{14}C] was purchased from New England Nuclear Corp. It was assayed and subjected to TLC before use and only one spot was observed. L-Sparteine-

Table 4. Transformation of ^{14}C alkaloids into amino acids in germinating *Lupinus angustifolius* seedlings

	Radioactivity		Compound(s) isolated (g)	Radioactivity mmole carbon (dpm)
	(dpm × 10^3)	(%)		
Introduced alkaloids	4560	100	0·1	760000
Isolated alkaloids	689	15·1	0·96	11960
Isolated crude amino acids	284	7·2	16·0	—
Isolated crystalline asparagine	196	4·3	12·0	612

[^{14}C] was biosynthesized in *L. luteus* from lysine-2-[^{14}C]. DL-Lupanine-[^{14}C] was biosynthesized in *L. albus* from lysine-2-[^{14}C], L-lupanine-[^{14}C] was biosynthesized in *L. angustofolius* from lysine-2-[^{14}C] and DL-thermopsine-[^{14}C] was biosynthesized in *L. nanus* from lysine-2-[^{14}C]. Each alkaloid was recrystallized to constant specific radioactivity. They were assayed by TLC.

Administration of alkaloids to germinating seeds. Lupanine HCl-[^{14}C] (100 mg) was dissolved in 10 ml H_2O and fed to 20 g of *L. angustifolius* seeds, variety "Frost", from the Florida Agricultural Experiment Station, Ganisville, Florida. The seeds were surface sterilized with 75% EtOH for 10 min before the alkaloid was added, then washed with sterile H_2O and then a 10 min sterilization treatment with 2% $HgCl_2$ was administered. The seeds were finally washed with sterile H_2O and placed into the solution to be fed. After 24 hr the seeds were treated with fungicide, planted in vermiculite, and kept in darkness for 10 days. At this time 3/4 of the plants were used for extraction of free amino acids and the rest for extraction of alkaloids.

Extraction of amino acids. The etiolated plantlets were cut into pieces, frozen with liquid N_2 and 100 ml of MeOH–$CHCl_3$–H_2O (6:2·5:1·5) was added. After the plants were sufficiently macerated 300 ml H_2O was added to the homogenate. The homogenate was heated at 10° for 3 hr and filtered. The residue was triturated in 300 ml H_2O, heated, and filtered. The solutions were combined, reduced to 75 ml and sufficient EtOH added to make the solution slightly turbid. Asparagine monohydrate crystallized out when the solution was left overnight at 4°; the crop of crystals was filtered off and recrystallized to constant specific radioactivity and to chromatographic purity. The mother liquor was used for chromatographic separation of other amino acids.

Synthesis of the pyridone. Ring labeled ricinine biosynthesized in previous experiments from nicotine acid-[6^{14}C] in young castor bean plants, was subjected to an alkaline hydrolysis in 1 N NaOH to produce *O*-demethylricinine [19]. It was subsequently hydrolyzed in 50% H_2SO_4 and yielded a compound with MW 125. The compound was purified by chromatography on Silica gel G TLC prep.

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