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USE OF THE METABOLIC GRID TO EXPLAIN THE METABOLISM OF QUINOLIZIDINE ALKALOIDS IN *LEGUMINOSAE**

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Key Word Index—Leguminosae; quinolizidine alkaloids; biosynthesis; metabolic grid.

Abstract—Evidence from feeding experiments with lysine-[2-^{14}C] and from metabolism experiments published previously suggest the operation of a gridlike conversion of quinolizidine alkaloids in *Leguminosae*. Using these results and the taxonomical distribution of alkaloids a metabolic grid was devised to explain the conversion of lysine into lupin alkaloids and their interconversions.

INTRODUCTION

The quinolizidine alkaloids (lupin alkaloids) are mainly restricted to three closely related tribes of the *Leguminosae*; Sophoreae, Podalyrieae and Genisteae. In addition, they occur in a few unrelated families, such as the Chenopodiaceae (*Anabasis aphylla*), Papaveraceae (*Chelidonium majus*), Solanaceae (*Solanum lycocarpum*), and Berberidaceae (*Caulophyllum robustum*). While the distribution outside the *Leguminosae* is apparently random, all three tribes of this family have lupin alkaloids in most species [1], although there are a few exceptions [2] (*Podalyria* which contains some tyramine derivatives [3] and *Crotalaria*, which have pyrrolizidine alkaloids). The three tribes are phylogenetically related and, besides having alkaloids with similar structure and biosynthetic origin, have species with similar proteins [4].

There is now sufficient evidence that all the quinolizidine alkaloids are synthesized from lysine [5], and that the alkaloids are converted from saturated, oxygen-free compounds into derivatives with various oxidation levels [6].

RESULTS AND DISCUSSION

By combining the biosynthetic data obtained in our earlier studies [5,6] with published evidence from the work of others and the distribution of alkaloids in the *Papilionaceae* (Schemes 1–4), a computer program to outline the possible pathways for the biosynthesis, conversion and ultimate degradation of quinolizidine alkaloids in plants was designed. The results are presented in the form of a "metabolic grid" of the type suggested by Bu Lock [7] (Table 1 and Figure 5).

Chemotaxonomical considerations

The "metabolic grid", together with the available data, permits some speculation on the origin of the alkaloids in species of Sophoreae, Podalyrieae and Genisteae. As mentioned earlier, these three tribes are closely related and all but a few species have lupin alkaloids. The absence of lupin alkaloids in *Podalyria* and *Crotalaria* may be secondary in character; both genera actually produce alkaloids but of different structures. While *Podalyria* has tyrosine derived amines, *Crotalaria* accumulates pyrrolizidine alkaloids which can be synthesized from ornithine on a pathway homologous to the synthesis of lupinine from lysine [8]. With these exceptions, all other lupin alkaloids can be derived from lysine (A11, Scheme 1). The next step in the biosynthesis must be a symmetrical compound since the distribution of radioactivity in

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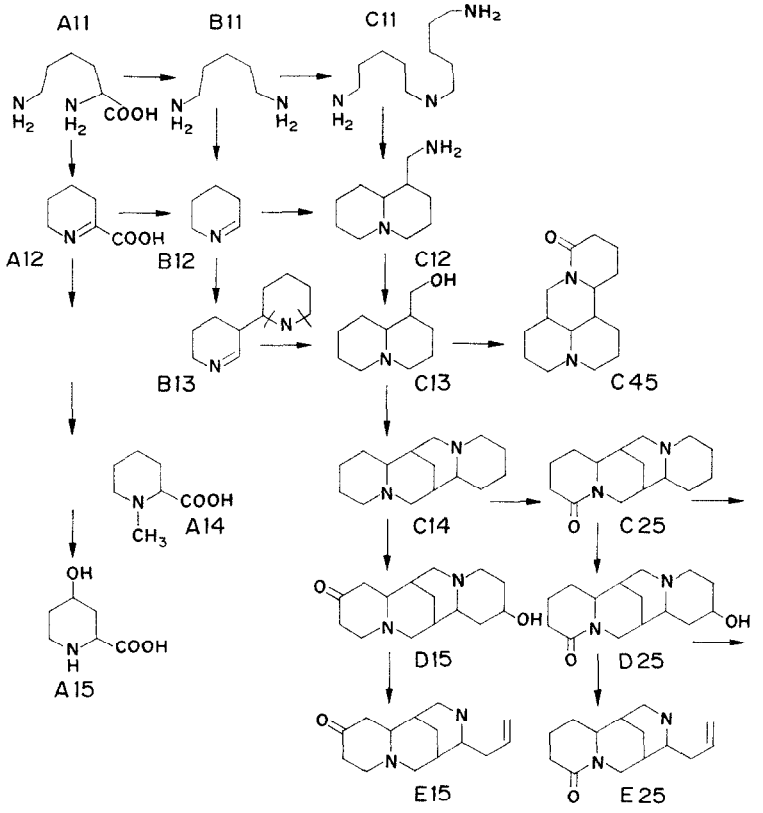
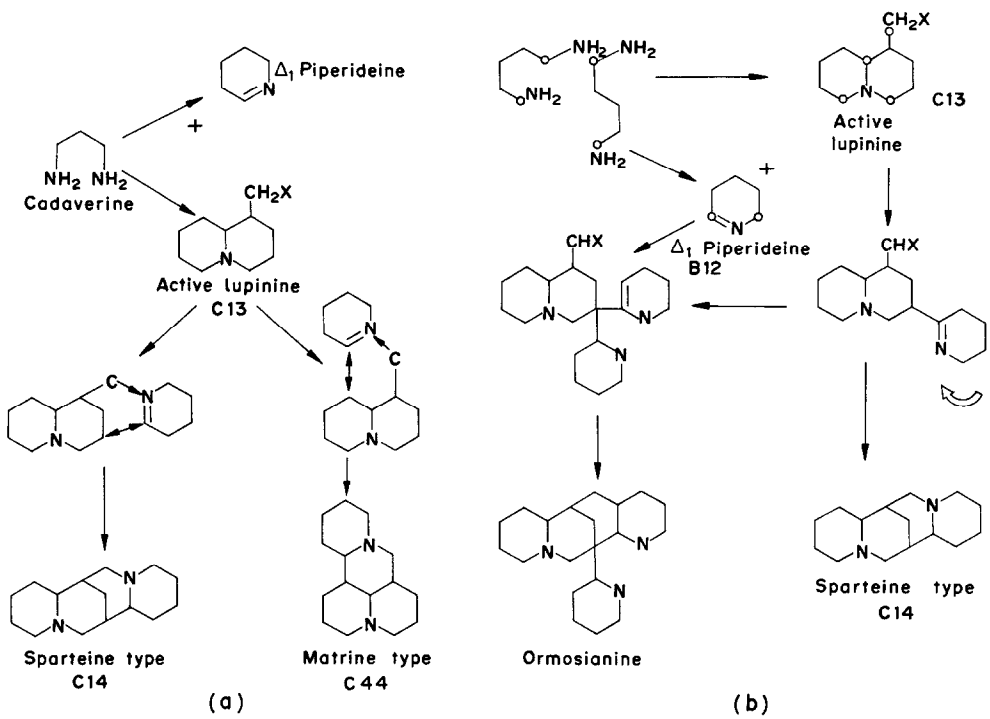


Scheme 1. Pathways for the conversion of lysine into alkaloids (see Table 1 for names of compounds).

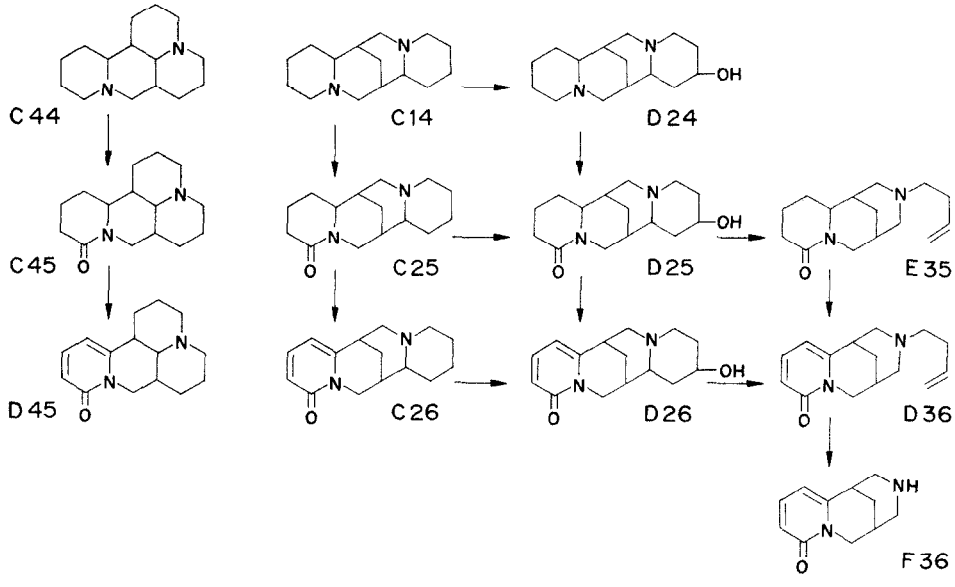
the rings is such that it is explainable only when a symmetrical intermediate is assumed [9–11]. The incorporation of cadaverine (B11) with a lower dilution factor than lysine and the decrease of lysine incorporation when fed simultaneously with a diamine makes the assumption highly probable that cadaverine is the proper intermediate. Cadaverine can be oxidized to form a piperidine ring both by a diamine oxidase and an amine transferase. The diamine oxidase can be excluded as the enzyme actively synthesizing the alkaloids, since no relation could be found between the actual alkaloid synthesis and the level of diamine oxidase in the plants. Some sweet lupin have higher levels of this enzyme than the bitter lupins [12]. The cyclic derivative of cadaverine can be dimerized or polymerized in the cell. Thus, the origin of the dipiperidine alkaloids such as ammodendrine (B14), sanquinarine, and hystrine (D22) is explainable without considering special enzyme systems being required to convert the piperidine nuclei. The dipiperidine alkaloids are encountered in the most primitive species of the tribes Sophoreae and Genisteae. A conversion of the dipiperidine into lupinine is possible only by means of a rearrangement in the molecule coupled with the loss of a nitrogen atom; unfortunately, the experimental data do not confirm this assumption. Actually, the dipiperidines are poorer substrates than cadaverine [13,14]. Lupinine is easily converted into sparteine in *L. luteus* [15] and should be the key compound leading to the diverse sparteine, matrine and ormosianine type of alkaloids. The condensation was thought to proceed on a pathway proposed by Bohlman *et al.* [16] through a condensation of lupinane with a piperidine molecule to form piperidinolupinane. However, this latter compound proved to be a poorer substrate than lupinine [17]. A different order of condensation could be proposed, omitting the piperidinolupinane stage such as is shown in Scheme 2. If lupinine is the key compound, then all the sparteines, both D and L isomers as well as the isosparteines, the matrine and ormosianine alkaloids should be derived from

lupinine or a related compound. Since the distribution of the main forms is quite well restricted to some taxonomical units, the conversion seems to be not only of taxonomical value but must reflect a very ancient branching of the pathways. Once the tetracyclic alkaloids are formed, they undergo a number of fates, some parallel. Both D and L sparteine are usually accompanied by corresponding lupanines, and α-isosparteine is accompanied by α-isolupanine. The derivatives of D-sparteine are readily transformed into dehydrogenated compounds which can lose the D ring. The conversion of L-sparteine to dehydrogenated compounds seems less common. The conversion of the *cis-trans* form into a *cis-cis* form can probably be achieved at all oxidation levels, i.e. sparteine, lupanine and anagirine (Scheme 4). The conversion of sparteine to lupanine involves a replacement of hydrogen by oxygen on C-2. When a replacement takes place on C-4 another series of alkaloids is formed, namely, the multiflorine derivatives.

Sparteine, lupanine and multiflorine can be hydroxylated on C-13. Some of the hydroxy compounds can be esterified with the same carboxylic acids which esterify tetrahydroanabasine in the more primitive species. The hydroxamine esters are probably the intermediate steps in the degradation of the D ring. An *in vitro* experiment by Bohlmann [18] indicated that it is possible to obtain both the angustifoline and rhombifoline ring opening via a 13-acetyloxyhydramine or 13-cinamonyloxylupanine. This reaction is easier with *cis-trans* alkaloids than with *cis-cis*. Thus, thermopsine should be more stable toward degradation of the D ring than anagirine and lupanine. In fact, only recently one 13-hydroxy derivative of isosparteine, argentamine was isolated [19]. Thermopsine, when introduced into *Baptisia*, did give rise to cytisine [16]. Introducing a second oxygen atom into a lupanine molecule at C-17 gives rise to oxolupanine, a neutral compound, which does not react with most alkaloid detection reagents. Oxolupanine was found, until recently, only in two species, *L. angustifolius* and *L. polyphyllus* [20]. It is possible that other alkaloids can be oxidized at C-17 following oxidation on C-2, and they will behave like oxolupanine and will thus escape attention. Oxidation of C-17 is very easy, at least,



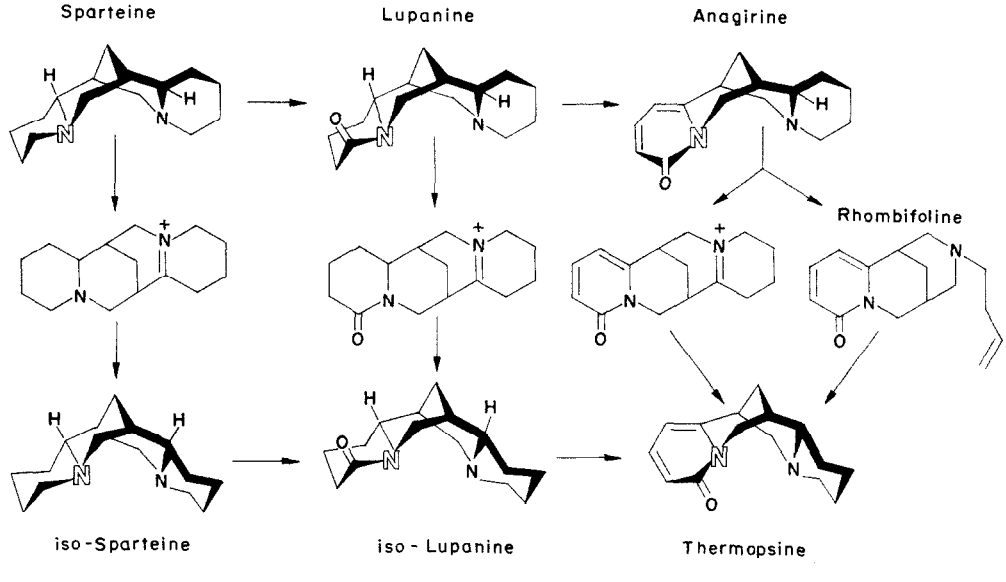
Scheme 2. (a) Branch point "active lupinine" leading to tetracyclic sparteine and matrine alkaloids; (b) branch point of *Lupinus* and *Ormosia* alkaloids.



Scheme 3. Metabolic grid pattern for the conversion of tetracyclic alkaloids.

chemically; however, biologically it is difficult. Our grid, therefore, reflects only one long pathway, which is the one leading to methylcytisine. All the compounds on this pathway are basic and none is oxidized at C-17. Other pathways in the grid end also with compounds not oxidized at C-17. A similar effect can be achieved with oxidation on C-15 and C-10 instead of C-17 and C-2.

After our experiment was completed, we became acquainted with the results of Cho and Martin [21] on the biosynthesis of lupin alkaloids from $^{14}CO_2$ by *Thermopsis* which suggested the primacy of lupanine with 5,6-dehydrolupanine as the intermediate between lupanine and anagirine. These results which were based on $^{14}CO_2$ kinetics indicated that sparteine could be on a side path in



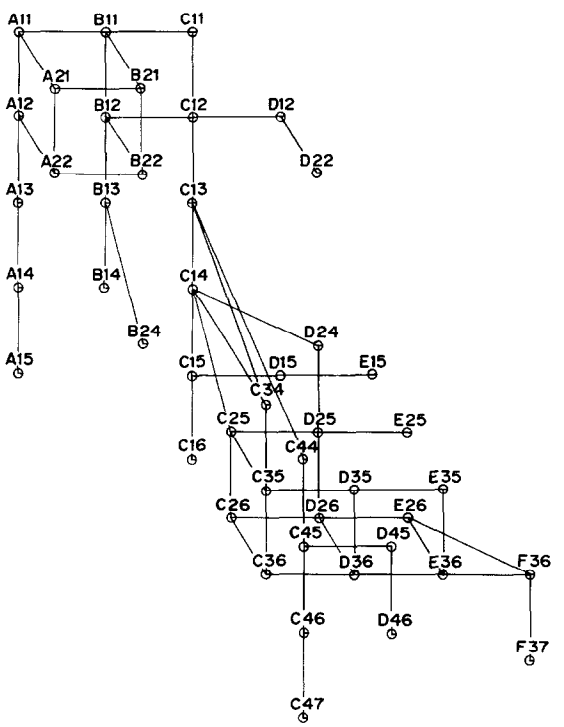
Scheme 4. Conversion of sparteine into L-*iso*-sparteine alkaloids.

the biosynthesis of the dehydrogenated and oxidized tetracyclic alkaloids. More direct evidence is thus needed to clarify the role of sparteine which is efficiently transformed into more oxidized lupine alkaloids when introduced into *L. angustifolius* and *L. nanus* plants and is an end product in *L. luteus* plants. The true intermediate in lupanine biosynthesis might be 1,2-dehydrosparteine, and for multiflorine 4,5-dehydrosparteine. Both predicted intermediates may originate directly from an "active lupinine" and a Δ_1 piperidine condensation. All other results, i.e. the order of production of the tricyclic bases, rhombifoline, cytisine and N-methylcytisine are consistent with the evidence[5,6,21].

In the metabolic grid it should be noted that although lupinine and epilupinine have, until now, been isolated from only six species, they should be in all species with tetracyclic alkaloids, since lupinine is the only known route for the formation of these compounds. Furthermore, despite numerous experiments, the intermediates between cadaverine and lupinine are also unknown. Lusitanine may be one of the possibilities, but this alkaloid has been found only once [22]. A clue may be found in the *Ormosia* alkaloids, which have the opposite stereochemistry for the C and D ring. In this case the tetracyclic alkaloids could originate from a condensation of a dehydro- or hydroxylupinine with piperidine. Lupinine would not be necessary. This still does not explain the biosynthesis of the simple quinolizidine ring.

There exists the possibility that lupinine is a degradation product of a tetracyclic alkaloid. This reaction was suggested by Nowacki *et al.* [23] and by Klyschew [24]. The conversion of lupinine into sparteine [25] is, however, against this.

Cyclic pathways are possible in other parts of the metabolic grid, e.g. lupanine, hydroxylupanine and hydroxylupanine esters. In most cases there is only circumstantial evidence, based on concentration changes of alkaloids during plant growth, and from evidence of *in vitro* chemical experiments.



Scheme 5. Proposed metabolic grid for the biosynthesis and interconversions of quinolizidine alkaloids in *papilionaceae*. For definition of symbols, see Table 1.

EXPERIMENTAL

Construction of the metabolic grid of lupine alkaloids. The data obtained in the experiments described previously [5,6], as well as data from published experiments [1–28] on the various conversions of the lupin alkaloids were used to construct short metabolic pathways. Data concerning the distribution of lupine alkaloids in Leguminoseae were drawn from Boit, Leonard, Steineger, Wiewiórowski, Faugerast, Balcer-Skrzydlewska, Cranmer, Mabry and Asłanow [26,28]. The pathways were connected when they overlapped. Tentative connections were drawn where no experimental data were available, but the structural formulas of the alkaloids enabled us to propose a conversion. In cases where the conversion of one substance to another was known to exist, but there were two possible intermediates and no proof was available that would permit us to choose one of the intermediates and disregard the other, both presumable intermediates were assigned the same probability. Each reported entry of an alkaloid was assigned a separate symbol (Table 1) in the computer program. Tracing backward from the substance to the common precursor —*lysine*—each substance on the pathway was assigned a separate symbol. In case there were two or three similar possibilities, e.g. thermopsine, which can arise theroretically from both anagyrine and isolupanine, and no data was available that indicated which of these alkaloids was the real substrate, both were assigned an equal chance for a symbol. To simplify the program, D and L isomers were treated together and the *Ormosia* alkaloids were disregarded.

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Table 1. Metabolic grid symbols and extent of occurrence of *papilionaceae* alkaloids

Grid symbol	Compound name	No. of times produced	No. of times occurred as end product
A11	Lysine	1002	0
A12	Pipecolic acid	28	3
A13	Methylpipecolic acid	24	20
A14	Homostachydrine	4	2
A15	Hydroxypipecolic acid	2	2
B11	Cadaverine	972	0
B12	Piperidine	498	1
B13	Tetrahydroanabasine	23	0
B14	Ammodendrine	7	7
C11	Dicadaverine	473	0
C12	Amino lupinine	947	1
C13	Lupinine	944	32
C14	Sparteine and dehydrosparteine	847	168
C15	Multiflorine	23	14
C16	Dehydromultiflorine	1	1
D12	Lusytanine	2	1
D15	Hydroxymultiflorine	8	2
E15	Albine	6	6
A21	N-methyl lysine	2	0
A22	Homostachydrine	3	2
B21	N-methyl cadaverine	1	0
B22	N-methyl piperidine	2	2
B24	Orensine	16	16
C25	Lupanine	478	123
C26	Anagirine	243	119
D22	Hystrine	1	1
D24	Hydroxysparteine	153	83
D25	Hydroxylupanine	141	26
D26	Baptifoline	217	9
E25	Angustifoline	6	6
E26	Rhombifoline	193	9
C34	Isosparteine	50	9
C35	Isolupanine	82	4
C36	Thermopsine	31	16
D35	Isohydroxylupanine	62	1
D36	Isobaptifoline	46	0
E35	Tetrahydrorhombifoline	46	0
E36	Rhombifoline	138	0
F36	Cystisine	276	154
F37	Methylcytisine	122	122
C44	Desoxymetrine	40	4
C45	Matrine	36	9
C46	Dehydromatrine	23	3
C47	Leontine	20	20
D45	D-Leontine derivative	4	0
D46	D-Leontine derivative	4	4

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