BIOSYNTHESIS OF LUPANINE FROM LYSINE AND OTHER LABELED COMPOUNDS

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Attempts were made previously by Reifer and Kleczkowska (1957) and Nowacki (1958) to study the biosynthesis of the lupine alkaloids by measuring the quantity of alkaloid synthesized following administration of several possible precursors to lupine plants. Because of the difficulty in interpreting the results of such experiments, studies were initiated to ascertain the incorporation of C^{14} labeled precursors into the lupine alkaloids. Schütte and Nowacki (1959) demonstrated that both cadaverine-C^{14} and lysine-C^{14} were incorporated into lupinine the most abundant alkaloid of Lupinus luteus L., and Schütte, Nowacki, Schäffer (1962) found that cadaverine was incorporated into lupanine in Lupinus angustifolius

There are still, however, many unanswered questions with regard to the pathway of carbon in the biosynthesis of the lupine alkaloids. The present study reports the results of incorporation of several C^{14} labeled compounds into these alkaloids by lupine plants.

Experimental Procedure: Five-week old L. angustifolius plants of the bitter variety "Wielkopolski Gorzki" were excised about a cm. above the roots and the excised end of the stem placed in about 1 ml. of an aqueous solution of the radioactive compound to be fed. The quantity of compound administered per plant was always less than 500 ug. and the radioactivity always less than 2 uc. It was assumed that the quantity of compound did not greatly effect the pool size of the

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compound within the plant. After the initial 1 ml. of solution had been taken up, the plants were given distilled water. About 20 plants were allowed to metabolize the radioactive compounds for a period of 48 hours in the light. They were then harvested and ground with about 20 mg. of KOH per plant. Next a 50:50 mixture of K_2CO_3 and K_2SO_4 was added until the plant material was dry. The dry mixture was extracted for 8 hours in a Soxhlet apparatus with chloroform. The chloroform extract was evaporated and the residue containing the alkaloids dissolved in about 10 ml. 0.01N HCl. This solution was made alkaline to phenolphthalein by adding solid K_2CO_3 and the aqueous solution was then extracted with petroleum ether (b.p. 40-60°). The petroleum ether extract, containing principally lupanine with traces of angustifoline and sparteine, was neutralized with 6% perchloric acid in methanol. The resulting alkaloid perchlorates were filtered, dried and weighed.

The radioactive alkaloid perchlorates were diluted with non-radioactive lupanine perchlorate and the mixture recrystalized 2 or 3 times from methanol to constant radioactivity. The lupanine perchlorate isolated in this manner showed a single component upon paper chromatography and radioautography with the same Rf value as authentic lupanine perchlorate, and had a m.p. of 210°C (reported m.p. 209-211°). The structural formula for lupanine is shown in Figure 1.

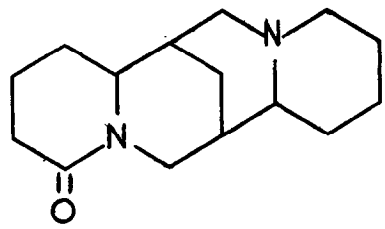

Fig.1.--Lupanine

Radioactivity of the isolated alkaloid salts was determined in a flow counter.

Results and Discussions: The radioactivity of lupanine after feeding several C^{14} labeled compounds is presented in Table 1. The greatest percentage incorpora-

tion of C^{14} into lupanine occurred after feeding lysine-2-C^{14}. Pipecolic-2-C^{14} acid, δ-aminovaleric acid-1-C^{14}, ornithine-2-C^{14} and formic acid-C^{14} provided decreasing quantities of radioactive carbon to lupanine in the order listed, with formic acid providing only about 2% of the C^{14} supplied by lysine-2-C^{14}.

Table I

Incorporation of Several C^{14} Labeled Compounds into Lupanine

Compound Fed	Specific Activity of Compound Administered cpm/mMol	Specific Activity of Lupanine cpm/mMol	Percent Incorporation into Lupanine of Compound Fed
Lysine	8.2×10^5	15600	1.90
Lysine	8.0×10^5	9800	1.23
Pipecolic Acid	6.6×10^5	3450	0.52
δ-Aminovaleric Acid	8.3×10^5	1330	0.16
Ornithine	5.1×10^5	642	0.12
Formic Acid	6.1×10^5	230	0.037

The experiments with ornithine confirmed previous results with uniformly labeled and guanidino labeled arginine (Nowacki and Przybylska, 1960) in which it was shown that this compound supplies little C^{14} to lupine alkaloids. Therefore, in spite of the fact that an inverse relationship exists between the concentration of alkaloids and arginine in several varieties of lupine (Przybylska, 1959) it seems clear that neither arginine nor ornithine is a direct precursor of lupanine.

The incorporation of δ-aminovaleric acid is of interest since this acid conceivably could be derived from either ornithine by deamination followed by reduction at position 2, or from lysine by decarboxylation and oxidation at position 2. δ-Aminovaleric acid could be converted to lupanine by reduction to the corresponding aldehyde followed by a condensation resulting in the formation of a 6-membered ring. In any event δ-aminovaleric acid, because it supplies less C^{14} to lupanine than lysine-2-C^{14}, cannot be considered on the direct pathway between lysine and lupanine.

The most probable pathway for the incorporation of lysine into lupanine would appear to be either through pipecolic acid or cadaverine. The results of the present study do not permit a clear choice between these alternatives, but the fact that C^{14} from pipecolic acid was incorporated to a relatively large extent establishes pipecolic acid as a possible intermediate.

It is conceivable that one of the final stages in the formation of lupanine might be the reaction between angustifoline and a one-carbon unit. The fact that formic acid is incorporated into lupanine in only relatively small quantities would seem to rule out this possibility.

A reasonable working hypothesis for the formation of lupanine, therefore, would point toward the utilization of three molecules of lysine for the formation of the ring systen of the alkaloid.

Summary: The biosynthesis of lupanine in Lupinus angustifolius has been studied by feeding lysine, δ-aminovaleric acid, pipecolic acid, ornithine and formic acid specifically labeled with C^{14}. Lysine-2-C^{14}, of all of these possible precursors, supplied the greatest quantity of C^{14} to lupanine.

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