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PYRROLIZIDINE ALKALOIDS. THE BIOSYNTHESIS OF RETRONECINE

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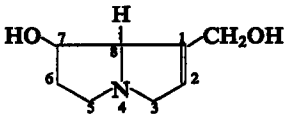
Abstract—The biosynthesis of the retronecine moiety of the pyrrolizidine alkaloids of *Senecio douglasii* has been studied with aid of radioactive tracers. It has been shown that putrescine and ornithine are used in the synthesis of the pyrrolizidine ring. When ornithine is used, at least one of the two molecules utilized in the synthesis of the base goes through a symmetrical precursor.

INTRODUCTION

ALKALOIDS containing the pyrrolizidine nucleus are found in the three plant families Leguminosae, Boraginaceae and Compositae. One of the most widely distributed of the natural bases, which are in most cases found as esters of C_5- to C_{10}-aliphatic acids, is retronecine (I).



(I)

Preliminary studies have been made of the biosynthesis of the acid[1] and base[2] moieties of alkaloids of this group, but there is still but little known about the biosynthetic route by which they are elaborated in the plant. Robinson's early suggestion[3] that the pyrrolizidine ring originates by the condensation of two ornithine residues finds support in the results of Nowacki and Byerrum,[2] who demonstrated that ornithine is incorporated into the retronecine derived from monocrotaline isolated from *Crotalaria spectabilis*.

This paper reports the results that have been obtained from the partial degradation of retronecine derived from the alkaloids of *Senecio douglasii*, after feeding the plants with [1,4-^{14}C]-putrescine, [2-^{14}C]-ornithine and [5-^{14}C]-ornithine.

RESULTS AND DISCUSSION

Senecio douglasii D.C. (Compositae) is a common perennial of the western United States. It has been shown[4–6] to contain the four alkaloids senecionine, seneciphylline, retrorsine and ridelliine, all of which are cyclic esters of C_{10}-dicarboxylic acids with the base retronecine (I).

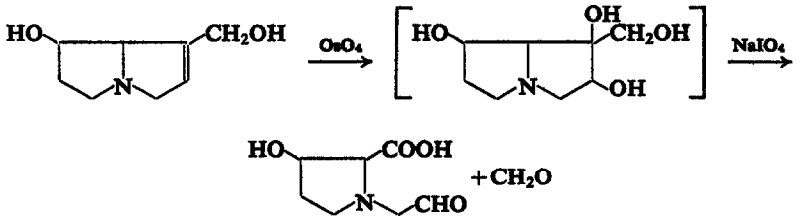
[1] C. HUGHES and F. L. WARREN, *J. Chem. Soc.* 34 (1962).
[2] E. NOWACKI and R. U. BYERRUM, *Life Sciences* 5, 157 (1962).
[3] R. ROBINSON, *The Structural Relations of Natural Products*, p. 72, Clarendon Press, Oxford (1955).
[4] R. ADAMS and T. R. GOVENDACHARI, *J. Am. Chem. Soc.* 71, 1956 (1949).
[5] F. L. WARREN, M. KROKMAN, R. ADAMS, T. R. GOVINDACHARI and J. H. LOOKER, *J. Am. Chem. Soc.* 72, 1421 (1950).
[6] R. ADAMS and J. H. LOOKER, *J. Am. Chem. Soc.* 73, 134 (1951).

Feeding of *Senecio douglasii* seedlings with ^{14}C-labeled putrescine or ornithine resulted in the incorporation of radioactivity in the retronecine portion of the alkaloids. Very little activity appeared in the acids derived from the alkaloids by hydrolysis. Table 1 shows the

Table 1. Incorporation of radioactive precursors into the alkaloids of *Senecio douglasii*

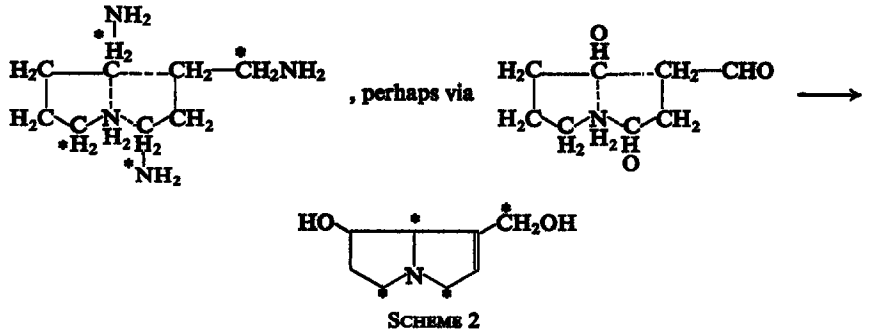
Precursor	Total incorporation into alkaloids (%)	Percentage of total activity of alkaloid found in	
		Acids	Retronecine
[1,4-^{14}C]-putrescine	0·18	5·0	98
[2-^{14}C]-ornithine	0·30	1·4	94
[5-^{14}C]-ornithine	0·75	2·4	94

efficiency of incorporation and the relative amounts of the radioactivity that were incorporated into the base and the C_{10}-acids. It is apparent that the carbon skeleton of the precursors remained intact and that the putrescine and ornithine were specifically incorporated into the pyrrolizidine moiety of the alkaloids.



Scheme 1

Treatment of the labeled retronecine, isolated from the total alkaloids by saponification, with osmium tetroxide and sodium metaperiodate converted the primary carbinol carbon atom to formaldehyde (Scheme 1), which was isolated as the dimedone derivative.



Scheme 2

If retronecine is formed in the plant from two molecules of a four-carbon-atom precursor, then [1,4-^{14}C]-putrescine would be expected to yield retronecine with 25% of its radioactivity in the side-chain carbon atom (Scheme 2). With ornithine labeled at the 2- or 5- position there

are several possibilities, but the present results do not permit conclusions to be drawn concerning all details of the manner in which ornithine is utilized. If ornithine is converted to a symmetrical intermediate, activity at the 2- or 5-position would become identical, and the same result would be obtained as with the use of [1,4-^{14}C]-putrescine. However, the incorporation of 25% of the activity of ornithine into the —CH_2OH group does not in itself prove that the ornithine is incorporated into the alkaloid entirely by way of a symmetrical intermediate, since it is possible to devise reasonable pathways in which the carbon atoms 5, 6, 7 and 8 of the pyrrolizidine ring system could be derived from ornithine in such a way as to impart radioactivity to 5 or 8, or to both of these positions. Further degradation of the pyrrolizidine ring system will have to be carried out to settle these questions.

TABLE 2. ACTIVITY OF THE FORMALDEHYDE OBTAINED BY DEGRADATION OF RETRONECINE

Precursor	Specific activity of retronecine (μc/mmole)	Specific activity of formaldehyde dimedone (μc/mmole)	Percentage of activity in —CH_2OH
[1,4-^{14}C]-putrescine	0·20	0·05	25
[2-^{14}C]-ornithine	0·21	0·05	24
[5-^{14}C]-ornithine	0·44	0·11	25

The results of the present study (Table 2) show that in each case one-quarter of the activity of the retronecine resided in the side-chain carbon atom. These results show that the biosynthesis of retronecine from ornithine in *S. douglasii* must proceed through a symmetrical intermediate to form that part of the ring system that contains the —CH_2OH group. The biosynthesis of nicotine from [2-^{14}C]-ornithine likewise proceeds through a symmetrical intermediate to yield alkaloid in which the pyrrolidine ring is labeled equally in both the 2- and 5-positions.[7–9]

Although these results show that two molecules of putrescine or orinthine are used for the synthesis of retronecine in *S. douglasii*, they do not disclose whether in the case of ornithine both molecules pass through a symmetrical intermediate.

EXPERIMENTAL

Radioactivity measurements were carried out with the use of a Nuclear-Chicago Scintillation Spectrometer Model No. 720. Dioxan was used as the solvent, and the solutions contained 0·7% of 2,5-diphenyloxazole (PPO) 0·05% of 2,2'-*p*-phenylene-bis-(5-phenyloxazole) (POPOP), and 5% of naphthalene.

Administration of Labeled Compounds

Six seedlings of *Senecio douglasii*, grown in soil for about two months, were transplanted to water culture in 300-ml beakers containing aerated Hoagland's solution.[10] After one to

[7] E. LEETE, *Chem. and Ind. (London)* 537 (1955).
[8] L. J. DEWEY, R. U. BYERRUM and C. D. BALL, *Biochim. Biophys. Acta* 18, 141 (1955).
[9] B. L. LAMBERTS, L. J. DEWEY and R. U. BYERRUM, *Biochim. Biophys. Acta* 33, 22 (1959).
[10] D. R. HOAGLAND and D. I. ARNON, *California Agricultural Experiment Station Circular* 347, 31 (1950).

two weeks the nutrient solution was replaced with fresh Hoagland's solution containing 100 μc of the labeled compound (approximate activity, 20 μc/mg). Over 90% of the radioactivity of the solution was taken up by the plants within two days. After seven days the tops of the plants were harvested and macerated in methanol. The filtered solution was evaporated to a small bulk, an equal volume of 2 N sulfuric acid was added and the solution filtered. An excess of zinc dust was added and the mixture stirred for two hours, the zinc removed by filtration, and the solution made alkaline with ammonia and extracted thoroughly with chloroform. The chloroform solution was extracted with 1 N sulfuric acid and the latter made basic and again extracted with chloroform. The dried chloroform solution was evaporated to dryness, leaving a crystalline residue of crude alkaloid. A weighed amount of the total alkaloids isolated from *S. douglasii* was added and the whole recrystallized from acetone.

Hydrolysis of the Alkaloid Mixture

A sample of 340 mg of the mixed alkaloids was refluxed for 90 min with a solution of 750 mg of barium hydroxide in 30 ml of water. The resulting solution was made acidic with dilute sulfuric acid, filtered through celite, and extracted continuously with ether (20 hr). The ether solution was dried and evaporated to yield a semi-solid mixture of the crude acids. The aqueous solution remaining after extraction of the acids was passed through a column of Dowex 2 (OH form), the effluent evaporated to dryness, and the residue sublimed at 110°/2 mm. The sublimate was recrystallized from acetone to yield retronecine, m.p. 119–120°, alone or mixed with authentic material.

Degradation of Retronecine

To a solution of 60 mg of retronecine in 3 ml of water was added a solution of 10 mg of osmium tetroxide in 0·2 ml of ether, and to this solution was added 150 mg of sodium metaperiodate. The mixture was stirred for 30 min, allowed to stand for a further 60 min, and filtered. A saturated aqueous solution of dimedone (50 ml) was added and the formaldehyde derivative allowed to crystallize. The product was filtered and dried; it had m.p. 188–189° alone or mixed with authentic formaldehyde dimedone. The yield was 10 mg.

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