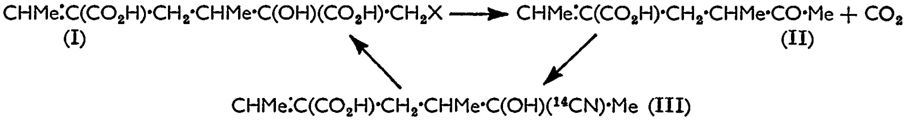
435. *The* Senecio *Alkaloids. Part XVII.*[1] *A Study of the Formation of the Total Alkaloid in the Plant in Relation to the Synthesis of the " Necine " Base and " Necic " Acid*

By C. A. Hughes, C. G. Gordon-Gray, F. D. Schlosser, and F. L. Warren

[*carboxy*-^{14}C]Intergerrinecic acid (I; X = H), synthesised and fed to *Senecio adnatus* D.C., is shown to be incorporated into rosmarinine (IV). [1,1′-*Ac*$_2$-^{14}C$_2$]Diacetylretonecine, fed to *S. isatideus* D.C. and *S. sceleratus* Schweikerdt, gave alkaloids of low activity distributed equally between the base and acid moieties.

The pyrrolizidine alkaloids are esters in which a " necine " base is esterified with either one or two monocarboxylic acids or a dicarboxylic acid.[2] In the biosynthesis of these alkaloids three possibilities exist, namely that (i) both acid and basic moieties are formed separately and then combine; (ii) the basic and acid halves are built in a single concerted enzyme system, or (iii) one or other is first formed and then the molecule is built up from this. The biosynthesis of the " necic " acids from two acetoacetates led Hughes and Warren [3] to draw attention to the possible relationship between the nature of the acid and the configuration of the 7-hydroxyl group which could be accommodated in either proposition (ii) or (iii) above.

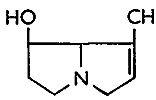
To ascertain whether the base could be built on to the acid, labelled integerrinecic acid was synthesised. Senecic acid (I; X = H; *cis*-form) was oxidised by lead tetra-acetate [4] to 5-methyl 6-oxohept-*cis*-2-ene-3-carboxylic acid (II), which, by the procedure for the synthesis of the inactive acid [5] with sodium [^{14}C]cyanide yielded the nitrile (III) which on hydrolysis gave [6-*carboxy*-^{14}C]5-methyl-7-hydroxyhept-*trans*-2-ene-3,6-dicarboxylic acid, integerrinecic acid (I; X = H; *trans*-form).



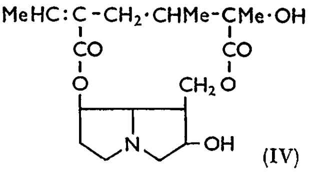
This acid was fed to *S. adnatus* D.C. and [^{14}C$_n$]rosmarinine (IV), isolated with only 0·02% incorporation, hydrolysed to integerrinecic acid (I; X = H; *trans*-form) [5] which had half the activity of the alkaloid. The acid from hydrolysis on oxidation with lead tetra-acetate gave carbon dioxide [5] which had 89% of the activity of the acid. It is surprising that the integerrinecic acid (I; X = H; *trans*-form) is incorporated into the alkaloid which contains senecic acid (I; X = H; *cis*-form). The incorporation was low and the [^{14}C$_1$]senecic acid may have been present in the synthesised acid as a result of incomplete

$HO_2C\cdot CMe(OH)\cdot CHMe\cdot CHMe\cdot C(OH)(CO_2H)\cdot CH_2X$

(VI)



(V)



(IV)

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[1] Part XVI, C. A. Hughes, R. Letcher, and F. L. Warren, *J.*, 1964, 4974.
[2] F. L. Warren *Progr. Chem. Org. Nat. Products*, **1955**, **12**, 198.
[3] C. A. Hughes and F. L. Warren, *J.*, 1962, 34.
[4] M. Kropman and F. L. Warren, *J.*, 1949, 2352.
[5] C. C. J. Culvenor and T. A. Geissman, *J. Amer. Chem. Soc.*, **1961**, **83**, 1647.

isomerisation during synthesis; but we were unable to detect such impurities on a paper chromatogram. It was not possible to isolate [^{14}C$_n$]rosmarinecine from the hydrolysis products.

The occurrence of some of the activity in the " necine " base must have resulted from the incorporation of degradation products of the labelled acid into the basic moiety. The occurrence of 89% of the activity of the " necic " acid in the carboxylic group indicates that the acid had been incorporated intact and only slight incorporation of any degradation products of the labelled acid had occurred in the acid.

To test whether a partially esterified base could build up to the total alkaloid, the acetylated base seemed a good choice, since the acid has been shown to arise from acetic acid.[3] For this purpose [1,1′-*Ac*$_2$-^{14}C$_2$]diacetylretronecine, prepared by acetylating retronecine (V) with [1-^{14}C]acetic anhydride, was fed to *S. isatideus* D.C. to give retrorsine [(V) + (I; X = OH)] and to *S. sceleratus* Schweikerdt to yield retrorsine, sceleratine [(V) + (VI; X = OH)], and sceleratinyl chloide [(V) + (VI; X = Cl)] with low activity which was distributed equally between the acid and basic moieties. The acetylretronecine accordingly must have hydrolysed to give acetic acid which was incorporated in the normal metabolic cycles.

EXPERIMENTAL

Preparation of Labelled Precursors.—[carboxy-^{14}C$_1$]*Integerrinecic Acid*.—Senecic acid (0·5 g.) was oxidised to 5-methyl-6-oxohept-*cis*-2-ene-3-carboxylic acid (0·3 g.), characterised as 2,4-dinitrophenylhydrazone, m. p. 190—191° (Found: C, 51·7; H, 5·5. Calc. for $C_9H_{14}O_3$: C, 51·3; H, 5·4%). The keto-acid (50 mg., 1 mol.) sodium [^{14}C]cyanide (3 mg.; 1 mc = 4·4 × 10^8 c.p.m. at 20% counting efficiency), and inactive sodium cyanide (14 mg.; total cyanide 1·1 mol.) in water (0·4 ml.) were set aside for 12 hr. Concentrated hydrochloric acid (3 ml.) was added, and the solution heated to 100° for 2½ hr. and evaporated, and this operation repeated twice. The residue was heated at 90° for 1 hr. with 2N-hydrochloric acid (3 ml.) and Girard reagent P (0·2 g.), and the mixture cooled and extracted with ether (5 × 25 ml.). The dried (Na_2SO_4) ethereal extract gave a gum which was dissolved in sodium hydrogen carbonate solution, which was then extracted with chloroform, acidified, and extracted with ether. The ether solution gave a gum which sublimed at 130°/0·5 mm. to give [*carboxy*-^{14}C]integerrinecic acid lactone (22·4 mg., 0·29 mol.; 6·8 × 10^7 c.p.m., *i.e.*, 6·56 × 10^8 c.p.m./mmole), m. p. 154° (Kropman and Warren [3] give m. p. 153°).

[1,1′-Ac$_2$-^{14}C$_2$]*Diacetylretronecine*.—[1-^{14}C]Acetic anhydride (26 mg.; 1 mc = 4·4 × 10^8 c.p.m. at 20% counting efficiency) was vaporised at 0·01 mm. into retronecine (15 mg.) and cooled in liquid air; dry air was admitted, and the tube sealed and heated at 130—140° for 45 min. The product distilled at 120°/0·5 mm. to give [1,1′-*Ac*$_2$-^{14}C]diacetylretronecine (15 mg.; 1·86 × 10^8 c.p.m./mmole). The product obtained from only inactive anhydride, by identical procedure, gave picrate, m. p. 146° (Barger [6] gives m. p. 146°).

Feeding with Labelled Compounds.—(*a*) [*carboxy*-^{14}C$_1$]Integerrinecic acid lactone (7·4 mg., 6·58 × 10^8 c.p.m./mmole) was heated with 2N-sodium hydroxide (0·5 ml.), cooled, and fed through capillary tubes into the stems of 15 plants of *S. adnatus* D.C. which were harvested after 10 days. The dried plant (60 g.) gave [^{14}C]rosmarinine (90 mg.; 1·12 × 10^5 c.p.m./mmole; 0·02% incorporated).

(*b*) [1,1′-*Ac*$_2$-^{14}C$_2$]Diacetylretronecine (3·75 mg.; 1·86 × 10^8 c.p.m./mmole) in water (0·5 ml.) fed as above to *S. isatideus* D.C. gave retrorsine (1·8 × 10^3 c.p.m./mmole). The same quantity fed to *S. sceleratus* Schweikerdt gave retrorsine (8·9, × 10^3 c.p.m./mmole) and a mixture of sceleratine and sceleratinyl chloride which was hydrolysis to retronecanol (2·3 × 10^3 c.p.m./mmole), sceleratinic acid (2·7 × 10^3 c.p.m./mmole), and sceleranecic acid (1·3 × 10^3 c.p.m./mmole).

Hydrolysis of [^{14}C]*Rosmarinine*.—Diluted [^{14}C]rosmarinine (1·79 × 10^4 c.p.m./mmole), obtained by diluting [^{14}C]rosmarinine (above) (40 mg.) with inactive rosmarinine (210 mg.), was hydrolysed to [^{14}C]integerrinecic acid, m. p. 150° (100 mg.; 8·4 × 10^3 c.p.m./mmole; 47% of the activity of the alkaloid).

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[6] G. Barger, *J.*, 1935, 11.

Oxidation of [^{14}C]*Integerrinecic Acid from* [^{14}C]*Rosmarinine.*—[^{14}C]Integerrinecic acid (50 mg.) and lead tetra-acetate (150 mg.) in glacial acetic acid (4 ml.) at 50° for 4 hr. gave carbon dioxide, precipitated as barium carbonate (38 mg., 0·8 mol.; 7·5 × 10^3 c.p.m./mmole; *i.e.*, 89% activity in CO_2H group).

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