

## 6. *The Senecio Alkaloids. Part XIV.\* The Biological Synthesis of the "Necic" Acids Using Carbon-14.*

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Sodium [1-<sup>14</sup>C]- and [2-<sup>14</sup>C]-acetate have been fed to *Senecio isatideus* to obtain labelled retrorsine which was then hydrolysed to [<sup>14</sup>C]<sub>4</sub>retronecic acids B and A, respectively. The two complementally labelled acids were oxidised systematically to give fission products which show that four acetic acid residues have been incorporated by way of two four-carbon chains. The biological synthesis envisaged from this pattern is extended to include several "necic" acids, and the possible connexion with the stereochemical structure of the pyrrolizidine part of the alkaloid. [2-<sup>14</sup>C]Mevalonic lactone, when fed under the same conditions, gave no active alkaloid.

THE acids associated with the pyrrolizidine alkaloids have been assigned general structures as substituted C<sub>10</sub>-adipic<sup>1,2</sup> and -glutaric acids,<sup>2</sup> and substituted monobasic C<sub>7</sub>-acids.<sup>2</sup> A route for the biological synthesis incorporating these several structures from acetoacetic acid was recently envisaged<sup>3</sup> as a development of Robinson's concept<sup>4</sup> of the formation of senecioid acid from acetic acid. We have now tested this idea that acetic acid is a precursor of these acids.

Plants of *Senecio isatideus*, grown under hydroponic conditions in the presence of sodium [2-<sup>14</sup>C]acetate, were extracted to give retrorsine which was hydrolysed to retronecic acid-A (activity A). The acid was then degraded stepwise<sup>5</sup> and the activities of the degradation products were determined and calculated as fractions of this activity.

Kuhn-Roth oxidation of the retronecic acid-A gave two mol. of [2-<sup>14</sup>C]acetic acid which was converted into barium acetate (activity 0.50A) and pyrolysed to yield barium carbonate (activity 0.024A) and [Me<sub>2</sub>-<sup>14</sup>C]acetone which was converted into [<sup>14</sup>C]iodoform (activity 0.240A). Ozonolysis of the retronecic acid yielded [2-<sup>14</sup>C]acetaldehyde, isolated as its dimedone derivative (activity 0.236A). Accordingly, the two methyl groups in the retronecic acid are active, whilst C<sub>(3)</sub> and C<sub>(6)</sub> are inactive. Oxidation of the retronecic acid with periodic acid gave formaldehyde, isolated as its dimedone derivative, with zero activity so that C<sub>(1)</sub> was inactive. Oxidation of the retronecic acid with lead tetra-acetate gave formaldehyde, which was not isolated, and carbon dioxide isolated as barium carbonate (activity 0.011A). A similar oxidation on the product (III) of ozonolysis after the removal of the acetaldehyde gave inactive carbon dioxide, so that both the carboxyl groups are non-radioactive.

These experiments are in agreement with incorporation of two acetic acid molecules; but these only account for half the activity of the retronecic acid-A, so that it seemed possible that two other acetic acid residues had been included in the latter.

The inactivity of the two carboxylic acid groups and the hydroxymethyl group in the above retronecic acid-A, left only C<sub>(2)</sub>, C<sub>(4)</sub>, and C<sub>(5)</sub> undetermined. The difficulty attending the determination of these groups was avoided by feeding sodium [1-<sup>14</sup>C]acetate whereupon an active retronecic acid-B (activity B) was obtained. This acid was then oxidised directly with lead tetra-acetate to give formaldehyde (activity 0.025B) and [<sup>14</sup>C]carbon dioxide (activity 0.219B) derived from the 2-carboxyl group. Ozonolysis followed by oxidation with lead tetra-acetate gave [<sup>14</sup>C]carbon dioxide (activity 0.487B) from both the original carboxyl groups, so that the activity of 5-carboxyl group was 0.268B. This activity shown by the two carboxyl groups in the second experiment suggests that C<sub>(2)</sub>

\* Part XIII, *J.*, 1958, 4574.

<sup>1</sup> Koekemoer and Warren, *J.*, 1951, 66.

<sup>2</sup> Warren, *Fortschr. Chem. organ. Naturstoffe*, 1955, **12**, 230.

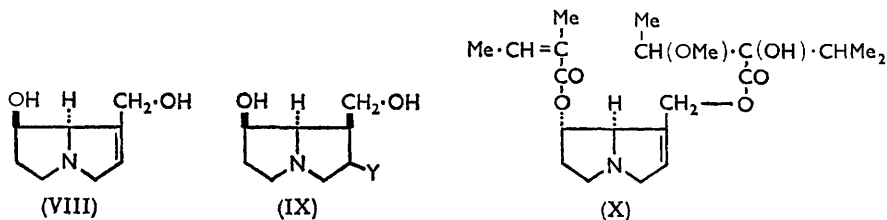
<sup>3</sup> Warren, *Record Chem. Progr.*, 1959, **20**, 18; cf. Adams and Gianturco, *Festschr. A. Stoll*, 1957, 72.

<sup>4</sup> Robinson, "The Structural Relations of Natural Products," The Clarendon Press, Oxford, 1955.

<sup>5</sup> Christie, Kropman, Novellie, and Warren, *J.*, 1949, 1703.



ring, as in retronecine (VIII) platynecine (IX; Y = H), and rosmarinine (IX; Y = OH),<sup>12</sup> the esters of which are C<sub>10</sub>-adipic or C<sub>10</sub>-glutaric acids. When, however, the 7-hydroxy-group has the opposite orientation, as in heliotridine,<sup>12</sup> then condensation is not possible as is seen in lasiocarpine (X).<sup>13</sup> Alternatively, condensation of the two acid



residues may result in the base assuming structure (VII) or (VIII), whilst non-condensation permits the less sterically hindered structure as in (X).

Numerous experiments were conducted to find the best conditions for the incorporation of labelled precursors. The attractive "wick" method of Leete<sup>14</sup> gave no incorporation in spite of the alkaloid's being found in the aerial portion only, in agreement with Glonti's observations.<sup>15</sup> The success attending root-feeding would indicate immediate transfer to the aerial portion of the plant. The optimum growing time seemed to be about 10 days, after which the yield of labelled alkaloid decreases, an observation made by Leete and Singfried for *Nicotiana* spp.<sup>16</sup> The minimum activity for effective feeding per plant was 10  $\mu$ C (1  $\times$  10<sup>6</sup> c.p.m.).

#### EXPERIMENTAL

All experiments were duplicated, except that the Kuhn-Roth degradation and periodic oxidation on retronecic acid-A were triplicated. All activities agreed within 10%.

*Determination of Activity.*—All counting was done on Nuclear Measurements Corporation 2 $\pi$ -proportional counter employing a 10% admixture of methane in argon. Samples were placed on 2.5 cm. diameter aluminium planchets. In view of the small quantities employed samples were counted at "infinite thinness." Sufficient counts were taken to give an efficiency of  $\pm$ 3%, and corrections were made for background only. The activity was calculated as c.p.m./mg. and recorded as for 100% yield of degradation product as "total activity." With the exception of the experiments involving ozonolysis, degradation products were obtained in theoretical yield.

*Feeding Experiments.*—Thirty plants of *S. isatideus* grown in vermiculite for 4 months were transferred to a solution of Leete's nutrient (750 ml.) and water (750 ml.) for 3 days, and then sodium [2-<sup>14</sup>C]acetate (13.8 mg.) and water (250 ml.) were added. The plants were subjected to 8 hours' sunlight per day with roots in the dark. After 10 days the plants were dried and extracted as previously described.<sup>1</sup> The aerial parts alone gave alkaloidal material, the roots being extracted only for materials balance. Identical conditions were adopted for feeding sodium [1-<sup>14</sup>C]acetate and [2-<sup>14</sup>C]mevalonic lactone, and the results are summarised in the Table.

	<sup>14</sup> CH <sub>3</sub> ·CO <sub>2</sub> H	CH· <sup>14</sup> CO <sub>2</sub> H	[2- <sup>14</sup> C]Mevalonic lactone
Activity fed (c.p.m.) .....	1.66 $\times$ 10 <sup>7</sup>	3.65 $\times$ 10 <sup>8</sup>	3.65 $\times$ 10 <sup>7</sup>
Incorporation in plants (%) .....	90	99.4	98.4
Yield of alkaloid (mg.) .....	166	200	120
Total alkaloid activity (c.p.m.) .....	4550	1930	66.7
Activity of aq. ammonia (c.p.m.) .....	2.64 $\times$ 10 <sup>4</sup>	6.75 $\times$ 10 <sup>4</sup>	2.64 $\times$ 10 <sup>4</sup>
Activity of fats (c.p.m.) .....	1.41 $\times$ 10 <sup>6</sup>	2.8 $\times$ 10 <sup>6</sup>	1.41 $\times$ 10 <sup>6</sup>

[<sup>14</sup>C]*Retronecic Acid.*—Retrorsine-A (207 mg.), ordinary retrorsine (894 mg.), and potassium hydroxide (360 mg.) in ethanol (15 ml.) were refluxed for 20 min. and cooled. The potassium

<sup>12</sup> Warren and von Klemperer, *J.*, 1958, 4574.

<sup>13</sup> Men'shikov and Zhdanovich, *Ber.*, 1936, 69, 1110.

<sup>14</sup> Leete, *Chem. and Ind.*, 1960, 692.

<sup>15</sup> Glonti, *Referat. Zhur. Khim., Biol. Khim.*, 1958, Abs. No. 3380 (*Chem. Abs.*, 1958, 52, 12,322).

<sup>16</sup> Leete and Singfried, *J. Amer. Chem. Soc.*, 1957, 79, 4529.

retronecate was filtered off, dried, dissolved in 2N-sulphuric acid, and extracted continuously with ether for 2 days. The ether solution gave [ $^{14}\text{C}_4$ ]retronecic acid-A which, crystallised from ethyl acetate, had m. p. 180—181° (250 mg., total activity 1537 c.p.m.).

*Kuhn-Roth Oxidation.*—Retronecic acid-A (29.6 mg., 182 c.p.m.) was oxidised under standard conditions and the acetic acid neutralised with 0.032N-barium hydroxide (3.70 ml., 2 equiv.). The barium acetate (34.3 mg., total activity 90 c.p.m.), when pyrolysed at 550° for 15 min. in a stream of nitrogen which passed into water, left barium carbonate (23.3 mg., 2.2 c.p.m., *i.e.*, total activity of carboxyl groups 4.4 c.p.m.). The aqueous solution gave iodoform, m. p. 119° (16.0 mg., total activity 43.8 c.p.m., *i.e.*, 87.6 c.p.m./2Me groups).

*Ozonolysis.*—Retronecic acid-A (58.8 mg., 362 c.p.m.) in ethyl acetate was ozonised at 0°. The ozonide was steam-distilled into dimedone solution, to give acetaldehyde dimedone, m. p. 137° (28.4 mg., total activity 85.5 c.p.m.).

*Lead Tetra-acetate Oxidation.*—(a) The residue after steam-distillation of the ozonide was treated with lead tetra-acetate (100 mg.) in a stream of nitrogen, and the carbon dioxide absorbed as barium carbonate (68.2 mg., total activity 2 c.p.m.).

(b) Retronecic acid-A (31.1 mg., 191.2 c.p.m.), water (5 ml.), and lead tetra-acetate (70 mg.) were treated as above, to give barium carbonate (28.3 mg., total activity 2 c.p.m.).

*Periodic Acid Oxidation.*—Retronecic acid-A (45.6 mg., 280.5 c.p.m.) in water (2 ml.), N-sodium hydrogen carbonate (2 ml.), and 7% periodic acid in water (2 ml.) were allowed to react for 1 hr. The solution was treated with N-hydrochloric acid (3 ml.) and 1.2N-sodium arsenite (2 ml.) and filtered. The filtrate was heated with M-sodium acetate (2 ml.) and an 8% solution of dimedone in 95% ethanol (1 ml.) at 100°. The precipitate (51.2 mg.), crystallised from ethanol-water, gave formaldehyde dimedone, m. p. 189° (activity zero).

[ $^{14}\text{C}_4$ ]Retronecic Acid-B.—Retrorsine-B (200 mg.), obtained from feeding sodium [ $^{14}\text{C}$ ]-acetate, gave [ $^{14}\text{C}_4$ ]retronecic acid-B (80 mg., total activity 512 c.p.m.).

Retronecic acid-B (25 mg., 160 c.p.m.) when oxidised with lead tetra-acetate as above gave formaldehyde dimedone (29.0 mg., total activity 4 c.p.m.) and barium carbonate (19.8 mg., total activity 35 c.p.m.).

Ozonolysis of acid-B (35 mg., 214 c.p.m.), followed by treatment with lead tetra-acetate as above, gave barium carbonate (58 mg., total activity 109 c.p.m.).

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