956. *The* Senecio *Alkaloids. Part XVI.* The Biosynthesis of the "Necine" Bases from Carbon-14 Precursors.*

By C. A. Hughes, R. Letcher and F. L. Warren.

[2-^{14}C]Ornithine hydrochloride and sodium [2-^{14}C]acetate have been fed to *Senecio isatideus* D.C. and to *S. sceleratus* Schweikerdt, respectively. The radio-active alkaloids isolated were hydrogenolysed to yield radioactive retronecanol-O and retronecanol-A, respectively. These bases were degraded, mainly by successive Hofmann reactions, and the reaction products further oxidised by the Kuhn–Roth technique. These methods showed that two ornithine units and four acetate units had been incorporated. This is discussed in the light of possible biosynthetic pathways.

The "necine" bases are 1-methylpyrrolizidines (I, R = H) of different stereochemical configurations and degrees of hydroxylation.[1] Robinson [2] suggested that ornithine is the precursor of this ring system and envisaged the symmetrical dialdehyde (II) as the key

* Part XV, *J.*, 1962, 953.

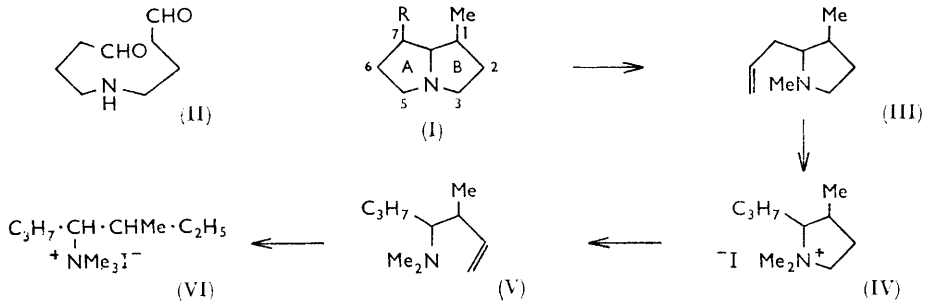
[1] Warren and von Klemperer, *J.*, 1958, 4574.

[2] Robinson, "The Structural Relations of Natural Products," The Clarendon Press, Oxford, 1955.

intermediate. Nowacki and Byerrum [3] have vindicated this concept by showing that [2-^{14}C]ornithine is incorporated into monocrotaline which contained almost all the activity in the retronecine. The biosynthetic route from labelled ornithine has been further investigated and a study made of the incorporation of labelled acetate.

The tracers were fed as previously described,[4] with the exception that the nutrient solution was omitted and aureomycin was used in the amino-acid experiment. [2-^{14}C]-Ornithine hydrochloride was fed to *Senecio isatideus* D.C. to give retrorsine-O * with 0·98% incorporation of the activity, which was hydrogenolysed to retronecanol-O. The sodium [2-^{14}C]acetate previously fed to *S. isatideus* [4] gave some of the activity in the retronecine. For the purpose of this study the acetate was fed to *S. sceleratus* Schweikerdt, and a mixture of labelled sceleratine, sceleratinyl chloride, and retrorsine was obtained. This was hydrogenolysed to retronecanol-A,* which was readily separated from the mixture of sceleranecic, sceleratinic and retronecic acid.

The Hofmann degradation of retronecanol, retronecanyl acetate, and heliotridene, investigated in an attempt to eliminate a number of steps in the degradation, gave intractable mixtures. The degradation of retronecanol-O and A (I, R = OH) was effected as previously described; [1,5] the intermediates were not necessarily isolated, but all compounds counted were checked for purity. Retronecanol (I, R = OH) was converted to heliotridane



(I, R = H) which was subjected to a Hofmann degradation to give the methine (III) which was reduced to the 1,3-dimethyl-2-propylpyrrolidine (IV). A second Hofmann reaction on (IV) gave the methine (V) which was reduced and converted to (VI).

Ozonisation of both methines (III) and (V) gave formaldehyde which was assayed as its dimedone derivative to obtain the activity of $C_{(5)}$ and $C_{(3)}$, respectively. Kuhn–Roth oxidation on the two reduced methine methiodides, (IV) and (VI), gave acetic acid assayed as its sodium salt to give the combined activity of ($C_{(1)}$, $C_{(1')}$, $C_{(5)}$, $C_{(6)}$) and ($C_{(1)}$, $C_{(1')}$, $C_{(2)}$, $C_{(3)}$, $C_{(5)}$, $C_{(6)}$), respectively. A similar oxidation of retronecanol gave acetic acid, assayed as the barium salt ($C_{(1)}$, $C_{(2)}$), the latter being pyrolysed to barium carbonate ($C_{(2)}$) and acetone, which was further oxidised by way of iodoform to carbon dioxide and assayed as barium carbonate ($C_{(1)}$). The combined activity of $C_{(7)}$ and $C_{(8)}$ was obtained by difference and confirmed by collecting the carbon dioxide from the Kuhn–Roth oxidation of (VI).

The labelling pattern from [2-^{14}C]ornithine follows Robinson's predictions; [2] but the unequal labelling of atoms $C_{(1')}$ and $C_{(8)}$ (labelling at $C_{(7)}$ is unlikely) indicates that ornithine is incorporated into the molecule in two distinct steps.

The specific labelling in retronecine recalls the results of feeding [2-^{14}C]ornithine [6] and

* The suffixes O and A are used to designate the alkaloid or its degradation product obtained from feeding [2-^{14}C]ornithine and [2-^{14}C]acetate, respectively.

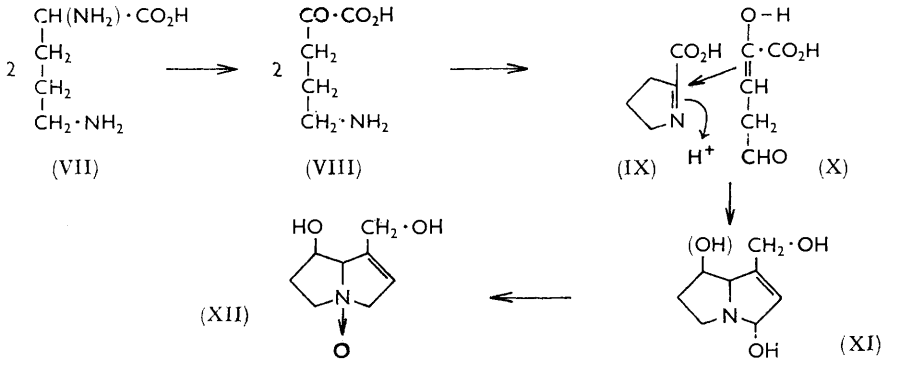
[3] Nowacki and Byerrum, *Life Sciences*, 1962, **5**, 157.
[4] Hughes and Warren, *J.*, 1962, 34.
[5] Men'shikov, *Ber.*, 1935, **68**, 1555; *Bull. Acad. Sci. U.R.S.S. Ser. Chim.*, 1937, 1035 (*Brit. Abs.*, AII, 1938, 162).
[6] Leete, Marion, and Spender, *Canad. J. Chem.*, 1954, **32**, 1116; *J. Amer. Chem. Soc.*, 1962, **84**, 55.

TABLE 1.

Percentage activity associated with the different carbon atoms of retronecanol derived from [2-^{14}C]ornithine and [2-^{14}C]acetate.

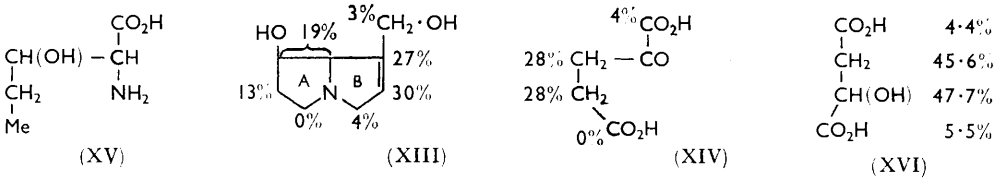
	Ring B				Ring A			
	$C_{(1')}$	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(5)}$	$C_{(6)}$	$C_{(7)} + C_{(8)}$	Total
Retronecanol-O	26	—	—	0	0	—	71	97
Retronecanol-A	3	27	30	4	0	13	19	96

[1-^{14}C]acetate,[7] regarded as a precursor of ornithine,[7] to *D. stramonium* to give hyoscyamine labelled in only one bridgehead. This is different from the incorporation of [2-^{14}C]-ornithine into nicotine, which results in the labelling of both $C_{(2)}$ and $C_{(5)}$ of the pyrrolidine ring [8] and is interpreted as proceeding by way of a mesomeric pyrroline anion.[8] The incorporation of ornithine (VII) into isatinecine (retronecine *N*-oxide) (XII) is envisaged as proceeding by way of 5-amino-penta-2-onoic acid (VIII) to Δ^1-pyrroline-2-carboxylic acid (IX) which condenses with a molecule of pentane-2,5-dionic acid (X), formed from a second molecule of ornithine, to give (XI) and hence (XII). This mechanism would be analogous to the formation of pipecolic acid from lysine [9] and would account for the unequal labelling of $C_{(1)}$ and $C_{(8)}$.



Robinson's theory [2] for the biosynthesis of lupinine from lysine is envisaged now as proceeding similarly.

The labelling pattern of retronecine (XIII) from [2-^{14}C]acetate finds a parallel in the studies by Griffith and Byerrum [10] of the pyrrolidine ring of nicotine, and recalls the relative distribution of carbon-14 in the formation of oxoglutarate and malate from the same



precursor. Ring A is envisaged as being formed by the incorporation of [2-^{14}C]acetate into the tricarboxylate cycle to give oxoglutaric acid (XIV) [11] which by amination can give glutamic acid and hence ornithine. It is significant that Adams and Gianturco [12] found 3-hydroxy-*N*-methylnorvaline (XV) in *Crotalaria* spp., which suggests the participation

[7] Bothner-By, Schulz, Dawson, and Solt, *J. Amer. Chem. Soc.*, 1962, **84**, 52.
[8] Ball, Byerrum, and Dewey, *Biochem. Biophys. Acta*, 1955, **18**, 141; Leete, *J. Amer. Chem. Soc.*, 1958, **80**, 2162.
[9] Meister and Buckley, *Biochem. Biophys. Acta*, 1957, **23**, 202.
[10] Griffiths and Byerrum, *Fed. Proc.*, 1959, **18**, 942.
[11] Goodwin, "Recent Advances in Biochemistry," Churchill, 1960, p. 167.
[12] Adams and Gianturco, *J. Amer. Chem. Soc.*, 1956, **78**, 4458.

of a prehydroxylated precursor in accordance with Robinson's suggestion.[2] Ring B can conceivably have resulted from the incorporation of [2-^{14}C]acetate in the glyoxalate cycle as exemplified by malic acid (XVI),[13] but it is realised that two different mechanisms for the building of ring B are then invoked.

Experimental

The degradations were carried out as previously described,[1,5] and all products were checked for purity by m. p. of the substance itself or a derivative. The activity was measured as previously.[3] The percentage figure in brackets is the activity recorded as a percentage of the total activity. The products from feeding [2-^{14}C]ornithine and sodium [2-^{14}C]acetate are represented as (O) and (A) respectively. All experiments were duplicated.

Retrorsine-O from Labelled Ornithine.—[2-^{14}C]Ornithine hydrochloride ($1{\cdot}8 \times 10^6$ c.p.m.) were fed to 36 three-month-old plants of *S. isatideus* growing in water containing 2 p.p.m. of aureomycin. The plants were harvested after 11 days, dried (31 g.), and extracted to give retrorsine-O (101 mg., $2{\cdot}974 \times 10^5$ c.p.m./mmole, 0·98% incorporation).

Retronecanol-A from Labelled Acetate.—Sodium [2-^{14}C]acetate (2 mc) was fed to *S. sceleratus* Schweikerdt and extracted as above to give a mixture of sceleratine, sceleratinyl chloride, and retrorsine, which mixture was hydrogenolysed to give retronecanol-A (196·6 mg., $2{\cdot}2123 \times 10^4$ c.p.m./mmole, 0·042% incorporation).

Calculations.—The activity of the acetic acid from the Kuhn–Roth oxidations was calculated in c.p.m./mmole, and this value was multiplied by the number of CMe groupings in the molecule being oxidised to obtain the total activity due to all the two-carbon groupings, and hence the percentage activity. For example, the oxidation of heliotridane dihydromethine methiodide (IV) (activity $1{\cdot}160 \times 10^4$ c.p.m./mmole) gave sodium acetate ($1{\cdot}752 \times 10^3$ c.p.m./mmole). The total activity due to the two CMe groups is $3{\cdot}504 \times 10^3$ c.p.m./2 mmole (30·2% of the original activity).

Barium-acetate values had to be halved to convert to c.p.m./mmole of acetic acid. The value obtained from barium carbonate from the iodoform had to be doubled since only one methyl group of the acetone is isolated.

Retronecanol-O and -A.—Retrorsine-O (96·7 mg.) was mixed with inactive retrorsine and hydrogenolysed to yield retronecanol-O (343·4 mg., $3{\cdot}66 \times 10^4$ c.p.m./mmole). For retronecanol-A, see above. Kuhn–Roth oxidation gave acetic acid, counted as barium acetate ($C_{(1)} + C_{(1')}$)

(O) 19,050 c.p.m./mmole, *i.e.*, 9530 c.p.m./mmole HOAc (26%).
(A) 13,280 c.p.m./mmole, *i.e.*, 6640 c.p.m./mmole HOAc (30%).

which was pyrolysed to barium carbonate ($C_{(1)}$),

(O) 500 c.p.m./mmole (1·4%).
(A) 5760 c.p.m./mmole (26%).

and acetone, which was converted to iodoform, and hence by oxidation to carbon dioxide, counted as barium carbonate ($C_{(1')}$),

(O) 9300 c.p.m./mmole (25·4%).
(A) 644 c.p.m./mmole (2·9%).

Diluted Retronecanol.—Retronecanol-O (191·3 mg.) mixed with inactive retronecanol (413 mg.) gave diluted retronecanol-O (11,600 c.p.m./mmole). Retronecanol-A (68·2 mg.) mixed with inactive retronecanol (618 mg.) gave diluted retronecanol-A (2200 c.p.m./mmole, calc.). These diluted retronecanols were used for the degradations below.

Heliotridane methiodide.—(O) 11350 and (A) 2090 c.p.m./mmole.

Heliotridane methine (III).—Ozonolysis gave formaldehyde ($C_{(5)}$) counted as its dimedone derivative: (O) and (A) gave zero activity.

Heliotridane dihydromethine methiodide.—Kuhn–Roth oxidation gave acetic acid (1·93 mole) isolated as sodium acetate ($C_{(1)}$, $C_{(1')}$, $C_{(5)}$, $C_{(6)}$).

(O) 1752 c.p.m./mmole, *i.e.*, 3504 c.p.m./2 mmole (30·2%).
(A) 473 c.p.m./mmole, *i.e.*, 946 c.p.m./2 mmole (43%).

[13] Cavin and Beevers, *J. Biol. Chem.*, 1961, **236**, 988.

Heliotridane dihydromethine methine (V).—Ozonisation gave formaldehyde ($C_{(3)}$) counted as its dimedone derivative.

(O) zero activity and (A) 88 c.p.m./mmole (4%).

4-*Dimethylamino*-3-*methylheptane methiodide* (VI).—Kuhn–Roth oxidation gave acetic acid (2·68 mole) counted as sodium acetate ($C_{(1)}$, $C_{(1')}$, $C_{(2)}$, $C_{(3)}$, $C_{(5)}$, $C_{(6)}$).

(O) 1110 c.p.m./mmole, *i.e.*, 330 c.p.m./3 mmole (28·8%).
(A) 563 c.p.m./mmole, *i.e.*, 1689 c.p.m./mmole (76·9%).

and carbon dioxide counted as barium carbonate ($C_{(7)}$, $C_{(8)}$, $C_{(NMe_3)}$).

(O) 1625 c.p.m./mmole, *i.e.*, 8125 c.p.m./5 mmole (70·4%).
(A) 83·6 c.p.m./mmole, *i.e.*, 418 c.p.m./5 mmole (19%).

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C.S.I.R. Natural Products Research Unit,
Department of Chemistry, University of Natal,
Pietermaritzburg, South Africa.

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