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TRACER STUDIES ON β-SITOSTEROL

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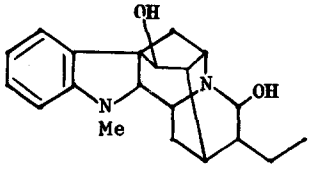
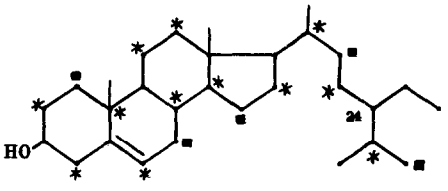
INCORPORATION of radioactivity from sodium [1-^{14}C]acetate into ajmaline (I) in Rauwolfia serpentina plants has recently been observed but those parts of the ajmaline molecule which were examined by degradation showed only random scatter of activity.[1] It was therefore important to study the labelling pattern of β-sitosterol (II) isolated from the same plants used for the work on ajmaline; the sterol was strongly radioactive (0.12% incorporation). β-Sitosterol was also isolated from R. serpentina plants to which (±)-[2-^{14}C]mevalonolactone had been administered and here the incorporation was 1.6%.



(I)



(II)

* From sodium [1-^{14}C]acetate

■ From [2-^{14}C]mevalonolactone.

There have been many reported incorporations of both precursors into plant sterols[2-4] but apart from two cases[2,3]

these substances have not been degraded to locate the labelled carbon atoms. No specific degradations of β-sitosterol have been recorded.

Standard methods were used for the isolation of β-sitosterol from the non-saponifiable fraction of the neutral substances and, after the crystalline material had been diluted with rigorously purified β-sitosterol,[5] it was chromatographed on alumina. The specific activity was proved to be constant across the band of β-sitosterol and the isolated material showed[6] m.p. 140-141° and $[\alpha]_D^{20}$ −35° (C, 1.6 in $CHCl_3$). Part of this product was converted into O-acetyl-β-sitosterol,[6] m.p. 129.5-130°, $[\alpha]_D^{20}$ −32.8° (C, 1.7 in $CHCl_3$) which had the same specific activity as β-sitosterol itself.

Kuhn-Roth oxidation of the β-sitosterol by the method which minimises methyl migration[7] afforded a mixture of acetic and propionic acids which were separated by chromatography in chloroform on a column of buffered silica gel. The pure acids were estimated by titration and the radioactivities of the sodium salts were determined by liquid scintillation counting. Schmidt degradation of the sodium acetate with sodium azide and polyphosphoric acid[8] yielded methylamine which, after steam distillation, was converted into N-methyl-3-benzyloxy-4-methoxybenzylamine hydrochloride.[9] The results are collected in the Table.

If β-sitosterol arises in the plant by the pathway firmly established[10] for cholesterol in animal systems, then the labelling patterns illustrated should be found. The acetic acid obtained by Kuhn-Roth oxidation of β-sitosterol derived from sodium [1-^{14}C]acetate should be labelled only at the

carboxyl group, and 90% of the activity was shown to be located there with only slight "scatter" into the methyl carbon (Table). In contrast, the acetic acid from the

Radioactive Materials	Precursor	
	Sodium [1-^{14}C] acetate	(±)-[2-^{14}C]-Mevalonolactone
	Relative molar activity	Relative molar activity
β-Sitosterol	100	100
Sodium acetate	4.3	2.7
Methylamine	0.43	2.75
Sodium propionate	0.7	0.4

experiment with [2-^{14}C] mevalonolactone should be labelled solely at the methyl group and this was found to be so (Table); there was no detectable "scatter" in this case.

It is thus demonstrated that specific labelling of β-sitosterol occurs from both precursors and the results are in agreement with the acetate—mevalonate pathway being followed for this higher plant sterol.

The propionic acid produced in the Kuhn-Roth oxidation can only arise from the ethyl group at position 24. Its very low activity shows that the ethyl group does not arise from acetic acid. This finding is in agreement with the recent work of Arigoni and his co-workers[11] who proved that the ethyl group in the related case of spinasterol is built from two methyl groups donated by methionine.

REFERENCES

1 A.R. Battersby, R. Binks, W. Lawrie, G.V. Parry and B.R. Webster, Proc. Chem. Soc., 369 (1963); cf. E. Leete, S. Ghosal and P.N. Edwards, J. Amer. Chem. Soc., 84, 1068 (1962).

2 E.G. Gros and E. Leete, Chem. and Ind., 698 (1963).

3 H.J. Nicholas and S. Moriarty, Fed. Proc., 22, 529 (1963).

4 H.J. Nicholas, J. Biol. Chem., 237, 1476, 1481, 1485 (1962); R.D. Bennett, E. Heftmann, A.E. Purcell and J. Bonner, Science, 134, 671 (1961); D.J. Baisted, E. Capstack and W.R. Nes, Biochemistry, 1, 537 (1962) and refs. therein.

5 We thank Dr. W. Lawrie (Glasgow) for a generous gift of pure β-sitosterol.

6 O.C. Musgrave, J. Stark and F.S. Spring, J. Chem. Soc., 4393 (1952).

7 J.W. Cornforth, R.H. Cornforth, A. Pelter, M.G. Horning and G. Popjak, Tetrahedron, 5, 311 (1959).

8 Grateful acknowledgement is made to Professor D. Arigoni (Zurich) for information concerning this method.

9 This convenient derivative was recommended to us by Prof. D.H.R. Barton and Dr. G.W. Kirby and we record our best thanks to them for the experimental details.

10 G. Popjak and J.W. Cornforth, Adv. Enzymology, 22, 281 (1960).

11 S. Bader, L. Guglielmetti and D. Arigoni, Proc. Chem. Soc., 16 (1964); cf. M. Castle, G. Blondin and W.R. Nes, J. Amer. Chem. Soc., 85, 3306 (1963).