Phytochemistry, 1968, Vol. 7, pp. 451 to 452. Pergamon Press. Printed in England

SHORT COMMUNICATION

ACCUMULATION OF SQUALENE-2,3-OXIDE DURING INHIBITION OF PHYTOSTEROL BIOSYNTHESIS IN *NICOTIANA TABACUM*

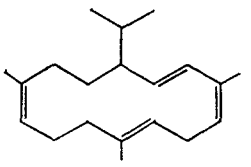
W. W. REID*

Research Department, British Tobacco Co. (Aust) Ltd., Box 2689, G.P.O. Sydney, Australia

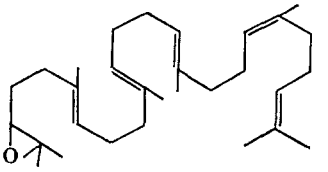
(*Received* 21 *October* 1967)

IN A previous note,[1] it was shown that the addition of tris(2-diethylaminoethyl)phosphate hydrochloride (SK&F 7997) to *Nicotiana* tissue slices fed with mevalonic acid-2-^{14}C, suppressed incorporation of activity into phytosterols with the accumulation of activity in a hydrocarbon designated Hydrocarbon 2.

It was originally shown that Hydrocarbon 2 had the spectral properties of cembrene (I)[2] but on chromatography of a radioactive sample on $AgNO_3/SiO_2$ it was resolved into three major components each of which was unlabelled (one of the components was cembrene, the two others appeared to be related hydrocarbons) and a trace component, which appeared to be more unsaturated than cembrene, which contained all the radioactivity.



Cembrene
(I)



Squalene-2,3-oxide
(II)

Recently, two groups[3,4] showed that squalene-2,3-oxide (II) was an intermediate in sterol synthesis in a rat liver enzyme system. The chromatographic properties of the radioactive component of Hydrocarbon 2 suggested it might be squalene-2,3-oxide. The ^{14}C-labelled component of Hydrocarbon 2 co-chromatographed with squalene-2,3-oxide on several TLC systems (Kieselgel G with benzene, R_f 0·28, squalene 0·66; with 5 per cent ethyl acetate/hexane, R_f 0·41, squalene 0·82; Kieselgel G/$AgNO_3$ with dichlorethane, R_f 0·05, squalene 0·05; with ethyl ether/benzene 1:4, R_f 0·52, squalene 0·60).

* Present address, 15 Taylor Road, Claremont, West Australia.

[1] W. W. REID, *Biochem. J.* **100** (1), 13P (1966).
[2] W. G. DAUBEN, W. F. THIESSEN and P. R. RESNICK, *J. Am. Chem. Soc.* **84**, 2015 (1962).
[3] E. J. CORY, W. E. RUSSEY and P. P. O. DE MONTELLANO, *J. Am. Chem. Soc.* **88**, 4750 (1966).
[4] E. E. VAN TAMELEN, J. D. WILLETT, R. B. CLAYTON and K. E. LORD, *J. Am. Chem. Soc.* **88**, 4757 (1966).

Hydrocarbon 2 diluted with racemic squalene-2,3-oxide was hydrated overnight in methanol + H_2SO_4 and chromatographed on Kieselgel G/CH_2Cl_2. Two components were obtained R_f 0·12 and 0·41, in approximately equal amounts and similar specific activity. Each component co-chromatographed with an identical component in a sample of 2,3-dihydrosqualene-2,3-diol prepared by Dr. Willett from perchloric acid hydration of racemic squalene-2,3-oxide.

The above evidence indicates that Hydrocarbon 2 is squalene-2,3-oxide and is most probably an intermediate in phytosterol synthesis in *Nicotiana* between squalene and 4,4-dimethyl sterols. When ^{14}C-Hydrocarbon 2 (5000 dpm) dissolved in mineral oil was applied to the surface of *Nicotiana* seedlings and left under illumination for 48 hr, approximately 31 per cent of the activity recovered co-chromatographed with squalene-2,3-oxide, 26 per cent co-chromatographed with methyl sterols, 21 per cent with phytosterols, and the remaining 21 per cent with polar material of low R_f. In a similar experiment with ^{14}C-squalene, the recovery was 44 per cent in squalene, 35 per cent in methyl sterols, 7 per cent in phytosterols, 14 per cent in polar materials. The composition of the methyl sterol and phytosterol fractions is complex[5–7] and it has been suggested[7] that the phytosterols of *Nicotiana* and the 9β,19-cyclopropane triterpenes are on separate biosynthetic pathways.

Leaf slices of *Nicotiana* fed with mevalonic acid-2-^{14}C incorporate considerable activity into methyl sterols and phytosterols. In the presence of the inhibitor SK&F 7997 activity in the phytosterols is reduced by a factor of 0·1–0·15 of the control value, that in squalene-2,3-oxide is increased by a factor of 9–17, yet the activity in the 4,4-dimethyl sterols and 4α-methyl sterols is reduced by only a factor of 0·5–0·7. These results may suggest that the decreased activity in the methyl sterols is due to suppression of biosynthesis of components in Path II,[7] whereas the biosynthesis of 9β,19-cyclopropane triterpenes in Path I is relatively little affected.

The 4,4-dimethyl sterol group may be separated into four discrete fractions by chromatography on $AgNO_3/SiO_2$ and the 4α-methyl sterols into six fractions by the same technique.[5,6] The main radioactive fraction of the 4,4-dimethyl sterols can be partially resolved by chromatography of the derived epoxides,[7] and three fractions of the 4α-methyl sterols can be shown by GLC of the trimethyl silyl ethers to be mixtures of 9β,19-cyclopropane triterpenes and derivatives of lophenol.

Existing techniques do not permit the complete separation and estimation of the specific activity of the four components of the 4,4-dimethyl sterol fraction and the six components of the 4α-methyl sterol fraction. Solution of this problem is essential if the probable existence of two biosynthetic pathways is to be resolved.

Since this paper was prepared, squalene-2,3-oxide has been identified in tissue cultures of *N. tabacum*.[8]

Acknowledgements—Thanks are due to Professor E. E. van Tamelen and Dr. Willett for samples of squalene-2,3-oxide and 2,3-dihydrosqualene-2,3-diol and to Dr. Holmes for a sample of SK&F 7997.

[5] M. BERNARD, R. G. NICHOLLS and W. W. REID, *Australian Biochem. Soc.*, Melbourne, Paper 63 (1965).
[6] M. BERNARD and W. W. REID, *Australian Biochem. Soc.*, Brisbane, Paper 65 (August 1966).
[7] M. BERNARD and W. W. REID, *Chem. & Ind.* 997 (1967).
[8] P. BENEVISTE and R. A. MASSEY-WESTROP, *Tetrahedron Letters* **37**, 3553 (1967).