Cyclisation of Squalene 2,3;22,23-Diepoxide by Microsomes from Bramble (*Rubus fruticosa*) Tissues grown *in Vitro*

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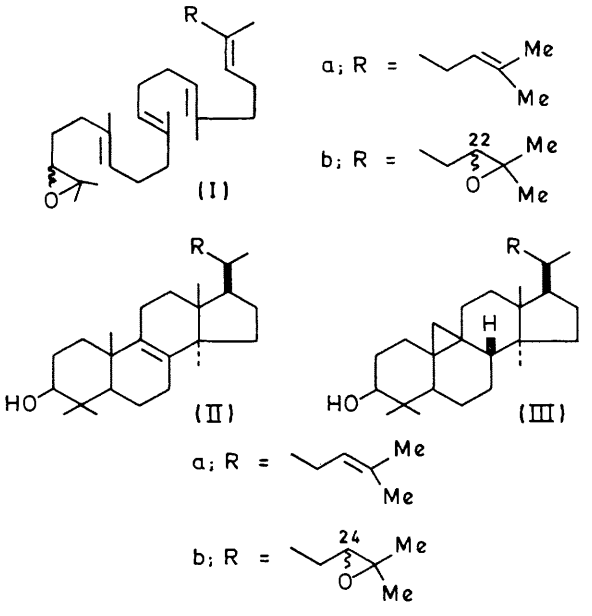
Summary Incubation of squalene 2,3;22,23-diepoxide with microsomes of *Rubus fruticosa* gives 24,25-epoxycycloartanol.

SQUALENE 2,3-EPOXIDE (Ia) is a precursor of animal and plant triterpenes:[1-4] rat-liver microsomes cyclise it to lanosterol (IIa),[1,2] whereas plant cell-free extracts convert it into cycloartenol (IIIa).[3,4] In order to elucidate the mechanism of enzymatic cyclisation, modified substrates, among them squalene 2,3;22,23-diepoxide (Ib) have been

used. Incubation of (Ib) with rat-liver enzymes produced 24,25-epoxylanosterol (IIb).[5] Therefore the 22,23-double bond of squalene is not involved in this cyclisation. To further the comparison between the two systems, we have studied the enzymatic activity of plant microsomes toward (Ib) as substrate.



[12,13-$^3H_2$]Squalene prepared by the method of Biellmann and Ducep [6a] with suitable modifications, [6b] was treated with *N*-bromosuccinimide in t-butyl alcohol–water (4 : 1) for 30 min. at room temperature. The products, 2-bromo-3-hydroxy-2,3-dihydrosqualene and 2,23-dibromo-3,22-dihydroxy-2,3;22,23-tetrahydrosqualene, were separated by t.l.c. The dibromohydrin was treated with K_2CO_3 in methanol for 5 hr.[7] The resulting squalene 2,3;22,23-diepoxide (Ib) was characterised by its mass spectrum (molecular ion *m/e* 442) and its n.m.r. spectrum (4 CH_3 on epoxide δ 1·24, 1·28; 2 H on epoxide δ 2·70, *J* 6 Hz). The specific radioactivity of (Ib) was 22 mc/mmol.

In a typical experiment, to 3 ml of microsomes (2 mg protein/ml) from bramble tissues grown *in vitro*[8,9] was added 6×10^6 d.p.m. (0·125 μmole) of squalene 2,3;22,23-diepoxide (Ib), dissolved in 50 μl acetone (0·5% Tween 80). After incubation for 5 hr. at 30°, the reaction was terminated by dilution with one volume of ethanolic KOH (10%). The mixture was extracted with three 18 ml portions of petroleum (40—60°). The organic phase was concentrated under reduced pressure and the residue was subjected to silica gel t.l.c. (eluant, 10% ethyl acetate in cyclohexane).

Two radioactive products were detected by the usual chromatographic and radiochromatographic techniques: squalene diepoxide (Ib) (R_F 0·54, $5·2 \times 10^6$ d.p.m.), and another product (R_F 0·27, 6×10^5 d.p.m.), which has the same R_F as endogenous sterols and 24,25-epoxycycloartanol. This band was eluted and the residue was acetylated after concentration. The resulting acetate (R_F 0·33, $2·7 \times 10^5$ d.p.m.) which co-chromatographed with (IIIb) acetate, was easily separated from steryl acetates (R_F 0·52) by t.l.c. (eluant, 5% ethyl acetate in cyclohexane). To the radioactive product was added 10 mg of non-radioactive 24,25-epoxycycloartanyl acetate, which was subsequently crystallised four times (see Table).

TABLE

Crystallisation	1	2	3	4
Specific radioactivity	20,300[a] ± 800	15,900 ± 600	15,500 ± 600	15,650 ± 600

[a] d.p.m./mg.

Synthetic squalene diepoxide is necessarily a mixture (probably 1 : 1 : 2) of *RR*, *SS*, and *RS* diastereoisomers of which the first is probably not cyclised. Reaction of the remaining two diastereoisomers would give rise to a mixture of 24(*R*),25- and 24(*S*),25-epoxycycloartanol. Since successive crystallisations led to products of equal specific radioactivity, it is therefore evident that the biosynthetic mixture† of 24(*RS*),25-epoxycycloartanol was very nearly the same as the chemically synthesised 24(*RS*),25-epoxycycloartanol, which has been shown to be approximately a 50 : 50 mixture of 24(*R*) and 24(*S*).[10]

The metabolite obtained on incubation is therefore 24,25-epoxycycloartanol (yield 5 nmol/mg of protein). These results demonstrate that plant enzymes, like rat-liver enzymes, can cyclise substrates modified in the terminal isoprenic unit.

Cycloartanol 24,25-epoxide (IIIb), which exists as a natural product in *Tillandsia usneoides*,[11] can arise either from epoxidation of cycloartenol (a possibility predictable on the basis of the known epoxidation of parkeol in position 24,25 by tobacco tissue cultures),[12] or from the direct cyclisation of squalene 2,3;22,23-diepoxide, in which case the diepoxide would be an intermediate between squalene 2,3-epoxide and 24,25-epoxycycloartanol.

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† Elucidation of this point will be the subject of a subsequent paper.

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