Assimilation of the Antipodal Forms of Squalene 2,3-Oxide by Mammalian, Yeast, and Plant Systems

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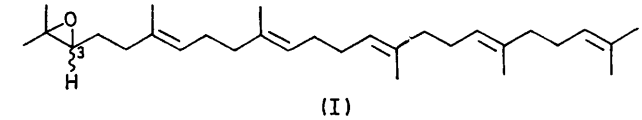
Summary (*S*)-Squalene 2,3-epoxide was found to be the exclusive precursor of lanosterol in pig liver, lanosterol, and ergosterol in yeast (*Saccharamyces cerevisiae*), and β-amyrin, lupeol and cycloartenol in pea seedlings.

SQUALENE 2,3 EPOXIDE (I) is well proven as the precursor of 3-oxygenated triterpenes in Nature.[1] The evidence available supports concerted mechanisms for the cyclisations and the enzymes which mediate some of these have been obtained in purified form.[2,3] It has been assumed, on the

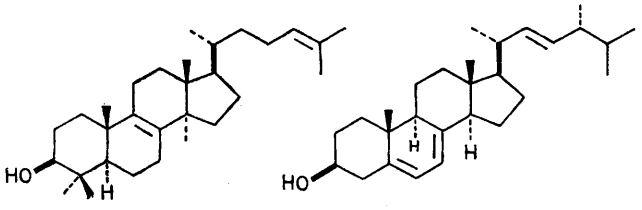
TABLE. Incorporation of squalene 2,3-epoxides

Organism	Substrate Chirality	Incorporation/% Lanosterol	Ergosterol	Cycloartenol	β-Amyrin	Lupeol
Liver	(*R*)	1·9				
	(*S*)	76·8				
	(*R*,*S*)	39·7				
Yeast						
(a) Whole cell	(*R*)	0·008	0·2			
	(*S*)	0·61	6·2			
	(*R*,*S*)	0·05	1·1			
(b) Cell-free ..	(*R*)	6·5				
	(*S*)	27·6				
	(*R*,*S*)	18·5				
Pea seedling ..	(*R*)			0·02	0·76	0·02
	(*S*)			0·57	26·9	0·59
	(*R*,*S*)			0·27	13·1	0·32

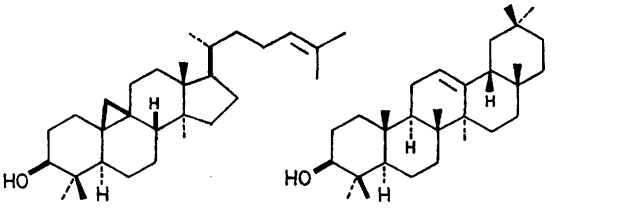
basis of this concertedness and the non-involvement of the C-3 centre in the accepted mechanisms of cyclisation, that (3 *S*)-squalene 2,3-epoxide is the precursor of the 3β-hydroxytriterpenes. We now present evidence which confirms this assumption and shows that the cyclase is stereoselective. The data also provide a biochemical test which shows the essential completeness of the resolution.



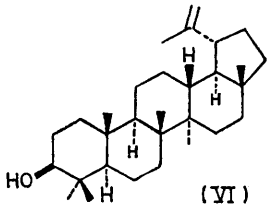
(I)



(II) (III)



(IV) (V)



(VI)

The resolution itself will be described elsewhere (R. B. Boar and K. Damps).

For initial studies with the resolved epoxides we chose a phylogenetically diverse range of organisms producing 3β-hydroxytriterpenes, *viz.*: pig liver [lanosterol (II)], *Saccharomyces cerevisiae* [lanosterol (II), ergosterol (III)], and *Pisum sativum* [cycloartenol (IV), β-amyrin (V), lupeol (VI)].

Microsome preparations from pig liver[3] were incubated at 37° with equal quantities respectively of (*R*)-, (*S*)-, and (*R*,*S*)-[4-$^3H_2$]squalene 2,3-epoxides dispersed in 0·5% Tween 80 solution under an atmosphere of nitrogen. After 2 h the feeding was quenched with 15% methanolic potassium hydroxide, inactive lanosterol added, and the mixture heated at 70° for 2 h. Conventional sterol extraction, benzoylation, and p.l.c. on 10% silver nitrate–silica plates gave lanosterol benzoate which was crystallised to constant activity. The results are given in the Table.

Whole yeast cells and a cell-free preparation were fed with the resolved and racemic epoxides for 4 h at 30° and 30 min at 37° respectively as described previously.[3–5] The solutions were worked up as above, after dilution with inactive materials, for lanosterol and ergosterol benzoates. The incorporations are given in the Table.

A cell-free homogenate of germinating peas (*Pisum sativum*) as previously described[5,6] except for the omission of sucrose, glutathione, and magnesium sulphate from the medium, was cultured with the precursors at ambient temperature for 24 h. The mixture was diluted with appropriate inactive triterpenes and worked up as before for cycloartenol,[7] β-amyrin and lupeol benzoates (see Table).

The results prove the intermediacy of (*S*)-squalene 2,3-epoxide in cyclic triterpene formation. The experiments with the liver and pea systems show the expected simple relationship between (*R*), (*S*), and (*R*,*S*) configurations. The results with yeast are somewhat different and will be more extensively discussed in a full paper.

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