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Comparison of the Behaviour of Oxidosqualene Cyclases from Pig Liver and Yeast toward Epoxy-Squalene Analogues Possessing a $\Delta^{18\text{-}19}$ *Z* or *E* C, C Double Bond.

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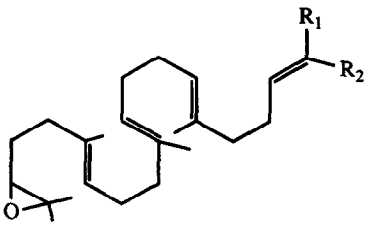
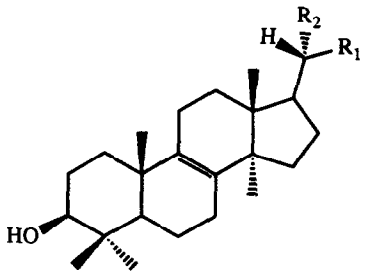
Abstract: 2,3-Oxidosqualene analogues possessing a $\Delta^{18\text{-}19}$ double bond with the natural E-stereochemistry **1b-d** *are cyclised by pig liver sterol cyclase or "ultrasonically stimulated" bakers' yeast (Saccharomyces cerevisiae) whereas their stereoisomers possessing a $\Delta^{18\text{-}19}$ double bond with the unnatural Z-stereochemistry* **1'** *possess a different behaviour toward the same cyclases. They are still cyclised by pig liver sterol cyclase but are inert toward "ultrasonically stimulated" bakers' yeast.*

It is well established [1] that pig liver 2,3-oxidosqualene cyclase catalyses the cyclisation of 2,3-oxidosqualene as well as of some analogues substituted at the $\Delta^{18\text{-}19}$ double bond **1** to produce lanosterol and the truncated analogues **2** in yields decreasing with the length of the side chain (Scheme 1). We described [2] that the same enzyme is also able to cyclise the oxidosqualene analogues **1'a** and **1'c** possessing $\Delta^{18\text{-}19}$ double bonds with the unnatural *Z*-stereochemistry to produce the norlanosterol bearing the unnatural 20S stereochemistry [2a] **2'c** or tricyclic compounds [2b] **3'a** and **4'a** depending upon the length of the chain attached on the C-19 carbon of **1'**.

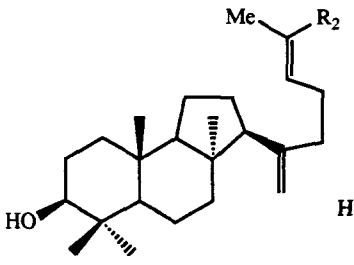
Scheme 1



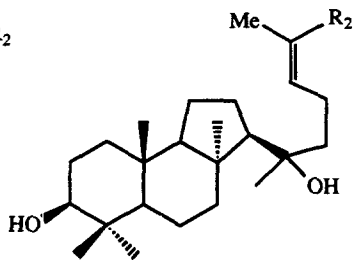
1a R_2 = Me $R_1 = CH_2CH_2CH{=}CMe_2$
1b $R_1 = CH_2CH_2Me$
1c $R_1 = CH_2Me$
1d R_1 = Me
1'a R_1 = Me $R_2 = CH_2CH_2CH{=}CMe_2$
1'b $R_2 = CH_2CH_2Me$
1'c $R_2 = CH_2Me$



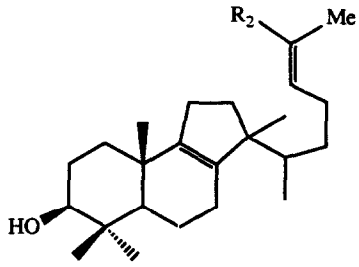
2a R_2 = Me $R_1 = CH_2CH_2CH{=}CMe_2$
2b $R_1 = CH_2CH_2Me$
2c $R_1 = CH_2Me$
2d R_1 = Me
2'c R_1 = Me $R_2 = CH_2Me$



3'a [2b] $R_2 = CH_2CH_2CH{=}CMe_2$



4'a [2b] $R_2 = CH_2CH_2CH{=}CH_2$



5c $R_2 = CH_2Me$
5d R_2 = Me

In the course of the latter work we required large quantities of **3'a** and **4'a** and tried to perform the same transformation on **1'a** using baker's yeast cyclase which was expected, from the elegant work of Kyler [3] to fulfill our needs. Unexpectedly we found that **1a'** does not cyclise at all under these [4b] (or even more drastic) conditions (which however worked in our hand as described previously [3] on the natural stereoisomer **1a**) and **1a'** was recovered unchanged after the usual work-up.

As a consequence of this finding we decided to study in more detail the behaviour of the various substrates **1a-d** and **1'a-c** possessing the natural *E*- or unnatural *Z*-stereochemistry toward baker's yeast. For comparison purposes, we have also treated the different substrates **1a-d** with pig liver oxidosqualene cyclase under standardized conditions. [4a] We report herein our preliminary results in this field.

We find that our previous observation with **1'a** and baker's yeast is not unique and that other 2,3-oxidosqualene analogues **1'** possessing a $\Delta^{18\text{-}19}$ double bond with the unnatural *Z* stereochemistry are not cyclised under similar conditions and are almost quantitatively recovered. In the natural *E* series however cyclisation does indeed occur producing norlanosterol derivatives [5a] **2** in yields decreasing dramatically with the length of the hydrocarbon skeleton of **1** (table 1). Increasing the amount of yeast per mole of **1** or the reaction time does not dramatically affect these results. On the other hand, in the case of the pig liver cyclase, we found that the same substrates **1** produce, under suitable conditions [4a] and regardless of their size, almost equal amounts (60-80%) of the norlanosterol derivatives **2** with, in some cases (**1c**, **1d**), small amounts (~10%) of rearranged tricyclic compounds to which structure **5** was tentatively assigned.[5b] Such structures have not been found when **1a** and **1b** were reacted with pig liver cyclase and when all the substrates **1** were reacted with yeast cyclase.

Table 1

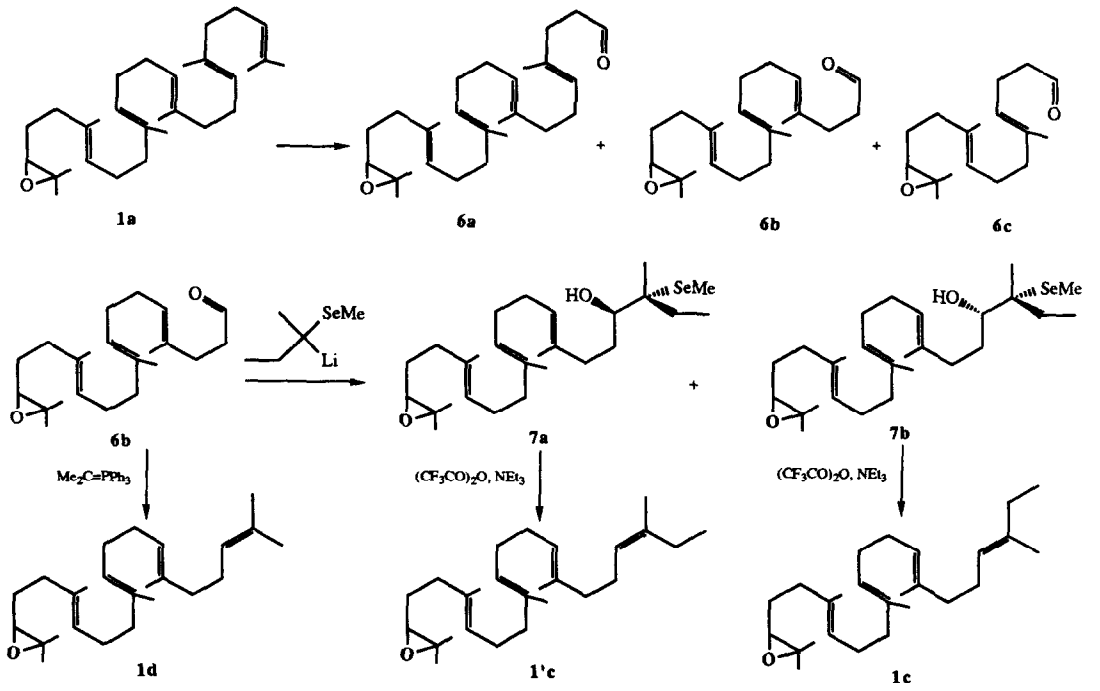
Oxidosqualene	Bakers' yeast		Pig liver			
		Yields (%)[+]		Yields (%)[+]		Yields (%)[+]
1a	**2a**	80	**2a**	80	-	0
1b	**2b**	40	**2b**	80	-	0
1c	**2c**	20	**2c**	77	**5c**	8
1d	**2d**	7	**2d**	60	**5d**	10

[+] Yield based on reaction of one enantiomer.

In conclusion it has been observed for the first time that oxidosqualene cyclases from bakers' yeast and pig liver show different behaviour towards oxidosqualene analogues bearing the unnatural *Z* stereochemistry at their $\Delta^{18\text{-}19}$ double bonds although they often exhibit similar characteristics toward those possessing the natural stereochemistry there.[3,6] It must be nevertheless pointed out that none of these transformations have been performed on a pure enzyme and therefore these results must be taken with caution.[7,8] Work is now in progress in our laboratory to determine whether or not other related cyclases exhibit such difference of behaviour.

The required radiolabelled 2,3-oxidosqualenes **1a** and **1'a** have been prepared as already described.[2] The syntheses of **1c**, **1'c** and **1d** have been performed in a straightforward manner from 2,3-oxidosqualene [9-11] as shown in scheme 2. The synthesis of **1b** and **1'b** which will be published soon [12] involves a strategy similar to the one described for of **1a**. It uses also a farnesyl unit but the other ones have been prepared from geraniol benzyl ether by a sequence of reactions which implies its ozonolysis and transformation of the resulting aldehyde to the desired olefins using the β-hydroxyalkyl selenide route [11] disclosed in the scheme 2.

Scheme 2



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(4) (a) ***Cyclisation by squalene sterol cyclase derived from pig liver.*** In a typical run, 250 µg of substrate (±)**1** (3.2 x 10^6 dpm),was anaerobically incubated at 20°C for 3h with 6 ml of a clear phosphate-buffered enzymatic solution (Fraction AII) [13] purified from the microsomal fraction of pig liver (SO cyclase EC 5.4.99.7). After saponification (10% KOH in ethanol, 20°C, 2.5 h) the crude radioactive material was extracted with ether. The lanosterol analogues were separated from unreacted **1** by thin layer chromatography (SiO_2, benzene/ethylacetate: 95 / 5) then purified by HPLC (RP-18, 7µ, methanol). -

(b) ***Cyclisation by squalene sterol cyclase derived from Bakers' yeast.*** In a typical run, 30 mg of substrate (±)**1** ,was anaerobically incubated at 20°C for 24h or 48h with 30 ml of bakers' yeast extract (pH : 7.4) (Saccharomyces cerevisiae) prepared as previously described.[3] After saponification (10% KOH in ethanol, 20°C, 2.5 h), the crude radioactive material was extracted with ether. The lanosterol analogues **2** were separated from the unreacted **1** and other by-products by tlc (SiO_2, benzene/ethylacetate 95 / 5).

(5) (a) Structural assignments were made on pure compounds **2a-d** by ^{1}H, ^{13}C, 2D-NMR, GC-MS and comparison with authentic samples in the cases of **2a-d**. (b) The MS spectrum of these compounds [5c] exhibit a pattern characteristic of a tricyclic structure (M^+: 289, 229, 214, 199, 147). (c) van Tamelen, E.E.; Sharpless, K.B.; Hanzlic R.; Clayton, R.B.; Burlingame, A.L.; Wszolék, P.C. *J. Am. Chem. Soc.* **1967**, *89*, 7150.

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(7) Preliminary results [8] involving reactions on pure cyclase derived from baker yeast tend to support our observation from the whole sonicated organism.

(8) We thank Prof. I. Scott for performing these reactions in his laboratory , Texas A &M University, USA.

(9) These transformations involve (i) ozonolysis of 2,3-oxidosqualene [10] [(a) 1.3 equiv. O_3, CH_2Cl_2, -78°C (b) Me_2S, CH_2Cl_2, 20°C, 3h; yield in **6**: 23% (**6a/6b/6c** ratio: 30/25/45)] and (ii) reconstruction of the C, C double bond with suitable substituents *(1) via the β-hydroxyalkyl selenide route* [11] for **1c** or **1'c** [(a) 2-methylseleno-2-propyllithium, THF,-78°C, 0.7h then aq. NH_4Cl, 79% yield in **7** (as a 1/1 mixture of the two stereoisomers **7a** and **7b** (b) separation of the two stereoisomers **7a** and **7b** on SiO_2 (c) $(CF_3CO)_2O$, NEt_3, CH_2Cl_2, 20°C, 1h, 70% yield in **1c** or **1'c**] or *(2) from a Wittig reaction* for **1d** [isopropylidenetriphenylphosphorane, THF, -78°C, 1h then 20°C, 0.7h, 70% yield in **1d**]. ^{3}H-radiolabelled analogues **1c** (2700 dpm/nmole), **1'c** (2800 dpm / nmole) and **1d** (3300 dpm/nmole) were also prepared along these lines. Their synthesis will appear in our full paper on this subject.[12]

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