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Novel Results on the Biocyclisation of 2,3-Oxidosqualene Analogs by Sterol Cyclase

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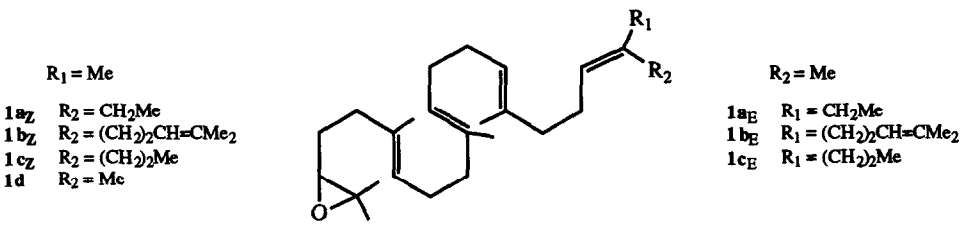
Although Oxidosqualenes analogs bearing a E-Δ^{18-19} C,C double bond are cyclized by 2,3-oxidosqualene cyclase (EC 5.4.99.7, "sterol cyclase") to produce 20-R norlanosterols, thoses possessing a Z-Δ^{18-19} C,C double bond instead produce, a mixture of 20-S norlanosterols and tricyclic derivatives whose ratio depends upon the length of the side chain.

A few years ago we reported that 2,3-oxidosqualene analogs possessing a Z-Δ^{18-19} C,C double bond **1a**$_Z$ [1] and **1b**$_Z$ [2] are cyclized by 2,3-oxidosqualene cyclase (EC 5.4.99.7, "sterol cyclase") to produce inter alia a tetracyclic compound with norlanosterol structure, **2a**$_S$, but inverted stereochemistry at C-20, or tricyclic derivatives **3b**$_Z$ or **4b**$_Z$, depending upon the length of the chain attached at C-19 of **1**$_Z$ (Scheme 1 and 2). These clear cut results led us to investigate the behavior of **1c**$_Z$ possessing the same stereochemistry but an alkyl side chain with intermediate structure between the one of **1a**$_Z$ and **1b**$_Z$ and to determine the structure of some minor compounds isolated in our previous work. [1,2]

Scheme 1



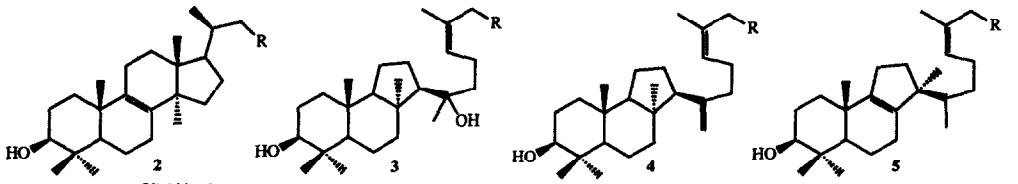
The (±) 2,3-oxidosqualene analog **1c**$_Z$ (302 dpm/nmol.) was synthesized [3,4] as described below and incubated with a purified preparation of 2,3-oxidosqualene cyclase from pig liver [5] (5 mg, 0.12 mmol., 3905292 dpm, 6 ml AII fraction, 20°C, 3h, under nitrogen). The resulting mixture was saponified, extracted with ether and purified on SiO_2 (preparative TLC plate, Merck, benzene-ethyl acetate 95-5) yielding recovered **1c**$_Z$ (70%, R_f 0.8), an intractable mixture of **2c**$_S$ and **5c**$_Z$ (ratio 40/60, R_f 0.45-0.34) and **3c**$_Z$ (R_f 0.20-0.14) each mixed with unlabeled compounds present in the enzymic preparation.[6-8]

2c$_S$ and **5c**$_Z$ were in turn separated as their acetates **2c**$_S$-Ac and **5c**$_Z$-Ac by sequential acetylation (excess Ac_2O, pyridine, 20°C, 17h, 93% yield) followed by HPLC purification (Lichrosorb, RP-18, 7µ, Merck, methanol) and have been obtained free from unlabeled compounds. **3c**$_Z$ was further freed from the high amount of exogenous cholesterol present by crystallization followed by HPLC purification (Lichrospher, Si-100, 5µ, Merck, hexane-ether 8-2). The structures of **2c**$_S$-Ac, **3c**$_Z$ and **5c**$_Z$-Ac were determined by ^1H, ^{13}C 1D and 2D NMR. Spectra (DQF-COSY, NOESY, Long range COSY, HMQC) of **3c**$_Z$ [9,10] were compared

with those of its related derivative $\mathbf{3b}_Z$ [9,10] and spectra of $\mathbf{2c}_S$-Ac were compared with those of an authentic sample.[11,12]

The presence of the tricyclic product $\mathbf{5c}_Z$ as the major cyclized component from the biotransformation involving $\mathbf{1c}_Z$ led us to suspect the presence of related derivatives among the various products isolated in small quantities in the reactions involving $\mathbf{1a}_Z$ and $\mathbf{1b}_Z$.[1,2] We therefore repeated both biosynthetic experiments, and in doing so confirmed our previous results and isolated one compound in each case to which we have assigned the structure $\mathbf{5a}_Z$ (12% yield) and $\mathbf{5b}_Z$ (1.4% yield). We recall that such tricyclic products have also been isolated in the biocyclization of 2,3-oxidosqualene analogs $\mathbf{1a}_E$ possessing a E Δ^{18-19} C,C double bond and **1d**.[13]

Scheme 2



Entry		Yield in 2	Yield in 3	Yield in 4	Yield in 5
a	R = H	62% (2d)	0% (3d)	0% (4d)	10% (5d)
b	R = Me	46% (2a)	0% (3a)	0% (4a)	13% (5a)
c	R = Et	16% (2c)	12% (3c)	0% (4c)	26% (5c)
d	R = 	0% (2b)	38% (3b)	20% (4b)	1.4% (5b)

The results of our work involving the reaction of four 2,3-oxidosqualene analogues $\mathbf{1}_Z$ possessing a Z Δ^{18-19} C,C double bond bearing a side chain of different length are assembled in scheme 2. Particularly impressive is the fact that all of these unnatural substrates are so well accepted and cyclized by 2,3-oxidosqualene cyclase from pig liver (EC 5.4.99.7, "sterol cyclase", $\mathbf{1a}_Z$-$\mathbf{c}_Z$,**d**: 80, 78, 60 and 76% yields)].[14] It can be observed that the shorter the side chain (i.e. the smallest the modification is compared to the natural substrate) the higher the percentage of 20(S)-lanosterol analogue **2** formed and the lower the amount of the tricyclic derivatives $\mathbf{3}_Z$, $\mathbf{4}_Z$ and $\mathbf{5}_Z$ produced. It is nevertheless difficult to rationalize the ratios of the different tricyclic derivatives formed. Why $\mathbf{4}_Z$ is so scarcely produced and why the percentage of the tricyclic products $\mathbf{5}_Z$ is so fluctuating is unclear. The fact that only one of the possible stereoisomers of $\mathbf{5}_Z$ is produced in each case suggests that they are each formed in a biosynthetic step and do not result, for example, from $\mathbf{3}_Z$ or $\mathbf{4}_Z$ via an acid catalyzed non enzymic pathway. [15]

The stereochemistry of the tricyclic skeleton in $\mathbf{3}_Z$ and $\mathbf{4}_Z$ and the β-orientation of their side chains suggest that the cyclization takes place through anti-periplanar additions of the C,C double bonds of the 2,3-oxidopolyenes folded in the enzyme in a chair-boat-chair conformation. The case of the tricyclic product $\mathbf{5}_Z$ is more intriguing since again the side chain is β-oriented. This can be rationalized by considering that $\mathbf{5}_Z$ is derived: (i) from a tricyclic skeleton bearing an α-oriented side chain and arising from anti-periplanar addition of C,C double bonds of 2,3-oxidopolyenes folded in the enzyme in a chair-boat-boat conformation. The (H, Me) migrations and the loss of the hydride which led finally to the olefinic compound $\mathbf{5}_Z$ are therefore expected to occur through concerted anti-periplanar 1,2-shifts, (ii) from the precursors of $\mathbf{3}_Z$ and/or $\mathbf{4}_Z$ bearing a β-oriented side chain arising from the 2,3-oxidopolyenes folded in the enzyme in a chair-boat-chair conformation. In order to explain the β-orientation of the side chain in $\mathbf{5}_Z$, we must assume, that a stepwise mechanism is operative involving a syn-periplanar migration of at least the first migrating Me group.[16,17] In

order to fully understand the intimate mechanism of transformation of $\mathbf{1}_Z$ to $\mathbf{3}_Z$, $\mathbf{4}_Z$ and $\mathbf{5}_Z$ it is important to determine unambiguously the stereochemistry at the remaining chiral center on the side chain of these compounds. We are currently working towards this end.

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References

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2. Krief, A.; Schauder, J.-R.; Guittet, E; du Penhoat, C.H.; Lallemand, J.-Y. *J. Amer. Chem. Soc.* **1987**, *109*, 7910.
3. The 2,3-oxidosqualene $\mathbf{1c}_Z$ and $\mathbf{1c}_E$ possessing a Z or a E Δ^{18-19} C,C bond were synthesized as shown in scheme 3, using the Biellmann coupling reaction, between the functionalized α-lithio allylic sulfide [2] **6c** and the allylic chlorides **12**. The latter were in turn prepared by a sequence of reactions which involved (i) the ozonolysis of geranyl benzyl ether [(a) 1.3 equiv. O_3, CH_2Cl_2, -78°C (b) Me_2S, 40°C, 2h, then 25°C, 12h, 60% yield in **7c**] and (ii) its further reaction with 2-lithio-2-methylseleno pentane **8** [4] [(a) 1.3 equiv., THF, -78°C, 1h (b) aq. NH_4Cl, 20°C, 77% yield in **9** as a 1/1 mixture of diasteroisomers $\mathbf{9c}_Z$ and $\mathbf{9c}_E$]. This mixture was resolved in its constituents by chromatography on silicagel [(Lobar, Lichroprep Si, Merck) eluted with a solution of hexane-ethyl acetate: 96-4, TLC (SiO_2, pentane-ether 80-20, four elutions, $\mathbf{9c}_Z$: 35% yield, R_f 0.73; $\mathbf{9c}_E$: 27% yield, R_f 0.67)]. Purified $\mathbf{9c}_Z$ and $\mathbf{9c}_E$ were in turn reacted with PI_3 [1.5 equiv., 9 equiv. NEt_3, CH_2Cl_2, 20°C, 1h] stereospecifically producing the 2E,6Z $\mathbf{10c}_Z$ and the 2E,6E $\mathbf{10c}_E$ 1-benzyloxy-3,7-dimethyl decadiene in 88 and 86% yields respectively [TLC, SiO_2, pentane-ether 94-06, $\mathbf{10c}_Z$ R_f 0.76 and $\mathbf{10c}_E$ R_f 0.75]. Deprotection of the benzyl ethers led to the corresponding alcohols [3.7 equiv. Li, ether-NH_3: 1-2.3, -78°C, 0.3h then i-PrOH then NH_4Cl, $\mathbf{11c}_Z$ or $\mathbf{11c}_E$: 95% yield, R_f 0.53 each, TLC, SiO_2, pentane-ether 60-40] which were in turn transformed to the corresponding E-allylic chlorides **12** by the Stork-Grieco method [(a) 1 equiv. MeLi, ether-HMPA: 2-1, 0°C (b) 1.5 equiv.TosCl, 20°C, 7 equiv. LiCl, 20°C, 15h, 99% yield each)]. The synthesis of **1c** was achieved from that stage by (i) alkylation of **6c** [from the corresponding sulfide (a) 0.9 equiv. n-BuLi, THF, -78°C, 2.5h and (b) 0.7 equiv. **12**, THF, -78°C, 2h, $\mathbf{13c}_Z$: 60% yield, $\mathbf{13c}_E$: 70% yield, R_f 0.6 each, TLC, SiO_2, pentane-ether 94-6) and (ii) further reduction of the resulting sulfide [30 equiv. Li, $EtNH_2$, -15°C, 0.5h then NH_4Cl, $\mathbf{14c}_Z$ or $\mathbf{14c}_E$: 67% yield and R_f 0.86 each, TLC, SiO_2, pentane-ether 94-6]. The dioxolane moiety in **14c** was removed by treatment with aqueous lithium perchlorate to provide the diols **15c** [3N, aq. $HClO_4$, THF, 20°C, 5h, $\mathbf{15c}_Z$ or $\mathbf{15c}_E$: 90% yield and R_f 0.8 each, TLC, SiO_2, pentane-ether 30-70] which were immediately cyclized to **1c** through their mesylates [(a) 10 equiv. $MeSO_2Cl$, pyridine, 20°C, 3h (b) K_2CO_3, MeOH, 20°C, 2h, $\mathbf{1c}_Z$ or $\mathbf{1c}_E$: 86% yield from **15** and R_f 0.48 each, TLC, SiO_2, benzene). Tritiated $\mathbf{1c}_Z$ or $\mathbf{1c}_E$ were obtained from $\mathbf{15c}_Z$ and $\mathbf{15c}_E$ by their sequential cleavage to the aldehydes **16c** ($NaIO_4$, MeOH, phosphate buffer pH 7.2, 0°C, 1h, 0°C, 3h, $\mathbf{16c}_Z$: 80% and $\mathbf{16c}_E$: 90% yield and R_f 0.76 each, TLC, SiO_2, benzene) followed by their tritiation [(1Ci/ml) T_2O, NEt_3, THF, 50°C, 17h, sealed tube, quantitative yield, $\mathbf{16c}_Z$*: 6036 and $\mathbf{16c}_E$*: 3398 dpm/nmole) and their further reaction with isopropylidene diphenylsulfurane [(a)Ph_2S-$CHMe_2$, BF_4, 1 equiv. LDA, 1 equiv. CH_2Cl_2, -78°C, 0.5h, THF, then addition of **16c***, -78°C, 3h, $\mathbf{1c}_Z$* 50% yield and $\mathbf{1c}_E$* 54% yield].
4. (a) Krief, A., Dumont, W.; Cravador, A.; Denis, J.N.; Halazy, S.; Hevesi, L.; Labar, D.; Lucchetti, J.; Rémion, J.; Sevrin, M.; Van Ende, D. *Bull. Soc. Chim. Fr.*, **1980**, *N° 11-12, II*, 519 (b) Labar, D.; Krief, A. *J. Chem. Soc. Chem. Commun.*, **1982**, 564 and references cited. (c) Krief, A. *Comprehensive Organic Synthesis*, Trost, B.M.; Fleming, I.; Schreiber, S. ed.**1992**, *vol 1*, Ch. 2.6, 629.
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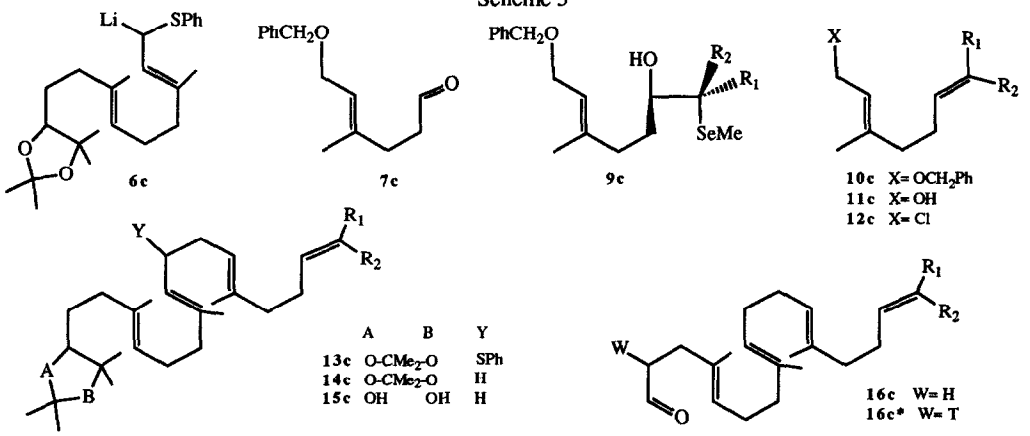
6. Unidentified compounds (a) 1% (b) 3% (Rf 0.05) are also present in this mixture.
7. For the purpose of comparison , we investigated the behavior of the stereoisomeric 2,3-oxidosqualene $\mathbf{1c}_E$ possessing a E-Δ^{18-19} C,C bond and observed that it led exclusively to the formation of the 20(R)-norlanosterol derivative $\mathbf{2c}_R$.[8]
8. This compound was compared with an authentic sample prepared (scheme 4) from lanosteryl acetate via ozonolysis of its 24-25 C,C double bond, Wolf-Kishner reduction of the resulting aldehyde **17** [(i) (a) O_3,CH_2Cl_2-pyridine, 0°C, 64% yield (b) 2.8 equiv. Me_2S, 20°C, 3h (ii) NH_2NH_2, KOH, ethylene glycol-ethanol, 120°C, 1h, 180°C, 2h, 200°C, 0.2h, quantitative yield in $\mathbf{2c}_R$, 96% purity].
9. The 2D NMR spectra of $\mathbf{3c}_Z$, which is for obvious reasons simpler than that of $\mathbf{3b}_Z$ shows that the side chain on the five membered ring lies on the β-side. Comparison of both spectra clearly suggested a

similar orientation for the side chain on $\mathbf{3b}_Z$ and on the basis of additional experiments (NOESY at 600 MHz among others) we have reassigned [10] its stereochemistry at that center.

10. Guittet, E.; du Penhoat, C. H.; Lallemand, J-Y.; Schauder, J-R.; Krief, A. *J. Amer. Chem. Soc.*, **1987**, *109*, 7911.
11. Prepared from 3β-tetrahydropyranyloxy lanosterol by a sequence of reactions which involved the intermediary formation of **18**,[12] its further reaction with ethyl magnesiumbromide [2.9 equiv., ether-benzene 3-1, 25°C, 1h, 88% yield, contaminated with 5% of its 20R stereoisomer] leading to the alcohol **19** which after oxidation and removal of the THP group produces **20** [(i) 6.3 equiv. PCC, 4.2 equiv. AcONa, CH_2Cl_2, 20°C, 3.5h (ii) 0.5 equiv. PTSA, MeOH-CH_2Cl_2 2-1, 20°C, 3h, 74% overall yield]. The latter was in turn transformed to the desired norlanosterol $\mathbf{2c}_S$ by reduction of the corresponding thioacetal **21** [(i) $(CH_2SH)_2$, AcOH, Et_2O-BF_3, 20°C, 24h, 86% yield (ii) Li, $EtNH_2$, -15°C, 0.25h, 92% yield including 19% of another compound to which we have not attributed a structure and which was separated from the desired compound after acetylation.
12. Schauder, J.R.; Krief, A. *Tetrahedron Lett.* **1982**, *23*, 4389.
13. Krief, A.; Pasau, P.; Quéré, L. *Biorg. & Med. Chem. Lett.* **1991**, *1*, 365.
14. This is not the case if Saccharomyces cerevisiae is used instead.[13]
15. We have observed during a ^1H NMR experiment performed in impure $CDCl_3$ containing a trace of acid, that $\mathbf{3c}_Z$ led (trace of HCl, $CHCl_3$, 20°C) to $\mathbf{4c}_Z$ (life time < 0.1h) which immediately rearranged to a large number of "by-products" containing traces of $\mathbf{5c}_Z$. We have ruled out a chemical isomerisation of $\mathbf{3c}_Z$ to $\mathbf{5c}_Z$ since we have not found such "by-products" besides $\mathbf{5c}_Z$ in the biosynthetic experiment and because we recovered $\mathbf{3c}_Z$ and not $\mathbf{5c}_Z$ when a pure sample of $\mathbf{3c}_Z$ was purified again under the same conditions used for its former purification.
16. This kind of assumption has been also used by Corey [17] to explain the stereochemistry of the β-oriented side chain at C-17 of a protosterol derivative occuring from cyclisation of an oxidosqualene analogue bearing a $\Delta^{20\text{-}21}$ extra C,C double bond.
17. Corey, E.J.; Virgil, S.C.; Sarshar S. *J. Amer. Chem. Soc.*, **1991**, *113*, 8171.

Scheme 3*



* *Series a: R_1=Me, R_2=Pr, Series b: R_1=Pr, R_2=Me*

Scheme 4