Further evidence that the polycyclization reaction by oxidosqualene-lanosterol cyclase proceeds *via* a ring expansion of the 5-membered C-ring formed by Markovnikov closure. On the enzymic products of the oxidosqualene analogue having an ethyl residue at the 15-position

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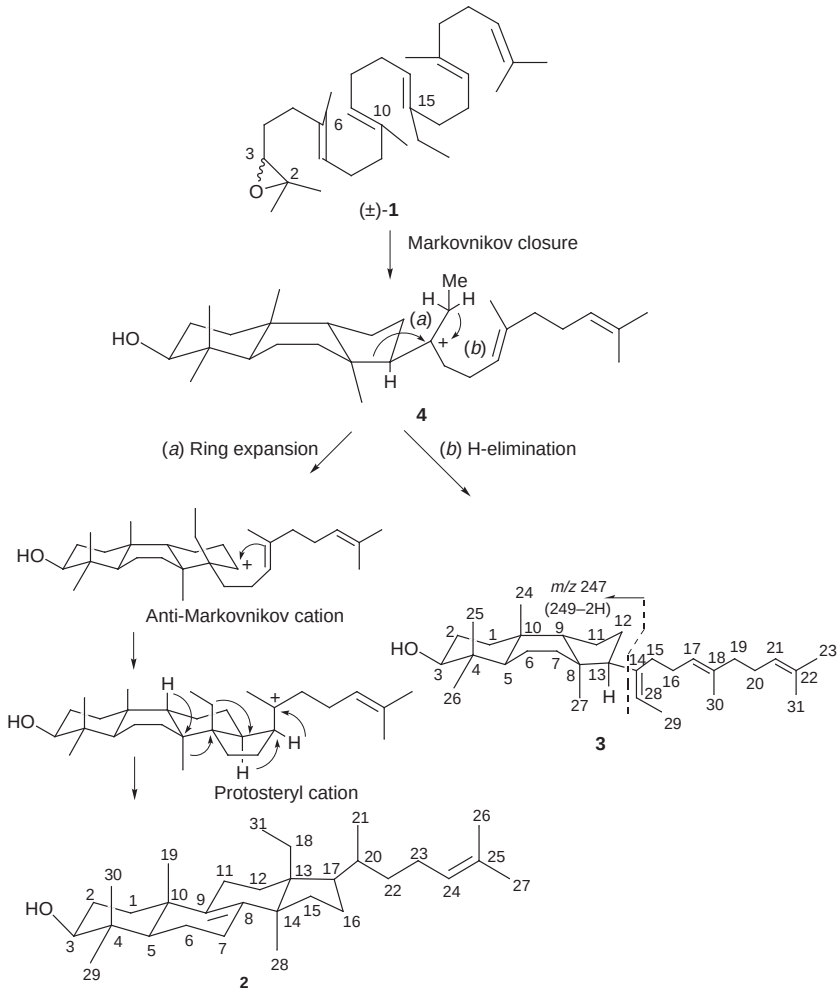
The incubation of the substrate analogue, (3*S*)-(6*E*,10*E*,14*E*,18*E*)-15-ethyl-2,6,19,23-tetramethyl-2,3-epoxytetracosa-6,10,14,18,22-pentaene (-)-1 with 2,3-oxidosqualene-lanosterol cyclase from pig liver gave products 2 (a lanosterol homologue) and 3 (a tricyclic product), definitively demonstrating that the cyclization of oxidosqualene proceeds *via* the expansion reaction from a 5- to a 6-membered ring for the C-ring formation of lanosterol.

In a preceding paper,[1] we reported the unprecedented cyclization products given by 2,3-oxidosqualene cyclase: substitution of the ethyl groups with methyl groups at both C-10 and C-15 of oxidosqualene halts the enzymic reaction at the monocyclic ring stage. This demonstrated that the cyclizing reaction to the protosterol cation proceeds *via* discrete carbocation intermediates, although usually this reaction has been assumed to be concerted, and gives the first example of the cyclase reaction being stopped at the stage of the initial A-ring formation. To gain more insight into the fine control of the polyolefin cyclization, we have now examined the enzymic reaction of the analogue **1**, in which only the methyl group at C-15 in the squalene backbone has been replaced by an ethyl group. Here, we report the tricyclic and tetracyclic formed products by the cyclase and give further evidence for the involvement of a C-ring expansion process in the formation of lanosterol.

The synthesis of (±)-**1** was essentially the same as previous papers.[1,2] The coupling reaction of (2*E*,6*E*)-1,10-dibromo-3,7,11-trimethyldodeca-2,6-diene-11-ol with (2*E*,6*E*)-1-bromo-



Scheme 1

3,7,11-trimethyldodeca-2,6,10-triene, by treatment of cuprous iodide with lithium pyrrolidide,[3] afforded the desired **1**, the squalene derivative having ethyl groups at C-10 and C-15, and 2,3:22,23-dioxidosqualene in the yields of 36, 29 and 6%, respectively, which could be easily separated by SiO_2 column chromatography due to remarkable differences of the products' polarity. Enzyme preparation from the microsomal fractions of pig liver was carried out according to the literature.[1,4] A sample of (±) **1** (38 mg) was anaerobically incubated for 24 h at 37 °C and lyophilized. Extraction of the reaction residues with hexane gave two new peaks (**2** and **3**) *via* GLC. The behavior of **2** and **3** on SiO_2 TLC was the same, irrespective of solvent system. By using an argentation SiO_2 column (10%), the metabolites **2** (solid) and **3** (oil) were isolated in yields of 5.8 and 2.2 mg, respectively. The total conversion was 42% based on one enantiomer of (±)-**1**. The specific rotations of **2**, **3** and the recovered **1** were +21.58 (*c* 0.417, $CHCl_3$), +13.25 (*c* 0.083, $CHCl_3$) and +1.93 (*c* 0.497, EtOH), respectively, indicating that the 3*S* form, (-)-**1**, was selectively transformed. The molecular composition of **2** was determined to be $C_{31}H_{52}O$ from EI-HRMS (*m/z* 440.4043; requires 440.4018) and the following characteristic mass fragments were observed: *m/z* 440 (M^+, 56%), 425 (M^+-Me, 100%), 407 (M^+-Me-H_2O, 21%); the fission patterns and peak intensities were the same as authentic lanosterol (*m/z* 426, 411, 393). The molecular composition of **3** was also determined to be $C_{31}H_{52}O$ from EI-HRMS (*m/z* 440.4035); *m/z* 440 (M^+), 247 (249-2H; tricyclic moiety). The hydroxy groups of **2** and **3** were confirmed to be equatorial from the coupling constants of H-3 (dd, *J* 4.5, 12 Hz). Detailed analyses of NMR spectra including ^1H–^1H COSY 45, HOHAHA, NOESY, DEPT, HMQC and HMBC experiments unambiguously verified the structures of **2** and **3** to be a lanosterol homologue and a tricyclic compound, respectively. In the HMBC spectrum of **2**, the crosspeak of 19-Me with the sp^2 quaternary C-9 and that of 28-Me with C-8 confirmed the position of the double bond, and the correlations of 28-Me and 31-Me to C-13 also proved the ethyl position. The clear NOEs of 31-Me with 21-Me and of 19-Me with 31-Me indicated that all the stereo-orientations of **2** were identical to those of lanosterol. The stereochemistry at C-20 has yet to be established, but it should have the same *R* configuration as natural lanosterol; the chemical shift (δ_C 35.1) of C-20 was homogenous. On the other hand, **3** had a partially cyclized 6/6/5 fused-ring system possessing a chair/boat geometry. The boat geometry of the B-ring was confirmed by the apparent correlation of H-5 with 27-Me and that of H-9 with 24-Me in the NOESY spectrum. The NOE correlation of 27-Me with H-13 indicated the α-orientation of H-13. The ethylidene moiety was also inferred from the HMBC data.

Formation of the two products **2** and **3** suggests that a common precursor **4**, the tricyclic cation having a 6/6/5-fused system, should be produced before the subsequent reactions occur (Scheme 1). The carbocation intermediate **4**, a thermodynamically favored product by Markovnikov closure, undergoes ring expansion toward the anti-Markovnikov cation of the 6-membered C-ring to form the protosterol cation, which is then subjected to four sequential antiparallel 1,2-shifts of two hydrogens, the ethyl group and the methyl group, followed by deprotonation leading to double bond formation [path (*a*)], just as does the natural substrate. It is noticeable that the *ethyl* group also underwent the migration reaction. If a proton is eliminated from the ethyl group, the tricyclic **3** is formed [path (*b*)]. The ring expansion competes with the deprotonation process from the ethyl group. The tricyclic fused-A/B/C ring system has also been isolated from the enzyme products of the $\Delta^{18,19}$ double bond *Z*-isomer,[5] the truncated substrate[6] and the 20-oxa analogue.[6] Recently, Corey proposed the idea, on the basis of the enzyme reactions of the truncated C_{20} and 20-oxa analogues, that the 6-membered C-ring of lanosterol is formed *via* ring expansion of the 5-membered cation, previously produced by Markovnikov closure. However, the enzyme reactions were halted at the intermediate stage without completion of the cyclase reaction, thus, the ring expansion process remained unclear. In contrast, analogue **1** afforded the complete lanosterol skeleton **2** along with capture of the intermediate **3**; therefore, this finding provides strong evidence for the involvement of a ring expansion process. The intermediate **4** would have been formed in a Markovnikov fashion and as a result of the longer lifetime of **4** due to the enhanced steric repulsion between the methyl at C-10 and the ethyl at C-15, compared to that between the two methyl groups of natural squalene, the detailed mechanism of which has been discussed.[6b] Kyler and co-workers have assumed that substitution on the β-side of the folded conformation interferes with the normal cyclization.[7] A good conversion of **1** to **2**, despite the ethyl moiety being situated on the β-face, was a contrast to this hypothesis inferred from Bakers' yeast. This inconsistency may originate from the difference in the biological source used. Indeed, no reaction was detectable for **1** or the diethylated oxidosqualene with the yeast cyclase.[1] The binding site and/or cavity size for the methyl group at C-15 may be different for the yeast and mammal enzymes. In the case of the diethylated analogue, only the initially cyclized A-ring was formed,[1] while the monoethylated **1** gave the subsequent tricyclic and tetracyclic reaction products. These results also imply that the 10-methyl group plays a critical role in the normal polycyclizaton reaction by the mammalian cyclase, which is consistent with a previous report.[8]

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Notes and References

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