Substitution of the Methyl Groups with Ethyl groups at C-10 and C-15 of 2,3-Oxidosqualene halts the Enzymatic Reaction of Oxidosqualene–Lanosterol Cyclase at the Monocyclic Ring Stage

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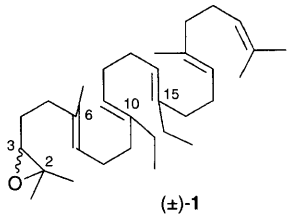
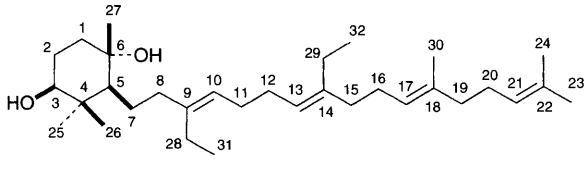
Incubation of the substrate analogue, (3*S*)-(*E*,*E*,*E*,*E*,*E*)-10,15-diethyl-2,6,19,23-tetramethyl-2,3-epoxytetracosa-6,10,14,18, 22-pentaene (−)-**1**, with 2,3-oxidosqualene–lanosterol cyclase from pigs liver gave unprecedented cyclization products **2** and **3** having a monocyclic skeleton.

Numerous studies on the reaction of 2,3-oxidosqualene–lanosterol cyclase with different substrates have been reported.[1] Among many investigations, special attention has been drawn by Tamelen,[2] Corey[3] and Kyler[4] to how the cyclization pathway is affected by the substitution of the methyl groups at C-10 or at C-15. The replacement of hydrogen at C-15[2] or of the vinyl group at C-10[4] led to the normal cyclization products, however the substrate analogue lacking methyl groups at both C-10 and C-15 gave an unusual cyclization product.[3] To gain more insight into the molecular recognition, we carried out the enzymic reaction on the substrate **1**, whose structure has two ethyl groups (slightly bulky substituents) at C-10 and C-15 in the squalene backbone. Here we report the unprecedented enzymic reaction where the polycyclization is terminated at the monocyclic stage.

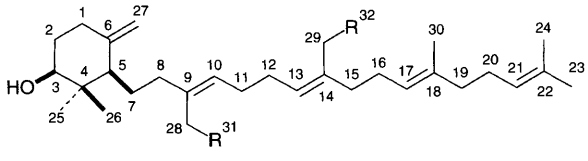
The synthetic methodology of (±)-**1** was essentially the same as that of oxidosqualene previously described[5] except that methyl 3-keto-valerate was the starting material. Microsome pellets from pigs liver[6,7] and Baker's yeast[8,9] were used as enzyme sources. Compound (±)-**1**† was anaerobically incubated at optimal pH[5,7] with the partially purified enzymes at 37 °C for 24–30 h. The product was analysed by GC. No conversion was observed with yeast cyclase, whereas two new peaks were found with pig liver cyclase. Large-scale incubation of (±)-**1** (40 mg) with the liver cyclase afforded **2** (4 mg) and **3** (5 mg) as oils, 45% conversion based on one enantiomer of (±)-**1**, after purification with SiO_2 column chromatography (hexane–EtOAc as eluent), $[\alpha]_D^{25}$ (EtOH) +6.25° (*c* = 0.21) and −6.1° (*c* = 0.36), respectively. The substrate **1** recovered after repetitive incubation showed an $[\alpha]_D^{25}$ +1.6° (*c* = 0.68, EtOH), verifying the fact that the 3*S*-substrate, (−)-**1**, was selectively converted to the new products as well as being naturally converted from 3*S* (−)-2,3-oxidosqualene to lanosterol.[10] From Lineweaver–Burk plots, the K_m values of (−) **1** were determined to be *ca.* 250 μmol dm^{-3} for each production of **2** and **3**, while that of (−)-oxidosqualene was 50 μmol dm^{-3},[7] indicating that the enzyme affinity of **1** was 2,3-times less. Compounds **2** and **3** were not produced in the presence of a specific inhibitor against mammalian cyclase of *N*-nonanoyl-8-aza-4α,10-dimethyl-*trans*-decal-3β-ol,[7,11] indicating that these conversions were only catalysed by the cyclase enzyme. The inhibitory effect of **1** on the conversion from oxidosqualene to lanosterol was small (IC_{50}, >4000 μmol dm^{-3} for baker's yeast and >420 μmol dm^{-3} for Pig liver cyclase).



(±)-**1**



(−)-**2**



(+)-**3** R = Me
Achilleol A R = H

The ^{1}H NMR spectrum of **2** in acetone-[$^2H_6$] showed four olefinic protons at 5.0–5.2 (m) (**1** had five), the absence of the oxide ring proton (2.63, 1 H, t, *J* = 6.3 Hz) (originally present for **1**) and the presence of new signals at 3.24 (1 H, m, 3-Hax) and 3.40 (1 H, d, *J* = 5.3 Hz, 3-O*H*). The presence of >C*H*-OH [δ_C 78.3(d)] indicated that cyclization had occurred. From EI-HRMS, the molecular formula was determined to be $C_{32}H_{56}O_2$ (M^+ *m*/*z* 472.4259, requires 472.4280). The other oxygen atom to be identified in **2** was that of the tertiary hydroxy group [δ_H 2.94 (1 H, s), δ_C 73.2 (s)]. In addition, the proton resonances of the three methyl groups [δ_H 1.64 (3 H, s, 6-Me), 1.21 (3 H, s, 1-H) and 1.23 (3 H, s, 2-Me)] in the spectrum of **1** shifted to δ_H 1.13, 1.02 and 0.78 (each 3 H, s, 27-H, 25-H and 26-H, respectively). Other methyl groups were left unchanged. These upfield chemical shifts further suggest that **1** lost one double bond to form a cyclized skeleton and **2** possessed dimethyl groups on C-4 of the cyclohexane ring. The detailed analyses of the HMBC spectrum, especially the hydroxy protons (*e.g.* cross peaks of 27CH_3/6OH and 4C/30H), revealed the positions of attachment of both the tertiary and secondary hydroxy groups mentioned above. The NOESY spectrum clarified the relative stereochemistry of **2**. The molecular composition of **3** was determined to be $C_{32}H_{54}O$ from CIMS (CH_4, *m*/*z* M^+ + H, 455) and EI-HRMS (*m*/*z* $M^+ - H_2O$, 436.4083; requires 436.4069). A comparison of the ^{1}H NMR spectrum of **3** in $CDCl_3$ with that of **1** showed seven and eight methyl groups for **3** and **1** respectively, and two new singlets at δ_H 4.58 (1 H, s, 27-H) and 4.85 (1 H, s, 27-H), both correlated to δ_C 108.3(d) in the HMQC spectrum, indicating the presence of an exomethylene group in **3**, which originated from the lost methyl group described above. As well as compound **2**, **3** was also cyclized, because four olefinic protons at δ_H 5.0–5.2(m) and a new signal at δ_H 3.39 [1 H, dd, *J* = 10.0 Hz, 4.1, 3-Hax, correlated to δ_C 77.2(d)] were observed; coupling constants of the latter signal showed an equatorial disposition for the hydroxy group. From HMBC and NOESY spectra, the structure of **3** was unequivocally determined.

The monocyclic carbocation, generated at C-6 by the first cyclization, reacted with a water molecule acting as a

nucleophile to produce **2**. The loss of a proton from the methyl group on C-6 generated the exomethylene of **3**. The formation of compounds **2** and **3** would occur if the ethyl residues lacked molecular recognition towards the methyl-binding sites of the enzyme, with which the methyl groups at C-10 and C-15 of the substrate strongly interact to give a folding boat–chair conformation, participating in the subsequent second and third cyclizations. The finding of Achilleol A,[12] an analogue of **3** (R=H), from *Achillea odorata* suggests that the plant's cyclase may lack the amino acid-alignments, which, though not yet known, are probably responsible for the methyl-binding sites. There are some indications that the methyl group at C-10 is crucial to the correct folding,[3] and that the structural modifications at the β-face regions of the folded conformation alter the cyclization pathway.[4,13]

Received, 25th August 1995; Com. 5/05642J

Footnote

† Satisfactory ^{1}H and ^{13}C NMR and HR-EIMS spectra were obtained for compound **1**.

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