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Conversion of a C_{20} 2,3-Oxidosqualene Analog to Tricyclic Structures with a Five-Membered C-Ring by Lanosterol Synthase. Further Evidence For a C-Ring Expansion Step in Sterol Biosynthesis

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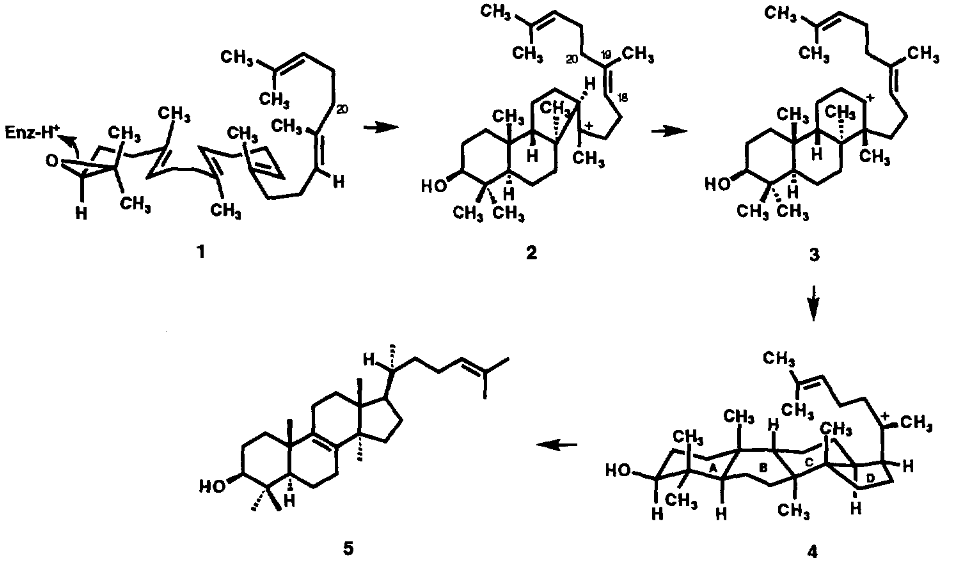
Summary: *Lanosterol synthase converts the truncated analog* ***6*** *of 2,3-oxidosqualene (**1**) into three tricyclic products,* ***7, 8*** *and* ***9****, each of which contains a five-membered C-ring. The results are in accord with expectations based on previous work indicating that the six-membered C-ring of sterols is formed via a five-membered predecessor.* Copyright © 1996 Elsevier Science Ltd

The molecular details of the enzymatic conversion by lanosterol synthase of (*S*)-2,3-oxidosqualene (**1**) to lanosterol (**5**),[1] via the intermediate 17β-protosterol cation (**4**)[2] are of great significance for the understanding of enzyme control in sterol biosynthesis. Recent work on gene cloning and the knowledge of amino acid sequences in a number of lanosterol synthases of diverse origin has added to the interest in this area.[3] Strong evidence has been obtained that the cyclization reaction occurs in discrete stages and specifically that the C-ring of the steroid nucleus is formed by ring closure to a *five-membered* structure **2** followed by ring expansion to **3**, as shown in Scheme 1.[4] In this paper we describe the results of an independent experimental test of the pathway shown in Scheme 1 using a substrate which is capable of forming tricyclic but not tetracyclic products, the C_{20} truncated 2,3-oxidosqualene analog **6**. It was anticipated, on the basis of the pathway shown in Scheme 1, that analog **6**

Scheme 1.

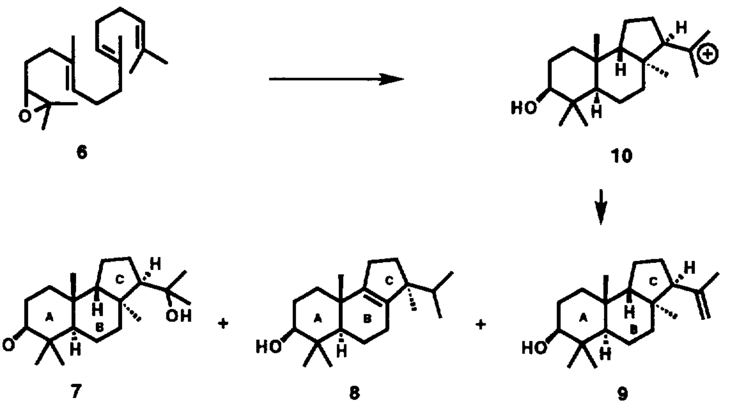


would be converted to 6/6/5-fused tricyclic product(s) if it underwent cyclization under the influence of lanosterol synthase. This surmise has been verified by the results reported below.

Racemic epoxide **6**[5] (100 mg) when incubated with 50 ml of a 2 μM solution of purified lanosterol synthase (from yeast)[3c] at pH 6.4 (sodium phosphate buffer[6]) for 24 hours underwent cyclization at a considerably slower rate than 2,3-oxidosqualene and afforded, in addition to recovered **6**, product of R_f similar to lanosterol (0.5 on silica gel plate using 1 : 1 ether–hexane for development) and product of lower R_f (0.3).[7] These cyclized materials (total 11.3 mg) were separated preparatively by flash chromatography on silica gel using 4 : 1 hexane–ethyl acetate for elution of the less polar fraction and 2 : 1 hexane–ethyl acetate for elution of the more polar product which was shown to be a single pure compound by ^{1}H NMR analysis (diol **7**). The less polar fraction was resolved into two mono hydroxy olefins (**8** and **9**) by chromatography on silica gel impregnated with silver nitrate using 4 : 1 hexane–ether for elution. The ratio of products **7**, **8** and **9** was 60 : 22 : 18, respectively. The structure of **7**, suggested by the ^{1}H NMR and mass spectra,[8] was confirmed by transformation to the crystalline *p*-bromobenzoate, mp 152.2 °C, and single crystal X-ray diffraction analysis. A computer rendering of the X-ray determined structure of **7** is shown in Figure 1.[9] The structures of **8**[10] and **9**[11] follow from the spectral data and the fact that they are formed from **7** upon storage for several weeks in $CDCl_3$ solution, as shown by isolation in pure form and spectroscopic/chromatographic comparison. The formation of **8** and **9** in a ratio of *ca.* 1 : 1 from **7** in $CDCl_3$ solution is clearly the result of an acid-catalyzed process via the cyclopentylcarbinyl carbocation (**10**) which parallels the enzymatic reaction leading from **6** to **7**, **8** and **9** (Scheme 2).

It is clear that in the enzymatic transformation of analog **6** to the tricyclic product **7**, the *trans-syn-trans* A/B/C arrangement of **7** corresponds to the protosterol stereochemistry and that lanosterol synthase controls the stereochemistry of cyclization of the C_{20} substrate **6** just as it does 2,3-oxidosqualene. Each of the cyclization products of **6**, i.e. **7**, **8**, and **9**, possesses a five-membered C-ring which supports the sterol biosynthetic pathway shown in Scheme 1 involving a five-membered ring C precursor **2**. As discussed previously, direct formation of

Scheme 2.



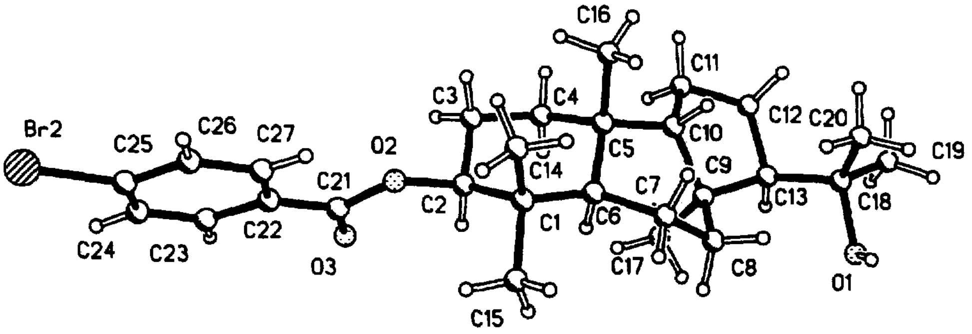


Figure 1. X-Ray crystallographically determined structure of the 3-*p*-bromobenzoate of diol **7**.

a six-membered C ring is disfavored by *both* Markovnikov and steric factors which lanosterol synthase may not be able to overcome. Thus, the indirect pathway to the protosterol cation via the tertiary cyclopentylcarbinyl cation **2** with ring expansion, though less direct, is kinetically preferred. The formation of a mixture of **7**, **8** and **9** in the enzymatic cyclization of **6** indicates that lanosterol synthase cannot control the final steps as well as it does the initial cyclization, a fact which is not surprising given the much smaller size of cation **10** relative to the protosterol cation (**4**), the likelihood of looser binding of **10** by the enzyme, and the high reactivity of cation **10** toward any available proton acceptor or nucleophile. The spontaneous formation of **8** and **9** from **7** in slightly acidic $CDCl_3$ at 23 °C clearly demonstrates that these products are kinetically preferred over C-ring expansion under non-enzymatic conditions. Therefore, it is entirely possible that the occurrence of **8** and **9** as coproducts in the lanosterol synthase-catalyzed cyclization of **6** may not even require channeling of the reaction by the enzyme. On the other hand, in the conversion of 2,3-oxidosqualene to lanosterol, the synthase enzyme must accelerate the ring expansion of cation **2** to form **3** relative to other reaction modes available to **2**

It has previously been reported that 20-thia-2,3-oxidosqualene is not a substrate for lanosterol synthase and is completely resistant to any change by the enzyme, in contrast to 20-oxa-2,3-oxidosqualene which is converted to tetracyclic products.[4] This fact has been interpreted as indicating that epoxide activation requires a conformational change after initial binding of the substrate and that the substitution of the C(20) methylene of **1** by a sulfur does not allow such a change, presumably because of the larger size of the 20-thia analog. Clearly, the truncated substrate **6**, which is smaller than 2,3-oxidosqualene, does not prevent epoxide activation, although the process is markedly slowed.

In conclusion, the conversion of the tetraprenoid oxidosqualene analog **6** to the 6/6/5 fused, tricyclic products **7**, **8** and **9** by yeast lanosterol synthase is fully consistent with the proposed 5 → 6 C-ring expansion pathway[4] for sterol biosynthesis and provides useful insights into the molecular details of the tetracyclization process of this remarkable biosynthesis.[12]

References and Notes

1. For a recent review, see Abe, I.; Rohmer, M.; Prestwich, G. D. *Chem. Rev.* **1993**, *93*, 2189.
2. For a revision of the stereochemistry of the tetracyclization reaction, see (a) Corey, E. J.; Virgil, S. C. *J. Am. Chem. Soc.* **1991**, *113*, 4025. (b) Corey, E. J.; Virgil, S. C.; Sarshar, S. *J. Am. Chem. Soc.* **1992**, *114*, 1524.
3. See (a) Kelly, R.; Miller, S. M.; Lai, M. H.; Kirsch, D. R. *Gene*, **1990**, *87*, 177 and Buntel, C. J.; Griffin, J. H. *J. Am. Chem. Soc.* **1992**, *114*, 9711 (*Candida albicans*). (b) Corey, E. J.; Matsuda, S. P. T.; Bartel, B. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11628 (*Arabidopsis thaliana*). (c) Corey, E. J.; Matsuda, S. P. T.; Bartel, B. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2211 (*Saccharomyces cerevisiae*). (d) Kasuno, M.; Shibuya, M.; Sankawa, U.; Ebizuka, Y. *Biol. Pharm. Bull. Japan* **1995**, *18*, 195 (*Rattus norvegieus*). (e) Baker, C. H.; Matsuda, S. P. T.; Liu, D. R.; Corey, E. J. *Biochem. Biophys. Res. Commun.* **1995**, *213*, 154 (*Homo sapiens*).
4. Corey, E. J.; Virgil, S. C.; Cheng, H.; Baker, C. H.; Matsuda, S. P. T.; Singh, V.; Sarshar, S. *J. Am. Chem. Soc.* **1995**, *117*, 11819.
5. Synthesized by the reaction of π–γ,γ-dimethylallyl barium with 2,3-oxidofarnesyl bromide; see Corey, E. J.; Noe; M. C.; Shieh, W.-C. *Tetrahedron Lett.* **1993**, *34*, 5995 and Corey, E. J.; Shieh, W.-C. *Tetrahedron Lett.* **1992**, *33*, 6435.
6. The solution also contained 20% glycerol, 0.2% triton X-100 and 3 mM dithiothreitol.
7. Substrate **6** was a weak competitive inhibitor of the cyclization of 2,3-oxidosqualene by lanosterol synthase. No time-dependent inactivation of the enzyme by **6** was observed.
8. Physical data for **7** *p*-bromobenzoate: mp 152.2 °C (recryst. from ethyl acetate–pentane); R_f 0.41 (silica gel, 1 : 1 diethyl ether–hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (s, 3 H, CH_3), 0.99 (s, 3 H, CH_3), 1.01 (s, 3 H, CH_3), 1.09 (s, 3 H, CH_3), 1.19 (s, 3 H, CH_3), 1.18 (s, 3 H, CH_3), 1.40-1.86 (m, 15 H, 3 x C*H*, 6 x CH_2), 2.67 (d, J = 6.2 Hz, 1 H, O*H*), 4.72 (dd, J = 5.2 Hz, J = 11.7 Hz, 1 H, C*H*OCO), 7.56 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 aryl *H*), 7.86 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 aryl *H*); MS (CI) *m/z* 508/510 (M + NH_4^+).
9. The coordinates of the *p*-bromobenzoate of **7** can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.
10. Physical data for **8** *p*-bromobenzoate: R_f 0.87 (silica gel, 1 : 3 diethyl ether–hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.66 (d, J = 6.8 Hz, CH_3), 0.82 (d, J = 6.8 Hz, CH_3), 0.94 (s, 3 H, CH_3), 0.95 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 1.01 (s, 3 H, CH_3), 1.23-2.13 (m, 14 H, 2 x CH, 6 x CH_2), 4.74 (dd, J = 4.8 Hz, J = 11.6 Hz, 1 H, CHOCO), 7.56 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 aryl *H*), 7.86 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 aryl *H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.84, 17.58, 18.07, 18.61, 19.72, 23.04, 24.10, 25.23, 28.00, 28.24, 29.90, 33.94, 35.00, 35.66, 38.13, 51.61, 52.36, 82.06, 127.78, 129.88, 131.07, 131.66, 138.33, 142.73, 165.52; MS (CI) *m/z* 490/492 (M + NH_4^+); mp 146-147 °C (crystallized from EtOAc/pentane).
11. Physical data for **9** *p*-bromobenzoate: R_f 0.87 (silica gel, 1 : 3 diethyl ether–hexane); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (s, 3 H, CH_3), 0.98 (s, 3 H, CH_3), 1.00 (s, 3 H, CH_3), 1.11 (s, 3 H, CH_3), 1.67 (s, 3 H, CH_3), 1.23-2.12 (m, 15 H, 3 x C*H*, 6 x CH_2), 4.56 (s, 1 H, vinyl *H*), 4.73 (dd, J = 4.8 Hz, J = 11.6 Hz, 1 H, C*H*OCO), 4.80 (s, 1 H, vinyl *H*), 7.56 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 H, aryl *H*), 7.88 (dd, J = 1.8 Hz, J = 8.4 Hz, 2 H, aryl *H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.17, 18.55, 20.89, 23.24, 24.81, 25.33, 27.67, 29.23, 29.56, 31.91, 33.85, 35.33, 38.52, 44.28, 47.10, 52.72, 57.79, 82.25, 111.32, 127.79, 129.85, 131.09, 131.66, 150.44, 165.60; MS (CI) *m/z* 490/492 (M + NH_4^+).
12. This work was supported by the National Institutes of Health. We are grateful to Mr. Mark C. Noe and Mr. Mihai Azimioara for the X-ray crystallographic analysis of the *p*-bromobenzoate of **7**.

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