Detection of 1,2-hydride shifts in the formation of euph-7-ene by the squalene–tetrahymanol cyclase of *Tetrahymena pyriformis*

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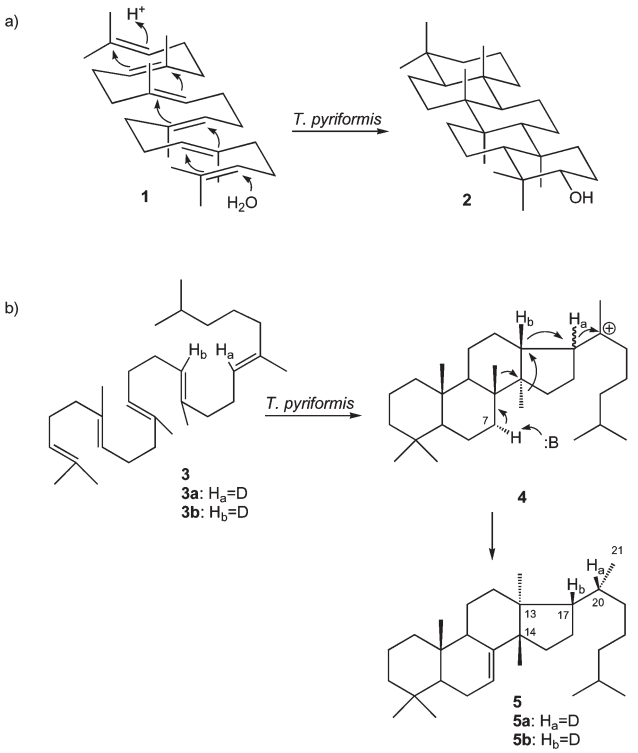
Received (in Cambridge, UK) 29th March 2005, Accepted 18th April 2005
First published as an Advance Article on the web 13th May 2005
DOI: 10.1039/b504300j

Incubation of samples of 2,3-dihydrosqualene, specifically labeled with deuterium at either carbon position 7 or 11, with an enzyme extract from *Tetrahymena pyriformis*, containing a squalene–tetrahymanol cyclase, provided specimens of euph-7-enes displaying deuterium patterns consistent with the biosynthetic operation of two consecutive 1,2-hydride shifts.

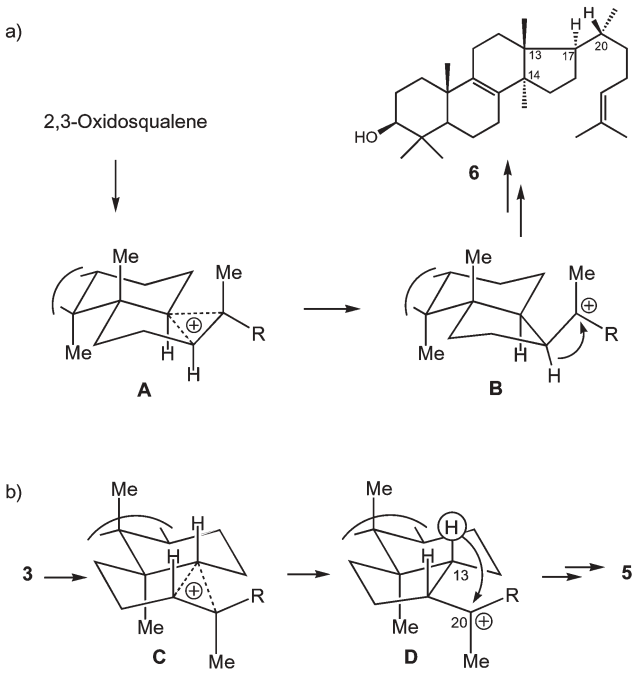
A cyclase from the ciliate protozoan *Tetrahymena pyriformis* is capable of cyclizing squalene in its all-chair conformation, **1**, to the pentacyclic triterpene alcohol tetrahymanol, **2**. According to the experimental evidence, the cyclization is initiated by protonation of a terminal double bond and the process terminated by the quenching of the resulting pentacyclic carbocation with water (Scheme 1a).[1] Later, Abe and Rohmer reported that the same cyclase can convert 2,3-dihydrosqualene, **3**, into euph-7-ene, **5** (Scheme 1b).[2,3] This unexpected result requires the formation of an intermediate tetracyclic carbocation, **4**, which in the original work was considered to subsequently undergo transformation into the final product *via* a sequence of two 1,2-hydride shifts and two 1,2-methyl migrations, followed by removal of the axial 7α-proton (Scheme 1b, **4** → **5**).[2]



Scheme 1 Cyclization reactions of *Tetrahymena pyriformis*: (a) Formation of tetrahymanol (**2**) from squalene (**1**). (b) Formation of euph-7-ene (**5**) from dihydrosqualene (**3**).

A similar set of hydride and methyl migrations had previously been demonstrated in the biosynthesis of lanosterol, **6**, from 2,3-oxidosqualene (Scheme 2a).[4] Moreover, the first tetracyclic, ionic intermediate in this process, **B**, has been shown to have the relative and absolute configuration imposed by the chair conformation, **A**, of the corresponding segment of the acyclic precursor. The (20*R*)-configuration of the resulting lanosterol has been interpreted as the outcome of a least-motion pathway for the first hydride shift.[5]

Compounds **5** and **6** display antipodal configurations at the three chiral centers C-13, -14 and -17, of their D-rings, while sharing an identical (*R*)-configuration at C-20. For this reason, a mirror image version of the scheme demonstrated for **6** (Scheme 2a) cannot be applied to the formation of **5**, since it



Scheme 2 (a) The stereochemical pathway of the reactions leading to the (20*R*)-configuration of lanosterol (**6**). (b) The hypothetical pathway leading to the (20*R*)-configuration of euph-7-ene (**5**).

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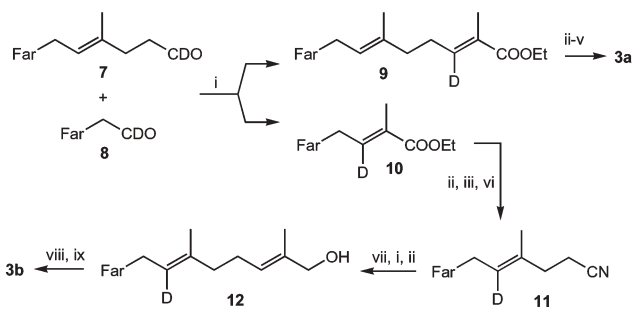
fails to predict its (20*R*)-stereochemistry. Accordingly, this "anomalous" C-20 configuration cannot be interpreted as the result of a least-motion pathway, and other factors must be at work in the specific generation of this chiral center. Nevertheless, an antipodal chair conformation, **C**, for the segment of **3** cyclizing to ring D on the way to **5** (Scheme 2b) seems to be a reasonable assumption, since it corresponds to the conformation required for the production of tetrahymanol in a normal cyclization process. Within this assumption, the operation of the 1,3-hydride shift indicated for the resulting tetracyclic ionic intermediate **D** would provide a simple way of explaining the (20*R*)-configuration of the final product. There is a strong precedence for similar 1,3-hydride shifts in sesquiterpene biosynthesis,[6] and the hydrogen bound to C-13 appears to be well positioned for such a process.

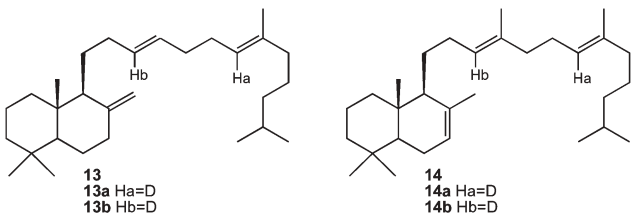
To test this hypothesis, we have now synthesized two specimens of dihydrosqualene, **3a** and **3b**, labeled specifically with deuterium at C-7 or C-11, and submitted them to the action of the *Tetrahymena* cyclase. A mixture of the unlabeled aldehydes, corresponding to **7** and **8**, is easily available from **1** using known methods.[7] A mixture of the labeled compounds, each containing 0.85 D, was prepared from **1** by reduction with $LiAlD_4$ followed by oxidation with PCC. As indicated in Scheme 3, this preparation was converted into a mixture of the *E*-esters **9** and **10**, which were easily separated by argentic silica gel chromatography. The ester **9** was transformed in four steps into the desired **3a**. The second labeled compound, **3b**, was obtained from **10** in eight steps including, *inter alia*, reaction of the appropriate allylic bromide with "cyanomethylcopper"[8] to give **11**, and a copper-catalyzed reaction of a Grignard reagent with the allylic acetate derived from **12**.[9]

The enzymatic cyclizations of 40 mg samples of **3a** and **3b** were carried out as previously described.[3] In each case, the desired product was obtained in *ca.* 40% yield and subjected to NMR and MS analysis. The product from the 7-D compound, **3a**, displayed a singlet at 0.82 ppm in its ^1H NMR spectrum ($CDCl_3$) instead of the doublet normally found for the C-21 methyl group (0.83 ppm, J = 6.6 Hz). The MS of the compound showed characteristic peaks at *m*/*z* 413 and 299, corresponding to the molecular ion



Scheme 3 Far = farnesyl. Reagents, conditions and yields as follows: i. $Ph_3P{=}C(CH_3)COOEt$ (1.2 equiv.), benzene, rt, 87%; ii. $LiAl(OEt)H_3$ (4 equiv.), Et_2O, 0 °C, 93%; iii. PBr_3 (1 equiv.), Et_2O, 0 °C, 73%; iv. $(CH_3)_2CHCH_2SO_2Ph$ (1.8 equiv.), *n*-BuLi (1.8 equiv.), THF–HMPT 10 : 1, −78 °C to rt, 89%; v. 6% Na–Hg (6 equiv.), Na_2HPO_4 (4 equiv.), MeOH, 0 °C, 73%; vi. CH_3CN (10 equiv.), *n*-BuLi (10 equiv.), CuI (1 equiv.), THF, −25 °C to rt, 83%; vii. DIBAL (1.1 equiv.), $PhCH_3$, 0 °C to rt, then 10% HOAc–H_2O, 97%; viii. Ac_2O–pyridine 1 : 1 (large excess), rt, 100%; ix. $(CH_3)_2CHCH_2CH_2MgBr$ (4 equiv.), Li_2CuCl_4 (0.04 equiv.), THF, −78 °C to rt, (29–60% recovered as **12**).

($C_{30}H_{51}D^+$) and a fragment arising from it by loss of a deuterated side chain ($M^+-C_8H_{16}D$) respectively. These results clearly demonstrate that the product of the enzymatic cyclization of **3a** bears its deuterium label at C-20 (**5a**). In keeping with this finding, the product from the 11-D compound, **3b**, shows the normal pattern of signals in the methyl region of its ^1H NMR spectrum and a peak at *m*/*z* 300 in the MS, consistent with the elimination of an unlabeled side chain from the molecular ion ($M^+-C_8H_{17}$). This is consistent with the presence of a deuterium label at C-17 (**5b**). The outcomes of the two complementary experiments rule out the operation of the putative 1,3-hydride shift and demonstrate that the rearrangement process leading to **5** is initiated, as in the case of **6**, by a 1,2-hydride shift from C-17 to C-20. Proof of the stereochemistry of the dammaranyl cation involved in this process will be presented in a separate communication.



In addition, two by-products, **13** and **14**, were produced in both enzyme experiments in their deuterated forms, each as *ca.* 5% of the total product. The two isomers could be separated from one another by argentic silica gel chromatography, and displayed molecular ions at *m*/*z* = 413 ($C_{30}H_{49}D$). Both compounds were shown to be bicyclic triterpene-trienes on the basis of their ^1H NMR spectra. ^1H NMR spectra ($CDCl_3$) of **13a** and **13b** showed seven methyl groups: two allylic methyl signals overlapping at 1.59 ppm, three methyl singlets at 0.87, 0.80 and 0.66 ppm, and two overlapping methyl doublets at 0.86 ppm (J = 6.6 Hz). Both **13a** and **13b** showed two olefinic singlets at 4.82 and 4.54 ppm, indicative of an *exo*-methylene group, as well as a signal at 5.11 ppm (triplet, J = 6 Hz). The NMR data for **14a** and **14b** were similar, but eight methyl groups were detected: three allylic methyl signals at 1.71, 1.61, and 1.58 ppm, three methyl singlets at 0.87, 0.85, and 0.74 ppm, and two overlapping methyl doublets at 0.86 ppm (J = 6.6 Hz). Both **14a** and **14b** exhibited an olefinic signal at 5.38 (multiplet). In addition, **14a** showed a signal at 5.14 (triplet, J = 6 Hz), while **14b** had a signal at 5.11 (triplet, J = 6 Hz). The new structures assigned to the type **13** and **14** compounds are closely related to those of α- and γ-polypodatetraene, two natural products from ferns, from which they differ only by lack of the isopropylidene double bonds.[10] Similar bicyclic compounds have previously been isolated from the incubation products of long chain polyprenyl ethers with the same cyclase.[11] The formation of these products is best visualized as being an interception of the bicyclic, ionic intermediates by the same base which mediates in the position 7 deprotonation during formation of **5**. In contrast, no corresponding bicyclic products can be detected from the incubation of natural substrate **1** with the cyclase. It seems noteworthy that a relatively minor change, such as the removal of the double bond at a remote center, is sufficient to influence the sequence of events which follow the second cyclization step.‡

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Notes and references

‡ An analogous situation has been detected in the case of the related squalene–hopene cyclase of *Alicyclobacillus acidocaldarius*. While polypodatetraenes are not generated from squalene by the wild type cyclase, their production has been detected with mutated forms of the enzyme.[12]

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