Nonoxidative Cyclization of Squalene by *Tetrahymena pyriformis*. The Incorporation of a 3β-Hydrogen (Deuterium) Atom into Tetrahymanol

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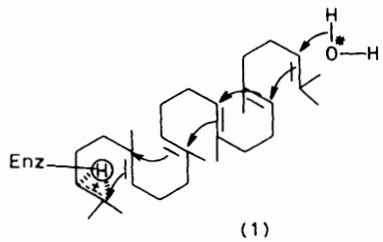
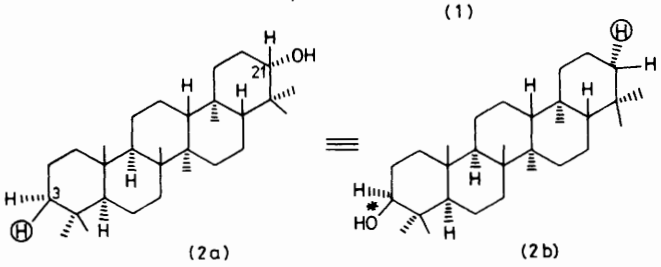
Summary It was shown by ^{2}H-n.m.r. spectroscopy that the proton introduced by *T. pyriformis* in the cyclization of squalene assumes the 3β-stereochemistry in the biosynthesized tetrahymanol.

WE have previously reported that the biosynthesis of tetrahymanol† (**2**) from squalene (**1**) in *Tetrahymena pyriformis* does not require molecular oxygen, and does not proceed through 2,3-oxido-squalene.[1] Evidence was also

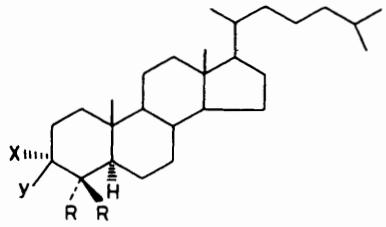
† Structures (**2a**) and (**2b**) are equivalent. For convenience of comparison of the ^{2}H-n.m.r. spectra of [^{2}H]tetrahymanol and the [3-$^2H_1$]-model compounds, the numbering system (**2a**) (*cf.* ref. 1) is used in the present paper. This is in contrast to the numbering system (**2b**) used in our previous paper, T. A. Wittstruck and E. Caspi, *J. Chem. Research*, 1977, (*S*), 180; (*M*) 2135.



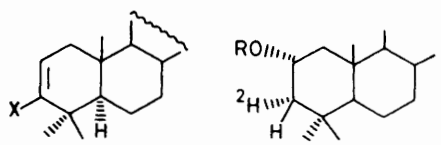


Ⓗ: hydrogen (deuterium) atom introduced in the biosynthesis from squalene.

presented that when the biosynthesis was carried out in a medium containing D_2O or $^{18}OH_2$, the tetrahymanol (**2**) produced contained one atom of deuterium, or one atom of oxygen-18, respectively.[1] Mass spectroscopy indicated that the deuterium was located in ring A or B of (**2**). The results fully supported the hypothesis that the biosynthesis of tetrahymanol proceeded by an enzyme-mediated attack on a terminal double bond of squalene.[1] We now present ^{2}H-n.m.r. evidence for the 3β-location of the incoming proton (deuteron) in tetrahymanol.



(3) R = H
(4) R = Me
a; X = ^{2}H; y = ^{1}H
b; X = ^{1}H; y = ^{2}H



(5)
a; X = ^{1}H
b; X = ^{2}H

(6)
a; R = H
b; R = *p*-$MeC_6H_4SO_2$

We first examined the separation of the chemical shifts of [3α-$^2H_1$]- (**3a**) and [3β-$^2H_1$]-cholestane[2] (**3b**). These showed a significant separation of 0·46 p.p.m. As a comparison model for tetrahymanol (**2**), we selected 4,4-dimethyl-5α-cholestane (**4**) in which rings A and B are structurally and conformationally analogous to those in the triterpene (**2**). The olefin (**5a**) was prepared[3] and treated with 1,2-dimethylpropyl[$^2H_1$]borane[4] to yield, among other products, 2α-hydroxy-[3α-$^2H_1$]-(**6a**). The derived tosylate (**6b**) on hydrogenolysis ($LiAlH_4$) gave approximately equal amounts of [3α-$^2H_1$]-(**4a**) and the [3-$^2H_1$]-olefin (**5b**). Hydrogenation of (**5b**) (platinum oxide–ethyl acetate) gave the [3β-$^2H_1$]-(**4b**) admixed with *ca.* 30% of the [3α-$^2H_1$]-isomer (**4a**). The chemical shifts of the 3α- and 3β-deuterons of (**4a**) and (**4b**) are in the Table. The difference in the chemical shifts of deuterons in the model compounds (**4a**) and (**4b**), 0·23 p.p.m., is sufficient for use in the unambiguous assignment of the stereochemistry of deuterium atoms of such compounds.

TABLE[a]

Compound	Chem. shift/p.p.m. 3α-$^2H_1$	3β-$^2H_1$
5α-Cholestane	1·21	1·67
4,4-Dimethyl-5α-cholestane	1·14	1·37
Tetrahymanol (from squalene in D_2O)	—	1·39

[a] The spectra were recorded on a Bruker HX-270 instrument at 41·44 MHz in $CHCl_3$ with $CDCl_3$ as internal standard, showing a deuterium chemical shift of 7·27 p.p.m.

A lyophilized enzyme powder of whole cells of *T. pyriformis* (2 g) was suspended in deuterium oxide [99·5%] (6 ml) and extracted gently with peroxide-free ether (5 × 50 ml) in the cold. Following decantation of the ether, the enzyme suspension was lyophilized in a cold room. The obtained powder was suspended in deuterium oxide (9 ml) then an emulsion of squalene (105 mg) and Triton X-100 (30 mg) in deuterium oxide (1 ml) was added and the mixture was incubated for 24 h. The tetrahymanol (**2**) was recovered and purified by t.l.c. (15% ethyl acetate–hexane). A pooled sample from several incubations (11·9 mg) showed a $^2H_1$ content of 41·0%, and its ^{1}H-n.m.r. spectrum was indistinguishable from that of authentic tetrahymanol. The ^{2}H-n.m.r. spectrum of the [$^2H_1$]-tetrahymanol was then recorded. Its deuteron chemical shift, 1·39 p.p.m., is essentially identical with that of the 3β-deuteron of (**4b**). It follows that the hydrogen (deuterium) atom incoming in the biosynthesis assumes the 3β-stereochemistry in tetrahymanol.

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