lactone,[9] have been reported; in each case, the isolation procedure included exposure to high temperatures. In view of the demonstrated transformation of germacranolide precursors to elemanolides by heating, the question has been raised as to whether some of the elemanolides may be artifacts.[8,9] In contrast, when an isolation procedure was devised which involved cold aqueous extraction of *V. hymenolepis*, vernolepin was isolated in a yield comparable to that obtained by hot ethanol extraction. This fact supports the view that vernolepin is, indeed, a naturally occurring compound.

Vernolepin and vernomenin appear to be the first recognized elemanolide dilactones.

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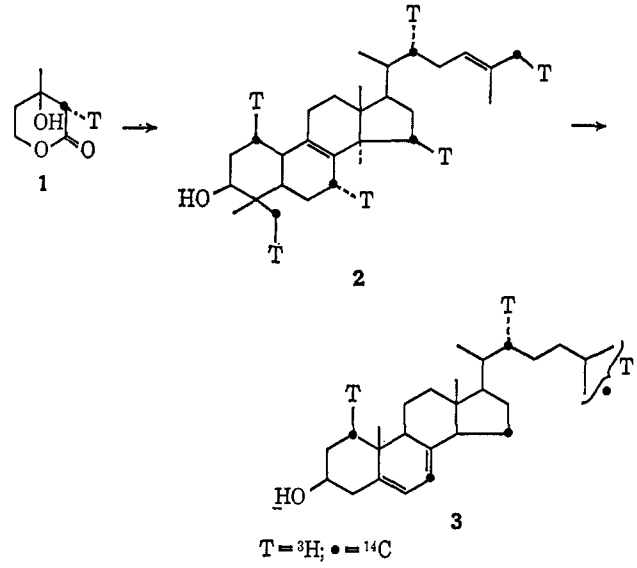
The Fate of the 15β Hydrogen of Lanosterol in Cholesterol Biosynthesis

Sir:

In cholesterol biosynthesis the three methyl groups, 4α, 4β, and 14α, of lanosterol are removed by oxidation to carbon dioxide.[1] The 14α-methyl group is believed to be transformed into a carboxyl group and eliminated by decarboxylation, which is facilitated by the 8,9 double bond.[2,3]

If the 8,9 double bond is not the only promoter of the elimination of the 14α-carboxyl group, this reaction could be facilitated by one of the following mechanisms: (a) oxidation of 14α-carboxylic acid with formation of a carboxyl radical and its elimination with the involvement of one of the hydrogen atoms in position 15, (b) oxidation in position 15 and concerted elimination of the carboxyl group, (c) previous formation of double bond in position 15,16 facilitating the elimination of carbon in 14. All these possibilities imply the removal of hydrogen atoms in position 15. The fate of these hydrogen atoms during cholesterol biosynthesis has been followed by determining the position of labeled hydrogens in lanosterol, 5α-cholest-7-en-3β-ol, and cholesta-5,7-dien-3β-ol biosynthesized from labeled mevalonic acids. Actual $^3H:^{14}C$ ratios and atom equivalents of all the significant compounds are shown in Table I.

Accumulation of labeled cholesta-5,7-dien-3β-ol (**3**) has been obtained by incubating liver homogenates[4] of rats pretreated with AY 9944,[5] in the presence of the same inhibitor[6] and of 3(±)-(2*S*)-[2-^{14}C-2-^{3}H]mevalonic acid lactone (**1**) (10 μCi of ^{14}C, $^3H:^{14}C$ 10.00).[7] Radioactive carbon atoms in cholesta-5,7-dien-3β-ol correspond to the positions shown in formula **3**.[8] Since the radioactive precursor is asymmetrically labeled, tritium should be localized at positions 1α, 15β, 22*R*, and 26 or 27, the 7 position being excluded on the basis of our previous results.[9] The $^3H:^{14}C$ ratio should be 4:5. If the 15β hydrogen is exchanged with the medium, this ratio should be 3:5.



The unsaponifiable residue from homogenates was acetylated, carrier cholesta-5,7-dien-3β-ol acetate was added, and the mixture was separated by column chromatography on silver nitrate–kieselgel G–Celite.[10] The obtained cholesta-5,7-dien-3β-ol acetate[11] was diluted again with nonradioactive material and hydrogenated in presence of tris(triphenylphosphine)rhodium chloride[6] to yield 5α-cholest-7-en-3β-ol acetate.[11] After column chromatography on silver nitrate–kieselgel G–Celite,[10] this compound (0.565 μCi of ^{14}C/mmole) showed a $^3H:^{14}C$ ratio of 6.07, corresponding to 3.02 labeled hydrogens out of 5 radioactive carbon atoms. The ^{14}C radioactivity and the $^3H:^{14}C$ ratio were constant after several crystallizations. Furthermore oxidation with osmium tetroxide of some of the radioactive 5α-cholest-7-en-3β-ol acetate produced the mixture of the epimeric *cis*-5α-cholestane-3β,7,8-triol 3β-acetates[6,11] (0.563 μCi of ^{14}C/mmole) which showed an unchanged $^3H:^{14}C$ ratio with respect to 5α-cholest-7-en-3β-ol acetate. The same ratio was found in the mixture of the epimeric *cis*-5α-cholestane-3β,7,8-triol 3β-acetates[6,10] (0.189 μCi of ^{14}C/mmole) obtained from radioactive 5α-cholest-7-en-3β-ol acetate which could be isolated in small amounts[6] from liver homogenates. The expected constant $^3H:^{14}C$ ratio was also found

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(11) The chemical purity of all compounds was established by comparing melting points, optical rotation values, mass spectra, and glpc retention times on a 1% phenylsilicone glass column with those of authentic samples.

in the mixture of the epimeric 5α-cholestane-3α,8-diol-7-one 3β-acetates[11] (0.564 μCi of ^{14}C/mmole) resulting from the mixture of the epimeric *cis*-5α-cholestane-3β,7,8-triol 3β-acetates.[6]

In order to establish if the missing tritium was originally from the 15 position, the remaining 5α-cholest-7-en-3β-ol acetate (0.565 μCi of ^{14}C/mmole) was oxidized with selenium dioxide[12] to 5α-cholest-8(14)-en-3β,7α-diol diacetate[11] which, adsorbed on silver nitrate–kieselgel G–Celite, gave rise to 5α-cholesta-7,14-dien-3β-ol acetate: mp 67–69°; uv max (C_2H_5OH) 242 mμ (ε 9700).[13] This diene was oxidized with chromium trioxide in acetic acid to yield 5α-cholest-8(14)-en-3β-ol-7,15-dione acetate; mp 153–155°; uv max (C_2H_5OH) 260 mμ (ε 6200); $[\alpha]^{25}D$ 65 (*c* 1, $CHCl_3$)[13] (0.567 μCi of ^{14}C/mmole). This compound showed a $^{3}H:^{14}C$ ratio identical with that of 5α-cholest-7-en-3β-ol acetate. The formation of a keto group in position 15, unaccompanied by a decrease of the $^{3}H:^{14}C$ ratio, shows that the 15β hydrogen of **3** is not radioactive.

In order to establish the stereospecificity of the exchange of hydrogens in position 15, a biosynthetic experiment has been performed starting from 3(±)-(2*R*)-[2-^{14}C-2-^{3}H]mevalonic acid lactone (10 μCi of ^{14}C, $^{3}H:^{14}C$ 9.20). Incubation and isolation procedures were the same as described above. Radioactive cholesta-5,7-dien-3β-ol acetate[11] was transformed and purified as in the previous experiment, and the 5α-cholest-7-en-3β-ol acetate[11] obtained (0.581 μCi of ^{14}C/mmole) showed a $^{3}H:^{14}C$ ratio 8.97 corresponding to 4.90 labeled hydrogens out of 5 radioactive carbon atoms. This result shows that the exchange of the 15β hydrogen is stereospecific.

The loss of hydrogen from the 15β position has been followed in cholesterol biosynthesis after the cyclization of squalene to lanosterol. Accumulation of labeled lanosterol (**2**) has been obtained by incubating rat liver homogenates with 3(±)-(2*S*)-[2-^{14}C-2-^{3}H]mevalonic acid lactone (10 μCi of ^{14}C, $^{3}H:^{14}C$ 10.16) in presence of 10^{-3} *M* sodium arsenite.[14] The unsaponifiable residue was acetylated, carrier lanosterol acetate (lanosta-8,24-dien-3β-ol acetate) was added, and the mixture was separated by tlc[15] on kieselgel G impregnated with silver nitrate. The isolated lanosterol acetate[11] showed a $^{3}H:^{14}C$ ratio corresponding to 5.74 labeled hydrogens out of 6 radioactive carbon atoms. This rather low ratio could have been caused by contamination of small amounts of demethyl analogs of lanosterol which are difficult to separate. Chromic acid oxidation[16] of radioactive lanosterol acetate gave rise to methyl 25,26,27-trisnor-3β-acetoxylanost-8-en-7,11-dione-24-oate[11] with a $^{3}H:^{14}C$ ratio corresponding to 3.95 labeled hydrogens out of 5 radioactive carbon atoms. This result proves that the lanosterol retains the radioactive 15β hydrogen.

It seems unlikely that the expulsion of the 4,4-dimethyl groups occurs with involvement of the 15β hydrogen. However the isomerization of 5α-cholest-8-en-3β-ol into 5α-cholest-7-en-3β-ol is proved to proceed through incorporation of only one hydrogen atom from the medium[17] and it is not intramolecular in nature.[6] Therefore it seems probable that the elimination of the 15β hydrogen accompanies the expulsion of the 14α-methyl group.

Further aspects of this elimination are under investigation.

Table I

Compound	$^{3}H:^{14}C$, μCi:μCi	Atom equiv Found	Atom equiv Calcd
3(±)-(2*S*)-[2-^{14}C-2-^{3}H]Mevalonic acid lactone	10.00		
5α-Cholest-7-en-3β-ol acetate	6.07	3.02/5	3/5
cis-5α-Cholestane-3β,7,8-triol 3β-acetate mixture	6.07	3.02/5	3/5
5α-Cholestane-3β,8-diol-7-one 3β-acetate	6.05	3.01/5	3/5
5α-Cholest-8(14)-en-3β-ol-7,15-dione acetate	5.90	2.94/5	3/5
3(±)-(2*S*)-[2-^{14}C-2-^{3}H]Mevalonic acid lactone	10.16		
Lanosta-8,24-dien-3β-ol acetate	9.70	5.74/6	6/6
25,26,27-Trisnor-3β-acetoxylanost-8-en-7,11-dion-24-oate	6.68	3.95/5	4/5
3(±)-(2*R*)-[2-^{14}C-2-^{3}H]Mevalonic acid lactone	9.20		
5α-Cholest-7-en-3β-ol acetate	8.97	4.90/5	5/5

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Halonium Ylides. I

Sir:

We wish to advocate the existence of bromonium ylides as reaction intermediates and to present preliminary findings concerning the chemistry of such ylides. Irradiation (>4800 Å, ~5–15°) of a degassed hexafluorobenzene solution 0.19 *M* in 3,5-di-*t*-butylbenzene 1,4-diazooxide[1] (**1**) and 0.94 *M* in 2,6-diisopropyl-4-bromophenol (**2**) leads to the precipitation of diarylbromonium bromide **3** as a reddish syrup isolable in *ca.* 15% yield after crystallization from hexafluorobenzene, *n*-hexane, and methylene chloride mixtures. The colorless salt has an elemental composition (*Anal.*

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