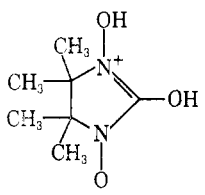
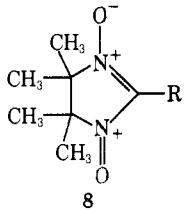
acidification the expected hydroxy nitronyl nitroxide **2**, R = OH, was not formed, and a quantitatively reversible disproportionation occurred to give the dihydroxyurea **5** [ν_{max}^{KBr} 1680 (C=O), 3200 cm^{-1} (OH); τ 8.95 (12 H), 1.97 (2 H) (DMSO); *m/e* 174 (M)][2] and the stable diamagnetic orange zwitterion **6** [ν_{max}^{KBr} 1765 cm^{-1} (C=O); λ_{max}^{EtOH} 310 mμ (ϵ 3600), 424 (7800); τ 8.46 ($CDCl_3$); *m/e* 172 (M)].[2] In alkaline solution air oxidation of **5** gave back the radical anion **4**, and on oxidation of **5** with lead dioxide the zwitterion **6** was formed. Quantitative reduction of **6** to the anion **4** could be achieved with alkaline hydrogen peroxide, and reduction to **5** was effected by neutral sodium thiosulfate.

The action of base on **2**, R = Cl or Br, likewise yields the anion **4**, but much smoother substitution of the halo radicals could be achieved with acid. Thus **2**, R = Br, was converted to the zwitterion **6** in 76% yield with 2 *N* hydrochloric acid at 25° for 30 min. Apparently nucleophilic attack by water on the halo nitronyl nitroxide is accelerated by protonation of a nitrone oxygen, and the resulting product **7** becomes air oxidized to give **6**.



7



8

Interestingly, in strong acids the zwitterion **6** is an exceptionally good oxidizing agent. Thus, for example, in 10% trifluoroacetic acid in methylene chloride this compound oxidized several hydrocarbons, including 9,10-diphenylanthracene, perylene, and tetraphenylethylene, to their respective radical cations. The active oxidant is presumably the cation **8**, R = OH, since the related cation **8**, R = C_6H_5, has also been found to have very strong oxidizing properties.[6] Since no nitronyl nitroxide esr signals are observed in these acidic solutions, **8** must be reduced to a species such as **7** which can disproportionate to a protonated dihydroxyurea **5** and the zwitterion **6**.

Further studies on the chemistry of the 1,3-dioxy-2-imidazolidone zwitterion **6** are in progress.

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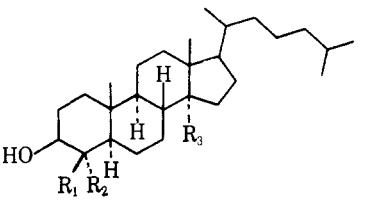
Biological Demethylation of 4,4-Dimethyl Sterols. Initial Removal of the 4α-Methyl Group

Sir:

One of the unresolved aspects of sterol biosynthesis[1] is the sequence of oxidative removal of the 4α- and 4β-methyl groups *en route* from lanosterol[2] to cholesterol. We herein report evidence that, contrary to earlier conclusions,[3] the equatorial 4α-methyl group is the site of initial attack in the demethylation process at C-4.

A preliminary study showed that 4,4-dimethylcholestanol (**1**)[4] was metabolized by rat liver homogenates to cholestanol (**2**) with 10–20% of the efficiency with which dihydrolanosterol (**3**)[5] was converted to cholesterol. The demethylation of each labeled substance was inhibited by a 20-fold concentration of the other in the unlabeled form, that of **1** to the extent of 86% and that of **3** to the extent of 38%. These results are to be expected if the 4,4-dimethyl Δ^8-sterol derived from lanosterol competes with 4,4-dimethylcholestanol for the same enzyme system. On the other hand, 4,4,14α-trimethylcholestanol (**4**)[6] was not measurably metabolized in this enzyme system. This finding implicates the Δ^8-olefinic linkage of lanosterol in the removal of the 14α-methyl group[7] and shows that this latter group inhibits enzymic attack on the 4,4-dimethyl substituents.



1, $R_1 = R_2 = CH_3$, $R_3 = H$
2, $R_1 = R_2 = R_3 = H$
4, $R_1 = R_2 = R_3 = CH_3$
10, $R_1 = R_3 = H$; $R_2 = CH_2OH$
11, $R_1 = CH_3$, $R_2 = CH_2OH$, $R_3 = H$
12, $R_1 = CH_2OH$, $R_2 = CH_3$, $R_3 = H$
13, $R_1 = R_3 = H$, $R_2 = CH_3$

The finding that **1** was metabolized to **2** permitted the use of readily synthesized and labeled substrate analogs in the cholestane series for more detailed studies of the demethylation process. The required model compounds were synthesized from 4α-carbomethoxycholestan-3-one (**5**),[8] which was conveniently prepared by reductive carbomethoxylation[9,10] of Δ^4-cholesten-3-one. Methylation of **5** (NaH, *t*-BuOH, and CH_3I in $H_3COCH_2CH_2OCH_3$) afforded 56% 4β-methyl-4α-carbomethoxycholestan-3-one (**6**), mp 100–101°,[11] and 19% 4α-methyl-4β-carbomethoxycholestan-3-one (**7**), mp 117–118°. That the expected[10,12] preponderance of β methylation had indeed been obtained was substantiated by the nmr spectra of **6** and **7**, in which the angular methyl group on C-10 of **7** appeared at a higher field (δ 0.97) than that of **6** (δ 1.06) owing to transannular shielding by the 4β-carbomethoxyl group.[10] Confirmation of these crucial stereochemical assignments was achieved by Clem-

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mensen reduction[12] of **6** and **7** to 4β-methyl-4α-carbomethoxycholestane (**8**), mp 95–97° (CH_3 on C-10: δ 0.90), and 4α-methyl-4β-carbomethoxycholestane (**9**), mp 79–80° (CH_3 on C-10: δ 0.68), respectively, and the demonstration that **9** was inert under basic hydrolysis conditions which sufficed to saponify **8**.[13]

Keto esters **5**–**7** were labeled by exchange with acidic tritium oxide in tetrahydrofuran[14] and then were reduced with lithium aluminum hydride to the desired diols **10** (mp 211–213°), **11** (mp 219–220°), and **12** (mp 209–210°), respectively.[15] Labeled sterols **1**, **3**, and **4** were prepared from their corresponding ketones by the same exchange–reduction sequence.

The results of incubations of these various labeled sterols are given in Table I. The most striking observation is that **11** is metabolized, but its 4β-hydroxymethyl epimer **12** is not. This result supports the involvement of a 4α-hydroxymethyl-4β-methyl sterol in cholesterol biosynthesis. Furthermore, the metabolism of **10** to cholestanol suggests that an analogous diol is an intermediate in the removal of the second C-4 methyl group from lanosterol.

Table I

Substrate[a]	% conversion to cholestanol
Dihydrolanosterol (**3**)	55.3 (cholesterol)
4,4-Dimethylcholestanol (**1**)	4.9
4,4,14α-Trimethylcholestanol (**4**)	0.0
4β-Methyl-4α-hydroxymethylcholestanol (**11**)	25.5 (+26.4% **13**)
4α-Methyl-4β-hydroxymethylcholestanol (**12**)	0.0
4α-Hydroxymethylcholestanol (**10**)	36.7

[a] Each substrate (46.5 nmol) was incubated aerobically with 4 ml of rat liver homogenate (N. L. R. Bucher and K. McGarrahan, *J. Biol. Chem.*, **222**, 1 (1956)). Products were isolated by saponification and extraction with ether. [^{3}H]Cholestanol and [^{3}H]4α-methylcholestanol (**13**) (F. Sondheimer and Y. Mazur, *J. Amer. Chem. Soc.*, **80**, 5220 (1958)) were characterized by (a) tlc mobility, (b) glpc retention time, (c) cocrystallization as the free sterol and as the acetate with authentic materials, and (d) demonstrated resistance to peracid treatment.

The reason why the results of this study are in apparent disagreement with those of Gaylor and Delwiche[3] is not obvious. These authors reported that lanosterol biosynthesized from [2-^{14}C]mevalonate yielded a 4-monomethyl sterol without loss of $^{14}CO_2$, whereas further metabolism of the monomethyl material to cholesterol released 1 equiv of $^{14}CO_2$. These findings were interpreted as indicating initial attack on the 4β-methyl group, since the 4α-methyl group has been found to be labeled with ^{14}C in all cases[16] of cyclic terpenoid natural products (soyasapogenol, gibberellic acid, and rosenonolactone) where the labeling pattern (from [2-^{14}C]mevalonate precursor) at C-4 has been determined. As an explanation for our discrepant results we have considered the possibility that Gaylor and Delwiche's reasonable assumption of labeling in the 4α-methyl group of lanosterol may have been incorrect. However, on the basis of a separate study[17] this explanation can be discounted. We cannot at present exclude an alternative possibility that the natural Δ^7 and Δ^8 substrates and the saturated substrates used in the present work are attacked by different enzymes, but this seems highly unlikely, particularly in view of the observed mutual inhibition of the metabolism of the natural and unnatural substrates. Further work is in progress in our laboratories that should clarify the situation.

Acknowledgments. The authors wish to thank Dr. D. A. Schooley (Stanford University) and Mr. P. P. Roller (Stanford University) for donating samples of **1** and **4**, respectively. Financial support was provided by the Alfred P. Sloan Foundation (to T. A. S.) and the American Heart Association (to R. B. C.).

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Spatial Distribution of Trapped Electrons in Alkaline Ice Produced by Photoionization

Sir:

The detection and study of trapped electrons in γ-irradiated and photoionized ices[1] and organic solids[2,3] has aroused much current chemical interest. The spatial distribution of the trapped electrons and the separation distance between positive ions and electrons is an important but only partially solved problem. Paramagnetic relaxation experiments can give much information about the spatial distributions of trapped species in frozen solutions. We have previously obtained results demonstrating the existence and approximate size of the spatial inhomogeneities associated with trapped electrons which were generated by γ rays in alkaline ices.[4,5] We report here the paramagnetic relaxation characteristics of trapped electrons in alkaline ice produced by photoionization, and contrast the different spatial distributions produced by γ irradiation and photoionization.

Ferrocyanide ion is known to be easily ionized by ultraviolet light at 254 nm, and the trapped electrons can be detected in 8 *M* NaOH at 77°K.[6] Reagent grade

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