The Reaction of Cholesterol with Ozone and Alcohols: a Revised Mechanism and Structure of the Principal Product

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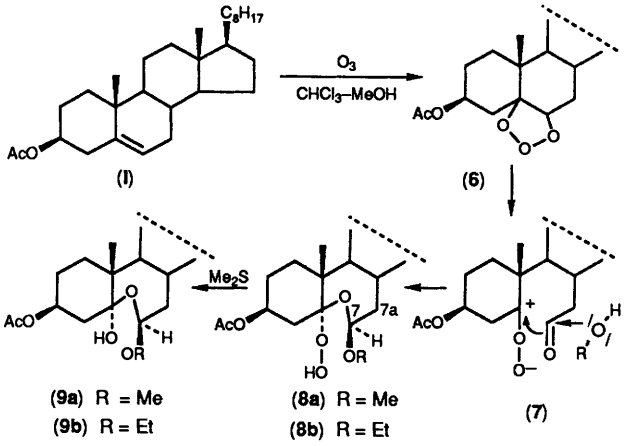
The structure of the product formed in the course of the reaction of cholesterol acetate with ozone in alcohol-containing solvents has been revised. In methanol–chloroform, it is shown to be the hydroperoxide, 3β-acetoxy-5α-hydroperoxy-7β-methoxy-5α-B-homo-6-oxacholestane (**8a**), and not the previously claimed cyclic hemiperacetal, 3β-acetoxy-5-hydroxy-7a-methoxy-B-dihomo-6,7-dioxacholestane (**1**; R^1 = Ac, R^2 = Me).

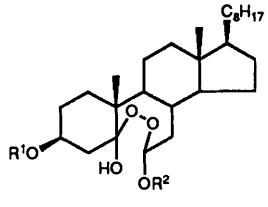
The reaction of cholesterol with ozone results in formation of the expected ozonide, which could be reduced to the 5,6-secosterol.[1] The 5,6-epoxides were also found among products of cholesterol ozonolysis in ethyl acetate and other polar solvents.[2] The reaction takes a different pathway in hydroxylic solvents, like water or alcohols. The major product of cholesterol ozonolysis carried out in non-polar solvents containing alcohols (MeOH, EtOH) was assigned structure (**1**)[3] on the basis of its chemical properties. Recently, Smith and co-workers published an extensive study on ozonation of cholesterol in participating[2] and non-participating[4] solvents. When our work was in progress Jaworski and Smith published an elaborate analysis of spectral data[5] in support of structure (**1**) for the products formed in reactions carried out in hydroxylic solvents. The presence of a lowfield signal, which was originally not observed,[2] at *ca.* δ 10 in the ^{1}H NMR spectra of compounds with the general structure (**1**) constitues a weakness in their structural assignment. The chemical evidence supports either structure (**1**) or a hydroperoxy structure (**2**). In general, hydroperoxides are characterized by a lowfield signal present in their ^{1}H NMR spectra at δ 7–10.[6] In oxabicyclic peroxides of partial structure (**3**), a lowfield signal at δ 8.3–9.3 was assigned to a hydrogen of a hydroperoxy group.[7] Recently, the hydroperoxide (**4**) (^{1}H NMR δ 9.80) and its reduction product (**5**) (^{1}H NMR δ 2.84) were described.[8] This result together with the literature data cast some doubt on the correctness of structure (**1**) and prompted us to reinvestigate the reaction of cholesterol acetate with ozone.

In the reaction of cholesterol acetate with ozone carried out in chloroform–methanol at −70 °C we obtained, quantitatively, a crystalline compound with spectral properties similar to that described;* this was previously assigned structure (**1**; R^1 = Ac, R^2 = CH_3).[2,3,5] In fact, this product is a hydroperoxide and has structure (**8a**). In the ^{1}H NMR spectrum of (**8a**), an important signal at δ 9.99 could be assigned to the OOH group. Also, the strong absorption at 3 300 cm^{-1} in the IR spectrum is consistent with the presence of a hydroperoxy group. In order to confirm

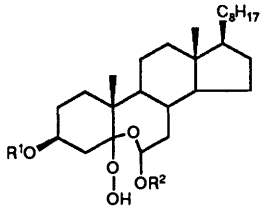


Scheme.

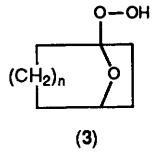
this, chemical evidence for the presence of a hydroperoxy group in (**8a**) was required. It was expected that mild reduction of the hydroperoxides (**8**) would enable the isolation of the hydroxy derivatives (**9**) without cleavage of ring B. Treatment of (**8a**) with lithium aluminium hydride in diethyl ether at −60 °C resulted in formation of compounds (**10**),[1] (**11**),[1] and (**12**).[5] The reaction of (**8a**) with potassium iodide in acetic acid gave, quantitatively, (**10**). However, brief treatment of (**8a**) with dimethyl sulphide without solvent gave, after evaporation of the excess of Me_2S, a product of higher polarity [R_F = 0.25 as compared with R_F = 0.51 of (**8**) (in benzene–ethyl acetate, 5:1)], which decomposed to the seco compound (**10**) on contact with silica gel. When the crude reduction product was dissolved in ethanol-free [$^2H_3$]chloroform, it gave a ^{1}H NMR spectrum perfectly consistent with structure (**9a**).‡ It showed the following



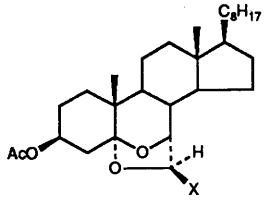
(1) R^1 = H or Ac
R^2 = H or alkyl



(2)



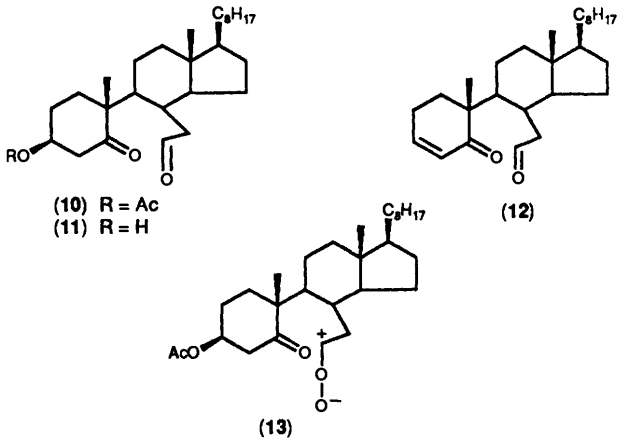
(3)



(4) X = OOH
(5) X = OH

† The spectral characteristics of compounds (**8a**) and (**8b**) were in agreement with the data given in ref. 2.

‡ (**9a**) oil, ν_{max} 3 555, 3 420, 1 732, and 1 237 cm^{-1}; δ 4.90 (1 H, m, 3α-H), 4.58 (1 H, t, *J* 7.7, 7α-H), 3.39 (3 H, s, OCH_3), 3.00 (1 H, s, 5-OH), 0.99 (s, 19-CH_3), and 0.66 (s, 18-CH_3); *m/z* 460 (M^+ − MeOH), 354, 318, 247, and 110.



characteristic signals: a singlet for the methoxy (δ 3.39), a singlet for the hydroxy(δ 3.00, the signal disappears after the addition of D_2O), and a triplet of one proton (δ 4.58, *J* 7.6 Hz) arising from the $CH(OR)_2$ group. The IR spectrum of (**9a**) showed absorption for both a free and an associated hydroxy group at 3 555 and 3 420 cm^{-1}. Similarly, the ethoxy-hydroperoxide (**8b**) was reduced to the bisacetal (**9b**)*. The acetals (**9**) were both very unstable and sensitive to hydroxylic solvents; they gave (**10**) on treatment with water, alcohols, or acetic acid.

In the ^1H NMR spectra, the similar position for the 7-H signal [δ 4.60 in (**8a**), δ 4.58 in (**9a**); δ 4.72 in (**8b**), δ 4.69 in (**9b**)] as well as a similar splitting pattern for this signal [for example: both triplets, *J* 7.8 and 7.6 Hz for (**8a**) and (**9a**), respectively] are indicative of the same size and conformation of ring B in compounds (**8**) and (**9**).

While there is no unequivocal evidence for the stereochemistry of (**8**) it is proposed that the 5-OOH and 7-OCH_3 groups have 5α- and 7β-stereochemistry, respectively. For such a stereochemistry a strain-free conformation of rings A and B may be envisaged. Such an arrangement of atoms in ring B is reflected in the coupling pattern of 7-H [an equal angle between this proton and both protons at C-7a is expected on the basis of a Dreiding model analysis].

* (**9b**) oil, ν_{max} 3 555, 3 400, 1 735, and 1 242 cm^{-1}; δ 4.96 (1 H, m, 3α-H), 4.69 (1 H, t, *J* 8.1, 7α-H), 3.46 and 3.86 (2 H, two q, *J* 7.2, 7-OCH_2CH_3), 2.98 (1 H, s, 5-OH), 1.20 (3 H, t, *J* 7.1, 7-OCH_2CH_3), 0.99 (s, 19-CH_3), and 0.66 (s, 18-CH_3); *m/z* 460 (M^+ − EtOH), 354, 318, 247, and 110.

Formation of (**8**) implies also, that in the reaction of cholesterol with ozone in alcohol-containing solvents, a more stable tertiary carbonyl oxide (**7**) is formed exclusively instead of the previously proposed (**13**).[2]

Our conclusions regarding the structure of the product formed during ozonolysis of cholesterol in methanol or ethanol has a precedence in the work of McCullough and co-workers on indene ozonolysis.[10] Moreover, a recent isolation of both types of products, a hydroperoxide and a hydroxyperoxide, from ozonation of indene derivatives,[11] as well as the comparison of their spectral properties, support our revision.

In view of our results it is proposed, that all structures (**1**) assigned to the principal products obtained in the ozonolyses of cholesterol in water or alcohols described in refs. 2, 3, and 5 have to be revised and isomeric structures (**2**) with a 5-hydroperoxy group are correct for those compounds. The dimeric and oligomeric structures proposed for products obtained during ozonation of cholesterol in non-participating solvents[4] have to be revised accordingly, since all these compounds show in their ^1H NMR spectra a lowfield signal of the proton characteristic of a hydroperoxy group.

Acknowledgements

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