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Note

Anomalous chromatographic behaviour of some C_{27}-steroid hydroperoxides

JON L. TENG and LELAND L. SMITH

Division of Biochemistry, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550 (U.S.A.)

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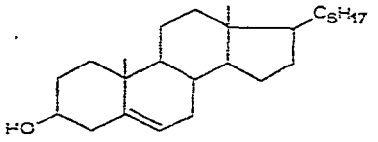
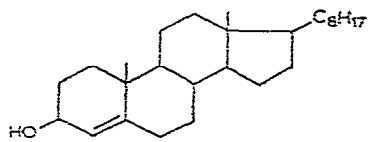
Our application of thin-layer chromatography (TLC) and gas chromatography to the analysis of autoxidized cholesterol (cholest-5-en-3β-ol) (I)[1–4] has led to the discovery of over ten sterol hydroperoxides formed by the direct attack of molecular oxygen on the sterol[5–10] and of other hydroperoxides associated with scission of the side-chain[11] and with additional oxidation of the A-ring[8]. We have utilized binary solvent mixtures exclusively for TLC of these hydroperoxides and their numerous thermal decomposition products, the manipulation of binary solvent mixture proportions heretofore serving our needs.

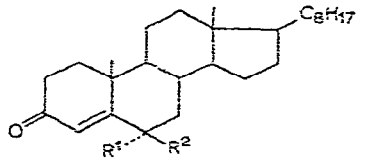
Occasionally positive hydroperoxide color tests[3] may be obtained overlaying the chromatographic zone nominally containing cholesterol. Although several of the side-chain hydroperoxides[5] are close to cholesterol and cholesterol 20α-hydroperoxide may be made to overlap the cholesterol zone by manipulation of solvents, it was obvious that the known side-chain hydroperoxides did not account for the positive peroxide test in all cases. We now provide explanation of these observations in the discovery of the anomalous chromatographic behaviour of the epimeric 6-hydroperoxycholest-4-en-3-ones III and IV whereby, depending on solvent proportions used, the mobility of these steroids may be greater than, less than, or equal to that of cholesterol.



I



II



III R^1=OOH, R^2=H
IV R^1=H, R^2=OOH
V R^1=R^2=H

It is fundamental to empirical chromatographic operations that the mobility of a given component may be altered by change of solvent and that relative mobilities of two components may be reversed as well. However, reversal of relative mobilities caused by the mere alteration of proportions of a binary solvent mixture is not common. Isolated examples of such behaviour for steroids have been reported[12–14], but by and large such matters have not received attention for either partition or adsorption modes of chromatography[15–18]. Accordingly, the marked reversal of mobilities of the epimeric 6-hydroperoxides III and IV relative to those of cholesterol and its isomer cholest-4-en-3β-ol (II) as exhibited in Fig. 1 merits attention both from the novelty and possible theoretical concern but also as a practical matter of the proper analysis of complex samples of autoxidized cholesterol.

TLC of the steroids was conducted using 20 × 20 cm chromatoplates of silica gel HF_{254} (Merck, Darmstadt, G.F.R.) 0.25 mm thick, using triple ascending solvent irrigation for optimal resolution of components. Steroids were detected by their UV light absorption, by N,N-dimethyl-*p*-phenylenediamine[3], and by 50% sulfuric acid[1]. Mobility data were obtained using cholesterol, generally near mid-plate, as unit mobility.

As demonstrated in Fig. 1, the mobilities of the Δ^4-3β-alcohol II and the corresponding Δ^4-3-ketone cholest-4-en-3-one (V) remain uniformly greater than cholesterol despite variation in solvent nature and proportions, although at the low solvent ratios (high ethyl acetate proportions) both II and V approach cholesterol in mobility. The several steroid hydroperoxides of our experience[5–11] have similarly exhibited a fixed relationship to cholesterol, being uniformly more polar. The related epimeric 6-hydroxy-Δ^4-3-ketones 6α-hydroxycholest-4-en-3-one and 6β-hydroxycholest-4-en-3-one likewise were uniformly resolved from one another and more polar than cholesterol.

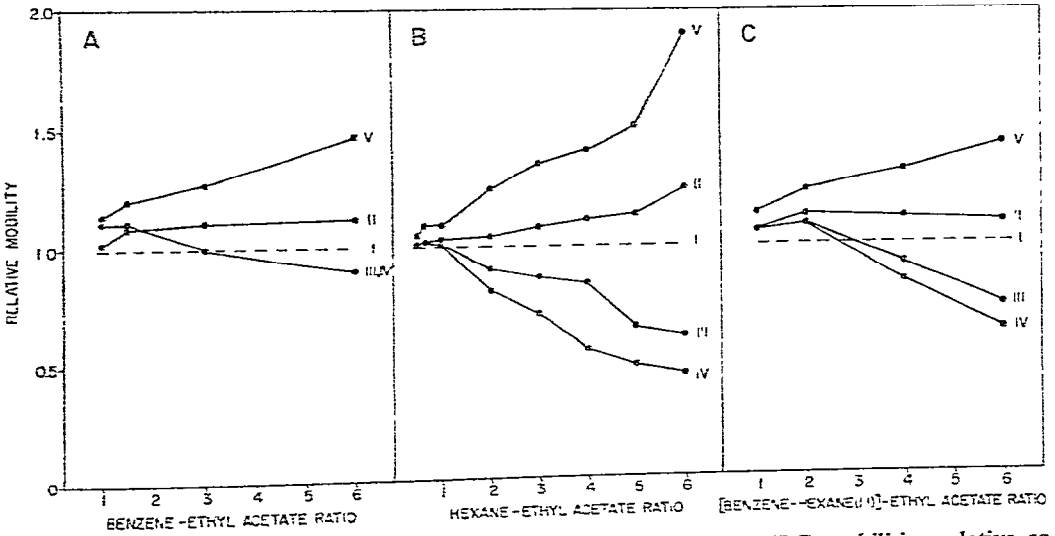


Fig. 1. Variation with changes in irrigation solvent composition of TLC mobilities relative to cholesterol (I) as unit mobility of the Δ^4-steroids. II = Cholest-4-en-3β-ol; III = 6α-hydroperoxycholest-4-en-3-one; IV = 6β-hydroperoxycholest-4-en-3-one; V = cholest-4-en-3-one.

The epimeric 6-hydroperoxides III and IV exhibit quite different properties. In benzene–ethyl acetate mixtures the epimeric 6-hydroperoxides were unresolved and their relationship to cholesterol and the isomeric Δ^4-sterol II varied as shown in Fig. 1A such that the 6-hydroperoxides could be manipulated to overlap the positions occupied by either sterol I or II. In hexane–ethyl acetate mixtures the 6-hydroperoxides III and IV were resolved except at the low solvent ratios, as shown in Fig. 1B. Again the 6-hydroperoxides could be manipulated such as to overlap either sterol I or II, but very high proportions of ethyl acetate were required and resolution of the epimeric 6-hydroperoxides III and IV was lost.

Very similar behaviour was also obtained using the ternary solvent mixture hexane–benzene–ethyl acetate as shown in Fig. 1C. Using the same ratios of mixed hydrocarbon solvents to ethyl acetate as for the binary solvent mixtures the epimeric 6-hydroperoxides III and IV were either resolved from one another and more polar than cholesterol or were unresolved and more mobile than cholesterol. In this case the more mobile 6β-hydroperoxide IV could be made to overlap the cholesterol zone with the less mobile 6α-hydroperoxide III as a more polar spot on the chromatogram, or the more polar 6α-hydroperoxide III could be made to overlap the cholesterol zone with the less polar 6β-hydroperoxide IV as a more mobile component ahead of cholesterol. The unresolved epimeric 6-hydroperoxides III and IV could also be made to overlap the isomeric Δ^4-sterol II.

The anomalous behaviour of the epimeric 6-hydroperoxides III and IV cannot be attributed to either Δ^4-3-ketone or 6-hydroperoxide moiety alone but must be a property of the Δ^4-3-ketone-6-hydroperoxide structural feature. Intramolecular hydrogen bonding between the 6-hydroperoxide and 3-carbonyl groups is unlikely for either epimeric 6-hydroperoxide III or IV, as the two structural features are remote from one another in all conformations examined using Dreiding molecular models. Intermolecular self association enhanced in the more polar solvent mixtures and diminished in the less polar mixtures appears improbable. Interaction with the aromatic solvent benzene is unlikely, for the effects were demonstrated with hexane as well. There remains interaction with a polar solvent enhanced in the solvent mixtures enriched in ethyl acetate. This possibility is supported by use of neat diethyl ether as solvent where both 6-hydroperoxides III and IV were substantially more mobile than cholesterol and unresolved from one another (relative mobility: I, 1.00; II, 1.05; III and IV, 1.12; V, 1.14).

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