Inhibition of sterol biosynthesis by 14α-hydroxy-Δ^7-sterols[1]

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Summary. 5α-Cholest-7-en-3β,14α-diol and 5α-cholest-7-en-14α-ol-3-one have been found to be potent inhibitors of the synthesis of digitonin-precipitable sterols in L cells in culture and to reduce the levels of HMG-CoA reductase activity in these cells.

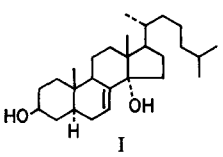
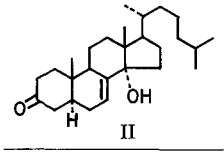
A number of oxygenated derivatives of cholesterol and of other sterols have been found to be potent inhibitors of sterol synthesis in animal cells in culture[2-6]. These inhibitors include sterols with oxygen functions at carbon atoms 6, 7, or 15 of the sterol nucleus as well as sterols with oxygen functions in the alkyl side chain[5]. A number of sterols with oxygen functions at C-15 or with a hydroxymethyl group at the 14α-position of the D ring of the sterol nucleus have been found to be particularly potent inhibitors of sterol synthesis[4,6-10]. We now wish to report the results of investigations of the possible inhibitory action on sterol synthesis of sterols with a hydroxyl group at the 14-position of ring D of the sterol nucleus. Specifically, we have studied the effects of 5α-cholest-7-en-3β,14α-diol (**I**) and 5α-cholest-7-en-14α-ol-3-one (**II**) on sterol synthesis and on the levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in L cells. The chemical syntheses of **I** and **II** have been described[11].

Mouse L cells (a subline of NCTC clone 929 mouse fibroblasts) were grown in serum-free medium as described previously[2,3]. The preparation of steroid-containing media and procedures for assaying the conversion of [1-^{14}C]-acetate into digitonin-precipitable sterols and fatty acids were as described previously[2,3]. The cultures were preincubated with the test compound for 4 h; then [1-^{14}C]-acetate was added at a concentration of 4 μmoles (4 μCi) per ml and the incubations were continued for 2 more h. To determine the effects of the sterols on HMG-CoA reductase levels of cultured L cells, the sterols were incubated with the cultures for 5 h prior to harvesting for the determination of HMG-CoA reductase activity by a modification[12] of the method of Brown, Dana, and Goldstein[13] using a higher concentration (80 μM) of RS-HMG-CoA and a 30-min incubation period. Studies of the rates of acetate metabolism to fatty acids were also made and indicated no significant effect of **I** and **II**. Minor variations in rates of fatty acid production in experimental flasks as well as in control flasks were considered to be due to technical error and to variation in the metabolic characteristics of individual cultures. In an effort to correct for the effects of these sources of variance upon estimates of inhibiting potency, concentrations required to inhibit sterol synthesis by 50% were estimated from plots of the ratio of [^{14}C] sterols to [^{14}C] fatty acids as a function of the concentration of the inhibitor[2].

The results presented in the table indicate that the 2 14α-hydroxy-Δ^7-sterols **I** and **II** are potent inhibitors of sterol synthesis and cause a reduction in the level of HMG-CoA reductase activity. The concentrations of the 14α-hydroxysterols required to cause 50% inhibition of sterol synthesis in the L cells were very similar to those required to cause a 50% reduction in the level of HMG-CoA reductase activity in the same cells.

These findings not only extend knowledge concerning structure-inhibitor relationships in the case of oxygenated sterols but also have possible important implications for the role of 14α-hydroxy-Δ^7-sterols in regulatory processes in other systems. Since the 14α-hydroxy-Δ^7-system is an essential structural feature of various ecdysones it is conceivable that these insect hormones (or 14α-hydroxy-Δ^7-sterol precursors of these hormones) may act by suppressing the level of HMG-CoA reductase in insects and, by virtue of the resulting control of mevalonate (or homomevalonate) formation, control the formation of juvenile hormones and other products of the metabolism of mevalonic acid in insects. It is important to note that the regulation of mevalonate formation and products derived therefrom by oxygenated sterols appears to be important in the control of replication of cells derived from higher organisms[5,14].

Inhibition of sterol synthesis and HMG-CoA reductase activity in L cells by 14α-hydroxy-Δ^7-sterols

Inhibitor	Concentration (μM) required for 50% inhibition	
	Sterol synthesis	HMG-CoA reductase
 I	7.0	5.0
 II	5.0	8.0

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2 A.A. Kandutsch and H.W. Chen, J. biol. Chem. *248*, 8408 (1973).
3 A.A. Kandutsch and H.W. Chen, J. biol. Chem. *249*, 6057 (1974).
4 G.J. Schroepfer, Jr, E.J. Parish, H.W. Chen and A.A. Kandutsch, J. biol. Chem. *252*, 8975 (1977).
5 A.A. Kandutsch, H.W. Chen and H.-J. Heininger, Science *201*, 498 (1978).
6 G.J. Schroepfer, Jr, R.A. Pascal, Jr, R. Shaw and A.A. Kandutsch, Biochem. biophys. Res. Commun. *83*, 1024 (1978).
7 G.J. Schroepfer, Jr, E.J. Parish and A.A. Kandutsch, J. Am. chem. Soc. *99*, 5494 (1977).
8 G.J. Schroepfer, Jr, D.L. Raulston and A.A. Kandutsch, Biochem. biophys. Res. Commun. *79*, 406 (1977).
9 G.J. Schroepfer, Jr, R.A. Pascal, Jr, and A.A. Kandutsch, Biochem. Pharmac. *28*, 249 (1979).
10 G.J. Schroepfer, Jr, E.J. Parish, M. Tsuda, D.L. Raulston and A.A. Kandutsch, J. Lipid Res. *20*, 994 (1979).
11 R.A. Pascal, Jr, and G.J. Schroepfer, Jr, J. Lipid Res. *21*, 118 (1980).
12 S.E. Saucier and A.A. Kandutsch, Biochim. biophys. Acta *527*, 541 (1979).
13 M.S. Brown, S.E. Dana and J.L. Goldstein, Proc. natl Acad. Sci., USA *70*, 2162 (1973).
14 H.W. Chen, H.-J. Heininger and A.A. Kandutsch, Proc. natl Acad. Sci., USA *72*, 1950 (1975).