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STEREOCHEMISTRY OF CHOLESTEROL HYDROXYLATION AT C-22 DURING PREGNENOLONE BIOSYNTHESIS

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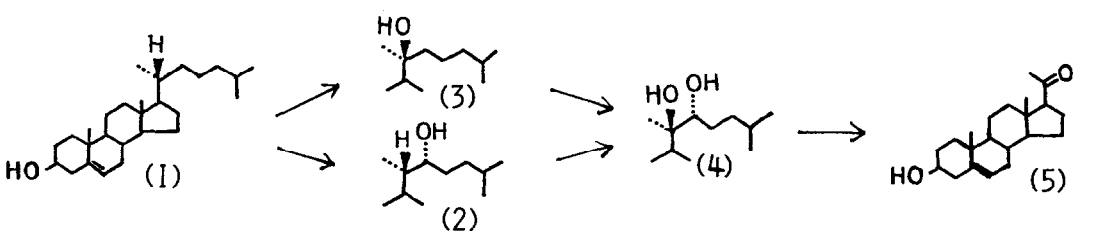
SUMMARY Cholesterol and 20-hydroxycholesterol labelled at C-22 stereospecifically by deuterium were incubated with the enzymes of bovine adrenocortical mitochondria. The results indicated that hydroxylation occurs with retention of the configuration of C-22 to give exclusively the 22R stereoisomers of 22-hydroxy- and/or 20,22-dihydroxycholesterol.

There is a great number of natural steroids containing oxygen function at C-22 position, e.g. ecdysones[1](insect moulting hormones), antheridiol[2](sex hormones of water mould) and withanolides[3](C_{28} steroidal lactones from Solanaceae, some of which have antitumor activity). The configurations at C-22 of these compounds are all 22R and the fact may be related to their characteristic biological activities. Furthermore, (22R)-22-hydroxycholesterol(2) and (20R,22R)-20,22-dihydroxycholesterol(4) have been postulated as the intermediates[4] of the enzymic conversion of cholesterol(1) into pregnenolone(5), which is the common precursor of all mammalian steroid hormones.



However, we recently showed[5] that not only the 22R isomer but the 22S compounds of 22-hydroxy- and 20,22-dihydroxycholesterols were efficiently converted to (5) on incubation with adrenocortical cytochrome P-450$_{scc}$. This raised the possibility of non-stereospecificity in the hydroxylation at C-22. In the present study, we have thus aimed to investigate the stereochemical course of 22-hydroxylation of (1) and (20S)-20-hydroxycholesterol(3) which is another possible precursor of(4).[5]

Cholesterol(10 μg) was incubated with the acetone powder[6](0.1 mg protein) of bovine adrenocortical mitochondria at 37^{o} for 10 min in the presence of NADP, glucose-6-phosphate and glucose-6-phosphate dehydrogenase. The products were extracted with CH_2Cl_2 and purified by t.l.c.(hexane-ethyl acetate, 2 : 1). The separated hydroxycholesterols were converted to their trimethylsilyl ethers and analyzed by GC-MS with an open tubular glass capillary column(SE-30, 30 m x 0.36 mm i.d., 290^{o}). Monitoring of the ions m/e 173 and m/e 461[5] revealed the peaks with the retention time of 8.9 min(2) and 12.3 min(4) respectively, without accompanying peaks of(22S)-22-hydroxycholesterol(r.t. = 8.6 min) and (20R,22S)-20,22-dihydroxycholesterol(r.t. = 12.8 min). These results indicate that the 22R stereoisomers are exclusively formed from cholesterol by the enzyme reaction.

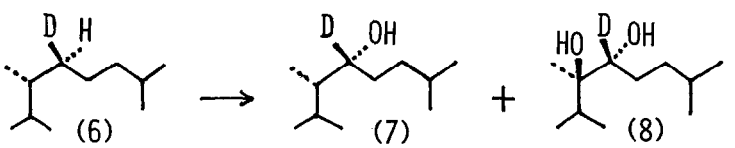
In order to examine whether the C-22 hydroxylation occurs with retention or inversion of the configuration of C-22, (22S)- (6) and (22R)-[22-^{2}H]-cholesterol (9) as well as (20S,22S)- (10) and (20S,22R)-[22-^{2}H]-20-hydroxycholesterols(11)[7] were chemically synthesized and then incubated with the enzyme. (22R)- and (22S)-22-Mesyloxycholesterol THP ethers were reduced with $NaB^{2}H_4$ in HMPA and the products were purified by $AgNO_3$-silica gel t.l.c. (hexane - benzene 2 : 1, 5 times) to remove the co-produced $\Delta^{22(23)}$-olefins. Acid hydrolysis of the purified products afforded (6) and (9). Compounds (10) and (11) were obtained by $LiAl^{2}H_4$ reduction of (20R,22R)- and (20R,22S)-20,22-epoxycholesterols.[8] [22-^{2}H]-22-Hydroxycholesterol and [22-^{2}H]-20,22-dihydroxycholesterol which were required for mass fragmentographic analysis of the incubation products, were synthesized as epimeric mixtures at C-22 by $NaB^{2}H_4$ reduction of 22-oxo- and 20-hydroxy-22-oxo-cholesterols,[9] respectively. Incubations of (6), (9), (10) and (11) were carried out as mentioned for (1). The trimethylsilyl ethers of the incutat-

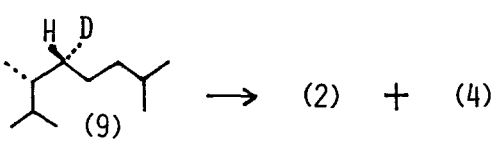
Table

Added substrate (^{2}H, mole %)	22-Hydroxycholesterol			20,22-Dihydroxycholesterol		
	Peak height of m/e174	Peak height of m/e173	No. of ^{2}H atoms per molecule*	Peak height of m/e290	Peak height of m/e289	No. of ^{2}H atoms per molecule*
(1)	1.5	7.3	0.04 ± 0.02	1.5	3.1	0.10 ± 0.05
(6) (83 %)	4.8	3.7	1.01*	4.1	1.4	0.71
(9) (88 %)	2.0	9.3	0.05	1.4	2.6	0.12
(3)	—	—	—	1.0	3.0	0.07
(10) (97 %)	—	—	—	2.4	0.5	1.00
(11) (95 %)	—	—	—	1.0	2.8	0.08
None	0.6	2.7	—	0.0	0.0	—
	Synthetic [22-^{2}H]-22-hydroxycholesterol (^{2}H, 80 mole %)			Synthetic [22-^{2}H]-20,22-dihydroxycholesterol (^{2}H, 81 mole %)		
	8.0	2.0	1.0	7.3	1.8	1.0

* Corrected number of ^{2}H atoms per molecule for example, 1.01 was calculated as follows :

$$\frac{\frac{4.8 - 0.6}{3.7 - 2.7} \times \frac{100}{83} \times 1.0}{\frac{8.0}{2.0} \times \frac{100}{80}}$$





ion products were analyzed on GC-MS(1.5 % OV-17, 280°) by the selected ion monitoring method. The ions m/e 173 and 174 comprising C-22 to C-27 of 22-hydroxycholesterol, and the ions m/e 289 and 290 comprising C-20 to C-27 of 20,22-dihydroxycholesterol were used. The results(Table) indicated that the 22-deuterium atoms of (9) and (11) were lost during the incubation, while those of (6) and (10) were retained. We thus concluded that the hydroxylation of (1) and (3) at C-22 preceeds by stereospecific displacement of the 22-pro-R hydrogen with retention of the configuration of C-22 to afford the 22R stereoisomers, (2) and/or (4), exclusively.

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