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STERIC CONSIDERATIONS REGARDING THE BIODEGRADATION OF CHOLESTEROL TO PREGNENOLONE.-EXCLUSION OF (22S)-22-HYDROXYCHOLESTEROL AND 22-KETOCHOLESTEROL AS INTERMEDIATES

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SUMMARY

(22S)-[22-$^{3}H_{1}$]-Cholesterol was incubated with an adrenocortical preparation and the isolated (22R)-[22-$^{3}H_{1}$]-22-hydroxycholesterol had a small loss of radioactivity, proving that direct replacement of the hydrogen from the now hydroxylated position occurred.

In addition [1-$^{3}H_{1}$]-4-methylpentanol was isolated, which also had incurred a relatively small loss of its specific activity, thereby excluding (20R)-3β,20-dihydroxycholest-5-en-22-one as an important metabolite in the degradation of cholesterol to pregnenolone by adrenal tissue.

INTRODUCTION

Upon incubation of cholesterol with adrenocortical mitochondrial acetone-dried powder preparations the sequential appearance of (22R)-22-hydroxy-cholesterol, (20R,22R)-20,22-dihydroxycholesterol, 3β-hydroxypregn-5-en-20-one (6) and 4-methylpentanol has been demonstrated[1]. It has also been shown[2,3] that these hydroxylations take place by utilization of molecular oxygen. The question whether the hydroxylation at C-22 takes place by either replacement of the H from the position to be hydroxylated or by a Walden type inversion with the removal of the H from the alternate position to the one to be hydroxylated has been answered by the sequence summarized in the next paragraph.

METHODS

The known (22R)-3β,22-dihydroxycholest-5-en-3β-benzoate (1)[4] was mesylated and the crude sulfonate was reduced with lithium aluminum tritide to give (22S)-[22-$^{3}H_{1}$]-cholesterol (3). The reduction product was purified on tlc and then recrystallized to a constant spec. activity. Incubation of this labeled cholesterol with an adrenocortical preparation yielded (22R)-[22-$^{3}H_{1}$]-22-hydroxycholesterol (4) which, after purification to constant spec. activity, had a spec. activity of ca. 90%[5] of the substrate, thereby proving substitution of the hydrogen by hydroxyl from the position to be hydroxylated. Hydroxylations in the cyclic moiety of steroids have been shown to occur by the same mechanism[6].

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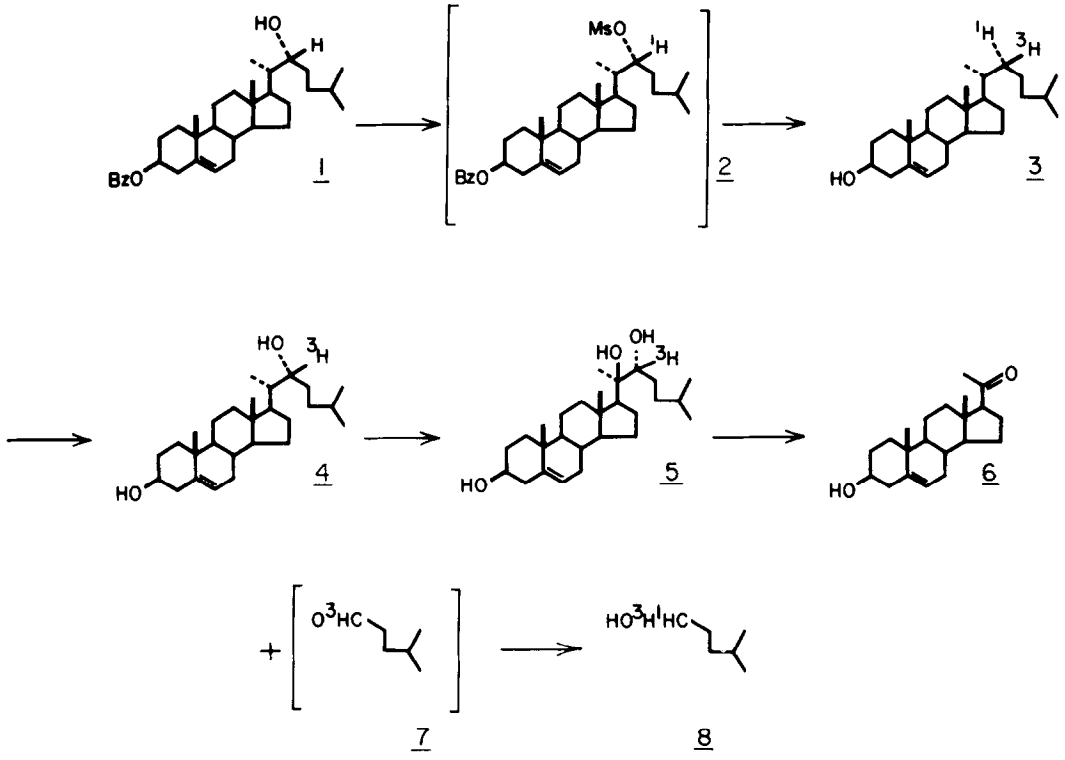
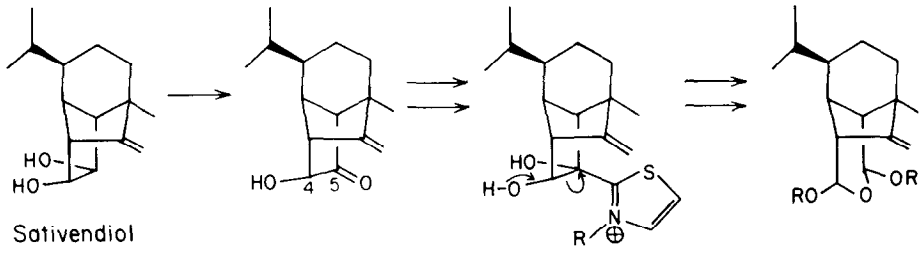


Although it has been demonstrated[7,8] that 3β-hydroxycholest-5-en-22-one is a considerably inferior substrate (in its ability to be metabolized by adrenomitochondrial preparations to 3β-hydroxypregn-5-en-20-one), compared to either cholesterol or (22R)-22-hydroxycholesterol, it has been suggested that, because of compartmentalizations and/or mixing, the 22-ketocholesterol could still be a metabolite. If the 22-ketone is indeed a metabolite, then one would expect to find pregnenolone and 4-methylpentanoic acid as products. However, Arigoni and Dorn[9] have convincingly proven that a non-oxidative reaction sequence, with e.g., thiaminpyrophosphate as cofactor, can yield a dioxo fission product. The case in point is the fermentation, by the fungus Helminthosporium, of a glycol (sativendiol) *via* a ketol to a di- aldehyde. That such a mechanism cannot pertain to the sidechain cleavage of cholesterol was shown in the following fashion:

In addition to (22R)-[22-$^3H_1$]-22-hydroxycholesterol, [1-$^3H_1$]-4-methylpentanol (8) was also isolated. This alcohol derives from the *in situ* reduction of the originally formed 4-methylpentanol (7), as already shown previously[10]. The isolated isohexanol 8 suffered a reduction of its specific activity by 22% thereby excluding the presence of the ketol, (20R)-3β,20-dihydroxycholest-5-en-22-one, as a major link in the metabolic degradation of cholesterol to pregnenolone with adrenal mitochondrial preparations.

EXPERIMENTAL

(22S)-[22-$^3H_1$]-Cholesterol (3) from 1.-The solution of 12 mg of (22R)-3β,22-dihydroxycholest-5-en 3β-benzoate[4] in 2 ml pyridine and 0.2 ml methane



Sativendiol

sulfonyl chloride was left at 25° for 25 hr. Then it was poured on ice and the material extracted with benzene. The benzene extract was washed with water and with sodium hydrogen carbonate solution, the solution dried over sodium sulfate and the solvent evaporated *in vacuo*. The crude residue was reduced at once with a solution of 5 mCi of [^{3}H]-lithium aluminum hydride (50 mCi/mmol) in tetrahydrofuran (5 h/25° followed by 15 h reflux). The usual work-up gave, after purification on tlc, followed by a partition column (heptane:80% methanol) 7 mg of pure 3 (90 μCi/mmol).

Incubation and Isolation.-The bovine adrenocortical mitochondrial acetone-dried powder preparations were obtained by methods previously described[11].

Incubations of 3 under O_2 and work-up were performed as described previously[3]. After 30 min incubation methylene chloride was added and the material extracted with the same solvent. Then the solvent was evaporated to a small volume and the desired metabolites isolated.

(22R)-[22-$^3H_1$]-22-Hydroxycholesterol (4).-This material was purified by chromatography on Celite partition chromatography columns, using systems already described[11]. The specific activity was determined as described by Rosenthal *et al.*[12].

[1-$^3H_1$]-4-Methylpentanol.-This product was isolated from the methylene chloride extract by gas-liquid chromatography as described previously[10]. The specific activity was determined by mass spectroscopy.

RESULTS

The total (22R)-22-hydroxycholesterol isolated amounted to 5 μg with a specific activity of 4.4 mCi/mmol, which represents a loss of approximately 10% of the 22S-hydrogen.

A total of 4 μg of [1-$^{3}H_{1}$]-4-methylpentanol with a specific activity of 3.9 mCi/mmol was isolated. In this case the apparent loss of ca. 12% (compared to the specific activity of its isolated precursor) is within the experimental errors.

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5. Since the starting material 1 was analytically pure, the 10% loss could be derived from the absence of stereospecificity of the displacement of the mesyloxy group by hydride or, less likely, from the hydroxylation step.

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