NONENZYMIC CONVERSION OF 17α-HYDROXYPREGNENOLONE INTO DEHYDROEPIANDROSTERONE

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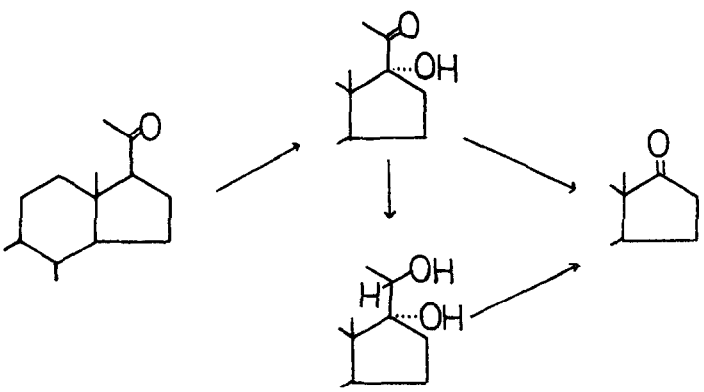
SUMMARY

The facile and quantitative conversion of 17α-hydroxypregnenolone into dehydroepiandrosterone by oxidation with leadtetraacetate in an aprotic medium is described. Thus, a model for the non-enzymic degradation of C_{21} to C_{19} steroids is presented involving a radical, rather than an ionic mechanism.

INTRODUCTION

The major metabolic pathway leading from cholesterol into the androstenes is generally recognized to proceed at some stage via pregnenolone as an obligatory intermediate (1-4). We have previously shown (5) that in certain microbial systems the C_2-side chain of progesterone is cleaved by a Baeyer-Villiger type enzymic oxidation to yield directly a 17β-acetoxy derivative. In mammalian systems (6-10), pregnenolone must undergo further transformation at C-17 and possibly C-20, prior to side chain scission, as shown in the following simplified scheme.



The key step in the above sequence is the formation of a 17α-hydroxy derivative. The α-ketol side chain can subsequently be oxidized directly

to the 17-ketone, or alternatively, via a C-17,C-20 glycol intermediate. All of the above reactions are assumed to be enzymatic and, because they occur in biological and therefore polar media, to be ionic in nature.

It is the purpose of the present study to demonstrate the easy and quantitative conversion of 3β,17α-dihydroxy-5-pregnen-20-one (17α-hydroxy-pregnenolone) into 3β-hydroxy-5-androsten-17-one (DHEA) by a non-enzymic, non-ionic reaction mechanism.

MATERIALS AND METHODS

17α-Hydroxypregnenolone and DHEA were purchased from Steraloids, Inc., Pawling, N.J. Leadtetraacetate (Fisher Scientific Co.) was recrystallized several times from glacial acetic acid and stored under anhydrous benzene, prior to use. Determination of melting points, infrared spectroscopy and thin-layer chromatography (TLC) was performed as described earlier (11). Gas liquid chromatography (GLC) was conducted with a F & M 402, high efficiency instrument, equipped with a hydrogen flame ionization detector under the following parameters: 180 x 0.3 cm glass column, packed with 3% OV-1 on 100-120 mesh Gaschrom Q (Applied Science Labs., State College, Pa.), injection port temperature 275°, oven 235°, detector 285°, He carrier gas flow 33 ml/min at 40 p.s.i., attenuation 10 x 16.

Preparation of 3β-Acetoxy-5-androsten-17-one (DHEA-acetate)

A solution of 400 mg of 17α-hydroxypregnenolone-3β-acetate (prepared by selective acetylation of 17α-hydroxypregnenolone) was dissolved in 40 ml of freshly distilled anhydrous benzene. Under nitrogen atmosphere was then introduced 200 mg of $CaCO_3$ and 800 mg of $Pb(OAc)_4$. The heterogenous mixture was refluxed under nitrogen by heating with a 500 Watt lamp. After 16 hours, the yellow coloured reaction mixture was cooled to room temperature and diluted with 200 ml of ethylacetate. The organic extract was filtered and washed successively with 5N H_2SO_4, 1N $Na_2S_2O_3$ and H_2O. After drying over anhydrous Na_2SO_4 and evaporation of the solvent there was obtained 440 mg of a yellow viscous oil. TLC of the crude product gave a single spot with R_f 0.70 (chloroform/methanol 9:1) and 0.63 (ethylacetate), and GLC a single peak with retention time of 0.57 (relative to 5α-cholestane), which gave peak enhancement upon coinjection of the same amount of sample with authentic DHEA-acetate. Recrystallization of the yellow oil from acetone/hexane gave 392 mg of colourless crystalls, m.p. 168-171° (a mixture melting point with authentic DHEA-acetate gave no depression). ν_{max}^{KBr}: 1728 cm^{-1} (acetate) and 1710 cm^{-1} (5-membered ring ketone).

RESULTS AND DISCUSSION

Although various corticosteroids with a Δ^4-3-ketone moiety have been subjected to oxidation with leadtetraacetate in polar media (12), the

specific interaction of 17α-hydroxypregnenolone with this reagent has not been investigated previously. On the other hand, the direct enzymatic conversion of cholesterol into DHEA in calf testis has been reported (13), thus suggesting a new pathway of the degradation of C_{27} to C_{19} steroids in mammals, bypassing pregnenolone. Recently, Lieberman. et al. (14) described the synthesis of pregnenolone by leadtetraacetate oxidation of 20α-hydroxycholesterol. It is of interest to note that of the other known properties of leadtetraacetate as an acetoxylating and methylating reagent (15), none of these reactions were observed with the above sterol and also with 17α-hydroxypregnenolone. The slightly acidic hydrogen of the OH-group suffices to direct a homolytic cleavage in aprotic media, leading in the case of 17α-hydroxypregnenolone to the smooth and quantitative formation of DHEA. The latter compound has recently (16) also been recognized among the various light induced autoxidation products of cholesterol, no doubt generated in a similar way by a radical reaction mechanism. Thus, the driving force of the degradation reaction must be sought in the formation of an oxy- or peroxy radical at C-17, which then stabilizes by expulsion of the acetyl side chain. In view of the facile generation of such a tertiairy oxygen radical, it may be reasonable to postulate that not all of the DHEA, found in biological material is necessarily of enzymatic origin, particularly, since none of the C-17(20)-lyases has been adequately purified and characterized thus far (17).

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