17-KETOSTEROIDS VIA A BASE INDUCED CLEAVAGE OF C-17-DIHYDROXY ACETONE SIDE CHAINS

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ABSTRACT

A new, base-catalyzed cleavage of C_{21}-steroids to give 17-keto-steroids is described. This reaction is specific for those steroids containing the C-17-dihydroxy acetone side chain. In the presence of oxygen, these same reaction conditions readily degrade corticosterone to the 17β-carboxylic acid.

INTRODUCTION

The transformation of C_{21}-steroids to C_{19}-17-ketosteroids is of importance in biosynthetic pathways and in steroid catabolism in normal and disease states. In particular, 17α,20-C_{21}-desmolase activity is involved in testosterone biosynthesis and cortisol degradation [1,2]. From these and other studies it has been established that desmolase substrates must contain a 17α-hydroxyl group and a C-20 ketone. As far as we can determine, all of the laboratory methods for accomplishing desmolase-type reactions involve oxidative cleavages [3-6]. In this paper we describe new, relatively mild, possibly non-oxidative conditions for the cleavage of C-17-dihydroxy acetone side chains to give C_{19}-17-ketosteroids. A relatively mild, oxidative cleavage of corticosterone to the 17β-acid by oxygen is also reported.

MATERIALS AND METHODS

Materials: Steroids 1, 4, 5, 7-9 were purchased from Sigma, 3 was obtained from Mann Research Laboratories and 2 was in the Steroid Reference Collection at NIH. The preparation [7] and reactivity [8] of cortisol-21-methanesulfonate (12) have been described elsewhere. Silica gel (GF) and neutral alumina (GF) TLC plates were purchased from Analtech. Hexanes (b.p. 68°-72°C) was obtained from Aldrich.

Instrumentation: Melting points were determined on a Fisher-Johns hot stage melting point apparatus and are uncorrected. A Perkin-Elmer 237B grating infrared spectrophotometer was used to record IR spectra. NMR spectra were acquired at 60 (Varian A-60) or 100 MHz (Varian HA-100 spectrometer). Low-resolution mass spectra were obtained on an Hitachi Perkin-Elmer RMU-6E mass spectrometer (electron impact [EI] mode) by Bill Landis or on a Finnigan 1015D mass spectrometer (chemical ionization [CI] mode) by Noel Whittaker. Analyses were performed by the Micro-analytical Section of the Laboratory of Chemistry.

11β-Hydroxy-4-androstene-3,17-dione (2): A solution of 0.25 M KOH in EtOH (16 ml) was added to 207 mg cortisol (1, 0.57 mmole) and stirred at room temperature for 100 min. The mixture was concentrated, extracted with CH_2Cl_2, concentrated and purified by preparative TLC on neutral alumina (2:1 benzene:ethyl acetate) to afford a 24% yield of crystalline product (m.p. = 197°-198°C). One recrystallization from acetone:hexanes yielded the analytical sample (m.p = 199°-200°C; lit. = 198°-200°C [9]). Ir (Nujol) 3534, 1733 and 1645 cm^{-1}. EI mass spectrum m/e (rel. intensities) 302 (P^+, 86), 284 ($P-H_2O$, 19), 269 (23), 189 (42), 163 (100), 124 (76), 123 (82).

"Oxygen-free" 0.25M KOH in EtOH was prepared with EtOH which had been refluxed for 2 hrs while bubbling argon through a gas dispersion tube into the solvent and then cooling the solvent under a positive pressure of argon. The KOH in an argon flushed flask was then dissolved in the "oxygen-free" EtOH and stored under argon. The reaction with cortisol (1) under "oxygen-free" conditions involved adding the "oxygen-free" KOH/EtOH to an argon flushed flask of cortisol and keeping a positive pressure of argon in the flask. For reactions with oxygen, just the oxygen in the ethanol and any oxygen picked up by the KOH/EtOH solution was used - no oxygen was added.

Identification of the 17α-hydroxy-17β-acid (11) was on the basis of TLC (R_f=0.68 with 3:1:1 CH_2Cl_2:benzene:CH_3OH on silica gel [10]) and the observation of the parent peak in the CI mass spectrum (MH^+=349; ionizing gas = ammonia).

4-Androstene-3,17-dione (4): A solution of 0.25 M KOH in EtOH (20.1 ml) was added to 249 mg of cortexolone (0.72 mmole) and stirred at room temperature for 130 min. The mixture was concentrated, extracted with CH_2Cl_2, concentrated and purified by preparative TLC on neutral alumina (4:1 benzene:ethyl acetate) to afford a 21% yield of a crystalline

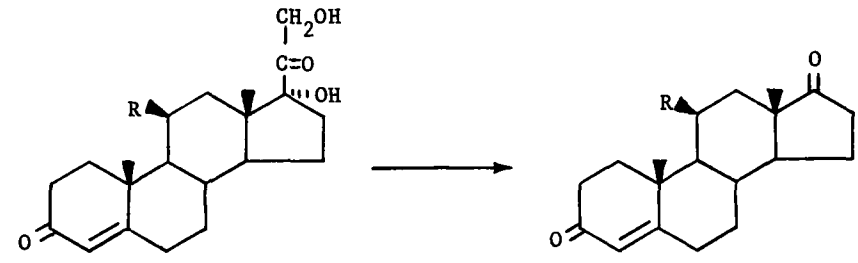
product (m.p. = 141°-143°C). This compound is dimorphous and another preparation gave, after recrystallization from hexanes, material melting at 171.5°-173°C (lit = 142°-144°C and 173°-174°C [11]). Ir (Nujol) 1729 and 1664 cm^{-1}. EI mass spectrum m/e (rel. intensities) 286 (P^+, 80), 271 (P-Me, 6), 258 (P-CO, 6), 244 (54), 164 (100), 148 (57), 124 (77).

11β-Hydroxy-1,4-androstadiene-3,17-dione (6): A solution of 0.25 M KOH in EtOH (19.5 ml) was added to 251 mg of prednisolone (5, 0.70 mmoles) and stirred at room temperature for 90 min. The mixture was concentrated, extracted with CH_2Cl_2, concentrated and purified by preparative TLC on neutral alumina (1:1 benzene:ethyl acetate) to afford a 23% yield of crystalline product (m.p. = 183°-184°C). Recrystallization from acetone gave analytically pure material as a 1:1 solvate with acetone (solid melted at 90°-100°C with evolution of gas, recrystallized at ∿130°C and remelted at 186.5°-189°C; lit = 185°-186°C [12]). Ir (Nujol) 3448, 1727 and 1650 cm^{-1}. EI mass spectrum m/e (rel. intensities) at low temperature (<200°) - 43 was the base peak with a large peak at 58. At high temperature (>200°) 300 (P^+, 12), 282 ($P-H_2O$, 2.5), 267 (3.3), 249 (4), 121 (100-off scale), 91 (44), 58 (9). Anal. calc'd for $C_{19}H_{24}O_3.C_3H_6O$ (mol. wt. 300.41 + 58.08): C, 73.71; H, 8.43. Observed: C, 73.57; H, 8.27.

11β-Hydroxy-3-oxo-4-androstene-17β-carboxylic acid (10): A solution of 0.25M KOH in EtOH (11.2 ml) was added to 138 mg of corticosterone (7, 0.40 mmoles) and stirred at room temperature for 3 hrs before being adjusted to pH∿3 with 0.5M HCl. The EtOH was removed under reduced pressure and the aqueous residue was extracted with EtOAc. After being dried over $MgSO_4$, the EtOAc was removed under reduced pressure and the residue (in 9:1 $CHCl_3$:MeOH) was purified by preparative TLC (2x2000μ silica gel plates developed with 12% MeOH in $CHCl_3$) to give 65 mg (49% yield) of an oil which soon crystallized (dec. 251-258°C). Recrystallization from acetone gave colorless rosettes (dec. 259-268°C) (lit. m.p. = 260-266 [13], 257-260 [14]). Ir (Nujol) 3395, 3185 (br), 1722 and 1650 cm^{-1}. EI mass spectrum m/e (rel. intensities) 332 (P^+, 33), 314 ($P-H_2O$, 18), 208 (16), 163 (100), 124 (71), 123 (60).

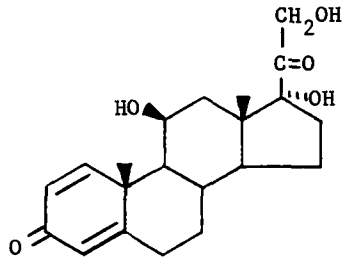
RESULTS AND DISCUSSION

During the preparation of various derivatives of cortisol [7], we discovered that cortisol (1) is readily degraded by ethanolic KOH solutions to give low, but synthetically useful yields of 11β-hydroxy-4-androstene-3,17-dione (2). Further studies indicated that the reaction also occurs with cortexolone (17,21-dihydroxy-4-pregnene-3,20-dione; 3) and prednisolone (11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione; 5) to give the respective 17-ketosteroids 4 and 6 in yields



1: R = OH
3: R = H

2: R = OH
4: R = H



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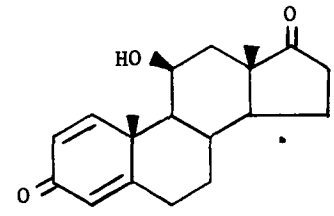

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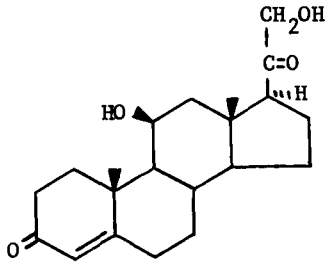
equal to that of 2 (i.e., 20-30%). Varying the temperature (from 0°C to r.t.) and the concentration of KOH (from 0.04M to 0.75M) did not significantly affect the course or yield of the reaction. Lower temperatures and base concentrations did decrease the rate of reaction, as was expected. The conditions we usually employed were 0.25M KOH in EtOH at room temperature. Sodium methoxide can replace KOH but a primary amine base (i.e., 0.2M n-propyl amine in EtOH) gave almost no reaction with cortisol, even after 16 hrs at room temperature.

To further define the structural requirements of this reaction we examined corticosterone (7), 17-hydroxyprogesterone (8) and progesterone (9). Very little if any, 3,17-dione could be detected (by comparison with authentic dione on TLC) in reactions of 7-9 using varying



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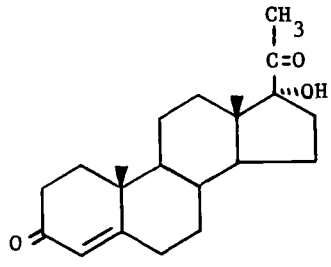

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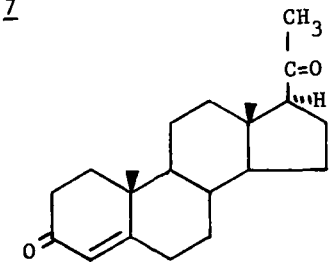

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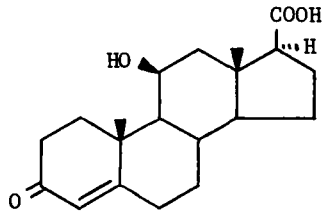

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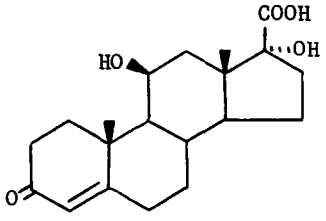
conditions - 0.04M KOH/EtOH at 0°C to 0.75M KOH/EtOH at room temperature for 22 hrs. Corticosterone (7) was readily degraded (about 3 hrs in 0.04M KOH/EtOH at 0°C) to the acid 10 but no dione was observed; 8 and 9 were relatively stable even in 0.75M KOH/EtOH at room temperature for 22 hrs. Thus this base-induced cleavage reaction to give 17-ketosteroids appears to be specific for the 17,21-diol-20-one structure.

In order to determine if the above observations involve an oxidative cleavage mechanism due to dissolved oxygen and/or oxygen absorbed from the air, we compared the course and rate of reactions run under the usual conditions but with and "without" oxygen (see Materials and Methods). The special reaction of corticosterone (7) to yield the acid 10 was quite sensitive to oxygen. In the "absence" of oxygen, the conversion of corticosterone (7) to acid 10 was only about 25% complete

after 3 hrs (by TLC analysis) when the reaction containing oxygen was finished. It is even possible that the observed reaction of corticosterone under supposedly oxygen-free conditions was due to adventitious oxygen.

Oxygen was noted to accelerate the rate of disappearance of cortisol (1) by a factor of about 2. However, no noticeable effect of oxygen was observed by TLC on the amount of ketone 2 that is formed from cortisol. The 17α-hydroxy-17β acid 11 is also formed in the reaction of cortisol; but, the final composition of reaction products from cortisol with and without oxygen appeared to be almost identical by TLC [15]. From this data, we cannot conclusively define the role of oxygen in the degradation of cortisol to the ketone 2. It appears that oxygen is not required but we can not rule out an effect of small amounts of oxygen. Nevertheless, regardless of the absolute effect of oxygen, this base induced cleavage



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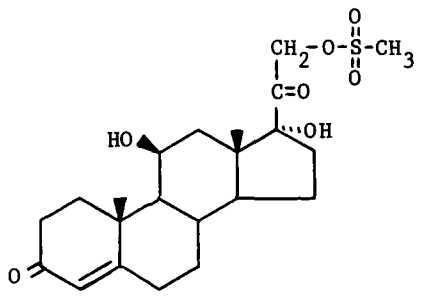

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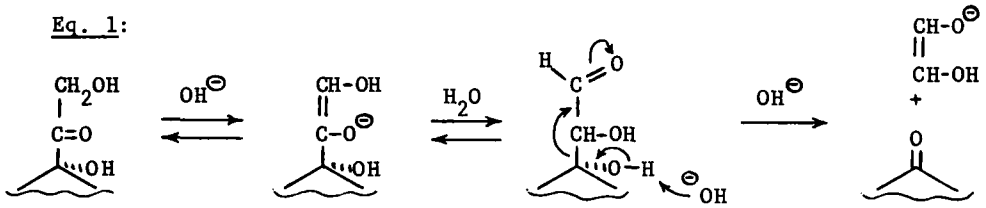
of C_{21}-steroids to 17-ketosteroids is much milder than the existing methods for this transformation [3-6].

Without knowing whether oxygen is required for the degradation of C-17-dihydroxy acetone substituted steroids, it is difficult to formulate a reaction mechanism. While some 17β-acid 11 is formed from cortisol

(1), the acid is not an intermediate because it is not degraded when the reaction (in 0.25M KOH/EtOH at room temperature) is continued for 17 hrs. Other mechanisms would predict that a more electronegative substituent at C-21 would accelerate the formation of 17-ketosteroid. In fact, when cortisol-21-methanesulfonate (12) was exposed to "oxygen-free" 0.25M KOH/EtOH at room temperature, no acid 11 was detected by TLC and only a small amount of ketone 2 (presumably formed from cortisol,

Eq. 1:



13 14

which is one of the products of the reaction of 12) was formed. Thus we feel that the mechanism of Eq. 1 is consistent with the observations and is worthy of further consideration. The final step in this scheme is a dealdolization, formally similar to the reverse reaction of aldolase [16]. Primary amines have been found to be very effective catalysts in the dealdolization of aqueous solutions of diacetone alcohol [17]. Since cortisol (1) was completely unreactive in the presence of n-propyl amine or n-propyl amine/n-propyl amine hydrochloride, tautomerization from the C-20-ketone (13) to the C-21-aldehyde (14) would have to be rate limiting if this mechanism is operative.

CONCLUSIONS

The base sensitivity of 17,21-dihydroxy-20-ketosteroids and one 21-hydroxy-20-ketosteroid (7) has been determined and investigated. In particular, corticosterone is easily degraded to the 17β-acid in a reaction that is at least accelerated by, and may require, oxygen. The 17,21-dihydroxy-20-ketosteroids are converted to 17-ketosteroids by a unique cleavage reaction using non-oxidizing reagents but which may require small amounts of oxygen. The yields are low but acceptable when considering the simplicity of the reaction and the fact that very few functional groups would need to be protected from these relatively mild conditions. This sequence should be particularly useful for molecules which contain groups that are unstable under the usual oxidative cleavage conditions [3-6], e.g., thio ethers, 1,2-diols and at least some α-hydroxy ketones, such as 8. Finally, this reaction to give 17-ketosteroids should be of some diagnostic utility since it is specific for the C-17-dihydroxy acetone functional group.

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FOOTNOTES

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NOTE ADDED IN PROOF

After this paper was accepted for publication, we discovered two recent patents describing the cleavage by alcoholic K_2CO_3 and added oxygen of C-17-dihydroxy acetone side chains to give 17β-carboxylic acids (Chem Abs., 93, 150494y, 168493g [1980]), e.g., 1→11 and 7→10.

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COMPOUND INDEX

1. Cortisol
2. 11β-Hydroxy-4-androstene-3,17-dione
3. 17,21-Dihydroxy-4-pregnene-3,20-dione
4. 4-Androstene-3,17-dione
5. 11β,17,21-Trihydroxy-1,4-pregnadiene-3,20-dione
6. 11β-Hydroxy-1,4-androstadiene-3,17-dione
7. Corticosterone
8. 17-Hydroxyprogesterone
9. Progesterone
10. 11β-Hydroxy-3-oxo-4-androstene-17β-carboxylic acid
11. 11β,17-Dihydroxy-3-oxo-4-androstene-17β-carboxylic acid
12. Cortisol-21-methanesulfonate