Tetrahedron, 1961, Vol. 16, pp. 271 to 282. Pergamon Press Ltd. Printed in Northern Ireland

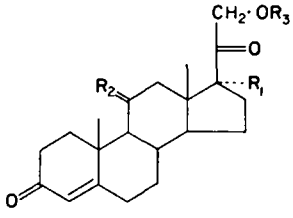
REACTION OF LEAD TETRAACETATE WITH STEROIDAL α,α'-DIHYDROXY KETONES IN ALCOHOLIC MEDIA[1]

E. CASPI, W. SCHMID[2] and T. A. WITTSTRUCK
Worcester Foundation for Experimental Biology
Shrewsbury, Mass.

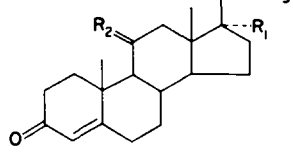
(*Received* 17 *June* 1961)

Abstract—The main product of the reaction of steroids with a dihydroxy acetone moiety and lead tetraacetate in the presence of methanol or ethanol was the corresponding etio-ester. The 17-keto-steroids were obtained as minor products. With higher alcohols in addition to 17-ketosteroids the etio-esters and 17α-formoxy-etio-esters were isolated. Partial acetylation of the C_{21} hydroxyl group was also observed. With tertiary-butyl alcohol, C-21 acetylation and cleavage to 17-ketosteroids occurred.

FOLLOWING the introduction of lead tetraacetate as reagent[3] for the cleavage of the carbon-carbon bond of α-glycols the usefulness of the method was almost immediately realized and thoroughly explored.[4] The scope of the reaction was soon extended and among other uses it found application to the cleavage of α-ketols, α-diketones and α-keto acids.[5] Evidence was presented that cleavage of the α-keto compounds proceeds via pseudo glycol formation and requires the presence of water or alcohols.[5] The application of the lead tetraacetate reagent to dihydroxy acetone systems in alcohol-containing media is the subject of the study to be described. Steroids with a dihydroxy acetone moiety at C-17 seemed to be particularly suitable model substrates for the investigation and were used throughout. The observed reactions, which were not anticipated, are presented in detail.



1



2

[1] This investigation was supported by grants from the American Cancer Society Inc. P-102 and P-103. A portion of the results was presented at the First International Congress of Endocrinology in Copenhagen July 1960; Abstracts of Papers p. 1025.
[2] Post doctoral fellow 1958–1960, present address Sandoz A.G., Basel, Switzerland.
[3] R. Criegee, *Ber. Dtsch. Chem. Ges.* **64**, 260 (1931).
[4a] R. Criegee, *Angew. Chem.* **70**, 173 (1958); [b] R. Criegee, L. Kraft and B. Rank, *Liebigs Ann.* **507**, 159 (1933); [c] H. Gilman, (Editor), *Organic Chemistry* Vol. 4, p. 1189. John Wiley, New York (1953); [d] M. S. Newman, (Editor), *Steric Effects in Organic Chemistry* p. 378. John Wiley, New York (1956).
[5] E. Baer, *J. Amer. Chem. Soc.* **62**, 1597 (1940); *Ibid* **64**, 416 (1942).

When a solution of cortisone (1a) in a mixture of benzene-methanol (1 : 1) was treated with an excess of lead tetraacetate and left for 16 hrs at room temperature the recovered neutral steroids contained about 10–15% of adrenosterone and gave (2a) in high yield. The pure (2a) m.p. 212–215° absorbed ultraviolet light, λ_{max}^{MeOH} 238 mμ (ε 15,500), and its infrared spectrum showed bands at 3400, 1740, 1710, 1665, 1620,

TABLE 1. STEROID ESTERS AND CERTAIN OF THEIR PHYSICAL CONSTANTS

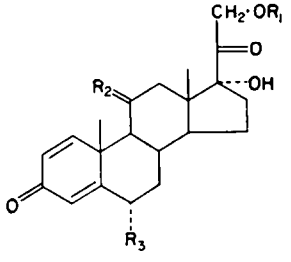
Ester	m.p.	λ_{max}^{MeOH} mμ	ε	Found		Calc. for		
				C	H	Element comp.	C	H
2a	212–215°	238	15,500	69·88	7·72	$C_{21}H_{28}O_5$	69·97	7·83
2e	192–194°	241	16,500	69·30	8·23	$C_{21}H_{30}O_5$	69·58	8·34
2f	182–183°	237	15,200	70·66	7·86	$C_{22}H_{30}O_5$	70·56	8·08
2h	195–198°	238	16,500	68·22	7·21	$C_{22}H_{28}O_5$	68·02	7·27
4a	206–209°	240	15,000	70·26	7·43	$C_{21}H_{26}O_5$	70·37	7·31
4b	249–254°	239	15,000	69·52	6·87	$C_{20}H_{24}O_5$	69·75	7·02
4c	194–197°	243	15,400	70·70	7·96	$C_{22}H_{30}O_5$	70·56	8·08
5a	162–164°	242	16,700	70·85	8·91	$C_{23}H_{34}O_5$	70·74	8·78
5b	186–188°	240	17,000	68·76	8·04	$C_{24}H_{34}O_6$	68·87	8·19
5c	165–167°	239	15,800	71·48	8·09	$C_{23}H_{32}O_5$	71·10	8·30
5d	111–113°	238	15,500	69·18	7·84	$C_{24}H_{32}O_6$	69·21	7·74
6a	126–130°	242	17,100	71·18	8·95	$C_{24}H_{36}O_5$	71·25	8·97
6b	124–128°	241	16,100	69·81	8·50	$C_{25}H_{36}O_6$	69·42	8·39
6c	205–207°	242	17,000	68·90	8·20	$C_{24}H_{34}O_6$	68·87	8·19
6d	156–158°	238	16,300	69·39	7·82	$C_{24}H_{32}O_6$	69·21	7·74

1270, 1220 cm^{-1}. The product did not react with blue tetrazolium and results of elemental analysis were best interpreted for a $C_{21}H_{28\pm2}O_5$ compound but a $C_{20}H_{24}O_5$ composition, although less satisfactory, could be considered. That the compound was not 2, 6 or 16 acetoxy adrenosterone, resulting from acetylation of the methylene group "α" to a ketone[6] or to a double bond[7] was evident from the presence of the hydroxyl

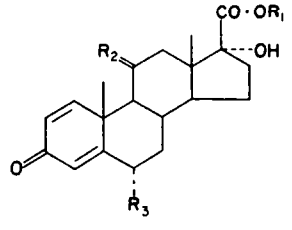
[6] E. Seebeck and T. Reichstein, *Helv. Chim. Acta* **27**, 948 (1944); G. Erhart, H. Ruschig and W. Aumüller, *Angew. Chem.* **52**, 363 (1939); T. Reichstein and C. Montigel, *Helv. Chim. Acta* **22**, 1212 (1939) F. Sondheimer, S. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *J. Amer. Chem. Soc.* **75**, 4712 (1953); R. L. Clarke, K. Dobriner, A. Mooradian, C. M. Martini, *Ibid.* **77**, 661 (1955); J. Herran, G. Rosenkranz and F. Sondheimer, *Ibid.* **76**, 5531 (1954); K. Yamakawa, *J. Org. Chem.* **24**, 897 (1959); M. Yanagita and K. Yamakawa, *Ibid.* **24**, 903 (1959).

[7] R. Criegee, *Liebigs Ann.* **481**, 263 (1930). H. Meerwein, *Ber. Dtsch. Chem. Ges.* **77**, 227 (1944).

band in the infrared spectrum. This was corroborated by the recovery of almost all of the starting material upon treatment of the product for 16 hr at room temperature with aqueous methanolic potassium bicarbonate[8,9] or boiling for 1 hr with aqueous methanolic sodium carbonate.[8] The negative reaction observed with blue tetrazolium[10] and the presence of a hydroxyl group, stable to chromium trioxide in pyridine,[11,12] presumably the C-17α hydroxyl, narrowed down the area of possible changes to carbons 20 and 21 of cortisone (1a). Results of elemental analysis indicated that the changes occurred without loss of oxygen, suggesting an oxidative cleavage of the C-20-21 bond and the concomitant formation of a neutral product probably an ester. That the substance was not a $C_{20}H_{24}O_5$ lactone formed by an attack of cationic oxygen[13] and extending e.g. from C-20 to C-18, was evident from the experiments described below and the already mentioned stability to both potassium bicarbonate and to sodium carbonate. The assigned methyl ester structure was proven by the saponification of (2a) in boiling aqueous methanolic sodium hydroxide to the etio acid[14] (2b) m.p. 270–273° and reesterification with diazomethane to (2a). The structure (2a) was also confirmed by an alternate route, whereby the ester was first saponified, then the excess sodium hydroxide neutralized, and the dried salt treated with acetyl chloride in benzene to yield 17-acetoxy-4-etiocholenyl-3,11-dione-acetyl anhydride (2c). The anhydride (2c) was sensitive to moisture and was hydrolyzed in aqueous acetone without purification, to the 17-acetoxy acid (2d) m.p. 136–138°. The acetoxy acid (2d) on treatment with sodium hydroxide gave the acid (2d) and this with diazomethane gave the ester (2a).



3



4

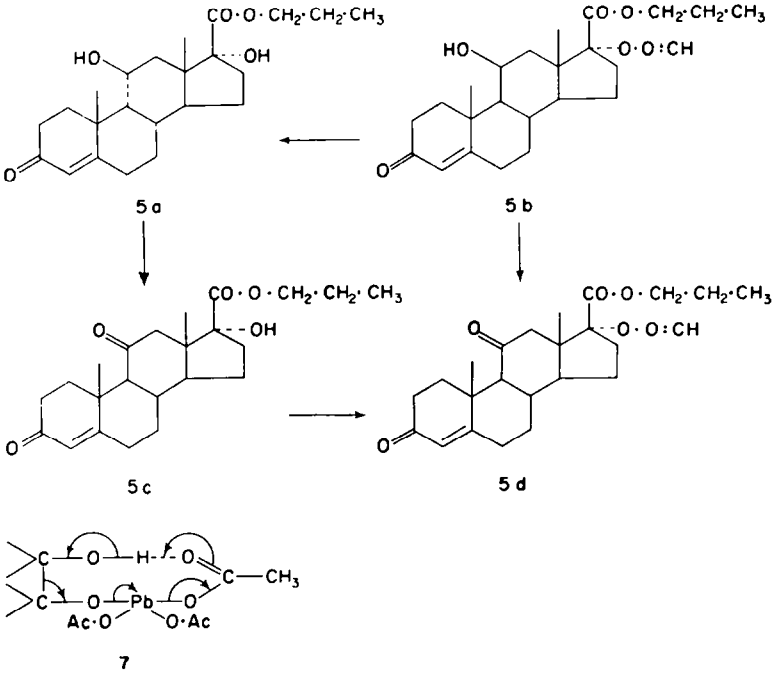
Several methyl and ethyl esters were prepared by this method, and are listed in Table 1 together with certain of their analytical and physical constants. With the low boiling alcohols, at the termination of the reaction, the volatile components were removed under reduced pressure at room temperature, and the redissolved residues were washed appropriately. When benzene was omitted from the reaction mixture essentially the same yield of ester was obtained, indicating that benzene, although it

[8] T. Reichstein and J. von Euv, *Helv. Chim. Acta* **21**, 1182 (1938).
[9] E. Caspi, *J. Org. Chem.* **24**, 669 (1959).
[10] E. Caspi, *J. Org. Chem.* **21**, 729 (1956). Ch. Chen and H. E. Tewell, *Fed. Proc.* **10**, 377 (1951).
[11] G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Amer. Chem. Soc.* **75**, 422 (1953).
[12] A. Vermuelen and E. Caspi, *J. Biol. Chem.* **234**, 2295 (1959).
[13] G. Cainelli, M. Lj. Mihailovic, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **42**, 1124 (1959); E. J. Corey and R. W. White, *J. Amer. Chem. Soc.* **80**, 6686 (1958).
[14a] J. von Euv and T. Reichstein, *Helv. Chim. Acta* **25**, 988 (1942); S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. von Euv, O. Schindler and T. Reichstein, *Ibid.* **37**, 1200 (1954); [b] E. Caspi, F. Ungar and R. I. Dorfman, *J. Org. Chem.* **22**, 326 (1957).

might increase the rate of reaction,[15] does not alter its course. Addition of water seemed to inhibit the esterification considerably as shown by the increase of the yield of the C_{19} steroid.

With higher alcohols, certain interesting side reactions were observed. Because of the higher boiling point of the homologous alcohols (C_3, C_4) it was impractical to attempt to remove these alcohols at room temperature in a manner described for methanol and ethanol. Accordingly, the processing of the reaction mixture was modified in such a manner that the excess lead tetraacetate was first decomposed with ethylene glycol, then the volatile components were removed and the residue washed.

When a mixture of cortisol (1b), n-propanol and benzene, was treated for sixteen hours at room temperature with lead tetraacetate, and the reaction was terminated with ethylene glycol, a complex mixture of products was recovered. The presence of at



least four major components was indicated by paper chromatography and these were separated in part by chromatography on silica gel. The most polar compound isolated gave a positive reaction with blue tetrazolium[10] and was identified as cortisol acetate (1c). Although the acetylation of hydroxyls with lead tetraacetate has been reported,[7,16] nevertheless the isolation of cortisol acetate was unexpected since it was thought that scission of the dihydroxyacetone moiety would proceed more readily. The next product was 11β-hydroxy-4-androstene-11,17-dione followed by the n-propyl ester (5a). The ester (5a) m.p. 162–164° was analyzed as expected for $C_{23}H_{34}O_5$, its ultraviolet and infrared spectra were in agreement with the assigned structure and on

[15] R. Criegee and E. Buchner, *Ber. Dtsch. Chem. Ges.* **73**, 563 (1940).
[16] K. Heusler, J. Kalvoda, Ch. Meystre, P. Wieland, G. Anner, A. Wettstein, G. Cainelli, D. Arigoni and O. Jeger, *Helv. Chim. Acta* **44**, 502 (1961).

saponification gave the free acid (2g). The NMR spectrum showed, among others, bands at τ 8·92 for the methyl and at τ 5·75, 5·88, 5·97 for the —O—CH_2— of the n-propyl group.

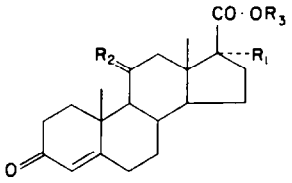
The least polar product isolated (5b) m.p. 186–188° absorbed ultraviolet light λ_{max}^{MeOH} 240 mμ (ε 17,000) and exhibited strong bands in the infrared at 3450, 1745, 1725, 1655, 1610, and 1155 cm^{-1}. Results of elemental analysis indicated a $C_{24}H_{34}O_6$ composition. The product, on treatment with aqueous methanolic potassium bicarbonate, gave the n-propyl ester (5a) and on boiling with aqueous methanolic sodium hydroxide the acid (2g). This suggested that esterification of one of the two hydroxyls of (5a) had occurred, presumably the C-17 hydroxyl, since the NMR spectrum showed a band at τ 5·58 of an 11α-equatorial proton.[17,18] The assumption was borne out, by oxidation of (5b) to the keto ester (5d), characterized by the absence of a hydroxyl band and appearance of a ketone band at 1700 cm^{-1} in the infrared. Further confirmation was provided by the disappearance of the 11α-proton band in the NMR accompanied by the appearance of two bands at τ 7·76, 7·72 ascribed to the C-2 and C-12 methylene groups "α" to C-3 and C-11 ketones.[19] Initially we suspected that (5b) was a 17-acetoxy ester but in view of the results of elemental analysis and the absence of bands ascribed to an acetate in the infrared and NMR spectra, this possibility was discarded. However, the NMR spectrum had, in addition to the band at τ 4·33, assigned to the proton on the double bond at C-4, a band at τ 2·08 of an intensity equivalent to a single hydrogen. The region at which the peak appeared indicated that the carbon-hydrogen bond was of a sp^2 type.[20] These results taken together with the presented evidence for the esterification of the C-17 hydroxyl and the presence of an ester band at 1155 cm^{-1} suggested the 17α-formoxy structure (5b). The presence in ethyl formate of a proton band at τ 1·97 supported the assumption and the proposed structure was proven by an unambiguous synthesis of (5d). The synthesis was first modeled on the more accessible 17α-hydroxy-methyl ester (2a) which on treatment with formic acid and *p*-toluenesulfonic acid[21] gave the 17α-formoxy ester (2h) m.p. 195–198°. The NMR spectrum of the ester (2h) showed among other bands a band at τ 2·03, assigned to the proton on the formoxy group. In analogous manner, (5d) was synthesized, by first oxidizing the 11β-hydroxy of (5a) to yield the 11-keto ester (5c) m.p. 615–167° which, when treated with formic acid and *p*-toluenesulfonic acid, gave the 17α-formoxy ester (5d). The ester (5d) showed a band in the NMR at τ 2·03 and its infrared spectrum was identical to the product of oxidation of (5b) with chromium trioxide in pyridine.

Upon treatment of cortisol in a mixture of benzene and n-butanol (1 : 1) with lead tetraacetate, again four compounds were obtained namely: cortisol acetate, 11β-hydroxy-4-androstene-3,17-dione, n-butyl ester (6a) m.p. 126–130° and 17α-formoxy-n-butyl ester (6b) m.p. 124–128°. The structure of the n-butyl ester was assigned to (6a) on the basis of elemental analysis, infrared, ultraviolet and NMR spectrometry and because on saponification it gave the acid (2g). The assignment of the 17α-formoxy-n-butyl ester structure (6b) was based on elemental analysis spectroscopic

[17] J. N. Shoolery and M. T. Rogers, *J. Amer. Chem. Soc.* **80**, 5121 (1958).
[18] E. Caspi, W. Schmid and B. T. Khan, *J. Org. Chem.* **26**, 3898 (1961). E. Caspi, B. T. Khan and W. Schmid, *J. Org. Chem.* **26**, 3894 (1961).
[19] J. S. G. Cox, E. O. Bishop and R. E. Richards, *J. Chem. Soc.* 5118 (1960).
[20] J. A. Pople, W. G. Schneider and H. J. Bernstein, *High Resolution Nuclear Magnetic Resonance* p. 165. McGraw-Hill, New York (1959).
[21] E. P. Oliveto, C. Gerold, R. Rausser and E. B. Hershberg, *J. Amer. Chem. Soc.* **77**, 3564 (1955).

evidence and NMR spectrometry which showed a band at τ 2·08 of the 17α-formoxy group.

It was then of interest to investigate the course of the reaction with a secondary and tertiary alcohol and the reaction was carried out with isopropanol and tert.-butanol. When cortisol was reacted with lead tetraacetate in a mixture of isopropanol and benzene, cortisol acetate, 11β-hydroxy-4-androstene-3,17-dione and 17α-formoxy-isopropyl ester (6c) were obtained. Although the 17α-hydroxy ester was probably also



6

present it was not isolated. The ester (6c) analyzed for a $C_{24}H_{34}O_6$ compound and had bands in the NMR at τ 8·99 of the C-18 methyl, τ 8·55, C-19 methyl and four bands at τ 8·68, 8·75, 8·80 and 8·85 arising from the two non equivalent methyls of the isopropyl group. Evidently the 17α-formoxy group hinders the rotation of the isopropyl group, resulting in splitting of the bands.[22] The incorporation of the isopropyl moiety was corroborated by the appearance of four bands at τ 4·83, 4·92, 5·01 and 5·08 assigned to the single hydrogen of the isopropyl group. An analogous quartet of bands is present in isopropyl acetate. In contrast to (6c) both methyl groups of isopropyl acetate are equivalent, their rotation not being hindered and they therefore contribute only two bands at τ 8·72 and 8·83. In addition the formoxy ester (6c) also had bands at τ 4·30 for the C-4 proton, τ 5·58, 11α-hydrogen and τ 2·06 hydrogen of the formoxy group. On oxidation, the 11-keto analog (6d) m.p. 156–158° was obtained. With cortisol, tertiary butyl alcohol, benzene and lead tetraacetate, cortisol acetate and 11β-hydroxy-4-androstene-3,17-dione were isolated.

The classical mechanism proposed for the clevage of α-glycols, requiring the formation of a five membered cyclic intermediate of tetravalent lead[23] has been recently questioned, and the cyclic[24] intermediate (7) or its linear[24a] modification were suggested. According to the proposed mechanism, lead glycolate is formed first by the attack of electrophilic[25,4a] $Pb(OAc)_3^+$ on the α-glycol, and then the reaction is brought to completion by an electron shift in the intermediate. Alternatively, it was suggested that the reaction might be initiated by the attack of the equivalent cation[26] CH_3COO^+, but the latter is expected to be less stable[27] and therefore less probable. Cleavage of

[22] Ref. 20, p. 377.
[23] W. Rigby, *J. Chem. Soc.* 1907 (1950).
[24] R. Criegee, E. Hoger, G. Huber, P. Kruck, F. Marktscheffel and H. Schellenberger, *Liebigs Ann.* **599**, 81 (1956).
[24a] R. P. Bell, V. G. Rivlin and W. A. Waters, *J. Chem. Soc.* 1696 (1958); C. A. Grob and P. W. Schiess, *Helv. Chim. Acta* **43**, 1546 (1960).
[25] L. S. Levitt, *J. Org. Chem.* **20**, 1297 (1955).
[26] W. A. Mosher and C. L. Kehr, *J. Amer. Chem. Soc.* **75**, 3172 (1953).
[27] W. A. Mosher and C. L. Kehr, *J. Amer. Soc.* **82**, 5342 (1960).

α-ketols, α-diketones and α-keto acids was considered to proceed through pseudo-glycols or pseudo hemiketals and evidence for such a mechanism, was derived from the acceleration of the bond scission by water and alcohols.[5]

The results reported in this paper indicate that the oxidative cleavage of the dihydroxy acetone moiety is most probably a stepwise process in which the initial attack takes place at the C-20-21 bond. The easier formation of a lead glycolate intermediate, encompassing the more accessible C-20-21 bond, rather than the congested C-17-20 bond and the tertiary, sterically rigid 17α-hydroxyl is undoubtedly the reason for the preferential attack on carbons-20-21. When the C-20-21 bond could not be cleaved because of the presence of an acetoxy group at C-21 i.e. in 6α-methyl-prednisolone acetate (medrol acetate), the C-17-20 link was severed and 6α-methyl-11β-hydroxy-4-androstene-3,17-dione was obtained in good yield. In this connection the survival of cortisol acetate formed during the reaction is of interest.

The formation of α-hydroxy esters can be rationalized by assuming an initial attack of the alcohols on the C-20 carbonyl, and the subsequent cleavage of the resulting hemiketals.[5] The reaction is arrested at that point, because of the stability of α-hydroxy esters to further attack by the reagent, as evidenced by the recovery of all the starting material upon treatment of a methanolic solution of (2e) with lead tetraacetate. There was also the possibility, although remote, that the reaction might have proceeded via the initial formation of the etio acid subsequently esterified by the alcohols present.[28] This possibility was excluded because, when the etio acid (2g) was treated with lead tetraacetate in a mixture of benzene and methanol (1 : 1), 11β-hydroxy-4-androstene-3, 17-dione was the sole product of the reaction. Isolation of 11β-hydroxy-4-androstene-3,17-dione as the main product of reaction of cortisol (1b) and lead tetraacetate in a solution of dry tertiary butanol and benzene, and the absence of the tertiary butyl-ester, brought up the question whether the presence of alcohol is prerequisite for the bond scission.[5] Indeed, the cleavage will take place in the absence of alcohol or water e.g. in dry tetrahydrofuran, the main products being adrenosterone, 11β-hydroxy-4-androstene-3,17-dione and cortisol acetate. Oxidation of the 11β-hydroxyl with lead tetraacetate at elevated temperature has been observed[29] but such an oxidation at room temperature has not been reported. Evidently, cleavage of a dihydroxy acetone moiety and presumably of other substances with similar structures can take place in the presence of nucleophilic solvents.

The formation of the 17-formoxy-etio-esters requires consideration. Initially it was thought that the compounds were artifacts, from the use of ethylene glycol for the decomposition of excess lead tetraacetate. To test this point, cortisol was treated with lead tetraacetate in isopropanol and benzene and at the termination of the reaction, the excess reagent was decomposed with sulfur dioxide instead of ethylene glycol. The major product isolated was again 17α-formoxy-isopropyl ester (6c), thus providing evidence that these esters were not artifacts and that carbon-21 was presumably the source of the formoxy group. It might be of interest, that no traces of the formoxy ester were found by NMR spectroscopy in the mother liquors of the preparation of (2e). The possibility that formaldehyde, derived from C-21, was first oxidized, and the so obtained formic acid then reacted preferentially with the 17α-hydroxyl is unlikely in

[28] We wish to thank Prof. W. von E. Doering for suggesting this possibility to us.
[29] A. Bowers and E. Denot, *J. Amer. Chem. Soc.* **82**, 4956 (1960).

view of the large amounts of alcohols present in the medium. Work is in progress on the elucidation of the mechanism of the observed reactions.

EXPERIMENTAL[30]

Methyl-17α-hydroxy-4-etiocholenate-3,11-dione (2a)

(a) To a solution of cortisone, 2 g, in methanol-benzene (1 : 1), 60 ml, lead tetraacetate, 10 g, was added and the mixture was left for 16 hr at room temperature in the dark. The volatile components were removed at room temperature under reduced pressure and the residue was dissolved in ethyl acetate and water. The organic phase was washed with water, 1 N sodium carbonate solution, saline solution, dried over sodium sulfate and concentrated to a crystalline solid (1·98 g). Chromatography on paper indicated that the crude residue consisted mainly of (2a) contaminated by about 10–15% of adrenosterone and traces of other substances. One crystallization from chloroform–methanol–ether removed the impurities.

A sample was crystallized several times from methanol–ether and showed a m.p. 212–215°; λ_{max}^{MeOH} 238 mμ (ε = 15,500), ν_{max}^{KBr} 3400, 1740, 1710, 1665, 1620, 1270, 1220 cm^{-1}.

(Found: C, 69·88, 70·00; H, 7·72, 7·88. Calc. for $C_{21}H_{28}O_5$: C, 69·97; H, 7·83%).

(b) A methanolic solution of 17α-hydroxy-4-etiocholenic acid-3,11-dione (2b) was treated with an excess of an etheral solution of diazomethane. Removal of solvent left a crystalline residue of (2a).

A solution of 50 mg of (2a) in 5 ml methanol was treated with a solution of 50 mg potassium bicarbonate in 1 ml of water. After 16 hr at room temperature all the starting material was recovered.

A mixture of 200 mg (2a), 20 ml methanol and 2 ml 2 N sodium carbonate was boiled for 1 hr, then most of the methanol was removed under reduced pressure and water was added. The aqueous phase was acidified, then extracted with ethyl acetate and the extract partitioned into neutral and acidic fractions to yield 166 mg of starting material and a small amount of the acid (2b).

A solution of 50 mg (2a) in 0·5 ml pyridine was added to a suspension of 50 mg of chromium trioxide in 0·5 ml of pyridine and was left for 16 hr at room temperature. The mixture was processed as previously described[12] to yield only starting material.

17α-*Hydroxy-4-etiocholenic acid-3,11-dione* (2b)

(a) A mixture of 136 mg of (2a) in 25 ml methanol and 5 ml 2 N sodium hydroxide was boiled under nitrogen for 2 hr. Processing of the mixture with ethyl acetate gave the acid 2b.

(b) A solution of 50 mg cortisone in 10 ml methanol was treated with 9 ml of a stock solution of sodium metaperiodate as previously described[14b] to yield the acid (2b).

(c) A mixture of 50 mg 17α-acetoxy-4-etiocholenic acid-3,11-dione (2d), in 5 ml methanol and 1 ml 2 N sodium hydroxide was boiled under nitrogen for 2·5 hr. Processing of the reaction mixture gave the acid (2b).

A sample of (2b) was crystallized several times from methanol and showed[14a] a.m.p. 270–274°. The infrared spectra of the three samples were identical and had bands at ν_{max}^{Kbr} 3580, 3200, 2650, 1720, 1705, 1640, 1600 cm^{-1}.

17α-*Acetoxy-4-etiocholenic acid-3,11-dione* (2c)

To a solution of 136 mg (2a) in 25 ml methanol, 5 ml 2 N sodium hydroxide was added and the mixture was boiled under nitrogen for 2 hr. The excess sodium hydroxide was neutralized with

[30] Commercial anhydrous methanol and ethanol were used. Other alcohols were distilled from anhydrous potassium carbonate prior to use. Benzene was redistilled and the middle fraction was utilized. Lead tetraacetate purchased from Araphoe Co. was employed throughout. The cortisol and cortisone were used as obtained. The crude reaction mixtures were first analyzed by paper chromatography in benzene–cyclohexane (1:1) formamide system and then were fractionated by chromatography on silica gel. Eluates were combined according to mobility on paper and infrared spectroscopy and the analytical samples were again tested for homogeneity by paper chromatography. Infrared spectra were taken in potassium bromide in paper blotters. Ultraviolet spectra were determined on methanolic solutions on a Cary Model 11 MS or 14 spectrophotometers. The NMR spectra were determined in deuterated chloroform with the use of tetramethylsilane as internal standard on a Varian High-Resolution NMR spectrometer Model V4300B. The spectra were calibrated using a Hewlett-Packard wide range oscillator, model 200 CDR together with a Hewlett-Packard electronic counter model 521CR. The results are expressed in τ units where $\tau = 10{\cdot}00 - \frac{\nu_{TMS} - \nu_X}{\nu_{TMS}} 10^6$. Analyses were made by Schwarzkopf Microanalytical Laboratories, New York and Dr. S. M. Nagy, Massachusetts Institute of Technology, Cambridge, Mass.

2 N hydrochloric acid, then the volatile components were removed under reduced pressure. The resulting mixture of salts was pulverized then dried for 16 hr at room temperature *in high vacuo* and the drying was continued for 5 more hours at 130–140°. Dry benzene, 25 ml, and acetyl chloride, 1 ml, were added and the mixture was boiled for 2 hr with the exclusion of moisture. The volatile components were removed *in vacuo*, and the remaining traces of acetyl chloride were removed by distilling the mixture several times with dry benzene under reduced pressure. The residue contained the mixed anhydride (2c) as evidenced by the infrared spectrum which showed bands at 1830, 1750, 1720, 1665, 1610, 1250 cm^{-1}.

The solid was dissolved in 20 ml acetone, 2 ml water, was added and the mixture was left for 16 hr at room temperature. The acetone was removed under reduced pressure and (2c) was recovered with ethyl acetate (160 mg).

A sample was crystallized several times from ethyl acetate and showed a m.p. 136–138°; λ_{max}^{MeOH} 238 mμ (ε = 15,000), ν_{max}^{KBr} 3200 (broad), 2650, 1760, 1715, 1665, 1615 cm^{-1}.

(Found: C, 67·83, 67·69; H, 7·53, 7·33. Calc. for $C_{22}H_{28}O_6$: C, 68·02; H, 7·27%).

Methyl-11β,17α-*dihydroxy*-4-*etiocholenate*-3-*one* (2e)

A mixture of cortisol, 57 mg, methanol:benzene (1:1), 4 ml, and lead tetracetate, 200 mg, was kept for 16 hr at room temperature and then was processed as described for (2a) to yield a crude crystalline residue. The residue was crystallized from ethyl acetate-ether-neohexane and 47 mg of (2e) was obtained.

A sample was crystallized several times from the same solvents and showed a m.p. 192–194°; λ_{max}^{MeOH} 241 mμ (ε = 16,500) ν_{Max}^{KBr} 3650, 3500, 1750, 1660, 1620, 1260 cm^{-1}.

(Found: C, 69·30; H, 8·23. Calc. for $C_{21}H_{30}O_5$: C, 69·58; H, 8·34%).

To a solution of 205 mg cortisol in 10 ml aqueous methanol (1:9), 1 g lead tetraacetate was added and the mixture was left for 16 hr at room temperature in the dark. The mixture was processed as described for (2a) to yield 165 mg of a neutral residue. The residue consisted of almost equal amounts of (2e) and 11β-hydroxy-4-androstene-3,17-dione as evidenced by paper chromatography. The quantities of the components were approximated from the darkening of the spots when observed under an ultraviolet lamp and were compared to standard mixtures.[31]

To a solution 100 mg of the ester (2e), in methanol–benzene (1:1), 10 ml, lead tetraacetate, 600 mg, was added and the mixture was left for 16 hr at room temperature. After processing of the mixture all of the starting material was recovered.

To a solution of cortisol, 210 mg, in methanol, 10 ml, lead tetraacetate, 1 g, was added and the mixture was left for 9 hr at room temperature in the dark. The methanol was removed in a stream of nitrogen at room temperature then ethyl acetate was added and the mixture was washed with water, 2 N sodium carbonate, a saline solution dried and concentrated (211 mg). The residue was crystallized from a mixture of methanol and ether to yield 170 mg of (2e) in two crops.

Ethyl-17α-*hydroxy*-4-*etiocholenate*-3,11-*dione* (2f)

A mixture of 200 mg cortisone (1a), 8 ml benzene:ethanol (1:1) and lead tetraacetate 1 g was kept in the dark for 16 hr at room temperature and then processed as described for (2a) to yield 201 mg of a neutral residue which was crystallized from ethyl acetate–ether.

A sample was crystallized several times from ethyl acetate and showed a m.p. 182–183°; λ_{max}^{MeOH} 237 mμ, (ε = 15,200); ν_{max}^{KBr} 3500, 1755, 1720, 1665, 1620, 1260, 1220 cm^{-1}.

(Found: C, 70·66; H, 7·86. Calc. for $C_{22}H_{30}O_5$: C, 70·56; H, 8·08%).

Methyl-17α-*hydroxy*-1,4-*etiocholadienate*-3,11-*dione* (4a)

A mixture of prednisone (3a), 265 mg, benzene:methanol (1:1), 20 ml, and lead tetraacetate, 1·3 g, was kept for 16 hr at room temperature and then was processed as described for (2a) to yield 258 mg of a neutral residue which was crystallized from a chloroform–methanol solution.

[31] R. Neher, *Chromatographie von sterinen Steroiden und verwandte Verbindungen* p. 66. Elsevier, Amsterdam (1958); R. Neher in *Chromatographic Review* (Edited by M. Lederer) Vol. 1, p. 163. Elsevier, Amsterdam (1959); R. Neher and A. Wettstein, *J. Clin. Invest.* **35**, 800 (1956); L. M. Reineke, *Analyt. Chem.* **28**, 1853 (1956); C. DeCourcey, I. E. Bush, C. H. Gray and J. B. Lunnon, *J. Endocrin.* **9**, 401 (1953).

A sample was recrystallized from methylene chloride ethanol to a m.p. 206–209°; λ_{max}^{MeOH} 240 mμ, (ε15,000); ν_{max}^{KBr} 3580, 1745, 1705, 1670, 1625, 1605 cm^{-1}.

(Found: C, 70·26; H, 7·43. Calc. for $C_{21}H_{26}O_5$: C, 70·37; H, 7·31%).

17α-Hydroxy-1,4-etiocholadienic acid-3,11-dione (4b)

(a) A solution of 26·7 mg of the ester (4a) in 5 ml methanol and 2 ml 0·5 N sodium hydroxide was boiled under nitrogen for 2 hr. After dilution with water the mixture was worked up with ethyl acetate and was partitioned into neutral and acidic fractions to yield 15 mg of the acid (4b).

(b) To a solution of prednisone, 100 mg, in methanol, 25 ml, a solution of periodic acid, 100 mg, in water, 3 ml, was added and the mixture was stored in the dark at room temperature for 3 hr. The solution was concentrated at reduced pressure at room temperature to yield the acid (4b).

A sample was crystallized from methanol and showed a m.p. 249–254° with prior softening at 246°; λ_{max}^{MeOH} 239 mμ, (ε15,000); ν_{max}^{KBr} 3580, 2700, (broad), 1720, 1700, 1650, 1600 (strong) cm^{-1}.

(Found: C, 69·52; H, 6·87. Calc. for $C_{20}H_{24}O_5$: C, 69·75; H, 7·02%).

Methyl-11β,17α-dihydroxy-6α-methyl-1,4-etiocholadienate-3-one (4c)

A solution of 6α-methyl prednisolone (3b), 212 mg, in benzene:methanol (1:1), 20 ml, and lead tetraacetate, 1 g, was left for 16 hr at room temperature. Ethylene glycol 0·2 ml was added and after 1 hr at room temperature the mixture was concentrated. Ethyl acetate was added and the organic phase was washed with water, a sodium carbonate solution, a saline solution, dried and concentrated to a residue (235 mg).

A sample was crystallized several times from ethyl acetate and showed a m.p. 194–197°; λ_{max}^{MeOH} 243 mμ, (ε15,400); ν_{max}^{KBr} 3580, 3500, 1735 (shoulder), 1725, 1655, 1620, 1600 (shoulder) cm^{-1}. NMR τ 2·63, 2·80, 3·65, 3·81, 3·95, 5·50, 6·23, 8·38, 8·56, 8·85, 8·94, 9·01.

(Found: C, 70·70; H, 7·96. Calc. for $C_{22}H_{30}O_5$: C, 70·56; H, 8·08%).

n-Propyl-11β-hydroxy-17α-formoxy-4-etiocholenate-3-one (5b)

A mixture of cortisol, 2 g, benzene:propanol (1:1), 200 ml, and lead tetraacetate, 10g, was kept for 16 hr at room temperature in the dark. The excess of lead tetraacetate was decomposed by addition of ethylene glycol, 2 ml, then the solvent was removed under reduced pressure, ethyl acetate was added and the mixture was washed dried and concentrated to leave 2·17 g of a neutral residue. Paper chromatography indicated the presence of at least four components in the crude residue. The residue was adsorbed on a column of silica gel (80 g) prepared with benzene, and the column was eluted with benzene, mixtures of benzene–chloroform, chloroform, mixtures of chloroform–ethyl acetate, ethyl acetate and mixtures of ethyl acetate–methanol. The eluates were scanned by paper chromatography and combined according to content into four fractions numbered in order of their elution from the column. The most polar fractions 4 and 3 were crystallized from ethyl acetate yield to cortisol acetate (1b) and 11β-hydroxy-4-androstene-3,17-dione respectively. The least polar fraction 1 was crystallized several times from ethyl acetate to yield (5b).

The formoxy ester (5b) showed a m.p. 186–188°; λ_{max}^{MeOH} 240 mμ, (ε = 17,000), ν_{max}^{KBr} 1750, 1725, 1655, 1615, 1155 cm^{-1}. NMR τ 2·08, 4·33, 5·58, 5·81, 5·92, 6·03, 7·67, 8·54, 8·99, 9·05.

(Found: C, 68·73, 68·76; H, 8·19, 8·04. Calc. for $C_{24}H_{34}O_6$: C, 68·87; H, 9·05%).

Propyl-11β,17α-dihydroxy-4-etiocholenate-3-one (5a)

(a) This substance was isolated from fraction 2 of the above experiment. After several crystallizations from ethyl acetate the ester (5a) showed a m.p. 162–164°; λ_{max}^{MeOH} 242 mμ, (ε = 16,700); ν_{max}^{KBr} 3570, 1740, 1650, 1610 cm^{-1}. NMR τ 4·33, 5·38, 5·75, 5·88, 5·97, 7·67, 8·56, 8·92, 9·01.

(Found: C, 70·85; H, 8·91. Calc. for $C_{23}H_{34}O_5$: C, 70·74; H, 8·78%).

(b) A mixture of (5b), 43 mg, methanol, 5 ml, potassium bicarbonate, 40 mg, and water, 0·8 ml, was kept under nitrogen for 16 hr at room temperature. The mixture was then diluted with water, extracted with ethyl acetate and the organic phase was washed with saline, dried and concentrated, to yield 43 mg of crude (5a).

A solution of the ester (5a), 25 mg, in methanol, 5 ml, containing 1 ml of 2 N sodium hydroxide was boiled for 2 hr under nitrogen, and the acid (2g) was recovered with ethyl acetate.

Propyl-17α-hydroxy-4-etiocholenate-3,11-dione (5c)

A solution of the ester (5a), 98 mg in pyridine 1 ml, was added to a suspension of chromium trioxide, 100 mg, in pyridine, 1 ml, and was left for 8 hr at room temperature. The solution was processed as previously described[15] to yield 76 mg of (5c).

A sample was crystallized from methylene chloride hexane and showed a m.p. 165–167°; λ_{max}^{MeOH} 239 mμ, (ε = 15,800); ν_{max}^{KBr} 3550, 1725, 1700, 1665, 1600 cm⁻¹. NMR τ4·28, 5·74, 5·83, 5·95, 7·71, 8·57, 8·92, 9·04, 9·17, 9·29.

(Found: C, 71·48; H, 8·09. Calc. for $C_{23}H_{32}O_5$: C, 71·10; H, 8·30%).

Propyl-17α-formoxy-4-etiocholenate-3,11-dione (5d)

(a) A solution of 22 mg (5b) in 0·2 ml pyridine was added to a suspension of 25 mg chromium trioxide in 0·3 ml pyridine and was left for 8 hr at room temperature. The mixture was processed as previously described and 15 mg of (5d) were obtained.

(b) A mixture of the 17α-hydroxy ester (5c), 48 mg, formic acid, 10 ml, *p*-toluenesulfonic acid, 20 mg, was refluxed for 3 hr and then was left for 16 hr at room temperature. The volatile components were removed in a stream of nitrogen, the residue was then dissolved in a mixture of ether–methylene chloride (3:1) washed with water, a sodium bicarbonate solution, a saline solution dried and reduced to a residue (52 mg). The syrup was dissolved in ethyl acetate treated with charcoal and on concentration a small amount of starting material was obtained. The mother liquor was concentrated to a syrup and the ester (5d) was crystallized from ether.

A sample was crystallized several times from ether and showed a m.p. 111–113° λ_{max}^{MeOH} 238 mμ, (ε15,500); ν_{max}^{KBr} 1720, 1700, 1660, 1605, 1150,cm⁻¹. NMR τ 2·02, 4·27, 5·82, 5·92, 6·03, 7·66, 7·77, 8·58, 8·98, 9·13, 9·29.

(Found: C, 69·18; H, 7·84. Calc. for $C_{24}H_{32}O_6$: C, 69·21; H, 7·74%).

n-Butyl-11β-hydroxy-17α-formoxy-4-etiocholenate-3-one (6b)

A mixture of cortisol (1b), 2 g, benzene-n-butanol (1:1), 200 ml, lead tetraacetate, 10 g, was kept for 16 hr at room temperature and then was processed and chromatographed on silica gel exactly as described for (5b). The eluates from the adsorption chromatography were combined into four major fractions. From the two most polar fractions 4 and 3, cortisol acetate (1c) and 11β-hydroxy-4-androstene-3,17-dione were isolated respectively. The least polar fraction 1 on crystallization from ethyl acetate gave (6b).

A sample was crystallized several times from ethyl acetate and showed a m.p. 124–128°; λ_{max}^{MeOH} 241 mμ, (ε = 16,100); ν_{max}^{KBr} 3550, 1740, 1655, 1605 cm⁻¹. NMR τ 2·02, 4·32, 5·54, 5·78, 5·88, 5·98, 8·08, 8·55, 9·00, 9·06.

(Found: C, 69·81; H, 8·50. Calc. for $C_{25}H_{36}O_6$: C, 69·42; H, 8·39%).

n-Butyl-11β,17α-dihydroxy-4-etiocholenate-3-one (6a)

This substance was crystallized from an ethyl acetate solution of fraction 2 of the above experiment.

A sample was crystallized several times, from the same solvent and showed a m.p. 126–130°; λ_{max}^{MeOH} 242 mμ, (ε = 17,100); ν_{max}^{KBr} 3580, 1730, 1640, 1600 cm⁻¹.

(Found: C, 71·18; H, 8·95. Calc. for $C_{24}H_{36}O_5$: C, 71·25; H, 8·97%).

Isopropyl-11β-hydroxy-17α-formoxy-4-etiocholenate-3-one (6c)

(a) A mixture of cortisol, 1·035 g, isopropanol, 35 ml, benzene, 30 ml, lead tetraacetate, 5 g, was left 16 hr at room temperature in the dark and then was processed and chromatographed on silica gel and treated subsequently exactly as described for (5b). The eluates from the silica gel column were combined according to content into three major fractions. Cortisol acetate was obtained from the most polar fraction, 11β-hydroxy-4-androstene-3,17-dione from the intermediately polar fraction and (6c) from a methylene chloride hexane crystallization of the least polar fraction.

(b) A mixture of cortisol, 441 mg, in isopropanol–benzene (1:1), 20 ml, lead tetraacetate, 2·5 g, was left for 16 hr at room temperature in the dark. The mixture was diluted with chloroform–ether (1:3), 200 ml, and a brisk stream of sulfur dioxide passed. The precipitated solid was filtered, washed with a mixture of chloroform ether (1:3) and discarded. The filtrate was washed with water, a sodium bicarbonate solution, a saline solution dried and concentrated. The residue was crystallized from ethyl acetate to yield 115 mg of (6c).

A sample was crystallized from methanol to a m.p. 205–207°; λ_{max}^{MeOH} 242 mμ, (ε = 17,000); ν_{max}^{KBr} 3480, 1735, 1750, 1620, 1160 cm^{-1}. NMR τ 2·08, 4·30, 4·92, 5·03, 5·54, 7·67, 8·56, 8·68, 8·73, 8·78, 8·84, 9·00.

(Found: C, 68·64, 68·90; H, 8·17, 8·20. Calc. for $C_{24}H_{34}O_6$: C, 68·87; H, 8·19%).

Isopropyl-17α-formoxy-4-etiocholenate-3,11-dione (6d)

A solution of the ester (6c), 43 mg, in pyridine, 0·3 ml, was added to a suspension of chromium trioxide, 48 mg, in pyridine, 0·4 ml, and was agitated for 3 hr at room temperature. Ethyl acetate was added, the mixture was filtered and the filtrate washed consecutively with water, 2 N hydrochloric acid, water, a solution of sodium bicarbonate, a saline solution then dried and reduced to a residue (35 mg). The residue was adsorbed on silica gel (1g) and the column was eluted with benzene, benzene–chloroform mixtures, chloroform and chloroform ethyl acetate mixtures. Eluates of benzene chloroform (1:3) gave 29 mg of (6d).

A sample was crystallized from ethyl acetate to a m.p. 156–158°; λ_{max}^{MeOH} 238 mμ, (ε = 16,300); ν_{max}^{KBr} 1745, 1695, 1650, 1605, 1155 cm^{-1}. NMR τ 2·03, 4·25, 7·64, 7·71, 8·59, 8·67, 8·75, 8·78, 8·85, 9·29.

(Found: C, 69·39; H, 7·82. Calc. for $C_{24}H_{32}O_6$: C, 69·21; 7·74%).

Methyl-17α-formoxy-4-etiocholenate-3,11-dione (2h)

A mixture of the methyl ester (2a), 200 mg, formic acid, 30 ml, *p*-toluenesulfonic acid, 25 mg, was boiled for 3 hr. The formic acid was removed in a stream of nitrogen, the residue dissolved in a mixture of ether–chloroform (3:1) and the solution was washed with water, a solution of sodium bicarbonate, a saline solution then dried and concentrated to yield 190 mg of (2h).

A sample was crystallized several times from methylene chloride ether to a m.p. 195–198°; λ_{max}^{MeOH} 238 mμ, ε = 16,500; ν_{max}^{KBr} 1750, 1725, 1700, 1670, 1610, 1155 cm^{-1}. NMR τ 2·03, 4·26, 6·25, 7·00, 7·23, 7·62, 7·70, 8·05, 8·99, 9·30.

(Found: C, 68·22; H, 7·21. Calc. for $C_{22}H_{28}O_6$: C, 68·02; H, 7·27%).

11β,17α-dihydroxy-4-etiocholenic acid-3-one (2g)

(a) To a solution of cortisol (1b), 1 g, in methanol, 50 ml, a solution of periodic acid, 1·05 g, in water, 10 ml was added and the mixture left for 3 hr at room temperature in the dark. The mixture was then gradually diluted with water and the crystalline (2g) collected.

(b) To a solution of the formoxy ester (6c), 34 mg, in methanol, 10 ml, 2 N normal sodium hydroxide, 1 ml, was added and the mixture was boiled under nitrogen for 2 hr. The cooled mixture was diluted with water and extracted with ethyl acetate and the extract discarded. The aqueous phase was acidified with 2 N hydrochloric acid, extracted with ethyl acetate and the organic phase washed, dried to yield on concentration (2g).

(c) The formoxy ester (5b) 30 mg was treated exactly as described above to yield (2g).

A sample was crystallized from ethyl acetate to a m.p. 240–244°; λ_{max}^{MeOH} 243 mμ, (ε = 15,300), ν_{max}^{KBr} 3600, 3200, 2700, 1725, 1640, 1600 cm^{-1}.

(Found: C, 68·62; H, 8·12. Calc. for $C_{20}H_{28}O_5$: C, 68·94; H, 8·10%).

To a solution of acid (2g), 50 mg, in benzene–methanol (1:1), 5 ml, lead tetraacetate, 250 mg, was added and after 16 hr at room temperature the reaction was worked up in the usual manner to yield 38 mg of 11β-hydroxy-4-androstene-3,17-dione.

11β-Hydroxy-6α-methyl-1,4-androstadiene-3,17-dione

A mixture of 6α-methyl-prednisolone acetate (medrol acetate) (3c) 250 mg, in benzene–methanol (1:1), 25 ml, and lead tetraacetate, 1·25 g, was kept for 16 hr at room temperature in the dark. The reaction was terminated by the addition of ethylene glycol 0·25 ml, and was processed as previously described to yield 210 mg of the crude 17-ketosteroid.

A sample was crystallized several times from ethyl acetate and showed a m.p. 260–262° with a change of crystalline structure at 215–225°; λ_{max}^{MeOH} 242 mμ, (ε = 15,100); ν_{max}^{KBr} 3600, 1725, 1650, 1620, 1600 cm^{-1}. NMR τ 2·63, 2·80, 3·66, 3·81, 3·95, 5·37, 7·92, 8·16, 8·53, 8·83, 8·92.

(Found: C, 76·27; H, 8·15. Calc. for $C_{20}H_{26}O_3$: C, 76·40; H, 8·34%).

Acknowledgements—The authors wish to thank Dr. H. J. Ringold of this Foundation for stimulating discussions of the results and reading of the manuscript. The steroids used in these investigations were obtained from the Cancer Chemotherapy National Service Center, U.S. Public Health Service.