yield of fluorohydrin IIIe was increased to 55%, and 28% of 9β,11-epoxide IVb was recovered.

9α-Fluoro-11β,16α,17α,21-tetrahydroxy-6α-methyl-1,4-pregnadiene-3,20-dione (IIIf). A solution of 4.10 g. of diacetate IIIe in 465 cc. of methanol in a nitrogen atmosphere was treated with 12.0 cc. of 10% potassium carbonate at 25° for 20 min. Acetic acid (4.56 cc.) was added and volume of solution was reduced to about 100 cc. by concentration under reduced pressure at a bath temperature of 40°. To the remaining solution was added slowly with stirring 800 cc. of ice water and the resultant white crystals were filtered and washed with water to yield 2.95 g. (87%), m.p. 229–231°. One recrystallization from acetone–petroleum ether raised the melting point to 252–253° (further recrystallizations gave inconsistent melting points); λ_{max} 239 mμ (ε 15,100); ν_{max} 3380, 1710, 1660, 1618 cm.$^{-1}$; $[\alpha]_D^{25}$ + 57° (methanol).

Anal. Calcd. for $C_{22}H_{29}O_6F \cdot 1/2H_2O$ (417.46): C, 63.29; H, 7.24; F, 4.55; O, 24.91; H_2O, 2.16. Found: C, 63.36; H, 7.35; F, 4.55; O, 24.18; H_2O, 1.15.

16α,21-Diacetoxy-9α-fluoro-17α-hydroxy-6α-methyl-1,4-pregnadiene-3,11,20-trione (Vb). A solution of 250 mg. of fluorohydrin IIIe in 6 cc. of pyridine was added to a slurry prepared by adding 2.5 cc. of pyridine to 188 mg. of chromic anhydride. The mixture was shaken vigorously and allowed to stand at room temperature for 18 hr. The mixture was diluted with 50 cc. of water and filtered. The residual brown solid was extracted several times with hot ethyl acetate and the combined extracts evaporated to a white solid under reduced pressure. Crystallization of the solid from acetone–petroleum ether yielded 170 mg. (68%) of white needles, m.p. 247–249°. Two recrystallizations from acetone-petroleum ether raised the melting point to 249–250°; λ_{max} 235 mμ (ε 14,800); ν_{max} 3325, 1730, 1665, 1620, 1610 (shoulder), 1230 cm.$^{-1}$; $[\alpha]_D^{25}$ + 59° (chloroform).

Anal. Calcd. for $C_{26}H_{31}O_8F$ (490.51): C, 63.66; H, 6.37; F, 3.87. Found: C, 63.95; H, 6.53; F, 4.10.

9α-Fluoro-11β,21-dihydroxy-16α,17α-isopropylidenedioxy-6α-methyl-1,4-pregnadiene-3,20-dione (VIb). To a suspension of 1.36 g. of tetrol IIIf in 68 cc. of acetone was added 0.138 cc. of 72% perchloric acid. Solution became complete after 10 min. of stirring. The reaction was allowed to stand at room temperature for 2 hr. and then treated with 35 cc. of water and 2.72 cc. of saturated aqueous sodium bicarbonate solution. Evaporation of the acetone gave a white solid which was filtered and washed with water to yield 1.41 g. (95%) of white crystals, m.p. 259–262°. A sample of this material was recrystallized four times from acetone-petroleum ether to give pure VIb, m.p. 258–260° dec., λ_{max} 240 mμ (ε 15,400); ν_{max} 3400, 1715, 1665, 1625, 1610 (shoulder) cm.$^{-1}$; $[\alpha]_D^{25}$ + 98.5° (chloroform).

Anal. Calcd. for $C_{25}H_{33}O_6F$ (448.51): C, 66.94; H, 7.42; F, 4.24. Found: C, 67.18; H, 7.56; F, 4.59.

21-Acetoxy-9α-fluoro-11β-hydroxy-16α,17α-isopropylidenedioxy-6α-methyl-1,4-pregnadiene-3,20-dione (VIc). To a suspension of 160 mg. of acetonide VIb in 7 cc. of pyridine was added 0.5 cc. of acetic anhydride. The mixture was heated on the steam-bath for 1 hr., solution being complete after several minutes. The solution was diluted with methanol and evaporated under reduced pressure. The residue was evaporated several times with toluene and the white solid residue crystallized from acetone–petroleum ether to yield 110 mg. of white needles, m.p. 307–308° dec. A second crop from the mother liquor was obtained 35 mg., m.p. 301–302° dec.

The first crop material was recrystallized twice from acetone–petroleum ether without changing the melting point; λ_{max} 238 mμ (ε 15,800); ν_{max} 3300, 1750, 1725, 1660, 1610, 1230 cm.$^{-1}$; $[\alpha]_D^{25}$ + 83° (chloroform).

Anal. Calcd. for $C_{27}H_{35}O_7F$ (490.55): C, 66.10; H, 7.19; F, 3.87. Found: C, 66.15; H, 7.31; F, 4.05.

Acknowledgment. We wish to thank Louis M. Brancone and associates for the analytical data, William Fulmor and associates for the spectral data and optical rotational data, and Charles Pidacks and associates for the partition chromatographic separations.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE DIVISION OF STEROID RESEARCH, THE JOHN HERR MUSSER DEPARTMENT OF RESEARCH MEDICINE, UNIVERSITY OF PENNSYLVANIA]

Investigations on Steroids. XXXII. Preparation of 14β,19-Dihydroxycortexone[1]

EDWARD J. BECKER AND MAXIMILIAN EHRENSTEIN

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By ozonolysis, strophanthidol (I) was converted into 3β,5,14,19,21-pentahydroxy-5β,14β-pregnan-20-one (IV), which in turn, by way of 5,14,19,21-tetrahydroxy-5β,14β-pregnane-3,20-dione (VIII), was transformed into 14β,19-dihydroxycortexone (IX) and its 19,21-diacetate (X). Data concerning the molecular rotation of X have been discussed. The rotatory dispersion curves of some pertinent compounds are recorded.

The syntheses of 19-hydroxy and 19-oxo analogs of a number of steroid hormones have been described by this laboratory.[2-7] During the course of these investigations it became desirable to prepare 14β,19-dihydroxycortexone[8] (IX) for the purpose of subjecting it to microbiological hydroxylation. The work on the synthesis and characterization of IX was concluded in May 1958. As the hydroxyla-

(1) This investigation was supported by research grants (CY757-C5 and CY757-C6) from the National Cancer Institute of the National Institutes of Health, Public Health Service. A part of the K-Strophanthin used in this investigation was kindly donated by S. B. Penick & Company, New York.

(2) (a) G. W. Barber and M. Ehrenstein, *J. Am. Chem. Soc.*, 76, 2026 (1954). (b) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 19, 1758 (1954).

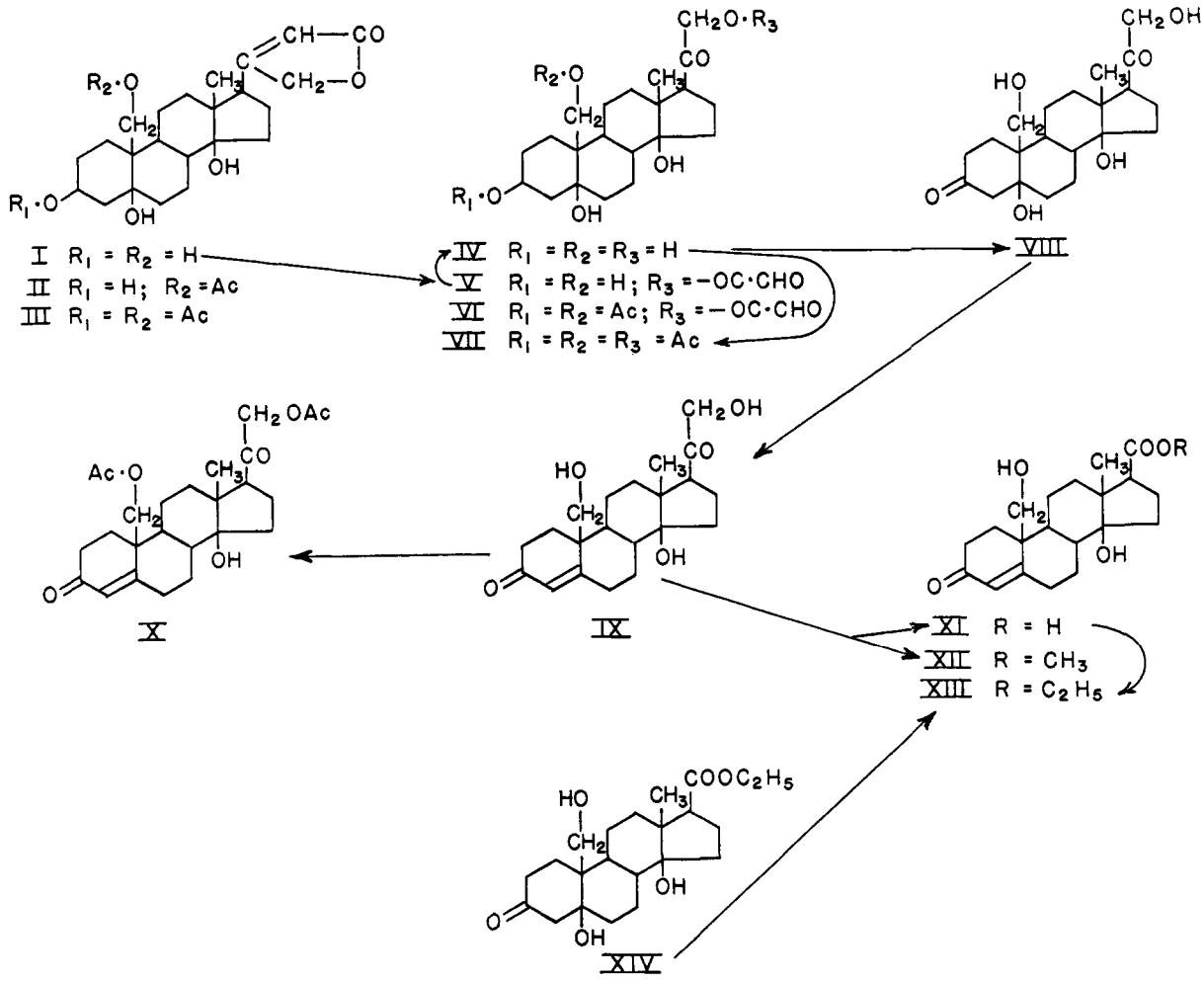
(3) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 20, 1253 (1955).

(4) M. Ehrenstein and M. Dünnenberger, *J. Org. Chem.* 21, 774 (1956).

(5) M. Ehrenstein and M. Dünnenberger, *J. Org. Chem.*, 21, 783 (1956).

(6) M. Ehrenstein and K. Otto, *J. Org. Chem.*, 24, 2006 (1959).

(7) G. W. Barber, D. H. Peterson, and M. Ehrenstein, *J. Org. Chem.*, 25, 1168 (1960).



tion attempts have not yielded encouraging results[9] and in view of the fact that the preparation of the diacetate of IX (*i.e.*, X) has meanwhile been described by Oliveto, *et al.*,[10] we now wish to report the synthesis of IX and X.

Starting material was strophanthidol (I), which is accessible by reduction of strophanthidin.[11] As was shown earlier,[12] ozonolysis of strophanthidol diacetate (III) and reductive cleavage of the resulting ozonide furnishes 3β,19-diacetoxy-21-glyoxyl-oxy-5,14-dihydroxy-5β,14β-pregnan-20-one (VI),[12a] which did not crystallize. At that time we did not succeed in securing by alkaline hydrolysis the corresponding free compound, *i.e.*, 3β,5,14,19,21-pentahydroxy-5β,14β-pregnan-20-one (IV), in a pure crystalline state. In these experiments it became evident that hydrolysis of VI even with mild alkali, such as potassium carbonate or potassium bicarbonate, produces epimerization at carbon atom 17 to some extent. More recently we have been able to obtain a fair yield of crystalline IV on hydrolysis of VI with sodium carbonate in aqueous methanol.[13]

It was later found more convenient to ozonize strophanthidol (I) directly. Subsequent reductive cleavage of the ozonide gave the crude amorphous 21-glyoxyloxy-3β,5,14,19-tetrahydroxy-5β,14β-pregnan-20-one (V).[12a] Without purification, it was sub-

(8) In agreement with the proposals of Fieser preference is given to the trivial name 14β,19-dihydroxycortexone rather than to 14β,19-dihydroxy-11-desoxycorticosterone. *Cf. Steroids* by Louis F. Fieser and Mary Fieser, Reinhold Publishing Corporation, New York, 1959, v. pp. 602, 706.

(9) Apparently fungi subject IX to extensive structural changes. In no instance was there an indication of the formation of a reasonable amount of hydroxylated IX. Organisms used for screening: *Cunninghamella blakesleeana; Sporotrichum sulfurescens; Rhizopus nigricans; Rhizopus arrhizus* (Experiments by D. H. Peterson and O. K. Sebek, Research Laboratories, The Upjohn Company, Kalamazoo, Mich.).

(10) E. P. Oliveto, L. Weber, C. G. Finckenor, M. M. Pechet, and E. B. Hershberg, *J. Am. Chem. Soc.*, **81**, 2831 (1959).

(11) *Cf.* M. Ehrenstein and A. R. Johnson, *J. Org. Chem.*, 11, 823 (1946).

(12) C. P. Balant and M. Ehrenstein, *J. Org. Chem.*, **17**, 1576 (1952).

(12a) In view of recent findings, the radical R_3 in formulas V and VI, given as—OC·CHO, has to be replaced by —OC·CH_2OH. With this change, the names of V and VI should be 21-glycolyloxy-3β,5,14,19-tetrahydroxy-5β,14β-pregnan-20-one and 3β,19-diacetoxy-21-glycolyloxy-5,14-dihydroxy-5β,14β-pregnan-20-one, respectively. *Cf.* M. Zingg and K. Meyer, *Helv. Chim. Acta*, **43**, 145 (1960).

(13) As this procedure was applied only once, it is not recorded in the experimental part.

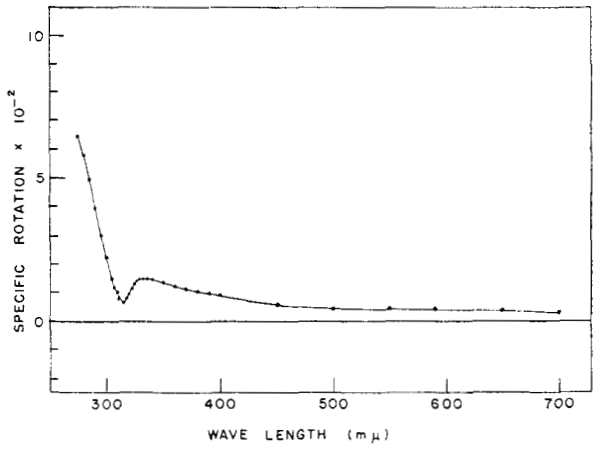


Fig. 1. Rotatory dispersion curve of ethyl 3-oxo-5,14,19-trihydroxy-5β,14β-etianate (XIV) (m.p. 150–152°) in dioxane (c = 0.089, 700 ~ 275 mμ)

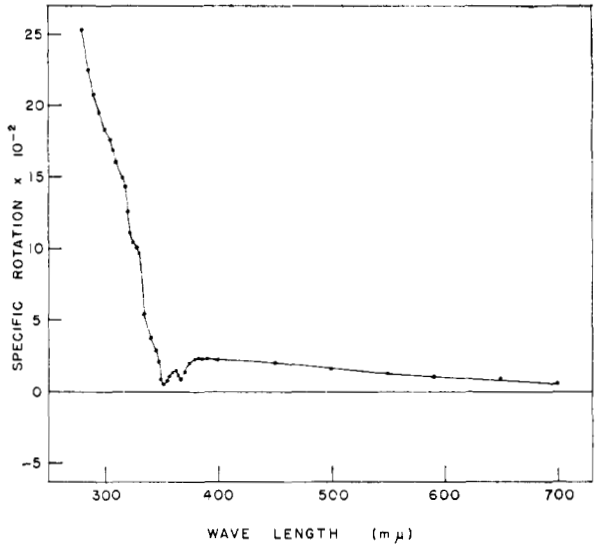


Fig. 2. Rotatory dispersion curve of ethyl 3-oxo-14,19-dihydroxy-Δ⁴-14β-etienate (XIII) (m.p. 187–189°) in dioxane (c = 0.085, 700 ~ 280 mμ)

jected to hydrolysis with sodium carbonate in aqueous ethanol or with potassium bicarbonate in aqueous methanol. In either case a satisfactory yield of crystalline 3β,5,14,19,21-pentahydroxy-5β,14β-pregnan-20-one (IV) resulted. In view of the observations mentioned earlier, it must be assumed that in these instances the C-17 epimer has remained in the mother liquors which were not investigated. The stereochemical identity of IV was established by conversion into the known 3β,-19,21-triacetoxy-5,14-dihydroxy-5β,14β-pregnan-20-one (VII).[12]

Treatment of IV with an amount of *N*-bromoacetamide slightly in excess of two equivalents gave 5,14,19,21-tetrahydroxy-5β,14β-pregnane-3,-20-dione (VIII). Dehydration of VIII by the action of Girard's reagent T furnished a good yield of 14β,-19-dihydroxycortexone[8] (IX) which by acetylation was converted into 14β,19-dihydroxycortexone 19,21-diacetate (X). As expected, X showed the typical hypsochromic shift of the ultraviolet absorption maximum associated with the acetylation of 19-hydroxyl groups in Δ⁴-3-keto steroids.[14]

As further corroboration of the stereochemical pattern in this series, IX was subjected to degradation with periodic acid in a solution of methanol. This yielded 3-oxo-14,19-dihydroxy-Δ⁴-14β-etienic acid (XI) and, in addition, the known methyl 3-oxo-14,19-dihydroxy-Δ⁴-14β-etienate (XII).[15] XI was further characterized by conversion with diazoethane into ethyl 3-oxo-14,19-dihydroxy-Δ⁴-14β-etienate (XIII). XIII had been prepared previously from ethyl 3β,5,14,19-tetrahydroxy-5β,14β-etianate by way of ethyl 3-oxo-5,14,19-trihydroxy-5β,14β-etianate (XIV).[16]

In a forthcoming publication from this laboratory it will be necessary, for purposes of comparison, to refer to the rotatory dispersion curves[17] of compounds XIV and XIII which were determined through the courtesy of Professor Carl Djerassi at Wayne State University (now at Stanford University). It appears convenient to record them in the present paper. The curve of XIV (Fig. 1) is typical of a 5β-3-keto steroid,[18] while the curve of XIII (Fig. 2) corresponds closely to a standard Δ⁴-3-ketone[19] which does not show any major conformational distortion.

As was mentioned, the preparation of 14β,19-dihydroxycortexone 19,21-diacetate (X) has recently been described by a group of investigators at the Schering Corporation.[10] In this synthesis strophanthidol 19-monoacetate (II) served as starting material, and the intermediates leading to X are clearly different from those reported in the present paper. It has not been firmly established, however, that the terminal product X of the Schering group is identical with the respective compound prepared in our laboratory. Oliveto, *et al.*[10] point out that the configurations assigned to compound X are supported by the value obtained for the molecular rotation. It is correctly stated that the introduction of a hydroxy group in the 19-position of a 3-keto-Δ⁴-steroid has no great influence on the molecular rotation. It is pointed out that the value found, M_D + 522° (in pyridine), is in good agreement with that reported for 14β-hydroxycortexone 21-monoacetate, M_D + 505° (in chloroform).[20] The authors have apparently overlooked the fact that the compound under consideration (X) has

(14) Lit. *cf.* ref. 16, v.p. 59; *cf.* also ref. 7.

(15) K. Florey and M. Ehrenstein, *J. Org. Chem.*, **19**, 1174 (1954).

(16) E. J. Becker and M. Ehrenstein, *Liebigs Ann. Chem.*, **608**, 54 (1957).

(17) For general literature *cf. Optical Rotatory Dispersion. Applications to Organic Chemistry* by Carl Djerassi, McGraw-Hill Book Company, Inc., New York, 1960.

(18) *Cf., e.g.*, ref. 17, pp. 50, 75.

(19) *Cf., e.g.*, ref. 17, pp. 17, 61, 65.

(20) K. Meyer and T. Reichstein, *Helv. Chim. Acta*, **30**, 1508 (1947).

an acetoxy group, rather than a hydroxy group, in the 19-position. As is shown in Table I, the introduction of an acetoxy group in the 19-position has a significant influence on the molecular rotation. It is evident that the value obtained with our compound X, M_D + 679° (in chloroform), is in agreement with expectations. It is to be noted that all rotations recorded in Table I were measured in chloroform and, hence, all ΔM_D values are comparable.

Passing mention should be made of investigations under way to prepare 14β,19-dihydroxy-1-dehydrocortexone 19,21-diacetate. In preliminary experiments it appears that treatment of X with selenium dioxide leads to a mixture consisting of at least four components (paper chromatogram).

Physiological activity. In assays carried out at the Worcester Foundation for Experimental Biology through the courtesy of Dr. Ralph I. Dorfman, 14β,19-dihydroxycortexone (IX), in doses of 6 to 100 μg., had no significant effect on the excretion of sodium or potassium in salt (sodium chloride) loaded adrenalectomized rats.

TABLE I

COMPARISONS OF MOLECULAR ROTATIONS
(All rotations measured in chloroform)

	Compound	Ref.	M_D	ΔM_D $(b - a)$	ΔM_D $(c - a)$
1.	(a) Progesterone	21	+641		
	(b) 19-Hydroxyprogesterone	2b	+611	−30	+188
	(c) 19-Acetoxyprogesterone	2b	+829		
2.	(a) Cortexone	22	+611		
	(b) 19-Hydroxycortexone	2b	+640	+29	+195
	(c) 19-Acetoxycortexone	2b	+835		
3.	(a) Cortexone acetate	21	+693		
	(b) 19-Hydroxycortexone 21-monoacetate	2b	+692	−1	+209
	(c) 19-Hydroxycortexone 19,21-diacetate	2b	+902		
4.	(a) 14β-Hydroxycortexone 21-monoacetate	20	+505		
	(b) 14β,19-Dihydroxycortexone 21-monoacetate	23	—	—	+174
	(c) 14β,19-Dihydroxycortexone 19,21-diacetate (X)	24	+679		

EXPERIMENTAL

Melting points. The melting points were determined with the Fisher-Johns melting point apparatus and are uncorrected.

Absorption spectra. Ultraviolet spectra were determined in 95% ethanol with a Beckman Model DU spectrophotometer. The infrared studies pertaining to this paper were carried out on a Perkin-Elmer Model 21 double beam spectrometer in the Division of Pure Chemistry of the National Research Council of Canada in Ottawa, Ontario. The interpretations[25] are by Dr. R. Norman Jones. The transposition of the original recordings for publication was done in the Eastern Regional Research Laboratory in Philadelphia through the courtesy of Dr. C. Roland Eddy.

Analyses. Unless stated otherwise, the microanalyses were performed by Dr. E. W. D. Huffman, Wheatridge, Colo., on samples which were dried to constant weight *in vacuo* (phosphorus pentoxide; 80°); according to Milner and Sherman.[26] The percentage loss of weight on drying is recorded. In no instance was there a gain of weight on exposure of the dried sample to the atmosphere.

Optical rotation. No correction for crystal solvent has been made. Unless stated otherwise, the sample was dissolved in chloroform to make 2 cc. of solution and the rotation was determined in a 2-dm. semimicro tube.

Chromatography. The alumina and silica gel used as adsorbents for chromatography have been described.[2b]

3β,5,14,19,21-Pentahydroxy-5β,14β-pregnan-20-one (IV) *from strophanthidol* (I). A stream of oxygen containing approximately 2.5% of ozone was passed at −80° through a solution of 2.49 g. of pure I in 500 cc. of redistilled ethyl acetate for 30 min. After keeping the blue colored solution at −80° for an additional 30 min. and removing the excess ozone by a stream of oxygen, the solvent was removed *in vacuo* at room temperature. The ozonide was dissolved in 50 cc. of glacial acetic acid and zinc dust was added in small portions with shaking until a negative starch-iodide test was obtained. After filtering and washing the precipitate with glacial acetic acid, the reaction mixture was brought to dryness *in vacuo* from the frozen state. The residue was dissolved in a total volume of 700 cc. of chloroform which was washed to neutrality with *N* sodium carbonate and with water. After drying over sodium sulfate and removing the solvent *in vacuo*, 1.920 g. of a colorless foam resulted, representing crude 21-glyoxyloxy-3β,5,14,19-tetrahydroxy-5β,14β-pregnan-20-one (V).[12a] From the carbonate phase there was isolated in the usual fashion 0.411 g. of resinous acidic material.

To 1.461 g. of crude V (preceding experiment) in 80 cc. of absolute ethanol was added 1.40 g. of anhydrous sodium carbonate in 8 cc. of water. The mixture was heated under reflux in a nitrogen atmosphere for 45 min. and was then filtered through cotton into chloroform, the final volume being 700 cc. The solution was washed twice with water, dried over sodium sulfate, and evaporated to a pale yellow foam, 1.261 g. From acetone-hexane, button-shaped crystalline aggregates (IV) separated; 0.790 g., m.p. 178–181° (yield from I: 34%). Repeated recrystallization from acetone gave clusters of plates of constant m.p. 186–188°. $[\alpha]_D^{21}$ +67° ± 1°; M_D^{21} +256° (14.40 mg. in 2 cc. of chloroform containing 5 drops of ethanol, α +0.96°).

Anal. Calcd. for $C_{21}H_{34}O_6$ (382.48): C, 65.94; H, 8.96. Found: C, 65.84; H, 9.17. Residue, 0.34.

As a variation of the saponification procedure, 0.460 g. of crude V (*v. supra*) in 16 cc. of methanol was mixed with a solution of 0.460 g. of potassium bicarbonate in 8 cc. of

(21) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez, and G. Rosenkranz, *J. Am. Chem. Soc.*, **75**, 4712 (1953).

(22) S. H. Eppstein, P. D. Meister, H. M. Leigh, D. H. Peterson, H. C. Murray, L. M. Reineke, and A. Weintraub, *J. Am. Chem. Soc.*, **76**, 3174 (1954).

(23) Compound not described.

(24) This paper.

(25) In these interpretations reference is made to: (a) Konrad Dobriner, E. R. Katzenellenbogen, and R. Norman Jones, *Infrared Absorption Spectra of Steroids. An Atlas*, Interscience Publishers, Inc., New York, 1953. (b) Glyn Roberts, Beatrice S. Gallagher, and R. Norman Jones, *Infrared Absorption Spectra of Steroids. An Atlas*, Volume II, Interscience Publishers, Inc., New York, 1958.

(26) R. T. Milner and M. S. Sherman, *Ind. Eng. Chem., Anal. Ed.*, **8**, 427 (1936).

water. The mixture was kept under an atmosphere of nitrogen at room temperature for 18 hr. and was then poured into 500 cc. of chloroform. The solution was washed to neutrality (litmus) with water, dried over sodium sulfate and evaporated to dryness, yielding 0.366 g. of a pale yellow foam. Crystallization from acetone-hexane and subsequent recrystallization from acetone gave clusters of plates (IV); 1st crop: 110 mg., m.p. 183–186°; 2nd crop: 101 mg., m.p. 180–183° (yield from I: 37.5%); no depression of melting point when mixed with an analytical sample (*v. supra*).

3β,19,21-Triacetoxy-5,14-dihydroxy-5β,14β-pregnan-20-one (VII). To 40 mg. of IV, m.p. 180–183°, in 3 cc. of dry pyridine was added 1.7 cc. of acetic anhydride. The solution was kept at room temperature overnight and was then poured onto a mixture of ice and hydrochloric acid. Working up as usual and crystallizing from acetone-hexane gave 45 mg. of stout needles, m.p. 185–190°. Repeated recrystallization raised the m.p. to 200–204°. There was no depression of the melting point when mixed with an authentic sample[12] of m.p. 195–200°.

5,14,19,21-Tetrahydroxy-5β,14β-pregnane-3,20-dione (VIII) *from 3β,5,14,19,21-pentahydroxy-5β,14β-pregnan-20-one* (IV). A total of 0.981 g. of IV, m.p. 179–183°, was dissolved in 54 cc. of *tert*-butyl alcohol, followed by the addition of 19 cc. of water. A stream of nitrogen[26] was passed through the solution and vessel, and then 750 mg. of *N*-bromoacetamide (96% by titration; slight excess of 2 equivalents) was added. The solution was kept at room temperature under an atmosphere of nitrogen[27] for 17 hr. The free bromine was then destroyed by the addition of sodium thiosulfate. After adding water, the mixture was extracted with chloroform-ethanol (3:1); total volume approximately 1 l. The solution was washed to neutrality with water, dried over sodium sulfate, and evaporated to a colorless foam, 1.071 g. On adding acetone, small prisms (VIII) separated overnight; 520 mg., m.p. 160–170° (yield 53%). To ensure its uniformity, this material was subjected to numerous recrystallizations, finally yielding 307 mg., m.p. 155–157° and 170 mg., m.p. 152–155°. On these crystallizations occasionally a polymorphic form of m.p. 165–168° was obtained. In a paper chromatogram (system: chloroform-formamide; development time: 18 hr.; indicator: triphenyltetrazolium chloride) the modifications of m.p. 165–168° and m.p. 155–157° showed the same behavior in that they gave identical R_f values. Both modifications were examined in the ultraviolet (concentrations: 0.5 mg. in 10 cc. of ethanol) in the range of 220–300 mμ and no absorption was observed. The analytical data were obtained with the product of m.p. 155–157°. $[\alpha]_D^{21}$ +62° ± 2°; M_D^{21} +236° (17.45 mg. in 2 cc. of chloroform containing 5 drops of ethanol, +1.08°).

Anal. Calcd. for $C_{21}H_{32}O_6$ (380.47): C, 66.29; H, 8.48. Found: C, 66.30; H, 8.40. Weight loss, 0.49.

14β,19-Dihydroxycortexone [*14,19,21-trihydroxy-Δ⁴-14β-pregnene-3,20-dione*] (IX) *from 5,14,19,21-tetrahydroxy-5β,14β-pregnane-3,20-dione* (VIII). To 142 mg. of VIII, m.p. 155–157°, in 6 cc. of absolute ethanol was added 215 mg. of Girard's reagent T and 0.1 cc. of glacial acetic acid. The mixture was refluxed for 1 hr.[28] and, after cooling to 0°, a solution of 65 mg. of sodium carbonate in 12 cc. of water was added. Extraction with four 20-cc. portions of chloroform in the cold yielded 14 mg. of resinous nonketonic material. The aqueous phase was then acidified to *p*H 2 with 1*N* hydrochloric acid and, after standing for 90 min., it was extracted with eight 25-cc. portions of chloroform. After washing with dilute aqueous sodium carbonate and with water, the combined chloroform extracts were dried over sodium sulfate and evaporated to dryness, yielding 84 mg. of crystalline ketonic material, m.p. 190–193°. Reextraction of the reaction mixture with eight 20-cc. portions of chloroform-ethanol, 3:1, gave 19 mg. of additional crystalline material, m.p. 190–194°; total yield of crude crystalline IX: 103 mg. (76%). Repeated recrystallization from acetone-hexane gave clusters of fine needles; constant m.p. 213–216°. $[\alpha]_D^{21}$ +120° ± 1°; M_D^{21} +435° (10.43 mg., α +1.25°). λ_{max}^{alc} 242 mμ, ε 15,100.

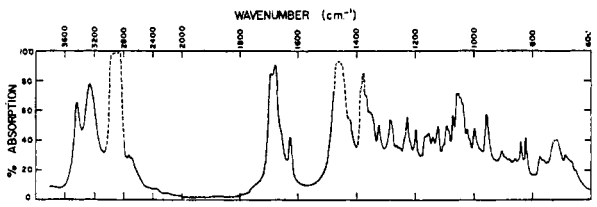


Fig. 3. Infrared spectrum of 14β,19-dihydroxycortexone (IX)

The infrared spectrum (Nujol mull; see Fig. 3) shows two OH bands at 3438 and 3260 cm.$^{-1}$, two C=O stretching bands at 1695 and 1673 cm.$^{-1}$ and a C=C stretching band at 1626 cm.$^{-1}$ These are all consistent with the proposed structure, the 1695 cm.$^{-1}$ band being assigned to the 20-ketone, and the 1673 and 1626 cm.$^{-1}$ bands to the Δ⁴-3-ketone group.

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.59; H, 8.34. Found: C, 69.39; H, 8.22.

14β,19-Dihydroxycortexone 19,21-diacetate [*19,21-diacetoxy-14-hydroxy-Δ⁴-14β-pregnene-3,20-dione*] (X) *from 14β,19-dihydroxycortexone* (IX). To 50 mg. of IX, m.p. 215–217°, was added 2 cc. of dry pyridine and 2 cc. of acetic anhydride. The solution was kept at room temperature for 17 hr. and was then poured into a suspension of ice in 15 cc. of 3*N* sulfuric acid. Extraction with chloroform in the usual fashion yielded 65 mg. of yellowish resin which was decolorized by treatment with charcoal in a solution of ethanol. Thus, 59.3 mg. of a colorless resin resulted which was crystallized from acetone-hexane; yield: 51.8 mg. (84%) of crude crystalline X. Recrystallization from the same solvents gave 49.3 mg. of shiny platelets of constant m.p. 137–139°. $[\alpha]_D^{21}$ +151° ± 3°; M_D^{21} +674° (8.12 mg., α +1.23°). $[\alpha]_D^{21}$ +153°; M_D^{21} +683° (21.18 mg., α +3.24°).[29] λ_{max}^{alc} 238 mμ, ε 15,000.

The infrared spectrum was determined as a Nujol mull (see Fig. 4) and also in a solution of carbon disulfide. As the solubility was not very good, the solution spectrum (carbon disulfide) is mainly of interest for the C=O stretching region. The spectra show free OH at 3380 cm.$^{-1}$ in Nujol and 3440 cm.$^{-1}$ in carbon disulfide, confirming a non-

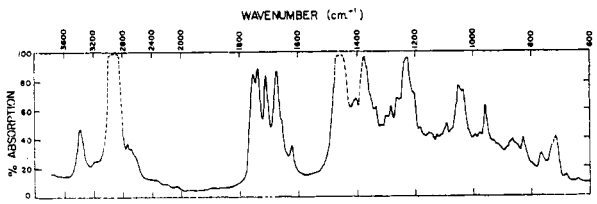


Fig. 4. Infrared spectrum of 14β,19-dihydroxycortexone 19,21-diacetate (X). The dotted lines in Figs. 3 and 4 indicate that the Nujol is absorbing in these regions

(27) It is essential that the oxidations with *N*-bromoacetamide be carried out in a nitrogen atmosphere. Papergram analysis of runs carried out in air demonstrated the presence of three substances: (1) a very polar acid (probably an etio acid); (2) the desired product (VIII); (3) a very nonpolar spot which has the same characteristics as 14β,19-dihydroxycortexone (IX). The system used was chloroform-formamide; development time: 21 hr.

(28) In subsequent experiments this was followed by concentrating the mixture *in vacuo* at room temperature to approximately one-fourth of its original volume. Otherwise the procedure was unchanged.

(29) The rotation was determined on samples resulting from two different acetylation experiments.

acetylated hydroxyl group. The absorption in the C=O and C=C stretching regions is complex, *viz.*,

Nujol (Fig. 4)	Carbon Disulfide	
1753 cm.$^{-1}$	1758 cm.$^{-1}$[a]	21-Acetate group
1736	1747[a]	Probably 19-acetate
1710	1720	20-Ketone
1672	1677	Δ^4-3-Ketone
1622	[b]	Δ^4—C=C—

[a] Unresolved doublet. [b] Obscured by solvent absorption.

This analysis is consistent with expectations for the proposed structure. The 19-acetate band at 1747 cm.$^{-1}$ (carbon disulfide) is high for an acetate group in an unperturbed position, but it is seen in a similar position in 19-acetoxy-Δ^4-androstene-3,17-dione (Ref. 25b, spectrum 530) where it overlaps the 17-ketone band completely, and also for 19,21-diacetoxy-Δ^4-pregnene-3,20-dione (Ref. 25b, spectrum 583) which in this region is very similar to the spectrum under consideration.

The band at 1710–1720 cm.$^{-1}$ assigned to the 20-ketone is displaced down from its position in most 21-acetoxy-20-ketones, and is nearer to its normal position in 21-methyl-20-ketones. This band is the most sensitive to the environmental effects, both intermolecular and intramolecular and this displacement could be rationalized in terms of the effect of the nearby 14β-hydroxyl group. The absorption of the Δ^4-3-ketone group is normal.

The finger-print region is normal, and may be compared with that of 19,21-diacetoxy-Δ^4-pregnene-3,20-dione (Ref. 25b, spectrum 583). The strong band at 960 cm.$^{-1}$ (Fig. 4) is of interest and might be associated with the 14β-hydroxyl group. A band at this position is seen also in the spectra of the cardiac aglycones (Ref. 25a, spectra 289, 290, 291, 292).

Anal. Calcd. for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68. Found: C, 67.06; H, 7.79. Weight loss, 4.03. Residue, 0.26. (Dried at room temp.)

3-Oxo-14,19-dihydroxy-Δ^4-14β-etienic acid (XI) *from 14β,19-dihydroxycortexone* (IX). To 87 mg. of fairly pure IX (pooled samples, melting points between 201° and 207°) in 8 cc. of methanol was added a solution of 153 mg. of periodic acid (H_5IO_6) in 1.1 cc. of water. The mixture was kept at room temperature for 19 hr. and was then poured into 150 cc. of chloroform. After the addition of 10 cc. of water, the aqueous phase was extracted with three 10-cc. portions of chloroform. The combined chloroform fractions were washed with three 10 cc. portions of aqueous *N* sodium carbonate and with water. After drying over sodium sulfate and removal of the solvent, 28 mg. of a pale yellow resin resulted (*neutral fraction*) which was chromatographed on 1.2 g. of alumina (act. III; diam. of column; 5 mm.). This furnished 17 mg. of crystalline material which, on recrystallizing from acetone-hexane, gave 14 mg. of feltlike needles, m.p. 177–178° (soluble in benzene). $[\alpha]_D^{21}$ +110° ± 2° (8.06 mg., α +0.73°). λ_{max}^{alc} 242 mμ, ε 13,500. This compound was recognized to be methyl 3-oxo-14,19-dihydroxy-Δ^4-14β-etienate (XII) (lit.[15]: m.p. 174–176°. $[\alpha]_D^{28}$ +109.3°. λ_{max}^{alc} 242 mμ, ε 13,800).

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.59; H, 8.34. Found: C, 70.21; H, 8.53. Weight loss, 0.27.

On acetylating approximately 1 mg. of the crystalline neutral material, m.p. 177–178°, with acetic anhydride in pyridine as usual, a crude amorphous reaction product resulted which was not subjected to purification, λ_{max}^{alc} 238 mμ; ε 16,100. (Note the expected hypsochromic shift of the absorption maximum.)

The carbonate phase was immediately acidified with 3*N* sulfuric acid and the acid material was extracted with ten portions of 15 cc. of chloroform. After washing with water and drying over sodium sulfate, the solvent was removed *in vacuo*, leaving 48 mg. of a pale yellow resin (*acid fraction*) which crystallized from acetone-hexane; yield: 35 mg. of crude XI, m.p. 200–205°. Repeated recrystallization from acetone-hexane gave 27 mg. of fine needles with the constant m.p. 213–215° dec. $[\alpha]_D^{21}$ +94° ± 1°. M_D^{21} +328° (8.82 mg. in 2 cc. of chloroform containing a few drops of ethanol, α +0.83°). λ_{max}^{alc} 242 mμ, ε 16,000.

Anal. Calcd. for $C_{20}H_{28}O_5$ (348.42): C, 68.94; H, 8.10. Found: C, 68.91; H, 8.20. Weight loss, 0.48.

Characterization of XI: *Preparation of ethyl 3-oxo-14,19-dihydroxy-Δ^4-14β-etienate* (XIII). To 6 mg. of XI, m.p. 213–215°, in 3 cc. of absolute ethanol was added an excess of an ethereal solution of diazoethane.[30] After standing for some time, the solution was evaporated to a colorless oil, which was dissolved in acetone. A small quantity of amorphous material was filtered off and crystallization was brought about from acetone-hexane; plates of m.p. 181–185°. Recrystallization gave 4 mg. of plates, m.p. 188–189°. There was no depression of the melting point when mixed with an authentic sample of XIII.[16] Papergram analysis in the system toluene–propylene glycol (development time: 6 hr.) gave single spots and identical R_f values for the reaction product and the authentic sample of XIII.[16]

Purification of ethyl 3-oxo-5,14,19-trihydroxy-5β,14β-etianate (XIV). Papergram analysis (system: toluene–propylene glycol; fluorescent spots visible in ultraviolet light after spraying with 85% phosphoric acid) of the product described previously[31] indicated the presence of a small amount of a more polar impurity. Purification was achieved by chromatography over silica gel and elution with chloroform–acetone. Recrystallization of pertinent chromatographic fractions from acetone-hexane gave needles; constant m.p. 150–152°. Papergram analysis of this material yielded only a single spot.

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(30) Prepared from ethylnitrosourea according to the *Organic Syntheses* directions for diazomethane. A. H. Blatt, *Org. Syntheses*, Coll. Vol. II, 165, 461 (1943).

(31) Compound XIX in the paper cited in ref. 16.