pentoxide and had m.p. 162–164°, $[\alpha]D$ +18 ± 2°, λ_{max}^{MeOH} 245 mμ (ε 12,500) and 385 (17,100).

Anal. Calcd. for $C_{27}H_{34}N_2O_3$: C, 74.62; H, 7.88; N, 6.45. Found: C, 74.69; H, 7.96; N, 6.44.

17-Hydroxy-3,20-dioxopregn-4-en-21-al 21-Phenylhydrazone (XIV) from Reichstein's Substance S.—To 173 mg. (0.5 mmole) of 17,21-dihydroxypregn-4-ene-3,20-dione in 12.5 ml. of methanol was added 25 mg. of cupric acetate in an equal volume of methanol. After 1 hr., during which time air was blown into the solution, the glyoxal was recovered in the usual fashion.[8] The product in 5 ml. of 85% acetic acid was treated with 150 mg. (1.04 mmoles) of phenylhydrazine hydrochloride in 5 ml. of the same solvent. After 15 min. at room temperature, the yellow phenylhydrazone (XIV) was precipitated with water, washed, and dried to give 174 mg. (80%) of product, m.p. 134–135.5°. The sample for analysis was recrystallized from methylene chloride–ether and had m.p. 184–186°, $[\alpha]D$ +375 ± 3°, λ_{max}^{MeOH} 241 mμ (ε 26,500) and 365 (21,100).

Anal. Calcd. for $C_{27}H_{34}N_2O_3$: C, 74.62; H, 7.88; N, 6.45. Found: C, 74.72; H, 7.88; N, 6.49.

3α-17-Dihydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (XV). A. From the Porter–Silber Reagent and THE Glyoxal.—To 182 mg. (0.5 mmole) of 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE) in 12.5 ml. of methanol was added an equal volume of 0.005 *M* methanolic cupric acetate. Air was blown into the solution for 50 min., and the resulting glyoxal was recovered in the usual manner.[8] It was dissolved in 25 ml. of methanol and, while being cooled in an ice bath, was treated with 50 ml. of 7:3 sulfuric acid–water which contained 100 mg. of phenylhydrazine hydrochloride. After 45 min. at room temperature, the reaction mixture was added to ice–water, and the solution was extracted with methylene chloride. The extract was washed with water and concentrated to dryness. The residue was reprecipitated from aqueous acetic acid and dried over anhydrous calcium chloride. The product (83 mg.) melted at 144–147.5°. It was purified by chromatography on a 1.8 × 36 cm. column in the system cyclohexane–benzene–methanol–water (300:200:80:20). Fractions (7 ml.) were numbered after 48 ml. of effluent had been discarded.

Fractions 16–27.—Precipitation from aqueous acetic acid gave 74 mg. (m.p. 147.5–149.5°) of XV. A sample for analysis was reprecipitated from acetic acid; the yellow solid was dried for 15 hr. at 100° at 1–2 mm.; the melting point was 147.5–148.5°; $[\alpha]D$ +185 ± 2°; λ_{max}^{MeOH} 241 mμ (ε 10,300), 298 (2300), and 365 (21,000).

Anal. Calcd. for $C_{27}H_{36}N_2O_4 \cdot H_2O$: C, 68.90; H, 8.14; N, 5.95. Found: C, 68.61; H, 8.07; N, 5.76.

B. Compound XV from Phenylhydrazine in Aqueous Acetic Acid and THE Glyoxal.—A solution of 1.09 g. (3 mmoles) of THE in 75 ml. of methanol was oxidized with 150 mg. of cupric acetate in the usual manner.[8] The amorphous, yellow methylene chloride extract weighing 855 mg. (79%) was dissolved in 25 ml. of 50% acetic acid and treated with a solution of phenylhydrazine hydrochloride (500 mg.) in 10 ml. of the same solvent. After 25 min. at room temperature the reaction mixture was added to 150 ml. of water. The resulting yellow precipitate was collected, washed with water, and dried over calcium chloride to give 810 mg. (60%) of XV, m.p. 146.5–148.5°. The infrared spectra in Nujol of this product and of the product of the reaction of the Porter–Silber reagent with THE glyoxal were identical.

3α-Hydroxy-5β-androstane-11,17-dione (XVI) from XV.—To a solution of 226 mg. (0.5 mmole) of 3α,17-dihydroxy-11,20-dioxo-5β-pregnan-21-al 21-phenylhydrazone (XV) in 10 ml. of methanol was added 7.6 ml. of a 1% solution of sodium borohydride in 50% methanol. After 30 min. at room temperature, the solution was added to dilute acetic acid. The resulting precipitate was collected, washed with water, and dried over calcium chloride. The yellow solid (200 mg.) melted at 143–145°. A mixture melting point with starting material was 135–140°. This product was assumed to be the 17,20-dihydroxy steroid 21-phenylhydrazone and was characterized as follows. An aliquot (100 mg. in 10 ml. of methanol and 4 ml. of water) was mixed with 6 ml. of 4% periodic acid in 0.2 *N* sulfuric acid. After 3 hr. at room temperature, the reaction mixture was added to water and extracted with methylene chloride. The extract was washed with dilute sodium thiosulfate solution and water, filtered through anhydrous sodium sulfate, and concentrated to dryness. The residue was chromatographed on a 1.8 × 36 cm. column prepared from 35 g. of Celite and 17.5 ml. of lower phase from the system cyclohexane–benzene–methanol–water (300:150:80:20). Fractions (7-ml. each) were collected after 40 ml. of effluent had been discarded.

Fractions 20–30.—Crystallization from acetone–petroleum ether gave two crops (20 mg., m.p. 189–191.5°; 10 mg., m.p. 186–189°) of colorless prisms in 45% yield. A mixture melting point with an authentic sample of 3α-hydroxy-5β-androstane-11,17-dione was 189.5–191.0°. The infrared spectrum of the isolated compound and that of authentic material were identical

11,19-Oxygenated Steroids Derived from Ouabagenin[1]

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The conversion of the cardiac aglycone, ouabagenin (**1a**), to cardanolides, 20(22)-cardenolides, and pregnanes oxygenated at C-11 and C-19, and to 11-oxo-19-norcard-20(22)-enolides is described.

The useful and unique action of the cardiac glycosides on the failing heart is well-documented.[2] In addition to their effects on cardiac muscle, data suggest that the cardiac glycosides and some of their aglycones might be important for their renal effects.[3] The latter possibility, therefore, prompted an investigation into the synthesis of derivatives of the aglycone, ouabagenin (**1a**), which can be obtained from the relatively abundant and potent cardiac stimulant, ouabain (**1c**).[4] Ouabagenin, by virtue of its polyhydroxylated steroid nucleus and potential hydroxyacetyl side chain in the 17β-position, derivable from the cleavage of the α,β-unsaturated lactone, might lend itself to conversion to a variety of oxygenated pregnane derivatives.[5,6] This consideration then provided a dual purpose for further investigation of the chemistry of ouabagenin.

The investigation began with the observation that the unsaturated lactone in **1a** is inert to catalytic hydrogenation in methanol with 5% palladium on charcoal at room temperature.[7] Therefore, now it was

(1) Presented in part as a preliminary communication in *Tetrahedron Letters*, **No. 13**, 425 (1961).

(2) (a) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 727; (b) R. P. Walton, "Pharmacology in Medicine," V. A. Drill, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1958, p. 451.

(3) J. C. Strickler, R. H. Kessler, and B. A. Knutson, *J. Clin. Invest.*, **40**, 311 (1961).

(4) Ref. 2a, p. 768.

(5) Previous studies on degradation of ouabagenin were directed toward structural determination. See leading reference, G. Volpp and C. Tamm, *Helv. Chim. Acta*, **46**, 219 (1963).

(6) The conversion of ouabagenin to some 14β-hydroxypregnane derivatives has been accomplished recently by C. Tamm and W. Zurcher, *ibid.*, **46**, 237 (1963).

(7) It has been observed that the hydrogenation of the α,β-unsaturated lactone in strophanthidin in aqueous methanol with colloidal palladium proceeds sluggishly; see W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **54**, 253 (1922).

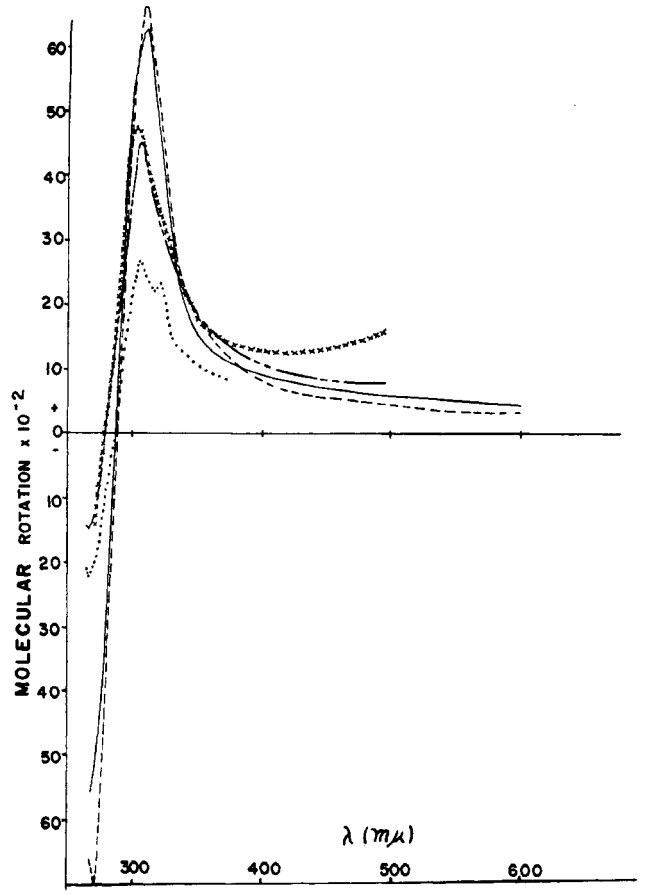
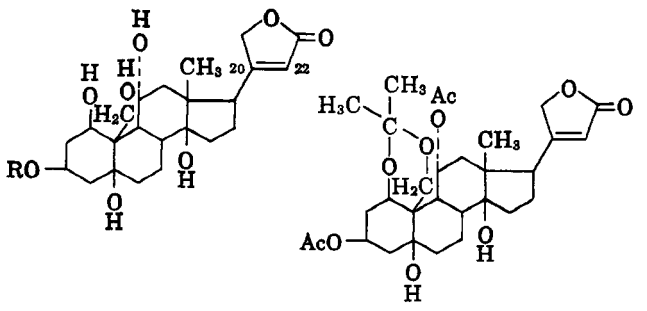


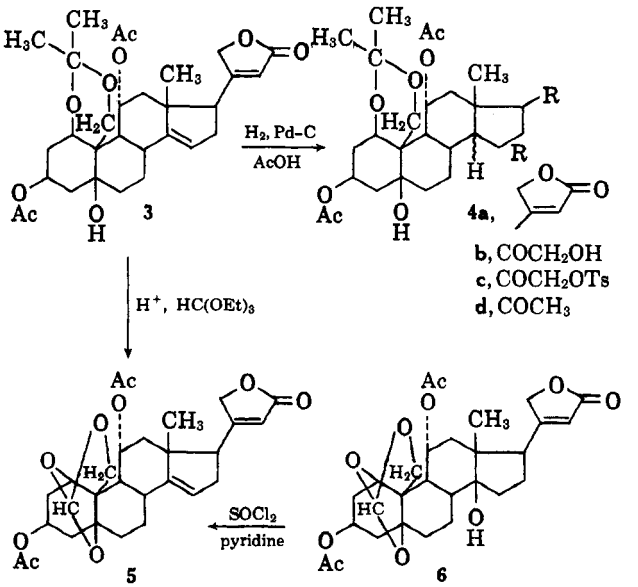
Fig. 1.—Optical rotatory dispersion curves in methanol solution: (———) 3β,11α-diacetoxy-1β,5β,19,21-tetrahydroxy-14ξ-pregnan-20-one 1,19-acetonide (**4b**); (– – – –) 3β,11α-diacetoxy-3β,5β,19-trihydroxy-14ξ-pregnan-20-one 1,19-acetonide (**4d**); (· · · ·) 1β,3β,11α,19-tetracetoxy-5β,14β,21-trihydroxypregnan-20-one (**22**)[13]; (x x x x) 21-hydroxy-3,3-11α-trimethoxy-11,19-epoxy-5β,14β-pregnan-20-one 21-acetate (**25a**); (— – –) 3,3,11α-trimethoxy-11,19-epoxy-5β,14β-pregnan-20-one (**25b**).



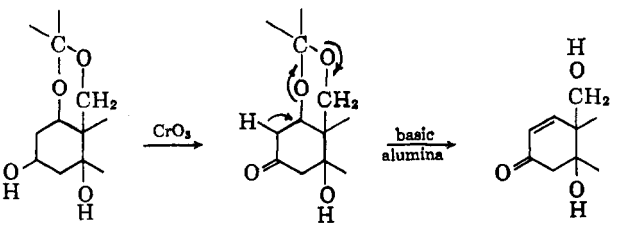
1a, R = H
b, R = H, 20(22)-Dihydro
c, R = L-Rhamnosyl

2

possible to consider the selective removal of hydroxyl groups and catalytic reduction of the generated double bonds with preservation of the α,β-unsaturated lactone. In particular, the hydroxyl group at C-14 in the 3,11-diacetate of ouabagenin 1,19-acetonide (**2**)[8] would be expected to dehydrate with thionyl chloride in pyridine in preference to the hydroxyl group at C-5, which, having strong 1,3-diaxial hydrogen-bonded interactions with oxygen atoms on C-1, 3, and 19, is less accessible for ester formation with thionyl chloride. The expectation was realized when **2** was dehydrated with an equivalent of thionyl chloride in pyridine at −15° in 89% yield. Structure **3** was substantiated by its n.m.r. spectrum (vinyl protons, τ 4.05 and 4.58) and its conversion to the orthoformate derivative (**5**), which was identical with that obtained by the dehydration of 3,11-diacetylouabagenin 1,5,19-orthoformate (**6**).[9]



Efforts were then directed toward the conversion of the cardadienolide (**3**) to oxygenated pregnane derivatives. Hydrogenation of **3** with 5% palladium on charcoal in acetic acid afforded the crystalline 14-desoxy derivative (**4a**) in 43% yield. Ozonolysis of the unsaturated lactone (**4a**) followed by hydrolysis of the resulting glyoxalate gave **4b**. Its *p*-toluenesulfonate (**4c**) was converted to its 21-iodo derivative which on treatment with zinc and acetic acid was reduced to the 20-oxo-14ξ-pregnane (**4d**).[10] The o.r.d. curves of **4b** and **4d** (see Fig. 1) display a positive Cotton effect (a = 118 and 136, respectively) expected of a 20-oxopregnane with a 17β side chain.[11,12] In light of the experience already available on the chemistry of ring A in ouabagenin,[5] an attempt was made to modify ring A in **4d** by the sequence of reactions, **a** → **b** → **c**. The diacetate (**4d**), therefore, was hydrolyzed to an



amorphous dihydroxy derivative which was oxidized with chromium trioxide–pyridine complex. The resulting product when treated with hot alcohol and basic alumina gave in low yield an intractable substance

(8) R. P. A. Sneeden and R. B. Turner, *J. Am. Chem. Soc.*, **75**, 3510 (1953).

(9) R. Tschesche and G. Snatzke, *Ber.*, **88**, 1558 (1955).

(10) T. Reichstein and H. G. Fuchs, *Helv. Chim. Acta*, **23**, 686 (1940).

(11) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 51.

(12) The low amplitudes of the curves **25a** and **25b** may be typical of 17β-acetyl steroids having the 14-isoconfiguration, see, *e.g.*, the o.r.d. curve of **22**[13] (see Fig. 1); the high amplitudes exhibited by the o.r.d. curves of **4b** and **4d** may indicate they have the normal (14α) pregnane skeleton (private communication, W. Klyne). Also see K. A. Jaeggi, E. K. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 494 (1963).

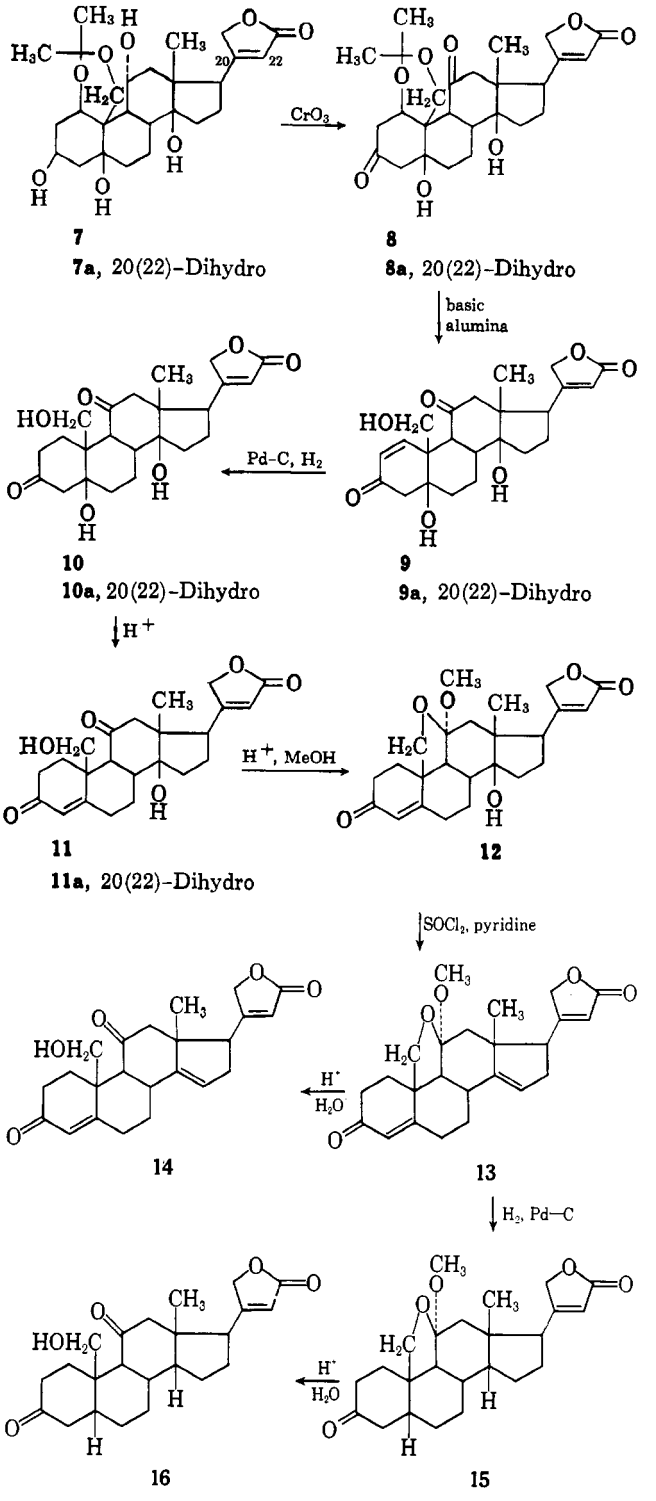
(13) R. F. Raffauf and T. Reichstein, *ibid.*, **31**, 2111 (1948).

whose infrared spectrum indicated that aromatization of ring A had occurred. The desire for a more practical route to pregnanes derived from ouabagenin led to the consideration of another scheme for degradation.

During the investigation of the synthesis of derivatives of 20(22)-dihydrouabagenin (**1b**), the substance **11a** was prepared in the following manner: hydrogenation of ouabagenin-1,19-acetonide (**7**)[14] with platinum catalyst gave **7a** which was oxidized to **8a** with chromium trioxide–pyridine complex; **8a** was then converted to **9a** with a mixture of hot ethanol and basic alumina; hydrogenation of **9a** in the presence of 5% palladium on charcoal yielded **10a** which was dehydrated with boiling acetic acid to 14,19-dihydroxy-3,11-dioxocard-4-enolide (**11a**).

The infrared spectrum in potassium bromide of **11a** indicated very weak absorption at 5.82 μ and, therefore, disclosed that the 11-ketone was masked in large part as an 11,19-hemiketal.[15] It was now possible to consider utilizing a single ketal group to protect the 19-hydroxy-11-keto group under certain reaction conditions. The possibility was reduced to practice when the reaction scheme for the conversion of **7a** to **11a** was applied to ouabagenin-1,19-acetonide (**7**). A study of the conditions for the base-catalyzed, selective elimination of acetone in **8** resulted in a modified procedure for the preparation of **9**,[16] in which **8** was treated with boiling ethanol and basic alumina to give **9** in an over-all 56% yield from **7**. Catalytic hydrogenation in methanol or dimethylformamide with 5% palladium on charcoal proceeded smoothly to yield **10** which upon dehydration with boiling acetic acid gave **11**. This substance, which also showed a weak carbonyl absorption band at 5.83 μ in its infrared spectrum, yielded 14-hydroxy-11α-methoxy-11,19-epoxy-3-oxocarda-4,20(22)-dienolide (**12**) when dissolved in methanol containing *p*-toluenesulfonic acid. Conversion of crude **9** to **12** also could be accomplished without isolation of intermediates in 72% yield. Dehydration of **12** with thionyl chloride in pyridine at $-15°$ proceeded in high yield to **13**. Acid-catalyzed hydrolysis of **13** gave the hydroxy ketone **14**, the infrared spectrum of which indicated only hydrogen bonding between the 19-hydroxyl and 11-carbonyl groups. The hydrogenation of **13** in ethyl acetate–methanol (4:1) with 5% palladium on charcoal proceeded rapidly at a constant rate until 2 equiv. of hydrogen was absorbed and yielded one product, **15** (Scheme I). The infrared, ultraviolet, and n.m.r. spectra had maxima characteristic of the structural features present in **15**. They were λ_{max}^{KBr} 5.83 μ, saturated ketone; λ_{max}^{MeOH} 216 mμ (ϵ 16,700), unsaturated lactone; τ 9.17 (methyl), 6.78 (methoxyl), 6.36, 6.19, 6.00, 5.87 (C-19 methylene), 5.24 (C-21 methylene), 4.20 (vinyl proton). Acid-catalyzed hydrolysis of **15** gave the hydroxy diketone (**16**). The optical rotatory dispersion studies on **15** and related keto steroids and comparison of other derivatives of **15** with compounds of known stereochemistry furnished evidence upon which the 5β,14β configuration in **15** is now assigned.[17]

SCHEME I



The syntheses of C-3 ketones related to **15** are described presently. The 20(22)-dihydro derivative (**21**) was obtained by the catalytic hydrogenation of **15** with platinum and subsequent reoxidation of the product with chromium trioxide–pyridine complex. Catalytic reduction of **12** with 5% palladium on charcoal

(14) C. Mannich and G. Siewert, *Ber. deut. chem. Ges.*, **75**, 737 (1942).

(15) The interaction between a 19-hydroxyl and 11-ketone functions apparently does not always result in hemiketal formation. The infrared spectrum of 3,20-diethylenedioxy-19-hydroxy-5α-pregnan-11-one only shows hydrogen bonding [H. Wehrli, M. S. Heller, K. Schaffner, and O. Jeger, *Helv. Chim. Acta*, **44**, 2162 (1961)]; also **26a** shows no tendency to form an 11α-O-methyl ether with methanol containing TsOH (private communication, K. Schaffner).

(16) C. Tamm, *ibid.*, **38**, 147 (1955).

(17) The previous conclusion[1] [based on stability relationships of C-17 acetyl steroids and the original o.r.d. curve in chloroform solution, which was similar to that of **21** (see Fig. 2) and was misinterpreted] that **15** possesses the 5α,14α skeleton was erroneous. The author is grateful to Professor W. Klyne for the o.r.d. curve of **15** in methanol solution and an interpretation of it.

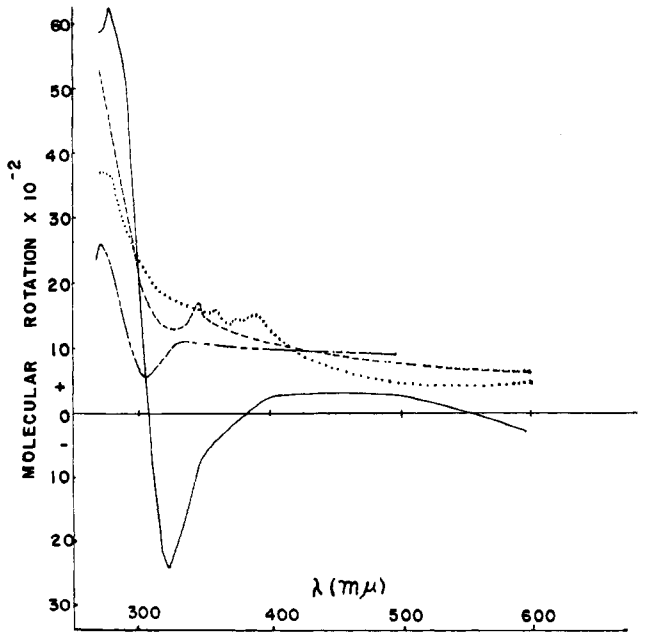
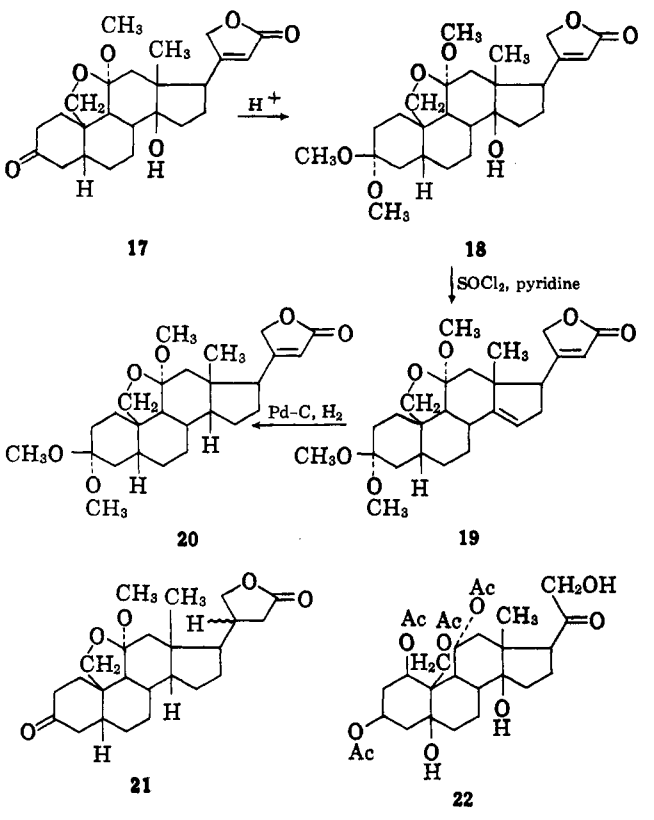


Fig. 2.—Optical rotatory dispersion curves in methanol solution: (· · · ·) 11α-methoxy-11,19-epoxy-3-oxo-5β-card-20(22)-enolide (**15**); (— — —) 14-hydroxy-11α-methoxy-11,19-epoxy-3-oxo-5β-card-20(22)-enolide (**17**); (– – – –) 11α-methoxy-11,19-epoxy-3-oxo-5β,20ξ-cardanolide (**21**); (———) 3α,19-diacetoxy-11-oxo-5β,14β-androstane-17β-carboxylic acid methyl ester (**28**).

in methanol gave **17**, which was converted by the sequence of reactions **17** → **18** → **19** → **20** to the dimethyl ketal (**20**) which also was obtained from **15**. Because of the nature of the chemical changes involved in these transformations, the stereochemistry at C-5 in **15**, **17**, and **21** must be identical; the fact that the o.r.d. curves of these ketones (see Fig. 2) display a negative Cotton effect establishes the configuration at C-5 in these compounds as β.[18,19] (See Scheme II.)

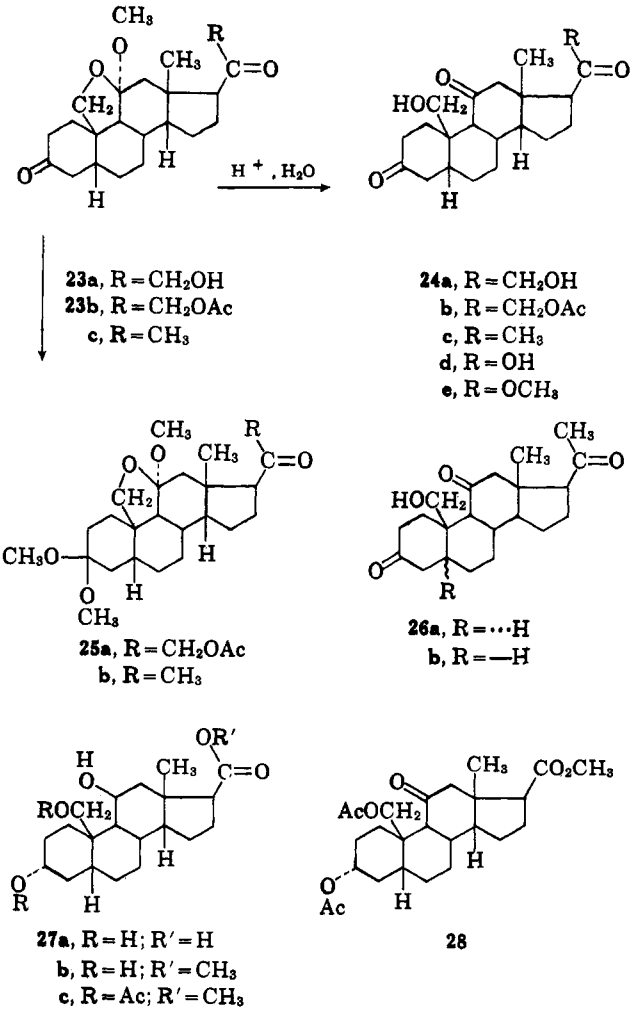
SCHEME II



17 **18** **19** **20** **21** **22**

The syntheses of C-20 and C-21 derivatives of the oxocardenolide (**15**) are outlined below. Ozonolysis of the unsaturated lactone in **15** and hydrolysis of the resulting 21-glyoxalate with aqueous methanolic potassium bicarbonate transformed it into the ketol (**23a**). Acetylation of the ketol with acetic anhydride and pyridine gave the acetate (**23b**). Acid-catalyzed hydrolysis of **23a** and **23b** with aqueous acetone yielded **24a** and **24b**, respectively. The *p*-toluenesulfonate of **23a** was converted to its 21-iodo derivative which, when reduced with zinc and acetic acid, gave the 21-desoxy derivative (**23c**).[10] Both **23b** and **23c** when dissolved in methanol containing trimethylorthoformate and *p*-toluenesulfonic acid were converted to their 3,3-dimethyl ketals, **25a** and **25b**, respectively. Their o.r.d. curves (see Fig. 1) exhibit the expected positive Cotton effect typical of a 17β-acetyl side chain[11,12] (a = 65 and 60, respectively). Acid-catalyzed hydrolysis of **23c** in aqueous acetone gave **24c** which, in a direct comparison with 19-hydroxy-5α-pregnane-3,11,20-trione (**26a**)[20] and 19-hydroxy-5β-pregnane-

SCHEME III



23a, R = CH_2OH
23b, R = CH_2OAc
c, R = CH_3

24a, R = CH_2OH
b, R = CH_2OAc
c, R = CH_3
d, R = OH
e, R = OCH_3

25a, R = CH_2OAc
b, R = CH_3

26a, R = ···H
b, R = —H

27a, R = H; R′ = H
b, R = H; R′ = CH_3
c, R = Ac; R′ = CH_3

28

(18) See ref. 11, p. 50.

(19) The o.r.d. curve of **15** exhibits what probably is a negative Cotton effect superimposed upon a positive rotation curve.

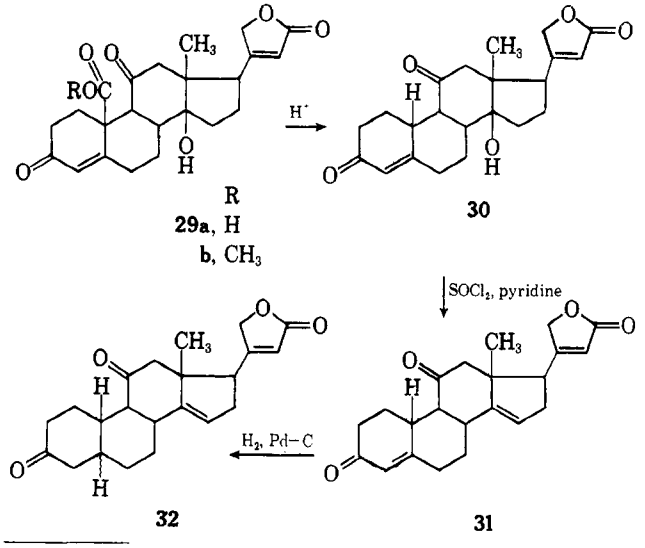
(20) See reference in ref. 15.

3,11,20-trione (**26b**), was found to be different.[21] (See Scheme III.)

Since **24c** and **26b** can only differ with regard to their stereochemistry at C-14, it must be concluded that **24c** possesses the 14-isopregnane nucleus and that the hydrogenation of **13** proceeded from the β-face of the molecule to yield 11α-methoxy-11,19-epoxy-3-oxo-5β-card-20(22)-enolide (**15**). Additional evidence to support this conclusion was obtained when **23a** was converted to **28** as follows: the ketol (**23a**) was oxidized and hydrolyzed with periodic acid in aqueous acetone to the diketoetianic acid (**24d**) which was reduced with sodium borohydride to **27a**; esterification to **27b** with diazomethane and acetylation of **27b** with pyridine and acetic anhydride to **27c** followed by oxidation with chromic acid gave the ketone (**28**); or alternately, the diketone (**24e**) was reduced selectively at C-3 with lithium tri-*t*-butoxyaluminum hydride to a diol which was converted by acetylation to **28**. The o.r.d. curve of the 11-ketone (**28**) exhibited a negative Cotton effect (see Fig. 2) which is consistent only with an 11-keto steroid having a 14-iso configuration.[22,23]

The syntheses of a variety of cardenolides derived from ouabagenin were concluded with the preparation of some 19-norcardenolides from **11** which is summarized below. The hydroxymethyl group in **11** was oxidized with chromium trioxide in acetic acid to the corresponding carboxylic acid (**29a**) which could be esterified with diazomethane to **29b** or decarboxylated by heating in the presence of acid to 14-hydroxy-3,11-dioxo-19-norcarda-4,20(22)-dienolide (**30**).[24] Dehydration of **30** with thionyl chloride and pyridine led to **31**. Hydrogenation of **31** with palladium on charcoal in methanol proceeded to give the 4,5-dihydro derivative (**32**) as the only identifiable product. (See Scheme IV.)

SCHEME IV



(21) The author is grateful to Professor O. Jeger, Dr. K. Schaffner, and Dr. H. Wehrli for having made these comparisons, and for a sample of **26b** (H. Wehrli, Diss. ETH, Zurich, 1962).

(22) C. Djerassi, O. Halpern, V. Halpern, O. Schindler, and C. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958).

(23) In steroids containing the 14-iso (14β) configuration, the more stable configuration at C-17 is also iso (α) [*cf.* K. Meyer, *ibid.*, **30**, 1976 (1947); A. Lardon, *ibid.*, **32**, 1517 (1949); C. P. Balant and M. Ehrenstein, *J. Org. Chem.*, **17**, 1576 (1952)]. In contrast, the 17β-acetyl group at **25b** appears to be stable in the β configuration since, when refluxed with 1 *N* potassium hydroxide in methanol, it is not epimerized. Dreiding models do indicate, however, that, when the 17-acetyl side chain in **25b** is α, there is a substantial steric interaction of the 17-acetyl group with the C-12α hydrogen and 11α-methoxyl group when ring C is either in a boat or chair conformation.

(24) It is assumed that the more thermodynamically stable isomer is formed and is 10β; see (a) A. J. Birch, *J. Chem. Soc.*, 367 (1950); (b) C. Djerassi, R. Riniker, and B. Riniker, *J. Am. Chem. Soc.*, **78**, 6362 (1956).

Experimental

Melting points were determined on a Fisher-Johns block and are not corrected. The ultraviolet spectra were taken in methanol. The n.m.r. data were obtained on a 40-Mc. Varian high resolution spectrometer using tetramethylsilane as standard. Rotations were taken at 25°. The analytical data were determined by R. T. Dillon and his staff of G. D. Searle and Co.

3,11-Diacetyl-14-anhydroouabagenin 1,19-Acetonide (3).—A solution of 2.51 g. (2.09 mmoles) of thionyl chloride in 20 ml. of pyridine was added, dropwise and with stirring over a 5-min. period, to a solution of 11.24 g. (2 mmoles) of 3,11-diacetyl-ouabagenin 1,19-acetonide in 35 ml. of pyridine maintained at −15°. The mixture was stirred for another 10 min. and then was diluted with chloroform. The chloroform solution was washed with excess saturated aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness *in vacuo*. The crystalline residue, when triturated with ether, or ethanol and ether, yielded 9.7 g. (89%) of crude product melting at 228–233°. Crystallization of the crude product from ethanol after treatment with charcoal yielded an analytical sample, m.p. 240–242°, $[\alpha]_D$ −4° (chloroform), n.m.r. at τ 4.05 and 4.58 (vinyl protons).

Anal. Calcd. for $C_{30}H_{40}O_9$: C, 66.16; H, 7.40. Found: C, 66.47; H, 7.25.

3,11-Diacetyl-14-anhydroouabagenin 1,5,19-Orthoformate (5). From 3.—A solution of 250 mg. (0.45 mmole) of 3,11-diacetyl-14-anhydroouabagenin 1,19-acetonide, 15 mg. of *p*-toluenesulfonic acid, and 1 ml. of triethyl orthoformate in 15 ml. of methanol was stirred for 15 min. The precipitate was collected by filtration and washed with ether. The cottony needles weighed 200 mg. (87%) and had m.p. 295–298° dec., $[\alpha]_D$ −20.2° (chloroform).

From 6.—A solution of 0.30 ml. of thionyl chloride in 3 ml. of pyridine was added with stirring to a solution of 200 mg. (0.37 mmole) of 3,11-diacetylouabagenin 1,5,19-orthoformate[9] in 5 ml. of pyridine at −15°. The solution was allowed to warm to room temperature and then was diluted with chloroform. The chloroform solution was washed with water, dilute hydrochloric acid, water, and aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness. The crystalline residue upon trituration with methanol yielded 180 mg. (95%), m.p. 295–298° dec. A mixture melting point with **5** gave no depression and an infrared spectrum in potassium bromide was identical with that of **5** obtained before.

Anal. Calcd. for $C_{28}H_{34}O_9$: C, 65.50; H, 6.68. Found: C, 65.16; H, 6.65.

1β,5β,19-Trihydroxy-3β,11α-diacetoxy-14ξ-card-20(22)-enolide 1,19-Acetonide (4a).—A solution of 99.0 g. (0.18 2mole) of 3,11-diacetyl-14-anhydroouabagenin 1,19-acetonide in 500 ml. of glacial acetic acid was shaken with 20 g. of 5% palladium on charcoal catalyst and hydrogen (3 atm.) in a Parr Shaker at 23° for 5.5 hr. during which 127% for 1 equiv. of hydrogen was absorbed. The hydrogenation mixture was separated by filtration and the filtrate was distilled to dryness *in vacuo*. The residue upon trituration with acetone and ether yielded 44.0 g. (43%) of product, m.p. 248–263°. Crystallization of the crude product from ether and petroleum ether gave an analytical sample, m.p. 260–263°, λ_{max} 215 mμ (ϵ 16,380), $[\alpha]_D$ +22.7° (chloroform).

Anal. Calcd. for $C_{30}H_{42}O_9$: C, 65.91; H, 7.74. Found: C, 66.12; H, 7.64.

When crude **3**, m.p. 237–240°, not completely free of sulfur was used, only a 24% yield of **4a** could be obtained.

3β,11α-Diacetoxy-1β,5β,19,21-tetrahydroxy-14ξ-pregnan-20-one 1,19-Acetonide (4b).—A solution of 27.2 g. (50 mmoles) of **4a** in 125 ml. of methylene chloride and 75 ml. of ethyl acetate cooled in a Dry Ice–acetone bath was saturated with ozone for 3 hr. The solution then was diluted with 10 ml. of acetone and stirred with 6 g. of zinc and 8 ml. of acetic acid for 45 min. with no external cooling. At this time the mixture had reached room temperature and was poured into methylene chloride and filtered. The filtrate was washed with excess aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness. The residue then was dissolved in 50% aqueous methanol containing 5.05 g. of potassium bicarbonate and was allowed to stand under

nitrogen for 18 hr. The solution then was concentrated at 30° *in vacuo* and extracted with chloroform. The chloroform solution was dried over sodium sulfate and distilled to dryness *in vacuo*. When the residue was dissolved in a small amount of acetone and then diluted with ether, two crops of product, m.p. 228–235°, weighing 13.5 g. (54%) were obtained. Crystallization of the crude product from acetone and ether gave an analytical sample, m.p. 241–243°, $[\alpha]_D$ +57.6° (chloroform).

Anal. Calcd. for $C_{28}H_{42}O_9$: C, 64.35; H, 8.10. Found: C, 63.95; H, 8.01.

3β,11α-Diacetoxy-21-(*p*-toluenesulfonoxy)-1β,5β,19-trihydroxy-14ξ-pregnan-20-one 1,19-Acetonide (4c).—A solution of 950 mg. (1.9 mmoles) of 3β,11α-diacetoxy-1β,5β,19,21-tetrahydroxy-14ξ-pregnan-20-one 1,19-acetonide and 560 mg. (2.9 mmoles) of *p*-toluenesulfonyl chloride in 5 ml. of pyridine was maintained at 0° for 1 day and then slowly was diluted with water at 0–5°. The crystalline material was collected by filtration, washed with water and then ether, and dried *in vacuo*. The analytically pure product, m.p. 205–208° dec., $[\alpha]_D$ +60.6°, (chloroform), weighed 1.1 g. (86%).

Anal. Calcd. for $C_{35}H_{48}O_{11}S$: C, 62.11; H, 7.15. Found: C, 61.95; H, 7.00.

3β,11α-Diacetoxy-1β,5β,19-trihydroxy-14ξ-pregnan-20-one 1,19-Acetonide (4d).—A solution of 500 mg. (0.74 mmole) of 3β,11α-diacetoxy-21-(*p*-toluenesulfonoxy)-1β,5β,19-trihydroxy-14ξ-pregnan-20-one 1,19-acetonide in acetone was mixed with a hot solution of 200 mg. of sodium iodide in acetone. The precipitate was separated by filtration, and the filtrate was distilled to dryness *in vacuo* at 30°. The residue was dissolved in chloroform and again the insoluble residue was separated by filtration and the filtrate was distilled to dryness at 30°. A solution of the residue in 1 ml. of acetone and 1 ml. of acetic acid was stirred for several minutes with 200 mg. of zinc and then poured into methylene chloride. The mixture was separated by filtration, and the filtrate was washed with excess aqueous sodium bicarbonate solution, dried over sodium sulfate, and evaporated to dryness. The residue when triturated with ether and acetone yielded 120 mg. (32%) of product, m.p. 226–230°. Crystallization of the crude product from acetone and petroleum ether gave an analytical sample, m.p. 229–230°, $[\alpha]_D$ +64.9° (chloroform).

Anal. Calcd. for $C_{28}H_{42}O_8$: C, 66.38; H, 8.36. Found: C, 66.12; H, 8.22.

A mixture of 3.5 g. of **4d**, m.p. 218–230°, and 5.0 g. of potassium carbonate in aqueous methanol was stirred at reflux for 4 hr. and cooled. The mixture was concentrated by distillation *in vacuo* and then extracted with chloroform. The chloroform extract was dried over sodium sulfate and distilled to dryness. An infrared spectrum in chloroform of the crude product showed the disappearance of the ester function in the 8-μ region. The crude amorphous product was dissolved in pyridine and stirred with 5.0 g. of chromium trioxide in 50 ml. of pyridine[25] overnight. The mixture was diluted with chloroform and filtered. The filtrate was distilled to dryness *in vacuo*, and the residue was dissolved in ethanol and stirred with 40 g. of basic alumina (Woelm, activity grade I) for 1 hr.; when the mixture was filtered and the filtrate was distilled to dryness, 1.3 g. of an amorphous product, $\lambda_{max}^{CHCl_3}$ 6.21, 6.28 (w), and 6.62 μ; λ_{max} 280 mμ (broad) (A = .03 at 1 mg.%), was obtained.

20(22)-Dihydrouabagenin 1,19-Acetonide (7a).—A mixture of 1.92 g. (4.0 mmoles) of ouabagenin-1,19-acetonide,[14] 160 mg. of platinum oxide, and 75 ml. of ethanol was stirred in an atmosphere of hydrogen for 6 hr. and then filtered. The filtrate was distilled to dryness and the residue when triturated with acetone yielded two crops of crystalline material, m.p. 285–290°, which showed no absorption in the ultraviolet. Crystallization of the crude product from acetone gave an analytical sample, m.p. 290–292°, $[\alpha]_D$ +17.5° (dioxane), λ_{max}^{KBr} 2.88 and 5.64 μ.

Anal. Calcd. for $C_{26}H_{40}O_8$: C, 64.97; H, 8.39. Found: C, 64.69; H, 8.26.

1β,5β,14,19-Tetrahydroxy-3,11-dioxocardanolide 1,19-Acetonide (8a).—A solution of 9.60 g. (20 mmoles) of **7a** in 100 ml. of pyridine was added to a freshly prepared mixture of 10.0 g. of chromic acid in 100 ml. of pyridine[25] and was stirred at room temperature for 18 hr. The mixture was filtered and the collected solid was washed with pyridine and then chloroform. The filtrate was diluted with 500 ml. of chloroform and washed with 400 ml. of water. The aqueous phase was separated and washed with 200 ml. of chloroform. The combined chloroform extract was evaporated to dryness *in vacuo*. The brown residue when triturated with acetone yielded 6.65 g. (70%) of crude product m.p. 256–270° dec. Crystallization of the crude product from acetone yielded an analytical sample, m.p. 270–274° dec.; $[\alpha]_D$ +10.5° (pyridine); λ_{max}^{KBr} 2.83, 5.68, 5.81, and 5.84 μ.

Anal. Calcd. for $C_{26}H_{36}O_8$: C, 65.53; H, 7.61. Found: C, 65.03; H, 7.61.

5β,14,19-Trihydroxy-3,11-dioxocard-1-enolide (9a).—A mixture of 1.0 g. (2.1 mmoles) of **8a**, 50 ml. of ethanol, and 8 g. of Woelm basic alumina (activity grade I) was stirred at reflux for 1.25 hr. and then was filtered. The collected alumina was stirred with hot ethanol for 30 min. and then was collected again by filtration. The combined ethanol filtrate was distilled to dryness *in vacuo*. The residue which weighed 450 mg. yielded upon trituration with acetone a crude product, m.p. 165–170°, λ_{max} 231.5 mμ (ε 10,700), weighing 250 mg. (28%). An analytical sample was prepared by purification of the crude product by column chromatography. Elution of the compound from a column of silica gel with chloroform–methanol (9:1) yielded crystals which, when crystallized from acetone, gave an analytical sample, m.p. 212–216°, $[\alpha]_D$ +131.9° (dioxane), λ_{max} 232 mμ (ε 11,000).

Anal. Calcd. for $C_{23}H_{30}O_7$: C, 66.01; H, 7.23. Found: C, 65.76 H, 7.04.

5β,14,19-Trihydroxy-3,11-dioxocardanolide (10a).—A mixture of 2.2 g. (5.3 mmoles) of **9a** and 400 mg. of 5% palladium on charcoal in methanol was stirred in an atmosphere of hydrogen until hydrogen ceased to be absorbed. The mixture was separated by filtration, and the filtrate was evaporated to dryness at room temperature. Slow evaporation at 35° of a solution of the residue in ethyl acetate–methanol (9:1) yielded 400 mg. (18%) of crystals, m.p. 232–234° dec. Crystallization of the crude product from ethyl acetate and methanol gave an analytical sample, m.p. 234–236° dec. (softening at 227°), $[\alpha]_D$ +36.2° (dioxane).

Anal. Calcd. for $C_{23}H_{32}O_7$: C, 65.69; H, 7.67. Found: C, 65.95; H, 7.77.

14,19-Dihydroxy-3,11-dioxocard-4-enolide (11a).—A solution of 100 mg. (0.24 mmole) of **10a** in 6 ml. of acetic acid was refluxed for 15 min. and distilled to dryness *in vacuo*. Trituration of the residue from acetone gave crystals, m.p. 245–250°, weighing 75 mg. (82%). Crystallization of the crude product from ethyl acetate and acetone gave an analytical sample, m.p. 246–250°; λ_{max} 242 mμ (ε 14,400); λ_{max} 2.80, 2.93, 5.63, 5.82 (w), and 6.08 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$: C, 68.63; H, 7.51. Found: C, 68.55; H, 7.71.

5β,14,19-Trihydroxy-3,11-dioxocarda-1,20(22)-dienolide (9).—A slurry of 30 g. (63 mmoles) of ouabagenin 1,19-acetonide (**7**) in 400 ml. of pyridine was added to a freshly prepared mixture of 30 g. of chromic acid in 400 ml. of pyridine,[25] and the mixture was stirred at room temperature for 1 day. The mixture was separated by filtration and the collected solid was washed with chloroform–pyridine (1–1). The filtrate was distilled to dryness *in vacuo*. The residue was dissolved in 1 l. of ethanol and stirred at reflux for 1 hr. with 290 g. of Woelm basic alumina (activity grade I). The alumina was collected by filtration and extracted twice by stirring with 1 l. of ethanol at reflux for 1 hr. The combined ethanol filtrate, when concentrated to a small volume *in vacuo*, yielded 14.75 g. (56%) of 5β,14,19-trihydroxy-3,11-dioxocarda-1,20(22)-dienolide,[16] m.p. 195–200°, λ_{max} 220 mμ (ε 23,800).

5β,14,19-Trihydroxy-3,11-dioxocard-20(22)-enolide (10).—A mixture of 2.0 g. (4.8 mmoles) of **9**, 100 ml. of methanol, and 200 mg. of 5% palladium on charcoal was stirred in an atmosphere of hydrogen until hydrogen absorption ceased. Approximately 1 equiv. of hydrogen was absorbed in 1.5 hr. The product which precipitated during the hydrogenation was dissolved by adding sufficient methanol to the mixture. The catalyst was separated by filtration and the filtrate was evaporated at 25°. The crude product weighed 2.0 g. (99%) and melted at 175–180°. Since the recrystallized product always contained a small amount of the 3-keto-Δ^4 derivative, a satisfactory analysis could not be obtained. The ultraviolet spectrum of the crude product had a maximum at 218 mμ (ε 17,300), and infrared spectrum in potassium bromide had maxima at 2.82, 2.90, 5.70–5.78, 5.87, and 6.13 μ.

14,19-Dihydroxy-3,11-dioxocarda-4,20(22)-dienolide (11).—A mixture of 2.0 g. (4.8 mmoles) of crude **10** obtained from the hydrogenation of **9** and 10 ml. of acetic acid was refluxed for 10

(25) G. I. Poos, G. E. Arth, R. E. Beyler, and L. H. Sarett, *J. Am. Chem. Soc.*, **75**, 422 (1953).

min. and then distilled to dryness *in vacuo*. Crystallization of the crude product from acetone and petroleum ether yielded three crops of material, the total of which weighed 1.7 g. (88%) and melted at 230–245°.

Crystallization of the crude product from acetone yielded an analytical sample, m.p. 248–250°; λ_{max} 218 (ϵ 20,000) and 244 mμ (shoulder) (ϵ 14,300); λ_{max}^{KBr} 2.89, 5.71, 5.83, (weak), 6.02, and 6.16 μ; $[\alpha]D$ +157° (pyridine).

Anal. Calcd. for $C_{23}H_{28}O_6$: C, 68.0; H, 7.05. Found: C, 68.1; H, 6.93.

14-Hydroxy-11α-methoxy-11,19-epoxy-3-oxocarda-4,20(22)-dienolide (12). From 11.—A mixture of 600 mg. (1.5 mmoles) of **11**, 50 mg. of *p*-toluenesulfonic acid hydrate, and 10 ml. of methanol were stirred for 6 hr. The mixture was concentrated by evaporation and the product, which was collected by filtration, washed with cold methanol, and dried, weighed 580 mg. (93%) and melted at 230–240°. Crystallization of the crude product from methanol gave an analytical sample, m.p. 258–260°, $[\alpha]D$ +149.8° (chloroform), λ_{max} 219 (ϵ 21,500) and 237 mμ (shoulder) (ϵ 19,000).

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 69.54; H, 7.30. Found: C, 69.77; H, 7.30.

From 9.—A mixture of 73.0 g. (175 mmoles) of **9**, 10.0 g. of 10% palladium on charcoal, and 900 ml. of dimethylformanide was hydrogenated in an atmosphere of hydrogen in a 2-l. Parr bomb. After 40 hr. when 3.85 l. of hydrogen had been absorbed, the catalyst was removed by filtration and the filtrate was distilled to dryness *in vacuo*. The residue was dissolved in 500 ml. of hot methanol containing 0.75 ml. of concentrated hydrochloric acid, and the solution was warmed just below reflux for 15 min. The solution was seeded with **12** and cooled at 0° overnight. The product which precipitated was collected by filtration, washed with cold methanol, and dried; it weighed 38.5 g. (53%) and melted at 240–250°. A second crop weighing 13.7 g. (19%), m.p. 255–260°, was obtained by concentration and cooling of the mother liquors. The infrared spectrum in potassium bromide of the total crude product was indistinguishable from that of **12** obtained previously.

11α-Methoxy-11,19-epoxy-3-oxocarda-4,14,20(22)-trienolide (13).—A solution of 12.0 g. of thionyl chloride (101 mmoles, freshly distilled) in 50 ml. of pyridine was added with stirring to a solution of 36.4 g. (88 mmoles) of **12**, m.p. 240–257°, in 300 ml. of pyridine at such a rate as to maintain the reaction mixture at −7° to −15°. The mixture was stirred below 0° for 10 min., allowed to rise gradually to 20°, and then diluted with 1 l. of chloroform. The organic solution was washed carefully and thoroughly with 900 ml. of saturated aqueous sodium bicarbonate solution. The aqueous bicarbonate solution was separated and then extracted with chloroform. The combined chloroform extract was dried over sodium sulfate and distilled to dryness *in vacuo*. A solution of the residue in methylene chloride and methanol was stirred with charcoal at reflux for about 10 min. and was filtered. When the methylene chloride was removed from the filtrate by distillation, the colorless crystalline product which precipitated was collected by filtration, washed with methanol, and dried. The crude product, which weighed 28.5 g. (82%), m.p. 205–210°, when crystallized from methylene chloride and methanol, yielded 27.0 g. of product, m.p. 211–214°. Crystallization of the substance from methanol and methylene chloride gave an analytical sample, m.p. 212–214°, $[\alpha]D$ +119.5° (chloroform), λ_{max} 217 (ϵ 20,200) and 241 mμ (shoulder) (ϵ 18,500).

Anal. Calcd. for $C_{24}H_{28}O_5$: C, 72.70; H, 7.12. Found: C, 72.43; H, 7.22.

19-Hydroxy-3,11-dioxocarda-4,14,20(22)-trienolide (14).—When a solution of 100 mg. (0.25 mmole) of **13** in acetone containing 5 mg. of *p*-toluenesulfonic acid and a little water was concentrated by distillation, the crystalline product which precipitated was collected by filtration, washed with acetone, and dried. The crude product weighed 50 mg. (52%) and melted at 220–225°. Crystallization of the crude product from acetone gave an analytical sample, m.p. 231–233°, λ_{max} 217 (ϵ 20,600) and 237 mμ (shoulder) (ϵ 18,200), λ_{max}^{KBr} 3.03 (bonded hydroxyl) and 5.84 μ (s, saturated carbonyl).

Anal. Calcd. for $C_{23}H_{26}O_5$: C, 72.23; H, 6.85. Found: C, 72.16; H, 7.01.

11α-Methoxy-11,19-epoxy-3-oxo-5β-card-20(22)-enolide (15).—A solution of 13.34 g. (33.9 mmoles) of **13**, m.p. 208–210°, in 800 ml. of ethyl acetate and 200 ml. of methanol was stirred with 3.5 g. of 5% palladium on charcoal at atmospheric pressure with hydrogen. Hydrogenation proceeded at a constant rate and then ceased after 55 min. when approximately 2 equiv. of hydrogen had been absorbed. The catalyst was removed by filtration and 0.5 ml. of pyridine was added to the filtrate. When the filtrate was distilled to dryness, the crystalline product which remained weighed 13.5 g. (100%) and melted at 188–194°. A thin layer chromatogram (silica gel G) spotted with a solution of the product in ethyl acetate, developed with ethyl acetate–benzene (1:1), and color tested with Zimmermann reagent indicated essentially that a single product was obtained. Crystallization of the crude product from methanol gave an analytical sample, m.p. 192–194° dec., $[\alpha]D$ +126.5° (chloroform), λ_{max} 216 mμ (ϵ 16,700).

Anal. Calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 72.33, H, 8.08.

19-Hydroxy-3,11-dioxo-5β-card-20(22)-enolide (16).—A solution of 100 mg. (0.25 mmoles) of **15** in acetone was diluted with a little water containing 5 mg. of *p*-toluenesulfonic acid. The solution was concentrated by distillation, and the crystalline precipitate which formed was collected by filtration, washed with water, and dried. The crude product weighed 90 mg. (93%) and melted at 217–222°. Crystallization of the crude product from methylene chloride gave an analytical sample, m.p. 220–224°, λ_{max} 215 mμ (ϵ 17,000).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.08; H, 7.67.

14-Hydroxy-11α-methoxy-11,19-epoxy-3-oxo-5β-card-20(22)-enolide (17).—A solution of 458 mg. (1.1 mmoles) of **12** in methanol was hydrogenated at atmospheric pressure over 50 mg. of 5% palladium on charcoal. After about 2 hr. 1 equiv. of hydrogen was absorbed.

The catalyst was removed by filtration and the filtrate was distilled to dryness. When the residue was crystallized from methanol containing a trace of pyridine, 400 mg. (86%) of crude product, m.p. 226–232°, was obtained. Crystallization of the crude product from methanol gave an analytical sample, m.p. 228–230° dec., $[\alpha]D$ +83.6 (chloroform).

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.21; H, 7.75. Found: C, 69.35; H, 7.91.

14-Hydroxy-3,3,11α-trimethoxy-11,19-epoxy-5β-card-20(22)-enolide (18).—A solution of 1 g. (2.4 mmoles) of **17**, m.p. 225–230°, 0.5 ml. of trimethyl orthoformate in 10 ml. of methanol containing 10 mg. of *p*-toluenesulfonic acid was allowed to remain overnight. The precipitate which was collected by filtration, washed with methanol, and dried, weighed 750 mg. (68%) and melted at 145–155°. Crystallization of crude product from methanol and methylene chloride containing a trace of *p*-toluenesulfonic acid gave an analytical sample, m.p. 154–155° dec., $[\alpha]D$ +54.5° (chloroform).

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28. Found: C, 67.61; H, 8.26.

3,3,11α-Trimethoxy-11,19-epoxy-5β-carda-14,20(22)-dienolide (19).—A solution of 5.4 g. (11.7 mmoles) of **18** in pyridine was treated with 2.8 g. of thionyl chloride in pyridine according to the procedure outlined for the preparation of **13**. The crude product which was obtained was purified by chromatography on Woelm basic alumina (activity grade I). When the column was eluted with ether–ethyl acetate (1:1), 1.0 g. (19%) of crude product, m.p. 150–160°, was obtained. Recrystallization from methanol gave an analytical sample, m.p. 159–160°.

Anal. Calcd. for $C_{26}H_{36}O_6$: C, 70.24; H, 8.16. Found: C, 70.51; H, 8.07.

3,3,11α-Trimethoxy-11,19-epoxy-5β-card-20(22)-enolide (20). From 19.—A solution of 400 mg. (0.9 mmole) of **19** in 75 ml. of methanol was hydrogenated over 80 mg. of 5% palladium on charcoal for 4 hr. when 1.1 equiv. of hydrogen were absorbed. The catalyst was recovered by filtration and the filtrate was distilled to dryness. Trituration of the residue with ether and petroleum ether yielded crystals, which when separated by filtration, washed with ether–petroleum ether, and dried, weighed 200 mg. (50%) and melted at 140–143°. Crystallization of the crude product from methanol containing a trace of *p*-toluenesulfonic acid gave an analytical sample, m.p. 154–155°.

Anal. Calcd. for $C_{26}H_{38}O_6$: C, 69.93; H, 8.58. Found; C, 69.71; H, 8.66.

From 15.—When 50 mg. of **15** was stirred with 2 mg. of *p*-toluenesulfonic acid and 0.2 ml. of trimethyl orthoformate in 2 ml. of methanol, concentration of the solution by evaporation yielded 20 mg. of product, m.p. 145–150°. Crystallization of

the crude product from methanol yielded crystals, m.p. 154–155°. A mixture melting point with **20** gave no depression and an infrared spectrum in potassium bromide was identical with that of **20**.

11α-Methoxy-11,19-epoxy-3-oxo-5β,20ξ-cardanolide (21).—A solution of 390 mg. (0.97 mmole) of **15** in 50 ml. of tetrahydrofuran and 50 ml. of ethanol was stirred in an atmosphere of hydrogen with 400 mg. of platinum oxide for 15 hr. The catalyst was collected by filtration and the filtrate was distilled to dryness. The crude product was stirred with a mixture of 600 mg. of chromium trioxide in 10 ml. of pyridine overnight. The oxidation mixture was diluted with chloroform and filtered. The filtrate was washed with aqueous sodium bicarbonate solution, dried over magnesium sulfate, and distilled to dryness. The residue (175 mg.) when recrystallized twice from acetone and petroleum ether after treatment with charcoal yielded 65 mg. of an analytical sample, m.p. 160–161°, $\lambda_{max}^{CHCl_3}$ 5.66 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.87; H, 8.43.

21-Hydroxy-11α-methoxy-11,19-epoxy-5β,14β-pregnane-3,20-dione (23a).—A solution of 10.0 g. (25 mmoles) of **15** in 75 ml. of methylene chloride and 75 ml. of ethyl acetate cooled in a Dry Ice–acetone bath was saturated with ozone for 3 hr. The cold solution then was stirred vigorously for 1 hr. with 4 g. of zinc, 10 ml. of acetic acid, and 10 ml. of methanol. During this time the mixture was allowed to rise gradually to about 30–40°. The mixture was diluted with methylene chloride and filtered. The filtrate was washed twice with excess saturated aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness *in vacuo*. The residue was dissolved in 150 ml. of methanol to which 30 ml. of water was added. To this solution then was added under nitrogen a solution of 2.5 g. of potassium bicarbonate in 80 ml. of water and 50 ml. of methanol. The solution was stirred under nitrogen for 18 hr. The product which precipitated was collected by filtration, washed with a little aqueous methanol, and dried. The crude product weighed 6.0 g. (64%) and melted at 170–175°. The filtrate, which was obtained when the product was separated, was extracted with chloroform. The chloroform solution was dried over sodium sulfate and distilled to dryness. Trituration of the residue with acetone and ether yielded an additional 800 mg. of crude product, m.p. 158–170°. Crystallization of the crude product from acetone gave an analytical sample, m.p. 178–180°, $[\alpha]_D$ +134.5° (chloroform).

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57. Found: C, 70.43; H, 8.28.

21-Hydroxy-11α-methoxy-11,19-epoxy-5β,14β-pregnane-3,20-dione 21-Acetate (23b).—A solution of 7.5 g. (19.5 mmoles) of **23a**, m.p. 170–173°, in 20 ml. of pyridine and 5 ml. of acetic anhydride was allowed to remain at room temperature for several hours and then was distilled to dryness *in vacuo*. The residue, upon crystallization from methanol containing 0.1 ml. of pyridine, yielded 4.0 g. (49%) of crude product, m.p. 172–176°. Crystallization of the crude product from methylene chloride and methanol containing a trace of pyridine gave an analytical sample, m.p. 180–182°, $[\alpha]_D$ +126° (chloroform).

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.87; H, 8.21. Found: C, 69.08; H, 8.41.

11α-Methoxy-11,19-epoxy-5β,14β-pregnane-3,20-dione (23c).—A solution of 573 mg. (1.53 mmoles) of **23a** and 400 mg. (1.53 mmoles) of *p*-toluenesulfonyl chloride in 3 ml. of pyridine was allowed to remain at 0° overnight. The solution was diluted at 0° with 0.5 ml. of water and then was added dropwise with vigorous stirring to 250 ml. of ice and water. The amorphous precipitate was collected by filtration, washed with water, and dried at 78° at 0.1 mm. The amorphous dried solid was then added to a solution of 1 g. of sodium iodide in 15 ml. of acetone. After the solution was warmed on the steam bath for 5–10 min., the mixture which resulted was evaporated to dryness. The residue was triturated with 50 ml. of benzene–methylene chloride (1:1). The mixture was filtered and the filtrate was evaporated to dryness. The residue, the crude iodo ketone, was shaken with 1.0 g. of zinc and a solution of 2 ml. of acetic acid in 4 ml. of methanol until the residue dissolved and the yellow color of the mixture disappeared (about 5 min.). The mixture was diluted with 100 ml. of methylene chloride and filtered. The filtrate was washed with excess saturated aqueous sodium bicarbonate solution, dried over magnesium sulfate, and distilled to dryness. The crude product weighed 389 mg (67%) and melted at 159–164°. Several recrystallizations of the crude product from methylene chloride and methanol containing a trace of pyridine gave an analytical sample, m.p. 168–169°, $[\alpha]_D$ +145° (chloroform).

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.05; H, 8.84.

19,21-Dihydroxy-5β,14β-pregnane-3,11,20-trione (24a).—A solution of 200 mg. (5.3 mmoles) of **23a** was dissolved in acetone and diluted with a small amount of water. After 10 mg. of *p*-toluenesulfonic acid was added to the solution, it was concentrated by distillation and cooled. The product which precipitated was collected by filtration, washed with aqueous acetone, and dried. The product weighed 100 mg. (52%) and melted at 158–161°. Crystallization of the crude product from acetone and petroleum ether gave an analytical sample, m.p. 165–167°, $[\alpha]_D$ +107° (chloroform).

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.70; H, 8.20.

19,21-Dihydroxy-5β,14β-pregnane-3,11,20-trione 21-Acetate (24b).—A solution of 500 mg. (1.2 mmoles) of **23b** in a solution of 6 ml. of water, 0.5 ml. of acetic acid, and 30 ml. of acetone was distilled slowly until the solution became cloudy (about 20 min.). Several drops of acetone then were added, and, when the solution was cooled and slowly allowed to evaporate, crystallization ensued. The crude product was collected by filtration and recrystallized from acetone and petroleum ether to yield 400 mg. (82%) of purified product, m.p. 133–135°. Crystallization of this substance from acetone and petroleum ether gave an analytical sample, m.p. 133–135° (softening at 132°).

Anal. Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.45; H, 7.88.

19-Hydroxy-5β,14β-pregnane-3,11,20-trione (24c).—A solution of 100 mg. of **23c** in 5 ml. of acetone containing 0.5 ml. of water and 50 mg. of *p*-toluenesulfonic acid was refluxed for 30 min., diluted with a little water, and concentrated by distillation. The crystals which precipitated were collected by filtration and dried. The crude product weighed 75 mg. and melted at 140–145°. Crystallization of the crude product twice from acetone and petroleum ether or purification by chromatography on silica gel by elution with benzene–ethyl (3:2) gave an analytical sample, m.p. 158–160°, $[\alpha]_D$ +103° (chloroform). A mixture melting point with **26b**,[20] m.p. 160°, was 140–145°.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.98; H, 8.67.

19-Hydroxy-3,11-dioxo-5β,14β-androstane-17β-carboxylic Acid (24d).—A solution of 2.0 g. (5.3 mmoles) of **23a** in 180 ml. of acetone was mixed with a solution of 5.0 g. of sodium metaperiodate and 30 ml. of water in 100 ml. of acetone. The solution was stirred for 20 hr., diluted with 100 ml. of water, and concentrated by evaporation to about 100 ml. The crystalline product which precipitated was collected by filtration and dissolved in aqueous sodium bicarbonate solution. The aqueous solution was filtered and acidified with dilute hydrochloric acid. The product which precipitated was collected by filtration, washed with water, and dried. The crude product, 1.35 g. (73%), m.p. 180–183°, upon crystallization from acetone yielded 1.0 g. of purified material, m.p. 184–186°. Crystallization of the purified material from acetone gave an analytical sample, m.p. 195–196°, $[\alpha]_D$ +88° (dioxane).

Anal. Calcd. for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 69.17; H, 8.23.

19-Hydroxy-3,11-dioxo-5β,14β-androstane-17β-carboxylic Acid Methyl Ester (24e).—To a solution of 1.7 g. (4.9 mmoles) of **24d** in about 200 ml. of methanol was added a solution of diazomethane in ether until a yellow color persisted. After the solution was evaporated to dryness, trituration of the residue with ether and petroleum ether yielded a crystalline product which was collected by filtration and dried. The crude product weighed 1.4 g. (81%) and melted at 126–133°. Crystallization of the crude product from ether and petroleum ether gave an analytical sample, m.p. 132–134°.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.50; H, 8.19.

21-Hydroxy-3,3,11α-trimethoxy-11,19-epoxy-5β,14β-pregnan-20-one 21-Acetate (25a).—A mixture of 400 mg. (0.96 mmole) of **23a**, 10 mg. of *p*-toluenesulfonic acid, 0.5 ml. of trimethyl orthoformate, and 10 ml. of methanol was stirred for about 30 min., diluted with 0.5 ml. of 25% trimethylamine in methanol, and poured into chloroform. The chloroform solution was washed with water, dried over sodium sulfate, and distilled to dryness *in vacuo*. The residue, upon crystallization from methanol, yielded

the product which weighed 320 mg. (72%) and melted at 100–108°. Crystallization of the crude product from methanol gave an analytical sample, m.p. 110°, $[\alpha]_D$ +96° (chloroform).

Anal. Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68. Found: C, 67.43; H, 8.72.

3,3,11α-Trimethoxy-11,19-epoxy-5β,14β-pregnan-20-one (25b).—A mixture of 500 mg. (1.4 mmoles) of **23c**, 50 mg. of *p*-toluenesulfonic acid, and 5 ml. of methanol was stirred until solution occurred. When the solution was concentrated and cooled at 0°, the crystalline product which precipitated was collected by filtration and washed with cold methanol. It weighed 350 mg. (70%) and melted at 100–105°. Several crystallizations of the crude product from methylene chloride and methanol containing a trace of pyridine gave an analytical sample, m.p. 111–112°; n.m.r. spectrum at τ 6.00, 6.14, 6.35, and 6.53 (quartet, C-19 methylene), 6.74 (methoxyl), 6.84 (two methoxyls), 7.86 (C-21 methyl), and 9.13 (C-18 methyl); $[\alpha]_D$ +107° (methanol) and $[\alpha]_D$ +105.8° (potassium hydroxide in methanol, after 1 hr.)

Anal. Calcd. for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42. Found: C, 71.04; H, 9.18.

25b, 100 mg., m.p. 111–112°, was refluxed 10 min. with 7 ml. of 1 *M* potassium hydroxide in methanol in 1 atm. of nitrogen. The solution was cooled and diluted with ether. The mixture was washed with saturated aqueous sodium chloride solution until neutral, dried over sodium sulfate, and distilled to dryness. Crystallization of the residue from 1 ml. of methanol at 0° gave 75 mg. of product, m.p. 111–112°. A mixture melting point with starting material was 111–112°. An infrared spectrum in potassium bromide of the product was identical with that of **25b**.

3α,11β,19-Trihydroxy-5β,14β-androstane-17β-carboxylic Acid (27a).—A mixture of 700 mg. (2 mmoles) of **24d**, 740 mg. of sodium borohydride, 25 ml. of dioxane, and 25 ml. of water was stirred at about 95° for 2 hr.

After another 500 mg. of sodium borohydride was added, the mixture was stirred for 2 hr., cooled, and acidified to pH 2. The product which precipitated was collected by filtration, washed with water, and dried. The crude product weighed 500 mg. (70%) and melted at 250–255°. Crystallization of the crude product from acetone gave an analytical sample, m.p. 254–256°, $[\alpha]_D$ +54° (chloroform).

Anal. Calcd. for $C_{20}H_{33}O_5$: C, 67.96; H, 9.41. Found: C, 67.53; H, 9.27.

3α,11β,19-Trihydroxy-5β,14β-androstane-17β-carboxylic Acid Methyl Ester (27b).—A solution of 300 mg. (0.85 mmole) of **27a**, m.p. 250–255°, in methanol was titrated with a solution of diazomethane in ether until a yellow color persisted in the solution. When the solution was evaporated to dryness, a crystalline residue, m.p. 190–195°, remained. Crystallization of the crude product from acetone and petroleum ether gave 200 mg. (65%) of analytical quality product, m.p. 198–199°, $[\alpha]_D$ +67° (chloroform).

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.66; H, 9.15.

3α,19-Diacetoxy-11β-hydroxy-5β,14β-androstane-17β-carboxylic Acid Methyl Ester (27c).—A solution of 200 mg. (0.55 mmole) of **27b** and 1 ml. of acetic anhydride in 2 ml. of pyridine was heated at 100° for 30 min. and cooled. The solution was diluted with water and the resulting mixture was extracted with ether. The ether solution, when washed successively with excess dilute hydrochloric acid, water, and aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness, yielded a crude crystalline product weighing 200 mg. (81%) and melting at 155–158°. Crystallization of the crude product from ether and petroleum ether gave an analytical sample, m.p. 158–160°, $[\alpha]_D$ +92° (chloroform).

3α,19-Diacetoxy-11-oxo-5β,14β-androstane-17β-carboxylic Acid Methyl Ester (28).—A solution of 200 mg. (0.55 mmole) of **24e** and 380 mg. (1.5 mmoles) of lithium tri-*t*-butoxyaluminum hydride in 50 ml. of tetrahydrofuran was stirred for 15 min. and then was acidified carefully with aqueous acetic acid. The mixture was extracted with chloroform. The chloroform solution was washed with water and aqueous sodium bicarbonate solution, dried over sodium sulfate, and evaporated to dryness. The amorphous residue, when acylated with pyridine and acetic anhydride at 100° for 30 min., yielded 100 mg. (41%) of crystalline crude product, m.p. 180–185°. Crystallization of the crude product from ether gave an analytical sample, m.p. 188–190°, $[\alpha]_D$ +39° (chloroform).

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94, H, 8.09. Found: C, 66.66; H, 8.13.

A solution of 20 mg. of **27c** in 10 ml. of acetone was stirred for 5 min., with 0.1 ml. of 8 *N* chromic–sulfuric acid mixture and diluted with water. The aqueous solution was extracted with ether. The ether solution was washed with water and aqueous sodium bicarbonate solution, dried over sodium sulfate, and evaporated to dryness. The residue upon crystallization from ether yielded 10 mg. of product, m.p. 189–190°, which gave no depression in melting point on admixture with **28** and had an identical infrared spectrum in potassium bromide with that of **28**.

14-Hydroxy-3,11-dioxocarda-4,20(22)-dienolid-19-oic Acid (29a).—To a solution of 2.0 g. (5.0 mmoles) of **11** in 90 ml. of acetic acid was added, with stirring over 10 min., 2.75 ml. of standard 8 *N* chromic–sulfuric acid solution. The excess chromic acid was destroyed with isopropyl alcohol and the mixture was diluted with about 100 ml. of water. After 10 min. the precipitated solid was collected by filtration, washed with water, and dried. The crude product weighed 1.05 g. (50%) and melted at 189–190° with gas evolution. Crystallization of the crude product from acetone gave an analytical sample, m.p. 189–191° dec., $[\alpha]_D$ +229.5° (methanol–dioxane, 1:1).

Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.32. Found: C, 66.69; H, 6.19.

14-Hydroxy-3,11-dioxocarda-4,20(22)-dienolid-19-oic acid Methyl Ester (29b).—A solution of diazomethane in ether (dried over potassium hydroxide) was added with stirring to a solution of 600 mg. (1.45 mmoles) of **29a** in 100 ml. of methanol until a yellow color persisted. The solution was evaporated to dryness, and the residue when triturated with methanol yielded crystals. The crude product which was collected by filtration and dried weighed 260 mg. (40%) and melted at 225–250°. Crystallization of the crude product from methylene chloride and methanol gave an analytical sample, m.p. 256–258° (softening at 254°).

Anal. Calcd. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.59. Found: C, 67.14; H, 6.67.

14-Hydroxy-3,11-dioxo-19-norcard-4,20(22)-enolide (30).—A mixture of 1.0 g. (2.4 mmoles) of **29a**, m.p. 180–187° dec., 15 ml. of methanol, 5 ml. of water, and 1 ml. of 3 *M* HCl was stirred at reflux for 65 min. The solution was concentrated by passing a stream of nitrogen over the solution heated at 90°. After cooling, the precipitated product was collected by filtration, washed with aqueous methanol, and dried; it weighed 670 mg. (75%) and melted at 228–245°. The crude product when crystallized twice from methylene chloride and ethyl acetate, after treatment with charcoal, gave an analytical sample, m.p. 255–257° (sintering at 240–250°), $[\alpha]_D$ +180° (chloroform).

Anal. Calcd. for $C_{22}H_{26}O_5$: C, 71.33; H, 7.08. Found: C, 71.46; H, 7.07.

3,11-Dioxo-19-norcarda-4,14,20(22)-trienolide (31).—To a solution of 2.08 g. (5.6 mmoles) of **30**, m.p. 245–254°, in 20 ml. of pyridine at −35° was added with stirring a solution of 730 mg. (6.1 mmoles) of thionyl chloride in 10 ml. of pyridine. After the addition, the solution was allowed to warm to about 10° in about 20 min. The solution was diluted with chloroform, washed with saturated aqueous sodium bicarbonate solution, dried over sodium sulfate, and distilled to dryness. The crude crystalline residue weighed 1.9 g. and when crystallized from acetone, after treatment with charcoal, yielded colorless crystal weighing 1.3 g. (65%) and melting at 230–235°. Crystallization of the crude product from acetone gave an analytical sample, m.p. 227–231°; λ_{max} 218.5 mμ (ε 22,000) and 240 mμ (broad) (ε 19,000); $\lambda_{max}^{CHCl_3}$ 5.72 (s), 5.85 (s), 6.02 (s), and 6.13 (w) μ; n.m.r. spectrum at τ 4.13 (multiplet, 2 vinyl protons) and 4.46 (multiplet, 1 vinyl proton).

Anal. Calcd. for $C_{22}H_{24}O_4$: C, 74.97; H, 6.86. Found: C, 75.15; H, 7.04.

3,11-Dioxo-19-nor-5ξ-carda-14,20(22)-dienolide (32).—A mixture of 590 mg. (1.65 mmoles) of **31**, 150 mg. of 5% palladium on charcoal, 40 ml. of methanol, and 40 ml. of ethyl acetate was stirred in an atmosphere of hydrogen. Although uptake of hydrogen was rapid during the initial 15 min., the uptake of hydrogen thereafter proceeded slowly and then almost stopped after another 85 min. when a total of 32 ml. of hydrogen was absorbed. The catalyst was separated by filtration and the filtrate was distilled to dryness. Column chromatography of the product on silica gel yielded 400 mg. (68%) of crude crystalline product, m.p. 198–205°, when the column was eluted with ethyl acetate–benzene (3:17). Several crystallizations of the crude product from acetone and petroleum ether gave an analytical sample, m.p.

215–218°; λ_{max} 211.4 mμ (ε 18,400); $\lambda_{max}^{CHCl_3}$ 5.72 (s), 5.86 (s), and 6.13 (w) μ; n.m.r. spectrum at τ 4.06 (multiplet, 1 vinyl proton) and 4.46 (multiplet, 1 vinyl proton).

Anal. Calcd. for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.45; H, 7.21.

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Carboxyl-Reduced Heparin. Monosaccharide Components[1]

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Commercial heparin (sodium salt) was purified through the cetyltrimethylammonium salt to a product readily convertible to the crystalline barium acid salt. The heparin polyelectrolyte molecule resists desulfation and *O*-acetylation. Partial desulfation of the so-purified heparin (sodium salt) with methanolic hydrogen chloride followed by *N*-acetylation allowed further desulfation. Subsequent acetylation converted most of the free hydroxyl groups to acetate esters. The nearly *O*-acetylated product contained two acetamido groups, two (free carboxyl) uronic acid residues, and approximately one ester acid sulfate group per tetrasaccharide unit. Reduction of the uronic acid carboxyl groups in this product with diborane gave the carboxyl-reduced analog with quantitative conversion of the uronic acid moiety to aldose. Acid hydrolysis of the carboxyl-reduced polysaccharide gave 2-amino-2-deoxy-D-glucose (isolated as the crystalline α-D-hydrochloride) and D-glucose (isolated as the crystalline penta-*O*-acetylglucose diethyl dithioacetal and previously as potassium hydrogen D-glucarate) as the only monosaccharide hydrolytic components. This establishment of D-glucose in the hydrolysate characterizes the uronic acid of heparin as D-glucuronic acid.

The polysaccharide heparin has been known for over forty years, and studies in relation to its blood anticoagulant properties have been extensive.[2] Chemical studies on heparin up to 1954 have been reviewed in detail,[3] and later work has been summarized.[4,5] Heparin has proved to be a remarkably intractable polysaccharide for chemical study. It is not amenable to many of the conventional procedures used in polysaccharide structure determination. Much inconclusive and conflicting data exists in the literature regarding the linkage sequence, and even the structural units, of the heparin molecule; a great deal of the supporting evidence presented is based on color reactions and indirect evidence. Work based on inhomogeneous preparations has added considerably to the confusion regarding precise chemical structure.

In this paper and the following papers from this laboratory we have endeavored to base our studies on well-purified material and to draw conclusions on classical proofs of structure, with isolation of crystalline degradation products identified by unequivocal methods.

The starting material we have used was a commercial heparin (sodium salt) of normal anticoagulant activity which we further purified by fractionation from salt solutions of the cetyltrimethylammonium salts of the sulfated polysaccharides present.[6,7] This fractionation was effected with considerable loss (about 50%), but that material with the highest degree of sulfation present in the commercial heparin was so obtained. Undoubtedly the yield could have been raised, but we wished to concentrate our efforts upon this fraction. That the mast cell, wherein heparin originates, contains a mixture[8] of polysaccharides cannot be doubted. We ourselves have described a galactan from this source[9] and dermatan sulfate (β-heparin) also has been found therein.[10]

The purified heparin was readily convertible to the crystalline polymeric barium acid salt first described by Charles and Scott,[11] which has been adequately characterized by chemical and physical methods.[12–14] The infrared spectrum of the purified polysaccharide (sodium salt) was identical with that described by Burson and co-workers[15] for "purified heparin." Our purified preparation contains five sulfate ester groups per tetrasaccharide unit[12,16] and even so undoubtedly contains some free amino groups from which the sulfuric acid residue has been removed by the mild acidity to which the material has been exposed during isolation and purification. It is known that the sulfoamino group of heparin is sensitive to acidity.[12,17,18] In the intact polysaccharide the anticoagulant activity is a function, among other things, of its degree of sulfation,[17] and indeed extrapolation of this activity to complete sulfation of the amino group leads to a predictable maximum activity of 190 I.U./mg. Whether such

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