the reaction product from a mixture of benzene and alcohol afforded 0.8 g. of hexestrol, m.p. and mixed m.p. 186°, which gave correct analyses for carbon and hydrogen.

3,3'-Diacetylhexestryl dimethyl ether. A solution of 4.5 g. (0.015 mole) of hexestryl dimethyl ether, m.p. 144°,[11] 5.9 g. (0.075 mole) of acetyl chloride and 60 ml. of nitrobenzene was cooled in an ice bath, and 10.5 g. (0.079 mole) of finely powdered aluminum chloride was added in small portions with stirring. After 4 hr. stirring at room temperature, the reaction mixture was poured onto crushed ice, acidified with hydrochloric acid, and the nitrobenzene was removed by steam distillation. The remaining solid was collected, dried, and recrystallized from a mixture of methanol and benzene to yield colorless prisms of 3,3'-diacetylhexestryl dimethyl ether, 5.4 g. (93%), m.p. 172°.

Other 3,3'-diacylhexestryl dimethyl ethers listed in Table II were prepared analogously by use of appropriate acyl chloride.

Dioxime of 3,3'-diacetylhexestryl dimethyl ether. A solution of 3.0 g. (0.008 mole) of 3,3'-diacetylhexestryl dimethyl ether, 1.5 g. (0.022 mole) of hydroxylamine hydrochloride and 2.0 g. of anhydrous sodium acetate in 15 ml. of ethanol was heated under reflux for 2 hr. on a water bath. After cooling, the mixture was diluted with cold water, whereupon 3.3 g. (100%) of crystals separated. Three recrystallizations from a mixture of ethanol and benzene gave colorless prisms melting at 228°.

3,3'-Diethylhexestryl dimethyl ether. The reduction was carried out according to the Wolff-Kishner method as modified by Huang-Minlon.[12] A mixture of 3.1 g. (0.008 mole) of 3,3'-diacetylhexestryl dimethyl ether, 7.0 g. (0.125 mole) of potassium hydroxide, 7 ml. of 85% hydrazine hydrate, and 70 ml. of diethyleneglycol was heated under reflux for 1.5 hr. After removal of the water formed, the mixture was heated at 195° for an additional 4 hr. The solution was diluted with cold water and poured into hydrochloric acid. After a few hours the resulting dark oil solidified, and the solid, 2.8 g., (98%), was recrystallized from a mixture of methanol and benzene to give colorless prisms melting at 108°.

3,3'-Diethylhexestrol. A mixture of 1.5 g. of 3,3'-diethylhexestryl dimethyl ether, 20 ml. of glacial acetic acid, and 7 ml. of 48% hydriodic acid was heated under reflux for 1.5 hr. The solution was poured into an aqueous solution of sodium bisulfite. The resulting crystals were collected by filtration and washed with water and then with a small amount of cold ethanol. Several recrystallizations from ligroin gave colorless prisms melting at 127.5–128.5°.

Other 3,3'-dialkylhexestrols listed in Table II were prepared by the similar reduction and demethylation processes.

3,3'-Diacetamidohexestryl dimethyl ether. To a suspension of 2.7 g. (0.007 mole) of the dioxime of 3,3'-diacetylhexestryl dimethyl ether in 50 ml. of absolute ether, 4.5 g. (0.022 mole) of phosphorus pentachloride was added gradually with stirring and cooling in an ice bath. After 10 min., the ice bath was removed, and the stirring was continued for an additional 20 min. at room temperature. The mixture was poured onto crushed ice and the ether layer separated and washed with water. After removal of the solvent, the crystalline residue was recrystallized from ethanol to yield 2.6 g. (96%) of product, m.p. 252°.

Anal. Calcd. for $C_{24}H_{32}N_2O_4$: C, 69.87; H, 7.82. Found: C, 69.58; H, 7.91.

A mixture of this product with the dioxime of 3,3'-diacetylhexestryl dimethyl ether showed a marked depression.

KYOTO, JAPAN
CHICAGO 37, ILL.

(11) Hexestryl dimethyl ether was supplied by the Chugai Pharmaceutical Co., Ltd., Tokyo, Japan.

(12) Huang-Minlon, *J. Am. Chem. Soc.*, 68, 2487 (1946).

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

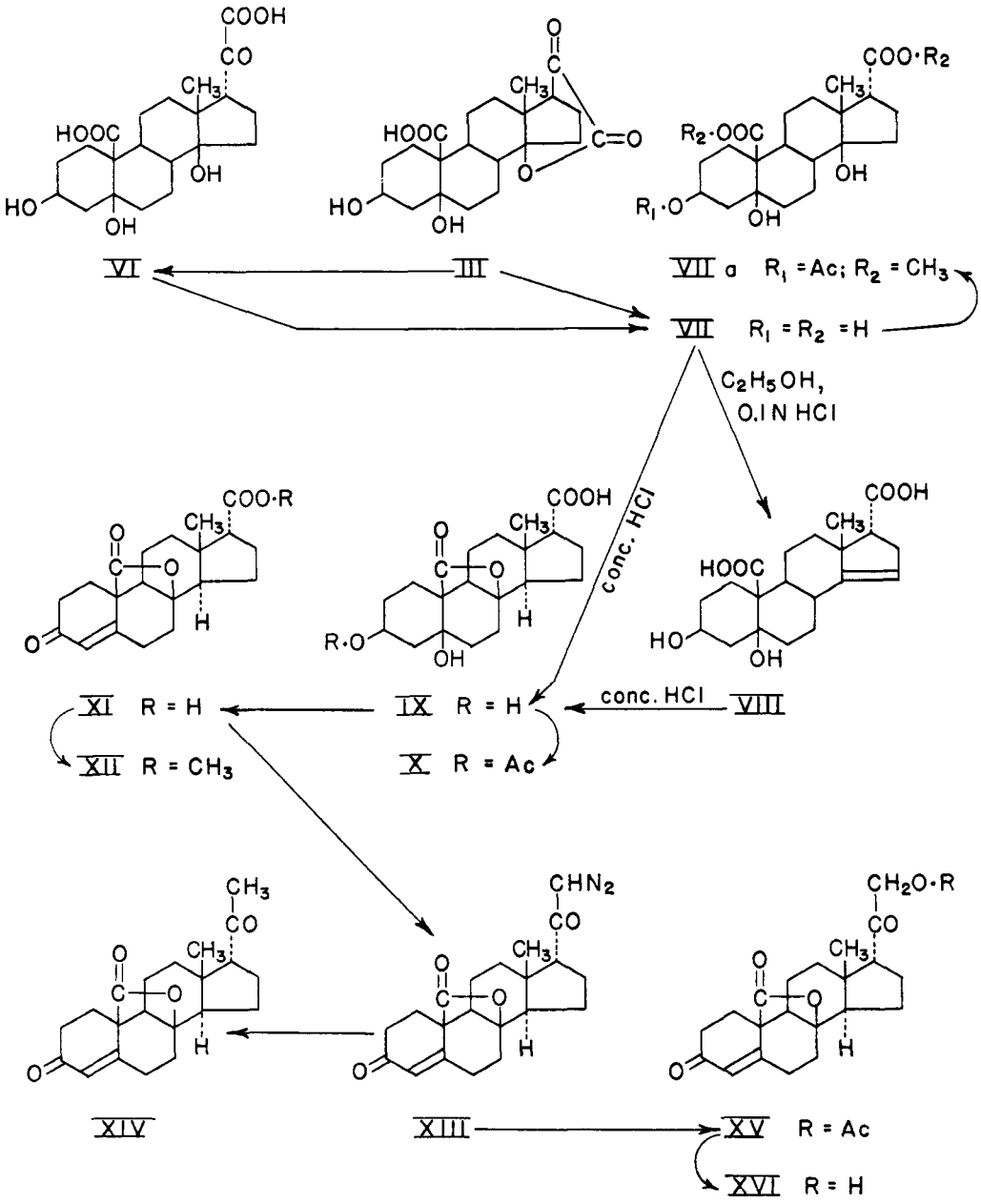
Investigations on Steroids. XXXIII. Conversion of Strophanthidin into 19:8-Lactone Analogs of Progesterone and Cortexone[1,2]

G. WINSTON BARBER AND MAXIMILIAN EHRENSTEIN

Received July 7, 1960

By oxidative procedures, strophanthidin (I) may be converted into 3β,5,14-trihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid (VII) or 3β,5,14-trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid (IV), which differ only regarding their configurations at carbon atom 17. Treatment of VII with concentrated hydrochloric acid gave 3β,5,8-trihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone (IX), whereas with IV the same reaction yielded 3β,5,8-trihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone (XVIII). The latter compound was also obtained by a different pathway, utilizing strophanthidinic acid (II) which on treatment with concentrated hydrochloric acid gave strophanthidinic acid 19:8-lactone (XX). The latter by ozonization yielded 3β,5,8,21-tetrahydroxy-20-oxo-5β-pregnan-19-oic acid 19:8-lactone (XXI), which by degradation with periodic acid furnished XVIII. Oxidation with chromium trioxide of IX and XVIII, and subsequent treatment of the reaction products with Girard's reagent T gave 8-hydroxy-3-oxo-21-nor-Δ⁴-17α-pregnene-19,20-dioic acid 19:8-lactone (XI) and 8-hydroxy-3-oxo-21-nor-Δ⁴-pregnene-19,20-dioic acid 19:8-lactone (XXII) respectively. The reaction of the acid chloride of XI with diazomethane gave 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone (XIII). By treatment with concentrated hydriodic acid, XIII was converted into 19:8-lacto-17α-progesterone (XIV), whereas with acetic acid 19:8-lacto-17α-cortexone acetate (XV) (amorphous) resulted which by saponification yielded 19:8-lacto-17α-cortexone (XVI). In identical fashion, XXII was converted into 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone (XXIII) which was transformed into 19:8-lactoprogesterone (XXIV) and 19:8-lactocortexone acetate (XXV). By saponification, the latter was converted into 19:8-lactocortexone (XXVI). The infrared absorption spectra of the terminal products XIV and XXIV, as well as XVI and XXVI have been compared. The results of the bioassays on these four compounds are also presented. XIV and XXIV produce only little, if any, progestational action. Both XVI and XXVI are devoid of mineralocorticoid activity.

In studies dealing with the degradation of strophanthidin, we encountered some time ago an etio acid which was first considered to possess a $\Delta^{8,14}$-double bond,[3] but was later[4,5] interpreted to contain a 19:8-lactone bridge (IX). In subsequent publications it was shown that 3β,5,8-trihydroxy-



21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone (IX) can be prepared in good yield by the action of concentrated hydrochloric acid either on 3β,5-dihydroxy-21-nor-Δ^{14}-5β,17α-pregnene-19,20-dioic acid (VIII)[5] or, more conveniently, on 3β,5,14-trihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid (VII).[6,7] VII is readily prepared from strophanthidin (I) by way of 3β,5,14-trihydroxy-20-oxo-5β,14β-pregnane-19,21-dioic acid 21:14-lactone (III), which is subjected in one operation to oxidation with hydrogen peroxide after treatment with alkali. The reaction proceeds through the intermediate 3β,5,14-trihydroxy-20-oxo-5β,14β,17α-pregnane-19,21-dioic acid (VI) which, because of the

(1) This investigation was supported by research grants (C757-C4, CY757-C5, CY757-C6 and CY757-C7) 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) The findings of this paper were presented on September 5, 1958, at the 4th International Congress of Biochemistry in Vienna (*cf.* Maximilian Ehrenstein: *Biochemistry of the Corticoids*, Proceedings of the Fourth International Congress of Biochemistry, Vol. 4 [Symposium: Biochemistry of Steroids], Pergamon Press, p. 259 (1959)).

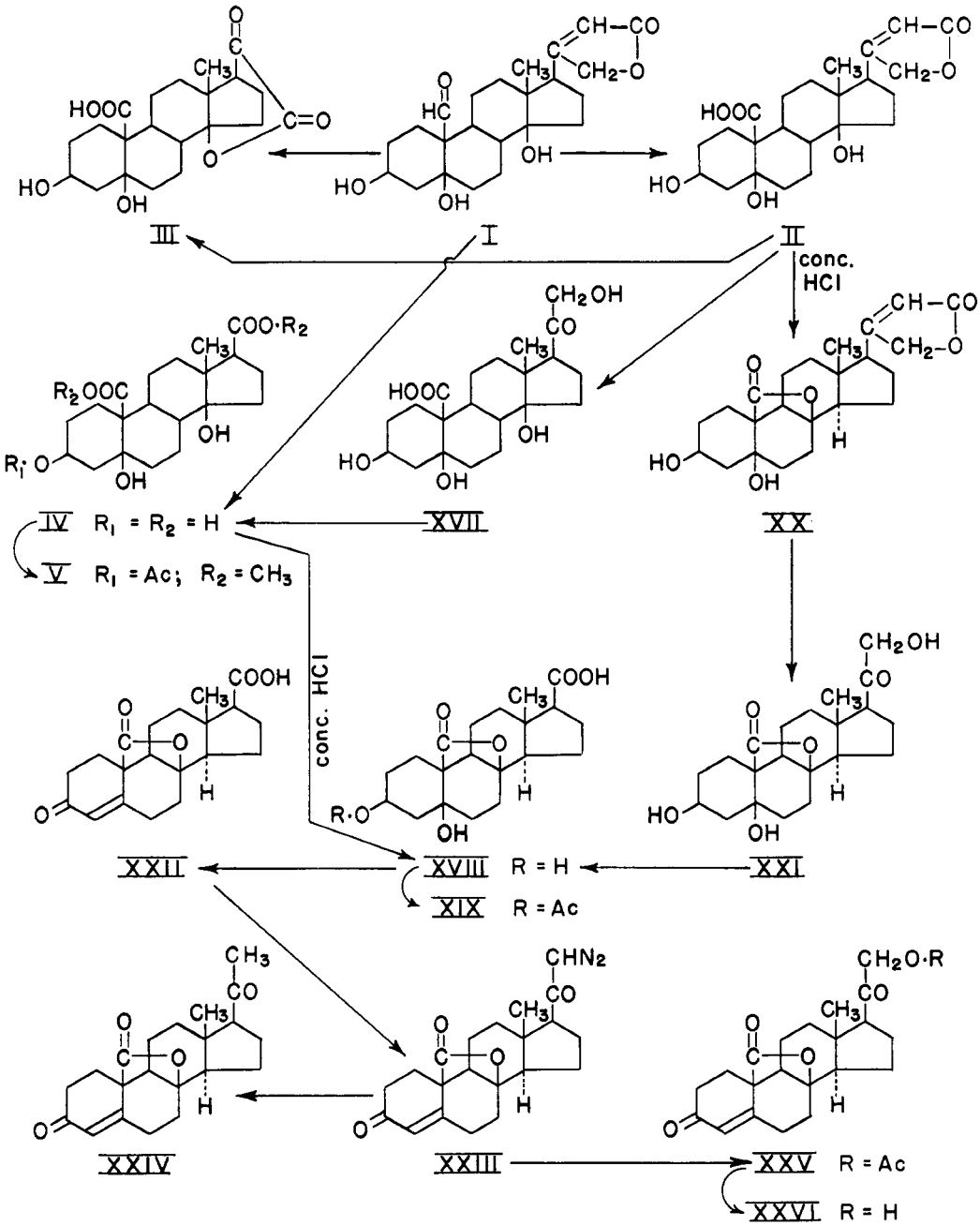
(3) M. Ehrenstein, *J. Org. Chem.*, 9, 435 (1944); *cf.* footnote on p. 446.

(4) M. Ehrenstein, G. W. Barber, and M. W. Gordon, *J. Org. Chem.* 16, 349 (1951); *cf.* p. 357.

(5) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, 16, 1615 (1951).

(6) G. W. Barber and M. Ehrenstein, *Liebigs Ann. Chem.*, 603, 89 (1957).

(7) In a previous publication (*cf.* Ref. 6, formula VII on p. 92) the hydrogen atom at C-14 had been assigned the β-configuration.



inversion at carbon atom 17, does not relactonize. It is to be noted that in VII, the carboxyl group at C-17 possesses the α-configuration. The conversion by concentrated hydrochloric acid of either VII or VIII into IX probably proceeds by way of a $\Delta^{8,14}$-unsaturated intermediate. Because of the presence of the free carboxyl group at C 10, it is unstable and immediately forms the γ-lactone IX. One may assume that this involves the *trans*-addition of the carboxyl group to the double bond and, consequently, the hydrogen atom at carbon atom 14 is denoted as having the α-configuration.

In consideration of the ready availability of the etio acid IX and in view of the recent interest in steroid lactones as potential aldosterone-blocking agents,[8] we decided to convert IX into the corresponding analogs of progesterone (XIV) and cortexone[9] (XVI). Previously, IX had been characterized by the ethyl[10] and methyl[10,5] esters. As an additional derivative, the 3-acetate, *i.e.* 3β-acetoxy-5,8-dihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8 lactone (X) was prepared. Interestingly, IX proved quite resistant to oxidation with *N*-bromoacetamide in *t*-butyl alcohol. By oxidation

(8) *Cf.* paper quoted in Ref. 2.

(9) In agreement with the proposals of Fieser, the trivial name cortexone is preferred to 11-desoxycorticosterone. *Cf. Steroids* by Louis F. Fieser and Mary Fieser, Reinhold Publishing Corporation, New York, 1959; v. pp. 602, 706.

(10) *Cf.* paper quoted in Ref. 4.

with chromium trioxide and subsequent treatment with Girard's reagent T, IX was converted into 8-hydroxy-3-oxo-21-nor-Δ^4-17α-pregnene-19,20-dioic acid 19:8-lactone (XI), which was characterized as the methyl ester (XII). The ethyl ester has been described earlier.[5] Treatment of the acid chloride of XI with diazomethane yielded the corresponding diazoketone, *viz.* 21-diazo-8-hydroxy-3,20-dioxo-Δ^4-17α-pregnen-19-oic acid 19:8-lactone (XIII). By treating XIII with concentrated hydriodic acid,[11-14,6] 8-hydroxy-3,20-dioxo-Δ^4-17α-pregnen-19-oic acid 19:8-lactone ["19:8-Lacto-17α-progesterone"] (XIV) resulted. On the other hand, heating of XIII with acetic acid gave 21-acetoxy-8-hydroxy-3,20-dioxo-Δ^4-17α-pregnen-19-oic acid 19:8-lactone ["19:8-Lacto-17α-cortexone acetate"] (XV) which did not crystallize. By saponification, XV was converted into 8,21-dihydroxy-3,20-dioxo-Δ^4-17α-pregnen-19-oic acid 19:8-lactone ["19:8-Lacto-17α-cortexone"] (XVI).

With the synthesis of 19:8-lacto-17α-progesterone (XIV) and 19:8-lacto-17α-cortexone (XVI) accomplished, it became desirable to prepare the corresponding epimers possessing at carbon atom 17 the normal, *i.e.*, the β-configurations. The logical starting material for such a project was 3β,5,14-trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid (IV) which has not been described before. We first obtained IV in amorphous form by a somewhat obscure approach. It was mentioned earlier that strophanthidin (I) may be converted into the 21:14-ketolactone III. This reaction is performed in a slightly alkaline medium by the action of potassium permanganate (*cf.* Experimental). It must be assumed that part of the material suffers further degradation leading to IV. In a first attempt to prepare IV, we subjected III to oxidation with hydrogen peroxide in a solution of acetic acid. Unfortunately, the starting material used in this experiment was not pure. Hence, it is difficult to judge whether substantial amounts of IV were present at the beginning of the experiment or whether the major part of IV resulted from the action of hydrogen peroxide on III. The identity of the amorphous IV follows from its chemical behavior as will be presented below.

Crystalline IV was obtained by a sequence of reactions using strophanthidinic acid (II) as starting material. II has been accessible from strophanthidin (I) by oxidation with potassium permanganate in a solution of acetone.[15] It can be more conveniently prepared by oxidizing I with hydrogen peroxide (*cf.* Experimental). II was subjected to ozonolysis[16] in a solution of ethyl acetate followed by reductive cleavage of the ozonide. The crude reaction product, representing the 21-glycolate of 3β,5,14,21-tetrahydroxy-20-oxo-5β,14β-pregnan-19-oic acid (XVII), was subjected to hydrolysis with potassium carbonate which furnished the crystalline ketol XVII. Oxidation of XVII with periodic acid gave crystalline IV in good yield. IV was characterized as the dimethyl ester acetate, viz. 3β-acetoxy-5,14-dihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid dimethyl ester (V), which showed the same melting point and optical rotation as a product of this structure described previously.[17] As expected, treatment of IV with concentrated hydrochloric acid gave a 19:8-lactone. It differs from IX in that the carboxyl group at C-17 possesses the β-configuration. In this instance, we also assume the formation of a $\Delta^{8,14}$-unsaturated compound as an intermediate which is followed by the *trans*-addition of the angular carboxyl group to the double bond. Hence the crystalline reaction product has to be assigned the structure of 3β,5,8-trihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone (XVIII). The same product XVIII was obtained on using either amorphous (see above) or crystalline IV as starting material.

For the preparation of XVIII from strophanthidinic acid (II) a second pathway has been worked out. By the action of concentrated hydrochloric acid on II, Jacobs[18] obtained a compound whose correct structure was recognized by Fieser.[19] The product, namely strophanthidinic acid 19:8-lactone (XX), was subjected to ozonolysis in a solution of methylene chloride. After reductive cleavage of the ozonide, the 21-glycolate of 3β,-5,8,21-tetrahydroxy-20-oxo-5β-pregnan-19-oic acid 19:8-lactone (XXI) was subjected to hydrolysis which yielded the free crystalline ketol XXI. On oxidizing XXI with periodic acid, a product resulted which was identical with XVIII previously obtained from either amorphous or crystalline IV by treatment with concentrated hydrochloric acid. XVIII was characterized as the 3-acetate, *i.e.* 3β-acetoxy-5,8-dihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone (XIX).

Oxidation of XVIII with chromium trioxide and

(11) R. D. H. Heard and P. Ziegler, *J. Am. Chem. Soc.*, **72**, 4328 (1950).

(12) G. W. Barber and M. Ehrenstein, *J. Org. Chem.*, **19**, 1758 (1954).

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

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

(15) W. A. Jacobs, *J. Biol. Chem.*, **57**, 553 (1923).

(16) For method and literature, *cf.* Ch. Tamm, *Neuere Ergebnisse auf dem Gebiete der glykosidischen Herzgifte: Grundlagen und die Aglykone* (Progress in the Chemistry of Organic Natural Products). **13**, 137 (1956). Springer Verlag, Wien. See p. 155. *Cf.* also the more recent pertinent publication by M. Zingg and K. Meyer, *Helv. Chim. Acta*, **43**, 145 (1960).

(17) A. Buzas and T. Reichstein, *Helv. Chim. Acta*, **31**, 84 (1948).

(18) W. A. Jacobs and A. M. Collins, *J. Biol. Chem.*, **65**, 491 (1925); *cf.* p. 499.

(19) Louis F. Fieser and Mary Fieser, *Natural Products Related to Phenanthrene*, 3rd Edition, Reinhold, New York, 1949; v. pp. 523–524; *cf.* also book cited in Ref. 9; v. pp. 742–743.

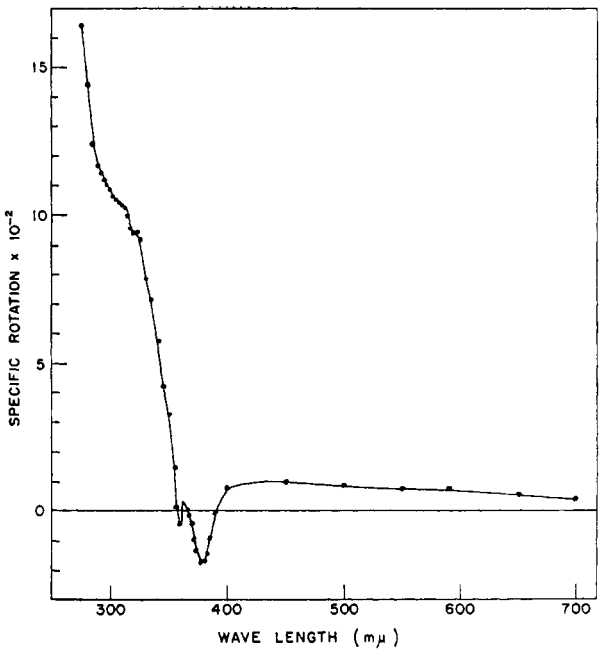


Fig. 1. Rotatory dispersion curve of 8-hydroxy-3-oxo-21-nor-Δ^4-17α-pregnene-19,20-dioic acid 19:8-lactone methyl ester (XII) (m.p. 165–166°) in dioxane (c = 0.084, 700 ~ 275 mμ)

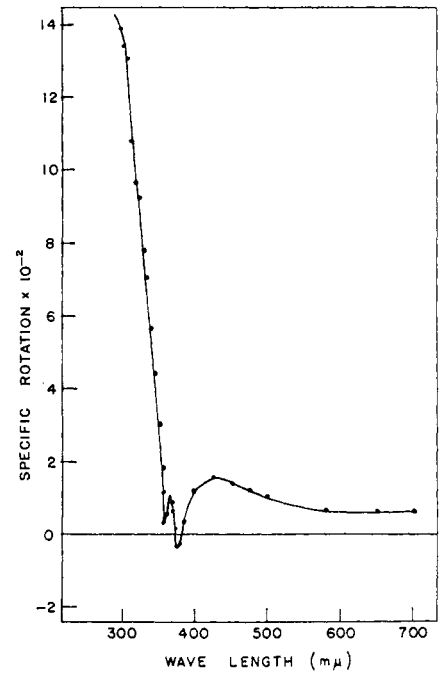


Fig. 2. Rotatory dispersion curve of 8-hydroxy-3-oxo-21-nor-Δ^4-pregnene-19,20-dioic acid 19:8 lactone (XXII) (m.p. 237–239°) in dioxane (c = 0.031, 700 ~ 300 mμ)

subsequent treatment of the reaction product with Girard's reagent T gave 8-hydroxy-3-oxo-21-nor-Δ^4-pregnene-19,20-dioic acid 19:8-lactone (XXII). On treating the acid chloride of XXII with diazomethane, 21-diazo-8-hydroxy-3,20-dioxo-Δ^4-pregnen-19-oic acid 19:8-lactone (XXIII) was obtained. By treatment of XXIII with concentrated hydriodic acid,[11–14,6] 8-hydroxy-3,20-dioxo-Δ^4-pregnen-19-oic acid 19:8-lactone ["19:8-Lactoprogesterone"] (XXIV) resulted in good yield. On heating XXIII with acetic acid, it was readily converted into 21-acetoxy-8-hydroxy-3,20-dioxo-Δ^4-pregnen-19-oic acid 19:8-lactone ["19:8-Lactocortexone acetate"] (XXV) which was obtained in crystalline form.[20] Saponification of XXV with potassium carbonate gave a satisfactory yield of 8,21-dihydroxy-3,20-dioxo-Δ^4-pregnen-19-oic acid 19:8-lactone ["19:8-Lactocortexone"] (XXVI). It should be stated that, although we believe that the reported 19:8-lactones possess the 14α-configurations, this cannot be considered proven. The correlation of the molecular rotations of the corresponding compounds of the 17α- and 17β-series does not permit any definite conclusions and is therefore omitted. Further experimental work will be undertaken to prove the configurations at C-14.

In a forthcoming publication from this laboratory, dealing with 8,19-epoxy compounds, it will be necessary, for purposes of comparison to refer to the rotatory dispersion curves[21] of compounds XII (Fig. 1) and XXII (Fig. 2) which were determined through the courtesy of Professor Carl Djerassi at Wayne State University (now at Stanford University). Both curves show a *negative* multiple Cotton effect and correspond closely to that of a standard Δ^4-3-ketone[22] which does not show any major conformational distortion. As expected, the difference of the configuration at C-17 has no influence.[23]

Of particular interest are the infrared absorption curves which were obtained with the terminal products of this investigation, namely: (a) XIV and XXIV; (b) XVI and XXVI. They were determined through the courtesy of Dr. R. Norman Jones in the Division of Pure Chemistry of the National Research Council of Canada in Ottawa, Ontario, and have previously been discussed to some extent.[24] Noteworthy are the slight displacements of both the C=O and C=C stretching

(20) In an experiment performed for the purpose of orientation, treatment of XXV with selenium dioxide in a solution of *t*-butyl alcohol containing acetic acid gave a crystalline product which possibly represents 21-acetoxy-8-hydroxy-3,20-dioxo-$\Delta^{1,4}$-pregnadien-19-oic acid 19:8-lactone ["19:8-lacto-1-dehydrocortexone acetate"]; m.p. 175–176°. $[\alpha]_D^{20}$ +189° ± 9°; M_D^{20} +753° ± 36° (3.33 mg.; α + 0.63°). λ_{max}^{ale} 250 mμ, ϵ 17,000. *Anal.* Calcd. for $C_{23}H_{26}O_6$ (398.44): C, 69.33; H, 6.58. Found: C, 68.95; H, 7.50. Residue, 0.58. Corrected: C, 68.7; H 7.1.,

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

(22) *Cf. e.g.*, Ref. 20, pp. 17, 61, 65.

(23) The authors had the privilege of discussing these aspects with Dr. W. Klyne (Postgraduate Medical School of London) on his visit to their laboratory on January 15, 1960.

(24) R. N. Jones and B. S. Gallagher, *J. Am. Chem. Soc.*, 81, 5242 (1959).

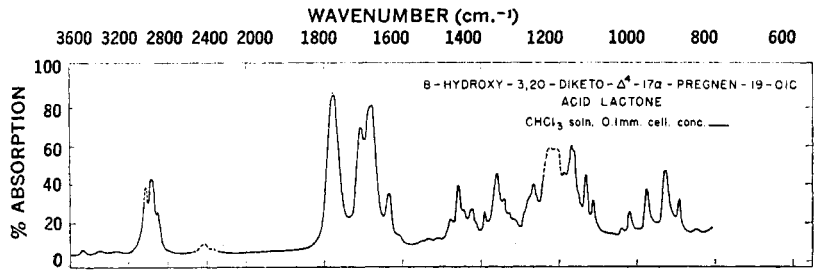


Fig. 3. Infrared spectrum of 19:8-lacto-17α-progesterone (XIV)

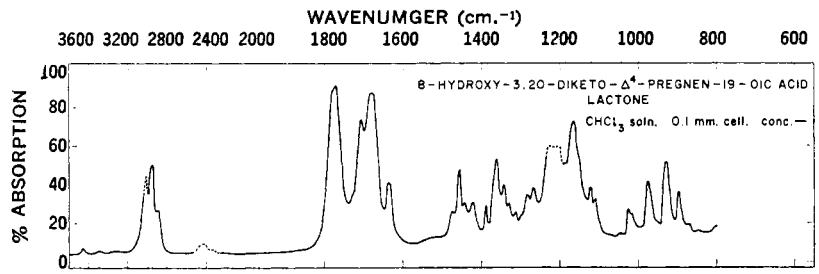


Fig. 4. Infrared spectrum of 19:8-lactoprogesterone (XXIV)

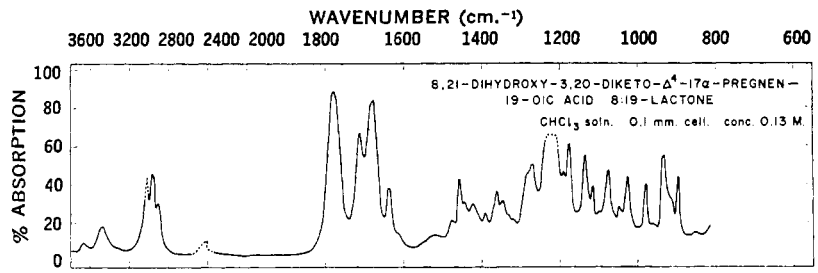


Fig. 5. Infrared spectrum of 19:8-lacto-17α-cortexone (XVI)

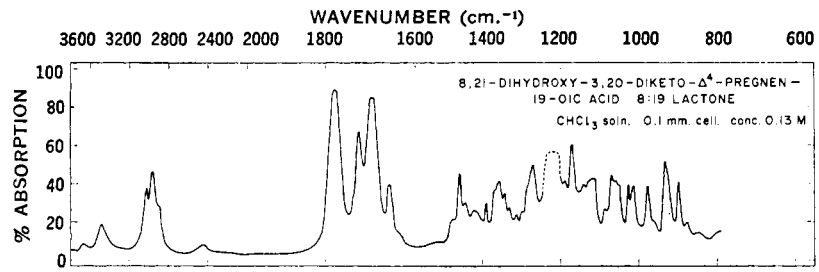


Fig. 6. Infrared spectrum of 19:8-lactocortexone (XXVI)

frequencies from their normal positions. In XIV and XXIV the carbonyl band of the Δ^4-3-ketone occurs at 1686 cm.$^{-1}$ in carbon tetrachloride solution. This is higher than the normal position at 1681–1677 cm.$^{-1}$ In chloroform solution XIV, XXIV, XVI, and XXVI absorb at 1675, 1677, 1678, and 1680 cm.$^{-1}$ respectively, while the normal range is 1668–1660 cm.$^{-1}$ The Δ^4 C=C stretching vibration is also displaced in these compounds from 1619–1613 to 1635 cm.$^{-1}$ (chloroform solution). It would appear therefore that the bridging of ring B by the lactone group increases the rigidity of the A/B ring system and so induces steric strains in the conjugated ketone structure. Dipole-dipole interactions between the two carbonyl groups could also raise the ketone and C=C stretching frequencies, but if this were so, the γ-lactone carbonyl frequency (observed for XIV, XXIV, XVI, and XXVI, in chloroform: 1772, 1772, 1777, and 1775 cm.$^{-1}$, respectively) would also be affected.

The infrared spectra of compounds XIV, XXIV, XVI, and XXVI, measured in chloroform on the Perkin Elmer Model 21 spectrophotometer, are presented in Figs. 3, 4, 5, and 6, respectively.[25] "It is interesting that there are small but still significant changes in the fingerprint regions of the spectra associated with isomerism at C 17. The main differences are:

(25) *Cf.* also: C. L. Angell, B. S. Gallagher, T. Ito, R. J. D. Smith, and R. N. Jones: *The Infrared Spectra of Lactones*, National Research Council Bulletin No. 7, Ottawa 1960; v, charts 51, 50, 53, 52.

(a) Compound XIV (Fig. 3)	Compound XXIV (Fig. 4)
1134 cm.$^{-1}$	1124 cm.$^{-1}$
1114	1110
1018	1022
890	894

(b) Compound XVI (Fig. 5)	Compound XXVI (Fig. 6)
1135 cm.$^{-1}$ singlet	1150–1110 cm.$^{-1}$ complex band group
1075 singlet	1070, 1058, 1050 triplet
—	1012 extra band

At the present state of our knowledge it is not possible to interpret these differences. They are interesting, however, in that they emphasize the importance of paying attention to small spectral differences when using the "fingerprint" spectra for identification purposes."[26]

Physiological activity. Bioassays for progestational activity were carried out by Dr. Roy Hertz, Chief of the Endocrinology Branch of the National Cancer Institute. With 19:8-lacto-17α-progesterone (XIV) the Clauberg test was negative in each of two rabbits with a total dose of 2.5 mg. With 19:8-lactoprogesterone (XXIV) a dosage level of 1.0 mg. per rabbit (two animals) was totally inactive. Since in this assay a maximal effect is obtained with 0.25 mg. of progesterone, the activities of XIV and XXIV, if any, are probably less than one-tenth that of progesterone.

19:8-Lacto-17α-cortexone (XVI) was tested for mineralocorticoid activity by two different groups. (1) In bioassays performed by Dr. Amos H. Lieberman in the laboratory of Dr. John A. Luetscher, Jr., at Stanford University School of Medicine, no sodium retaining or potassium excreting activity was noted in a dose of 30 μg. As rats are usually sensitive to as little as one microgram of cortexone or cortexone acetate,[27] these bioassays show that the mineralocorticoid activity of XVI, if any, is less than one-thirtieth that of cortexone acetate. (2) In assays conducted at the Worcester Foundation for Experimental Biology by Dr. Ralph I. Dorfman, XVI, in doses of 25 and 50 μg., had no significant effect on the excretion of sodium or potassium in salt (sodium chloride) loaded adrenalectomized rats.[28] 19:8-Lactocortexone (XXVI) was bioassayed through the courtesy of Dr. Ralph I. Dorfman in the same fashion. In doses of 6 and 50 μg., XXVI had no effect on the excretion of sodium or potassium. On the basis of these findings it appears that both XVI and XXVI are devoid of mineralocorticoid activity.

(26) The sentences in quotation marks are comments supplied by Dr. R. Norman Jones.

(27) *Cf.* e.g. John A. Luetscher, Jr., and Quentin B. Deming: "Bioassay of sodium-retaining corticoids and some changes in excretion of these substances in disease" in *Renal Function*, Transactions of the Second Conference, 155–178, Josiah Macy, Jr., Foundation, New York, 1951, v. p. 159.

(28) The 6 μg. dose level seemed to have caused minimum sodium excretion, an observation which has to be studied further.

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.

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.[29] The percentage loss of weight on drying is recorded; there was in no instance a gain of weight on exposure of the dried sample to the atmosphere.

Optical rotation. No correction for crystal solvent has been made. 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,[12] silica gel,[12] and Florisil[30] used as adsorbents for chromatography have been described.

3β,5,14-Trihydroxy-20-oxo-5β,14β-pregnane-19,21-dioic acid 21:14-lactone (III) *from strophanthidin* (I).[31] Crystalline strophanthidin (I) was converted, in 10-g. batches, into brittle foam by dissolving in acetone and evaporating to dryness *in vacuo*. The foam was suspended in 1000 cc. of 0.1*N* aqueous sodium hydroxide and 250 cc. of 5% potassium permanganate solution was added dropwise while vigorous stirring was maintained. The addition required 1 hr., after which 20 cc. of 6*N* hydrochloric acid was added, and the manganese dioxide was filtered by suction and washed with hot water. The filtrate was concentrated *in vacuo* to a volume of approximately 75 cc. and 25 cc. of 6*N* hydrochloric acid was added. After 2 days in the icebox, the resulting precipitate was filtered, washed with water, and dried. The tan powder, thus isolated, was suspended in a mixture of 10 cc. of acetone and 20 cc. of ether. After standing in the icebox overnight, filtration yielded from 3.56 g. to 3.92 g. of crude III as a white powder. The material obtained in this way from 38.376 g. of strophanthidin (I) was repeatedly recrystallized from methanol to give a total of 12.739 g. (yield, 34.2%) of crystalline III with melting points above 270°.

3β,5,14-Trihydroxy-20-oxo-5β,14β,17α-pregnane-19,21-dioic acid (VI) *from 3β,5,14-trihydroxy-20-oxo-5β,14β-pregnane-19,21-dioic acid 21:14-lactone* (III). A total of 100 mg. of III, m.p. 270–272°, dissolved in 5 cc. of 2% aqueous sodium hydroxide, was heated on a steam bath for 45 min. After standing at room temperature overnight, the solution was acidified to Congo Red with 6*N* hydrochloric acid (no precipitate) and was then extracted with five 25-cc. portions of ethyl acetate. After drying over sodium sulfate and evaporating the solvent, 110.5 mg. of a solid white residue resulted. Crystallization from acetone-ether gave 78.4 mg. of minute needles, m.p. 180–182° (foaming), representing 3β,5,14-trihydroxy-20-oxo-5β,14β,17α-pregnane-19,21-dioic acid (VI). By recrystallization from methanol-ether the m.p. was raised to 184° (foaming).

3β,5,14-Trihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid (VII) *from 3β,5,14-trihydroxy-20-oxo-5β,14β-pregnane-19,21-dioic acid 21:14-lactone* (III).[32] The purity of the

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

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

(31) For alternate method, using strophanthidin acetate as starting material, *cf.* Ref. 6, p. 101.

(32) *Cf.* also Ref. 6, p. 101.

starting material (III) is of crucial importance in that the yield of VII is drastically reduced when samples of III melting below 270° are employed. A solution of 6.021 g. of III, m.p. 273–275°, in 120 cc. of 2% aqueous sodium hydroxide was left at room temperature overnight, and 60 cc. of 30% hydrogen peroxide was then added. After 2 more days, 100 cc. of *N* sulfuric acid was added, whereupon VII began to crystallize slowly. After 2 more days at room temperature and a night in the icebox, the product (prisms) was filtered and washed with water. The yield was 4.348 g. (76%) of VII, m.p. 275–278°.

3β-Acetoxy-5,14-dihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid dimethyl ester (VIIa) *from 3β,5,14-trihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid* (VII). To a suspension of 20 mg. of VII, m.p. 275–276°, in 2 cc. of acetone was added an excess of ethereal diazomethane. On evaporating to dryness, a crystalline residue was obtained which was dissolved in 1 cc. of pyridine and then 0.5 cc. of acetic anhydride was added. After standing overnight, 10 cc. of 3*N* hydrochloric acid was added and the reaction product was isolated in the usual fashion. Yield of crude crystalline material: 24.7 mg. Recrystallization from ether–petroleum ether (b.p. 30–60°) gave 18.5 mg. of colorless prisms, m.p. 182–184°. Renewed crystallization from acetone-water raised the m.p. to 186° (sharp). $[\alpha]_D^{21}$ +18° ± 2°; M_D^{21} +82° ± 9° (10.45 mg., α + 0.19°). Lit.[17]: m.p. 183–185°. $[\alpha]_D^{20}$ +20.4° ± 2° (chloroform).

3β,5,8-Trihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone (IX) *from 3β,5,14-trihydroxy-21-nor-5β,14β,17α-pregnane-19,20-dioic acid* (VII).[32] To 548.6 mg. of VII, m.p. 270–272°, was added 25 cc. of concd. hydrochloric acid. After standing overnight, the resulting solution was diluted with 100 cc. of water and extracted with four 100-cc. portions of ethyl acetate. The ethyl acetate extract was washed with water, dried over sodium sulfate, and evaporated *in vacuo*, leaving 548.6 mg. of a yellow crystalline residue. Repeated recrystallization from methanol-ether gave 252.1 mg. of IX; sparkling colorless prisms, m.p. 307–308°. The product was identical with material prepared by treatment of 3β,5-dihydroxy-21-nor-Δ[14]-5β,17α-pregnene-19,20-dioic acid (VIII) with hydrochloric acid.[5]

3β-Acetoxy-5,8-dihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone (X) *from 3β,5,8-trihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone* (IX). A solution of 50 mg. of IX, m.p. 308–309°, in 2 cc. of pyridine and 1 cc. of acetic anhydride was left at room temperature. After 18 hr., 20 cc. of 3*N* hydrochloric acid was added, and after 15 min., the mixture was extracted with ethyl acetate. The extract was washed with 3*N* hydrochloric acid and with water and was then dried over sodium sulfate and evaporated *in vacuo*, yielding a crystalline residue; wt. 59.0 mg. Recrystallization from acetone-hexane and twice from acetone-water gave 48.1 mg. of X as colorless needles, m.p. 258–259°. $[\alpha]_D^{21}$ +47° ± 4°; M_D^{21} +191° ± 16° (11.04 mg., α + 0.52°).

Anal. Calcd. for $C_{22}H_{30}O_7$ (406.46): C, 65.00; H, 7.44. Found: C, 64.96; H, 7.54. Weight loss, 3.98.

8-Hydroxy-3-oxo-21-nor-Δ⁴-17α-pregnene-19,20-dioic acid 19:8-lactone (XI) *from 3β,5,8-trihydroxy-21-nor-5β,17α-pregnane-19,20-dioic acid 19:8-lactone* (IX). To 487.7 mg. of IX, m.p. 305–307°, in 25 cc. of glacial acetic acid, a solution of 98 mg. of chromic anhydride (10% excess) in 50 cc. of 90% acetic acid was added in five 10-cc. portions at half-hour intervals. After standing at room temperature overnight, the reaction mixture was evaporated *in vacuo*, and the residue was taken up in ethyl acetate. After washing the solution with *N* sulfuric acid and water, it was dried over sodium sulfate and evaporated to dryness, leaving 427.1 mg. of a colorless crystalline residue. This was dissolved in 10 cc. of absolute ethanol, 1 g. of Girard's reagent T, and 1 cc. of glacial acetic acid were added, and the mixture was refluxed for 1 hr. Dilution to 100 cc. with water and extraction with ethyl acetate yielded 61.4 mg. of amorphous nonketonic material. The aqueous phase was acidified to approximately 1*N* by the addition of hydrochloric acid and was left overnight. Extraction with ethyl acetate produced 333.0 mg. of slightly brown crystalline ketonic material. The ketonic fraction was combined with 58.9 mg. of ketonic material obtained from another run, and the total of 391.9 mg. was chromatographed over 20 g. of silica gel (18 × 105 mm.). Pure chloroform first eluted small amounts of two resinous colored impurities, which were followed by 364 mg. of colorless crystalline XI. Recrystallization from acetone-ether and acetone-water gave the analytical sample as long slender needles, m.p. 232–236° when heated from room temperature. When placed on the melting point block at 210°, the crystals melted at once with bubbling, promptly resolidified, and then melted at 236–238°. $[\alpha]_D^{22}$ +83.5° ± 2°; M_D^{22} +289° ± 7° (10.06 mg. in 2.0 cc. of chloroform containing 5 drops of ethanol, α + 0.84°). λ_{max}^{alc} 244 mμ, ε 13,300.

Anal. Calcd. for $C_{20}H_{24}O_5$ (344.39): C, 69.75; H, 7.02. Found: C, 69.80; H, 7.16. Weight loss, 6.67.

8-Hydroxy-3-oxo-21-nor-Δ⁴-17α-pregnene-19,20-dioic acid 19:8-lactone methyl ester (XII). To 52.7 mg. of XI, m.p. 230–235°, in 2 cc. of acetone was added an exce ss of ethereal diazomethane. After standing 10 min., the solution was evaporated to dryness. Recrystallization of the residue from acetone-hexane gave 36.8 mg. of clusters of yellow needles, m.p. 160–162°. This product was recrystallized several times alternately from acetone-water and acetone-hexane to give 24.1 mg. of colorless, long, slender needles, m.p. 165–166°. $[\alpha]_D^{21}$ +77° ± 3°; M_D^{21} +276° ± 11° (11.67 mg., α + 0.90°). λ_{max}^{alc} 244 mμ, ε 13,600.

Anal. Calcd. for $C_{21}H_{26}O_5$ (358.42): C, 70.37; H, 7.31. Found: C, 70.13; H, 7.35.

21-Diazo-8-hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone (XIII) *from 8-hydroxy-3-oxo-21-nor-Δ⁴-17α-pregnene-19,20-dioic acid 19:8-lactone* (XI). To a solution of 198.8 mg. of XI, m.p. 229–231°, in 10 cc. of ethanol was added 48.5 mg. of sodium bicarbonate in 1 cc. of water. The mixture was frozen and evaporated *in vacuo*, and the residue was thoroughly dried and suspended in a mixture of 10 cc. of dry benzene and 5 drops of pyridine. This was cooled in ice until partly frozen, and 1 cc. of oxalyl chloride was added. After standing for 15 min., the mixture was frozen and evaporated to dryness *in vacuo*. The residue was dissolved in 10 cc. of dry benzene and the solution was brought to dryness from the frozen state. Finally, the odorless residue was taken up in 10 cc. of dry benzene, and the suspension was filtered through sintered glass under nitrogen pressure into ethereal diazomethane which had been freshly prepared from 8 g. of methylnitrosourea[33] and dried over sodium. The residue of salts was washed with two 5-cc. portions of dry benzene, and the reaction mixture was left at room temperature for 1 hr. and was then evaporated to dryness *in vacuo*. The yellow solid residue was taken up in 100 cc. of ethyl acetate, and the solution was washed with *N* sodium carbonate, dried over sodium sulfate and evaporated, yielding 217.3 mg. of yellow foam. This was chromatographed on 20 g. of alumina (activity I–II, 18 × 80 mm.). Benzene-ether, range 19:1 to 2:3, eluted a total of 127.6 mg. of crystalline fractions of XIII. Successive recrystallization from acetone-ether and acetone-water gave the analytical sample as long, slender, pale-yellow needles with no clearly defined melting point. The crystals decrepitated at 145–150° to give a powder which sintered at 195–200° but never really liquefied. $[\alpha]_D^{25}$ +38° ± 2°; M_D^{25} +141° ± 8° (10.21 mg., α + 0.39°). λ_{max}^{alc} 247 mμ, ε 23,400.

Anal. Calcd. for $C_{21}H_{24}N_2O_4$ (368.42): C, 68.46; H, 6.57; N, 7.60. Found: C,[34] 67.94; H,[34] 6.69; N,[35] 7.67. Residue, 0.14. Weight loss, 0.41.

In repeated runs of this experiment, the chromatographic purification occasionally yielded a second, more polar

(33) A. H. Blatt, *Org. Syntheses*, Coll. Vol. II, 165, 461 (1943).

(34) Dried at 70°.

(35) Not dried.

product; recrystallization from acetone-hexane and acetone-water; colorless elongated prisms, m.p. 288–289°. Possibly this represents the amide of the starting material XI.[36]

Anal. Calcd. for $C_{20}H_{25}NO_4$ (343.41): C, 69.94; H, 7.34; N, 4.08. Found: C, 70.10; H, 7.56; N, 4.54.

8-Hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone ["*19:8-Lacto-17α-progesterone*"] (XIV) *from 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone* (XIII). A solution of 50 mg. of the analytical sample of the diazo ketone XIII in 20 cc. of chloroform was shaken for 2 min. with 2 cc. of 48% hydriodic acid (Baker's Analyzed Reagent). The chloroform layer was then shaken successively with three 5-cc. portions of saturated aqueous sodium iodide and with 5 cc. of *N* sodium thiosulfate. After drying over sodium sulfate, the chloroform was evaporated *in vacuo*, leaving 50.1 mg. of colorless needles. Recrystallization from acetone-hexane gave 45.1 mg. of needles of m.p. 204–205°. Renewed recrystallization from acetone-water yielded 40 mg. of XIV as fan-shaped clusters of flat needles, m.p. 204–205°. $[\alpha]_D^{25}$ +49° ± 2°; M_D^{25} +168° ± 6° (10.83 mg., α + 0.53°). λ_{max}^{alc} 243 mμ, ε 15,900.

Anal. Calcd. for $C_{21}H_{26}O_4$ (342.42): C, 73.66; H, 7.65. Found: C, 73.33; H, 7.58.

8,21-Dihydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone ["*19:8-Lacto-17α-cortexone*"] (XVI) *from 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone* (XIII). A solution of 110.4 mg. of the diazoketone XIII, m.p. 205–210°, in 10 cc. of glacial acetic acid was heated on the steam bath for 1 hr. Evaporation to dryness *in vacuo* gave a brownish amorphous residue, representing crude 21-acetoxy-8-hydroxy-3,20-dioxo-Δ⁴-17α-pregnen-19-oic acid 19:8-lactone ["19:8-lacto-17α-cortexone acetate"] (XV).[37] This was dissolved in 3 cc. of methylene chloride, and 7.5 cc. of 0.05*M* potassium carbonate in 75% aqueous methanol was added.[38] After standing for 15 min., the reaction mixture was diluted with 40 cc. of water and extracted with four 50-cc. portions of ethyl acetate. After drying the extract over sodium sulfate, evaporation left 89.8 mg. of yellow crystalline residue. This was chromatographed over 10 g. of Florisil (18 × 90 mm.). Chloroform containing from 3% to 16% of acetone, eluted a total of 71.8 mg. of crystalline XVI with melting points in the range 188–208°. Recrystallization from acetone-hexane and then from acetone-water gave colorless needles, m.p. 206–207°. $[\alpha]_D^{21}$ +60° ± 6°; M_D^{21} +215° ± 20° (11.21 mg., α + 0.67°). λ_{max}^{alc} 244 mμ, ε 13,300.

Anal. Calcd. for $C_{21}H_{26}O_5$ (358.42): C, 70.37; H, 7.31. Found: C, 70.06,[39] 69.93[40]; H, 7.40,[39] 7.43.[40] Residue: 0.23.[40]

3β,5,14,21-Tetrahydroxy-20-oxo-5β,14β-pregnan-19-oic acid (XVII) *from strophanthidinic acid* (II). A solution of 975 mg. of crystalline II[15] (colorless prisms; m.p. 160–175°) in 200 cc. of ethyl acetate was cooled in Dry Ice–acetone and oxygen containing approx. 2.5% of ozone was passed in until a permanent deep blue color was obtained. After standing at Dry Ice–acetone temperature for 30 min., the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in 10 cc. of glacial acetic acid, zinc dust was added, and the mixture was heated gently and swirled until a starch-iodide test was negative. The mixture was filtered, the residue was washed with acetic acid, and the filtrate was evaporated *in vacuo*, leaving 1.150 g. of colorless brittle foam. This was taken up in 100 cc. of 0.1*N* potassium carbonate in 75% methanol. After 15 min., this solution was barely acidified to Congo Red with hydrochloric acid, and sodium chloride was added to saturation. Extraction with twenty 100-cc. portions of ethyl acetate yielded 782 mg. of colorless foam which was leached with several portions (total: 10 cc.) of hot water, leaving a small amount of yellow resin. Evaporation of the aqueous extract left 728 mg. of crude XVII. Crystallization from acetone-ether and recrystallization from methanol-ether gave 201 mg. of colorless granules, m.p. 235–237° with effervescence. Chromatography of the mother liquor material on 100 g. of silica gel gave only 84 mg. more of crystalline material. The analytical sample (colorless prisms) melted at 235–237° with effervescence when heated from room temperature and at 238–239° when placed on the block at 230°. $[\alpha]_D^{24}$ +41° ± 3°; M_D^{24} +163° ± 12° (10.44 mg. in 2.0 cc. of chloroform containing 10% of ethanol, α + 0.43°).

Anal. Calcd. for $C_{21}H_{32}O_7$ (396.47): C, 63.62; H, 8.14. Found: C, 63.50; H, 8.12.

3β,5,14-Trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid (IV). A. *From a mixture of products containing 3β,5,14-trihydroxy-20-oxo-5β,14β-pregnane-19,20-dioic acid 21:14-lactone* (III). To a solution of 6.833 g. of impure III, m.p.'s below 200°, in 100 cc. of glacial acetic acid was added 50 cc. of 30% hydrogen peroxide. After standing for 2 weeks, the reaction mixture was concentrated *in vacuo* to about 25 cc., diluted to 200 cc. with water, and extracted with five 200-cc. portions of ethyl acetate. The ethyl acetate extract was washed with water and with saturated aqueous sodium chloride and was then dried and evaporated *in vacuo*, leaving 5.663 g. of yellow foam. This was combined with 3.824 g. of material obtained in exactly the same way from 4.544 g. of pure III, m.p.'s 269–271° and 266–269°, and the total (9.487 g.) was chromatographed on 200 g. of silica gel (38 × 285 mm.). Chloroform containing from 1% to 10% of acetone eluted only traces of yellow resin. Chloroform containing from 12% to 80% of acetone eluted a total of 8.590 g. of colorless foam in a single broad peak. The center fractions of the peak were combined (5.685 g.). Paper chromatographic examination in the system toluene–propylene glycol revealed the presence of a contaminant which was assumed to be the 3-acetate, so this material was dissolved in 10 cc. of 95% ethanol and 100 cc. of *N* ethanolic potassium hydroxide was added. After standing overnight, concentration *in vacuo*, dilution with water, and acidification, thorough extraction with ethyl acetate yielded 4.990 g. of yellow foam. This was chromatographed on 200 g. of silica gel (38 × 270 mm.). Chloroform containing from 20% to 40% of acetone eluted 4.299 g. as a single, fairly sharp peak (twelve 500-cc. fractions). Most of this material resisted all attempts at crystallization, but it was assumed to consist essentially of the desired IV, as treatment with concentrated hydrochloric acid yielded 19:8-lactone XVIII (see below).

From two of the center fractions of the above peak, a crystalline product was obtained, and repeated recrystallization from methanol-ether gave 438 mg. of clusters of glistening prisms, m.p. 223–225° with foaming, following decrepitation at 195–210°. $[\alpha]_D^{25}$ +3° ± 2° (10.35 mg. in 2.0 cc. of 95% ethanol, α + 0.03°). *Anal.* Found: C, 62.22; H, 7.20. This was first thought to be IV, but it was quite different from authentic IV, subsequently prepared from 3β,5,14,21-tetrahydroxy-20-oxo-5β,14β-pregnan-19-oic acid (XVII) (*cf.* B), and treatment with concentrated hydrochloric acid gave a product (m.p. 263–264°. *Anal.* Found: C, 63.52; H, 6.90. Weight loss, 3.76. Dried at room temp.) which was not identical with the 19:8-lactone XVIII.

B. *From 3β,5,14,21-tetrahydroxy-20-oxo-5β,14β-pregnan-19-oic acid* (XVII). To a suspension of 50 mg. of XVII, m.p. 235–237°, in 5 cc. of water was added a solution of 43 mg. of periodic acid (H_5IO_6) in 5 cc. water. After 3 days, the crystals had disappeared and the solution was extracted with five portions of 50 cc. of ethyl acetate, yielding 46 mg. of colorless foam. Three successive crystallizations from acetone-hexane gave 40.3 mg. of IV as clusters of needles.

(36) For a similar observation *cf.* Ref. 12.

(37) In a preliminary experiment, 50 mg. of XIII furnished 49.0 mg. of crude XV. Chromatography over 5 g. of Florisil gave, by eluting with chloroform, 33.1 mg. of a colorless resin, representing pure XV. All attempts at crystallization failed.

(38) For method, *cf.* J. Schmidlin, G. Anner, J.-R. Billeter, K. Heusler, H. Ueberwasser, P. Wieland, and A. Wettstein, *Helv. Chim. Acta*, **40**, 2291, v. p. 2319 (1957).

(39) Dried at room temperature.

(40) Dried at 100°.

The crystals foamed without melting at approximately 125–135° and the resulting solid foam melted at 179–181°. $[\alpha]_D^{24}$ +3° ± 2°; M_D^{24} +11° ± 8° (10.74 mg. in 2 cc. of chloroform containing 5% of ethanol, α + 0.03°).

Anal. Calcd. for $C_{20}H_{30}O_7$ (382.44): C, 62.81; H, 7.91. Found: C, 62.34; H, 8.43. Weight loss, 2.36.

3β-Acetoxy-5,14-dihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid dimethyl ester (V) *from 3β,5,14-trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid* (IV). To approximately 20 mg. of IV, m.p. 179–181° (*cf.* preceding expt.), in 2 cc. of acetone was added an excess of freshly prepared ethereal diazomethane. After 5 min., the solution was evaporated to dryness, leaving a partly crystalline resin. This was dissolved in 1 cc. of pyridine and, after the addition of 0.5 cc. of acetic anhydride, the solution was kept overnight. Subsequently, 10 cc. of 3*N* hydrochloric acid was added and, after standing for 15 min., the mixture was extracted with four 25-cc. portions of ether. The ether extract was washed with *N* hydrochloric acid, water, and a saturated aqueous solution of sodium chloride. After drying, the ether was evaporated, leaving 22.8 mg. of a crystalline residue. Recrystallization from ether–petroleum ether and acetone-water gave 12.0 mg. of transparent rods; m.p. 165° (sharp). $[\alpha]_D^{21}$ + 78° ± 2°; M_D^{21} +355° ± 9° (7.90 mg., α + 0.62°). Lit.[17]: m.p. 164–165°. $[\alpha]_D^{15}$ +72.9° ± 2° (chloroform).

Strophanthidinic acid 19:8-lactone (XX) *from strophanthidinic acid* (II).[18] A solution of 500 mg. of II,[41] m.p. 180–185°, in 5 cc. of methanol was evaporated *in vacuo* to form a brittle foam which quickly dissolved on adding 10 cc. of concd. hydrochloric acid. The solution was allowed to stand overnight whereby it turned yellow. Subsequent dilution with water to a volume of 100 cc. produced a flocculant precipitate which was removed by filtration. The filtrate was extracted with four 100-cc. portions of ethyl acetate and the extract was then washed with water and saturated aqueous sodium chloride. After drying over sodium sulfate, evaporation to dryness gave 403.4 mg. of a colorless crystalline residue. On recrystallizing repeatedly from acetone–petroleum ether and acetone-water, finally 276.1 mg. of XX resulted as colorless transparent plates; m.p. 225–230°, when placed on block at room temperature; m.p. 234–236° when placed on block at 220°. Legal test positive. Lit.[18]: m.p. 235–236°.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.74; H, 7.52. Weight loss, 3.83.

3β,5,8,21-Tetrahydroxy-20-oxo-5β-pregnan-19-oic acid 19:8-lactone (XXI) *from strophanthidinic acid 19:8-lactone* (XX). A solution of 898 mg. of XX, m.p. 238–240°, in 100 cc. of methylene chloride was cooled in a salt-ice bath (approximately −20°).[42] A stream of oxygen containing approx. 2.5% of ozone was passed through for 1 hr., after which time the solution had assumed a persisting, although not very intense, blue color. After allowing it to warm to room temperature, 10 cc. of glacial acetic acid and 1 g. of zinc dust were added, and the mixture was stirred and heated on a steam bath until the starch-iodide test had become negative (approximately 1 hr.). Filtration, washing of the solids with acetone, and evaporation of the filtrate to dryness gave a residue which was taken up in acetone. After renewed filtering and evaporating to dryness, 925.8 mg. of a colorless foam resulted. This was taken up in 10 cc. of ethanol and 200 cc. of ethyl acetate and the solution was shaken with three 10-cc. portions of *N* sodium carbonate and then with 10 cc. each of water and saturated aqueous sodium chloride. The aqueous phases were re-extracted with ethyl acetate. From the carbonate phase was isolated in the usual fashion 61.8 mg. of acidic material representing a yellow resin. The combined ethyl acetate extracts yielded, after drying and evaporating to dryness, 669.5 mg. of neutral product as colorless foam.

One-fourth of the neutral material was taken up in 10 cc. of methanol, followed by the addition of 10 cc. of 0.1*M* potassium carbonate in 50% methanol. After standing for 1 hr., 20 cc. of water was added and the mixture was extracted with three 100-cc. portions of ethyl acetate. The ethyl acetate extracts were washed with *N* sodium carbonate and with water. From the aqueous phases was isolated in the usual fashion 46.3 mg. of acidic material as a yellow resin. The ethyl acetate phase yielded, after drying and evaporating to dryness, 109.3 mg. of neutral product as colorless resin. Crystallization from acetone-ether gave 50.1 mg. of yellowish prisms, m.p. 220–224°, which produced rapidly a positive blue tetrazolium test. By repeated recrystallizations from acetone-ether and acetone-hexane the m.p. was raised to 229–231°. $[\alpha]_D^{18}$ +126° ± 3°; M_D^{18} +477° ± 12° (17.45 mg. in 2.0 cc. of chloroform containing 5 drops of ethanol, α + 2.20°).

Anal. Calcd. for $C_{21}H_{30}O_6$ (378.45): C, 66.64; H, 7.99. Found[43]: C, 66.75; H, 8.34.

3β,5,8-Trihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone (XVIII). A. *From amorphous 3β,5,14-trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid* (IV). The amorphous material from the reaction of impure III with hydrogen peroxide in acetic acid (see above), 3.652 g. of pale yellow foam dissolved at once on addition of 25 cc. of concd. hydrochloric acid. After standing overnight, dilution with 175 cc. of water and extraction with six 200-cc. portions of ethyl acetate, 3.370 g. of brown brittle foam was obtained. This was chromatographed on 200 g. of silica gel (38 × 280 mm.). Chloroform containing up to 10% of acetone eluted 563.4 mg. of resinous material which could not be crystallized. Chloroform containing 10% to 50% of acetone then eluted 2.581 g. of material as a single broad peak of twenty 500-cc. fractions. On dissolving in acetone and adding ether, eleven of these fractions, totaling 1.932 g. crystallized. Recrystallization from acetone-ether-hexane gave a total of 1.089 g. of crystalline fractions with melting points in the range 260–270°. These fractions were combined and recrystallized from acetone-ether to give 840.4 mg. of colorless prisms, m.p. 272–274°. The analytical sample melted at 273–274° (effervescence). $[\alpha]_D^{24}$ +107° ± 2°; M_D^{24} +391° ± 8° (9.50 mg. in 2.0 cc. of 95% ethanol, α + 1.02°).

Anal. Calcd. for $C_{20}H_{28}O_6$ (364.42): C, 65.91; H, 7.75. Found: C, 65.51; H, 7.64.

B. *From crystalline 3β,5,14-trihydroxy-21-nor-5β,14β-pregnane-19,20-dioic acid* (IV). A solution of 19.2 mg. of crystalline IV, m.p. 176–180°, in acetone was evaporated *in vacuo* yielding a white foam to which was added 1 cc. of concd. hydrochloric acid. After standing overnight, the yellow solution was diluted with 9 cc. of water, whereby it became colorless. It was then extracted with five 25-cc. portions of ethyl acetate and the combined extracts were washed with water and saturated aqueous sodium chloride. After drying and evaporating the solvent, 17.3 mg. of a colorless resin resulted which crystallized from acetone-ether-hexane; 13.9 mg. of yellowish crystals, m.p. 268–271°. The solution of the latter in acetone was decolorized by treatment with Norit. Addition of ether to the filtered and concentrated

(41) The strophanthidinic acid (II) used in this experiment was prepared from strophanthidin (I) by a new method as follows: To 1 g. of crystalline I in 10 cc. of methanol were added 2 cc. of 30% hydrogen peroxide and 2 drops of saturated aqueous ferrous ammonium sulfate. After standing for 4 days, the mixture was worked up by partitioning between *M* sodium carbonate and ethyl acetate. The ethyl acetate phase yielded 236.2 mg. of neutral material. From the carbonate phase resulted 827.4 mg. of acidic product which was recrystallized from acetone-water, yielding 530.3 mg. of II as colorless prisms, m.p. 180–185° (effervescence); Legal test positive.

(42) In a preliminary experiment the solution was cooled in Dry Ice–acetone, but the ozonization did not proceed well. For the use of methylene chloride as an ozonolysis solvent, *cf.* G. Slomp, *J. Org. Chem.*, **22**, 1277 (1957).

(43) Dried at 60°.

solution gave colorless rods of constant m.p. 277° (sharp). The mixture m.p. with the preceding analytical sample (method A) was 275–277°.

C. *From 3β,5,8,21-tetrahydroxy-20-oxo-5β-pregnan-19-oic acid 19:8-lactone* (XXI). A total of 17.4 mg. of XXI, m.p. 229–231°, was suspended in a solution of 80 mg. of periodic acid (H_5IO_6) in 2 cc. of water. After standing for 3 days, all crystals had disappeared. An amount of solid sodium bicarbonate was added to the clear solution sufficient to produce alkalinity to litmus. Extraction with ethyl acetate yielded no neutral product. The combined aqueous phases were acidified to Congo Red and the acid material was extracted with four 10-cc. portions of ethyl acetate. After drying and evaporating the combined extracts, 16.3 mg. of a crystalline residue was obtained. Recrystallization from acetone-hexane gave 14.8 mg. of minute granular crystals, m.p. 271–274°. The mixture melting point with the analytical sample (method A) was 271–274°.

Infrared analysis[44]: The sample obtained by method C was compared with the analytical sample obtained by method A. These substances produced identical spectra in the following regions, 4000–2750 cm.$^{-1}$, 1800–1600 cm.$^{-1}$, 1500–1280 cm.$^{-1}$, 1400–650 cm.$^{-1}$ The spectra were obtained from potassium bromide disks using Perkin-Elmer Spectrophotometers (Model 21) with sodium chloride and calcium fluoride prisms.

3β-Acetoxy-5,8-dihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone (XIX) *from 3β,5,8-trihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone* (XVIII). A solution of 50 mg. of XVIII, m.p. 273–274° (analytical sample, prepared by method A; see above), in 2 cc. of pyridine and 1 cc. of acetic anhydride was left at room temperature for 44 hr. Addition of 50 cc. of 3*N* hydrochloric acid was followed, after 15 min., by extraction with ethyl acetate. After washing with 3*N* hydrochloric acid and with water, the ethyl acetate extract was dried and evaporated *in vacuo*, leaving 49.1 mg. of crystalline residue. Recrystallization from acetone-hexane and then from acetone-water gave 30.1 mg. of XIX as fan-like clusters of needles, m.p. 274° (sharp), depressed on admixture with XVIII. $[\alpha]_D^{24}$ +93° ± 2°; M_D^{24} +379° ± 8° (10.73 mg., α 1.00°).

Anal. Calcd. for $C_{22}H_{30}O_7$ (406.46): C, 65.00; H, 7.44. Found: C, 64.97; H, 7.38. Weight loss, 0.42.

8-Hydroxy-3-oxo-21-nor-Δ⁴-pregnene-19,20-dioic acid 19:8-lactone (XXII) *from 3β,5,8-trihydroxy-21-nor-5β-pregnane-19,20-dioic acid 19:8-lactone* (XVIII). A solution of 10 mg. of chromic anhydride in 5 cc. of 90% acetic acid was added, in five 1-cc. portions at half-hour intervals, to 50 mg. of XVIII, m.p. 274–275° (prepared by method A; see above), dissolved in 5 cc. of glacial acetic acid. After standing for 4 days, the reaction mixture was evaporated *in vacuo*. The residue was taken up in ethyl acetate and washed with *N* sulfuric acid and with water. Drying and evaporation of the ethyl acetate left 50 mg. of colorless crystalline residue. To this were added 5 cc. of absolute ethanol, 100 mg. of Girard's reagent T and 0.5 cc. of glacial acetic acid. After refluxing for 1 hr., the solution was diluted with ice water to 50 cc. and extracted with ethyl acetate, yielding 10.2 mg. of resinous, nonketonic material. The aqueous phase was made acid to approximately *p*H 1 by the addition of 5 cc. of concd. hydrochloric acid. After standing overnight, extraction with ethyl acetate yielded 33.4 mg. of crystalline ketonic fraction. Successive recrystallization from acetone-ether and acetone-water gave 16.9 mg. of XXII as long, slender, shining needles, m.p. 237–239° (heated from 230°), depressed on admixture with XI. $[\alpha]_D^{24}$ +110° ± 2°; M_D^{24} +379° ± 8° (7.89 mg., α + 0.87°). λ_{max}^{alc} 244 mμ, ε 13800.

Anal. Calcd. for $C_{20}H_{24}O_5$ (344.39): C, 69.75; H, 7.02. Found: C, 69.84; H, 7.32. Weight loss, 2.15. Residue, 0.55.

(44) Courtesy of Mrs. Beatrice S. Gallagher, Division of Steroid Metabolism, Sloan-Kettering Institute for Cancer Research in New York.

21-Diazo-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone (XXIII) *from 8-hydroxy-3-oxo-21-nor-Δ⁴-pregnene-19,20-dioic acid 19:8-lactone* (XXII). The sodium salt was prepared from a solution of 195 mg. of XXII, m.p. 237–239°, in 10 cc. of ethanol by adding 47.6 mg. of sodium bicarbonate in 2 cc. of water and evaporating to dryness from the frozen state. The dried salt was suspended in 10 cc. of dry benzene containing 5 drops of pyridine, and this mixture was cooled until partly frozen, whereupon 2 cc. of oxalyl chloride was added. After standing for 15 min., the reaction mixture was frozen in Dry Ice and evaporated to dryness *in vacuo*. Addition of 5 cc. of dry benzene, freezing, and evaporating again left an odorless residue. This was taken up in 10 cc. of dry benzene and filtered under nitrogen pressure through sintered glass into ice cold ethereal diazomethane, which was freshly prepared from 10 g. of methylnitrosourea[33] and dried over sodium. The residue of salts was washed with dry benzene and the reaction mixture was left at room temperature for 1 hr. and was finally evaporated to dryness *in vacuo*, yielding 266.8 mg. of yellow oil. This was chromatographed over 20 g. of alumina (activity I–II, 15 × 130 mm.). Benzene and benzene-ether eluted 131.6 mg. of crystalline XXIII. Successive recrystallization from acetone-hexane and acetone-water gave 96.8 mg. of XXIII as pale yellow needles with no definite melting point. The crystals foamed without melting at 145–155° and the foam liquefied at 198–205°. $[\alpha]_D^{25}$ +220° ± 2°; M_D^{25} +810° ± 8° (9.99 mg.; α + 2.20°). λ_{max}^{alc} 247 mμ, ε 23,400.

Anal. Calcd. for $C_{21}H_{24}N_2O_4$ (368.42): C, 68.46; H, 6.57; N, 7.60. Found: C,[34] 68.78; H,[34] 6.83; N,[35] 7.21. Weight loss, 0.73.

In repeated runs of this experiment, the chromatographic purification yielded a second, more polar product, m.p. 272–274°, which has not yet been identified.[36]

8-Hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone ["19:8-Lactoprogesterone"] (XXIV) *from 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone* (XXIII). A solution of 30 mg. of the analytical sample of the diazo ketone XXIII in 10 cc. of chloroform was shaken for 2 min. with 1 cc. of 48% hydriodic acid (Baker's Analyzed Reagent). The chloroform layer was then shaken successively with three 5-cc. portions of saturated aqueous sodium iodide and with 5 cc. of *N* sodium thiosulfate. After drying over sodium sulfate, evaporation of the chloroform *in vacuo* left 29.5 mg. of colorless crystalline residue. Recrystallization from acetone-hexane gave 25.5 mg. of colorless needles, m.p. 156–159°. Repeated recrystallization from acetone-water yielded 18.8 mg. of XXIV as long shining needles; constant m.p. 162–163°. $[\alpha]_D^{22}$ +147° ± 2°; M_D^{22} +504° ± 7° (11.07 mg., α + 1.63°). λ_{max}^{alc} 244 mμ, ε 14,900.

Anal. Calcd. for $C_{21}H_{26}O_4$ (342.42): C, 73.66; H, 7.65. Found: C, 73.25; H, 7.75. Weight loss, 0.18. Residue, 0.24. (Corr. for residue: C, 73.43; H, 7.77.)

21-Acetoxy-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone ["19:8-Lactocortexone acetate"] (XXV) *from 21-diazo-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone* (XXIII). A solution of 53 mg. of the analytical sample of the diazo ketone XXIII in 4 cc. of glacial acetic acid was heated on the steam bath for 45 min. and was then evaporated to dryness *in vacuo*, leaving 53 mg. of yellow foam. On suspending in ether and adding 10 drops of acetone, the product slowly crystallized; yield: 45.9 mg.; m.p. 177–179°. Recrystallization from acetone-water gave 37 mg. of XXV as colorless needles, m.p. 181–183°. The analytical sample, derived from a repeat experiment, melted at 183–184°. $[\alpha]_D^{22}$ +159° ± 2°; M_D^{22} + 643° ± 8° (11.17 mg., α + 1.78°). λ_{max}^{alc} 244 mμ, ε 12,900.

Anal. Calcd. for $C_{23}H_{28}O_6$ (400.45): C, 68.98; H, 7.05. Found[39]: C, 68.78; H, 6.92. Residue, 0.25. (Corr. for residue: C, 68.99; H, 7.02.)

8,21-Dihydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone ["19:8-Lactocortexone"] (XXVI) *from 21-acetoxy-8-hydroxy-3,20-dioxo-Δ⁴-pregnen-19-oic acid 19:8-lactone ["19:8-*

Lactocortexone acetate"] (XXV). To a solution of 40 mg. of XXV, m.p. 183–184°, in 1 cc. of methylene chloride was added 2.5 cc. of 0.05*M* potassium carbonate in 75% methanol.[38] After 5 min., 1 cc. of water and a piece of Dry Ice were added, and the mixture was concentrated *in vacuo* to about 1 cc., yielding a crop of colorless needles. After filtering and washing the crystals (28.2 mg.), the filtrate was extracted with ethyl acetate, yielding 7.5 mg. of crystalline residue. These two portions were combined and chromatographed over 5 g. of Florisil (10 × 115 mm.). Chloroform containing from 2% to 25% of acetone eluted a total of 31.6 mg. of crystalline material which was recrystallized from acetone-water and acetone-hexane to give 24.2 mg. of XXVI as clusters of minute needles, m.p. 199–201°, depressed on admixture with XVI. $[\alpha]_D^{21}$ +158° ± 3°; M_D^{21} +566° ± 10° (9.95 mg., α + 1.57°). λ_{max}^{alc} 244 mμ, ϵ 14,000.

Anal. Calcd. for $C_{21}H_{26}O_5$ (358.42): C, 70.37; H, 7.31. Found[39]: C, 70.54; H, 7.50. Residue, 1.38.

PHILADELPHIA 4, PA.

[CONTRIBUTION FROM THE AGRICULTURAL CHEMISTRY DEPARTMENT EXPERIMENT STATION, MAX C. FLEISCHMANN COLLEGE OF AGRICULTURE AND THE CHEMISTRY DEPARTMENT, UNIVERSITY OF NEVADA]

Isolation, Purification, and Structural Identity of an Alfalfa Root Saponin

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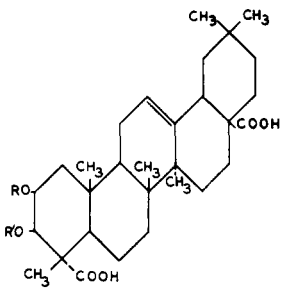
The isolation and structural identity of a pure alfalfa root saponin nucleus was accomplished. The probable structural formula was shown by experimentation to be a β-linked D-glucoside of 2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid; the aglycone is known as medicagenic acid. Specific enzyme hydrolysis was used to establish the linkage involved in the glucose attachment. Other evidence for the reported structure was prepared from chemical constants, elemental analysis, hydroxyl determinations, infrared interpretations and comparisons, and an NMR study.

In the last fifteen years the water soluble saponins isolated from alfalfa (Medicago sativa) have been indicated as a factor in inhibiting growth of chicks[2] and contributing to ruminant bloat.[3,4] From current structural investigations of these saponins isolated from alfalfa tops has come valuable information confirming the presence of a number of hexose and pentose sugars attached in some order to a triterpenoid nucleus.[5,6] The final absolute structure of the aglycone was reported in 1957 and is now known as medicagenic acid.[7]

Previous structural investigations[5–7] have not included the isolation and structural determination of a pure saponin but have concentrated on the individual identity of the monosaccharides and the triterpenoid nucleus following hydrolysis. It was therefore considered expedient to attempt the isolation, purification, and structural determination of a compound representative of a composite of carbohydrate and sapogenin from alfalfa. Such a compound would help establish the proper sequence of hexoses and pentoses in some saponin formations, furnish information on the glycosidic linkage and permit the complete structural proof of component parts. The information obtained should be useful in a better understanding of saponin formation and suggest a useful method for synthesis of these products.



I R,R'= H
II R,R'= CH_3CO
III R=H, R'= β-D-GLUCOSE

Figure 1

EXPERIMENTAL

Saponin isolation and purification. Preliminary investigations of the occurrence of saponins in alfalfa plants showed a potentially rich concentration in the roots. This portion of the plant body was therefore selected for this study.

Samples of alfalfa roots from Ranger and Lahontan varieties were gathered as plowed, cleaned of leaf material, and dried in a forced-draft oven until the roots became brittle. These dried roots were then ground to a fine powder and stored until needed.

The saponin isolation from root powder follows:

Four hundred and fifty grams of this powder was placed in a 4-l. steel beaker together with 1300 ml. of water and 1500 ml. of 95% ethanol. The slurry was maintained at 80° on a hot plate under constant agitation for 6 hr. This mixture was filtered with gentle suction through a large Buchner funnel so packed that it retained about a quarter of an inch

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