[CONTRIBUTION FROM THE DIVISION OF BIOCHEMISTRY, MAYO FOUNDATION, UNIVERSITY OF MINNESOTA]

Steroids Derived from Bile Acids. XV. The Formation of a Glyoxal Side Chain at C-17 from Steroids with Dihydroxyacetone and Δ^{16}-Ketol Side Chains[1]

BY VERNON R. MATTOX

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It is shown (Fig. 1) that in methanolic hydrogen chloride steroids with dihydroxyacetone side chains (I and IV) or with Δ^{16}-ketol side chains (III and VI) give 20-keto-21,21-dimethoxy derivatives (II and V). By treatment of the 20-keto-21,21-dimethoxy compound (V, Fig. 2) with bromine in the presence of hydrogen bromide a 17α,21-dibromo derivative (XII) is obtained which undergoes solvolysis in 80% acetic acid to give the 17α-bromoglyoxal (XIII). The bromo glyoxal (XIII) can be converted into the hydrazone (XVIII), or on treatment with sodium iodide it undergoes reductive enolization to yield the enol (XIV). The treatment of the 20-keto-21,21-dimethoxy substance (V) with hydrogen bromide in chloroform gave XV, in which the side chain had undergone inversion to the α-configuration. This 20-keto-21-bromo-21-methoxy substance (XV) could be brominated to give the 17α,21-dibromo derivative (XII), or it could be converted, with retention of configuration at C-17, into the 21,21-dimethoxy or 21,21-dihydroxy derivative (XVI and XVII). The 17-epimeric 20-keto-21,21-dimethoxy compounds (V and XVI) are interconvertible through enolization at C-17 with methanolic potassium hydroxide. The various ketones and glyoxals were characterized through their hydrazones and osazones.

When cortisone acetate was hydrolyzed in aqueous methanolic hydrogen chloride there were obtained about 80% of cortisone and from 5 to 8% of a by-product, $C_{23}H_{32}O_5$, which contained two methoxyl groups.[2] The present study was undertaken to determine the structure of this dimethoxy derivative.

Treatment of cortisone acetate (I) (Fig. 1) with methanolic hydrogen chloride in the absence of water resulted in a yield of 75% of the dimethoxy compound (II). When it was shown that II could be obtained by treatment of 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione[3] (III) with methanolic hydrogen chloride, it seemed probable that the dimethoxy derivative was 16α,21-dimethoxy-Δ^4-pregnene-3,11,20-trione. This structure seemed most likely since the addition of methanol to the Δ^{16}-bond of 3β-hydroxy-$\Delta^{5,16}$-pregnadien-20-one to form the 16α-methoxy derivative in methanolic alkali had been demonstrated by Fukushima and Gallagher[4] and subsequently the same reaction had been achieved in this Laboratory with acid catalysis.[5] However, the two 20-keto-Δ^{16}-steroids are not strictly comparable, since one of them, 3β-hydroxy-$\Delta^{5,16}$-pregnadien-20-one, is unsubstituted at C-21 and the other (III) has an acetate group at C-21. Further work has shown that, when C-21 is substituted with a hydroxyl or acetoxyl group, methanol does not add to the double bond at C-16 but a rearrangement occurs with formation of a dimethyl acetal at C-21.

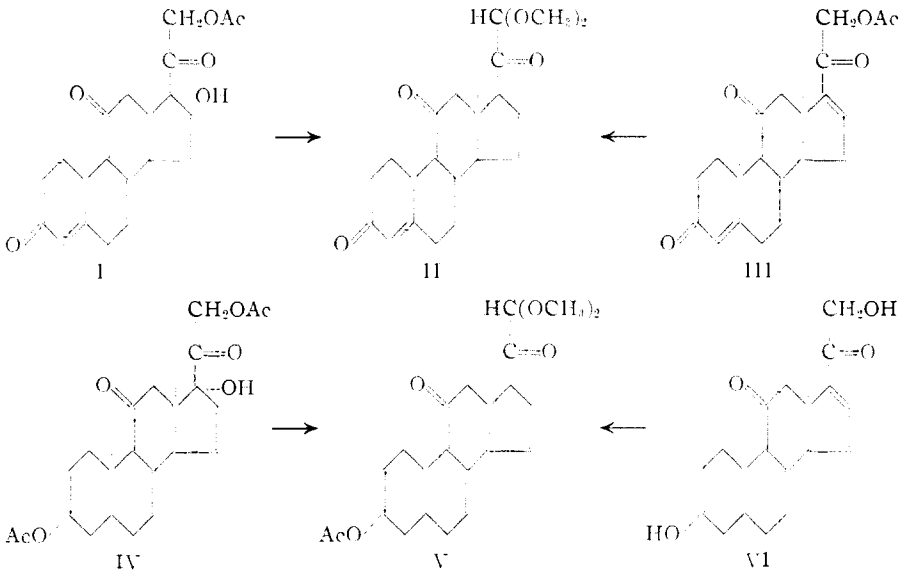

Fig. 1.

After a few preliminary experiments had been performed with the dimethoxy product from cortisone acetate it appeared that the α,β-unsaturated 3-keto group in ring A was a complicating factor and that the problem could be attacked more directly by using a compound that did not contain this grouping. Consequently 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione (IV) was prepared and used as a model compound.

When the model substance (IV) was treated with dry methanolic hydrogen chloride and subsequently acetylated, a 3α-acetoxydimethoxy derivative (V) was obtained in 87% yield along with 5% of unchanged starting material (IV). Furthermore, the same dimethoxy derivative (V) was obtained from 3α,21-dihydroxy-Δ^{16}-pregnene-11,20-dione[3] (VI) in 71% yield by similar treatment. The ultraviolet spectrum on the whole solution from VI indicated that less than 3% of the 20-keto-Δ^{16}-

(1) Presented at the Gordon Research Conference on Steroids, New Hampton, N. H., August 13 to 15, 1951.

(2) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).

(3) This compound was kindly supplied by Mr. Warren F. McGuckin. Its preparation will be published in the near future.

(4) D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **73**, 196 (1951).

(5) Samples of 3β-hydroxy-$\Delta^{5,16}$-pregnadien-20-one and 3β-acetoxy-16α-methoxypregnan-20-one were supplied through the courtesy of Dr. T. F. Gallagher.

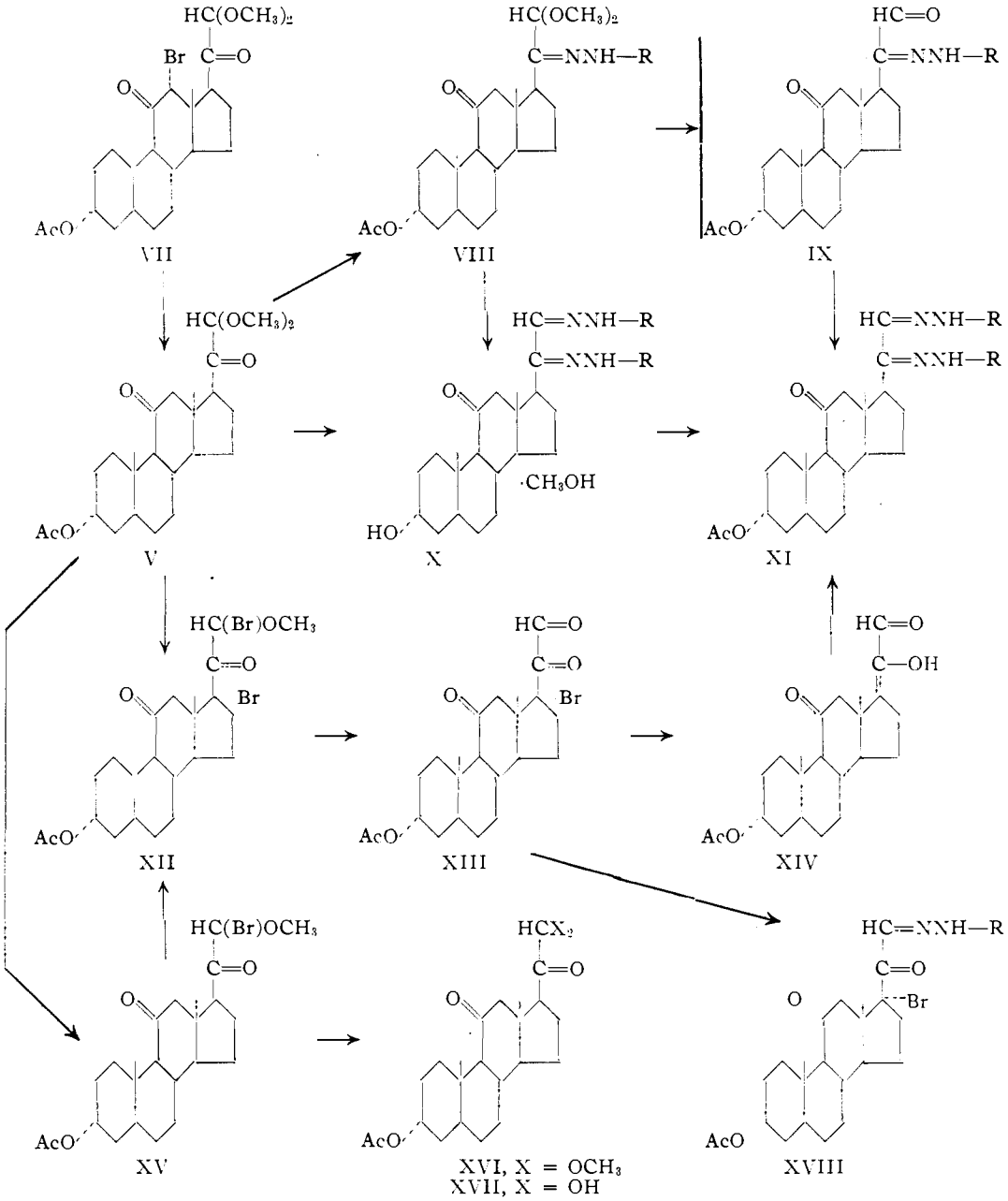

Fig. 2.

chromophore remained unchanged. That the 3α-acetoxydimethoxy steroid (V) contained an active carbonyl group was shown by formation of a cyanhydrin and a 2,4-dinitrophenylhydrazone (VIII). In aqueous methanol V yielded a 3α-hydroxysteroidal osazone (X) with methanol of crystallization which was not lost *in vacuo* at 155° but was removed by crystallization from acetic acid. The same osazone (X) was obtained by treatment of 3α,21-dihydroxypregnane-11,20-dione 20-(2,4-dinitrophenylhydrazone) with 2,4-dinitrophenylhydrazine. Acetylation of the 3α-hydroxy osazone (X) gave the 3α-acetoxyosazone (XI).

With dilute mineral acid in acetic acid the dimethoxyhydrazone (VIII) gave a hydrazone (IX) which did not contain a methoxyl group. The structure of this hydrazone (IX) was immediately evident from the similarity of its ultraviolet spectrum to that of the known 12α-bromo analog[6] and from its conversion to the osazone (XI). However, these findings did not definitely establish the structure of V, since a 20-hydrazone of a 16α,21-dimethoxy compound might undergo the same reactions.

When the transformations which have been described failed to show conclusively the structure of the 3α-acetoxydimethoxy product, (V), 3α-acetoxy-12α-bromo-21,21-dimethoxypregnane-11,20-dione[7] (VII, Fig. 2) was debrominated catalytically and the product was found to be identical

(6) G. A. Fleisher and E. C. Kendall, *J. Org. Chem.*, **16**, 556 (1951).
(7) G. A. Fleisher and E. C. Kendall, *ibid.*, **16**, 573 (1951).

with the 3α-acetoxydimethoxy steroid (V) in question. By analogy the dimethoxy product from cortisone is assigned the structure represented by II.[8]

From an electronic point of view the conversion of both the 17α-hydroxy and Δ^{16}-steroids into derivatives of the steroidal 20,21-glyoxal is straightforward, and the following scheme (Fig. 3) may be suggested to represent the transient intermediate steps.

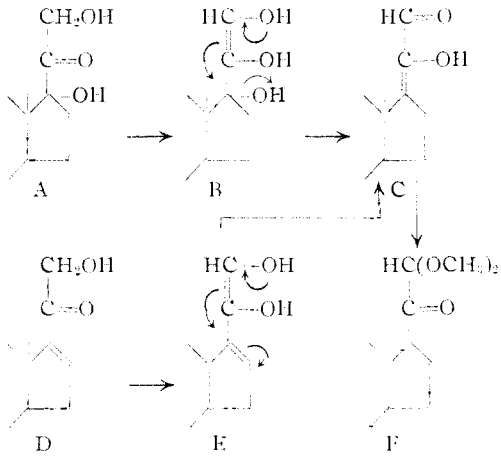

Fig. 3.

The formation of C from A represents a dehydration and that of C from D a rearrangement. It is possible, however, that C may be formed from A *via* D and E, although electronically B appears to be more favorable toward dehydration than A. It is not known whether the dimethyl acetal (F), which is the final product, is formed at C-21 before ketonization of the enol occurs or whether ketonization is the primary step.

Reich and Reichstein[9] and Fleisher and Kendall[7] have prepared steroids with a glyoxal side chain; further reactions of this type of compound will now be described in this paper.

Hydrogen bromide was passed over the surface of a solution of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione (V, Fig. 2) in chloroform at 0° for 10 minutes and then one mole of bromine was added. The bromine was consumed within 2 minutes to give a product which failed to crystallize. When the amorphous dibromo compound was treated in acetic acid solution with sodium iodide, iodine was liberated and 3α-acetoxy-11-keto-20-hydroxy-Δ^{17}-pregnen-21-al (XIV), was formed. Its optical and chemical properties are similar to those of the corresponding 12α-bromo compound, which is known.[7]

When the crude brominated product was dissolved in 80% acetic acid–20% water, solvolysis occurred, one equivalent of bromide ion was lost and an amorphous product was obtained, the spectrum of which (λ_{max} 433 mμ, ε 20–33) suggested that it was a glyoxal. With 2,4-dinitrophenylhydrazine the compound gave 3α-acetoxy-11,20-diketo-17α-bromopregnan-21-al 21-(2,4-dinitrophenylhydrazone) (XVIII), the structure of which was deduced from its analysis and the similarity of its ultraviolet spectrum to that of the known 12α-bromo analog.[6] This indicated that the glyoxal was 3α-acetoxy-11,20-diketo-17α-bromopregnan-21-al (XIII). By treatment of the 17α-bromo glyoxal (XIII) with sodium iodide in acetic acid reductive enolization occurred and the enol (XIV) was formed.[10]

The enol (XIV), with two moles of 2,4-dinitrophenylhydrazine, slowly gave the osazone (XI); however, XIII, which is substituted at C-17 with an atom of bromine, combined with only one mole of 2,4-dinitrophenylhydrazine and yielded the hydrazone XVIII.

Although it was not possible to isolate the intermediates (XII and XIII) in crystalline form, it seems probable that in the preparation of XIV from V hydrogen bromide displaced a methoxyl group at C-21 and bromination introduced an atom of bromine at C-17 to give a 17α,21-dibromo-20-keto-21-methoxy derivative (XII) which was the precursor of the 17α-bromo glyoxal (XIII).

A crystalline monobromomonomethoxy derivative (XV) could be obtained in about 50% yield by treatment of the dimethoxy derivative (V) with hydrogen bromide in chloroform at 0° for 4.5 hours. Its conversion with quantitative loss of bromide ion in 80% acetic acid to a glyoxal hydrate (XVII) and in methanol containing ethylmorpholine to a dimethoxy derivative (XVI) indicated that the atom of bromine was at C-21. Furthermore, a correlation of the changes in molecular rotation in the preparation of XVII and XVI from V *via* XV with the differences in molecular rotation of several pairs of 20-keto steroids which are epimeric at C-17 indicated that the side chain in the glyoxal and dimethoxy derivatives (XVII and XVI) was oriented in the α-configuration.

The average value of $M^{17\beta}{}_D - M^{17\alpha}{}_D$ for ten pairs of 20-keto steroids[11] which are epimeric at C-17 is +561° with the extremes represented by +448 and +720°. For the two 21,21-dimethoxy compounds (V and XVI) the delta value is +595°. In these calculations $[M]_D = ([\alpha]_D \times \text{mol. wt.}) \div 100$.

Although a crystalline 17β-epimer of XVII has not been prepared, a crude solution had M_D +497° and the delta value for the two glyoxal hydrates is +541°. On this basis and on its method of preparation from XV the glyoxal hydrate (XVII) appears to be a 17α-pregnane.

Since it is known that solvolysis of 20-keto-21-bromo-21-acetoxysteroids does not cause inversion[7] at C-17, it follows that the side chain in XV is oriented in the α-configuration and that this configuration was established during the treatment of V with hydrogen bromide in chloroform.

The dimethoxy derivative (XVI) with the 17α side chain gives an osazone which is different from the previously prepared osazone (X) with the β side chain. That the two 3α-acetoxydimethoxy

(8) This type of structure was first suggested by Dr. G. A. Fleisher by analogy to the formation of methyl glyoxal from dihydroxyacetone in dilute sulfuric acid. Beilstein, "Handbuch der organ. Chemie," Vol. I, Julius Springer, Berlin, 1918, p. 762.

(9) H. Reich and T. Reichstein, *Helv. Chim. Acta*, **22**, 1124 (1939).

(10) G. A. Fleisher and E. C. Kendall[6] have described the reduction, with sodium bisulfite, of 3α-acetoxy-11,20-diketo-12α,17α-dibromopregnan-21-al to the corresponding 12α-bromo enol.

(11) These data are tabulated by C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **179**, 1265 (1949).

steroids, (V) and (XVI), are in fact isomeric at C-17 was shown by their conversion one into the other in 0.4 *N* potassium hydroxide in methanol. Mutarotation occurred with each of the epimers and within about 3 hours the value for the optical rotation was constant. After reacetylation the specific rotation of the mixture in chloroform indicated the presence of about 66% of the β-epimer and 34% of the α-epimer, and it was possible to separate both V and XVI from either starting material after treatment of either with methanolic potassium hydroxide. The equilibrium mixtures of two other pairs of 20-ketopregnanes which are unsubstituted in the neighborhood of the 20-keto group contain 66 and 75% of the 17β-epimers.[11]

Although one mole of bromine was absorbed by the dimethoxy derivative (V) in chloroform in the presence of hydrogen bromide within less than 2 minutes, the 21-bromo-21-methoxy-17α-pregnane derivative (XV) required about 8 hours for consumption of a mole of bromine. Despite this difference the product of the bromination of XV, although not separated in crystalline form, was found to give derivatives (XIII, XIV and XVIII) identical with those of the dibromo compound XII obtained from V. It is noteworthy that V and XV, which are epimeric at C-17, give the same 17α-bromo derivative (XIII).

Finally it was shown that in the usual method of preparation of XII from V a methoxyl group at C-21 was replaced with bromine during the initial 10 minute treatment of V with hydrogen bromide. Although the 21-bromo-21-methoxy intermediate was not obtained in crystalline form, its presence could be demonstrated. In 80% acetic acid it lost 84% of an equivalent of bromide ion, and the amorphous glyoxal (λ_{max} 450 mμ, ϵ 23) was formed. The optical rotation of the glyoxal, when compared to that of the 17α-epimer (XVII), suggested that only a small amount of the compound had undergone inversion at C-17 during the 10-minute treatment with hydrogen bromide. Treatment of the crude glyoxal with 2,4-dinitrophenylhydrazine gave the osazone (XI).

The ultraviolet spectra of the hydrazones (VIII, IX and XVIII) and osazones (X and XI) were similar to those of corresponding compounds which have been reported by Fleisher and Kendall.[6]

Experimental

All melting points were taken on a Fisher–Johns apparatus. The ultraviolet absorption spectra were determined on a Beckman model DU quartz spectrophotometer which had been standardized against mercury lines. Optical rotations were taken in chloroform at a concentration of about 1% and at 27 ± 2° unless otherwise designated. Analyses were performed by Mr. J. F. Alicino, Metuchen, N.J. After a water-immiscible solvent had been washed with water the solvent was always dried by filtration through a pad of sodium sulfate before concentration and all concentrations were *in vacuo*.

21,21-Dimethoxy-Δ^4-pregnene-3,11,20-trione (II) from Cortisone Acetate (I).—One millimole of cortisone acetate[2] was treated with methanolic hydrogen chloride as described under the preparation of V from IV except that the acidic solution stood for 30 min. with 10 ml. of water before neutralization with sodium acetate. The acetylation also was omitted. On crystallization from ether, II was obtained (160 mg., m.p. 160–162°; 138 mg., m.p. 155–158°) in 76% yield. It did not depress the melting point of the product that was prepared from cortisone acetate in aqueous methanol.[2]

II from III.—By treatment of 192 mg. of 21-acetoxy-$\Delta^{4,16}$-pregnadiene-3,11,20-trione[3] with methanolic hydrogen chloride, as described in the previous paragraph, 62 mg. of crude dimethoxy derivative (m.p. 149–154°) was obtained. After recrystallization from methanol the product (30 mg.) melted at 161.5–162.5° and did not depress the melting point of a sample of II prepared from I. The infrared spectrum of the product was identical with that of II prepared from I; λ_{max} 238 mμ, ϵ15,100 (methanol).

21,21-Dimethoxy-Δ^4-pregnene-3,11,20-trione 3,20-Bis-(2,4-dinitrophenylhydrazone) from II.—21,21-Dimethoxy-Δ^4-pregnene-3,11,20-trione[12] (194 mg.) was added to a solution of 297 mg. of 2,4-dinitrophenylhydrazine in 50 ml. of methanol, 50 ml. of chloroform and 1.0 ml. of 5.0 *N* hydrochloric acid at room temperature. After 20 hours red crystals (346 mg., m.p. 239–243°) were filtered off and the filtrate was concentrated to yield an additional 28 mg. of product, m.p. 235–237°. After several crystallizations from a mixture of chloroform and methanol a product was obtained which melted at 242–244°; λ_{max} 378 mμ, ϵ 57,200 (chloroform).

Anal. Calcd. for $C_{35}H_{40}O_{11}N_8$: C, 56.14; H, 5.39; CH_3O, 8.29. Found: C, 55.73; H, 5.40; CH_3O, 7.91.

3α,21-Diacetoxy-16α,17α-epoxypregnane-11,20-dione from 3α,21-Diacetoxy-12α-bromo-16α,17α-epoxypregnane-11,20-dione.—3α,21-Diacetoxy-12α-bromo-16α,17α-epoxypregnane-11,20-dione (m.p. 163–164°) was prepared[13,14] in about 80% yield by acetylation of 11,20-diketo-12α-bromo-16α,17α-epoxypregnane-3α,21-diol[13] and crystallization from aqueous methanol. A sample of the diacetate (44.1 g.) was debrominated[13] according to the procedure described under IV from 3α,21-diacetoxy-16β-bromo-17α-hydroxypregnane-11,20-dione. Crystallization from methanol gave 30.4 g., m.p. 158.5–160°, and 2.0 g., m.p. 156–157°, $[\alpha]_D$ +119 ± 2°.

Anal. Calcd. for $C_{24}H_{34}O_7$: C, 67.24; H, 7.67. Found: C, 67.46; H, 7.79.

3α,21-Diacetoxy-16β-bromo-17α-hydroxypregnane-11,20-dione from 3α,21-Diacetoxy-16α,17α-epoxypregnane-11,20-dione.—A solution of 9.38 g. of 3α,21-diacetoxy-16α,17α-epoxypregnane-11,20-dione in 245 ml. of chloroform at −15° was added to a solid mass of 122 ml. of 0.73 *N* hydrogen bromide in glacial acetic acid at −15°. After thorough mixing the solution became homogeneous. After 24 hours at −15° water and chloroform were added, and the chloroform solution was washed with water and concentrated to about 30 ml. On addition of 150 ml. of absolute ether, crystals formed; yield 7.58 g., m.p. 198–200°. From the filtrate additional crops of crystals separated (2.13 g., m.p. 200–201° and 0.26 g., m.p. 198–200°) bringing the total yield to 90%. Several recrystallizations from ethyl acetate did not raise the melting point; $[\alpha]_D$ +59 ± 2°. The product begins to turn brown within a few days when stored at room temperature.

Anal. Calcd. for $C_{25}H_{35}O_7Br$: C, 56.93; H, 6.69. Found: C, 57.07; H, 6.90.

3α,21-Diacetoxy-17α-hydroxypregnane-11,20-dione (IV) from 3α,21-Diacetoxy-16β-bromo-17α-hydroxypregnane-11,20-dione.—A solution of 9.34 g. of 3α,21-diacetoxy-16β-bromo-17α-hydroxypregnane-11,20-dione in 400 ml. of methanol was stirred in an atmosphere of hydrogen in the presence of 28 g. of 2.0% palladous hydroxide on calcium carbonate until no further hydrogen was absorbed (1 hour) and then for an additional hour. The catalyst was filtered off and the filtrate was concentrated to 50 ml., diluted with water and filtered. The filtrate contained 96% of the theoretical amount of bromide ion. The precipitate was dissolved in chloroform, the solution was washed with water and evaporated to a small volume, and crystals (1.37 g., m.p. 228–230°; 0.17 g., m.p. 218–220°) were obtained from methanol. At this point it was observed that most of the product had crystallized during the reduction and had been filtered off with the catalyst. By extraction of the spent

(12) A generous sample of this compound was supplied by Dr. Jacob van de Kamp.

(13) Prepared by Mr. A. J. LaVine.

(14) Prepared according to the procedure of F. B. Colton, W. R. Nes, D. A. Van Dorp, H. L. Mason and E. C. Kendall, *J. Biol. Chem.*, **194**, 235 (1952).

catalyst with chloroform and subsequent crystallization from methanol additional product (5.55 g., m.p. 228–230°) was obtained. When a sample of the material was recrystallized from a mixture of chloroform and methanol it melted at 233–236° when placed on the apparatus at 225° and did not depress the m.p. of a sample of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione,[15,16] $[\alpha]_D$ +91 ± 2° (acetone).

3α-Acetoxy-21,21-dimethoxypregnane-11,20-dione (V) from IV.—Twenty grams of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione was suspended in 260 ml. of chloroform, and 608 ml. of methanol was added. The homogeneous solution was then diluted with 172 ml. of 1.22 *N* hydrogen chloride in dry methanol. After 48 hours at room temperature 20 g. of sodium acetate and 400 ml. of water were added, and the solution was concentrated to remove the bulk of the methanol. Chloroform was added, and the solution was washed with water and concentrated to dryness. In order to acetylate the residue it was dissolved in 20 ml. each of acetic anhydride and pyridine at room temperature and allowed to stand for 5 hours. Ice was added, the product was extracted with chloroform, and the solution was washed with dilute hydrochloric acid, and with water. After the solution had been evaporated to dryness 50 ml. of absolute ether was added. When crystals started to separate 450 ml. of petroleum ether was added and 976 mg. of product (m.p. 224–226°) was filtered off and identified as IV by a mixture melting point with an authentic sample.

When the filtrate was cooled in ice 10.90 g. of crystals separated, m.p. 107–107.5°. By concentrating the filtrates 5.75 g. of crystals (m.p. 105.5–106.5°) was obtained. The material did not reduce ammoniacal silver nitrate. The ultraviolet spectrum indicated that there was no α,β-unsaturated ketone present; $[\alpha]_D$ + 131 ± 2°, $[\alpha]_D$ +124 ± 2° (acetone).

Anal. Calcd. for $C_{25}H_{36}O_5$: C, 69.09; H, 8.81; CH_3O, 14.28. Found: C, 69.00; H, 9.05; CH_3O, 14.14.

V from VI.—3α,21-Dihydroxy-Δ[16]-pregnene-11,20-dione[3] (346 mg.) was treated with methanolic hydrogen chloride according to the procedure for the preparation of V from IV. The ultraviolet spectrum of the whole solution indicated that less than 3% of the 20-keto-Δ[16]-chromophore remained intact. After acetylation and crystallization from ether–petroleum ether 184 mg. of crystals, m.p. 101–102°, was obtained. By chromatography of the filtrate on 10 g. of a 1:1 mixture of magnesium silicate and infusorial earth and elution with benzene and with benzene containing 0.1% of methanol an additional 126 mg. of crystals, m.p. 106–107°, was obtained. The chromatographed material did not depress the m.p. of V prepared from IV, VII or XVI, and the infrared spectra (nujol) of the material prepared from the four sources were identical, $[\alpha]_D$ +130 ± 2°.

V from VII.—A solution of 257 mg. of 3α-acetoxy-12α-bromo-21,21-dimethoxypregnane-11,20-dione[7] in 20 ml. of methanol and 2 ml. of ethylmorpholine was shaken in the presence of 257 mg. of 2.0% palladous hydroxide suspended on calcium carbonate under an atmosphere of hydrogen for 20 minutes (hydrogen uptake ceased after 10 minutes). The catalyst was filtered off, and the filtrate was concentrated almost to dryness. Benzene and water were added. The aqueous phase contained 0.49 millimole of bromide ion. The organic phase was washed with dilute sulfuric acid, with water, and concentrated to dryness. Crystals (53 mg. m.p. 103–105°) were obtained from ether–petroleum ether. The product did not depress the m.p. of samples of V prepared from IV and from VI, $[\alpha]_D$ +131 ± 2°. By chromatography of the filtrate on 10 g. of a 1:1 mixture of magnesium silicate and infusorial earth and elution with benzene containing 0.1% of methanol additional material was obtained; yield 71 mg., m.p. 106–107°; 41 mg., m.p. 105–106°.

V from XVI.—3α-Acetoxy-21,21-dimethoxy-17α-pregnane-11,20-dione (434 mg.) was treated in 0.40 *N* methanolic potassium hydroxide as described under XVI from V. After 2.5 hours the rotation became constant at +0.65°; after acetylation $[\alpha]_D$ +86 ± 2° in chloroform. This corresponds to a mixture of 67% of V and 33% of XVI. By chromatographing the mixture 16 mg. of XVI, m.p. 138–138.5°, $[\alpha]_D$ −4 ± 4°, and 90 mg. of V, m.p. 106.5–107°, $[\alpha]_D$ +131 ± 2° were obtained.

(15) L. H. Sarett, THIS JOURNAL, **70**, 1454 (1948).

(16) Kindly supplied by Dr. L. H. Sarett.

20-Cyanhydrin of 3α-Acetoxy-21,21-dimethoxypregnane-11,20-dione from V.—This compound was prepared according to the method used by Sarett[15] for the preparation of the 20-cyanhydrin of 3α-acetoxypregnane-11,20-dione. Four millimoles of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione gave 926 mg. (50%) of cyanhydrin which melted at 188–190° and 46 mg. which melted at 195–197°. By retreatment of the mother liquors with hydrogen cyanide an additional 320 mg. (m.p. 196–198°) was obtained. Several recrystallizations from ethyl acetate–petroleum ether did not raise the melting point above 196–198°, $[\alpha]_D$ +65 ± 2°.

Anal. Calcd. for $C_{26}H_{39}O_6N$: C, 67.65; H, 8.52; N, 3.03. Found: C, 67.71; H, 8.74; N, 3.37.

In an attempt to dehydrate the cyanhydrin according to Sarett's procedure[15] at room temperature, starting material was recovered in 76% yield. Treatment with pyridine and phosphorus oxychloride at the boiling point for 15 minutes produced an intractable gum.

3α-Acetoxy-21,21-dimethoxypregnane-11,20-dione 20-(2,4-Dinitrophenylhydrazone) (VIII) from V.—Acetic acid (136 ml.) was added to 1.48 g. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione and 814 mg. of 2,4-dinitrophenylhydrazine, and after the mixture had been shaken occasionally for 8 hours at room temperature 400 mg. of 2,4-dinitrophenylhydrazine was added. After 27 hours the solution was concentrated to dryness and 25 ml. of chloroform was added. β-Acetyl-2,4-dinitrophenylhydrazine (460 mg., m.p. 197–199°) was filtered off and identified. Methanol was added to the filtrate and it was concentrated to yield the yellow hydrazone (1.94 g., m.p. 199–204°; 125 mg., m.p. 201–203°). A sample that had been crystallized several times from chloroform–methanol melted at 201–204°, λ_{max} 367 mμ, ε 25,300 (chloroform).

Anal. Calcd. for $C_{31}H_{42}O_9N_4$: C, 60.57; H, 6.89; CH_3O, 10.10. Found: C, 60.94; H, 6.73; CH_3O, 10.20.

3α-Acetoxy-11,20-diketopregnan-21-al 20-(2,4-Dinitrophenylhydrazone) (IX) from VIII.—To 614 mg. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione 20-(2,4-dinitrophenylhydrazone) in 90 ml. of acetic acid was added 10.0 ml. of 0.1 *N* perchloric acid in acetic acid (prepared by diluting 72% perchloric acid with acetic acid), and the solution was maintained at 60 ± 2° for 4.5 hours. The solution was concentrated to about 10 ml., water and chloroform were added, and the organic phase was concentrated. Yellow crystals (391 mg., m.p. 241–242°; 111 mg., m.p. 238–240°) were obtained from acetic acid. Acetone–ether yielded an additional crop of crystals (34 mg., m.p. 225–227°). After the crystals had been combined, a small amount of insoluble material (12 mg., m.p. 307–309° dec.) was separated from about 20 ml. of acetic acid at room temperature, and an additional 13 mg. (m.p. 306–308° dec.) was obtained by removing the acetic acid and suspending the residue in 20 ml. of benzene. After the insoluble material had been recrystallized from chloroform–acetic acid it melted at 315–317° dec. and was identified by means of a mixture melting point and infrared spectrum (nujol) as the osazone, XI. After several recrystallizations of the more soluble material from acetic acid it melted at 237–239°. $\lambda_{max\ 1}$ 371 mμ, ε 21,500; $\lambda_{max\ 2}$ 402 mμ, ε 22,700 (chloroform). In acetone containing aqueous bicarbonate the product gave a pink color.[6]

Anal. Calcd. for $C_{29}H_{36}O_8N_4 \cdot {}^1/_2H_2O$: C, 60.30; H, 6.46. Found: C, 60.40; H, 6.56; CH_3O, 0.0.

3α-Hydroxy-11,20-diketopregnan-21-al 20,21-Bis-(2,4-dinitrophenylhydrazone) (X) from V.—A mixture of 434 mg. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione, 792 mg. of 2,4-dinitrophenylhydrazine, 50 ml. of methanol, 50 ml. of chloroform and 4.0 ml. of 5.0 *N* hydrochloric acid remained at room temperature for 28 hours, and no crystals of osazone separated. The solution was refluxed for 2 hours (crystals began to form after 1 hour) and cooled, and 266 mg. of osazone (m.p. 307–310°) was filtered off. Two grams of sodium acetate was added to the filtrate, and the solution was concentrated to dryness. Water and chloroform were added, and 322 mg. of 2,4-dinitrophenylhydrazine was filtered off. After addition of methanol and concentration of the filtrate 112 mg. of pale orange osazone (m.p. 300–302°) was obtained. By retreatment of the residue from the filtrate in 50 ml. of chloroform, 50 ml. of methanol, 4.0 ml. of 5.0 *N* hydrochloric acid and 792 mg. of 2,4-dinitrophenylhydrazine at the boiling point for 11 hours, additional material (215 mg., m.p. 310–312°; 41 mg., m.p.

302–305°) was obtained. The sample for analysis was recrystallized several times from chloroform–methanol and dried at 0.1 mm. and 100°, m.p. 309–311° dec., $\lambda_{max\ 1}$ 349 mμ, ε 33,000; $\lambda_{max\ 2}$ 397 mμ, ε 24,200; $\lambda_{max\ 3}$ 450 mμ, ε 22,900 (chloroform).

Anal. Calcd. for $C_{33}H_{38}O_{10}N_8 \cdot CH_3OH$: C, 55.28; H, 5.73; CH_3O, 4.20. Found: C, 55.66; H, 5.65; CH_3O, 3.94.

The loss in weight of a sample in 1.5 hours at 0.1 mm. and 155° was only 0.85%; calcd. for CH_3OH, 4.34%. A sample which had been recrystallized from acetic acid contained no methoxyl group (Zeisel).

X from VIII.—A solution containing 615 mg. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione 20-(2,4-dinitrophenylhydrazone), 792 mg. of 2,4-dinitrophenylhydrazine, 50 ml. of methanol, 50 ml. of chloroform and 4.0 ml. of 5.0 *N* hydrochloric acid was refluxed for 9 hours, cooled, and the crystals were collected; yield 566 mg., m.p. 307–310° dec., $\lambda_{max\ 1}$ 349 mμ, ε 32,400; $\lambda_{max\ 2}$ 397 mμ, ε 24,000; $\lambda_{max\ 3}$ 450 mμ, ε 22,600 (chloroform). The infrared spectrum of this sample in nujol was identical with that of X prepared from V and from 3α,21-dihydroxypregnane-11,20-dione 20-(2,4-dinitrophenylhydrazone).

Two grams of sodium acetate was added to the filtrate, and the solution was concentrated to dryness. After addition of 25 ml. of water and 25 ml. of chloroform 546 mg. of 2,4-dinitrophenylhydrazine was filtered off. The chloroform phase was separated, concentrated to a small volume and diluted with methanol. Orange yellow crystals (58 mg., m.p. 297–300° dec.) were obtained.

X from 3α,21-Dihydroxypregnane-11,20-dione 20-(2,4-Dinitrophenylhydrazone).—One millimole of 3α,21-dihydroxypregnane-11,20-dione 20-(2,4-dinitrophenylhydrazone)[6] was converted into the osazone by the procedure used for preparing X from VIII except that the solution was refluxed for 24 hours. The product (441 mg., m.p. 310–311° dec.; 39 mg., m.p. 308–309° dec.) did not depress the m.p. of X prepared from V or VIII, and their infrared spectra were identical, $\lambda_{max\ 1}$ 349 mμ, ε 32,500; $\lambda_{max\ 2}$ 397 mμ, ε 24,300; $\lambda_{max\ 3}$ 449 mμ, ε 22,700 (chloroform).

3α-Acetoxy-11,20-diketopregnan-21-al 20,21-Bis-(2,4-dinitrophenylhydrazone) (XI) from IX.—A solution of 180 mg. of 3α-acetoxy-11,20-diketopregnan-21-al 20-(2,4-dinitrophenylhydrazone) and 80 mg. of 2,4-dinitrophenylhydrazine in 50 ml. of acetic acid stood at room temperature for 20 hours, and crystals (204 mg., m.p. 313–315° dec.) were filtered off: $\lambda_{max\ 1}$ 349 mμ, ε 31,700; $\lambda_{max\ 2}$ 397 mμ, ε 23,600; $\lambda_{max\ 3}$ 447 mμ, ε 22,200 (chloroform).

Anal. Calcd. for $C_{35}H_{40}O_{11}N_8$: C, 56.14; H, 5.39. Found: C, 56.65; H, 5.72.

XI from X.—3α-Hydroxy-11,20-diketopregnan-21-al 20,21-bis-(2,4-dinitrophenylhydrazone) (74 mg.) was dissolved in 3.0 ml. each of acetic anhydride and pyridine. After 1 hour at room temperature, the mixture was worked up, and 71 mg. (95%) of the 3-acetate (XI), m.p. 312–314° dec., was obtained. It did not depress the m.p. of XI prepared from IX and XIV and the infrared spectra (nujol) of the samples from the three sources were identical. When the time of acetylation was extended to 15 hours the yield was only 55%.

XI from XIV.—To a solution of 53 mg. of 3α-acetoxy-11-keto-20-hydroxy-Δ^{17}-pregnen-21-al in 5.0 ml. of acetic acid was added 81 mg. of 2,4-dinitrophenylhydrazine. After 2 days at room temperature 29 mg. of yellow osazone, m.p. 317–318° dec., had separated. The osazone forms slowly and the excess of 2,4-dinitrophenylhydrazine is removed by acetylation. By three additional treatments for 3 days each with 81-mg. portions of 2,4-dinitrophenylhydrazine an additional 61 mg. of osazone, m.p. 317–318° dec., was obtained. This osazone did not depress the melting point of XI prepared from IX.

3α-Acetoxy-11,20-diketo-17α-bromopregnan-21-al (XIII) from V.—Dry hydrogen bromide was passed over the surface of a solution of 434 mg. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione at 0° for 10 minutes, and then 2.00 ml. of 1.00 *N* bromine in chloroform was added. The bromine was consumed within 2 minutes, and the solution was concentrated to about 3 ml. Chloroform was added, and the solution was washed and concentrated to dryness to give the crude dibromo intermediate.

In several experiments the intermediate dibromo derivative was allowed to stand in 80% acetic acid (10 ml. per millimole of steroid) at room temperature for 30 minutes. In every case 1 millimole of steroid lost 0.97–0.99 millimole of bromide ion. On extraction with chloroform a yellow solution was obtained; λ_{max} 434 mμ, ε 27–33. The absorption at 284 mμ (ε 176–186) showed that no more than a trace of the enol (XIV) was present. Attempts to crystallize the glyoxal were not successful.

The following experiment indicated that a methoxyl group at C-21 of V was replaced with bromine during the preliminary saturation of the solution with hydrogen bromide. A solution of 434 mg. of V in 10 ml. of chloroform was cooled to 0°, and dry hydrogen bromide was passed over the solution for 10 minutes. The solution was concentrated to about 3 ml., chloroform was added, and the solution was washed with water and filtered through a pad of sodium sulfate. In 43.4 ml. of chloroform α_D +0.99° (*l* = 1 dm.). The solvent was removed, and the residue was dissolved in 10 ml. of 80% acetic acid. After 30 minutes at room temperature water was added, and the solution was extracted with chloroform. The aqueous phase contained 0.84 millimole of bromide ion. For the organic phase (40 ml.), α_D +1.24° (*l* = 1 dm.), which corresponds to $[\alpha]_D$ +122° (calculated as the glyoxal hydrate); λ_{max} 450 mμ, ε 18. ε increased as dehydration occurred and after 2 hours was approximately constant at 23. The glyoxal hydrate was converted into the osazone XI by the procedure described under XI from XIV.

XIII from XV.—Six ml. of 0.88 *N* hydrogen bromide in chloroform at 0° was added to a solution of 725 mg. of 3α-acetoxy-21-bromo-21-methoxy-17α-pregnane-11,20-dione at 0°, and then 3.00 ml. of 1.02 *N* bromine in chloroform was added. When, after 15 minutes, it appeared that the bromine was not being consumed, dry hydrogen bromide was passed over the solution for 30 minutes, and the solution was maintained at 0°. Most of the bromine had been consumed after 5 hours. However, a small amount was still present after 8 hours. The solution was then concentrated to about 5 ml., diluted with chloroform, washed with water and concentrated to dryness. The colorless residue was dissolved in 15 ml. of 80% acetic acid–20% water, and after 30 minutes at room temperature water was added. Chloroform was added. The aqueous phase contained 1.45 millimoles (97%) of bromide ion. The yellow chloroform solution was filtered through a pad of sodium sulfate: λ_{max} 433 mμ, ε 22; at 284 mμ, ε = 154. Attempts to crystallize the glyoxal were unsuccessful.

3α-Acetoxy-11-keto-20-hydroxy-Δ^{17}-pregnen-21-al (XIV) from V.—One millimole of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione was converted into the intermediate dibromo derivative (XII) as described under XIII from V.

Reduction.—The solution was taken to dryness, and with the residue in 5.0 ml. of acetic acid and under an atmosphere of carbon dioxide 1.50 g. of sodium iodide was added. After 5 minutes at room temperature, the iodine which was liberated was titrated with 0.1 *N* sodium thiosulfate (17.6 ml. required) after addition of chloroform. The chloroform solution was concentrated to dryness, and crystals (122 mg., m.p. 179–182°; 36 mg., m.p. 178–180°) were obtained from absolute ether. A purified sample melted at 184–186°.

The material reduced Tollens reagent rapidly and gave a bluish-green color with ferric chloride. The infrared spectrum showed the presence of a hydroxyl group, $[\alpha]_D$ +86 ± 4°, λ_{max} 284 mμ, ε 12,800 (95% ethanol).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.11; H, 8.30. Found: C, 71.03; H, 7.83; CH_3O, 0.0.

XIV from XIII. A.—When the non-crystalline 17α-bromo glyoxal which is described under XIII from V was treated with sodium iodide in acetic acid as described under the preparation of XIV from V, 1.00 to 1.04 ml. of 1.0 *N* iodine was liberated per millimole of glyoxal and λ_{max} 284 mμ in chloroform with ε varying from 6,300 to 6,500, regardless of whether the time of reaction with sodium iodide was 5 minutes, 20 minutes or 1 hour. The enol (XIV) was isolated in about 13% yield and identified by its melting point and spectrum, λ_{max} 284 mμ, ε 14,200 (95% ethanol). In several experiments in which the 17α-bromo glyoxal was treated with sodium iodide the amount of enol formed (determined spectrophotometrically) was equivalent to the amount of iodine liberated. Retreatment of the solution in acetic acid and with sodium iodide gave an additional amount of iodine.

XIV from XIII. B.—The 17α-bromo glyoxal (XIII) (0.5 millimole) prepared from XV *via* XII, was treated in 2.5 ml. of acetic acid under carbon dioxide at room temperature with 750 mg. of sodium iodide for 10 minutes. Chloroform and water were added, and titration with thiosulfate indicated that 4.45 ml. of 0.1 *N* iodine had been formed. The chloroform solution was washed, separated and filtered through sodium sulfate. The spectrum (λ_{max} 286 mμ, ε 5,440) indicated that about 42% of enol had been formed. When the chloroform solution stood at room temperature for 0.5 hour 0.6 ml. of 0.1 *N* iodine was liberated. Crystals of the enol (41 mg., m.p. 181–182°) were obtained from ether and identified by a mixture melting point, λ_{max} 284 mμ, ε12,600 (95% ethanol).

3α-Acetoxy-21-bromo-21-methoxy-17α-pregnane-11,20-dione (XV) from V.—Dry hydrogen bromide was passed over the surface of a solution of 1.304 g. of 3α-acetoxy-21,21-dimethoxypregnane-11,20-dione in 30 ml. of chloroform at 0° for 30 minutes. The reaction mixture was kept at 0° for 4 hours, concentrated to about 5 ml., diluted with chloroform and washed with water. The chloroform solution was concentrated to dryness, and then two portions of absolute ether were evaporated under diminished pressure to remove the last of the chloroform. Crystals (667 mg., m.p. 165–168°; 55 mg., m.p. 180–182°) were obtained from absolute ether–petroleum ether at 0°. By retreatment of the mother liquor with hydrogen bromide in chloroform an additional amount of product (133 mg., m.p. 165–172°; 44 mg., m.p. 176–177°) was obtained. Purified material melted at 179–180° and showed no selective absorption (in absolute ether) between 220 and 285 mμ, $[\alpha]_D$ +36 ± 2°; after 50 minutes at room temperature $[\alpha]_D$ + 27 ± 2°.

Anal. Calcd. for $C_{24}H_{35}O_5Br$: C, 59.62; H, 7.30; Br, 16.53; CH_3O, 6.42. Found: C, 59.97; H, 7.58; Br, 16.25; CH_3O, 6.24.

3α-Acetoxy-21,21-dimethoxy-17α-pregnane-11,20-dione (XVI) from V.—3α-Acetoxy-21,21-dimethoxypregnane-11,20-dione (434 mg.) was dissolved in 43.4 ml. of 0.40 *N* methanolic potassium hydroxide at room temperature, and the rotation of an aliquot was observed in a 1-dm. tube. The rotation became constant at −0.65° after 2 hours. After 4.5 hours 2.0 ml. of acetic acid was added, and the solution was concentrated almost to dryness. Chloroform was added, and the solution was washed and concentrated to dryness. The residue was acetylated at room temperature for 2 hours in 3.0 ml. each of acetic anhydride and pyridine and then washed up in the usual way. For the total product in 43.4 ml. of chloroform $[\alpha]_D$ +85°. This corresponds to a mixture of 66% of V and 34% of XVI. By crystallization, 179 mg. of V, m.p. 104–105°, and 38 mg. of XVI, m.p. 127–128°, were obtained. The filtrate was chromatographed on 16 g. of a 1:1 mixture of magnesium silicate and infusorial earth. The material eluted with benzene and with benzene containing 0.1% of methanol gave 63 mg. of product which melted at 137.5–138°. This product did not depress the melting point of XVI, $[\alpha]_D$ −6 ± 2°.

The material which was eluted with benzene containing 1% of methanol gave 51 mg. of crystals (m.p. 104–106°) from ether–petroleum ether. After combining this product with the 179 mg. of crystals obtained without chromatographing, the total product was purified from ether–petroleum ether. The crystals melted at 106–106.5°; no depression on admixture with V, $[\alpha]_D$ +131 ± 2°.

XVI from XV.—3α-Acetoxy-21-bromo-21-methoxy-17α-pregnane-11,20-dione (242 mg.) was dissolved in a mixture of 10 ml. of methanol and 1.0 ml. of ethylmorpholine. After 1 hour the solution was concentrated to a small volume, benzene was added, and the solution was washed with water. The aqueous phase contained 100% of theory of bromide ion. The benzene solution was washed with dilute sulfuric acid, with water and concentrated to dryness. Crystals (179 mg., m.p. 137–138°; 6 mg., m.p. 135–136°) were obtained from petroleum ether. The ultraviolet spectrum showed that no α,β-unsaturated ketone was present. A purified sample melted at 138.5–139°, $[\alpha]_D$ −6 ± 2°.

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81; CH_3O, 14.28. Found: C, 69.43; H, 8.73; CH_3O, 14.24.

3α-Hydroxy-11,20-diketo-17α-pregnan-21-al 20,21-Bis-(2,4-dinitrophenylhyrazone) from XVI.—By refluxing a solution of 40 mg. of 3α-acetoxy-21,21-dimethoxy-17α-pregnane-11,20-dione, 80 mg. of 2,4-dinitrophenylhydrazine, 5 ml. of chloroform, 5 ml. of methanol and 0.4 ml. of 5.0 *N* hydrochloric acid for 24 hours and cooling, 47 mg. of yellow crystals, m.p. 307–308° dec., was obtained. After removal of 34 mg. of 2,4-dinitrophenylhydrazine from the filtrate by its insolubility in about 3 ml. of chloroform, an additional 13 mg. of osazone (m.p. 305–307° dec.) was obtained from methanol. After several recrystallizations from chloroform–methanol the product melted at 308–310° dec. and depressed the melting point of X. The infrared spectra of this compound and X in nujol were different in the fingerprint region; $\lambda_{max\ 1}$ 349 mμ, ε 33,000; $\lambda_{max\ 2}$ 397 mμ, ε 25,400; $\lambda_{max\ 3}$ 450 mμ, ε 24,800 (chloroform).

Anal. Calcd. for $C_{33}H_{38}O_{10}N_8 \cdot CH_3OH$: C, 55.28; H, 5.73; CH_3O, 4.20. Found: C, 55.75; H, 5.64; CH_3O, 3.11.

3α-Acetoxy-21,21-dihydroxy-17α-pregnane-11,20-dione (XVII) from XV.—3α-Acetoxy-21-bromo-21-methoxy-17α-pregnane-11,20-dione (242 mg.) was dissolved in 10 ml. of 80% acetic acid at room temperature. After 30 minutes the solution was diluted with water, and crystals (187 mg., m.p. 114–115°) were filtered off. The filtrate contained 4.95 ml. of 0.1 *N* bromide ion (99%). A sample which was purified from dilute acetic acid and dried *in vacuo* at room temperature was colorless and melted at 117–119° with effervescence, $[\alpha]_D$ −11 ± 2°. A freshly prepared chloroform solution of the substance was colorless, but within a few minutes it began to turn yellow, λ_{max} 450 mμ, ε 11 after 45 minutes. ε increased with time to a constant of 25 after about 8 hours (chloroform). The infrared spectrum in nujol showed the presence of a hydroxyl group.

Anal. Calcd. for $C_{21}H_{32}O_6$: C, 67.95; H, 8.43. Found: C, 67.96; H, 8.51.

3α-Acetoxy-11,20-diketo-17α-bromopregnan-21-al 21-(2,4-Dinitrophenylhydrazone) (XVIII) from XIII. A.—To 0.5 millimole of amorphous glyoxal (XIII) (which had been prepared from V by way of XII) in 50 ml. of acetic acid was added 300 mg. of 2,4-dinitrophenylhydrazine. After 92 hours at room temperature the solution was concentrated to dryness. The β-acetyl-2,4-dinitrophenylhydrazine was removed from the steroidal hydrazone by extraction of a solution of the residue in chloroform with dilute alkali and, after washing the chloroform solution and evaporating to a small volume, yellow needles were obtained by addition of methanol; yield: 110 mg., m.p. 216–217°; 21 mg., m.p. 215–217°. A purified sample melted at 217–219°, $\lambda_{max\ 1}$ 272 mμ, ε 8,650; $\lambda_{max\ 2}$ 359 mμ, ε 23,400; $\lambda_{max\ 3}$ 392 mμ, ε 24,400.

Anal. Calcd. for $C_{29}H_{35}O_8BrN_4$: C, 53.79; H, 5.45; Br, 12.34. Found: C, 53.61; H, 5.73; Br, 12.83; CH_3O, 0.0.

XVIII from XIII. B.—The bromo glyoxal (XIII) (0.5 millimole) which had been prepared from XV *via* XII was dissolved in 20 ml. of acetic acid, and 110 mg. of 2,4-dinitrophenylhydrazine was added. After 72 hours at room temperature the product was worked up as described in the previous paragraph. Yellow crystals (142 mg., m.p. 195–197°) were obtained from methanol. Several crystallizations from chloroform–methanol gave a product which melted at 219–220° dec. and did not depress the m.p. of XVIII which had been prepared according to the sequence V, XII, XIII; $\lambda_{max\ 1}$ 270 mμ, ε 9,130; $\lambda_{max\ 2}$ 358 mμ, ε 24,000; $\lambda_{max\ 3}$ 392 mμ, ε 25,200 (chloroform).

3β-Acetoxy-16α-methoxy-Δ⁵-pregnen-20-one from 3β-Hydroxy-Δ⁵,¹⁶-pregnadien-20-one.—3β-Hydroxy-Δ⁵,¹⁶-pregnadien-20-one[5] (314 mg., λ_{max} 239 mμ, ε 8,300) was dissolved in 5.82 ml. of chloroform and 15.7 ml. of methanol, and 1.78 ml. of 2.62 *N* hydrogen chloride in methanol was added at room temperature. Aliquots were removed, diluted with chloroform and washed with water, and the spectrum was taken in methanol. After 17 hours λ_{max} 239 mμ, ε 2,040; after 48 hours λ_{max} 239 mμ, ε 2,010. These values indicate that approximately 24% of the 20-keto-Δ¹⁶-chromophore was still present. At the end of 48 hours 450 mg. of sodium acetate and 9 ml. of water were added. The solvent was removed, chloroform was added and the solution was washed with water. After acetylation of the product it was chromatographed on 16 g. of a 1:1 mixture of infusorial earth and magnesium silicate. The material eluted with 1:1 and 3:1 benzene–petroleum ether yielded, after two crystallizations from ether–petroleum ether, 28 mg. of 3β-acetoxy-Δ⁵,¹⁶-pregnadien-20-one (m.p.

175–177°), which was identified by a mixture melting point with an authentic sample; λ_{max} 239 mμ, ε 9,100 (methanol). An intractable gum was eluted with 9:1 benzene–petroleum ether. Benzene and benzene containing increasing concentrations of methanol up to 1% removed material which, after three crystallizations from ether–petroleum ether, weighed 137 mg. and melted at 159–160°. It was identified by mixture melting point and its infrared spectrum (nujol) as 3β-acetoxy-16α-methoxy-Δ⁵-pregnen-20-one,[5] $[\alpha]_D$ −29 ± 2°, at 239 mμ ε = 25 (methanol).

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ROCHESTER, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE MOUNT SINAI HOSPITAL]

The Mechanism of the Hydrolysis of Salicyl Phosphate. I[1]

BY J. D. CHANLEY, E. M. GINDLER AND HARRY SOBOTKA

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The hydrolysis of salicyl phosphate shows an unusual *p*H dependency, the compound being most unstable at *p*H 5.3. The kinetics of this hydrolysis have been investigated. These studies and comparison with related cases lead to the conclusion that it is the ionized carboxyl group which brings about the rapid scission of the O–P bond. The *p*H dependency is discussed and a mechanism for the reaction is suggested involving the formation of a cyclic transition state. The energy and entropy of activation have been evaluated.

The rate of hydrolysis of salicyl phosphate (A) to salicylic and phosphoric acids varies with *p*H in an unexpected manner[2]; hydrolysis is most rapid at *p*H 5.3, while the compound is very stable in the extreme acid region and completely stable in strong alkali. A similar behavior between *p*H 2 and 8 has been described by Desjobert[3] for ethyl phosphate and glycol phosphate. However, the maximum rate of hydrolysis of these phosphates around *p*H 3–5 at 100°, is about 10^5 times slower than that of salicyl phosphate extrapolated to the same temperature. Salicyl phosphate has a half-life period of 2.1 hours at *p*H 5.3 and 37°. The observed ease of hydrolysis is in contrast to that of phenyl phosphate,[4a,b] *m*- and *p*-carboxyphenyl phosphate[2] and salicylaldehyde phosphate[2b] which remain virtually unchanged under the extremely mild conditions that effect complete hydrolysis of salicyl phosphate. Moreover, the maximum of the *p*H *vs.* hydrolysis rate curve is in contrast to the usual hydrogen ion and hydroxyl ion catalysis, operative in the hydrolysis of carboxyl acid esters, and to the very acid conditions usually necessary to effect the rapid hydrolysis of phosphoric acid esters.[3,4b]

We have observed the same type of *p*H dependency in the hydrolysis of 3-carboxynaphthyl-2-phosphate.[5] The aim of this investigation was to explain the *p*H dependency, and suggest a mechanism for this hydrolysis.

p*H Dependency.—We have determined, by analysis for liberated phosphoric acid, the rate of hydrolysis of salicyl phosphate at three temperatures (37.2°, 42.0°, 47.4°) over the *p*H range 2–10. The same *p*H dependency was observed at all temperatures and in each instance first order kinetics obtained throughout the course of this reaction (Fig. 1).

$$-d[SP]/dt = d[P]/dt \quad (1)$$

$$-d[SP]/dt = k_{obsd}[SP] \quad (2)$$

where SP stands for salicyl phosphate, P for phosphoric acid and *k* for the observed rate constant.

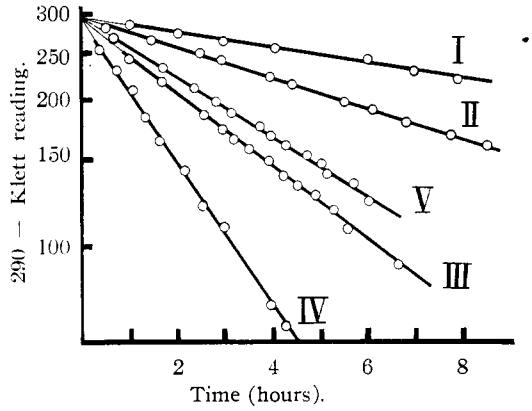
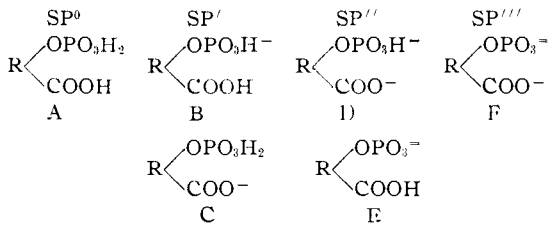


Fig. 1.—Hydrolysis of 0.00130 molar salicyl phosphate at 37° at various *p*H values: I, *p*H 2.32; II, *p*H 2.99; III, *p*H 3.76; IV, *p*H 5.67 (experiment at *p*H 4.92 gave substantially the same figure as (IV)); V, *p*H 6.93 (experiment at *p*H 7.67 gave substantially the same figure as I).

In the elucidation of the *p*H dependency the following considerations are necessary. Salicyl phosphate at *p*H values 2–10 exists predominantly in one or more of these ionic species



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(2) (a) C. Manaka, *J. Biochem.*, **14**, 191 (1931); (b) **14**, 481 (1932); (c) J. Arai, *ibid.*, **20**, 465 (1934).

(3) A. Desjobert, *Compt. rend.*, **224**, 575 (1945); *Bull. soc. chim.*, 809 (1947).

(4) (a) Unpublished observations; (b) *cf.* R. H. Plimmer and W. Burch, *J. Chem. Soc.*, 290 (1929); R. H. Plimmer, *Biochem. J.*, **7**, 72 (1913).

(5) To be reported in a subsequent communication.