of ethereal diazomethane (obtained from 10 g. of precursor). The product, recovered in the usual manner, was chromatographed on a 1.8 × 63 cm. column of 100 g. of activated silica gel. The mobile phase was ethyl acetate and the fraction volume was 5.5 ml. Fractions 49–65 gave 56 mg. of needles, m.p. 122.5–126.5°, λ_{max}^{MeOH} 238 mμ (ε 23,800), from acetone–ether. By determination of the melting point of a mixture and comparison of infrared spectra, the product was shown to be identical with V formed in the incomplete Porter–Silber reagent.

B. Indirect Synthesis of V from 3,20-Bis(ethylenedioxy)-21-methoxy-17-hydroxypregn-5-en-11-one (XII).—To a chilled solution of 150 mg. of XII in 5 ml. of pyridine was added 1 ml. of thionyl chloride.[11] After 16 hr. at −20°, the reaction mixture was added to ice–water and extracted with methylene chloride. The organic phase was washed successively with 1 *N* hydrochloric acid, dilute sodium bicarbonate, and water, filtered through sodium sulfate, and concentrated to dryness. The reaction mixture was fractionated on a 1.8 × 63 cm. column of 100 g. of activated silica gel. The mobile phase was ethyl acetate; 5-ml. fractions were collected.

The residue from fractions 21–34 gave plates from aqueous methanol (67 mg., m.p. 104–107.5°; 8 mg., m.p. 99–105°) and was 3,20-bis(ethylenedioxy)-21-methoxypregna-5,16-dien-11-one (XIII). Several recrystallizations from aqueous methanol raised the melting point to 107.5–109.5°, $[\alpha]^{29}D$ −16.1 ± 2° (*c* 0.433, chloroform), ν^{CHCl_3} 1700 cm.$^{-1}$.

Anal. Calcd. for $C_{26}H_{36}O_6$: C, 70.24; H, 8.16; CH_3O, 6.98. Found[27]: C, 70.11; H, 8.24; CH_3O, 7.19.

A solution of 22.2 mg. of XIII (0.05 mmole) in 3 ml. of methanol and 0.25 ml. of 8% sulfuric acid was refluxed for 50 min.; the product was recovered in the usual manner. Crystallization from acetone–hexane gave rosettes (5 mg., 122.5–126.5°; 5 mg., m.p. 121–124°) in a yield of 56%. A melting point when mixed with V obtained from the reaction of cortisone with the incomplete Porter–Silber reagent was 121.5–125°. Paper chromatographic mobilities, color reactions, and infrared spectra were identical.

The Mechanism of the Porter–Silber Reaction. II. Formation of 17-Deoxysteroidal 21-Phenylhydrazones[1]

MARVIN L. LEWBART[2] AND VERNON R. MATTOX

The Section of Biochemistry, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

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The product formed from the reaction between the Porter–Silber reagent and a 17,21-dihydroxy-20-keto steroid is a 17-deoxy-20-keto steroid 21-phenylhydrazone. The same phenylhydrazone is obtained by treatment of the analogous Δ^{16}-20-keto-21-ol and the 17-deoxy-20-keto-21-al with the Porter–Silber reagent. Several additional 20-keto steroid 21-phenylhydrazones as well as a Δ^{16}-20-keto steroid 21-phenylhydrazone and two 17-hydroxy-20-keto steroid 21-phenylhydrazones have been prepared. The spectra of these three classes of compounds have been obtained in methanol, in the Porter–Silber reagent, and in methanolic alkali. The spectra of most of the hydrazones are very similar in the acidic Porter–Silber reagent and in alkali. Although molar absorptivities of the crystalline hydrazones are of similar magnitude in the Porter–Silber reagent, absorptivities of the corresponding 20-keto-21-als are considerably less. In addition, large differences in molar absorptivities are found among the 20-keto-21-als in the Porter–Silber reagent. The structures of the tautomeric modifications of the 20-keto steroid 21-phenylhydrazones in acid and alkali are discussed.

Porter and Silber[3] found that compounds which have a dihydroxyacetone grouping associated with C-17 of the steroid nucleus give a characteristic yellow color with phenylhydrazine in a mixture of sulfuric acid, water, and methanol. Subsequently,[4] they showed that only one phenylhydrazine group was associated with the side chain and suggested that this group was attached to C-21 as a phenylhydrazone. We have investigated the mechanism of the reaction and the structure of the principal product which is formed. After our work was finished, Barton, McMorris, and Segovia[5] reported an investigation of this reaction in which it was shown that the principal product was the 20-keto steroidal 21-monophenylhydrazone. We are presenting our data since certain aspects of our approach to the problem were different from those of Barton, *et al.*

To study the structure of the product formed in the Porter–Silber reaction, we chose a compound, 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE, I), which has no carbonyl group in the nucleus active toward phenylhydrazine. Treatment of THE with the Porter–Silber reagent for 18 hr. at room temperature gave a yellow-orange solution from which a crude, amorphous product was obtained. After column chromatography, the major component crystallized readily from chloroform as a yellow solvate (1:1). On treatment with sulfuric acid–water–methanol, this substance showed the same spectral properties as are observed for the product obtained following treatment of 17,21-dihydroxy-20-keto steroids with the Porter–Silber reagent. For reasons which will be presented, this yellow compound is formulated as the steroid-21-al 21-phenylhydrazone (IVa).

The major product obtained by treatment of either the Δ^{16}-ketol (Δ^{16}-THA, II) or the steroidal glyoxal (III) with the Porter–Silber reagent was identical with the yellow product (IVa) from THE (I). It is known that, in methanolic hydrogen chloride, both THE (I) and the Δ^{16}-ketol (II) are converted to the acetal[6] of glyoxal III. In sulfuric acid–water–methanol, cortisone is converted into a mixture from which six substances, including the glyoxal and the Δ^{17}-enol glyoxal, can be isolated.[7] These results establish the fact that, in the Porter–Silber reaction, a Mattox rearrangement is a necessary prerequisite to formation of the yellow product with maximal absorption at 410 mμ from 17,21-dihydroxy-20-keto steroids.

The phenylhydrazone (IVa) could be obtained more readily and in better yield by treatment of glyoxal III

(1) Abridgment of thesis submitted by M. L. Lewbart to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biochemistry, June, 1961.

(2) This investigation was carried out during the tenure of a fellowship from the Division of General Medical Sciences, Public Health Service.

(3) C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185**, 201 (1950).

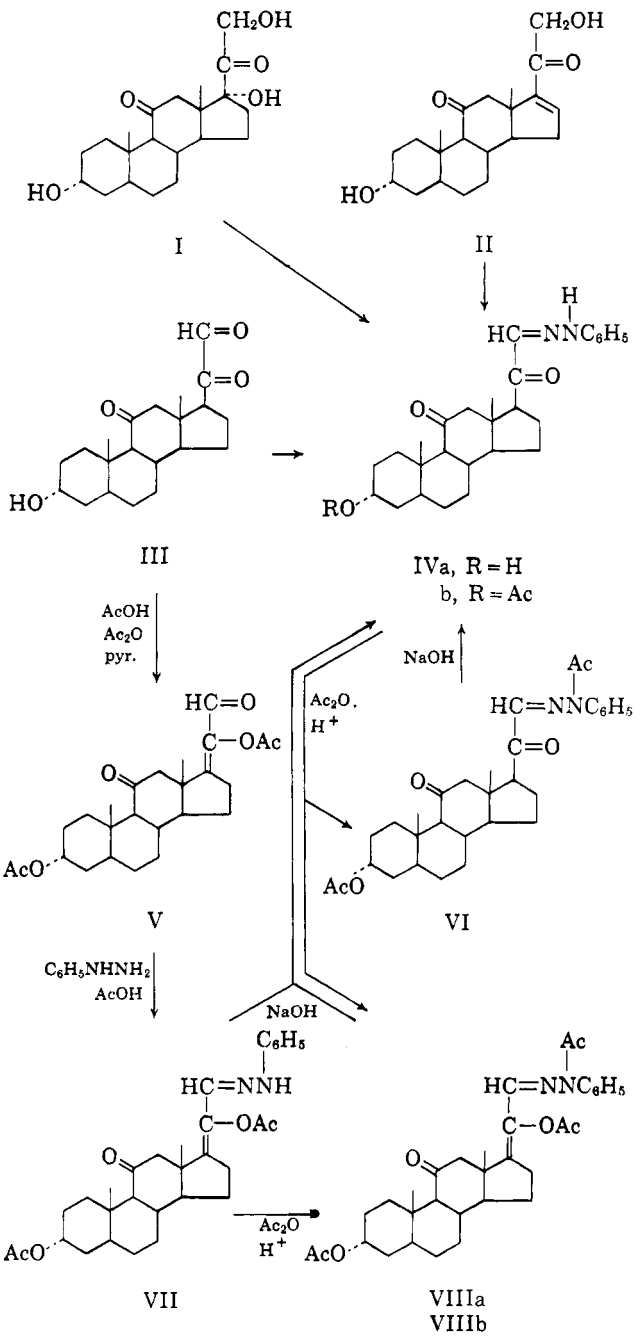
(4) R. H. Silber and C. C. Porter, "Methods of Biochemical Analysis," Vol. 4, David Glick, Ed., Interscience Publishers, Inc., New York, N. Y., 1957, p. 139.

(5) D. H. R. Barton, T. C. McMorris, and R. Segovia, *J. Chem. Soc.*, 2027 (1961).

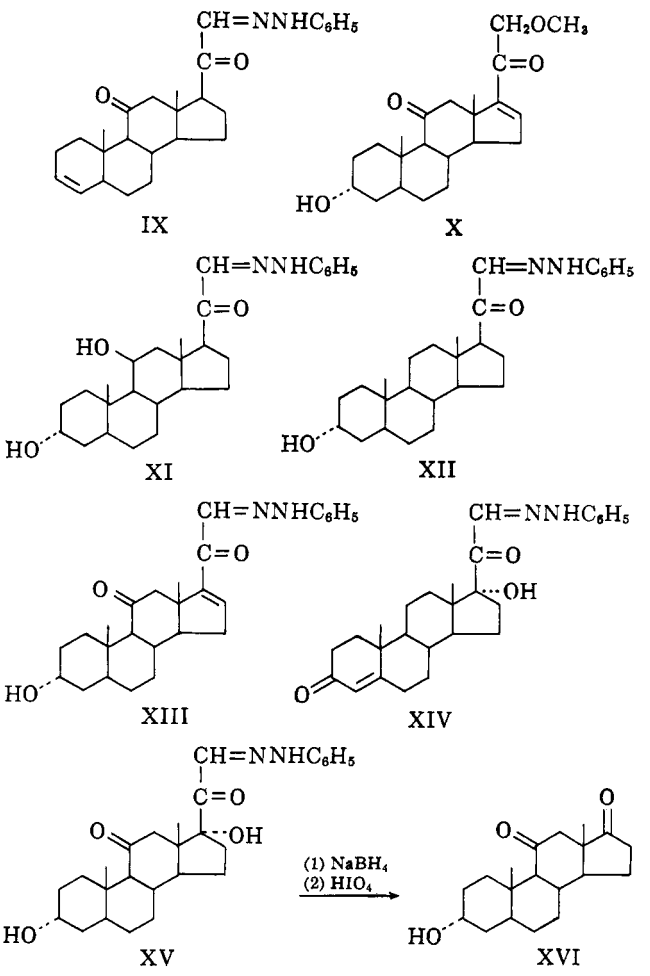
(6) V. R. Mattox, *J. Am. Chem. Soc.*, **74**, 4340 (1952).

(7) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **29**, 513 (1964).

SCHEME I



SCHEME II



with phenylhydrazine in aqueous acetic acid than by the reaction between the Porter–Silber reagent and THE. Complete reaction occurred within a few minutes at room temperature. Even in the presence of excess phenylhydrazine, under the conditions which were employed, the phenylhydrazone (IVa) was the only product isolated.

Treatment of phenylhydrazone IVa with pyridine and acetic anhydride gave the monoacetate (IVb). Analysis of both the phenylhydrazone (IVa) and its acetate (IVb) confirmed the report of Silber and Porter[4] that a single phenylhydrazine residue is associated with the side chain.

The following results indicate that the phenylhydrazine moiety of IVa is attached at C-21 rather than at C-20.

(1) The hydrazone (IVa) did not reduce Tollens reagent; its acetylated derivative (IVb) gave no acidic product after treatment with 5 molar equiv. of chromic acid in 90% acetic acid for 3 hr. at room temperature. A steroid-21-al 20-phenylhydrazone would be expected to react with either ammoniacal silver oxide or chromic acid to give a pregnan-21-oic acid.

(2) After treatment of IVa with 0.25 *N* hydrogen chloride in dry methanol for 48 hr. at room temperature, starting material could be recovered in 96% yield. If the substance had been a steroid-21-al 20-phenylhydrazone, it should have formed a 21,21-dimethyl acetal.

(3) Glyoxal III failed to form an osazone in the presence of an excess of phenylhydrazine. It is well-known that a bulky substituent at C-21 in 20-keto pregnanes greatly decreases the reactivity of the C-20 carbonyl group. On the other hand, a steroid-21-al 20-phenylhydrazone should react readily with another molecule of phenylhydrazine to give an osazone, since C-21 is not subject to appreciable steric hindrance.

(4) The phenylhydrazone (XV) from the analogous (and presumably similarly reacting) steroidal 17-hydroxy glyoxal was shown conclusively to be a 21-phenylhydrazone.

Additional evidence for the location of the phenylhydrazine residue at C-21 was obtained as follows. The $\Delta^{17(20)}$-enol acetate (V), prepared by enol acetylation of the glyoxal (III), was converted to its 21-phenylhydrazone (VII). Alkaline hydrolysis of VII gave phenylhydrazone IVa. If the phenylhydrazine group does not migrate during this treatment, the series of reactions firmly establishes the structure of IVa.

TABLE I

MOLAR ABSORPTIVITIES OF 21-PHENYLHYDRAZONES DERIVED FROM STEROIDAL GLYOXALS

21-Phenylhydrazone from glyoxal of	λ_{max}^{MeOH}, mμ	ε	λ_{max}^{P-S}, mμ[a]	ε[b]	ϵ_g[c]	λ_{max}^{NaOH}, mμ[d]	ε
THA	238	12,000					
(3α,21-Dihydroxy-5β-pregnane-11,20-dione)	295	4,250	410	29,100	24,600	410	27,800
	350	22,000		(27,200)			
THB	238	11,900					
(3α,11β,21-Trihydroxy-5β-pregnan-20-one)	295	4,500	415	27,000	12,600	410	24,200
	350	21,500		(25,700)			
THQ	238	12,000					
(3α,21-Dihydroxy-5β-pregnan-20-one)	295	4,550	415	28,200	16,100	410	24,200
	350	22,000		(26,200)			
Δ[16]-THA	245	12,500				400	11,000
(3α,21-Dihydroxy-5β-pregn-16-ene-11,20-dione)	385	17,100					
THE	241	10,250					
(3α,17,21-Trihydroxy-5β-pregnane-11,20-dione)	298	2,300	425	28,800		410	32,800
	365	21,000		(25,400)			
Substance S	241	26,500[e]	425	26,800		410	32,700
(17,21-Dihydroxypregn-4-ene-3,20-dione)	365	21,100		(25,300)			

[a] λ_{max}^{P-S} was calculated from a reading taken 15 min. after dissolving hydrazone in the Porter–Silber reagent. The reagent was prepared by dissolving 50 mg. of phenylhydrazine hydrochloride in 100 ml. of an 8:2 mixture of sulfuric acid and water and adding 0.5 volume of methanol. [b] Values in parentheses refer to molar absorptivities in the blank reagent. [c] ϵ_g values were obtained by mixing crystalline glyoxals and the Porter–Silber reagent.[10] [d] λ_{max}^{NaOH} was calculated from a reading taken 15 min. after dissolving hydrazone in 1 *N* sodium hydroxide in 50% aqueous methanol. [e] The Δ[4]-3-keto chromophore contributes about 16,000 to this molar absorptivity.

Acetylation of the phenylhydrazone (VII) of the enol acetate under strenuous conditions afforded a product which contained three acetyl groups and is formulated tentatively as VIIIa. Acetylation of IVa under forcing conditions gave an isomeric triacetoxy phenylhydrazone provisionally assigned structure VIIIb, in low yield. The infrared spectra of these compounds were similar, but their ultraviolet absorption spectra were markedly different. It is probable that VIIIa and VIIIb are geometric isomers. Alkaline hydrolysis of both VIIIa and VIIIb gave unacetylated hydrazone IVa.

The principal product from the forced acetylation of phenylhydrazone IVa was colorless (λ_{max} 274 mμ), and lacked NH and OH bands in the infrared region. This compound, formulated as the diacetoxy phenylhydrazone (VI) was converted into the unacetylated phenylhydrazone (IVa) by alkaline hydrolysis.

Treatment of the glyoxal (III) of THE (I) and of Δ[16]-THA (II) with the complete Porter–Silber reagent gave, in addition to the 21-phenylhydrazone (IVa), three by-products in small amounts. One of these substances was a less polar yellow compound which was not acetylated by mild treatment with acetic anhydride–pyridine. It is believed to be the Δ[2] or Δ[3] dehydration product (IX) of IVa. The remaining two products were colorless and both were more polar than the phenylhydrazone (IVa); on paper chromatograms they absorbed 254-mμ radiation. The more polar compound of the two reduced blue tetrazolium; the less polar one gave a yellow color with 2 *N* sodium hydroxide. On the basis of paper chromatographic mobilities and color reactions, these by-products were provisionally identified[7] as the Δ[16]-ketol (II) and the Δ[16]-ketol ether (X), respectively. Their formation, even in the presence of phenylhydrazine, is noteworthy and has been commented on in a previous paper.[7]

Additional steroidal 21-phenylhydrazones (XI, XII, and XIII) were prepared by treatment of the corresponding glyoxals with phenylhydrazine in aqueous acetic acid. Their spectral constants are listed in Table I.

We wished to investigate the reaction of 17-hydroxysteroidal glyoxals with the Porter–Silber reagent and to compare the products with those obtained from 17-deoxysteroidal glyoxals. The 17-hydroxy glyoxal[8] obtained by cupric acetate oxidation of 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE) was chosen for this study. Treatment of this glyoxal either with the complete Porter–Silber reagent or with phenylhydrazine in aqueous acetic acid gave the corresponding phenylhydrazone (XV). When this phenylhydrazone (XV) was treated with sodium borohydride and the resulting product was oxidized with periodic acid, 3α-hydroxy-5β-androstane-11,17-dione (XVI) was obtained. This compound could have been formed only if the phenylhydrazine moiety of XV was attached at C-21 and if a hydroxyl group was present at C-17. (See Scheme II.)

Treatment of the aldehyde of Reichstein's substance S with 2 equiv. of phenylhydrazine hydrochloride in aqueous acetic acid gave the 21-phenylhydrazone (XIV) in 80% yield in spite of the fact that the carbonyl function at C-3 was not protected. The analysis and the properties of the product indicate that the hydroxyl group at C-17 was retained.

Absorptivities of the phenylhydrazones in methanol, in the Porter–Silber reagent, and in 1 *N* sodium hydroxide are included in Table I. The absorption spectrum of the phenylhydrazone from THA glyoxal in each of the three solvents is shown in Fig. 1. All 17-deoxy-20-keto steroid 21-phenylhydrazones, with exception of the Δ[16] compound (XIII), exhibited absorption maxima at 238, 295, and 350 mμ. These findings are in excellent agreement with the maxima reported[9] for pyruvaldehyde 1-phenylhydrazone (λ_{max} at 237 and 350 mμ), the simplest analog of 20-keto steroid 21-phenylhydrazones. The absorption maxima of the two 17-hydroxy-20-keto steroid 21-phenylhydrazones (XIV and XV) were at slightly longer wave lengths

(8) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 2001 (1963).
(9) R. E. Bowman and C. S. Franklin, *J. Chem. Soc.*, 1583 (1957).

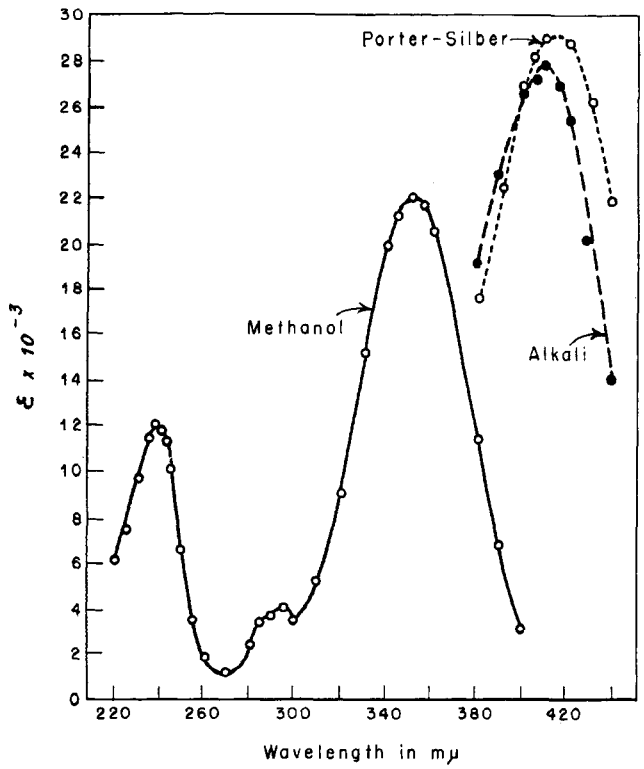


Fig. 1.—Absorption spectrum of the phenylhydrazone from THA glyoxal in three different solvents: solid line with open circles, in methanol; dotted line with open circles, in Porter–Silber reagent; and broken line with solid circles, in alkali.

(λ_{max} at 241, 298, and 365 mμ) than those of the 17-deoxy derivatives (IVa, XI, and XII). The ultraviolet absorption spectrum of the Δ^{16}-20-keto steroid 21-phenylhydrazone (XIII) differed appreciably from that of its analog saturated in ring D. It contained no absorption maximum at 295 mμ, and there was a bathochromic displacement of the other two maxima (λ_{max} at 245 and 385 mμ).

With the exception of the Δ^{16} compound (XIII) which gave no color, all phenylhydrazones immediately gave nearly as much color with the blank reagent (lacking phenylhydrazine) as with the complete Porter–Silber reagent. The absorption maxima of the crystalline phenylhydrazones were the same as previously obtained[10] after treatment of the glyoxals with the Porter–Silber reagent in the microanalytical procedure. Whereas there was considerable variation in the chromogenicity of the glyoxals in the Porter–Silber reagent, their respective crystalline 21-phenylhydrazones had approximately the same molar absorptivities. In addition, the data in Table I show that the 21-phenylhydrazones of glyoxals produced more color with the Porter–Silber reagent than did the glyoxals themselves. This difference was 15 to 20%, even for the 11-keto steroid glyoxals which were the most chromogenic in the microanalytical procedure. This finding suggests that, under the conditions employed in the analytical procedure, the yield of 21-phenylhydrazone from the 11-keto steroid glyoxals is approximately 80%.

In view of the finding[11] that the rate of hydrolysis of steroidal C-21 ethylene ketals is dependent on the nature of the function which is present at C-11, it was of interest to determine whether there was a significant difference between hydrazones IVa, XI, and XII in the rates of loss of absorbance in the blank Porter–Silber reagent. Readings were taken at 410 mμ at 1.5, 15, and 60 min. after dissolving the hydrazones in the acidic reagent. After 15 min. the readings on IVa, XI, and XII were 97, 90, and 97%, respectively, compared to the initial readings; after 60 min., values were 92, 85, and 93% of the initial values. Thus, the rates of loss of absorbance are not markedly different. It seems probable that differences in chromogenicity of the three glyoxals are due to extent of formation of the phenylhydrazones in the complete Porter–Silber reagent.

More color is obtained from a glyoxal by treating it with phenylhydrazine in acetic acid and then dissolving the product in the blank Porter–Silber reagent than is obtained by treating the glyoxal directly with the complete reagent. The data obtained on 35-μg. amounts of the 21-aldehyde of Substance S are shown in Table II.

TABLE II

COMPARISON OF COLOR YIELDS AFTER DIFFERENT METHODS OF TREATMENT OF 35-μg. AMOUNTS OF 21-ALDEHYDE OF SUBSTANCE S

No.	Treatment	λ, mμ	—Absorbance— 15 min.	75 min.	ε 15 min.
1	1 ml. of methanol + 2 ml. of P–S reagent	425	0.162	0.161	4860
2	0.1 ml. of 85% HOAc contg. 1 mg. of $C_6H_5NHNH_2 \cdot HCl$. After 5 min., 0.9 ml. of CH_3OH + 2 ml. of blank reagent added	425	0.840	0.665	26,400
3	1 ml. of HOAc contg. 1 mg. of $C_6H_5NHNH_2 \cdot HCl$. After 5 min., 2 ml. of blank reagent added	425	0.900	0.649	27,000
4	0.1 ml. of HOAc contg. 1 mg. of $C_6H_5NHNH_2 \cdot HCl$. After 5 min., 0.9 ml. of CH_3OH + 2 ml. of 2 *N* aqueous NaOH added	410	0.998	0.785	30,000

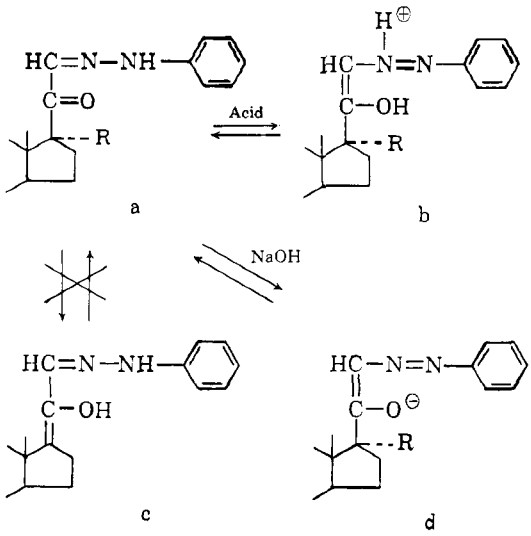
An unexpected finding was that, in 1 *N* sodium hydroxide in 50% methanol, the 17-deoxy-20-keto steroidal 21-phenylhydrazones exhibit spectra almost identical with those produced in the Porter–Silber reagent. The 17-hydroxy compounds, which show maxima at 425 mμ in the acidic reagent, exhibited maxima at 410 mμ in alkali (a hypsochromic shift of 15 mμ) and were 25 to 30% more absorptive in this medium than in the Porter–Silber reagent. In alkali, the 21-phenylhydrazone (XIII) of the Δ^{16} compound possessed a spectrum different from that of the other two types of 20-keto steroid 21-phenylhydrazones. The absorbance at the maximum (400 mμ) was only 40% of that of the corresponding saturated compound (IVa).

The maxima in the absorption spectra of the phenylhydrazones occur at considerably longer wave lengths in the acidic Porter–Silber reagent and in *N* alkali than they do in methanol. The bathochromic increment with the Porter–Silber reagent is 60 mμ, giving maxima at 410 and 425 mμ for the 17-deoxy- and 17-hydroxyphenylhydrazones, respectively. With alkali, on the

(10) M. L. Lewbart and V. R. Mattox, *Anal. Chem.*, **33**, 559 (1961).

(11) S. Bernstein, M. Heller, and W. S. Allen, *J. Org. Chem.*, **26**, 1333 (1961).

SCHEME III



other hand, both types have absorption maxima at 410 mμ. The presence of a double bond in the 16,17-position of the 20-keto steroid 21-phenylhydrazones (XIII) completely absolishes absorption at 410 mμ in the Porter–Silber reagent,[12] but does not prevent intense color development in alkali.

Although a 20-keto steroid 21-phenylhydrazone structure can be assigned with certainty to the crystalline yellow products of the Porter–Silber reaction, it is obvious from the spectra that there is an alteration of the chromophoric group when the hydrazones are dissolved in the strongly acidic Porter–Silber reagent. The very similar spectra obtained in strong acid and in alkali suggest that similar chromophores are formed at the two extremes of pH. A tautomeric shift can convert the hydrazone (a, Scheme III) into an azo structure with a system of double bonds identical in acid (b) and in alkali (d). It would be predicted that conversion of structure a to structures b and d, whereby lengthening of the conjugated system occurs, would result in an intensification of the absorption and a marked bathochromic shift. Because of the similar spectra given by 17-deoxy- and 17-hydroxyphenylhydrazones with the Porter–Silber reagent, it is unlikely that enolization between C-17 and C-20 to form c can account for the formation of the 410-mμ chromophore from 17-deoxy phenylhydrazones. The slight change in location of the absorption maxima of 17-hydroxy phenylhydrazones in acid and alkali can be ascribed to differences in the nature and distribution of the electrical charges at the extremes of pH.

Experimental

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were taken in methanol at a, concentration of about 1% and at a temperature of 24 ± 2°, unless otherwise indicated. Analyses were by Mr. J. F. Alicino, Metuchen, N. J.

3α-Hydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVa). A. From the Porter–Silber Reagent and THA Glyoxal Hydrate (Hydrate of III).—To a solution of 2.25 g. (6.2 mmoles) of 3α,21,21-trihydroxy-5β-pregnane-11,20-dione[8] in 333 ml. of methanol was added, while cooling in an ice bath, 667 ml. of 8:2 sulfuric acid–water which contained 1.5 g. (10.3 mmoles) of phenylhydrazine hydrochloride. After 1 hr. at room temperature, the solution was added to 2 l. of ice–water and the mixture was extracted four times with a total volume of 900 ml. of methylene chloride. The extract was washed twice with 100 ml. of water, dried, and concentrated to dryness. Paper chromatography of an aliquot in isooctane–toluene–methanol–water (140:60:160:40) showed the presence of four compounds. The major component (R_f 0.36) was yellow and absorbed long-wave (366 mμ) ultraviolet illumination more intensely than it did short-wave (254 mμ) illumination. A small amount of a second yellow compound (R_f 0.88) also was present. The two remaining compounds (R_f 0.05 and 0.12) were visible only under 254-mμ illumination. The less mobile compound reduced blue tetrazolium; the more mobile one gave a yellow color with 2 *N* sodium hydroxide. The reaction mixture was applied to a 6 × 44 cm. column containing 400 g. of Celite which had been pretreated with 200 ml. of formamide. The mobile phase was a 4:3 mixture of cyclohexane and benzene, saturated with formamide. Collection of the 25-ml. fractions was begun after 750 ml. of effluent had been discarded.

Dehydration Product (IX) of 3α-Hydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVa). Fractions 1–10.—The residue was precipitated from aqueous methanol. After being dried to constant weight over anhydrous calcium chloride, the yellow compound (45 mg.) melted at 104.5–107.5°. After recrystallization from aqueous methanol, it had m.p. 105–107°, $[\alpha]_D$ −172 ● 3°.

Anal. Calcd. for $C_{27}H_{34}N_2O_2$: C, 77.47; H, 8.19; N, 6.70. Found: C, 76.70; H, 8.35; N, 6.91.

The product was recovered unchanged after treatment with acetic anhydride–pyridine at room temperature.

3α-Hydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVa). Fractions 20–100.—This compound descended the chromatographic column as a broad yellow band. The crude product, after precipitation from methanol with water, weighed 1.57 g. (m.p. 149.5–150.5° and m.p. 193–195°). It crystallized as yellow needles from chloroform. When dried in air and then dried further for 3 hr. at 100° and 1–2 mm., it lost 22.2% of its weight (calcd. for loss of 1 mole of chloroform, 21.5%). The melting point was 195–197°; $[\alpha]_D$ −198 ± 2°; λ_{max}^{MeOH} 238 mμ (ε 12,000), 295 (4250), 350 (22,000).

Anal. Calcd. for $C_{27}H_{36}N_2O_3$: C, 74.27; H, 8.31; N, 6.42. Found: C, 73.90; H, 8.24; N, 6.34.

B. Compound IVa from Phenylhydrazine and THA Glyoxal (III) in Aqueous Acetic Acid. 1. With 1.2 Molar Equiv. of Phenylhydrazine.—A solution of 6.96 g. (20 mmoles) of 3α,21-dihydroxy-5β-pregnane-11,20-dione (THA) in 250 ml. of methanol was oxidized in the usual manner[8] for 20 min. with 1 g. of cupric acetate in an equal volume of methanol. The reaction mixture was added to 1 l. of water which contained 1.5 g. of EDTA and was extracted with methylene chloride. The yellow solution of THA glyoxal was washed successively with dilute sodium bicarbonate and water, dried, and concentrated to dryness. The residue, in 40 ml. of 85% acetic acid, was treated with a solution of phenylhydrazine hydrochloride (3.5 g., 24 mmoles) in 50% acetic acid (40 ml.). After 15 min. at room temperature, 200 ml. of water was added, and the yellow precipitate was collected, washed with water, and dried. Recrystallization from chloroform–cyclohexane gave the yellow chloroform solvate in 78% yield (7.3 g., m.p. 196.5–198°; 1.41 g., m.p. 194–196°). The infrared spectrum in Nujol was identical with that of the product formed from THA glyoxal and the Porter–Silber reagent.

2. With 5 Molar Equiv. of Phenylhydrazine.—A solution of 72.8 mg. (0.2 mmole) of THA glyoxal hydrate and 145 mg. (1 mmole) of phenylhydrazine hydrochloride in 5 ml. of 85% acetic acid stood for 15 min. at room temperature. The product, recovered as before, gave yellow needles from chloroform–hexane in 77% yield (77 mg., m.p. 192.5–195°; 9 mg., m.p. 189–193°).

3α-Hydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVa) from THE (I) and from Δ16-THA (II).—To separate 100-mg. quantities of 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (I) and of 3α,21-dihydroxy-5β-pregn-16-ene-11,20-dione (II) in 33 ml. of methanol was added, while cooling in ice, 67 ml. of 8:2 sulfuric acid–water which contained 67 mg. of phenylhydrazine hydrochloride. After 18 hr. at room temperature, the reaction mixtures were added to ice–water and the products were recovered by extraction with methylene chloride. Paper chromatography of aliquots showed patterns identical with the pattern obtained after treatment of THA glyoxal with the Porter–Silber reagent.

(12) This statement refers to hydrazones of structure XIII and should not be confused with Δ16-ketolic steroids (such as II) which give hydrazones which absorb maximally at 410 mμ in the Porter–Silber reagent.

The respective residues were chromatographed on a 1.8 × 36 cm. column prepared from 40 g. of Celite pretreated with 20 ml. of formamide. The mobile phase was a 1:1 mixture of cyclohexane and benzene, saturated with formamide. Only the major yellow band from each extract was collected. The crude product from THE (I), which weighed 43 mg., was recrystallized from chloroform–petroleum ether (b.p. 50–70°) to give 20 mg. of product, m.p. 196–197.5°. The corresponding product from Δ^{16}-THA (II) weighed 62 mg. and was recrystallized from chloroform–petroleum ether to give 35 mg. of product, m.p. 197–198°. The infrared spectra of both compounds in Nujol were identical with the spectrum of the phenylhydrazone (IVa) which was prepared from THA glyoxal (III).

3α-Acetoxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVb) from IVa.—To 250 mg. of 3α-hydroxy-11,20-dioxo-5β-pregnan-21-al 21-phenylhydrazone was added 0.5 ml. each of pyridine and acetic anhydride. After 12 hr. at room temperature, the reaction mixture was added to ice and dilute hydrochloric acid. The yellow precipitate which formed was washed with water and dried over anhydrous calcium chloride. The product (265 mg., m.p. 134.5–136°) was recrystallized from benzene and gave yellow needles which melted at 181.5–182.5°; $[\alpha]_D$ −148 ± 1°; λ_{max}^{MeOH} 238 mμ (ε 12,400), 295 (4300), 350 (22,200).

Anal. Calcd. for $C_{29}H_{38}N_2O_4$: C, 72.77; H, 8.05; N, 5.86. Found: C, 72.33; H, 8.06; N, 5.90.

Acetylation of 3α-Hydroxy-11,20-dioxo-5β-pregnan-21-al 21-Phenylhydrazone (IVa) under Forcing Conditions.—To a solution of 278 mg. (0.5 mmole) of chloroform-solvated phenylhydrazone (IVa) in 2 ml. of acetic anhydride and 10 ml. of acetic acid was added 200 mg. of *p*-toluenesulfonic acid. After 20.5 hr. at room temperature, the mixture was added to water and the product was extracted with methylene chloride. The extract was washed with 5% sodium bicarbonate solution and water, dried, and concentrated to dryness.

A. 3α-Acetoxy-11,20-dioxo-5β-pregnan-21-al 21-(N-Acetylphenylhydrazone) (VI).—Crystallization from methanol gave two crops (130 mg., m.p. 224–226°; 16 mg., m.p. 220–223°) of the colorless diacetate (VI) in 56% yield. When the reaction was stopped after 2 hr., the yield of VI was 78%. A purified sample melted at 224–225°, λ_{max}^{MeOH} 274 mμ (ε 17,100), no NH or OH bands in the infrared spectrum.

Anal. Calcd. for $C_{31}H_{40}N_2O_5$: C, 71.59; H, 7.75; CH_3CO, 16.53; N, 5.38. Found: C, 71.73; H, 7.94; CH_3CO, 14.88; N, 5.51.

The residue from the mother liquor (138 mg.) was chromatographed on a 1.8 × 36 cm. column prepared by pretreating 35 g. of Celite with 17.5 ml. of lower phase from cyclohexane–methanol–water (400:80:20). After 40 ml. of effluent had been discarded, 5-ml. fractions were collected.

B. 3α,20-Diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al 21-(N-Acetylphenylhydrazone) (VIIIb). Fractions 11–19.—The residue gave a colorless product (30 mg., m.p. 118–120°) from aqueous acetic acid. On paper chromatography in isooctane–methanol–water (200:160:40), compounds VIIIa and VIIIb had identical mobilities (R_f 0.28). No NH band was present in the infrared spectrum. A sample for analysis was obtained by reprecipitation from acetic acid and drying for 4 hr. at room temperature and 0.1 mm. over phosphorus pentoxide. The melting point was 117–120°, λ_{max}^{MeOH} 269 mμ (ε 13,200).

Anal. Calcd. for $C_{33}H_{42}N_2O_6 \cdot H_2O$: C, 68.25; H, 7.64; N, 4.83; CH_3CO, 22.3. Found: C, 68.41, 68.50; H, 7.71, 7.72; N, 5.05; CH_3CO, 23.3.

3α-20-Diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al (V) from Hydrate of THA Glyoxal (III).—A solution of 300 mg. of 3α,21,21-trihydroxy-5β-pregnane-11,20-dione in 1 ml. each of acetic acid, acetic anhydride, and pyridine was heated for 30 min. at 60°. The mixture was added to water and extracted with methylene chloride. The organic solvent was washed successively with dilute hydrochloric acid, 5% sodium bicarbonate solution and water, filtered through anhydrous sodium sulfate, and concentrated to dryness. The residue was chromatographed on a 1.8 × 47 cm. column prepared from 50 g. of Celite which has been pretreated with 25 ml. of formamide. The mobile phase was cyclohexane saturated with formamide. The 6-ml. fractions were collected after 50 ml. of effluent had been discarded.

Fractions 24–37.—Colorless crystals of V were obtained from ether in 41% yield (192 mg., m.p. 144–146°; 48 mg., m.p. 142–144°). The analytical sample had m.p. 144.5–146.5°, $[\alpha]_D$ +67 ± 2° (chloroform), λ_{max}^{ether} 246 mμ (ε 15,200).

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.67; H, 8.05.

3α,20-Diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al 21-Phenylhydrazone (VII) from V.—To 100 mg. of 3α,20-diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al (V) in 2.5 ml. of 85% acetic acid was added 40 mg. of phenylhydrazine hydrochloride in an equal volume of the same solvent. After 20 min. at room temperature, the solution was added to water, the resulting precipitate was collected, washed with water, and dried over calcium chloride. The product weighed 107 mg. (88.6%) and melted at 129.5–130.5°. Crystallization from methanol afforded clusters of fine needles which, in the same solvent, spontaneously changed into large, prismatic needles (78.5 mg., m.p. 215–218°). The sample for analysis was recrystallized from methanol to give m.p. 217–219°; $[\alpha]_D$ +85 ± 2° (chloroform); λ_{max}^{MeOH} 248 mμ (ε 10,000), 304 sh (14,900), and 336 (29,600); ν_{max}^{Nujol} 3293 cm.$^{-1}$ (NH band).

Anal. Calcd. for $C_{31}H_{40}N_2O_5$: C, 71.51; H, 7.74; N, 5.38; CH_3CO, 16.53. Found: C, 71.32; H, 8.10; N, 5.28; CH_3CO, 13.85.

3α,20-Diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al 21-(N-Acetylphenylhydrazone) (VIIIa) from VII.—To 52 mg. (0.1 mmole) of 3α,20-diacetoxy-11-oxo-5β-pregn-17(20)-en-21-al 21-phenylhydrazone in acetic acid (5 ml.) and acetic anhydride (1 ml.) was added *p*-toluenesulfonic acid (10 mg.). After 30 min. at room temperature, the solution was added to water, and the product was collected and dried over calcium chloride. The crude product (53 mg., 98.2%, m.p. 125–128°) was crystallized from methanol to give yellow plates (22 mg., m.p. 205–207°; 8.5 mg., m.p. 202.5–203.5°). No NH band was present in the infrared spectrum. Recrystallization from methanol gave the analytical sample with m.p. 206–208°, $[\alpha]_D$ +25 ± 1° (chloroform), λ_{max}^{MeOH} 281 mμ (ε 34,200).

Anal. Calcd. for $C_{33}H_{42}N_2O_6$: C, 70.43; H, 7.52; N, 4.98; CH_3CO, 22.95. Found: C, 70.56; H, 7.91; N, 5.02; CH_3CO, 22.70.

Regeneration of Phenylhydrazone IVa from Acetylated Phenylhydrazones VI, VII, VIIIa, and VIIIb.—To 40-mg. quantities of VI, VII, VIIIa, and VIIIb in 2 ml. of methanol was added 0.25 ml. of 2 *N* sodium hydroxide. After 12 hr. at room temperature, the products were recovered after dilution of these mixtures with water and extraction with methylene chloride. Crystallization of the four residues from chloroform–petroleum ether gave products which, as shown by their infrared spectra, were identical with phenylhydrazone (IVa).

3α,11β-Dihydroxy-20-oxo-5β-pregnan-21-al 21-Phenylhydrazone (XI) from THB Glyoxal Hydrate.—To a solution of 100 mg. of 3α,11β,21,21-tetrahydroxy-5β-pregnan-20-one[8] in 2.5 ml. of 85% acetic acid was added 50 mg. of phenylhydrazine hydrochloride in an equal volume of the same solvent. After 15 min. at room temperature, the yellow product (XI) was precipitated with water, filtered, washed, and dried over calcium chloride to yield 113 mg. (94.5%) of product, m.p. 152.5–153.5°. A sample for analysis was recrystallized from ether and had m.p. 203–205°; $[\alpha]_D$ −78 ± 2°; λ_{max}^{MeOH} 238 mμ (ε 11,900), 295 (4500), and 350 (21,500).

Anal. Calcd. for $C_{27}H_{38}N_2O_3$: C, 73.94; H, 8.73; N, 6.36. Found: C, 74.03; H, 8.69; N, 6.39.

3α-Hydroxy-20-oxo-5β-pregnan-21-al 21-Phenylhydrazone (XII) from THQ Glyoxal Hydrate.—3α,21,21-Trihydroxy-5β-pregnan-20-one[8] (50 mg.) was treated with 25 mg. of phenylhydrazine hydrochloride in 4 ml. of 85% acetic acid. After 15 min. at room temperature 56 mg. (93.7%) of the phenylhydrazone (XII), m.p. 129–130°, was recovered in the usual manner. A sample for analysis was recrystallized from ether and had m.p. 219–219.5°; $[\alpha]_D$ −91 ± 2°; λ_{max}^{MeOH} 238 mμ (ε 12,000), 295 (4550), and 350 (22,000).

Anal. Calcd. for $C_{27}H_{38}N_2O_2$: C, 73.73; H, 9.06; N, 6.63. Found: C, 76.74; H, 9.02; N, 6.59.

3α-Hydroxy-11,20-dioxo-5β-pregn-16-en-21-al 21-Phenylhydrazone (XIII) from Δ^{16}-THA Glyoxal Hydrate.—3α,21,21-Trihydroxy-5β-pregn-16-en-11,20-dione[8] (100 mg.) was treated with 50 mg. of phenylhydrazine hydrochloride in 5 ml. of 85% acetic acid. After 15 min. the yellow-orange phenylhydrazone (XIII) was recovered in the usual manner (121 mg., m.p. 144–150°). The analytical sample was precipitated from ether–petroleum ether and dried for 4 hr. at 100° and 1 mm. over phosphorus

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

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

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

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

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

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

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

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

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

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

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

JOHN S. BARAN

The Laboratories of G. D. Searle and Company, Chicago, Illinois

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

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

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

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

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

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

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

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

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

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