THE JOURNAL OF Organic Chemistry®

VOLUME 29, NUMBER 3

MARCH 12, 1964

The Mechanism of the Porter–Silber Reaction. I. Rearrangement of the Dihydroxyacetone Group of Steroids[1]

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Received September 5, 1963

When steroids with a dihydroxyacetone group at C-17 are treated with the Porter–Silber reagent (phenylhydrazine in sulfuric acid–water–alcohol), 17-deoxy-21-phenylhydrazones are formed. After treatment of cortisone with incomplete (no phenylhydrazine) Porter–Silber reagent, it was possible to isolate the glyoxal intermediate as well as five other related compounds: a Δ^{16}-ketol, an enol, a Δ^{16}-ketol ether, a 17β(21-acetal), and a 17α(21-acetal). These compounds are interconvertible in the incomplete Porter–Silber reagent, but they are not convertible into cortisone. The chemical nature of the products is correlated with their order of formation from cortisone and the mechanism of the reaction is discussed. It is concluded that, in the Porter-Silber reaction, a rearrangement of the dihydroxyacetone grouping precedes condensation with phenylhydrazine to yield the final yellow product.

In 1950 Porter and Silber[3] described a reaction in which steroids with a dihydroxyacetone grouping give a yellow color, with maximal absorption at 410 mμ when treated with a mixture of sulfuric acid, water, alcohol, and phenylhydrazine. Subsequently, they proposed[4] that in the course of the reaction ". . .compounds bearing the dihydroxyacetone side chain first undergo a Mattox rearrangement[5] with the production of a 20,21-ketal." In addition, they suggested that this intermediate reacts with phenylhydrazine to form the steroidal 21-phenylhydrazone (D, Scheme I), and that this substance is responsible for the yellow color which is obtained. This postulate was based on the following evidence: (1) the acidic conditions employed in the reaction are similar to those used for the rearrangement[5] of cortisone to a glyoxal derivative, (2) glyoxals give a yellow color with the Porter–Silber reagent more rapidly than do the corresponding 17-hydroxy ketols, and (3) overnight treatment of cortisone with the incomplete reagent (sulfuric acid–water–alcohol), followed by addition of phenylhydrazine, results in a rapid appearance of the yellow color. These findings strongly suggest, but do not conclusively prove, that a glyoxal is an intermediate in the reaction.

To test the hypothesis that a rearrangement occurs before reaction with phenylhydrazine to form the 410-mμ chromophore, studies were carried out in which cortisone was treated with the incomplete reagent. It was assumed that the product or products of this reaction would be intermediates in the reaction that occurs in the presence of phenylhydrazine. In preliminary experiments, cortisone was treated at room temperature with a mixture of 2 parts of 7:3 sulfuric acid–water and 1 part of 95% ethanol. Aliquots, withdrawn from the reaction mixture at intervals, were analyzed by paper chromatography. There was a rapid conversion of cortisone into a mixture which contained at least six substances. All of these compounds retained the Δ^4-3-keto grouping in ring A as evidenced by fluorescence after treatment with sodium hydroxide.[6] The structures and order of appearance of these substances were determined so that information could be obtained on the course of the rearrangement reaction. The time at which each compound first could be detected after initiation of the reaction was as follows: "enol," 30 sec.; "glyoxal," 75 sec.; "Δ^{16}-ketol," 75 sec.; "Δ^{16}-ketol ether," 5 min.; and "acetals," 10 min. The first three of these substances were shown to be compounds IV, III, and II, respectively, in Scheme II. The Δ^{16}-ketol ether and the acetals are analogous to V, VI, and VII, which were obtained when methanol was substituted for ethanol in the reaction mixture. After 8 hr. in the reaction mixture, only a trace of starting material remained.

On paper chromatograms, III, IV, and the ethyl analogs of VI and VII gave yellow spots immediately

(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. This investigation was supported in part by Research Grant AM 01255 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service.

(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).

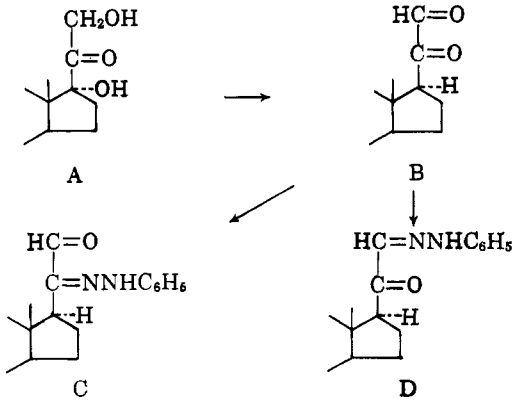
(4) R. H. Silber and C. C. Porter, "Methods of Biochemical Analysis," David Glick, Ed., Interscience Publishers, Inc., New York, N. Y., 1957, p. 147.

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

(6) I. E. Bush, *Biochem. J.*, **50**, 370 (1952).

SCHEME I

POSTULATED STEPS IN PORTER-SILBER REACTION



after treatment with Porter–Silber reagent B.[7] Compound II and the ethyl analog of V also gave a yellow color after elution from paper and treatment with the Porter–Silber reagent overnight at room temperature. Compound II gave a purple spot with alkaline blue tetrazolium. The ethyl analog of V appeared as a light yellow spot with the same reagent; it later was found that the yellow color was formed with alkali in the absence of blue tetrazolium. After elution of each of the six compounds and retreatment with the incomplete reagent, paper chromatographic patterns were obtained that were identical with those observed for the original reaction mixture except that no cortisone was formed from any of the products.

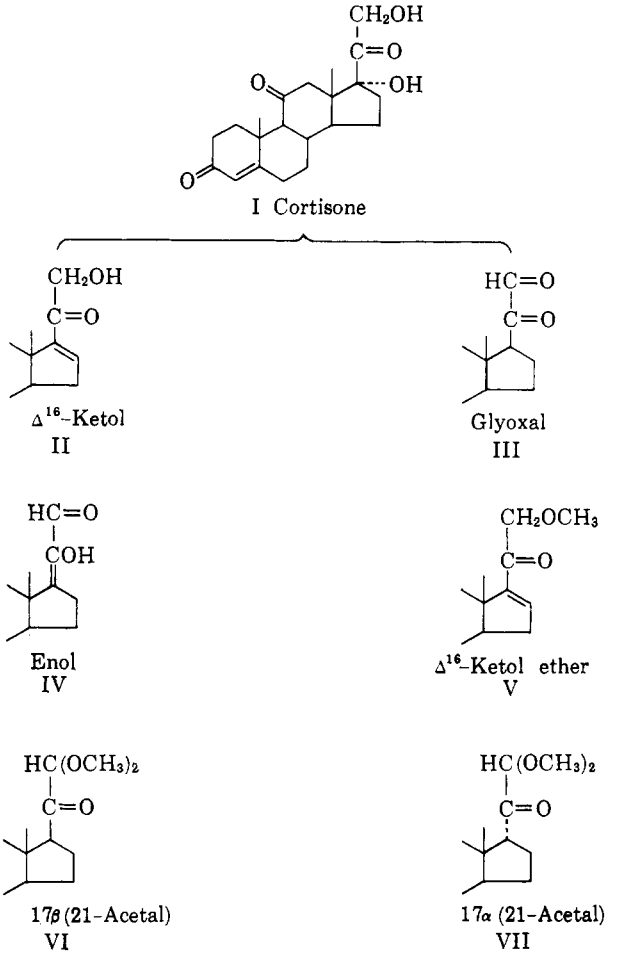
When the relative amounts of sulfuric acid–water and ethanol were varied, striking changes were noted in the relative quantities of the various products obtained. With an ethanol–acid ratio[8] of 2:1, the major products were the glyoxal and the acetals, but, with an ethanol–acid ratio of 1:4, the Δ^{16}-ketol was the major (and almost exclusive) recognizable product. However, much intractable material was formed under the latter conditions. With intermediate ratios of alcohol to acid, the yields of enol and enol ether became maximal, although significant amounts of the other compounds also were formed.

For preparative experiments, an alcohol–acid ratio of 1:2 was chosen because this ratio gives all products in isolable yields and because it is the ratio ordinarily employed in quantitative analysis with the complete Porter–Silber reagent. Methanol was substituted for ethanol in the incomplete Porter–Silber reagent. This substitution resulted in greater ease of crystallization of the products which contained alkoxyl groups and a more favorable mobility of the products during chromatography in benzene–formamide.

Column chromatography of the products in Bush-type systems on Celite[9] or cellulose was unsatisfactory; the enol was unstable and its decomposition products contaminated the other compounds. The mixture from the reaction of 2 g. of cortisone[10] with sulfuric acid–water–methanol was successfully fractionated in 500-mg. portions on sheets of paper in formamide–benzene. Components of the eluates from individual zones were purified by direct crystallization (when possible) and by column chromatography. The pertinent physical constants and yields of the purified compounds are listed in Table I; their chromatographic mobilities in several systems, and the reagents ordinarily used for detecting them are shown in Table II.

SCHEME II

PRODUCTS ISOLATED AFTER TREATMENT OF CORTISONE WITH SULFURIC ACID–WATER–METHANOL (INCOMPLETE PORTER–SILBER REAGENT)



In order to determine the efficiency of isolation of the various products from the reaction mixture, two experiments were performed under the same conditions except, in the first, 2 g. of cortisone was used and, in the second, 25 mg. was used. The reaction mixture from 25 mg. of cortisone was fractionated by paper chromatography in benzene–formamide, and the eluates from the five major zones were analyzed by their absorption in the ultraviolet region and by the Porter–Silber reaction. Of the cortisone used as starting material, 75.5% could be accounted for in these fractions, as compared with 64.3% in the crystalline products recovered in the large-scale experiment.

The Δ^{16}-ketol (II) has been prepared previously.[11] Its structure was immediately suggested by its ultraviolet spectrum, reduction of blue tetrazolium, and chromatographic mobility.

Recovery of the glyoxal (III) was complicated by its formation of an addition product[12] with formamide

(7) M. L. Lewbart and V. R. Mattox, *Anal. Chem.*, **33**, 559 (1961).
(8) Acid refers to 7:3 sulfuric acid–water (v./v.).
(9) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **28**, 1779 (1963).
(10) Donated by Merck and Co., Rahway, N. J.
(11) W. S. Allen and S. Bernstein, *J. Am. Chem. Soc.*, **77**, 1028 (1955).
(12) The reactions of aldehydes with formamide, especially those activated by a negative group in the α-position, have been cited in the literature [H. L. du Mont and G. Rätzel, *Chem. Ber.*, **72**, 1500 (1939), and references cited therein].

TABLE I

PHYSICAL CONSTANTS AND YIELDS OF PRODUCTS OF REACTION OF CORTISONE WITH SULFURIC ACID–WATER–METHANOL

Steroid	M.p., °C.	$[\alpha]_D$	Molar absorbtivity λ_{max}^{MeOH}, mμ	ε	ε[a]	% yield from cortisone 2 g.	% yield from cortisone 25 mg.
Δ16-Ketol (II)	205–209	+230	238	23,800	25,900	7.5	7.5
Glyoxal hydrate (III)	117–119	+228	238	15,500	26,000	17.3	20.6
Enol[b] (IV)	189–191	+196	238	15,600	25,600		
			282	13,100			
Enol acetate[c]	232–234	+154	242	23,500	27,100	3.4	6.3
Δ16-Ketol ether (V)	123–126	+224	238	23,400	17,900	21.4	20.7
17β(21-Acetal) (VI)	161–163	+240	238	15,900	27,000	11.5	20.4[d]
17α(21-Acetal) (VII)	152–153	+89	238	15,600	28,100	3.2	

[a] Molar absorbtivities determined at 410 mμ after treatment of the steroid for 20 hr. at room temperature in Porter–Silber reagent A.[7] [b] Prepared independently. [c] Enol from the reaction of cortisone and sulfuric acid–water–methanol was isolated as the acetate. [d] Value for combined acetal fraction.

TABLE II

PAPER CHROMATOGRAPHIC MOBILITIES AND COLOR REACTIONS OF PRODUCTS FROM CORTISONE AFTER TREATMENT WITH SULFURIC ACID–WATER–METHANOL

Compound	Solvent system[a]	R_f	Detection[b] D_1	D_2	D_3
Δ16-Ketol (II)	1	0.38	+	+	+S
	3	0.38			
Glyoxal (III)	1	0.62	+	−	+F
	3	0.05 (streaked)			
Enol (IV)	1	0.71	+	−	+F
	3	0.63			
Enol acetate	2	0.22	+	−	+F
Δ16-Ketol ether (V)	1	0.71	+	Y	+S
	2	0.16			
	3	0.71			
17β(21-Acetal) (VI)	1	0.87	+	−	+F
	2	0.59			
	3	0.89			
17α(21-Acetal) (VII)	1	0.87	+	−	+F
	2	0.67			
	3	0.89			

[a] Solvent system 1 is toluene–isooctane–methanol–water (275:225:400:100); solvent system 2 is toluene–isooctane–methanol–water (50:150:160:40); and solvent system 3 is benzene–formamide (papers were impregnated with 40% formamide in acetone). [b] Detection: D_1, sodium hydroxide-induced fluorescence[6]; D_2, blue tetrazolium, Y, yellow color; D_3, Porter–Silber reagent,[7] S, slow, F, fast; +, positive; −, negative.

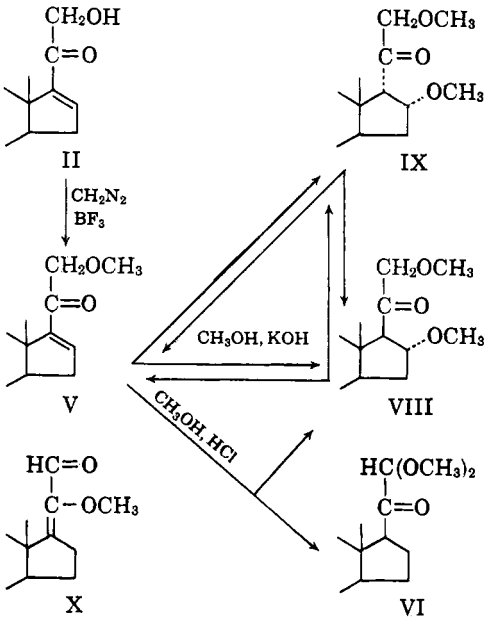
during paper chromatography. The adduct was cleaved readily with dilute mineral acid, and the glyoxal hydrate could be recovered. Its properties are in agreement with those published for this compound by Beyler and Hoffman.[13] In addition, this product was identical with the hydrate of the glyoxal prepared by hydrolysis of 21,21-dimethoxypregn-4-ene-3,11,20-trione.[5]

The enol (IV) in the eluate from the paper chromatograms had absorption maxima at 238 and 282 mμ and gave a blue-black color with ferric chloride. These properties are consistent with a $\Delta^{17(20)}$-20-hydroxy-21-aldehydic structure.[14] Because of the previously encountered instability of this enol, it was acetylated immediately on recovery. Independent synthesis of the enol acetate from the glyoxal by the method of Beyler and Hoffman[13] gave a product identical with the acetylated enol. In contrast to their findings, however, the compound did not reduce blue tetrazolium.

(13) R. E. Beyler and F. Hoffman, *J. Am. Chem. Soc.*, **79**, 5297 (1957).
(14) G. A. Fleisher, and E. C. Kendall, *J. Org. Chem.*, **16**, 573 (1951).

SCHEME III

REACTIONS OF THE Δ16-KETOL ETHER

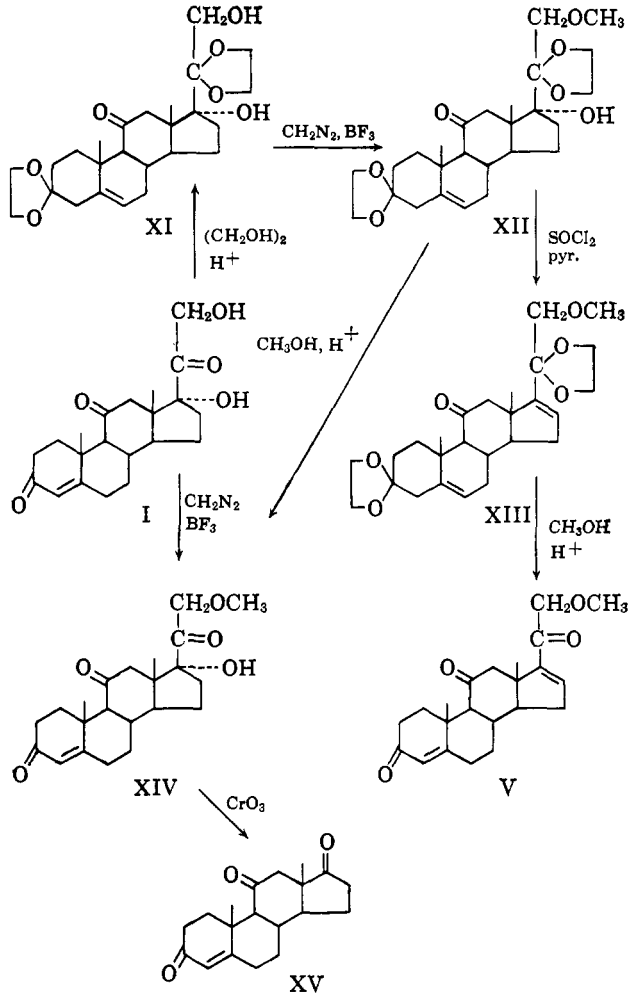


The free enol, which was prepared by treatment of the glyoxal with pyridine and acetic acid, was stable in crystalline form. Its properties, shown in Table I, are in agreement with those reported for analogous compounds.

Initially, the Δ16-ketol ether (V) was thought to be one of two structures (V or X, Scheme III). It contained one methoxyl group and had a molar absorptivity [λ_{max} 238 mμ (ε 23,400)] that indicated the presence of another chromophore in addition to the one in ring A. It gave no color with ferric chloride or the Schiff reagent and did not reduce Tollens reagent or alkaline tetrazolium blue. Attempts to prepare V by treatment of enol IV with diazomethane (without BF_3) and with dimethyl sulfate and alkali were unrewarding. Compound V had an absorption band at 1592 cm.$^{-1}$ which falls within the range 1588 to 1600 that is characteristic[11] of Δ16-20-keto steroids.

Treatment of V with methanolic hydrogen chloride gave acetal VI and a dimethoxy compound (VIII). Since the infrared spectrum of VIII did not show a hydroxyl band, the compound could not be the hemiacetal of X. Treatment of V with methanolic potassium hydroxide gave two dimethoxy compounds, VIII and IX, in which only ring A was chromophoric

SCHEME IV
INDEPENDENT SYNTHESIS OF THE Δ^{16}-KETOL ETHER



to ultraviolet light. Compounds VIII, IX, and V were interconvertible in methanolic potassium hydroxide. These results indicate that V is a Δ^{16}-ketol ether and that substances VIII and IX are 16-methoxy compounds. Addition of methanol to 20-keto-Δ^{16} steroids to give saturated 20-keto-16-methoxy derivatives has been shown to occur under both alkaline[15] and acidic[5] conditions.

The orientation of the groups at C-16 and C-17 in VIII and IX are assigned as indicated in Scheme III because (1) the group entering C-16 under the experimental conditions used becomes α-oriented, (2) the compound with the 17β side chain is more dextrorotatory[16] than the 17α isomer, and (3) the derivative with the 17β side chain is present in greater quantity at equilibrium.[16,17]

More direct evidence for the structure of V was obtained through its preparation from the Δ^{16}-ketol (II) by treatment with diazomethane in the presence of boron fluoride.[18] The yield in this reaction was 31%. In addition, it was possible to prepare V by another route. The 3,20-bisethylene ketal[19] of cortisone (XI, Scheme IV) was converted into the corresponding 21-methoxy bisketal (XII) by methylation with diazomethane. Elimination of water from XII by treatment with thionyl chloride and pyridine[11] gave the 16-dehydro derivative (XIII) which could be hydrolyzed to yield V.

Hydrolysis of XII itself gave the 21-methyl ether of cortisone (XIV) in 70% yield. This substance could be obtained also by treatment of cortisone (I) with diazomethane in the presence of boron fluoride. It is concluded that substance XIV is a 21-O-methyl derivative, rather than a 17-O-methyl compound, because it does not reduce alkaline tetrazolium blue, it is not acetylated by treatment with acetic anhydride and pyridine at room temperature, and it is oxidizable with chromic acid to adrenosterone (XV) in good yield. The melting point of our sample of XIV (chromatographically pure in three different solvent systems) was about 15° lower than that reported for this substance by Huang-Minlon, *et al.*[20]

The identity of the 17β(21-acetal) (VI) was readily established by comparison with an authentic sample of the dimethyl acetal prepared from cortisone.[5]

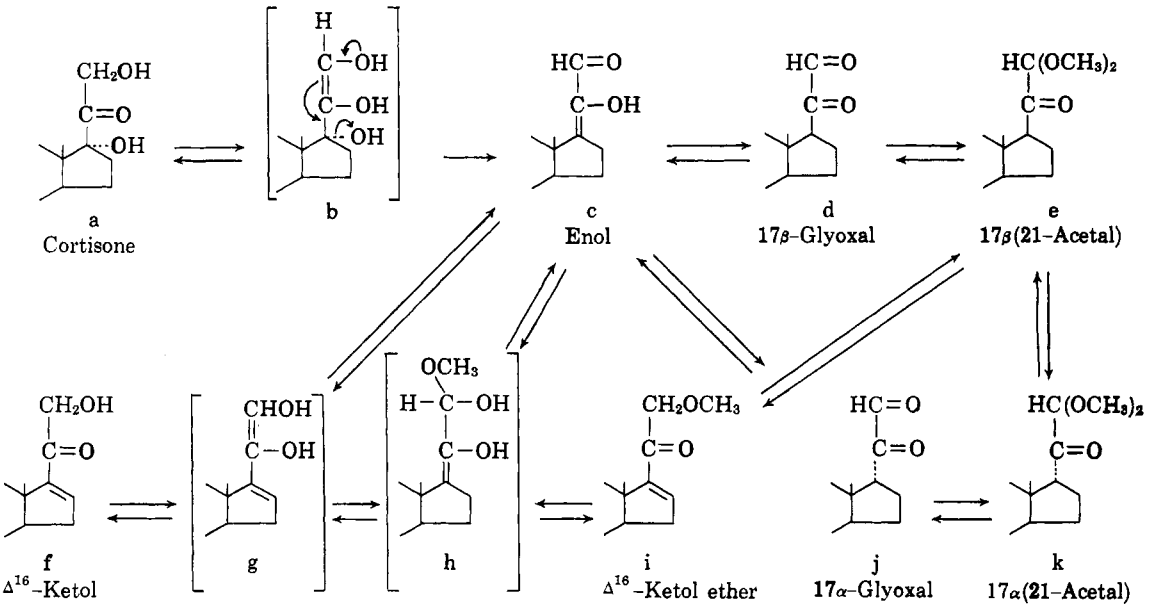
The 17α(21-acetal) (VII) was not separated from its 17β epimer during paper chromatography in the benzene–formamide system but was obtained subsequently by column chromatography of the mixture in a less polar solvent system. The per cent composition, methoxyl content, and weak positive rotation of VII indicated that it was the 17α epimer of acetal VI. The value, $M_D^{VI} - M_D^{VII} = -586$ units, is in close agreement with that ($M_D^{17\beta} - M_D^{17\alpha} = -595$ units) obtained for two 20-keto-21,21-dimethoxy compounds epimeric at C-17 which were studied previously.[5] The structure of the 17α(21-acetal) (VII) was confirmed by an independent synthesis through isomerization of the 17β(21-acetal) (VI) with methanolic potassium hydroxide.[5]

The scheme illustrated in Scheme V is postulated as the mechanism of the formation of the various products from cortisone by treatment with sulfuric acid–water–methanol. All of the reactions shown, except b → c, are reversible. Enolization of cortisone (a) between C-20 and C-21 gives the enetriol (b). Removal of a proton at C-21 followed by a simultaneous shift of electrons and β-elimination of the hydroxyl group at C-17 gives the enol (c). Ketonization of c forms the glyoxal (d) which, on reaction with methanol, is converted to the dimethyl acetal (e). Epimerization of e at C-17 results in the formation of a dimethyl acetal (k) with a side chain in the α-orientation. The Δ^{16}-ketol ether (i) may arise by reaction of the enol (c) with methanol *via* the hemiacetal (h) or it may be formed by loss of methanol from e or k. Similarly the Δ^{16}-ketol (f) may be formed from c by way of the dienediol (g), directly or with the hemiacetal of the enol (h) as an intermediate. It is probable that the relative amounts of acid, water, and alcohol in the reaction mixture not only determine the ratios of products formed but also influence the quantitative contri-

(15) D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951).
(16) C. W. Marshall and T. F. Gallagher, *J. Biol. Chem.*, **179**, 1265 (1949).
(17) R. B. Moffett and W. M. Hoehn, *J. Am. Chem. Soc.*, **66**, 2098 (1944).
(18) E. Müller and W. Rundel, *Angew. Chem.*, **70**, 105 (1958); M. C. Caserio, J. D. Roberts, M. Neeman, and W. S. Johnson, *J. Am. Chem. Soc.*, **80**, 2584 (1958).
(19) R. Antonucci, S. Bernstein, M. Heller, R. Lenhard, R. Littell, and J. H. Williams, *J. Org. Chem.*, **18**, 70 (1953).
(20) Huang-Minlon, R. Tull, and J. Babcock, *J. Am. Chem. Soc.*, **76**, 2396 (1954).

SCHEME V

POSTULATED MECHANISM FOR THE REACTION OF CORTISONE WITH SULFURIC ACID–WATER–METHANOL



bution of certain intermediates involved in the transformations.

The enol (c) should ketonize to give both the 17β-glyoxal (d) and the 17α-glyoxal (j). Although the 17α-glyoxal (j) could not be demonstrated in the reaction, it is reasonable to assume that the 17α(21-acetal) arises, in part, from it (j → k) as well as from inversion of the 17β(21-acetal) side chain (e → k). A possibility that the Δ16-ketol can arise, in part, directly from cortisone (a → f) cannot be excluded, although the finding (Fig. 1) that Δ16-ketols give color with the Porter–Silber reagent much more slowly than do 17-hydroxy ketols suggests that direct dehydration (a → f) prior to rearrangement is not an important pathway in the Porter–Silber reaction.

The five products which were isolated in addition to the glyoxal itself are related closely to the glyoxal postulated as the intermediate of the Porter–Silber reaction. These findings support the belief of Silber and Porter[4] that a Mattox-type[5] rearrangement occurs in this reaction. Furthermore, it appears that, of the various products formed, the glyoxal and the enol are the most important intermediates in the reaction as ordinarily performed in the presence of phenylhydrazine.

It was demonstrated that compounds II–VII of Scheme II are interconvertible in the incomplete Porter–Silber reagent. In the complete Porter–Silber reagent, the product responsible for the yellow color has structure D in Scheme I (actually a tautomeric form of D) as shown by Barton and co-workers[21a] and confirmed by us.[21b] Furthermore, when a steroid with the dihydroxyacetone grouping is treated with the complete Porter–Silber reagent and the product is chromatographed, small amounts of the Δ16-ketol and Δ16-ketol ether could be detected along with the principal product (which was D of Scheme I). It seems probable that differences in chromogenicity of various

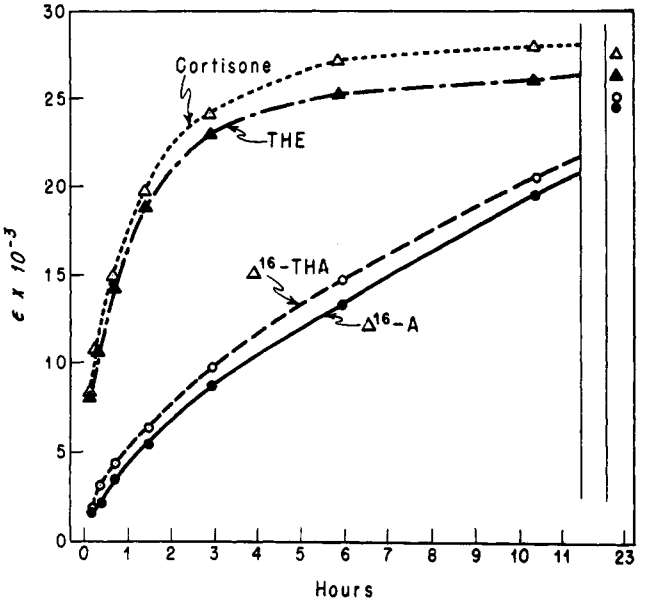


Fig. 1.—Rates of color development in the Porter–Silber reaction with 17-hydroxy ketols and Δ16-ketols. Equivalent amounts of cortisone, 3α,17,21-trihydroxy-5β-pregnane-11,20-dione (THE), 21-hydroxypregna-4,16-diene-3,11,20-trione (Δ16-A), and 3α,21-dihydroxy-5β-pregn-16-ene-11,20-dione (Δ16-THA) were treated with Porter–Silber reagent A[7] at room temperature. Readings at 410 mμ were made on aliquots removed at intervals.

glyoxals[7] in the Porter–Silber reagent are due to formation of different ratios of yellow phenylhydrazones and colorless intermediates derived from the glyoxals.

The findings of several groups of investigators are pertinent to a discussion of the mechanism of the rearrangement outlined in Scheme V. Norymberski[22a] found that when steroids with a dihydroxyacetone group (a, in Scheme V) were treated with zinc in aqueous acetic acid, the 17-hydroxyl group was removed. Slates and Wendler[22b] suggested that this process involved more than a simple reductive removal

(21) (a) D. H. R. Barton, T. C. McMorris, and R. Segavia, *J. Chem. Soc.*, 2027 (1961); (b) M. L. Lewbart and V. R. Mattox, *J. Org. Chem.*, **29**, 521 (1964).

(22) (a) J. K. Norymberski, *J. Chem. Soc.*, 517 (1956); (b) H. L. Slates and N. L. Wendler, *J. Org. Chem.*, **22**, 498 (1957).

of the 17-hydroxyl group; they postulated that a rearrangement was involved and that reduction occurred at the stage of the intermediate glyoxal (d). Herzog and associates[23] thought that zinc acetate, which is formed in the Norymberski reaction, had a beneficial effect on the rearrangement, and they were able to demonstrate that zinc acetate catalyzes the rearrangement of the dihydroxyacetone group (a) to the enol aldehyde (c). They showed that certain compounds which have the dihydroxyacetone grouping (a) yield little or no enol aldehyde (c) when heated in acetic acid alone, but that, in the presence of zinc acetate, yields of the enol aldehyde are 20–40%. Rearrangement of the dihydroxyacetone function does not occur in acetic acid (in the absence of zinc) if C-21 is acetylated. It seems probable that zinc acetate is functioning as a Lewis acid in catalyzing the rearrangement.

Tsuda, Ohki, and Suzuki[24] isolated both 17-isodeoxycorticosterone and 17-isoprogesterone after treating Reichstein's substance S (a, of Scheme V) with zinc in acetic acid. Herzog and co-workers[23] suggest that the enol aldehyde (c) is an intermediate in this reaction. The production of these two compounds from their precursor is analogous to the formation, in our experiments, of the 17α(21-acetal) (k) from cortisone by way of an initial intermediate (c) and subsequent intermediates (j and e), or both.

Experimental

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

Treatment of Cortisone with Sulfuric Acid–Water–Methanol.—To a solution of 500 mg. of cortisone in 165 ml. of methanol was added, while cooling in an ice bath, 335 ml. of a mixture of 7 parts of concentrated sulfuric acid and 3 parts (v./v.) of water. The clear yellow solution stood at room temperature for 18 hr. and then was poured into 2 l. of ice–water. The mixture was extracted with four 150-ml. portions of methylene chloride; the extract was washed twice with 100 ml. of water, dried, and concentrated *in vacuo* to dryness. The residue was dissolved in a small volume of methylene chloride and applied evenly to 20 sheets (18 by 55 cm.) of unwashed Whatman No. 1 paper which had been impregnated previously with 40% (v./v.) of formamide in acetone. After chromatography for 3–3.5 hr. with benzene saturated with formamide as the mobile phase, the chromatograms were scanned under illumination at 254 mμ. Ultraviolet absorbing zones were marked, cut into small pieces, placed in glass-stoppered bottles which contained either water or aqueous methanol, and stored at 4°.

Since the enol was found to be unstable in preliminary experiments, this fraction was processed immediately after the chromatograms were completed. The combined enol zones were eluted by pulping the paper with 150 ml. of water, filtering, and washing the pulp with water. The combined eluate and washings were extracted with methylene chloride and the solvent was removed *in vacuo*. The residue had maximal absorption at 282 mμ in methanol and gave a blue-black color with ferric chloride solution. It was treated with 0.2 ml. each of pyridine and acetic anhydride at room temperature for 1 hr.

The entire procedure was repeated three more times on successive days in order to process a total of 2.0 g. of cortisone. The other four zones were eluted after the reaction mixture from 2.0 g. of cortisone had been chromatographed. Solvents and volumes used in elution of steroids from filter paper follow.

Zone	R_f	No. in Scheme II	Eluent	Volume, ml.
Δ^{16}-Ketol	0.38	II	1:1 H_2O–MeOH	400
Glyoxal	0.05	III	H_2O	600
Enol	0.63	IV	H_2O	600
Δ^{16}-Ketol ether	0.71	V	1:1 H_2O–MeOH	700
Acetals	0.89	VI, VII	1:1 H_2O–MeOH	700

The respective eluates were extracted directly with methylene chloride; the extracts were washed with water, filtered through anhydrous sodium sulfate, and concentrated to dryness. Components of the individual zones were crystallized and characterized.

A. 21-Hydroxypregna-4,16-diene-3,11,20-trione (II).—Two crops of needles (128 mg., m.p. 204–207°; 14 mg., 201–204°) were obtained from acetone. A purified sample melted at 205–209°, $[\alpha]_D$ +230° ± 3°, λ_{max}^{MeOH} 238 mμ (ϵ 23,800); lit.[11] m.p. 223–228°, $[\alpha]_D$ +236° (chloroform), λ_{max}^{MeOH} 238 mμ (ϵ 23,900). The infrared spectrum[25] was identical with that of 21-hydroxypregna-4,16-diene-3,11,20-trione prepared by Allen and Bernstein.[11]

Treatment of this product (II) with pyridine and acetic anhydride gave a substance which had an infrared spectrum identical with that of 21-acetoxypregna-4,16-diene-3,11,20-trione.[26]

B. 21,21-Dihydroxypregn-4-ene-3,11,20-trione (Hydrate of III).—The major component of this fraction was a glyoxal-formamide adduct (R_f 0.46 in system C of Bush[6]). There also was present a small amount of free steroidal glyoxal (R_f 0.60 in system 1 of Table II). To cleave the adduct, the residue was dissolved in 10 ml. of methanol and mixed with 10 ml. of 1 *N* hydrochloric acid. Paper chromatography of an aliquot removed after 16 hr. at room temperature revealed the presence of only one product, and it had the mobility of the glyoxal. After 19.5 hr., 60 ml. of water was added, the solution was extracted with methylene chloride, and the extract was washed with water and concentrated to dryness. The residue was chromatographed on a 4.6 × 35 cm. column prepared by treating 200 g. of Celite with 100 ml. of the lower phase from the system cyclohexane–benzene–methanol–water (1200:800:1400:600). After 400 ml. of effluent had been discarded, 15-ml. fractions were collected. The residues from fractions 7–18 crystallized from aqueous methanol to give 290 mg., m.p. 113–115°, and 55 mg., m.p. 108–112°. A sample, recrystallized from wet ether and dried to constant weight over calcium chloride, melted at 117–119°, $[\alpha]_D$ +228 ± 1°, λ_{max}^{MeOH} 238 mμ (ϵ 15,500); lit.[13] m.p. 121–124°, λ_{max}^{MeOH} 238 mμ (ϵ 15,300). The compound reduced Tollens reagent rapidly and gave a positive test with the Schiff reagent.

C. 21,21-Dihydroxypregn-4-ene-3,11,20-trione (Hydrate of III) from VI.—To a solution of 388 mg. of 21,21-dimethoxypregn-4-ene-3,11,20-trione (VI) in 25 ml. of methanol was added 75 ml. of hot water and 5 ml. of concentrated hydrochloric acid. The mixture was heated on the steam bath for 20 hr. in a glass-stoppered flask, cooled, and extracted with methylene chloride. The extract was washed with water and evaporated to dryness. Crystallization from wet ether gave 300 mg. (84%, m.p. 112–114°) of yellow rosettes. The product did not depress the melting point of the glyoxal hydrate described in the previous paragraph.

D. 20-Acetoxy-3,11-dioxopregna-4,17(20)-dien-21-al (Acetate of IV).—The combined acetylated enol fractions from the benzene–formamide chromatograms were treated with charcoal in acetone. Concentration of the solvent gave rosettes (49 mg., m.p. 222–223° dec.). The residue from the mother liquor was chromatographed on a 1.8 × 48 cm. column prepared by treating 50 g. of Celite with 22.5 ml. of formamide. The mobile phase was cyclohexane–benzene (1:1) saturated with formamide. After 60 ml. of effluent had been discarded, 6 ml. fractions were collected. The residue from fractions 23–50 gave an additional 23 mg. (m.p. 224–226° dec.) of enol acetate to bring the total yield to 72 mg. (3.4%). The product reduced Tollens reagent slowly. Tests with the Schiff reagent, alkaline blue tetrazolium, and ferric chloride were negative. A sample, recrystallized from acetone and dried to constant weight at 100° and 1–2 mm., melted at 232–234° (on stage at 226°), $[\alpha]_D$ +154 ± 1°, λ_{max}^{MeOH} 241 mμ (ϵ 23,500); lit.[13] m.p. 226–232°, $[\alpha]_D$ +163° (chloroform), λ_{max}^{MeOH} 241 mμ (ϵ 23,700).

(23) H. L. Herzog, M. J. Gentles, H. Marshall, and E. B. Hershberg, *J. Am. Chem. Soc.*, **83**, 4073 (1961).

(24) K. Tsuda, E. Ohki, and J. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **7**, 552 (1959).

(25) We are indebted to Dr. Seymour Bernstein for determining and comparing these spectra.

(26) An authentic sample was supplied by Dr. Warren F. McGuckin.

E. 20-Acetoxy-3,11-dioxopregna-4,17(20)-dien-21-al from III.—A solution of 300 mg. of 21,21-dihydroxypregn-4-ene-3,11,-20-trione (hydrate of III) in 1.0 ml. each of pyridine and acetic acid was heated at 60° for 3 hr., cooled, and added to ice–water. The solution was extracted with methylene chloride and the extract was washed with dilute hydrochloric acid, dilute sodium bicarbonate, and water, and then taken to dryness. The residue was chromatographed on eight sheets of paper in formamide–benzene in the manner described previously. The combined enol zones were eluted with 100 ml. of 50% aqueous methanol, and the residue from the methylene chloride extract was treated with 0.3 ml. each of pyridine and acetic anhydride for 1.5 hr. at room temperature. The product was recovered and crystallized from acetone–ether to give 26 mg., m.p. 222.5–223.5° dec. Its melting point was not depressed on admixture with the enol acetate described in the previous paragraph.

F. 20-Hydroxy-3,11-dioxopregna-4,17(20)-dien-21-al (IV).—The experiment in the previous paragraph was repeated through elution of the product from the paper chromatogram. The free enol was crystallized from aqueous methanol to give 64 mg., m.p. 186–188°. It gave a blue-black color with ferric chloride (0.5 mg. of steroid in 5 drops of methanol treated with 1 drop of aqueous 1% ferric chloride), reduced Tollens reagent, and gave an immediate yellow color with the Porter–Silber reagent; it did not react with the Schiff reagent. A hydroxyl band was present in its infrared spectrum. A sample for analysis was recrystallized from methanol to give m.p. 189–191°; $[\alpha]_D$ +196 ± 3° (chloroform); λ_{max}^{MeOH} 238 mμ (ε 15,600), 282 (13,100).

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.65; H, 7.65. Found: C, 73.17; H, 7.88.

G. 21-Methoxypregna-4,16-diene-3,11,20-trione (V).—The material eluted from the preparative chromatograms was treated with charcoal in acetone, and the solvent was evaporated almost to dryness. Addition of 10 ml. of absolute ether gave cubes (277 mg., m.p. 119–121°). The residue from the mother liquor was chromatographed on the same column used for the acetylated enol. Crystallization of the residue from fractions 32–50 gave an additional 147 mg., m.p. 120–125°, of enol ether to bring the yield to 21.4%. It gave negative tests with Tollens and with the Schiff reagent. Ten micrograms spotted on paper gave a light yellow color with 2 *N* aqueous sodium hydroxide. The analytical sample had m.p. 123–126°; $[\alpha]_D$ +224 ± 2°; λ_{max}^{MeOH} 238 mμ (ε 23,400); ν^{CHCl_3} 1593, 1618, 1671, and 1701 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.91; CH_3O, 8.71. Found: C, 74.37; H, 7.87; CH_3O, 9.04.

H. Acetals (VI and VII).—The mixture comprising the least polar fraction from the benzene–formamide paper chromatograms was fractionated on the previously described 1.8 × 48 cm. Celite column in the system cyclohexane–benzene (600:200) saturated with formamide. Numbering of the 6-ml. fractions was begun after 60 ml. of effluent had been discarded.

Crystallization of the residue of fractions 7–9 from ether gave needles of 21,21-dimethoxy-17α-pregn-4-ene-3,11,20-trione (VII, 56 mg., m.p. 151–152°; 14 mg., 149–150°) in 3.2% yield from cortisone. Recrystallization from ether gave the analytical sample with m.p. 152–153°, $[\alpha]_D$ +89 ± 1, λ_{max}^{MeOH} 238 mμ (ε 15,600).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.13; H, 8.30; CH_3O, 15.97. Found: C, 71.01; H, 8.30; CH_3O, 16.60.

Crystallization of residues from fractions 11–18 from acetone–ether and from ether gave the corresponding 17β(21-acetal) (VI, 195 mg., m.p. 161–163°; 53 mg., m.p. 158–162°) in 11.5% yield from cortisone. It did not depress the melting point of 21,21-dimethoxypregn-4-ene-3,11,20-trione from another source[5]; their infrared spectra were identical.

21,21-Dimethoxy-17α-pregn-4-ene-3,11,20-trione (VII) from VI.—To 388 mg. (1 mmole) of 21,21-dimethoxypregn-4-ene-3,-11,20-trione was added 38.8 ml. of 0.4 *N* methanolic potassium hydroxide. A portion of the solution was used to fill a 1-dm. polarimeter tube, and readings of optical rotation were taken at intervals (temperature, 22.5°C). After 3 hr., the readings had decreased and become constant at α_D +1.83°, which indicated the presence of a 2:1 mixture of 17β and 17α epimers. After 4.5 hr., 2 ml. of acetic acid was added to the reaction mixture which then was diluted with 125 ml. of water and extracted with methylene chloride. The extract was washed, dried, and concentrated to dryness. The residue was chromatographed on a 1.8 × 48 cm. Celite column in the system cyclohexane–benzene (300:100) saturated with formamide. Numbering of 5-ml. fractions was begun after 60 ml. of effluent had been discarded.

The residue from fractions 7–10 was crystallized from ether to give needles (93 mg., 24.0%, m.p. 149.5–151°). The melting point on admixture with the product (VII) formed from the incomplete Porter–Silber reagent and cortisone was 150–151.5°.

The residue from fractions 12–17 was crystallized from acetone–ether and ether–petroleum ether (b.p. 50–70°) and gave two crops (217 mg., m.p. 159.5–161°; 12 mg., m.p. 159–161°) of crystals which did not depress the melting point of the 17β(21-acetal) (VI). The yield was 229 mg. (59.0%).

Treatment of 21-Methoxypregna-4,16-diene-3,11,20-trione (V) with Methanolic Hydrogen Chloride.—A solution of V (212 mg.) in 20 ml. of 0.8 *N* hydrogen chloride in dry methanol stood at room temperature for 62 hr. and then was diluted with 75 ml. of water and extracted with three portions of methylene chloride. The extract was washed with water and taken to dryness. Paper chromatography of an aliquot in solvent system 1 (Table II) showed the presence of three compounds with absorption maxima in the ultraviolet (R_f 0.61, 0.73, and 0.83, respectively). The compounds with R_f 0.61 and 0.73 gave a yellow color with alkaline blue tetrazolium; only the compound with R_f 0.83 gave an immediate yellow color with the Porter–Silber reagent. The mixture was fractionated on a 1.8 × 48 cm. column (50 g. of Celite plus 22.5 ml. of formamide) with cyclohexane–benzene (500:200) saturated with formamide. Fractions of 6.5 ml. each were collected after the first 60 ml. of effluent was discarded. After fraction 27 had been collected, 1:1 cyclohexane–benzene saturated with formamide was used as the mobile phase.

A. 21,21-Dimethoxypregn-4-ene-3,11,20-trione (VI).—Fractions 9–13 gave 37 mg. (m.p. 161–162°) of crystals from ether. The infrared spectrum of the product was identical with that of authentic 17β(21-acetal) (VI).

B. 16α,21-Dimethoxypregn-4-ene-3,11,20-trione (VIII).—Fractions 38–43 yielded 76.5 mg. (m.p. 137–139°) of light yellow rosettes. The product gave a yellow spot on paper with 2 *N* aqueous sodium hydroxide. It gave no color with ferric chloride and did not reduce Tollens reagent. The analytical sample had m.p. 141–143°, $[\alpha]_D$ +161° ± 2°, λ_{max}^{MeOH} 238 mμ (ε 16,500).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.13; H, 8.30; CH_3O, 15.97. Found: C, 70.90; H, 7.91; CH_3O, 16.36.

C. 21-Methoxypregna-4,16-dien-3,11,20-trione (V).—Fractions 53–63 gave a product (44 mg., m.p. 123–126°; 9 mg., m.p. 120–122°) on crystallization from ether–petroleum ether which did not depress the melting point of starting material V.

D. 21-Methoxypregna-4,16-diene-3,11,20-trione (V) from VIII.—When 19.9 mg. of 16α,21-dimethoxypregn-4-ene-3,11,20-trione in 1.4 ml. of methanol and 1.35 ml. of water was heated on a steam bath with 0.25 ml. of concentrated hydrochloric acid for 20 hr., a product with the paper chromatographic mobility of the Δ^{16}-ketol ether (V) was obtained. Preparative paper chromatography of the reaction mixture in benzene–formamide permitted isolation of material which had an infrared spectrum identical with that of the Δ^{16}-ketol ether (V).

Treatment of 21-Methoxypregna-4,16-diene-3,11,20-trione (V) with Methanolic Potassium Hydroxide.—A solution of 21-methoxypregna-4,16-diene-3,11,20-trione (250 mg.) in 20 ml. of 0.5 *N* methanolic potassium hydroxide stood at room temperature for 2 hr. The mixture then was diluted with 75 ml. of methylene chloride, washed three times with water, and taken to dryness. Paper chromatography of an aliquot in toluene–isooctane–methanol–water (70:130:160:40) showed the presence of three ultraviolet absorbing spots (R_f 0.42, 0.59, and 0.70, respectively), all of which gave a yellow color with alkaline blue tetrazolium. The slowest component had the same mobility as starting material.

The mixture was fractionated on a 1.8 × 65 cm. column of 60 g. of Celite plus 27 ml. of formamide. The mobile phase was *n*-hexane–benzene (500:400) saturated with formamide, and 7.5-ml. fractions were collected.

A. 16α,21-Dimethoxy-17α-pregn-4-ene-3,11,20-trione (IX).—The crystalline residue from fractions 26–39 weighed 44.9 mg. (18.0%). Recrystallization from acetone–ether gave prismatic needles (26 mg., m.p. 140–141.5°; 5 mg., m.p. 139–141°). A melting point when mixed with compound VIII was 125–140°. There was no hydroxyl band in the infrared spectrum.

The sample for analysis was recrystallized from acetone–ether and had m.p. 141.5–142°, $[\alpha]_D$ +107 ± 2°, λ_{max}^{MeOH} 238 mμ (ε 16,000).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.13; H, 8.30; CH_3O, 15.97. Found[27]: C, 70.94; H, 8.50; CH_3O, 15.76.

B. 16α,21-Dimethoxypregn-4-ene-3,11,20-trione (VIII).—From fractions 41–49 was obtained 125 mg. (50%) of colorless material. Crystallization from ether gave needles (106 mg., m.p. 141.5–143°; 9 mg., m.p. 137–138°). The product did not depress the melting point of VIII obtained from the reaction of V with methanolic hydrogen chloride, and their infrared spectra were identical.

C. 21-Methoxypregna-4,16-diene-3,11,20-trione (V).—Fractions 81–95 afforded 71 mg. (28.2%) of residue. Crystallization from acetone–ether gave 52 mg. (m.p. 121.5–125.5°) of starting material.

Interconvertibility of V, VIII, and IX in Methanolic Potassium Hydroxide.—One-milligram amounts of compounds V, VIII, and IX were treated separately with 0.2 ml. of 0.5 *N* methanolic potassium hydroxide for 2 hr. at room temperature. When 10-μl. aliquots from each reaction mixture were chromatographed on paper in toluene–isooctane–methanol–water (70:130:160:40), three ultraviolet absorbing spots with the same qualitative and quantitative distribution of products described under the preparation of VIII and IX from V were observed.

A solution of 16α,21-dimethoxypregn-4-ene-3,11,20-trione (VIII) (194 mg., 0.5 mmole) in 19.4 ml. of 0.5 *N* methanolic potassium hydroxide stood at room temperature for 2 hr. The product was recovered and chromatographed under the same conditions used in the preparation of VIII and IX from V. The residue from fractions 21–27 gave a product from ether (19 mg., m.p. 140.5–142°; 3 mg., m.p. 139.5–141.5°) identical with IX prepared from V.

Fractions 37–47 gave 87.5 mg. (45.1%) of residue. Crystallization from ether gave 67 mg., m.p. 140.5–142°, and 12 mg., m.p. 138–140°, of starting material VIII.

The residue from fractions 78–94 weighed 53.7 mg. (30.2%). Crystallization from ether gave rosettes (37 mg., m.p. 119–122; 6 mg., m.p. 119–124°) which did not depress the melting point of V obtained from reaction of cortisone with the incomplete Porter–Silber reagent. The infrared spectra of the two compounds were identical.

Direct Synthesis of 21-Methoxy-17-hydroxypregn-4-ene-3,11,-20-trione (XIV) from Cortisone.—To 360 mg. (1 mmole) of cortisone in 200 ml. of methylene chloride containing 1.0 ml. of boron trifluoride etherate–methylene chloride (1:40), equivalent to 0.10 mmole of BF_3, was added slowly 62 mmoles of ethereal diazomethane (100 ml.) prepared from 20 g. of N-methyl-N′-nitro-N-nitrosoguanidine.[28] After 55 mmoles of CH_2N_2 had been added, the yellow color was no longer discharged. The addition required 20 min. After 30 min. more, the solution was washed with 0.1 *N* sodium hydroxide and with water, filtered through sodium sulfate, and concentrated to dryness. The residue was fractionated on a 3.0 × 70 cm. column of 300 g. of activated silica gel. The mobile phase was 2% methanol in methylene chloride, and 6.5-ml. fractions were collected. The residue from fractions 244–310 gave 210 mg. (56.2%) of colorless prisms from methanol, m.p. 234.5–238° dec. The product was homogeneous by paper chromatography in toluene–isooctane–methanol–water (120:80:150:50), with R_f 0.26. It gave a gray-yellow color with alkaline blue tetrazolium. Treatment of 2.4 mg. with 0.1 ml. each of pyridine and acetic anhydride for 20 hr. at room temperature did not change its paper chromatographic mobility.

A sample, recrystallized from methanol, melted at 235–237°, $[\alpha]_D$ +198 ± 2° (*c* 0.402, chloroform), λ_{max}^{MeOH} 238 mμ (ε 16,300); lit.[20] m.p. 250–253°, λ_{max}^{MeOH} 238 mμ ($E_{1\%}^{1cm}$ 400, equivalent to ε 15,000); ν^{Nujol} 3518, 1701, 1653, and 1620 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08; CH_3O, 8.28. Found[27]: C, 70.56; H, 8.23; CH_3O, 8.08.

4-Androstene-3,11,17-trione (XV) from XIV.—To 74.8 mg. (0.2 mmole) of XIV in 9.5 ml. of glacial acetic acid was added 40 mg. (0.4 mmole) of chromic anhydride in 0.5 ml. of water. After 45 hr. at room temperature, paper chromatography in toluene–isooctane–methanol–water (120:80:150:50) showed only a trace of starting material (R_f 0.25). The major less-polar product (R_f 0.41) had the same mobility and color reactions as adrenosterone. The excess chromic anhydride was decomposed with methanol; the reaction mixture was diluted with water and extracted with methylene chloride. The organic phase was washed with 0.1 *N* sodium hydroxide and water, filtered through sodium sulfate, and concentrated to dryness. Repeated crystallizations from acetone and ethanol failed to free the product from starting material. This mixture was adsorbed on a 1.8 × 48 cm. column of 80 g. of activated silica gel and eluted with 2.5% methanol in methylene chloride; fraction volumes were 4.0 ml. An opaque, narrow band with a yellow front emerged sharply at fraction 58. The residue from fractions 58 and 59 afforded plates from ethanol (36 mg., m.p. 221–223°; 4 mg., m.p. 218–222°) in a yield of 67%. A mixture with an authentic sample of 4-androstene-3,-11,17-trione melted at 220–225.5°, and the infrared spectrum was identical with that of 4-androstene-3,11,17-trione.

Cortisone acetate (80.4 mg.) was treated, as described for the preparation of XV from XIV, for 46 hr. at room temperature. The product was recovered and chromatographed on four sheets of Whatman No. 1 paper (19 × 55 cm., previously impregnated with 40% formamide in acetone). The mobile phase was benzene–hexane (2:1) saturated with formamide. The ultraviolet absorbing material at R_f 0.4 was eluted, and transparent plates (13.2 mg., m.p. 220–223°; 3.0 mg., m.p. 218–221°) were obtained in a yield of 27%. There was no depression of the melting point on admixture with authentic adrenosterone.

Indirect Synthesis of XIV from Cortisone. A. 3,20-Bis(ethylenedioxy)-21-methoxy-17-hydroxypregn-5-en-11-one (XII) from XI.—3,20-Bis(ethylenedioxy)-17,21-dihydroxypregn-5-en-11-one (XI) was prepared from cortisone by the method of Bernstein and co-workers[19] in 37% yield, m.p. 237–240°, $[\alpha]^{27}D$ −10.8 ± 2° (*c* 0.458, chloroform); lit.[19] m.p. 234–238.5°, $[\alpha]^{20}D$ −7.5° (*c* 0.805, chloroform).

To 448 mg. (1 mmole) of XI in 200 ml. of methylene chloride containing 0.05 mmole of BF_3 was added excess ethereal diazomethane prepared from 20 g. of N-methyl-N′-nitro-N-nitrosoguanidine.[28] After approximately two-thirds of the total diazomethane had been added, a permanent yellow color resulted; an addition 0.025 mmole of BF_3 was introduced and the remainder of the diazomethane then was added. The product was recovered as in the preparation of IX from cortisone.

The reaction mixture was fractionated on a 1.8 × 65 cm. column of 80 g. of Celite plus 40 ml. of formamide. The mobile phase was *n*-hexane–benzene (1200:300) saturated with formamide, and the fraction volume was 5.3 ml. The residue from fractions 25–45 gave rectangular prisms from methanol (198 mg., m.p. 175–177°; 16 mg., m.p. 173–175°, 46.4% yield). A sample recrystallized from methanol melted at 175–177°; it possessed no intense absorption band in the ultraviolet region, ν^{CHCl_3} 3518 and 1700 cm.$^{-1}$, $[\alpha]^{29}D$ −14.0 ± 2° (*c* 0.430, chloroform).

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28; CH_3O, 6.71. Found[27]: C, 67.62; H, 8.49; CH_3O, 6.48.

Treatment of 1.1 mg. of XII with 0.1 ml. each of pyridine and acetic anhydride for 20 hr. at room temperature did not alter its paper chromatographic mobility (R_f 0.35) in toluene–isooctane–methanol–water (20:180:160:40).

B. 21-Methoxy-17-hydroxypregn-4-ene-3,11,20-trione (XIV) from XII.—A solution of 46.2 mg. (0.1 mmole) of XII in 5 ml. of methanol and 0.5 ml. of 8% sulfuric acid was refluxed for 1 hr., diluted with water, and extracted with methylene chloride. The organic solvent was washed with water and concentrated to dryness. Crystallization from methanol gave 8 mg. of rosettes, m.p. 235–240° dec. The product did not depress the melting point of XIV which was prepared by direct methylation of cortisone. The infrared spectra of the sample of XIV from the two sources were identical.

Paper chromatography in toluene–isooctane–methanol–water (120:80:150:50) of an aliquot of the mother liquor showed only a trace of additional XIV (R_f 0.26). The major component absorbed ultraviolet light and was less polar (R_f 0.72) than XIV. Direct treatment of the paper chromatogram with alkaline blue tetrazolium gave neither a purple nor a yellow spot. After preliminary spraying of a duplicate chromatogram with 0.4 *N* sulfuric acid in 80% ethanol and heating at 50° for 5 min., the less polar component gave a gray-yellow spot with the blue tetrazolium reagent. This presumed 20-monoketal was retreated with methanolic sulfuric acid, as before, for 5 hr., and the reaction mixture stood overnight at room temperature. The product, recovered as before, gave an additional 18 mg. of XIV, m.p. 234–238° dec. The total yield of XIV was 26 mg. (70%).

21-Methoxypregna-4,16-diene-3,11,20-trione (V). A. Direct Synthesis from 21-Hydroxypregna-4,16-diene-3,11,20-trione (II).—A solution of 171 mg. of II (0.5 mmole) in 100 ml. of methylene chloride containing 0.05 mmole of BF_3 was treated with an excess

(27) Analysis by E. Thommen, Basle, Switzerland.

(28) A. F. McKay and G. F. Wright, *J. Am. Chem. Soc.*, **69**, 3028 (1947).

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]

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Received September 5, 1963

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.

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