Reaction of Benzaldehyde with the Aminopyrazoline (VII).—Two grams (0.0048 mole) of (VII) was mixed with 3.1 g. (0.29 mole) of benzaldehyde, 5 ml. of ethyl alcohol and 1 ml. of glacial acetic acid and heated under reflux for eight hours. Cooling the reaction mixture gave a product which, after recrystallization from absolute alcohol and then a benzene–absolute alcohol mixture, was a pale-yellow crystalline material (VIII). Calcd. for $C_{36}H_{32}N_3O$: mol. wt., 523.6. Found: mol. wt., 510. When a sample of (VIII) was heated for five hours with 20% sulfuric acid, pyrazole (III) resulted in a 60% yield.

TABLE II

ABSORPTION SPECTRA OF PHENYLHYDRAZINE DERIVATIVES IN 95% ALCOHOL

Derivative	Fig. no.	Molar concn. × 10^5	Maxima λ(mμ)	Maxima ε×10^{-3}	Minima λ(mμ)	Minima ε×10^{-3}
(V)	1	14.3	360	10.7	335	9.09
			288	15.4	272	13.4
			244	20.3	231	18.2
(VI)	1,2	9.06	365	10.5	359	10.3
			324	12.8	313	12.5
			287	14.8	275	14.3
			245	21.8	230	20.5
(VII)	1,3	4.77	361	22.9	282	4.27
			245	18.9	230	15.1
(IX)	1	4.95	365	22.4	317	5.50
			312	6.28	284	3.87
			245	20.4	228	14.0
(III)	2	6.19	255	31.4	227	16.9
Mixture of [a] (III) + (IX)	2	4.65	365	11.5	320	3.08
			253	28.8	227	17.5
(VIII)	3	5.03	365	20.2	320	5.80
			308	6.32	284	3.50
			245	17.7	235	16.3
1,5-Diphenyl-[b] 3-*p*-tolyl-pyrazoline	3	2.40	355	19.9	278	5.08
			242	17.9	223	12.7
(X)	3	5.93	333	21.9	267	5.32
			240	18.3	223	15.1

[a] A 1.93-mg. sample of (III) and 2.72 mg. of (IX) were dissolved in 200 ml. of 95% alcohol. The molar concn. of this solution was calculated assuming a molecular weight of 501.6 (value for (IX)). [b] Prepared according to the directions of Raiford and Peterson, ref. 9.

Acetylation of Aminopyrazoline (VII).—A 1.0-g. sample of (VII) was refluxed for two hours with 15 ml. of acetic anhydride. Most of the acetic anhydride was distilled under vacuum and the residue treated with sodium bicarbonate solution. The solid residue was recrystallized from benzene and petroleum ether and finally from 90% alcohol. The pale-yellow product was dried under vacuum at 80° for three hours to give (IX).

Benzyl-*p*-methylacetophenone Phenylhydrazone (X).—This phenylhydrazone (X) was prepared from benzyl-*p*-methylacetophenone in the usual way in 90% yields and recrystallized from ethyl alcohol, m.p. 77–79°. This compound decomposed to a tar after exposure to light and air for a few days.

Anal. Calcd. for $C_{22}H_{22}N_2$: N, 8.91. Found: N, 9.10, 8.94.

Absorption Spectra Measurements.—These measurements were made using a Beckman Model DU Photoelectric Quartz Spectrophotometer. At the sensitivity used, the maximum band width was one millimicron, with a rated accuracy of 0.1%. Pure 95% alcohol was used as a solvent medium since most of these compounds were not sufficiently soluble in a non-polar solvent such as heptane (see Figs. 1, 2 and 3, and Table II).

Summary

1. *trans* Ethylene imine ketones react with phenylhydrazine to give the corresponding pyrazoles and N-acetylphenylhydrazones of the ethylene imine ketones.

2. *cis* Ethylene imine ketones give the intermediate 4-aminopyrazolines which are stable in neutral or basic solutions but lose benzylamine to give the pyrazole when a solvolytic proton becomes available in acid solutions.

3. The absorption spectra of these compounds have been compared to aid in the elucidation of their structures. The pyrazolines have similar but not identical spectra with those of related phenylhydrazones.

LINCOLN, NEBRASKA RECEIVED AUGUST 2, 1948

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, SOUTHWESTERN MEDICAL COLLEGE]

16-Substituted Steroids. VI. The Steric Structure of Steroidal 16,17-Ketols and 16,17-Glycols[1]

BY MAX N. HUFFMAN AND MARY HARRIET LOTT

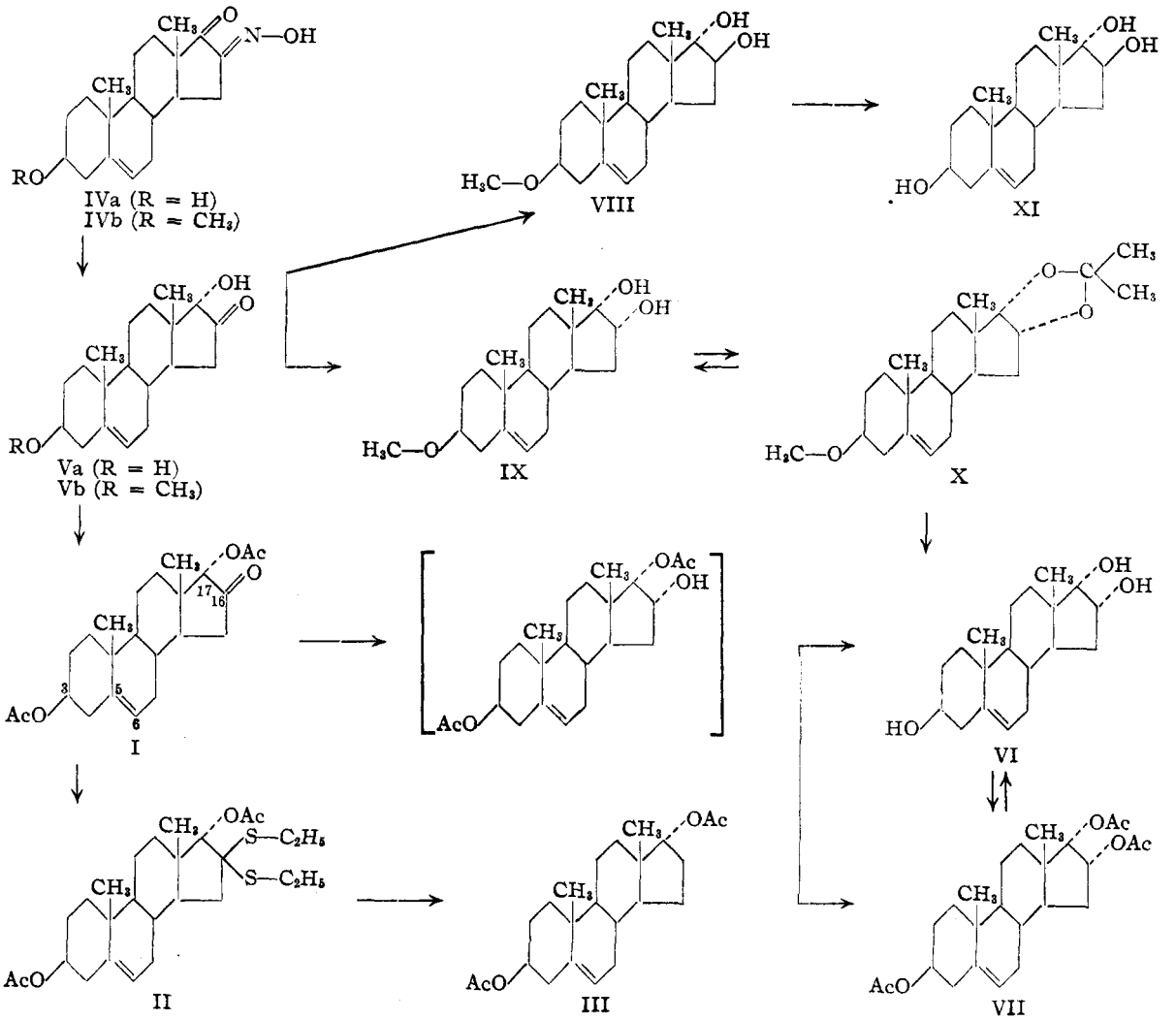
Stodola, Kendall and McKenzie[2,3] have shown that the zinc–acetic acid reduction of 16-oximino-17-ketosteroids followed by acetylation results in the formation of 16-keto-17-acetoxysteroids. The Stodola reduction of 16-oximinodehydroisoandrosterone, for instance, was found to give, after acetylation with acetic anhydride in pyridine, an androstenediolone diacetate in which the ketone group clearly occupied the C_{16} position. We have confirmed this finding of Stodola and co-workers and furthermore shown that the acetoxyl at C_{17} has the α spatial configuration. If the 3(β),17-diacetoxy-16-keto-Δ^5-androstene (I) of Stodola is treated with ethyl mercaptan in the presence of fused zinc chloride and anhydrous sodium sulfate[4] a crystalline diethyl thioacetal (II) is readily obtained in good yield. This thioacetal loses its mercaptyl residues on hydrogenolysis with modified Raney nickel, and there is obtained the known 3(β),17(α)-diacetoxy-Δ^5-androstene (III). Since in these reactions there can be no question of

(1) The announcement of the findings described in this publication was presented as a Communication by Huffman and Lott, THIS JOURNAL, 69, 1835 (1947).

(2) Stodola, Kendall and McKenzie, *J. Org. Chem.*, 6, 841 (1941).

(3) Stodola and Kendall, *ibid.*, 7, 336 (1942).

(4) Bernstein and Dorfman, THIS JOURNAL, 68, 1152 (1946).



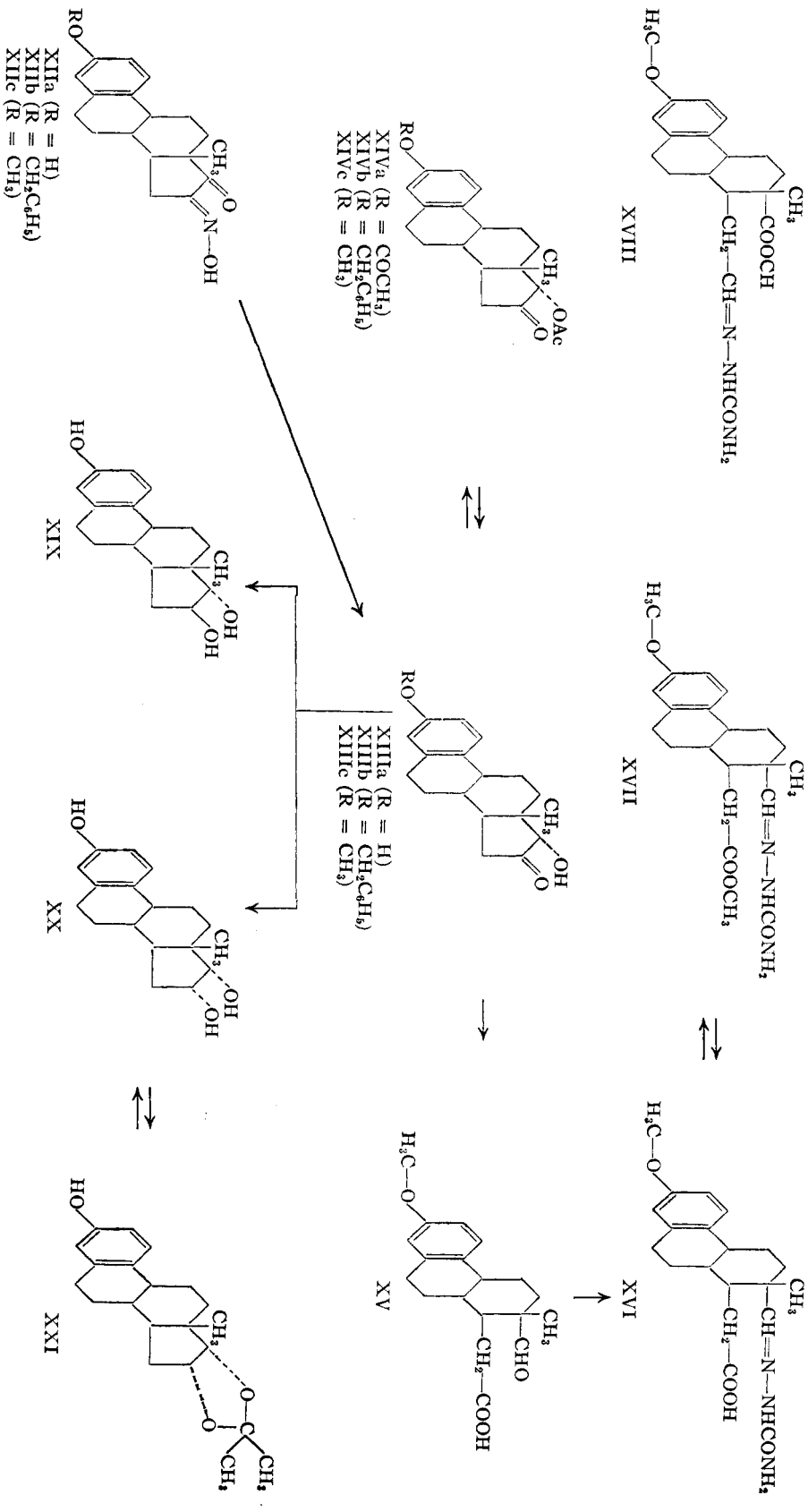
keto–carbinol isomerization, it is certain that the original androstenediolone diacetate had its carbonyl at position 16 and its C_{17} acetoxyl in the α spatial configuration.

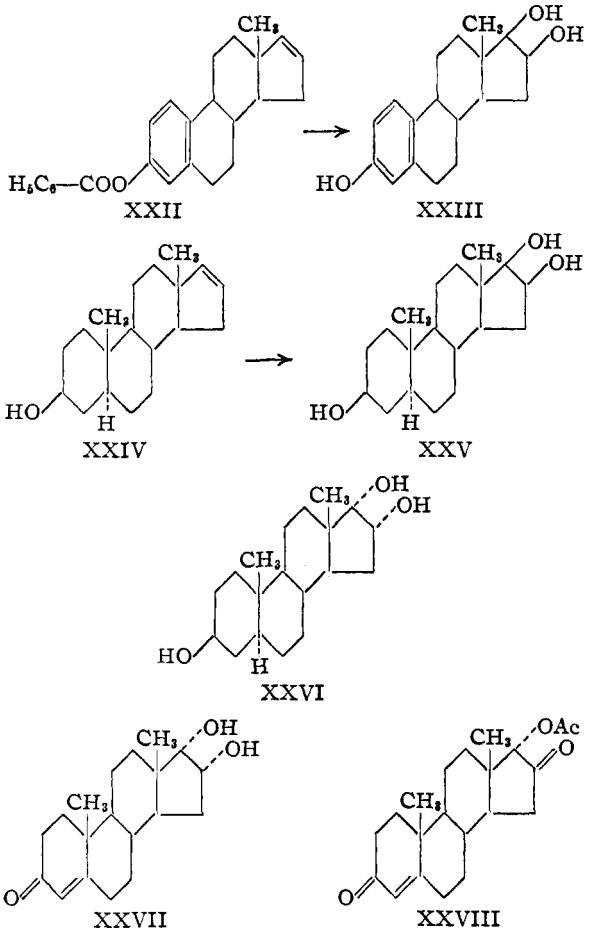
It seems clear that the method of carbonyl reduction by thioacetal hydrogenolysis (discovered by Wolfrom and Karabinos[5]) should find wide application in structural studies in the general group of vicinal ketols where the Clemmensen and Wolff-Kishner methods of reduction are decidedly unsuited.

The Stodola reduction of 16-oximino-17-ketosteroids is peculiar in that there is produced, instead of the expected amine, a 16,17-ketol. We feel certain that such ketols are actually 16-keto-17(α)-hydroxysteroids and that no rearrangement occurs upon acetylation. The zinc–acetic acid reduction of 16-oximinodehydroisoandrosterone (IVa) gives an androstenediolone[6] (Va) which furnishes 3(β),17(α)-diacetoxy-16-keto-Δ^5-androstene (I) either upon long standing with acetic anhydride and pyridine or upon very rapid acetylation with acetic anhydride and phosphoric acid according to Ciusa and Sollazzo.[7] The yields are virtually identical in both methods of esterification. These facts suggest that no rearrangement of the ketol takes place during esterification. Furthermore, ketols prepared by Stodola reduction of 16-oximino-17-ketosteroids may be acetylated and the resulting acetate then saponified to produce the identical ketol which was originally esterified. For instance, 3-benzyloxy-17(α)-acetoxy-16-keto-$\Delta^{1,3,5}$-estratriene (XIVb) may be saponified to produce the same estradiolone-3-benzyl ether (XIIIb) from which the 17-acetoxy derivative was formed.

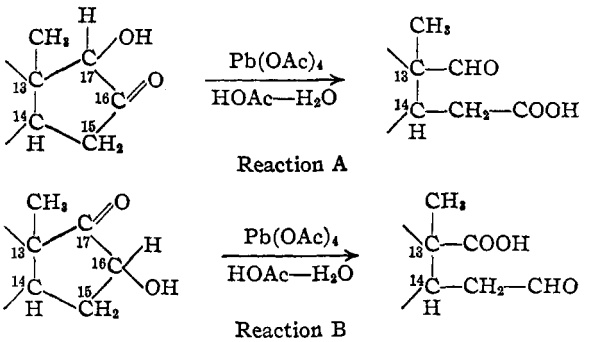
The most decisive evidence in regard to the structure of Stodola-prepared 16,17-ketols comes, in our opinion, from the studies on the oxidation products of these ketols resulting from lead tetraacetate cleavage of steroid Ring D. Baer[8,9] has

(5) Wolfrom and Karabinos, THIS JOURNAL, **66**, 909 (1944).
(6) Butenandt, Schmidt-Thomé and Weise, *Ber.*, **72**, 417 (1939).
(7) Ciusa and Sollazzo, *Ann. chim. applicata*, **33**, 72 (1943).
(8) Baer, THIS JOURNAL, **62**, 1597 (1940).
(9) Baer, *ibid.*, **64**, 1416 (1942).


XIIa (R = H)
XIIb (R = CH2C6H5)
XIIc (R = CH3)
XIVa (R = COCH3)
XIVb (R = CH2C6H5)
XIVc (R = CH3)
XIIIa (R = H)
XIIIb (R = CH2C6H5)
XIIIc (R = CH3)
XVIII
XVII
XIX
XX
XXI
XV
XVI



shown that vicinal ketols (both acyclic and cyclic) can be oxidized with lead tetra-acetate in aqueous acetic acid with rupture of the carbon to carbon bond between the functional groups to produce an aldehyde and a carboxylic acid. The aldehyde arises from the original carbinol carbon and the carboxylic acid from the original carbonyl carbon of the vicinal ketol so treated. After lead tetra-acetate cleavage of Ring D of Stodola-prepared 16,17-ketols it has been found that the carboxylic acid so produced is not the well-known tertiary one attached to steroid position C_{13}. If the 16,17-ketol (XIIIc) prepared by the zinc–acetic acid reduction of 3-methoxy-16-oximino-17-keto-$\Delta^{1,3,5}$-estratriene (XIIc) is treated briefly with lead tetra-acetate in aqueous acetic acid, there is obtained an aldehyde–acid (XV) which furnishes an easily crystallizable semicarbazone-acid (XVI). Treatment of this semicarbazone-acid with diazomethane destroys its acidic properties with formation of the methyl ester (XVII). This semicarbazone-methyl ester is readily saponified to regenerate the original semicarbazone-acid (XVI) under conditions which would not hydrolyze a carbomethoxy group at C_{13}. This finding seems interpretable only upon the basis that the original carbonyl on the intact Ring D occupied position 16, for had this carbonyl been at position 17, the carboxylic acid produced by lead tetra-acetate cleavage would have been attached to tertiary position 13 (Contrast Reaction A and Reaction B).



Furthermore, had the original estradiolone-3-methyl ether been a 16-hydroxy-17-ketosteroid, the aldehyde–acid produced on lead tetra-acetate cleavage (as in Reaction B) would have led to the same semicarbazone-methyl ester (XVIII) as that recently prepared by Miescher[10] and which melts at 212–213° (cor.). Our semicarbazone-methyl ester (XVII) decomposes at 166–167.5° (uncor.) with evolution of gas.

From the foregoing evidence, and from evidence shortly to be presented, we feel justified in stating that Stodola-prepared 16,17-ketols possess the structure of 16-keto-17(α)-hydroxysteroids. It is noteworthy that 16,17-diketosteroids likewise form 16-keto-17(α)-hydroxysteroids upon reduction with zinc or with titanium trichloride.[11,12]

Stodola, Kendall and McKenzie[2] found that catalytic reduction of 3(β),17(α)-diacetoxy-16-keto-Δ^5-androstene (I) in ethanol yielded, after further acetylation, the triacetate (VII) of the androstenetriol originally prepared by Butenandt.[6] We have repeated the experiment of Stodola, *et al.*, except that reduction with hydrogen and Raney nickel was effected in a medium of ethyl acetate–ethanol (2.5:1) in order to obviate any danger of saponification of the diacetate (I), the structure of which is now quite securely known. We likewise found that Butenandt's androstenetriol is formed (reduction of I followed by acetylation to give the triacetate VII). Now Butenandt[6] clearly showed that his androstenetriol (VI) possessed the C_{16}–OH and C_{17}–OH in *cis* relationship from the standpoint of geometric isomerism, and we have confirmed this point as will be seen later; there is, therefore, no doubt that Butenandt's triol is 3(β),16(α),17(α)-trihydroxy-Δ^5-androstene (VI).

If a 16-keto-17(α)-hydroxysteroid be reduced with 2% sodium amalgam in excess dilute ethanolic acetic acid (40°), there is produced a mixture of *cis*-oid and *trans*-oid 16,17-glycols. (At temperatures above 40° there is apparently produced relatively more of the *cis*-oid form.) Thus if 3(β)-

(10) Heer and Mierscher, *Helv. Chim. Acta*, **29**, 1895 (1946).
(11) Huffman, *J. Biol. Chem.*, **167**, 273 (1947).
(12) Huffman and Lott, *ibid.*, **172**, 325 (1948).

methoxy-17(α)-hydroxy-16-keto-Δ^5-androstene (Vb) is reduced with sodium amalgam as described, there is obtained a mixture of geometrically isomeric 16,17-glycols (VIII and IX). In this mixture the *trans*-oid component (VIII) is less soluble and may readily be obtained in pure form by repeated crystallization. The remaining isomer (IX) is obtained by transformation to the cyclic acetonide (X) and by purification in this form. Hydrolysis of this acetonide (X) followed by demethylation furnishes Butenandt's triol[6] (VI) —known with certainty to be 3(β),16(α),17(α)-trihydroxy-Δ^5-androstene. Demethylation of the *trans*-oid 3-ether (VIII) yields Hirschmann's triol[13] (XI) which must from the foregoing evidence be 3(β),16(β),17(α)-trihydroxy-Δ^5-androstene as it is the only *trans*-oid glycol possible from the 16-keto-17(α)-hydroxy configuration. (At this point it should be carefully noted that proof of the α spatial configuration of the carbinol group in unesterified Stodola-prepared 16,17-ketols is now complete, for such a ketol has been shown to give rise to a known 16(α),17(α)-glycol).

Upon sodium amalgam reduction as just described, 3,17(α)-dihydroxy-16-keto-$\Delta^{1,3,5}$-estratriene (XIIIa) yields a mixture of geometrically isomeric 16,17-glycols (XIX and XX). If this mixture be treated briefly with anhydrous hydrogen chloride in absolute acetone, the *cis*-oid isomer (XX) reacts to furnish a cyclic acetal (XXI) whereas the *trans*-oid (XIX) does not. It was found that the acetal could be readily separated from the *trans*-oid glycol by partitioning between chloroform and 0.1 *N* sodium hydroxide, the free glycol passing to the alkaline phase and the acetal remaining in the chloroform. The *trans*-oid isomer (XIX), recovered from the alkali, proved to be the sex hormone estriol (theelol); and the acetal, after hydrolysis, furnished a substance (XX) identical with the estrogenic triol originally named isoestriol-A.[14] Estriol is, therefore, 3,16(β),17(α) trihydroxy-$\Delta^{1,3,5}$-estratriene (XIX), and isoestriol-A is 3,16-(α),17(α)-trihydroxy-$\Delta^{1,3,5}$-estratriene (XX).

It is to be observed that catalytic hydrogenation[6,2,14] of a 16-keto-17(α)-hydroxysteroid has, so far, always given the 16(α),17(α)-glycol, whereas sodium amalgam reduction yields a mixture of 16(β),17(α)- and 16(α),17(α)-glycols.

In 1945 Prelog, Ruzicka and Wieland[15] submitted 3-benzoxy-$\Delta^{1,3,5,16}$-estratetraene (XXII) to reaction with osmium tetroxide and obtained, after saponification, an isomer (XXIII) of estriol to which was assigned the structure 3,16(α),17(α)-trihydroxy-$\Delta^{1,3,5}$-estratriene. It is certain that this estriol possesses its C_{16} and C_{17} hydroxyl groups in *cis* arrangement as the latter groups were introduced by the known osmium tetroxide reaction; however, Prelog, Ruzicka, and Wieland assumed that the C_{16}–C_{17} hydroxyl groups would form on the least sterically hindered side of the steroid skeleton (*i. e.*, opposite to the C_{13}–methyl group). For the same reasons, Ruzicka, Prelog and Wieland[16] assigned the structure 3(β),16(α),-17(α)-trihydroxyandrostane to the triol (XXV) which results from the osmium tetroxide oxidation of 3(β)-hydroxy-Δ^{16}-androstene (XXIV).

Since we have already determined the structure of isoestriol-A as that of 3,16(α),17(α)-trihydroxy-$\Delta^{1,3,5}$-estratriene (XX), it follows that the isomeric estriol of Ruzicka, *et al.*, must be a 3,16(β),-17(β)-trihydroxy-$\Delta^{1,3,5}$-estratriene (XXIII) as it is not identical with isoestriol-A, yet must have the C_{16}–OH and C_{17}–OH in *cis* arrangement. For similar reasons the androstanetriol of Ruzicka, *et al.*,[16] must be 3(β),16(β),17(β)-trihydroxyandrostane (XXV) since this compound is not identical with 3(β),16(α),17(α)-trihydroxyandrostane (XXVI) prepared by hydrogenation of Butenandt's triol.

In Table I is presented a résumé of the steric structures of steroidal 16,17-ketols and 16,17-glycols. Only key compounds in these series are included, functional derivatives in general being omitted. While a certain amount of speculation has heretofore been advanced concerning the stereochemistry of these structures, the authors hope now to have placed this subject within the realm of rigorous organic chemistry.

Experimental[17,18]

PART I. Proof of Structure of 3(β),17(α)-Diacetoxy-16-keto-Δ^5-androstene (I)

Preparation of the Diethyl Thioacetal of 3(β),17(α)-Diacetoxy-16-keto-Δ^5-androstene.—3(β),17(α)-Diacetoxy-16-keto-Δ^5-androstene[2] (I), 0.25 g. (m.p. 124.5-125°), was mixed with 0.50 g. of anhydrous sodium sulfate and 0.25 g. of freshly fused zinc chloride and the whole at once covered with 10 cc. of ethyl mercaptan (Eastman Kodak Co. No. 958). The reaction flask was stoppered, its contents mixed until solution of the diacetate had become complete, and then placed in the ice box for seventy-two hours. The excess mercaptan was removed *in vacuo* at room temperature and the resulting solid residue taken up alternately in 1.0 *N* sodium hydroxide (200 cc.) and ethyl ether (200 cc.) containing a slight amount of pyridine. After the two phases had been shaken together and the alkali separated off, the ether was washed three times with water, dried over sodium sulfate, and evaporated at 50°. The almost colorless oil was crystallized in the ice box from aqueous acetone containing a trace of pyridine. A second crystallization from this medium gave 253 mg. of flakes melting at 136.5–138° (II). An analysis of this material, which contained a touch of yellow color, indicated that it was already substantially pure.

Anal. Calcd. for $C_{27}H_{42}O_4S_2$: S, 12.96. Found: S, 12.74, 12.68.

Hydrogenolysis of 3(β),17(α)-Diacetoxy-16-keto-Δ^5-androstene Diethyl Thioacetal (II).—Approximately 3 g. of modified Raney nickel catalyst in absolute ethanol was added to 0.21 g. of thioacetal (m.p. 136.5–138°), and after the addition of 30 cc. of dioxane, the mixture was heated for seven hours on the steam-bath (air condenser). After having been allowed to stand overnight at room tempera-

(13) Hirschmann, *J. Biol. Chem.*, **150**, 363 (1943).

(14) Huffman and Darby, THIS JOURNAL, **66**, 150 (1944).

(15) Prelog, Ruzicka and Wieland, *Helv. Chim. Acta*, **28**, 250 (1945).

(16) Ruzicka, Prelog and Wieland, *ibid.*, **28**, 1609 (1945).

(17) All melting points are uncorrected unless otherwise specified.

(18) All 16-keto-17-hydroxysteroids were prepared by zinc–acetic reduction of the corresponding 16-oximino-17-ketosteroid according to Stodola, *et al.*[1,2]

TABLE I

Original name	Author	M. p., °C.
	Androgen Series	
Androstenediolone	Butenandt, Schmidt-Thomé and Weiss[a]	197
Androstenediolone diacetate	Butenandt, Schmidt-Thomé and Weiss[a]	123
	Stodola, Kendall and McKenzie[b]	124–125
16-Ketotestosterone acetate	Stodola and Kendall[c]	194–195
Androstenetriol	Butenandt, Schmidt-Thomé and Weiss[a]	273–275
	Stodola, Kendall and McKenzie[b]	
16-Oxytestosterone	Butenandt, Schmidt-Thomé and Weiss[a]	172–173
Urinary androstenetriol	Hirschmann[d] (isolation)	266–270 cor.
	Huffman and Lott[e]	
"Androstan-triol-(3β,16α,17α)"	Ruzicka, Prelog and Wieland[f]	265–266 cor.
Androstane-3(β),16(α),17(α)-triol	Huffman and Lott (this publication)	251–253
	Estrogen Series	
16-Hydroxyestrone	Huffman[g]	234–237
16-Oxyestrone methyl ether	Butenandt and Schäffler[h]	169–169.5 cor. ?
	Huffman[i]	167–168[j]
16-Acetoxyestrone methyl ether	Butenandt and Schäffler[h]	149 cor. ?
	Marrian[k] (isolation)	
Estriol (theelol)	Doisy, Thayer, Levin and Curtis[l] (isolation)	280–282 cor.
	Huffman[i]	
	Butenandt and Schäffler[h]	
		267–269
Isoestriol-A	Huffman and Darby[m]	274–276[n]
"$\Delta^{1,3,5}$-Oestratrien-triol-(3,16α,17α)"	Prelog, Ruzicka and Wieland[o]	236.5–237 cor.

ture, the mixture was filtered free of catalyst and the filtrate distilled *in vacuo* to a volume of 5–10 cc. Addition of several volumes of water precipitated the hydrogenolysis product, which was filtered after several hours at 5°. The well-washed and dried crystals were dissolved in acetone, filtered from a slight amount of insoluble material, and the acetone evaporated to dryness. Solution was next effected in methanol and crystallization allowed to take place at 5°. The sequence of solution in acetone, filtration from insoluble residue, evaporation of filtrate, and recrystallization from methanol was repeated until no more matter insoluble in acetone was observed. Several further recrystallizations from methanol yielded finally 34 mg. of plates (III) melting at 157–158°. A mixed melting point test with authentic 3(β),17(α)-diacetoxy-Δ^5-androstene[19] (m.p. 158–158.5°) supplied by Dr. Hans Hirschmann showed a melting point value of 157.5–158.5°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.92, 73.84; H, 9.11, 9.16.

From the methanolic mother liquors were recovered an additional 30 mg. of diacetate melting at 153–154.5°.

PART II. Proof that Stodola-Prepared 16,17-Ketols Are 16-Keto-17(α)-hydroxysteroids.

3(β),17(α)-Dihydroxy-16-keto-Δ^5-androstene (Va).—This material, prepared according to Stodola, Kendall and McKenzie,[2] was recrystallized once from aqueous acetic acid to give tiny leaves melting at 197–199°. Butenandt and co-workers,[6] who first prepared this androstenediolone (Va) by another method, reported a melting point of 197°.

Acetylation of 3(β),17(α)-Dihydroxy-16-Keto-Δ^5-androstene.—Androstenediolone (400 mg., as above) (Va) was dissolved in 5 cc. of dry pyridine in the cold. Acetic anhydride (3 cc.) was then added, the phases mixed well and left to react for twenty-four hours at room temperature in a stoppered flask. The diacetate was then precipitated by the addition of 200 cc. of ice water, and, after three hours in the ice box, filtered and washed copiously with water. The diacetate, without drying, was dissolved in methanol and recrystallized from boiling aqueous methanol, final crystallization being allowed to take place overnight at 5°. After filtration and drying in the desiccator, the product was treated with charcoal in acetone and the solvent evaporated to leave a colorless oil. This oil was redissolved in 9.0 cc. of 95% ethanol and 3.0 cc. of water. After 24 hours at 5°, 332 mg. of silky needles (I) (desiccator dried) were obtained. These melted at 124.5–125°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.22, 71.28; H, 8.30, 8.37.

Another acetylation of androstenediolone (identical batch as above, m.p. 197–199°) was effected according to the method of Ciusa and Sollazzo.[7] Acetic anhydride (10 cc.) was added to 393 mg. of the ketol, and then 1.0 cc. of 90% phosphoric acid (Mallinckrodt) gradually added with continuous swirling. Solution of the steroid took place at once and much heat was evolved. The reaction flask was allowed to cool for thirty minutes with continuous swirling of its contents. Then 200 cc. of ice water was added and the isolation and purification of the diacetate were carried out exactly as in the case of the acetic anhydride–pyridine acetylation. There were obtained 313 mg. of silky needles (I) which melted at 124.5–125° either by themselves or after admixture with the diacetate obtained in the preceding paragraph. The yields by the pyridine method and the phosphoric acid method were thus 65 and 62%, respectively.

Acetylation of 3-Benzyloxy-17(α)-hydroxy-16-keto-$\Delta^{1,3,5}$-estratriene (XIIIb) and Saponification of the 17-Acetoxy Derivative (XIVb).—3-Benzyloxy-17(α)-hydroxy-16-keto-$\Delta^{1,3,5}$-estratriene (XIIIb) (m.p. 197–200°, with yellow color),[12] prepared as previously described,[12] (0.37 g.) was treated with pyridine (5 cc.) and acetic anhydride (3 cc.) as usual. The acetate, precipitated with ice water, was recrystallized from hot acetone–ethanol–water, final crystal formation being allowed to take place at 5°. The yield of felt-like needles (XIVb) was 0.38 g., melting at 140–141°. Further recrystallization of this acetate did not improve its melting point.

Anal. Calcd. for $C_{27}H_{30}O_4$: C, 77.48; H, 7.23. Found: C, 77.50, 77.57; H, 7.23, 7.16.

3-Benzyloxy-17(α)-acetoxy-16-keto-$\Delta^{1,3,5}$-estratriene (125 mg., m.p. 140–141°) (XIVb) was saponified by re-

(19) Ruzicka and Wettstein, *Helv. Chim. Acta*, **18**, 1264 (1935); Butenandt and Hanisch, *Ber.*, **68**, 1859 (1935).

TABLE I (*Continued*)

Optical rotation	Steric structure	Formula
	3(β),17(α)-Dihydroxy-16-keto-Δ⁵-androstene	Va
	3(β),17(α)-Diacetoxy-16-keto-Δ⁵-androstene	I
$[\alpha]^{25}_{4561}$ −56°	17(α)-Acetoxy-3,16-diketo-Δ⁴-androstene	XXVIII
	3(β),16(α),17(α)-Trihydroxy-Δ⁵-androstene	VI
	16(α),17(α)-Dihydroxy-3-keto-Δ⁴-androstene	XXVII
	3(β),16(β),17(α)-Trihydroxy-Δ⁵-androstene	XI
$[\alpha]^{16}D$ −19 ± 4°	3(β),16(β),17(β)-Trihydroxyandrostane	XXV
$[\alpha]^{26}D$ +18°	3(β),16(α),17(α)-Trihydroxyandrostane	XXVI
$[\alpha]^{29.5}D$ −102°	3,17(α)-Dihydroxy-16-keto-Δ[1,3,5]-estratriene	XIIIa
	3-Methoxy-17(α)-hydroxy-16-keto-Δ[1,3,5]-estratriene	XIIIc
	3-Methoxy-17(α)-acetoxy-16-keto-Δ[1,3,5]-estratriene	XIVc
$[\alpha]^{27}D$ +61°	3,16(β),17(α)-Trihydroxy-Δ[1,3,5]-estratriene	XIX
$[\alpha]^{29.5}D$ +88°	3,16(α),17(α)-Trihydroxy-Δ[1,3,5]-estratriene	XX
$[\alpha]^{22}D$ +58 ± 5.5°	3,16(β),17(β)-Trihydroxy-Δ[1,3,5]-estratriene	XXIII

[a] Butenandt, Schmidt-Thomé and Weiss, *Ber.*, **72**, 417 (1939). [b] Stodola, Kendall and McKenzie, *J. Org. Chem.*, **6**, 841 (1941). [c] Stodola and Kendall, *ibid.*, **6**, 837 (1941). [d] Hirschmann, *J. Biol. Chem.*, **150**, 363 (1943). [e] Huffman and Lott, *ibid.*, **172**, 789 (1948). [f] Ruzicka, Prelog and Wieland, *Helv. Chim. Acta*, **28**, 1609 (1945). [g] Huffman, THIS JOURNAL, **64**, 2235 (1942). [h] Butenandt and Schäffler, *Z. Naturforsch.*, **1**, 82 (1946). [i] Huffman, *J. Biol. Chem.*, **169**, 167 (1947). [j] It is not certain whether there exists a lower-melting crystalline modification (m. p. 162–163°) of this ketol [*cf.* Huffman, *ibid.*, **167**, 273 (1947).] [k] Marrian, *Biochem. J.*, **24**, 435 (1930). [l] Doisy, Thayer, Levin and Curtis, *Proc. Soc. Exp. Biol. Med.*, **28**, 88 (1930); Thayer, Levin and Doisy, *J. Biol. Chem.*, **91**, 655 (1931). [m] Huffman and Darby, THIS JOURNAL, **66**, 150 (1944). [n] Isoestriol-A in the form of tiny, flat needles melting at 274–276° has been obtained; it is possible that these represent a crystalline modification of the microscopic crystals melting at 267–269°. [o] Prelog, Ruzicka and Wieland, *Helv. Chim. Acta*, **28**, 250 (1945).

fluxing for one hour in 0.25*N* aqueous methanol. After the methanol had been largely removed by distillation on the steam-bath, the cooled mixture was taken up in ethyl ether, and the latter extracted well with alkali and then washed with water. Evaporation of the ether yielded a crystalline residue which was crystallized once from aqueous ethanol and once from absolute methanol to give 63 mg. of tiny needles (XIIIb) melting at 198.5–200.5°. This material showed no mixed melting point depression with authentic 3-benzyloxy-17(α)-hydroxy-16-keto-Δ[1,3,5]-estratriene.[12]

Acetylation of 3,17(α)-Dihydroxy-16-keto-Δ[1,3,5]-estratriene (XIIIa) and Saponification of the 3,17-Diacetoxy Derivative (XIVa).—This ketol, 302 mg. (m.p. 233–235°) (XIIIa) prepared from 16-oximinoestrone (XIIa) as previously described,[12] was acetylated with acetic anhydride (3 cc.) in pyridine (3 cc.) during forty-eight hours. The excess anhydride was decomposed with ice water and the diacetate taken up in benzene. The benzene phase was washed with dilute sulfuric acid, with bicarbonate, and washed finally with water. Evaporation of the benzene solution yielded a semicrystalline residue which was saponified without further purification. To effect saponification, the crude diacetate (XIVa) was dissolved in 24 cc. of hot methanol and, after cooling, 15 cc. of 1.5*N* potassium hydroxide added; the phases were mixed well and left in a stoppered flask for twenty-four hours at room temperature. The clear solution was diluted with water, acidified with hydrochloric acid and then extracted with ethyl ether. After having been washed with bicarbonate and water, the ethereal phase was evaporated, and the resulting crystalline residue was leached with cyclohexane, treated with charcoal, recrystallized once from aqueous methanol and finally recrystallized from 50% acetic acid to give 187 mg. of ketol (XIIIa) melting at 232–235°. A mixed melting point test performed with this material and authentic 3,17(α)-dihydroxy-16-keto-Δ[1,3,5]-estratriene gave a value of 234–236°.

A 76-mg. portion of this ketol (187 mg. batch, m.p. 232–235°) gave upon oximation (120 mg. of hydroxylamine hydrochloride, 240 mg. of crystalline sodium acetate, 10 cc. of aqueous ethanol, two hour reflux) 70 mg. of oxime decomposing at 224–225°.[12]

Lead Tetraacetate Cleavage of 3-Methoxy-17(α)-hydroxy-16-keto-Δ[1,3,5]-estratriene (XIIIc).—To 352 mg. of 3-methoxy-17(α)-hydroxy-16-keto-Δ[1,3,5]-estratriene[19a] (m.p. 164–166°) (XIIIc) was added 18 cc. of 0.0705 molar lead tetraacetate in acetic acid. The steroid soon dissolved at room temperature, whereupon 4 cc. of 50% acetic acid was added. After the solution had been swirled continuously for thirty minutes, 200 cc. of 1% ethylene glycol in water was added. The cleaved steroid (XV) was allowed to crystallize at 5° for a day and then filtered, washed well with water, and dried *in vacuo* over sulfuric acid. Semicarbazone formation was effected without further purification.

7-Methylmarrianolic Acid Hemialdehyde Semicarbazone (XVI).—The crude aldehyde-acid (of the preceding paragraph) (XV) was dissolved in 40 cc. of methanol and to this solution were added 0.40 g. of semicarbazide hydrochloride and 0.56 g. of crystalline sodium acetate dissolved in 5 cc. of water. This mixture was warmed to dissolve all components and then distilled on the steam-bath during

(19a) Huffman, *J. Biol. Chem.*, **169**, 167 (1947).

thirty minutes until the volume had been reduced to 10 cc. Ice water (50 cc.) was added in one portion to insure precipitation of the semicarbazone-acid in a finely divided state. The mixture of crude semicarbazone-acid was transferred to a separatory funnel using 100 cc. of ethyl acetate, and after the addition of 40 cc. of 0.21*N* hydrochloric acid, partitioned well. After removal of the aqueous phase, the ethyl acetate phase was extracted briefly with 100 cc. of ice-cold 1.0*N* potassium hydroxide. Acidification (ice-bath) of the potassium hydroxide with 85% phosphoric acid (acid to congo red) re-precipitated the semicarbazone-acid which, after a day in the ice box, was filtered and washed copiously with water. The yield of nicely crystalline air-dried 7-methylmarrianolic acid hemialdehyde semicarbazone (XVI) was 316 mg. decomposing at 185–186° with evolution of gas. Recrystallization of a 50-mg. portion of this semicarbazone-acid from aqueous isopropyl alcohol gave 46 mg. decomposing at 188–189°. A second recrystallization from the same medium yielded 41 mg. decomposing at 192–193°; but, oddly, a third recrystallization gave 35 mg. decomposing at the original value of 185–186°. Analysis was performed upon the original 316-mg. batch (XVI).

Anal. Calcd. for $C_{20}H_{27}O_4N_3$: C, 64.32; H, 7.29; N, 11.25. Found: C, 64.17, 64.06; H, 7.33, 7.30; N, 11.10, 11.16.

Esterification of 7-Methylmarrianolic Acid Hemialdehyde Semicarbazone (XVI).—To a solution of 199 mg. of semicarbazone-acid (dec. 185–186°) (XVI) in 5 cc. absolute ethanol plus 5 cc. of ethyl acetate was added an excess of a concentrated ethereal solution of freshly prepared diazomethane. The yellow solution was then diluted with 100 cc. of ethyl ether and shaken well with 100 cc. of 1.0*N* hydrochloric acid. The ethereal phase was extracted twice with 100-cc. portions of 1.0 *N* potassium hydroxide and washed twice with 50-cc. portions of water. After the addition to the ether of 5 cc. of ethyl acetate and 5 cc. of absolute ethanol, the solution was distilled *in vacuo* at 50°. The nicely crystalline residue of methyl ester (dried *in vacuo* over sulfuric acid and potassium hydroxide) weighed 194 mg. It was recrystallized once from aqueous methanol (containing 1 drop of acetic acid) to give 184 mg. of material decomposing at 166.5–167.5° with evolution of gas. A second crystallization from absolute methanol gave 128 mg. of tiny needles (XVII) in rosettes which decomposed at 166–167.5° with evolution of gas.

Anal. Calcd. for $C_{21}H_{29}O_4N_3$: C, 65.09, H, 7.54; N, 10.85. Found: C, 64.99 64.91; H, 7.58, 7.53; N, 10.86, 10.95.

7-Methylmarrianolic acid hemialdehyde (*primary*) semicarbazone methyl ester (XVIII), prepared by Heer and Miescher,[10] melts at 212–213° (cor.), apparently with no decomposition.

Saponification of 7-Methylmarrianolic Acid Hemialdehyde (tertiary) Semicarbazone Methyl Ester (XVII).—To a solution of 90 mg. of semicarbazone-methyl ester (dec. 166–167.5°) in 6.0 cc. of methanol was added 2.0 cc. of aqueous potassium hydroxide (from a solution of 4.0 g. of potassium hydroxide in 20 cc. of water) and the mixture swirled continuously for thirty minutes at room temperature. The mixture was then heated for a few seconds on the steam-bath to redissolve a portion of ester which had crystallized from the medium, and the mixture then swirled at room temperature for another thirty minute interval. The mixture was again heated for a few seconds and swirled at room temperature during a third thirty minute interval, after which it was transferred to a separatory funnel using 50 cc. of 1.0 *N* potassium hydroxide, freed of a slight turbidity with 50 cc. of ethyl ether, and the alkaline layer carefully acidified (ice-bath) with 2.6 cc. of 85% phosphoric acid. The semicarbazone soon crystallized; and after a night in the ice box, it was filtered and washed well with water. The yield of white, granular crystals (XVI) was 59 mg. decomposing at 186–187° with evolution of gas. A mixed melting point, performed with this product and the original semicarbazone (dec. 185–186°), showed no depression.

According to Heer and Miescher,[10] the carbomethoxy group at C_{13} is not saponified by refluxing for 2.5 hours in a solution of 4 g. of potassium hydroxide in 60 cc. of methanol and 20 cc. of water. Our semicarbazone-methyl ester actually may be saponified by refluxing in 0.4 molar potassium carbonate in aqueous methanol, although the heat promotes some destruction of the sensitive semicarbazone.

PART III. Proof of Steric Structure of Steroidal 16,17-Glycols.

Reduction of 3(β),17(α)-Diacetoxy-16-keto-Δ⁵-androstene (I) to the Glycol Stage.—To a solution of 147 mg. of 3(β),17(α)-diacetoxy-16-keto-Δ⁵-androstene (m.p. 123–124°) (I) in 25 cc. of ethyl acetate contained in the Cheronis[20] semimicro hydrogenation apparatus was added 1 cc. of Raney nickel catalyst (completely free of alkali) and the latter rinsed down with 10 cc. of absolute ethanol. A stream of hydrogen was blown through the solution for eight hours at a bath temperature of 60–65°. The solution, freed of catalyst by filtration, was evaporated on the steam-bath, and the cooled semi-crystalline residue was acetylated with acetic anhydride (2 cc.) in dry pyridine (3 cc.) at room temperature for two days. The triacetate, obtained in the usual manner, was recrystallized once from aqueous methanol and four times from ethanol to give 30 mg. of plates (VII) melting sharply at 208–209°[21]; these showed no mixed melting point depression with a sample of androstenetriol triacetate (m.p. 206.5–208.5°) furnished by Dr. Stodola. The X-ray patterns of the Stodola triacetate and our triacetate are identical.[22]

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.27, 69.27; H, 8.40, 8.31.

An additional 23 mg. of crude triacetate was obtained from the mother liquors.

Stodola, Kendall, and McKenzie obtained their triacetate by acetylation of the product resulting from the four-hour hydrogenation of 3(β),17-(α)-diacetoxy-16-keto-Δ⁵-androstene (I) in alcohol using Raney nickel catalyst. A consideration of the parallel findings of Stodola, *et al.*,[2] and Butenandt, *et al.*,[6] in this series leaves no doubt that both groups were dealing with the same androstenetriol.

3(β)-Methoxy-17(α)-hydroxy-16-keto-Δ⁵-androstene (Vb).—Pure 3(β)-methoxy-16-oximino-17-keto-Δ⁵-androstene[23] (0.99 g.) (IVb) was reduced in the usual way with zinc in aqueous acetic acid (3.0 g. of zinc dust, 75 cc. of 50% acetic acid, one hour reflux) and the 16,17-ketol precipitated by dilution with water. Recrystallization from aqueous ethanol, aqueous methanol, and from aqueous acetone yielded 0.58 g. of ketol melting at 180–183° (with yellow turning).[12] Of this product 50 mg. was treated with charcoal and recrystallized from aqueous ethanol to give 43 mg. melting at 188–191° (Vb).

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.45; H, 9.44.

Sodium Amalgam Reduction of 3(β)-Methoxy-17(α)-hydroxy-16-keto-Δ⁵-androstene (Vb).—3(β)-Methoxy-17(α)-hydroxy-16-keto-Δ⁵-androstene (1.22 g., m.p. 180–183°) (Vb) was dissolved in 150 cc. of 95% ethanol plus 15 cc. of acetic acid and reduced with 300 g. of 2% sodium amalgam, as previously described,[23] the temperature being carefully maintained at 40°, and 10 cc. of 50% acetic acid being added as soon as sodium acetate commenced to precipitate. The reaction mixture was distributed between ether (750 cc.) and water (600 cc.) and the ether further washed, once with 1.0 *N* sodium hydroxide and twice with water. Evaporation of the ether yielded a crystalline residue which was recrystallized twice from acetone–cyclohexane–Skellysolve B and once from aqueous ethanol to give 574 mg. of Hirschmann's triol methyl ether[23] (m.p. 195–196°) (VIII).

(20) Cheronis and Koeck, *J. Chem. Ed.*, **20**, 488 (1943).

(21) With Dr. Stodola's melting point apparatus these crystals melted at 213–214.5° (personal communication, Dr. Stodola).

(22) Personal communication, Dr. Stodola.

(23) Huffman and Lott, *J. Biol. Chem.*, **172**, 789 (1948).

The combined mother liquors were taken up in ether and the solution washed three times with water. The crystalline residue resulting from the evaporation of the ethereal phase was dissolved in 40 cc. of 75% propanol containing 0.40 g. of carboxymethoxylamine hemihydrochloride and 0.60 g. of potassium acetate. This mixture was refluxed for one hour and the ketonic fraction removed from the non-ketonic material in the usual way. There resulted 0.46 g. of crystalline non-ketones. The non-ketonic fraction was dissolved in 42 cc. of absolute acetone at room temperature and 6 cc. of acetone saturated with anhydrous hydrogen chloride added. The acetalizing medium was swirled continuously during fifteen minutes and then added in one portion to 300 cc. of ice-cold 3% potassium carbonate, and thorough mixing effected at once. After a day in the ice box, the precipitated acetonide was filtered, washed with water, and dried *in vacuo* to give 0.42 g. of material. This crude acetonide was recrystallized twice from aqueous methanol containing a trace of pyridine (treatment with charcoal). The yield of glistening plates was 265 mg. melting at 144–145.5°. Of this material 101 mg. was recrystallized from absolute methanol (trace of pyridine) to give 55 mg. of long, flat colorless needles (X) melting at 146.5–148°.

Anal., Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.47, 76.56; H, 10.01 10.05.

The Transformation of 3(β)-Methoxy-16(α),17(α)-dihydroxy-Δ⁵-androstene Acetonide (X) to 3(β),16(α),17(α)-Triacetoxy-Δ⁵-androstene (VII).—To a solution of 0.34 g. of acetonide (m.p. 144–145.5°), prepared as above, in 50 cc. of ethanol was added 10 cc. of 1.2 molar phosphoric acid and the solution refluxed for thirty minutes and then left overnight at room temperature. The water-clear solution was distributed between ethyl ether and water, and the organic phase then washed with dilute sodium bicarbonate and with water. Evaporation of the ether gave a crystalline residue which was acetylated in pyridine (5 cc.) with acetic anhydride (3 cc.) in the usual manner. The diacetate, procured by precipitation with ice water, was dried thoroughly and then treated with 0.21 g. of *p*-toluenesulfonic acid monohydrate in 21 cc. of acetic anhydride for thirty minutes on the steam-bath by the method previously described.[18] The reaction mixture, after having been cooled in the ice-bath, was diluted with 100 cc. of ice water and then left for three days at 5°. The precipitated triacetate was recrystallized from ethanol to give 199 mg. of waxy plates melting at 204–206°. Another crystallization from 5 cc. of ethanol (ice box) gave 152 mg. melting at 207–208.5°. A third crystallization from 5 cc. of ethanol (ice box) yielded 119 mg. of plates (VII) melting at 208–209.5°. A mixed melting point test performed with androstenetriol triacetate furnished by Dr. Stodola gave no depression.

The Sodium Amalgam Reduction of 3,17(α)-Dihydroxy-16-keto-Δ¹,³,⁵-estratriene (XIIIa).—This 16,17-ketol (m.p. 232.5–234°) (XIIIa) was reduced with sodium amalgam in the manner previously described (0.44 g. of ketol in 50 cc. of ethanol plus 5 cc. of acetic acid, 100 g. of 2% sodium amalgam, finally 5 cc. of 50% acetic acid, temperature 40°). The supernatant fluid was decanted from the mercury and the latter rinsed twice with ethanol. To the combined alcoholic solutions was added 50 cc. of water and the diluted liquid distilled (steam-bath) until ebullition had nearly ceased. More water (50 cc.) was then added to the mixture, and after a day in the ice box, the glycols were filtered and dried at 90°. The yield of crystalline material was 0.36 g. Any trace of ketonic material was separated from this residue with carboxymethoxylamine in the fashion previously described (0.25 g. of carboxymethoxylamine hemihydrochloride, 0.50 g. of potassium acetate, 40 cc. of 75% propanol, 1.5 hours reflux).

Separation of *cis*-oid and *trans*-oid 16,17-Glycols Formed by Sodium Amalgam Reduction of 3,17(α)-Dihydroxy-16-keto-Δ¹,³,⁵-estratriene (XIIIa).—The crystalline non-ketonic fraction of the preceding section was dissolved in 35 cc. of absolute acetone and 5 cc. of acetone saturated with anhydrous hydrogen chloride added. After the acetalizing medium had been swirled continuously for eighteen minutes, 50 cc. of aqueous potassium carbonate (≈ 1.50 g. of potassium carbonate) was added in one portion, mixed at once, and then diluted to a volume of 400 cc. with water. After a day in the ice box the precipitated material was filtered, washed with water containing 0.5% pyridine, and then dried in the vacuum desiccator. The resulting 0.29 g. of dried material was dissolved in 300 cc. of chloroform and partitioned with 300 cc. of 0.1 *N* sodium hydroxide. The alkaline phase was separated and again washed with 300 cc. of chloroform. The sodium hydroxide fraction was then acidified with 3 cc. of concentrated hydrochloric acid and extracted with 400 cc. of ethyl ether. The ether was washed once with 200 cc. of water, and after the addition of a few drops of pyridine, evaporated to a white crystalline residue. This residue was recrystallized twice from acetone–cyclohexane–Skellysolve B, treated with charcoal, and then finally recrystallized from aqueous acetone. White microscopic crystals of estriol (theelol) (XIX) were obtained (85 mg.). These melted at 268–269.5° and showed no depression of melting point after admixture with authentic theelol supplied by Dr. D. W. MacCorquodale of The Abbott Laboratories. A mixed melting point test performed with authentic isoestriol-A gave a value of 251.5–256°.

Methylation of a portion of this 85-mg. batch of estriol gave **estriol methyl ether** (34 mg. of triol in 25 cc. of 0.5 *N* sodium hydroxide shaken one hour with 0.6 cc. of methyl sulfate). This derivative (13 mg.) melted at 158–161° and showed no mixed melting point depression with authentic theelol methyl ether.

Acetylation of another portion of this estriol (32 mg. in 1 cc. of pyridine and 1 cc. of acetic anhydride) gave a product (42 mg.) melting at 124.5–126°. **Estriol triacetate** melts at 126°.[24]

The 600 cc. of solution comprising the combined chloroform phases in the above separation was washed with water (350 cc.) and evaporated to dryness. There resulted an oily residue which was taken up in 50 cc. of ethanol, and after the addition of 10 cc. of 1.2 molar phosphoric acid, refluxed on the steam-bath for thirty minutes. To the hydrolysis medium was then added 25 cc. of water and boiling allowed to continue without the condenser until most of the ethanol had been removed. The mixture was then left overnight in the ice box and the precipitated material filtered, washed well with water, and dried in the oven (100°); yield, 0.12 g. of white crystals. These were treated with charcoal and recrystallized from aqueous methanol to give 94 mg. of small flat needles (XX) melting at 272–275°. A mixed melting point test performed with known isoestriol-A (m.p. 274–276°)[25] showed no depression.

Isoestriol-A Acetonide (XXI).—Isoestriol-A (91 mg., from the preceding experiment) (XX) was covered with 10 cc. of absolute acetone and then 1.5 cc. of acetone saturated with anhydrous hydrogen chloride added. The mixture was swirled for eight minutes, although solution was effected within one minute time. Then, in one portion, 10 cc. of aqueous potassium carbonate (≈ 0.45 g. of potassium carbonate) was added, mixed in at once, and then diluted to a volume of 50 cc. with water. After a day in the ice box, the precipitated acetonide was filtered and washed well with water containing a trace of pyridine. The air-dried product was recrystallized once from water–acetone–pyridine and once from water–methanol–pyridine to give 91 mg. of flakes which nearly melted at 98° but formed a liquid melt only at 182–184°. After several recrystallizations from water–methanol–pyridine it was finally learned that this compound actually possesses a double melting point. The final product (28 mg.) cleared almost completely at 103.5–104.5°, then solidified, and again melted sharply at 183.5–184.5° (XXI). Analysis indicates that it is hydrated with one molecule of water.

(24) Thayer, Levin and Doisy, *J. Biol. Chem.*, **91**, 655 (1931).

(25) Isoestriol-A in the form of tiny, flat needles melting at 274–276° has been obtained; it is possible that these represent a crystalline modification of the microscopic crystals melting at 267–269°.

Anal., Calcd. for $C_{21}H_{28}O_3 \cdot H_2O$: C, 72.80; H, 8.73. Found: C, 72.77, 72.63; H, 8.69, 8.61.

Attempted Acetonide Formation with Estriol-3-methyl Ether.—Estriol-3-methyl ether (350 mg., m.p. 157–158°) was covered with 35 cc. of anhydrous acetone and treated with 5 cc. of 8% hydrogen chloride in absolute acetone as usual. After thirty minutes swirling, 50 cc. of aqueous potassium carbonate (≈ 1.5 g. of potassium carbonate) was added in one portion, mixed at once, and placed at 5°. After a day at 5°, the precipitated white plates were filtered, washed well with water (0.5% pyridine), and then dried in the oven. The recovery of estriol-3-methyl ether melting at 158–159.5° was 334 mg.

3(β),16(α),17(α)-Trihydroxyandrostane (XXVI).—Adams catalyst (0.10 g.) was suspended in 10 cc. of acetic acid and reduced with hydrogen; then 155 mg. of pure 3(β),16(α),17(α)-triacetoxy-Δ⁵-androstene (VII) in 25 cc. of absolute ethanol was added. Hydrogenation was carried out for thirty minutes at atmospheric pressure, the catalyst removed by filtration, and the solution of hydrogenated steroid evaporated *in vacuo*. Saponification of the crystalline residue was accomplished by refluxing for one hour in a solution of 20 cc. of 2 *N* sodium hydroxide and 60 cc. of ethanol. The neutral residue was recrystallized consecutively from aqueous ethanol, aqueous methanol, absolute acetone, aqueous methanol, absolute acetone, and from aqueous ethanol to give 64 mg. of triol melting at 251–253° (XXVI).

Anal. Calcd. for $C_{19}H_{32}O_3$: C, 73.98; H, 10.46. Found: C, 73.97, 73.87; H, 10.49, 10.41; $[\alpha]^{26}D$ + 18° (c = 0.53 in 95% ethanol)[26].

Acknowledgment.—The authors wish to thank Dr. Frank Stodola for his aid in determining the identity of his and their samples of androstanetriol triacetate. They wish also to extend their appreciation to Dr. Hans Hirschmann for his sample of androstenediol diacetate and for determining the optical rotation of their androstane-3(β),16(α),17(α)-triol. To the United States Standard Products Company, who gave generous financial support to this project, the authors are greatly indebted. Micro-analyses were performed by Dr. E. W. D. Huffman, Denver.

Summary

The 16,17-ketols resulting from the zinc–acetic acid reduction of 16-oximino-17-ketosteroids and 16,17-diketosteroids have been found to be 16-keto-17(α)-hydroxysteroids.

The steric structures of steroidal 16,17-glycols have been determined.

(26) This optical rotation was determined by Dr. Hans Hirschmann.

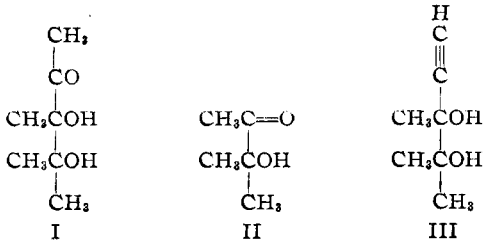
DALLAS, TEXAS RECEIVED JUNE 30, 1948

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

2,3-Dimethyl-4-pentyne-2,3-diol as a Source of C-Methyl Sugars

BY CHARLES D. HURD AND JAMES MOFFAT

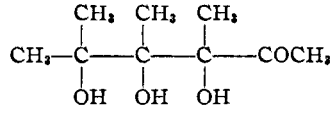
At the outset of this work it was planned to synthesize a monosaccharide whose alcohol groups were tertiary. 3,4-Dimethyl-3,4-dihydroxy-2-pentanone (I) was selected. Such a structure obviously may be regarded as 1,2,3,3-C-tetramethylglycerose. These steps were visualized in the synthesis: acetone to dimethylethynylcarbinol to 3-methyl-3-hydroxy-2-butanone (II) following known procedures, then to 2,3-dimethyl-4-pentyne-2,3-diol (III) by condensation with acetylene, and finally to I by hydration of III.



Steps through the synthesis of III were completed when the work was interrupted by war. Shortly thereafter, Favorskii and Onishchenko[1] announced their synthesis of both I and III, preparing III by pyrolysis of 2,3,6,7-tetramethyl-4-octyne-2,3,6,7-tetraol (IV), $CH_3(CMeOH)_2C{\equiv}C(CMeOH)_2CH_3$. IV was prepared by gradual addition of II into a mixture of potassium hydroxide and dry ether previously saturated with acetylene. They produced I by hydrating the triple bond of III with mercuric sulfate and dilute sulfuric acid.

We prepared III in about 90% yields directly from II by use of sodium acetylide with liquid ammonia as solvent. The acetic ester of III was prepared readily with ketene as the acetylating agent, whereas it could not be prepared with acetic anhydride and pyridine.

The present synthesis seems general. In other words, just as I is obtainable from II, a similar sequence of steps from I should give rise to a C-pentamethyltetrose:



Experimental

Dimethylethynylcarbinol and 3-methyl-3-hydroxy-2-butanone (II) were made in accordance with previously established directions.[2] 2,3-Dimethyl-4-pentyne-2,3-diol (III) was synthesized from the latter as follows.

To 300 ml. of dry liquid ammonia at −78° in a protected 3-necked flask was added 10.3 g. of sodium. Dry

(1) A. E. Favorskii and A. S. Onishchenko, *J. Gen. Chem. (U. S. S. R.)*, **11**, 1111 (1941); *C. A.*, **37**, 3735 (1943).

(2) Hurd and McPhee, THIS JOURNAL, **69**, 239 (1947); **71**, 398 (1949).