that monomer can replace MB as a hydrogen acceptor from the system XO + HCHO. This conforms with the known fact that XO has no pronounced specificity so far as hydrogen acceptors are concerned.

The mechanism for the initiation of addition polymerization reactions by the system XO + HCHO + MMA can therefore be explained following the accepted scheme for the interaction of the system XO + HCHO + MB. The reaction occurring in the system XO + HCHO + MMA can be represented by the generalized equation

$$H_2C(OH)_2 + 2m \underset{+2H}{\overset{-2H}{\rightleftharpoons}} XO \quad 2Hm^* + HCOOH \quad (1)$$

where m is a monomer molecular and Hm* the monomer free radical formed. Reaction (1) can be visualized as occurring through the possible steps

$$+ H_2C(OH)_2 \rightleftharpoons XO - H + H_2C(OH)O\cdot \quad (2)$$

$$XO - H + m \longrightarrow Hm^* + XO \quad (3)$$

$$H_2C(OH)O\cdot + m \longrightarrow Hm^* + HCOOH \quad (4)$$

Reactions (3) and (4) can initiate polymerization chains. The occurrence of reaction (3) depends mostly on the thermodynamic oxidation–reduction potential of the system XO–H + m. The mechanism represented by equation (1) is in accord with the Haber and Willstätter[3] theory for chain processes in enzymic systems. The outstanding features of this theory are: (1) hydrogen atom transfer should occur in single steps; (2) reaction is propagated by radicals produced by monovalent dehydrogenation. Both requirements are strikingly demonstrated by the present experiments. It should be added that it has already been shown by Michaelis, *et al.*,[4] that during the two stages of reduction of riboflavine to leucoriboflavine free radicals of the semiquinone type are involved. From the results obtained in the presence of MB it can be concluded that it is a faster or more specific hydrogen acceptor from XO than MMA.

As can be seen from Table II no polymer can be obtained in the presence of oxygen. This can be accounted for on the assumption that the action of XO is blocked by hydrogen peroxide formed by reduction of molecular oxygen, unless catalase is present, as is the case for living systems.

(3) Haber and Willstätter, *Ber.*, **64**, 2844 (1931).

Summary

1. Enzymic systems such as *B. coli* in formic acid and xanthine oxidase in formaldehyde can initiate polymerization of methyl methacrylate present in aqueous solutions freed from oxygen.

2. Methylene blue is a better acceptor than methyl methacrylate in xanthine oxidase–formaldehyde solutions.

3. Oxygen inhibits the polymerization in presence of xanthine oxidase.

4. The mechanism proposed for the polymerization process is in accord with the Haber–Willstätter theory of chain processes in enzymic systems.

(4) Michaelis, *J. Biol. Chem.*, **116**, 587 (1936); *Chem. Revs.*, **16**, 243 (1935); **22**, 437 (1938); THIS JOURNAL, **60**, 1678 (1938); Kuhn and Ströbele, *Ber.*, **70**, 753 (1937).

PRINCETON, NEW JERSEY RECEIVED JULY 5, 1950

[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Partial Synthesis of Compounds Related to Adrenal Cortical Hormones. XIV. Preparation of the Dihydroxyacetone Side Chain; 17α-Hydroxyprogesterone and "Substances L and P"[1]

BY THEODORE H. KRITCHEVSKY AND T. F. GALLAGHER

Current investigations have focused attention on the profound influence of the structure of the side chain on the biological activity of the adrenocortical hormones. The dihydroxyacetone structure present in cortisone and related hormones has from a biochemical and medical standpoint the greatest interest because these compounds exhibit the most striking chemotherapeutic action on the metabolic processes altered by disease. The primary problem in the synthesis of the side chain of these compounds is the formation of a tertiary alcohol in the proper orientation at C-17 with the retention of a ketone function at C-20. A corollary problem, then, is the introduction of a hydroxyl group at C-21. We have been able to accomplish both of these objectives smoothly and in high yield from readily available materials. We have exemplified these procedures by the partial synthesis of the representative adrenal hormones, 17α-hydroxyprogesterone and Reichstein's "Substances L and P."[2] The reactions involved are generally applicable and permit the preparation of a series of cortical hormones with or without oxygen at C-11.[3] We have utilized them for the elaboration of isotopically labeled compounds for future biochemical investigation. At the same time, however, they afford a convenient means for the technical preparation of biologically important hormones and structurally related compounds of immediate interest in medical research.

We found[4] that when an enol ester of a 20-keto-

(1) This investigation was supported by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, the Lillia Babbit Hyde Foundation and the National Cancer Institute, United States Public Health Service.

(2) The isolation and chemical identification of these hormones have been reviewed by Reichstein and Shoppee in Harris and Thimann, "Vitamins and Hormones," Vol. 1, p. 359, New York, N. Y., 1943. The "recorded constants" in the experimental section have been taken from this article.

(3) Koechlin, Garmaise, Kritchevsky and Gallagher, THIS JOURNAL, **71**, 3262 (1949).

(4) Kritchevsky and Gallagher, *J. Biol. Chem.*, **179**, 507 (1949).

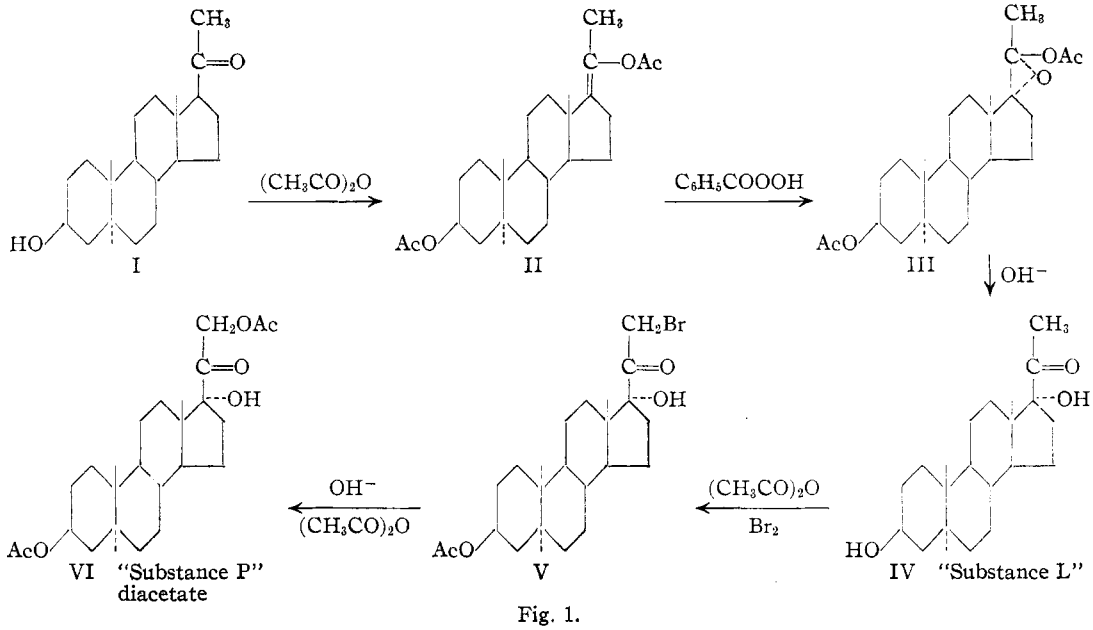

Fig. 1.

steroid was treated with a concentrated solution of perbenzoic acid, a vigorous exothermic reaction occurred, and the product after mild alkaline hydrolysis was a 17α-hydroxy-20-ketone. 3β-Hydroxyallopregnan-20-one (I) was converted in 86% yield to the adrenal steroid "Substance L" (IV) by these reactions. Perbenzoic acid oxidation of the enol diacetate of 3α-hydroxypregnan-20-one and hydrolysis yielded 3α,17α-dihydroxypregnan-20-one (VII), a steroid metabolite found in human urine, isolated and identified for the first time by Lieberman and Dobriner.[5] 3β,17α-Dihydroxypregnan-20-one (VIII), hitherto undescribed, was prepared by similar means. Oxidation of a 20-ketosteroid enol ester by means of chromium trioxide likewise led to the formation of a 17α-hydroxy-20-ketone, but in view of the excellent results with the peracid, this approach was not exhaustively explored. The facile preparation of the side chain ketol structure by these simple procedures established the essential features of the reaction which in turn permitted a more detailed study of the mechanism and opened the way to the synthesis of more complicated natural products.

The preparation of 17α-hydroxyprogesterone (X) from pregnanolone is illustrative of the application of these investigations to adrenal steroids with an α,β-unsaturated ketone system in ring A. The compound was originally isolated from adrenal glands by Pfiffner and North[6] and was prepared by partial synthesis from dehydroisoandrosterone by Hegner and Reichstein and Prins and Reichstein.[7] In our process 3α, or β,17α-dihydroxypregnan-20-one (VII, VIII) was oxidized with N-bromoacetamide to 17α-hydroxypregnane-3,20-dione (IX). This product was in turn selectively brominated at C-4 without difficulty, and the elements of hydrogen bromide were readily eliminated by a modification[8] of the excellent method of Mattox and Kendall.[9] These reactions were accomplished smoothly and in an over-all yield of 41% from pregnanolone without taking recoverable product into account.

The completion of the dihydroxyacetone side chain was accomplished by an interesting reaction with this relatively sensitive structure; the general reactions can be well illustrated by the partial synthesis of Reichstein's "Substance P" (VI). 3β-Acetoxy-17α-hydroxyallopregnan-20-one was brominated at C-21 in either chloroform or acetic acid solution. Trial investigations proved that replacement of the halogen by hydroxyl could be achieved with alkali in dilute aqueous ethanol if the proper precautions were employed. The most important variables were time and the concentration of base. It was found that with 0.05 *N* NaOH all of the halogen was present as bromide ion after ten minutes and a negligible fraction of the steroid was converted to acidic products during this time. We considered that the exclusion of oxygen and the highest concentration of water compatible with the solubility of the compound would minimize other undesirable side reactions. Under these conditions the bromoketone (V) was very satisfactorily converted to "Substance P" (VI), isolated and characterized as the diacetate. It was in all respects identical with the natural product. When 3β-acetoxy-17α-hydroxyallopregnan-20-one was brominated, hydrolyzed and acetylated to "Substance P" acetate without purification or isolation of intermediates, the yield was 64% of the theoretical; that is, the over-all yield in the side chain synthesis of "Substance P" was 55%.

It is noteworthy that alkaline hydrolysis at two stages in the side-chain synthesis did not result in

(5) Lieberman and Dobriner, *J. Biol. Chem.*, **161**, 269 (1945). The melting point was given as 219–219.5°, but the specific rotation was incorrectly recorded in this reference. It was, however, corrected to +64.5° (ethanol) in *J. Biol. Chem.*, **172**, 263 (1948).

(6) Pfiffner and North, *ibid.*, **139**, 855 (1941).

(7) Hegner and Reichstein, *Helv. Chim. Acta*, **24**, 824 (1941); Prins and Reichstein, *ibid.*, **24**, 945 (1941).

(8) Koechlin, Kritchevsky and Gallagher, *J. Biol. Chem.*, **148**, 393 (1950).

(9) Mattox and Kendall, THIS JOURNAL, **70**, 882 (1948).

the production of any considerable amount of D-homo steroid. This is doubtless a result of the relatively short time of exposure of the compounds to an alkaline medium, since more prolonged treatment or higher temperatures is known to effect this transformation. Prolonged heating in the dry state, as in the determination of a melting point, can, however, transform a very appreciable fraction to a D-homo steroid. This accounts, in part, for the wide deviations reported in the literature for the melting point of these 17-hydroxy compounds.

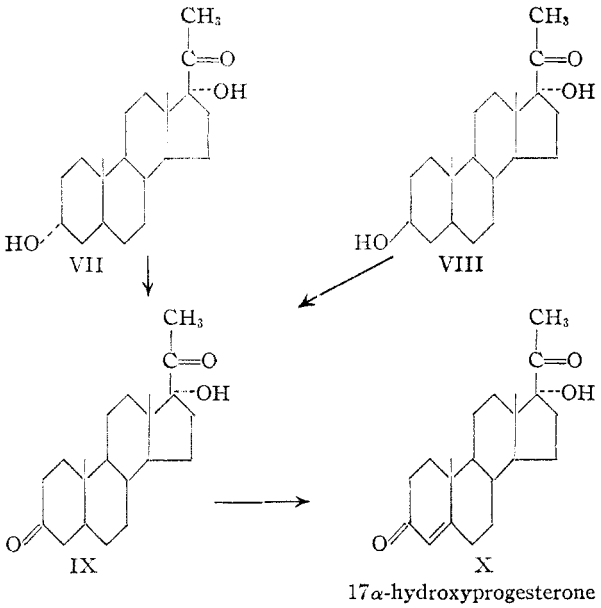

Fig. 2.

The advantages of the procedure described are to be found in the accessibility of the starting material, the relatively few steps in the reaction sequence, and the simplicity and availability of the reagents. Only recently has the preparation of a 17α-hydroxyl group been possible by means other than osmium tetroxide reaction with an ethylene. This reaction, introduced by Criegee,[10] has been widely used[11] and in one of its most efficient applications was an essential step in Sarett's preparation of cortisone.[12] In general, however, it is a reaction with many disadvantages, not the least of which are the extreme toxicity of the reagent and the circuitous routes often necessary to reach a suitably unsaturated compound. An essentially new and different procedure for the preparation of 17α-hydroxy C_{21} steroids was described by Plattner, Heuser and Feurer,[13] who found that reduction of a 16,17-epoxy-20-ketosteroid by lithium aluminum hydride resulted in two glycols (Reichstein's Substances "J" and "O"), each with a 17α-hydroxyl. Julian, Meyer and Ryden[14] independently employed a similar reduction of the epoxy steroids and in addition described the protection of the 20-ketone by formation of a cyclic ketal, so that the very useful ketol structure was preserved. Moreover, a second novel procedure for the preparation of 17α-hydroxy C_{21} steroids was described by Julian, Meyer, Karpel and Ryden[15] who found that the 16,17-epoxy-20-ketosteroids were converted into 17α-hydroxy-20-ketosteroids by treatment with hydrogen bromide, followed by reductive dehalogenation with Raney nickel. These investigators were able to effect a new partial synthesis for 17α-hydroxyprogesterone from Δ^5-pregnenolone as a result of these studies and have applied similar reactions to the preparation of other adrenocortical steroids.

Some features of the reaction of organic peracids with enol esters should be noted. The direct product of reaction is an epoxide with an intact acyloxy group. This was demonstrated by isolation of this intermediate (III) in both normal and allo series. The structure of these compounds was proved by elementary analysis, the saponification equivalent and by the absence of a hydroxyl band in the infrared spectrum. The configuration of the epoxide was predictable from the "rule of the rear"[16] and was experimentally verified by the orientation of the tertiary alcohol formed on hydrolysis, since only the configuration at C-17 has significance. One of the referees of this manuscript drew our attention to the significant fact that the enol acetate of 3β-hydroxyallopregnan-20-one, which has been shown to be a mixture of two geometrical isomerides, affords the 17α-hydroxy-20-keto steroid in an 86% yield. This is clear evidence that both forms react to yield the same product and is doubtless true of the other examples studied. Since 20-ketosteroids without other bulky substituents in the vicinity are unquestionably mixtures of both geometrical isomers and as a consequence difficult to obtain in crystalline form, it is apparent that the orientation of the substituents in the side chain has relatively little influence on the attack of C-17. We wish to express our appreciation for this critical comment. This reaction with perbenzoic acid is general for unhindered enol derivatives and has been used by Mousseron, Winternitz and Jacquier[17] to characterize the direction of enolization in methylcyclohexanone. These authors also obtained a ketol after hydrolysis. The isolation of the epoxide intermediate is unnecessary for preparative purposes; its properties, however, substantiate the conclusion of Turner[18] that this structure is not intermediate in the oxidation of ketones to esters by means of peracids.

A few experimental details deserve brief comment. In the initial experiments[19] the enol acetates were chromatographed on a short alumina column in order to remove the dark brown material formed from acetic anhydride in the presence of acid. Some hydrolysis of the enol ester un-

(10) Criegee, *Ann.*, **522**, 75 (1936).

(11) Summarized in Fieser and Fieser, "Natural Products Related to Phenanthrene," Chapter V, third edition, Reinhold Publishing Corp., New York, N. Y., 1949.

(12) Sarett, THIS JOURNAL, **71**, 2443 (1949).

(13) Plattner, Heuser and Feurer, *Helv. Chim. Acta*, **31**, 2210 (1948).

(14) Julian, Meyer and Ryden, THIS JOURNAL, **71**, 736 (1949); **72**, 367 (1950).

(15) Julian, Meyer, Karpel and Ryden, *ibid.*, **71**, 3574 (1949).

(16) Gallagher and Kritchevsky, *ibid.*, **72**, 882 (1950).

(17) Mousseron, Winternitz and Jacquier, *Bull. soc. chim., France*, 83 (1947).

(18) Turner, THIS JOURNAL, **72**, 878 (1950).

(19) Marshall, Kritchevsky, Lieberman and Gallagher, *ibid.*, **70**, 1837 (1948).

doubtedly occurred on alumina; later experience proved that chromatography was unnecessary, and somewhat better yields of a white product were obtained when the brown amorphous enol esters reacted directly with the peracid. The concentration of the peracid appears to be important since more dilute solutions gave smaller yields of product. The preparation of the peracid in benzene as recommended by Kolthoff[20] is superior to that in chloroform. When larger quantities of enol ester are used, it is advisable to provide cooling, since the reaction is rapid and vigorous.

The hormones described in this report were also prepared with deuterium in a chemically stable position in the molecule. 3β-Acetoxy-d_2-5,6-allopregnan-20-one was obtained by complete reduction of Δ^5-pregnenolone acetate with 99% deuterium gas in acetic acid-*d* and reoxidation with chromium trioxide to the saturated ketone. 3α-Hydroxy-d_2-11,12-pregnan-20-one was prepared by the procedure described earlier from this Laboratory.[5] These were converted to the adrenal steroids by the same methods described for the usual materials, but since the products differed in no essential details from the normal compounds, they have not been included in the experimental section. These substances will, however, serve for a portion of our investigations on steroid metabolism.

Acknowledgments.—We wish to express our appreciation to Dr. K. Dobriner and Mrs. Phyllis Humphries of this Institute, who determined and interpreted the infrared spectra for us. We gratefully acknowledge a generous gift of pregnenenolone from Dr. C. R. Scholz, Ciba Pharmaceutical Company, Summit, New Jersey, as well as the help of Dr. David K. Fukushima of this Institute, who prepared the 3β-acetoxy-d_2-5,6-allopregnan-20-one.

Experimental

17α,20-Epoxypregnane-3α,20-diol Diacetate.—Five and three-tenths grams of 3α-hydroxypregnan-20-one was converted to its enol acetate by the procedure used by Marshall, *et al.*,[19] and after chromatography the colorless sirupy product in benzene solution was treated with 50 cc. of 3.5 *M* perbenzoic acid in benzene. The temperature increased spontaneously and cooling was necessary. After 1 hour ethyl acetate was added and the solution was extracted with 5% sodium hydroxide and with water in the usual procedure. A small portion of the residue was removed for characterization. This was crystallized from petroleum ether and recrystallized from acetone–petroleum ether as irregular prisms, m.p. 167–168°, $[\alpha]^{25}D$ −6.3° (chloroform). The product did not exhibit a hydroxyl band in the infrared spectrum. Calcd.: neut. equiv., 209. Found: neut. equiv., 213.

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.73; H, 9.15. Found: C, 71.82; H, 9.41.

3α,17α-Dihydroxypregnan-20-one (VII).—The principal portion of the reaction product from the preceding preparation was saponified with 500 cc. of 0.3 *N* NaOH in 50% ethanol at room temperature for 1 hour. The reaction product was isolated by the usual procedure and 5.03 g. was obtained which after crystallization from acetone yielded 4.05 g., m.p. 208–210°. The analytically pure sample melted 213–214°, $[\alpha]^{25}D$ +63° (ethanol), and the infrared spectrum was identical with an authentic sample obtained by Lieberman and Dobriner[4] from human urine. The total yield, including the product isolated as the acetate, was 79%.

3α-Acetoxy-17α-hydroxypregnan-20-one.—The residues from the crystalline product described above (0.99 g.) after acetylation and chromatography yielded an additional 360 mg. of 3α-acetoxy-17α-hydroxypregnan-20-one, m.p. 198–199.5°.

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.63; H, 9.69.

3α,17α-Dihydroxypregnan-20-one by Chromium Trioxide Oxidation.—A cold solution of 1.148 g. of chromium trioxide in 25 cc. of 90% acetic acid was added dropwise with stirring to a solution of 1.146 g. (2.85 m.e.) of sirupy $\Delta^{17,20}$-pregnene-3α,20-diol diacetate in 25 cc. of chloroform and 25 cc. of 90% acetic acid at ice-bath temperature. After stirring for 5.5 hours, the unreacted chromium trioxide was reduced with sodium bisulfite, the reaction product was extracted with ether and isolated in the usual manner; 1.145 g. was obtained and was saponified in 200 ml. of 0.25 *N* sodium hydroxide solution in 70% ethanol at room temperature for 30 minutes. The oily saponification product (1.09 g.) was dissolved in benzene–petroleum ether and three crops of crystalline material, m.p. 195–210°, $[\alpha]^{25}D$ +66° (ethanol), were obtained. The infrared spectrum was for all practical purposes identical with that of 3α,17α-dihydroxypregnan-20-one. The crystalline material was acetylated with acetic anhydride and pyridine at room temperature, and after chromatography over 15.2 g. of aluminum oxide, 81 mg., m.p. 181–185°, was obtained which was shown by infrared analysis to be identical with 3α-acetoxy-17α-hydroxypregnan-20-one. From the non-crystalline mother liquors of the original reaction product 65 mg. of 3α-acetoxy-17α-hydroxypregnan-20-one was obtained after similar treatment. Recrystallization from acetone gave thin prisms, m.p. 198.5–199.5°. The yield of 3α-acetoxy-17α-hydroxypregnan-20-one from the enol acetate was 13.6%.

3α-Acetoxyetiocholan-17-one.—One hundred and twenty-six mg. of 3α-acetoxy-17α-hydroxypregnan-20-one was dissolved in 1.8 cc. of glacial acetic acid, and 2.4 cc. of a 2% solution of chromium oxide in 90% acetic acid was added. After 5 hours at room temperature the neutral oxidation product was isolated by the usual procedure and chromatographed on alumina. Thirty-two mg. of pure 3α-acetoxyetiocholan-17-one, m.p. 94–95°,[19] was obtained which upon admixture with an authentic sample showed no depression of melting point; infrared analysis confirmed the identity of the product.

3β,17α-Dihydroxypregnan-20-one (VIII).—Eight and three-hundredths grams of 3β-acetoxypregnan-20-one, m. p. 115–116°, was converted to the enol acetate which, after chromatography, was treated with 25 ml. of a 1.75 *M* solution of perbenzoic acid in benzene. The solution became hot, and cooling was necessary. The reaction product was diluted with ether and isolated by the usual procedure. The oily product was dissolved in 600 ml. of 95% ethanol, and 450 ml. of 0.5 *N* NaOH was added. After 1 hour at room temperature the product was diluted with ether and isolated in the usual manner. After recrystallizations from ethyl acetate, 5.031 g. (62%) of prisms was obtained which melted at 204–207°. The melting point of the analytical sample was 209–210.5°; $[\alpha]D$ +35° (ethanol).

Anal. Calcd. for $C_{21}H_{34}O_3$: C, 75.40; H, 10.25. Found: C, 75.33; H, 10.27.

3β-Acetoxy-17α-hydroxypregnan-20-one.—This compound was prepared from the hydroxy ketone with acetic anhydride and pyridine at room temperature. The acetate crystallized as needles and melted 171–173°; $[\alpha]^{25}D$ +32° (ethanol).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.37; H, 9.64. Found: C, 73.31; H, 9.09.

17α,20-Epoxyallopregnane-3β,20-diol Diacetate (III).—Five hundred mg. of 3β-acetoxyallopregnan-20-one was converted to the enol acetate, and the brown amorphous product, without chromatography, was dissolved in 20 cc. of a 3 *M* solution of perbenzoic acid in benzene. After 1 hour at room temperature, the solution was diluted with ether, and the product was isolated by the usual procedure. The product crystallized from petroleum ether and melted 173–176°. One hundred mg. was removed and recrystallized from acetone–petroleum ether as plates, m.p. 190–193°; $[\alpha]^{28}D$ −37° (chloroform); calcd.: neut. equiv., 209. Found: neut. equiv., 207.

(20) Kolthoff, Lee and Mairs, *J. Polymer Sci.*, **2**, 199 (1947).

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.73; H, 9.15. Found: C, 71.81; H, 9.34.

3β,17α-Dihydroxyallopregnan-20-one (Reichstein's "Substance L"[21] (IV).—The remainder of the material from the preceding preparation was saponified with 200 ml. of 0.5 *N* NaOH in 50% alcohol at room temperature for one hour. The saponification product was a white crystalline solid, and on recrystallization from methanol-acetone, 318 mg. (86%, taking into account the 100 mg. removed for the preceding experiment) of Reichstein's "Substance L" was obtained, m.p. 255–259°. Recrystallization yielded hexagonal plates, m.p. 257–259°; $[\alpha]^{25}D$ +32° (ethanol). Upon admixture with an authentic sample of Reichstein's "Substance L" obtained from Professor Reichstein, there was no depression of melting point. The acetate, prepared by room temperature acetylation with acetic anhydride and pyridine, melted at 188–190°; $[\alpha]^{25}D$ +16° (acetone).

3,20-Diketopregnan-17α-ol (IX).—To a solution of 100 mg. (0.299 mM.) of 3α,17α-dihydroxypregnan-20-one in 2 cc. of *t*-butyl alcohol was added 82.5 mg. of N-bromoacetamide (0.598 mM.), 0.1 cc. of water and 0.15 cc. of redistilled pyridine. After 18 hours at room temperature the reaction mixture turned from light yellow to orange; 117% of the stoichiometric amount of Br^+ was consumed in the reaction as evidenced by iodimetric titration. The reaction product was extracted with acid, base and sodium chloride in the usual procedure. The product weighed 96 mg., m.p. 203–207°. After recrystallization from acetone, irregular prisms were obtained, m.p. 215–217°; $[\alpha]^{25}D$ +53.9° (ethanol).

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.05; H, 9.60.

3,20-Diketo-4-bromopregnan-17α-ol.—Three hundred seventy and six-tenths mg. of 3,20-diketopregnan-17α-ol (1.108 mM.) was dissolved in 12 ml. of redistilled acetic acid, and 0.5 ml. of a 0.2457 *M* solution of bromine in acetic acid was added dropwise to the solution with shaking and cooling. When the solution was decolorized, dropwise addition was continued with 4.12 ml. of a 0.2526 *M* bromine solution in acetic acid, also 0.25 *M* with respect to sodium acetate. The reaction product was diluted with ethyl acetate and isolated by the usual procedure. A white crystalline compound was obtained, and after two recrystallizations from chloroform–ether, 328 mg. of rhombic prisms was isolated; $[\alpha]^{25.5}D$ +34.0° (chloroform), m.p. 186–187° (dec.).

Anal. Calcd. for $C_{21}H_{31}O_3Br$: C, 61.16; H, 7.82; Br, 19.38. Found: C, 60.92; H, 8.02; Br, 19.74.

The crystalline residues were more levorotatory and were reduced with zinc in acetic acid at room temperature. The yield of pure 4-bromo derivative was 72% without taking into account recovered starting material.

17α-Hydroxyprogesterone (X). (a) Pyridine Dehydrobromination.—Ninety-one mg. of 3,20-diketo-4-bromopregnan-17α-ol was dissolved in 5 ml. of redistilled pyridine. The reaction mixture was sealed in a glass tube in nitrogen atmosphere and was heated at 110–130° for 12 hours. The reaction product was diluted with ether and isolated by the usual procedure; 50 mg. of a white crystalline product was obtained which melted 205–207°; ϵ_{2420} = 14,100 (ethanol). After two recrystallizations from acetone, platelets were obtained, m.p. 218–220°; ϵ_{2420} = 16,500 (ethanol), which on admixture with an authentic sample obtained from adrenal glands showed no depression of melting point.

(b) With Semicarbazide.—One hundred and thirty-three mg. of 3,20-diketo-4-bromopregnan-17α-ol in 27.5 cc. of acetic acid was heated at 67° with 110 mg. of sodium acetate, 108 mg. of semicarbazide hydrochloride and a few drops of water for two hours in a nitrogen atmosphere. Then 2 cc. of water, 1 cc. of redistilled pyruvic acid and 300 mg. of sodium acetate were added, and heating was continued for two hours. After cooling, 200 cc. of water was added, and a precipitate of fine needles formed. These were filtered and 49 mg. was obtained which, after one recrystallization from acetone, yielded 22 mg. of platelets, m.p. 209–212°; ϵ_{2420} = 16,400 (ethanol). The mother liquors were combined with the aqueous filtrate which was extracted with ethyl acetate, washed with dilute sodium hydroxide and with water. Removal of the solvent yielded 90 mg. of product which after recrystallization from acetone yielded 63 mg. of platelets, m.p. 207–210°. The combined crystalline crops were recrystallized from acetone as plates, m.p. 218–219°; ϵ_{2420} = 16,500 (ethanol).[22] The yield was 80%.

3β-Acetoxy-17α-hydroxy-21-bromoallopregnan-20-one (V).—Three hundred and eighteen-thousandths mM. of bromine in 1.27 ml. of acetic acid was added to 100 mg. (0.266 mM.) of 3β-acetoxy-17α-hydroxyallopregnan-20-one in 4 cc. of acetic acid after the addition of a small amount of dry hydrogen bromide. The solution was yellow after 10 minutes and was completely decolorized after fifteen minutes. The acetic acid was removed *in vacuo* on the steam-bath. The crystalline residue weighed 122.2 mg. After recrystallization from ether and ethyl acetate, needles were obtained, m.p. 194–195°; $[\alpha]^{27}D$ +34.5° (chloroform). Upon admixture with the starting material, the melting point was depressed.

Anal. Calcd. for $C_{23}H_{35}O_4Br$: C, 60.65; H, 7.75; Br, 17.55. Found: C, 60.27; H, 7.60; Br, 17.84.

Twenty mg. of the 21-bromo derivative was dissolved in 2.5 cc. of glacial acetic acid and heated at 100° with 60 mg. of zinc dust for one hour. The crystalline reaction product after recrystallization from acetone melted at 186–187.5° and did not depress the melting point of an authentic sample of 3β-acetoxy-17α-hydroxyallopregnan-20-one upon admixture.

3β,21-Diacetoxy-17α-hydroxyallopregnan-20-one (Reichstein's "Substance P" Diacetate) (VI).—Two hundred one and five-tenths mg. of 3β-acetoxy-17α-hydroxy-21-bromoallopregnan-20-one was dissolved in 100 ml. of 95% ethanol, and the solution was flushed with a stream of nitrogen; 100 cc. of 0.1 *N* sodium hydroxide solution was added at room temperature, and after ten minutes the solution was acidified with dilute nitric acid, diluted with ether and the ether washed with small amounts of 5% sodium hydroxide and 5% sodium chloride solution. After removal of the solvent, 157 mg. of white crystalline product was obtained. This was acetylated with acetic anhydride and pyridine overnight. The diacetate after recrystallization from methanol melted 206–207°; $[\alpha]^{25}D$ +47.6° (chloroform).[23]

3β,21-Diacetoxy-17α-hydroxyallopregnan-20-one was also prepared from 3β-acetoxy-17α-hydroxyallopregnan-20-one by bromination in chloroform solution followed by alkaline hydrolysis and acetylation without isolation of intermediates; 4.45 cc. of 0.266 *M* bromine solution was added to 426 mg. of 3β-acetoxy-17α-hydroxyallopregnan-20-one in 5 cc. of reagent chloroform. The solution was diluted with ethyl acetate, washed in the usual procedure and the solvent removed. The crystalline solid was hydrolyzed with base as in the preceding experiment and the reaction product isolated and acetylated as before. Crystallization from methanol yielded 252 mg., m.p. 202–203°. The mother liquors after chromatography yielded an additional 62 mg., m.p. 196–199°. The combined products were recrystallized once from methanol as needles and melted 206–207°; $[\alpha]^{25}D$ +48° (chloroform). The yield was 64% in this experiment.

Summary

A new procedure has been described for the introduction of a tertiary alpha hydroxyl group at C-17 into 20-ketosteroids. Treatment of the enol esters of 20-ketosteroids with perbenzoic acid results in the formation of epoxy derivatives, which upon alkaline hydrolysis are converted into 17α-hydroxy-20-ketopregnanes.

The complete dihydroxyacetone side chain, characteristic of the most active adrenal hormones, can be elaborated from the 17α-hydroxy-20-ketopregnanes by bromination at C-21 and subsequent replacement of the bromo function with hydroxyl by mild alkaline hydrolysis.

These procedures have been exemplified by the partial synthesis of 17α-hydroxyprogesterone and

(21) Recorded constants: m. p. 264; $[\alpha]D$ +38° (ethanol).

(22) Recorded constants: m. p. 222–223°; $[\alpha]D$ +105.6° (chloroform).

(23) Recorded constants: m. p. 208–209°; $[\alpha]D$ +38.4°, +46.1° (chloroform).

Reichstein's "Substances L and P." In addition, these compounds have been prepared with deuterium in chemically stable positions.

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Partial Synthesis of Compounds Related To Adrenal Cortical Hormones. XV. 17α,21-Dihydroxy-Δ⁴-pregnene-3,20-dione (Reichstein's "Substance S")[1]

BY BERNARD A. KOECHLIN, THEODORE H. KRITCHEVSKY AND T. F. GALLAGHER

As a consequence of the striking effectiveness of cortisone (17α,21-dihydroxy-Δ⁴-pregnene-3,11,20-trione) in rheumatoid arthritis, leukemia and other disorders, considerable interest has been drawn to closely related adrenal hormones, notably 17α,21-dihydroxy-Δ⁴-pregnene-3,20-dione (XIV) (Reichstein's "Substance S"), since the molecule embodies all of the structural features of cortisone save the C-11 oxygen function. "Substance S" was isolated for the first time by Reichstein and von Euw, and the same authors[2] effected an ingenious partial synthesis from dehydroisoandrosterone. A partial synthesis was also described by Sarett.[3]

The biological activity of "Substance S" was previously found to be of a low order as judged by the tests used at that time.[4] Since the recent advances were made possible by the administration of comparatively large amounts of material, a preparative method better suited to these demands was necessary. One of our aims was to stimulate research with the intrinsically interesting hormone by elaboration of a practical method for partial synthesis.[5] More important, however, were two other considerations. Since "Substance S" is the simplest representative of adrenal hormones with the dihydroxy acetone side chain as well as the α,β-unsaturated ketone at C-3, it offered a convenient model for the study of many reactions applicable to more complicated congeners. Further, by the incorporation of an isotopic label, it would be possible to obtain this representative cortical hormone for a critical study of its biochemical action and metabolic fate.

It was apparent that the procedure for the elaboration of the side chain, which has been treated in detail in the preceding paper[6] was applicable to the synthesis of "Substance S" from 3-hydroxy-20-ketopregnanes. The basic new problem, then, involved preparation of the reactive side chain in such a way that selective oxidation of the C-3 hydroxyl group was possible without interference with the rest of the molecule. With this in view, 21-bromo-3α- or β,17α-dihydroxypregnan-20-one (VI or X) appeared to be the most promising key intermediate, since oxidation with N-bromoacetamide would yield a 3-keto compound (XI) easily converted to (XII), the saturated analog of the hormone. The introduction of the 4–5 conjugated double bond would then offer no difficulty and was, in fact, accomplished in high yield. This in outline was the procedure which was eventually proved practical; it has been described briefly in a preliminary communication.[7] Since then, Julian, Meyer, Karpel and Ryden[8] have also reported an interesting new partial synthesis for "Substance S" from 3β-hydroxy-Δ⁵,¹⁶-pregnadien-20-one. As starting materials for the synthesis of "Substance S" we used both 3β- and 3α-hydroxypregnan-20-one. 3α-Hydroxypregnan-20-one was particularly advantageous because it is prepared in good yield with isotope in the chemically stable 11- and 12-positions.[9] The 3β-epimer, on the other hand, as a derivative of the plant steroid sarsasapogenin[10] can be obtained in potentially unlimited amounts.

Both 20-ketosteroids were converted to the corresponding 17α-hydroxy compounds (I and VII) as described in the preceding paper.[6] The first objective was bromination of the C-21 methyl group. In the early phases of our investigation it was thought that protection of the C-3 hydroxyl was obligatory. Both the acetoxy and formoxy group was used for this purpose; either was successfully removed from the 21-bromo compound, without attack of the halogen, by an ester exchange with methanol in the presence of hydrogen chloride. The formate was superior in that acid catalyzed cleavage was more easily accomplished. A significant improvement was made when it was found that the formoxy group was completely removed with dilute sodium bicarbonate solution before reaction of the halogen at C-21. This sharply defined differential hydrolysis provided a satisfactory and useful preparation of the intermediate VI.

Halogenation of C-21 proceeded in the expected manner when the acetoxy derivatives were treated with bromine in acetic acid, but with the formoxy derivatives under the same conditions a more than negligible quantity of the formate was transformed to the acetate. This needlessly complicated puri-

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(2) Reichstein and von Euw, *Helv. Chim. Acta*, **21**, 1197 (1938); von Euw and Reichstein, *ibid.*, **23**, 1258 (1940); **24**, 1140 (1941).

(3) Sarett, *J. Biol. Chem.*, **162**, 627 (1946).

(4) Reichstein and Shoppee in Harris and Thimann, "Vitamins and Hormones," Vol. I, Academic Press, New York, N. Y., 1943, p. 359.

(5) As a result of these investigations we were able to supply Dr. Randall Sprague of the Mayo Foundation with a sufficient quantity of the hormone to test its effectiveness in the treatment of human rheumatoid arthritis.

(6) Kritchevsky and Gallagher, THIS JOURNAL, **73**, 184 (1951).

(7) Koechlin, Garmaise, Kritchevsky and Gallagher, *ibid.*, **71**, 3262 (1949).

(8) Julian, Meyer, Karpel and Ryden, *ibid.*, **71**, 3574 (1949).

(9) Koechlin, Kritchevsky and Gallagher, *J. Biol. Chem.*, **184**, 393 (1950).

(10) Marker, THIS JOURNAL, **62**, 3350 (1940); Marker and Rohrmann, *ibid.*, **62**, 521 (1940).