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]

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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-

(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) 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.

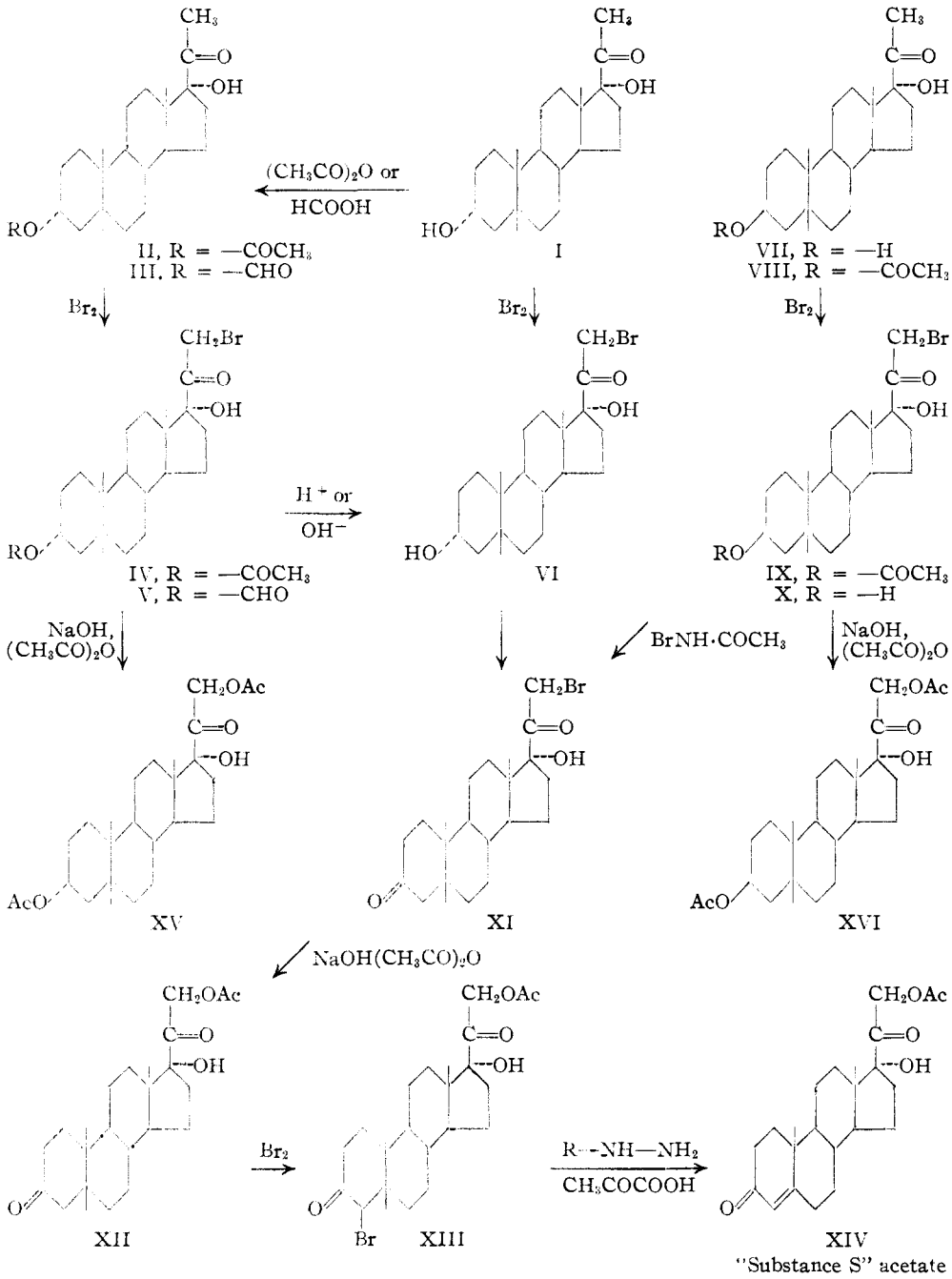
(6) Kritchevsky and Gallagher, THIS JOURNAL, **73**, 184 (1951).

(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).



fication of the reaction product and led to additional minor difficulty in later cleavage of the ester. This was prevented by brominating the formate in dry pure chloroform. In this solvent the reaction proceeded very smoothly and addition of hydrogen bromide was not necessary. Reaction with the stoichiometrical amount of bromine still yielded some dibromoketone which was found in the crystalline crop together with unreacted starting material. The most uniform product was obtained when the rate of the reaction was controlled by cooling and slow addition of the reagent.

The most advantageous reaction sequence to the key intermediate VI at this point was bromination of the formate (III) followed by hydrolysis of the ester with aqueous sodium bicarbonate. Although these successive stages were accomplished in good yield at each step, it was still possible to simplify the over-all process when it was found that, if the proper precautions were observed, esterification of the 3-hydroxyl group was not necessary. When 3α,17α-dihydroxypregnan-20-one (I) was brominated in reagent grade chloroform, the corresponding 21-bromo compound (VI) was formed without loss in yield compared to the longer reaction sequence. When acid-washed freshly redistilled chloroform was used instead of the analytical reagent, the yield of VI was lower, and in addition a small amount of a compound tentatively identified as 3-bromo-17α-hydroxypregnan-20-one was isolated. This finding suggested that the ethanol in the reagent grade chloroform was ef-

fective in preventing the formation of this side product.

Oxidation of the 3-hydroxyl was achieved readily with N-bromoacetamide at low temperature. It was found advantageous to chromatograph this intermediate (XI) rather than the 3-hydroxy-21-bromo compound (VI) and to carry small amounts of side product from previous steps through to this stage. Magnesium silicate was more suitable for this purpose than alumina since some samples of alumina produced secondary changes in the steroid. The pure 21-bromo compound (XI) was then hydrolyzed with dilute sodium hydroxide by the procedure reported in the preceding paper[6] and the product directly acetylated without isolation of the intermediate dioldione.

The introduction of the conjugated double bond was accomplished by the procedure we have described in detail elsewhere,[9] essentially a modification of the method of Mattox and Kendall.[11] As we had shown for analogous compounds, it was found that rigorous purification of the 4-bromoketone (XIII) was essential for a high yield in the subsequent reaction and that the optical rotation was the most suitable criterion for characterization of the product. The dehydrobromination was most effectively achieved by formation of the semicarbazone followed by cleavage with pyruvate in buffered solution. The product (XIV), obtained in 80% yield, was colorless and essentially pure in contrast to the reaction using dinitrophenylhydrazine, where persistent highly colored impurities necessitated repeated crystallization. In view of the excellent results obtained when 3-ketocholane derivatives are converted to α-β unsaturated 3-ketones by this procedure, saturated steroids offer many advantages in synthesis. This is especially important if consideration must be given to protection of C-5,6 unsaturation at several stages of an extended sequence of reactions in order to preserve a structure that can be oxidized and rearranged to an α,β-unsaturated ketone.

The over-all yield of "Substance S-acetate" from pregnanolone through this six-step procedure was over 20%. The product was identical in all respects with the compound described by Reichstein.[1,3]

The partial synthesis of isotopically labelled "Substance S" was effected starting from 3α-hydroxy-$d_2$11,12-pregnan-20-one.[9] Since the preparation differed in no essential detail from that described for the non-isotopic compound, it has not been included in the experimental section. The final product contained 5.0 atom per cent. excess deuterium. As an extension of this investigation we also prepared and characterized two new compounds which are potential metabolites of "Substance S," *i. e.*, 3α,20-diacetoxy-17α-hydroxypregnan-20-one (XV) and the 3β-epimer (XVI) of this substance.

Acknowledgments.—We wish to express our sincere appreciation for the considerable assistance we have received in the course of these studies. Dr. Konrad Dobriner and Phyllis Humphries of this Institute determined and interpreted many infrared spectra for us; Dr. Willard Hoehn of the University of Kansas City gave us a generous supply of 3α-hydroxypregnan-20-one; Dr. Leon A. Sweet of Parke, Davis and Company supplied the 3β-hydroxypregnan-20-one.

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

Experimental[12]

3α-Formoxy-17α-hydroxypregnan-20-one (III).—A mixture of 3.01 g. of 3α,17α-dihydroxypregnan-20-one (m.p. 207–209°) (I), 100 cc. of benzene and 50 cc. of formic acid (98–100%) was slowly distilled for 1.5 hours under reduced pressure at 50–60°. The solution was diluted with ethyl acetate, and after extraction with 5% sodium bicarbonate in the usual procedure, crystallization from ethyl acetate yielded 2.82 g. (85%) of m.p. 178–182°. Two recrystallizations from ethyl acetate gave hexagonal plates of m.p. 184–186° after rearrangement at 160°; $[\alpha]^{33}D$ +67.7° (ethanol).

Anal. Calcd. for $C_{22}H_{34}O_4$: C, 72.89; H, 9.45. Found: C, 73.00; H, 9.63.

After saponification of the mother liquors most of the remainder of the starting material was recovered.

3α-Acetoxy-21-bromo-17α-hydroxypregnan-20-one (II).—At room temperature 1.70 cc. of a 0.501 *M* solution of bromine, together with a small amount of hydrogen bromide in the same solvent, was added to 315 mg. (0.84 mM.) of 3α-acetoxy-17α-hydroxypregnan-20-one in 5 cc. of pure acetic acid. The solution decolorized after 35 minutes. It was diluted with ether, and after extraction with sodium carbonate in the usual procedure the product was crystallized from ether; 261 mg. (70%) of prisms was obtained. After two recrystallizations from ether, the m.p. was 230–235° with decomposition; $[\alpha]^{33}D$ +93° (ethanol).

Anal. Calcd. for $C_{23}H_{35}O_4Br$: C, 60.65; H, 7.75; Br, 17.55. Found: C, 60.90; H, 7.58; Br, 17.66.

3α-Formoxy-21-bromo-17α-hydroxypregnan-20-one (V). (a) **By Bromination of III in Chloroform.** At room temperature 11.5 cc. of a freshly prepared 0.24 *M* bromine solution in pure chloroform was added dropwise within 15 minutes to 950 mg. (2.62 mM., m.p. 181–182°) of 3α-formoxy-17α-hydroxypregnan-20-one (III) in 10 cc. of pure chloroform. The solution was diluted with ether and extracted with sodium bicarbonate in the usual procedure. After crystallization from ether, 870 mg. (75%), m.p. 180–205°, was obtained. Three recrystallizations from ether yielded elongated prisms, m.p. 205–210° (dec.); $[\alpha]^{27}D$ +77° (ethanol).

Anal. Calcd. for $C_{22}H_{33}O_4Br$: Br, 18.10. Found: Br, 17.91.

(b) **By Bromination of III in Acetic Acid.**—To 7.29 g. (22 mM., m.p. 177–181°) of 3α-formoxy-17α-hydroxypregnan-20-one (III) in 80 cc. of pure acetic acid, 40.5 cc. of 0.495 *M* bromine in pure acetic acid was added at room temperature. Ten cc. of approximately 3 *M* HBr in acetic acid was added, and decolorization was complete after 30 minutes. The solution was poured into a large volume of water and ether and extracted with sodium bicarbonate solution in the usual procedure; 6.19 g. of a crystalline product, m.p. 170–190° (c) (dec.) was obtained from ether. The three-times recrystallized product melted at 188–190° and appeared to be contaminated with the acetoxy compound formed by ester exchange during the reaction. The acetoxy group was not completely removed by the mild hydrolysis effective with the formoxy derivative and, therefore, a small portion of the acetate was carried through subsequent stages of the synthesis. It was thus possible to isolate 3α,21-diacetoxy-17α-hydroxypregnan-20-one (XV) as described subsequently in this report.

(12) Melting points, unless otherwise indicated, were determined upon a Kofler block; those followed by a (c) were taken in a capillary tube and are corrected for stem exposure. "Pure" solvents, where indicated, were prepared as follows: glacial acetic acid was redistilled from chromium trioxide, and the first third of the distillate was discarded; chloroform was extracted thoroughly with concentrated sulfuric acid, washed with water, dried with calcium chloride and redistilled. Purification by the "usual procedure" indicates extraction of the solution with either aqueous acid or base or both, as indicated in the text, followed by washing with water and drying the solution over sodium sulfate before distillation of the solvent.

3α,17α-Dihydroxy-21-bromopregnan-20-one (VI). (a) **From the 3-Formate (V) by Ester Exchange.**—To a solution of 3.745 g. (11 mM., m.p. (c) 180–190°) of 3α-formoxy-21-bromo-17α-hydroxypregnan-20-one (V) in 300 cc. of dry methanol, 80 cc. of methanol containing 3 g. of dry hydrochloric acid was added, and the reaction mixture was kept at 10° for 18 hours. The solution was diluted with a large volume of ether, and after extraction with sodium carbonate in the usual procedure, 3.38 g. (85%), m.p. 190–192°, (c) was obtained. The product appeared to be completely crystalline and depressed the melting point of the formate (V) upon admixture. Repeated recrystallization gave plates, m.p. 206–209° (c); $[\alpha]^{30}D$ +79° (ethanol).

Anal. Calcd. for $C_{21}H_{33}O_3Br$: Br, 19.33. Found: Br, 19.29.

(b) **VI from the 3-Formate (V) by Alkaline Hydrolysis.**—To a solution of 800 mg. of 3α-formoxy-21-bromopregnan-20-one (V) (1.8 mM., m.p. 180–207°) in 80 cc. of methanol, 8 cc. of aqueous 1 *N* potassium bicarbonate was added. An initial precipitation disappeared after 10 minutes. After 1 hour at room temperature the reaction was complete, and no bromide ion was found on titration. The solution was diluted with ether, washed with cold sodium chloride solution, and evaporated. The crystalline residue, after recrystallization from ethyl acetate–ether yielded 680 mg. melting at 188–197° (dec.). This product was contaminated with some 21-dibrominated as well as some unbrominated material, carried through from the previous step. These side products were separated more easily by chromatography after oxidation to the 3-keto compounds (see below).

(c) **VI from the 3-Acetate (IV) by Ester Exchange.**—Seven cc. of methanol containing 300 mg. of dry hydrochloric acid was added to a solution of 210 mg. of 3α-acetoxy-21-bromo-17α-hydroxy-20-ketopregnane (**IV**) in 35 cc. of dry methanol, and the reaction mixture was stored at room temperature. A sample worked up after 24 hours still contained starting material as judged from the presence of an acetate band in the infrared spectrum; after 48 hours at room temperature, the reaction appeared to be complete. The solution was concentrated under reduced pressure, taken up in ether and extracted with sodium bicarbonate solution in the usual procedure. Crystallization from methanol–ether yielded 163 mg. of crude crystalline product which, after recrystallization from acetone–ether, melted at 210° (dec.); $[\alpha]^{30}D$ +79° (ethanol) and gave no depression on admixture with an authentic sample of VI.

(d) **VI from the 3-Hydroxy Compound (I) by Direct Bromination.**—To 800 mg. (2.4 mM.) of 3α,17α-dihydroxypregnan-20-one (I) dissolved in 30 cc. of untreated reagent grade chloroform, 9.7 cc. of a 0.253 *M* bromine solution in the same solvent was added within 20 minutes. The chloroform solution was diluted with ether and subjected to the usual procedure using sodium bicarbonate. Crystallization from ethyl acetate–ether yielded 685 mg. (66%) of m.p. 198–205° (dec.). From the mother liquor 135 mg. of starting material (I) was recovered after debromination with zinc in acetic acid.

With methanol-free chloroform purified and dried as described previously, the bromination proceeded less satisfactorily in that replacement of the 3-hydroxyl group by halogen occurred. This was evidenced by the isolation of an impure product, m.p. 195–203°; $[\alpha]^{27}D$ +56° (ethanol), the analysis of which was in fair agreement with 3-bromo-17α-hydroxypregnan-20-one.

Anal. Calcd. for $C_{21}H_{33}O_2Br$: C, 63.46; H, 8.37; Br, 20.11. Found: C, 63.09; H, 8.83; Br, 18.89.

3β-Acetoxy-21-bromo-17α-hydroxypregnan-20-one (IX).—To 3.55 g. (9.5 mM., m.p. 169–173°) of 3β-acetoxy-17α-hydroxypregnan-20-one (VIII) in 20 cc. of pure acetic acid, 19 cc. of a 0.501 *M* solution of bromine in pure acetic acid was added at room temperature in the presence of a small amount of hydrogen bromide in the same solvent. Decolorization was complete after one hour. Most of the solvent was removed at 45° under reduced pressure. Ether was added to the remaining solution, and after extraction with dilute sodium carbonate in the usual procedure, the oily residue crystallized slowly from ether–petroleum ether to yield 3.26 g. (75%) as elongated prisms. After two recrystallizations from the same solvent, the product melted at 156° but underwent rearrangement on storage to melt at 202–210° (dec.); $[\alpha]^{30}D$ +65° (ethanol).

Anal. Calcd. for $C_{23}H_{33}O_3Br$: C, 60.65; H, 7.75; Br, 17.55. Found: C, 60.90; H, 7.45; Br, 17.35.

3β,17α-Dihydroxy-21-bromopregnan-20-one (X).—A solution of 3.1 g. of dry hydrogen chloride in 70 cc. of methanol was added to 3.20 g. (7 mM.) of 3β-acetoxy-17α-hydroxy-21-bromopregnan-20-one (VIII) dissolved in 350 cc. of methanol and 50 cc. of chloroform. After 48 hours at room temperature, the reaction was worked up as described for the 3α compound (VI). 2.63 g. of a crystalline product was obtained on crystallization from ether–petroleum ether. After recrystallization the product melted at 185–190°; $[\alpha]^{35}D$ +68° (ethanol), but was very hygroscopic, and the analysis for bromine gave a low value. The identity of the product was established by oxidation to the 3-ketone (XI) as well as conversion to 3β,21-diacetoxy-17α-hydroxypregnan-20-one (XVI).

17α-Hydroxy-21-bromopregnane-3,20-dione (XI). (a) **From 3α-Hydroxy (VI).**—3.26 g. of crude 3α,17α-dihydroxy-21-bromopregnan-20-one (VI) (7.9 mM., m.p. 190–192° (c)) was dissolved in 35 cc. of *t*-butanol, and 2.1 g. of N-bromoacetamide (15.2 mM.), 0.7 cc. of pyridine and 0.5 cc. of water were added. The solution was kept at 10° for 16 hours and was partially solid at the end of this period. Titration with thiosulfate indicated that about 1.2 equivalents of the oxidizing agent had been used. It was diluted with ether and subjected to the usual procedure; 1.09 g. of heavy prisms, m.p. 203–204° (c) was obtained from ethyl acetate in the first crop. The mother liquors yielded crystals of slightly lower melting point and, in all, 2.495 g. (77%) was obtained. After two recrystallizations from ethyl acetate, the pure compound melted at 206–209° (c); $[\alpha]^{35}D$ +81° (ethanol).

Anal. Calcd. for $C_{21}H_{31}O_3Br$: C, 61.31; H, 7.60; Br, 19.43. Found: C, 61.02; H, 7.65; Br, 19.59.

(b) **XI from 3β-Hydroxy (X).**—To 2.36 g. of crude 3β,17α-dihydroxy-21-bromopregnan-20-one (5.7 mM.) in 30 cc. of *t*-butanol was added 1.5 g. of N-bromoacetamide (10.9 mM.), 0.77 cc. of pyridine and 0.4 cc. of water. After 16 hours at 10° the reaction was diluted with ether and subjected to the usual procedure; 1.7 g. of the residue remaining after a technical loss was crystallized from ether. 1.4 g. of crude crystalline product which decomposed at 175–185° was obtained. Infrared analysis showed that this product was essentially identical with the pure bromoketone (XI) obtained from the 3α-compound (VI). It was converted without further purification to 21-acetoxy-17α-hydroxypregnane-3,20-dione (XII), as described in a later section.

17α-Hydroxy-21-bromopregnane-3,20-dione (XI) was conveniently purified by chromatography on magnesium silicate–celite mixture; with alumina the separation and recovery was less satisfactory. Since the diketo-21-bromo compound (XI) could be readily separated from other substances, it was often expedient to proceed in the earlier stages of the synthesis with materials of less than analytical purity. This fact has been noted earlier in the experimental section, and therefore a typical chromatogram is described in detail.

Six hundred and fifty mg. of crude 17α-hydroxy-21-bromopregnane-3,20-dione (obtained by bromination of the 3α-formate in chloroform and aqueous bicarbonate hydrolysis, followed by N-bromoacetamide oxidation) was dissolved in 150 cc. of benzene and poured on a column of 50 g. of magnesium silicate–celite (2:1). The chromatogram was developed with benzene which eluted a small amount of amorphous material. Mixtures of benzene and 5–10% ether resulted in the elution of 70 mg. of the dibromo-ketone described below. The eluates obtained with benzene and 15–20% ether yielded 415 mg. (65%) of pure 17α-hydroxy-21-bromopregnane-3,20-dione (XI), m.p. 203–206°. Finally, 35 mg. (6%) of unbrominated 17α-hydroxypregnane-3,20-dione, m.p. 215°, was obtained and identified by the melting point of a mixture with authentic material as well as by infrared spectroscopy.

21,21-Dibromo-17α-hydroxypregnane-3,20-dione was recrystallized from chloroform–ether, m.p. 213–215°; $[\alpha]^{28}D$ +108° (ethanol).

Anal. Calcd. for $C_{21}H_{30}O_3Br_2$: Br, 32.60. Found: Br, 32.33.

21-Acetoxy-17α-hydroxypregnane-3,20-dione (XII).—A solution of 510 mg. of 21-bromo-17α-hydroxypregnane-3,20-dione (XI) (1.24 mM., m.p. 203–206°) in 125 cc. of eth-

anol was diluted with 75 cc. of water, and a stream of nitrogen was bubbled through the solution for 25 minutes; 20 cc. of 0.55 *N* aqueous sodium hydroxide was added at 28°, and the solution was kept in a nitrogen atmosphere for 9 minutes. After acidification with a slight excess of dilute acid, the solution was poured into a large volume of ether, sodium chloride was added and the ether extract was well washed with 5% sodium bicarbonate solution. The ether solution was dried with sodium sulfate and the solvent removed at a low temperature. The crystalline residue without isolation was acetylated in 5 cc. of pyridine and 3 cc. of acetic anhydride overnight at room temperature. Following concentration under reduced pressure, ether was added and the acetate was worked up in the usual manner, using base and acid. The residue weighed 450 mg.; 305 mg. of plates (m.p. 193–196°) crystallized from ethyl acetate–ether, and after two recrystallizations the product melted at 198–200° following rearrangement at 190° to elongated prisms; $[\alpha]^{28}D$ +81° (ethanol). An additional 30 mg. of pure product was obtained after chromatographic purification of the crystalline mother liquors on alumina for a total yield of 70%.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.52. Found: C, 70.81; H, 8.77.

3α,21-Diacetoxy-17α-hydroxypregnan-20-one (XV).—This compound was obtained from the mother liquors of 21-acetoxy-17α-hydroxy-3,20-diketopregnane prepared from a contaminated bromoketone (XI) after chromatography on a column of magnesium silicate–celite. It crystallized from ethyl acetate as hexagonal plates, melting at 205–206° (rearrangement at 195°); $[\alpha]D$ +88° (ethanol).

Anal. Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.86. Found: C, 68.74; H, 8.83.

Thirty-five mg. was oxidized with chromium trioxide in acetic acid, and 18 mg. of 3α-acetoxyetiocholan-17-one, m.p. 95–97°, crystallized after inoculation. It was identical in all respects with an authentic sample.

3β,21-Diacetoxy-17α-hydroxypregnan-20-one (XVI).—In an atmosphere of nitrogen 6 cc. of 0.55 *N* aqueous sodium hydroxide was added to 80 mg. of 3β,17α-dihydroxy-21-bromopregnan-20-one in 35 cc. of ethanol and 20 cc. of water. After 9 minutes the solution was neutralized with dilute hydrochloric acid, diluted with ether and extracted with ice-cold sodium chloride solution and sodium carbonate in the usual manner. The dry residue was acetylated in 3 cc. of pyridine and 2 cc. of acetic anhydride at room temperature overnight and the acetate isolated as described above. The crystalline product (75 mg.) was chromatographed on 1.5 g. of alumina. Benzene–petroleum ether, benzene and low concentration of ether in benzene eluted 35 mg. of crystals. After three recrystallizations from ether–petroleum ether and benzene–petroleum ether, 8.5 mg. of hexagonal plates was obtained, m.p. 154–157°; $[\alpha]^{28}D$ +51° (ethanol).

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.09; H, 8.81. Found: C, 69.14; H, 8.76.

4-Bromo-17α-hydroxy-21-acetoxypregnane-3,20-dione (XIII).—A few drops of a 0.493 *M* solution of bromine in pure acetic acid was added to a solution of 1.50 g. of 17α-hydroxy-21-acetoxypregnane-3,20-dione (XIII) in 25 cc. of pure acetic acid. After decolorization the solution of the ketone was chilled to incipient crystallization, and the addition of bromine was continued at a rate such that no considerable excess was present at any time. About 40 minutes was required for the addition of 7.8 cc., and at the end of the reaction a crystalline precipitate had formed. Chloroform and ether were added and the solution was washed with sodium carbonate in the usual procedure. 1.2 g. (68%) $[\alpha]D$ +78° was obtained upon crystallization from ether; 550 mg. of crystalline products; $[\alpha]D$ +68° was obtained from the mother liquors. After recrystallization of the first fraction from chloroform–ether, 1.05 g. (58%) of pure product; $[\alpha]^{30}D$ +81° was obtained. The analytical sample was prepared by recrystallization from chloroform as heavy prisms, m.p. 205–210° (dec.); $[\alpha]^{30}D$ +82° (chloroform).

Anal. Calcd. for $C_{23}H_{33}O_5Br$: Br, 17.03. Found: Br, 17.17.

The more levorotatory crystalline fractions together with the mother liquors were debrominated by heating in acetic acid with an equal weight of zinc dust for one hour; 300 mg. of 21-acetoxy-17α-hydroxypregnane-3,20-dione (XII) (20% of the amount originally brominated) was recovered. When the bromination was carried out at room temperature, the yield of pure monobromoketone was found to be 5–10% lower.

17α-Hydroxy-21-acetoxy-Δ⁴-pregnene-3,20-dione (Reichstein's Compound "S" Acetate) (XIV). (a) Dehydrobromination over the Semicarbazone.—One hundred and four mg. of 4-bromo-17α-hydroxy-21-acetoxypregnane-3,20-dione (XIII); $[\alpha]D$ +80° (0.22 mM.) was dissolved in 5 cc. of pure acetic acid; 75 mg. of semicarbazide hydrochloride (0.66 mM.) mixed with 80 mg. of sodium acetate and two drops of water was added to the solution of the bromoketone with 15 cc. of acetic acid. The mixture was heated to 65° in a nitrogen atmosphere for 2 hours and a crystalline precipitate separated. One cc. of pyruvic acid, 300 mg. of sodium acetate and 2 cc. of water were added to this suspension, and the mixture was kept at 70° for 1.5 hours and then at 80° for 15 minutes. After the addition of 0.4 cc. of pyruvic acid, the solution was stored for 18 hours at room temperature. The addition of two volumes of water caused the separation of a crystalline precipitate which was filtered, washed with water, and dried by distillation of benzene. The solid residue, 58 mg., was recrystallized from acetone, and 50 mg. of colorless flat prisms (m.p. 239–241°) was obtained. The aqueous filtrate from the cleavage solution was extracted with a mixture of ether and chloroform. The organic layer was washed with 5% sodium hydroxide, which removed most of the yellow color, and with water, and the solvent was removed after drying over sodium sulfate. The residue weighed 24 mg. and crystallized on the addition of acetone. It was pooled with the mother liquor of the main fraction, and by direct crystallization and chromatography on alumina an additional 21 mg. of colorless crystals, m.p. 230–237°, was obtained. The total yield was 71 mg. (80%). The product was identical in all respects with the acetate of Reichstein's Compound "S"; $[\alpha]^{35}D$ +116° (acetone) +147° (chloroform); ϵ_{2410} 17,400 (methanol).

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.11; H, 8.30. Found: C, 71.06; H, 8.46.

(b) **Debromination over the 2,4-Dinitrophenylhydrazone.**—Seventy mg. of 2,4-dinitrophenylhydrazine (1.2 equivalents) and 30 mg. of sodium acetate were added to 240 mg. of 4-bromo-17α-hydroxy-21-acetoxypregnane-3,20-dione (XIII); $[\alpha]D$ +82° (0.30 mM.) dissolved in 12 cc. of pure acetic acid at 60°. The solution was kept at 60° in a nitrogen atmosphere for 25 minutes. After initial darkening of the yellow solution, the red crystalline 2,4-dinitrophenylhydrazone precipitated. The solution was concentrated under reduced pressure and 8 cc. of chloroform, 2 cc. of pyruvic acid and 1 cc. of water were added. The solution was heated at 65–70° under nitrogen with the addition of 1 cc. of pyruvic acid at hourly intervals. After 4 hours the cleavage was completed as recognized by the color change from red to yellow. The solution was diluted with ether and washed thoroughly with sodium carbonate in the usual procedure. The residue was crystalline but contaminated with reddish impurities. Crystallization from acetone yielded 99 mg. of flat prisms. Two recrystallizations were required to obtain 45 mg. of a fairly white product, m.p. 239–240°; $[\alpha]D$ +112° (acetone), identical with the product obtained from the previous procedure. From the various mother liquors, more colored crystals of somewhat lower melting point were obtained by crystallization from chloroform–benzene or acetone. The over-all yield was 99 mg. (83%). The preparation of a colorless compound in high yield was seriously complicated by the presence of the persistent red impurity.

Summary

A procedure has been described for the partial synthesis of 17α-hydroxy-21-acetoxy-Δ⁴-pregnene-3,20-dione (acetate of Reichstein's "Substance S") from both 3α- and 3β-epimers of 3,17α-dihydroxypregnan-20-one based upon the following reactions: (a) bromination at C-21, (b) selective oxidation at C-3 by means of N-bromoacetamide, (c) hydrolysis with dilute alkali, (d) bromination at C-4, and (e) dehydrobromination with semicarbazide and

cleavage with pyruvic acid. Several variations of the process and intermediates have been described. By the same procedure "Substance S" was prepared with 5.0 atom per cent. excess deuterium in chemically stable positions.

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[FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Formation of an Acrylal in Side Chain Degradation of the Bile Acids[1]

BY T. F. GALLAGHER AND ELISE ELISBERG

In the course of preparation of 3α,12α-dihydroxypregnan-20-one by oxidation of 24,24-diphenyl-$\Delta^{20,23}$-choladiene-3α,12α-diol diacetate, we obtained a considerable amount of a product that was not the expected 20-ketosteroid. The oxidation was carried out with purified diene essentially according to the procedure of Miescher and Schmidlin[2]; subsequent separation of the 20-ketone by means of the Girard Reagent T was omitted in order to avoid discoloration of the product with the azine of diphenylacrolein. The neutral fraction after oxidation was saponified with alkali to remove one or both of the acetoxy groups prior to chromatographic separation of the mixture. When the alkaline hydrolysis was effected on the steam-bath, a yellow compound, m. p. 148–154°; $[\alpha]_D$ +99° (chloroform) crystallized readily. The substance had two maxima in the ultraviolet, ϵ_{2420} 12,300, ϵ_{2375} 29,000, and the analysis was in agreement with the molecular formula $C_{36}H_{44-46}O_3$.

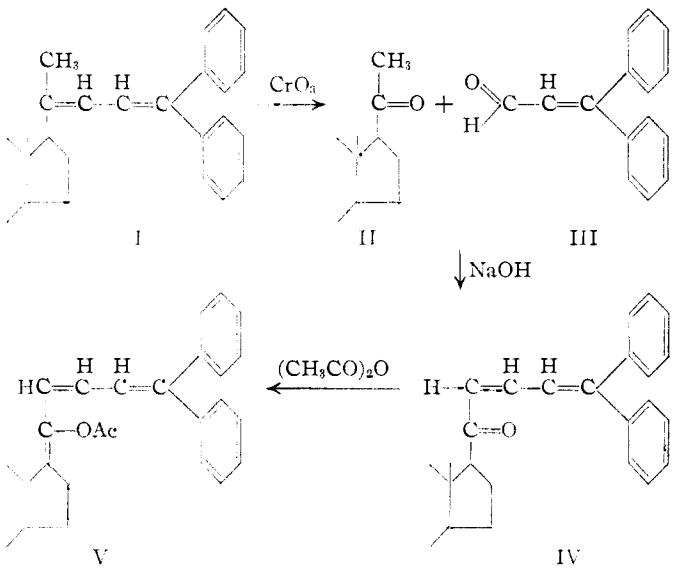

Fig. 1

Acetylation at 0° with perchloric acid as a catalyst[3] yielded oily material for the most part, together with a crystalline product, m. p. 253–253.5°; $[\alpha]_D$ +114° (chloroform); ϵ_{2450} 18,500, ϵ_{3350} 51,000, which was correctly recognized as an enol acetate.

It was thought that the oxidation had taken an atypical course with the derivative of desoxycholic acid, and therefore, the diphenyl diene from lithocholic acid was submitted to the same procedure. The ultraviolet absorption ($E^{1\%}_{1cm.}$ = 258 at 3375 Å. in ethanol) demonstrated that a similar product was present, and chromatography on alumina resulted in the isolation of three products. The first eluates yielded a pale yellow compound, m. p. 151.5–153°; $[\alpha]_D$ +55.5° (chloroform); ϵ_{2420} 13,900, ϵ_{3375} 31,800 (ethanol), the analysis of which was in agreement with $C_{36}H_{44-46}O_2$. ($E^{1\%}_{1cm.}$ 624 at 3375 Å.), immediately followed by a second crystalline product, m. p. 160–162° ($E^{1\%}_{1cm.}$ 374 at 3375 Å.). We suspected that this second substance was a molecular compound of pregnanolone with the product isolated from the earlier fractions of the chromatogram. The anticipation was confirmed when an equimolar mixture of pregnanolone (m. p. 147–148°) and the compound melting at 151.5–153° crystallized from acetone and melted at 163°. The infrared spectrum of the molecular compound from the pure components was identical with that of the 160–162° melting product obtained from the chromatogram. The last fractions from the chromatogram were essentially pure 3α-hydroxypregnan-20-one.

It was clear from these results that the same type of reaction product could be obtained from steroids with and without the 12 hydroxyl group. A clue to the nature of the reaction was provided when the ultraviolet absorption was determined at successive stages of the process. It was found that the oxidation product of the diacetoxydiene exhibited slight absorption ($E^{1\%}_{1cm.}$ 23 at 3000 Å.) prior to saponification with alkali. After alkaline hydrolysis the maximum shifted to longer wave length with a considerable increase in the extinction coefficient ($E^{1\%}_{1cm.}$ 370 at 3375 Å.). These results were consistent with the interpretation that in the presence of aqueous alkali, condensation of the aldehyde fragment of the side chain with the C-21 methyl group of the 20-ketosteroid had occurred as outlined in Fig. 1. The products isolated then were diphenylacrylal derivatives, IV, of the respective 20-ketosteroids, and as could be anticipated, these were easily converted to the enol acetates V. Yields of the 3α,-12α-dihydroxy derivative of IV in excess of 60% based on crystalline product were readily obtained, and the mother liquors still contained acrylal as

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(2) Miescher and Schmidlin, *Helv. Chim. Acta*, **30**, 1405 (1947).

(3) Whitman and Schwenk, THIS JOURNAL, **68**, 1865 (1946).