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 $C_{36}H_{44-46}O_3$.

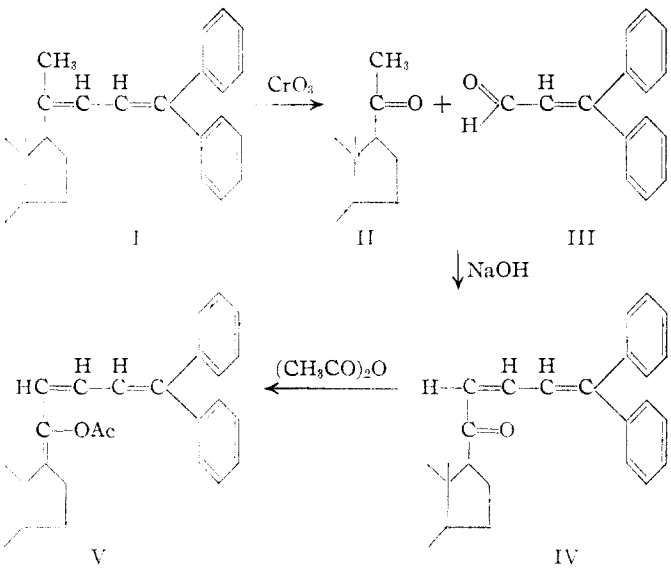

Fig. 1

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

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

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

evidenced by the ultraviolet absorption. Proof for the structure was provided by chromic acid oxidation of 3α,12α-diacetoxy-21-(1,1-diphenylacrylal)-pregnan-20-one which yielded methyl 3α,12α-diacetoxyetiocholanate identical in all respects with the product obtained by independent methods.

These results show that the condensation of diphenylacrolein and a 20-ketosteroid is a facile reaction which proceeds readily in an aqueous medium. It is interesting and important that so large an amount of diphenylacrolein persists in the presence of excess chromic acid, since the amount of aldehyde is the limiting factor in the formation of the acrylal derivative IV. For the isolation of 20-ketosteroids, efficient removal of the aldehydic fragment is highly desirable. While this can be accomplished to a certain extent by formation of the bisulfite compound,[2] the reaction is reversible, and a certain amount of the aldehyde remains with the steroid fraction. Alkaline hydrolysis, even under mild conditions, will then form the acrylal with consequent loss of product. These facts explain, we believe, the frequently disappointing yields encountered in the oxidation of diphenyl dienes to 20-ketosteroids and the somewhat better results obtained when an acetoxy group is situated at C-21 prior to the oxidation. They likewise direct attention to a means whereby the over-all yield in the side chain degradation may be significantly improved. The ultraviolet spectra are shown in Fig. 2.

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.

Experimental

3α,12α-Dihydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—A solution of 11.69 g. (19.6 millimoles) of 24,24-diphenyl-$\Delta^{20,23}$-choladiene-3α,12α-diol diacetate [m.p. 139–142°; $[\alpha]^{25}D$ +191°; (chloroform); ϵ_{3050} 25,500 in ethanol] in 90 cc. of ethylene dichloride was added to 70 cc. of 80% acetic acid and chilled to −1°. Chromium trioxide (9.85 g., 98.5 millimoles) in 140 cc. of 80% acetic acid was added dropwise with vigorous stirring during 50 minutes while the temperature was maintained below 1.5°. Stirring was continued for an additional 2.5 hours at 0°, and then 10 g. of sodium bisulfite in 100 cc. of water was added at such a rate that the temperature never exceeded 3°. An approximately equal volume of water was added, and most of the solvent was removed under diminished pressure. The residue was extracted with ether and the ether solution was washed with acid, base and water, dried with sodium sulfate and evaporated to dryness. The residual oil ($E^{1\%}_{1cm.}$ 23 at 3000 Å.) was dissolved in 150 cc. of ethanol, and 7.9 g. (197 millimoles) of sodium hydroxide in 100 cc. of water was added. Most of the alcohol was distilled off on the steam-bath during approximately thirty minutes. The reaction product was extracted with ether. After washing the ether solution with acid and water the solvent was removed and the product crystallized from acetone; yellow platelets (4.91 g., 48%), m.p. 145–150°; $E^{1\%}_{1cm.}$ 605 at 3375 Å. in ethanol, were obtained in the first crop. Concentration of the mother liquors yielded 0.80 g. (8%), m.p. 141–146°; $E^{1\%}_{1cm.}$ 477 at 3375 Å. in ethanol. Chromatography of the residue on alumina resulted in the separation of 0.90 g. of 3α,12α-dihydroxypregnan-20-one, m.p. 169–172°, together with other materials showing considerable absorption in the ultraviolet.

The acrylal derivative crystallized readily from most organic solvents when acetone was added. From the melting point and from the infrared spectrum these products contained acetone which could be removed after heating several hours at 100° *in vacuo*. When crystallized slowly from either ethyl acetate or benzene, similar solvates were obtained whose melting points varied somewhat with the rate of heating from 142 to 153°; $[\alpha]D$ +99° (chloroform); ϵ_{2420} 12,300, ϵ_{3375} 29,000 (ethanol).

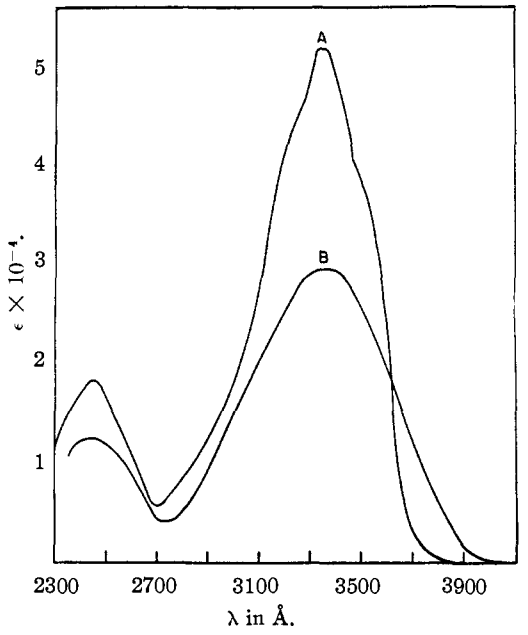

Fig. 2.—Ultraviolet spectra of steroid diphenylacrylals: A, triacetate of the enol of 3α,12α-dihydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one in ethanol; B, 3α,12α-dihydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one in ethanol.

Anal. Calcd. for $C_{36}H_{44}O_3$: C, 82.39; H, 8.45. Found: C, 81.79; C, 8.58.

Triacetate of the Enol of 3α,12α-Dihydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—This compound was obtained in about 10% yield when the dihydroxy ketone was acetylated at 0° in the presence of perchloric acid. It was more readily prepared in yield of over 70% by the procedure of Marshall, *et al.*,[4] and after recrystallization from ethyl acetate formed needles which melted at 253–253.5°; $[\alpha]^{24}D$ +114° (chloroform); ϵ_{2450} 18,500, ϵ_{3350} 51,000 (ethanol).

Anal. Calcd. for $C_{42}H_{50}O_6$: C, 77.51; H, 7.74. Found: C, 77.71; H, 7.94.

Methyl 3α,12α-Diacetoxyetiocholanate from 3α,12α-Diacetoxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—Two grams of sirupy 3α,12α-diacetoxy-21-(1,1-diphenylacrylal)-pregnan-20-one was dissolved in 30 cc. of glacial acetic acid, and in the course of six hours at room temperature 155 cc. of 0.85 *N* chromium trioxide in 90% acetic acid was added. After standing overnight the excess chromic acid was destroyed by the addition of methanol. The solution was poured into water and extracted with a 1:1 mixture of ether and ethyl acetate. The organic layer was washed with dilute sodium chloride solution, dilute hydrochloric acid and then with 5% sodium hydroxide. The alkaline extract was allowed to stand at room temperature for 45 minutes and was then added dropwise with stirring to an excess of dilute sulfuric acid. The crystalline product was filtered off and recrystallized from methanol, yield, 350 mg. (29%), m.p. 293–295°. It was esterified with diazomethane and acetylated with acetic anhydride in the presence of perchloric acid. The product upon recrystallization from methanol melted at 152.5–153°; $[\alpha]^{25}D$ +152° (acetone).[5]

3α-Hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—A solution of 13.02 g. (24.3 millimoles) of 24,24-diphenyl-

(4) Marshall, Kritchevsky, Lieberman and Gallagher, THIS JOURNAL, **70**, 1837 (1948).

(5) Lardon and Reichstein, *Helv. Chim. Acta*, **26**, 607 (1943); melting point 149–150°, $[\alpha]D$ +149.8° (acetone).

$\Delta^{20,23}$-choladien-3α-ol acetate (m.p. 150°; $[\alpha]^{25}D$ +89° (chloroform); ϵ_{3050} 24,900 in ethanol) was oxidized with 12.1 g. (121 millimoles) of chromium trioxide precisely as in the foregoing experiment, and the subsequent saponification was likewise identical. The amorphous product, $E^{1\%}_{1cm.}$ 258 at 3375 Å., was chromatographed on alumina. After several mobile oily fractions, 3.918 g. of yellow product was obtained which on recrystallization from benzene–acetone yielded 1.26 g., $E^{1\%}_{1cm.}$ 547 at 3375 Å. in alcohol. This substance was a solvate which became amorphous when dried at 100° *in vacuo*. It was recrystallized three times from ethyl acetate and formed thin prisms melting 151.5–153°; $[\alpha]^{24}D$ +55.5° (chloroform); ϵ_{2420} 13,900, ϵ_{3375} 31,800.

Anal. Calcd. for $C_{36}H_{44}O_2$: C, 85.19; H, 8.72. Found: C, 84.95; H, 8.86.

1:1 Molecular Compound of Pregnanolone and 3α-Hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—Later fractions from the chromatogram yielded pale yellow crystals from acetone which melted 160–162°; $E^{1\%}_{1cm.}$ 374 at 3375 Å.; calculated for a 1:1 molecular compound of the acrylal and pregnanolone is 373. A mixture of 5.1 mg. (0.01 millimole) of 3α-hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one (m.p. 151.5–153°) and 3.2 mg. (0.01 millimole) of 3α-hydroxypregnan-20-one was dissolved in acetone and the solvent allowed to evaporate spontaneously. After drying at 100° the crystalline residue melted 163–163.5° and exhibited an infrared spectrum indistinguishable from the product isolated from the chromatogram.

Diacetate of the Enol of 3α-Hydroxy-21-(1,1-diphenylacrylal)-pregnan-20-one.—Three hundred mg. of the 1:1 molecular compound of the acrylal and pregnanolone was acetylated by the procedure of Marshall, *et al.*[4] Crystallization from acetone–methanol and from ethyl acetate yielded 130 mg. of needles, m.p. 132–135°; $[\alpha]^{25}D$ +100° (chloroform); ϵ_{2450} 16,200, ϵ_{3350} 47,600.

Anal. Calcd. for $C_{40}H_{48}O_4$: C, 81.04; H, 8.16. Found: C, 81.43; H, 8.18.

Summary

After oxidation of 24,24-diphenyl-$\Delta^{20,23}$-choladiene with an excess of chromic acid, the 20-ketosteroid and diphenylacrolein condensed in the presence of aqueous alkali with the formation of a 21-diphenylacrylal. The constitution of the product was proved by degradation, and some of the properties of these derivatives were studied.

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[CONTRIBUTION FROM THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

The Action of Alcoholic Potassium Hydroxide on Δ^{16}-20-Ketosteroids[1]

BY DAVID K. FUKUSHIMA AND T. F. GALLAGHER

An attractive means for the preparation of steroid hormones of the adrenocortical type was advanced by Marker[2] as a result of his finding that a Δ^{16}-20-ketosteroid by addition of the elements of water in the presence of methanolic alkali formed a 17-hydroxy-20-ketosteroid. The reaction was presumed to account for the fact that heating the oxidation product of pseudobotogenin diacetate, 3β-acetoxy-$\Delta^{5,16}$-pregnadiene-12,20-dione, with potassium hydroxide in methanol yielded a compound having an additional atom of oxygen in the molecule. Since the newly introduced oxygen function could not be acetylated, Marker concluded that it was present as a tertiary alcohol group at C-17. Because of our interest in the preparation of 17-hydroxy cortical hormones, we wished to confirm and extend this important reaction, so novel in its simplicity and so readily applicable to the synthesis of adrenal steroids from a widely distributed class of natural products. We therefore investigated the products obtained when a pure sample of 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one (I) was heated with a methanolic solution of potassium hydroxide, and found that the reaction resulted in the formation of a quite different compound than that postulated by Marker.

The ultraviolet absorption proved an extremely useful tool in following the reaction. From the decrease in the extinction coefficient at 2390 Å., it was apparent that in the presence of alkali more than half the product had been converted to a substance that no longer had an α,β-unsaturated ketone system. The mixture proved difficult to separate by fractional crystallization, but after acetylation, chromatography yielded two easily distinguished products. One was 3β-acetoxy-$\Delta^{5,16}$-pregnadien-20-one and the other a white crystalline compound, m. p. 158.5–159.5°, $[\alpha]D$ −28°, that had no absorption above 2250 Å. The infrared spectrum in the region 3000 to 4000 cm.$^{-1}$ showed that no free hydroxyl group was present in the new compound. This fact alone would have eliminated either diastereoisomer of 3β-acetoxy-17-hydroxy-Δ^5-pregnen-20-one from consideration but, fortunately, the physical constants of both known epimers[3,4] were sufficiently different from one another and from the unknown product to confirm this conclusion. It was possible that a 17-hydroxy-20-ketopregnane formed initially had been subsequently transformed to a D-homosteroid by alkali; this structure, too, was unlikely from the physical constants[4] as well as from the absence of the hydroxyl band in the infrared spectrum. The elementary analysis and a Zeisel determination showed that there was an additional carbon atom present as a methoxyl group. Therefore, the compound isolated was probably formed by the addition of a molecule of methanol to the unsaturated ketone. This conclusion was confirmed when an *ethanol* solution of potassium hydroxide was used for the reaction, and a different product was isolated, which proved to be the corresponding ethoxy derivative by elementary analysis and ethoxyl determination. A Δ^{16}-20-ketosteroid, therefore, formed an alkoxy derivative with loss of the α,β-unsaturation when treated with base in alcohol solutions. The base-

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

(2) Marker, THIS JOURNAL, **71**, 4149 (1949).

(3) Hegner and Reichstein, *Helv. Chim. Acta*, **24**, 828 (1941).

(4) Shoppee and Prins, *ibid.*, **26**, 201 (1943).