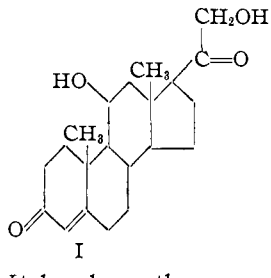
[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

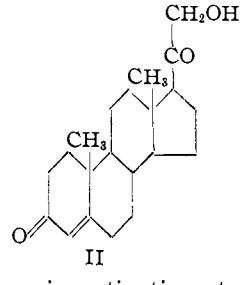
Studies on the Partial Synthesis of Corticosterone. I

BY PURNENDU NATH CHAKRAVORTY[1] AND EVERETT S. WALLIS

Through the pioneering investigations of Rogoff,[2] Swingle[3] and Hartman[4] the existence of a powerful hormone in the adrenal gland, other than adrenalin, was established. The method of Swingle and Pfiffner[5] for the preparation of concentrates of this hormone has been widely used, and has culminated in the isolation of several crystalline products, notably by three groups of investigators led by Reichstein[6] at Zürich, Kendall[7] at the Mayo Clinic and Wintersteiner[8] at Columbia University. Among these crystalline materials which have been isolated some have been found to be inactive and the structures of the few physiologically active compounds are still uncertain on certain points. Corticosterone, the most potent of the crystalline compounds, has been assigned formula I.



I



II

It has been the purpose of our investigations to find a method of synthesis of the above substance, thereby settling the structural question and also providing adequate amounts of the hormone for clinical investigations.

Stieger and Reichstein[9] have prepared a very active compound, "desoxycorticosterone" (formula II) but the starting material is rather difficult to obtain, and recent clinical tests have thrown great doubt on its value for medicinal use. In our efforts to obtain corticosterone we started from desoxycholic acid which is easily available. Of the several methods tried, that shown in the diagram seems to be most promising.

Desoxycholic acid was oxidized in the cold directly to 3-oxy-12-ketocholanic acid III by a method first described by Kaziro and Shimada.[10] The yield was very good and the material so obtained was easy to purify. The methyl ester of this acid prepared for the first time melted at 143° The acetoxy derivative (m. p. 197°) on treatment with bromine either in chloroform or acetic acid solution in the presence of hydrogen bromide gave a bromide, IV, which could be used directly for dehalogenation. The introduction of the double bond was effected with sodium ethylate, the unsaturated keto acid so obtained melting at 173°. Its acetate melts at 199°. An analysis and determination of its absorption spectra showed that it was the desired 3-oxy-12-keto-9,11-cholenic acid, V. If dehalogenation was effected with zinc and acetic acid, the saturated 3-oxy-12-ketocholanic acid was produced in good yields. It is also to be recorded that dehalogenation with sodium acetate and acetic acid failed to give the desired product.

Treatment of the above acid with semicarbazide acetate gave a semicarbazone which melted at 221°. It is interesting to note in this connection that whereas 3-oxy-12-ketocholanic acid gives a semicarbazone only after twelve hours of refluxing with the reagent, this unsaturated acid requires but two hours similar treatment. Thus, as one might expect, the conjugated double bond makes the 12-keto group more reactive. The semicarbazone thus obtained was reduced by heating it with sodium ethylate in a sealed tube for fifteen hours at 180°. On working up the reaction product the corresponding methylenic compound, VI, is obtained in readily crystallizable form. It is contaminated, as would be expected, with a small amount of its epimer, β-3-oxy-9,11-cholenic acid, but this latter compound is removed

(1) Research Assistant on Special Funds from the Rockefeller Foundation.

(2) Rogoff and Stewart, *Amer. J. Physiol.*, **84**, 660 (1928); *J. Am. Med. Assoc.*, **92**, 1569 (1929).

(3) Swingle and Pfiffner, *Science*, **71**, 321 (1930).

(4) Hartman and Brownel, *ibid.*, **72**, 76 (1930); Hartman, *Am. J. Physiol.*, **95** 670 (1930).

(5) Swingle and Pfiffner, *Am. J. Physiol.*, **96** 153, 164, 180 (1930); *ibid.*, **98**, 144 (1931). See also Pfiffner, Vars and Taylor, *J. Biol. Chem.*, **106**, 625 (1934), and Cartland and Kuizenga, *ibid.*, **116**, 57 (1936).

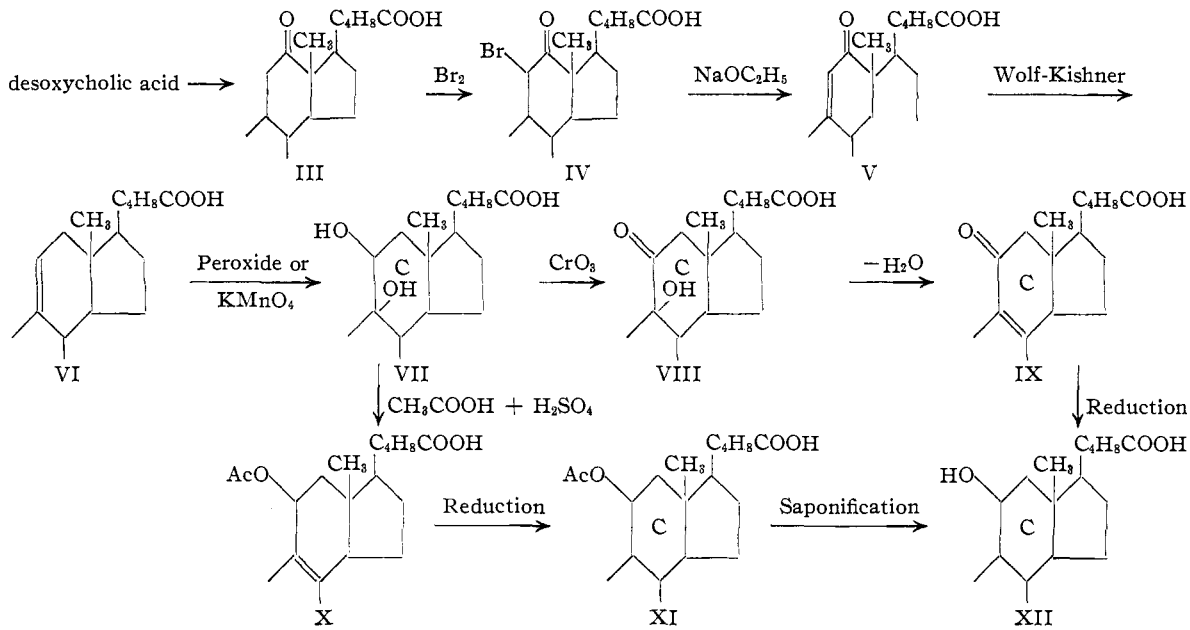
(6) Reichstein et alia, *Helv. Chim. Acta*, **19**, 29, 223, 401, 402, 979 (1936).

(7) Kendall and co-workers, *Proc. Staff Meetings Mayo Clinic*, **8**, 90 (1933).

(8) Wintersteiner, Vars and Pfiffner, *J. Biol. Chem.*, **109**, c (1934). See also Pfiffner, Vars, Bott and Swingle, *Proc. Soc. Exp. Biol. Med.*, **29**, 998, 1267 (1931–1932).

(9) Stieger and Reichstein, *Nature*, **139**, 925 (1937); *Helv. Chim. Acta*. **20**, 1164 (1937).

(10) Kaziro and Shimada, *Hoppe-Seyler, Z. physiol. Chem.*, **249**, 220 (1937). See also Bergström and Haslewood, *J. Chem. Soc.*, 541 (1939).



by recrystallization from acetone. α-3-Oxy-9,11-cholenic acid crystallizes in needles from acetone which melt at 183–184°. Further reactions of this compound will be reported in a later paper.

Experimental Part

Preparation of 3-Acetoxy-12-ketocholanic Acid.—This acid was prepared according to the method of Kaziro and Shimada[10] as described by Bergström and Haslewood.[10] It was converted into its acetoxy derivative in the usual manner.

Preparation of 3-Hydroxy-12-keto-9,11-cholenic Acid.—Five grams of 3-acetoxy-12-ketocholanic acid was dissolved in 15 cc. of glacial acetic acid, and treated with 15 cc. of a 1.05 *N* acetic acid solution of bromine. A few drops of a 34% aqueous solution of hydrogen bromide were added as a catalyst. The solution was then kept at 70° for four hours and allowed to stand overnight at room temperature. After this time it was poured into ice water and the amorphous powder which precipitated was filtered. After thorough washing with water it was dissolved in ether, and the ether solution again washed to remove all traces of hydrobromic acid. After drying with sodium sulfate the ether was evaporated to dryness and the bromide, which was obtained as a sticky reddish residue, was taken up immediately in absolute alcohol and poured into a boiling solution of sodium ethylate (prepared from 6 g. of sodium dissolved in 70 cc. of absolute alcohol). Immediately the mixture became very dark brown and some sodium bromide precipitated. Refluxing was continued for two hours, after which time a considerable amount of sodium bromide had precipitated. The condenser was then removed and after sufficient water had been added to dissolve the salt the boiling was continued to remove as much of the alcohol as possible. The reaction mixture was then cooled with ice water and to it was added an ice cold solution of 20% sulfuric acid. After standing in the icebox for one-half hour, the precipitated acid so obtained was filtered and washed free from sulfuric acid. It was then taken up in ether and the dried ether solution was decolorized with charcoal. The solution was then concentrated to a small volume and allowed to stand. (If the ether solution is perfectly dry and if the removal of hydrogen bromide has been complete, crystallization of the dissolved acid begins when the solution has reached about half its original volume.) The crystalline product so obtained was filtered and washed with cold ether. A sample of it melted at 160–165°. Recrystallization from ethyl acetate gave a product which melted at 172–173°. A melting point depression was observed when a sample of the material was mixed with a specimen of α-3-hydroxy-12-ketocholanic acid, yield 30%.

An absorption spectra study showed an absorption at wave length 242.5, a wave length which is characteristic for α,β-unsaturated ketones.

Anal. Calcd. for $C_{24}H_{36}O_4$: C, 74.2; H, 9.4. Found: C, 74.2; H, 9.8.

Preparation of 3-Hydroxy-9,11-cholenic Acid.—A portion of the above acid (0.2 g.) was refluxed for two hours with an aqueous alcoholic solution of 0.2 g. of semicarbazide acetate prepared from carbazide hydrochloride and 0.2 g. of anhydrous sodium acetate. The product was worked up in the usual manner. From benzene it came down as a gelatinous precipitate which became crystalline when filtered and washed with ether. A specimen of the crystals melted at 221°. The semicarbazone was not further purified but was directly employed for the Wolf–Kishner reduction. The heating with sodium ethylate (made from 1 g. of sodium and 15 cc. of absolute alcohol) was carried out in a sealed tube at 180° for fifteen hours. After this time the reaction product was taken up in water and boiled for one-half hour on the water-bath. After cooling with ice it was acidified with ice-cold dilute sulfuric acid. The precipitate so obtained was filtered, washed thoroughly with water and then taken up in acetone. On

crystallization needles were obtained which melted unsharply at 175°. Several recrystallizations from acetone gave a product which showed a strong positive Liebermann reaction and which melted at 183–184°, $[\alpha]^{25}D$ +27.0 (63 mg. in 5 cc. of absolute alcohol solution gave a reading of +0.68). When mixed with a specimen of 3-hydroxy-12-keto-9,11-cholenic acid a depression of the melting point was observed.

Anal. Calcd. for $C_{24}H_{34}O_3$: C, 76.96; H, 10.23. Found: C, 76.7; H, 10.5.

We wish to take this opportunity to express our thanks to the Rockefeller Foundation for a grant-in-aid which in part was used in support of this work.

Summary

1. A new route has been suggested to the preparation of sterol derivatives having a hydroxyl group C_{11}. Progress in this direction has been reported.

2. By the debromination followed by saponification of 3-acetoxy-11-bromo-12-ketocholanic acid, an unsaturated acid has been prepared which proved to be 3-hydroxy-12-keto-9,11-cholenic acid. This acid melts at 173° and possesses the characteristic absorption of the α,β-unsaturated keto derivatives.

3. The crude semicarbazone of the unsaturated keto-hydroxy acid, m. p. 221°, has been reduced according to the Wolf–Kishner method to 3-hydroxy-9,11-cholenic acid of m. p. 183–184°.

PRINCETON, NEW JERSEY RECEIVED OCTOBER 23, 1939

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF NEW YORK UNIVERSITY]

Steric Hindrance in Ketone–Phenol Condensations. The Condensation of Guaiacol with Cyclic Ketones

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In a previous publication[2] the effect of steric hindrance in ketone–phenol condensations has been investigated. It was established that, while the usual condensation product is an alkylidene-diphenol,[3] this is not formed when a reaction system is selected which would lead to the formation of a cycloalkylidene-diphenol in which there is a substituent group in *ortho* position to either one of the two reacting carbon atoms involved in the condensation. In these cases only equimolar condensation is possible, leading to the formation of cycloalkylenephenols or coumarans, respectively.

For a practical application of this hypothesis, a phenolic compound having an alkoxy group in *ortho* position to the free hydroxyl group was condensed with cyclic ketones. Condensation products analogous to the ones isolated in condensations involving an ortho alkylated phenol should be obtainable. As shown in the following, similar types of condensation products could actually be isolated.

Thus, the condensation of guaiacol with cyclohexanone itself and the *m*- and *p*-methylcyclohexanone isomers, yielded the expected alkylidene-diphenols (I, II, III), while the reaction of guaiacol with *o*-methylcyclohexanone resulted in the unsaturated cyclohexenyl-guaiacol (IV).

Of the four primary condensation products (I–IV) the following derivatives were prepared and verified by analysis: crystalline acetates (Ic, IIc, IIIc), benzoates (Id, IId, IIId) and phenylurethans (Ib, IIb, IIIb) of the three alkylidene-diphenols (I, II, III) and a crystalline aryloxyacetic acid (IVa) of the unsaturated monomolecular condensation product of guaiacol with *o*-methylcyclohexanone (IV).

Experimental

Condensation Method

1,1-*bis*-(3'-Methoxy-4'-hydroxyphenyl)-cyclohexane (I).—Molar quantities of guaiacol and half molar quantities of cyclohexanone were dissolved in 100 cc. of glacial acetic acid and the mixture placed in a 1-liter, triple-necked, round-bottomed flask which was provided with a reflux condenser, a thermometer and a gas inlet tube extending to the bottom of the vessel. A vigorous stream of dry hydrogen chloride gas was passed into the system for six hours at room temperature on two consecutive days, then the flask was stoppered and left standing at room temperature for four weeks. Crystals deposited which were filtered and recrystallized from benzene; m. p. 174°; yield, 31%.

Attempts to hydrolyze the ether groups in this compound by refluxing the compound with hydrobromic acid (48%)

(1) The material presented in this paper is taken from the thesis presented by Joseph Grumer to the Graduate School of New York University in partial fulfilment of the requirements for the degree of Master of Science.

(2) J. B. Niederl and co-workers, THIS JOURNAL, **61**, 1785 (1939).

(3) M. E. McGreal and co-workers, *ibid.*, **61**, 345 (1939).