fect the acetylation of the α-hydrogen of cyclohexanone or acetophenone with acetic anhydride to form the β-diketone; instead some of the ketone underwent self-condensation. Moreover, neither boron chloride nor aluminium chloride brought about acetylation of the α-hydrogen of even *p*-nitrophenylacetone, which is relatively reactive.

Similar observations were reported recently by Perfetti and Levine[5] who showed that neither aluminum chloride nor stannic chloride can effect the acetylation of acetophenone to form benzoylacetone. However, these workers did realize this acetylation with zinc chloride and ferric chloride, although the temperature employed (110°) is much higher than that (0°) known to effect the reaction with boron fluoride.

Experimental

Acetoacetylations by Boron Fluoride.—A mixture of the aromatic compound (0.20 mole) and acetic anhydride (0.80 mole) was saturated with boron trifluoride at 0–10° in two to three hours and then stirred for an additional period to make a total reaction time of four hours. A solution of 100 g. of sodium acetate in 500 ml. of water was added and the reaction mixture refluxed 15–30 minutes. The mixture was cooled and extracted two or three times with 30–60° ligroin. The combined ligroin solution was washed three times with small portions of water and once with saturated sodium bicarbonate solution. The ligroin solution was then extracted several times with cold 2% sodium hydroxide solution until the ligroin phase no longer gave a positive enol test. The combined alkaline solution was acidified at 0° and the β-diketone taken up in ether, from which it was recovered by fractionation of the dried solution (Table I). The ligroin phase was dried and fractionated, yielding the monoketone and some high-boiling residue.

Experiments with Boron Chloride and Aluminum Chloride.—A mixture of toluene (0.20 mole) and acetic anhydride (0.8 mole) was saturated with boron chloride at 10° and the reaction mixture worked up as described above for boron fluoride. There was obtained a 27% yield of *p*-methylacetophenone, b.p. 101–102° at 13 mm.

A mixture of cyclohexanone (0.15 mole) and acetic anhydride (0.30 mole) was saturated with boron chloride in 40 minutes at 10°. After stirring 30 minutes longer, the reaction mixture was decomposed with excess sodium acetate in ice-water. The mixture was extracted with ligroin and, after drying, the ligroin solution was fractionated to give 2-cyclohexylidenecyclohexanone[6] (59%), b.p. 142–143° at 17 mm.[6]; semicarbazone, m.p. 178–179°.[6]

Mixtures of acetophenone and acetic anhydride and of *p*-nitrophenylacetone and this anhydride were treated similarly with boron chloride. There were obtained some dypnone and tarry material, respectively.

A mixture of *p*-nitrophenylacetone (0.02 mole), acetic anhydride (0.06 mole), aluminum chloride (0.14 mole) and 40 ml. of carbon disulfide was stirred 12 hours at room temperature. After distilling most of the solvent, the mixture was poured onto ice and hydrochloric acid to give the original ketone (colored).

In all of these experiments, the products gave negative enol tests with ferric chloride showing the absence of β-diketones.

(5) B. M. Perfetti and R. Levine, THIS JOURNAL, **75**, 626 (1953).

(6) A. D. Petrov, *Bull. soc. chim.*, [IV] **43**, 1272 (1928).

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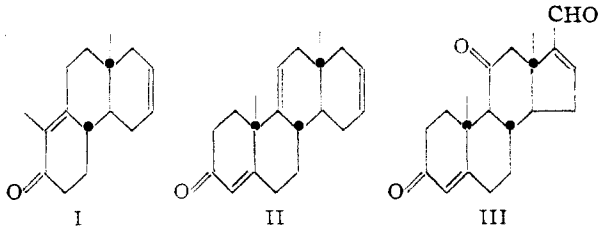
COMMUNICATIONS TO THE EDITOR

A SYNTHESIS OF *dl*-CORTISONE ACETATE

Sir:

We wish to report a direct synthesis of *dl*-cortisone acetate from the Woodward tricyclic ketone,[1] *dl*-1,14-dimethyl-2-keto-$\Delta^{1(11),6,9}$-octahydrophenanthrene. A distinguishing feature of this synthesis is that the cortical side chain and the eleven oxygen function are introduced without protecting the α,β-unsaturated ketone in ring A.

Selective hydrogenation of the Woodward tricyclic ketone with palladium on strontium carbonate gave the oily dihydrotricyclic ketone I ($\lambda_{max}^{alc.}$ 250 mμ, ϵ15,300. Found: C, 83.5; H, 9.5). I was blocked in the 3 position by the methylanilinomethylene group[1] (m.p. 124–125°. Found: C, 82.8; H, 8.3.). The protected ketone was condensed with β-propiolactone[2] in the presence of potassium amide in ether. Removal of the blocking group yielded *dl*-1-(β-carboxyethyl)-1,14-dimethyl-2-keto-$\Delta^{6,10}$-decahydrophenanthrene as a crystalline isomer[3] (m.p. 171–173°. Found: C, 75.2; H, 8.7). This keto-acid was converted to the enol lactone (m.p. 100–102°) and thence by treatment with methylmagnesium bromide followed by cyclization[1] to the tetracyclic ketone II (m.p. 147–148°. Found: C, 84.9; H, 9.2). II was oxidized with iodine and silver acetate in wet acetic acid[4] to give a β-*cis*-glycol. Reaction with acetone gave *dl*-3-keto-16β,17β-dihydroxy-$\Delta^{4,9(11)}$-D-homoandrostadiene acetonide[5] (m.p. 174–175°). The structure of the acetonide was proved by conver-



(1) *Cf.* R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952).

(2) *Cf.* T. L. Gresham, J. S. Jansen, F. W. Shaver, M. R. Frederick and W. L. Beears, *ibid.*, **73**, 2345 (1951), and earlier papers.

(3) The mother liquor from the isolation of this material undoubtedly contained the epimeric compound.

(4) A reagent described in a private communication from R. B. Woodward; *cf.* S. Winstein and R. E. Buckles, THIS JOURNAL, **64**, 2787 (1942).

(5) It is to be noted that our acetonide differs from Woodward's[1] in that it was derived from a β-*cis*-glycol whereas his was from an α-*cis*-glycol, where α and β designate configuration corresponding to standard steroid convention.

sion to *dl*-$\Delta^{9(11),16}$-21-norprogesterone previously prepared by Woodward.[1]

The acetonide was converted in excellent yield to *dl* - 3 - keto - 11β,16β,17β - trihydroxy - Δ^4 - 9α-bromo-D-homoandrostene acetonide (m.p. 194–196°) with N-bromosuccinimide and sulfuric acid in aqueous acetone.[6] The crude bromohydrin was converted by alkali to *dl*-3-keto-9β,11β-oxido-16β,17β-dihydroxy-Δ^4-D-homoandrostene acetonide (m.p. 191–193°. Found: C, 74.3; H, 8.7). This crude bromohydrin was also oxidized with pyridine–chromium trioxide complex[7] to give a crude bromoketone (m.p. 195–198° dec.) which without purification was debrominated with zinc and aqueous acetic acid to give *dl*-3,11-diketo-Δ^4-16β,17β-dihydroxy-D-homoandrostene acetonide (m.p. 198–200°). Treatment with periodic acid followed by benzene and piperidine acetate[1] gave *dl*-11-keto-Δ^{16}-21-norprogesterone III (m.p. 207–209°. Found: C, 76.5; H, 7.7). Reaction with alkaline hydrogen peroxide[8] produced *dl*-11-keto-16α,17α-oxido-21-norprogesterone (m.p. 243–245°). Oxidation with silver oxide gave *dl*-3,11-diketo-16α,-17α-oxido-Δ^4-etiocholenic acid (m.p. 217–220° dec.). Reaction of the dry sodium salt with oxalyl chloride yielded an acid chloride which on treatment with diazomethane[9] gave a crystalline diazoketone (m.p. 193–195°) having strong infrared absorption at 4.75μ. Reaction of the diazoketone with hot acetic acid gave non-crystalline *dl*-16α,-17α - oxido - 3,11,20 - triketo - 21 - hydroxy - Δ^4-pregnene acetate. Opening with hydrogen bromide[8] produced *dl*-16β-bromocortisone acetate (m.p. 238–240° dec.). Debromination with Raney nickel[8] gave *dl*-cortisone acetate[10] (m.p. 240–243°) whose infrared spectrum was identical with natural cortisone acetate.

We thank Dr. R. H. Munch, Mr. G. W. Ashworth and Mr. O. E. Kinast for help with the numerous infrared and ultraviolet spectra needed in this work. In addition, we acknowledge the invaluable advice and assistance of Dr. R. B. Woodward.

(6) After the completion of our work, J. Fried and E. F. Sabo [THIS JOURNAL, **75**, 2273 (1953)] reported that they added hypobromous acid in good yield to a 3-keto-$\Delta^{4,9(11)}$ steroid. It now appears that the low yield obtained by Hicks and Wallis [*J. Biol. Chem.*, **162**, 641 (1946)] may be attributed to the fact that in their case rings A and B were *cis*.

(7) A reagent first announced at the Gordon Research Conferences, A.A.A.S., New Hampton, N. H., August 4–8, 1952; *cf.* G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(8) *Cf.* P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Waller, *ibid.*, **72**, 5145 (1950).

(9) *Cf.* A. L. Wilds and C. H. Shunk, *ibid.*, **70**, 2427 (1948).

(10) L. H. Sarett, G. E. Arth, R. M. Lukes, R. E. Beyler, G. I. Poos, W. F. Johns and J. M. Constantin, *ibid.*, **74**, 4974 (1952).

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RECEIVED JULY 7, 1953

THE RECONSTRUCTION OF THE FATTY ACID OXIDIZING SYSTEM OF ANIMAL TISSUES

Sir:

A system including seven enzymes has been shown to catalyze the following sequence

$$\text{Butyrate} \xrightarrow[\text{(I)}]{\text{ATP, CoA}^1} \text{BuCoA} \xrightarrow[\text{(II)}]{-2\text{E}}$$

$$\text{Crotonyl CoA} \xrightarrow[\text{(III)}]{\text{H}_2\text{O}} \beta\text{-Hydroxy}$$

$$\text{BuCoA} \xrightarrow[\text{(IV)}]{\text{DPN}} \text{AcAcCoA} \xrightarrow[\text{(V)}]{\text{CoA}}$$

$$2\,\text{AcCoA} \xrightarrow[\text{(VI, VII)}]{\text{2 Malate, DPN}} 2\,\text{Citrate}$$

where (I) represents the fatty acid activating enzyme,[2,3] (II) fatty acyl CoA dehydrogenase, (III) unsaturated acyl CoA hydrase, (IV) β-hydroxyacyl CoA dehydrogenase,[4] (V) AcAcCoA cleavage enzyme,[4,5,6] (VI) malic dehydrogenase[7] and (VII) AcCoA-oxalacetate condensing enzyme.[8] Enzymes (I–V) have been isolated from beef liver mitochondria. Tz is the final electron acceptor with pyocyanine as intermediary carrier. Diaphorase[9] (VIII) catalyzes the oxidation of DPNH. The over-all balanced reaction is

$$\text{BuO}^- + 4\text{Tz} + 2\,\text{Malate} + \text{ATP} \longrightarrow 2\,\text{Citrate} + 4\text{Fz} + \text{AMP} + \text{PP}_\text{I} \quad (1)$$

The observed citrate:Fz ratio of 1:2.2 is in good agreement with the 1:2 ratio of equation (1).

Preparations of (I) at the highest purity level are homogeneous in the ultracentrifuge. At *p*H 10 with heptanoate as substrate, 1 mg. of (I) catalyzes the formation of 3.8 μ mole of acyl CoA per min. at 38°. (I) activates a wide variety of odd or even, straight (C_4–C_{12}), branched chain, or substituted fatty acids as well as α,β- and β,γ-unsaturated acids. (I) has proved invaluable for preparation of all acyl CoA derivatives required as substrates for (II–IV). The mechanism of activation by ATP is the same as for the acetate activation enzyme system.[10]

(IIg) a green copper flavoprotein[11] has been isolated in a form which is homogeneous in both the ultracentrifuge and Tiselius apparatus. The riboflavin content of the homogeneous enzyme is 1.2%. The prosthetic flavin has the same absorption spectrum and enzymatic activity as FAD.[12,13] (IIg) can be converted into an apoenzyme at *p*H 3.7

(1) The following abbreviations will be used: adenosinetriphosphate (ATP); adenosine-5'-phosphate (AMP); coenzyme A (CoA); di- and triphosphopyridine nucleotide (DPN, DPNH and TPN, TPNH); flavin adenine dinucleotide (FAD); acetyl (Ac); acetoacetyl (AcAc); butyryl (Bu); triphenyltetrazolium (Tz); formazan (Fz); and inorganic pyrophosphate (PP_I).

(2) H. R. Mahler, "Phosphorus Metabolism," Vol. 2, 286, Johns Hopkins Press, Baltimore, 1953; H. R. Mahler, S. J. Wakil and R. M. Bock, *J. Biol. Chem.*, in press.

(3) G. Drysdale and H. A. Lardy, "Phosphorus Metabolism," Vol. 2, p. 281, Johns Hopkins Press, Baltimore, Md., 1953.

(4) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(5) J. R. Stern, M. J. Coon and A. del Campillo, *Nature*, **171**, 28 (1953).

(6) D. E. Green, D. S. Goldman, S. Mii and H. Beinert, *J. Biol. Chem.*, **202**, 137 (1953).

(7) F. B. Straub, *Z. physiol. Chem.*, **275**, 63 (1942).

(8) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).

(9) J. G. Dewan and D. E. Green, *Biochem. J.*, **32**, 626 (1938).

(10) H. Beinert, D. E. Green, P. Hele, H. Hift, R. W. Von Korff and C. V. Ramakrishnan, *J. Biol. Chem.*, **203**, 35 (1953).

(11) H. R. Mahler, THIS JOURNAL, **75**, 3288 (1953).

(12) E. Negelein and H. Brömel, *Biochem. Z.*, **300**, 225 (1939).

(13) O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150 (1938).