[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX S. A.]

Steroids. LXVII.[1] The Decarboxylation of Unsaturated Steroidal Acids. Synthesis of 17-Epitestosterone and of 17-Methylepitestosterone[2]

BY FRANZ SONDHEIMER, O. MANCERA, M. URQUIZA AND G. ROSENKRANZ

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3β-Hydroxy-$\Delta^{5,16}$-etiadienic acid (V) on being refluxed with quinoline in the presence of copper chromite is shown to decarboxylate smoothly to $\Delta^{5,16}$-androstadien-3β-ol (VI). Such Δ^{16}-androstene derivatives had been obtainable previously only from the rare 17α-hydroxyandrostanes. Oppenauer oxidation of VI, followed by preferential oxide formation in ring D, lithium aluminum hydride reduction and manganese dioxide oxidation at C-3, furnished 17-epitestosterone (Ia). Similarly $\Delta^{5,17(20)}$-pregnadien-3β-ol-21-oic acid (XII) is decarboxylated with copper chromite and quinoline to 17-methylene-Δ^{5}-androsten-3β-ol (XIII), which by the sequence of reactions indicated before was converted to 17-methylepitestosterone (II).

17-Epitestosterone (Ia) and 17-methylepitestosterone (II), isomeric at C-17 with the highly androgenic testosterone and 17-methylestosterone, both have been shown to possess only little androgenic activity.[3,4] We were interested in making available these 17α-hydroxy compounds in order that their possible utility as anabolic agents could be investigated. They had been prepared previously[3,4] in very small over-all yield by transformations involving Δ^{5}-androstene-3β,17α-diol and 17β-methyl-Δ^{5}-androstene-3β,17α-diol, obtained as by-products from dehydroepiandrosterone through C-17 reduction and reaction with methylmagnesium iodide, respectively. However, these cannot be considered preparative methods, since attack of steroidal C-17 ketones takes place almost exclusively from the α-side of the molecule with the consequent predominant formation of the 17β-hydroxy compounds.[5] In the present paper we describe syntheses of both Ia and II by methods which are stereospecific and by which these compounds may be prepared in quantity.

For the synthesis of epitestosterone (Ia) it was necessary to investigate routes leading to 17α-hydroxyandrostane derivatives. Apart from the one mentioned above which involves reduction of a C-17 ketone, three methods have been described previously. The first proceeds from the 17β-ol, which on conversion to the *p*-toluenesulfonate and subsequent acetolysis and saponification furnishes the 17α-ol in about 5% yield.[6] We have applied this scheme to testosterone, but again only obtained the 17α-ol, epitestosterone in this case, in only 5% yield. Another substance isolated (21%) was the ψ-androstene derivative IV[7] which appears to be the main product in this type of reaction.[6] The second method involves perbenzoic acid oxidation of a 17-isopregnane derivative,[8] but seems of little preparative value in view of the rather poor yield obtained in the oxidation and the fact that in the equilibrium between pregnanes and 17-isopregnanes the former predominate.[9] The third method proceeds from the Δ^{16}-unsaturated androstene, which on transformation to the 16α,17α-oxide and subsequent reduction with lithium aluminum hydride produces the 17α-ol in satisfactory yield.[10] Since, however, the only known preparation of Δ^{16}-androstene derivatives necessitates the required 17α-ols themselves as starting materials (pyrolysis of benzoates or hexahydrobenzoates[11a,b] or *via* the xanthates[11c]), this method is of no preparative utility unless a new route to Δ^{16}-androstenes be found. We now have found a new route to these compounds and the path to epitestosterone was therefore opened.

The starting material, 3β-hydroxy-$\Delta^{5,16}$-etiadienic acid (V), may be prepared from dehydroepiandrosterone acetate through cyanohydrin formation, dehydration at C-17 and vigorous alkaline hydrolysis,[12] or more directly (though, in poorer yield), by reaction of $\Delta^{5,16}$-pregnadien-3β-ol-20-one acetate (the degradation product of diosgenin) with potassium hypoiodite.[13] It was hoped that the unsaturated acid V with boiling quinoline would decarboxylate to VI, but in fact the starting material was recovered. However, when the reaction was catalyzed by addition of a small amount of copper chromite,[14] decarboxylation occurred smoothly and the required $\Delta^{5,16}$-androstadien-3β-ol (VI) could be isolated in 65–70% yield. That no unexpected rearrangement had occurred during the reaction was shown through catalytic hydrogenation of VI, when hydrogen equivalent to about 2 double bonds was absorbed and the known androstan-3β-ol (VII)[15] was produced. The Δ^{16}-position of the double bond in ring D of VI follows from the further transformations. Moreover VI had a definite odor of urine, which appears to be characteristic of certain Δ^{16}-androstene derivatives.[11]

(1) Paper LXVI, A. Zaffaroni, C. Casas Campillo, F. Cordoba and G. Rosenkranz, *Experientia*, in press. In paper LXIII (A. Zaffaroni, H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, THIS JOURNAL, **76**, 6210 (1954)) the ultraviolet spectral data in footnote 9 should read "$\lambda_{max.}$ 302 mμ, log ε 4.15" instead of "$\lambda_{max.}$ 320 mμ, log ε 4.15."

(2) Presented in part at the New York Meeting of the American Chemical Society, September, 1954.

(3) L. Ruzicka and H. Kägi, *Helv. Chim. Acta*, **19**, 842 (1936).

(4) K. Miescher and W. Klarer, *ibid.*, **22**, 962 (1939).

(5) *Cf.* L. F. Fieser, *Experientia*, **6**, 312 (1950).

(6) (a) J. Elks and C. W. Shoppee, *J. Chem. Soc.*, 241 (1953); (b) O. S. Madaeva and F. A. Lur'i, *Doklady Akad. Nauk S.S.S.R.*, **84**, 713 (1952) (*C. A.*, **47**, 3326 (1953)).

(7) See K. Miescher and H. Kägi, *Helv. Chim. Acta*, **32**, 761 (1949), for proof of structure of ψ-androstenes and earlier references.

(8) T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950).

(9) *Inter al.* A. Butenandt, *et al.*, *Ber.*, **68**, 1847 (1935); **70**, 96 (1937).

(10) H. Heusser, M. Feuer, K. Eichenberger and V. Prelog, *Helv. Chim. Acta*, **33**, 2243 (1950).

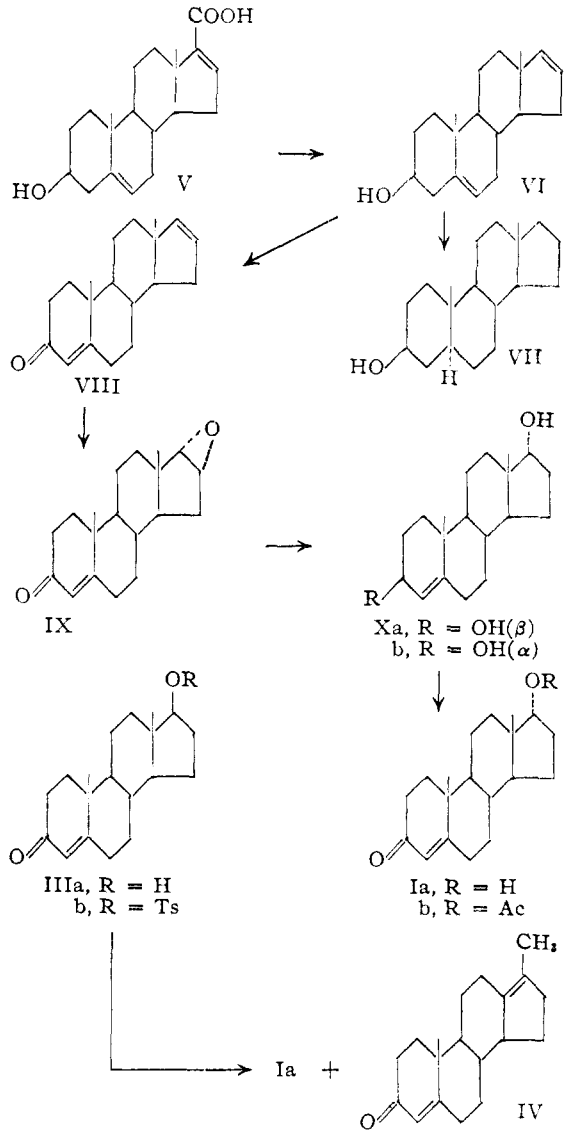
(11) (a) V. Prelog, L. Ruzicka, P. Meister and P. Wieland, *ibid.*, **27**, 66 (1944); (b) **28**, 618 (1945); (c) H. Kägi and K. Miescher, *ibid.*, **22**, 683 (1939).

(12) (a) L. Ruzicka, E. Hardegger and C. Kauter, *ibid.*, **27**, 1164 (1944); (b) A. Butenandt and J. Schmidt-Thomé, *Ber.*, **71**, 1487 (1938); (c) **72**, 182 (1939).

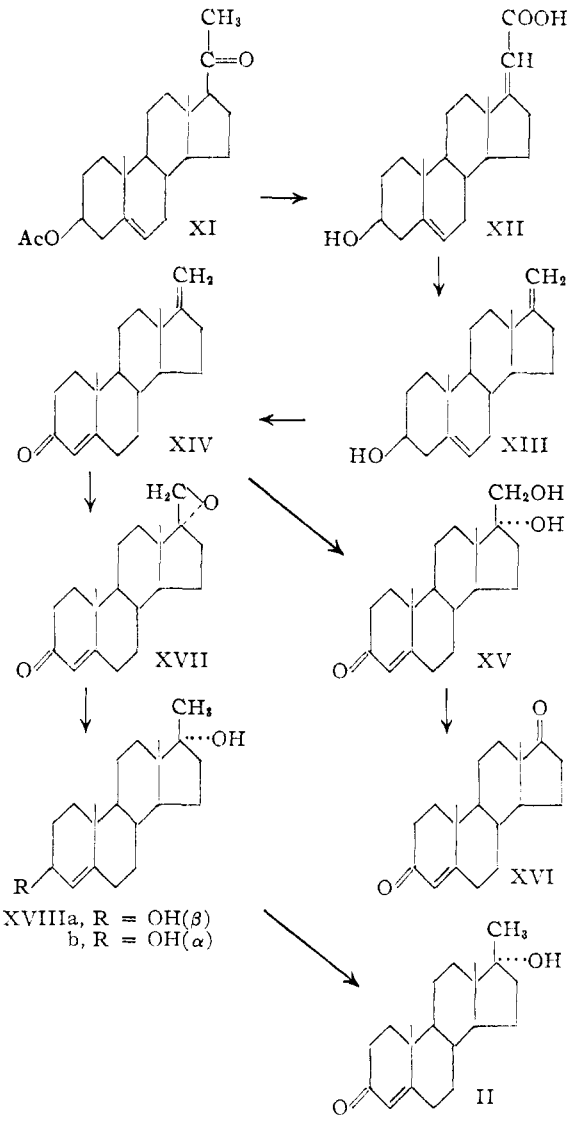
(13) R. E. Marker and R. B. Wagner, THIS JOURNAL, **64**, 1842 (1942).

(14) *Cf.* M. L. Sherrill and E. S. Matlack, *ibid.*, **59**, 2134 (1937).

(15) L. Ruzicka, V. Prelog and P. Meister, *Helv. Chim. Acta*, **28**, 1651 (1945).



The next step involved Oppenauer oxidation of VI, whereby $\Delta^{4,16}$-androstadien-3-one (VIII) was formed (86%). This compound agreed well in properties with those described for VIII as obtained by pyrolysis of the benzoate of epitestosterone.[11b] Moreover it had the very strong odor of urine, as reported.[11b] The preferential oxide formation in ring D of VIII by means of perbenzoic acid to yield 16α,17α-oxido-Δ^4-androsten-3-one (IX) and the lithium aluminum hydride reduction of the latter to Δ^4-androstene-3β,17α-diol (Xa) already have been described.[10] We subjected the total reduction product (probably containing some of the 3α,17α-diol Xb in addition to Xa) to preferential oxidation at C-3 with manganese dioxide, as had been the corresponding 17β-epimer.[16,17] There resulted a 68% yield (based on the oxido-ketone IX) of epitestosterone (Ia), further characterized as its acetate (Ib). Both these compounds exhibited properties in good agreement with those reported[3] and final structure proof was obtained by direct comparison of the manganese dioxide oxidation product with an authentic sample of Ia.



The above described successful synthesis of epitestosterone, based on the decarboxylation of the 3β-hydroxy-$\Delta^{5,16}$-etadienic acid (V), led us to investigate the synthesis of 17-methylepitestosterone (II) by an analogous route based on the decarboxylation of 3β-hydroxy-$\Delta^{5,17,(20)}$-pregnadien-21-oic acid (XII). This latter acid is prepared most conveniently from Δ^5-pregnen-3β-ol-20-one acetate (XI) through conversion to the 5,6,17,21-tetrabromide, followed by treatment with sodium iodide to 17-bromo-21-iodo-Δ^5-pregnen-3β-ol-20-one acetate and Favorskii rearrangement with potassium hydrox-

(16) F. Sondheimer, C. Amendolla and G. Rosenkranz, THIS JOURNAL, **75**, 5930 (1953).

(17) The manganese dioxide was prepared as described by O. Mancera, G. Rosenkranz and F. Sondheimer (*J. Chem. Soc.*, 2189 (1953)) with the exception that the precipitated reagent was washed only with water (the methanol and ether washings being omitted) before drying at 120–130°. This reagent has been found superior in a number of cases to that obtained by methanol washing, since the dioxide oxidizes methanol to formaldehyde and thereby becomes partially deactivated. It is recommended in general that the methanol washing be omitted.

ide.[18] When the resulting crude acid XII (or the purified product) was heated with quinoline in the presence of copper chromite under the conditions used with V, smooth decarboxylation occurred and 17-methylene-Δ^5-androsten-3β-ol (XIII) was produced in 85% yield. In this instance the reaction took place even in the absence of copper chromite, although the yield of XIII was then only 46%. The formulation of the decarboxylation product as XIII, rather than the corresponding 17-methyl-Δ^{16}-compound or a structure arising from rearrangement of the carbon skeleton, follows from the infrared spectrum which showed the bands at 1652 and 890 cm.$^{-1}$ characteristic of a terminal methylene group[19] and from its eventual transformation to Δ^4-androstene-3,17-dione (XVI) (*vide infra*). This method for producing 17-methyleneandrostane derivatives appears to be superior both as regards yield and ease of operation to that involving pyrolysis of 17β-hydroxy-17α-methylandrostanes or 17α-hydroxy-17β-methylandrostanes over copper sulfate.[4,20,21]

Oppenauer oxidation of XIII led to the corresponding Δ^4-3-ketone, 17-methylene-Δ^4-androsten-3-one (XIV) (79%). This substance agreed fairly well in properties with those reported for a compound believed to be XIV (*cf.* ref. 20) prepared in poor yield by the pyrolysis of either of the isomeric 17-methyltestosterones.[4] The structure of the product was confirmed through osmium tetroxide hydroxylation to 17β-hydroxymethyl-Δ^4-androsten-17α-ol-3-one (XV), which on lead tetraacetate cleavage (*cf.* ref. 20) furnished Δ^4-androstene-3,17-dione (XVI), identified with an authentic specimen.[22]

Oxidation of XIV with 0.9 equivalent of perbenzoic acid proceeded smoothly and produced the 17,20-monoxide, 17α,20-oxido-17β-methyl-Δ^4-androsten-3-one (XVII),[23] in 62% yield as the only transformation product to be isolated.[24] Finally the oxide XVII was reduced with lithium aluminum hydride to what is presumably a mixture of 17β-methyl-Δ^4-androstene-3β,17α-diol (XVIIIa) and the 3α,17α-diol XVIIIb, which without purification was re-oxidized at C-3 with manganese dioxide[17] in chloroform at room temperature. The resulting 17-methylepitestosterone (II), produced in 64% yield, was shown to be a tertiary carbinol as it was recovered unchanged on attempted acetylation and it agreed well in properties with those reported for the known compound.[4] The production of the 17α-hydroxy-17β-methyl compound, rather than the 17β-hydroxymethyl isomer, by lithium aluminum hydride reduction of the 17α,-20-oxide was to be expected since such reductions appear to give rise mainly to the axial hydroxy compounds.[10,25]

Experimental[26]

17-Epitestosterone (Ia) and 18-Nor-17-methyl-$\Delta^{4,17(18)}$-androstadien-3-one (IV) from Testosterone (IIIa).—Testosterone (15 g.) dissolved in 60 cc. of dry pyridine was allowed to stand at room temperature with 15 g. of *p*-toluenesulfonyl chloride for 24 hours at room temperature. The solution then was poured into ice-water and the precipitate was collected and washed well with dilute hydrochloric acid and water. In this way 21.3 g. (92.5%) of the crude *p*-toluenesulfonate IIIb with m.p. 163–168° was obtained (reported[6b] m.p. 169–171°).

A solution of 10 g. of the crude derivative IIIb in 200 cc. of glacial acetic acid was refluxed with 10 g. of potassium acetate for 24 hours. Addition of water and extraction with ether yielded a residue which on crystallization from ether–pentane–ether or from aqueous acetone furnished a total of 0.75 g. of the ψ-androstene derivative IV with m.p. 105–108°. The oily mother liquors were saponified by being refluxed in 200 cc. of methanol with 5 g. of potassium hydroxide dissolved in 5 cc. of water for 1 hour. Water then was added and the product was extracted with ether. The solvent was evaporated and the residue was chromatographed on 300 g. of neutral alumina. The fractions eluted with hexane–benzene (8:2 to 4:6) on crystallization from pentane produced another 0.52 g. of IV with m.p. 100–107° (total, 1.27 g. or 21%). The analytical sample was obtained by crystallization from pentane–ether and finally sublimation *in vacuo*. It showed m.p. 113–114°, $[\alpha]D$ + 84°, λ_{max} 240 mμ, log ϵ 4.23, ν_{max} 1660 cm.$^{-1}$, no free hydroxyl band, strong positive reaction in the Miescher test for ψ-androstenes.[7,11c] It may be identical with the substance of m.p. 108–111° obtained by Madaeva and Lur'i[6b] by acetolysis of IIIb.

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.07; H, 9.58.

The next crystalline chromatography fractions were eluted with benzene–ether (8:2 to 6:4) and after crystalliza-

(18) (a) R. E. Marker, H. M. Crooks, E. M. Jones and A. C. Shabica, THIS JOURNAL, **64**, 1276 (1942); (b) P. L. Julian and W. J. Karpel, *ibid.*, **72**, 362 (1950). The latter authors reported the Favorskii rearrangement to proceed in practically quantitative yield, without, however, divulging experimental details. We have found the reaction (when carried out under conditions similar to those described by Marker, *et al.*[18a]) to produce in about 85% yield a crude acid with a m.p. about 30° lower than that of pure XII, from which the latter could be obtained by repeated crystallization. It is possible that the low m.p. of the crude acid is due to the presence of the isomeric $\beta\gamma$-unsaturated acid (3β-hydroxy-$\Delta^{5,16}$-pregnadien-21-oic acid) since both the crude Favorskii product and the pure acid XII gave very similar yields on decarboxylation (*cf.* R. T. Arnold, R. W. Amidon, O. C. Elmer and R. M. Dodson, *ibid.*, **72**, 2871, 4359 (1950)).

(19) N. Sheppard and D. M. Simpson, *Quart. Revs.*, **6**, 26 (1952).

(20) L. Ruzicka, P. Meister and V. Prelog, *Helv. Chim. Acta*, **30**, 867 (1947).

(21) 17-Methyleneandrostanes also have been obtained from the corresponding 17β-hydroxy-17α-methyl derivatives, albeit in very poor yield, by dehydration with boiling acetic anhydride–pyridine or with phosphorus oxychloride–pyridine (S. A. Julia and H. Heusser, *ibid.*, **35**, 2080 (1952)).

(22) It is of interest to note that the $\Delta^{17(20)}$-ethylenes XIII and XIV, the glycol XV, as well as the oxide XVII, are transformed to new products when treated with mineral acids (*e.g.*, the glycol XV on attempted fission with periodic acid yielded a substance with m.p. 170–172° which differed from Δ^4-androstene-3,17-dione). The structures of these products are now under investigation.

(23) The α-configuration of the oxide function is assigned on the basis of the general α-attack at C-17.[5] *Cf.* also the formation of 17α,20-oxides by perbenzoic acid oxidation of 20-acetoxy-$\Delta^{17(20)}$-pregnenes (T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, THIS JOURNAL, **73**, 184 (1951); **74**, 483 (1952)).

(24) This despite the fact that 17-ethylidene-Δ^4-androsten-3-one, the 20-methyl homolog of XIV, has been shown to yield at least 3 different products (none isolated in the pure state in more than a few per cent. yield) on oxidation with 1.5 equivalents of monoperphthalic acid (L. Ruzicka, M. W. Goldberg and E. Hardegger, *Helv. Chim. Acta*, **25**, 1297 (1942)). The reaction of this ethylidene compound with 0.9 equivalent of perbenzoic acid now has been shown also to lead predominantly to the 17α,20-monoxide (E. Batres, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **77**, 4155 (1955)).

(25) *Cf.* P. A. Plattner, A. Fürst, F. Koller and H. H. Kuhn, *Helv. Chim. Acta*, **37**, 258 (1954), and reference cited there. See also A. H. Soloway, W. J. Considine, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **76**, 2941 (1954), for the lithium aluminum hydride reduction of a 20-acetoxy-17α,20-oxidoallopregnane to the 17α,20-diol.

(26) Melting points are uncorrected. Unless noted otherwise rotations were determined at 20° in chloroform and ultraviolet absorption spectra in 95% ethanol solution. We are grateful to Mrs. P. Lopez and staff for these measurements as well as for the infra-red spectra which were determined in chloroform solution on a Perkin–Elmer model 12C single beam spectrophotometer with sodium chloride prism. Thanks also ar due to Mrs. A. Gonzalez for the microanalyses.

tion from acetone–hexane yielded a substance with m.p. 173–174°, which has not been investigated further. Finally the fractions eluted with benzene–ether (2:8) and ether on crystallization from chloroform–hexane produced 0.31 g. (5%) of 17-epitestosterone with m.p. 212–216° (Kofler), raised to 220–222° (Kofler) on further purification. It proved to be identical (mixture m.p., infrared comparison) with the material prepared by the method described below.

$\Delta^{5,16}$-Androstadien-3β-ol (VI).—A solution of 10 g. of 3β-hydroxy-$\Delta^{5,16}$-etiadienic acid (V) (m.p. 248–253°, reported m.p. 248–250°, [12a] 256°[12b]) in 100 cc. of freshly distilled quinoline was refluxed with 1 g. of copper chromite for 4 hours (590 mm.). Water then was added, the product was extracted with ether and the ether layer was washed with dilute hydrochloric acid, sodium carbonate solution and water. The residue obtained by evaporation of the dried extract could be purified by direct crystallization, but it was found more efficient to chromatograph it on 500 g. of neutral alumina. The fractions eluted with benzene–ether (8:2 to 4:6) on crystallization from acetone–hexane furnished 5.81 g. (67.5%) of $\Delta^{5,16}$-androstadien-3β-ol with m.p. 133–137°. The analytical specimen showed m.p. 140–141°, $[\alpha]_D$ −68°, ν_{max} free hydroxyl band, no carbonyl bands, yellow color with tetranitromethane, marked urine-like odor.

Anal. Calcd. for $C_{19}H_{28}O$: C, 83.77; H, 10.36. Found: C, 84.07; H, 10.05.

Almost the same yield was obtained when the decarboxylation was carried out with a crude acid (m.p. 229–233°), obtained by the hydrolysis of 17-cyano-$\Delta^{5,16}$-androstadien-3β-ol acetate and probably contaminated with the 16α-methoxy compound.[12a] When the reaction was performed in the absence of copper chromite, no crystalline VI could be isolated and the acid V was recovered in 86% yield.

Androstan-3β-ol (VII).—A solution of 200 mg. of $\Delta^{5,16}$-androstadien-3β-ol (VI) in 20 cc. of acetic acid was shaken in hydrogen over 50 mg. of a pre-reduced platinum oxide catalyst at 586 mm. and 22°. After 50 minutes 2.1 equivalents of hydrogen had been absorbed and uptake ceased. Removal of catalyst and solvent followed by crystallization of the residue from acetone–pentane furnished 164 mg. of androstan-3β-ol with m.p. 148–150°, $[\alpha]_D$ −3° (reported[15] m.p. 151–152°, $[\alpha]^{22}_D$ 0°). There was no depression in m.p. on admixture with an authentic specimen (m.p. 147–150°).

$\Delta^{4,16}$-Androstadien-3-one (VIII).—A solution of 10 g. of $\Delta^{5,16}$-androstadien-3β-ol (VI) in 475 cc. of toluene and 75 cc. of cyclohexanone was distilled (*ca.* 50 cc. of distillate collected) to eliminate moisture, 5 g. of aluminum isopropoxide in 50 cc. of toluene was added and the solution was refluxed for 1 hour. Water then was added, the volatile components were removed by steam distillation and the residue was extracted with chloroform. Evaporation of the dried extract, followed by crystallization of the residue from chloroform–hexane, yielded 7.53 g. of $\Delta^{4,16}$-androstadien-3-one with m.p. 128–131°. Another 0.97 g. (total, 8.5 g., 86%) with m.p. 130–133° was obtained by chromatography of the mother liquors on neutral alumina. Further crystallization from the aforementioned solvent pair produced a pure specimen with m.p. 133–134°, $[\alpha]_D$ +121°, λ_{max} 240 mμ, log ϵ 4.22, ν_{max} 1660 cm.$^{-1}$, no free hydroxyl band, very strong odor of urine (reported[11b] m.p. 131.5–133.5°, $[\alpha]^{16}_D$ +123°).

Anal. Calcd. for $C_{19}H_{26}O$: C, 84.39; H, 9.69. Found: C, 84.48; H, 9.58.

16α,17α-Oxido-Δ^4-androsten-3-one (IX).—This compound was obtained in *ca.* 75% yield by the reaction of $\Delta^{4,16}$-androstadien-3-one (VIII) with 1 equivalent of perbenzoic acid, essentially as described previously.[10] It showed m.p. 208–209°, $[\alpha]_D$ +115°, λ_{max} 240 mμ, log ϵ 4.22, ν_{max} 1660 cm.$^{-1}$ (reported[10] m.p. 204–205°, $[\alpha]_D$ +112°).

Anal. Calcd. for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15. Found: C, 79.58; H, 9.19.

Δ^4-Androsten-17α-ol-3-one (17-Epitestosterone) (Ia).—The oxide IX (6 g.), dissolved in 250 cc. of dry tetrahydrofuran, was reduced by being refluxed for 2 hours with 6 g. of lithium aluminum hydride in 60 cc. of tetrahydrofuran. The excess reagent was destroyed by the careful addition of ethyl acetate, and the total reduction product was isolated by the sodium sulfate procedure.[16] The unpurified diols Xa and Xb (no appreciable absorption in the ultraviolet) then were dissolved in 600 cc. of chloroform and shaken with 30 g. of manganese dioxide[17] for 14 hours at room temperature. Another 15 g. of manganese dioxide was added and shaking was continued for a further 22 hours. The dioxide was removed, washed well with chloroform and the combined filtrates were evaporated. The solid residue could be crystallized directly, but was best purified by chromatography on 300 g. of neutral alumina. Crystallization of the fractions eluted with benzene–ether (8:2 to 6:4) from chloroform–hexane produced 4.12 g. (68%) of epitestosterone with m.p. 216–218° (capillary), 222–223° (Kofler), $[\alpha]_D$ +69° (ethanol), +86° (chloroform), λ_{max} 240 mμ, log ϵ 4.23, ν_{max} 1660 cm.$^{-1}$ and free hydroxyl band; reported[3] m.p. 220–221°, $[\alpha]^{22}_D$ +71.5° (ethanol). It was identical with a specimen (m.p. 217–219°) obtained from industrial testosterone mother liquors as evidenced by the non-depression of m.p. on admixture and infrared comparison.

Anal. Calcd. for $C_{19}H_{28}O_2$: C, 79.12; H, 9.78. Found: C, 79.23; H, 9.70.

The acetate (acetic anhydride–pyridine, 1 hour, steam-bath) after crystallization from acetone–hexane showed m.p. 114–115°, λ_{max} 240 mμ, log ϵ 4.22 (reported[3] m.p. 115.5–116.5°).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.55; H, 9.33.

$\Delta^{5,17(20)}$-Pregnadien-3β-ol-21-oic Acid (XII).[18]—A solution of 50 g. of 17-bromo-21-iodo-Δ^5-pregnen-3β-ol-20-one acetate (m.p. 154–157° dec.; prepared in *ca.* 65–70% yield from Δ^5-pregnen-3β-ol-20-one acetate)[18b] in 2 l. of methanol was refluxed for 2 hours with 100 g. of potassium hydroxide, previously dissolved in 100 cc. of water. The mixture was poured into ice-water, excess concentrated hydrochloric acid was added and the precipitate was collected, washed with water, dried and crystallized from chloroform–hexane. In this way 24.55 g. (84%) of the crude acid with m.p. 215–222° was obtained, which was of sufficient purity to be used for the next step.[18] On recrystallization from the above solvent pair the m.p. steadily rose and finally reached the constant value m.p. 252–254° (Marker, *et al.*,[18a] give m.p. 252–253°).

17-Methylene-Δ^5-androsten-3β-ol (XIII).—Crude $\Delta^{5,17(20)}$-pregnadien-3β-ol-21-oic acid (30 g., m.p. 215–222°) dissolved in 120 cc. of freshly distilled quinoline was refluxed with 1.5 g. of copper chromite for 4 hours. The product was isolated with ether, as described for VI and was then chromatographed on 400 g. of neutral alumina. The fractions eluted with benzene on crystallization from acetone–hexane produced 22.1 g. (85%) of 17-methylene-Δ^5-androsten-3β-ol with m.p. 128–131°. The analytical sample exhibited m.p. 133–134°, $[\alpha]_D$ −65°, ν_{max} 1652 and 890 cm.$^{-1}$ and free hydroxyl band, yellow color with tetranitromethane.

Anal. Calcd. for $C_{20}H_{30}O$: C, 83.86; H, 10.56. Found: C, 83.77; H, 10.33.

Very similar yields were obtained when the reaction was carried out with the purified acid XII (m.p. 247–250°). When the decarboxylation was performed by refluxing the acid (m.p. 215–222°) with quinoline for 4 hours, without added copper chromite, 46% of the decarboxylation product XIII with m.p. 129–134° was obtained and 26% of the acid XII with m.p. 247–252° was recovered.

17-Methylene-Δ^4-androsten-3-one (XIV).—The Oppenauer oxidation was carried out with 10 g. of 17-methylene-Δ^5-androsten-3β-ol (XIII) and 5 g. of aluminum isopropoxide in 75 cc. of cyclohexanone and 475 cc. of toluene as described above for VI (refluxing 1 hour). Isolation as before, followed by crystallization from acetone–pentane, furnished 7.80 g. (79%) of the Δ^4-3-one XIV with m.p. 129–131°. Further crystallization from acetone–hexane or from methanol yielded a specimen with m.p. 134–135°, $[\alpha]_D$ +124° (ethanol), +131° (chloroform), λ_{max} 240 mμ, log ϵ 4.23, yellow color with tetranitromethane (Miescher and Klarer[4] give m.p. 135–136°, $[\alpha]^{21}_D$ +136° (ethanol) for a compound believed to possess this structure).

Anal. Calcd. for $C_{20}H_{28}O$: C, 84.45; H, 9.92. Found: C, 84.14; H, 9.97.

17β-Hydroxymethyl-Δ^4-androsten-17α-ol-3-one (XV).—A solution of 0.64 g. of osmium tetroxide in 5 cc. of ether was added to a solution of 0.60 g. of 17-methylene-Δ^4-androsten-3-one (XIV) in 15 cc. of ether, containing 2 drops of pyri-

dine. After being allowed to stand overnight, the black precipitate was collected, washed well with ether, suspended in 75 cc. of ethanol and refluxed for 5 hours with 5 g. of sodium sulfite, previously dissolved in 25 cc. of water. The precipitate was removed, washed with ethanol and the filtrate was concentrated to 50 cc. Dilution with water, thorough extraction with chloroform and finally crystallization from acetone yielded 0.23 g. of the diolone XV with m.p. 222–228°. A further purified sample showed m.p. 234–236°, $[\alpha]_D$ +57°, λ_{max} 240 mμ, log ε 4.23, ν_{max} 1660 cm.$^{-1}$ and free hydroxyl band. This substance is probably identical with that of m.p. 238°, $[\alpha]^{20}D$ +51° (ethanol) described by Miescher and Klarer.[4]

Anal. Calcd. for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.80; H, 9.70.

Δ⁴-Androstene-3,17-dione (XVI) from XV.—Lead tetraacetate (420 mg.; *ca.* 90% pure), previously dissolved in 8 cc. of glacial acetic acid, was added to a solution of 100 mg. of the diolone XV in 4 cc. of acetic acid and the mixture was kept at room temperature for 24 hours. Water was added, the product was extracted with ether and the extract was washed with sodium carbonate solution and water, dried and evaporated. The oily residue was chromatographed on 8 g. of neutral alumina and the fractions eluted with benzene were crystallized from a little ether. The Δ⁴-androstene-3,17-dione thus produced showed m.p. 168–171° and this m.p. was undepressed on admixture with an authentic specimen (m.p. 169–171°).

17α,20-Oxido-17β-methyl-Δ⁴-androsten-3-one (XVII).—17-Methylene-Δ⁴-androsten-3-one (15 g.) dissolved in 90 cc. of chloroform was treated with 6.55 g. (0.9 equivalent) of perbenzoic acid in 110 cc. of chloroform and the solution was allowed to stand at room temperature for 18 hours. By this time all the peracid had been consumed. The solution was washed with aqueous sodium carbonate and water, dried and evaporated to dryness. The residue was purified most efficiently by chromatography on 500 g. of neutral alumina. The fractions eluted with benzene–hexane (8:2) and benzene on crystallization from acetone produced 9.8 g. (62%) of the oxide XVII with m.p. 179–182°. Further crystallization from acetone or methanol led to the analytical sample with m.p. 189–191°, $[\alpha]_D$ +112°, λ_{max} 240 mμ, log ε 4.24, ν_{max} 1660 cm.$^{-1}$.

Anal. Calcd. for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39. Found: C, 79.87; H, 9.40.

The above conditions were the best ones found in a series of experiments carried out with different proportions of monoperphthalic and perbenzoic acid at different temperatures. The use of 0.7 equivalent of perbenzoic acid at room temperature gave some recovery of starting material, while 2.0 equivalents gave mainly a polar hydroxylic material (*cf.* ref. 24) and only little of the mono-oxide XVII.

17β-Methyl-Δ⁴-androsten-17α-ol-3-one (17-Methylepitestosterone) (II).—The oxide XVII (5 g., m.p. 179–182°) dissolved in 300 cc. of dry tetrahydrofuran was reduced with 5 g. of lithium aluminum hydride in 250 cc. of tetrahydrofuran (30 minutes refluxing). The diol mixture XVIIIa and XVIIIb was isolated as described above for Xa, b and without purification was dissolved in 500 cc. of chloroform and oxidized by being shaken with 30 g. of manganese dioxide[17] for 16 hours. The dioxide was removed, washed with chloroform and the filtrate was evaporated. The crystalline residue was purified best by chromatography on 250 g. of neutral alumina. Crystallization of the fractions eluted with benzene–ether (6:4) from acetone–hexane furnished 3.21 g. (64%) of 17-methylepitestosterone with m.p. 175–177°. The analytical sample showed m.p. 181–182°, $[\alpha]_D$ +68° (ethanol), +82° (chloroform), λ_{max} 240 mμ, log ε 4.23, ν_{max} 1660 cm.$^{-1}$ and free hydroxyl band; reported[4] m.p. 182–183°, $[\alpha]^{21}D$ +66° (ethanol), +72° (chloroform).

Anal. Calcd. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.25; H, 9.86.

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[CONTRIBUTION FROM THE DEPARTMENT OF PLANT NUTRITION, UNIVERSITY OF CALIFORNIA, BERKELEY]

Photosynthesis by Isolated Chloroplasts. III. Evidence for Complete Photosynthesis[1]

BY M. B. ALLEN, DANIEL I. ARNON,[2] J. B. CAPINDALE, F. R. WHATLEY AND LOIS J. DURHAM

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Isolated chloroplasts, when illuminated, were found to fix CO_2 with simultaneous evolution of oxygen. This is regarded as complete extracellular photosynthesis since the observed CO_2/O_2 ratio was found to be approximately 1/1 in agreement with the well-known photosynthetic quotient of intact cells and among the products of photochemical CO_2 fixation by isolated chloroplasts were starch, phosphorylated sugars, amino acids and organic acids. Some of the factors influencing CO_2 fixation by isolated chloroplasts are described.

Heretofore, it has generally been accepted that "complete photosynthesis—that is, reduction of carbon dioxide to carbohydrates, and oxidation of water to oxygen, at low temperature and with no energy supply except visible light—has never been achieved outside the living cell."[3] The discovery of two new light-dependent reactions of isolated chloroplasts, CO_2 fixation[4] and photosynthetic phosphorylation[4-7] (the anaerobic esterification of inorganic phosphate, forming the pyrophosphate bonds of adenosine triphosphate), raised the question whether this extracellular photosynthetic activity of chloroplasts could be regarded as complete photosynthesis. To meet the criteria laid down by the above definition, evidence was required that CO_2 is reduced to carbohydrates by isolated chloroplasts, and that CO_2 fixation is accompanied by the evolution of oxygen. This paper presents evidence on both these points, leading to the conclusion that chloroplasts are the cytoplasmic structures in which the complete photosynthetic process is carried out, both inside and, under suitable conditions, outside the living cell. The relation of CO_2 fixation to other light-dependent reactions of isolated chloroplasts and a discussion of a general concept of photosynthesis supported by these findings will be given in another paper.[8]

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(8) D. I. Arnon, M. B. Allen and F. R. Whatley (manuscript in preparation).