Steroids. Part XIII. The Conversion of Ergosterol into Progesterone.*

By FRANCIS JOHNSON, G. T. NEWBOLD, and F. S. SPRING.

[Reprint Order No. 4886.]

The conversion of ergosterol into 3β-hydroxypregnan-20-one is described, thus completing a route from the yeast sterol to progesterone (cf. *Chem. and Ind.*, 1953, 1230).

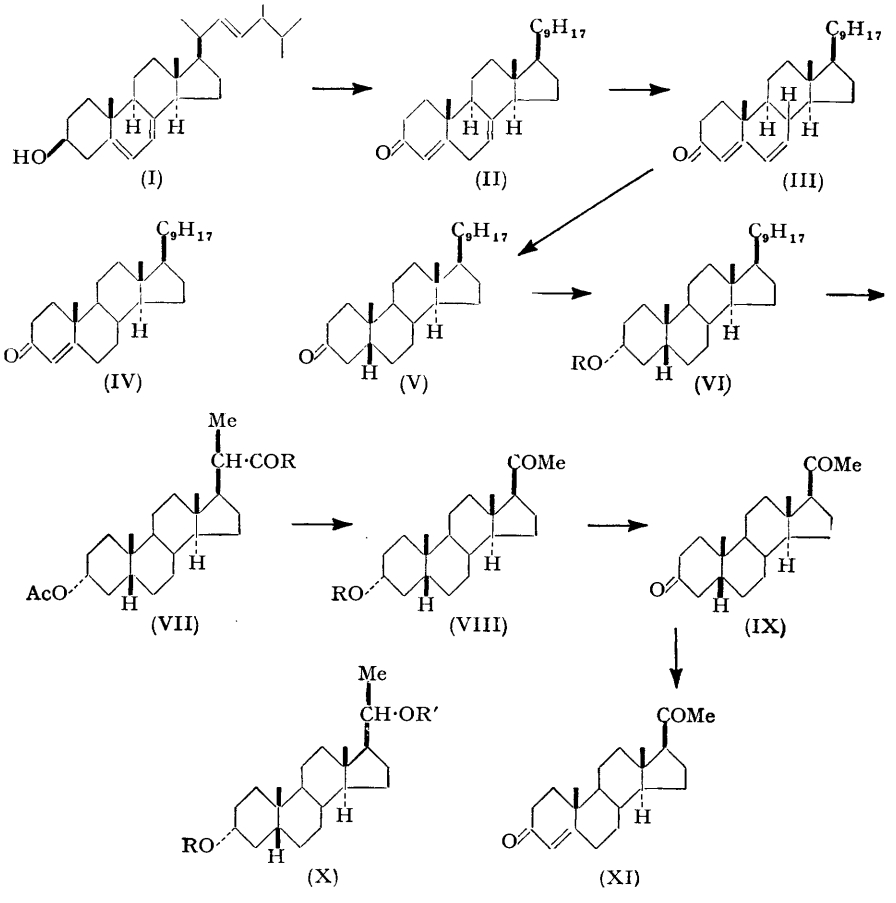
MICROBIOLOGICAL oxidation of progesterone (XI) gives 11α-hydroxyprogesterone (Peterson, Murray, Eppstein, Reineke, Weintraub, Meister, and Leigh, *J. Amer. Chem. Soc.*, 1952, **74**, 5933) which can be transformed into cortisone by a ten-step route of high overall efficiency (Mancera, Ringold, Djerassi, Rosenkranz, and Sondheimer, *ibid.*, 1953, **75**, 1286). The limited availability of progesterone led us to explore the possibility of employing ergosterol (I) as a starting material for its partial synthesis.

Oxidation of ergosterol to ergosterone (II) by the Oppenauer method (*Rec. Trav. chim.*, 1937, **56**, 137) gave a highly coloured product requiring extensive purification. A considerable improvement was made by using aluminium *tert.*-butoxide in toluene with *cyclo*hexanone as hydrogen acceptor. The method of Barton, Cox, and Holness (*J.*, 1949, 1771) was used for the rearrangement of (II) to *iso*ergosterone (ergosta-**4** : **6** : 22-trien-3-one) (III). Selective hydrogenation of *iso*ergosterone, with a palladium catalyst in the presence of alkali, gave 5β-ergost-22-en-3-one (V) in high yield.

Reduction of *iso*ergosterone (III) to ergosta-4 : 22-dien-3-one (IV) was also accomplished by partial hydrogenation in the presence of a palladium catalyst in benzene. Reduction

* Part XII, *J.*, 1954, 1223.

of Δ^8-11-oxo-steroids with lithium in liquid ammonia gives the corresponding saturated-11-oxo-steroid (Turnbull, Chamberlin, Reinhold, Erickson, Ruyle, Chemerda, and Tishler, *J. Amer. Chem. Soc.*, 1952, **74**, 2696; Sondheimer, Yashin, Rosenkranz, and Djerassi, *ibid.*, p. 2696), whereas with excess of lithium in liquid ammonia in the presence of methanol the saturated 11α-hydroxy-steroid is obtained (Sondheimer *et al.*, *loc. cit.*). More recently Barton, Ives, and Thomas (*Chem. and Ind.*, 1953, 1180) found that reduction of Δ^4-3-oxo-steroids with lithium in liquid ammonia leads to the saturated 5α-ketones. We find that reduction of the conjugated dienone (III) with excess of lithium in liquid ammonia in the presence of methanol gives ergosta-4 : 22-dien-3-one (IV) in good yield. Hydrogenation of this in the presence of palladium in an alkaline medium also gives 5β-ergost-22-en-3-one (V). When (V) is crystallised from methanol containing a trace of mineral acid, it readily



forms a ketal from which the parent ketone is re-formed by hydrolysis with aqueous mineral acid.

Reduction of 5β-ergost-22-en-3-one with lithium aluminium hydride, followed by acetylation, gives 5β-ergost-22-en-3α-yl acetate (VI; R = Ac) in good yield, and hydrolysis then gives 5β-ergost-22-en-3α-ol (VI; R = H); the last compound has been prepared by Barton, Cox, and Holness (*loc. cit.*) by sodium–propanol reduction of 5β-ergost-22-en-3-one (V) which they obtained together with (IV) by careful fractionation of the complex mixture obtained by partial hydrogenation of *iso*ergosterone (III) in presence of platinum in ethyl acetate.

Ozonolysis of 5β-ergost-22-en-3α-yl acetate (VI; R = Ac) in chloroform at −45° and decomposition of the ozonide with zinc dust and acetic acid gave a mixture of 3α-acetoxy-

bisnorcholan-22-al (VII; R = H) and 2 : 3-dimethylbutyraldehyde, and each was characterised as its 2 : 4-dinitrophenylhydrazone. The aldehyde (VII; R = H) was further characterised and identified by oxidation to 3α-acetoxybisnorcholanic acid (VII; R = OH) which had previously been obtained by side-chain degradation of lithocholic acid (Reindel and Niederlander, *Ber.*, 1935, **68**, 1969; Sawlewicz and Reichstein, *Helv. Chim. Acta*, 1937, **20**, 949).

Treatment of 3α-acetoxybisnorcholan-22-al (VII; R = H) with acetic anhydride and potassium acetate gave an oily enol acetate, ozonolysis of which, followed by decomposition with zinc dust and acetic acid, gave 20-oxopregnan-3α-yl acetate (VIII; R = Ac) as major product; hydrolysis yielded the alcohol (VIII; R = H). The last compound has been prepared from lithocholic acid (Meystre and Miescher, *Helv. Chim. Acta*, 1946, **29**, 33) and also from pregnane-3 : 20-dione (IX) by preferential reduction either catalytically (Marker, Kamm and Wittle, *J. Amer. Chem. Soc.*, 1937, **59**, 1841; Butenandt and Müller, *ibid.*, 1938, **71**, 191) or with sodium borohydride (Mancera *et al.*, *loc. cit.*). A second, amorphous (minor) product from the ozonolysis of the enol-acetate was readily separated by chromatography, being much more strongly held by alumina. On acetylation it yielded 3α : 20α-diacetoxypregnane (X; R = R′ = Ac). The isolation of this diacetate is attributed to the formation of a small quantity of 3α-acetoxypregnan-20α-ol (X; R = Ac, R′ = H) during the zinc treatment of the ozonolysis product. The diol (X; R = R′ = H) has been isolated from pregnancy urine by Hartman and Locher (*Helv. Chim. Acta*, 1935, **18**, 160) and has been obtained by sodium and *iso*propyl alcohol reduction of 3α-hydroxypregnan-20-one (VIII; R = H) (Butenandt and Müller, *loc. cit.*).

Oxidation of 3α-hydroxypregnan-20-one (VIII; R = H) yielded pregnane-3 : 20-dione (IX) (Marker and Kamn, *J. Amer. Chem. Soc.*, 1937, **59**, 1373) from which progesterone (XI) may be obtained by Butenandt and Schmidt's method (*Ber.*, 1934, **67**, 1901).

Since this work was completed, similar partial syntheses of progesterone from ergosterol have been briefly described by the Upjohn group (*Chem. Eng. News.*, 1953, 3977) and Daglish, Green, and Poole (*Chem. and Ind.*, 1953, 1207).

Experimental

For general instructions see *J.*, 1954, 1219, 1224.

Ergosterone (*Ergosta*-4 : 7 : 22-*trien*-3-*one*).—A solution of ergosterol (50 g.) in dry toluene (500 c.c.) and *cyclo*hexanone (375 g.) was distilled until 50 c.c. of distillate had been collected. A solution of freshly prepared aluminium *tert.*-butoxide (50 g.) in dry toluene (400 c.c.) was then added rapidly and the solution heated under reflux for 2 hr. The cooled mixture was washed with sulphuric acid (4 × 1000 c.c.; 4%), water, 10% aqueous sodium hydrogen carbonate, and water, and dried (Na_2SO_4). The solvents were removed under reduced pressure and the residue heated at 138—140°/10^{-4} mm. to remove self-condensation products of *cyclo*hexanone; neglect of this precaution caused a marked reduction of the yield. The residue, crystallised from acetone–light petroleum (b. p. 60—80°), gave ergosterone (40·5 g.) as pale yellow needles, m. p. 123—126°, which was used for the next stage without further purification. A specimen, twice recrystallised from the same solvent, yielded ergosterone, m. p. 134°, $[\alpha]_D$ −12° (*c*, 4·5) (Found: C, 84·8; H, 10·5. Calc. for $C_{28}H_{42}O$: C, 85·2; H, 10·7%). Light absorption: Max. at 2400 Å (ε 13,300). It gives a yellow colour with tetranitromethane in chloroform. Oppenauer (*loc. cit.*) gives m. p. 131·5—132°, $[\alpha]_D$ −15·7°; Heilbron, Kennedy, Spring, and Swain (*J.*, 1938, 869) give m. p. 132°, $[\alpha]_D$ −0·8°; Wetter and Dimroth (*Ber.*, 1937, **70**, 1665) give m. p. 132°, $[\alpha]_D$ −0·5°, −0·8°.

iso*Ergosterone* (*Ergosta*-4 : 6 : 22-*trien*-3-*one*).—Ergosterone (16 g.) in dry chloroform (150 c.c.) was treated with a stream of dry hydrogen chloride at 0° for 1 hr. The red solution was washed with water, 10% aqueous potassium carbonate, and water, and dried (Na_2SO_4). Recrystallisation of the product from ethyl acetate–methanol yielded *iso*ergosterone, m. p. 100° (14·5 g.), which was used for the next stage. A sample twice crystallised from the same solvent separated as thick rods, m. p. 106—107°, $[\alpha]_D$ −24·5° (*c*, 1·0) (Found: C, 84·9; H, 10·9%). Light absorption: Max. at 2840 Å (ε = 28,000). It gives a pale yellow colour with tetranitromethane in chloroform. Heilbron, Kennedy, Spring, and Swain (*loc. cit.*) give m. p. 108°, $[\alpha]_D$ −30°; Wetter and Dimroth (*loc. cit.*) give m. p. 110°; Barton, Cox, and Holness (*loc. cit.*) give m. p. 105°.

Ergosta-4 : 22-dien-3-one.—(*a*) *iso*Ergosterone (0·5 g.) in thiophen-free benzene (20 c.c.) was shaken with a suspension of palladised strontium carbonate (2%; 0·25 g.) in benzene (10 c.c.) under hydrogen for 45 min.; absorption then approximated to 1 mol. The product was crystallised from aqueous acetone, to yield ergosta-4 : 22-dien-3-one (0·2 g.) as needles, m. p. 129—130°, $[\alpha]_D$ +42° (*c*, 1·4) (Found : C, 84·9; H, 11·3. Calc. for $C_{28}H_{44}O$: C, 84·8; H, 11·2%). Light absorption : Max. at 2400 Å (ε 18,700). It gives a pale yellow colour with tetranitromethane in chloroform. Barton, Cox, and Holness (*loc. cit.*) give m. p. 127·5—128·5°, $[\alpha]_D$ +43°, +44°.

(*b*) *iso*Ergosterone (1·0 g.) in dry ether (50 c.c.) and methanol (5 c.c.) was added during 3 min. to a solution of lithium (0·5 g.) in liquid ammonia (200 c.c.). Stirring was continued for 35 min. and the ammonia allowed to evaporate overnight. The residue was diluted with water, and the product isolated with ether and crystallised from aqueous acetone, to give ergosta-4 : 22-dien-3-one (0·6 g.) as needles, m. p. and mixed m. p. 129—130°, $[\alpha]_D$ +41° (*c*, 0·9) (Found : C, 84·8; H, 11·2%). Light absorption : Max. at 2420 Å (ε 18,000).

5β-Ergost-22-en-3-one.—(*a*) *iso*Ergosterone (2·0 g.) in dry ethanol (50 c.c.) and a solution of potassium hydroxide (1·5 g.) in dry ethanol (30 c.c.) were shaken in hydrogen with freshly prepared 10% palladised charcoal (0·25 g.) in dry ethanol (10 c.c.). After 40 min., 2 mols. of hydrogen had been absorbed and the filtered mixture was worked up with ether, to give 5β-ergost-22-en-3-one in almost quantitative yield; this separates from ethanol as plates, m. p. 110°, $[\alpha]_D$ −5·2° (*c*, 2·1) (Found : C, 84·6; H, 11·8. Calc. for $C_{28}H_{46}O$: C, 84·35; H, 11·6%). Light absorption : Max. at 2070 (ε 1100) and 2700 Å (ε 107). 5β-Ergost-22-en-3-one gives a pale yellow colour with tetranitromethane in chloroform. Barton, Cox, and Holness (*loc. cit.*) give m. p. 110·5°, $[\alpha]_D$ −2°, −1°.

(*b*) Ergosta-4 : 22-dien-2-one (1·0 g.) was hydrogenated as described in (*a*) until absorption approximated to 1 mol. The product was 5β-ergost-22-en-3-one (0·45 g.) and it separated from ethanol as plates, m. p. and mixed m. p. 109—110°, $[\alpha]_D$ −5° (*c*, 2·0) (Found : C, 84·2; H, 11·7%). The 2 : 4-dinitrophenylhydrazone separates from benzene–ethanol as yellow needles, m. p. 197—198° (Found : N, 9·8. Calc. for $C_{34}H_{50}O_4N_4$: N, 9·7%).

3 : 3-*Dimethoxy-5β-ergost-22-ene.*—Crystallisation of 5β-ergost-22-en-3-one from methanol containing a trace of mineral acid gave 3 : 3-*dimethoxy-5β-ergost-22-ene* (72%) as blades, m. p. 84—85·5°, $[\alpha]_D$ −5·5° (*c*, 2·5) (Found : C, 81·4; H, 11·9; OMe, 14·7. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8; OMe, 14·0%). Light absorption : Max. at 2040 Å (ε 1900).

Hydrolysis. The dimethyl ketal (100 mg.) in dioxan (40 c.c.) was heated in aqueous sulphuric acid (0·5 c.c. of concentrated acid in 10 c.c. of water) under reflux for 1 hr. The product was isolated with ether and crystallised from ethanol, to give 5β-ergost-22-en-3-one (65 mg.) as plates, m. p. and mixed m. p. 109—111°, $[\alpha]_D$ −5·4° (*c*, 1·7).

5β-Ergost-22-en-3α-yl Acetate.—5β-Ergost-22-en-3-one (5·92 g.) in dry ether (100 c.c.) was added during 15 min. to a boiling solution of lithium aluminium hydride (6·0 g.) in dry ether (250 c.c.). After 1 hr., acetone (50 c.c.) was added, and the product isolated in the usual manner. Acetylation by warm acetic anhydride and pyridine followed by crystallisation from either ethanol or ethyl acetate–methanol gave 5β-ergost-22-en-3α-yl acetate (4·5 g.) as plates, m. p. 114—115°, $[\alpha]_D$ +9·2° (*c*, 1·3) (Found : C, 81·6; H, 11·7. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·4%). Barton, Cox, and Holness (*loc. cit.*) give m. p. 114—115°, $[\alpha]_D$ +16°.

The acetate (136 mg.) was refluxed for ¾ hr. with methanolic potassium hydroxide (25 c.c.; 4%); isolation through ether gave 5β-ergost-22-en-3α-ol in quantitative yield; it separates from methanol as needles, m. p. 149—150°, $[\alpha]_D$ −6·2° (*c*, 1·6) (Found : C, 83·6; H, 12·3. Calc. for $C_{28}H_{48}O$: C, 83·9; H, 12·1%); Barton and Holness (*loc. cit.*) give m. p. 149—150°, $[\alpha]_D$ −4°. Reacetylation of the alcohol with acetic anhydride–pyridine at 80° gave 5β-ergost-22-en-3α-yl acetate, m. p. and mixed m. p. 114—115°, $[\alpha]_D$ +10° (*c*, 1·4).

3α-Acetoxybisnorcholan-22-al.—Ozonised oxygen was passed through a solution of 5β-ergost-22-en-3β-yl acetate (5·0 g.) in dry chloroform (150 c.c.) at −45° until a blue colour persisted. The solution was allowed to attain room temperature, glacial acetic acid (50 c.c.) and zinc dust (10 g.) were added, and the mixture was stirred for 2 hr. The filtered mixture was distilled in steam. After 1 l. of distillate had been collected, the non-volatile product was isolated by means of ether and crystallised from ethanol, to give the aldehyde (3·0 g.) as plates, m. p. 115—118°. Recrystallisation from ethanol or aqueous acetone gave *3α-acetoxybisnorcholan-22-al* as plates, m. p. 121—123°, sintering at 115°, $[\alpha]_D$ +36° (*c*, 1·25) (Found : C, 77·1; H, 10·4. $C_{24}H_{38}O_3$ requires C, 77·0; H, 10·2%). The aldehyde does not show high-intensity light absorption above 2200 Å or give a colour with tetranitromethane in chloroform. The 2 : 4-*dinitrophenylhydrazone* of 3α-acetoxybisnorcholan-22-al, prepared in the usual manner, was purified by filtration of its benzene solution through a short column of Grade II alumina; it

separates from ethyl acetate as yellow needles, m. p. 205° (Found : N, 9·8. $C_{30}H_{42}O_6N_4$ requires N, 10·1%).

Extraction of the steam-distillate with chloroform gave an oil which on treatment with Brady's reagent yielded the 2 : 4-dinitrophenylhydrazone of 2 : 3-dimethylbutyraldehyde, separating from methanol as orange blades, m. p. 125 (lit., m. p. 124—125°).

3α-*Acetoxybisnorcholanic Acid*.—3α-Acetoxybisnorcholan-22-al (225 mg.) in glacial acetic acid (20 c.c.) was treated at 15° with a solution of chromium trioxide (50 mg.) in glacial acetic acid (10 c.c.) during 10 min. Next morning, methanol was added and the acidic product, which forms an insoluble sodium salt, was isolated in the usual manner. 3α-Acetoxybisnorcholanic acid (175 mg.) separates from acetone–light petroleum (b. p. 60—80°) as needles, m. p. 221—222°, $[\alpha]_D$ +21° (*c*, 1·4) (Found : C, 73·8; H, 9·8. Calc. for $C_{24}H_{38}O_4$: C, 73·8; H, 9·8%). Sawlewicz and Reichstein (*loc. cit.*) give m. p. 213—219° for this acid. The *methyl ester*, prepared by using ethereal diazomethane, separates from aqueous acetone as plates, m. p. 108—109°, $[\alpha]_D$ +29° (*c*, 2·8) (Found : C, 74·3; H, 10·2. $C_{25}H_{40}O_4$ requires C, 74·2; H, 10·0%).

20-*Oxopregnan*-3α-*yl Acetate*.—A mixture of 3α-acetoxybisnorcholan-22-al (m. p. 115—118°, 3·4 g.), freshly fused potassium acetate (1·75 g.), and acetic anhydride (25 c.c.) was kept at 135° for 6 hr., then poured into water containing some pyridine, and the product, isolated by use of ether as a brown gum, was dissolved in benzene–light petroleum (b. p. 60—80°) (200 c.c.; 1 : 9) and adsorbed on a column of Grade II alumina (50 g.). The column was washed with the same solvent mixture (400 c.c.), and the combined eluates were evaporated under reduced pressure, to give a colourless viscous oil (2·63 g.); in contrast to the parent aldehyde, the enol-acetate gives a yellow colour with tetranitromethane. The enol-acetate (2·6 g.) in chloroform (120 c.c.) was ozonised and the ozonide treated with zinc by the method described for the preparation of 3α-acetoxybisnorcholan-22-al, the steam-distillation being omitted. The product (2·1 g.), isolated by means of chloroform, was adsorbed from light petroleum (80 c.c.; b. p. 60—80°) on grade II alumina (20 × 2·5 cm.), and the column washed with light petroleum (160 c.c.; b. p. 60—80°) and benzene–light petroleum (b. p. 60—80°) (160 c.c.; 1 : 9). Evaporation of the combined eluates gave a gum (300 mg.) which was not examined. Further elution with benzene–light petroleum (b. p. 60—80°) (1·7 l.; 1 : 9 and 300 c.c.; 1 : 1) gave 20-oxopregnan-3α-yl acetate (1·2 g.) which separates from light petroleum (b. p. 60—80°) as thick rods, m. p. 100—101°, $[\alpha]_D$ +123° (*c*, 0·7) (Found : C, 76·8; H, 10·1. Calc. for $C_{23}H_{36}O_3$: C, 76·6; H, 10·1%). The keto-acetate was undepressed in m. p. when mixed with a specimen, m. p. 99—100°, $[\alpha]_D$ +121° (*c*, 1·0), prepared by reduction of pregnane-3 : 20-dione, followed by acetylation (Mancera, Ringold, Djerassi, Rosenkranz, and Sondheimer, *loc. cit.*).

3α : 20α-*Diacetoxypregnane*.—Continued elution of the alumina column described above, with benzene–methanol (200 c.c.; 1 : 1), gave an amorphous solid (280 mg.). This was acetylated (acetic anhydride and pyridine at 80°), and the product chromatographed in light petroleum (30 c.c.; b. p. 60—80°) on grade II alumina (10 × 1·0 cm.). Light petroleum (b. p. 60—80°) eluted a solid (170 mg.), crystallisation of which from light petroleum (b. p. 60—80°) gave 3α : 20α-diacetoxypregnane as needles, m. p. 180°, $[\alpha]_D$ +35° (*c*, 1·1) (Found : C, 74·0; H, 10·0. Calc. for $C_{25}H_{40}O_4$: C, 74·2; H, 10·0%). Hartman and Locher (*loc. cit.*) give m. p. 182—183°, $[\alpha]_D$ +35·3° (in C_6H_6).

3α-*Hydroxypregnan*-20-*one*.—20-Oxopregnan-3α-yl acetate (237 mg.) in methanol (20 c.c.) were heated with potassium carbonate (400 mg.) in water (5 c.c.) and methanol (10 c.c.) under reflux for 1 hr. Isolation by means of ether gave 3α-hydroxypregnan-20-one (214 mg.), needles [from acetone–light petroleum (b. p. 60—80°)], m. p. 147—148°, $[\alpha]_D$ +110° (*c*, 0·9) (Found : C, 79·2; H, 10·9. Calc. for $C_{21}H_{34}O_2$: C, 79·2; H, 10·8%). Meystre and Miescher (*loc. cit.*) gave m. p. 154°, $[\alpha]_D$ +110°. A mixture with a specimen prepared by the method of Mancera *et al.* (*loc. cit.*) {m. p. 145—147°, $[\alpha]_D$ +108° (*c*, 0·75)} was undepressed in m. p. Oxidation of 3α-hydroxypregnan-20-one with chromium trioxide gave pregnane-3 : 20-dione (85% yield), crystallising from light petroleum (b. p. 60—80°) as thick rods, m. p. 120—121° (lit., 123°), $[\alpha]_D$ +112° (*c*, 1·0) (Found : C, 79·5; H, 10·5. Calc. for $C_{21}H_{32}O_2$: C, 79·7; H, 10·2%).

We are glad to express our thanks to Glaxo Laboratories Ltd. for grants in aid of this work and for gifts of material; the receipt of a Maintenance award (to F. J.) from the Department of Scientific and Industrial Research is gratefully acknowledged.

THE ROYAL TECHNICAL COLLEGE, GLASGOW. [*Received, December 14th*, 1953.]