593. *Steroids. Part V.* Partial Synthesis of* 9 : 11-*Anhydrocorticosterone Acetate* [21-*Acetoxypregna*-4 : 9(11)-*diene*-3 : 20-*dione*].

By R. CASANOVA, C. W. SHOPPEE, and (in part) G. H. R. SUMMERS.

Deoxycholic acid has been converted through *ætio*deoxycholic acid into methyl 3-oxoetia-4 : 9(11)-dienoate, which was also obtained from 9 : 11-anhydrocorticosterone by oxidation with periodic acid, and from corticosterone by oxidative degradation of the side-chain and dehydration of the resulting 11β-hydroxy-*ætio*-ester. By the diazo-ketone synthesis 3-oxoetia-4 : 9(11)-dienoic acid gave 21-acetoxypregna-4 : 9(11)-diene-3 : 20-dione (9 : 11-anhydrocorticosterone acetate),† which is one of the most active adreno-cortical hormones in respect of influence on mineral metabolism.

CORTICOSTERONE ACETATE (I) was found by Shoppee and Reichstein (*Helv. Chim. Acta*, 1943, **26**, 1316) to afford an anhydrocorticosterone † acetate, m. p. 159°, on gentle treatment with hydrochloric–acetic acid. This compound is now regarded as arising by an ionic 1 : 2-*trans*-elimination process (11β-OH/9α-H) and is formulated as (II*b*). Under more energetic conditions the anhydro-compound, m. p. 159°, was accompanied by a second anhydrocorticosterone acetate, m. p. 142°, which was shown to result from isomerisation of the former and was provisionally formulated as (III).

11 : 12-Anhydrocorticosterone acetate (III) was subsequently prepared by three different methods (ref. c ‡) and differs from both the above anhydro-compounds. It seemed therefore desirable to attempt to elucidate the structures of the compounds of m. p. 159° and 142°, and the present paper deals with the former of these, which is shown to be (II*b*). A future communication will deal with the anhydro-compound, m. p. 142°, which we regard provisionally as 8 : 9-dehydro-11-deoxycorticosterone acetate, although it has been formulated as 14 : 15-dehydro-11-deoxycorticosterone acetate by Fieser and Fieser (" Natural Compounds Related to Phenanthrene," Reinhold Publ. Corpn., New York, 3rd Edn., 1949, p. 409).

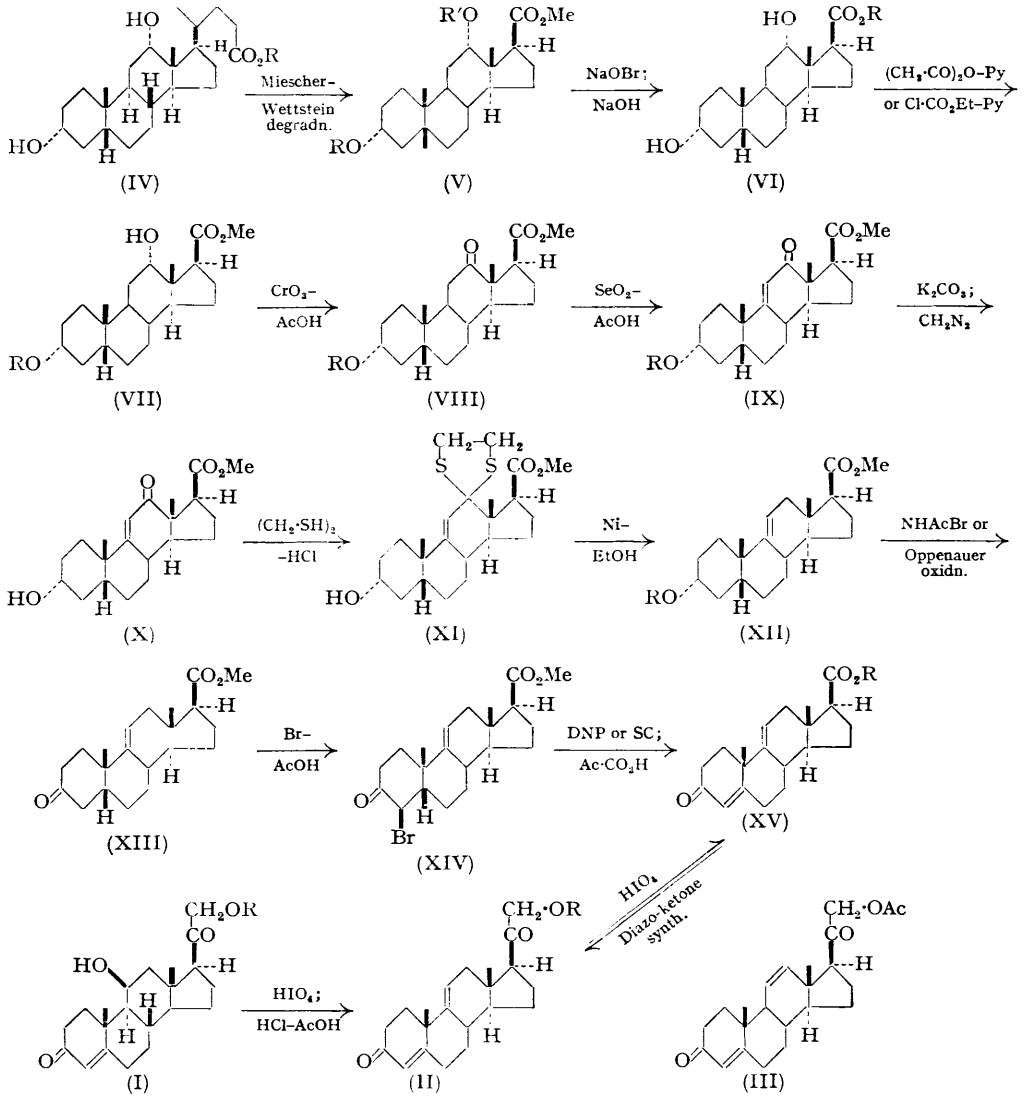
At the time (1949) that this work began, deoxycholic acid (IV*a*) was the only available starting material. Its methyl ester (IV*b*) with phenylmagnesium bromide gave 24 : 24-diphenylcholane-3α : 12α : 24-triol; acetic anhydride–pyridine at 100° converted this into a diacetate which, without purification, was dehydrated with hot acetic acid to 3α : 12α-diacetoxy-24 : 24-diphenylchol-23-ene. Application of the elegant degradation procedure of Meystre, Frey, Wettstein, and Miescher as modified by Meystre, Ehmann, Neher, and Miescher (ref. g) gave 12α-acetoxy-24 : 24-diphenylchola-20(22) : 23-dien-3α-ol, which was converted into the diacetate and oxidised with chromium trioxide at 15—20° to give, after partial hydrolysis with potassium carbonate, 12α-acetoxy-3α-hydroxypregnan-20-one (V*b*).

The further degradation of 3α : 12α-diacetoxypregnan-20-one (V*c*) has previously been carried out in five stages: conversion into the 21-benzylidene derivative, reacetylation, ozonolysis to the 20-keto-21-aldehyde, oxidation with periodic acid, and alkaline hydrolysis to give 36—44% yields of *ætio*deoxycholic acid (VI*a*) (refs. e, f). We have found that by oxidation with sodium hypobromite in aqueous dioxan at 0°, 3α : 12α-diacetoxypregnan-20-one gives in a single operation a 70% yield of pure *ætio*deoxycholic acid (VI*a*).

Methyl *ætio*deoxycholate (VI*b*), as its 3-(methyl succinate) (VII*a*), was oxidised with chromium trioxide to the 12-oxo-ester (VIII*a*) as described by M. Sorkin and Reichstein (ref. j; cf. Schwenk, Riegel, Moffett, and Stahl, *J. Amer. Chem. Soc.*, 1943, **65**, 549); this

* Part IV, *J.*, 1953, 1709. † Use of the prefix " anhydro " in this series to denote formation of an ethylenic bond by loss of H and OH from within one molecule is, of course, irregular. The systematic use of " anhydro " is to denote formation of an –O– bridge by loss of water between two OH in separate molecules (cf. acetic anhydride) or in one molecule (cf. anhydro-sugars). However, since the irregular use has been customary for some time in certain steroid papers and shows the relation of the products to corticosterone, these anhydro-names are used in the Introduction to this paper, but systematic names are used in the Experimental section.

‡ References given thus are to be found on p. 2985 annexed to the reaction scheme.



[Py = pyridine; DNP = 2 : 4-$(NO_2)_2C_6H_3$·NH·NH_2; SC = NH_2·CO·NH·NH_2].

	M. p.	$[\alpha]_D$†	Ref.‡
(I*a*) R = H	180°	+223° E	a
(I*b*) R = Ac	145	+195 A	a, b
(II*a*) R = H	[not isolated]		
(II*b*) R = Ac	159	+129 A	b, k
(III)	180	+178 A	c
(IV*a*) R = H	176	+ 53 E	d
(IV*b*) R = Me	81	+ 56 A	d
(V*a*) R = R′ = H	167	+165 A	e, f, g
(V*b*) R = H; R′ = Ac	214	+148 A	e, g
(V*c*) R = R′ = Ac	118	+190 A	e, f, g
(VI*a*) R = H	298	—	e, h
(VI*b*) R = Me	115/145	+105 M	h, i
(VII*a*) R = CO·$[CH_2]_2$·CO_2Me	99	—	j
(VII*b*) R = CO_2Et	Amorph.	—	k
(VIII*a*) R = CO·$[CH_2]_2$·CO_2Me	152°	+115° C	k
(VIII*b*) R = CO_2Et	125/156	+138 C	k
(IX*a*) R = CO·$[CH_2]_2$·CO_2Me	193	+116 C	k
(IX*b*) R = CO_2Et	161	+103 C	k
(X)	178	+109 C	k
(XI)	178	—	k
(XII*a*) R = H	131	+ 69 C	k
(XII*b*) R = Ac	128/136	+ 88 A	l, m, n, o
(XIII)	125	+ 56 C	k, l
(XIV)	154	+ 84 C	k
(XV*a*) R = H	240 *	—	k
(XV*b*) R = Me	105	+132 C	k

* With decomp; † A = acetone; C = chloroform; D = dioxan; E = ethanol; M = methanol.

‡ See p. 2985.

by further oxidation with selenium dioxide in boiling acetic acid (Schwenk and Stahl, *Arch. Biochem.*, 1949, **14**, 125) gave the 3-(methyl succinate) of 3α-hydroxy-12-oxoeti-9(11)-enoate (IX*a*), with an absorption maximum at 239 mμ (log ε 4·14), hydrolysed by potassium carbonate to methyl 3α-hydroxy-12-oxoeti-9(11)-enoate (X) with an absorption maximum at 240 mμ (log ε 4·10). This sequence of reactions was also carried through with the 3α-hydroxyl group protected by condensation with ethyl chloroformate (cf. Borsche, *Ber.*, 1924, **57**, 1620; Fieser and Rajagopalan, *J. Amer. Chem. Soc.*, 1950, **72**, 5530; 1952, **74**, 3309); the 3α-ethoxycarbonyloxy-12α-hydroxy-ester (VII*b*) failed to crystallise but by oxidation with chromium trioxide gave the beautifully crystalline 3α-ethoxycarbonyloxy-12-oxo-ester (VIII*b*), converted by selenium dioxide–acetic acid into methyl 3*a*-ethoxycarbonyloxy-12-oxoeti-9(11)-enoate (IX*b*), showing maximum absorption at 239 mμ (log ε 4·07) and giving (X) by complete hydrolysis and re-esterification with diazomethane.

Reduction of the 12-oxo-ester (X) was unsuccessfully attempted by the Wolff–Kishner procedure, but was smoothly accomplished by conversion into the ethylene thioketal (XI) which by desulphurisation with W-7 Raney nickel gave methyl 3α-hydroxyeti-9(11)-enoate (XII*a*), characterised as the 3α-acetate (XII*b*). These compounds were first described by Lardon and Reichstein (ref. l); methyl 3α-acetoxy-11-oxoetianoate by energetic hydrogenation gave methyl 3α-acetoxy-11β-hydroxyetianoate, dehydrated by phosphorus oxychloride–pyridine at 18° to methyl 3α-acetoxyeti-9(11)-enoate (XII*b*), which by partial hydrolysis gave crude (XII*a*). The acetoxy-ester (XII*b*) has also been described, subsequently to our work, by Heymann and Fieser (ref. m), and, as an intermediate in their steroid total synthesis, by Woodward, Sondheimer, Taub, Heusler, and McLamore (refs. n, o). Acetylation of our 3α-hydroxy-ester (XII*a*) gave the 3α-acetate (XII*b*), double m. p. 127—128° and 135—136°, identical with specimens provided by Professor Reichstein and Professor Fieser.

Mild oxidation of 9 : 11-unsaturated steroids with chromium trioxide leads to 9 : 11-unsaturated 12-ketones and 9α : 11α-epoxides (Alther and Reichstein, *Helv. Chim. Acta*, 1943, **26**, 492; Seebeck and Reichstein, *ibid.*, 1943, **26**, 536; Lardon and Reichstein, ref. l; Reich and Lardon, *ibid.*, 1947, **30**, 329; Shoppee, *ibid.*, 1947, **30**, 766). Formation of such by-products was avoided by oxidation with aluminium *iso*propoxide–*cyclo*hexanone or, better, *N*-bromoacetamide in aqueous *tert.*-butanol (cf. Kritchevsky and Gallagher, *J. Amer. Chem. Soc.*, 1951, **73**, 184) whereby the 3α-hydroxy-ester (XII*a*) gave methyl 3-oxoeti-9(11)-enoate (XIII). Bromination in acetic acid furnished the crystalline 4β-bromo-ester (XIV) in which the configuration of the bromine atom is assigned by analogy with the conclusions of R. N. Jones *et al.* (*ibid.*, 1952, **74**, 2828; cf. Djerassi, *J. Org. Chem.*, 1947, **12**, 823; *J. Amer. Chem. Soc.*, 1949, **71**, 1003), and of Fieser and Ettore (*ibid.*, 1953, **75**, 1700). The 4β-bromo-ester (XIV) was recovered unchanged after treatment with dry pyridine at 100—120° for 12 hours, but was smoothly dehydrobrominated by treatment with 2 : 4-dinitrophenylhydrazine (Mattox and Kendall, *ibid.*, 1948, **70**, 882; *J. Biol. Chem.*, 1950, **185**, 601; McGuckin and Kendall, *J. Amer. Chem. Soc.*, 1952, **74**, 3951) or, better,

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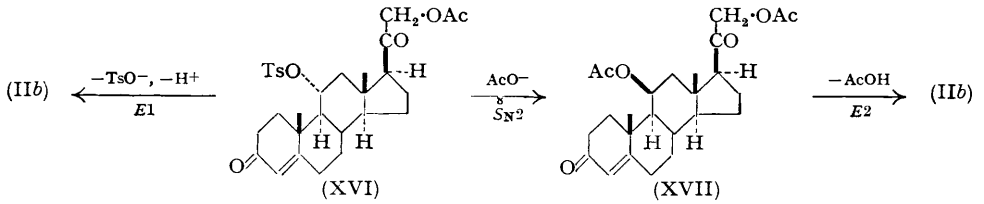
a, Reichstein, *Helv. Chim. Acta*, 1937, **20**, 953.
b, Shoppee and Reichstein, *ibid.*, 1943, **26**, 1316.
c, Meystre and Wettstein, *ibid.*, 1948, **31**, 1890; 1949, **32**, 880; von Euw and Reichstein, *ibid.*, 1948, **31**, 2076.
d, Reichstein and M. Sorkin, *ibid.*, 1942, **25**, 797.
e, Reichstein and von Arx, *ibid.*, 1940, **23**, 747.
f, Hoehn and Mason, *J. Amer. Chem. Soc.*, 1938, **60**, 1493, 2824; 1939, **61**, 1614.
g, Meystre, Frey, Wettstein, and Miescher, *Helv. Chim. Acta*, 1944, **27**, 1815; Meystre, Ehrmann, Neher, and Miescher, *ibid.*, 1945, **28**, 1252.
h, Mason and Hoehn, *J. Amer. Chem. Soc.*, 1938, **60**, 2824; 1939, **61**, 1614.
i, Wenner and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 965.
j, M. Sorkin and Reichstein, *ibid.*, 1946, **31**, 1218.
k, This paper.
l, Lardon and Reichstein, *Helv. Chim. Acta*, 1945, **30**, 1420.
m, Heymann and Fieser, *J. Amer. Chem. Soc.*, 1951, **73**, 4054.
n, Woodward, Sondheimer, and Taub, *ibid.*, p. 4057.
o, Woodward, Sondheimer, Taub, Heusler, and McLamore, *ibid.*, 1952, **74**, 4223.
p, Mason, Hoehn, McKenzie, and Kendall, *J. Biol. Chem.*, 1937, **120**, 719.
q, von Euw and Reichstein, *Helv. Chim. Acta*, 1947, **30**, 205.

with semicarbazide (Hershberg, *J. Org. Chem.*, 1948, **13**, 542; Koechlin, Kritchevsky, and Gallagher, *J. Biol. Chem.*, 1950, **184**, 393) to give, after exchange with pyruvic acid–acetic acid buffered with sodium acetate (Sidgwick, " Organic Chemistry of Nitrogen," Oxford Univ. Press, 1942, p. 394; Conant and Bartlett, *J. Amer. Chem. Soc.*, 1932, **54**, 2881; Hershberg, *loc. cit.*), methyl 3-oxoetia-4 : 9(11)-dienoate (XV*b*), m. p. 104—105°, $[\alpha]_D$ +132°, $\lambda_{max.}$ 240 mμ (log ε 4·1).

3-Oxoetia-4 : 9(11)-dienoic acid (XV*a*) was obtained in the first instance in quantity insufficient to permit its conversion by the diazo-ketone synthesis into 9 : 11-anhydrocorticosterone acetate (II*b*). The latter was therefore hydrolysed with potassium hydrogen carbonate to 9 : 11-anhydrocorticosterone (II*a*) and this degraded by oxidation with periodic acid, to give after esterification with diazomethane methyl 3-oxoetia-4 : 9(11)-dienoate (XV*b*) identical with the synthetic product. Alternatively, corticosterone (I*a*) was oxidised with periodic acid to 11β-hydroxy-3-oxoeti-4-enoic acid (refs. p, q), dehydrated as the methyl ester by hot hydrochloric–acetic acid to give, after re-esterification, methyl 3-oxoetia-4 : 9(11)-dienoate (XV*b*). These reactions establish the structure of 9 : 11-anhydrocorticosterone acetate (II*b*).

{(Added in proof, June 15th, 1953). Since this paper was written, another preparation of 9 : 11-anhydrocorticosterone acetate has been described by Fried and Sabo (*J. Amer. Chem. Soc.*, 1953, **75**, 2273). 11-*epi*Corticosterone was successively converted into the 21-acetate and the 21-acetate 11α-toluene-*p*-sulphonate (XVI), which by treatment with sodium acetate in boiling acetic acid gave 9 : 11-anhydrocorticosterone acetate (II*b*), m. p. 160°, $[\alpha]_D$ +128°. Superficially the process appears to involve *cis*-9 : 11-elimination [9α-H(polar)/11α-TsO(equatorial)] of toluene-*p*-sulphonic acid; it seems more probable that the reaction proceeds either by heterolysis of the 11α-toluene-*p*-sulphonate group to give a carbonium ion which achieves neutrality by elimination of the tertiary 9α-hydrogen atom as a proton, or by acetolysis of the 11α-toluene-*p*-sulphonate group to give, with inversion of configuration, the unknown corticosterone 11β : 21-diacetate (XVII), which then undergoes synchronous ionic *trans*-9 : 11-elimination [9α-H(polar/11β-AcO(polar)] of acetic acid (cf. Reichstein and Shoppee, *Helv. Chim. Acta*, 1940, **23**, 729; Shoppee, *ibid.*, p. 740)}.



Subsequently the partial synthesis was completed by Dr. Summers. 3-Oxoetia-4 : 9(11)-dienoic acid (XV*a*) with oxalyl chloride (cf. Sheehan, *J. Amer. Chem. Soc.*, 1952, **74**, 360) gave the acid chloride (cf. Wilds and Shunk, *J. Amer. Chem. Soc.*, 1948, **70**, 2427), which with diazomethane yielded the diazo-ketone, converted by treatment with acetic acid into 9 : 11-anhydro-corticosterone acetate (II*b*), m. p. 159°, $[\alpha]_D$ +129°, $\lambda_{max.}$ 240 mμ (log ε 4·12) (for infra-red spectrum see Experimental section).

9 : 11-Anhydrocorticosterone acetate (II*b*) was found to exhibit in the Everse–de Fremery test 2—3 times the activity of 11-deoxycorticosterone acetate, and to show approximately equal activity in the life-maintenance test in adrenalectomised young rats. Similarly 11 : 12-anhydrocorticosterone acetate (III) showed slightly lower activity than 11-deoxycorticosterone acetate in the life-maintenance test in rats and approximately equal activity in adrenalectomised dogs (ref. c; Wettstein, personal communication). It seemed therefore of interest to examine 9 : 11-anhydrocorticosterone (II*a*) by the new micro-bioassay for mineralocorticoid activity (Tait, Simpson, and Grundy, *Lancet*, 1952, 122) which is based on the urinary ^{24}Na : ^{42}K ratio in adrenalectomised rats; Dr. Tait and Mrs. Simpson find that 9 : 11-anhydrocorticosterone (II*a*) and its acetate (II*b*) yield single spots by paper chromatography, running slightly more polar than 11-deoxycorticosterone and its acetate respectively, and that 1 and 2 μg. of (II*a*) per rat give the same response

as 1 and 2 μg. of 11-deoxycorticosterone respectively, whilst a balanced assay on three groups of 8 rats gave the following values for the ^{24}Na : ^{42}K ratio :

Quantity administered (μg.)	1	2	4
11-Deoxycorticosterone	3·16	2·92	2·36
9 : 11-Anhydrocorticosterone	3·18	2·90	2·33

9 : 11-Anhydrocorticosterone is thus one of the most active of the adrenocortical hormones in respect of influence on mineral metabolism.

Finally, 9 : 11-anhydrocorticosterone (II*a*) may be an intermediate in the biochemical oxygenation of steroids at $C_{(11)}$ if the conversion, by perfusion through isolated adrenal glands, by treatment with adrenal homogenates, or by incubation with species of *Streptomyces*, of 11-deoxycorticosterone or its acetate into corticosterone (I*a*) * or of substance S acetate to substance M (cortisol) * (Pincus *et al.*, *J. Amer. Chem. Soc.*, 1949, **71**, 3261; *Arch. Biochem.*, 1950, **25**, 457; Sweat *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 4056; McGinty *et al.*, *Science*, 1950, **112**, 506; Kahnt and Wettstein, *Helv. Chim. Acta*, 1951, **34**, 1790; Haines *et al.*, *J. Amer. Chem. Soc.*, 1952, **74**, 2381), or the transformation, by fermentation with *Aspergillus niger* or *Rhizopus nigricans*, of 11-deoxycorticosterone or its acetate into 11-*epi*corticosterone * or of substance S into 11-*epi*-substance M * (Peterson, Murray, *et al.*, *J. Amer. Chem. Soc.*, 1951, **73**, 1871; 1953, **75**, 408, 412; Fried *et al.*, *ibid.*, 1952, **74**, 3962; Wettstein *et al.*, *Experientia*, 1952, **8**, 422), takes place by enzymic dehydrogenation succeeded by a biochemical non-Markovnikov hydration process. This possibility is analogous to the transformation in the Krebs cycle of succinic acid ⟶ fumaric acid ⟶ malic acid, and is being investigated by Dr. R. I. Dorfman (Worcester Foundation, Shrewsbury, Mass.) using 9 : 11-anhydrocorticosterone acetate (II) supplied by us. Similar experiments have recently been performed by Miescher, Wettstein, and Kahnt (*Acta Physiol. Latino Amer.*, 1953, **1**, in the press), who treated the 9 : 11-"anhydro"-derivative of substance M acetate with adrenal homogenates and demonstrated the formation of substance M (cortisol).

Experimental

For general directions see *J.*, 1953, 243, 540, 1709. $[\alpha]_D$ are in $CHCl_3$, ultra-violet absorption spectra were determined in EtOH on a Unicam SP.500, and infra-red spectra on a Perkin–Elmer double-beam instrument.

3α : 12α-*Diacetoxy*-24 : 24-*diphenylchol*-23-*ene*.—Methyl 3α : 12α-dihydroxycholanoate (IV*b*) (50 g.) (ref. d) by treatment with phenylmagnesium bromide (ref. f) gave 24 : 24-diphenylcholane-3α : 12α : 24-triol, m. p. 109° after recrystallisation from methanol; acetic anhydride–pyridine (3 hr. at 100°) and then refluxing acetic acid (Morsman, Steiger, and Reichstein, *Helv. Chim. Acta*, 1937, **20**, 1) afforded 3α : 12α-diacetoxy-24 : 24-diphenylchol-23-ene, m. p. 157—159° (56 g., 76%) after several recrystallisations from methanol. Another run furnished a 75% yield of product of m. p. 156—159°.

3α : 12α-*Diacetoxy*-24 : 24-*diphenylchola*-20(22) : 23-*diene*.—Much difficulty was experienced in obtaining this compound as described by Miescher and Wettstein (*loc. cit.*), but the following procedure † finally proved satisfactory. 3α : 12α-Diacetoxy-24 : 24-diphenylchol-23-ene (10 g.; m. p. 157—159°), dissolved in carbon tetrachloride (90 c.c.; purified and dried), was refluxed with *N*-bromosuccinimide (30 g.), with irradiation (3 × 100-w. bulbs) in sunlight and with vigorous mechanical stirring for 10 min. The yellow solution was cooled with ice and filtered, and the filtrate refluxed for 4 hr. with exclusion of moisture. After removal of carbon tetrachloride the residue was dissolved in acetone, from which 3α : 12α-diacetoxy-24 : 24-diphenylchola-20(22) : 23-diene slowly separated in needles, m. p. 138—141° (2·37—4·06 g. in 5 runs). Partial hydrolysis of the material from the acetone mother-liquors with hot aqueous-methanolic potassium carbonate precipitated the 12α-monoacetate, forming needles, m. p., 228—230°, on recrystallisation from benzene, and chromatography on aluminium oxide of the material from the alkaline mother-liquors gave, on elution with ether–benzene (1 : 4 and 1 : 1), further quantities of the 12α-monoacetate, reacetylated with acetic anhydride–pyridine at 100° to the 3α : 12α-diacetate. One run under apparently identical conditions gave no yellow colour and was a failure; another, with 45 g. of starting material, on the other hand, furnished

* No instance of 11α-hydroxylation occurring in any mammalian tissue has been reported.

† Adopted after discussion with Dr. A. Wettstein in Basle, for which we are most grateful.

the 3α : 12α-diacetate, m. p. 136° (27·7 g.), and the 12α-monoacetate, m. p. 220—230° (6·56 g.), which together correspond to a yield of 75%.

3α : 12α-*Diacetoxypregnan*-20-*one* (V*a*).—Miescher and Wettstein's procedure (*loc. cit.*, p. 1823) was slightly modified by working at 5—10° for 4 hr. with mechanical stirring, and keeping the solution for 16 hr. at 15—20°. 3α : 12α-Diacetoxy-24 : 24-diphenylchola-20(22) : 23-diene (m. p. 138—142°; 35·6 g.) thus gave an oxidation product (32·3—32·5 g.), separated by use of Girard's reagent T into non-ketonic material (7·7—7·0 g.) and crude partly crystalline 3α : 12α-diacetoxypregnan-20-one (V*c*) (23·2—23·5 g.). The latter with aqueous methanolic potassium carbonate for 16 hr. at 20° gave 12α-acetoxy-3α-hydroxypregnan-20-one (V*b*), m. p. 203—207° (6·9—8·5 g.) after recrystallisation from methanol; the material from the mother-liquors (11·5—12·5 g.), by chromatography on aluminium oxide (300 g.) and elution with ether–benzene (1 : 4; 5 × 600 c.c.), gave a further quantity of crude crystalline 12α-monoacetate (V*b*) (3·5—3·3 g.) (total yield, 47—50%). For purification it was found advantageous to hydrolyse the 12α-monoacetate (15·4 g.) with 5% aqueous-methanolic potassium hydroxide for 2 hr.; saturation with carbon dioxide, vacuum-evaporation, extraction with ether, washing, drying, concentration, and addition of pentane yielded pure 3α : 12α-dihydroxypregnan-20-one (V*a*), m. p. 169—170° (9·6 g.) and m. p. 165—167° (1·4 g.) (83% isolation). Similarly the crude crystalline 12α-monoacetate (6·8 g.) by hydrolysis gave the diol (V*a*), m. p. 168—169° (3·7 g.) and m. p. 165—168° (600 mg.). The mother-liquors from these hydrolyses were united and the resulting material (4·26 g.) chromatographed on aluminium oxide (120 g.) prepared in benzene; elution with ether (4 × 400 c.c.) gave the diol (V*a*), m. p. 166—169° (1·70 g.). Reacetylation of the pure diol (17 g.) with acetic anhydride (80 c.c.)–pyridine (60 c.c.) for 3 hr. at 100° gave the 3α : 12α-diacetate, m. p. 112—114° (19 g.), after crystallisation from pentane (overall yield, 42%).

To avoid the large-scale use of Girard's reagent T, oxidations were carried out, in 5 runs, with 3α : 12α-diacetoxy-24 : 24-diphenylchola-20(22) : 23-diene (m. p. 138—142°) in amounts ranging from 2 to 24 g., and the resultant oils partially hydrolysed with potassium carbonate, to give crude partly crystalline products which by chromatography on aluminium oxide and elution with benzene and ether–benzene (1 : 4) yielded the 12α-monoacetate (V*b*) (yields, 48—58%), purified by conversion into the diol (V*a*). In another run 50 g. of diene gave, after partial hydrolysis, 42 g. of crude product yielding by chromatography 19 g. (65%) of the 12α-monoacetate (Vb) m. p. 203—210°.

3α : 12α-*Dihydroxy*ætio*cholane*-17β-*carboxylic Acid* (ætio*Deoxycholic Acid*) (VI*a*).—3α : 12α-Diacetoxypregnan-20-one (V*c*) (m. p. 112—114°; 7·0 g.) in dioxan (70 c.c.) at 0° was treated dropwise with an ice-cold solution of sodium hypobromite [from sodium hydroxide (9·1 g.) in water (70 c.c.) and bromine (4·20 c.c.)] during 0·75 hr., with mechanical stirring. The mixture was stirred for 3 hr. at 0—15°, excess of sodium hypobromite destroyed with sodium hydrogen sulphite, 170 c.c. of water added, and ~200 c.c. of distillate removed in a vacuum. The solution was extracted with ether; the ethereal extract, after being shaken with 2N-sodium carbonate and water, gave a neutral oil (100 mg.). The alkaline solution, alkaline extract, and washings (with addition of a little 2N-sodium hydroxide), were heated for 2 hr. at 100° to ensure hydrolysis of acetyl groups, and added dropwise with mechanical stirring to ice-cold concentrated hydrochloric acid. The precipitated acid was filtered off, washed with water, and dried; recrystallisation from acetone–methanol of a specimen gave 3α : 12α-dihydroxy*ætio*cholane-17β-carboxylic acid (VI*a*), m. p. 294—296°. The whole of the material was treated with excess of ethereal diazomethane at 0° for 1 hr. to give, after washing, etc., methyl 3α : 12α-dihydroxy-*ætio*cholane-17β-carboxylate (VI*b*), double m. p. 114—116° and 140—143° (4·06 g., 69%). Earlier runs [with 10 g. of (V*c*)] gave yields of 55%; the mother-liquors from all runs were united and by chromatography on aluminium oxide and elution with ether and chloroform–ether (1 : 1) gave further amounts of the ester, m. p. 108° and 140°.

Methyl 3α-*Hydroxy*-12-*oxo*ætio*chol*-9(11)-*ene*-17β-*carboxylate* (X).—(a) *Use of* 3-(*methyl succinates*). Methyl 3α : 12α-dihydroxy*ætio*cholane-17β-carboxylate (VI*b*) by the method of M. Sorkin and Reichstein (ref. j) gave the 3-(methyl succinate) (VIII*a*), m. p. 151—153°, $[\alpha]_D^{16}$ +115° ± 2° (*c*, 1·38), in 92% yield. The united mother-liquors from several runs, by chromatography, yielded further material, m. p. 147—150°.

The dimethyl ester (VIII*a*) (3·0 g.) was refluxed in acetic acid (15 c.c.) with selenium dioxide (1·5 g.) for 18 hr. with exclusion of moisture. The hot solution was filtered, and the filtrate evaporated in a vacuum. The product, dissolved in ether–chloroform, was washed with acid, alkali, and ice-water, dried, and evaporated, to give a crystalline residue, m. p. 182—195° (1·3 g.); recrystallisation from ether–methanol gave the dimethyl ester (IX*a*) as yellowish

needles, m. p. 193°, $\lambda_{max.}$ 239—240 mμ (log ε 4·15). The mother-liquors, by evaporation and chromatography on aluminium oxide with elution with ether–benzene (1 : 1), gave a small quantity of the dimethyl ester (IX*a*), m. p. 185—193° after recrystallisation from acetone–methanol. The alkaline washings by acidification and extraction with ether yielded an oil; with ethereal diazomethane this gave a crystalline yellow product (1·61 g.), which was chromatographed on aluminium oxide (50 g.). Elution with benzene (4 × 150 c.c.) and with ether–benzene (1 : 1; 3 × 150 c.c.) gave, after recrystallisation from acetone–methanol or ether–methanol, the 3-(*methyl succinate*) (IX*a*) (534 mg.) of methyl 3α-hydroxy-12-oxo*ætio*chol-9(11)-ene-17β-carboxylate as colourless needles, m. p. 190—193°, $[\alpha]_D^{18}$ +116° ± 3° (*c*. 0·59), $\lambda_{max.}$ 239 mμ (log ε 4·14) (Found, after drying at 100°/0·01 mm. for 1 hr.: C, 67·8; H, 7·9. $C_{26}H_{36}O_7$ requires C, 67·8; H, 7·9%), giving no depression with the material from the neutral fraction and no yellow colour with tetranitromethane.

The dimethyl ester (IX*a*) (400 mg.) was refluxed with 2% methanolic potassium hydroxide (20 c.c.) for 4 hr. After addition of a few drops of water and saturation with carbon dioxide, methanol was removed completely in a vacuum, and the product acidified with hydrochloric acid and extracted with ether, to give a crude crystalline product which was esterified directly with ethereal diazomethane. The crystalline product, by chromatography on aluminium oxide (12 g.) and elution with ether–benzene (1 : 1), ether, and chloroform–ether (1 : 1), gave *methyl* 3α-*hydroxy*-12-*oxo*æ*tiochol*-9(11)*ene*-17β-*carboxylate* (X) as colourless needles (from ether), m. p. 175—177°, $[\alpha]_D^{19}$ +109° ± 2° (*c*, 1·605), $\lambda_{max.}$ 242 mμ (log ε 4·10) (Found, after drying at 70°/0·01 mm. for 2 hr.: C, 72·5; H, 8·6. $C_{21}H_{30}O_4$ requires C, 72·8; H, 8·7%), giving no yellow colour with tetranitromethane.

In subsequent larger-scale oxidations the dimethyl ester (IX*a*) was not isolated. The hot acetic acid solution (30 c.c.) obtained by oxidation of the methyl succinate (VIII*a*) (m. p. 150—152°; 9·37 g.) with selenium dioxide (3·0 g.) for 48 hr. was filtered, and the black precipitate of selenium washed with chloroform (~300 c.c.); the filtrate and washings were united and stirred rapidly with a solution of potassium chromate (6 g.) in 50% acetic acid (~200 c.c.) at 15° for 3 hr. The chloroform layer was separated, the aqueous layer was extracted with chloroform, and the united chloroform extracts were shaken with aqueous chromium trioxide at 15° for 2 hr. This procedure removed most of the colloidal selenium; the chloroform solution was washed thrice with water, dried, and evaporated. The resulting oil (8·0 g.) was dissolved in methanol (64 c.c.), a solution of sodium hydroxide (4·6 g.) in water (6·4 c.c.) added, and the mixture set aside at 15° for 2 hr. After addition of water, saturation with carbon dioxide, and removal of methanol in a vacuum, the alkaline liquid was allowed to flow slowly into a 10% solution of acetic acid (15 c.c.) at 100°. After 0·5 hr. on the steam-bath, the suspension was cooled and the precipitated needles were filtered off and dried (4·33 g.); esterification with ethereal diazomethane and crystallisation from ether gave methyl 3α-hydroxy-12-oxo*ætio*chol-9(11)-ene-17β-carboxylate (X) (3·24 g.) in needles, m. p. 176—178°. The materials from the mother-liquor, together with that obtained by ether-extraction of the aqueous acetic acid filtrate and esterification with diazomethane, by chromatography on aluminium oxide and elution with benzene–ether (1 : 1), ether, and chloroform–ether gave a further quantity (334 mg.), m. p. 174—178° (total yield, 51%).

(b) *Use of* 3α-*ethoxycarbonyloxy-derivatives*. Methyl 3α : 12α-dihydroxy*ætio*cholane-17β-carboxylate (VI*b*) (5 g., dried by azeotropic distillation with benzene and at 20°/0·01 mm. for 0·25 hr.), dissolved in dry pyridine (20 c.c.), was treated with ethyl chloroformate (4 c.c.; freshly distilled) dropwise at 0° with exclusion of moisture, and the mixture was kept at 15—20° for 17 hr. Working up gave a colourless oil, which did not crystallise, even after chromatography. After being dried at 40°/0·01 mm., this (5·8 g.) was treated in acetic acid (30 c.c.) with a 4% solution of chromium trioxide in acetic acid (40 c.c.) for 1·5 hr. at 15—20°. Excess of chromium trioxide was destroyed with methanol, and the mixture worked up to give a crystalline product, m. p. 112—120° (4·8 g.); a small portion recrystallised from methanol in long needles, double m. p. 125—130° and 150—155°. The material was purified by chromatography on aluminium oxide (150 g.) in pentane, whereby elution with benzene–pentane (1 : 1, 5 × 500 c.c.) and with benzene (6 × 500 c.c.) gave *methyl* 3α-*ethoxycarbonyloxy*-12-*oxo*æ*tiocholane*-17β-*carboxylate* (VIII*b*) (3·26 g.) as hexagonal prisms (from ether–pentane), m. p. 155—156°, $[\alpha]_D$ +138° ± 2° (*c*, 2·862) [Found, after drying at 65°/0·05 mm., for 2 hr.: C, 68·6; H, 8·6. $C_{24}H_{36}O_6$ requires C, 68·55; H, 8·6%). Further elution with ether–benzene (2 : 3; 4 × 500 c.c.) gave methyl 3 : 12-dioxo*ætio*cholane-17β-carboxylate (550 mg.), needles (from methanol), m. p. 169—171°.

The 3α-ethoxycarbonyloxy-derivative (VIII*b*) (2 g.) was refluxed in acetic acid (50 c.c.) with selenium dioxide for 18 hr., then filtered hot, and the dark red solution was evaporated completely in a vacuum. The residue was dissolved in ether, washed with acid, water, alkali, and water,

dried, and evaporated. The neutral product was dissolved in benzene, filtered through a column of aluminium oxide, and washed through with benzene and ether–benzene (1 : 1); the filtrate by evaporation yielded reddish needles, which were recrystallised several times from ether until only slightly coloured (m. p. 158—160°; 800 mg.). A sample by sublimation at 170—200°/0·05 mm. and recrystallisation from ether gave *methyl* 3α-*ethoxycarbonyloxy*-12-*oxo*-æ*tiochol*-9(11)-*ene*-17β-*carboxylate* (IX*b*) as colourless needles, m. p. 160—161°, $[\alpha]_D$ +103·5° + 2° (*c*, 0·612), $\lambda_{max.}$ 239 mμ (log ε 4·07) (Found, after drying at 95°/0·05 mm. for 2 hr.: C, 68·6; H, 8·2. $C_{24}H_{34}O_6$ requires C, 68·85; H, 8·2%), giving no yellow colour with tetranitromethane. The alkaline washings were acidified with concentrated hydrochloric acid and extracted with ether; the extract was washed repeatedly with water, dried, and evaporated, to give an acidic oil (800 mg.), which was esterified with ethereal diazomethane at 0°. The product obtained by working up was chromatographed on aluminium oxide (30 g.) prepared in pentane; elution with benzene–pentane (1 : 1; 2 × 80 c.c.) and with benzene (2 × 80 c.c.) gave the ethoxycarbonyloxy-derivative (IX*b*) (210 mg.), m. p. 160—161° after recrystallisation from ether (total yield, 50%).

This product (210 mg.) was refluxed with 1% methanolic potassium hydroxide (20 c.c.) for 1·5 hr.; after addition of a little water and saturation with carbon dioxide, methanol was removed completely in a vacuum, and the residue acidified and extracted with ether, to give a crystalline product, which was esterified by treatment with ethereal diazomethane at 0°. The resulting ester was chromatographed on aluminium oxide (5 g.) prepared in pentane, whereby elution with benzene, ether–benzene mixtures, and ether (20-c.c. eluates) gave the methyl ester (X) (158 mg.), m. p. 174° after recrystallisation from ether.

Methyl 3α-*Hydroxy*æ*tiochol*-9(11)-*ene*-17β-*carboxylate* (XII*a*).—Methyl 3α-hydroxy-12-oxoæ*tiochol*-9(11)-ene-17β-carboxylate (X) (m. p. 175—176°; 4·95 g.) was dissolved in pure dry chloroform (5 c.c.), ethanedithiol (6 c.c.) added, and the mixture cooled to −15° and treated with dry hydrogen chloride (~300 c.c.) with exclusion of moisture. After 16 hr. at 0°, the mixture was neutralised with solid sodium carbonate and extracted with ether. The ethereal extract was washed with ice-cold 5N-sodium hydroxide, water, 2N-hydrochloric acid, and water, dried, and evaporated. Recrystallisation from ether gave square plates of *methyl* 12-(*ethylenedithio*)-3α-*hydroxy*æ*tiochol*-9(11)-*ene*-17β-*carboxylate* (XI) (3·88 g.), m. p. 177—178° (Found, after drying at 65°/0·02 mm. for 2 hr.: S, 15·25. $C_{23}H_{34}O_3S_2$ requires S, 15·2%); a second crop (791 mg.), m. p. 175°, and a third crop (653 mg.), m. p. 174°, were obtained, whilst the mother-liquors gave material (625 mg.) which by chromatography on aluminium oxide (20 g.) and elution with ether–benzene (1 : 4, 3 × 60 c.c.; and 1 : 1, 3 × 60 c.c.) gave a further quantity (320 mg.), m. p. 172—176° after recrystallisation from ether (total yield, 94%).

The pure dithioketal (XI) (2·20 g.) was refluxed with W-7 Raney nickel (15 g.; Mozingo *et al.*, *J. Amer. Chem. Soc.*, 1943, **65**, 1013) in pure dry ethanol (100 c.c.) for 7 hr. After being kept overnight at 15—20°, the solution was filtered through aluminium oxide on a sintered-glass funnel and evaporated in a vacuum. Crystallisation from pentane gave *methyl* 3α-*hydroxy*-æ*tiochol*-9(11)-*ene*-17β-*carboxylate* (XII*a*) as needles (1·69 g., 94%), m. p. 131—132°, $[\alpha]_D^{19}$ +69° ± 2° (*c*, 1·79) (Found, after drying at 70°/0·02 mm. for 2 hr.: C, 75·95; H, 9·55. $C_{21}H_{32}O_3$ requires C, 75·85; H, 9·7%), giving a yellow colour with tetranitromethane–chloroform. Lardon and Reichstein (ref. l.) record double m. p. 102° and 124° for a crude product. The material (850 mg.) from the mother-liquors of this and other runs, by chromatography on aluminium oxide and elution with ether–benzene (1 : 5, 1 : 1), gave a further quantity (437 mg.) of the hydroxy-ester (XII*a*), m. p. 125—130°. Acetic anhydride–pyridine at 20° gave the acetoxy-ester (XII*b*) as prismatic needles from ether–pentane, with partial transformation at 126° into thin needles, m. p. 132°; this had m. p. 132—134°, remelting at 134°, when mixed with Lardon and Reichstein's preparation (ref. l.), and 132—134°, remelting at 134—135°, with that of Heymann and Fieser (ref. m.).

Methyl 3-*Oxo*æ*tiochol*-9(11)-*ene*-17β-*carboxylate* (XIII).—(*a*) Methyl 3α-hydroxyæ*tiochol*-9(11)-ene-17β-carboxylate (XII*a*) (m. p. 126—130°; 500 mg.), dissolved in *tert.*-butanol (10 c.c.), was treated with *N*-bromoacetamide (386 mg.), water (0·5 c.c.), and pyridine (0·75 c.c.), and the mixture kept at 15° for 16 hr. Excess of *N*-bromoacetamide was destroyed with sodium thiosulphate, and the solution evaporated in a vacuum; the product was extracted with ether, the extract washed with 2N-sodium carbonate, 2N-hydrochloric acid, and water, dried, and evaporated. The residue (m. p. 85—110°; 480 mg.) was chromatographed on aluminium oxide (15 g.) prepared in pentane. Elution with benzene–pentane (1 : 1; 3 × 50 c.c.) and with benzene (50 c.c.) gave methyl 3-oxoæ*tiochol*-9(11)-ene-17β-carboxylate (XIII) (200 mg.), m. p. 125—126°, $[\alpha]_D^{19}$ +56° ± 2° (*c*, 2·20), after recrystallisation from pentane (Found, after drying at 65°/0·01 mm. for 2 hr.: C, 76·35; H, 9·0. Calc. for $C_{21}H_{30}O_3$: C, 76·3; H, 9·15%) (Lardon

and Reichstein, ref. l., record m. p. 123°, $[\alpha]_D$ +63° in ethanol), which depressed the m. p. of the hydroxy-ester (XII*a*) to 101°; the *semicarbazone*, plates (from methanol), had m. p. 210—212° (decomp.) (Found, after drying at 75°/0·01 mm. for 1·5 hr.: N, 11·2. $C_{22}H_{33}O_3N_3$ requires N, 10·85%). Further elution with ether–benzene (1 : 4, 3 : 7, and 1 : 1) yielded unoxidised hydroxy-ester (XII*a*) (125 mg.), m. p. and mixed m. p. 125—129°. The total yield, allowing for recovered (XII*a*), was 53%. The above procedure, based on that of Kritchevsky and Gallagher (*J. Amer. Chem. Soc.*, 1951, **73**, 184), could not be completely standardised. Other runs gave yields of 35% and 40%; a four-fold increase in the quantity of pyridine and use of 250 mg. of (XII*a*), m. p. 125—130°, afforded a yield of 64%, but repetition with 1·69 g. of (XII*a*) gave only 32%. In two runs, a small amount of an unidentified substance, m. p. 240—250°, eluted with ether–benzene (1 : 9), was encountered.

(*b*) Methyl 3α-hydroxy*æt*iochol-9(11)-ene-17β-carboxylate (XII*a*) (500 mg.), dried by repeated azeotropic distillation with toluene and dissolved in pure dry toluene, with *cyclo*hexanone (10 c.c.), and aluminium *iso*propoxide (2 g., freshly distilled at 0·01 mm.), was refluxed under anhydrous conditions for 16 hr. After removal of toluene and *cyclo*hexanone by steam-distillation, the solution was acidified with 2N-sulphuric acid and extracted with ether. The semisolid product (411 mg.) was chromatographed on aluminium oxide (12 g.) in pentane. Elution with benzene–pentane mixtures gave oils, but use of benzene (2 × 40 c.c.) and ether–benzene (1 : 9; 2 × 40 c.c.) gave methyl 3-oxo*æt*iochol-9(11)-ene-17β-carboxylate (XIII) (82 mg.), m. p. 122° [mixed m. p. 125—127° with the specimen described under (*a*)]. After elution with ether–benzene (1 : 4; 40 c.c.) had yielded an oil (8 mg.), use of ether–benzene (3 : 7, 2 × 40 c.c.; and 1 : 1, 2 × 40 c.c.) gave unchanged hydroxy-ester (XII*a*) (76 mg.), m. p. 125—130° (20%).

Methyl 3-*Oxo*æti*ochola*-4 : 9(11)-*diene*-17β-*carboxylate* (XVb).—Methyl 3-oxo*æti*ochol-9(11)-ene-17β-carboxylate (XIII) (m. p. 120—122°; 306 mg.) was treated in acetic acid, whilst cooled and shaken, with 5·76 c.c. of a 0·161M-solution of bromine in acetic acid, added dropwise at such a rate that no considerable excess of bromine was present at any time (1 hr.). Sufficient ether was added, acetic acid removed by washing with water, 2N-sodium carbonate, and water, and the ethereal solution dried and concentrated to give, by addition of pentane, the bromo-ketone (220 mg.), m. p. 135—145°, raised by recrystallisation from ether to 145—150°. A specimen by repeated recrystallisation from ether gave *methyl* 4β-*bromo*-3-*oxo*æti*ochol*-9(11)-*ene*-17β-*carboxylate* (XIV) in plates, m. p. 151—154°, $[\alpha]_D^{20}$ +84° ± 2° (*c*, 0·477) (Found, after drying at 50°/0·01 mm. for 3 hr.: Br, 19·8. $C_{21}H_{29}O_3Br$ requires Br, 19·5%). The residue from this and other brominations, by reduction with zinc–acetic acid at 100° for 1 hr., furnished material which on chromatography gave the pure oxo-ester (XIII), m. p. 119—123°.

The 4β-bromo-ester (XIV) (m. p. 149—153°; 85 mg.) was treated in acetic acid (8 c.c.) with 2 : 4-dinitrophenylhydrazine (25 mg.) and anhydrous potassium acetate (12 mg.) at 65° in nitrogen for 1 hr. The red solution was evaporated in a vacuum, and the residue dissolved in chloroform; pyruvic acid (1·5 c.c.) and water (0·8 c.c.) were added and the mixture was kept at 65—70° in nitrogen. After 1 hr. and then 2 hr. pyruvic acid (0·8 c.c.) was added, and after 5 hr. the yellow-brown solution was cooled and extracted with ether. The extract was well washed with 2N-sodium carbonate and water, dried, and evaporated, to give a yellow oil (85 mg.), which was chromatographed on aluminium oxide (2·5 g.) prepared in pentane. Elution with benzene–pentane (1 : 1; 3 × 10 c.c.) and benzene (2 × 10 c.c.) gave crystalline fractions, m. p. ~100°, which were united and, recrystallised twice from ether–pentane (1 : 4), gave the 3-oxo-4 : 9(11)-diene-ester (XV*b*) as rods, m. p. 99—102°, $\lambda_{max.}$ 240 mμ (log ε 4·07).

Alternatively, the 4β-bromo-ester (XIV) (m. p. 146°; 100 mg.) in acetic acid (10 c.c.) was treated similarly with semicarbazide acetate [prepared by grinding together semicarbazide hydrochloride (75 mg.) and sodium acetate trihydrate (120 mg.) with a few drops of water and filtration]. After exchange with pyruvic acid (3 c.c.), the product was isolated in the usual way and crystallised partially by scratching (75 mg.). Chromatography as under (*a*) (p. 2990) gave the 3-oxo-4 : 9(11)-diene-ester (23 mg.) (XV*b*) as rods [from ether–pentane (1 : 4)], m. p. 100—102°. Recrystallisation from ether–pentane gave *methyl* 3-*oxo*æti*ochola*-4 : 9(11)-*diene*-17β-*carboxylate*, (XV*b*), m. p. 104—105°, $[\alpha]_D^{20}$ +132° ± 2° (Found, after drying at 50°/0·02 mm. for 4 hr.: C, 76·7; H, 8·55. $C_{21}H_{28}O_3$ requires C, 76·8; H, 8·6%). In a repetition, (XIV) (320 mg.) gave (XV*b*) (75 mg.), which was hydrolysed with hot 4% methanolic potassium hydroxide (2 c.c.) for 2 hr. After being worked up in the usual way, the crystalline acid fraction (65 mg.) was recrystallised from acetone–pentane, to give 3-oxo*æti*ochola-4 : 9(11)-diene-17β-carboxylic acid (XV*a*) as prisms m. p. 240—250° (decomp.).

21-*Acetoxypregna*-4 : 9(11)-*diene*-3 : 20-*dione* (II*b*).—3-Oxo*æti*ochola-4 : 9(11)-diene-17β-carb-

oxylic acid (XV*a*) (63 mg.) in benzene was treated with oxalyl chloride (50 mg.), and the mixture shaken for 0·5 hr. at 15—20° and set aside for 1 hr. Benzene and excess of oxalyl chloride were removed at 35—40°/10 mm., then, thrice, the residual yellow oil was dissolved in benzene and the benzene evaporated in a vacuum. The acid chloride, dissolved in ether–benzene (1 : 1, 5 c.c.), was treated with dry ethereal diazomethane at −5° for 1 hr.; evaporation furnished the diazo-ketone as an oil which solidified when rubbed with acetone–pentane. It was refluxed in acetic acid (5 c.c.) for 5 min. Acetic acid was removed in a vacuum, and the residue dissolved in ether, washed with water, 2N-sodium carbonate (twice), and water (thrice), and dried, and the solution evaporated. The resulting oil (44 mg.) solidified on trituration with ether–pentane and was chromatographed on neutralised aluminium oxide (1 g.) in pentane, with 5-c.c. eluates. Elution with pentane and benzene–pentane gave no material, whilst benzene–pentane yielded only oils; elution with benzene, and ether–benzene (1 : 19) gave material which crystallised when moistened with pentane and seeded with 21-acetoxypregna-4 : 9(11)-diene-3 : 20-dione (II). These fractions were united and sublimed at 180°/0·01 mm.; the sublimate (20 mg.), which crystallised on nucleation, was recrystallised from ether–pentane, to give (II) as prisms, m. p. and mixed m. p. 159°, giving a yellow colour with tetranitromethane and reducing ammoniacal silver solution immediately at 20°, and yielding an orange colour with concentrated sulphuric (green fluoresence against a dark background). The infra-red spectrum [in CS_2: bands at 1230 and 1756 (21-acetate), 1730 (20-carbonyl), 1677 (4 : 5-unsaturated 3-ketone), 804 and 823 cm.$^{-1}$ (>CR·CH·)] was identical with that of a genuine specimen prepared from corticosterone acetate.

Degradation of 21-*Acetoxypregna*-4 : 9(11)-*diene*-3 : 20-*dione* (II) *to Methyl* 3-*Oxo*æ*tiochola*-4 : 9(11)-*diene*-17β-*carboxylate* (XV*b*).—The dione (II) (m. p. 158—159°; 25 mg.), in methanol (2·5 c.c.) was hydrolysed by treatment with potassium hydrogen carbonate (25 mg.) in water (0·5 c.c.) at 20° for 16 hr. (cf. Reichstein and von Euw, *Helv. Chim. Acta*, 1938, **21**, 1181); addition of a little water and removal of methanol in a vacuum gave the crude hydroxy-dione (II*a*) (19 mg.), which was oxidised in dioxan (2 c.c.; freshly distilled over sodium) with periodic acid (25 mg.) in water (0·5 c.c.) at 20° for 16 hr. (ref. q; cf. Shoppee, *Helv. Chim. Acta*, 1940, **23**, 925). After removal of some dioxan in a vacuum, acidification gave a precipitate which was extracted with ether; the extract was washed with water, dried, and evaporated to give a solid acid, which was esterified with ethereal diazomethane at 0°. The product, by chromatography on aluminium oxide and elution with benzene, gave an oil (10 mg.) which gradually crystallised (m. p. 95—98°) and was sublimed at 100°/0·01 mm.; the crystalline sublimate, by recrystallisation from ether–pentane gave methyl 3-oxo*ætio*chola-4 : 9(11)-diene-17β-carboxylate (XV*b*), m. p. and mixed m. p. 105°.

Degradation of Corticosterone (I*a*) *to Methyl* 3-*Oxo*æ*tiochola*-4 : 9(11)-*diene*-17β-*carboxylate* (XV*b*).—Corticosterone (I*a*) (m. p. 178—180°; 150 mg.) was oxidised with periodic acid at 20° to 11β-hydroxy-3-oxo*ætio*chol-4-ene-17β-carboxylic acid, m. p. 253—255° (decomp.), according to the directions of von Euw and Reichstein (ref. q), and converted into the methyl ester with ethereal diazomethane at 0°. This (m. p. 180—182°; 100 mg.) was refluxed with concentrated hydrochloric acid–acetic acid (1 : 9; 1 c.c.) for 0·5 hr.; the solution was evaporated in a vacuum, and the residue thoroughly dried by repeated azeotropic distillation with benzene and esterified with ethereal diazomethane at 0°. The resulting oil (100 mg.) was chromatographed on aluminium oxide (3 g.) in pentane, with 10-c.c. eluates. Elution with benzene–pentane (4 : 1) (fraction 14) and benzene (fractions 15—19) gave material, m. p. 95—102°, which was united and recrystallised from ether–pentane to give methyl 3-oxo*ætio*chola-4 : 9(11)-diene-17β-carboxylate (XV*b*) (23 mg.), m. p. 103°, giving no depression by admixture with a synthetic specimen, m. p. 104—105°. Elution with benzene (fractions 20—23) and with ether–benzene (1 : 49 and 1 : 19) (fractions 24, 25) gave partly crystalline material (25 mg.). Further elution with ether–benzene (1 : 9, 1 : 4, 3 : 7, 2 : 3, and 1 : 1) (fractions 26—30) gave material (45 mg.), m. p. 182° after recrystallisation from acetone–ether, consisting of the unchanged 11β-hydroxy-ester.

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