76. *Experiments on the Synthesis of Substances related to the Sterols. Part LI.* Completion of the Syntheses of Androgenic Hormones and of the Cholesterol Group of Sterols.*

By H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, Hugo Holtermann, and Sir Robert Robinson.

The formal total synthesis of *epi*androsterone has been completed. The first stage was transformation of Reich's tetradecahydro-1 : 7-diketo-2 : 13-dimethylphenanthrene (I), which had been synthesised by Cornforth and Robinson (*J.*, 1949, 1855), into Köster and Logemann's $\Delta^{9(14)}$-dodecahydro-7-hydroxy-1-keto-2 : 13-dimethylphenanthrene (XII; R = H). The latter was used as the first relay; † it was converted into ætio*allo*bilianic acid (X), which was securely characterised and then used, as the second relay, for conversion into *epi*androsterone (XXXIII; R = H). The conversion of this hormone into androstenedione had already been realised and, in order to bring in a further series of steroids, androstenedione has been changed into dehydroandrosterone. From the latter *allo*pregnanolone had already been obtained and this substance has been converted into cholestanol by way of a 17 : 20-dehydro-derivative. Manifold known transformations enable other members of the steroid group to be embraced in the synthetic scheme and examples are testosterone, cholesterol, vitamin D_3, and cortisone.

A preliminary account of the synthesis of *epi*androsterone has already been published (*Chem. and Ind.*, 1951, 389).

The complete synthesis of the tricyclic diketone (I) identical with a ketone obtained from the oxidative degradation of cholesterol and bile acids (Reich, *Helv. Chim. Acta*, 1945, **28**, 892) was described by Cornforth and Robinson (*Nature*, 1947, **160**, 173; Part XLVIII, *J.*, 1949, 1855) ‡ who foreshadowed that the further elaboration of substances containing the tetracyclic ring system of the steroids and male sex hormones might follow the lines adumbrated in earlier parts of this series (cf. Pedler Lecture, *J.*, 1936, 1087).

In fact several new methods for the construction of ring D were studied and will be discussed in a subsequent paper. Eventually, however, we decided to rely upon one of the earliest methods contemplated in this series, that involving a Reformatsky reaction for the construction of ring D.

The stages, with the exception of the first (II to III) are those described by Robinson and Walker (*J.*, 1938, 183) and Litvan and Robinson (*ibid.*, p. 1997), and used by Kuwada and Nakamura (*J. Pharm. Soc. Japan*, 1938, **58**, 235, 257) for the partial synthesis of dehydro*epi*androsterone (XI) from ætiobilienic acid (X) (stages VI to IX), by Bachmann, Cole, and Wilds (*J. Amer. Chem. Soc.*, 1940, **62**, 824) for the synthesis of equilenin (stages III to IX), and by Anner and Miescher (*Helv. Chim. Acta*, 1948, **31**, 2173) for the synthesis of œstrone (stages III to IX).

This route has the advantage that relay can be made from the tetracyclic ketone (IX) by oxidative ring fission; in addition, the appropriate ætiobilienic acid (X) of type (VI) can be isolated from the acid residues from the oxidation of cholesteryl acetate dibromide. Substantial quantities of this acid were obtained by the latter means, and its conversion into dehydro*epi*androsterone (XI; R = H) (Kuwada and Nakamura, *loc. cit.*; Hershberg, Schwenk, and Stahl, *Arch. Biochem.*, 1948, **19**, 300) was confirmed.

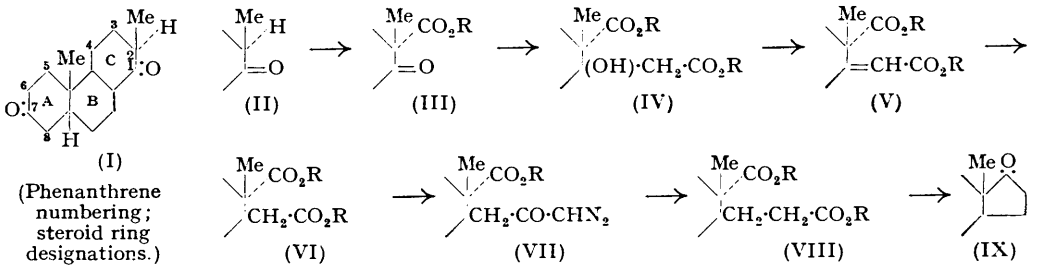
* Part L. *J.*, 1952, 1224.

† When, in a long synthesis, an intermediate is prepared by complete synthesis in small quantity and further supplies are drawn from natural sources or are derived from natural products, that intermediate may be described as a relay.

‡ The following errata occur in that paper (Part XLVIII):

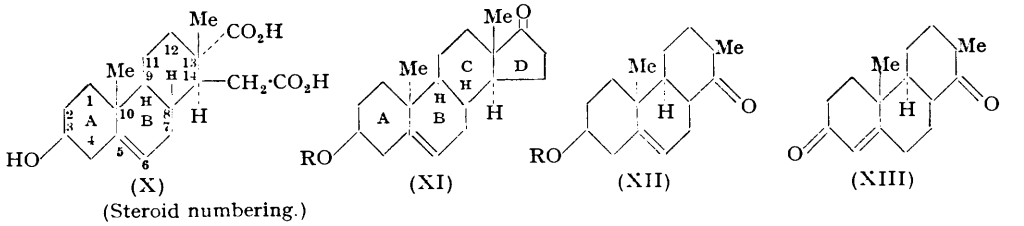
p. 1846.	*For* Morton *read* Martin.
p. 1857, l. 5 from bottom.	*For* +33·5° *read* +38·5°.
p. 1863, l. 44.	*For* −33·5° *read* −38·5°.
p. 1864, ll. 8 & 9.	*For* 11·319 g. *and* 13·231 g. *read* 1·1319 g. and 1·3231 g.
l. 31.	*For* +9·3° *read* +9·8°.

The most readily available tricyclic (ABC) substance which could stand in the direct line of our projected synthesis was clearly the hydroxy-ketone (XII; R = H), obtained as its acetate as a by-product of the oxidation of cholesteryl acetate dibromide by Köster and Logemann (*Ber.*, 1940, **73**, 299) and termed below the K.L. ketone; in view of its accessibility we decided to rely upon it as a relay. Its relation to (X) is obviously very



close but consideration of later stages showed that it was unlikely that full advantage could be taken of this. And so it proved, since the double bond was perforce saturated in the course of our operations.

Another circumstance that made the decision to use K.L. ketone difficult was that the synthesis of the substance had not been accomplished although it had been converted into a synthetic tricyclic ketone, namely, the Reich diketone (I). Fortunately these doubts were soon dissipated by the brilliant work of Birch, and of Dauben, who indicated different methods for the conversion of cholestenone into cholesterol, a transformation which is a model for that required to bridge the gap between Reich diketone (I) and K.L. ketone (XII; R = H). As the work proceeded we became more and more committed to our selected, if not preferred, route, which was eventually carried to the end. The formal synthesis which we can now describe admits of many improvements and should be regarded as scaffolding.



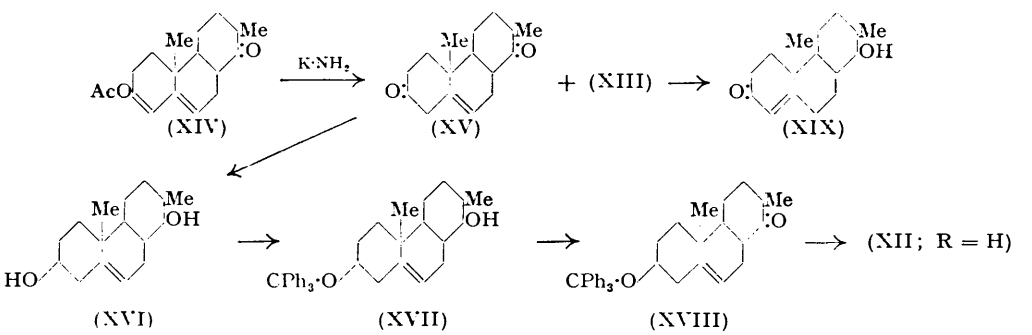
The following are the chief groups of stages. (1) Conversion of the Reich diketone (I) into the K.L. ketone (XII; R = H); (2) carboxylation of a derivative of the K.L. ketone; (3) synthesis of 3β-hydroxyætio*allo*bilianic acid from the end-product of (2); (4) conversion of the ætio*allo*bilianic acid into *epi*androsterone; (5) conversion of androstenedione into dehydro*epi*androsterone; and (6) synthesis of cholestanol from pregnenolone.

Stage (1).—Oppenauer oxidation of the K.L. ketone (XII; R = H) furnished the unsaturated diketone (XIII) from which Cornforth and Robinson (Part XLVIII, *loc. cit.*) obtained the Reich diketone (I). The latter was a product of degradation of deoxycholic acid by means of processes not involving the A : B ring fusion, which must therefore be of *cis*-decalin type. The method of preparation from (XIII) would also be expected to give the *cis*-decalin configuration on the grounds of numerous analogies, *e.g.*, the behaviour of cholestenone. Instances, however, are accumulating where the rule is not strictly observed, but in these there is usually a polar group in ring C or D, or its vicinity; and in the Experimental section we describe the catalytic reduction of androstenedione (XXXIV) which, contrary to earlier findings, gives the *cis*- and *trans*-decalin (A–B) derivatives in almost equal amount.

If, as seemed certain, the Reich diketone (I) is a *cis*-decalin (A–B), then it should be brominated in position 8 (phenanthrene) and afford (XIII) on dehydrobromination. Whilst we were studying these changes the processes were described by Billeter and Miescher (*Helv. Chim. Acta*, 1950, **33**, 388). It remained to convert (XIII) into (XII) and we were greatly assisted by the publication of methods for the conversion of cholestenone into cholesterol (Birch, *J.*, 1950, 2325; cf. Dauben and Eastham, *J. Amer. Chem. Soc.*, 1950, **72**, 2305; also Shoppee and Summers, *J.*, 1950, 687).

The ingenious methods of Birch, and of Shoppee and Summers, were followed in principle. It might be still better to use the more recently disclosed method of Dauben and Eastham (*J. Amer. Chem. Soc.*, 1951, **73**, 4463) whereby a 75% yield of cholesterol is obtained by the reduction of the enol acetate of cholestenone with sodium borohydride.

Treatment of (XIII) with a mixture of acetyl chloride and acetic anhydride (cf. Inhoffen, *Ber.*, 1936, **69**, 2144; Westphal, *Ber.*, 1937, **70**, 2128) gave the enol acetate (XIV) which was ammonolysed by means of potassamide in liquid ammonia, followed by ammonium chloride. The product was a mixture of recovered (XIII) with the new $\beta\gamma$-unsaturated ketone (XV). Attempts to isolate (XV) were defeated by its lability and hence the crude product was at once reduced by means of lithium aluminium hydride in ether. As a preliminary to the chromatographic separation of the reduction products we examined the reduction of K.L. ketone (XII) under similar conditions. Only one isomeride was produced and the reduction at position 1 (phenanthrene) was thus stereospecific. This implies the survival of the B–C *trans*-decalin system and also a particular orientation of the methyl and new *sec.*-alcoholic group in relation to it. Further comment on this matter is made below in connexion with the analogous results in the tetracyclic series.



We made use of the method of fractional elution of adsorbates (cf. Reichstein and Shoppee, *Discuss. Faraday Soc.*, 1949, **7**, 305) and always employed activated alumina and material to be purified in the ratio 30 : 1, so that experience with the diol from K.L. ketone enabled us to look in the right place for the required stereoisomeride (XVI).

Available analogies made it clear that reduction at position 7 would probably take place in the two possible directions, though the desired isomeride should preponderate.

On reduction of the crude mixture of (XIII) and (XV) made by the ammonolysis of the enol acetate (XIV) we obtained the diol (XVI), identical with that furnished by reduction of K.L. ketone, together with the unsaturated diketone (XIII) and an unsaturated keto-alcohol (XIX) derived from (XIII). The keto-alcohol had the ultra-violet absorption of an $\alpha\beta$-unsaturated ketone.

The diol (XVI) isolated in this way was not quite homogeneous but it afforded a 7-triphenylmethyl ether (XVII) identical with the same derivative of the diol from the reduction of K.L. ketone.

This ether was prepared in order to protect the hydroxyl at position 7 after other devices (acetylation, hydrogen succinoylation, hydrogen phthaloylation) had been tried with unsatisfactory results.

The general idea was to assist the differential between positions 1 and 7 by using a sterically hindered substituent in the hope that the hindrance factors would be not merely

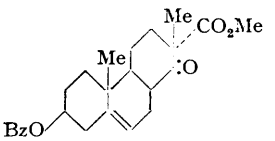
added but multiplied. Whether this was correct or not the method succeeded; on oxidation the ether (XVIII) of the K.L. ketone was obtained and by hydrolysis of this the K.L. ketone (XII; R = H) itself was produced.

In regard to the stereochemistry of this substance there can be no reason to suppose that the 3β-hydroxyl (7β- in XII) of cholesterol suffers inversion and similarly the B–C *trans*-ring fusion has surely survived. It is there in the first place and, even if it were not, the α-keto-group would induce its formation, the configuration at 9 (steroid; ≡ 12 phenanthrene) being assumed unalterable under usual circumstances. A doubt arises, however, in regard to the methyl group which is 13β in cholesterol.

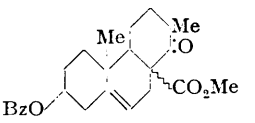
This could easily change to 13α (2α in the phenanthrene skeleton) under the influence of catalysts and the methyl is in the thermodynamically stable form because K.L. ketone is obtained by the hydrolysis of its acetate with methanolic potassium hydroxide. On the basis of Barton's reasoning (*Experientia*, 1950, 316; cf. Hassel, *Research*, 1950, 504), 2α being equatorially bound should be more stable than 2β (polar).

The formation of a single substance on reduction of K.L. ketone (see above) appears to support the 2β-configuration in which steric hindrance and tendency to produce equatorial bonds work in the same direction; whereas in the 2α-configuration they are opposed. Nevertheless it may very well be that one of these effects is predominant. In the case of the ketone, in the presence of powerful catalysts, the reversible processes should perhaps favour the emergence of the 2α-configuration. If the configuration of the new hydroxyl group could be ascertained by independent means it would throw light on the relative importance of the various factors which have been indicated.

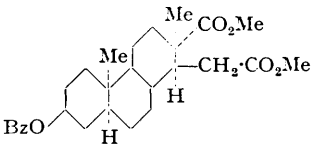
Stage (2).—A technique for the carboxylation of alicyclic ketones by treatment with sodium triphenylmethide in ether under nitrogen, followed by addition to carbon dioxide, was worked out. The product was carefully acidified and the carboxylic acid formed was extracted and esterified by diazomethane. An account of such model experiments with 2-methyl*cyclo*hexanone and *trans*-2-methyldecal-1-one will be submitted later. In the case of K.L. ketone the hydroxyl group was protected as benzoyloxy, so as to avoid the introduction of a new site of possible reactivity, such as the methyl of an acetoxy-derivative. Applying the method to K.L. ketone benzoate we obtained two isomeric keto-esters, separated by chromatography on alumina. These are designated (A) and (B) and were obtained in approximately the ratio 1 : 2 (A : B) (60—70%) yield.



(XX)



(XXI)



(XXII)

(A) is the more strongly adsorbed on alumina and assuming that (A) and (B) were stereoisomerides we concluded that (B) would be the isomeride required for our synthesis. It appeared to us that the *cis*-relation of polar groups, in this case BzO and CO_2Me, should tend to strong adsorption and we needed these groups in *trans*-relation. This argument was probably sound, given the premise, but it did not prove a reliable guide, and the outcome of our further work has been that (A) is certainly (XX), structurally and stereochemically the desired compound, whilst (B) is probably (XXI). The reasons for these conclusions are given below. As we only obtained a single stereoisomeride (XX) we were very fortunate to get the right one. This knowledge was acquired in a later phase of the work.

Stage (3).—The first attempt to carry out the stages (III to VI) with the keto-esters (A) and (B) was made in early days when the starting material available was exiguous.

The target was a derivative of ætio*allo*bilianic acid which Kuwada and Miyasaka (*J. Pharm. Soc. Japan*, 1936, **56**, 631) had already obtained by reduction of the accesssible ætiobilienic acid (X); we have examined some of the derivatives of the saturated acid. The dimethyl ester (XXII) of the *O*-benzoate crystallises well and exhibits a characteristic double melting point on the microscope hot-stage.

Selection of this objective implied that we would hydrogenate the 5 : 6-double bond (steroid numbering) in the course of the synthesis and thus increase the number of stages necessary in order to re-introduce it. This compromise was expedient in the first instance but there is no theoretical necessity for it and we hope to devise a method for the retention of the double bond through the course of the synthesis.

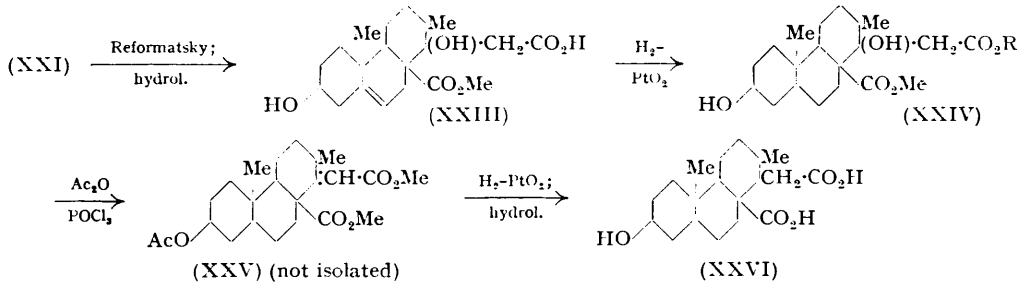
As we erroneously believed that (B) was the desired isomeride, (A) was used first. No attempts to isolate pure intermediates were made, and in the catalytic reduction the 14 : 15- and 5 : 6-double bonds (steroid numbering) were reduced together. The final products were isolated (by chromatography) as the 3-benzoate dimethyl esters. Four crystalline substances, (A) (i) m. p. 106—107°, (A) (ii) m. p. 153—155°, (A) (iii) m. p. 129—132°, $[\alpha]_D$ −11·5°, and (A) (iv) m. p. 121—123°, $[\alpha]_D$ +83·8°, were isolated in very small quantities (1—11 mg.). All these isomers appeared to be different from the natural methyl 3β-benzoyloxy-ætio*allo*bilianate (XXII), m. p. 144° and 158°, $[\alpha]_D$ −16·2° (but see below). The same series of reactions was then applied to the keto-ester (B). The overall yield was much lower. Chromatography of the benzoate dimethyl esters finally gave two crystalline substances, (B) (i) m. p. 148·5—149°, $[\alpha]_D$ +83°, and (B) (ii) m. p. 161—161·5°; both substances were different from the natural product.

As the isolation of four substances from the (A) series had suggested, contrary to general experience in the steroid field, that reduction of the 5 : 6-double bond was giving both *cis*- and *trans*-isomers (A–B linkage) it appeared possible that the desired compound was present in the (B) series, but had not been isolated owing to the small quantity of material available. Later we were able to dispose of adequate quantities of material as the result of generous gifts of residues from the oxidation of cholesteryl acetate dibromide and after the development of a simple method of isolation of K.L. ketone acetate from them. Preparation of suitable quantities of the A- and B-keto-esters was then undertaken and the later stages were re-investigated.

The Reformatsky reaction with methyl bromoacetate and the keto-ester (B) was first studied in detail. It was noted that appreciable debenzoylation occurred, which might account for the complex mixtures obtained in our earlier attempts to synthesise the ætio-*allo*bilianic acid derivative. The free 3-hydroxy-group would be partly removed, as well as the 14-hydroxy-group, in the dehydration. Accordingly the product was hydrolysed and isolated as the 3 : 14-dihydroxy half ester (XXIII, anticipating the argument given below). Attempts to convert the hydroxy-ester, after protection of the 3-hydroxy-group, *via* the 14-chloro-compound, into the bilienic acid were unsuccessful. Reduction of the 5 : 6-double bond was therefore performed before dehydration of the hydroxy-ester. These and subsequent steps are illustrated below. The following considerations had weight in the allocation of the structure (XXI) to the keto-ester-B. (i) The Reformatsky reaction gave a single stereoisomer; Anner and Miescher (*loc. cit.*) obtained approximately equal quantities of two stereoisomers in a similar reaction in the synthesis of œstrone. Similarly we obtained two steroisomers as products of the Reformatsky reaction applied to keto-ester-A. (ii) Hydrogenation of the crude unsaturated ester (XXV) gave substantially one product. Anner and Miescher obtained two isomers in equivalent quantities. (iii) The principal stereoisomeride (XXVI) finally isolated had a considerable positive rotation. (Another and still more dextrorotatory acid was isolated but the small quantity available did not enable us to establish its composition with certainty.) The last point is perhaps the most convincing. Fieser and Fieser ("Natural Products related to Phenanthrene," Reinhold Publ. Corpn., New York, 1949, p. 214) have concluded from an analysis of optical rotatory data that in the natural steroids $C_{(13)}$ is a dextro- and $C_{(14)}$ is a lævo-rotatory centre of approximately equal power. 3β-Hydroxyætio*allo*bilianic acid 17-monomethyl ester had a specific rotation of −19°. The free acid would have practically the same rotation (cf. Heer and Miescher, *Helv. Chim. Acta*, 1947, **30**, 786). If the B-series is assumed to be epimeric at $C_{(13)}$ with the natural series, then even the more dextrorotatory ætio*allo*bilianic acid ($C_{(13)}$ −; $C_{(14)}$ +) of this series should have a negative rotation only slightly weaker than the "natural" acid, and this is in marked disagreement with the observed difference in rotation (ΔM_D 170) between the "natural" and the B-acids.

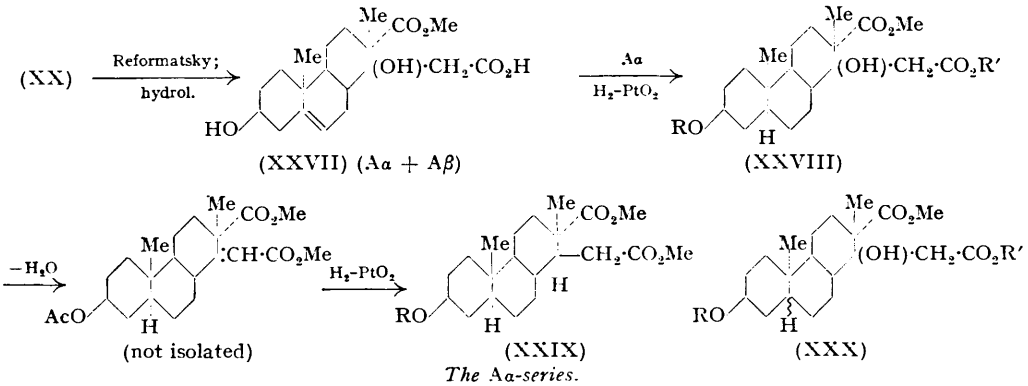
The rotations of the marrianolic and doisynolic acids (Fieser and Fieser, *op. cit.*, pp.

347—349) support these conclusions. It follows that isomer (B) cannot have a carbomethoxy-group at $C_{(13)}$ (steroid numbering). The alternative position is in the angle (XXI) and if this is so it is not surprising that the derivatives of the substance behave quite differently from those of the keto-ester used by Anner and Miescher (*loc. cit.*) in the synthesis of œstrone.



The B-keto-ester series.

The experience gained was now applied to the A-keto-ester series. After the Reformatsky reaction with methyl bromoacetate and mild hydrolysis of the product, two isomeric, lævorotatory dihydroxy half esters were obtained, namely Aα, m. p. 221° (XXVII) and Aβ, m. p. 182° (XXVII, inverted at $C_{(1)}$). When the Aα-hydroxy-ester was carried through the stages illustrated below, an oily dimethyl acetoxyætio*allo*bilianate was obtained. The crude product crystallised when seeded with the ester of natural origin. It was converted into the *O*-benzoate which was rigorously proved to be identical with the substance of natural origin. The specimens exhibited the characteristic double melting point, alone or mixed together, each had $[\alpha]_D$ −16·0°, and they were crystallographically identical. We are greatly indebted to Dr. D. M. Crowfoot Hodgkin for her invaluable co-operation throughout this research.



The Aα-series.

The Aβ-hydroxy-ester [XXVII, inverted at position 1 (phenanthrene)] was catalytically reduced to a dihydro-derivative (XXX; R = R′ = H). The related methyl ester acetate (XXX; R = Ac, R′ = Me) was not dehydrated under the conditions which succeeded in the Aα-series.

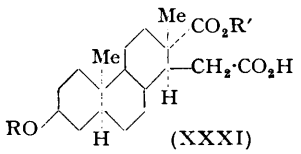
Two matters of more general interest emerge from these results. First, the K.L. ketone benzoate is carboxylated to some extent in the angle (position 11 of the phenanthrene ring) whereas 2-methyl-*trans*-decal-1-one was substituted in position 2 only. The chief difference between the tricyclic substance and its bicyclic model is the presence of the double bond in the former. This is in the βγ-position to the activated centre (γδ to carbonyl group) of the K.L. ketone benzoate and hence would not normally be regarded as exerting an electromeric effect. The reaction is quite normal in one sense because enolisation, or incipient enolisation, is possible on either side of the carbonyl group. The preference for one side or the other is a second-order phenomenon and may well be swayed by stereo-

chemical conditions determined to a sufficient extent by the presence or absence of the double bond. In addition, it is not out of the question that the double bond may exert its own direct polar effect on the activating system. This would be entirely analogous to very numerous secondary effects established in the study of the orientation of substitutions in aromatic compounds. The second point is that the carboxylation in both positions led to a single isomer in each case, or to a great preponderance of one isomer, if another substance was contained in the 30—40% not isolated. In the case of the (A) compound we know that the entering carboxyl is α-oriented. This could be due to the β-orientation of the neighbouring methyl group in position 2 (phenanthrene) which probably exists in the enolate ion. It is equivalent to assume that the bond-forming electrons on position 2, which may have a fractional value, are α-oriented. Such bond-forming electrons correspond to the displacements (a) in $^{-}O{-}C{=}C(Me)$ and there is reason to believe that a small value of (a) suffices to preserve the tetrahedral environment of the carbon atom, the electron or electron moiety functioning as the fourth group. These considerations do not solve the problem but they tend to put it one stage further back and it is beyond our present scope to pursue the matter further.

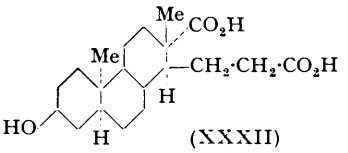
Summarising, the evidence bearing on the orientation of the 2-methyl group in K.L. ketone is conflicting. The theoretical argument, based on the influence of steric factors on stability, favours the α-configuration but two reactions (reduction and carboxylation), also discussed from a speculative theoretical angle, favour the β-configuration. Not one of these arguments is conclusive.

The question of the configuration of the B-keto-ester also remains open. Reasoning on stereochemical grounds leads to the conclusion that in this case also the carbomethoxy-group should be α-oriented.

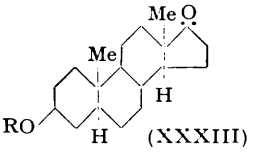
Stage (4).—The dimethyl ætio*allo*bilianate benzoate (XXII) was hydrolysed by methanolic potassium hydroxide to a hydroxy half-ester (XXXI; R = H, R′ = Me) which afforded an acetoxy half-ester (XXXI; R = Ac, R′ = Me) on acetylation. This acid was then homologated and carried through the stages (VI to IX) by the Arndt–Eistert and Blanc



(XXXI)



(XXXII)



(XXXIII)

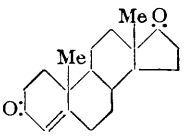
methods. Successive treatment of (XXXI; R = Ac) with oxalyl chloride, diazomethane, ammoniacal silver nitrate in aqueous ethanol, and boiling methanolic potassium hydroxide (16 hours) gave the partly crystalline homo-acid (XXXII). This was not further examined but was heated with acetic anhydride, and the acetate-anhydride pyrolysed. *epi*Androsterone acetate (XXXIII; R = Ac) was produced and purified by distillation and subsequent chromatography. The specimen (m. p. 103—104°; $[\alpha]_D$ +64·5° ± 2°) was identical in all respects with one made by catalytic hydrogenation and re-oxidation of dehydro*epi*androsterone acetate (m. p. 101·5—103·5°; $[\alpha]_D$ +68·5° ± 2°). As a punctilio *epi*androsterone was prepared by hydrolysis of its acetate.

Walden inversion at position 3 of *epi*androsterone leads to androsterone (Marker, Whitmore, Kamm, Oakwood, and Blatterman, *J. Amer. Chem. Soc.*, 1936, **58**, 338), from which 2-bromoandrostanedione, androstenedione, and testosterone have been obtained (Ruzicka, Plattner, and Aeschbacher, *Helv. Chim. Acta*, 1938, **21**, 866). The formation of androstenedione involves an abnormal dehydrobromination and a more straightforward method has recently been disclosed (Rosenkranz, Mancera, Gates, and Djerassi, *J. Amer. Chem. Soc.*, 1950, **72**, 4077). In this process the 2 : 4-dibromo-ketone is treated with sodium iodide to form the 2-bromo-4-iodo-derivative. Heating the latter with collidine affords the desired Δ^4-ketone by simultaneous dehydrobromination and de-iodination; alternatively these processes can be carried out successively.

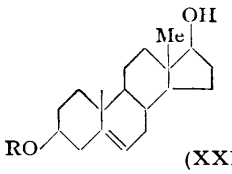
It should be noted that Marker and Rohrmann (*ibid.*, 1939, **61**, 2722) have prepared

ætiobilianic acid (A–B *cis*) by oxidative degradation of sarsasapogenin and have converted the acid (*ibid.*, 1940, **62**, 900) into 3β-hydroxyætiocholan-17-one, its 3β-epimeride, and testosterone. It might have been feasible to set up a *cis*-A–B series leading to ætiobilianic acid but this was not necessary since androstenedione (XXXIV) has been found to yield almost equal amounts of ætio- and ætio*allo*-cholane-3 : 17-dione on catalytic hydrogenation.

Stage (5).—In order to use dehydro*epi*androsterone (XI) as a relay, it was necessary to obtain it from androstenedione and this is the ring-homologue of the problem of converting (XIII) into (XII).



(XXXIV)



(XXXV)

The methods employed were the same as those used in Stage (1). The enol acetate of androst-4-ene-3 : 17-dione (Ruzicka and Fischer, *Helv. Chim. Acta*, 1936, **19**, 1371) was ammonolysed with potassamide in liquid ammonia, the solution acidified with ammonium chloride, and the products reduced with lithium aluminium hydride. We isolated unchanged androstenedione, testosterone, and androst-5-en-3β : 17β-diol (XXXV; R = H).

The diol was converted into dehydro*epi*androsterone by the 3-triphenylmethyl ether method, the various stages being parallel with those used in the tricyclic series. The final product was identical in all respects with authentic dehydro*epi*androsterone.

We take this opportunity to comment briefly on the mechanism of reduction by lithium aluminium hydride. The course of the reduction of carbonyl groups taken in the steroid series has been carefully studied by a number of workers and views concerning the mechanism have been advanced (Shoppee and Summers, *loc. cit.*, Trevoy and Brown, *J. Amer. Chem. Soc.*, 1949, **71**, 1675; Kenner and Murray, *J.*, 1950, 406; Brown, "Organic Reactions," Wiley, Vol. VI, p. 471; cf. Dostrovsky, Hughes, and Ingold, *J.*, 1946, 173). At this date it is a commonplace to state that the carbon atom of a carbonyl group is cationoid (or electrophilic) in the first phase of a reaction and it is also self-evident that the stereochemical effect of substituents will often determine the side of entry of the addenda. The only question which we now propose to discuss is the nature of these addenda.

Shoppee (*loc. cit.*) and others have assumed that these are Li^+ (or appearance of an electrovalent anionic change on oxygen) and AlH_4^-. However, the attack on the cationoid carbon atom by AlH_4^- can at best be a mere preliminary electrovalent connexion—any covalency formed must be between carbon and hydrogen. A more direct mechanism would clearly be the attachment of oxygen to aluminium and hydrogen to carbon.

Very instructive parallel reactions have been discovered by Ziegler and Gellert and their co-workers (cf. *Angew. Chemie*, 1952, **64**, 523) who find that ethylene and lithium aluminium hydride afford lithium tetraethylaluminium $LiAlEt_4$. This focuses attention on the >Al–H link, and in fact AlH_3 is much more reactive than $LiAlH_4$ and is converted by ethylene into triethylaluminium. This reaction, and similar reactions with other olefins of the form $CH_2{:}CRR'$, is reversible. It was already known (Meerwein, *J. pr. Chem.*, 1937, **147**, 226) that triethylaluminium reduces chloral with the formation of ethylene: $3CCl_3{\cdot}CHO + AlEt_3 \longrightarrow (CCl_3{\cdot}CH_2{\cdot}O)_3Al + 3C_2H_4$.

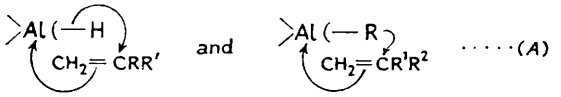
An important extension of the Ziegler reaction consists in the addition of olefins, $CH_2{:}CRR'$, to alkylaluminiums, a process which in the case of ethylene proceeds many times with formation of waxes (up to the present polythene analogues have not been so obtained). For example,

$$>Al{\cdot}CH_2{\cdot}CH_3 + CH_2{:}CH_2 \longrightarrow >Al{\cdot}Bu^n \xrightarrow{n C_2H_4} >Al{\cdot}[CH_2]_{2n}Bu^n$$

In the case of the higher olefins the Ziegler reaction follows the Markownikoff rule :

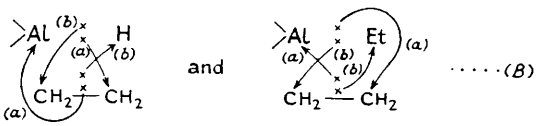
$$>Al{\cdot}Bu^n + CH_2{:}CHEt \longrightarrow >Al{\cdot}CH_2{\cdot}CHEtBu^n \rightleftharpoons >AlH + CH_2{:}CEtBu^n$$

Hence the mechanism is clearly either



.....(*A*)

or, preferably, corresponding mechanisms, travelling along the same lines in the first phases, but only involving transfer of one electron from the olefin to the aluminium atom, for example :



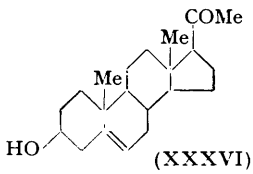
.....(*B*)

This essentially homopolar process is supposed to be *initiated* by the displacements (*a*)(*a*), which are followed by (*b*)(*b*). Any intermediate between the fully polar mechanism (*A*) and the homopolar mechanism (*B*) can be envisaged. In the case of the interaction of >AlH and >C=O we imagine there is a close approach to the (*A*) type, whereas that of >AlEt and CH_2:CH_2 is close to the (*B*) type.

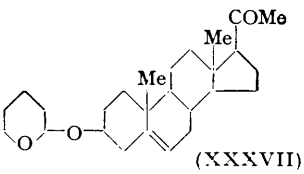
Such schemes in no way affect the validity of the conclusions respecting the influence of stereochemical conditions because these hold even if the larger group goes to oxygen. The determining circumstances are the direction of approach of the reagent and the location of electrons involved in the first stages of the addition.

Stage (6).—It was always our intention to use one of the androgenic hormones and its known transformation products as stepping stones to the cholesterol series, and the appropriate method has long been obvious. The submission of our preliminary note (*loc. cit.*) on the synthesis of the androgenic hormones was quickly followed by verbal disclosure of the very different syntheses by R. B. Woodward and his colleagues. A little later, a note was published (Woodward, Sondheimer, and Taub, *J. Amer. Chem. Soc.*, 1951, **73**, 3548) which anticipates our work in respect of the conversion of *allo*pregnanolone into cholestanol. However, we submit our own results in this field, because they are an integral part of the research initiated twenty years ago, and also because, up to the time of writing, the intermediate products have not been described.

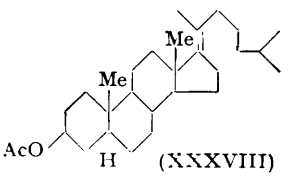
The chief reason for undertaking the reconversion of androstenedione into dehydro*epi*androsterone (see above) was to take advantage of the transformation of the latter hormone into 3-hydroxypregn-5-en-20-one (XXXVI) which had been accomplished by Butenandt and Schmidt-Thomé (*Ber.*, 1938, **71**, 1487; 1939, **72**, 182). A Grignard reaction with a suitably protected derivative of this substance was evidently the most convenient method for the introduction of the full sterol side-chain. As a preliminary we studied the methods available for the preparation of *iso*hexyl alcohol and submit a procedure which we regard as an improvement on all of them for the purpose in view. Unfortunately the reaction, neither of pregnenolone acetate, nor of the new dihydropyran adduct (XXXVII) of pregnenolone (cf. Woods and Kremer, *J. Amer. Chem. Soc.*, 1949, **71**, 1840; Greenhalgh, Henbest, and Jones, *J.*, 1950, 1190) with *iso*hexylmagnesium bromide gave satisfactory results. [(XXXVII) was crystalline in spite of the introduction of a new asymmetric centre, but no attempt was made to separate stereoisomerides.]



(XXXVI)



(XXXVII)



(XXXVIII)

In view of the abortive nature of these experiments only an outline of the reaction sequence is included in the Experimental section. The work had definite value to us in drawing attention to the character of the difficulties likely to be encountered, some of which were clearly due to the 5 : 6-double bond. As this could so easily be eliminated and as the

final product, namely, cholestanol, gives access to the whole cholesterol group, we turned to the case of *allo*pregnanolone (made from pregnenolone by Plattner, Heusser, and Angliker, *Helv. Chim. Acta*, 1946, **29**, 468; cf. Huang-Minlon, *J. Amer. Chem. Soc.*, 1949, **71**; 3301) and thus followed Woodward *et al.* (*loc. cit.*). The reaction between 3β-acetoxy*allo*pregnan-20-one and *iso*hexylmagnesium bromide was carried out in anisole, and the product dehydrated and acetylated (cf. Butenandt and Cobler, *Z. physiol. Chem.*, 1935, **234**, 218). Analysis and the infra-red spectrum of the pure product isolated by chromatographic procedures showed that it was 3β-acetoxycholest-17(20)-ene (XXXVIII).

This constitution applies to the 8% of solid isolated (absorption band at 12 μ absent) but the oily part of the material may have contained $\Delta^{20(21)}$- or $\Delta^{20(22)}$-isomerides. This was to be expected from the work of Reichstein and Koechlin (*Helv. Chim. Acta*, 1944, **27**, 549) on the dehydration of 20-methyl*allo*pregnane-3β : 20-diol. Both the solid and the oily product were hydrogenated (PtO_2): the solid (XXXVIII) afforded cholestanyl acetate in moderately good yield, but the oil gave only a small amount of cholestanyl acetate and the major part remained oily and gave no digitonide. The cholestanyl acetate was rigorously compared with a specimen of natural origin and proved identical with it in all respects, including the crystallographic data. The further transformations of cholestanol to cholestanone, cholest-4-enone, and cholesterol, and many other substances are well known. The newer methods of Birch and of Dauben for the conversion of cholestenone into cholesterol are mentioned above.

Experimental

M. p.s are uncorrected. Petrol refers to light petroleum of b. p. 40—60°.

7-Acetoxy-$\Delta^{7:9(14)}$*-decahydro-1-keto-2 : 13-dimethylphenanthrene* (XIV).—A solution of Δ^{8}-dodecahydro-1 : 7-diketo-2 : 13-dimethylphenanthrene (XIII) (2·5 g.) in acetic anhydride (20 c.c.) and acetyl chloride (20 c.c.) was refluxed for 3 hours, then concentrated to 5 c.c., and, on cooling, *7-acetoxy-*$\Delta^{7:9(14)}$*-decahydro-1-keto-2 : 13-dimethylphenanthrene* (2·3 g.) crystallised. Recrystallisation from ethanol gave plates, m. p. 114°, $[\alpha]_D^{17}$ −133° (*c* 0·96 in EtOH) (Found : C, 75·1; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·3%).

$\Delta^{9(14)}$*-Dodecahydro-1 : 7-dihydroxy-2 : 13-dimethylphenanthrene* (XVI).—(*a*) The above enol acetate (6·0 g.) in ether (120 c.c.) was added to potassamide (from 6·0 g. of potassium) in liquid ammonia (600 c.c.). After 1 hour ammonium chloride (12·0 g.) was added, the solution kept for an hour, and the ammonia evaporated off. The residue was extracted with ether (5 × 200 c.c.). Evaporation of a portion of this solution gave an oil whose negative rotation ($[\alpha]_D^{15}$ −33°) (*c* 0·9 in EtOH) showed that it consisted largely of the βγ-unsaturated ketone.

The ethereal solution of the unsaturated diketones was added slowly to a solution of lithium aluminium hydride (4·0 g.) in ether (200 c.c.). After 2 hours the mixture was cooled in ice, and the excess of lithium aluminium hydride decomposed by the addition of 20% hydrochloric acid. The ethereal layer was washed with sodium hydrogen carbonate solution, then with water, and dried (Na_2SO_4). The solvent was removed yielding an oil (4·4 g.) which was chromatographed in benzene (100 c.c.) on an alumina (50 g.) column, prepared in benzene. Three main fractions were obtained, *viz.*, (i) (1·5 g.) eluted by benzene, (ii) (397 mg.) eluted by benzene–ether (4 : 1), and (iii) (310 mg.) eluted by benzene–ether (7 : 3—1 : 4). These fractions were rechromatographed as follows :

(i) In benzene–petrol (2 : 3; 100 c.c.) on alumina (30 g.). The fraction eluted by benzene–petrol (1 : 1) yielded a white solid crystallising from ether in needles, m. p. 135—136°, $[\alpha]_D^{22}$ +116° (*c* 1·49 in EtOH) (Found: C, 77·6; H, 8·8. Calc. for $C_{16}H_{22}O_2$: C, 78·0; H, 8·9%). The m. p. was not depressed on admixture with Δ^{8}-dodecahydro-1 : 7-diketo-2 : 13-dimethylphenanthrene (XIII), m. p. 140°, $[\alpha]_D$ +128° in $CHCl_3$.

Fraction (ii) proved homogeneous, crystallising from ether in triangular prisms, m. p. 142°, $[\alpha]_D^{22}$ +85° (*c* 0·965 in EtOH). The ultra-violet absorption ($\lambda_{max.}$ 240 mμ, log $\epsilon_{max.}$ 4·2) was characteristic of an αβ-unsaturated ketone (Found : C, 77·1; H, 9·3. $C_{16}H_{24}O_2$ requires C, 77·4; H, 9·6%). The product is therefore Δ^{8}*-dodecahydro-1-hydroxy-7-keto-2 : 13-dimethylphenanthrene* (XIX).

(iii) In benzene (20 c.c.) on alumina (9 g.). The fraction eluted by benzene–ether (7 : 3) yielded a white solid crystallising from ether in rosettes of needles, m. p. (capillary) 169—174° with softening from 120°, $[\alpha]_D^{20}$ −71° (*c* 1·09 in EtOH) (Found : C, 76·5; H, 10·4. $C_{16}H_{26}O_2$ requires C, 76·8; H, 10·4%). On the microscope hot-stage the needles started to sublime at

120°. The sublimate condensed in fronds of needles on the cover-slip. At 165—174° the needles contracted and changed into rods, the rods finally melting at 183°. The same phenomena were observed with a specimen made by the reduction of K.L. ketone (see below) or when the specimens were mixed. This substance is the *diol* (XVI) named in the heading of this section.

(*b*) The K.L. ketone (2·5 g.) in ether (250 c.c.) was added slowly to an ice-cold solution of lithium aluminium hydride (1·0 g.) in ether (100 c.c.), and the mixture set aside at the room temperature for an hour. The excess of lithium aluminium hydride was decomposed by the addition of 5% hydrochloric acid. The ethereal layer was washed with sodium hydrogen carbonate solution, then with water, and dried (Na_2SO_4). The solvent was removed and the product (1·5 g.) crystallised from ether in fine needles, m. p. 183°, showing the polymorphic changes on heating described under (*a*, iii) and $[\alpha]_D^{16}$ −70° (Found : C, 76·1; H, 10·3%). Fractional crystallisation of the diol, or of its dihydrogen disuccinate followed by hydrolysis, gave no indication of the presence of an isomeride.

$\Delta^{9(14)}$-*Dodecahydro*-2 : 13-*dimethylphenanthr*-1 : 7-*ylene Dihydrogen Disuccinate*.—Dodecahydrodihydroxydimethylphenanthrene (1·0 g.) and succinic anhydride (1·0 g.) were heated with pyridine at 120° for 2 hours. The product was dissolved in ether (100 c.c.), washed with 20% hydrochloric acid until free from pyridine, then with water, and dried. The solvent was removed, and the product set aside in ether (5 c.c.) to crystallise. The derivative (50 mg.) was recrystallised from ether and obtained in needles, m. p. 154°, $[\alpha]_D^{16}$ −32° (*c* 1·3 in EtOH) (Found : C, 64·2; H, 7·4. $C_{24}H_{34}O_8$ requires C, 64·0; H, 7·6%). Further crops (200 mg.) were obtained from the mother-liquors but evaporation of the solvent gave an oily residue. Hydrolysis of the above *dihydrogen disuccinate* (130 mg.) with hot aqueous potassium hydroxide (5 c.c. of 3%) for 15 minutes gave the pure diol.

$\Delta^{9(14)}$-*Dodecahydro*-1-*keto*-2 : 13-*dimethylphenanthr*-7-*yl Hydrogen Succinate*.—The K.L. ketone (1·0 g.), succinic anhydride (1·0 g.), and pyridine (2 c.c.) was heated at 120° for 2 hours. The product was dissolved in ether, and washed with 20% hydrochloric acid till free from pyridine, and with water. The dried ethereal solution was concentrated to 10 c.c. and cooled, whereupon the *hydrogen succinate* (1·1 g.) crystallised in plates, m. p. 151—152°, $[\alpha]_D^{16}$ −57° (*c* 1·15 in EtOH) (Found : C, 69·0; H, 8·3. $C_{20}H_{28}O_5$ requires C, 69·0; H, 8·0%). Hydrolysis of the succinate by 5% aqueous potassium hydroxide yielded the theoretical quantity of the keto-alcohol.

$\Delta^{9(14)}$-*Dodecahydro*-1-*keto*-2 : 13-*dimethyl*-7-*triphenylmethoxyphenanthrene* (XVIII).—(*A*) The diol (XVI) (1·5 g.) and triphenylmethyl chloride (1·7 g.) in pyridine (5 c.c.) were heated on the steam-bath for 4 hours. The product was dissolved in ether and washed with 20% hydrochloric acid, with sodium hydrogen carbonate solution, then with water, and dried. Removal of the ether left a dark oil containing a few crystals. The oil was dissolved in benzene–petrol (300 c.c.; 1 : 4) and purified by chromatography on an alumina column (90 g.) prepared in petrol. Two main fractions were obtained; the first (623 mg.) was eluted by benzene–petrol (400 c.c., 1 : 4), and the second (313 mg.) by benzene–petrol (100 c.c., 2 : 3). The former was a waxy solid of indeterminate m. p. which was not purified by further chromatography, and was probably a mixture of the isomeric 1- and 7-ethers. It was oxidised as described below. On further purification by chromatography on alumina (10 g.) the second fraction, m. p. 135—145°, yielded triphenylmethanol, m. p. and mixed m. p. with an authentic specimen, 160°.

The waxy mixture of ethers (0·43 g.) was heated in toluene (15 c.c.) and *cyclo*hexanone (2 c.c.) to the b. p. Aluminium *iso*propoxide (0·2 g.) in toluene (5 c.c.) was added, and the solution was refluxed for 1½ hours and then distilled in steam. The residue was acidified with dilute hydrochloric acid, and the product isolated by means of ether. The oil (310 mg.), in benzene–petrol (20 c.c.; 2 : 3), was purified by chromatography on alumina (6·5 g.) prepared in the same solvent. The fraction eluted by benzene–petrol (80 c.c.; 1 : 1) on crystallisation from ether gave the $\Delta^{9(14)}$-*dodecahydro*-1-*keto*-2 : 13-*dimethyl*-7-*triphenylmethoxyphenanthrene*, m. p. 168—170°, $[\alpha]_D^{20}$ −66° (*c*, 2·6 in $CHCl_3$) (Found : C, 85·2; H, 7·8. $C_{35}H_{38}O_2$ requires C, 85·7; H, 7·8%). The m. p. was undepressed on admixture with an authentic specimen prepared by the reduction of the K.L. ketone triphenylmethyl ether (see below). Dr. D. Crowfoot Hodgkin and Dr. P. M. Cowan kindly carried out experiments on the *X*-ray diffraction patterns (powder photographs) of the two substances, and reported that they were identical.

(*B*) The K.L. ketone (330 mg.) and triphenylmethyl chloride (375 mg., 1 mol.) were heated in pyridine (1 c.c.) on the steam-bath for 4 hours. The product was isolated in the usual manner and chromatographed in benzene–petrol (10 c.c.; 1 : 1) on alumina (10 g.) prepared in benzene–petrol (2 : 3). The second fraction eluted by benzene–petrol (10 c.c.; 1 : 1) gave a white solid (200 mg.) crystallising from ether in plates, m. p. 160—164°, and the third fraction a further 100

mg. The fractions were combined and purified by chromatography, the triphenylmethoxy-ketone being obtained from benzene or ether in transparent plates, m. p. 168—170°, $[\alpha]_D^{17}$ −54° (*c* 1·19 in $CHCl_3$) (Found: C, 85·5, 86·4; H, 8·6, 7·9. $C_{35}H_{38}O_2$ requires C, 85·7; H, 7·8%). In later experiments repeated chromatography and crystallisation raised the m. p. to 176°.

$\Delta^{9(14)}$-*Dodecahydro*-1-*hydroxy*-2 : 13-*dimethyl*-7-*triphenylmethoxyphenanthrene* (XVII).—The above triphenylmethoxy-ketone (200 mg.) in ether (10 c.c.) was added to lithium aluminium hydride (100 mg.) in ether (250 c.c.), kept for an hour, and worked up as usual. The crystalline *product* (200 mg.) was chromatographed in benzene–petrol (20 c.c.; 1 : 9) on an alumina column (6 g.) prepared in petrol, and eluted by benzene–petrol (1 : 4). It was chromatographed once more, and crystallised from petrol in transparent plates, m. p. 80—81°, $[\alpha]_D^{17}$ −3° (*c* 1·07 in $CHCl_3$) (Found: C, 87·6, 87·9, 86·8; H, 6·5, 7·1, 6·5. $C_{35}H_{40}O_2$ requires C, 85·4; H, 8·1%). These results may be explicable on the grounds that the material is a molecular compound of the ether with triphenylmethane.

Attempts to repeat this experiment furnished the monoether as an oil which could not be crystallised but was directly oxidised to the ether of the hydroxy-ketone. To a boiling solution of the oily mono(triphenylmethyl) ether of the diol (XVI) (450 mg.) in toluene (15 c.c.) and *cyclo*hexanone (2 c.c.), aluminium *iso*propoxide (300 mg.) in toluene (15 c.c.) was added. The mixture was refluxed for 3 hours and distilled in steam. The oily product (200 mg.), isolated as usual, was purified by chromatography on alumina (6 g.) prepared in petrol. The fraction eluted by benzene–petrol (2 : 3) crystallised from petrol in rods, m. p. 120—135°. These were dissolved in benzene–petrol (1 : 4) and again purified by chromatography on an alumina column (5 g.) prepared in petrol. The fraction eluted by benzene–petrol (20 c.c.; 2 : 3) yielded the triphenylmethyl ether of the K.L. ketone which crystallised from petrol in rods, m. p. 170—172° (20 mg.). The mixed m. p. with an authentic specimen (m. p. 176°) was 170—172°.

$\Delta^{9(14)}$-*Dodecahydro*-7-*hydroxy*-1-*keto*-2 : 13-*dimethylphenanthrene* (K.L. *Ketone*) (XII).—$\Delta^{9(14)}$-Dodecahydro-1-keto-2 : 13-dimethyl-7-triphenylmethoxyphenanthrene (750 mg.) in chloroform (20 c.c.), cooled in ice and hydrochloric acid, was treated with an ice-cold saturated solution of hydrogen chloride in chloroform (50 c.c.). The mixture was kept at the room temperature for 30 minutes, washed with sodium hydrogen carbonate solution, then with water, and dried and the solvent removed. The product was chromatographed in benzene–petrol (100 c.c.; 3 : 7) on alumina (20 g.) prepared in petrol. The fractions eluted by benzene–petrol (2 : 3 and 1 : 1) yielded triphenylmethanol (351 mg.), m. p. 160°, and that eluted by benzene–petrol (7 : 3) yielded dodecahydrohydroxyketodimethylphenanthrene (110 mg.), m. p. 131—132°, $[\alpha]_D^{20}$ −87° (*c* 1·82 in $CHCl_3$); the m. p. was not depressed on admixture with authentic K.L. ketone, m. p. 133—134°, $[\alpha]_D^{20}$ −88°.

Isolation of Ketones from Residues from the Oxidation of Cholesteryl Acetate Dibromide.—*Materials.* British Drug Houses Ltd. divide the material obtained on treatment of the neutral fraction with semicarbazide into three crops. Crop 1 contains the majority of the dehydro*epi*-androsterone acetate semicarbazone. Crop 2 contains mixed semicarbazone. Crop 3, which is obtained by dilution of the mother-liquors from Crop 2 with water, concentration to small bulk, and absorption of the resulting oil on kieselguhr, does not contain an appreciable quantity of semicarbazones. 12 Kg. of Crop 2 and 17 kg. of Crop 3 were kindly supplied by British Drug Houses Ltd. In addition Dr. Hershberg of the American Schering Corporation kindly provided 5 kg. of residues which appeared to be very similar to B.D.H. Crop 2 residues. 2 Kg. of Crop 2 were examined in detail by a laborious process which is only outlined below. The tricyclic ketone thus isolated (15 g.) was used for the initial attempt to prepare the bilianic acids. The remainder of Crop 2 was not worked up in the same manner because a simple process for the isolation of the tricyclic ketone from Crop 3 was developed. This simple process could, with minor modifications, be applied to Crop 2. The material from Crop 3 was used for the second and successful attempt to prepare the bilianic acids.

Crop 2. A brief examination revealed that the tricyclic hydroxy-ketone (XII) of Köster and Logemann (*loc. cit.*) was not present as a semicarbazone. The dry residues were therefore continuously extracted with hot petrol. The extract was evaporated and the resulting mixture of acetates and lactones was hydrolysed with methanolic potassium hydroxide. The recovered neutral moiety, now lactone free, was triturated with ether, leaving relatively large quantities of cholesterol. The ether-soluble oil (190 g. from 2 kg. of residues) was dissolved in benzene (800 c.c.) and diluted with petrol (800 c.c.). This solution in two equal portions was passed through two alumina columns (1 kg. each) which were successively eluted with petrol, benzene, and chloroform, over 200 l. of solvents being used in all. Benzene–chloroform (7 : 1) eluted most of the desired K.L. ketone, contaminated with appreciable quantities of cholesterol. The

K.L. ketone was purified by conversion into the acetate and crystallisation from methanol. The total yield of K.L. ketone acetate was 14 g.

The process will, it is hoped, be described in greater detail in connexion with the description by one of us (H. H.) of the isolation of norcholesterolone (Ruzicka, Werner, and Fischer, *Helv. Chim. Acta*, 1937, **20**, 1291) from eluate-V (benzene–chloroform) and of a hydroxycholesterol from eluates-VI and -VII (benzene–chloroform). The latter substance crystallised from methanol in slender needles, m. p. 177—178·5° (Found : C, 80·7; H, 11·3. Calc. for $C_{27}H_{26}O_2$: C, 80·5; H, 11·5%). Its *dibenzoate*, crystallised several times from ethanol, formed leaflets, m. p. 129—131° (Found : C, 81·0; H, 8·8. $C_{41}H_{54}O_4$ requires C, 80·6; H, 8·9%). This hydroxycholesterol is probably identical with a substance, m. p. 177°, of unknown constitution described by Windaus, Bursian, and Riemann (*Z. physiol. Chem.*, 1941, **271**, 177).

The semicarbazone fraction of Crop 2 was also examined in detail. It contained no tricyclic ketone, and the examination revealed no ketonic products other than those already isolated by other workers (Fieser and Fieser, " Natural Products related to Phenanthrene," Reinhold Publ. Corpn., New York, 1949, pp. 364—367). It was observed, during this work, that norcholesterol-24-one reacted quantitatively with Girard reagent-P in hot ethanol during 15 minutes. Under the same conditions pregnenolone was partly converted into a Girard derivative whilst dehydro*epi*androsterone was unaffected. The K.L. ketone acetate was unaffected even in the presence of naphthalene-β-sulphonic acid, and with continuous removal of water by azeotropic distillation with benzene–ethanol.

Crop 3. This product contained no semicarbazones and unlike Crop 2 consisted largely of 3-hydroxy(not 3-acetoxy)-compounds. The free-flowing powder (750 g.) was extracted with boiling methanol (2 l.), and the solution filtered from kieselguhr. On cooling, large quantities of crude cholesterol separated. The filtrate was treated with cold methanol (2 l.) containing potassium hydroxide (100 g.). After 48 hours a further crop of cholesterol was collected. The filtrate was then concentrated to a small bulk under reduced pressure on the steam-bath and the residue was shaken continuously with ether and water. The aqueous layer on acidification, extraction with chloroform, refluxing of the chloroform solution for 1 hour, and extraction with aqueous sodium carbonate gave a chloroformic solution of lactones. Removal of lactones by this alkaline hydrolysis was found to be essential for the subsequent smooth distillation of ketones. The ethereal extract was freed from solvent, and the residual oil was acetylated with acetic anhydride–pyridine in the usual manner. The mixture of acetoxy-compounds was isolated by ether and heated to 140—150° and the vacuum was progressively applied until there was no frothing at 0·1 mm. The hot oil was rapidly transferred to a 500-c.c. glass retort packed with glass wool, and the retort was connected to a receiver and mercury-vapour pump by all-glass joints; full vacuum was then rapidly applied. Unless the oil was still quite mobile, inconvenient frothing occurred. The retort was then heated in an air-bath, which completely enclosed the distillation bend, to 170°/0·001 mm. for 6 hours; the golden distillate partly solidified. One or two crystallisations from methanol gave pure K.L. ketone acetate, m. p. 126—127°. 17 Kg. of Crop 3 residues, worked up in this manner, gave approx. 200 g. of this ketone.

Redistillation of the volatile material collected in the cold traps during the distillation of K.L. ketone acetate (in the hope that some products of profound degradation of the sterol nucleus might be obtained) gave fine elongated plates or needles (subliming readily at 50°/0·001 mm.). The substance crystallised readily from petrol, had m. p. 78—80°, and proved to be acetamide.

Carboxylation of 7-Benzoyloxy-$\Delta^{9(14)}$*-dodecahydro-1-keto-2 : 13-dimethylphenanthrene.*—The K.L. ketone acetate did not react appreciably with 2 : 4-dinitrophenylhydrazone in aqueous-alcoholic hydrochloric acid during 0·5 min. at the b. p. The ketone appears to yield a digitonide which formed colourless needles but these were not closely examined.

The K.L. ketone was prepared from its acetate by hydrolysis with 5% methanolic potassium hydroxide at the room temperature for 4 days. The hydroxy-ketone was usually isolated by means of ether (solution dried over $MgSO_4$) and directly benzoylated.

The *benzoate* was prepared from the K.L. ketone by pyridine–benzoyl chloride at room temperature. It crystallised from ethanol in colourless prisms, m. p. 155—156°, $[\alpha]_D^{20}$ −39·6° (*c* 1 in $CHCl_3$) (Found : C, 78·6; H, 8·0. $C_{23}H_{28}O_3$ requires C, 78·4; H, 8·0%).

Into a solution of the K.L. ketone benzoate (4·0 g.) in dry benzene (40 c.c.), in a separatory funnel, a solution of sodium triphenylmethide in ether was transferred under pressure of nitrogen until a faint pink colour persisted. The suspension of the sodium enolates was then run on chopped solid carbon dioxide. After 10 mins., distilled water was added and the mixture was shaken mechanically The benzene layer was extracted three times with ice-cold 2N-sodium

hydroxide (if emulsions were formed the mixture was acidified and extracted again with aqueous sodium hydroxide). The combined ice-cold aqueous extracts were acidified with ice-cold 2N-hydrochloric acid and rapidly extracted with ether. The ethereal extract was then treated with an excess of ethereal diazomethane. After 0·5 hour the excess of diazomethane was decomposed with 2N-hydrochloric acid. The ethereal extract was washed with aqueous sodium hydrogen carbonate, and with water and dried ($MgSO_4$). On removal of the ether a white crystalline residue of keto-esters (3·0 g.) was obtained. The yield was considerably less when the experiment was scaled up (to 40 g. of K.L. ketone benzoate).

Separation of Methyl 7-Benzoyloxy-$\Delta^{9(14)}$*-dodecahydro-1-keto-2 : 13-dimethylphenanthrene-2-carboxylate* (XX) *and -11-carboxylate* (XXI). (*The A- and B-Keto-esters, respectively.*)—The mixed keto-esters (8·0 g.) were chromatographed in benzene (40 c.c.)–petrol (160 c.c.) on alumina (160 g.). The following fractions (eluant in parentheses) were obtained: (i) 0·15 g., m. p. 91—92°, triphenylmethane (benzene–petrol, 3 : 7); (ii) m. p. 141—147° (benzene–petrol, 1 : 1); (iii) 3·41 g., m. p. 147—148° (benzene); (iv) oil (benzene); (v) 1·91 g., m. p. 177—179° (benzene–chloroform, 7 : 3); (vi) oil (chloroform). Fractions (ii), (iv), and (vi) were rechromatographed, giving the following fractions: (*a*) 0·24 g., m. p. 160—177° (benzene–petrol, 3 : 7); (*b*) 75 mg. (benzene–petrol, 3 : 7); (*c*) 1·02 g., m. p. 146—148° (benzene–petrol, 7 : 3); (*d*) 0·1 g., m. p. 105—148° (benzene); (*e*) m. p. 153—155° (benzene–chloroform, 9 : 1); (*f*) 74 mg., m. p. 177—179° (benzene–chloroform, 1 : 1). Fractions (iii) and (*c*) (4·43 g.) consisted of the B or 11-*carbomethoxy*-isomer (XXI), which crystallised from methanol in colourless, thick prisms, m. p. 148—149°, $[\alpha]_D$ −80° (*c* 1 in $CHCl_3$) (Found: C, 73·3; H, 7·3. $C_{25}H_{30}O_5$ requires C, 73·1; H, 7·3%). Fractions (v) and (*f*) (1·98 g.) consisted of the A or 2-*carbomethoxy*-isomer (XX), which crystallised from methanol in colourless needles, m. p. 177—179°, $[\alpha]_D$ −100° (*c* 1 in $CHCl_3$) (Found: C, 73·0; H, 7·3%). Fraction (*a*) after three recrystallisations from methanol had m. p. 183—185° and consisted of methyl triphenylacetate (Found: C, 83·0; H, 6·0. Calc. for $C_{21}H_{18}O_2$: C, 83·4; H, 6·0%). Fraction (*e*) did not depress the m. p. of a specimen of the starting material (K.L. ketone benzoate).

Attempted Preparation of the Bilianic Acids from Substance-A.—The keto-ester A (1·5 g.), activated zinc (4·5 g.), ether (2·5 c.c.), benzene (25 c.c.), and methyl bromoacetate (1·5 c.c.) were stirred and heated under nitrogen. On addition of a crystal of iodine a vigorous reaction was initiated. The mixture was stirred and refluxed for 24 hours with occasional addition of methyl bromoacetate (total 3·0 c.c.) and zinc (total 4·5 g.). The mixture was cooled in ice and shaken with ice-cold aqueous acetic acid. The organic layer was washed with cold dilute aqueous ammonia, and with water, and was dried ($MgSO_4$). The residue (1·68 g.) crystallised readily but only 0·2 g. of a pure isomer was obtained on three crystallisations from methanol; this formed colourless prisms, m. p. 164—165°, of *methyl 3-benzoyloxy-*$\Delta^{9(14)}$*-decahydro-1-hydroxy-2 : 13-dimethylphenanthrene-1-acetate-2-carboxylate* (Found: C, 69·2; H, 7·6. $C_{28}H_{36}O_7$ requires C, 69·4; H, 7·5%). The crude ester (1·6 g.) was dissolved in dry pyridine (16 c.c.); phosphoryl chloride (1·6 c.c.) was added with caution, and the mixture was refluxed gently for 45 minutes. The dark brown reaction mixture was cooled and poured on chopped ice. The mixture was shaken with ether and ice-cold 2N-hydrochloric acid. The ethereal layer was washed with more acid, and water, and dried ($MgSO_4$). On evaporation of the solvent a solid (1·12 g.) was obtained which crystallised from methanol as fine, white needles, m. p. 143—153° with sintering at 135°. This was assumed to be a mixture of geometrical isomerides of the unsaturated *benzoate dimethyl* ester (cf. V) (Found: C, 71·9; H, 7·6. $C_{28}H_{34}O_6$ requires C, 72·0; H, 7·3%).

The crude unsaturated esters (1·1 g.) were dissolved in 10% methanolic potassium hydroxide (10 c.c.), and the mixture was refluxed for 6 hours. After concentration *in vacuo*, water was added and neutral material was removed by ether-extraction. The acids, on acidification of the aqueous solution, were extracted with ether. The ethereal extract was treated with ethereal diazomethane. The resulting methyl esters (0·6 g.), freed from methyl benzoate at 110°/0·01 mm. during an hour, were hydrogenated in glacial acetic acid in the presence of platinic oxide. Analysis of the product after benzoylation suggested that hydrogenation was incomplete. The substance was therefore hydrolysed, esterified, hydrogenated, and re-benzoylated. The mixture of benzoate dimethyl esters (0·36 g.) was then chromatographed on acid-washed alumina. The fractions eluted with benzene–petrol (1 : 1) crystallised on slow evaporation of a light petroleum (b. p. 60—80°) solution. The product (70 mg.) was crystallised several times from light petroleum (b. p. 60—80°). *The benzoate dimethyl* ester (cf. VI) crystallised in elongated prisms, m. p. 106—107° (microscope hot-stage) (Found: C, 70·8; H, 7·4. $C_{28}H_{38}O_6$ requires C, 71·5; H, 8·1. $C_{28}H_{38}O_7$ requires C, 69·1; H, 7·8%). This sample was unfortunately lost before its specific rotation could be determined. The fraction eluted with benzene–petrol

(4 : 1) deposited a few crystals A (ii) on evaporation of the solvent. The substance, after one recrystallisation from light petroleum (b. p. 60—80°), melted at 153—155° (microscope hot-stage). There was insufficient material (<1 mg.) for rotation or analysis. A crystal on admixture with authentic natural bilianate showed a strong depression of the m. p. Two further isomers were isolated in such small quantities that a rotation or analysis could be determined but not both. The former only was determined. A(iii), crystallised in fine, hair-like needles from light petroleum (b. p. 60—80°), had m. p. (microscope hot-stage) 129—132°, $[\alpha]_D^{20}$ −11·5° (*c* 1 in $CHCl_3$). A(iv) crystallised from light petroleum (b. p. 60—80°) as colourless, imperfect prisms, m. p. 121—123° (microscope hot-stage), $[\alpha]_D^{20}$ +83·8° (*c* in $CHCl_3$).

Attempted Preparation of the Bilianic Acids from Substance-B.—The same procedure was used as for substance-A. The Reformatsky reaction proceeded less smoothly, and the dehydration was unsatisfactory. The benzoate dimethyl esters were finally purified by chromatography. The fraction eluted with methanol and crystallisation from light petroleum (b. p. 60—80°) gave colourless prisms (11 mg.), m. p. 148·5—149° (microscope hot-stage), $[\alpha]_D^{20}$ +83° (*c* 1 in $CHCl_3$) (Found: C, 71·7; H, 8·0. $C_{28}H_{38}O_6$ requires C, 71·5; H, 8·1%). The rotation alone was sufficient to indicate that this was not the *natural* bilianate. The fraction eluted with benzene eventually gave a few colourless, rectangular plates (<1 mg.), m. p. 161—161·5° (microscope hot-stage).

After a considerable interval these experiments were resumed.

11-*Carbomethoxy*-$\Delta^{9(14)}$-*dodecahydro*-1 : 7-*dihydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetic Acid* (XXIII).—A mixture of the B-keto-ester (2·001 g.), benzene (20 c.c.), ether (20 c.c.), granulated zinc (6 g.; activated according to Fieser and Johnson, *J. Amer. Chem. Soc.*, 1940, **62**, 576), coarsely ground Pyrex glass (15—20 g.; 20-mesh), and methyl bromoacetate (2 c.c.) was stirred and refluxed under nitrogen. Addition of iodine (0·2 g.) initiated the reaction; a sticky greenish solid was formed and adhered to the glass, leaving the zinc surface clean. After 8 hours, more methyl bromoacetate (0·75 c.c.) was added; after 10 hours, heating and stirring were stopped. Next day, ice-cold 2N-acetic acid (50 c.c.) was added, and the glass and residual zinc were removed and washed with dilute acetic acid and ether. The ether–benzene layer was washed with water and then four times with dilute, ice-cold aqueous ammonia; after a final washing with water it was dried ($MgSO_4$) and evaporated at low pressure, finally at 40°/0·5 mm. The residue was dissolved in a little methanol, seeded with the keto-ester, and left at 0° for 2 days with occasional shaking. Unchanged keto-ester (582 mg.) was removed and the filtrate evaporated, leaving a gum (1853 mg.) smelling of methyl benzoate. Hot methanol (88 c.c.) was added, followed gradually by 0·2N-sodium hydroxide (44 c.c.). The mixture was boiled for 19 hours, the methanol was largely removed at low pressure, and the residue after dilution with water was extracted with ether. The ethereal extract smelled strongly of acetophenone. Acidification of the aqueous solution, freed from ether, gave a crystalline precipitate (853 mg.), m. p. 188—192°. A small further quantity was obtained by extracting the filtrate with ether and removing benzoic acid from the crude acid by extraction with light petroleum. Recrystallisation from aqueous methanol gave the *half-ester* as slender needles, m. p. 195—196°, $[\alpha]_D^{20}$ −57° (*c* 3·3 in EtOH) (Found: C, 65·7; H, 8·2. $C_{20}H_{30}O_6$ requires C, 65·6; H, 8·2%). The mother-liquors were examined for a stereoisomer, but without result. The *methyl* ester, prepared with diazomethane in the usual manner, crystallised from ether in silky needles, m. p. 164—165°, $[\alpha]_D^{20}$ −57° (*c* 4·0 in $CHCl_3$) (Found: C, 65·7; H, 8·3. $C_{21}H_{32}O_6$ requires C, 66·3; H, 8·4%).

The half-ester (478 mg.) in acetic acid (5 c.c.; purified) with platinum oxide (63 mg.) was shaken with hydrogen at room temperature and pressure, one molecular proportion being absorbed in 15 minutes. Ethanol was added to redissolve the crystalline product, the catalyst was removed, and the solvent evaporated. Recrystallisation of the residue from acetone gave stout prisms, m. p. 216°, of 11-*carbomethoxyperhydro*-1 : 7-*dihydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetic acid* (XXIV; R = H) * (90%), $[\alpha]_D^{19}$ −15·8° (*c* 5·0 in EtOH) (Found: C, 65·1; H, 8·7. $C_{20}H_{32}O_6$ requires C, 65·2; H, 8·7%).

This product (500 mg.), dissolved in a little dioxan, was esterified with ethereal diazomethane. The *methyl* ester (XXIV; R = Me), crystallised from light petroleum (b. p. 60—80°) in silky needles (487 mg.), m. p. 132°, $[\alpha]_D^{19}$ −18·2° (*c* 2·6 in $CHCl_3$) (Found: C, 65·2; H, 8·9. $C_{21}H_{34}O_6$ requires C, 66·0; H, 8·9%). It was dissolved in dry pyridine (2 c.c.), and acetic anhydride (1 c.c.) added. After 20 hours at 37° the product, a gum, was isolated in the normal manner, dissolved in dry pyridine (5·5 c.c.), cooled in ice, and treated dropwise with freshly distilled phosphoryl chloride (0·55 c.c.). After 3 hours' refluxing the solution was cooled, poured on ice, acidified, and extracted with ether. Evaporation of the ether left a gum (470

mg.), $[\alpha]_D^{22}$ −45° (in MeOH). This was dissolved in pure ether and the solution passed through acid-washed alumina (5 g.). The filtrate was divided into two parts.

One half was evaporated, and the residue was hydrogenated in purified acetic acid (5 c.c.) at room temperature and pressure in presence of platinum oxide (60 mg.). After 48 hours the catalyst was removed and the solution, which showed $[\alpha]_D^{22}$ +8°, was evaporated. The residue was dissolved in methanol (12 c.c.) and neutralised; 0·2N-sodium hydroxide (7 c.c.) was then added and the mixture was refluxed overnight, The resulting acidic product (200 mg.) was isolated in the usual way; it deposited the undehydrated half-ester (XXIV; R = R′ = H) (marked * above) (31 mg.) when kept in ethyl acetate–light petroleum. The rest of the product (" residue X ") was saponified (see below).

The other half of the alumina filtrate was evaporated and the residue dehydrated again (3 hours' reflux) with pyridine (2·5 c.c.) and phosphoryl chloride (0·25 c.c.). The product was isolated as before, passed through alumina, and hydrogenated in acetic acid (5 c.c.), over platinum oxide (50 mg.), for 18 hours. The solution, $[\alpha]_D^{22}$ +13°, was evaporated and the residue in hexane was chromatographed on acid-washed alumina (20 g.), the effluents being examined polarimetrically. Hexane and hexane–benzene (3 : 1) eluted only a trace of material; with hexane–benzene (1 : 1), after a fore-run (31 mg.) of $[\alpha]_D^{22}$ +3° (these rotations are approximate), a gum (125 mg.) of +10° was obtained. After this, a fraction (34 mg.) of $[\alpha]_D^{22}$ +20° was slowly eluted; the column was then washed out with ether to give a final fraction, $[\alpha]_D^{22}$ +30° (37 mg.). After attempts to crystallise these " acetate dimethyl ester " fractions as such or after conversion into "hydroxy monomethyl ester," "hydroxy dimethyl ester," and "3 : 5-dinitrobenzoate dimethyl ester " had all failed, they were saponified to the " hydroxy-dicarboxylic acid " state, either by refluxing them overnight with 10% potassium hydroxide in methanol or by fusion for a few minutes with potassium hydroxide and water at 150—160°. The acids were isolated by means of ether and crystallised from aqueous acetic acid. From the +3° and +10° fractions, solvated *perhydro-7-hydroxy-2 : 13-dimethylphenanthrene-1-acetic-11-carboxylic acid* (XXVI) (~60 mg.) was obtained and recrystallised from acetic acid as well-formed prisms, m. p. about 140°, $[\alpha]_D^{20}$ +26·5° (*c* 4·67 in EtOH) (Found : C, 63·0; H, 8·5%; equiv., 132. $C_{19}H_{30}O_5,C_2H_4O_2$ requires C, 63·3; H, 8·5%; equiv., 133). Another, and perhaps unsolvated, form crystallised poorly from ethyl acetate, with m. p. 215°, $[\alpha]_D^{22}$ +33° (*c* 1 in MeOH); crystallisation of this from acetic acid gave the solvated form. From the +30° fraction another *acid* was obtained; it crystallised from ethyl acetate in small leaflets (2·5 mg.), m. p. 242°, $[\alpha]_D^{22}$ +72° (*c* 0·5 in $CHCl_3$); after recovery of the specimen from the rotation tube only 1·47 mg. were available for analysis (Found : C, 69·5; H, 8·7. $C_{19}H_{30}O_5$ requires C, 67·5; H, 8·9. $C_{19}H_{30}O_4$ requires C, 70·8; H, 9·3%). Accordingly this acid may lack the 7-hydroxy-group.

Saponification (by potassium hydroxide fusion) of " residue X " without chromatography, and crystallisation of the product from aqueous acetic acid, gave a first crop of the solvated acid, a second crop (6 mg.) consisting largely of the acid, m. p. 242° (2 mg. after recrystallisation from ethyl acetate), and further crops of the solvated acid (total about 60 mg.). The latter acid was also obtained from the residues of a previous experiment where dehydration had been far from complete and a considerable amount of undehydrated material had been separated as the acid.

2-Carbomethoxy-Δ⁹⁽¹⁴⁾-dodecahydro-1 : 7-dihydroxy-2 : 13-dimethylphenanthrene-1-acetic Acid α and β (XXVII).—A mixture of the A keto-ester (XX) (1·499 g.) with benzene (25 c.c.), ether (20 c.c.), methyl bromoacetate (1·5 c.c.), ground glass (15 g.), and activated zinc (4·5 g.) was heated and stirred under nitrogen, and reaction was initiated by addition of iodine (0·2 g.). The mixture was refluxed for 16 hours, methyl bromoacetate (1·5 c.c.) being added after 6 and 12 hours, and zinc (3·5 g.) after 12 hours. The total neutral product (1·967 g. after drying at 80°/1 mm.) was isolated as described above for the B series. It was dissolved in hot methanol (100 c.c.); 0·2N-sodium hydroxide (55 c.c.) was gradually added and the mixture was refluxed for 27 hours; a strong smell of acetophenone was noticed. Most of the methanol was removed at low pressure, and the residue was twice extracted with ether, freed from dissolved ether, brought to a volume of 120 c.c., and acidified. The oily precipitate was extracted several times with ether. The extract was treated with magnesium sulphate and a little charcoal, filtered, and evaporated. The residue was extracted repeatedly with hot petrol to remove benzoic acid, then dissolved in a little ether and cooled, whereupon *2-carbomethoxy-Δ⁹⁽¹⁴⁾-dodecahydro-1 : 7-dihydroxy-2 : 13-dimethylphenanthrene-1-acetic acid* α (240 mg.) separated. Recrystallisation from ethyl acetate gave colourless rods, m. p. 221°, $[\alpha]_D^{21}$ −50° (*c* 2·5 in MeOH) (Found, in material dried at 135° *in vacuo* : C, 65·5; H, 8·2. $C_{20}H_{30}O_6$ requires C, 65·6; H, 8·2%).

The ethereal filtrate from the α acid left a residue (966 mg.) which eventually crystallised but

could not easily be purified. Some of it (800 mg.) was esterified with ethereal diazomethane. The resulting crude ester was treated in a little benzene with pyridine (0·4 c.c.) and 3 : 5-dinitrobenzoyl chloride (0·8 g.). Next day the product was isolated by normal procedures; crystallisation from ethyl acetate gave two light yellow 3 : 5-*dinitrobenzoates* of methyl $\Delta^{9(14)}$-dodecahydro-1 : 7-dihydroxy-2 : 13-dimethylphenanthrene-2-carboxylate-1-acetate. The Aα derivative, prisms from ethyl acetate, had m. p. 187—188° (Found: C, 58·3; H, 6·0. $C_{28}H_{34}O_{11}N_2$ requires C, 58·5; H, 5·9%) undepressed by a specimen prepared from the Aα acid of m. p. 221°. The Aβ derivative separated from ethyl acetate in fine needles, m. p. 186—187° depressed by the Aα isomer (Found: C, 58·5; H, 5·7%). The Aβ ester was saponified with 0·4N-aqueous-methanolic sodium hydroxide, and dinitrobenzoic acid then removed by reduction with sodium dithionite. This was not a good procedure and the recovery of saponified product was poor. Recrystallisation from aqueous acetic acid gave hydrated 2-*carbomethoxy*-$\Delta^{9(14)}$-*dodecahydro*-1 : 7-*dihydroxy*-2 : 13-*methylphenanthrene*-1-*acetic acid* β in nodules, m. p. 182—183°, $[\alpha]_D^{22}$ −67°(*c* 2 in MeOH) (Found: C, 63·8; H, 7·8. $C_{20}H_{30}O_6,\frac{1}{2}H_2O$ requires C, 64·0; H, 8·3%).

2-*Carbomethoxyperhydro*-1 : 7-*dihydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetic Acid* α (XXVIII; R = R′ = H).—The above monomethyl ester α (127·6 mg.) was hydrogenated in acetic acid (5 c.c.) over platinum oxide (15 mg.). Uptake of hydrogen was rapid but shaking was continued for several hours. The catalyst and solvent were removed and the residue crystallised from aqueous acetic acid in fine needles (72 mg.). The *monomethyl ester* α (XXVIII; R = R′ = H) appeared to be solvated; it softened at 160°, resolidified, and melted at 192° (decomp.); it had $[\alpha]_D^{22}$ −8° (*c* 1·4 in MeOH) (Found, in material dried *in vacuo* at 80°: C, 62·2; H, 9·3. $C_{20}H_{32}O_6,H_2O$ requires C, 62·2; H, 8·8%). A further 25—30 mg. were obtained from the mother-liquors.

Methyl Perhydro-1 : 7-*dihydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetate*-2-*carboxylate* α (XXVIII; R = H, R′ = Me).—The monomethyl ester (XXVIII) above (95 mg.) was dissolved in a little methanol and esterified with ethereal diazomethane. The *dimethyl* ester (85 mg.) separated from light petroleum (b. p. 60—80°) in blades, pointed at one end, m. p. 141—142°, $[\alpha]_D^{22}$ −11·5° (*c* 1·6 in MeOH) (Found: C, 65·7; H, 9·0. $C_{21}H_{34}O_6$ requires C, 66·0; H, 8·9%). From mother-liquors, and by esterification of the mother-liquors of the monomethyl ester (XXVIII), a further 15 mg. were collected.

Methyl 7-*Acetoxyperhydro*-1-*hydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetate*-2-*carboxylate* α and β (XXVIII; R = Ac, R′ = Me).—A mixture of the above dimethyl ester (93·5 mg.), pyridine (0·4 c.c.), and acetic anhydride (0·2 c.c.) was kept for 24 hours and the neutral product then isolated. The *dimethyl acetoxy*-ester α (90 mg.) crystallised from light petroleum (b. p. 60—80°) in prisms, m. p. 117·5—118·5°, $[\alpha]_D^{23}$ −15° (*c* 1·6 in MeOH) (Found: C, 65·1; H, 8·8. $C_{23}H_{36}O_7$ requires C, 65·1; H, 8·5%). Hydrogenation of 2-carbomethoxy-$\Delta^{9(14)}$-dodecahydro-1 : 7-dihydroxy-2 : 13-dimethylphenanthrene-1-acetic acid β (110 mg.) afforded the monomethyl ester β (XXX; R = R′ = H) as diamond-shaped leaflets, m. p. 196°, from dilute acetic acid. Esterification with diazomethane gave the dimethyl ester (XXX; R = H, R′ = Me), leaflets (from ether), m. p. 141—142°, $[\alpha]_D^{22}$ −4° (*c* 1·2 in MeOH); and on acetylation *methyl* 7-*acetoxyperhydro*-1-*hydroxy*-2 : 13-*dimethylphenanthrene*-1-*acetate*-2-*carboxylate* β (XXX; R = Ac, R′ = Me) (75 mg.) was obtained. It crystallised from petrol in plates, m. p. 116—117°, strongly depressed by admixture with the α isomer, and had $[\alpha]_D^{23}$ −12° (*c* 1 in MeOH) (Found: C, 65·6; H, 8·1. $C_{23}H_{36}O_7$ requires C, 65·1; H, 8·5%).

Methyl 3(β)-*Benzoyloxyætio*allo*bilianate* (XXIX; R = Bz).—The dimethyl acetoxy-ester α (80 mg.) in pyridine (3 c.c.) was cooled in ice and treated with phosphoryl chloride (0·3 c.c.); the mixture was then refluxed for 1½ hours, and the product isolated as described above (B series). A gum (75 mg.) of $[\alpha]_D^{23}$ −12° (in Et_2O) was obtained. It was passed in pure ether through alumina (2 g.); the ether was replaced by acetic acid (3 c.c.), platinum oxide (34 mg.) added, and the mixture shaken with hydrogen for 20 hours. The specific rotation (in ether–acetic acid) was then about +3°. The product crystallised partly when seeded with methyl 3(β)-acetoxyætio*allo*bilianate (XXIX; R = Ac), and the crystals were lævorotatory; but this derivative did not seem satisfactory for purification. The total product was therefore dissolved in methanolic hydrogen chloride (2 c.c. of 1·5N); the solution was kept overnight, refluxed for 0·5 hour, and treated as usual for recovery of the neutral product. This was treated in a little pyridine with benzoyl chloride (0·1 c.c.). After 1 hour water was added and the neutral product isolated, dissolved in a few drops of light petroleum (b. p. 60—80°), and seeded with a trace of methyl 3(β)-benzoyloxyætio*allo*bilianate; crystallisation set in. Next day there were two kinds of crystals: fine, hairy needles and thick plates. On gentle warming, the needles dissolved; from the decanted solution a further crop of plates separated after 48 hours, and the needles

did not reappear. These two crops were recrystallised from light petroleum (b. p. 60—80°) and united; the whole (13 mg.) was recrystallised once more, the double melting point (in a capillary) remaining unchanged at 140° and 150—151°. The final yield of twice recrystallised material was 9 mg. The double melting points (in a capillary) were not depressed on mixing with the " natural " substance (XXII) (see p. 579). On the microscope hot-stage fine hair-like needles started growing from the elongated plates of the " synthetic " substance at 140°, and at 141·5° the unchanged plates melted, the needles finally melting at 154—155°. On admixture with the " natural " substance (which underwent these changes at 142°, 145°, and 158—159° respectively) the same phenomena occurred, the needles finally melting at 154—159°. The " synthetic " substance had $[\alpha]_D$ −16° and the " natural " $[\alpha]_D^{15}$ −16·2°. The rotations were also determined in a micro-tube by Dr. F. B. Strauss, who found: " synthetic " $[\alpha]_D^{20}$ −21·9° (*c* 2 in $CHCl_3$); natural $[\alpha]_D^{20}$ −18·3° (*c* 3 in $CHCl_3$). Analyses of the same quantities of both *esters* gave: " Synthetic " C, 72·0; H, 8·1. " Natural " C, 71·4; H, 7·5% ($C_{28}H_{38}O_6$ requires C, 71·5; H, 8·1%).

We are very grateful to Dr. Cowan for the following report.

" The ' natural ' and ' synthetic ' specimens examined were both nicely crystalline, growing as needles. The natural crystals were quite large, the ' synthetic ' consisting of rather small, slender needles. The crystals were monoclinic with *b* parallel to the needle axis. The (100) and (001) faces were almost equally developed in most crystals giving a roughly diamond-shaped cross section. Crystal shape and optics were similar for both specimens.

" *X*-Ray powder photographs of the ' natural ' and ' synthetic ' materials showed a majority of lines having spacing and relative intensity the same but each photograph appeared to have a few weak lines which did not correspond to any of those of the other. This observation could either be interpreted as due to slight, but significant, differences in crystal structure, or to traces of impurities in the crystals. It was therefore necessary to take single crystal photographs to check this result.

" *X*-Ray photographs taken for crystals of each specimen mounted about the [010] and [001] axes were found to be identical, thus confirming that the non-correspondence of certain lines in the powder photographs was due to traces of impurities, mainly in the synthetic specimen. The dimensions of the monoclinic unit cell are as follows: $a = 14{\cdot}07$, $b = 6{\cdot}04$, $c = 15{\cdot}05$ Å, $\beta = 94{\cdot}7°$. The space group is P_{2_1}. P. M. Cowan. D. M. Crowfoot Hodgkin. [Laboratory of Chemical Crystallography, University Museum, Oxford. May 1951.] "

When the dimethyl acetoxy-ester β (XXX; R = Ac, R′ = Me) (63 mg.) was submitted to the same process of dehydration, hydrogenation, alcoholysis, and benzoylation, the product crystallised partly. On recrystallisation from light petroleum (b. p. 60—80°), colourless plates, m. p. 155°, were obtained; on recrystallisation of these from methanol, pearly plates, m. p. 146°, resulted. This product on analysis was found to have retained the tertiary hydroxyl group, and was therefore *methyl 7-benzoyloxyperhydro-1-hydroxy-2 : 13-dimethylphenanthrene-1-acetate-2-carboxylate* β (Found: C, 69·0; H, 7·3. $C_{28}H_{38}O_7$ requires C, 69·1; H, 7·9%).

Carboxylation of 7-Benzoyloxyperhydro-1-keto-2 : 13-dimethylphenanthrene.—The *benzoyl* derivative, prepared in the usual manner from the hydroxy-ketone (Achtermann, *Z. physiol. Chem.*, 1934, **225**, 141; Billeter and Miescher, *Helv. Chim. Acta*, 1950, **33**, 388), crystallised from methanol in colourless prisms, m. p. 137—138°, $[\alpha]_D^{20}$ +10·7° ± 5° (*c* 0·4 in $CHCl_3$) (Found: C, 77·4; H, 8·2. $C_{23}H_{30}O_3$ requires C, 78·0; H, 8·5%).

This Achtermann ketone benzoate (820 mg.) in benzene (10 c.c.) was treated with a slight excess of ethereal sodium triphenylmethide. The suspension was then added to chopped solid carbon dioxide. When evolution of carbon dioxide had ceased the mixture was shaken with ice-water, and the resulting emulsion was broken in a refrigerated centrifuge. The aqueous extract was extracted with a further quantity of ether and was then acidified with ice-cold 2N-sulphuric acid. The liberated keto-acids were extracted with ether–benzene and esterified with ethereal diazomethane. The methyl esters, after removal of the ether, were chromatographed on alumina. Benzene–petrol (1 : 1) eluted methyl triphenylacetate, m. p. 182—183°. Benzene–chloroform (4 : 1) eluted two *keto-esters*, (i) colourless prisms (from methanol), m. p. 135—136°, $[\alpha]_D^{20}$ −55·6° ± 2° (*c* 0·7 in $CHCl_3$) (Found: C, 72·8; H, 8·0. $C_{25}H_{32}O_5$ requires C, 72·8; H, 7·8%), and (ii) elongated plates (from methanol), m. p. 164—165°, $[\alpha]_D^{20}$ −48·8° ± 3° (*c* 0·8 in $CHCl_3$) (Found: C, 72·5; H, 8·8%). The overall yield was low and an accurate estimate of the relative amounts of the isomeric esters could not be made, but they appeared to be present in comparable amount, the ester (ii) possibly being the more abundant.

Preparation of bilianic acid and syntheses of tetracylic ketones.

Reduction of Androst-4-ene-3 : 17-dione.—Androstenedione (5·0 g.) in ethanol (50 c.c.) was hydrogenated at room temperature and pressure in the presence of palladised strontium carbonate (850 mg.); one mol. of hydrogen was absorbed in 19 minutes. The product readily crystallised from *n*-hexane in elongated plates, m. p. 90—92°, not raised on repeated crystallisation. Occasionally it crystallised in prisms of two kinds that could be separated mechanically; both had m. p. 126—128°, but much lower on admixture. The entire product was then separated into these two substances by repeated chromatography on alumina. First androstane-3 : 17-dione, m. p. 128—129° (eluted with benzene–petrol, 1 : 9), and then testane-3 : 17-dione, m. p. 128—129° (eluted with benzene–petrol, 1 : 1), were obtained in approximately equal quantities. Paland, quoted by Butenandt, Tscherning, and Hanisch (*Ber.*, 1935, **68**, 2099), stated that reduction of androstenedione gave androstane-3 : 17-dione.

3β-*Hydroxyætiobilienic Acid* (X).—This acid was isolated in 0·9% overall yield from crude acids obtained as by-products of the oxidation of cholesteryl acetate dibromide *via* the water-soluble potassium salts. This material was kindly supplied by British Drug Houses Ltd. The dipotassium salt was precipitated from methanolic solution (cf. Kuwada, *J. Pharm. Soc. Japan*, 1936, **56**, 75; Wieland and Miescher, *Helv. Chim. Acta*, 1948, **31**, 211), and the recovered and purified acid had m. p. 240—241° (decomp.), alone or mixed with an authentic specimen kindly provided by Dr. E. B. Hershberg. The dimethyl ester, m. p. 112—112·5° (Kuwada, *loc. cit.*, gives m. p. 112°), had $[\alpha]_D^{20}$ −77° (*c* 5·2 in $CHCl_3$). Neither the acid nor the ester decolorised bromine in acetic acid at room temperature but did so rapidly at 50—60°.

3β-*Hydroxyætio*allo*bilianic Acid* (XXXI; R = R′ = H).—The bilienic acid (3·9 g.) in glacial acetic acid (60 c.c.) was hydrogenated in the presence of platinic oxide (200 mg.). After 4 hours the solution was filtered from the catalyst, and the solvent removed under reduced pressure. The bilianic acid crystallised from aqueous methanol in colourless needles, m. p. 238° (decomp.) (Kuwada and Miyasaka, *J. Pharm. Soc. Japan*, 1936, **65**, 631, give m. p. 239°), which did not decolorise bromine in acetic acid at 50—60°. The *dimethyl* ester, prepared by means of diazomethane, crystallised from benzene–light petroleum (b. p. 60—80°) in colourless prisms, m. p. 111—112°, $[\alpha]_D^{20}$ −23·7° (*c* 1·13 in $CHCl_3$) (Found: C, 68·6; H, 9·5. $C_{21}H_{34}O_5$ requires C, 68·8; H, 9·4%). This ester was also obtained by direct hydrogenation of the dimethyl bilienate. It was then less easy to purify, presumably owing to contamination by the isomeric bilianic ester (rings A–B *cis*). The *benzoate* (XXII) of the dimethyl ester crystallised from light petroleum (b. p. 60—80°) in colourless, elongated prisms, m. p. (capillary) 149—150° with softening at 140°. On a microscope hot-stage, long needles started to grow out of the prisms at 142°, at 145—146° those prisms which had not changed into needles melted, and the needles finally melted at 158—159°. Very slow heating was essential for observation of this highly characteristic phenomenon. The derivative had $[\alpha]_D^{15}$ −16·2° (*c* 5·3 in $CHCl_3$), hence $[M]_D$ 3-benzoate *minus* 3-hydroxy, +15° (Barton, *J.*, 1945, 813, gives +2° ± 3° for this change in stanols) (Found: C, 71·4; H, 8·4. $C_{28}H_{38}O_6$ requires C, 71·5; H, 8·1%).

3β-*Hydroxyætio*allo*bilianic Acid* 17-*Methyl Ester* (XXXI; R = H, R′ = Me).—The dimethyl 3-hydroxy-ester (1·6 g.), potassium carbonate (0·5 g.), water (2 c.c.), and methanol (18 c.c.) were heated on a steam-bath for 24 hours with occasional addition of water (total 25 c.c.). The hot solution was acidified with 2N-hydrochloric acid. The crystalline *half-ester* (yield 95%) was recrystallised from aqueous methanol, forming colourless needles, m. p. 175—177° after being dried at 80° in a high vacuum, $[\alpha]_D^{17}$ −19·0° (*c* in MeOH) (Found: C, 68·1; H, 8·9. $C_{20}H_{32}O_5$ requires C, 68·2; H, 9·1%). The dimethyl benzoyloxy-ester was hydrolysed in a similar manner and the resulting 3-hydroxy-half-ester, after removal of benzoic acid by sublimation, had m. p. 176—177°, $[\alpha]_D^{17}$ −19·0°, alone or mixed with the above half-ester.

3β-*Acetoxyætio*allo*bilianic Acid* 17-*Methyl Ester* (XXXI; R = Ac, R′ = Me).—The above half-ester was acetylated (acetic anhydride–pyridine), and the product refluxed with aqueous acetic acid to hydrolyse any mixed anhydrides. The *acetoxy-half-ester* (yield, 80%) crystallised from aqueous acetone in colourless needles, m. p. 92—97°, $[\alpha]_D^{15}$ −28·1 (*c* 1 in EtOH), whence $[M]_D$ 3-acetate *minus* 3-hydroxy = −34° (Barton gives −34° ± 11° for stanols) (Found: C, 64·6; H, 8·8. $C_{22}H_{34}O_6,H_2O$ requires C, 64·1; H, 8·7%).

3β-*Acetoxyandrostan*-17-*one* (epi*Androsterone Acetate*) (XXXIII; R = Ac).—The acetoxy-half-ester (4·3 g.) was warmed with dry benzene (20 c.c.) and oxalyl chloride (10 c.c.) until evolution of hydrogen chloride ceased. The mixture was evaporated to dryness under reduced pressure on the steam-bath, and this process was thrice repeated with fresh quantities of dry benzene. The residual oil was dissolved in light petroleum (b. p. 60—80°) and filtered from some unchanged acetoxy-half-ester into an ice-cold ethereal solution of diazomethane (1 g.).

The mixture was kept overnight at −5° and the solvent then removed under diminished pressure at room temperature. The oily diazo-ketone did not crystallise and was treated in 95% ethanol at 50° with silver nitrate (2 g.) in aqueous ammonia (20 c.c.; *d* 0·880). After 2½ hours' refluxing the solution was concentrated *in vacuo* and extracted with ether. The oily amide, obtained on evaporation of the solvent, was dissolved in methanol (40 c.c.) containing potassium hydroxide (4 g.). The mixture was refluxed for 16 hours and then concentrated *in vacuo*. On acidification an oil separated from which a few crystals separated slowly. These were recrystallised several times from aqueous methanol and found to be the parent acid, m. p. 236—238° (Found : C, 67·2; H, 8·9. Calc. for $C_{19}H_{30}O_5$: C 67·5; H, 8·9%). The remaining oily acid (XXXII) was refluxed in acetic acid (200 c.c.) containing acetic anhydride (13 c.c.) for 6 hours. The solvent was removed by distillation, and the residue was heated to 250—260°/15 mm. for 15 minutes and then distilled at 0·001 mm. The clear distillate slowly crystallised, and was purified by chromatography. *epi*Androsterone acetate (overall yield 15%) crystallised from petrol in thick, colourless prisms, m. p. 103—104°, $[\alpha]_D^{18}$ +64·6° ± 2° (*c* 1 in $CHCl_3$), whence $[M]_D$ 3-acetate *minus* 3-hydroxy = −30° (Barton gives −34° ± 11° for stanols) (Found : C, 75·3, 75·6, 76·5; H, 9·4, 10·5, 9·5. Calc. for $C_{21}H_{32}O_3$: C, 75·9; H, 9·6%). Its m. p. was not depressed on admixture with a sample (prepared by the hydrogenation and re-oxidation of dehydro*epi*androsterone acetate), m. p. 101·5—103·4°, $[\alpha]_D^{18}$ +68·5° ± 2° (Found : C, 76·2; H, 9·6%). Barton (*J.*, 1946, 1116) gives $[\alpha]_D$ +69°. Ruzicka, Goldberg, and Brüngger (*Helv. Chim. Acta*, 1934, **17**, 1389, 1395) give m. p. 96—97° (but not a rotation) for a sample prepared by chromic acid oxidation of cholestanyl acetate; Butler and Marrian (*J. Biol. Chem.*, 1938, **124**, 237) give m. p. 115—118° for an acetate prepared from *epi*androsterone which had been isolated from the urine of a patient suffering from an adrenal tumour, but they give neither an analysis nor the optical rotatory power of the specimen. Drs. Hodgkin and Cowan reported that the two specimens were crystallographically identical.

Hydrolysis of the above synthetic acetate gave *epi*androsterone, m. p. 171—172° (Ruzicka *et al.*, *loc. cit.*, give m. p. 174—174·5°), alone or mixed with a specimen prepared by hydrolysis of the acetate obtained by degradation of tetracyclic material (see above).

*Preparation of Dehydro*epi*androsterone Acetate from Ætiobilienic Acid.*—Our results agreed with one exception, with those given by Kuwada and Nakamura (*loc. cit.*), by Hershberg, Schwenk, and Stahl (*loc. cit.*), and by Heer and Miescher (*Helv. Chim. Acta*, 1947, **30**, 786). These authors state respectively that 3-acetoxyætiobilienic acid 17-methyl ester melted at 168·5—169·5°, 170—171·5°, and 167°. Our specimen crystallised from aqueous acetone in colourless, rectangular plates which, after being dried at 100° *in vacuo* for 2 hours, melted (capillary or micro-hot-stage) at 156—157°, then resolidified, and finally melted at 167° (Found : C, 67·8; H, 8·2. Calc. for $C_{22}H_{32}O_6$: C, 67·3; H, 8·2%).

Reduction of Androst-4-ene-3 : 17-dione Enol Acetate.—A mixture of androstenedione (11·0 g.), acetyl chloride (80 c.c.), and acetic anhydride (80 c.c.) was refluxed for 3 hours and then concentrated to about 20 c.c. under reduced pressure. On cooling, the enol acetate (10 g.) solidified. It crystallised from methanol in needles, m. p. 124—125° (Ruzicka and Fischer, *Helv. Chim. Acta*, 1936, **19**, 1371, give m. p. 127—129°).

This (10·0 g.) in ether (200 c.c.) was added, with stirring, to a solution of potassamide (from 10·0 g. of potassium) in liquid ammonia (750 c.c.), and the solution kept for an hour. Ammonium chloride (20 g.) was added, the solution set aside for a further hour, and the ammonia evaporated off. The residue was extracted with ether (5 × 200 c.c.), and the ethereal extract added immediately to a solution of lithium aluminium hydride (5·0 g.) in ether (500 c.c.). After 5 hours, more lithium aluminium hydride (2·0 g.) was added, and at the end of an hour the excess of lithium aluminium hydride and the complex were decomposed by the addition of ice-cold 10% hydrochloric acid, the solution also being cooled in ice. The ethereal layer was separated, the aqueous layer was extracted with ether, and the ethereal fractions were combined, washed with dilute sodium carbonate solution and with water, and dried. Removal of the solvent afforded first a yellow solid, m. p. 120—128°, $[\alpha]_D^{20}$ −20° in EtOH, and then a glass (total yield, 9 g.). The product was treated with ether–benzene (500 c.c.; 1 : 4) and purified by chromatography on alumina (150 g.) prepared in the same solvent (giving fraction A). A portion (1·7 g.; m. p. 115—120°, $[\alpha]_D^{23}$ −23° in EtOH) did not dissolve and was treated separately (fraction B).

The fraction (A), eluted by benzene–ether (4 : 1 and 7 : 3), was a solid (750 mg.), m. p. 100—105°, $[\alpha]_D^{12}$ +85° in EtOH. It was chromatographed in benzene (75 c.c.) on alumina (22·5 g.) prepared in benzene. The fraction eluted by benzene–ether (4 : 1) yielded a white solid crystallising from the solvent in needles (50 mg.), m. p. 150—154°, $[\alpha]_D^{19}$ +171° in EtOH. It could not be purified further, but appeared to be mainly androst-4-enedione (m. p. 174°, $[\alpha]_D$ +190°).

The portion eluted by benzene–ether (7 : 3) gave a solid crystallising from ether in needles (300 mg.), m. p. 105—108°, $[\alpha]_D^{21}$ +85° in EtOH. Further chromatographic purification yielded testosterone, m. p. 150°, $[\alpha]_D^{20\cdot5}$ +113° (*c* 2·5 in EtOH), ultra-violet absorption max. at 242·5 mμ (log ε 4·2) (Found : C, 78·4; H, 9·7. Calc. for $C_{19}H_{28}O_2$: C, 79·1, H, 9·7%). The physical constants for testosterone are m. p. 155°, $[\alpha]_D$ +109°, $\lambda_{max.}$ 238 mμ (log ε 4·1) (Ruzicka, *Helv. Chim. Acta*, 1935, **18**, 1264).

Further fractions were eluted by benzene–ether (3 : 2 and 1 : 1) and these afforded a white crystalline solid (1·42 g.; m. p. 123—128°, $[\alpha]_D^{21}$ −34° in EtOH). The solid was dissolved in benzene–ether (600 c.c.; 7 : 3) and chromatographed on alumina (45 g.) prepared in the same, solvent. The fractions eluted by benzene–ether (300 c.c.; 3 : 2) yielded a white solid crystallising from ether in needles, m. p. 158—162°, $[\alpha]_D^{20}$ −36° in EtOH. This was recognised as impure androst-5-enediol.

The solid B (1·7 g.; see above) was triturated with benzene–ether (600 c.c.; 7 : 3). A portion C (250 mg.) did not dissolve. The solution was chromatographed on alumina (45 g.) and a portion eluted by benzene–ether (300 c.c.; 3 : 2) yielded a white solid crystallising from ether in needles, m. p. 167—169°, $[\alpha]_D^{20}$ −44° in EtOH (750 mg.), but this material was not further examined.

The sparingly soluble residue C crystallised from ether in needles, m. p. 174°, $[\alpha]_D^{20}$ −46° in EtOH (Found : C, 74·2; H, 10·1. Calc. for $C_{19}H_{30}O_2,H_2O$: C, 74·0; H, 10·4%). The m. p was undepressed on admixture with androst-5-ene-3β : 17β-diol prepared by the reduction of dehydro*epi*androsterone, as follows.

A solution of dehydro*epi*androsterone (5·0 g.) in dry ether (200 c.c.) was added slowly, with stirring, to one of lithium aluminium hydride (2·0 g.) in ether (200 c.c.). After 16 hours the excess of lithium aluminium hydride and the complex were decomposed by the addition of ice-cold 10% hydrochloric acid. The product partly separated from the solution and was collected (3·5 g.; m. p. 175—176°). The ethereal layer and subsequent ethereal extracts were combined, washed with dilute potassium carbonate solution and with water, and dried. Removal of the solvent yielded the diol (XXXV; R = H) (400 mg.) which crystallised from ether in needles, m. p. 178°, $[\alpha]_D^{20}$ −50° in EtOH (Found : C, 74·3; H, 10·3. Calc. for $C_{19}H_{30}O_2,H_2O$: C, 74·0; H, 10·4%).

3-*Triphenylmethoxyandrost-5-en*-17-*one*.—A solution of dehydro*epi*androsterone (1·0 g.) and triphenylmethyl chloride (900 mg.) in pyridine (2 c.c.) was heated on the steam-bath for 4 hours and then added to water. The product was isolated by means of ether and freed from pyridine. The oil (1·9 g.) was dissolved in benzene–petrol (200 c.c.; 1 : 9) and purified by chromatography on alumina (60 g.), prepared in petrol. The fraction eluted by benzene–petrol (2 : 3) yielded the required *ether* (657 mg.) as clusters of fine needles (from petrol), m. p. 198°, $[\alpha]_D^{19}$ −7° (*c* 1·08 in $CHCl_3$) (Found : C, 85·8; H, 7·6. $C_{38}H_{42}O_2$ requires C, 86·0; H, 7·9%).

3-*Triphenylmethoxyandrost-5-en*-17-*ol* (XXXV; R = CPh_3).—(*a*) The foregoing ketone ether (444 mg.) in ether (30 c.c.) was added to a solution of lithium aluminium hydride (200 mg.) in ether (30 c.c.) and kept for 20 hours. The excess of lithium aluminium hydride was decomposed by the addition of ice, and the complex by ice-cold 10% hydrochloric acid. The ethereal layer (A) was separated, the aqueous layer shaken with ether (B), and the extracts A and B were combined, washed with dilute potassium carbonate solution and with water, and dried. On removal of the solvent the *product* crystallised (335 mg.) in needles, m. p. 210°, $[\alpha]_D^{19}$ −43° (*c* 1·04 in $CHCl_3$) (Found : C, 84·2; H, 8·2. $C_{38}H_{44}O_2,0{\cdot}5H_2O$ requires C, 84·3; H, 8·3%). On admixture with the starting material the m. p. was depressed to 175—192°.

(*b*) Androst-5-ene-3 : 17-diol (4·1 g.) and triphenylmethyl chloride (4·0 g.) in pyridine (8 c.c.) were heated on the steam-bath for 6 hours. The product was isolated by means of ether (2·0 g. of solid separated). On removal of the dried solvent, the product crystallised in the successive fractions: (1) 680 mg., m. p. 135—150°; (2) 150 mg., m. p. 155—176°; (3) 560 mg., m. p. 150—153°; (4) 700 mg., m. p. 149—151°; and (5) 1·44 g., m. p. 145—150°. The first three fractions were unchanged androstenediol. Fractions (4) and (5) were combined and chromatographed in benzene–petrol (200 c.c.; 1 : 4) on alumina (60 g.) prepared in petrol. The fractions eluted by benzene–petrol (2 : 3), changing to pure benzene, yielded triphenylmethanol (800 mg.), and those by benzene–ether (2 : 3) yielded 3-triphenylmethoxyandrost-5-en-17-ol (121 mg.), m. p. 211°, $[\alpha]_D^{20}$ −42° (*c* 1·14 in $CHCl_3$), the m. p. being undepressed by admixture with an authentic specimen of this compound, prepared as described above (Found : C, 84·4; H, 8·2%). By recrystallisation in a dry atmosphere from carefully dried ether the anhydrous *compound*, m. p. 211°, was obtained (Found : C, 85·2; H, 8·4. $C_{38}H_{44}O_2$ requires C, 85·5; H, 8·3%).

Oxidation of 3-*Triphenylmethoxyandrost-5-en*-17-*ol*.—To a boiling mixture of the foregoing

alcohol (373 mg.), *cyclo*hexanone (3 c.c.), and toluene (16 c.c.), aluminium *iso*propoxide (300 mg.) in toluene (6 c.c.) was added. The solution was refluxed for 3 hours, and then distilled in steam. The residue was made acid with dilute hydrochloric acid, and extracted with ether with the usual technique. The oily product was chromatographed in benzene–petrol (30 c.c.; 1 : 4) on alumina (9 g.) prepared in petrol. The fractions eluted by benzene–petrol (2 : 3) yielded the ketone as clusters of white needles (180 mg.), m. p. 198°, $[\alpha]_D^{21}$ −6° (*c* 1·14 in $CHCl_3$), from petrol (Found : C, 86·0; H, 7·8. Calc. for $C_{38}H_{42}O$: C, 86·0, H, 7·9%). The m. p. was undepressed on admixture with an authentic specimen.

Hydrolysis.—The triphenylmethoxy-ketone (200 mg.) in dry chloroform (10 c.c.) was mixed with chloroform (25 c.c.) saturated with hydrogen chloride, the solution being cooled in ice. Hydrogen chloride was passed in for 15 minutes, and after 0·5 hour at the room temperature the chloroform solution was washed with dilute potassium carbonate solution and with water, and dried. The solvent was removed, and the product (200 mg.) chromatographed in benzene–petrol (20 c.c.; 1 : 4) on alumina (6 g.) prepared in petrol. The fractions eluted by benzene–petrol (2 : 3) yielded unchanged starting material (11 mg.), and those by benzene–petrol (1 : 1) and by benzene gave triphenylmethanol (45 mg.), m. p. 158° undepressed on admixture with a genuine specimen. The fraction eluted by benzene–ether (1 : 1) yielded dehydro*epi*androsterone (59 mg.), m. p. 147°, undepressed on admixture with an authentic specimen.

Androst-4-ene-3α : 17β-diol and -3β : 17β-diol.—Lithium aluminium hydride (35 mg.) in ether (20 c.c.) was added to androst-4-ene-3 : 17-dione (700 mg.) in ether (100 c.c.) during 10 minutes. After a further 15 minutes the complex was decomposed by dilute hydrochloric acid, the ethereal layer separated, the aqueous layer extracted with ether, and the ethereal extracts were combined, washed with sodium hydrogen carbonate solution and with water, and dried. Removal of the solvent left a solid (0·7 g.) which was chromatographed in benzene–petrol (100 c.c., 1 : 1) on alumina (30 g.) prepared in the same solvent. The fraction eluted by benzene–ether (400 c.c., 9 : 1) was unchanged androst-4-ene-3 : 17-dione (206 mg.), m. p. 168°, $[\alpha]_D$ +198° (*c* 1·0 in $CHCl_3$); that eluted by benzene–ether (400 c.c.; 1 : 1) gave androst-4-ene-3α : 17β-diol, crystallising from ether in needles (170 mg.), m. p. 149—150°, $[\alpha]_D$ +78° (*c* 0·98 in $CHCl_3$) (Found : C, 76·4; H, 10·3. Calc. for $C_{19}H_{30}O_2$,0·5H_2O : C, 76·2; H, 10·4%).

The fractions eluted by benzene–ether (300 c.c., 2 : 1) and ether (100 c.c.) yielded androst-4-ene-3β : 17β-diol, crystallising from ether in needles (100 mg.), m. p. 212—215°, $[\alpha]_D^{19}$ +102° (*c* 2·22 in EtOH) (Found : C, 79·2; H, 10·0. Calc. for $C_{19}H_{30}O_2$: C, 78·7; H, 10·3%). Butenandt and Heuser (*Ber.*, 1938, **71**, 198) give m. p. 154°, $[\alpha]_D^{19}$ +48·5° (in EtOH) for androst-4-ene-3α : 17β-diol, and m. p. 202—206°, $[\alpha]_D^{22}$ +187·5° (in pyridine) for androst-4-ene-3β : 17β-diol.

iso*Hexylation of Pregnenolone Acetate.*—The reaction of *iso*hexylmagnesium bromide with 3-acetoxypregn-5-en-20-one was carried out in the usual manner and the isolated product was re-acetylated. Chromatography on alumina afforded a product, m. p. 98—100°, $[\alpha]_D^{14}$ −13° in $CHCl_3$ (Found : C, 79·1, 79·8; H, 9·6, 9·4%). This was probably a mixture (or compound) of pregna-3 : 5-diene-20-one (Calc. : $[\alpha]_D$ −69°; C, 84·6; H, 10·1%) and 20-hydroxycholesteryl 3-acetate or its 17 : 20-stereoisomeride (Calc. : $[\alpha]_D$ *ca.* −38°; C, 78·4; H, 10·8%).

After dehydration with toluene-*p*-sulphonyl chloride and pyridine, and isolation (chromatography), a product, m. p. 108°, $[\alpha]_D$ −21°, $\lambda_{max.}$ 236 and 265 (log ε 4·1 and 2·9 respectively) (Found : C, 83·5, 82·3; H, 10·1, 9·9%), was obtained. This may be a mixture of pregnadienone [$\lambda_{max.}$ 235 (log ε 4·2)] with 17 : 20-dehydrocholesteryl acetate (Calc. : $[\alpha]_D$ −38°; C, 81·7; H, 10·8%). Hydrogenation over palladium afforded material, m. p. 87°, $[\alpha]_D$ +98° (Found : C, 83·5; H, 10·8%), and then over platinic oxide material, m. p. 109—111°, $[\alpha]_D$ +77·5° (Found : C, 81·8, 84·2; H, 10·4, 11·2%). This showed a carbonyl band in the infra-red spectrum and was doubtless impure *allo*pregnan-20-one (Shoppee, Lewis, and Elbs, *Chem. and Ind.*, 1950, 454, give m. p. 129°, $[\alpha]_D$ +99°. Calc. : C, 83·4; H, 11·2%). Our impression was that the initial Grignard reaction had not worked well and that such *iso*hexylated product as was produced gradually disappeared in the extensive adsorption processes used for purification at all stages.

Dihydropyran Adduct of Pregnenolone (XXXVII).—Pregnenolone (1·0 g.) was dissolved in dihydropyran (15 c.c.) and two drops of concentrated hydrochloric acid were added. The solution was warmed on the steam-bath for 1½ hours, cooled, and made alkaline with methanolic potassium hydroxide. The oily product was isolated with the aid of ether and solidified when rubbed with petrol. The *derivative* crystallised from methanol in white, glistening plates (870 mg.), m. p. 124—125°, $[\alpha]_D^{19}$ +18° (*c* 1·18 in $CHCl_3$) (Found : C, 77·9; H, 10·1. $C_{26}H_{40}O_3$ requires C, 78·0; H, 10·0%). Reaction of this substance with *iso*hexylmagnesium bromide and chromatography of the product gave material, m. p. 95—98°, $[\alpha]_D^{18}$ +11° (Found : C, 77·9; H, 10·2%); acetylation and dehydration by phosphoryl chloride and pyridine gave a compound,

m. p. 117°, $[\alpha]_D$ +40°, and, after hydrogenation over platinic oxide, *allo*pregnan-20-one, m. p. 127°, $[\alpha]_D$ +81° (carbonyl band in the infra-red spectrum), was the only product isolated by chromatography.

Preparation of iso*Hexyl Bromide* (*1-Bromo-4-methylpentane*).—A solution of *iso*amyl bromide (112 g.; reputedly pure commercial specimen, refractionated) in ether (500 c.c.) was added to magnesium (17·6 g.) at such a rate that the ether refluxed gently. The solution was warmed for a further 30 minutes, cooled in ice–salt, and powdered solid carbon dioxide was gradually added so that the temperature did not rise above 0° (3 hours). The mixture was stirred for 2 hours at the room temperature, cooled, and decomposed with 20% hydrochloric acid (250 c.c.). The acidic product (53 g.), distilled through an efficient fractionating column, had b. p. 118—120°/20 mm. (yield, 35 g.).

The *iso*hexanoic acid (35 g.) in ether (500 c.c.) was added to a slurry of lithium aluminium hydride (12·0 g.) in ether (400 c.c.), cooled in methanol and solid carbon dioxide. When the addition was complete (1 hour) the mixture was stirred for 2 hours at the room temperature, then cooled and worked up in the known manner. The yield of *iso*hexanol, b. p. 148—150°, was 21 g. The bromide, prepared by Sabetay and Bléger's method (*Bull. Soc. chim.*, 1930, **47**, 885), had b. p. 144° (Found : C, 43·4; H, 7·8; Br, 48·5. Calc. for $C_6H_{13}Br$: C, 43·6; H, 7·9; Br, 48·5%).

3β-*Acetoxycholest*-17(20)-*ene* (XXXVIII).—3β-Acetoxy*allo*pregnan-20-one (2·6 g.) in anisole (25 c.c.) was added with vigorous stirring to a solution of *iso*hexylmagnesium bromide (from 1·0 g. of *iso*hexyl bromide and 1·02 g. of magnesium) in anisole (50 c.c.), and the solution refluxed for 3 hours. After the addition of 50% hydrochloric acid (5 c.c.), the anisole was removed by steam-distillation. The product was isolated by means of ether, and the extract washed with sodium hydrogen carbonate solution, then with water, and dried (Na_2SO_4). Removal of the solvent yielded a viscous oil (3·0 g.; probably cholestane-3 : 20-diol) which was refluxed for 2½ hours with glacial acetic acid (25 cc.). The solvent was removed under reduced pressure, acetic anhydride (25 c.c.) added, and the solution again refluxed for ½ hour. The acetic anhydride was removed under reduced pressure, the product dissolved in ether, and the ethereal solution washed with sodium hydrogen carbonate solution and with water, and dried. Removal of the solvent left an oil (3 g.) which was chromatographed in petrol (100 c.c.) on alumina (30 g.). The fractions eluted by petrol (200 c.c.) and by petrol–benzene (400 c.c.; 9 : 1) yielded an oil which was chromatographed again in petrol (100 c.c.) on alumina (90 g.). Elution by 100-c.c. portions of petrol yielded ten fractions which could not be crystallised; ten 100-c.c. portions of benzene–petrol (1 : 9) eluates behaved similarly. The combined oils (A) (1·75 g.) had $[\alpha]_D$ +10° (*c* 0·72 in $CHCl_3$). The column was washed with petrol–ether (100 c.c.; 1 : 1), and ether, and yielded semicrystalline pastes (0·35 g. and 0·18 g.) which were combined and chromatographed in petrol (60 c.c.) on alumina (15 g.). Elution by 60-c.c. portions of petrol yielded as the third fraction, a waxy solid (0·5 g.), which was triturated repeatedly with methanol, finally being cooled in solid carbon dioxide–methanol, powdered, and allowed to warm to the room temperature; the solvent was replaced, and the process repeated. 3β-Acetoxycholest-17(20)-ene was obtained as a white waxy solid (B) (0·23 g.), m. p. 65—74°, which crystallised from methanol in needles, m. p. 86—88°, $[\alpha]_D$ +2° (*c* 0·92 in $CHCl_3$) (Found : C, 81·1; H, 11·4. $C_{29}H_{48}O_2$ requires C, 81·3; H, 11·2%).

Reduction of 3β-*Acetoxycholest*-17(20)-*ene*.—(*a*) The oil (A) (1·75 g.) from the previous experiment was hydrogenated in acetic acid (100 c.c.) with a trace of perchloric acid over Adams's platinum oxide. The solution was then poured into water (150 c.c.) and extracted with chloroform. The chloroform extract was washed with sodium hydrogen carbonate solution and dried (Na_2SO_4). Removal of the solvent yielded an oil (1·3 g.) which was chromatographed in petrol (100 c.c.) on alumina (60 g.), prepared in petrol. Elution by 100-c.c. portions of petrol yielded three oily fractions. Elution with ether–petrol (1 : 19) yielded only traces of oil, but on washing of the column with ether (100 c.c.) a waxy solid (150 mg.) was obtained, which crystallised from methanol in glistening needles, m. p. 109—110°, $[\alpha]_D^{17}$ +12·8° (*c* 0·94 in $CHCl_3$) (Found : C, 81·4, 80·6; H, 11·6, 11·5. Calc. for $C_{29}H_{50}O_2$: C, 80·9; H, 11·6%). The m. p. was undepressed on admixture with an authentic specimen of cholestanyl acetate, m. p. 110—111°, $[\alpha]_D^{16}$ +13°, obtained by the reduction of chlolesterol (Nace, *J. Amer. Chem. Soc.*, 1951, **73**, 2379) followed by acetylation. The infra-red spectra of the natural and synthetic specimens were identical. The following is a report on the *X*-ray diffraction properties.

"*Cholestanyl acetate*. Single crystals from the natural and synthetic samples have been examined, and found to be identical.

"The unit cell is monoclinic, with the approximate dimensions a = 10·2 Å, b = 39·6 Å, c = 7·6 Å, β = 103°. March, 1952. Mrs. D. M. Crowfoot Hodgkin and Dr. B. M. Oughton."

The oil from which the cholestanyl acetate had been removed was hydrolysed by boiling 10% methanolic potassium hydroxide (100 c.c.). Water (150 c.c.) was added, and the solution worked up as usual to yield an oil (0·61 g.) which gave no precipitate with digitonin in alcohol. The infra-red absorption spectrum of this oil showed that it contained ketonic material.

(*b*) 3β-Acetoxycholest-17(20)-ene (B) (168 mg.) in acetic acid (20 c.c.) containing a trace of perchloric acid was hydrogenated during 15 minutes over Adams's catalyst. The product, isolated as usual, crystallised from methanol in needles, m. p. 108° $[\alpha]_D^{17}$ +14·3° (*c* 0·7 in $CHCl_3$) (Found : C, 81·1 ; H, 11·3. Calc. for $C_{29}H_{50}O_2$: C, 80·9, H, 11·6%). The m. p. was undepressed on admixture with an authentic specimen of cholestanyl acetate, and the infra-red spectra of the two materials were identical.

From the mother-liquors two further crops, m. p. 72—74°, $[\alpha]_D$ +16·5°, and m. p. 55—60°, were obtained. They could not be purified by crystallisation, and were combined and hydrolysed by boiling 10% methanolic potassium hydroxide (25 c.c.) for an hour. On dilution with water the product was precipitated; it crystallised from methanol in needles, m. p. 106—110°, $[\alpha]_D^{16}$ +17·3° (*c* 0·99 in $CHCl_3$), which gave no digitonide in methanol.

At this culmination of an investigation commenced in 1932, it is appropriate to express gratitude to the Rockefeller Foundation of New York, both for the provision of funds that made possible the building of the extension of this laboratory in which much of the work was prosecuted, and also for grants and gifts over a long period. We are also grateful to Imperial Chemical Industries Limited for a considerable grant for equipment, as well as for recurrent grants and gifts of material.

We are deeply indebted to British Drug Houses Ltd. for generous gifts of valuable residues and of a large specimen of androstenedione. We thank Dr. E. B. Hershberg and the Schering Corporation of America Inc. for various specimens and for residues from cholesteryl acetate dibromide oxidations. We thank Messrs. CIBA Limited for a generous gift of the pure Köster–Logemann keto-acetate. Grateful acknowledgment is made of the support given by Nyegaard and Co A/S, Oslo, and by the Royal Norwegian Council for Scientific Research to one of us (H. H.). This work was carried out during the tenure of an I.C.I. Fellowship (H. M. E. C.) and of an 1851 Overseas (Eire) Scholarship (S. R. D.).

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