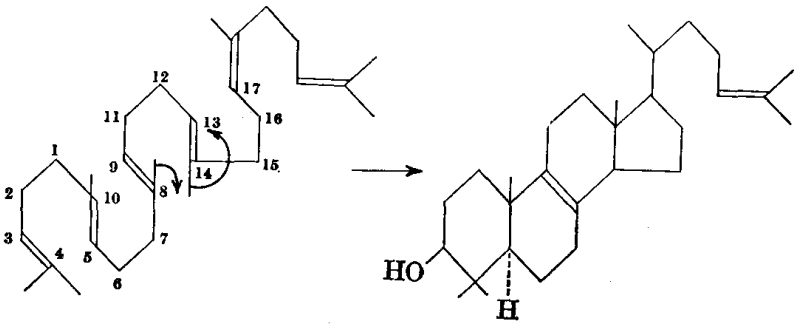
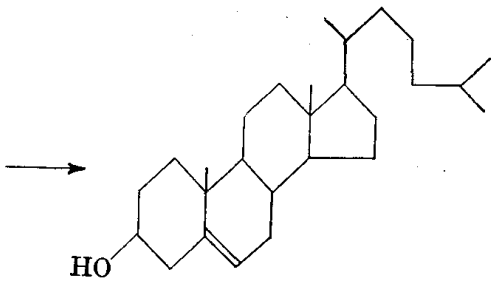


squalene lanosterol



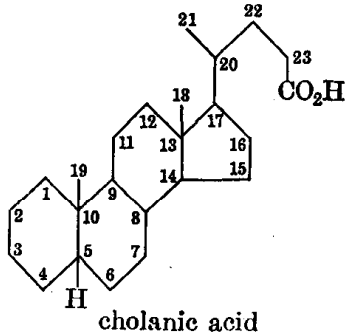
cholesterol

Bloch *et al.* (1957) also found that the three methyl groups of lanosterol are eliminated as carbon dioxide (*via* oxidation to carboxyl groups). Several intermediates and new precursors which function between lanosterol and cholesterol have now been identified (Cornforth, 1959; Crabbé, 1959). Finally, studies with yeast extracts have shown the mevalonic acid 5-pyrophosphate, *iso*pentenyl pyrophosphate, geranyl pyrophosphate and farnesyl pyrophosphate are successive intermediates in the biosynthesis of squalene (see §32a. VIII).

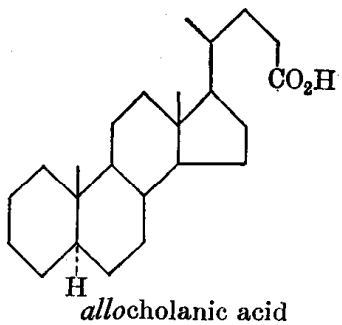
The biosynthesis of ergosterol from acetate has been carried out by Bloch *et al.* (1951), and the distribution pattern corresponds to that of cholesterol. Bloch *et al.* (1957) also showed that formate is an efficient source for the methyl group at C_{28}.

BILE ACIDS

§8. Introduction. The bile acids occur in bile (a secretion of the liver which is stored in the gall-bladder) of most animals combined as amides with either glycine ($NH_2{\cdot}CH_2{\cdot}CO_2H$) or taurine ($NH_2{\cdot}CH_2{\cdot}CH_2{\cdot}SO_3H$), *e.g.*, glycocholic acid (= glycine + cholic acid), taurocholic acid (= taurine + cholic



cholanic acid



*allo*cholanic acid

acid). The bile acids are present as sodium salts, and they function as emulsifying agents in the intestinal tract, *e.g.*, fats, which are insoluble in water, are rendered " soluble ", and so may be absorbed in the intestine.

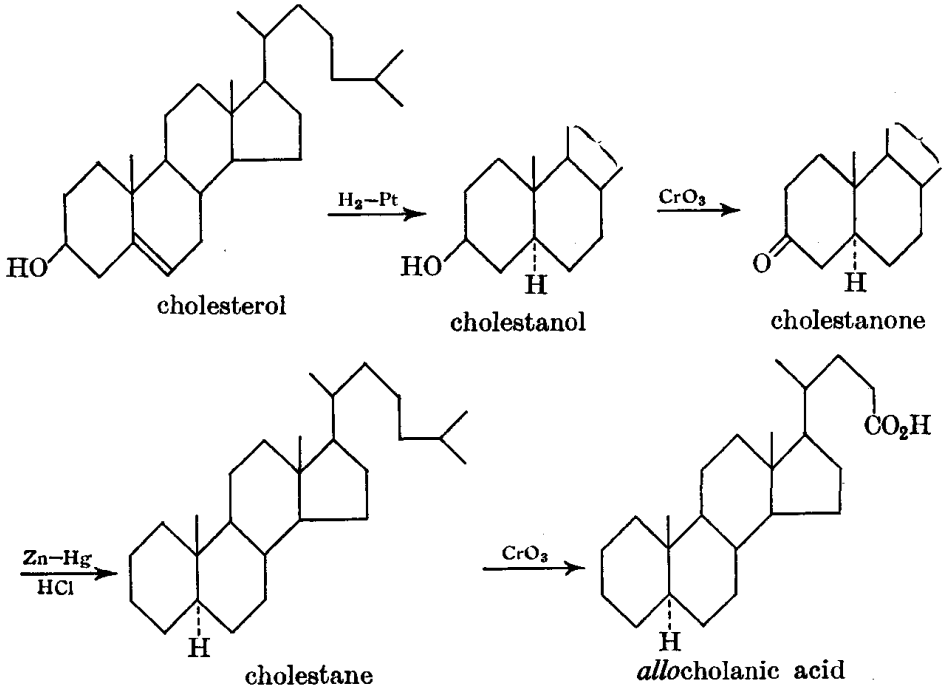
The bile acids are hydroxy derivatives of either cholanic acid or *allo*-cholanic acid (but see §10). Dehydration of a bile acid by heating in a vacuum, followed by catalytic reduction, gives either cholanic or *allo*cholanic acid.

About twelve natural bile acids have been characterised, and a number of others are synthetic. The positions of the hydroxyl groups are any of the following: 3, 6, 7, 11, 12 and 23, and in almost all of the natural bile acids the configurations of the hydroxyl groups are α (see §4b). Some of the more important natural bile acids are:

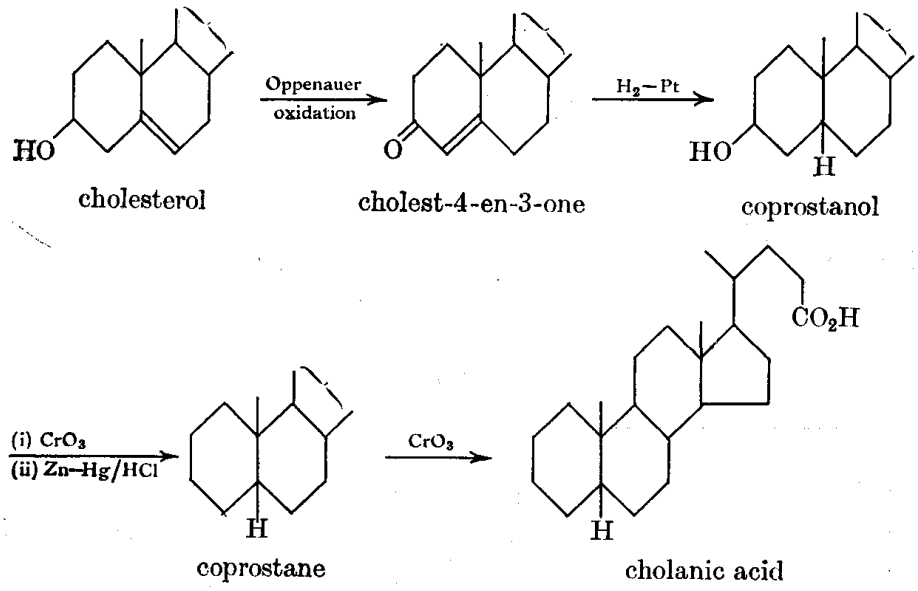
Name	*M.p.*	*Hydroxyl groups*	*Source*
Cholic acid	195°	3α : 7α : 12α	Man, ox
Deoxycholic acid	172°	3α : 12α	Man, ox
Lithocholic acid	186°	3α	Man, ox
Chenodeoxycholic acid . .	140°	3α : 7α	Man, ox, hen
Hyodeoxycholic acid . . .	197°	3α : 6α	Pig

§9. The structures of cholanic acid and *Allo*cholanic acid. These acids may be derived from coprostane and cholestane, respectively, as follows (*cf.* §4c). At the same time, these reactions show the relationship between the bile acids and the sterols (Windaus, 1919).

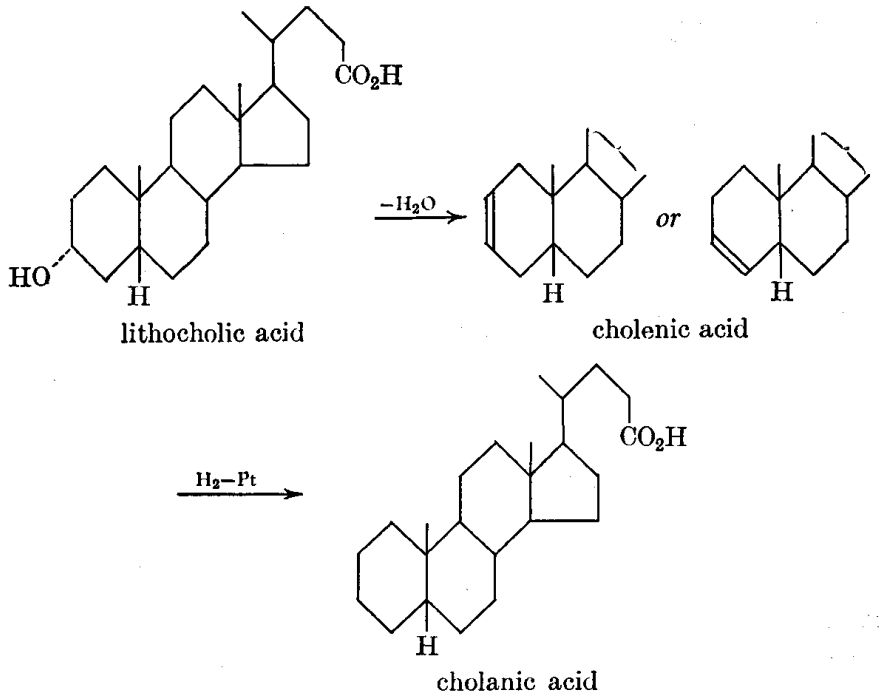
***Allo*cholanic acid.**



Cholanic acid.



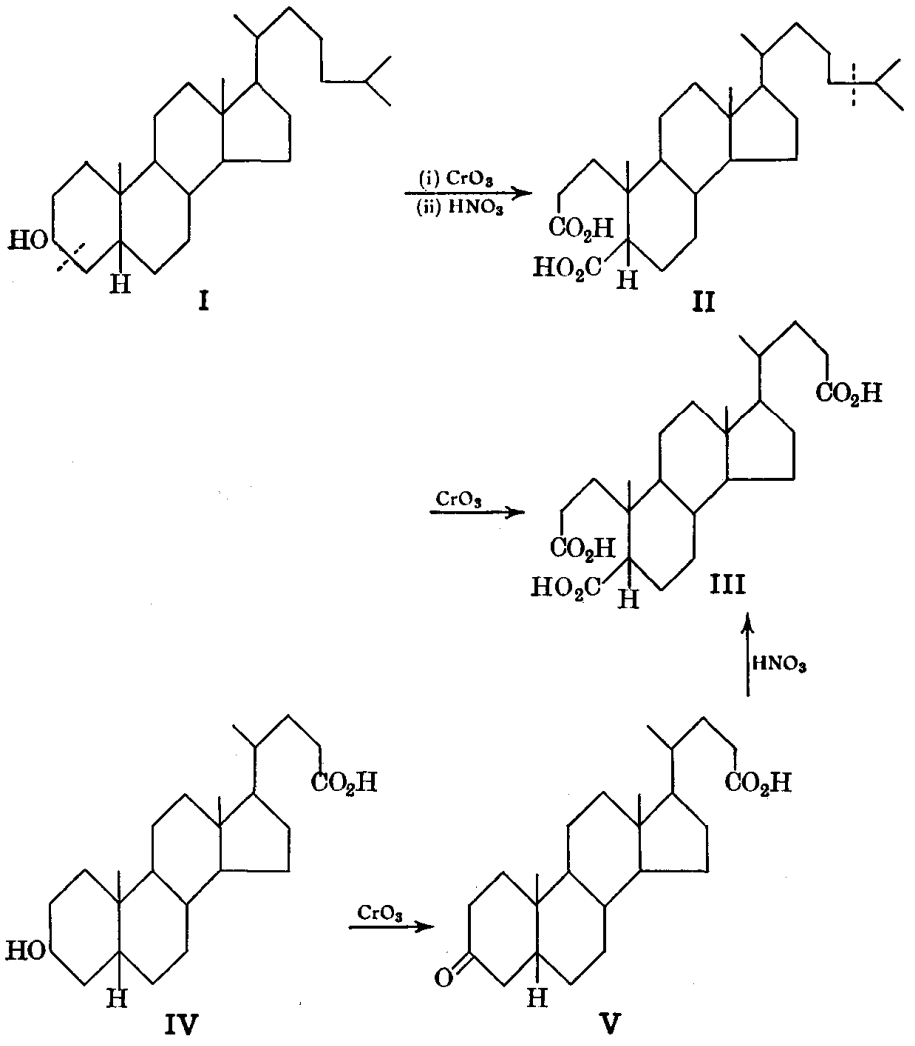
§10. Structure of the bile acids. Since all the bile acids can be converted into either of the cholanic acids, the former are therefore hydroxy derivatives of the latter, *e.g.*, lithocholic acid can be converted into cholanic acid as follows:



According to Fieser *et al.* (1955), cholenic acid is a mixture of the two compounds shown, the chol-3-enic acid being the main constituent.

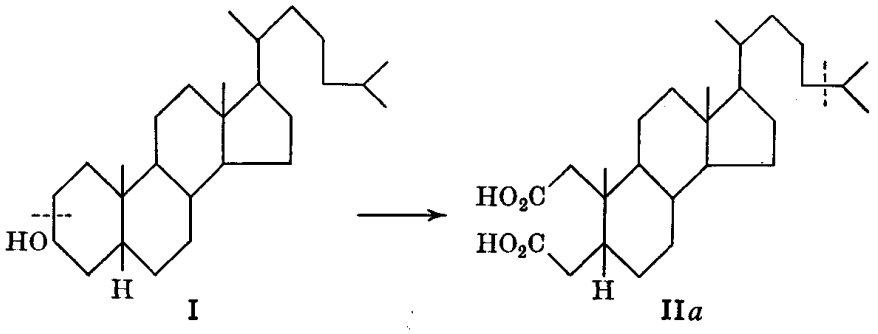
The positions of the hydroxyl groups in the bile acids have been determined by means of oxidative degradation, *e.g.*, the position of the hydroxyl group in lithocholic acid is shown to be at 3 as follows. Cholesterol can be

converted into coprostanol I (see, *e.g.*, §9) which, on oxidation with chromium trioxide, forms a ketone and this, when oxidised with nitric acid, gives a dicarboxylic acid, II. II, on further oxidation with nitric acid, produces the tricarboxylic acid, lithobilianic acid, III. Lithocholic acid, IV, on oxidation with chromium trioxide, forms dehydrolithocholic acid, V, and this, when oxidised with nitric acid, forms III. It therefore follows that the hydroxyl group in lithocholic acid is probably in the same position as in coprostanol, *viz.*, position 3. Thus:

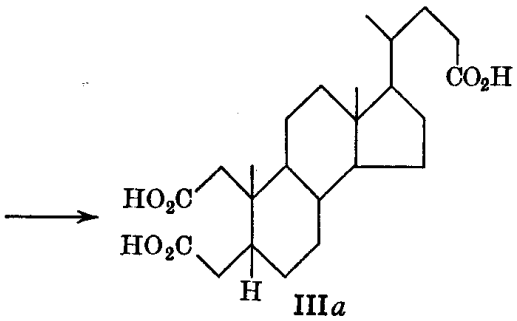


The above evidence is not conclusive, since had the hydroxyl group in lithocholic acid been at position 4, III could still have been obtained. In practice, however, the oxidation of I produces two isomeric acids for II, one being II as shown, and the other II*a*, in which the ring A is opened between C_2 and C_3; this acid, on further oxidation, gives *iso*lithobilianic acid, III*a*. Since the oxidation of lithocholic acid, IV, also produces a mixture of the *same* two acids, III and III*a*, there can be no doubt that the hydroxyl group is at position 3.

The configuration of the hydroxyl group in lithocholic acid has been shown to be α by, *e.g.*, the oxidative degradation of the acetates of lithocholic acid and epicoprostanol to 5-*iso*androsterone (formerly known as 3α-hydroxy-ætiocholan-17-one). Since all of the natural bile acids except one ("β"

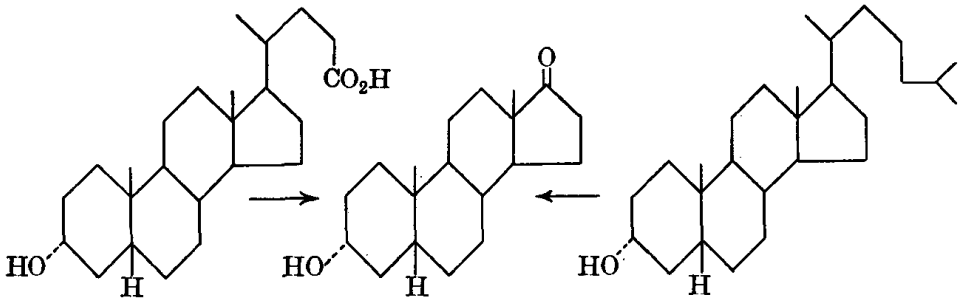


I IIa



IIIa

hyodeoxycholic acid) can be converted into lithocholic acid, all have therefore the α-configuration for the hydroxyl group at C_3.



lithocholic acid 5-*iso*androsterone epicoprostanol

The bile acids form molecular compounds with various substances. Cholic acid, in particular, forms these molecular compounds with such compounds as fatty acids, esters, alcohols, etc.; these are known as the **choleic acids.** These choleic acids are of the channel complex type (like urea complexes; see Vol. I).

The bile acids discussed in the foregoing account are all derivatives of cholanic or *allo*cholanic acid. There are, however, some bile acids which are not derivatives of the cholanic acids, *e.g.*, in the bile of crocodiles there is the bile acid 3α : 7α : 12α-trihydroxycoprostanic acid, $C_{27}H_{46}O_5$.

SEX HORMONES

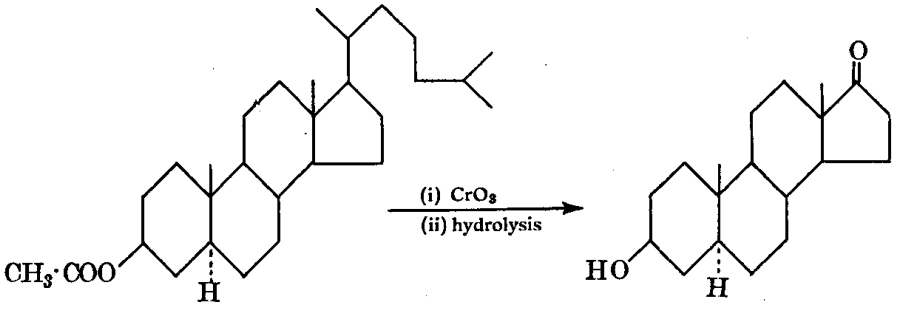
§11. Introduction. Hormones are substances which are secreted by the ductless glands, and only minute amounts are necessary to produce the various physiological reactions in the body. As a group, hormones do not resemble one another chemically, and their classification is based on their physiological activity. There appear to be about 60 different hormones recognised so far, and more than half of these are steroids. The sex hormones

belong to the steroid class of compounds, and are produced in the gonads (testes in the male, and ovaries in the female). Their activity appears to be controlled by the hormones that are produced in the anterior lobe of the pituitary gland. Because of this, the sex hormones are sometimes called the secondary sex hormones, and the hormones of the anterior lobe of the pituitary (which are protein in nature) are called the primary sex hormones.

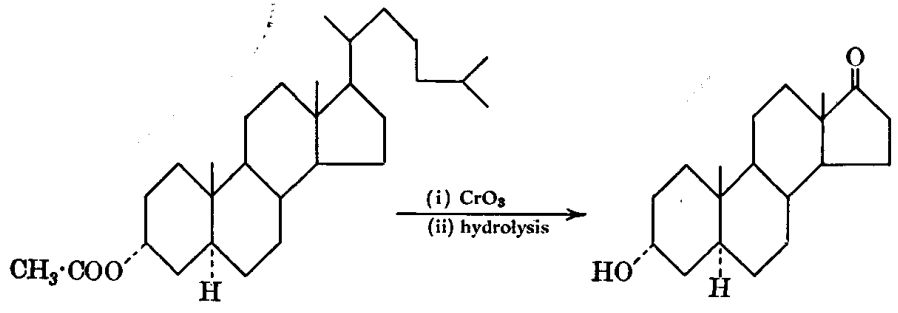
The sex hormones are of three types: the **androgens** (male hormones), the **œstrogens** (female or follicular homones) and **progesterone** (the corpus luteum hormone). The sex hormones are responsible for the sexual processes, and for the secondary characteristics which differentiate males from females.

ANDROGENS

§12. Androsterone, $C_{19}H_{30}O_2$, m.p. 184–185°, is dextrorotatory. It was first isolated by Butenandt *et al.* (1931) from male urine (about 15 mg. from 15,000 litres of urine). Androsterone behaves as a saturated compound, and since it forms mono-esters, *one* oxygen atom is present as a hydroxyl group. The functional nature of the other oxygen atom was shown to be oxo, since androsterone forms an oxime, etc. The parent hydrocarbon of androsterone, $C_{19}H_{30}O_2$, is therefore $C_{19}H_{32}$, and since this corresponds to the general formula C_nH_{2n-6}, the molecule is tetracyclic. This led to the suggestion that androsterone probably contains the steroid nucleus, and since it is a hydroxyketone, it was thought that it is possibly related to



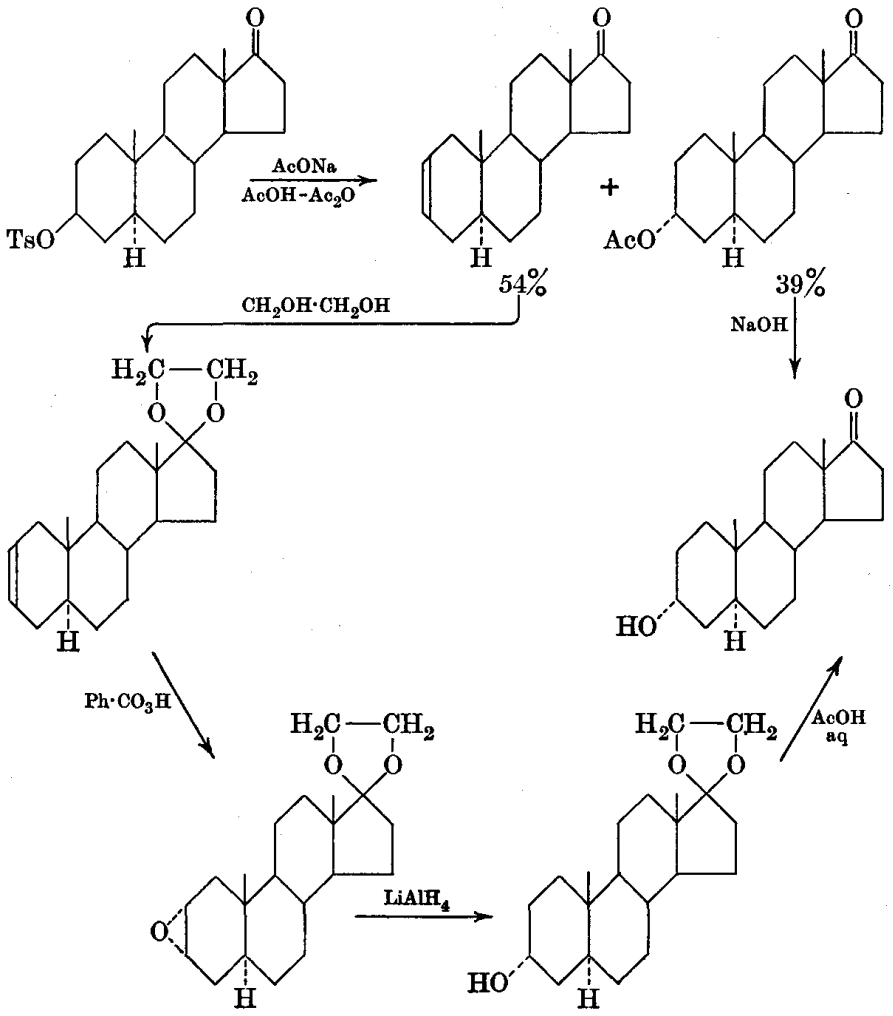
cholestanyl acetate — epiandrosterone



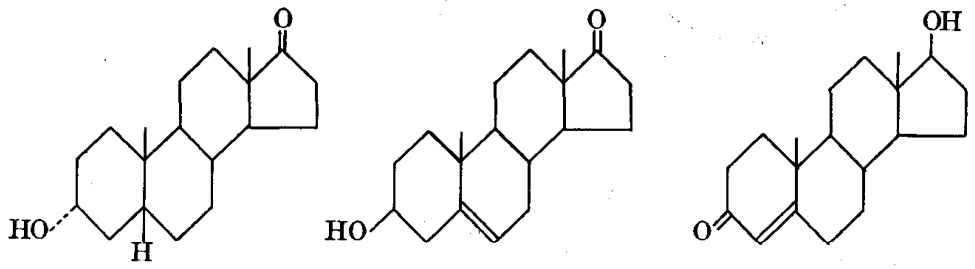
epicholestanyl acetate — androsterone

œstrone (§14). Butenandt (1932) therefore proposed a structure which was proved correct by Ruzicka (1934) as follows. Ruzicka oxidised cholestanyl acetate with chromium trioxide in acetic acid to **epiandrosterone,** a hydroxyketone with the structure proposed for androsterone by Butenandt. When, however, epicholestanyl acetate was oxidised, the product was androsterone. Thus the configuration of the hydroxyl group at C_3 is α and not β as Butenandt suggested. Epiandrosterone (formerly known as *iso*androsterone) has about one-eighth of the activity of androsterone.

Sondheimer *et al.* (1955) have converted epiandrosterone into androsterone, starting with epiandrosterone *p*-toluenesulphonate (*cf.* tosyl esters of sugars, §9. VII).



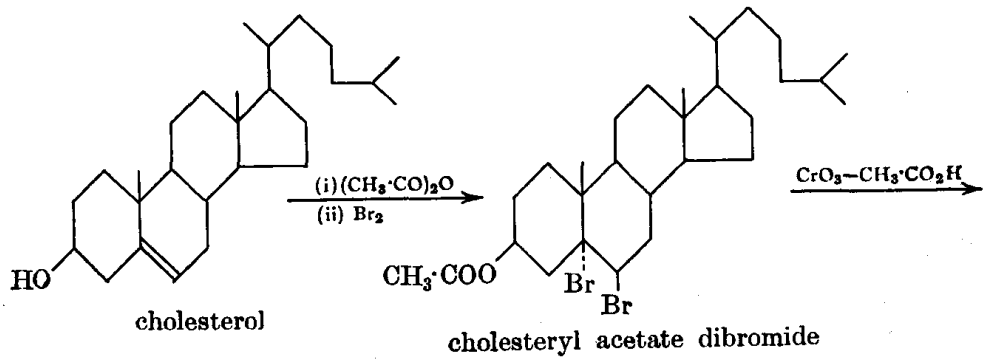
Soon after the discovery of androsterone, Butenandt *et al.* (1934) isolated two other hormones from male urine, 5-*iso*androsterone and dehydroepiandrosterone. Then Laqueur (1935) isolated the hormone testosterone from steer testes (10 mg. from 100 kg. of testes).



5-*iso*androsterone dehydroepiandrosterone testosterone

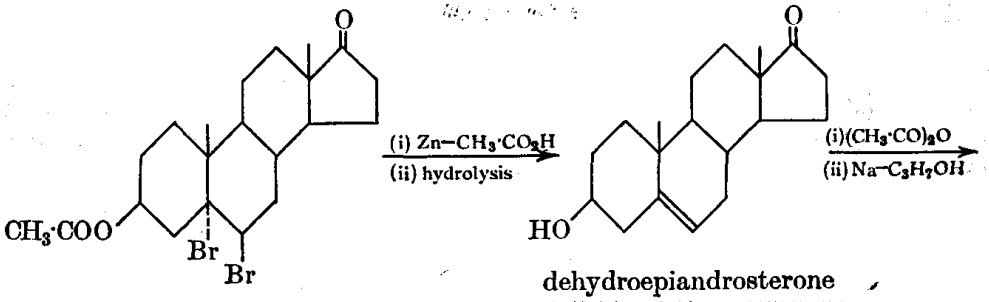
§13. Testosterone, $C_{19}H_{28}O_2$, m.p. 155°, is dextrorotatory. Testosterone has been produced commercially by the following method of Butenandt

(1935) and Ruzicka (1935); the Oppenauer oxidation step in this method was introduced by Oppenauer (1937). This preparation of testosterone establishes the structure of this hormone. This method has been improved

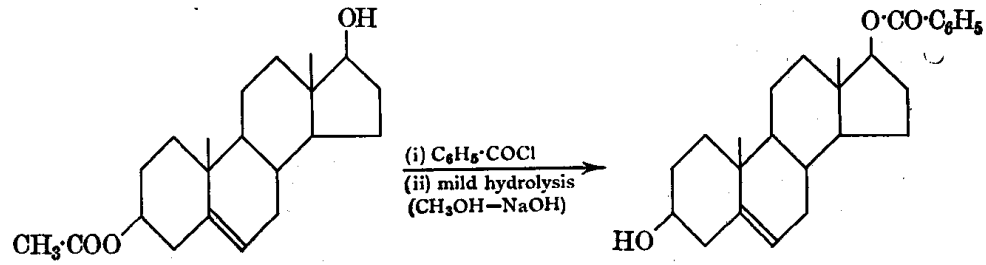


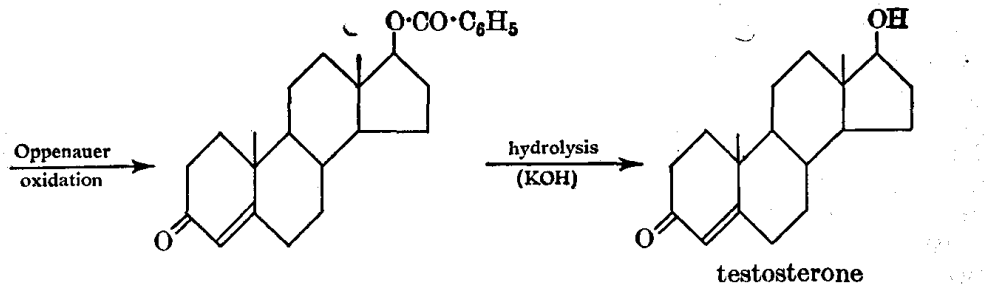
cholesterol

cholesteryl acetate dibromide



dehydroepiandrosterone

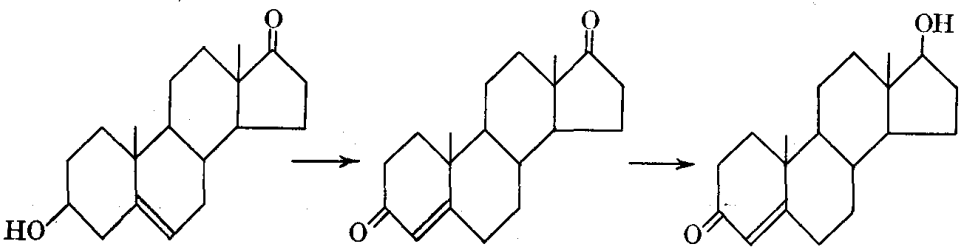




testosterone

by Mamoli (1938), who converted dehydroepiandrosterone into testosterone by means of micro-organisms; the first stage uses an oxidising yeast in the presence of oxygen, and the second stage a fermenting yeast.

Elisberg *et al.* (1952) have shown that sodium borohydride selectively reduces the 3-keto group in the presence of others at 11, 12, 17 or 20. On



dehydroepiandrosterone androst-4-ene-3:17-dione testosterone

the other hand, Norymberski *et al.* (1954) have shown that if there is a double bond in position 4 : 5, then the keto group at 17 or 20 is preferentially reduced to that at 3. Thus androst-4-ene-3 : 17-dione is reduced to testosterone by sodium borohydride (*cf.* §3 i). Johnson *et al.* (1960) have adapted Johnson's synthesis of equilenin (§17) to provide an improved synthesis of testosterone.

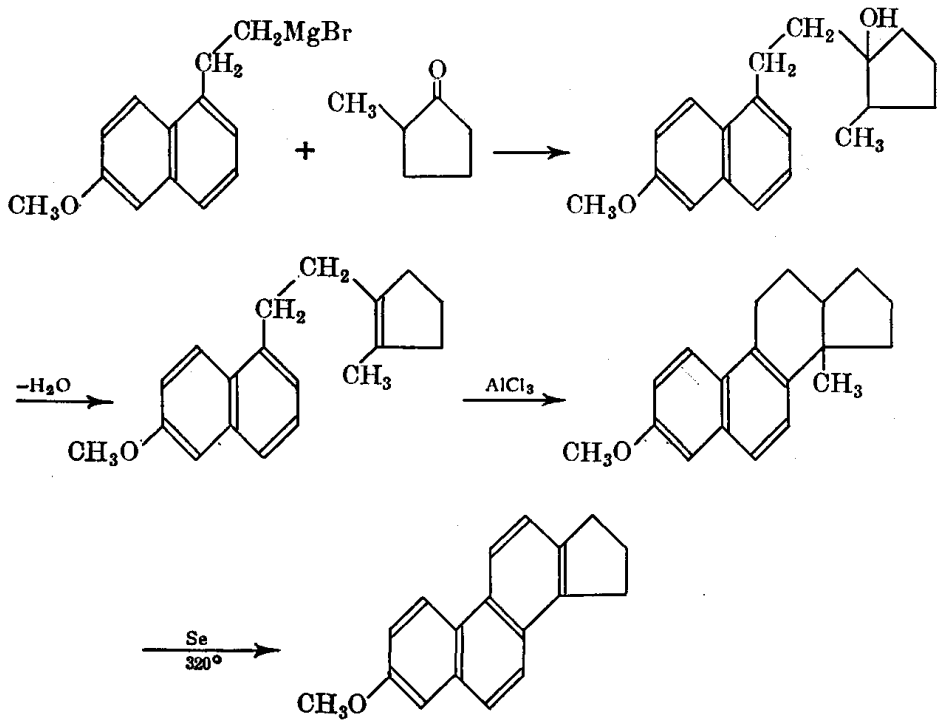
It appears that testosterone is the real male sex hormone in the body; the others are metabolic products of testosterone. The ketonic steroids are separated from the non-ketonic steroids (all from urine) by means of Girard's reagents (P and T); the ketonic compounds form *soluble* derivatives, and may be regenerated by hydrolysis (see also Vol. I). Many other hormones have also been isolated from urine.

ŒSTROGENS

§14. Œstrone. It has been known for a long time that there are hormones which control the uterine cycle, but it was not until 1929 that Butenandt and Doisy independently isolated the active substance **œstrone** from the urine of pregnant women. Œstrone is the first known member of the sex hormones, and soon after its discovery two other hormones were isolated, œstriol and œstradiol.

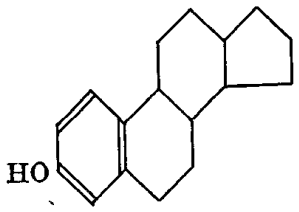
(+)-Œstrone, m.p. 259°, has the molecular formula $C_{18}H_{22}O_2$. It behaves as a ketone (forms an oxime, etc.), and contains one hydroxyl group (it forms a monoacetate and a monomethyl ether). Furthermore, this hydroxyl group is *phenolic*, since œstrone couples with diazonium salts in alkaline solution (this reaction is typical of phenols). When distilled with zinc dust, œstrone forms chrysene; this led to the suggestion that œstrone is related to the steroids (*cf.* §1). The X-ray analysis of œstrone also indicates the presence of the steroid nucleus, and at the same time showed that the keto group and the hydroxyl group are at the opposite ends of the molecule (Bernal, 1932). On catalytic hydrogenation, œstrone forms octahydro-œstrone, $C_{18}H_{30}O_2$. This compound contains two hydroxyl groups (two hydrogen atoms are used for converting the keto group to an alcoholic group), and so six hydrogen atoms are used to saturate *three* double bonds. If these three double bonds are in one ring, *i.e.*, there is a benzenoid ring present, then the phenolic hydroxyl group can be accounted for. The presence of one benzene ring in the structure of œstrone is supported by measurements of the molecular refractivity and the ultraviolet absorption spectrum.

When the methyl ether of œstrone is subjected to the Wolff–Kishner reduction, and the product distilled with selenium, 7-methoxy-1 : 2-*cyclo*-pentenophenanthrene is formed. The structure of this compound was established by the following synthesis (Cook *et al.*, 1934):

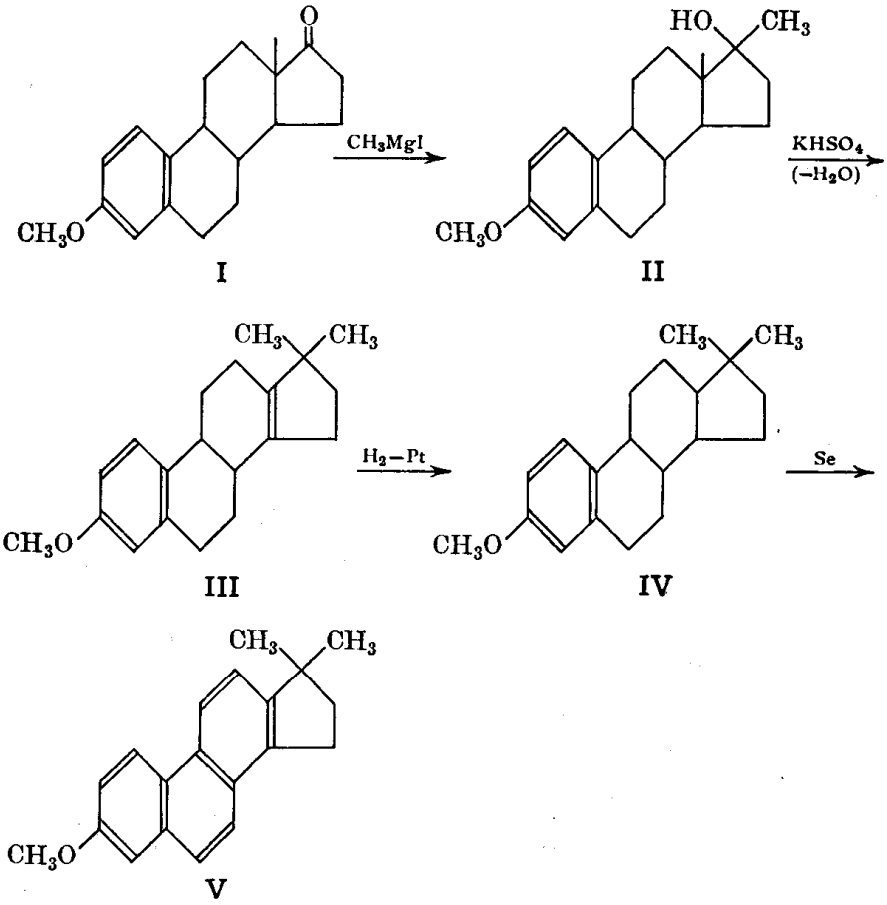


7-methoxy-1:2-*cyclo*pentenophenanthrene

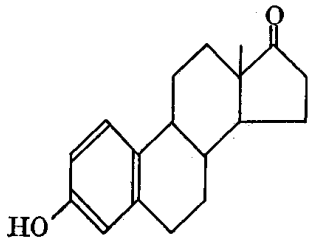
Thus the benzene ring in œstrone is ring A, and the (phenolic) hydroxyl group is at position 3; hence the skeleton of œstrone is:



Into this skeleton we must fit the keto group, and since this skeleton contains only 17 carbon atoms, another carbon atom must also be placed. The position of the keto group was shown to be at 17, and the extra carbon atom was shown to be an angular methyl group at position 13, as follows (Cook *et al.*, 1935). When the methyl ether of œstrone, I, is treated with methylmagnesium iodide, compound II is obtained. When II is dehydrated with potassium hydrogen sulphate to III, this catalytically reduced to IV, and then IV distilled with selenium, the product is 7-methoxy-3′ : 3′-dimethyl-1 : 2-*cyclo*pentenophenanthrene, V. The formation of V can be explained only if there is a keto group at position 17 and an angular methyl group at position 13. It should be noted that in the given equations, the dehydration is accompanied by the migration of the angular methyl group; this assumption is based on the analogy with known examples in which this occurs (see overleaf).



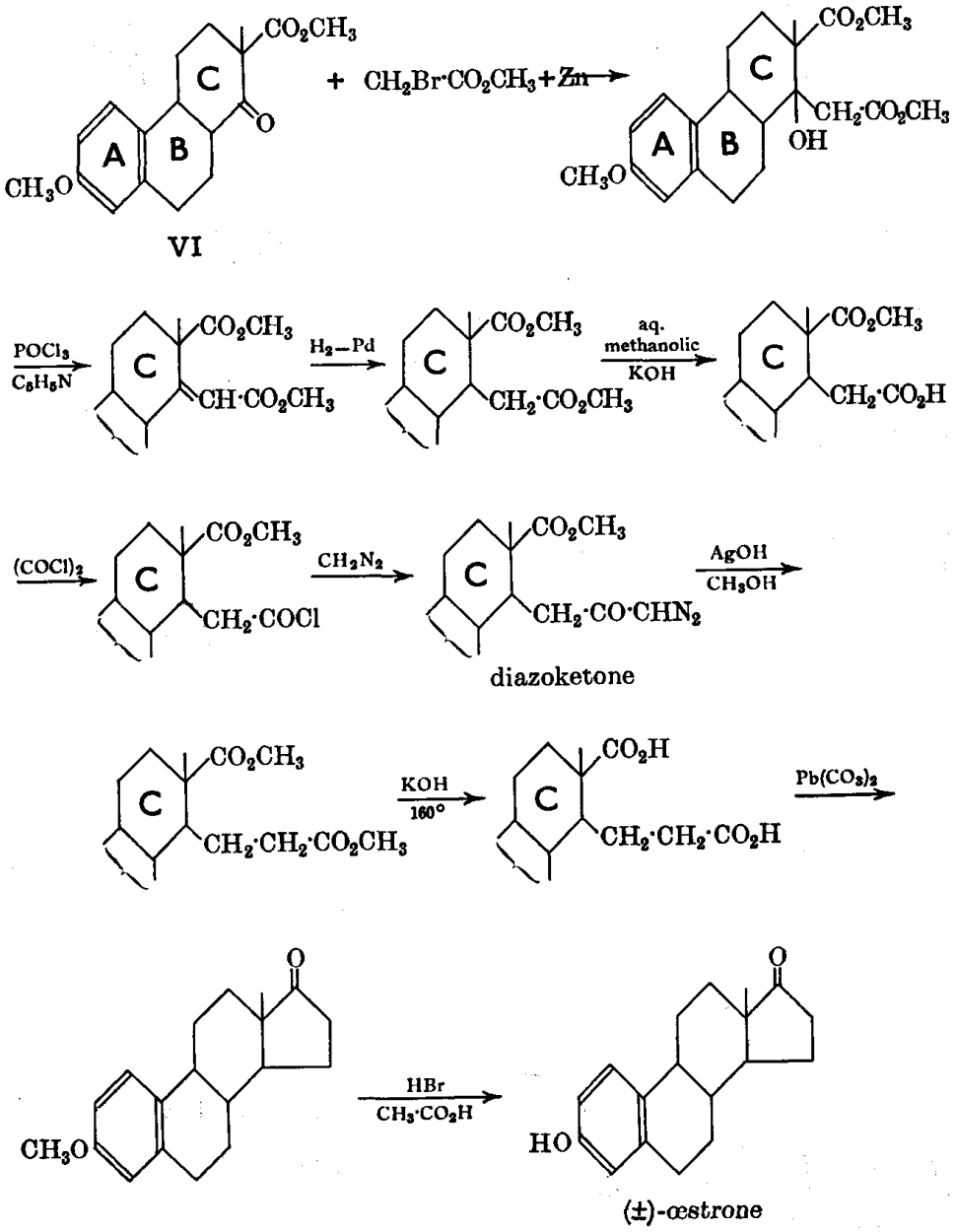
The structure of V has been confirmed by synthesis (Cook *et al.*, 1935). Thus the structure of œstrone is:



œstrone

This has been confirmed by the synthesis of Anner and Miescher (1948). These authors started with the phenanthrene derivative VI, which had been prepared previously by Robinson *et al.* (1938), and by Bachmann *et al.* (1942). The first step of the Anner–Miescher synthesis involves the Reformatsky reaction, and a later one the Arndt–Eistert synthesis.

The stereochemical problems involved in the synthesis of œstrone are not so complicated as in cholesterol, since only four asymmetric carbon atoms are present in the hormone (*cf.* §3). VI contains 3 asymmetric carbon atoms, and so four racemates are possible. Three have been isolated by Anner and Miescher, and one of these was converted into (±)-œstrone (C/D *trans*) and the stereoisomer (C/D *cis*) as shown above. These were separated and the



(±)-œstrone resolved with (−)-menthoxyacetic acid. The (+)-enantiomorph that was obtained was shown to be identical with the natural compound.

Johnson *et al.* (1958, 1962) have carried out a total synthesis of œstrone; each step in their synthesis was stereoselective. Hughes *et al.* (1960) have reported total syntheses of œstrone which appear to be simpler than any previous method and just as efficient.

§15. Œstriol, $C_{18}H_{24}O_3$, m.p. 281°, was isolated from human pregnancy urine by Marrian (1930). Since œstriol forms a triacetate, three hydroxyl groups must be present in the molecule. One was shown to be phenolic (*cf.* œstrone), and the other two secondary alcoholic, since, on oxidation, a diketone is produced. Furthermore, X-ray analysis indicates that the two alcoholic groups are in the *vicinal* position (*i.e.*, 1 : 2-). When œstriol is heated with potassium hydrogen sulphate, one molecule of water is removed