Anal. Calcd. for $C_{19}H_{32}O_2$: C, 78.0; H, 11.0. Found: C, 78.2; H, 10.7.

To a solution of 100 mg. of 3,17-androstanediol in 20 cc. of acetic acid, 100 mg. of chromic oxide was added. The solution remained at 20° overnight and was then diluted with water. It was extracted with ether and the ethereal solution was washed with a saturated sodium carbonate solution. The ether was evaporated and the residue distilled in high vacuum. It was crystallized from petroleum ether (b. p. 80–90°) and ethyl acetate; m. p. 128–129°. A mixture with 3,17-androstanedione prepared by the oxidation of *iso*-androsterone with chromic oxide gave no depression in the melting point.

17-Aminoandrostane Hydrochloride.—To a solution of 400 mg. of α-3-chloroandrosterone oxime in 100 cc. of dry amyl alcohol, was added 8 g. of sodium in small pieces. The amyl alcohol was refluxed until the sodium had dissolved. Water was added and the amyl alcohol layer separated. This was concentrated and the amine distilled in high vacuum at 110°. A portion of this oil was dissolved in ether and treated with dry hydrogen chloride. The amine hydrochloride was filtered and crystallized from alcohol-ether; m. p. 345° (dec.).

Anal. Calcd. for $C_{19}H_{34}NCl$: C, 73.3; H, 10.9. Found: C, 73.2; H, 10.9.

Androstanol-17.—(a) A solution of 100 mg. of 17-aminoandrostane in 5 cc. of glacial acetic acid was treated with 15 cc. of water, a few drops of sulfuric acid and a cooled solution of 2 g. of sodium nitrite. After standing for three hours, the solution was warmed to 60°. This was extracted with ether, and the residue after evaporation of the ether was sublimed in a high vacuum at 110°. It was crystallized from ligroin (b. p. 80–90°); m. p. 166°.

Anal. Calcd. for $C_{19}H_{32}O$: C, 82.5; H, 11.6. Found: C, 82.4; H, 11.3.

(b) To prove the identity of androstanol-17 prepared from 17-aminoandrostane, the product was prepared by reduction of α-3-chloroandrosterone.

A solution of 300 mg. of α-3-chloroandrosterone in 100 cc. of *n*-amyl alcohol was heated to boiling and treated with 5 g. of sodium. After solution, water was added and the amyl alcohol layer was separated and concentrated *in vacuo*. The residue was crystallized from dilute alcohol, sublimed in high vacuum at 100–105°, then crystallized from petroleum ether (b. p. 80–90°); m. p. 166°. This gave no depression in melting point when mixed with androstanol-17 prepared from 17-aminoandrostane.

Anal. Calcd. for $C_{19}H_{32}O$: C, 82.5; H, 11.6. Found: C, 82.2; H, 11.3.

Summary

17-Aminoandrosterone and 17-aminoandrostane were prepared by reduction of the corresponding oximes. These on diazotization gave the same hydroxy compounds as the sodium reductions of androsterone and α-3-chloroandrosterone.

STATE COLLEGE, PA.
DETROIT, MICH.

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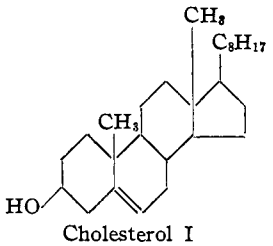
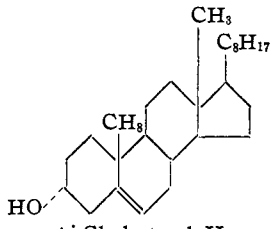
Studies on Sterols. V. *epi*-Cholesterol

BY RUSSELL E. MARKER, THOMAS S. OAKWOOD AND HARRY M. CROOKS

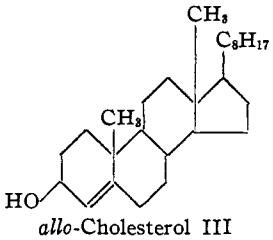
Many workers have attempted the synthesis of *epi*-cholesterol, which differs from cholesterol only in the configuration of the –OH group in the 3-position. The nearest approach to this was the work of Evans and Schoenheimer,[1] who prepared *epi-allo*-cholesterol, an isomer of *epi*-cholesterol, by the reduction of cholestenone with aluminum isopropylate. The *epi*-forms of the saturated sterols of dihydrocholesterol and coprosterol have been prepared by the catalytic reduction of cholestanone[2] and coprostanone[3] as well as by the separation of γ-cholestanol into its components of beta-cholestanol and *epi*-cholestanol by Windaus.[4] This was done by the precipitation of beta-cholestanol by digitonin and recovery of the *epi*-cholestanol from the filtrate of the digitonide.



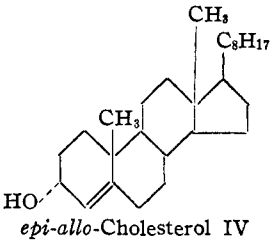
Cholesterol I



epi-Cholesterol II



allo-Cholesterol III



epi-allo-Cholesterol IV

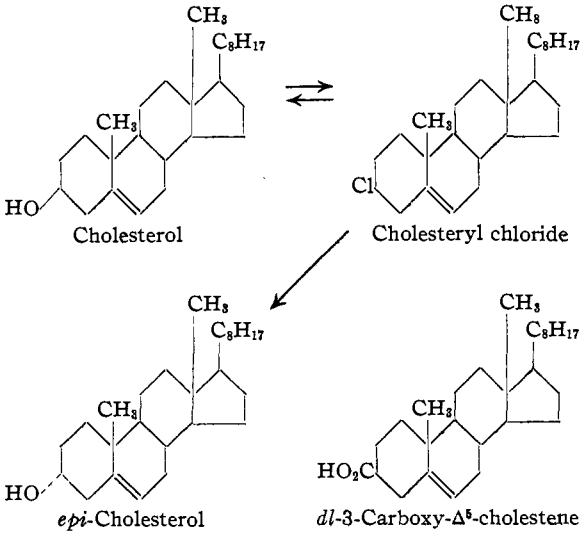
The preparation of the *epi*-form of cholesterol is of the greatest importance from the physiologi-

(1) Evans and Schoenheimer, THIS JOURNAL, **58**, 182 (1936).
(2) Vavon and Jakubowicz, *Bull. soc. chim.*, [4] **53**, 584 (1933).
(3) Ruzicka and co-workers, *Helv. Chim. Acta*, **17**, 1413 (1934).
(4) Windaus, *Ber.*, **49**, 1726 (1916).

cal standpoint since Ruzicka and co-workers[5] have shown that androsterone and *iso*-androsterone prepared from *epi*-cholestanol and beta-cholestanol are radically different in physiological activity, androsterone being seven times as active as *iso*-androsterone. This is noteworthy in that they differ only in the configuration of the –OH group at C_3. Therefore it is logical to expect the physiological activity of *epi*-cholesterol to differ greatly from that of cholesterol.

One of us had observed that in attempting to make an optically active acid from an optically active secondary halide through the Grignard reagent and carbon dioxide, the resulting acid was completely inactive. Porter[6] has also recently observed the inactivation of Grignard reagents prepared from active halides. Advantage was taken of this series of changes to synthesize *epi*-cholesterol from cholesterol.

Cholesterol was converted into cholesteryl chloride[7] by means of phosphorus pentachloride. A Grignard reagent was prepared from this and oxygen passed into it. The product contained an equal mixture of cholesterol and *epi*-cholesterol, due to racemization of C_3 during the process. These sterols were separated by precipitating the cholesterol out of alcoholic solution by means of digitonin. *epi*-Cholesterol is not precipitated by digitonin.



Whereas cholesterol on catalytic reduction with platinum oxide gives beta-cholestanol, *epi*-cholesterol on reduction gave *epi*-cholestanol quantitatively, showing the double bond to be in the 5,6-position. If it had shifted in the course of the reaction to the 4,5-position, the reduction product would have been a mixture of *epi*-cholestanol and *epi*-coprostanol. *epi*-Cholesterol forms an acetate with acetic anhydride.

dl-3-Carboxy-Δ^5-cholestene was prepared from cholesterylmagnesium chloride and carbon dioxide. This on catalytic reduction gave the corresponding *dl*-3-carboxycholestane. These acids were converted into their methyl and ethyl esters. They and their derivatives have not yet been resolved into their separate *d*- and *l*-forms.

These products were also made from cholesterylmagnesium bromide as well as the chloride. We are now preparing the *epi*-forms of the other unsaturated sterols and converting *epi*-cholesterol into Δ^5-androsterone. The physiological properties of these compounds are being studied.

Experimental

***epi*-Cholesterol.**—A Grignard reagent was prepared from 0.124 mole of cholesteryl chloride. This was cooled to −7° and a slow stream of dry oxygen was passed over the surface of the vigorously stirred solution for eight hours under a pressure of 40 mm. of mercury. During this time the temperature was allowed to slowly rise to 0°. The Grignard complex was decomposed by pouring into about 500 cc. of ice-cold 5% sulfuric acid. The ethereal solution was separated, washed with water, filtered and then evaporated. The residue was extracted with 50-cc. portions of 95% alcohol, giving 5 g. of white powder which upon recrystallization from alcohol gave a melting point of 127–132° (uncorr.). This racemic mixture was sublimed in high vacuum at 150°. A solution of 1.2 g. of the sublimate in 50 cc. of alcohol was added to a solution of 2 g. of digitonin in 200 cc. of alcohol. After standing for one hour, the precipitate was filtered and the alcoholic filtrate was evaporated to dryness. The residue was extracted with ether, filtered, and the ether evaporated. The residue, 480 mg., was crystallized four times from alcohol, m. p. 141°. The product does not give a precipitate with digitonin. It absorbs bromine readily. A mixture with a small mount of cholesterol (m. 147–148°) gave a depression in melting point of 12°.

Anal. Calcd. for $C_{27}H_{46}O$, C, 83.9; H, 12.0. Found: C, 84.1; H, 11.8.

Reduction of *epi*-Cholesterol.—A solution of 70 mg. of *epi*-cholesterol in 50 cc. of dry ether was shaken with 100 mg. of platinum oxide catalyst under hydrogen at 3 atm. pressure for one hour. The catalyst was filtered, the ether was evaporated and the remaining white solid was recrystallized twice from alcohol, m. p. 182–183°. A mixture with *epi*-cholestanol from the reduction of cholestanone gave no depression in melting point.

Anal. Calcd. for $C_{27}H_{48}O$: C, 83.4; H, 12.5. Found: C, 83.4; H, 12.7.

(5) Ruzicka and co-workers, *Helv. Chim. Acta*, **17**, 1395 (1934).
(6) C. W. Porter, THIS JOURNAL, **57**, 1436 (1935).
(7) Marker, Whitmore and Kamm, *ibid.*, **57**, 2358 (1935).

***dl*-3-Carboxy-Δ^5-cholestene.**—A Grignard reagent prepared from 0.3 mole of cholesteryl chloride was cooled to 0° and dry carbon dioxide was passed over the surface of the solution for five hours with vigorous stirring under a pressure of 40 mm. of mercury. The Grignard complex was decomposed by pouring into 300 cc. of ice-cold 10% sulfuric acid with shaking. The layers were separated and the ethereal solution filtered, removing most of the ether insoluble cholesteryl acid. The residue was washed with ether and then extracted in a Soxhlet extractor. It was then crystallized from benzene, m. p. 222–225°.

The acid adds bromine readily. The sodium salt is only slightly soluble in water. The free acid is sparingly soluble in ether, but may be crystallized from benzene. The same acid was prepared from cholesterylmagnesium bromide.

Anal. Calcd. for $C_{28}H_{46}O_2$: C, 81.1; H, 11.2. Found: C, 81.0; H, 11.4.

***dl*-3-Carboxycholestane.**—A solution of 2.8 g. of *dl*-3-carboxy-Δ^5-cholestene in 500 cc. of dry ether and 5 cc. of glacial acetic acid was shaken with 500 mg. of platinum oxide under hydrogen at 3 atm. pressure for one hour. The catalyst was filtered off, the acetic acid washed out with three 50-cc. portions of water and the ether evaporated. The solid was crystallized from benzene, m. p. 209–211°. A mixture with the unsaturated acid gave a melting point depression of 11°.

Anal. Calcd. for $C_{28}H_{48}O_2$: C, 80.7; H, 11.6. Found: C, 80.8: H, 11.8.

Methyl Ester of *dl*-3-Carboxy-Δ^5-cholestene.—To a solution of 1.0 g. of the recrystallized unsaturated acid in 25 cc. of absolute methyl alcohol was added 4 drops of sulfuric acid. The product was refluxed for forty hours. Water was added and the product filtered. The residue was taken up in 25 cc. of ether, washed with 5% sodium hydroxide solution, and finally with water. The ether solution was filtered and the ether distilled off, leaving a solid residue. This was crystallized from ether–methyl alcohol; yield 0.9 g., m. p. 101.5–102.5°. The ester is readily soluble in ether.

Anal. Calcd. for $C_{29}H_{48}O_2$: C, 81.2; H, 11.3. Found: C, 81.1; H, 11.3.

Ethyl Ester of *dl*-3-Carboxy-Δ^5-cholestene.—A solution of 5 g. of recrystallized acid was refluxed for eight hours with 80 cc. of absolute ethyl alcohol and 20 drops of sulfuric acid. The reaction mixture was poured into 200 cc. of water and 100 cc. of ether added. The ether layer was washed with 5% sodium hydroxide solution, then with water. It was filtered and then the ether was evaporated. The product was crystallized from ether–ethyl alcohol; yield 4.0 g., m. p. 82.5–83.0°.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.4; H, 11.4. Found: C, 81.4; H, 11.2.

Methyl Ester of *dl*-3-Carboxycholestane.—A solution of 25 cc. of absolute methyl alcohol containing 5 drops of sulfuric acid was added to 1 g. of *dl*-3-carboxycholestane. This was refluxed for twenty hours. The mixture was then added to 50 cc. of water. To this was added 30 cc. of ether and the ethereal solution was washed with 5% sodium hydroxide solution and then with water. The ester was crystallized from ether–methyl alcohol; yield 0.85 g., m. p. 70–71°.

Catalytic reduction of the methyl ester of *dl*-3-carboxy-Δ^5-cholestene gave the same ester.

Anal. Calcd. for $C_{29}H_{50}O_2$: C, 80.9; H, 11.7. Found: C, 80.8; H, 11.8.

Ethyl Ester of *dl*-3-Carboxycholestane.—A solution of 1.0 g. of the ethyl ester of *dl*-3-carboxy-Δ^5-cholestene in 100 cc. of ether with 2 cc. of acetic acid was reduced catalytically by shaking with platinum oxide under hydrogen at a pressure of 3 atm. The catalyst was filtered, the ethereal solution was washed with 5% sodium hydroxide solution, and then the ether was evaporated. The residue was crystallized from ether–ethyl alcohol; yield 0.8 g., m. p. 66.5–67.5°.

Anal. Calcd. for $C_{30}H_{52}O_2$: C, 81.0; H, 11.8. Found: C, 81.0; H, 11.7.

Summary

epi-Cholesterol has been prepared from cholesterylmagnesium chloride, giving a mixture of cholesterol and *epi*-cholesterol which was separated by means of digitonin. The unsaturated cholesteryl-*dl*-3-carboxylic acids were prepared from cholesterylmagnesium chloride and bromide and carbon dioxide. These were converted into their esters and reduced to the saturated acids and esters.

STATE COLLEGE, PA.
DETROIT, MICH.

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