Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.6; H, 12.0.

When this acetate was hydrolyzed with alcoholic potassium hydroxide it gave a product which, after crystallization from alcohol, melted at 139°. It showed no depression in melting point when mixed with stigmastanol, prepared by the hydrogenation and subsequent hydrolysis of stigmasteryl acetate.

Summary

Upon dehydrogenation with copper, sitosterol and stigmasterol give sitostenone and stigmastenone, respectively. Catalytic hydrogenation of either of these ketones gives 24-ethyl-*epi*-coprostanol, which can be oxidized to 24-ethylcoprostanone. The reduction of the latter with aluminum isopropylate gives a mixture of 24-ethyl-*epi*-coprostanol and 24-ethyl-coprostanol (β). The latter is epimerized by sodium in boiling xylene, yielding 24-ethyl-*epi*-coprostanol. 24-Ethylcoprostanone forms a monobromo derivative which, under the action of pyridine, may be converted into sitostenone.

A new approach to the preparation of sterols unsaturated only in the side chain has been found. By the action of sodium in amyl alcohol on stigmastenone, for example, 5,6-dihydrostigmasterol is formed. This can be reduced to stigmastanol.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE]

Sterols. XXV. The Allostigmasterols and Allositosterols

BY RUSSELL E. MARKER AND THOMAS S. OAKWOOD

Recently Schoenheimer and Evans[1] have prepared allocholesterol ($\Delta^{4,5}$-cholesterol) and *epi*-allocholesterol by reducing cholestenone with aluminum isopropylate, the isomers being separated by the use of digitonin. They found that allocholesterol and, to an even greater extent, *epi*-allocholesterol, are readily dehydrated by acids.

We have now prepared the corresponding allositosterols and allostigmasterols, following the method of Schoenheimer and Evans,[1] by the reduction of sitostenone[2,3] and stigmastenone[3] with aluminum isopropylate. The tendency of these substances to undergo dehydration is in complete accord with the similar observations of Schoenheimer and Evans on allocholesterol and *epi*-allocholesterol. In the case of *epi*-allostigmasterol this tendency is so marked that we have been only able to obtain it mixed with its dehydration product, a stigmasterylene. After reducing stigmastenone with aluminum isopropylate, the reduction products were separated by means of digitonin. Only impure *epi*-allostigmasterol, admixed with its dehydration product, could be obtained even when attempts were made to separate these by means of the half succinic ester. That *epi*-allostigmasterol was present is shown by the fact that when the mixture was hydrogenated and then treated with sodium in boiling xylene, 24-ethyl-*epi*-coprostanol was formed. In the case of *epi*-allositosterol, allositosterol and allostigmasterol, the pure sterols could be isolated. Their structures are proved by the analytical data, their mode of preparation and their reduction (and epimerization) to 24-ethyl-*epi*-coprostanol.

(1) Schoenheimer and Evans *J. Biol. Chem.*, **114**, 567 (1936).

(2) Heiduschka and Gloth, *Arch. Pharm.*, **253**, 415 (1915); *C. A.*, **10**, 1182 (1916).

(3) Marker and Wittle, THIS JOURNAL, **59**, 2704 (1937).

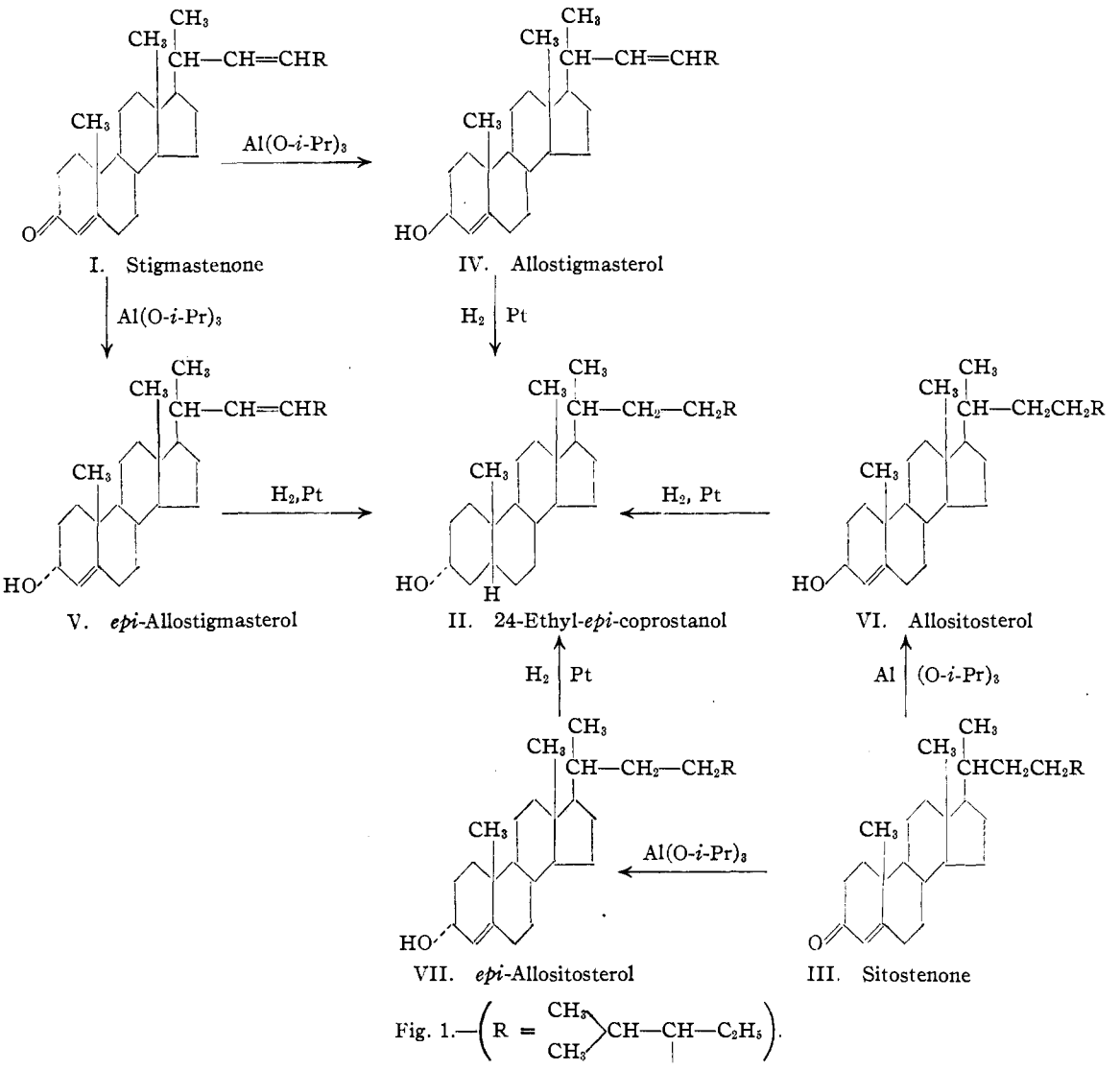
Experimental

Allostigmasterol.—A mixture of 5 g. of stigmastenone,[3] 7.5 g. of aluminum isopropylate, and 200 cc. of dry isopropyl alcohol was refluxed for six hours, and then distilled over a period of six hours to half its original volume. Water and ether were added and then the mixture was shaken with dilute sulfuric acid. The ether layer was separated and evaporated, and the residue dissolved in 1 liter of hot alcohol. A solution of 15 g. of digitonin in 500 cc. of alcohol was added. The next day the digitonide was filtered and washed well with alcohol.

The insoluble digitonide was dried, pulverized and allowed to stand overnight with 100 cc. of dry pyridine. The next day the pyridine solution was poured into 500 cc. of ether, the precipitated digtonin filtered and the filtrate washed free of pyridine with dilute sulfuric acid. The ether was evaporated and the residue, after crystallization from methanol and alcohol, gave allostigmasterol, m. p. 137°.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.4; H, 11.7. Found: C, 84.2; H, 11.6.

Allostigmasteryl Acetate.—To a solution of 500 mg. of allostigmasterol in 10 cc. of pyridine was added 5 cc. of acetic anhydride. After the solution had stood overnight, ice water was added and the precipitated crude acetate



filtered. Crystallization of this crude acetate from methanol and alcohol gave the pure acetate, m. p. 132°.

Anal. Calcd. for $C_{31}H_{50}O_2$: C, 81.9; H, 11.1. Found: C, 81.9; H, 11.1.

Catalytic Reduction of Allostigmasteryl Acetate.—A suspension of 100 mg. of platinum oxide catalyst in a solution of 500 mg. of allostigmasteryl acetate in 100 cc. of ether was shaken with hydrogen at 45 lb. (3 atm.) pressure for six hours. The catalyst was filtered, the ether evaporated and the residue hydrolyzed by warming with an excess of alcoholic potassium hydroxide solution. The crude sterol was isolated by neutralizing the alkaline solution with acetic acid, diluting with water, and filtering the precipitated sterol. Since the reduction gave a mixture of sitostanol and 24-ethylcoprostanol (β), these were separated by epimerizing the mixture and thereby converting the 24-ethyl-coprostanol (β) into 24-ethyl-*epi*-coprostanol, the sitostanol remaining largely unaffected by this treatment. The crude sterol was refluxed for six hours with 1 g. of sodium in 25 cc. of xylene. Water was added, the xylene layer separated and evaporated, and the residue dissolved in alcohol. A solution of 1 g. of digitonin in 50 cc. of alcohol was added. The next day the digitonide was filtered and the filtrate evaporated to dryness. The residue was extracted with ether and the ether evaporated, leaving a residue which was crystallized from methanol and alcohol. This substance, m. p. 137°, was 24-ethyl-*epi*-coprostanol, since it showed no depression in melting point when mixed with an authentic sample prepared by the reduction of stigmastenone.[3]

Fifty milligrams of the above product was acetylated by the method used for preparing allostigmasteryl acetate. After crystallization from methanol, the acetate melted at 94° and did not depress the melting point of 24-ethyl-*epi*-coprostyl acetate prepared from stigmastenone.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.4; H, 12.1.

***epi*-Allostigmasterol.**—The mother liquor from the insoluble digitonide obtained as described in the preparation of allostigmasterol was evaporated to dryness under re-

duced pressure, the residue extracted with ether, and the ethereal extract evaporated. Although the residue was crystallized repeatedly from ether–alcohol and acetone, no homogeneous product could be obtained. Apparently, some of the *epi*-allostigmasterol had dehydrated to an unsaturated hydrocarbon, which could not be separated from the sterol by crystallization. Attempts to purify the sterol by means of its half succinic ester failed for more hydrocarbon was formed upon acidification of the ester. Accordingly, the mixture was hydrogenated using 0.5 g. of platinum oxide catalyst and 100 cc. of ether. The catalyst was filtered, the ether evaporated and the residual mixture of reduced sterols and hydrocarbons separated by the usual procedure using 1 g. of succinic anhydride and 2 cc. of pyridine. The sterol mixture so obtained was epimerized in the usual manner with sodium in boiling xylene. Although the starting material, the mixture of *epi*-allostigmasterol and its dehydration product, gave no insoluble digitonide, an appreciable amount of digitonide was formed when the epimerized reduced sterol mixture was treated with alcoholic digitonin solution, showing thereby the presence of *epi*-allostigmasterol in the original material before hydrogenation. The filtrate from the digitonide was evaporated to dryness, extracted with ether, the ether evaporated, and the resulting residue crystallized from methanol and alcohol. The product, m. p. 137°, was 24-ethyl-*epi*-coprostanol since it showed no depression in melting point when mixed with an authentic sample prepared from stigmastenone.

The product formed an acetate, m. p. 93°, which showed no depression in melting point when mixed with 24-ethyl-*epi*-coprostyl acetate.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.2; H, 12.0.

The proof of the presence of *epi*-allostigmasterol in the crude original mixture is therefore based on the formation of a dehydration product, and the formation of reduced sterols both of the allo and regular series on hydrogenation.

Allositosterol.—A mixture of 5 g. of sitostenone,[2,3] 7.5 g. of aluminum isopropylate and 200 cc. of dry isopropyl alcohol was refluxed for six hours and then slowly distilled over a period of six hours to half its original volume. The reaction mixture was diluted with ether, washed with dilute sulfuric acid, and the ether layer separated and evaporated. To the residue dissolved in 1 liter of alcohol was added a solution of 15 g. of digitonin in 500 cc. of alcohol. The next day the digitonide was filtered, washed well with alcohol and dried.

The insoluble digitonide was pulverized and allowed to stand overnight with 100 cc. of dry pyridine. The next day the digitonin was removed by adding 300 cc. of ether and filtering the suspension. The filtrate was shaken with sulfuric acid, and the ether layer separated and evaporated. The residue was crystallized from methanol, ethanol and acetone to give allositosterol, m. p. 158°.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.5; H, 12.2. Found: C 83.6; H, 12.3.

Allositosteryl Acetate.—To a solution of 50 mg. of allositosterol in 1 cc. of pyridine was added 0.5 cc. of acetic anhydride. After standing overnight, water was added and the crude acetate filtered. It was crystallized from methanol. Allositosteryl acetate melts at 88°.

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.5; H, 11.5. Found: C, 81.2; H, 11.7.

Catalytic Reduction of Allositosteryl Acetate.—A solution of 500 mg. of allositosteryl acetate in ether was hydrogenated, epimerized and the product purified as described for the reduction of allostigmasteryl acetate. The product, 24-ethyl-*epi*-coprostanol, m. p. 136°, gave no depression with an authentic sample. It gave an acetate, m. p. 94°, which showed no depression in melting point when mixed with 24-ethyl-*epi*-coprostyl acetate. The acetate was analyzed.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.0; H, 11.8.

***epi*-Allositosterol.**—The alcoholic filtrate from the digitonide of allositosterol was evaporated to dryness, extracted with ether, and the ether evaporated. This residue was crystallized from methanol and alcohol, and gave *epi*-allositosterol, m. p. 138°.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.5; H, 12.2. Found: C, 83.5; H, 12.5.

***epi*-Allositosteryl Acetate.**—This acetate was prepared from 500 mg. of *epi*-allositosterol by the same method as that used for the preparation of allostigmasteryl acetate. After crystallization from methanol and alcohol, the acetate melted at 92°.

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.5; H, 11.5. Found: C, 81.2; H, 11.7.

Catalytic Reduction of *epi*-Allositosteryl Acetate.—A solution of 500 mg. of *epi*-allositosteryl acetate in ether was hydrogenated, epimerized and the product purified as described for the reduction of allostigmasteryl acetate. The product, m. p. 136°, was shown by mixed melting point determinations to be 24-ethyl-*epi*-coprostanol. Its acetate, m. p. 93°, gave no depression in melting point when mixed with 24-ethyl-*epi*-coprostyl acetate.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.0; H, 12.0.

Summary

The reduction of sitostenone and of stigmastenone with aluminum isopropylate in isopropyl alcohol gives allositosterol and *epi*-allositosterol, and allostigmasterol and *epi*-allostigmasterol, respectively. The latter has not been obtained pure, but only in the form of a mixture with its dehydration product. All of these allosterols are readily dehydrated, and on reduction they give mixtures of the corresponding cholestanol and coprostanol homologs.

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