the organisms did not grow after exposures of six, ten, sixteen and twenty minutes, respectively.

Klebsiella Pneumoniae.—With 0.00008 molar solutions of *n*-butyl, *n*-propyl, ethyl, and methylmercuric acetates the organisms did not grow after exposures of four, eight, fourteen, and eighteen minutes, respectively.

E. Coli.—Using solutions (concentration 1:100,000) of benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl, and *o*-nitrophenylmercuric acetates the organisms did not grow after exposures of fifteen, fifteen, eighteen, eighteen, eighteen, and eighteen minutes, respectively.

Summary

The organomercuric acetates containing the radicals methyl, ethyl, *n*-propyl, *n*-butyl, benzyl, *p*-tolyl, *p*-methoxyphenyl, phenyl, *o*-chlorophenyl and *o*-nitrophenyl have been prepared and the bactericidal action determined with several organisms.

In the aliphatic series the toxicity of the compounds increases uniformly from methylmercuric acetate to *n*-butylmercuric acetate.

The toxicity of the aromatic mercuric acetates is greater than that of the aliphatic compounds. There is no significant difference in the toxicities of the six aromatic organomercuric acetates used.

IOWA CITY, IOWA RECEIVED AUGUST 16, 1937

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

Sterols. XXIV. Sitostenone and Stigmastenone

BY RUSSELL E. MARKER AND EUGENE L. WITTLE

Tallol-sitosterol is one of the most readily available sterols since, in contrast to the difficulty of isolation of pure phytosterols from wheat germ oil, it may be obtained easily in a pure state from pine oil.[1] Although their identity has not been established with certainty, β-sitosterol from wheat germ oil,[2] cinchol from cinchona bark,[3] and tallol-sitosterol are probably the same substance. Therefore, we shall, for convenience, designate tallol-sitosterol simply as sitosterol.

Bengtsson[4] found no depression in melting point on mixing comparable derivatives of stigmastanol and sitostanol; since these derivatives included the ketones and hydrocarbons as well as a number of esters, it is highly probable, if not certain, that sitosterol is 22-dihydrostigmasterol.

We have compared the melting points of a number of new substances related to stigmasterol and sitosterol and find no depression in melting point on mixing corresponding derivatives. The accompanying chart (Fig. 1) shows the relationship of these derivatives to the parent sterols. It may now be said with certainty that sitosterol from pine oil is identical with 22-dihydrostigmasterol; since the complex interconversions reported here exclude any chance coincidences.

Stigmasterol (I) and sitosterol (X), upon dehydrogenation with copper powder at 200° under reduced pressure, yield stigmastenone (II) and sitostenone (IX), respectively. The latter has been prepared previously by Heiduschka and Gloth[5] by the oxidation and subsequent debromination of sitosterol dibromide. α-Fucostenone, prepared in a somewhat similar manner from α-dihydrofucosterol,[6] is probably identical with sitostenone. Both stigmastenone (II) and sitostenone (IX), on catalytic hydrogenation, and subsequent treatment with sodium in boiling xylene to render small amounts of allo compounds precipitable with digitonin, yield 24-ethyl-*epi*-coprostanol (V).[7] This, upon oxidation with chromium trioxide in acetic acid, gives 24-ethylcoprostanone (VII). The latter, when reduced with aluminum isopropylate, gives a mixture of 24-ethyl-*epi*-coprostanol (V) and 24-ethylcoprostanol (β) (VI) which may be separated by the use of digitonin. 24-Ethylcoprostanol (β) (VI) on treatment with sodium in boiling xylene, is epimerized to give as the chief product, 24-ethyl-*epi*-coprostanol (V). The bromination of 24-ethylcoprostanone (VII) in acetic acid gives 4-bromo-24-ethylcoprostanone (VIII), which, on treatment with pyridine, gives sitostenone (IX) identical with that obtained by the dehydrogenation of sitosterol (X).

(1) Sandqvist and Bengtsson, *Ber.*, **64**, 2167 (1931).

(2) Anderson, Burr and Shriner, THIS JOURNAL, **48**, 2987 (1926).

(3) Strain, in "Organic Chemistry," ed. by H. Gilman, John Wiley and Sons, Inc., New York, 1937, Vol. II, Chap. 15.

(4) Bengtsson, *Z. physiol. Chem.*, **237**, 46 (1935).

(5) Heiduschka and Gloth, *Arch. Pharm.*, **257**, 415 (1915).

(6) Coffey, Heilbron and Spring, *J. Chem. Soc.*, 738 (1936).

(7) The name coprostanol seems preferable to coprosterol since the former, but not the latter name, corresponds to the epimer, cholestanol. The suffix sterol should, perhaps, be applied only to carbinols unsaturated in the first or second ring. It should be noted that the only known naturally occurring substance affected by this suggestion is coprostanol.

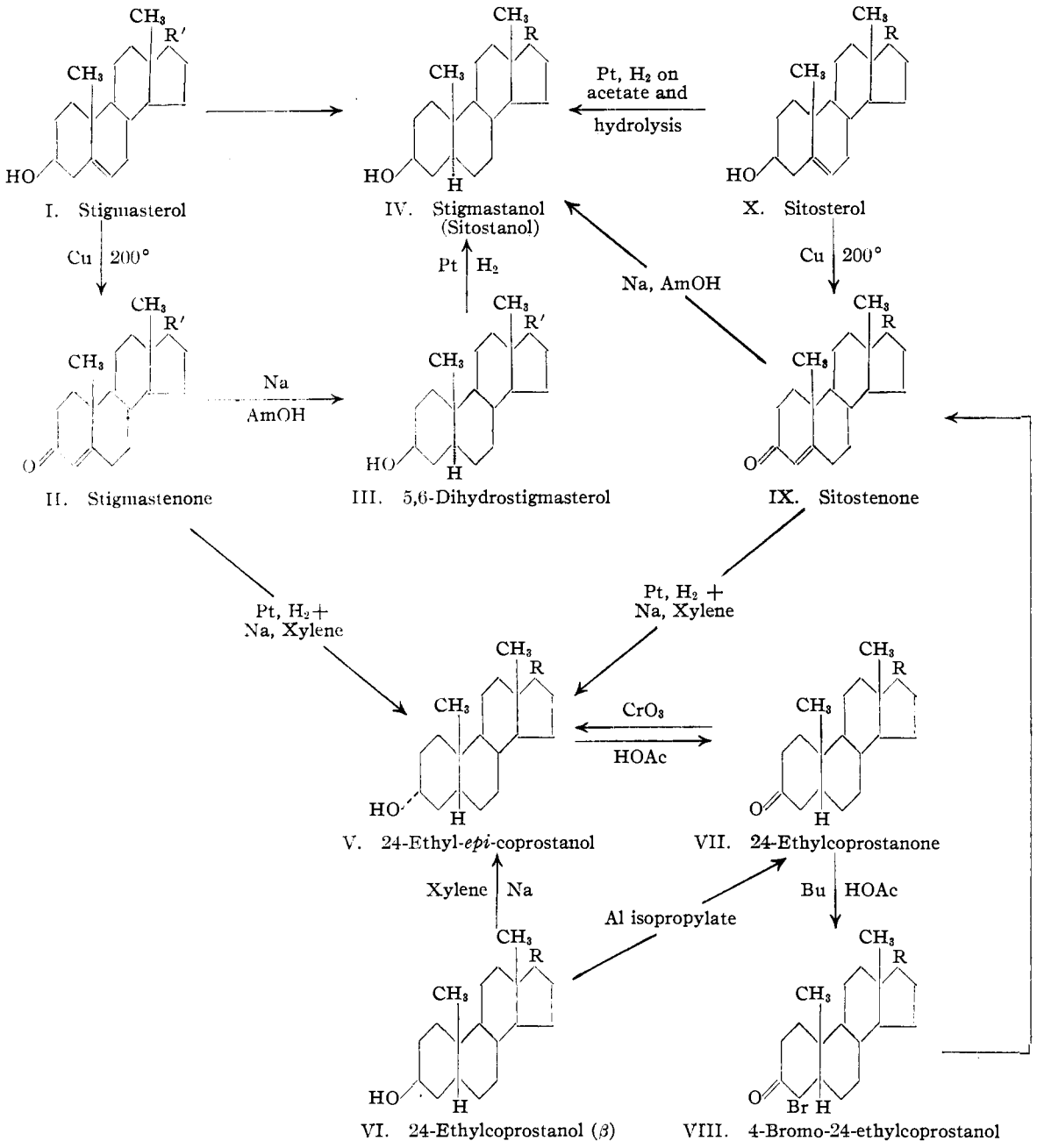

Fig. 1.

The degradation of sterol side chains is greatly facilitated if side-chain double bonds are present. Since the two sterols, stigmasterol and ergosterol, which are available for this purpose, have nuclear as well as side-chain double bonds, it has been necessary to protect the nuclear double bonds by partial bromination. The necessarily lengthy process of bromination, oxidation and debromination results in low yields of the degradation product. Thus, Fernholz[8] obtained by this method only 1 g. of 3-acetoxybisnorcholenic acid from 6 g. of stigmasteryl acetate. An alternative approach to the preparation of sterols unsaturated only in

(8) Fernholz *Ann.* **507**, 128 (1934).

the side chain was suggested to us by the observation of Diels and Abderhalden[9] that β-cholestanol may be obtained by the reduction of cholestenone with sodium and amyl alcohol. We find that stigmastenone (II), treated in a like manner, yields 5,6-dihydrostigmasterol (III) which contains a β-3 hydroxyl group since it is precipitated with digitonin. The catalytic hydrogenation of 5,6-dihydrostigmasterol (III) yields stigmastanol (IV), identical with sitostanol (IV) prepared from sitosterol (X). The treatment of sitostenone (IX) with sodium in amyl alcohol also yielded sitostanol (IV).

We wish to thank Dr. George H. Fleming for the microanalyses reported in this paper.

Experimental

The tallol used in this work was obtained from the Gaylord Bag and Paper Company of Bogolusa, Louisiana. Sitosterol was obtained from this tallol by essentially the method outlined by Sandqvist and Bengtsson.[1] The purified sterol melted at 139°.

A non-saponifiable fraction from soya bean oil, corresponding to about 55% sterols, was purchased from Hansa-Mühle A. G., Hamburg, Germany. Stigmasterol was isolated[10] from this fraction as the acetate tetrabromide. The purified stigmasterol melted at 170°.

Sitostenone.—A mixture of 15 g. of sitosterol and 15 g. of Baker precipitated copper in a retort was heated *in vacuo* at 2 mm. pressure and 150–200° for thirty minutes. At the end of this time hydrogen had ceased to be evolved; so the temperature was raised to 275° to distil the product. The distillate was crystallized from methanol–ether and methanol–acetone mixtures followed by sublimation at high vacuum to give sitostenone, m. p. 82°. Heiduschka and Gloth[5] report that sitostenone melts at 82°, and gives a semicarbazone sintering at 243° and melting at 254°.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.4; H, 11.8. Found: C, 84.0; H, 11.5.

A mixture of 50 mg. of sitostenone, 20 mg. of semicarbazide hydrochloride and 35 mg. of sodium acetate in 20 cc. of alcohol was heated for one hour. The semicarbazone which precipitated after dilution and cooling was recrystallized from alcohol to give sitostenone-semicarbazone, sintering at 243° and melting at 252°.

Anal. Calcd. for $C_{30}H_{51}ON_3$: C, 76.6; H, 10.9. Found: C, 76.4; H, 11.3.

Stigmastenone.—A mixture of 10 g. of stigmasterol and 12 g. of Baker precipitated copper was heated in a retort to 180–200° at 2 mm. pressure for thirty minutes. Then the temperature was raised to 250–275° to distil the product. The distillate was crystallized from acetone, methanol and alcohol to give stigmastenone, m. p. 94°.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.8; H, 11.3. Found: C, 84.3; H, 11.6.

24-Ethyl-*epi*-coprostanol from Sitostenone.—A solution of 5 g. of sitostenone in 200 cc. of ether was shaken with 0.5 g. of platinum oxide catalyst in a hydrogen atmosphere at 45 lb. (3 atm.) pressure for four hours. After filtering the ethereal solution, the ether was distilled, and the residue refluxed for nine hours with 5 g. of sodium and 250 cc. of xylene. This step served to convert small amounts of allo compounds almost completely into their β-3-OH forms which could be removed by precipitation with digitonin. Alcohol and water were added to the reaction mixture, and the xylene layer was separated and evaporated under reduced pressure. To the residue, dissolved in 1 liter of boiling alcohol, was added a solution of 10 g. of digitonin in 500 cc. of alcohol. After standing overnight, the digitonide was filtered. The filtrate was evaporated to dryness and the sterol extracted from the excess digitonin with ether. The ether was evaporated and the residue crystallized from methanol and alcohol to give 24-ethyl-*epi*-coprostanol, m. p. 137°.

Anal. Calcd. for $C_{29}H_{52}O$: C, 83.3; H, 12.6. Found: C, 83.8; H, 12.8.

A solution of 100 mg. of 24-ethyl-*epi*-coprostanol in 5 cc. of acetic anhydride was refluxed for thirty minutes. After evaporating the excess acetic anhydride, the residual 24-ethyl-*epi*-coprostyl acetate was crystallized from methanol. This acetate melts at 94°.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.5; H, 11.9.

24-Ethyl-*epi*-coprostanol from Stigmastenone.—A solution of 5 g. of stigmastenone in 200 cc. of ether was shaken with 0.5 g. of platinum oxide catalyst in a hydrogen atmosphere at 45 lb. (3 atm.) pressure for three hours. After filtering the ethereal solution, and evaporating the ether, the residue was treated with sodium and boiling xylene, and with digitonin as described for the preparation of 24-ethyl-*epi*-coprostanol from sitostenone. The resulting product was crystallized from methanol and alcohol to give 24-ethyl-*epi*-coprostanol, m. p. 137°. It showed no depression in melting point when mixed with 24-ethyl-*epi*-coprostanol from sitostenone.

Anal. Calcd. for $C_{29}H_{52}O$: C, 83.3; H, 12.6. Found: C, 83.4; H, 12.4.

It gave an acetate, m. p. 94°, which gave no depression in melting point when mixed with 24-ethyl-*epi*-coprostyl acetate from sitostenone.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 81.5; H, 11.7.

24-Ethylcoprostanone.—To a solution of 4 g. of 24-ethyl-*epi*-coprostanol in 200 cc. of acetic acid was added 1 g. of chromium trioxide in 10 cc. of 90% acetic acid. After the solution had stood overnight at room temperature, it was diluted with water and filtered. The crude ketone was crystallized from methanol–ether and alcohol–ether mixtures to give 24-ethylcoprostanone, m. p. 114°.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.5; H, 12.2. Found: C, 83.9; H, 12.0.

24-Ethylcoprostanol (β).—A mixture of 2 g. of 24-ethylcoprostanone, 3 g. of aluminum isopropylate, and 100 cc. of dry isopropyl alcohol was refluxed for six hours, and then slowly distilled over a period of six hours to half its former volume. The mixture was diluted with ether and then shaken with dilute sulfuric acid. The ether layer was separated, the ether evaporated, and to the residue,

(9) Diels and Abderhalden, *Ber.*, **39**, 884 (1906).

(10) Bonstedt, *Z. physiol. Chem.*, **176**, 269 (1928).

dissolved in 100 cc., was added 5 g. of digitonin in 200 cc. of alcohol. The next day the precipitated digitonide was filtered, dried, and then heated on a steam-bath for thirty minutes with 15 cc. of dry pyridine. The digitonin was precipitated as usual by the addition of ether and filtered. The filtrate was washed with dilute hydrochloric acid and the ether layer separated and evaporated. The residue was crystallized from methanol to give 24-ethylcoprostanol (β), m. p. 127°.

Anal. Calcd. for $C_{29}H_{52}O$: C, 83.3; H, 12.6. Found: C, 83.7; H, 12.7.

One hundred milligrams of 24-ethylcoprostanol (β) was acetylated by refluxing it with 5 cc. of acetic anhydride. The acetate, after crystallization from methanol, melted at 89°.

Anal. Calcd. for $C_{31}H_{54}O$: C, 81.2; H, 11.8. Found: C, 81.4; H, 11.9.

The filtrate from the digitonide was worked up as described before and gave 24-ethyl-*epi*-coprostanol, m. p. 137°.

Epimerization of 24-Ethylcoprostanol (β) to 24-Ethyl-*epi*-coprostanol.—A solution of 200 mg. of 24-ethylcoprostanol (β) in 50 cc. of xylene was refluxed for eight hours with 1 g. of sodium. After alcohol and water had been added, the xylene layer was separated and then evaporated. The residue, after crystallization from methanol, melted at 137° and showed no depression in melting point when mixed with samples of 24-ethyl-*epi*-coprostanol prepared from sitostenone and stigmastenone. No depression in melting point was observed when its acetate was mixed with 24-ethyl-*epi*-coprostyl acetate from sitostenone or stigmastenone.

4-Bromo-24-ethylcoprostanone.—To a solution of 1 g. of 24-ethylcoprostanone in 15 cc. of acetic acid was added a drop of 48% aqueous hydrobromic acid and 2.5 cc. of a 1 *M* solution of bromine in acetic acid. The brominated ketone which precipitated was crystallized from acetic acid and alcohol, giving 4-bromo-24-ethylcoprostanone, m. p. 149°.

Anal. Calcd. for $C_{29}H_{49}OBr$: C, 70.5; H, 10.0. Found: C, 69.9; H, 10.2.

Sitostenone from 4-Bromo-24-ethylcoprostanone.—A solution of 500 mg. of 4-bromo-24-ethylcoprostanone in 15 cc. of pyridine was refluxed for eight hours. After diluting the reaction mixture with ether, the pyridine was removed by washing with dilute hydrochloric acid. The ether was evaporated and the residue, after crystallizing from methanol–acetone and methanol–ether mixtures, gave sitostenone, m. p. 102°. This product showed no depression in melting point when mixed with sitostenone, m. p. 102°, prepared by the dehydrogenation of sitosterol. After sublimation, the sitostenone melted at 82°, but on recrystallization from methanol–ether it again melted at 102°.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.4; H, 11.8. Found: C, 84.2; H, 11.7.

Sitostanol from Sitostenone.—Three grams of sodium in small pieces were added to a boiling solution of 500 mg. of sitostenone in 50 cc. of dry amyl alcohol. The solution was refluxed for one hour, cooled, and water added. The amyl alcohol layer was separated and then evaporated and the residue dissolved in alcohol. A solution of 2 g. of digitonin in 100 cc. of alcohol was added, and the solution allowed to stand overnight. The next day the digitonide was filtered, dried and warmed on a steam-bath for thirty minutes with 10 cc. of pyridine. Ether was added, and the precipitated digitonin filtered. After washing the ethereal filtrate with dilute hydrochloric acid to remove pyridine, the ether was evaporated and the residue crystallized from alcohol. The sitostanol obtained in this manner melted at 140° and showed no depression in melting point when mixed with authentic sitostanol, m. p. 140°, obtained by the reduction and subsequent hydrolysis of sitosteryl acetate.

It gave an acetate, m. p. 136°, which showed no depression in melting point when mixed with authentic stigmastyl acetate, m. p. 136°.

Anal. Calcd. for $C_{31}H_{54}O_2$: C, 81.2; H, 11.8. Found: C, 80.8; H, 11.7.

5,6-Dihydrostigmasterol.—Ten grams of sodium was added in small pieces to a boiling solution of 2 g. of stigmasterol in 100 cc. of dry amyl alcohol. The solution was refluxed for thirty minutes after the sodium had dissolved. Then water was added, and the amyl alcohol layer was separated and distilled under reduced pressure. The residue was heated on a steam-bath for one hour with 5 g. of succinic anhydride and 10 cc. of pyridine. The mixture was cooled, diluted with ether, and washed with dilute hydrochloric acid, and then with sodium carbonate solution to remove the half-succinic ester. The sodium carbonate extract was acidified with hydrochloric acid, and extracted with ether. After distilling the ether, the residue was hydrolyzed by warming it with alcoholic potassium hydroxide solution. This solution was diluted with water, extracted with ether and the ethereal extract evaporated. The residue was crystallized from alcohol, to give 5,6-dihydrostigmasterol, m. p. 187°. It forms an insoluble digitonide.

The ether layer from the sodium carbonate extraction was distilled and the residue crystallized from alcohol. This substance, m. p. 72°, absorbed bromine, and was thought to be a hydrocarbon. However, no satisfactory analysis could be obtained.

A solution of 100 mg. of 5,6-dihydrostigmasterol in 10 cc. of acetic anhydride was refluxed for thirty minutes. After removing the excess acetic anhydride by distillation, the residue was crystallized from methanol and alcohol, giving 5,6-dihydrostigmasteryl acetate, m. p. 122°.

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.5; H, 11.5. Found: C, 81.6; H, 11.6.

This acetate gave a dibromide when treated with bromine in acetic acid.

Catalytic Hydrogenation of 5,6-Dihydrostigmasteryl Acetate.—A solution of 100 mg. of 5,6-dihydrostigmasteryl acetate in 25 cc. of acetic acid was shaken with 100 mg. of platinum oxide catalyst in a hydrogen atmosphere at 45 lb. (3 atm.) pressure. The catalyst was filtered and the acetic acid distilled under reduced pressure. The residue was crystallized from methanol and alcohol to give sitostyl acetate (stigmastyl acetate), m. p. 136°. This substance gave no depression in melting point when mixed with authentic stigmastyl acetate obtained by the catalytic reduction of stigmasteryl acetate.

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.

STATE COLLEGE, PENNA. RECEIVED OCTOBER 30, 1937

[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 Cloth, *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