[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH, DIVISION OF ORGANIC CHEMISTRY]

The Elimination of Hydrogen Bromide from Stigmasterol 22,23-Dibromide

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Under certain conditions the tetrabromides of stigmasteryl acetate[1] and brassicasteryl acetate[2] can be converted into dibromides containing the halogen in the side-chain on carbon atoms 22 and 23. In view of the fact that sterols with more than one double bond in the side-chain are not known, it seemed of interest to prepare such compounds from the new dibromides by the elimination of hydrogen bromide. The bromine atoms in the 22,23-dibromides of stigmasterol and brassicasterol are firmly bound and resist boiling methyl alcoholic alkali for several hours. While short boiling of stigmasteryl acetate 22,23-dibromide with 20% alcoholic potassium hydroxide removed only the acetyl group, prolonged boiling resulted in the formation of a mixture of halogen-free sterols from which only stigmasterol could be obtained in sufficiently pure form to assure its identity. This reducing action of alcoholic potassium hydroxide on the dibromide is somewhat analogous to its action on neopentyl iodide.[3]

Somewhat to our surprise the only product isolated in pure form from the action of boiling pyridine (116°) or quinoline (234°) on the dibromide was stigmasteryl acetate.

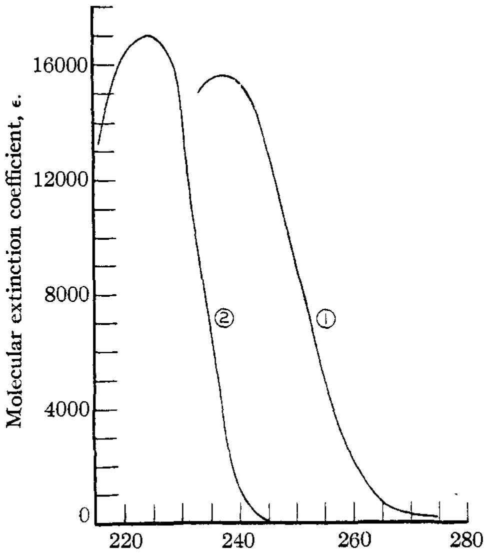

Fig. 1.—(1) Acetate of stigmastatrienol in alcohol, N. H. Coy; (2) 2,3-dimethylbutadiene in hexane, "I. C. T.," Vol. V, p. 364.

When the dibromide was refluxed in 2-ethylhexanol (b. p. 183°) in the presence of an excess of anhydrous potassium acetate the sole reaction product isolated was the acetate of a triene, m. p. 128–129°, which on hydrolysis yielded the triene alcohol, m. p. 125–126°. The new compound reacted with 3 moles of hydrogen yielding a saturated sterol which was identical with stigmastanol in melting point and rotation and gave an acetate and *m*-dinitrobenzoate with correct constants. This indicates that no rearrangement has occurred in the carbon skeleton of the side-chain.

The presence of a conjugated system of double bonds is indicated by the characteristic ultraviolet absorption spectrum[4] with a λ max. = 237.5 mμ, illustrated in Fig. 1. The shifting of the absorption maxima toward the longer wave length with the increase in the number of alkyl substituents, especially on the 1 and 4 position of a conjugated system, is illustrated by this compound. Butadiene has a λ max. = 217 mμ and, 2,3-dimethylbutadiene (see Fig. 1) has λ max. = 225.[5] The compound reacted with maleic anhydride, but resisted hydrogenation with sodium and alcohol.

In order to obtain information regarding the location of the two double bonds the substance was ozonized, and the volatile carbonyl compounds thus obtained transformed into *p*-nitrophenylhydrazones. By means of chromatographic adsorption the hydrazone of acetaldehyde was obtained in pure form, indicating that the ethyl group of the side-chain carries one of the double bonds.

Having fixed the position of one of the double bonds in the conjugated system the other must occupy the position between 22 and 23 or 25 and 26. The latter position seems most improbable since it would involve a shift of double bonds along four carbon atoms. Further, it is unlikely

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[1] E. Fernholz and H. E. Stavely, THIS JOURNAL, **61**, 2956 (1939).

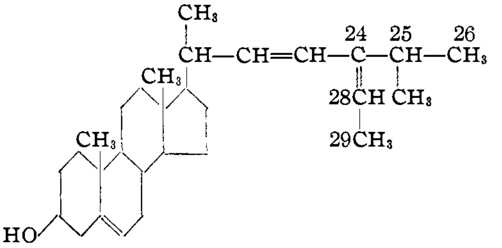
[2] E. Fernholz and H. E. Stavely, *ibid.*, **62**, 428 (1940).

[3] F. C. Whitmore, E. L. Wittle and A. H. Popkin, *ibid.*, **61**, 1586 (1939).

[4] For the measurement of the absorption spectrum we are indebted to Dr. N. H. Coy of the Biological Laboratories of E. R. Squibb and Sons.

[5] Dimroth, *Angew. Chem.*, **52**, 545 (1939).

that formaldehyde *p*-nitrophenylhydrazone would remain undetected in the products from ozonolysis. The new stigmastatrienol is thus Δ-5,22,24:28-stigmastatrienol-(3) shown in formula I.



I

The compound autoxidized very readily, and showed peroxide reactions after short exposure to air. A sample took up 14% of a mole of oxygen within eighteen hours. The substance was definitely more sensitive to oxygen than ergosterol which has a conjugation in the ring system.

Experimental

Reactions of Stigmasteryl Acetate 22,23-Dibromide

(a) **Alcoholic Potassium Hydroxide.**—One gram of the acetoxydibromide was refluxed with 50 cc. of 20% ethyl alcoholic potassium hydroxide for twenty-one hours. Water was added and the mixture extracted with ether. The ether residue was crystallized from alcohol. A yield of 0.6 g. of material was obtained melting at 140–148°. Repeated crystallizations raised the melting point to 162°. This material was acetylated by means of acetic anhydride in the presence of pyridine at room temperature. The product melted at 139° and gave no depression with stigmasteryl acetate (141°). Also the rotation $[\alpha]_D$ −56° agreed with that of stigmasteryl acetate, and on bromination a tetrabromide of m. p. 205° was obtained. *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.09. Found: C, 81.95; H, 10.93.

(b) **Pyridine.**—Three grams of stigmasteryl acetate 22,23-dibromide were refluxed with 20 cc. of dry pyridine for seven hours. After addition of water the mixture was extracted with ether, the ether washed with dilute acid, then with water, dried, and evaporated. The residue was recrystallized repeatedly from ethanol, and 1.5 g. of stigmasteryl acetate (m. p. 139°, $[\alpha]_D$ −54° in chloroform) was obtained; 1.0 g. of this acetate was dissolved in 15 cc. of ether and 25 cc. of 5% bromine in acetic acid was added. After two hours the precipitate was filtered and washed with ether; 1.2 g. of the characteristic stigmasteryl acetate tetrabromide of m. p. 205° was thus obtained.

A similar experiment in boiling quinoline (b. p. 234°) also yielded stigmasteryl acetate although in lower yield.

Preparation of the Acetate of Stigmastatrienol.—Fifty grams of fused potassium acetate was added to 550 cc. of 2-ethylhexanol, and 50 cc. of the alcohol was distilled off to remove traces of water; to the hot solution was rapidly added 50 g. of stigmasteryl acetate 22,23-dibromide and one gram of hydroquinone as anti-oxidant. The mixture was refluxed for one hour while a stream of dry carbon dioxide was passed in through a tube reaching the bottom of the flask. After cooling, water was added and the octyl alcohol layer washed several times with water. The solvent was then removed on the steam-bath in a stream of carbon dioxide in a good vacuum. The oily residue was dissolved in 75 cc. of hot *n*-butyl alcohol containing 0.1 g. of hydroquinone and the solution allowed to crystallize in the icebox. The solid was filtered off and washed with cold alcohol and recrystallized from a mixture of 50 cc. of butanol and 25 cc. of ethanol; yield 13.8 g., m. p. 123–125°. This material still contained traces of unchanged dibromide. This could be removed by sublimation in a high vacuum. The pure material forms shining leaflets from 95% alcohol, m. p. 128–129° (sealed capillary under CO_2), $[\alpha]^{24}_D$ −47° (26.0 mg. in 2 cc. chloroform solution, 1-dm. tube, α_D −0.61°).

Anal. Calcd. for $C_{31}H_{48}O_2$: C, 82.24; H, 10.69. Found: C, 81.74; H, 10.58. The carbon values are low because of rapid autoxidation.

Bromination of the Triene Acetate.—To a solution of 0.5 g. of the crude acetate m. p. 123–125° in 5 cc. of ether, 14 cc. of a 5% bromine solution in acetic acid was added. A small precipitate of stigmasteryl acetate tetrabromide (25 mg.) was obtained, largely due to the presence of some stigmasteryl acetate dibromide in the starting material. The acetate of the trienol does not form an insoluble bromide.

Reaction with Maleic Anhydride.—One gram of the acetate was refluxed with 0.5 g. of maleic anhydride in 10 cc. of dry toluene for ninety minutes. The solution was then evaporated to dryness, hydrolyzed with methyl alcoholic potassium hydroxide on the steam-bath, diluted with water and extracted with ether. Only 172 mg. of a neutral material was obtained probably from the oxidation of the substance before reaction with maleic anhydride. No attempt was made to purify the addition product.

Saponification.—To 0.618 g. of the triene acetate was added 15 cc. of 0.5 *N* KOH in 95% alcohol and this solution, as well as a blank, refluxed for one hour. The solutions were then titrated to determine the consumption of alkali. Calcd. for 1 acetyl: 1.35 cc. *N* KOH; found: 1.34 cc. The sterol was isolated by means of ether, crystallized from alcohol, and obtained in the form of leaflets, m. p. 125–126° (sealed capillary under CO_2).

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 84.33; H, 11.62.

Reduction with Sodium and Alcohol.—The trienol (3.5 g.) was dissolved in absolute alcohol (350 cc.) and heated with a flame. In the course of two hours 35 g. of sodium was added in portions. The hot solution was then poured into water and the sterol extracted with ether. It consisted largely of starting material of which 80% could be recovered. An examination of the mother liquors failed to disclose the presence of a dihydro derivative, particularly stigmasterol.

Catalytic Hydrogenation.—The acetate of stigmastatrienol (2.08 g.) was suspended in acetic acid (50 cc.) and hydrogenated with platinum oxide catalyst. An amount of hydrogen corresponding to three moles was taken up within a few hours. Shaking was continued for twenty-four hours and the hydrogenation product isolated. It gave a faint Liebermann reaction and was therefore dissolved in 50 cc. of chloroform, 40 cc. of acetic anhydride, cooled and treated with 15 cc. of concentrated sulfuric acid. The acetate was then recrystallized from alcohol and melted at 130–131°, $[\alpha]^{24}\mathrm{D}$ +17°. These are also the constants for stigmastyl acetate. The acetate was saponified and converted into the 3,5-dinitrobenzoate, m. p. 213–214°, $[\alpha]^{24}\mathrm{D}$ +15°. This derivative yielded after saponification stigmastanol, m. p. 135–136°, $[\alpha]^{24}\mathrm{D}$ +24°.

Ozonization.—Two grams of the acetate of stigmastatrienol suspended in 20 cc. of acetic acid was ozonized for one hour; 100 cc. of water was then added and the mixture distilled through a column. The receiver contained *p*-nitrophenylhydrazine in 50% acetic acid. The distillation was discontinued when about 20 cc. had come over. The yield of crude hydrazone was 341 mg. On digestion with benzene it left undissolved 81 mg. of an unknown orange-yellow compound, m. p. 204°. The benzene soluble fraction was chromatographed on aluminum oxide and developed with benzene. The lower, orange layer was eluted with ether, giving a crude hydrazone of m. p. 118–123°. This was recrystallized from hot water and also from benzene–petroleum ether and melted then at 128–129°. Its identity with acetaldehyde *p*-nitrophenylhydrazone was shown by a nitrogen determination and mixed melting point determination. *Anal.* Calcd. for $C_8H_9O_2N_3$: N, 23.47. Found: N, 23.65.

Summary

Stigmasteryl acetate 22,23-dibromide yields stigmasterol or its acetate when treated with alcoholic potassium hydroxide, pyridine or quinoline. With potassium acetate in a high boiling alcohol a new stigmastatrienol acetate is obtained. Observations are reported indicating that the new compound is Δ-5,23,24:28-stigmastatrienol.

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[Contribution from the William H. Chandler Chemistry Laboratory of Lehigh University]

The Saponification of Ethyl Esters of Aliphatic Acids

By H. S. Levenson and Hilton A. Smith

In a previous paper it was demonstrated that the effect of chain length on the velocity of acid catalyzed esterification of normal acids was quite similar to its effect on the velocity of saponification of the ethyl esters of the same acids.[1] The purpose of the present paper is to supplement the work on the saponification of aliphatic esters which is already available in the literature[2] so that a complete comparison of the effect of an aliphatic radical on the velocity of saponification of ethyl esters may be made with the recently published material on the catalyzed esterification of their corresponding acids.[3]

Experimental

Ethyl laurate was obtained from Eastman Kodak Co. The ester was fractionated under reduced pressure in a 5 ft. (1.5 meter) Vigreux column, and constant-distilling fractions were used.

Ethyl β-methylvalerate and ethyl isocaproate were prepared by esterification of the corresponding acids[4] with ethanol using sulfuric acid as a catalyst. The preparation of these acids has already been described.[3,5] The esters were purified by fractionation in a small 2-foot (0.6 meter) Vigreux column.

The ethyl esters of diethylacetic acid, dipropylacetic acid, dibutylacetic acid and di-isobutylacetic acid were prepared by esterification of the acids. Diethylacetic acid was obtained from Eastman Kodak Co. and the other acids were prepared by malonic ester syntheses.[3] The diethyl and di-isobutylacetic acid esters were purified by fractionation in a 5 foot (1.5 meter) Vigreux column, while the 2-foot column was used for purification of the other two.

Each ester was analyzed by complete saponification of weighed samples with excess alkali, and back titration with standard acid. All of the esters gave analyses between 99.5 and 100.5%, the precision of this method being about 0.5%.

The velocity determinations were carried out in 85% aqueous ethanol, this medium being prepared in the manner already described.[1] The method of making velocity determinations was similar to that previously employed.

Experimental Calculations and Results

In general, the rate constants were calculated using the second order reaction rate equation

$$k = x/t(a - x)a \qquad (1)$$

where a is the initial concentration of each reactant, and x represents the concentration of each reactant at the time t. The results of a typical run are given in Table I.

The runs with the esters of diethyl, dipropyl, and dibutyl acetic acids were so slow that, at higher temperatures, the action of alcoholic sodium hydroxide on the Pyrex glass reaction flasks was appreciable. This was indicated by an

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[1] Smith and Levenson, This Journal, **61**, 1172 (1939).

[2] Evans, Gordon and Watson, *J. Chem. Soc.*, 1439 (1938).

[3] Smith, This Journal, **62**, 1136 (1940).

[4] The authors are greatly indebted to Mr. A. S. Raff for his aid in the preparation of these acids.

[5] Smith, This Journal, **61**, 1176 (1939).