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Stigmasterol 22,23-Dibromide

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Stigmasterol possesses two double bonds, one in the ring system (5,6), the other in the side-chain (22,23). These differ in reactivity, and it is possible to add preferentially various reagents to one or the other. Bromine,[1] hydrogen bromide,[2] hydrogen chloride,[2] and perbenzoic acid[3] first react with the nuclear double bond, while catalytic hydrogenation leads primarily to the saturation of the side-chain.[4]

We have now succeeded in preparing a dibromide of stigmasterol in which the bromine is situated in the side-chain. Its acetate is obtained by removal of one mole of bromine from the tetrabromide with sodium iodide. Schoenheimer,[5] who first debrominated sterol bromides by this method, stated that the bromine atoms in the side-chain of stigmasterol tetrabromide do not react readily. However, it seems that no efforts were made to investigate the reaction products more thoroughly. The yield in which the new dibromide can be isolated depends to a great extent on the experimental conditions, and a comparatively small yield is obtained when the reaction is carried out in boiling solution as described in the literature.[5] The best yield (60%) is obtained by letting the reactants stand at room temperature for about twenty hours. It was not possible to replace sodium iodide by zinc dust, since even at room temperature in moist ether or in boiling alcohol the debromination did not stop at the dibromide but resulted in the formation of stigmasteryl acetate.

The new dibromide is remarkably stable and has a high melting point (212–213°). It resembles stigmasteryl acetate tetrabromide in this respect and is different from the 4,5-dibromide, which has a low melting point and cannot be recrystallized without decomposition. Although there was little doubt that we were dealing with the 22,23-dibromide and not a 5,6-dibromide isomeric with the one obtained by addition of one mole of bromine to stigmasteryl acetate, we made experiments to prove our supposition.

By saponification with methyl alcoholic potassium hydroxide stigmasterol 22,23-dibromide, m. p. 210°, was obtained without difficulties. The halogen in the side-chain was not noticeably attacked. This compound could be dehydrogenated by Oppenauer's method[6] and a ketone was obtained, m. p. 182–184°, which had an absorption spectrum with a maximum at 240 mμ (log E [molar] 4.08 in absolute alcohol)[7] characteristic for α,β-unsaturated ketones. This proves conclusively the anticipated structure of the new dibromide.

Treatment of the above ketone with zinc dust and acetic acid yielded a ketone of m. p. 125°, stigmastadienone. Under the name stigmastenone Marker and Wittle[8] have described a compound prepared from stigmasterol by catalytic dehydrogenation with copper. This compound correctly should be called stigmastadienone and should be identical with our compound; it melts, however, at 94° which is much lower. The preparation of stigmastadienone from stigmasterol by Oppenauer's method has been described in the patent literature[9] where a melting point of 107° has been recorded. We have prepared stigmastadienone by this method and the substance obtained had a constant melting point of 125°, and was identical with the stigmastadienone prepared from the dibromide.

Experimental

Stigmasteryl Acetate 22,23-Dibromide.—To 5 g. of stigmasteryl acetate tetrabromide in 75 cc. of benzene was added 3 g. of sodium iodide in 25 cc. of ethanol, and the mixture allowed to stand at room temperature for twenty hours. The mixture was then shaken with sodium sulfite to remove free iodine, washed with water, dried over sodium sulfate, concentrated, and filtered. The precipitate was crystallized from benzene–ethanol. The yield of pure acetoxy dibromide was 60%; fine needles, m. p. 212–213°, $[\alpha]^{25}D$ 30 (15.3 mg. in 2.0 cc. chloroform, 1-dm. tube, α_D −0.23). Mixed m. p. with stigmasteryl acetate tetrabromide showed a depression of 20°.

Anal.[10] Calcd. for $C_{31}H_{50}O_2Br_2$: C, 60.58; H, 8.21; Br, 26.01. Found: C, 60.73; H, 8.25; Br, 25.91, 26.06.

(1) E. Fernholz, *Ann.*, **507**, 128 (1933).
(2) L. Ruzicka, W. Fischer and J. Meyer, *Helv. Chim. Acta*, **18**, 1486 (1935).
(3) E. Fernholz, *Ann.*, **508**, 215 (1934).
(4) S. Bernstein and E. S. Wallis, *J. Org. Chem.*, **2**, 341 (1937).
(5) *J. Biol. Chem.*, **110**, 461 (1935).
(6) Oppenauer, *Rec. trav. chim.*, **56**, 141 (1937).
(7) This measurement was carried out by Dr. N. H. Coy of the Biological Laboratories of E. R. Squibb and Sons.
(8) Marker and Wittle, THIS JOURNAL, **59**, 2704 (1937).
(9) *C. A.*, **32**, 4174[3] (1938).
(10) The micro-analyses reported in this paper were carried out by Mr. J. F. Alicino, Fordham University.

Two hundred milligrams of the product was debrominated with zinc dust and acetic acid. The bromine-free product had a m. p. of 138–139°; mixed m. p. with stigmasterol acetate gave no depression.

Runs on a larger scale gave poorer yields. A lower melting mixture was isolated from the mother liquor which was reconverted to stigmasterol tetrabromide and used over.

Stigmasterol 22,23-Dibromide.—One gram of stigmasteryl acetate 22,23-dibromide was refluxed with 5% potassium hydroxide in methanol for two hours. After addition of water it was extracted with ether, the ether washed, dried over sodium sulfate, evaporated to dryness and crystallized twice from benzene–ethanol; needles, m. p. 209–210°. Mixed melting point with the starting material gave a depression of 25°.

Anal. Calcd. for $C_{29}H_{48}OBr_2$: Br, 27.95. Found: Br, 27.65.

Stigmastadienone 22,23-Dibromide.—Three and six-tenths grams of stigmasterol 22,23-dibromide, 5 g. of aluminum *t*-butylate, 150 cc. of dry benzene, and 40 cc. of dry acetone were refluxed together overnight. The mixture was washed with dilute sulfuric acid, then with water until neutral, dried over sodium sulfate and evaporated to dryness in a vacuum. The residue was crystallized from acetone, giving 2.6 g. of material, m. p. 173–178°. After recrystallizing many times the m. p. became constant at 182–184°. $[\alpha]^{22}D$ +53° [18.8 mg. in 2.0 cc. chloroform, 1-dm. tube, αD +0.50°].

Anal. Calcd. for $C_{29}H_{46}OBr_2$: C, 61.05; H, 8.13. Found: C, 60.78, 60.87; H, 8.18, 8.11.

Stigmastadienone.—Two hundred milligrams of stigmastadienone 22,23-dibromide was heated on the steam-bath with 250 mg. of zinc dust and 10 cc. of glacial acetic acid for one hour, decanted from the zinc, water added and extracted with ether. The ether was washed with dilute sodium carbonate solution, then with water, dried over sodium sulfate, and evaporated to dryness. The residue was crystallized from ethanol.

The product had a melting range of 80–110° and was probably partially reduced. The semicarbazone was prepared by heating with semicarbazide hydrochloride and potassium acetate in boiling ethanol. It was recrystallized twice from chloroform–ethanol; crystals turned brown at 227°, m. p. 235–237°. The semicarbazone was decomposed by boiling with 3 cc. of 5 *N* sulfuric acid and 6 cc. of ethanol for two hours. Water was added and the precipitate filtered and crystallized from ethanol. The product weighed 45 mg.; narrow leaflets, m. p. 123–124°. After two more crystallizations from ethanol and decolorizing with norit the m. p. was constant at 124–125°.

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 84.80, 84.66; H, 11.24, 11.34.

Stigmastadienone from Stigmasterol.—A mixture of 5 g. of stigmasterol, 6 g. of aluminum isopropylate, 50 cc. of cyclohexanone and 150 cc. of toluene was refluxed for two hours and then poured into cold water. Concentrated hydrochloric acid was added and the aqueous layer discarded. The organic solvents were removed by steam distillation. The crude stigmastadienone was purified by crystallizing from ethanol; narrow leaflets, m. p. 125°, $[\alpha]^{25}D$ +63° (27.7 mg. in 2.0 cc. chloroform, 1-dm. tube, αD +0.87°).

Anal. Calcd. for $C_{29}H_{46}O$: C, 84.81; H, 11.29. Found: C, 84.78; H, 11.08.

A mixed m. p. with stigmastadienone prepared from the dibromide gave no depression.

Summary

Stigmasteryl acetate 22,23-dibromide has been prepared by the removal of one mole of bromine from stigmasteryl acetate tetrabromide.

The position of the bromine atoms has been established by conversion into a α,β-unsaturated ketone, stigmastadienone 22,23-dibromide.

The preparation of stigmastadienone from this dibromide and from stigmasterol has been described.

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