[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

STUDIES ON THE DEGRADATION OF THE SIDE CHAIN OF STIGMASTEROL[1]

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The degradation of the side chain of stigmasterol (I) was first accomplished by Fernholz (1) who found that the acetate could be selectively brominated with one mole of bromine to give the 5,6-dibromo compound (II). Ozonization of this compound followed by treatment with zinc dust and glacial acetic acid gave 3-acetoxy-5-bisnorcholenic acid (III) in about 20% yield. This latter compound served as the starting material in the partial synthesis of the female hormone, progesterone. More recent work by Heyl, *et al.* (2) has shown that under proper conditions this same acetoxy dibromide can be ozonized to give the corresponding aldehyde.

In this paper we wish to report on some experiments we have carried out by means of which greatly increased yields of III can be obtained. Some years ago it was shown in this laboratory (3) that stigmasteryl benzoate can be selectively chlorinated in the 5,6-position with iodobenzene dichloride to give two isomeric 5,6-dichlorostigmastan-3β-yl benzoates. It was, therefore, considered appropriate to investigate the behavior of such dichlorides upon ozonization. Consequently, in a similar manner, the corresponding 5,6-dichloroacetates (IV) were prepared, and, for characterization, were separated by fractional crystallization. Two isomeric products were obtained of melting points 202.5–204.5° and 144–146°, respectively. Ozonization of the unseparated isomeric mixture gave 3-acetoxy-5-bisnorcholenic acid (III) in 77% yield. Similar experiments[3] were also carried out on each isomer separately in order to determine whether configurational relationships play any significant role in the ozonization process. No essential difference in yields of III obtained from each isomer was observed.

Other methods of nuclear inactivation have also been studied and we are reporting on them at this time. Riegel, *et al.* (4) have reported that the double bond and the hydroxyl group of stigmasterol can be protected by conversion to the *i*-methyl ether (V) and can be converted by ozonization into an impure 6-methoxy-*i*-bisnorcholenic acid (VI) in a 62% yield. We thought it would be of interest if a similar ozonization were carried out on *epi-i*-stigmasterol (XI), a compound not yet reported in the literature. A paper from this laboratory (5) describing the preparation of *epi-i*-cholesterol by the action of lithium aluminum hydride on *i*-cholestan-6-one suggested the preparation of this highly interesting compound. For this purpose, stigmasteryl chloride (VII) was converted to 6-

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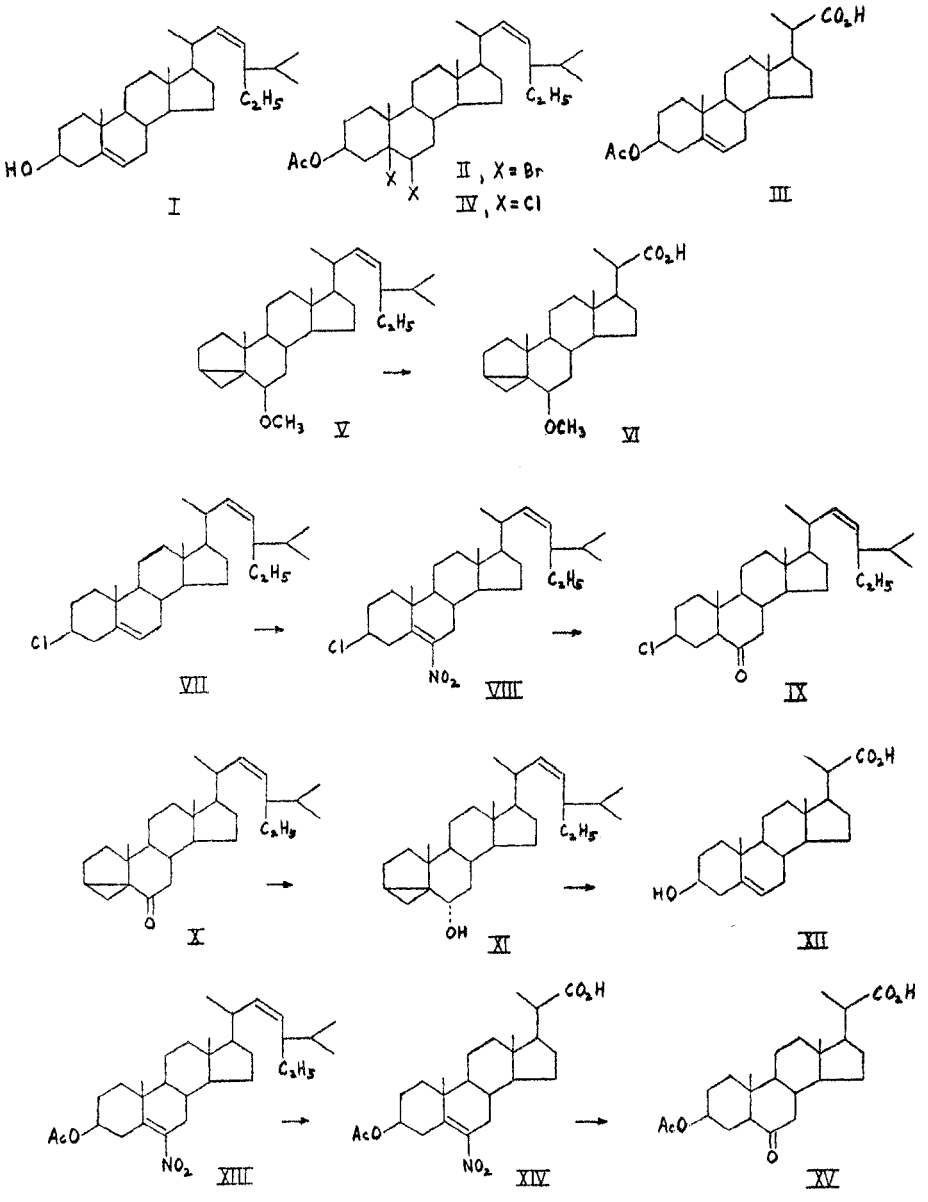

nitrostigmasteryl chloride (VIII) by the action of fuming nitric acid. Reduction with zinc dust and acetic acid yielded 3-chlorostigmasten-22-one-6 (IX) in 62% yield. On refluxing IX with alcoholic potassium hydroxide, a 90% yield of *i*-stigmasten-22-one-6 (X) was obtained. Reduction of X with lithum aluminum hydride gave *epi-i*-stigmasterol (XI) in essentially a quantitative yield. This latter compound was ozonized in chloroform solution and the ozonide was treated with acetic acid. No attempt was made to isolate the intermediate *epi-i*-bisnorcholenic acid, the product being directly rearranged to the normal structure

with sulfuric acid in glacial acetic acid. A 75% yield of crude 3-acetoxy-5-bisnorcholenic acid (III) was obtained. Hydrolysis of this product produced 3-hydroxy-5-bisnorcholenic acid (XII) identical with an authentic specimen prepared by the method of Fernholz (1).

Finally, we have found that the nuclear double bond can be made inactive toward ozonization by direct nitration. A sample of 6-nitrostigmasteryl acetate (XIII) (6) was prepared and submitted to the action of ozone. A 64.5% yield of 3-acetoxy-6-nitro-5-bisnorcholenic acid (XIV) was obtained. For the preparation of 3-acetoxy-6-ketobisnorcholanic acid (XV), which is obtainable by the reduction of XIV with zinc dust in acetic acid solution, it was found that this compound could be obtained directly from XIII without isolation of XIV as an intermediate. The crystalline 3-acetoxy-6-ketobisnorcholanic acid was so prepared in a yield of 40%.

EXPERIMENTAL

Preparation of the isomeric 5,6-dichlorostigmastan-3β-yl acetates (IV). To one liter of dry chloroform were added 50.0 g. (0.110 mole) of stigmasteryl acetate and 30.2 g. (0.110 mole) of iodobenzene dichloride (7). The solution was kept at 40–45° for 30 minutes. The temperature was then raised to the boiling point to remove the chloroform. The dark residue was poured into 300 ml. of ethanol, and another portion of 250 ml. was added. The white precipitate which formed immediately was filtered and washed with 100 ml. of ethanol. On further addition of 450 ml. of ethanol to the mother liquor, a second crop of crystals was obtained. After this was filtered and washed with 100 ml. of ethanol, additional solid material was isolated by allowing the mixture to stand after careful addition of 100 ml. of water. Further attempts to procure more product yielded only a reddish-brown oil. Thus a total of 49.1 g. (85%) of crystalline solid was isolated.

The least-soluble fraction was recrystallized several times from ethanol-benzene to yield 5α,6α-dichlorostigmastan-3β-yl acetate,[4] m.p. 202.5–204.5°, $[\alpha]_D^{25}$ −30.3° (20.6 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{31}H_{50}Cl_2O_2$: C, 70.83; H, 9.59.
Found: C, 70.60; H, 9.46.

The more soluble fractions were recrystallized from acetone-water to give 5α,6β-dichlorostigman-3β-yl acetate, m.p. 144–146°, $[\alpha]_D^{20}$ −38.6° (19.7 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{31}H_{50}Cl_2O_2$: C, 70.83; H, 9.59.
Found: C, 70.74; H, 9.54.

Ozonization of the isomeric 5,6-dichlorostigmastan-3β-yl acetates. An ice-cold solution of 10.5 g. (20 mmol.) of the mixed isomeric 5,6-dichlorostigmastan-3β-yl acetates in 150 ml. of chloroform was ozonized for a period of 45 minutes; 35.1 mmoles of ozone were absorbed. After this time, 150 ml. of glacial acetic acid was added, together with 4 g. of zinc dust. The mixture was then heated on the water-bath for one hour. After filtration, the solution was diluted with water and extracted with ether. The ethereal solution was then treated with 2 *N* sodium hydroxide and the voluminous, gelatinous precipitate which formed between the layers was isolated. The free 3-acetoxy-5-bisnorcholenic acid (III) was liberated from its sodium salt with 2 *N* sulfuric acid. This was extracted with ether and from the ether solution, 5.95 g. (77%) of the acid was obtained. One crystallization from acetone gave a product which melted at 232–237° (dec.). The melting point of this compound as reported by Fernholz (1) is 235° (unsharp with decomposition).

Similar experiments[3] were carried out on each of the two isomeric dichloroacetates

[4] The indicated configurations are based on the analogous cholesterol derivatives (8).

described above, in order to determine whether the configuration of the two chlorine atoms in the 5,6-position plays any significant role in the ozonization process. No significant difference in the yield of the 3-acetoxy-5-bisnorcholenic acid (III) obtained from each isomer was observed.

Preparation of 6-nitrostigmasteryl chloride (VIII). The stigmasteryl chloride (VII) used in this experiment was prepared from stigmasterol by its reaction with pure thionyl chloride. This method in our hands was found to be much more preferable to that of either Windaus and Hauth (9) or Marker and Lawson (10) who used phosphorus pentachloride. Essentially a quantitative yield of product of m.p. 95–96.5° was obtained, $[\alpha]_D^{20}$ −44.1° (19.9 mg. in 1.95 ml. of chloroform; 1-dm. tube).

To 9.0 g. of stigmasteryl chloride (VII) suspended in 60 ml. of glacial acetic acid was rapidly added 15 ml. of fuming nitric acid (*sp. gr.* 1.49). The mixture was stirred vigorously for 24 hours at 20–25° and then poured into cold water. The solid was filtered and recrystallized from methanol and from acetic acid. Yield 7.4 g. (75%), m.p. 131–133°, $[\alpha]_D^{20}$ −47.8° (20.0 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{29}H_{46}ClNO_2$: C, 73.15; H, 9.74.

Found: C, 72.90; H, 9.84.

Preparation of 3-chlorostigmasten-22-one-6 (IX). A solution of 20.0 g. of 6-nitrostigmasteryl chloride (VIII) in 400 ml. of glacial acetic acid and 25 ml. of water was refluxed with stirring for four hours. During this time 40 g. of zinc dust was added in small portions. The hot solution was filtered, cooled, and one liter of water was added. The oil so formed was taken up in ether. Concentration of the ethereal solution gave a crystalline material. A recrystallization from methanol gave 11.6 g. (62%) of product which melted at 103–104.5°, $[\alpha]_D^{20}$ −8.7° (20.2 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{29}H_{47}ClO$: C, 77.90; H, 10.56.

Found: C, 78.20; H, 10.63.

Preparation of i-stigmasten-22-one-6 (X). A solution of 1.0 g. of 3-chlorostigmasten-22-one-6 (IX) in 100 ml. of 5% ethanolic potassium hydroxide was refluxed for one hour. The solution was poured into 300 ml. of water, and the solid was filtered and recrystallized from methanol. Yield 0.85 g. (93%), m.p. 108–109°, $[\alpha]_D^{20}$ +27.0° (15.2 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{29}H_{46}O$: C, 84.81; H, 11.29.

Found: C, 84.95; H, 11.19.

Preparation of epi-i-stigmasterol (XI). To 4.0 g. of *i*-stigmasten-22-one-6 (X) in 10.0 ml. of anhydrous ether was added 50 ml. of a 0.15 *molar* ethereal solution of lithium aluminum hydride. After standing overnight, the excess hydride was destroyed. The mixture was treated with 10% aqueous potassium hydroxide solution. The alkaline solution was then washed with ether. The combined dried ether solution was evaporated and the residue was taken up in ethanol from which the solid product was obtained. Yield 3.72 g. (93%), m.p. of crystals from ethanol 52–53°, $[\alpha]_D^{25}$ +54.4° (20.0 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{29}H_{48}O$: C, 84.40; H, 11.72.

Found: C, 84.00; H, 11.46.

A portion of this product (100 mg.) was dissolved in 10 ml. of glacial acetic acid at room temperature and rearranged in acid medium in the usual manner. Stigmasteryl acetate in quantitative yield was obtained.

Ozonization of epi-i-stigmasterol. An ice cold solution of 3.72 g. (9.01 mmoles) of *epi-i*-stigmasterol (XI) in 125 ml. of chloroform was treated with ozone for a period of ten minutes and 9.4 mmoles of ozone were absorbed. On working up the product, 2.73 g. (75%) of oily yellow-white solid was obtained which could not be satisfactorily recrystallized. However, on saponification with 5% ethanolic potassium hydroxide followed by acidification with hydrochloric acid, a crystalline material was isolated which, after recrystallization from acetic acid, melted at 282–291° (dec.), and did not depress the melting point of an authentic sample of 3-hydroxy-5-bisnorcholenic acid (XII) of m.p. 291–295° (dec.).

Preparation of 3-acetoxy-5-nitro-5-bisnorcholenic acid (XIV). This compound was prepared from 6-nitrostigmasteryl acetate (XIII) of m.p. 125–127° (6). Ozonization of this material was carried out in a manner essentially the same as that described for *epi-i*-stigmasterol. A yield of 64.5% of the crude acid (XIV) was obtained. Recrystallization from dilute ethanol gave long, white needles of 3-acetoxy-6-nitro-5-bisnorcholenic acid (XIV), m.p. 193–194°, $[\alpha]_D^{25}$ −102.8° (20.7 mg. in 1.95 ml. of chloroform; 1-dm. tube).

Anal. Calc'd for $C_{24}H_{35}NO_6$: C, 66.48; H, 8.14.

Found: C, 66.80; H, 7.85.

Preparation of 3-acetoxy-6-ketobisnorcholanic acid (XV). This compound was prepared from 6-nitrostigmasteryl acetate (XIII) without isolation of the above intermediate compound XIV. An ice-cold solution of 0.53 g. (1.06 mmoles) of 6-nitrostigmasteryl acetate was dissolved in 20 ml. of chloroform and ozonized with 1.97 mmoles of ozone in 2.5 minutes. Then 8 ml. of glacial acetic acid and 1.0 g. of zinc dust were added. The mixture was warmed on the steam-bath for one hour. The chloroform was removed *in vacuo* at room temperature and the solution at reflux temperature was treated with 3 g. of zinc dust added in small portions during a period of three hours. On working up the product in the usual manner, 0.17 g. (40%) of 3-acetoxy-6-ketobisnorcholanic acid (XV) was isolated. The crystallized product from ethanol-water melted at 258–260° (dec.), $[\alpha]_D^{20}$ −14.6°. (20.1 mg. in 1.95 ml. of dioxane; 1-dm. tube).

Anal. Calc'd for $C_{24}H_{36}O_5$: C, 71.27; H, 8.97.

Found: C, 71.22; H, 9.12.

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SUMMARY

1. Ozonization of the side chain double bond of stigmasteryl acetate has been found to give an increased yield of 3-acetoxy-5-bisnorcholenic acid if the nuclear double bond is blocked with chlorine, rather than with bromine, as is commonly used.

2. *Epi-i*-stigmasterol has been prepared and shown to be capable of being ozonized and rearranged to 3-acetoxy-5-bisnorcholenic acid.

3. Nitration has been shown to suitably deactivate the nuclear double bond of sigmasteryl acetate to permit ozonization of the side chain.

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