Syntheses of 23-Ketositosterol and Its Derivatives

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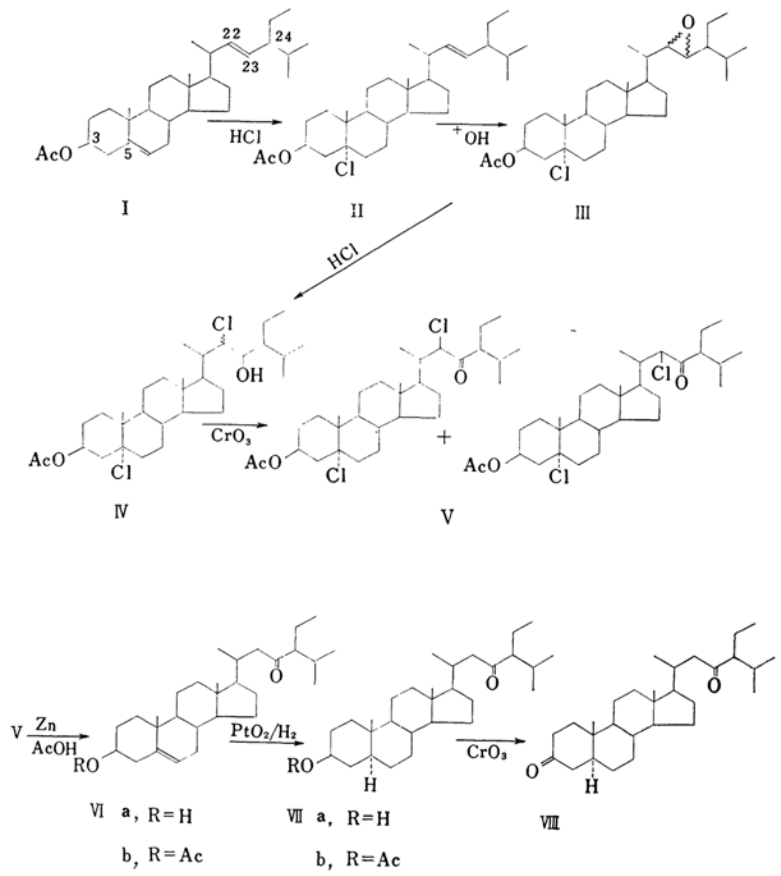
In connection with other studies, it was necessay to prepare sitosterol derivatives with oxygen functions at the C_{22} and C_{23} carbon atom, respectively. This article describes a method for the introduction of oxygen functions into the C_{23} carbon atom of sitosterol.

The peroxybenzoic acid oxidation of 3β-acetoxy-5α-chlorostigmast-22-ene (II)[1] proceeded stereospecifically and gave a single isomer of 5α-chloro-3β-acetoxy-22ξ, 23ξ-epoxystigmastane (III). Bladon et al.[2] have reported that the peroxybenzoic acid oxidation of 3β-acetoxy-5α, 8α-epidioxyergosta-6, 9 (11), 22-triene gives two isomers of 3β-acetoxy-22ξ, 23ξ-epoxy-5α-hydroxyergosta-7, 9(11)-diene. The difference is probably due to the alkyl side chains at C_{24}. The side chain at C_{24} is an ethyl group with an α-configuration[3] in the stigmastane series, whereas it is a methyl group with a β-configuration[4] in the ergostane series. The oxide ring of compound III probably has the α-configuration, since an examination of the



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1) E. M. Chamberlin, E. Tristram, T. Utne and J. M. Chemerda, *J. Am. Chem. Soc.*, **79**, 456 (1957).

2) P. Bladon, P. B. Clayton, C. W. Greenhalgh, H. B. Henbest, E. R. H. Jones, B. J. Lovell, G. Silverstone, G. W. Wood and G. F. Wood, *J. Chem. Soc.*, **1952**, 4883.

3) Y. Kishida, *Chem. Pharm. Bull.*, **8**, 357 (1960).

4) L. F. Fieser, "Steroids," Reinhold Publishing Co., New York (1959), p. 339.

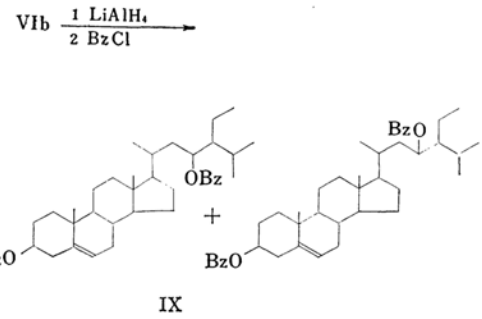
Dreiding model[5] seems to indicate that the α-side of the Δ^{22}-double bond of compound II is less hindered.

The treatment of compound III with hydrogen chloride in acetic acid gave a chlorohydrin mixture. The chromic acid oxidation of the chlorohydrin gave two isomeric chloroketones: isomer A [(m. p. 161~162°C, $[\alpha]_D$+43°, λ_{max} 305 mμ (ε, 57), and isomer B (m. p. 178~179°C $[\alpha]_D$+17°, λ_{max} 298 mμ (ε, 60)]. It is possible that one, after formed, was in part epimerized to the other. Both the isomers possess the 22-chloro-23-keto structure (V), as the following evidence shows. Both gave the same keto acetate on reduction with zinc dust and acetic acid. The hydrogenation of the keto acetate on an Adam platinum catalyst in an acidic medium, followed by an alkaline hydrolysis, gave a saturated keto alcohol. The chromic acid oxidation of the saturated keto alcohol gave a diketone (m. p. 159~160°C, $[\alpha]_D$+54.4°) which was different from the stigmastane-3, 22-dione (m. p. 176~177°C, $[\alpha]_D$+21.8°) obtained by Fischer and Mägerlein.[6] Therefore, the only possible structure for the diketone is VIII (stigmastane-3, 23-dione). When the crude oxidation product of the chlorohydrin (IV) was treated with zinc dust and acetic acid, the only isolable ketone was 3β-acetoxystigmast-5-en-23-one (VIb). The attack of the chloride ion upon the oxide III occurred exclusively at the C_{22} carbon atom.

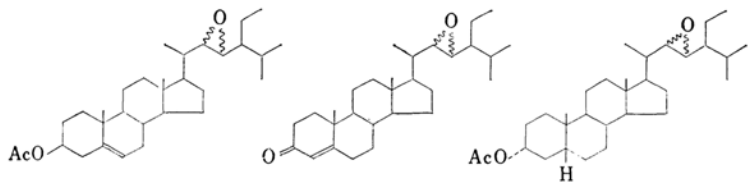
It is noteworthy that the prolonged hydrogenation of 3β-acetoxystigmast-5-en-23-one (VIb) on a platinum oxide catalyst in an acidic medium furnished saturated ketone VIIb in a good yield. No hydroxy compound was formed.[7] The treatment of 3β-acetoxystigmast-5-en-23-one (VIb) with hydroxylamine hydrochloride in pyridine[8] uner reflux for 30 hr., under the condition which give oximes from hindered ketones, gave the unchanged starting material. The attempted esterification of 3β-acetoxy-5α, 22ξ-dichlorostigmastan-23-one (V) by Baeyer-Villiger reaction (peroxybenzoic acid in chloroform for one month) also failed. These facts indicate that the C_{23}-keto group in a stigmastane series is sterically highly hindered.

The reduction of compound VIb with lithium aluminum hydride gave epimeric diols. Two isomers were separated as benzoates (IX) (major one: m. p. 130~130.5°C, $[\alpha]_D$ −9°; minor one: m. p. 160~162°C, $[\alpha]_D$ −31°). According to Cram's rule[9] for the steric control of asymmetric induction, the major isomer may have the α-configuration.

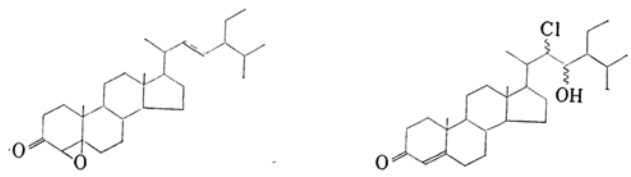
The treatment with an alkali and the reacetylation of compound III gave 3β-acetoxy-22ξ,



IX



X XI XII



XIII XIV

5) A. S. Dreiding, *Helv. Chim. Acta*, **42**, 1339 (1959).
6) F. G. Fischer and H. Mägerlein, *Ann.*, **636**, 88 (1960).
7) In this connection, it may be noted that the reduction of sargasteryl acetate (3β-acetoxy-20-isostigmasta-5, 24(28)-diene) with platinum oxide in ethyl acetate gave 5, 6-dihydrosteryl acetate. Cf. K. Tsuda, R. Hayatsu, Y. Kishida and S. Akagi, *J. Am. Chim. Soc.*, **80**, 921 (1958), footnote 8.
8) E. B. Hershberg, E. P. Oliveto and R. Rausser, *Chem. & Ind.*, **1958**, 1477.
9) D. J. Cram and F. A. A. Elhafez, *J. Am. Chem. Soc.*, **74**, 5828 (1952).

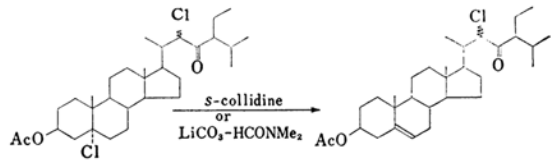
23ξ-epoxystigmast-5-ene (X). The peracid oxidation of stigmasta-4, 22-dien-3-one[10] and 3α-acetoxy-5β-stigmast-22-ene[11] gave the corresponding oxides, XI and XII respectively. The oxidation of stigmasta-4, 22-dien-3-one with alkaline hydrogen peroxide gave 4, 5-oxide XIII. The treatment of XI with hydrogen chloride gave a corresponding chlorohydrin XIV. The 22ξ-chloro-23ξ-hydroxy structure was assigned by analogy with a previous reaction.

The dehydrohalogenation of V was attempted as the means of obtaining $\Delta^{20(22)}$-23-ketone by treatment with *s*-collidine or lithium carbonate in dimethylformamide, but no such compound could be isolated, although the crude product showed a slight ultraviolet absorption around 230~240 mμ. The only isolable compound was epimeric 3β-acetoxy-22ξ-chlorostigmast-5-en-23-one (XV.)

The purity of the compounds described in this article was examined by thin-layer chromatography; the R_f values of some of the compounds are recorded in Table I.

TABLE I. THIN-LAYER CHROMATOGRAPHY OF STIGMASTANE DERIVATIVES (BENZENE, SILICA GEL G)

No	Compound	R_f
1	Stigmast-5-ene-3β, 23ξ-diol	0.02
2	3β-Hydroxy-stigmastan-23-one (VIIa)	0.03
3	3β-Hydroxy-stigmast-5-en-23-one (VIa)	0.03
4	Stigmast-4-ene-3, 23-dione	0.03
5	Cholesterol	0.07
6	Stigmasterol	0.07
7	Stigmastane-3, 23-dione (VIII)	0.19
8	3β-Acetoxystigmastan-23-one (VIIb)	0.29
9	3β-Acetoxystigmast-5-en-23-one (VIb)	0.30
10	3β-Acetoxy-5α-chloro-22ξ, 23ξ-epoxystigmastane (III)	0.30
11	3β-Acetoxy-22ξ, 23ξ-epoxystigmast-5-ene (X)	0.34
12	3β-Acetoxy-5α, 22ξ-dichlorostigmastan-23-one (V) (Isomer A, m. p. 161~162°C)	0.33
13	3β-Acetoxy-5α, 22ξ-dichlorostigmastan-23-one (V) (Isomer B, m. p. 178~179°C)	0.30
14	3β-Acetoxy-22ξ-chlorostigmast-5-en-23-one (XV) (Isomer A, m. p. 166.5~168°C)	0.37
15	3β-Acetoxy-22ξ-chlorostigmast-5-en-23-one (XV) (Isomer B, m. p. 177.5~180.5°C)	0.33
16	3β-Acetoxy-5α-chlorostigmast-22-ene (II)	0.48
17	Cholesteryl acetate	0.51
18	Stigmasteryl acetate (I)	0.51
19	3β, 23ξ-Dibenzoxystigmastane (IX) (Isomer A, m. p. 159~162°C)	0.62
20	3β, 23ξ-Dibenzoxystigmastane (IX) (Isomer B, m. p. 130~130.5°C)	0.67



V XVa, b

Experimental[12]

3β-Acetoxy-5α-chlorostigmast-22-ene (II).—This material was prepared essentially by the procedure previously described.[1] The presence of acetic acid (100 ml. for 100 g. of stigmasteryl acetate) increased the yield. The product was obtained as needles; m. p. 183~184°C, $[\alpha]_D^{19}$ −7° (*c* 0.997) (reported m. p. 183~185°C, $[\alpha]_D^{24}$ −8.1°)

3β-Acetoxy-5α-chloro-22ξ, 23ξ-epoxystigmastane (III).—To a cold solution of 18.4 g. of II in 184 ml. of chloroform was added 45 ml. of a chloroform solution of peroxybenzoic acid containing 1.3 molar equivalents of the peracid. The mixture was then allowed to react at 0~3°C for fifteen days. The excessive peracid was decomposed with 10% aqueous potassium iodide, and the liberated iodine was titrated with 0.1N sodium thiosulfate (1.05 mol. of peracid were consumed). The chloroform solution was washed with 10% aqueous potassium carbonate and water and dried over anhydrous sodium sulfate. The chloroform was expelled under reduced pressure (the inner temperature was kept below 50°C). Recrystallization from ethyl acetate gave 12.5 g. of needles; m. p. 137~138°C, $[\alpha]_D^{22}$ −15° (*c* 0.46).

Found: C, 73.49; H, 10.34; Cl, 7.34. Calcd. for $C_{31}H_{51}O_3Cl$: C, 73.41; H, 10.14; Cl, 6.99%.

3β-Acetoxy-22ξ, 23ξ-epoxystigmast-5-ene (X).—To a solution of 3.2 g. of III in 150 ml. of ethyl alcohol was added a solution of sodium carbonate (3.2 g.) in water (18 ml.); the mixture was then heated under reflux for 5 hr. the usual manipulation gave 2.9 g. of an oil which was acetylated by treating it with acetic anhydride (5 ml.) and pyridine (11 ml.) overnight at room temperature; 3 g. of an sticky oil was thus obtained. Chromatography and recrystallization form methanol gave crystals (m. p. 114.5~115°C, $[\alpha]_D^{17}$ −71° (*c* 0.47)).

Found: C, 79.09; H, 10.71. Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%.

22ξ, 23ξ-Epoxystigmast-4-en-3-one (XI).—Stigmasta-4, 22-dien-3-one (500 mg., m. p. 125~126°C) was dissolved in 2 ml. of chloroform containing 170 mg. of perbenzoic acid. The mixture was allowed to react at room temperature for three days in the

10) G. Slomp, Jr., Y. F. Shealy, J. L. Johnson, R. A. Donia, B. A. Johnson, R. P. Holysz, R. L. Pederson, A. O. Jensen and A. C. Ott, ibid., 77, 1216 (1955).
11) D. H. R. Barton and C. J. W. Brooks, ibid., 72, 1633 (1950).
12) All melting points were uncorrected. Rotations were measured in chloroform solution. Infrared spectra were determined with a Perkin-Elmer Model 21. Ultraviolet spectra were determined with a Hitachi Type EPU-2. We are indebted to Miss Sachiko Yoshikawa for measurements of rotations and some technical assistance and to Sankyo Co. for analyses.

dark. The usual manipulation gave 600 mg. of a cake which was chromatographed on 16 g. of alumina. Elution with a mixture of petroleum-ether and benzene (2 : 8) gave 200 mg. of the starting material. Further elution with chloroform gave 250 mg. of crystals (m. p. 117.5~118.5°C) after recrystallization from methanol. The infrared spectrum showed the absence of a Δ^{22}-double bond (970 cm^{-1}).

Found: C, 81.73; H, 10.83. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10. 86%.

4β, 5β-Epoxystigmast-22-en-3-one (XIII).—To a stirred solution of 500 mg. of stigmasta-4, 22-dien-3-one in 20 ml. of methanol, 1.2 ml. of 4 N sodium hydroxide and 3 ml. of 30% hydrogen peroxide were added at 0~3°C. The mixture was then left to stand for 48 hr., poured into water and extracted with ether. The evaporation of ether and the crystallization of the residue from methanol gave 350 mg. of crystals (m. p. 120~122°C). The infrared spectrum showed the presence of a Δ^{22}-double bond (970cm^{-1}). The β-configuration of the oxide was assumed, since cholest-4-en-3-one gave 4β, 5β-epoxycholestan-3-one when similarly treated.[13]

Found: C, 81.25; H, 11.32. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.86%.

3α-Acetoxy-22ξ, 23ξ-epoxy-5β-stigmastane (XII).—This material was prepared by a procedure similar to that described for compound III. Recrystallization from methanol gave crystals (m. p. 105~107°C).

Found: C, 78.32; H, 11.34. Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.08%.

5α-Chlorostigmast-22-en-3-one. — Two hundred milligrams of 5α-chlorostigmast-22-en-3β-ol [m. p. 153.5~155°C (from benzene-methanol)], prepared from stigmasterol and hydrochloric acid by a procedure similar to that employed in the preparation of 3β-acetoxy-5α-chlorostigmast-22-ene (II), was dissolved in 5 ml. of chloroform and treated with 100 mg. of chromic acid in a mixture of 0.5 ml. of water and 10 ml. of acetic acid at room temperature overnight. Crystallization of the product from benzene-methanol gave 100 mg. of crystals (m. p. 136~136.5°C, $[\alpha]_D^{18}$ −5° (*c* 0.41)).

Found: C, 77.78; H, 10.62; Cl, 7.75. Calcd. for $C_{29}H_{47}OCl$: C, 77.90; H, 10.60; Cl, 7.93%.

Semicarbazone; m. p. 216~218°C.

Found: C, 71.73; H, 9.54; Cl, 7.02; N, 8.35. Calcd. for $C_{30}H_{50}OClN_3$: C, 71.46; H, 9.99; Cl, 7.03; N, 8.33%.

3β-Acetoxy-5α, 22ξ-dichlorostigmastan-23ξ-ol (IV).—To a solution of 21.3 g. of 3β-acetoxy-5α-chloro-22ξ, 23ξ-epoxystigmastane (III) in 200 ml. of chloroform, 270 ml. of a 4% solution of hydrogen chloride in glacial acetic acid was added, and the mixture was allowed to stand for 24 hr. at room temperature. The reaction mixture was then poured into water and extracted with chloroform three times. The combined chloroform extract was washed with 5% aqueous potassium carbonate and water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give 23 g. of a yellow foam.

Crystallization from a 1 : 1 mixture of ethyl acetate and ethyl alcohol gave 10.0 g. of the first crop (m. p. 157~160°C) and 6.5 g. of the second crop (m. p. 152~156°C). The analytical sample was recrystallized from acetone; m. p. 162~164°C, $[\alpha]_D^{29}$ 0° (*c* 0.69).

Found: C, 68.71; H, 9.70; Cl, 12.73. Calcd. for $C_{31}H_{52}O_3Cl_2$: C, 68.48; H, 9.64; Cl, 13.04%.

22ξ-Chloro-23ξ-hydroxystigmast-4-en-3-one (XIV).—One gram of 22ξ, 23ξ-epoxystigmast-4-en-3-one (XI) was dissolved in 15 ml. of a 5% solution of hydrogen chloride in glacial acetic acid, and the mixture was allowed to react for 24 hr. at 0~5°C. The product was crystallized from methanol to 0.65 g. of crystals (m. p. 216~218°C).

Found: C, 75.51; H, 10.27; Cl, 7.86. Calcd. for $C_{29}H_{47}O_2Cl$: C, 75.20; H, 10.23; Cl, 7.65%.

3β-Acetoxy-22ξ-chlorostigmastan-23-one (V).—To a stirred solution of 11.5 g. of 3β-acetoxy-22ξ-chlorostigmastan-23ξ-ol (IV) in a mixture of 80 ml. of chloroform and 80 ml. of glacial acetic acid, a solution containing 6 g. of chromic acid in a mixture of 6 ml. of water and 90 ml. of acetic acid was added at 20~30°C over a 3-hr. period. The mixture was continuously stirred for a further twenty hours at room temperature. Excessive chromic acid was decomposed with 30 ml. of ethanol (stirred for 1 hr.), and the mixture was poured into water. The chloroform extracts were combined, washed with 5% aqueous sulfuric acid (three times) and water (three times), dried over anhydrous sodium sulfate, and concentrated. The residue was crystallized from acetone to 8.5 g. of crystals (m. p. 145~150°C). The crude material was used for the next reaction.

The fractional recrystallization of the crude crystals gave two compounds that were found to be epimeric isomers.

Isomer A: m. p. 161~162°C; $[\alpha]_D^{14}$ +43° (*c* 1.0).

Found: C, 69.13; H, 9.52; Cl, 12.85. Calcd. for $C_{31}H_{50}O_3Cl_2$: C, 68.74; H, 9.36; Cl, 13.09%.

UV $\lambda_{max}^{CHCl_3}$ 305 mμ (ε, 57); IR (KBr) 1732, 1714, 1247 cm^{-1}.

Isomer B: m. p. 178~179°C; $[\alpha]_D^{13}$ +17° (*c* 1.0).

Found: C, 68.77; H, 9.24; Cl, 13.16. Calcd. for $C_{31}H_{50}O_3Cl_2$: C, 68.74; H, 9.36; Cl 13.09%.

UV $\lambda_{max}^{CHCl_3}$ 298 mμ (ε, 60); IR (KBr) 1733, 1707, 1241 cm^{-1}.

3β-Acetoxy-22ξ-chlorostigmast-5-en-23-one (XV).—Five grams of crude V (m. p. 145~150°C) were dissolved in 60 ml. of *s*-collidine, and the mixture was heated under reflux for 24 hr. The precipitated collidine hydrochloride was then removed by filtration and washed with ether. The mother liquor was diluted with more ether and washed with 10% sulfuric acid and water. The evaporation of the ether gave 4.5 g. of a brown oil. Crystallization from ethyl alcohol gave 1.5 g. of needles (m. p. 160~163°C). Further recrystallization from methylenechloride-acetone gave isomer A, 166.5~168°C, $[\alpha]_D^{19}$ −31° (*c* 0.67).

Found: C, 73.33; H, 9.80; Cl, 7.49. Calcd. for $C_{31}H_{49}O_3Cl$: C, 73.70; H, 9.78; Cl, 7.02%.

UV $\lambda_{max}^{CHCl_3}$ 296 mμ (ε, 93), IR (Nujol) 1736, 1256, 802 cm^{-1}.

13) P. A. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1822 (1948).

The mother liquor of the first crystallization of the above experiment was concentrated to a brown oil, which was then chromatographed on 60 g. of florisil packed in petroleum ether. Elution with 1% acetone in petroleum ether and recrystallization from acetone gave 230 mg. of isomer B; m. p. 177.5~180.5°C; $[\alpha]_D^{19}$ −63° (*c* 0.67).

Found: C, 73.78; H, 9.78; Cl, 7.47. Calcd. for $C_{31}H_{49}O_3Cl$: C, 73.70; H, 9.78; Cl, 7.02%.

UV $\lambda_{max}^{CHCl_3}$ 297 mμ (ε, 65); IR (Nujol) 1729, 1251, 800 cm^{-1}.

Further elution gave no crystalline material.

3β-Acetoxystigmast-5-en-23-one (VIb). — Seven grams of crude V (m. p. 145~150°C) were dissolved in 140 ml. of glacial acetic acid, and 7 g. of zinc dust was added with swirling. The mixture was then heated under reflux for 3 hr. After cooling, excessive zinc dust was removed and washed with benzene. The mother liquor was diluted with water and extracted with benzene. The combined benzene extract was washed with a saturated solution of sodium bicarbonate in water, dried over anhydrous sodium sulfate, and concentrated. The residue (6.9 g.) was dissolved in a small amount of methylene chloride and the solution was put on 200 g. of a florisil column packed in petroleum ether (b. p. 35~70°C). Elution with petroleum ether (fractions Nos. 1 and 2) and recrystallization from benzene-methanol gave 189 mg. of aliphatic saturated hydrocarbon (paraffin), which was present in the stigmasterol extracted from soybean oil[14] as an impurity; m. p. 67~69°C; IR (KBr) 2590, 2870, 1475, 1470, 734, 723 cm^{-1}. (Found: C, 84.78; H, 14.67%.) The Liebermann-Burchard and Beilstein reactions were both negative. Futher elution with 1% ethyl acetate in petroleum ether (Nos. 19—24) and recrystallization from ethyl acetate gave 1.53 g. of an unidentified material; m. p. 136~138.5°C (sintering at 127°C). The infrared spectrum showed the presence of an acetate and the absence of a hydroxyl group and of a Δ^{22}-double bond and no ketone band other than that of an acetate (Found: C, 77.01; H, 10.33). Further elution with 1% and 2% ethyl acetate in petroleum ether (Nos. 44—107) and recrystallization from acetone gave 2.4 g. of 3β-acetoxystigmast-5-en-23-one (VIa); m. p. 153~154°C, $[\alpha]_D^{18}$ −16° (*c* 0.69).

Found: C, 79.22; H, 10.39. Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%.

UV $\lambda_{max}^{CHCl_3}$ 291 mμ (ε, 46); IR (KBr) 1733, 1709, 1249, 1037, 800 cm^{-1}.

3β-Hydroxystigmast-5-en-20-one (VIa). — The alkakine hydrolysis of VIb gave a free corresponding alcohol of VIb. Recrystallization from acetone gave crystals; m. p. 157~157.5°C, $[\alpha]_D^{18}$ −11° (*c* 0.62).

Found: C, 80.85; H, 11.39. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29%.

UV $\lambda_{max}^{CHCl_3}$ 291 mμ, (ε, 67); IR (KBr) 3465, 1716, 1700 (shoulder), 794 cm^{-1}.

Stigmast-4-ene-3,23-dione.—The Oppenauer oxidation of VIa gave stigmast-4-ene-3,23-dione; m. p. 122~123°C, $[\alpha]_D^{19}$ +93° (*c* 0.68).

Found: C, 81.08; H, 10.61. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87%.

UV λ_{max}^{MeOH} 241 mμ (ε, 16300); IR (Nujol) 1712, 1678, 1623 cm^{-1}.

3β,23α(and β)-Dibenzoxystigmast-5-ene (IX).—To a solution of 500 mg. of 3β-acetoxystigmast-5-en-23-one (VIb) in dry tetrahydrofuran, 0.4 g. of lithium aluminum hydride was added; the mixture was then heated under reflux for 5 hr. After the mixture had been allowed to stand overnight, the product was isolated in a conventional way to give 490 mg. of a solid. A portion of the solid was recrystallized from methylene chloride-ethyl alcohol to form crystals; m. p. 178~180°C, $[\alpha]_D^{19}$ −34° (*c* 0.69).

Found: C, 80.52; H, 11.56. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H. 11.70%.

The remaining solid (445 mg.) was converted into a benzoate by treating it with benzoyl chloride-pyridine at 50°C for 6 hr.; it was then allowed to stand at room temperature overnight after which the benzoate was chromatographed on 100 g. of alumina (Sumitomo Kagaku Co., 100 mesh). Elution with 1% ethyl acetate in petroleum ether and recrystallization from ethyl acetate-methanol gave 282 mg. of needles; m. p. 130~130.5°C, $[\alpha]_D^{18}$ −9° (*c* 0.67).

Found: C, 80.45; H, 9.10. Calcd. for $C_{43}H_{58}O_4$: C, 80.83; H, 9.15%.

Further elution with the same solvent and recrystallization from ethyl acetate-methanol gave 54 mg. of needles; m. p. 159~162°C, $[\alpha]_D^{19}$ −31° (*c* 0.86).

Found: C, 80.21; H, 8.95. Calcd. for $C_{43}H_{58}O_4$: C, 80.83; H, 9.15%.

The two isomers gave similar infrared spectra.

3β-Acetoxystigmastan-23-one (VIIb). — 3β-Acetoxystigmast-5-en-23-one (VIb) (942 mg.) was dissolved in a mixture of 40 ml. of dry ether and 20 ml. of acetic acid (distilled over potassium permanganate), and then 44 mg. of platiunm oxide was added. The mixture was agitated with hydrogen. After 58 ml. of hydrogen had been absorbed during a 30-min. period, the absorption of hydrogen ceased. The product was isolated in the usual way and was recrystallized from ethanol to give 747 mg. of crystals; m. p. 151~152°C, $[\alpha]_D^{17}$ +27° (*c* 0.68).

Found: C, 78.47; H, 10.97. Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09%.

UV $\lambda_{max}^{CHCl_3}$ 291 mμ (ε, 54); IR (Nujol) 1733, 1704, 1250 cm^{-1} (no absorption at 800 cm^{-1}). The tetranitromethane test was negative.

3β-Hydroxystigmastan-23-one (VIIa).—To a solution of 540 mg. of 3β-acetoxystigmastan-23-one (VIIb) in a mixture of 10 ml. of methylene chloride and 10 ml. of methanol, a solution of 0.1 g. of potassium hydroxide in 1 ml. of water was added; the mixture was allowed to stand overnight. The usual manipulation gave 525 mg. of crystals; m. p. 175~175.5°C; $[\alpha]_D^{17}$ +40° (*c* 0.65).

Found: C, 80.53; H, 11.49. Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70%.

UV $\lambda_{max}^{CHCl_3}$ 290 mμ (ε, 49); IR (Nujol) 3460, 3333, 1714, 1695 cm^{-1}.

Stigmastane-3,23-dione (VIII).—To a solution of 200 mg. of 3β-hydroxystigmastan-23-one (VIIa)

14) For the isolation of stigmasterol from phytosterol, see, T. Yamada and K. Morita, *J. Japan Oil Chemist's Association*, **11**, 290 (1962).

in a mixture of 1 ml. of methylene chloride and 10 ml. of acetic acid, 0.1 g. of chromic acid in 0.5 ml. of water was added; the mixture was allowed to stand overnight. After the decomposition of the excessive chromic acid with ethyl alcohol, the product was extracted with methylene chloride. The extract was washed with aqueous sodium carbonate and water, dried over anhydrous sodium sulfate, and concentrated. The residue was crystallized from ethanol to give 118 mg. of crystals; m. p. 159~160°C; $[\alpha]_D^{17}$ +54° (*c* 0.68).

Found: C, 80.97; H, 11.17. Calcd. for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29%.

UV $\lambda_{max}^{CHCl_3}$ 290 mμ (ε, 71); IR (Nujol) 1724, 1709 cm^{-1}.

3β-Acetoxy-22ξ-chlorostigmast-5-en-23ξ-ol.—*From 3β-Acetoxy-5α, 22ξ-dichlorostigmastan-22ξ-ol.*—A solution of 800 mg. of 3β-acetoxy-5α, 22ξ-dichlorostigmastan-23ξ-ol (IV) in 16 ml. of *s*-collidine was heated under reflux for 17 hr. The mixture was poured into water and extracted with chloroform. The chloroform extract was washed with diluted sulfuric acid and water, dried with anhydrous sodium sulfate, and concentrated to dryness. The residual crude material (780 mg.) was chromatographed on 24 g. of florisil. Elution with benzene and recrystallization from acetone gave crystals (m. p. 168.5~169.5°C). The Beilstein test was positive.

Found: C, 73.48; H, 9.86; Cl, 7.04. Calcd. for $C_{31}H_{51}O_3Cl$: C, 73.41; H, 10.13; Cl, 6.99%.

From 3β-Acetoxy-22ξ, 23ξ-epoxystigmast-5-ene (X).—To a solution of 300 mg. of oxide X in 20 ml. of glacial acetic acid, 6 ml. of a 5% solution of hydrogen chloride in acetic acid was added; the mixture was then heated at 65~70°C for 2 hr. The reaction mixture was poured into water, and the precipitated solid was collected by filtration. Recrystallization of the dried sample from acetone gave crystals with a m. p. 166~168°C. Mixed melting point determination with the 3β-acetoxy-22ξ-chlorostigmast-5-en-23ξ-ol obtained above showed no depression, and the infrared spectra were identical.

Thin-layer Chromatography.—Silica gel G was used as the adsorbent (0.25 mm. thick) and benzene was used as the eluant. A spray reagent was prepared by dissolving potassium permanganate (0.1%) in 5% aqueous sodium carbonate.[15] Slightly different R_f values were obtained for each run. The values in Table I are averages of several runs. A mixture of stigmasterol, 3β-acetoxystigmast-5-en-23-one and stigmasteryl acetate was chosen as the reference mixture; it was run each time with the other compounds.

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