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79. Ryoichi Hayatsu: Steroid Studies. VII. Syntheses of Sargasterol, Fucosterol, and C_{20}-Isocholesterol.

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A new sterol, sargasterol, was isolated from a brown algae, *Sargassum Ringgoldianum* HARVEY, and its structure was concluded[1] by chemical degradation most likely to be C_{20}-isofucosterol.[2] The present study was undertaken to confirm this structure by synthesis. Further, preparation of fucosterol[1,3] from several brown algae and C_{20}-isocholesterol, derived from sargasterol, are also described.

C_{20}-Isosterol has been unknown in past literature except for two kinds of natural sterol. Bergmann, *et al.*[4] reported that the methyl group at C-20 in haliclona- and palysterol has a configuration reverse of that present in cholesterol but this configuration was only supported by the determination of molecular rotation.

In bisnorsteroids, it has been reported that methyl 3α,12α-dihydroxy-bisnorcholanate,[5] methyl bisnorcholanate,[6] and 3-oxo-Δ^4-bisnorcholen-22-al[7] were converted to the corresponding 20-isobisnorsteroids by treatment with alkali or acid. Plattner[8] also obtained 3β-hydroxy-20-iso-Δ^5-norcholenic acid and 3β-hydroxy-20-isonorcholanic acid from 3β-hydroxy-$\Delta^{5,20(22)}$-norcholadienic acid by catalytic reduction.

The starting material, 3β-hydroxy-20-iso-Δ^5-cholenic acid (IIa), for the synthesis of sargasterol, was prepared by treatment of methyl 3β-acetoxy-Δ^5-cholenate (Ic) with potassium hydroxide in diethylene glycol. The yield of (IIa) is 70% but that of treatment of (Ib) is poor. Treatment of (IIb) with thionyl chloride in ether solution gave (IIIa) and the reduction of (IIIa) with sodium borohydride in dioxane-methanol for 3 hours afforded 3β-acetoxy-22-hydroxy-20-iso-Δ^5-bisnorcholene (IVa). However, longer reduction time than the above gave 3β,22-dihydroxy-20-iso-Δ^5-bisnorcholene. (IVa) was converted to its tosylate (Va) with *p*-toluenesulfonyl chloride. On heating with sodium diethylmalonate in xylene and subsequent saponification, the tosylate was converted to 22-dicarboxylic acid (VIa). When (VIa) was kept for one hour in an oil bath of 180~190°, 3β-hydroxy-20-iso-Δ^5-cholenic acid (VIIa), m.p. 249~251°, was formed.

On the other hand, 3β-hydroxy-Δ^5-cholenic acid (VIIb), m.p. 236~239°——20-normal series——was also obtained from 3β-acetoxy-Δ^5-bisnorcholenic acid by the same procedure as described for the 20-iso series. (VIIb) and its derivatives were found to be

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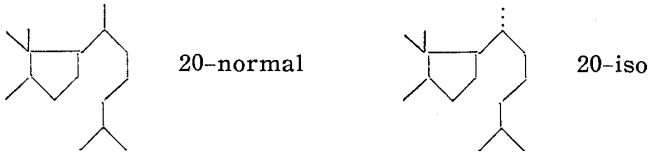
1) Part VI. K. Tsuda, R. Hayatsu, S. Akagi, Y. Kishida: J. Am. Chem. Soc., **79**(1957), in press; a brief report was made as a Communication to the Editor of this Bulletin, **5**, 85(1957).
2) In the present paper the configuration same as that present in cholesterol at C_{20}-methyl group is named 20b or 20-normal and the reverse configuration is named as 20a or 20-iso.



3) I.M. Heilbron, *et al.*: J. Chem. Soc., **1934**, 1572; **1935**, 1205; Biochem. J.(London), **29**, 1376(1935); J. Chem. Soc., **1936**, 738.
4) W. Bergmann, R.J. Feeney, A.N. Swift: J. Org. Chem., **16**, 1337(1951).
5) M. Sorkin, T. Reichstein: Helv. Chim. Acta, **27**, 1631(1944); **28**, 875(1945).
6) H. Wieland: Z. physiol. Chem., **161**, 100(1926).
7) M.E. Herr, F.W. Heyl: J. Am. Chem. Soc., **74**, 3627(1952).
8) Pl. A. Plattner, J. Pataki: Helv. Chim. Acta, **26**, 1241(1943).

identical in physical constants and infrared absorptions with authentic samples[9] obtained by chromic oxidation of cholesterol.

Riegel and Kaye[10] have shown that 3β-acetoxy-Δ^5-cholenic acid chloride (chloride of (VIIIb)-acetate) reacts with diisopropylcadmium to form 24-oxo-cholesteryl acetate (VIIIb), m.p. 128.5~129.5, $[\alpha]_D$ −43.5°. 24-Oxo-20-isocholesteryl acetate (VIIIa), m.p. 117~118°, $[\alpha]_D$ −26.5°, was obtained from 3β-acetoxy-20-iso-Δ^5-cholenic acid chloride (chloride of (VIIa)-acetate) by a similar manner as above. After the Grignard reaction (ethylmagnesium bromide) and dehydroxylation of 24-oxo-20-iso (VIIIa) and 24-oxo-20-normal (VIIIb) compounds, sargasterol and fucosterol were respectively obtained and these synthetic sterols and their derivatives were found to be identical with natural sargasterol and fucosterol, and their derivatives in melting points, optical rotation,and infrared absorption. The physical constants of synthetic and natural sterols are shown in Table I.

Table I.

	Natural product		Synthesized product	
	m.p.(°C)	$[\alpha]_D$(in $CHCl_3$)	m.p.(°C)	$[\alpha]_D$(in $CHCl_3$)
Sargasterol[1]	132~133.5	−47.5°	134~135	−48.0°
acetate[1]	138~139	−52.9	136.5~137	−53.5
benzoate[1]	114~115	−22.5	114~115	−21.5
Fucosterol[1,3]	125~126	−39.5	127.5~129	−39.0
acetate[1,3]	120~122	−42.0	119~120	−42.5
benzoate[1,3]	118~119	−19.9	118.5~119	−19.0
20-Isocholesterol[a]	154~155 (133~134.5)[b]	−43.0	155.5~156 (132~134)[b]	−43.0
acetate[a]	123~124	−47.9	121.5~122	−48.5
benzoate[a]	164~165 (132~134)[b]	−19.2	163.5~165 (134~136)[b]	−19.5

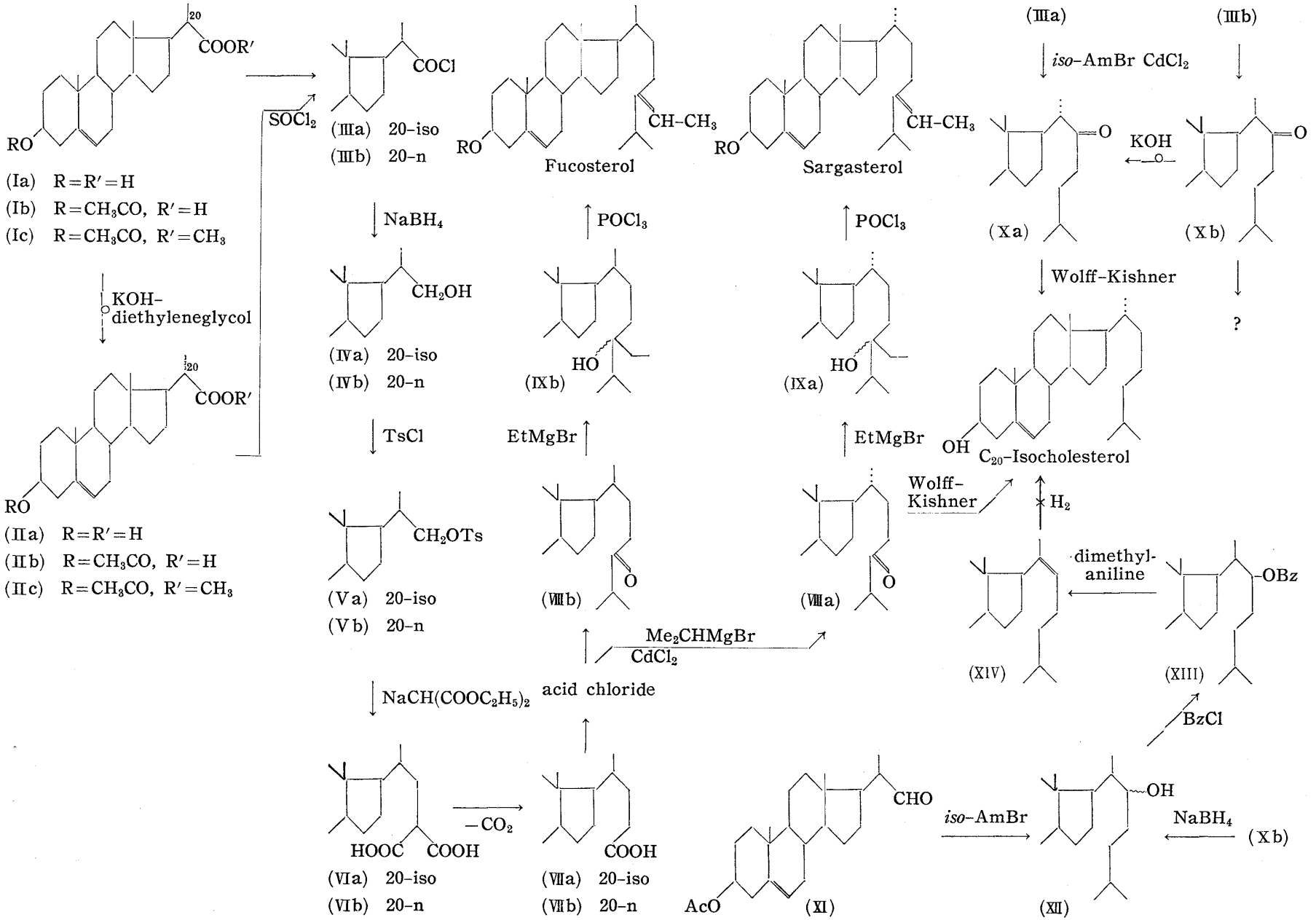
a) The compounds prepared from sargasterol by ozonization and Wolff-Kishner reduction.
b) Crystallized from EtOH.

In the previous paper,[1] it was reported that 20-isocholesterol was obtained from sargasterol by ozonization and Wolff-Kishner reduction, but there has been no report on 20-isocholesterol in the literature. Therefore, the synthesis of this compound was conducted in the following manner to confirm its structure.

22-Oxo-20-isocholesterol (Xa) was obtained from the acid chloride (IIIa) by the Grignard reaction (diisoamylcadmium). This ketone was also obtained from 22-oxo-cholesterol (Xb) by isomerization with potassium hydroxide in diethylene glycol. The isomerization was effected in a good yield without influence of reaction time (yield of 20-iso-22-oxo compound : 8 hours, 85%; 12 hours, 81%; 16 hours, 83%). From the result of the above and bisnor-type, it is concluded that 20-iso series is more likely to be stable than 20-normal series. Cole, *et al.*[11] reported that 3β-hydroxy-ternorcholenyl methyl (or ethyl, phenyl) ketone was converted to the corresponding 20-iso compound with potassium hydroxide. The 20-iso type could revert to 20-normal type again but reversion yield was not shown. The 22-position in the sterol molecule is definitely a hindered position and therefore, 22-oxo- and 22-oxo-20-isocholesterol were diffiicult to react with ketonic reagents. Wolff-Kishner reduction of 22-oxo-20-isocholesterol (Xa) only yielded a hydrazone in 50% yield. The hydrazone was converted to 20-isocholesterol by refluxing with potassium hydroxide in diethylene glycol under anhydrous condition.

22-Oxocholesterol (Xb) gave an oily product by a similar treatment as described above. Although, the product seemed to be identical with cholesterol in infrared absorption, it was not crystallized even by chromatographic purification. The product probably contains cholesterol and 20-isocholesterol.

9) L. Ruzicka, A. Wettstein : Helv. Chim. Acta, **18**, 986(1935); E.S. Wallis, E. Fernholz : J. Am. Chem. Soc., **57**, 1505(1935).
10) B. Riegel, J.A. Kaye : *Ibid.*, **66**, 723(1944).
11) W. Cole, *et al.* : *Ibid.*, **67**, 1369(1945).



The synthesized 20-isocholesterol was identical with a sample prepared from ozonization and Wolff-Kishner reduction of sargasterol.

The Grignard reaction of 3β-acetoxy-Δ^5-bisnorcholen-22-al (XI) with isoamylmagnesium bromide or the reduction of 22-oxocholesterol (Xb) with sodium borohydride gave 22-hydroxycholesterol (XII).[12] The hydroxy compound was separated into two kinds of sterol by chromatographic purification of its dibenzoate. One of them, dibenzoate of m.p. 252~253°, $[\alpha]_D$ −10.2, was identical with a sterol isolated from a lily, *Narthecium ossifragum* by Stabursvik.[13] The other dibenzoate showed m.p. 167~169°/237~239° (double melting point), $[\alpha]_D$ −27.5°.

3β,22ξ-Dibenzoxy-Δ^5-cholestene (XIII) was converted to 3β-benzoxy-$\Delta^{5,20(22)}$-cholestadiene (XIV)[14] by refluxing with dimethylaniline. (XIV) was reduced by catalytic hydrogenation in the presence of platinum oxide, palladium-charcoal, or Raney nickel in an acidic or neutral medium, but 20-isocholesterol was not obtained. Robinson[15] and Woodward[16] also reported that 20-isocholestanol was not obtained by the catalytic hydrogenation of $\Delta^{17(20)}$-cholestenol or $\Delta^{20(22)}$-cholestenol.

The comparison of infrared spectra of 20-iso and its corresponding 20-normal compound[17] differed slightly only in 800~1000 cm^{-1} region. It was established by the foregoing syntheses and reactions that sargasterol was 20-isofucosterol.

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Experimental*

Methyl 3β-Acetoxy-Δ^5-bisnorcholenate (Ic)—To a suspension of 70 g. of Ag salt of (Ib) in 300 cc. of EtOH 40 g. of MeI was added and the reaction mixture was refluxed for 3 hrs. on a steam bath, filterd, and the filtrate was distilled off *in vacuo*. The residual yellow crystals were recrystallized from MeOH to colorless needles (Ic), m.p. 156~158°; $[\alpha]_D$ −61.0°(c=1.7); yield, 42 g. (Ia) m.p. 287~290°, (Ib) m.p. 234~236°.

Isomerization of (Ic)—To a solution of 40 g. of (Ic) in 200 cc. of diethylene glycol 25 g. of KOH was added and the mixture was heated at 190° for 6 hrs. The reaction mixture was poured into water, the product was collected by filtration, and washed with 10% HCl and water. The white solid dissolved in boiling MeOH-$CHCl_3$ (5:1) and insoluble solid (Ia)(20-normal acid) was removed by filtration. The filtrate was allowed to stand at room temperature for 8 hrs., the separated crystals (Ia) were removed by filtration, and the filtrate was distilled under reduced pressure to remove most of the solvent. The residual crystals were recrystallized 4 times from MeOH to colorless plates, m.p. 265~267°; yield, 23 g.

3β-Acetate (IIb), m.p. 197~198°; $[\alpha]_D$ −44.7°(c=1.5). *Anal.* Calcd. for $C_{24}H_{36}O_4$: C, 74.19; H, 9.34. Found: C, 73.72; H, 9.60.

Methyl 3β-acetate (IIc), m.p. 142~143°; $[\alpha]_D$ −53.6°(c=2.7). *Anal.* Calcd. for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.66; H, 9.38.

3β-Acetoxy-22-hydroxy-20-iso-Δ^5-bisnorcholene (IVa)—A mixture of 10 g. of the acid (IIb), 200

* Optical rotation was measured in $CHCl_3$.

12) Further studies on the stereochemistry of 22-hydroxychoxycholesterol and other position in the side chain of sterol will be reported shortly.

13) Arnulv Stabursvik: Acta Chem. Scand., **7**, 1220(1953).

14) This structure was established by Oppenauer oxidation and ozonization to progesterone and its study will be reported shortly.

15) J. W. Cornforth, R. Robinson: J. Chem. Soc., **1949**, 1885; **1953**, 361.

16) R. B. Woodard, *et al.*: J. Am. Chem. Soc., **74**, 4223(1952).

17) For the molecular rotation of 20-normal- and 20-isosteroids (cortical steroids, bisnorcholanic acid, and bile acid) cf. L. F. Fieser, M. Fieser: Experientia, 285(1948); W. Klyne, W. M. Stokes: J. Chem. Soc., **1954**, 1979; L. F. Fieser, M. Fieser: "Natural Products related to Phenanthrene," Reinhold Publ. Corp., N. Y., 279, 418(1949).

cc. of dehyd. ether, 50 cc. of dehyd. benzene, 3.5 cc. of freshly distilled $SOCl_2$, and 1 drop of pyridine was allowed to stand at 25° for 5 hrs. with occasional swirling. The solvent was removed completely *in vacuo*, the pale yellow crystalline residue was washed with pentane, leaving 10 g. of chloride which was satisfactory for the following preparations. It may be recrystallized from benzene, from which it separates as colorless stout needles, m.p. 122~125°. *Anal.* Calcd. for $C_{24}H_{35}O_3Cl$: Cl, 8.71. Found: Cl, 8.16.

To a solution of 8 g. of the acid chloride (IIIa) dissolved in 50 cc. of dioxane, 4 g. of $NaBH_4$ in 40 cc. of dioxane-MeOH (1:1) was added with stirring at room temperature. After 3 hrs.' stirring, 10% AcOH solution was added and the mixture was poured into water. The colorless oil that separated and solidified on standing, was collected, washed with water, dried, and recrystallized from MeOH to colorless needles, m.p. 135.5~137°; $[\alpha]_D$ −50.9°(c=1.3); yield, 6.1 g. *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.98; H, 10.10.

When the reaction time is longer than the above (10 hrs.), 3β,22-dihydroxy-Δ^5-bisnorcholene was obtained, m.p. 186~188°; yield, 1.2 g.(from 1.9 g. of (IIIa)). *Anal.* Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found: C, 79.77; H, 10.63.

22-Tosylate (Va)—To 5 g. of (IVa) in 40 cc. of pyridine 3 g. of *p*-toluenesulfonyl chloride was added and the mixture was allowed to stand for 1 day at room temperature. It was poured into water, the collected product was washed with 10% HCl and water, dried, and recrystallized from AcOEt-MeOH to colorless plates, m.p. 125~128°; $[\alpha]_D$ −39.7°(c=1.0); yield, 4.8 g. *Anal.* Calcd. for $C_{31}H_{44}O_5S$: C, 70.40; H, 8.39. Found: C, 70.53; H, 8.48.

3β-Hydroxy-20-iso-Δ^5-norcholene-23-dicarboxylic Acid (VIa)—A mixture of 1.15 g. of Na in 20 cc. of dehyd. xylene was heated in an oil bath of 115°. Through the dropping funnel a mixture consisting of 10 g. of diethyl malonate and 10 cc. of dehyd. xylene was added slowly with constant stirring. When the addition was complete and all Na had dissolved, a solution of 10.5 g. of 22-tosylate (Va) in 25 cc. of xylene was poured into the flask. The temperature of the oil bath was maintained at 115° while heating and stirring for 10 hrs., during which time a voluminous precipitate of Na tosylate formed. The color of the reaction mixture turned brown. After cooling to room temperature the precipitate was filtered off and washed with xylene. The filtrates were combined and xylene was distilled off *in vacuo* on a steam bath. The residue was a very viscous oil, which consisted of diethyl 22-dicarboxylate and unreacted diethyl malonate. The residue was dissolved in 80 cc. of *iso*-PrOH(50 cc.)-MeOH(30 cc.)-KOH(6 g.) and refluxed for 5 hrs. The reaction mixture was poured into water, extracted with ether, and the aq. layer was acidified with 10% HCl. The crystalline precipitate was washed with water, dried, and recrystallized from MeOH to colorless microcrystals, m.p. 189~196°(decomp.); yield, 6.8 g. *Anal.* Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.66; H, 9.73.

3β-Hydroxy-20-iso-Δ^5-cholenic Acid (VIIa)—Five grams of (VIa) was kept for 1.5 hrs. in an oil bath of 190~200°. During this time evolution of CO_2 took place and the substance changed into a clear brown melt. This melt was cooled, dissolved in 100 cc. of $CHCl_3$, the solution was treated with charcoal, filtered, and the solvent was distilled off *in vacuo*. The residue was crystallized from $CHCl_3$-MeOH(1:1). (VIIa)(2.1 g.) was obtained as colorless microcrystals, m.p. 249~251°(decomp.).

3β-Acetate: m.p. 213~215°; $[\alpha]_D$ −42.7°(c=1.2). *Anal.* Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found: C, 74.71; H, 9.87.

Methyl 3β-acetoxy-20-iso-Δ^5-cholenate: m.p. 169~170°; $[\alpha]_D$ −50.3°(c=1.2). *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.30; H, 9.84. Found: C, 75.46; H, 9.47.

24-Oxo-20-isocholesterol (VIIIa)—3β-Acetoxy-20-iso-Δ^5-cholenyl chloride, prepared from 1 g. of 3β-acetoxy-20-iso-Δ^5-cholenic acid, was not isolated and it was dissolved in dehyd. benzene and then added to a diisopropylcadmium solution (prepared from 600 mg. of Mg, 5 g. of *iso*-PrBr, dehyd. ether, and 4 g. of $CdCl_2$). The reaction mixture was refluxed (55~60°) for 2 hrs. and after standing overnight, it was worked up in the usual way. The resulting product was dissolved in petr. ether-benzine (1:1) and chromatographed on alumina. Colorless needles, m.p. 117~118°(from MeOH); $[\alpha]_D$ −26.5° (c=1.3); yield, 320 mg. *Anal.* Calcd. for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.77; H, 10.30.

24-Oxo-20-isocholesterol: Colorless plates, m.p. 147~148°(from MeOH); $[\alpha]_D$ −25.0°(c=1.4). The ketosterol and keto-acetate gave no depression of m.p. on admixture with corresponding compounds prepared by ozonization of sargasterol. The synthesized keto-acetate was also identical in infrared absorption with a sample from sargasterol.

3β,24ξ-20-Isodihydroxy Compound (IXa)—A Grignard solution, prepared from 500 mg. of Mg, 3.5 cc. of EtBr, and 25 cc. of ether, was cooled in an ice bath and reacted with 1.2 g. of 22-oxo-20-isocholesterol in 20 cc. of benzene-ether (1:1). The mixture was refluxed for 3 hrs., and cold 10% HCl was added dropwise. The organic layer was washed with water, dried, and evaporated under reduced pressure. The resulting product (m.p. 140~150°) was crystallized from MeOH and 800 mg. of (IXa) was obtained as colorless plates, m.p. 182~184°. *Anal.* Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found: C, 80.38; H, 11.42.

3β-Acetate of (IXa) : Prepared by the treatment of (IXa) with Ac_2O-pyridine (1:2) on a steam bath for 1.5 hrs. Recrystallization from MeOH gave colorless needles, m.p. 153～155°. *Anal.* Calcd. for $C_{31}H_{50}O_3$: C, 78.76; H, 11.09. Found : C, 78.55; H, 11.21.

Sargasterol from (IXa)—To a solution of 600 mg. of 3β-acetoxy-24ξ-hydroxy-20-iso-Δ^5-stigmastane (IXa : OH=OAc) in 10 cc. of pyridine, 3 cc. of $POCl_3$ was added, the solution was heated on a steam bath for 1 hr., and then allowed to stand overnight. The reaction mixture was poured into 10% K_2CO_3 solution with ice cooling. The product which soon solidified was dissolved in petr. ether-benzene (1:1) and passed through a column containing alumina. The residue obtained from earlier fractions was recrystallized from EtOH to colorless needles, m.p. 136.5～137°; $[\alpha]_D$ −53.5°(c=2.8); yield, 120 mg. Mixed m.p. 137～138° with natural sargasteryl acetate, m.p. 138～139°. *Anal.* Calcd. for $C_{31}H_{50}O_2$: C, 81.88; H, 11.08. Found : C, 81.62; H, 11.32.

Sterol : m.p. 134～135°; $[\alpha]_D$ −48.0(c=1.8); mixed m.p. 133～134° with natural sargasterol, m.p. 132～133.5°.

Steryl benzoate : m.p. 114～115°; $[\alpha]_D$ −21.5°(c=2.3); mixed m.p. 114～115° with natural sargasteryl benzoate, m.p. 114～115°. The synthetic sterol and their derivatives were identical with corresponding natural sargasterol and their derivatives in infrared absorptions.

3β-Acetoxy-Δ^5-cholenic Acid Chloride (IIIb)—3β-Acetoxy-Δ^5-bisnorcholenic acid was treated with $SOCl_2$ according to the procedure of Cole.[11] Recrystallization from benzene gave colorless needles, m.p. 128.5～130.

3β-Acetoxy-22-hydroxy-Δ^5-bisnorcholene (IVb)—Six grams of (IIIb) was reduced with 2 g. of $NaBH_4$ in dioxane-MeOH (1:1) for 3 hrs. as described for 20-iso series. Recrystallization from a mixture of Me_2CO-MeOH gave 2.4 g. of colorless needles, m.p. 153～154°; $[\alpha]_D$ −60.7°(c=1.4); m.p. 127.5～129° when crystallized from hydrous acetone. *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.78; H, 10.46.

When (IIIb) was reduced with $NaBH_4$ at room temperature for 10 hrs., 3β,22-dihydroxy-Δ^5-bisnorcholene formed and crystallization from MeOH gave colorless rosette needles, m.p. 195～197°; $[\alpha]_D$ −53.8°(c=1.2); yield, 45%. *Anal.* Calcd. for $C_{22}H_{36}O_2$: C, 79.46; H, 10.92. Found : C, 79.01; H, 10.78.

3β-Acetoxy-22-tosyloxy-Δ^5-bisnorcholene (Vb)—The 22-tosylate (Vb) was prepared from (IVb) with *p*-toluenesulfonyl chloride in pyridine, as described for 20-iso series. Recrystallization from acetone gave colorless needles, m.p. 132～134°; yield, 2.2 g.(from 2 g. of (Vb)). *Anal.* Calcd. for $C_{31}H_{44}O_5S$: C, 70.40; H, 8.39. Found : C, 70.90; H, 8.32.

3β-Hydroxy-Δ^5-bisnorcholene-23-dicarboxylic Acid (VIb)—Diethyl ester of (VIb) was prepared from (Vb) with sodium diethylmalonate in xylene and was converted to (VIb) by saponification with KOH in *iso*-PrOH-MeOH (1:1), as described for 20-iso series. After crystallization from MeOH, it melted at 204～207°(decomp.); yield, 3.7 g.(from 4.2 g. of (Vb)). *Anal.* Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found : C, 71.26; H, 9.20.

3β-Hydroxy-Δ^5-cholenic Acid (VIIb)—When 3 g. of (VIb) was heated in an oil bath of 190～200° for 2 hrs., 1.2 g. of (VIIb) was formed. Crystallization from AcOEt gave colorless fine crystals, m.p. 236～239°(decomp.).

3β-Acetate : Needles (from EtOH), m.p. 184～186°. *Anal.* Calcd. for $C_{26}H_{40}O_4$: C, 74.96; H, 9.68. Found : C, 75.12; H, 10.00.

Methyl 3β-acetoxy-Δ^5-cholenate : Crystallized from EtOH in needles, m.p. and mixed m.p. 154～155°. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.30; H, 9.84. Found : C, 75,12; H, 9.62.

24-Oxocholesterol (VIIIb)—Diisopropylcadmium was reacted with 3β-acetoxy-Δ^5-cholenyl chloride, prepared from 3β-acetate of (VIIb) according to the procedure of Riegel and Kaye.[10] The product was purified by chromatography on alumina, m.p. 128.5～129.5°; $[\alpha]_D$ −43.5°, mixed m.p. 129° with the ozonolysis product of fucosterol. *Anal.* Calcd. for $C_{29}H_{46}O_3$: C, 76.68; H, 10.48. Found : C, 76.82; H, 10.33.

Sterol : Recrystallized from MeOH to colorless needles, m.p. 138～139°; $[\alpha]_D$ −36.5°(c=1.3), which was identical with a product of ozonization from fucosterol in m.p. and infrared absorption.

3β,24ξ-Dihydroxy-Δ^5-stigmastene (IXb)—Treatment of 1 g. of 24-oxocholesteryl acetate with EtMgBr as describes for 20-iso series gave 850 mg. of (IXb) and recrystallization from MeOH gave colorless plates, m.p. 167～169°. *Anal.* Calcd. for $C_{29}H_{50}O_2$: C, 80.87; H, 11.70. Found : C, 80.40; H, 11.44.

The 3β-acetate was obtained by acetylation with Ac_2O and pyridine (1:2), and crystallized from MeOH to needles, m.p. 148.5～150°. *Anal.* Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.92; H, 11.02.

Fucosterol—To a solution of 700 mg. of 3β-acetate of (IXb) dissolved in 10 cc. of pyridine, 3 cc. of $POCl_3$ was added, the reaction mixture heated on a steam bath for 1 hr., and allowed to stand overnight. The oily product was adsorbed on a column (1.5×20 cm.) of alumina (Brockmann Grade II), the adsorbent was washed with 400 cc. of petr. ether-benzene (2:1), and the eluate was discarded. Fucosterol (220 mg.), prepared by hydrolysis of the acetate, melted at 127.5～129°, $[\alpha]_D$ −39.0°(c=2.3), mixed m.p. with natural fucosterol (m.p. 126°), 128°. The infrared spectrum was identical with that

of the natural product. *Anal.* Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72. Found : C, 84.71; H, 11.53.

Acetate : Crystallized from ethanol in needles, m.p. 119~120°, $[\alpha]_D$ −42.5°(c=1.3); mixed m.p. 120° with the natural product.

Benzoate : Prepared in the usual manner and crystallized from AcOEt in plates, m.p. 118.5~119°; $[\alpha]_D$ −19.0°(c=1.3); mixed. m.p. 119° with the natural product.

22-Oxo-20-isocholesteryl (Xa) Acetate—A Grignard solution, prepared from 1.7 g. of Mg, 12 cc. of *iso*-AmBr, and 120 cc. of ether, was cooled in an ice-bath and reacted with 7.6 g. of powdered anhyd. $CdCl_2$. After 30 mins.' stirring, the diisoamylcadmium solution was reacted with a benzene solution (40 cc.) of the acid chloride (IIIa), prepared from 5 g. of 3β-acetoxy-20-iso-Δ^5-bisnorcholenic acid(IIb). The mixture was refluxed for 2 hrs. with stirring and allowed to stand at room temperature for 10 hrs. Usual treatment, including steam distillation to remove diisoamyl, gave 5 g. of the ketone, m.p. 132~135°. Recrystallization from MeOH gave colorless needles, m.p. 134.5~136°; $[\alpha]_D$ −46.7° (c=2.0). *Anal.* Calcd. for $C_{29}H_{46}O_3$: C, 78.69; H, 10.47. Found : C, 78.30; H, 10.81.

22-Oxo-20-isocholesterol (Xa)—(A) Six grams of 22-oxocholesterol (Xb)* was isomerized by refluxing for 8 hrs. with 3.5 g. of KOH in 70 cc. of diethylene glycol, giving 5.2 g. of a crystalline product and the product was fractionally crystallized from MeOH to 4.9 g. of the pure hydroxy-ketone as colorless needles, m.p. 109~110°; $[\alpha]_D$ −40.4°(c=1.2). *Anal.* Calcd. for $C_{27}H_{44}O_2$: C, 80.94; H, 11.07. Found : C, 80.98; H, 10.89.

(B) A mixture of 1 g. of the acetoxy-ketone (Xa-acetate), 1 g. of Na_2CO_3, 1 cc. of water, and 20 cc. of MeOH was refluxed for 5 hrs. on a steam bath, cooled, and diluted with ether. The washed ether solution was evaporated and the residue was crystallized from MeOH. The hydroxy-ketone was obtained as colorless needles, m.p. 110~111.5°; mixed m.p. 109~111° with a sample prepared by (A).

Wolff-Kishner Reduction of (Xa)—Two grams of (Xa) was refluxed for 10 hrs. with 2 g. of KOH and 7 cc. of 80% hydrazine hydrate in 25 cc. of diethylene glycol. The reaction mixture was poured into water, the product was collected by filtration, washed with 10% HCl and water, and recrystallized 5 times from MeOH to pale yellow, silky crystals, m.p. 236~238°; yield, 1.1 g. *Anal.* Calcd. for $C_{27}H_{46}ON_2$: N, 6.76. Found : N, 6.49. This product is 22-hydrazone.

One gram of the hydrazone was refluxed in 20 cc. of diethylene glycol with 1.5 g. of KOH for 8 hrs., the reaction mixture was poured into water, and the product was collected by filtration and dried. After the benzoylation of resulting product (m.p. 120~140°), it was dissolved in petr. ether-benzene (1:1) and chromatographed through alumina. The white solid obtained from earlier fractions was recrystallized from AcOEt to colorless prisms, m.p. 163.5~165°; $[\alpha]_D$ −19.5°(c=1.2); yield, 610 mg.; mixed m.p. 164° with 20-isocholesteryl benzoate derived from sargasterol. *Anal.* Calcd. for $C_{34}H_{50}O_2$: C, 83.21; H, 10.27. Found : C, 83.43; H, 10.44. When recrystallized from EtOH, the steryl benzoate separated in plates which melted at 134~136°.

Sterol : Colorless plates (from petr. ether), m.p. 155.5~156°; $[\alpha]_D$ −43.0°(c=1.3); mixed m.p. 155° with 20-isocholesterol derived from sargasterol. Recrystallized from EtOH to plates, m.p. 132~134°. *Anal.* Calcd. for $C_{27}H_{46}O$: C, 83.87; H, 11.99. Found : C, 83.50; H, 11.97.

Steryl acetate : m.p. 121.5~122°; $[\alpha]_D$ −48.5°(c=1.2); mixed m.p. 123° with 20-isocholesteryl acetate derived from sargasterol.

The synthetic sterol, acetate, and benzoat were identical with the sterol and their derivatives derived from sargasterol in infrared absorptions.

22ξ-Hydroxycholesterol (XII)—i) From (Xb) : To a solution of 1 g. of (Xb) in 50 cc. of dioxane 700 mg. of $NaBH_4$ in 40 cc. of dioxane-MeOH (1:1) was added and the reaction mixture was allowed to stand overnight at room temperature. A solution of 10% HCl was dropped into the reaction mixture, the reaction mixture was poured into water, and filtered. The product was saponified with methanolic alkali (300 mg. of KOH in 30 cc. MeOH) on a steam bath for 3 hrs. After the usual manner, the dihydroxy compound was obtained and recrystallized from MeOH to colorless needles, m.p. 165~169°(mixture of 22-hydroxy isomer ?); yield, 810 mg. *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found : C, 80.38; H, 11.29.

ii) From (XI) : A Grignard solution, prepared from 1.5 g. of Mg, 12 cc. of *iso*-AmBr, and 120 cc. of ether, was cooled in an ice bath and reacted with a solution of 5 g. of 3β-acetoxy-Δ^5-bisnorcholen-22-al (XI)** in 40 cc. of ether-benzene (1:3). The mixture was refluxed for 7 hrs. with stirring and worked up in the usual manner. The resulting product was recrystallized from MeOH to colorless needles, m.p. 158~164°(mixture of 22-hydroxy isomer ?). *Anal.* Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found : C, 80.46; H, 11.69.

22-Benzoxycholesteryl Benzoate (XIII)—To a solution of 700 mg. of (XII) from (Xb) in 10 cc. of pyridine, 500 mg. of BzCl was added and the reaction mixture was allowed to stand overnight at

* (Xb) was prepared from (IIIb) according to the method of W. Cole and P. L. Julian.[11]

** (XI) was prepared from stigmasteryl acetate according to the method of A. P. Centolella, *et al.*: J. Am. Chem. Soc., **70**, 2953(1948).

room temperature. By working up in the usual manner, the dibenzoate was obtained and recrystallized from AcOEt to colorless needles, m.p. 167~179°. A solution of 500 mg. of this dibenzoate dissolved in petr. ether-benzene (1:1) was passed through an alumina column (1.5×25 cm., Grade II), which was developed with the same solvent and the effluent was fractionated in 20-cc. portions.

Fractions 2~3 : Colorless needles (from AcOEt-EtOH=2:1), m.p. 252~253°; $[\alpha]_D$ −10.2°(c=2.3); yield, 120 mg.; mixed m.p. 252~253° with 22-benzoxy-cholesteryl benzoate isolated from *Narthecium*. *Anal.* Calcd. for $C_{41}H_{54}O_4$: C, 80.61; H, 8.91. Found : C, 80.72; H, 9.03.

Fractions 5~7 : m.p. 167~169/237~239°(double melting point)†; $[\alpha]_D$ −27.5°(c=2.3); yield, 60 mg. *Anal.* Calcd. for $C_{41}H_{54}O_4$: C, 80.61; H, 8.91. Found : C, 80.28; H, 8.66. This dibenzoate is the isomer of natural dibenzoate, m.p. 252~253°. Two kinds of dibenzoate were also obtained from dihydroxy compound (XII) from (XI) in a similar manner.

$\Delta^{5,20(22)}$-Cholestadienyl Benzoate (XIV)—A solution of 500 mg. of (XIII), m.p. 237~239°, in 15 cc. of freshly distilled dimethylaniline was refluxed for 10 hrs. The reaction mixture was poured into 10% HCl and the product was crystallized from AcOEt to colorless prisms, m.p. 152~153°(clear at 190°). *Anal.* Calcd. for $C_{34}H_{48}O_2$: C, 83.55; H, 9.90. Found : C, 83.40; H, 10.21. Hydrogenation of (XIV) was not successful and 20-isocholesterol was not obtained.

Summary

Synthesis of sargasterol (isolated from *Sargassum ringgoldianum* HARV.) was carried out from 3β-acetoxy-20-iso-Δ^5-bisnorcholenic acid as a starting material. Fucosterol (isolated from several brown algae) was also prepared from 3β-acetoxy-Δ^5-bisnorcholenic acid by the same procedure. These synthesized sargasterol and fucosterol were respectively identical with natural sargasterol and fucosterol. Wolff-Kishner reduction of 22-oxo-20-isocholesterol, prepared from 22-oxocholesterol by isomerization, afforded 20-isocholesterol. 22-Hydroxycholesterol was prepared from 22-oxocholesterol by reduction with sodium borohydride and this sterol was identical with a sample isolated from a lily sterol.

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† Benzoylation of the isomer of natural sterol is difficult.