ture was filtered hot and the filtrate concentrated to a gum *in vacuo*. The gum was crystallized from 60% aqueous isopropyl alcohol, yielding 680 mg. of the ketal, m.p. 214–215° (capillary); λ_{max} 239 mμ (ε 14,600); mobility R_f 0.45 (system V) compared with 20β-dihydrotriamcinolone at R_f 0 and with triamcinolone 16α,17α-acetonide at R_f 0.16; negative to tetrazolium blue reagent; λ_{max}^{KBr} 2.91, 3.41, 6.01, 6.17, 6.23, 6.91, 8.60, 9.06, 9.36, 9.45, 10.70, 11.24 μ, etc.

Anal. Calcd. for $C_{27}H_{37}O_6F$: C, 68.04; H, 7.83; F, 3.99. Found: C, 66.36; H, 7.99; F, 3.84.

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[CONTRIBUTION FROM THE INSTITUTE OF APPLIED MICROBIOLOGY, UNIVERSITY OF TOKYO]

Steroid Studies. XVI.[1] Isolation of 22-Dehydrocholesterol from *Hypnea japonica*

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22-Dehydrocholesterol was isolated from a species of red algae, *Hypnea japonica*.

Previous reports from these laboratories[3] and others[4] have indicated that only fucosterol (C_{29}-sterol) is generally to be isolated from various species of brown algae,[5] while more recently cholesterol (C_{27}-sterol), a characteristic animal sterol, was found to occur without exception in all fifteen species of red algae so far examined in these laboratories.[3b,6]

It would thus appear that fucosterol is the characteristic sterol of the brown algae and cholesterol is the common sterol not only in the animal kingdom but in red algae. It is, therefore, of considerable biogenetic interest to report in this paper the isolation of a cholesterol analog, 22-dehydrocholesterol, from a species of red algae, *Hypnea japonica* Tanaka.

The sterol reported in this paper was isolated as a non-saponifiable matter from the extract of the dried and powdered alga and showed m.p. 134–135.5°, $\alpha^{29}D$ −56.3°, on purification by successive chromatography of its acetate and of the free sterol on alumina.

The homogeneity of the sterol was confirmed by the appearance of a single orange colored zone on a silicic acid–Celite column when the *p*-phenylazobenzoyl ester was subjected to chromatography according to the method of Idler, *et al.*;[7] the melting point and optical rotation value of the sterol regenerated from the azoyl ester thus purified remained unchanged.

The sterol formed a monoacetate, m.p. 127–128°, and a monobenzoate; the latter melted at 145–146° to milky white, showing a characteristic fluorescence, and clears at 167–170° with sudden disappearance of a play of colors.

The steryl acetate afforded on treatment with bromine a tetrabromide, m.p. 186–187° dec., and on hydrogenation over platinum oxide in acetic acid at 1 atm. pressure gave a saturated acetate, m.p. 109–110°, which was identical with cholestanyl acetate by mixed melting point and infrared spectra. The sterol must be therefore a doubly unsaturated cholestanol.

Oppenauer oxidation of the sterol gave a stenone, m.p. 69–70°, absorbing at 240.5 mμ (ε 18.000) in the ultraviolet region, and on treatment with ozone the steryl acetate furnished isovaleraldehyde, whose 2,4-dinitrophenylhydrazone, m.p. 121–123°, was identified with an authentic sample by mixed melting point and infrared spectra.[8] Two ethylenic linkages were thus shown to be at C_5 and C_{22}, and this was also supported by the infrared absorption bands at 798, 840 (Δ^5) and 970 cm.$^{-1}$ (Δ^{22}).[9]

From these results the sterol in *Hypnea japonica* was clearly shown to be 22-dehydrocholesterol, already synthesized by Bergmann and Dusza who expected its natural occurrence on biogenetic grounds.[10] The physical properties of synthetic and natural sterols and some of their derivatives are in good agreement as shown in Table I.

Experimental[11]

Extraction and Purification of 22-Dehydrocholesterol.—Dried, powdered *Hypnea japonica* (8.5 kg.) was extracted twice with 40 l. of boiling benzene for 25 hours. The dark brown oil (44 g.) obtained after removal of the solvent was saponified with a mixture of methanol (50 ml.), benzene (10 ml.) and 40% aqueous sodium hydroxide solution (20 ml.) by refluxing for 2.5 hours. The cooled reaction mixture was diluted with 150 ml. of methanol and 100 ml. of water and then extracted four times with benzene. The combined extract was washed twice with 40% aqueous methanol solu-

(1) Preceding paper, E. Ohki, *Chem. Pharm. Bull. (Tokyo)*, in press.

(2) Takamine Laboratory, Sankyo Co., Ltd., Shinagawa, Tokyo, Japan.

(3) (a) K. Tsuda, R. Hayatsu, Y. Kishida and S. Akagi, THIS JOURNAL, **80**, 921 (1958); (b) K. Tsuda, S. Akagi, Y. Kishida, R. Hayatsu and K. Sakai, *Chem. Pharm. Bull. (Tokyo)*, **6**, 724 (1958); (c) R. Hayatsu, *Pharm. Bull. (Tokyo)*, **5**, 452 (1957).

(4) I. M. Heilbron, R. F. Phipers and H. R. Wright, *Nature*, **133**, 419 (1934); *J. Chem. Soc.*, 1572 (1934); D. H. Coffey, I. M. Heilbron, F. S. Spring and H. R. Wright, *ibid.*, 1205 (1935); I. M. Heilbron, E. G. Parry and R. F. Phipers, *Biochem. J.*, **29**, 1376 (1935); D. H. Coffey, I. M. Heilbron and F. S. Spring, *J. Chem. Soc.*, 738 (1936); P. W. Carter, I. M. Heilbron and B. Lythgoe, *Proc. Roy. Soc. (London)*, **B128**, 82 (1939); H. B. MacPhillamy, THIS JOURNAL, **64**, 1732 (1942); D. H. Hey, J. Honeyman, W. G. Peal, *J. Chem. Soc.*, 2881 (1950); W. Bergmann and M. Klosty, THIS JOURNAL, **73**, 2935 (1951).

(5) We have also found that a new sterol, sargasterol, 20-isofucosterol[3] occurred concomitantly with fucosterol in two species of brown algae (*Sargassum ringgoldianum* and *Eisenia bicyclis*).

(6) K. Tsuda, S. Akagi and Y. Kishida, *Science*, **126**, 927 (1957); K. Tsuda, S. Akagi and Y. Kishida, *Pharm. Bull. (Tokyo)*, **6**, 101 (1958).

(7) D. R. Idler, S. W. Nicksic, D. R. Johnson, V. W. Meloche, H. A. Schuette and C. A. Baumann, THIS JOURNAL, **75**, 1712 (1953).

(8) We have noticed that α-methylisovaleraldehyde 2,4-dinitrophenylhydrazone (m.p. 120–121°) obtained on ozonolysis of ergosteryl acetate showed only slight depression (*ca.* 1°) of melting point on admixture with isovaleraldehyde 2,4-dinitrophenylhydrazone; however, the infrared spectra were different in the finger-print region, the former exhibiting bands at 829, 834 and 980 cm.$^{-1}$ and the latter at 833, 840 and 960 cm.$^{-1}$.

(9) A. R. H. Cole, "Progress in the Chemistry of Organic Natural Products," Vol. XIII, Springer-Verlag, Wien, 1956, p. 41.

(10) W. Bergmann and J. P. Dusza, *J. Org. Chem.*, **23**, 1245 (1958).

(11) All melting points are uncorrected. The optical rotations are for chloroform solutions unless otherwise noted.

TABLE I

	Synthetic		Natural	
	M.p., °C.	$[\alpha]_D$	M.p., °C.	$[\alpha]_D$
Sterol	133.5–134	−57.3°	134.5–135.5	−56.9°
Acetate	126–126.5	−63.2	127–128	−61.1
Benzoate	146–147	−29.2	145–146	−29.0
Tetrabromide	188–189 d.		186–187 d.	

tion. The solvent was removed and the residual non-saponifiable substance (10.5 g.) was treated with acetic anhydride and pyridine. On chromatographing the resulting acetate on an alumina (500 g.) column, 6.2 g. of brownish crystalline acetate of m.p. 121–125° was obtained from an eluate of a mixture of petroleum ether and benzene (5:1). The acetate was saponified with 5% methanolic potash to afford 4.9 g. of crude sterol, which was chromatographed on a column of alumina (250 g.). The recovered sterol (3.0 g.) of m.p. 130–132° eluted with a mixture of benzene and methanol (1:1) was recrystallized once from methanol to give pure 22-dehydrocholesterol (2.4 g.) of m.p. 134–135.5°, $[\alpha]^{29}D$ −56.3° (*c* 1.10).

The sterol (500 mg., m.p. 134–135.5°) was heated on a steam-bath for two hours with *p*-phenylazobenzoyl chloride (450 mg.) in 10 ml. of pyridine. The cooled mixture was diluted with water, and the precipitated crude azoyl ester was filtered, washed throughly with water, and dried, yield 750 mg. The ester was dissolved in 15 ml. of warm benzene and filtered to remove most of the pyridinium salt.

The filtered solution was adsorbed on a column, 5 cm. inside diameter and 60 cm. length, which was prepared by packing with a slurry of silicic acid[12]–Celite 503 (2:1) in petroleum ether under slight pressure. A mixture of petroleum ether (b.p. 60–80°) and benzene (4:1) was passed through the column at the flow rate of 5 ml. per minute for 15 hours, until a single orange colored band had come down about in the middle of the column. The azoyl ester recovered from this band was recrystallized from benzene–ethanol to orange needles, m.p. 192–193°, yield 550 mg.

Anal. Calcd. for $C_{40}H_{52}O_2N_2$: C, 81.04; H, 8.84; N, 4.73. Found: C, 81.38; H, 8.92; N, 4.84.

The azoyl ester (500 mg.) was refluxed for one hour on a steam-bath with a mixture of benzene (20 ml.), water (8 ml.), ethanol (25 ml.) and 8% potash solution (12 ml.) in 70% ethanol. The cooled mixture was diluted with water, extracted with benzene, the extract was washed with water, dried, and the solvent was evaporated. The crystalline residue was recrystallized from benzene–methanol to give 22-dehydrocholesterol, m.p. 134.5–135.5°, yield 300 mg., $[\alpha]^{29}D$ −56.9° (c 1.32); ν_{max}^{KBr} 3400 (OH), 798, 840 (Δ^5) and 970 cm.$^{-1}$ (Δ^{22}).

Anal. Calcd. for $C_{27}H_{44}O$: C, 84.31; H, 11.53. Found: C, 84.35; H, 11.36.

The acetate of the sterol crystallized from methanol in leaflets, m.p. 127–128°, $[\alpha]^{31.5}D$ −61.1° (*c* 1.06).

Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.56; H, 10.59.

The benzoate crystallized from benzene–ethanol in needles, m.p. 145–146°, $[\alpha]^{30.5}D$ −29.0° (c 1.08).

Anal. Calcd. for $C_{34}H_{48}O_2$: C, 83.55; H, 9.90. Found: C, 83.51; H, 9.90.

Steryl Acetate Tetrabromide.—To a solution of 210 mg. (0.5 mmole) of the steryl acetate in 0.8 ml. of dry ether was added 1.6 ml. of 10% solution of bromine in acetic acid. The precipitated crystalline material was collected and recrystallized from ethyl acetate to afford tetrabromocholestanyl acetate of m.p. 186–187° dec., yield 190 mg.

Anal. Calcd. for $C_{29}H_{46}Br_4O_2$: C, 46.62; H, 6.16; Br, 42.83. Found: C, 46.91; H, 6.33; Br, 43.02.

The tetrabromide (430 mg.) in 9 ml. of acetic acid and 4 ml. of ether was refluxed with 400 mg. of zinc dust on a water-bath for 1.5 hours. The cooled mixture was diluted with water and extracted with benzene. The extract was washed with aqueous sodium carbonate solution, dried, and the solvent was removed. The residue was recrystallized from methanol to give the parent steryl acetate of m.p. 127–128°.

Stenone.—A mixture of 390 mg. of the sterol, 4.5 ml. of freshly distilled cyclohexanone and 14 ml. of toluene was distilled until 5 ml. of toluene had been removed. A solution of 600 mg. of aluminum isopropoxide in 4 ml. of toluene was added and the mixture was refluxed for two hours. To the cooled mixture was added 7 ml. of a saturated aqueous solution of Rochelle salt and the volatile solvent was removed by steam distillation. The oily residue was extracted twice with benzene, the extract was dried and the solvent was removed under reduced pressure. The residue, was, after chromatography on alumina with a mixture of petroleum ether and benzene (3:1), recrystallized from aqueous methanol to give 22-dehydrocholestenone, m.p. 69–70°, colorless needles, yield 140 mg., $[\alpha]^{28}D$ +69.5° (c 1.03), λ_{max}^{EtOH} 240.5 mμ (ε 18.000); ν_{max}^{KBr} 1682 (3 CO), 1620 (Δ^4) cm.$^{-1}$.

Anal. Calcd. for $C_{27}H_{42}O$: C, 84.75; H, 11.07. Found: C, 84.65; H, 10.96.

Ozonolysis.—The steryl acetate (85 mg.) in 20 ml. of methylene chloride was treated at −30° with ozonized air (0.73 mmole of ozone per minute) for 70 seconds. Zinc dust (200 mg.) and 20 ml. of acetic acid was then added and the mixture was stirred at room temperature for two hours. The mixture was steam distilled into a solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The hydrazone was chromatographed on a silicic acid–Celite column (2:1)[13] with a mixture of petroleum ether and benzene (9:1), showing a single yellow colored zone, which was eluted and recrystallized from methanol to give isovaleraldehyde 2,4-dinitrophenylhydrazone, m.p. 121–123°, yield 26 mg. (49.4 %), which was identified by mixed melting point and infrared spectra with an authentic sample prepared by oxidation of isoamyl alcohol.

Cholestanyl Acetate.—A solution of 120 mg. of the steryl acetate in 40 ml. of acetic acid was shaken with platinum oxide at 1 atm. pressure in an atmosphere of hydrogen. The catalyst was removed by filtration and the solvent was evaporated under reduced pressure. The residue was recrystallized twice from methanol to give cholestanyl acetate, m.p. 109–110°, yield 75 mg., which showed no depression of the melting point on admixture with an authentic sample. Their infrared spectra were completely superimposable.

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(12) Analytical reagent (100 mesh) prepared for chromatography; Mallinckrodt Chemical Works.

(13) B. E. Gordon, F. Wopat, Jr., H. D. Burnham and L. C. Jones, Jr., *Anal. Chem.*, **23**, 1754 (1951).