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The Identification of Fucosterol in the Marine Brown Algae *Hizikia fusiformis*

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During the course of our studies on the activator of pig pancreatic lipase (E.C. 3.1.1.3),[1] the sterol isolated from the unsaponifiable matter of *Hizikia fusiformis* has been found to possess an accelerating action on lipase activity *in vitro*.[2]

Many reports have been published on the occurrence of fucosterol in marine brown algae,[3~5] but very little information is available on sterols in *H. fusiformis* (Japanese name, hiziki), long used in Japan as an edible seaweed. Shirahama (1936)[6] suggested that the sterol, "pelvesterol," isolated from hiziki might be fucosterol, solely on the basis of the chemical constants compared with those reported by Heilbron *et al.*[3] Therefore, we considered it would be of interest, because of its activity as a lipase activator,[2] to reinvestigate the sterol by means of the more refined techniques now available.

In the present work, the major component of the sterol fraction in hiziki was identified as fucosterol, the typical sterol of marine brown algae,[4] by means of chemical constants, TLC, GLC, and IR and MS spectrometries. The NMR spectrum suported this conclusion, thereby excluding the possible occurrence of 28-iso-fucosterol. An additional minor peak suggested to be 24-methylenecholesterol was detected by GLC. The lipase activating activity of fucosterol has been reported elsewhere.[2]

The extraction procedure of the unsaponifiable matter (18 g) from the crude lipids (110 g) in hiziki (10 kg), harvested on the Sanriku coast of Japan in autumn of 1968, has been described previously.[1] Sterol content of the unsaponifiable matter determined by precipitation with tomatine as well as digitonin was 55%, corresponding to 0.1% of the dry weight. From a portion (12 g) of the unsaponifiable matter, crude sterols (4 g) were obtained by recrystallization from MeOH, and acetylated with pyridine and acetic anhydride. The major fraction of steryl acetate was separated by preparative TLC (1 mm layer of silica gel G; petroleum ether/ether/AcOH=80/20/1 as the solvent), and recrystallized from MeOH. After saponification, the free sterol was purified again by preparative TLC and recrystallized from MeOH to the constant mp. A crystalline sterol (700 mg) which gave a positive Liebermann-Burchard reaction (λ_{max} 620 nm) was obtained.

The mp (123.5~124°C; 119~120°C) and optical rotations ($[\alpha]_D$: −36°; −41°) of the hiziki sterol thus isolated and its acetate, respectively, proved to be in good agreement with those of authentic fucosterol (kindly supplied by Ikekawa *et al.*[7]). By TLC (0.25 mm thick; benzene/EtOAc=5/1), it was also found that the hiziki sterol gave a single spot with an *Rf* value (=0.40) identical to that of fucosterol and it occupied a major part of the "total sterol*" in hiziki. Further, the GLC on OV-17 as well as SE-30 as the stationary phases showed almost a single peak (97%) with the same relative retention time (Rt_R) as that of fucosterol, except for an additional minor peak (3%) (Table I). This minor peak might be taken to that of 24-methylenecholesterol by comparing its Rt_R with that of this sterol recently reported by Ikekawa *et al.*[7] and Patterson,[8] as shown in parentheses in Table I. On the other hand, GLC revealed that the hiziki sterol content of the "total sterol" was about 80% (as fucosterol). The IR spectrum of the hiziki sterol was in complete agreement with that of fucosterol, with peaks at

TABLE I. RELATIVE RETENTION DATA FOR HIZIKI STEROL AND STEROL STANDARDS

Compound	Rt_R[a]	
	SE-30[b]	OV-17[c]
	TMSi[d] (Acetate[8])	TMSi[d] (Free[7])
Cholesterol	1.00 (1.00)	1.00 (1.00)
24-Methylene-cholesterol	— (1.26)	— (1.33)
Fucosterol	1.65 (1.63)	1.68 (1.59)
Hiziki sterol	1.65	1.68
Unidentified sterol[e]	1.27	1.35

a) Relative to cholesterol; values in parentheses are for Rt_R reported in literature.[7,8]
b) Column 0.3×150 cm, 2% SE-30 on 60~80 mesh Chromosorb W; column temp. 250°C; nitrogen 40 ml/min; t_R of cholesterol 6.75 min.
c) Column 0.4×150 cm, 1% OV-17 on 60~80 mesh Chromosorb W; column temp. 260°C; nitrogen 45 ml/min; t_R of cholesterol 6.05 min.
d) TMSi=trimethylsilyl ether.
e) Minor sterol (3%) contained in the hiziki sterol fraction.

* The "total sterol" in hiziki was obtained by digitonin precipitation method from the unsaponifiable matter.

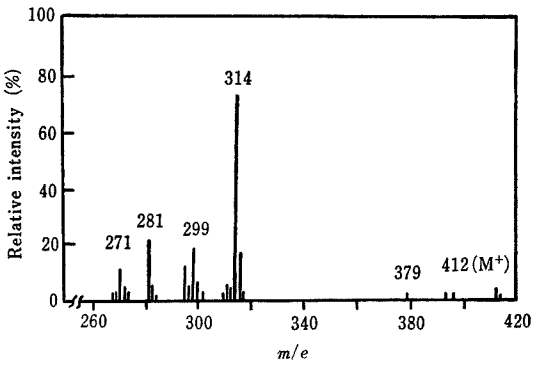

FIG. 1. Mass Spectrum of the Hiziki Sterol.

3420 cm^{-1} and 840 cm^{-1} (Δ^5) and a peak at 820 cm^{-1} (Δ^{24}).

Furthermore, the NMR spectrum of the hiziki sterol was essentially the same as that of fucosterol with peaks at 0.73 δ (3H, singlet, Me), 1.63δ (3H, doublet, J=7Hz, C=CH-Me), 3.53δ(1H, broad, CHOH), 5.20δ(1H, multiplet, C=CH-Me) and 5.37δ(1H, multiplet, C=CH). In this spectrum, a multiplet at 2.8 δ as observed in that of 28-iso-fucosterol, an epimer of fucosterol, found in some green algae recently,[9] was not detected. Also the MS spectrum gave the fragmentation pattern, being in complete accord with that of fucosterol with a parent ion (M^+) at m/e 412 (4.3%) and a fragment ion at m/e 314 [M^+—part of side chain (C_7H_{14}), 74%], which is characteristicof $\Delta^{24(28)}$-sterol[10] (Fig. 1).

In the NMR spectrum, however, an additional peak was observed at 1.9δ (1.5H, singlet) which was not found in that of fucosterol. Since this peak did not diminish after recrystallization, it might be due to the possible presence of the isomer of fucosterol such as discussed in the reference,[11] in which a double bond at C-24(28) is rearranged to C-24 (25); the isomer is expected to be indistinguishable from fucosterol in its physical properties, such as IR and MS spectra. The possibility of occurrence of this isomer is under investigation.

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