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Determination of Fucosterol in the Marine Algae *Pelvetia siliquosa* by Gas Chromatography

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Abstract – A new method for the quantitative determination of fucosterol in the marine algae *Pelvetia siliquosa* by gas chromatography was established. A HP-1 column programmed as 200°C (5 min) → 5/min → 280°C (10 min) was developed. The analysis of fucosterol in *P. siliquosa* was successfully carried out within 30 min.
Keywords – *Pelvetia siliquosa*, Fucaceae, fucosterol, gas chromatography.

Introduction

Pelvetia siliquosa belongs to the family Fucaceae. *P. siliquosa* is a marine algae endemic to the Korean peninsula and self-grown on the craggy surfaces near of the southern seashores (Yoon, 1995). It has traditionally been used as seasoned sea greens for religious services or as health food (Oh *et al.*, 1990). In the previous paper, we reported that the ether fraction of *P. siliquosa* exhibits the hepato-protective and anti-diabetic effects (Lee *et al.*, 2002), and fucosterol from *P. siliquosa* have an anti-oxidant activity (Lee *et al.*, 2003).

In this study, fucosterol in *P. siliquosa* was quantified by gas chromatography. The results are of value for quality control of the marine algae *P. siliquosa* and its preparations.

Experimental

Instruments and reagents – IR spectrum was recorded with a Jasco FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AVANCE 400 NMR spectrometer in $CDCl_3$ using TMS as internal standard. MS spectrum was measured with a Jeol JMS-AX505WA mass spectrometer. GC chromatogram was recorded with a YoungLin M600D GC instrument equipped with HP-1 column. All other chemicals and reagents were analytical grade.

Materials – *Pelvetia siliquosa* Tseng et Chang (Fucaceae) was collected at Jindo area, Jeonnam Province in 2002 and botanically identified by Prof. Jong-Ahm Shin, Yosu National University, Korea. The voucher specimen has been deposited at the Seokwon Life Science Research Institute, World Sea Green Co. Ltd., Korea.

Extraction and isolation – The air-dried and powdered whole parts (4 kg) of *P. siliquosa* were extracted three times with *n*-hexane and MeOH (1:5) under reflux. The resultant extract was combined and concentrated under reduced pressure to afford 59 g of the residue. The MeOH extract was suspended in water and then extracted successively with equal volumes of *n*-hexane, $CHCl_3$, EtOAc, and *n*-BuOH. Each fraction was evaporated *in vacuo* to obtain *n*-hexane (39 g), $CHCl_3$ (1 g), EtOAc (1 g), and *n*-BuOH (1 g) fractions.

A portion of the *n*-hexane fraction (30 g) was chromatographed on silica gel column eluting with a gradient of *n*-hexane-EtOAc to afford fucosterol (756 mg).

Fucosterol; EI-MS (70 eV, rel. int., %): *m/z* 412 $[M]^+$ (11.9), 397 (3.8), 394 (2.8), 379 (3.7), 314 (100), 299 (23.9), 296 (16.7), 281 (24.5), 271 (12.6), 255 (5.6), 253 (5.1), 229 (21.4), 213 (11.5), 145 (10.7), 55 (25.3); IR ν_{max} (KBr): 3439, 2936, 1626, 823 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.36 (1H, br d, *J* = 5.2 Hz, H-6), 5.19 (1H, q, *J* = 6.7 Hz, H-28), 3.53 (1H, m, H-3), 1.58 (3H, d, *J* = 6.7 Hz, H-29), 1.02 (3H, s, H-19), 1.01 (3H, br s, H-21), 1.00 (3H, d, *J* = 1.2 Hz, H-27), 0.98 (3H, d, *J* = 1.2 Hz, H-26), 0.70 (3H, s, H-18); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 146.9 (C-24), 140.7 (C-5), 121.6 (C-6), 115.5 (C-28), 71.7 (C-3), 56.7 (C-14), 55.7 (C-17), 50.1 (C-9), 42.3 (C-13), 42.2 (C-4), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.4 (C-20), 35.2 (C-22), 34.7 (C-25), 31.8 (C-7,8), 31.5 (C-2), 28.2 (C-16), 25.6 (C-23), 24.3 (C-15), 22.2 (C-

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Table 1. Condition of GC for fucosterol analysis in *P. siliquosa*

Items	Condition
Instrument	YoungLin M600D GC
Column	HP-1 (30 m × 0.32 mm × 0.25 μm)
Detector	F. I. D.
Carrier gas	N_2
Injection port temperature	285°C
Detector temperature	280°C
Temperature program	200°C (5 min)→5°C/min→280°C (10 min)

Table 2. Fucosterol contents in the marine algae

Samples	Amounts of extracts (mg/g)	Fucosterol contents (mg/g)
Pelvetia siliquosa	21.6	0.426±0.012[a)]
Sagassum piluliferum	14.7	0
Enteromorpha prolifera	4.2	0
Geildium amansii	0.9	0

[a)]The Mean ± S.D. of triplicate determinations.

26), 22.1 (C-27), 21.0 (C-11), 19.4 (C-19), 18.7 (C-21), 13.1 (C-29), 11.8 (C-18).

Standard and sample preparation – For the quantification of fucosterol, a standard solution was prepared as 0.5, 1.0, 2.0, 4.0, and 8.0 μg/ml in DMSO. Each 1 g of the marine algae was extracted with 20 ml of MeOH, stirred at room temperature for 12 hr and evaporated *in vacuo*. The residue was dissolved in 2 ml of DMSO and filtered. The resulting solution was as a sample solution for quantification by GC analysis.

GC analysis – The analysis condition for the identification and quantification of fucosterol in GC was shown at Table 1. The injection volume was 10 μl. All injection was performed in triplicate.

Results and Discussion

A chromatographic separation of the *n*-hexane fraction from *P. siliquosa* led to the isolation of sterol (Fig. 1). It was elucidated as fucosterol by comparison with an authentic sample as described in the literature (Atta-ur-

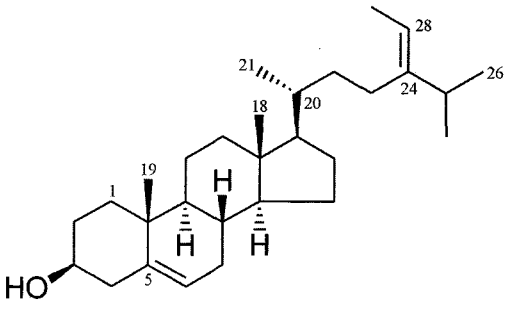

Fig. 1. Structure of fucosterol isolated from *P. siliquosa*.

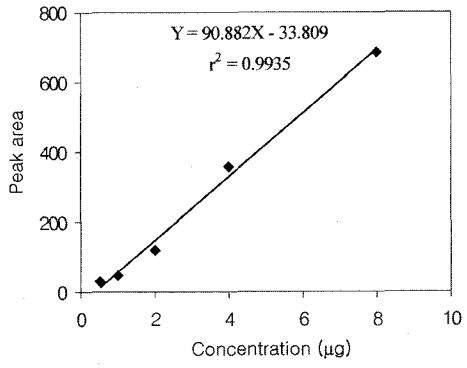

Fig. 2. Calibration curve for fucosterol.

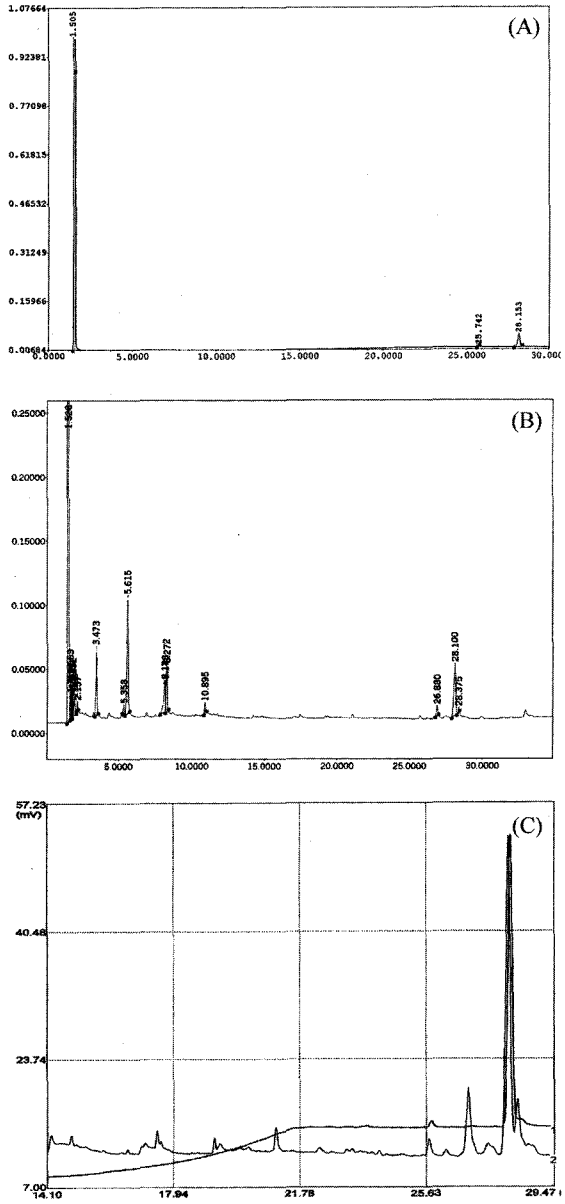

Fig. 3. GC chromatograms of standard fucosterol (**A**), the MeOH extract of the marine algae *P. siliquosa* (**B**) and overlap with standard (**C**).

Rahman *et al.*, 1999). It has been reported that fucosterol decrease angiotensin-converting enzyme levels with reduction of glucocorticoid receptor in endothelial cells (Hagiwara *et al.*, 1986). Also it inhibited the lymphatic absorption of cholesterol in rats (Ikeda *et al.*, 1988). It exhibited antifungal activity against *Curvularia lunata, Stachybotrys atra* and *Microsporum canis* (Atta-ur-Rahman *et al.*, 1997). It also exhibited cytotoxic activity against P-388 cancer cell lines (Tang *et al.*, 2002). Fucosterol was the main component responsible for various activities of the algae.

Fucosterol in *P. siliquosa* was quantified by GC. As shown in Fig. 2, the standard curve for fucosterol was Y = 90.882X - 33.809 and correlation constant was 0.9935. Fig. 3 demonstrates the satisfactory resolution achieved for the major component of the extract. Standard fucosterol was retained at about 28.1 min. In the GC profile of sample solution, the expected fucosterol peak was retained at about 28.1 min. Table 2 showed the contents of fucosterol in the marine algae. The content of fucosterol in *P. siliquosa* is 0.426±0.012 mg/g. It was not detected in *Sagassum piluliferum, Enteromorpha prolifera,* and *Geildium amansii.*

Based on the results, it may be concluded that GC remains the method of choice for the assay of a most relevant component of the marine algae. Direct analysis by GC represents a valuable alternative to obtain typical fingerprints of the marine algae and a reliable identification of a number of the marine algae components.

Acknowledgments

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