

Note

Antitumor Promoters in Leaves of Jute (*Corchorus capsularis* and *Corchorus olitorius*)

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Two antitumor promoters against tumor promoter-induced Epstein-Barr virus activation were isolated from the leaves of jute (*Corchorus capsularis* L.). The antitumor-promoting activity was examined by an immunoblotting analysis. Their active components were identified as phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and monogalactosyldiacylglycerol (1,2-di-*O*- α -linolenoyl-3-*O*- β -D-galactopyranosyl-*sn*-glycerol) by spectroscopic data and chemical and enzymatic reactions. The content of the latter in four cultivars of *C. capsularis* L. and *C. olitorius* L. was found to vary with the cultivar. The detectable amount of each active component increased by treatment of the leaves with hot water.

Keywords: *Corchorus capsularis* L., *Corchorus olitorius* L., jute, phytol, monogalactosyldiacylglycerol, antitumor promoter, cancer chemoprevention

Recent epidemiological investigations have been providing increasing evidence that a high consumption of vegetables and fruits could be associated with a reduced risk of cancer, especially that in the gastrointestinal tract (Steinmetz & Potter, 1991a, 1991b, 1996; Block *et al.*, 1992; Ziegler, 1991). These epidemiological predictions suggest that vegetables and fruits contain anticancer or antitumor-promoting components. Thus the physiologically functional components in vegetables could be good sources of chemopreventive agents with low-toxicity available in our daily food supply.

Jute (white jute, *Corchorus capsularis* L.; nalta jute or tossa jute, *C. olitorius* L.; Tiliaceae family), known as a fiber plant, is a time-honored medicinal vegetable in North Africa, the Middle and Near East and Southeast Asia. The young leaves of *C. olitorius* that have been introduced into Japan as a healthy vegetable, moroheiya, are rich in vitamins, carotenoids, calcium, potassium and dietary fiber (Resources Council, Science and Technology Agency, Japan, 2000).

During our investigation of edible plants as sources of functional constituents, we found that jute leaves contain two active components against tumor promoter-induced Epstein-Barr virus (EBV) activation in Raji cells. We report herein the identification of the active components (phytol and monogalactosyldiacylglycerol), variation of their contents among four cultivars of jute and change in their detectable amounts by treatment with hot water.

Materials and Methods

General procedure NMR spectra were recorded with a JEOL A400 FT NMR spectrometer at 400 MHz for ¹H and 100.4 MHz for ¹³C. NMR chemical shifts were referenced to the signal of the deuterated chloroform (CDCl₃, δ_{H} 7.24, δ_{C} 77.0).

Mass spectra were obtained with a JEOL JMS-SX102AQQ hybrid mass spectrometer. *m*-Nitrobenzylalcohol was the matrix for FABMS, and in EIMS the ionization voltage was 70 eV.

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) and D-galactose oxidase were purchased from Sigma Chemical Co. (St. Louis, MO) and phytol from Nacalai Tesque (Kyoto). Dulbecco's modified Eagle's medium was purchased from ICN Biomedicals (Ohio, U.S.A.) and fetal calf serum (FCS) from Boehringer Mannheim K.K. (Tokyo). Chromatographic materials used were silica gel 60 for column chromatography from Nacalai Tesque and Kieselgel 60 F₂₅₄ for TLC from Merck (Darmstadt, Germany).

Plant materials Several cultivars of *Corchorus capsularis* L. were cultivated in a field on our faculty farm in 1998, and the leaves were used for isolation of the active components.

The seeds of *C. olitorius* L., tentatively called cultivar M, were purchased from Ubagai Seed Co. (Ehime), and the other seeds of *C. capsularis* L. (cultivars A-38 and C-443) and *C. olitorius* L. (O-9897) were provided from the Bangladesh Institute of Nuclear Agriculture, Bangladesh. Four of the cultivars were grown in the same field in 2000. Two months after sowing the seeds, the leaves were harvested, freeze-dried and powdered; these leaves were stored at -30°C until used.

Extraction and isolation of phytol (1) and monogalactosyldiacylglycerol (2) The fresh leaves of jute (3.7 kg) were treated twice with methanol at room temperature. The combined methanol solution was concentrated under reduced pressure, and the aqueous residue obtained was partitioned with benzene to give a benzene-soluble fraction (32.4 g). A portion of this fraction (10 g) was subjected to silica gel column chromatography that was successively developed with 0, 2, 5, 10, 20, 30, 40, 50 and 100% acetone in hexane. The 10% fraction (830 mg) was purified by preparative TLC [acetone/chloroform (1 : 9)] to afford **1** (166 mg). The 50 and 100% fractions (2.1 g) were combined and sub-

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jected to silica gel column chromatography (5% methanol in chloroform). The fractions containing compound **2** were further purified by preparative HPLC [column, Cosmosil 5C₁₈-AR-II (250×10 mm i.d.); solvent, methanol; flow rate, 2 ml/min; detection, refractive index (Shimadzu RID-6A)] to give **2** (178 mg).

Compound 1 Colorless oil. ¹H-NMR δ_H (CDCl₃): 0.82 (3H, d, *J*=6.6 Hz, H-18 or H-19), 0.83 (3H, d, *J*=6.3 Hz, H-18 or H-19), 0.84 (6H, d, *J*=6.6 Hz, H-16, H-17), 0.99–1.44 (18H, m, overlapping NMR signal, H-5 to H-14), 1.50 (1H, hept-like, H-15), 1.65 (3H, br. s, H-20), 1.97 (2H, br. t, *J*=7.6 Hz, H-4), 4.13 (2H, br. d, *J*=7.1 Hz, H-1), 5.39 (1H, ttq, *J*=7.1, 1.3, 1.3 Hz, H-2). ¹³C-NMR δ_C (CDCl₃): 16.2 (q), 19.70 (q), 19.74 (q), 22.6 (q), 22.7 (q), 24.5 (t), 24.8 (t), 25.1 (t), 28.0 (d), 32.7 (d), 32.8 (d), 36.6 (t), 37.28 (t), 37.35 (t), 37.42 (t), 39.4 (t), 39.9 (t), 59.4 (t), 123.1 (d), 140.3 (s). The multiplicity of the ¹³C signals was demonstrated by the DEPT experiment. EIMS *m/z*: 296 (M⁺). The molecular formula is regarded as C₂₀H₄₀O.

Compound 2 Colorless oil. [α]_D²⁸ –4.0° (*c* 0.6, chloroform). ¹H-NMR δ_H (CDCl₃): 0.96 (6H, t, *J*=7.6 Hz, H-18', H-18''), 1.23–1.37 (16H, m, H-4' to H-7', H-4'' to H-7''), 1.59 (4H, m, H-3', H-3''), 2.01–2.09 (8H, m, H-8', H-17', H-8'', H-17''), 2.29 (2H, t, *J*=7.5 Hz, H-2'), 2.30 (2H, t, *J*=7.5 Hz, H-2''), 2.78 (8H, t-like, H-11', H-14', H-11'', H-14''), 3.52 (1H, ddd, *J*=6.0, 4.3, 1.0 Hz, H-5), 3.57 (1H, dd, *J*=9.4, 3.2 Hz, H-3), 3.63 (1H, dd, *J*=9.4, 7.4 Hz, H-2), 3.72 (1H, dd, *J*=11.2, 6.3 Hz, H-*sn*-3), 3.83 (1H, dd, *J*=12.0, 4.3 Hz, H-6), 3.89 (1H, dd, *J*=11.2, 5.5 Hz, H-*sn*-3), 3.94 (1H, dd, *J*=12.0, 6.0 Hz, H-6), 3.99 (1H, dd, *J*=3.2, 1.0 Hz, H-4), 4.19 (1H, dd, *J*=12.1, 6.5 Hz, H-*sn*-1), 4.25 (1H, d, *J*=7.4 Hz, H-1), 4.37 (1H, dd, *J*=12.1, 3.5 Hz, H-*sn*-1), 5.25–5.41 (12H, m, H-9', H-10', H-12', H-13', H-15', H-16', H-9'', H-10'', H-12'', H-13'', H-15'', H-16''), 5.29 (1H, overlapping NMR signal, H-*sn*-2). ¹³C-NMR δ_C (CDCl₃): 14.3 (q, C-18', C-18''), 20.5 (t, C-17', C-17''), 24.8 and 24.9 (t, C-3', C-3''), 25.5 and 25.6 (t, C-11', C-14', C-11'', C-14''), 27.2 (t, C-8', C-8''), 29.04 (t, C-4'), 29.09 (t, C-4''), 29.13 (t, C-6', C-6''), 29.18 (t, C-5', C-5''), 29.6 (t, C-7', C-7''), 34.1 (t, C-2'), 34.3 (t, C-2''), 62.6 (t, C-6), 62.7 (t, C-*sn*-1), 68.3 (t, C-*sn*-3), 69.4 (d, C-4), 70.2 (d, C-*sn*-2), 71.6 (d, C-2), 73.4 (d, C-3), 74.5 (d, C-5), 104.0 (d, C-1), 127.1 (d, C-15', C-15''), 127.75 and 127.76 (d, C-10', C-10''), 128.2 and 128.3 (d, C-12', C-13', C-12'', C-13''), 130.20 and 130.21 (d, C-9', C-9''), 132.0 (d, C-16', C-16''), 173.5 (s, C-1''), 173.8 (s, C-1'). FABMS *m/z*: 797 (M⁺+Na). The molecular formula is regarded as C₄₅H₇₄O₁₀.

Hydrolysis of compound 2 A solution of **2** (20 mg) in 0.1 M NaOH (1 ml) was allowed to stand for 2 h at 65°C. The reaction mixture was acidified with 0.1 M HCl, washed with ethyl acetate and evaporated to dryness. The residue was treated overnight with 1 M HCl at 110°C to give a mixture of galactose and glycerol. The resulting solution was analyzed by TLC [acetone/H₂O (9 : 1), sprayed with the naphthoresorcinol-H₂SO₄ reagent and heated] and HPLC [column, Gelpack GL-C611 (300×10.7 mm i.d.); solvent, 10⁻⁴ M NaOH; flow rate, 1 ml/min; detection, refractive index].

A solution of 0.2% sodium methoxide in methanol (1 ml) was added to **2** (10 mg), and the whole was left for 15 min at room temperature. The reaction mixture was diluted with acetic acid (1 ml) and H₂O (5 ml) and treated with hexane. The hexane solution was concentrated to afford methyl α-linolenate, which was analyzed by GLC [column, HP-1 (10 m×0.1 mm i.d., 0.1 μm film

thickness); oven temperature, 100–240°C, 5°C/min; carrier gas, N₂, 0.5 ml/min; detection, FID] and EIMS [*m/z* 292 (M⁺)]. The aqueous solution was concentrated to dryness. A solution of the residue in 1 M HCl (1.5 ml) was allowed to stand overnight at 120°C. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The hydrolysate obtained was purified by cellulose TLC [Merck; 1-butanol/acetic acid/H₂O (4 : 1 : 5)] to furnish galactose, the configuration of which was determined by the enzymatic reaction with D-galactose oxidase (Sturgeon, 1994).

Antitumor-promoting activity The antitumor-promoting activity was tested by an immunoblotting analysis using a mouse antiserum against EBV producer P3HR-1 cells. In Raji cells carrying the genome of the EBV early antigen (EA), the antiserum could detect the 48-kDa polypeptide (EBV EA-diffuse component) induced by TPA, and an antitumor promoter, curcumin, inhibited the induced polypeptide synthesis as previously described (Kondo *et al.*, 1998). The Raji cells were grown in Dulbecco's modified Eagle's minimum essential medium supplemented with 20 mM HEPES and 10% FCS at 37°C in a CO₂ (5%) incubator. The cells (2×10⁵ cells/ml) were incubated in 20 ml of the medium with or without inducers (1 mM sodium *n*-butyrate and 65 nM TPA) and the isolated components for 48 h, and then solubilized with an extraction buffer (about 10⁶ cells/25 μl) containing 1% Tween 20, 1% sodium deoxycholate and 1 mM EDTA. The solubilized extracts (20 μg of protein) of the cells were electrophoresed on 10% polyacrylamide slab gel containing 0.1% SDS. The proteins in the gel were electrophoretically transferred to a nitrocellulose sheet, and then the sheet was immunostained with the antiserum against P3HR-1 cells (1 : 50 dilution), biotinylated goat anti-mouse IgG (1 : 500 dilution, Amersham Pharmacia Biotech, Buckinghamshire, England) and peroxidase conjugated streptavidin (1 : 500 dilution, Zymed Lab., San Francisco, CA). The cytotoxicity of the isolated components was assessed by the number of viable cells measured with the trypan blue exclusion test.

Quantitative analysis of 1 and 2 The lyophilized leaf powder (1 g) of each cultivar (A-38 and C-443 of *C. capsularis*; O-9897 and M of *C. olitorius*) in methanol/chloroform (1 : 1, 10 ml) was sonicated for 30 min, after which the suspension was filtered. This treatment was repeated twice. The combined filtrate was concentrated to dryness. The residue was dissolved in 5% NaCl (10 ml), and the solution was treated with ethyl acetate (5 ml×3). The ethyl acetate solution obtained was concentrated and subjected to HPLC analysis [column, YMC-Pack Pro C18 (75×4.6 mm i.d.); solvent, methanol/H₂O (95 : 5); flow rate, 0.5 ml/min; detection, refractive index]. The quantity was estimated by comparison of the peak area with that of an authentic sample.

Treatment of the leaves with hot water A suspension of the leaf powder (cultivar A-38, 100 mg) in H₂O (5 ml) was incubated at 80°C. After incubation for 0, 10, 30 or 60 min, the suspension was cooled instantly in an ice bath. Methanol (5 ml and 3 ml) and methanol/chloroform (1 : 1, 3 ml) were used to extract the constituents, and the amount of each component was estimated in the manner described above.

Results and Discussion

Identification of the active components Two potent antitumor promoters, compounds **1** and **2**, were isolated by several

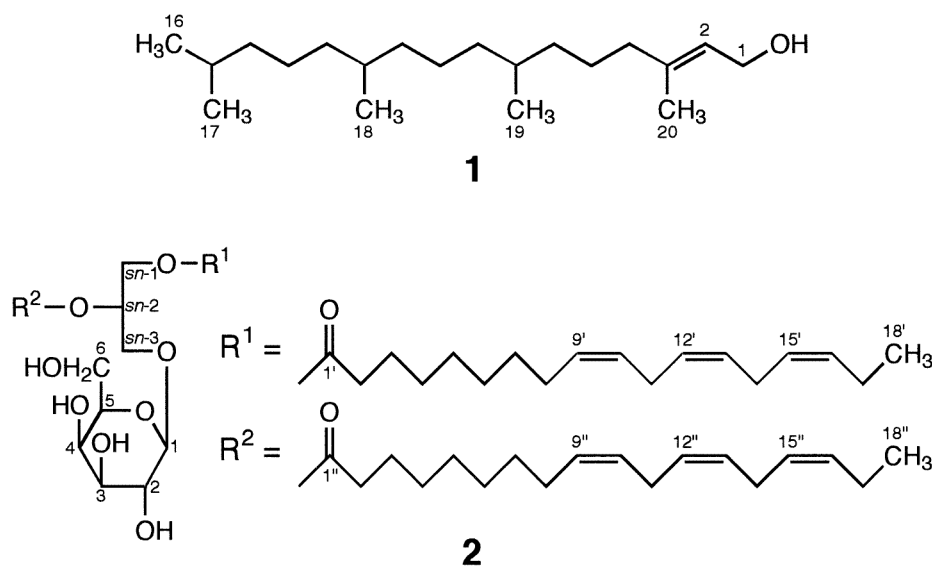


Fig. 1. Structures of phytol (1) and monogalactosyldiacylglycerol (2).

chromatographies as colorless oils from fresh leaves of jute (*Corchorus capsularis* L.). Compound **1** (Fig. 1) was identified as phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) by direct comparison of the NMR and mass spectra and behavior on TLC and HPLC with those of an authentic sample. Phytol (**1**) is a well-known acyclic diterpene and is present as the ester side chain in the molecule of chlorophylls (Harborne, 1998).

Compound **2** had an ion peak at m/z 797 ($M^+ + Na$) in the FAB mass spectrum. The ¹H- and ¹³C-NMR spectra of **2** suggested that this active component is a glycolipid having two unsaturated fatty acid moieties (δ_H 5.25–5.41, 12H, m; δ_C 173.5 and 173.8) in the molecule. Hydrolysis of **2** with sodium methoxide gave a single methyl α -linolenate, indicating that two fatty acid moieties in **2** are the α -linolenoyl group. Alkaline hydrolysis and subsequent acid hydrolysis of **2** afforded glycerol and galactose, and the D configuration of galactose was confirmed by the enzymatic reaction with D-galactose oxidase (Sturgeon, 1994). Furthermore, the stereochemistry of the anomeric carbon atom in a galactose moiety, β -anomer, was deduced from DIFNOE data for compound **2**. The attachment of galactose and α -linolenic acid to glycerol was deduced from the long-range ¹³C-¹H correlation detected in the LR-HETCOR (HMBC and COLOC) spectra (H-1/C-*sn*-3; H-*sn*-3/C-1; H-*sn*-1/C-1'; H-*sn*-2/C-1''). Therefore, the structure of compound **2** was established as 1,2-di-*O*- α -linolenoyl-3-*O*- β -D-galactopyranosyl-*sn*-glycerol (Fig. 1). Such galactolipids as **2** are known to occur in plant tissues, especially in chloroplast membranes of photosynthetic tissues (Douce & Joyard, 1980).

Antitumor-promoting activity To confirm the antitumor-promoting activity of phytol (**1**) and monogalactosyldiacylglycerol (**2**), we examined the effects of these components on the expression of EBV EA in Raji cells treated with inducers (TPA and sodium *n*-butyrate) using an immunoblotting analysis (Kondo *et al.*, 1998), a useful confirmation method for detecting antitumor promoters. In this assay, one polypeptide of 48 kDa (EBV EA) was detected in the inducer-treated cells, but not in the untreated cells by the antiserum against P3HR-1 cells. Com-

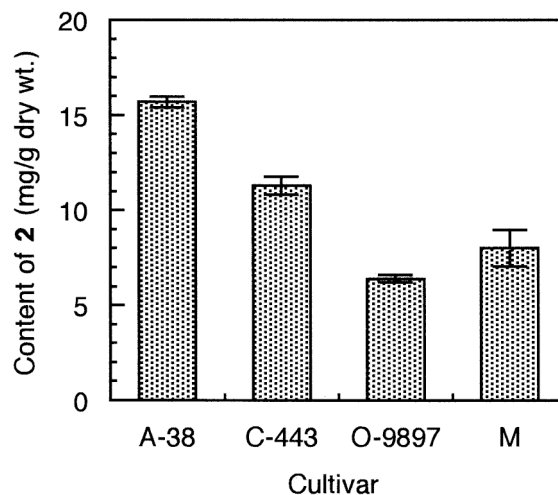


Fig. 2. Content of monogalactosyldiacylglycerol (**2**) in jute cultivars. Each value represents the mean \pm standard deviation of triplicate experiments. A-38 and C-443, cultivars of *C. capsularis*; O-9897 and M, cultivars of *C. olitorius*.

pounds **1** and **2** inhibited completely the induction of EBV EA at respective concentrations of 15 μ g/ml (50.7 μ M) and 30 μ g/ml (38.8 μ M) in the medium. However, the inhibitory effects of **1** and **2** at respective concentrations of 10 μ g/ml and 25 μ g/ml were insufficient. The number of viable cells in the inducer-treated Raji cells decreased to approximately 20% of that in the untreated cells. The number of viable cells in the inducer- and active component-treated Raji cells decreased to 81% (10 μ g/ml, **1**), 47% (15 μ g/ml, **1**), 67% (25 μ g/ml, **2**) and 42% (30 μ g/ml, **2**) of that in the inducer-treated cells.

So far, the same monogalactosyldiacylglycerol as **2** and a mixture of two other galactolipids have been isolated from the leaves of *Citrus hystrix* as potent inhibitors of EBV activation in Raji cells induced by teleocidin B-4 (Murakami *et al.*, 1995). The former has also been found to possess an *in vivo* antitumor-pro-

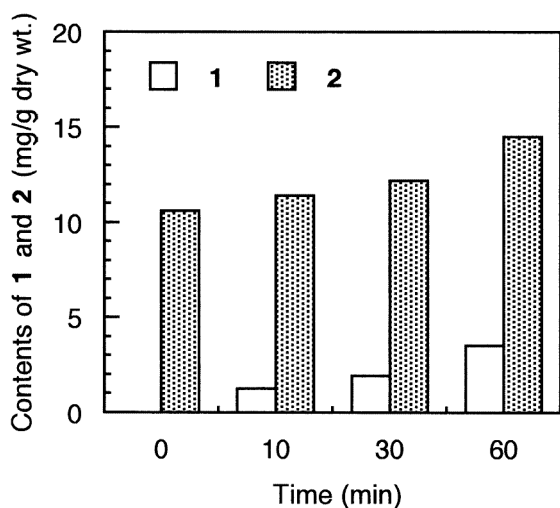


Fig. 3. Change of the detectable amounts of phytol (1) and monogalactosyldiacylglycerol (2) in jute leaves by treatment with hot water. Each value represents the mean of duplicate experiments.

moting effect on ICR mouse skin and anti-inflammatory activity on ICR mouse ear. In addition, there have been some reports of various antitumor-promoting glycolipids: monogalactosyldiacylglycerols and digalactosyldiacylglycerols from the green alga, *Chlorella vulgaris* (Morimoto *et al.*, 1995), digalactosyldiacylglycerols from the freshwater cyanobacterium, *Phormidium tenue* (Tokuda *et al.*, 1996), synthetic 1-*O*-acyl-3-*O*-(6'-*O*-acyl- β -D-galactopyranosyl)-*sn*-glycerols (Shirahashi *et al.*, 1996) and synthetic monogalactosyldiacylglycerols together with *sn*-2 lyso derivatives and galactosylglycerol (Nagatsu *et al.*, 1994). Furthermore, the anti-inflammatory effect of phytol has been demonstrated (Shimizu & Tomoo, 1994). These findings suggest that these constituents in plant leaves could play an important part in suppressing tumor-promotion.

Contents of the active components among cultivars of jute

To examine the contents of phytol (1) and galactolipid (2) among the cultivars of jute, four cultivars (A-38 and C-443 of *C. capsularis*; O-9897 and M of *C. olitorius*) were grown in the same field and harvested at the same time. In HPLC analysis of the extracts obtained from the lyophilized leaves, the content of 2 varied with the cultivar from 6.4 to 15.7 mg/g dry weight (Fig. 2), almost a 2.5 times difference between the lowest and highest values. Since these cultivars were grown in the same field and for the same period, this result indicates that the variation of the content of 2 depends on the cultivars, but not on the growth conditions. In contrast, no peak of 1 was detected in all the leaf extracts, although 1 was obtained in the isolation experiment. This probably was due mainly to the release of 1 from chlorophylls by chemical and/or enzymatic hydrolysis during harvest and isolation processes.

Elsewhere, we reported the variation of the content of chlorogenic acid derivatives among the cultivars of lettuce and burdock and between the different culture conditions (Amimoto *et al.*, 1996; Wang *et al.*, 2001). These facts suggest that the contents of functional constituents in vegetables would differ with cultivar as well as with culture conditions.

Change of the active component contents by treatment with hot water To investigate the change of phytol (1) and galacto-

lipid (2) contents in jute leaves during the treatment with hot water which is one of the cooking conditions, we used the lyophilized leaves of cultivar A-38 that showed the highest content of 2 among the cultivars. The contents of both components increased gradually with increasing period of the treatment (Fig. 3), indicating that the components are not easily decomposed by this treatment. The increments of these components are possibly caused by hydrolyses of chlorophyll and digalactosyldiacylglycerol, another galactolipid in plant tissue (Douce & Joyard, 1980). The stability of 1 and 2 in the human digestive system has yet to be resolved, but this fact suggests that the treatment of vegetables with hot water makes it effective to ingest directly these active components from food materials.

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References

- Amimoto, K., Yamasaki, A., Tokoro, K., Kudou, R. and Fukui, H. (1996). Component analysis of vegetables for chemical breeding (Part 1). Comparison of components between cultivars of lettuce (*Lactuca sativa* L.). *J. SHITA*, **8**, 146–153 (in Japanese).
- Block, G., Patterson, B. and Subar, A. (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer*, **18**, 1–29.
- Douce, R. and Joyard, J. (1980). Plant galactolipids. In "The Biochemistry of Plants, Vol. 4," ed. by P.K. Stumpf. Academic Press, New York, pp. 321–362.
- Harborne, J.B. (1998). "Phytochemical Methods, 3rd edition." Chapman & Hall, London.
- Kondo, A., Morimoto, T. and Okazaki, K. (1998). Detection of antitumor promoting activity in Raji cells carrying Epstein-Barr virus genome by immunoblotting analysis. *Biosci. Biotechnol. Biochem.*, **62**, 377–379.
- Morimoto, T., Nagatsu, A., Murakami, N., Sakakibara, J., Tokuda, H., Nishino, H. and Iwashima, A. (1995). Anti-tumour-promoting glyceroglycolipids from the green alga, *Chlorella vulgaris*. *Phytochemistry*, **40**, 1433–1437.
- Murakami, A., Nakamura, Y., Koshimizu, K. and Ohigashi, H. (1995). Glyceroglycolipids from *Citrus hystrix*, a traditional herb in Thailand, potentially inhibit the tumor-promoting activity of 12-*O*-tetradecanoylphorbol 13-acetate in mouse skin. *J. Agric. Food Chem.*, **43**, 2779–2783.
- Nagatsu, A., Watanabe, M., Ikemoto, K., Hashimoto, M., Murakami, N., Sakakibara, J., Tokuda, H., Nishino, H., Iwashima, A. and Yazawa, K. (1994). Synthesis and structure-anti-tumor-promoting activity relationship of monogalactosyl diacylglycerols. *Bioorg. Med. Chem. Lett.*, **4**, 1619–1622.
- Resources Council, Science and Technology, Japan (ed.) (2000). "Standard Tables of Food Composition in Japan, 5th revised edition." Printing Bureau, Ministry of Finance, Tokyo (in Japanese).
- Shimizu, M. and Tomoo, T. (1994). Anti-inflammatory constituents of topically applied crude drugs. V. Constituents and anti-inflammatory effect of Aoki, *Aucuba japonica* THUNB. *Biol. Pharm. Bull.*, **17**, 665–667.
- Shirahashi, H., Morimoto, T., Nagatsu, A., Murakami, N., Tatta, K., Sakakibara, J., Tokuda, H. and Nishino, H. (1996). Antitumor-promoting activities of various synthetic 1-*O*-acyl-3-*O*-(6'-*O*-acyl- β -D-galactopyranosyl)-*sn*-glycerols related to natural product from freshwater cyanobacterium *Anabaena flos-aquae* f. *flos-aquae*. *Chem. Pharm. Bull.*, **44**, 1404–1406.
- Steinmetz, K.A. and Potter, J.D. (1991a). Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control*, **2**, 325–357.

- Steinmetz, K.A. and Potter, J.D. (1991b). Vegetables, fruit, and cancer. II. Mechanisms. *Cancer Causes Control*, **2**, 427–442.
- Steinmetz, K.A. and Potter, J.D. (1996). Vegetables, fruit, and cancer prevention: A review. *J. Am. Diet. Assoc.*, **96**, 1027–1039.
- Sturgeon, R.J. (1994). Enzymic determination of D-galactose. In “Methods in Carbohydrate Chemistry, Vol. X,” ed. by J.N. BeMiller, D.J. Manners and R.J. Sturgeon. John Wiley & Sons, New York, pp. 15–16.
- Tokuda, H., Nishino, H., Shirahashi, H., Murakami, N., Nagatsu, A. and Sakakibara, J. (1996). Inhibition of 12-*O*-tetradecanoylphorbol-13-acetate promoted mouse skin papilloma by digalactosyl diacylglycerols from the fresh water cyanobacterium *Phormidium tenue*. *Cancer Lett.*, **104**, 91–95.
- Wang, R., Ayano, H., Furumoto, T., Kondo, A. and Fukui, H. (2001). Variation of the content of chlorogenic acid derivatives among cultivars and market items of burdock (*Arctium lappa* L.). *Nippon Shokuhin Kagaku Kogaku Kaishi.*, **48**, 857–862 (in Japanese).
- Ziegler, R.G. (1991). Vegetables, fruits, and carotenoids and the risk of cancer. *Am. J. Clin. Nutr.*, **53**, 251S–259S.