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

Toshio FURUMOTO,1 Rong WANG,1 Katsuchihiro OKAZAKI,2 A.F.M. Feroj HASAN,1 M. Idris ALI,3 Akira KONDO1 and Hiroshi FUKUI1*

1Department of Biochemistry and Food Science and 2Department of Life Sciences, Faculty of Agriculture, Kagawa University, Kagawa 761-0795, Japan
3Bangladesh Institute of Nuclear Agriculture, Mymensingh 2200, Bangladesh

Received December 14, 2001; Accepted March 18, 2002

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 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.

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

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, δH 7.24, δC 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-35 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 aqeous 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-
ected 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 δH (CDCl3): 0.82 (3H, d, J = 6.6 Hz, H-18’), 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 δC (CDCl3): 16.2 (q), 19.70 (q), 19.74 (q), 22.6 (q), 22.7 (t), 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 δH (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-8”, H-17’, 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-11”, H-14’, H-14”), 3.52 (1H, ddd, J = 12.0, 6.0 Hz, H-6), 3.99 (1H, ddd, J = 9.4, 7.4 Hz, H-3), 3.63 (1H, ddd, J = 9.4, 7.4 Hz, H-2”), 3.72 (1H, dd, J = 11.2, 6.3 Hz, H-18”, 3.83 (1H, dd, J = 12.0, 4.3 Hz, H-6), 3.89 (1H, dd, J = 11.2, 5.5 Hz, H-18”), 3.94 (1H, dd, J = 12.0, 6.0 Hz, H-6), 3.99 (1H, dd, J = 9.4, 1.0 Hz, H-4), 4.19 (1H, dd, J = 12.1, 6.5 Hz, H-18”), 4.25 (1H, d, J = 7.4 Hz, H-1), 4.37 (1H, ddd, J = 12.1, 3.5 Hz, H-18”, 5.25–5.41 (12H, m, H-9’, H-9”’, H-10’, H-10”’, H-12’, H-12”’, H-13’, H-13”’, H-15’, H-15”’, H-16’, H-16”’, H-9”, H-9”, H-12”, H-12””, H-13”, H-13””, H-15”, H-15””), 5.29 (1H, overlapping NMR signal, H-2”). 13C-NMR δC (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-3’, C-3”), 68.3 (t, C-3, C-3”), 69.4 (d, C-4), 70.2 (d, C-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-12”, C-12’, C-12”), 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: 479 (M+Na). The molecular formula is regarded as C25H39O12.

**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 dissolved in 5% H2O (9 : 1), sprayed with the naphthoresorcinol-H2SO4 reagent and then solubilized with an extraction buffer (about 106 cells/ml) containing 0.1% 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 antisera 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 cytoxicity 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 antitu- mor promoters, compounds 1 and 2, were isolated by several
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 \(^1\)H- and \(^13\)C-NMR spectra of 2 suggested that this active component is a glycosylglycerolipid having two unsaturated fatty acid moieties (\( \delta_{\text{H}} 5.25–5.41, 12\text{H}, \text{m; \delta}_{\text{C}} 173.5 \) and 173.8) in the molecule. Hydrolysis of 2 with sodium methoxide gave a single methyl \(-\text{linolenate, indicating that two fatty acid moieties in 2 are the \(-\text{linolenyl group. Alkaline hydrolysis and subsequent acid hydrolysis of 2 afforded glycerol and galactose, and the } \text{D configuration of galactose was confirmed by the enzymatic reaction with } D\text{-galactose oxidase (Sturgeon, 1994). Furthermore, the stereochimistry of the anemic carbon atom in a galactose moiety, \( \beta \)-anomer, was deduced from DIFNOE data for compound 2. The attachment of galactose and \(-\text{linolenic acid to glycerol was deduced from the long-range } ^{13}\text{C}-^1\text{H correlation detected in the LR-HETCOR (HMBC and COLOC) spectra (H-1/C-sn-3; H-sn-3/C-1’; H-sn-2/C-1’). Therefore, the structure of compound 2 was established as 1,2-di-\( \text{O-}\text{-\text{linolenoyl-3-O-}\text{-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. Compounds 1 and 2 inhibited completely the induction of EBV EA at respective concentrations of 15 \( \mu\text{g/ml} \) (50.7 \( \mu\text{M} \)) and 30 \( \mu\text{g/ml} \) (38.8 \( \mu\text{M} \)) in the medium. However, the inhibitory effects of 1 and 2 at respective concentrations of 10 \( \mu\text{g/ml} \) and 25 \( \mu\text{g/ml} \) 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 \( \mu\text{g/ml, 1} \)), 47% (15 \( \mu\text{g/ml, 1} \)), 67% (25 \( \mu\text{g/ml, 2} \)) and 42% (30 \( \mu\text{g/ml, 2} \)) 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-promoting activity.
To investigate the change of phytol (1) and monogalactosyldiacylglycerol (2) in jute leaves by treatment with hot water. Each value represents the mean of duplicate experiments.

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.

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 (Aminimoto 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 galactolipid (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 (Doucet & 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.

Acknowledgments
We are grateful to Prof. K. Izumori of our faculty for help in the galactose analysis, to Ms. R. Kadono of this laboratory for her technical assistance and to Mr. M. R. Haider and Mr. K. M. Shamsuzzaman of the Bangladesh Institute of Nuclear Agriculture for providing the seeds. This work was supported in part by a Grant-in-Aid for Scientific Research (C) to H. F. from the Japan Society for the Promotion of Science (No. 11660113).

References
Morimoto, T., Nagatsu, A., Murakami, N., Sakakibara, J., Tokuda, H., Nishino, H. and Iwashima, A. (1995). Anti-tumour-promoting glycerolipids from the green alga, Chlorella vulgaris (Morimoto et al., 1995), digalactosyldiacylglycerols from the freshwater cyanobacterium, Phormidium tenue (Tokuda et al., 1996), synthetic 1-O-acetyl-3-O-(6′-O-acetyl-β-D-galactopyranosyl)-sn-glycerols (Shirahashi et al., 1996) and synthetic monogalactosyldiacylglycerols together with sn-2 lyso derivatives and galactosyldiacylglycerol (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.


