JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 10515–10520 **10515**

α-Eleostearic Acid and Its Dihydroxy Derivative Are Major Apoptosis-Inducing Components of Bitter Gourd

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Bitter gourd (*Momordica charantia* L.) pericarp, placenta, and seed extracts were previously shown to induce apoptosis in HL60 human leukemia cells. To determine the active component that induces apoptosis in cancer cells, bitter gourd ethanol extract was fractionated by liquid−liquid partition and silica gel column chromatography. Several fractions obtained by silica gel column chromatography inhibited growth and induced apoptosis in HL60 cells. Among them, fraction 7 had the strongest activity in inhibiting growth and inducing apoptosis in HL60 cells. A component that induced apoptosis in HL60 cells was then isolated from fraction 7 by another silica gel column chromatography and high-performance liquid chromatography (HPLC) using a C18 column and was identified as (9*Z*,11*E*,13*E*)-15,16-dihydroxy-9,11,13-octadecatrienoic acid (15,16-dihydroxy α-eleostearic acid). 15,16-Dihydroxy α-eleostearic acid induced apoptosis in HL60 cells within 5 h at a concentration of 160 μM (50 μg/mL). (9*Z*,11*E*,13*E*)-9,11,13-Octadecatrienoic acid (α-eleostearic acid) is known to be the major conjugated linolenic acid in bitter gourd seeds. Therefore, the effect of α-eleostearic acid on the growth of some cancer and normal cell lines was examined. α-Eleostearic acid strongly inhibited the growth of some cancer and fibroblast cell lines, including those of HL60 leukemia and HT29 colon carcinoma. α-Eleostearic acid induced apoptosis in HL60 cells after a 24 h incubation at a concentration of 5 μM. Thus, α-eleostearic acid and the dihydroxy derivative from bitter gourd were suggested to be the major inducers of apoptosis in HL60 cells.

KEYWORDS: Bitter gourd; 15,16-dihydroxy α-eleostearic acid; α-eleostearic acid; apoptosis; HL60 cells

INTRODUCTION

Bitter gourd is known as a tropical vegetable with healthful effects, which has been traditionally used as a bitter stomachic and an antidiabetic agent (*1*−*3*). A bitter gourd extract or juice was reported to reduce the blood glucose level in streptozotocin- or alloxan-induced diabetic rats or type 2 diabetic mice (*4*−*6*). Bitter gourd reduces the blood glucose or triglyceride level and improves insulin sensitivity in high-fat-fed mice (*7*−*10*). Many cucurbitane triterpenoids and their glycosides have been identified from bitter gourd (*11*−*17*). The triterpenoid momordicines were identified as bitter principles (*11*). Some triterpenoids and their glycoside were reported to enhance glucose disposal in insulin-sensitive and insulin-resistant mice and have a hypoglycemic effect in alloxane-induced diabetic mice (*14, 15*). Other triterpenoids were shown to suppress 7,12-dimethylbenz[*a*]anthracene- and peroxynitrite-induced skin carcinogenesis in mice (*16*).

We previously demonstrated that bitter gourd placenta extract suppressed lipopolysaccharide (LPS)-induced TNFα production in RAW264.7 macrophage-like cells (*17*). Bitter gourd placenta extract suppressed the expression of LPS-induced inflammatory genes, such as those for TNF, IL1α, IL1β, and Ccl5 in RAW264.7 cells (*18*). The extract was suggested to suppress inflammatory gene expressions through inhibiting the activities of the nuclear transcriptional factor NF-κB and MAP kinases (MAPKs) (*18*). Moreover, bitter gourd juice was suggested to suppress LPS and anticollagen antibody-induced arthritis of Balb/c mice (*18*). Our results suggested that bitter gourd suppresses inflammatory responses *in vitro* and *in vivo*. We have identified the active components from bitter gourd as 1-α-linolenoyl lysophosphatidylcholine (LPC), 2-α-linolenoyl LPC, 1-lynoleoyl LPC, and 2-linoleoyl LPC (*18*).

In our study on the physiological functions of bitter gourd, we also found that bitter gourd ethanol extract but not other vegetable extracts inhibited growth and induced apoptosis in HL60 human leukemia cells (*17, 19, 20*). All of the pericarp,

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10.1021/jf8020877 CCC: $40.75
Published on Web 10/30/2008

placenta, and seed extracts of unripe fruits of bitter gourd cultivars that we examined suppressed growth and induced apoptosis in HL60 cells (*17*). Here, we identified the active component that induced apoptosis in HL60 cells as 15,16-dihydroxy-(9*Z*,11*E*,13*E*)-9,11,13-octadecatrienoic acid (15,16-dihytroxy α-eleostearic acid). (9*Z*,11*E*,13*E*)-9,11,13-Octadecatrienoic acid (α-eleostearic acid), which is the major fatty acid in bitter gourd seeds, inhibited the growth of HL60 leukemic cells, as well as its dihydroxy derivative, and of HT29 colorectal carcinoma and other cancer and fibroblast cell lines. We also examined the suppressive effect of bitter gourd juice and the control 3% glucose water on the growth of HT29 human colon carcinoma transplanted in mice.

MATERIALS AND METHODS

Materials. Bitter gourd (*Momordica charantia* L.) cultivar ("Sadowara 3") grown in Miyazaki, Japan, was kindly provided by the Miyazaki Agricultural Experiment Station. Bitter gourd juice produced from bitter gourd cultivar ("Sadowara 3") grown in Miyazaki was kindly prepared by Miyazaki Nokyo Kajyu Co., Ltd. α-Eleostearic acid was purchased from Larodan Fine Chemicals AB (Malmo, Sweden).

Cells and Cell Culture. HL60 human promyelocytic leukemia cells (JCRB0085) were provided by the Human Science Research Resources Bank (Osaka, Japan). HT29 human colorectal adenocarcinoma cells (ATCC HTB38) were purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). WI38 human embryonic lung fibroblasts (RCB702), CACO-2 human colon carcinoma cells (RCB0988), COLO-320 human colon carcinoma cells (RCB1193), and B16 melanoma 4A5 cells (RCB0557) were provided by the RIKEN Cell Bank (Tsukuba, Japan). HL60 and COLO-320 cells were maintained in RPMI1640 medium (Invitrogen Corp., Carlsbad, CA). HT29 cells were grown in McCoy 5A medium (Invitrogen), and WI38 fibroblasts were maintained in MEM (Invitrogen). CACO-2 cells were maintained in MEM medium added to 0.1 mM non-essential amino acids (Invitrogen). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air in medium supplemented with 10% heat-inactivated fetal calf serum (ICN Biochemicals, Inc., Aurora, OH). Cell viability was determined using WST-1 reagent (Cell Counting Kit, Dojindo Laboratories, Kumamoto, Japan). Data are expressed as the arithmetic mean ± standard deviation (SD) of triplicate determinations.

Determination of Apoptosis. Apoptosis of HL60 cells was determined by microscopy as induction of typical apoptotic cell bodies after 5−24 h. To analyze the nuclear fragmentation typical of apoptosis, cells were stained with 1 mM bisbenzimide (Hoechst 33258) in phosphate-buffered saline (PBS). We also determined nucleosomal DNA fragmentation typical of apoptosis by the following method. HL60 cells were lysed in 20 μL of lysis buffer [50 mM Tris-HCl at pH 8.0, 100 mM ethylenediaminetetraacetic acid (EDTA), and 0.5% sodium dodecyl sulfate (SDS)] and incubated with 0.5 mg/mL RNase (Sigma Chemicals, St. Louis, MO) at 50 °C for 30 min and then with 0.5 mg/mL Proteinase K (Sigma) at 50 °C for 60 min. The samples were electrophoresed in Tris-borate buffer (pH 8.0) on a 2% agarose gel, and the DNA was stained with ethidium bromide.

Extraction of Bitter Gourd. Bitter gourd unripe fruits were lyophilized and extracted with 80% ethanol or acetone. Ethanol extract of bitter gourd was concentrated by an evaporator and lyophilized. The extract was then dissolved in distilled water and partitioned between water and ethyl acetate. After the solvent was removed, the ethyl-acetate- and water-soluble fractions and the acetone extract were dissolved in dimethyl sulfoxide before being added to a culture medium of HL60 cells. The final concentration of dimethyl sulfoxide in the medium was less than 0.2%.

Isolation of an Active Component from the Bitter Gourd Ethyl Acetate Fraction. The ethyl acetate fraction (22 g from 8 kg of bitter gourd unripe fruit) of bitter gourd was applied to a silica gel column (4 × 39 cm, Wakogel C-200, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and eluted with hexane/ethyl acetate (v/v) of 4:1 (fractions 1 and 2), 3:1 (fractions 3 and 4), 1:1 (fractions 5−7), 1:3 (fractions 8 and 9), and 1:4 (fractions 10 and 11), 100% ethyl acetate (fraction 12), and 100% methanol (fraction 13). Fraction 7 (658 mg), which induced apoptosis in HL60 cells, was then applied to a silica gel column (2.5 × 21 cm, Wakogel C-200, Wako) and eluted with hexane/ethyl acetate (v/v) of 2:3 [fractions 7-1 and 7-2 (330 mg)] and 1:3 (fraction 7-3) and ethyl acetate (fraction 7-4). Fraction 7-2 was applied on a high-performance liquid chromatography (HPLC) column (YMC-Pack Pro C18 RS column, 250 × 4.6 mm i.d., 5 μm, YMC Co., Ltd., Kyoto, Japan) and eluted with 80% methanol (fractions 7-2-1−7-2-5) at a flow rate of 1.0 mL/min. Constituents were detected using a diode array detector (SPD-M10AVP, Shimadzu Corp., Kyoto, Japan) in the range of 200−400 nm. We obtained 0.8 mg of fraction 7-2-4 eluted at 6.8 min in the HPLC of 50 mg of fraction 7-2.

Identification of an Active Component from Bitter Gourd Ethyl Acetate Fraction. Fraction 7-2-4 (1 mg) was dissolved in acetone-d_6 (0.6 mL), and nuclear magnetic resonance (NMR) spectra were measured at 298 K on an Avance 800 spectrometer (Bruker Biospin, Karlsruhe, Germany). The active component of this fraction was identified using ^{1}H NMR, ^{13}C NMR, and two-dimensional NMR, such as double-quantum-filtered correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-bond correlation (HMBC). Electrospray ionization (ESI) mass spectra were recorded on a LCQclassic mass spectrometer (Thermo Fisher Scientific, Waltham, MA) in the positive- and negative-ion modes. Fast atom bombardment (FAB) mass spectra were on a HX-110/110A mass spectrometer (JEOL Ltd., Tokyo, Japan) in the positive-ion mode, using Magic Bullet (3:1 mixture of dithiothreitol/dithioerythritol, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) as the matrix.

Determination of the Effect of Bitter Gourd Juice on the Growth of HT29 Human Colon Carcinoma Cells Transplanted in Balb/c Mice. Female Balb/cAnNCrj-nu/nu mice were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and maintained three per cage on a NMF diet (Oriental Yeast Co., Ltd., Tokyo, Japan). Food and water were provided *ad libitum* to the mice. The vivaria were maintained at 24 ± 1 °C, 55 ± 5% humidity, and 12 h light/dark phtocycles. The animals were treated in accordance with the basic guidelines of the Ministry of Agriculture, Forestry, and Fisheries for laboratory animal studies. Mice (7 weeks old) were subcutaneously injected with HT29 cells (2 × 10^6 cells/mouse). After 1 day, mice were divided into three groups, and these groups were given 200 μL of 50 or 100% bitter gourd juice or 3% glucose in water every day.

RESULTS

Identification of a Component from Bitter Gourd That Suppressed Growth and Induced Apoptosis in HL60 Human Leukemia Cells. We previously showed that the ethanol extract of bitter gourd induced apoptosis in HL60 human leukemia cells (*17, 19, 20*). To determine the active component that induced apoptosis in HL60 cells, we extracted the lyophilized bitter gourd unripe fruit with 80% ethanol or acetone. The ethanol extract but not the acetone extract induced apoptosis in HL60 cells after a 24 h incubation at a concentration of 50 μg/mL. The acetone extract did not induce cell death at concentrations of 200 μg/mL or less. The ethanol extract was partitioned between water and ethyl acetate, and it was the ethyl-acetate-soluble fraction but not the water-soluble fraction that induced apoptosis in HL60 cells. The ethyl acetate fraction was then applied onto a silica gel column and fractionated into 13 fractions. Several fractions obtained by silica gel column chromatography inhibited the growth of HL60 cells at concentrations of 25 and 50 μg/mL (**Figure 1**). After a 24 h incubation, fractions 2, 7, and 9 induced apoptosis in HL60 cells, while fractions 12 and 13 only slightly induced apoptosis in HL60 cells (**Figure 1**). In place of apoptotic cell bodies, the loss of integrity of many cell membranes was observed after the treatment of fractions 12 and 13. We then further fractionated fraction 7, which strongly inhibited growth and induced apoptosis of HL60 cells at a concentration of 25 μg/mL, into 4

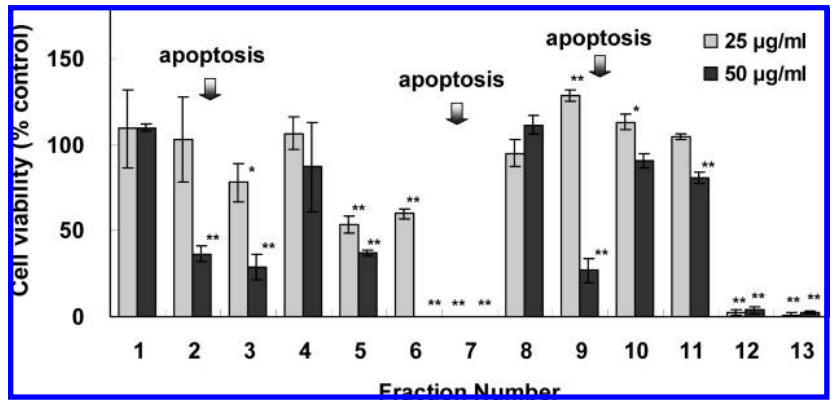

Figure 1. Apoptosis-inducing effect of bitter gourd fractions purified by silica gel column chromatography. The bitter gourd ethyl acetate fraction was applied to a silica gel column and eluted with hexane/ethyl acetate (v/v) of 4:1 (fractions 1 and 2), 3:1 (fractions 3 and 4), 1:1 (fractions 5−7), 1:3 (fractions 8 and 9), and 1:4 (fractions 10 and 11), 100% ethyl acetate (fraction 12), and 100% methanol (fraction 13). HL60 cells (1×10^5 cells/mL) were treated with bitter gourd fractions for 24 h. Cell viability was quantified using WST-1 reagent (Cell Counting Kit, Dojindo Laboratories). Apoptotic cells induced by bitter gourd fractions were detected using a microscope. All assays were performed in triplicate, and data are expressed as means ± SD. (∗) $p < 0.05$ and (∗∗) $p < 0.01$ (two-sided) are significantly different from the nontreated control group by Bonferroni-type multiple *t* test.

Table 1. Assignment of NMR Signals[a] of 15,16-Dihydroxy α-Eleostearic Acid

position	^{1}H NMR δ (ppm)	multiplicity	*J* (Hz)	^{13}C NMR δ (ppm)
1				174.6
2	2.27	dd	7.4, 7.4	34.1
3	1.58	br m		25.6
4−6	1.30−1.35			29.7
7	1.39	br m		30.2
8	2.21	ddd	7.5, 7.5, 1.2	28.3
9	5.44	ddd	11.3, 7.5, 7.5	133.0
10	6.02	dd	11.4, 11.3	129.6
11	6.53	dd	14.8, 11.4	128.4
12	6.22	dd	14.8, 11.0	133.3
13	6.34	dd	15.3, 11.0	132.0
14	5.82	dd	15.3, 6.6	134.8
15	4.00	br m		75.9
16	3.42	br m		76.6
17	1.54	m		26.2
	1.35	m		
18	0.93	t	7.5	10.6

[a] Obtained at 800.02 MHz for ^{1}H NMR and 201.19 MHz for ^{13}C NMR in acetone-d_6 at 298 K.

fractions, designated as 7-1−7-4, by silica gel column chromatography. Fractions 7-2 and 7-3 induced apoptosis in HL60 cells. We further purified fraction 7-2, which showed a larger peak on HPLC at about 270 nm, from HPLC using a C18 column. The peaks were detected at the maximum absorbance wavelength in the range of 200−400 nm using a diode array detector. All of the five fractions (fractions 7-2-1−7-2-5) completely induced the death of HL60 cells after the 24 h treatment. Fraction 7-2-4 as well as fractions 7-2-1 and 7-2-2 had higher activity than fractions 7-2-3 and 7-2-5, in that they induced apoptosis in HL60 cells after only a 5 h treatment at a concentration of 50 μg/mL.

Because of the high purity of fraction 7-2-4, we obtained spectroscopic data on this compound. ^{1}H NMR spectrum of the component of fraction 7-2-4 showed six signals of olefinic protons at 5.44−6.53 ppm, which revealed the presence of a conjugated triene structure on DQF-COSY and TOCSY spectra (**Table 1**). ^{1}H at one of the ends of the conjugated triene appeared at 5.82 ppm coupled with a ^{1}H at 4.00 ppm, and ^{1}H−^{1}H coupling could be traced sequentially from the signal of the latter ^{1}H to those at 3.42, 1.35, and 0.93 ppm. The ^{1}H resonating at 1.35 ppm strongly coupled with that at 1.54 ppm.

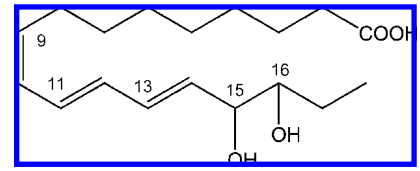

Figure 2. Structure of 15,16-dihydroxy α-eleostearic acid.

^{1}H at the other end of the conjugated triene appeared at 5.44 ppm coupled with two ^{1}H signals at 2.21 ppm, which also coupled with ^{1}H in methylene at 1.39 ppm. This series of coupled ^{1}H signals suggested a partial structure of $-CH_2-CH_2-CH=CH-CH=CH-CH=CH-CH(O)-CH(O)-CH_2-CH_3$. A carbonyl signal was observed in ^{13}C NMR spectra at 174.6 ppm. HMBC correlation was detected from this carbonyl to ^{1}H signals at 2.27 and 1.58 ppm. The latter ^{1}H showed coupling with a methylene cluster observed at 1.30−1.35 ppm, which suggested another partial structure of $-CH_2-CH_2-CH_2-C(O)=O$. From these partial structures and the presence of a methylene cluster, this compound was considered to be a fatty acid having a conjugated triene. The negative ESI mass spectrum of this compound gave a peak at *m/z* 309. In the positive-ion mode, a peak was observed at *m/z* 311 accompanied by several peaks corresponding to $[M + Na]^+$, $[M - H_2O]^+$, and $[M - 2H_2O]^+$. The FAB mass experiment in the presence of NaI gave a peak at *m/z* 377 corresponding to $[M - 2H + 3Na]^+$. These data suggested a molecular weight of 310. When results of NMR and MS analyses were combined, the compound was identified as 15,16-dihydroxy-9,11,13-octadecatrienoic acid, connecting the above two partial structures with two methylenes. On the basis of the values of the coupling constant between the olefinic protons, the geometry of the double bonds was determined as (9*Z*,11*E*,13*E*). Because (9*Z*,11*E*,13*E*)-9,11,13-octadecatrienoic acid is known as α-eleostearic acid, one of the conjugated linolenic acids, the identified active compound (9*Z*,11*E*,13*E*)-15,16-dihydroxy-9,11,13-octadecatrienoic acid can be called 15,16-dihydroxy α-eleostearic acid (**Figure 2**).

α-Eleostearic Acid Suppressed the Growth of Some Cell Lines and Induced Apoptosis in HL60 Cells. Bitter gourd seeds typically contain the conjugated linolenic acid α-eleostearic acid. Because the structure of the isolated active component, 15,16-dihydroxy α-eleostearic acid, is closely related to that of α-eleostearic acid, we examined the apoptosis-inducing effect of α-eleostearic acid in HL60 cells. α-Eleostearic acid strongly suppressed the growth of HL60 cells. After a 24 h

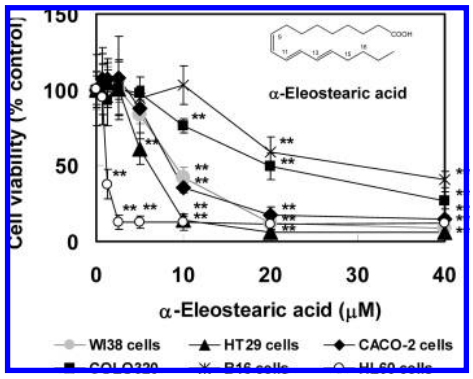

Figure 3. Effect of α-eleostearic acid on the growth of cancer and normal cell lines. HL60 cells (1×10^5 cells/mL) were incubated with α-eleostearic acid for 24 h. Other cells were seeded at 5×10^4 cells/mL, incubated for 24 h to allow the cells to adhere to the bottom of the culture plate, and then treated with α-eleostearic acid for 24 h. Cell viability was quantified using WST-1 reagent (Cell Counting Kit, Dojindo Laboratories). All assays were performed in triplicate, and data are expressed as means ± SD. (**) $p < 0.01$ (two-sided) are significantly different from the nontreated control group by Bonferroni-type multiple *t* test.

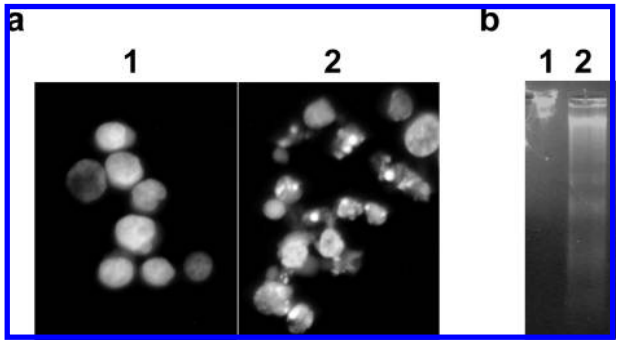

Figure 4. α-Eleostearic acid induced apoptosis in HL60 leukemia cells. (a) HL60 cells (1×10^5 cells/mL) were untreated or treated with 20 μM α-eleostearic acid for 6 h and then stained with Hoechst 33258. (1) Control cells and (2) cells treated with α-eleostearic acid. (b) Cells (1×10^5 cells/mL) were (1) untreated or (2) treated with 5 μM α-eleostearic acid for 24 h, and then DNA was extracted and analyzed by agarose gel electrophoresis.

incubation, α-eleostearic acid at a concentration of 1.25 μM had reduced cell viability to 38% of the value for control cells (**Figure 3**). α-Eleostearic acid induced cellular and nuclear fragmentation typical of apoptosis after a 6 h treatment at a concentration of 20 μM (**Figure 4a**). α-Eleostearic acid induced nucleosomal DNA fragmentation typical of apoptosis (**Figure 4b**). We also examined the effect of α-eleostearic acid on the growth of other cancer and fibroblast cell lines. α-Eleostearic acid strongly inhibited the growth of HT29 and CACO-2 human colon carcinoma cells as well as the growth of WI38 normal human fibroblast cells (**Figure 3**).

Bitter Gourd Juice Did Not Suppress the Growth of HT29 Human Colon Carcinoma Cells in Mice. α-Eleostearic acid strongly inhibited the growth of HT29 cells (**Figure 3**). To determine the suppressive effect of bitter gourd juice on tumor cell growth *in vivo*, we subcutaneously injected HT29 cells to Balb/c-nu/nu mice and then orally administered bitter gourd juice daily after injection. The tumor volume in mice administered 0, 50, and 100% bitter gourd juice (200 μL mouse^{-1} day^{-1}) was 105.6 ± 14.7, 111.4 ± 17.2, and 81.2 ± 16.2 mm^3, respectively. The tumor weight in mice administered 0, 50, and 100% bitter gourd juice (200 μL mouse^{-1} day^{-1}) was 146.3 ± 18.3, 135.6 ± 20.3, and 131.0 ± 20.1 mg, respectively. There were no significant differences in tumor size between control and bitter-gourd-administered mice. Bitter gourd juice did not affect body and liver weights of mice.

DISCUSSION

Although the seed extract was the most effective in inhibiting HL60 cell growth, bitter gourd pericarp, placenta, and seed extracts equally inhibited the growth and induced apoptosis of HL60 cells (*17*). Therefore, we fractionated the extract of bitter gourd unripe fruit, including three parts, which were the pericarp, placenta, and seeds. Previously, we obtained the active fraction that suppressed the LPS-induced inflammatory response in RAW264.7 cells, and linoleoyl- and liolenoyl-lysophosphatidylcholines were found to be the active components (*18*). In this study, we showed that several bitter gourd fractions induced apoptosis in HL60 cells, suggesting that bitter gourd contains several components that induce apoptosis in HL60 cells. Here, we first isolated 15,16-dihydroxy α-eleostearic acid and showed that it is one of the major active components in the bitter gourd ethanol extract, although many triterpenoids of bitter gourd have been isolated as physiologically active components. Fraction 7−2, which strongly induced apoptosis in HL60 cells, showed several peaks other than 15,16-dihydroxy α-eleostearic acid on HPLC at about 270 nm. This suggests that some other components related to α-eleostearic acid exist in the active fraction. Because the triterpenoids typically contained in bitter gourd were reported to be in fractions eluted with hexane/ethyl acetate of silica gel column chromatography (*12*), some active fractions of our silica gel column chromatography may contain the terpenoids typical of bitter gourd.

α-Eleostearic acid is the conjugated linolenic acid typically contained in bitter gourd seeds (*21*) and was suggested to be synthesized from linoleoyl phosphatidylcholine (*22*). Seed oil of bitter gourd contains more than 50% α-eleostearic acid (*21*). 15,16-Dihydroxy α-eleostearic acid may be enzymatically synthesized from linoleoyl phosphatidylcholine or linonenoic acid as reported for lipoxin (*23*). 15,16-Dihydroxy α-eleostearic acid induced apoptosis in HL60 cells after a 5 h treatment at a concentration of 160 μM. On the other hand, α-eleostearic acid induced apoptosis in HL60 cells after a 6 h treatment at a concentration of 20 μM. This result indicates that the structure of α-eleostearic acid bears the apoptosis-inducing effect of 15,16-dihydroxy α-eleostearic acid in HL60 cells.

α-Eleostearic acid has been reported to have anticancer effects *in vivo*. Diets containing 0.01% bitter gourd oil (0.006% as α-eleostearic acid), were shown to suppress azoxymethane-induced rat colon carcinogenesis (*24*). α-Eleostearic acid was reported to be converted to conjugated linoleic acid, (9*Z*,11*E*)-9,11-octadecadienoic acid, in plasma, liver, kidney, and small intestinal mucosa within 24 h (*25*). Elevation of PPARγ expression in colon mucosa and alteration of the lipid composition in colonic mucosa and liver were suggested to be mechanisms of the suppressive effect of bitter gourd oil on colon carcinogenesis (*24*). α-Eleostearic acid was also suggested to suppress the growth and angiogenesis of DLD-1 colorectal carcinoma cells through induction of apoptosis and activation of PPARγ (*26, 27*). Because we identified an α-eleostearic-acid-related component as an apoptosis-inducing component in HL60 cells, we examined the effect of α-eleostearic acid on the growth of HL60 and other cancer and normal cell lines. The α-eleostearic acid content of bitter gourd unripe fruit was 6−70 mg/100 g of fresh weight. α-Eleostearic acid, which is extracted with chloroform, probably was not contained in any fraction other than the acetone extract. Although α-eleostearic

acid strongly induced apoptosis in HL60 cells, the acetone extract, which is probably rich in α-eleostearic acid, did not induce apoptosis in the cells. Lipophilic components of pericarp and placenta in the acetone extract may blunt the apoptosis of HL60 cells.

α-Eleostearic acid, which is converted to conjugated linoleic acid *in vivo*, had a stronger suppressive effect than the conjugated linoleic acid on tumor cell growth *in vitro* and *in vivo* (*27*). It was shown to suppress the growth of DLD-1 human colon cancer cells by inducing apoptosis via lipid peroxidation (*27*). Islam et al. showed that *trans*, *trans* conjugated linoleic acids were incorporated into the membrane and induced mitochondria-mediated apoptosis in MCF-7 human breast cancer cells (*28*). The mitochondria-mediated pathway is common in apoptosis of HL60 cells. α-Eleostearic acid may induce apoptosis by altering the lipid composition of membrane and/or by causing oxidative stress and induce the following mitochondrial pathway in HL60 cells.

Our results showed that α-eleostearic acid induced apoptosis in HL60 cells and inhibited the growth of colon cancer cells. However, bitter gourd juice did not produce a sufficiently effective suppressive effect on HT29 colon carcinoma cell growth in mice. Because the moisture content of bitter gourd was about 94%, the estimated concentration of α-eleostearic acid in bitter gourd juice is <66 mg/100 g. The amount of α-eleostearic acid administered to mice in our experiment was probably somewhat less than that in the study of Kohno et al. (*24*).

In this study, we found that α-eleostearic acid, a typical conjugated linolenic acid in bitter gourd seeds, and its dihydroxy derivative had apoptosis-inducing activities. Although we did not show a suppressive effect of bitter gourd itself on tumor cell growth *in vivo*, identification of the physiologically active component is important for a better understanding of the physiological function of bitter gourd that has been proposed to prevent some diseases, such as diabetes, cancer, and inflammatory diseases. Our results show that not only triterpenoids and the glycosides but also polyunsaturated fatty acids, including conjugated fatty acids, in bitter gourd have physiological activities, leading to beneficial effects on our health.

ABBREVIATIONS USED

(9*Z*,11*E*,13*E*)-9,11,13-octadecatrienoic acid, α-eleostearic acid; 15,16-dihydroxy-(9*Z*,11*E*,13*E*)-9,11,13-octadecatrienoic acid, 15,16-dihytroxy α-eleostearic acid; LPS, lipopolysaccharide.

ACKNOWLEDGMENT

We express our thanks to Ms. T. Sato and Mr. Y. Mori of the National Food Research Institute for help in MS analyses and to Ms. I. Maeda of the National Food Research Institute for help in NMR experiments.

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Received for review July 9, 2008. Revised manuscript received September 24, 2008. Accepted September 25, 2008. This work was financially supported in part by grants from the Ministry of Agriculture, Forestry, and Fisheries.

JF8020877