Food Chemistry 130 (2012) 547–551

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Isolation and identification of an antiproliferative substance from fructose–tyrosine Maillard reaction products

In Guk Hwang [a], Hyun Young Kim [b], Sang Hoon Lee [b], Koan Sik Woo [c], Jung Ok Ban [d], Jin Tae Hong [d], Kwang Won Yu [e], Junsoo Lee [b], Heon Sang Jeong [b,*]

[a] Department of Agrofood Resources, National Academy of Agricultural Science, RDA, Suwon 441-853, Republic of Korea
[b] Department of Food Science and Technology, Chungbuk National University, Cheongju 361-763, Republic of Korea
[c] Department of Functional Crop, National Institute of Crop Science, RDA, Miryang 627-803, Republic of Korea
[d] College of Pharmacy, Chungbuk National University, Cheongju 361-763, Republic of Korea
[e] Division of Food and Biotechnology, Chungju National University, Chungbuk 368-701, Republic of Korea

ARTICLE INFO

Article history:
Received 12 April 2011
Received in revised form 6 May 2011
Accepted 19 July 2011
Available online 26 July 2011

Keywords:
Maillard reaction
Fructose–tyrosine MRPs
Antiproliferative effect
2,4-Bis(*p*-hydroxyphenyl)-2-butenal

ABSTRACT

We isolated a substance from fructose–tyrosine Maillard reaction products (MRPs) and investigated its antiproliferative effect on six human cancer cell lines. The ethyl acetate fraction of fructose–tyrosine MRPs showed a strong antiproliferative effect; this fraction was isolated and purified using silica gel column chromatography, semipreparative RP-HPLC, and recycling HPLC. The structure of the purified compound was determined using spectroscopic methods. The isolated compound was identified as 2,4-bis(*p*-hydroxyphenyl)-2-butenal ($C_{16}H_{14}O_3$, HPB242). HPB242 inhibited cell growth in a dose-dependent manner (10–80 μg/ml) on the six human cancer cell lines. The IC_{50} values of HPB242 on the six human cancer cell lines were 17.34 μg/ml (MCF-7), 29.21 μg/ml (HCT-116), 34.57 μg/ml (H-460), 34.87 μg/ml (HepG2), 48.77 μg/ml (PC-3), and 55.83 μg/ml (MKN-45).

1. Introduction

Cancer, one of the most prominent diseases in humans, poses a serious clinical problem. It affects millions of patients worldwide, reducing their quality of life, and it is one of the leading causes of death in the world (Cheng et al., 2005; Kinghorn et al., 2003). Under normal conditions, the cells in which DNA or other components are irreversibly damaged by various causes undergo apoptotic cell death a self-destructive metabolism triggered by a genetically encoded cell-death signal (Hooper, Toft, Webb, & Bird, 1999). However, cancer cells, which are already irreversibly developed, obtain the capability to evade apoptosis in various ways. Therefore, cell death via induction of apoptosis in cancer cells is considered to be a cancer preventive and therapeutic strategy (Bold, Termuhlen, & McConkey, 1997). Many studies have been carried out to identify anticancer materials in natural product sources such as fruits, vegetables, and medicinal herbs (Lee, Hwang, & Lim, 2004; Wu, Wu, & Yang, 2002).

The Maillard reaction (MR), a common and complex reaction, mainly takes place in foods during thermal processing (Yilmaz & Toledo, 2005). The MR is a nonenzymatic reaction between carbonyl groups of reducing sugars and amino groups, and it develops into a complex set of reactions that may generates numerous products, such as aroma compounds, ultraviolet absorbing intermediates, and high-molecular-weight melanoidins (Jing & Kitts, 2004; Morales & Jiménez-Pérez, 2001; Yilmaz & Toledo, 2005). Many studies have reported beneficial effects associated with MR products (MRPs), including antioxidative (Lertittikul, Benjakul, & Tanake, 2007; Maillard, Billaud, Chow, Ordonaud, & Nicolas, 2007; Rufián-Henares & Morales, 2007a), antimicrobial, antihypertensive, anticarcinogenic, and antimutagenic properties (Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001; Rufián-Henares & Morales, 2007b; Yen & Tsai, 1993). In particular, much research has focused on the antioxidant activity of MRPs in model systems and foods (Lertittikul et al., 2007; Maillard et al., 2007).

However, little effort has been made toward isolating the active compounds of MRPs. We previously evaluated various biological activities of MRPs from sugar–amino acid model systems and confirmed that fructose–tyrosine MRPs show antiproliferative effects (Hwang, Kim, Woo, Lee, & Jeong, 2011). In this work, we sought to isolate and identify the antiproliferative substance from fructose–tyrosine MRPs. We also investigated its antiproliferative effect on six human cancer cell lines of the isolated compound.

* Corresponding author. Tel.: +82 43 261 2570; fax: +82 43 271 4412.
E-mail address: hsjeong@chungbuk.ac.kr (H.S. Jeong).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.07.068

2. Materials and methods

2.1. Chemicals and reagents

D-Fructose, L-tyrosine, and 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). RPMI 1640 mediums and foetal bovine serums (FBSs, whose origin were New Zealand) were purchased from GIBCO® of Introgen™ (Seoul, Korea). HPLC-grade water was purchased from J.T. Baker (Phillipsburg, NJ, USA). Reagents were highest grade quality.

2.2. Preparation of Maillard reaction products (MRPs)

We prepared 100 ml of 0.05 M fructose–tyrosine mixture as follows. The amounts of 0.05 M tyrosine (0.9060 g) was accurately weighed in cap-glass tubes and then added with 100 ml of 0.05 M fructose solution. The MR took place in a temperature-controlled autoclave apparatus (Jisico, Seoul, South Korea) at 130 °C for 2 h determined in previous studies on food model systems as an optimum condition (Hwang et al., 2006; Kwon et al., 2006). After 2 h heating, the reaction mixture was filtered through a 0.45 μm membrane and used to isolate the active compounds.

2.3. Purification and identification of antiproliferative substance

Fructose–tyrosine MRPs were partitioned consecutively in a separating funnel using solvents of increasing polarity: ethyl acetate (EtOAc), *n*-butanol, and water. The solvent was evaporated using a rotary evaporator (Eyela N-1000; Tokyo Rikakikai Co., Tokyo, Japan) at 40 °C. The EtOAc fraction (957.0 mg) was subjected to open column chromatography (500 × 35 mm, i.d.) using a silica gel (Kiesel gel 60, 70–230 mesh; Merck, Darmstadt, Germany); elution was carried out using a mixture of dichloromethane (DCM): methanol (MeOH) with an increasing amount of MeOH (20:1, 10:1, 5:1, 2:1, 1:1, 0:1, v/v, 500 ml). Six fractions were collected and assayed for antiproliferative effect and then loaded onto a silica gel 60 F254 glass plates (0.25 mm thick, 20 × 20 cm; Merck), which then developed with DCM:MeOH = 20:1. The plate was then sprayed with 20% sulphuric acid solution in 10% vanillin/ethanol solution to analyse the spot pattern. The active fraction A2 (415.9 mg) was further purified by silica gel column (300 × 10 mm, i.d.) chromatography; elution was carried out using a mixture of DCM:MeOH with increasing amounts of MeOH (100:0, 95:5, 90:10, 85:15, 80:20, 0:100, v/v, 400 ml). The twenty-three sub-fractions were collected and assayed for antiproliferative effect and then loaded onto a plate of silica gel TLC in the same order as mentioned above. The fraction B14–B16 showing antiproliferative activity was further purified by semi-preparative RP-HPLC (column: Discovery® C18 column, 250 × 10 mm, i.d., 5 μm, Supelco, Bellefonte, PA, USA; mobile phase: 30% acetonitrile; flow rate: 3.0 ml/min; detection: 206 nm) on a Younglin SP930D Instrument (Anyang, Korea) and the preparative recycling HPLC system (column: JAIGEL-GS310, 500 × 20 mm, i.d., 5 μm; mobile phase: 100% MeOH; flow rate: 3.0 ml/min; detection: 206 nm) on a JAI LC-9201 model (Tokyo, Japan). The structure of the purified compound was determined using several spectroscopic methods.

The UV spectrum in methanol was recorded on a spectrophotometer (UV-1650; Shimadzu, Kyoto, Japan). The ESI-MS spectra were acquired in positive ion mode using a Thermo Scientific LCQ Fleet Ion Trap Mass Spectrometer instrument (Thermo Scientific, CA, USA) equipped with an electrospray ion source (ESI) and an Xcalibur® system manager data acquisition software. Sample solutions (0.5 mg/ml) were infused in the ESI source using a syringe pump at a flow rate of 0.25 ml/min and the mass scan range was *m*/*z* 200–1000. Operating conditions on the ion trap mass spectrometer in positive polarity were as follows: spray voltage, 5.0 kV; capillary temperature, 275 °C; capillary voltage, 35 V; tube lens offset, 120 V; multipole 0 offset, −4.89 V; multipole 1 offset, −12.5 V; sheath gas flow (N_2), 10 A.U. The ^{1}H nuclear magnetic resonance (NMR, 500 MHz), ^{13}C NMR (125 MHz), Distortionless Enhancement by Polarisation transfer (DEPT), Heteronuclear Multiple Bond Correlation (HMBC), and Heteronuclear Multiple Quantum Correlation (HMQC) spectra were recorded on a spectrometer (Avance 500; Bruker, Madison, WI, USA) using CD_3OD as a solvent.

2.4. Antiproliferative effects (MTT-assay)

Human colon cancer cells (HCT-116), mammary cancer cells (MCF-7), lung cancer cells (H-460), liver cancer cells (HepG2), prostate cancer cells (PC-3), and stomach cancer cells (MKN-45) lines were obtained from the American Type Culture Collection (Cryosite, Lane Cove NSW, Australia). Cancer cells were grown in RPMI1640 with 10% foetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2 humidified air. Serial dilutions of samples (100 μl) were added into each of 96-well plates, then, cancer cells were plated at a density of 10^4 cells in 96-well plates per 100 μl medium and cells incubated for 24 h. After incubation, the medium was removed and cells in each well were incubated with MTT solution (15 μl) at 37 °C for 4 h. Again the supernatant was carefully removed and 100 μl of dimethylsulphoxide was added into each well. The resulting colour was assayed at 540 nm using a microplate absorbance reader (Sunrise, Tecan, Switzerland). The inhibition of cell growth by the samples was calculated as the percentage cell viability and was calculated using the following formula: percentage cell viability (A_1/

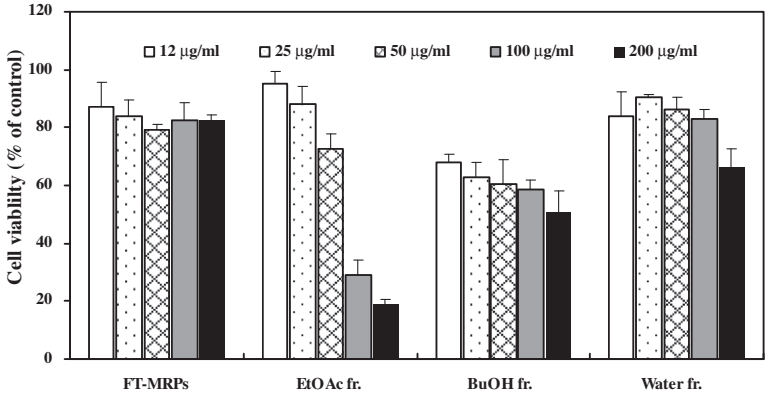

Fig. 1. Antiproliferative effects of solvent fractions from MRPs in a fructose–tyrosine on cells of a human colon cancer cell line (HCT-116).