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New monoterpene glycosides from sunflower seeds and their protective effects against H_2O_2-induced myocardial cell injury

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ABSTRACT

Three new monoterpene glycosides (**1**–**3**) and eleven known compounds (**4**–**14**) were isolated from seeds of *Helianthus annuus* L. (sunflower). Their structures were determined by spectroscopic and chemical methods. All the compounds were isolated from sunflower seeds for the first time. Protective effects of compounds **1**–**14** against H_2O_2-induced H9c2 cardiomyocyte injury were evaluated, and compounds **1** and **2** showed some cell-protective effects. No significant DPPH radical scavenging activity was observed for compounds **1**–**14**.

1. Introduction

Helianthus annuus L. (sunflower) is an important oilseed crop (Sackston, 1981). Due to its great capability of adaptation to different climatic and soil conditions (Villamide & San Juan, 1998), the crop is cultivated worldwide, and the total production of seeds was 44.7 million metric tons in 2013 according to the Food and Agriculture Organization (FAO) of the United Nations. Along with rape seed, soybean, peanut, and palm oil, sunflower oil is one of the most important vegetable oils and is widely used in the food and nutraceutical industries (Schmidt, 2013).

Sunflower seeds have excellent nutritional properties. The quality of its edible oil ranks it as one of the best vegetable oils among the cultivated plant oils. Up to 90% of the fatty acids in conventional sunflower oils are typically unsaturated fatty acids, namely oleic and linoleic, palmitic, stearic (British Pharmacopoeia, 2005), and minor amounts of myristic, myristoleic, palmitoleic, arachidic, behenic and other fatty acids account for the remaining 10% (Skoric, Jocic, Sakac, & Lecic, 2008). Sunflower seeds, containing high amounts of proteins and significant contents of tocopherols (British Pharmacopoeia, 2005), copper (USDA, 2012), zinc (USDA, 2012), crude fiber (Skrbic & Filipcev, 2008), sesquiterpenes (Macias et al., 2002), diterpenes (Suo et al., 2007), triterpenes (Ukiya et al., 2007), and phenols (Žilić et al., 2010), are used to prepare breads, biscuits and snack foods. Kernels of sunflower seeds are eaten raw or roasted as a rich source of protein and vitamins B, D, E and K. Other studies have shown its benefits in the reduction of cardio-vascular diseases (Zhu, Liu, Xia, & Ma, 2003). In addition to being used as food, sunflower seeds were reported to be used for medicinal purpose. The Indians of North America use the seeds for the treatments of cold, cough, and the ailments related to throat and lung (Putt, 1978). In Venezuela, the flowers and seeds are used in folk medicine for treating cancer (Hartwell, 1982).

Sunflower seeds are a rich and renewable natural resource with high economic value. The seeds are primarily utilized for production of vegetable oil. However, the pressed cake (or the residue resulting from oil extraction), a by-product from the oil production, is underutilized (Weisz, Carle, & Kammerer, 2013). In order to fully utilize this rich resource, more investigations on the chemical constituents in sunflower seeds are needed. In addition to proteins, other chemicals such as phenolic compounds, organic acids, phospholipids, tocopherols, and phytosterols have been found in the seeds (Amakura, Yoshimura, Yamakami, & Yoshida, 2013; Baydar

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& Erbas, 2005; Martinez-Force, Alvarez-Ortega, Cantisan, & Garces, 1998; Rashid, Anwar, & Arif, 2009; Weisz, Kammerer, & Carle, 2009). Recent studies reported more than twenty compounds identified from the seeds, and some of the compounds were found for the first time from *Helianthus* genus (Amakura et al., 2013; Fei, Chen, Li, Xu, & Yang, 2014). In this paper, we report the isolation and structure identification of three new monoterpene glycosides from sunflower seeds, together with eleven other compounds. In addition, the results about the protective effects of these compounds against cardiomyocytes injury induced by H_2O_2, as well as their DPPH radical scavenging activities are included.

2. Materials and methods

2.1. Materials and chemicals

Melting points of the isolates were determined using the XT5 micro-melting-point system (Beijing Optical Instrument Factory, Beijing, China). Specific optical rotation values were determined using a 241 polarimeter (Perkin–Elmer Inc., Waltham, MA, USA). IR spectra were recorded on a 983 G spectrometer (Perkin–Elmer Inc., Waltham, MA, USA). ^{1}H NMR, ^{13}C NMR, and 2D NMR spectra were obtained from an Inova 500 spectrometer (Varian Inc., Palo Alto, CA, USA) in C_5D_5N using tetramethylsilane (TMS) as the internal standard. HR-ESI-MS spectra were determined on a Q-TOF2 spectrometer (Micromass Corp., London, UK). EI-MS was determined on a Micromass Zabspec spectrometer (Micromass Corp., London, UK). High performance liquid chromatography (HPLC) analysis and purification were performed with an Agilent Zorbax SB-C18 semipreparative HPLC column (250 × 9.4 mm i.d., 5 μm, Agilent Corp. Palo Alto, CA, USA) on a Shimadzu HPLC system composed of a LC-20AT pump with an SPD-20A detector (Shimadzu Corp., Kyoto, Japan), the flow rate was 2 mL/min, and the wavelength for detection was 203 nm. Medium pressure liquid chromatography (MPLC) purification was performed on a Büchi Flash Chromatography system composed of a C-650 pump with a flash column (460 mm × 26 mm i.d., Büchi Corp., Flawil, Switzerland). GC analysis was conducted on a GC-14C (Shimadzu Corp., Kyoto, Japan) instrument with a flame ionization detector and the analytical conditions were as follows: DB-5 column (i.d. 0.25 mm, length 30 m; Suzhou Huitong Chromatography Technology Co., Ltd., Suzhou, China), column temperature at 210 °C; injector temperature at 270 °C; and detector temperature at 300 °C. The samples (1 μL) were injected manually into the column.

Silica gel (200–300 mesh) for column chromatography and precoated silica gel TLC plates were purchased from Qingdao Marine Chemical Factory. ODS for MPLC was purchased from Merck KGaA (Darmstadt, Germany). Sephadex LH-20 for column chromatography was purchased from GE Healthcare Corp. (Beijing, China). Compounds on TLC were colored by spraying 10% sulfuric acid alcohol solution and heating. Vitamin E was purchased from J&K Scientific Ltd. (Beijing, China). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and phosphate buffered saline (PBS) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), mitochondrial dehydrogenase and fetal bovine serum (FBS) were purchased from Gibco™ (Grand Island, NY, USA). Rat H9c2 cardiomyocytes were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). 96-Well plates were obtained from Corning Costar (Corning Costar, Cambridge, MA, USA). CD_3OD and $CDCl_3$ were obtained from Merck (Darmstadt, Germany). Methanol (MeOH) for HPLC analysis and purification was HPLC grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Standard (+)-campholenol and (−)-myrtenol were obtained from Jiangxi Bencaotiangong Technology Co., Ltd. (Nanchang, China). All of the other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Plant materials

The sunflower seeds (*H. annuus* L.) were purchased from a farmers' market in Qiqihar city (Heilongjiang province, China) in September 2011 and authenticated by Prof. Xiaoran Li (Department of Pharmacognosy, College of Pharmaceutical Science, Soochow University) using morphological identification. A voucher specimen (No. 10-09-08-01) was deposited in the herbarium of the College of Pharmaceutical Science at Soochow University.

2.3. Extraction and isolation

Sun-dried seeds (20 kg) were crushed into fine powder and extracted twice in MeOH (200 L). The solvent was removed under reduced pressure. The resulting residue (150 g) was dissolved in distilled water and fractionated successively with petroleum ether, chloroform, and *n*-butyl alcohol. The chloroform fraction (34 g) was dissolved in chloroform, loaded onto a silica gel column, and eluted with $CHCl_3$–MeOH (80:20, 60:40, 40:60, 0:100; 4.0 L each) under reduced pressure. The $CHCl_3$–MeOH (60:40) eluate (6.2 g) was subjected to separation on a MPLC/ODS column and eluted with MeOH–H_2O (40:60, 60:40, 80:20, and 90:10; 1000 mL each) at 20 mL/min to provide five fractions. Fraction 1 (196 mg) was separated by Sephadex LH-20 gel column chromatography (100 cm × 3 cm i.d.) eluting with MeOH to give compounds **6** (43 mg) and **10** (32 mg). Compounds **7** (31 mg, t_R 13.27 min), **8** (7.2 mg, t_R 29.35 min) and **9** (6.4 mg, t_R 37.15 min) were obtained from fraction 2 (163 mg) after separating by semi-preparative RP-HPLC eluting with MeOH–H_2O (67:33) at 2.0 mL/min. Compounds **4** (15 mg, t_R 37.14 min), **3** (13 mg, t_R 14.61 min), **2** (15 mg, t_R 16.00 min), **5** (15 mg, t_R 26.61 min), and **1** (9.2 mg, t_R 32.70 min) were obtained from fraction 3 (120 mg) after the purification using semi-preparative RP-HPLC with MeOH–H_2O (56:44) for elution. Fraction 4 (90 mg), which was subjected to separation by semi-preparative RP-HPLC and eluted with MeOH–H_2O (86:14), yielded compounds **11** (32 mg, t_R 12.42 min) and **12** (19 mg, t_R 15.62 min). Compounds **13** (51 mg, t_R 28.16 min) and **14** (13 mg, t_R 37.43 min) were obtained from fraction 5 (102 mg) after purification by using semi-preparative RP-HPLC with MeOH–H_2O (89:11) as the eluent.

2.3.1. (+)-Campholenol-10-O-β-D-glucopyranoside (**1**)

(4S)-2,2,3-trimethyl-3-cyclopentene-1-ethanol-10-O-β-D-glucopyranoside. white powder; $[\alpha]_D^{25}$ −36 (c = 0.015, MeOH); ^{1}H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) spectroscopic data are shown in Table 1; HR-ESI-MS (negative ion mode) m/z 315.1813 ([M−H]$^-$, calcd for $C_{16}H_{28}O_6$; 315.1808).

2.3.2. (+)-Campholenol-10-O-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside (**2**)

(4S)-2,2,3-trimethyl-3-cyclopentene-1-ethanol-10-O-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside. white powder; $[\alpha]_D^{25}$ −55 (c = 0.016, MeOH); ^{1}H NMR (500 MHz, MeOD) and ^{13}C NMR (125 MHz, MeOD) spectroscopic data are shown in Table 1; HR-ESI-MS (negative ion mode) m/z 447.2236 ([M−H]$^-$, calcd for $C_{21}H_{36}O_{10}$; 447.2230).