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Isolation and characterization of Maclura (*Maclura pomifera*) extracts obtained by supercritical fluid extraction

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ABSTRACT

The chemical composition of the CO_2 extract of *Maclura* fruit (Osage orange) was analyzed. The major constituents of the CO_2 extracts were triterpene alcohol esters, along with the triglycerides of C_{16} and C_{18} fatty acids. Column chromatography of the CO_2 extract yielded lupeol ester of hexadecanoic acid (**2**), lupeol ester of 3-hydroxyhexadecanoic acid (β-hydroxy palmitic acid) (**3**) trace of lupeol (**1**), butyrospermol ester of hexadecanoic acid (**5**), butyrospermol acetate (**6**) and trace of butyrospermol (**4**) as the least polar components. By consequent extraction with acetone of the lipid free *Maclura* material an extract was obtained, in which isoflavonoids osajin (**7**) and pomiferin (**8**) (natural antioxidant) were found in high yields. The structure of isolated compounds was established by spectroscopic data (IR, ^1H and ^{13}C NMR and MS) and some chemical reactions. The **3** is new natural product, not yet described in literature.

1. Introduction

Maclura pomifera (Rafin.) Schneider belongs to the *Moraceae* or the mulberry family (Smith and Perino, 1981). It is commonly known as the Osage orange tree which grows extensively throughout the Midwestern and Southwestern regions of the United States and also cultivated in different parts of the world. The common name is derived from its fruit, which resembles the shape of an orange (Prokudina et al., 2011). The fruit is not edible for humans, its extract exhibits antimicrobial, anti-inflammatory, cytotoxic, antidiabetic, estrogenic, antimalarial and anti-insect activities, and the Native Americans have used *M. pomifera* for cancer treatment (Franova and Pavlik, 2007; Hay et al., 2004; Küpeli et al., 2006; Mahmoud, 1981; Maier et al., 1995; Peterson et al., 2000, 2002). Several phenolic compounds have been isolated and identified from various parts of this plant, namely isoflavonoids from fruit (Delle Monache et al., 1994; Tian et al., 2006; Wolfrom et al., 1946), flavonols and xanthones from the heartwood and stem bark (Deshpande et al., 1975; Laidlaw and Smith, 1959), and flavanones and xanthones from the root bark (Delle Monache et al., 1984; Wolfrom et al., 1965). Among the phytochemicals in the fruit of the *Maclura*, isoflavones are the predominant group and are perhaps the most studied. Osajin and pomiferin have antimicrobial activity but of greater interest is the antioxidant activity of this isoflavones (Budincevic and Vrbaski, 1991; Scall and Quackenbush, 1956; Tsao et al., 2003).

Maclura fruit is also, rich source of triglycerides, phospholipids and triterpenes (Djarmati et al., 1998). They have not been frequently investigated. The previous papers (Gearien and Klein, 1975; Lewis, 1959; Wagner and Harris, 1952a,b) focused on the investigation of crude non-saponifiable material, which resulted in isolation of the products of hydrolysis, mainly triterpene alcohols. The new investigation of *Maclura* fruit is in field as source of oil edible for biodiesel production (Moser et al., 2011).

The extraction of phenolic compounds from plant has been traditionally performed using solvent extraction or steam distillation. Traditional methods for extraction require large volumes of solvents, time consuming and obtained extracts are with toxic organic residues (Martins Teixeira and Teixeira da Costa, 2005). Supercritical fluid extraction (SFE) has gained increasing attention over conventional techniques because it is generally recognized as safe (GRAS) (Reverchon and De Marco, 2006).

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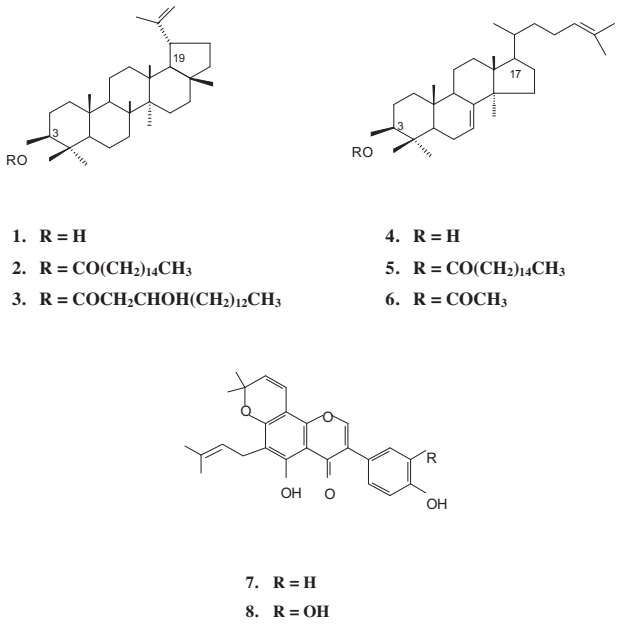

Fig. 1. Chemical structures of compounds lupeol (**1**), lupeol hexadecanoate (**2**), lup-20(29)-en-3-(3β-hydroxyhexadecanoate) (**3**), butyrospermol (**4**), butyrospermol hexadecanoate (**5**), butyrospermol acetate (**6**), osajin (**7**), and pomiferin (**8**).

SFE with CO_2 as a solvent is used for selective and mild extraction of sensitive natural products, so we used this technique for the extraction of lipids from the dried and ground *Maclura* fruit. The paper reports the extraction of *Maclura* fruit with supercritical carbon-dioxide and the isolation and determination of a new ester of lupeol, we called "maclura ester" (**3**) Fig. 1. To the best of our knowledge, this is the first time to find long chain hydroxy fatty acid esters of lupeol as natural products. Following the CO_2 extraction, we utilized the extraction with acetone to obtain the extract with high content of isoflavonoids osajin (**7**) and pomiferin (**8**). Pomiferin with catechol unite in its structure is responsible for the antioxidant activity of the fruit and extracts obtained there from.

2. Materials and methods

2.1. Chemicals

Acetone, ethyl acetate, benzene, petroleum ether, methanol, potassium hydroxide, acetone, pyridine, benzoyl chloride, HCl, diethyl ether, and vanillin with p.a. All chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany).

Jones reagent was prepared by dissolving 66.7 g CrO_3 in 57.5 mL H_2SO_4 and water up to 250 mL.

2.2. Plant material and sample preparation

The plant material was collected on the surrounding area of Zrenjanin (district of Vojvodina, Serbia). The unripe fruit (ranged 500–850 g) were sliced in thin foils (1–2 mm) and oven dried at maximum 50 °C. The dried material was ground to 1 mm particles and stored at room temperature.

2.3. General experimental procedure

^1H NMR (250 MHz) and ^{13}C NMR spectra (62.9 MHz) were recorded in $CDCl_3$ soln., with TMS as an internal standard. ^{13}C NMR signals were assigned by off-resonance and noise decoupled ^{13}C NMR spectra and heteronuclear ^{13}C–^1H and homonuclear COSY ^1H–^1H correlation. Experiments were done on a Bruker AC 250E instrument. EI mass spectra were obtained on a Finningan MAT Mass spectrometer Model 8230. IR spectra in KBr was obtained on PerkinElmer FT-IR 1725X. The method of HPLC (HP-model 1090) analyses of acetone extracts was similar to that used by Schwarz and Ternes (1992) and Schwarz et al. (1992).

For column chromatography (CC), Merck silica gel (0.063–0.2 mm and under 0.08 mm in ratio 1:1, 1000 g) was used and 25 g of sample was eluted using benzene. Preparative thin-layer chromatography (TLC) was carried out using Merck silica gel HF. The plates were sprayed with vanillin (1 g vanillin in 30 mL EtOH and 1 mL cc H_2SO_4) and detected visually. For eluting the plates as mobile phase EtOAc–C_6H_6 (1:22, v/v) was used.

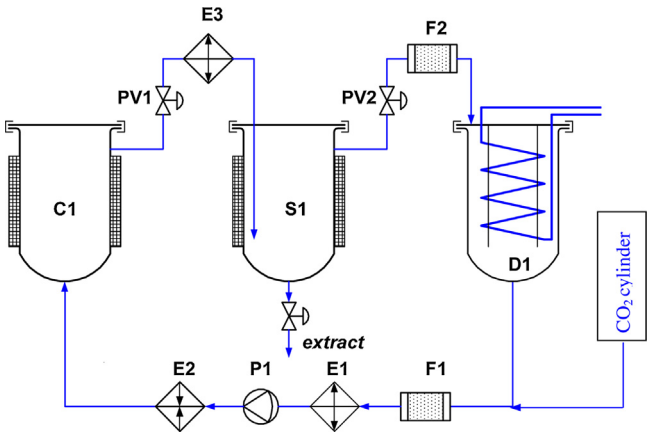

Fig. 2. Schematic flow sheet of supercritical extraction pilot plant (Uhde Germany) (C1 – extractor, S1 – separator, D1 – CO_2 tank, P1 – CO_2 pump, E1–E3 – heat exchanger, F1, F2 – flow rate control, PV1, PV2 – control valve).

2.4. Extraction and isolation

The supercritical CO_2 extraction was carried out using an UHDE GmbH pilot scale plant (extractor volume 4 L) (Fig. 2). The total ground material (10.5 kg), with moisture content of 12.7%, was extracted with CO_2 in portions of 1.5 kg. Supercritical fluid extraction was started at pressure of 210 bar, temperature 40 °C and time of 6 h, with flow rate 20 kg CO_2/h and bed porosity 0.61. After the extractions were finished, the first extract was obtained (SFE-1). The extraction was continued at pressure of 350 bar, temperature 60 °C, time 6 h, flow rate 20 kg CO_2/h, and the second extract was obtained (SFE-2). In both cases the separation conditions were the same, temperature 25 °C, pressure 50–55 bar. After extraction, obtained extracts were placed in glass bottles, sealed and stored at +4 °C to prevent any possible degradation.

The CO_2 extracted plant material was divided into three parts and each part was extracted with 10 L of acetone at room temperature with occasional stirring, and was left overnight. The mixture was filtered and the residue was extracted with 15 L of fresh acetone in the same manner as the initial extraction. The combined filtrates were concentrated by rotary evaporator under reduced pressure to heavy viscous yellow product which crystallized spontaneously.

The CO_2 extract of *M. pomifera* obtained at 350 bar, 60 °C (SFE-2) was chromatographed on silica gel column (50 × 1440 mm, 25 g). The column was eluted with benzene continually, and collected fraction of about 1.5 L. The collected fractions were monitored by TLC. The CC yield a mixture of **2** and **5** (2.51 g), **6** (300 mg), **3** (840 mg), **1** (60 mg) and **4** (170 mg) respectively. By repeated CC on silica gel (0.063–0.2 mm and 0.063–0.032 mm in ratio 1:1, 1000 g) eluted with petroleum ether, the mixture of two compounds (**2** and **5**) were separeted and 500 mg of **2** and 330 mg of **5** were obtained.