Chemistry of Natural Compounds, Vol. 43, No. 4, 2007

NEUTRAL LIPIDS FROM SEEDS OF *Cercis siliquastrum, Sapium sebiferum,* AND *Koelreuteria paniculata*

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The chemical composition of neutral lipids from seeds of Cercis siliquastrum, Sapium sebiferum, *and* Koelreuteria paniculata *were studied. Characteristic features of their individual classes were established.*

Key words: *Cercis siliquastrum*, *Sapium sebiferum*, *Koelreuteria paniculata*, seeds, neutral lipids.

Cercis siliquastrum L. (Judas tree), *Sapium sebiferum* L. Roxb. (Chinese tallow), and *Koelreuteria paniculata* Laxm. (goldenrain tree) grow well in Georgia, are easily propagated, and bear abundant fruit [1-3].

Polar lipids from seeds of these plants were found at the Kutateladze Institute of Pharmaceutical Chemistry, Academy of Sciences of Georgia, to be active against growth of xenograft tumors of cancer cells [4]. We have previously reported the chemical composition of phospholipids from seeds of *C. siliquastrum* and *S. sebiferum* [4-6].

Herein we report results from a study of neutral lipids (NL) from seeds of *C. siliquastrum* (ID), *S. sebiferum* (SD), and *K. paniculata* (MD).

Air-dried ground seeds of ID, SD, and MD that were collected when fully ripe were extracted exhaustively with *n*-hexane at room temperature to produce the NL. The total NL were obtained as oily yellow liquids up to 10% for ID and up to 20% for SD and MD.

The physicochemical properties of NL from ID, SD, and MD, respectively, were as follows. Acid number (mg/KOH) 2.80, 23.39, 2.00; d_4^{20} 0.918, 0.924, 0.909; n_D^{20} 1.475, 1.479, 1.473; saponification number (mg/KOH) 119.22, 142.40, 183.20; iodine number (%I_2) 145.33, 90.78, 88.80; ester number (mg/KOH) 116.42, 119.01, 181.20; moisture and volatiles (%) 3.1, 2.0, 1.8.

TLC analysis using various solvent systems detected bands in the studied NL corresponding to hydrocarbons (HC), sterol esters (SE), triacylglycerides (TAG), free fatty acids (FFA), free sterols (FS), diacylglycerides (DAG), and unidentified components [5]. The NL of MD did not contain SE although bands for fatty-acid methyl esters (FAME) were detected.

Adsorption chromatography of the total NL followed by preparative TLC (PTLC) of the mixed fractions isolated the individual classes.

Fatty acids (FA) were isolated from the main acyl-containing lipids by alkaline hydrolysis. Their composition was determined by GC of the methyl esters [7-10].

Table 1 shows that NL of the studied seeds had a FA composition consisting of only 5-6 components. The FA of total lipids and TAG of ID were dominated by linoleic acid (66-64%); FFA and SE, palmitic. Total FA and FFA of SD were dominated by linoleic and oleic; TAG and SE, palmitic. NL of MD had a high (80%) content of oleic acid.

FA of NL from ID and SD were converted to the methyl esters and separated by preparative TLC (Ag^+) using system 3. Fractions of saturated, monoenoic, dienoic, and trienoic acids were obtained.

According to GC, FAME of saturated (1), monoenoic (2), dienoic (3), and trienoic (4) NL fractions from ID and SD seeds had the following composition (mass %): 1) ID: 16:0, 63.8; 18:0, 29.5; 18:1, 6.7; SD: 12:0, 7.0; 14:0, 2.0; 16:0, 56.0; 18:0, 8.0; 18:1, 27.0; 2) ID: 18:1, 100; SD: 16:0, 5.4; 18:0, 10.6; 18:1, 84.0; 3) ID: 18:2, 100; SD: 14:0, 1.1; 18:2, 98.9; 4) ID: 18:2, 5.2; 18:3, 94.8; SD: 16:0, 5.9, 18:1, 3.4; 18:2, 9.3; 18:3, 81.4.

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 0009-3130/07/4304-0384

TABLE 1. Fatty Acid Composition and Individual Neutral Lipid Classes of Seeds from *Cercis siliquastrum* (ID), *Sapium sebiferum* (SD), and *Koelreuteria paniculata* (MD), %, GC

Fatty acid	Total			TAG			FFA			SE		FAME
	ID	SD	MD	ID	SD	MD	ID	SD	MD	ID	SD	MD
12:0	2.6	-	-	-	-	-	5.8	-	-	2.6	-	-
14:0	1.7	-	-	-	-	-	6.2	-	-	-	-	-
16:0	5.8	20.3	8.0	6.5	32.5	9.8	42.3	27.7	53.9	39.5	33.0	32.2
17:0	-	Tr.	-	-	Tr.	-	-	-	-	-	-	-
18:0	1.7	6.9	4.0	-	-	Tr.	-	-	5.2	5.9	19.6	19.4
18:1	21.8	26.5	80.1	29.3	21.4	66.9	16.9	59.2	4.9	32.5	27.7	18.8
18:2	66.4	38.6	6.7	64.2	26.5	20.2	28.9	11.3	36.1	19.4	16.3	29.6
18:3	Tr.	7.6	1.1	Tr.	19.7	3.2	-	1.8	-	-	3.4	-

Nonanedioic acid was the only dicarboxylic acid found after periodate—permanganate destruction of the ME of monoenoic, dienoic, and trienoic acids of NL from ID and SD with subsequent GC and TLC analysis of the acidic products (system 4). The monocarboxylic acid extracted from the aqueous reaction mixture containing the monoenoic acids was nonanoic acid (9:0); dienoic, caproic acid (6:0); and mainly trienoic, propionic acid (3:0).

The formation of these fragments upon oxidation of unsaturated FA showed that the mixture of unsaturated FA in NL of ID and SD contained Δ^9-oleic, $\Delta^{9,12}$-linoleic, and $\Delta^{9,12,15}$-linolenic acids.

The sterol composition was found by analyzing total sterols, fractions of free sterols, and sterol esters. Total sterols of NL from ID contained stigmasterol (4.3%) and β-sitosterol (95.67%). The free sterols consisted mainly of β-sitosterol.

Total sterols of NL from SD contained mainly β-sitosterol with stigmasterol and cholesterol observed in trace quantities. Sterol esters contained stigmasterol (26.4%), cholesterol (5.4%), and β-sitosterol (68.1%) [4, 7, 8].

EXPERIMENTAL

TLC detection of individual lipid classes and isolation and identification of FA were performed as before [4, 7, 8]. TLC used silufol and silica-gel plates (L 100/160) with added gypsum (10%); TLC (Ag^+), with added $AgNO_3$ (20%) and solvent systems hexane:Et_2O:CH_3CO_2H (87:14:1, 1); petroleum ether:Et_2O:CH_3CO_2H (80:20:1, 2); hexane:Et_2O (4:1, 3); and butan-1-ol:NH_4OH:H_2O (20:1:4, 4). GC was carried out in a Chrom-4 chromatograph with a flame-ionization detector using a stainless-steel column (2.5 m × 3 mm) packed with Reoplex 400 (15%) on Chromaton N-AW (0.160-0.200 mm) at thermostat temperature 220°C with He carrier gas (40 mL/min). For sterols, the column was the same size but had SE-30 (3%) on Chromaton N-AW at 280°C.

Sterols were recrystallized from methanol.

Acylglycerols were hydrolyzed using KOH (5.6%) in ethanol calculated as alkaline solution (10 mL) per TAG (0.2-0.25 g) with stirring for 30 min [9]. FA were methylated using CH_2N_2 in Et_2O. Diazomethane was produced as before [11].

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