Chemistry of Natural Compounds, Vol. 40, No. 6, 2004

LIPIDS OF *Thermopsis alterniflora* BEAN SEEDS AND SHELLS

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UDC 547.915:665.3

The lipid groups and fatty-acid composition of Thermopsis alterniflora *(Leguminosae) bean seeds and shells were established. It was shown that shell lipids have a greater content of unsaponified substances, galactolipids, and esterfied unsaturated 18:1, 18:2, and 18:3 acids (75% total) than seed lipids.*

Key words: Leguminosae, *Thermopsis alterniflora*, neutral lipids, glycolipids, phospholipids, fatty acids.

Thermopsis alterniflora Regel et Schmalh. (Leguminosae) is a known medicinal plant endemic to western Tyan-Shan [1, 2]. The herb contains alkaloids [3]; flavonoids [4]; vitamin C; macroelements (mg/g): K (20.8), Ca (4.6), Mg (2.0), Fe (0.1); and microelements (μg/g): Mn (0.1), Cu (0.35), Zn (0.49), Co (0.15), Mo (1.2), Cr (0.02), Al (0.06), Ba (0.1), V (0.01), Se (24.0), Ni (0.28), Pb (0.04), I (0.3), Br (15.9), and especially Se and Br [5].

The air-dried aerial part is used as a medicinal raw material for a cytisine preparation. The seeds contain 10.5 dry mass % fatty oil consisting mainly of glycerides of saturated (11.9%), oleic (39%), linoleic (42%), and linolenic acids (7.0%) [6]. However, data on the individual lipid classes of seeds or other organs of this plant have not been published.

We present results from a comparison of seed and shell lipids of *T. alterniflora* beans. Before the investigation of the lipids, specimens were evaluated gravimetrically for moisture and mass of 1000 pieces of beans and seeds. Separation of beans into seeds (specimen 1) and shells (specimen 2) gave a mass ratio of 1:2, which was 58.2 and 30.5 g, respectively.

Repeated extraction of ground specimens 1 and 2 with low-boiling hydrocarbons (40-60°C) gave the free lipids. Then, Folch extraction with $CHCl_3$:CH_3OH (2:1, v/v) produced bound lipids.

TLC (silica gel, systems 1-3) showed that free lipids of both specimens contained almost no polar lipids and consisted of a known component set of neutral lipids (NL). Bound lipids of seeds included NL, glycolipids (GL), and phospholipids (PL). Bound lipids of shells, in contrast with seeds, contained only traces of PL.

Extracts of bound lipids of specimens 1 and 2 were separated by column chromatography (CC) into NL, GL, and PL fractions [7, p. 122]. Then, free lipids of each specimen were combined with the corresponding NL fractions isolated from the column to give NL of seeds (NL 1) and shells (NL 2).

The contents of unsaponified substances were determined by saponifying aliquots of NL 1 and NL 2 under strong alkaline hydrolysis conditions [7, p. 87]. Unsaponified substances were extracted from the lipid hydrolysis products by diethylether. Their contents in NL 1 and NL 2 were determined gravimetrically. Table 1 gives the experimental results and shows that *Th. alterniflora* seeds contain significantly more free and bound lipids than shells. NL dominate in the total lipids of 1 and 2. The shell lipids contain more GL than seed lipids.

The investigation of the qualitative composition of the polar lipids of specimens 1 and 2 showed that seed GL consisted of a known component set (TLC, system 1): mono- and digalactosyldiacylglycerides and sterylglycosides and their fatty-acid esters. In contrast with the seed lipids, galactolipids dominated the shell GL. The components of seed PL (TLC, system 2) included phosphatidylethanolamines, phosphatidylcholines, and phosphatidylinosites and traces of phosphatidic acids.

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 0009-3130/04/4006-0532

TABLE 1. Properties and Ratios of Separate Lipid Groups of *Thermopsis alterniflora* Bean Seeds and Shells

Property	Seeds	Shells
Moisture, %	6.5	7.2
Mass of 1000 pieces, g	30.5	-
Content, dry mass % of lipids:		
free	7.96	3.56
bound	1.4	0.32
Composition of total lipids, %:		
neutral	98.2	94.9
glycolipids	0.7	5.1
phospholipids	1.1	Tr.
Content of unsaponified substances in neutral lipids, %	4.89	14.1

*Tr. < 0.1%.

TABLE 2. Composition of Neutral Lipids of *Thermopsis alterniflora* Seeds

Component	Content, mass % of neutral lipids
Hydrocarbons	0.9
Carotinoids	Tr.
Waxy esters, esters of triterpenols and sterols	2.7
Triacylglycerides	94.5
Free fatty acids	0.6
Fatty alcohols	0.3
Triterpenols and sterols	0.9
Unidentified	0.1

TABLE 3. Fatty-acid Composition of *Thermopsis alterniflora* Bean Seed and Shell Lipids, % by GC

Acid	Seed				Shell	
	NL	TAG	PL	GL	NL	GL
12:0	Tr.	0.2	Tr.	0.9	0.3	Tr.
14:0	1.4	0.2	1.7	7.5	7.0	0.5
15:0	Tr.	0.1	Tr.	0.1	0.2	-
16:0	9.5	8.4	19.0	31.5	14.3	21.8
16:1	Tr.	0.6	2.6	7.2	-	-
17:0	0.5	0.3	0.6	0.1	1.8	-
18:0	2.7	2.8	8.0	14.4	7.9	2.7
18:1	31.1	34.9	25.4	19.7	22.3	23.6
18:2	48.8	47.3	40.5	15.9	39.3	38.2
18:3	4.2	4.2	2.2	Tr.	2.4	13.2
20:0	1.8	1.0	Tr.	2.7	4.5	Tr.
$\Sigma_{sat.}$	15.9	13.0	29.3	57.2	36.0	25.0
$\Sigma_{unsat.}$	84.1	87.0	70.7	42.8	64.0	75.0

NL, neutral lipids; GL, glycolipids; PL, phospholipids; TAG, triacylglycerides; Tr. <0.1%.

The qualitative similarity of the lipid and lipophilic-component composition of *Th. alterniflora* seeds and shells was established by comparing analyses (TLC, system 3) of aliquots of NL 1 and NL 2 of identical concentration. Triacylglycerides were the principal components. However, shell lipids differed in that there was a large content of waxy esters and triterpenol and sterol esters. According to TLC, NL2 contained almost three times more unsaponified components than NL 1 (Table 1). The unsaponified substances of NL from both specimens were hydrocarbons, carotenes, high-molecular-weight alcohols, triterpenols, and sterols with fatty and cyclic alcohols dominating.

Next, seed NL were fractionated into pure components using CC. Table 2 presents the results and indicates that seed NL contain more fatty-acid esters of aliphatic alcohols, triterpenols, and sterols than free ones. The unidentified seed NL component was preliminarily assigned as an isoprenoid alcohol but was not further studied owing to its low content.

Acids were isolated from the products of strong hydrolysis of NL 1 and NL2 and also from mild alkaline hydrolysis of GL of both specimens, PL, and seed triacylglycerides in order to establish the fatty-acid composition. The fatty acids were analyzed by GC as the methyl esters [7, p. 244 and 7].

Substantial differences were observed in the fatty-acid classes of seed-lipid components. NL (including triacylglycerides) and PL of seeds (Table 3) were mostly esterified by unsaturated acids with linoleic acid dominating. On the other hand, seed GL contained more saturated acids with palmitic dominating. Shell GL contained a higher level of unsaturated acyls, including linolenic acid, than seed GL.

Thus, a comparison of the composition of NL and PL of *Th. alterniflora* seeds and shells showed that shell polar lipids consist of GL that differ from seed GL by a higher content of galactosyldiacylglycerides with unsaturated acids.

EXPERIMENTAL

The conditions for CC, TLC of lipids on silica gel, and GC of fatty-acid methyl esters were analogous to those described previously [8].

TLC of lipids used the following systems: $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (1, 65:20:10:10:3, for GL), $CHCl_3$:CH_3OH:NH_3 (25%) (2, 13:5:1, for PL), and C_6H_{12}:$(C_2H_5)_2O$:CH_3CO_2H (3, 70:30:1, for NL).

Lipids were extracted and purified by known methods [7, p. 74].

Th. alterniflora Regel et Schmalh. was collected in 2002 near Nanai in Tashkent District and was supplied by A. M. Nigmatullaev.

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