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# POLAR LIPIDS OF *Hippophae rhamnoides* LEAVES

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A large number of studies have been devoted to the chemical investigation of sea buckthorn, but they have all been restricted to interest in the fatty oil of the fruit. It has been established that it contains about 1% of phospholipids (PhLs) consisting mainly of lecithin [1]. Eight PhL components have been detected in sea buckthorn seeds [2].

Continuing a systematic study of the lipids of vegetative organs of plants of the Eleagnaceae family [3], we have investigated the polar compounds of sea buckthorn leaves; namely, the PhLs and the glycolipids (GLs). The yields of GLs and PhLs after purification of the polar lipids on Molselekt and their separation into individual groups amounted to 36.5 and 12.1% on the weight of the extract. Individual homogeneous classes of GLs and PhLs were obtained with the aid of PTLC in various solvent systems.

The main GL classes of the lipids were monogalactosyl-, digalactosyl-, and sulfoquinovosyldiglycerides (MGDGs, DGDGs, and SQVDGs, respectively), making up a total of 85.2% of the weight of the GLs (Table 1). The ratio by weight of the MGDGs, DGDGs, and SQVDGs was 6:4:1, respectively, which agrees with that found for the leaves of some higher plants [4]. In addition to the three main classes, we detected free and esterified sterol glycosides (SGs) and two unidentified GLs.

The most unsaturated class was the MGDGs, which contained 72% of linolenic acid, followed by the DGDGs, with 64% of this acid. As in the leaves of other plant materials, the SQVDGs were enriched with palmitic acid, which made up more than 50% of the weight of the acids.

Under the conditions that we used for the GLC analysis of the fatty acid (FA) compositions of individual classes of polar lipids (Tables 1 and 2), we were unable to separate the isomeric 16:1 acids. It is known that for photosynthesizing tissues the composition of the FAs acylating phosphatidylglycerol (PG) is characterized by a considerable level of the unusual hexadec-*trans*-3-enoic acid, which was first identified by Debuch [5], its structure being confirmed later by Klenk and Knipprat [6]. This acid is localized specifically in the second position of the GLs of the chloroplasts of the lamellae [7].

In view of these facts, the total FAs isolated from the individual classes of PhLs and GLs were separated in a thin layer of silica gel impregnated with 20% of $AgNO_3$; the monoene, diene, and triene fractions were subjected to periodate oxidation and the degradation products were identified by GLC [8].

It was established that each class of GLs contained two isomeric 16:1 acids: 16:1(9) and 16:1(3). Among the trienic acids, hexadeca-7,10,13-tridecenoic was concentrated mainly in the SQVDGs, although it is known that it is usually found in the MGDGs. In the sea buckthorn leaves, the amount of the 16:3 acid was least in the MGDGs (1%), which distinguishes this plant from the other species investigated [4].

Among the PhLs, four main classes were identified: phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), PGs, and phosphatidylinositols (PIs), and also traces of diPGs (see Table 2). The FAs of the PCs, PEs, and PIs had approximately the same qualitative and quantitative compositions, their main acids being the 16:0, 18:3, and 18:2 species. The 18:3, 16:0, and 16:1 acids predominated in the PGs. The usual 16:1(9) isomer — palmitoleic acid — was found in all the PhL classes, and the 16:1(3) isomer only in the PGs. The differences in the FA compositions of the PCs, PEs, PGs, and PIs of sea buckthorn leaves and the leaves of some other plant species are mainly quantitative [4, 8, 9].

The polar lipids investigated differed from the neutral lipids of the leaves, studied previously [3], in which — as in the fatty oil of the pericarp [10] — a high content of the 16:1(9) acid was found, together with the 16:0 acid. On comparing the PhL classes of sea buckthorn leaves and seeds [2], we see that, in contrast to the PhLs of the seeds, where the main esterifying acids are the 18:2 and 16:0 species, in the leaf PhLs they are, in the main, the 16:0 and 18:3 acids.

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TABLE 1. Glycolipids of Sea Buckthorn Leaves and Their Fatty Acid Compositions

| Class of GLs | Amount, % by weight | Acid, GLC, % by weight | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 15:0 | 16:0 | 16:1(3)+ 16:1(9) | 16:2 | 16:3 | 18:0 | 18:1 | 18:2 | 18:3 | Σ of other acids | $\Sigma_s$ | $\Sigma_u$ |
| SQVDGs | 7.5 | 3.3 | 1.8 | 55.4 | 3.2 | – | 4.8 | 6.1 | 6.0 | 3.6 | 15.8 | – | 66.1 | 33.4 |
| DGDGs | 32.5 | 0.3 | 0.2 | 24.4 | 1.5 | 1.3 | 1.1 | 1.2 | 1.4 | 2.7 | 64.2 | 1.7[a] | 26.1 | 73.9 |
| $X_1$ | 1.2 | 0.4 | – | 18.5 | 1.5 | – | 1.1 | – | 6.1 | 10.4 | 58.0 | 4.0[b] | 20.1 | 79.9 |
| $X_2$ | 1.7 | – | 0.4 | 20.0 | 1.8 | – | 1.1 | – | 8.3 | 9.0 | 57.7 | 1.7[a] | 20.9 | 79.1 |
| SGs | 5.7 | – | – | – | – | – | – | – | – | – | – | – | – | – |
| MGDGs | 45.2 | 0.3 | 0.2 | 9.1 | 1.3 | – | 0.9 | – | 2.9 | 11.7 | 72.2 | – | – | – |
| Ac-SGs | 6.2 | 1.4 | 0.8 | 28.5 | 2.9 | – | 0.9 | 1.4 | 14.4 | 10.8 | 31.4 | 7.5[c] | 33.7 | 66.3 |

a) 12:1, 13:1; b) 9:0, 11:1, 13:0, 14:1; c) 13:0, 14:1; c) 13:0, 14:1, 14:2, 20:1.

TABLE 2. Phospholipids of Sea Buckthorn Leaves and Their Fatty Acid Compositions

| Class of GLs | Amount, % by weight | Acid, GLC, % by weight | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | Σ of other acids | $\Sigma_s$ | $\Sigma_u$ |
| PCs | 32.8 | 0.6 | 30.4 | 0.8 | 2.5 | 5.3 | 19.5 | 38.2 | 2.7[a] | 34.7 | 65.3 |
| PEs | 29.7 | 1.2 | 35.2 | 2.5 | 2.2 | 4.8 | 15.2 | 32.3 | 6.6[a] | 40.0 | 60.0 |
| PGs | 21.5 | 0.8 | 21.5 | 12.6 | 1.2 | 2.4 | 8.5 | 48.6 | 4.4[b] | 25.5 | 74.5 |
| PIs | 16.0 | 1.0 | 37.4 | 1.9 | 3.6 | 4.2 | 16.8 | 27.5 | 7.6[c] | 44.6 | 55.4 |

a) 9:0, 10:0, 12:0, b) 10:0, 10:1, 14:1; c) 10:0, 12:0, 15:0.

This is the first time that the GLs of this plant material have been investigated.

## EXPERIMENTAL

The plant material was gathered in September in the berry-ripening period in the Paltau mountain area. After comminution, the air-dry sea buckthorn leaves were extracted with chloroform–methanol (2:1). The extract was separated into neutral and polar lipids by countercurrent distribution in a two-phase 85% ethanol–petroleum ether (1:1) [11].

The total polar lipids were purified on a column filled with Molselekt G-25, separation into GLs and PhLs was carried out by CC and PTLC, and the separation of the GLs and PhLs as in [12].

The yields of neutral and polar lipids amounted to (in % of the total extract) 51.4 and 48.6%, respectively.

The polar lipids were saponified by the procedure of [12].

The preliminary identification of the fatty acid methyl esters (FAMEs) on GC chromatograms was achieved by comparing relative retention times (RRTs) in relation to the 16:0 species, and also by plotting a graph of the dependence of the logarithm of the retention time on the number of C atoms in the chain.

The total FAs were oxidized by the periodate–permanganate method, as in [11].

For the conditions of recording the MEs of dicarboxylic acids in GLC, see [8].

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