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LIPIDS FROM LEAVES OF *Ephedra equizetina*

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The content and composition by class and fatty acid of neutral (NL), glyco- (GL), and phospholipids (PL) in leaves of Ephedra equizetina *Bunge (Ephedraceae) are determined. The acid composition of NL, GL, and PL includes saturated 12:0-32:0 acids and unsaturated 15:1, 16:1, 18:1, 18:2, and 18:3 acids. Unsaponified components of the total lipids also contained biologically active substances such as α-tocopherol, carotenoids, high-molecular-weight fatty alcohols, triterpenes, and sterols.*

Ephedra is a genus of gymnospermous conifers in the Ephedraceae family that includes more than 40 species found in deserts, steppes, and thin forests of Eurasia, northern Africa, and North and South America [1]. *E. equizetina* and other species contain the alkaloid ephedrine, which has medicinal applications. The extract of *E. equizetina* plants is used as a cold remedy and for treating stomach ulcers. The extract of its fruits possesses antipyretic activity. Branches of *E. distachya* or *E. kuzmichovii* are used in folk medicine to cure rheumatism. The presence of biologically active phenolic compounds in ephedra and the rather abundant raw material make a more rational use of the plant in the medical and food industries promising [2].

We investigated lipids in the leaves of *E. equizetina* collected in the Alimtau mountains of Yuzhno-Kazakhstan district during the growth phase. The lipid composition of *E. equizetina* has not previously been reported in the literature available to us. Only the fatty-acid composition of the seed lipids is known. High-molecular-weight unsaturated 20:2, 20:3, and 20:4 acids were detected [3].

Total lipids from air-dried leaves of 7.4% moisture were extracted by the Folch method, purified of nonlipidic components, and washed with 0.04% aqueous $CaCl_2$ solution. The extract was separated into neutral lipids (NL) and polar lipids by counter-current techniques [4]. The polar lipids were fractionated into glycolipids (GL) and phospholipids (PL) by preparative TLC.

Leaves of *E. equizetina* contained 4.7% total lipids in which the fractions of NL, GL, and PL were 3.0, 1.6, and 0.1%, respectively, of the dry weight. Thus, NL dominate (63.6%) in the total lipids; GL (34.7%), in the polar lipids. This is expected based on the literature [5].

The qualitative composition of the lipids was established using analytical TLC under the conditions used to separate NL, GL, and PL. The lipids were identified by comparison with known classes of compounds, literature data, and qualitative reactions [4].

TLC using systems 2 and 3 identified in NL hydrocarbons, waxy ethers, triacylglycerines, free fatty acids, tocopherols, triterpenes, sterols, diacyl- and monoacylglycerides, and pigments such as carotenes, chlorophylls, and their oxidation products.

Monogalactosyldiacylglycerines, digalactosyldiacylglycerines, and steroid glycosides were detected in the GL fraction using system 4.

According to TLC in system 5, the PL contain mainly three components: phosphatidylglycerines, phosphatidylinosites, and phosphatidic acid.

The fatty-acid compositions of NL, GL, and PL were determined by GLC of their methyl ethers (Table 1).

Table 1 shows that the contents of saturated and unsaturated acids in NL and PL of the leaves are almost identical. However, the ratios of components are different. The most unsaturated acid (18:3) is present in all three groups of lipids in small quantities (2.3-3.9%). Of the unsaturated acids in the PL and GL, the 18:1 acid dominates. The amounts of the 18:1 and 18:2 acids in the NL are almost identical.

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TABLE 1. Fatty-Acid Composition of *E. equizetina* Leaves (%, GLC)

Acid	Neutral lipids	Glycolipids	Phospholipids
12:0	2.3	Tr.	Tr.
13:0	0.4	0.6	0.4
14:0	1.8	1.6	1.0
15:0	7.4	1.0	0.6
15:1	0.4	Tr.	Tr.
16:0	15.3	58.7	38.9
16:1	4.1	Tr.	Tr.
17:0	0.9	3.7	4.7
18:0	8.5	4.2	3.6
18:1	18.5	13.6	28.5
18:2	19.4	6.5	14.9
18:3	3.9	3.1	2.3
20:0	12.3	7.0	5.1
22:0	4.8	Tr.	Tr.
Σ_{sat}	53.7	76.8	54.3
Σ_{unsat}	46.3	23.2	45.7

The saturated 23:0-32:0 acids were identified in mass spectra in addition to the saturated acids listed in Table 1 for NL in the leaves of *E. equizetina*. The acids 20:0-30:0 (6.2% total) were detected previously in lipids from process wastes of the underground part of this plant [6]. Furthermore, high-molecular-weight unsaturated 20:2, 20:3, and 20:4 acids were found in the seeds of *E. campilopodia* [3]. Data from Ag^+-TLC, GLC, and mass and UV spectra of the methyl ethers indicate that lipids of the leaves studied by us do not contain such acids.

Thus, the unsaturated 15:1, 16:1, 18:1, 18:2, and 18:3 acids occur in lipids of *E. equizetina* leaves. The most saturated class is GL, mainly owing to the 16:0 acid. The 20:0 and 22:0 acids are more prominent in NL.

Unsaponified components of *E. equizetina* leaves were isolated by strong alkaline hydrolysis [7] in 22.9% yield (based on total lipid mass). The carotenoid content in them, according to UV spectra, was 6.1 mg/g. The carotenoids include β-carotene and xanthophylls. The latter were probably identified by UV spectroscopy as violoxanthine and luteine [8].

The unsaponified components also contained (TLC, systems 2 and 3) hydrocarbons, high-molecular-weight fatty alcohols, triterpenes, steroids, and α-tocopherol. These components were separated by preparative TLC using system 6 and were analyzed by UV spectroscopy and mass spectrometry.

According to TLC in system 7, the hydrocarbons include zones of substances belonging to alkanes and olefins (R_f 0.90) in addition to aromatic (R_f 0.67) hydrocarbons. The hydrocarbon composition was established by UV and mass spectra. The alkanes include the homologs $C_{21-33}H_{44-68}$, with $C_{29}H_{60}$ predominating. The olefinic hydrocarbons were identified as $C_{21-33}H_{42-66}$. The aromatic hydrocarbons corresponded to alkylbenzenes of the series $C_{21-33}H_{36-60}$. The structures of other unsaturated components occuring in small quantities were not established owing to the complicated hydrocarbon composition.

High-molecular-weight fatty alcohols of *E. equizetina* leaves consisted of even homologs $C_{22-30}H_{45-61}OH$ according to mass spectra.

Mass spectra of the tocopherols revealed peaks of M^+ (430) and fragments formed via decomposition of α-tocopherol [9]. The characteristic ions M^+ at 402 and 416 that are due to the δ-, β-, and/or γ-isomers were not observed.

The steroids included the usual set of components consisting of cholesterol, campesterol, stigmasterol, and β-sitosterol with the last predominating. These components were accompanied by small quantities of the triterpenes lupeol, cycloartanol, and cycloartenol.

Thus, *E. equizetina* leaves contain lipids enriched with such biologically active components of neutral lipids as β-carotene, fatty alcohols, α-tocopherol, and 18:2 and 18:1 fatty acids [10].

EXPERIMENTAL

UV spectra were recorded on a Perkin—Elmer Lambda-16 instrument in hexane. Mass spectra were measured on an MX-1310 instrument by direct probe sample introduction at 50 V ionizing potential, 40 μA collector current, 170°C ionization chamber temperature, and 80°C vaporization temperature.

GLC was performed under the conditions reported in the literature [11].

TLC of lipids was carried out on L 5/40 silica gel and Silufol plates (Czech Republic) in solvent systems acetone (1), hexane—diethyl ether (98:2) (2), heptane—methylethylketone—acetic acid (43:7:1) (3), acetone—benzene—water (91:30:8) (4), $CHCl_3$—CH_3OH—NH_4OH (25%) (65:25:5) (5), hexane—diethyl ether (1:1) (6), and heptane—benzene (9:1) (7).

Moisture was determined according to the literature method [7].

Total Carotenoids. UV spectrum, λ_{max}(hexane): 420, 443, 471 (violoxanthine), 420, 445, 475 (luteine), 425, 450, 476 nm (β-carotene).

Hydrocarbons. UV spectrum, λ_{max}(hexane): 221 nm. Mass spectrum: *m/z* 292-460 (M^+, alkanes), 290-458 (M^+, olefins), 288-456 (M^+, alkylbenzenes) [9].

High-Molecular-Weight Alcohols. Mass spectrum: $[M - 18]^+$, 308-420.

α-Tocopherol. Mass spectrum, *m/z* (*I*, %): 430 (M^+, 100), 205 (57.7), 176 (30.8), 166 (44.2), 165 (94.0), 164 (46.2) [9].

Sterols. Mass spectrum, *m/z* (*I*, %): M^+ 386 (15.6, cholesterol), M^+ 400 (37.5, campesterol), M^+ 412 (21.9, stigmasterol), M^+ 414 (100, β-sitosterol) [12].

Triterpenes. Mass spectrum, *m/z* (*I*, %): M^+ 426 (3.9), 411 (2.6), 207 (20.5), 189 (39.5), 95 (100) (lupeol) [13]; M^+ 428 (3.1), 413 (5.2), 410 (3.1), 395 (6.8), 367 (14.6), 341 (26.6), 288 (11.5), 69 (100) (cycloartanol); M^+ 426 (3.1), 411 (4.2), 408 (3.1), 393 (4.2), 365 (12.0), 339 (28.6), 286 (15.6), 69 (100) (cycloartenol) [14].

Fatty acids. Mass spectrum: ([M^+] 368, 382, 396, 410, 424, 438, 452, 466, 480, 504; $[M - 31]^+$ 337, 351, 365, 379, 393, 407, 421, 435, 449, 473; $[M - 74]^+$ 294, 308, 322, 336, 350, 364, 378, 392, 406, 430) [15].

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