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LIPIDS OF *Acorus calamus**

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Lipids of the leaves, stems, and rhizomes with roots of Acorus calamus *L. were investigated. The neutral-lipid classes and fatty-acid composition of neutral-, glyco-, and phospholipids were determined. The essential oil content in the total lipids of A. calamus was established by steam distillation.*

Key words: *Acorus calamus*, neutral lipids, glycolipids, phospholipids, fatty acids, essential oil.

Acorus calamus L. (sweet flag) is a member of the Araceae (aroid) family. The rhizomes contain starch, the bitter glucoside acorin, the alkaloid calamine, vitamin C, and essential oil (4.8%) [1]. The essential oil is known to contain eugenol, borneol, azarone, palmitic acid, the diketone acorone, isoacorone, anisoxide, etc. [1, 2].

The rhizome extract possesses sedative, antiflatulative, and stimulant activity and is used in many areas of medicine [1].

We found no data in the literature for the lipids of sweet flag.

Essential oils are extracted together with lipids from the plants by various solvents such as $CHCl_3$ [3], petroleum ether [4], $CHCl_3$—CH_3OH (2:1, v/v) [5], and others.

We extracted finely ground air-dried leaves with stems (**1**) and rhizomes from roots (**2**) with a $CHCl_3$—CH_3OH mixture (2:1, v/v). The yield of extracted substances from **1** was 2.9%; from **2**, 5.6%, after removal of nonlipidic components by $CaCl_2$ solution (0.04%).

The isolated fractions have pleasant odors typical of essential oils. Column chromatography on silica gel with elution by $CHCl_3$ gave neutral lipids (NL); by CH_3OH, polar lipids (PL). Preparative TLC on silica gel with elution by system 1 of the PL isolated glycolipids (GL) and phospholipids (PhL). Essential oil was found mainly in the NL by repeating TLC of the isolated fractions using system 2.

Below we give the quantitative composition of the isolated lipid fractions.

Component	Content, %	
	1	**2**
NL	64.5	75.4
GL	28.2	17.4
PL	7.3	7.2

The NL are the principal lipid components of both the aerial part and the rhizomes. PhL were distributed almost equally in the investigated plant parts. GL were concentrated mainly in **1**.

TLC of NL on silica gel using systems 2 and 3 showed that lipid classes of **1** and **2** were qualitatively the same. They include hydrocarbons, fatty-acid esters of sterols and triterpenols, fatty-acid methyl esters, triacylglycerides (TAG), free fatty-acid (FFA), triterpenols, and sterols.

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TABLE 1. Fatty-Acid Composition of *A. calamus* Lipids, % GLC

Lipids	Acids									
	12:0	14:0	16:0	18:0	16:1	18:1	18:2	18:3	Σ_{sat}	Σ_{unsat}
Leaves and stems										
NL	Tr.	5.1	25.5	7.2	Tr.	6.8	26.3	29.1	37.8	62.2
GL	0.6	0.8	40.4	13.3	5.8	6.9	12.9	19.3	55.1	44.9
PL	Tr.	1.3	36.2	15.1	2.4	11.1	10.4	23.5	52.6	47.4
Rhizomes from roots										
NL	9.7	22.0	14.1	Tr.	14.3	20.9	19.0	Tr.	45.8	54.2
GL	1.3	1.5	34.9	10.9	Tr.	14.7	34.3	2.4	48.6	51.4
PL	Tr.	0.6	35.6	8.1	Tr.	11.1	41.7	2.9	44.3	55.7

Alkaline hydrolysis of the separate lipid classes gave the total fatty acids, which were converted to the methyl esters by diazomethane and analyzed by GLC (Table 1).

Table 1 shows that lipids from these specimens have qualitatively identical fatty-acid compositions. Palmitic acid dominates in the GL and PhL. The very unsaturated linolenic acid is present in a significant amount in lipids from the leaves and stems. This is characteristic of photosynthetic tissue. Its fraction in lipids from rhizomes is only 3%. Palmitoleinic acid is concentrated mainly in NL of the rhizomes, in which the content of 12:0 and 14:0 acids is also high.

Essential oil (51.7%) was isolated from the total lipids of rhizomes with roots by steam distillation. This was 2.9% calculated on the basis of air-dried material.

Solvent systems that are usually used to separate NL were used to analyze the essential oils by TLC [6].

TLC on silica gel and Silufol using system 2 gave the starting lipids, a fraction of lipids remaining after steam distillation, and essential oil. The essential oil was separated into eight spots that varied significantly in chromatographic mobility: R_f 0.23, 0.29, 0.36, 0.46, 0.50, 0.76, 0.84, and 0.92. The spot with R_f 0.84 corresponded to the fatty-acid methyl esters.

The presence of the methyl esters in the essential oil was proved by saponifying them with alcoholic KOH (10%). The unsaponified fraction was isolated with ether. The saponified part was decomposed with H_2SO_4 (20%). The products isolated by ether were analyzed by TLC. The chromatogram detected fatty acids and another two unidentified spots. The literature contains reports of fatty acids and their methyl esters in essential oil [7, 8]. According to GLC, the composition of fatty acids (%) isolated from the essential-oil fractions is: 14:0, 3.7; 16:0, 29.1; 18:0, 10.8; 18:1, 21.2; 18:2, 35.2. Palmitic, oleinic, and linoleic acids dominated.

The unsaponified fraction of essential oil was separated by TLC using system 2 into a mixture of components, the strongest spots of which corresponded to R_f 0.27 and the hydrocarbon fraction. The hydrocarbon fraction was isolated by preparative TLC on silica gel using system 2 and separated by TLC using petroleum ether [6]. A large part of the hydrocarbons was located close to the solvent front. Three components with R_f 0.63, 0.71, and 0.81 represented an insignificant fraction.

Thus, the study of lipids from the aerial part and rhizomes with roots of *A. calamus* showed the presence of several biologically active substances such as GL, PhL, triterpenols, sterols, and essential oil.

EXPERIMENTAL

GLC was performed on a Khrom instrument using a stainless-steel column packed with PEGS (17%) on Chromaton W, temperature thermostatted at 198°C, and N_2 carrier gas. TLC was carried out on KSK silica gel and Silufol plates in the solvent systems: acetone—toluene—acetic acid—water (60:60:2:1) (1) and hexane—diethylether (7:3, 2 and 1:1, 3). Plates were visualized using $KMnO_4$ (0.5%) in H_2SO_4 (0.5%).

Steam distillation of the lipid extract was carried out in a 250-mL round-bottom flask containing the material by adding steam. The essential oil that distilled after 1 h into the receiving flask was extracted three times with ether. The ether was removed at 25°C. The essential oil was weighed. The remainder of the lipids in the flask was re-extracted with $CHCl_3$. The

$CHCl_3$ was removed in a rotary evaporator. The yield was 48.3% of the mass of starting lipids.

A. calamus was grown on the experimental plot and analyzed within four months of its collection.

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