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LIPIDS OF *Calendula officinalis*

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The lipids of the seeds and of extracts of the leaves and flowers of Calendula officinalis *L., fam. Asteraceae, have been analyzed. The amount of neutral lipids in the seeds was 15.7%, that of phospholipids 0.6%, and that of glycolipids 0.9%. Among the fatty acids of the neutral lipids of the seeds, in addition to the usual components, we found the 18:3* (trans-8, trans-10, cis-12) *acid that is characteristic for the Calendulae tribe.*

Calendula officinalis L. – marigold – is a well-known plant of the Asteraceae family. A dye used in the food industry is obtained from the flowers of this plant [1]. Extracts and tinctures of the flowers are used in medical practice as bactericidal, wound-healing, antiphlogistic, disinfectant, astringent, cholagogic, and diuretic agents. An oil extract of the flowers is used in the treatment of wounds, ulcers, bedsores, and burns. An extract of the flowers is a component of the "cn" tablets (calendula with nicotinic acid) used as a symptomatic agent in some forms of cancer [2].

Flavonoids, resins, organic acids, an essential oil, vitamin K, carotenoids, and tanning and pectin substances have been detected in the flower heads [2, 3].

The main components of a petroleum ether extract of the flowers are free steroids, triterpene monools and diols, and esters of the latter with the 2:0, 10:0, 12:0, and 16:0 fatty acids [4, 5]. In the fatty acids of marigold seeds have been found about 59% of an 18:3 conjugated trienic (*trans*-8, *trans*-10, *cis*-12) acid – calendic acid and about 5% of a 9-hydroxy-18:2 (*trans*-9, *cis*-11) acid – dimorphecolic acid [6]. These acids are classified as uncommon ones. Among them, calendic acid is characteristic only for some genera of Asteraceae belonging to the Calendulae tribe [7].

In the present paper, we give mainly the results of an investigation of the neutral lipids (NLs) and polar lipids (PLs) of marigold seeds. The amounts of lipids in the seeds were, %: NLs – 15.7; glycolipids (GLs) – 0.9; and phospholipids (PhLs) – 0.6. The yield of extracts from the flowers (EF) was 17.1%, and from the leaves (EL) 9.3%,

The total NLs were separated into individual classes by column chromatography and preparative thin-layer chromatography on silica gel. The lipids were identified by qualitative reactions and chemical transformations. The amounts of the various classes were determined gravimetrically.

The following classes of lipids were revealed (% by weight): hydrocarbons (HCs) – 0.9; esters of sterols and triterpenols with fatty acids (SEs) – 0.5; triacylglycerols (TAGs) – 20.0 (TAGs-1 – 59.0; TAGs-2 – 11.8; TAGs-3 – 4.3); free fatty acids (FFAs) – tr.; hydroxy-TAGs – 0.8; free sterols (FSs) and triterpenols (FTs) – 0.4; diacyl- and monoacylglycerols (DAGs and MAGs) – 1.2; unidentified components – 1.1.

As we see, the common TAGs were separated into four fractions with R_f 0.72, 0.61, 0.49, and 0.39 in solvent system 1.

The UV spectra of the TAGs-1, TAGs-2, and TAGs-3 each showed three peaks, at 264, 272, and 288 nm, which confirmed the presence of a conjugated trienic acid in these TAG fractions.

The unusual polarity of the TAG fractions is explained by their different levels of calendic acid, as shown by the GLC of the fatty acids (Table 1). The TAGs-1 contained 32.2% of the 18:3 (8,10,12) acid, i.e., one position in them was occupied by this acid. In the TAGs-2 there was 46% of this acid, which is somewhat less than if two positions were occupied by it. The low content of the conjugated trienic acid is apparently explained by its rapid polymerization during the separation and purification of the TAGs on silica gel. For this reason, it was impossible to isolate the TAGs-3 when the corresponding fraction was rechromatographed in a thin layer of silica gel.

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TABLE 1. Composition of the FFAs of *Calendula officinalis* Lipids (GLC, % by weight)

Acid	Leaves	Flowers	Seeds				
			NLs	GLs	PhLs	TAGs-1	TAGs-2
10:0	0.2	0.5	–	tr.	tr.	–	–
12:0	1.8	3.0	1.5	tr.	tr.	1.7	1.5
13:0	0.5	–	–	–	–	–	–
14:0	3.4	13.7	1.5	3.8	1.0	1.7	3.0
15:0	0.6	–	tr.	tr.	tr.	–	–
16:0	12.8	28.9	8.0	23.5	22.4	7.8	4.7
16:1	2.0	tr.	2.1	0.9	0.5	2.6	3.2
17:0	3.5	2.8	2.0	tr.	0.6	2.0	2.1
18:0	7.3	5.3	5.4	3.8	7.1	5.2	4.7
18:1	6.5	4.2	11.3	20.6	43.9	11.0	8.2
18:2	13.4	22.4	33.3	47.4	24.5	27.7	17.0
18:3	11.9	17.1	4.7	tr.	tr.	4.6	5.3
20:0	13.8	tr.	3.6	–	–	3.5	4.4
21:0	2.8	tr.	–	–	–	–	–
22:0	6.2	tr.	–	–	–	–	–
23:0	1.5	tr.	–	–	–	–	–
24:0	2.8	tr.	–	–	–	–	–
25:0	1.6	tr.	–	–	–	–	–
18:3 (conj.)	7.4	2.0	26.6	–	–	32.2	45.9
Σ_{sat}	58.8	54.2	22.0	31.1	31.1	21.9	20.4
Σ_{unsat}	41.2	45.7	78.0	68.9	68.9	78.1	79.6

The quantitative compositions of the PhLs and GLs were determined by TLC on silica gel in solvent systems 2 and 3. It was found that the PhL complex of marigold seeds consisted of eight classes, which may be arranged in order of content by weight as follows: PCs > PIs > N-acyl-PEs > N-acyl-lyso-PEs > lyso-PIs > PSs > lyso-PCs > PEs. We must mention the low level of PEs in the seeds of the plant investigated, which is uncharacteristic for plant PhLs.

On analyzing the total PhLs by TLC on silica gel in solvent system 4, we detected a component rarely encountered – a phosphonolipid. From its mobility in a thin layer of silica gel and from qualitative reactions this phosphonolipid was assigned to the phosphono analogs of the PCs [8].

The glycolipids of the seeds were represented by four components (TLC, solvent system 3) forming the following sequence by mass content: SGs > ESGs > MGDGs > DGDGs.

The compositions of the fatty acids of the NLs, GLs, and PhLs differed with respect to both the sets of components and their amounts. The FAs of the NLs were more unsaturated and included about 4.7% of the 18:3 (9,12,15) acid and 26.6% of calendic acid, while the latter was absent from the GLs and PhLs. Although the ratios of the weights of saturated and unsaturated acids in the GLs and PhLs were the same, differences were observed in the ratios of 18:1 and 18:2 acids, linoleic predominating in the GLs and oleic in the PhLs.

Analysis of the FEs and LEs on Silufol plates in solvent systems 1 and 5 revealed the presence of the following components: HCs, SEs, TAGs, DAGs, MAGs, FFAs, FSs, FTs, carotenoids, chlorophylls, and unidentified substances. We may note that, of the above-mentioned components, in the FEs the free triterpene alcohols and sterols predominated considerably by weight. In the LEs no clearly predominating class of lipids was observed. As the figures in Table 1 show, the leaf and flower extracts had richer sets of fatty acids than the seed lipids. More than half the weight of the FFAs of these extracts consisted of saturated acids, the 16:0 and 20:0 acids predominating in the LEs and the 16:0 and 14:0 acids in the FEs.

EXPERIMENTAL

The UV spectra of the TAGs were taken on a Hitachi spectrophotometer.

TLC was conducted on L5/40 mesh silica gel with 10% of gypsum. Solvent systems: 1) hexane–diethyl ether–acetic acid (7:3:0.1); 2) chloroform–methanol–ammonia (65:35:5); 3) acetone–benzene–water (91:30:8); 4) methanol–water (2:1); 5) hexane–diethyl ether (1:1).

The GLC of the FFAs was conducted on a Chrom-41 instrument with a flame-ionization detector at a column temperature of 198°C with helium as the carrier gas. A 4 × 2000 mm column filled with PEGS on Chromaton W was used.

The neutral lipids were extracted from the previously ground seeds with hexane by steeping. The GLs and PhLs were extracted together from the residual meal by Folch's method [9]. The flower and leaf extracts were obtained by the same

method. The total PhLs were freed from hydrocarbons by CC on Molselekt G-25 as described in [10] and were then separated into GLs and PhLs by TLC in dry acetone.

The CC of the neutral lipids was conducted on L100/160 mesh silica gel, the various classes being eluted with hexane containing ether in concentrations gradually increasing from 0 to 100%.

The GLs were identified with α-naphthol, the PhLs with ninhydrin and the Vas'kovskii and Dragendorff reagents [11], and the NLs with iodine vapor and 50% sulfuric acid.

Alkaline hydrolysis of the lipids was carried out as in [12].

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