LIPIDS OF THE PETALS OF *Carthamus tinctorius*

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The following compounds have been identified in the lipids of the petals of *Carthamus tinctorius:* C_{32} and C_{29} isoparaffins; free fatty acids, the main component of which is palmitic acid; 33 esters of phytol, esterified with three groups of fatty acids — paraffinic, isoparaffinic, and monoenoic of the C_9-C_{26} series; and β-sitosterol and its β-D-glucopyranoside.

Plants of the genus *Carthamus*, family Asteraceae are rich in pigments, flavonoids, vitamins, proteins, and other valuable substances [1], and their seeds contain an edible oil [2]. The structures of the substances in the leaves of these plants, apart from the pigments, have not been studied. We have investigated some lipids from the petals of *C. tinctorius* (safflower).

The hydrocarbons, according to the results of IR and mass spectroscopy, form a mixture of paraffins with molecular weights of 436 (C_{31}) and 408 (C_{29}), the first of them being present in greater amount. The presence of a doublet and a triplet at 0.8 and 0.86 ppm in the PMR spectrum of these substances and the total integral intensities of these signals in comparison with the integral intensity of the "methylene hump" (9 H as compared with 54 H, respectively) show branching of the paraffins at the second carbon atom. Consequently, the hydrocarbons isolated are isoparaffins: 2-methyltriacontane and 2-methyloctacosane. Similar branched paraffins have been detected previously as trace impurities in refined safflower oil [2].

Esters of phytol migrate in a thin layer of silica gel similarly to waxes. The IR spectrum of these substances confirms the presence in the molecules of an ester bond, of a long hydrocarbon chain, of a cis-ethylenic bond, of a long hydrocarbon chain, of a cis-ethylenic bond, and of methyl branching. The PMR spectrum indicates the presence of five or six methyl groups, about one olefinic proton, and two protons of an esterified primary alcohol group (=CHC$\underline{H_2}$OCOR). The mass spectrum of the combined esters showed the presence of 28 homologs with molecular weights of 674-436.

The alkaline hydrolysis of the esters gave two products in a weight ratio close to 1:1.3 migrating in a thin layer of silica gel in a similar manner to higher alcohols and fatty acids. From the results of IR, PMR, and mass spectroscopy the first product was identified as phytol and the second as fatty acids. The latter were methylated with diazomethane and the methyl esters were separated quantitatively by gas-liquid chromatography. From the results of IR and PMR spectroscopy and the GLC of the fatty methyl esters, higher fatty acids with even and odd numbers of carbon atoms were identified: 23 saturated, from $C_{29:0}$ to $C_{9:0}$, and 10 monenoic, from $C_{26:1}$ to $C_{18:1}$ and $C_{16:1}$. Of them, the 12:0, 14:0, 20:0, and 26:0 acids and part of the 16:0, 18:0, 20:0, and 24:0 acids were branched, since the logarithms of the relative times of the methyl esters of these acids on a graph of their dependence on the number of carbon atoms lay on a straight line passing parallel to and below the straight line for the methyl esters for the corresponding unbranched saturated acids [3].

It is possible to summarize the results obtained in the form of a structural formula of esters of phytol and fatty acids:

$$H[CH_2-\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2CH_2]_3CH_2C=\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2OCOR,$$

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TABLE 1. Composition of the Fatty Acids of the Phytol Esters according to GLC, % of the Total

Acid	Amount	Acid	Amount	Acid	Amount
Iso-26:0	7,2	26:0	Tr.	26:1	Tr.
—		25:0	Tr.	25:1	Tr.
Iso-24:0	5,9	24:0	5 2	24:1	Tr.
—		23:0	Tr.	23:1	Tr.
Iso-22:0	2,1	22:0	29 5	22:1	Tr.
—		21:0	Tr.	21:1	Tr.
Iso-20:0	3,0	—		20:1	Tr.
—		19:0	9,0	19:1	Tr.
Iso-18:0	Tr.	18:0	6 8	18:1	4,5
—		17:0	Tr.	—	
Iso-16:0	Tr.	16:0	12,3	16:1	2,4
—		15:0	1,2	—	
Iso-14:0	0,9	—		—	
—		13:0	Tr.	—	
Iso-12:0	10,0	—		—	
—		11:0	Tr.	—	
		10:0	Tr.	—	
		9:0	Tr.	—	
Total: Iso acids 29.1		Saturated acids 64.0		Monoenoic acids 6.9	

where R is the hydrocarbon residues of the acids (Table 1).

The free fatty acids from a petroleum ether extract consisted of myristic (0.8% of the total), myristoleic (0.7), palmitic (71.1), palmitoleic (1.3), stearic (15.1), oleic (1.8), linoleic (3.5), linolenic (1.2), and arachidic (4.5). The main component of these acids was palmitic.

The sterols from a methanolic extract consisted mainly of β-sitosterol according to melting points, molecular weight, and characteristic fragmentation (mass spectrum). Bound β-sitosterol in the form of the β-D-glucopyranoside was isolated simultaneously and was identified from the results of acid hydrolysis, GLC, [4, 5], melting point, specific rotation, and migration in a thin layer of adsorbent.

EXPERIMENTAL

IR spectra were recorded on a UR-10 instrument, PMR spectra on a JNM-4H-100/60 MHz instrument (in 10-14% solutions in CCl_4 with HMDS as standard), and mass spectra on a MKh-1303 instrument.

The gas-liquid chromatography of the fatty acid methyl esters was carried out on a Khrom-4 instrument and that of the sugars, in the form of the methylsilyl ethers of the methyl glycosides, on a Tsvet-4 instrument.

Isolation of the Hydrocarbons and Esters. Distilled water was added in a ratio of 1:5 to the combined substances of an ethanolic extract from safflower petals (20.4%). From the resulting mass were extracted the substances passing into petroleum ether (7.4%), chloroform (1.2%), ethyl acetate (1.9%), butanol (2.6%), and water (7.2%). The combined substances extracted by petroleum ether were subjected to column chromatography. A series of fractions of substances was isolated in the first of which hydrocarbons were detected (0.7%), and in the second esters (0.1%). The substances of each fraction were purified by rechromatography on a thin layer of silica gel in suitable solvent systems, 1-3 and 3-5.

Column Chromatography. Silica gel L 100/160 μ. Diameter and height of the column of adsorbent 3.5 × 120.0 cm. Ratio of weight of substance to adsorbent 1:10. Elution was carried out with petroleum ether containing chloroform (systems 1-4). The eluates were collected in a volume of 200 ml. The solvents were replaced after the evaporated eluates had become free from the components of the mixture of substances being separated. The composition of the fractions of substances was checked by analytical thin-layer chromatography in the corresponding solvent systems.

To separate the substances of the methanolic extract we used alumina (Brockman activity V). Solvent systems 8 and 9.

Thin-layer Chromatography. A. Silica gel LS 5/40 μ. Glass plates 6 × 9 cm for analytical and 18 × 24 cm for preparative purposes. The zones of the substances were revealed by keeping the chromatogram in iodine vapor and by spraying them with 50% sulfuric acid followed by the carbonization of the substances.

B. Type LS 5/40 μ silica gel impregnated with a 0.3 M solution of sodium dihydrogen phosphate. Solvent system 11. Revealing agent: 25% methanolic solution of tungstophosphoric acid for sterol glycosides, o-toluidine salicylate in ethanol (4 g of salicyclic acid, 2.5 ml of o-toluidine, and 100 ml of ethanol) for free sugars.

Solvent Systems. Petroleum ether–chloroform: 1) 10:0; 2) 9:1; 3) 8:2; 4) 7:3; 5) 6:4. Petroleum ether–diethyl ether: 6) 8:2; 7) 7:3. Chloroform–methanol: 8) 10:0; 9) 9:1. Benzene–ethyl acetate: 10) 3:1. Chloroform–methanol–water: 11) 65:35:8.

Hydrocarbons. IR spectra (ν_{max}^{KBr}, cm^{-1}): 2965 s, 2880 s, 1380 m, (–CH_3); 2935 v.s, 2855 v.s, 1465 s, 730, and 725 m, (–$(CH_2)_n$–), PMR spectrum (δ, ppm): d 0.8 and t 0.86 (9 H, 3 CH_3–); m 1.2, (∿54 H, –(CH_2) ∿ 27); m 3.8-3.9. (1 H, ⩽ CH). Mass spectrum (m/z, % rel.) M^+M^+ 436 (1.5) – C_{31} 408 (5.4) – C_{29}; $(M - 15)^+$ 421 (w), 393 (4.5); $(M - 15 - 14\ n)^+$ 407 (3.0), 379 (8.1), 365 (4.5), 351 (4.0), 337 (4.1), 323 (4.2), 309-211 (6-18), 197 (24)-141 (54)-57 (100). R_f 1.0 in system 7.

Esters. IR spectrum (ν_{max}^{film}, cm^{-1}): 3150 m; 3050 m, 1675 w, –CH=CH–, cis; 2960 s, 2860 s, doublet 1385 and 1370 m, (–$CH(CH_3)_2$); 2925 s, 2875 s, 1470 s, doublet 725 and 730, (–$(CH_2)_n$–); 1745 s, 1420 m, 1170 m, –OCOR. PMR spectrum (δ, ppm); d 0.8 >CH–CH_3); t 0.86 (3 H, CH_3–CH_2–); m 1.22, (–CH_2–)$_n$, superposed signals, (∿2H, $-CH_2C=$ and $-CH_2CH=CHCH_2-$); t 2.38(2.28(2H, RCH_2COO-); m 4-5 (>CH–CH_3); d 4.55 :2H, $=CHCH_2OCOR$), m 5.25 (∿1H, $-C=CH-$).

Mass spectrum (m/z, % rel.): M^+M^+ 674 (11.0), 672 (2); 660 (w), 658 (w); 646 (44.4), o44 (w); 632 (w), 630 (w); 618 (100.0), 616 (w); 604 (w), 602 (w); 590 (94.5), 588 (w); 576 (w); 574 (w); 562 (77.8), 560 (w); 548 (w); 534 (w), 532 (w); 520 (55.9), 506 (w), 492 (72.2), 478 (w), 464 (61.1), 450 (w), 436 (88.9). The peak of maximum intensity was taken among the peaks corresponding to the molecular ions. The remaining peaks correspond to the fragments $(M - 15)^+$, $(M - 15 - 14n)^+$ and to β-cleavage with transfer of the γ-hydrogen atom. R_f 96·0 in system 6 and 0.33 in system 2.

The alkaline hydrolysis of the esters was carried out with a 30% solution of caustic potash in methanol – 50 ml to 300 mg of esters. The mixture was kept at 50°C for 6 h with constant stirring by means of a magnetic stirrer. The methanol was evaporated off to a volume of 25 ml. The remaining concentrate was diluted with hot distilled water (1:10). The phytol was extracted from the resulting solution of potassium soaps with petroleum ether. The mixture of fatty acids was extracted with diethyl ether from the aqueous layer after its acidification with dilute sulfuric acid.

Phytol. IR spectrum (ν_{max}^{film}, cm^{-1}): 3400-3250 m, (–OH); 2970 s, 2865 s, doublet 1385 and 1370 s, (–$CH_2(CH_3)_2$); 2935 s, 2880 s, 1470 s, 740 m, (–$(CH_2)_4$–); 1020 s, (–CH_2OH); 1675 w (–CH=C<). PMR spectrum (δ, ppm): d 0.8 (12 H, >CH–CH_3); m 1.23, –$(CH_2)_n$–; superposed 7 H signals in the form of s 1.6 and t 1.9 (2H, $-CH_2C=$) d 3.95 (2H, $=CHCH_2OH$); m-5:3H, –CH– CH_3); m 5.3 (1H, –C=CH–).

Mass spectrum (m/z, % rel.): M^+ 296 (0.84), $(M - 15)^+$ 281 (0.85), $(M - 18)^+$, 278 (1.26), $(M - 18 - 15)^+$ 263 (0.84), $(CH_3C=CH-CH_2OH)^+$ 71 (100). R_f 2.5 in system 7.

Fatty Acids Esterifying the Phytol. IR spectra of the fatty acid methyl esters (ν_{max}^{film}, cm^{-1}): 3050 w, 1675 w, 2970 v.s, 2865 v.s, 1375 m, 2935 v.s, 288 v.s, 1470 s, 725 and 730 s, 1745 s, 1420 m, 1170 s.

PMR spectrum of the fatty acid methyl acids (δ, ppm): t 0.85 (4 H, $-CH_3$); m 1.24 (–CH_2–)$_n$; t 2.28 (2 H, $-CH_2OCOR$); s 3.6 (3 H, $-OCH_3$); m 4-4.2 (<1H, > CH–CH_3); m 5.22 (2 H, –CH=CH–). R_f 0.27 in system 6.

Isolation of the Free and Bound β-Sitosterol. A methanolic extract from the petals was obtained at room temperature. The sum of the extractive substances was chromatographed on a column of alumina in systems 8 and 9. The addition of methanol (1:1) to the chloroform fractions I-X led to the crystallization of the combined sterols. By chromatography on Silufol

in system 10 the main component of the combined sterols was isolated, with R_f 0.67 [11]; it was identical with β-sitosterol according to mass spectroscopy (mol. wt. 414, characteristic fragmentation, and melting point 139.5-140°C).

When elution of the substances from the column was continued with system 9, a glycoside with R_f 0.4 in the same system was isolated.

The glycoside had mp 285-287°C (from methanol), $[\alpha]_D$ −30° (c 1.4; pyridine). After the acid hydrolysis of the glycoside, the reaction mixture was diluted with water 1:1, the bulk of the methanol was distilled off, and the precipitate that had deposited was filtered off. The combined substances obtained in the precipitate were subjected to column chromatography on alumina in systems 8 and 9. β-Sitosterol, identified as described above, was isolated from the chloroform fractions.

On continuing elution, approximately 50% of unhydrolyzed initial glycoside was isolated. The filtrate was boiled for 2 h to decompose the methyl glycoside and was neutralized with barium acetate. The precipitate was filtered off and the filtrate was evaporated to dryness. D-Glucose was detected in the residue by thin-layer chromatography in system 11. These results were confirmed by gas-liquid chromatography [4] and the presence of a single sugar residue in the glycoside molecule was established.

Isolation of the Volatile Substances and Free Fatty Acids. On steeping at room temperature for a day the leaves yielded 6.6% of a petroleum ether extract. The amount of volatile substances in the dry extract after the evaporation of the solvent (1.6%) was determined by adding ethanol to it and then distilling it off. At the same time, the amount of essential oil in the leaves, which we determined by Ginzburg's method [7] amounted to 1.1%.

From the residual 5% of nonvolatile components, on crystallization from ethanol (−5°C), 0.5% of combined free fatty acids was isolated with R_f 0.35 in system 6, like that of a model sample of palmitic acid. After methylation with diazomethane, fatty acid methyl esters were obtained the composition of which was determined by gas-liquid chromatography.

CONCLUSION

From the leaves of *Carthamus tinctorius* L. have been isolated and identified lipids belonging to four classes of organic compounds: C_{31} and C_{29} isoparaffins (0.7%), esters of phytol (0.1%), free fatty acids (0.5%) the main component of which is palmitic acid (71% of the total), and free β-sitosterol and its β-D-glucopyranoside.

Esters of phytol esterified with 33 higher fatty acids — paraffinic, isoparaffinic, and monoenoic of the C_9-C_{26} families — have been isolated from the flower petals for the first time.

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