5-10 ml of aqueous acetone and the mixture was shaken for 10-15 min, filtered into measuring flasks, and made up to the mark with aqueous acetone.

SUMMARY

A rapid spectrophotometric semimicro method of determining free gossypol in cotton seeds and the products of their processing is proposed which permits thousandths of a percentage part of gossypol in a sample to be detected.

Calibration curves are given for determining the amount of gossypol in ethanolic and aqueous acetone solutions.

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NONGLYCERIDE COMPLEX OF THE SEED OIL OF *Onopordum acanthium*

N. T. Ul'chenko, É. I. Gigienova, U. A. Abdullaev, and A. U. Umarov

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Extraction of the comminuted seeds has yielded an oil from which have been isolated: C_{33}-C_{25}, C_{18} and C_{17} paraffinic hydrocarbons, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{17:1}$, $C_{17:2}$ and $C_{17:3}$ olefinic hydrocarbons, ethyl esters of $C_{32:0}$, $C_{31:0}$, $C_{30:0}$, $C_{29:0}$, and $C_{28:0}$ fatty acids, sterols with molecular weights of 414, 412, and 400, and the alcohols α-amyrin and lupeol with their natural acetates. Extraction of the uncomminuted seeds has shown that the paraffinic hydrocarbons, ethyl esters, and alcohol acetates pass into the oil from the husks of the seeds. This is the first time that the $C_{31:0}$ and $C_{29:0}$ fatty acids have been detected as natural compounds, and it is the first time that the ethyl esters of C_{34}, C_{33}, C_{32}, C_{31}, and C_{30} fatty acids have been isolated from seed oils of higher plants.

In a study of the seed oils of *Onopordum acanthium* (Scotch thistle), family Compositae, growing in Central Asia, attention is attracted by the considerable variation in the amount of "unsaponifiables" in it (0.5-1.5% on the oil). To explain this fact, we have investigated the nonglyceride complex of the oil. The oil was subjected to column chromatography (CC) with a mixture of hexane and ether. Three fractions of oil were collected. The migration of the substances of these fractions on Silufol plates (systems 1, 2, and 3) corresponded to the migration of model samples of hydrocarbons, pentacyclic alcohols, and sterols (results of one of the experiments) (Table 1).

The total substances of fraction I were rechromatographed by the CC method (systems 1 and 4). When system 1 was used, as was shown by IR and NMR spectra, only the hydrocarbons passed into the first portions of the eluate. The mass spectra of the readily volatile substances when a sample of hydrocarbons was present in the glass tube for direct introduction and the reaction with iodine on Silufol showed the presence in the oil of two groups of hydrocarbons, C_{17} and C_{18}. The hydrocarbons within each group differed from one another by their degree of unsaturation, i.e., they contained from 0 to 3 ethylenic bonds. On complete introduction, saturated paraffins were detected in the mass spectrum with molecular weights

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

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Table 1

Fraction	Class of substances	Solvent system	Amount, % on the oil
I	Hydrocarbons	1	0.04
	Esters	1	0.13
II	Oxytriglycerides	2	1.58
	Pentacyclic alcohols	2	0.04
III	Oxytriglycerides	3	0.30
	Diglycerides	3	0.55
	Free sterols	3	0.30

of 464, 450, 436, 422, 408, 394, 380, 366, and 352, respectively corresponding to C_{33}, C_{32}, C_{31}, C_{30}, C_{29}, C_{28}, C_{27}, C_{26}, and C_{25} aliphatic chains.

Solvent system 4 isolated a homologous mixture of esters of high-molecular-weight saturated fatty acids (IR spectrum). The alkaline hydrolysis of this mixture gave ether-soluble products which migrated on Silufol plates in system 5 like model samples of C_{18} fatty acids. The mass spectrum of the esters contained the peak of a McLafferty ion with m/e 88 (100%) [1] and peaks with M/e 508 (M^+), 494, 480, 466, and 452, and two homologous series of fragments $[M - 45]^+$ and $[M - 43]^+$ differing by two mass units. Their appearance is due to the successive cleavage of carbon–carbon bonds in saturated chains formed as the result of the splitting out of ethoxyl ($-OCH_2CH_3$) on the one hand and the appearance of fragments with m/e 43, 57, 71... (protonated forms of cyclopropane and its homologs [1]) from the methyl end of the molecule, on the other hand. For the strongest ion with M^+ 480 we observed the peak of a metastable ion with m/e 397.85, confirming the formation of the fragment with m/e 437 or $[M - 43]^+$, and, consequently, a whole series of subsequent fragments $[M - 57]^+$, $[M - 71]^+$, etc. Thus, the mass spectrum of the esters corresponds to the spectrum of the ethyl esters of the saturated high-molecular-weight C_{34}, C_{33}, C_{32}, C_{31}, and C_{30} fatty acids. Of these, the derivatives of acids with odd numbers of carbon atoms are new, and so are the acids themselves — hentriacontanoic acid, $C_{31:0}$, and nonacosanoic acid, $C_{29:0}$ [1, 3].

The pentacyclic alcohols were isolated from fraction II by thin-layer chromatography (TLC) in system 3. The alcohols were separated preparatively in the form of acetates in a thin layer of silica gel in system 6 [4] and were hydrolyzed with alkali. This gave the alcohols α-amyrin (I) and lupeol (II) [5]. They did not give the Lieberman–Burchard reaction, but in the Salkowski reaction compound (I) behaved as an unsaturated steroid and (II) slowly imparted an orange color to the phase-separation boundary. The IR and NMR spectra of the alcohols and their acetyl derivatives confirmed the structures (I) and (II). The acetate of (I) was obtained in the form of acicular crystals with an orange tinge [7], mp 220-222°C (according to the literature: 221-225°C [5]). Molecular weight (from the mass spectrum) 468. The acetate of (II) was obtained in the form of acicular crystals with mp 221-221.5°C, correspoding to the value given in the literature. Molecular weight 468 according to its mass spectrum.

The free sterols were isolated from fraction III by crystallization: 10 times from methanol and twice from acetone. The mass spectrum at 170°C showed the presence of a mixture of homologs — β-sitosterol with a molecular weight of 414 and campesterol with a molecular weight of 400 — and also traces of sitosterol or stigmasterol with a molecular weight of 412. The latter is more likely, since it is more frequently found in natural materials.

From the unground seeds petroleum ether extracted paraffinic hydrocarbons, fatty acid ethyl esters, and the natural acetates of alcohols (I) and (II). Consequently, the olefinic hydrocarbons, alcohols (I) and (II), and the sterols, like the oil, are concentrated in the seed kernel, and the amount of "unsaponifiable" substances in it changes according to the depth of extraction of the oil from the broken-down seeds, since the latter dissolve in petroleum ether with greater difficulty than the glycerides.

EXPERIMENTAL

The authenticity of the molecular ions was determined by comparison with the mass spectra at an ionizing voltage of 17 V. The mass spectra were taken on a MKh-1303 instrument (at an ionizing voltage of 40 V and a temperature of the inlet tube of 135°C), the IR spectra were recorded on a UR-10 instrument, and the NMR spectra on a JNM-4H-100/100-MHz instrument (10-12% solutions in carbon tetrachloride with HMDS as internal standard).

The oil was extracted from the ground seeds with hexane at room temperature by the steeping method. The substances from the husks of the seeds were extracted by low-boiling fractions of petroleum ether (40-60°C).

Chromatography. The adsorbent for CC was silica gel of type L 100/250 μ. TLC was performed on Silufol plates and on glass plates, 18 × 24 cm, coated with KSK silica gel, 150 mesh, with 3% of gypsum and 10% of $AgNO_3$. The solvent systems used were mixtures of hexane and ether in ratios of 1) 10:1; 2) 6:4; 3) 1:1; 4) 9.5:0.5; and 5) 8:2, and 6) chloroform–carbon tetrachloride (1:1).

Alkaline hydrolysis was carried out at room temperature with a 30% solution of caustic potash in methanol.

The alcohols were acetylated for 12 h with acetic anhydride in pyridine at room temperature [2].

The C_{17} and C_{18} hydrocarbons were identified from the results of analytical TLC (Silufol, hexane, R_f 0.97, spots revealed in iodine vapor) and of mass spectroscopy (20-25°C, 40 V), m/e (%): M^+ 254, 252, 250, 248 –C_{18} (1), M^+ 240, 238, 236, 234 –C_{17} (I), 239, 237, 235, 233 (0.5); 225, 223, 221, 219 (2), 211, 209, 207, 205 (2, 5), 197–191 (3, 4), 183–177 (4), 169–163 (6), 155–149 (8), 141–135 (13), 127–121 (19), 111–110 (31), 97, 95 (100), 85, 83 (68), 57, 55 (63), 43–41 (60).

The paraffinic hydrocarbons were identified from their migration characteristics in a thin layer (Silufol, hexane, R_f 0.98) and on the basis of a mass spectrum (17 V); m/e (%): M^+ 464 (4), M^+ 450 (1), M^+ 436 (10), M^+ 422(2), M^+ 408(47), M^+ 394(7), M^+ 380(30), M^+ 366(10), M^+ 352(5), 435 (5), 421 (3), 407 (23), 393(7), 379(13), 365(17), 351(7), 337(7.2)–225(16), 211(16)–127(38), 113, 111(60), 99(100), 97(80), 85(63), 83(59), 71.69(25), 43(48), 41(29).

Ethyl Esters of Fatty Acids. IR spectrum ν_{max}^{film}, cm^{-1}: 2970 s, 2880 s, 1380 m, $-CH_3$, 2940 v.s, 2865 s, 1465 s, doublet 730 m $-CH_2-$, 1740 s, 1440 m, 1250 m, 1200 m, 1180 s, 1130 m, 1020 m –OCOR. Mass spectrum (150°C, 40 V), m/e (%): M^+ 508 (22), M^+ 494 (11), M^+ 480 (63), M^+ 466(15), M^+452(13), 493(3), 479(26), 465(5), 453(3), 437(16), 435(13), 423(8). 421(6), 409(7), 407(6), 395(6), 393(3), 381(5), 379(4), 367, 365–171. 169(4-3), 157(22), 155(14), 143 (23), 141(6), 129(16), 127(8), 115(10), 113(8), 111(25), 101(76), 99(25), 97(73), 89(45), 88 (100), 85(48), 83(53), 73(25), 71(67), 69(41), 59(14), 57(32), 55(24), 43(17), 41(11).

Pentacyclic Alcohols. The IR spectra showed absorption bands common for compounds (I) and (II) (ν_{max}^{NaCl}, cm^{-1}: 3600-3200 s, 1190 s, 1045 m, 2930 v.s, 2875 v.s, 2855 v.s, 1455 s –1467 s, 1385 s, and 1370 s, 990 w, 975 m, 940 w, 910 w) and bands characteristic for (I) (1660 w, 830 w, 815 m). The NMR spectra of compounds (I) and (II) correspond to the complex spectra of three terpene skeletons without aliphatic branching [4, 6].

The protons of the terminal methylene group of (II) resonated at 4.5 ppm (2 H, δ scale, deuterochloroform). Apart from M^+ 426 (40 and 50%, respectively), $[M - 15]^+$ (10 and 15%), $[M - 18]^+$ (8 and 10%), and $[M - 33]^+$ (5 and 8%), which are characteristic for monohydric triterpene alcohols, the mass spectrum of (I) contained peaks characteristic of α-amyrin with m/e numbers of 218 (100%), 203 (37%), and 189 (17%), and the spectrum of (II) contained peaks characteristic for lupeol with m/e numbers of 189 (100%), 207 (70%), and 95 (49%) in [7].

Acetates of the Alcohols (I) and (II). The IR spectra of the esters isolated from the seed husks (ν_{max}^{NaCl}, cm^{-1}: 1730 v.s, 1250 v.s, 1030 m, bands at 3600-3200, 1190, and 1045 absent), and their mass spectra (M^+ 468, 3%; $[M - 15]^+$, 2%; $[M - 60]^+$, 1.5%; $[M - 75]^+$, 1.5%) were identical with the IR and mass spectra of the monoacetyl derivatives obtained by the acetylation of alcohols (I) and (II).

SUMMARY

Homologous mixtures of normal C_{33}-C_{25} paraffins, the ethyl esters of the C_{34}-C_{30} aliphatic acids, and sterols with molecular weights of 414, 412, and 400 have been isolated from the seed oil of *Onopordum acanthium*. Two groups of hydrocarbons have been found — C_{17} and C_{18} — each of which consists of four components differing from one another by the number of ethylenic bonds (from 0 to 3). The simultaneous presence of the pentacyclic alcohols α-amyrin and lupeol, with their natural acetates, has been established.

This is the first time that the $C_{31:0}$ and $C_{29:0}$ aliphatic acids have been detected as natural compounds, and it is the first time that the ethyl esters of the C_{32}, C_{31}, C_{30}, C_{29}, and C_{28} fatty acids have been isolated from the seed oils of higher plants.

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PHOSPHOLIPIDS OF *Goebelia* SEEDS

Kh. Karshiev, Kh. S. Mukhamedova, and S. T. Akramov

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The qualitative and quantitative compositions of the phospholipids of the seeds of *Goebelia pachycarpa* and the fatty-acid composition of the total phospholipids and of homogeneous fractions have been studied. The molecular weights of the main fractions have been calculated.

Goebelia pachycarpa Bunge. (*Sophora pachycarpa* C. A. Mey.), family Leguminosae (Fabaceae) is a perennial herbaceous plant which is widely distributed in the desert-steppe territories of Central Asia [1].

A few representatives of this family have been studied for their phospholipid (PL) content [2-8] and the total phospholipids of their seeds proved to be extremely diverse both in the qualitative and in the quantitative respect. The detailed molecular compositions of the individual classes of PLs have been studied for only two representatives of this family — *Glycine max* (an industrial soybean crop) [7] and *Psoralea drupaceae* (scurf pea) [8].

We have investigated the fractional and fatty-acid compositions of the PLs of the seeds of *Goebelia pachycarpa* collected in the environs of Tashkent.

The total PLs were obtained and freed from accompanying impurities by a known procedure [9, 10]. The yield of purified total material was 1.5% on the weight of the air-dry seeds. By two-dimensional TLC in systems 1 and 2 we determined the qualitative compositions of the total PLs and, by determining the amounts of phosphorus in the spots [11], the quantitative distribution of the individual PL fractions in the total. The mean results of three determinations were as follows (%): phosphatidylcholines (PCs), 41.7; phosphatidylinositols (PIs), 25.6; phosphatidylethanolamines (PEs), 18; N-acyllysophosphatidylethanolamines (N-acyllyso-PEs), 5.5; lysophosphatidylcholines (lyso-PCs), 5.1; and N-acylphosphatidylethanolamines (N-acyl-PEs), 4.1.

The individual groups of phospholipids were separated by column chromatography on silica gel, being eluted with mixtures of chloroform and methanol in various ratios, followed by subfractionation in a thin layer of silica gel in system 1.

The structures of the phospholipids were confirmed by the results of determinations of their contents of P, N, and ester groups, and also by identifying the water-soluble products of acid hydrolysis.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.

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