rated solution of sodium bicarbonate, the upper layer was separated off, and the lower — chloroform — layer was distilled to small volume. After purification by preparative TLC the IR spectrum of N-acetylphosphatidylethanolamine was recorded.

SUMMARY

Three minor components have been isolated from the total PLs of kenaf seeds of variety Kuban'-333 by means of column and preparative thin-layer chromatography and they have been characterized by lysophosphatidylcholine and N-acylphosphatidylethanolamine and its lyso analog.

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NAPHTHOQUINONES OF *Lithospermum erythrorhizon*

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In recent years, investigations of the chemical composition of plants of the family Boraginaceae have been carried on intensively in view of the discovery of physiologically active compounds in them. Naphthoquinones from the roots of species of *Lithospermum* and *Arnebia* possess an antimicrobial action [1], and they have been reported to have an antitumoral activity [3].

The roots of *Lithospermum erythrorhizon* Sieb. et Zucc., collected in Maritime Territory in July, 1975, contained about 3% of naphthoquinone pigments (on the dry weight of the roots). The aim of the present work was to determine their structure. From the air-dry roots of *L. erythrorhizon* by extraction with petroleum ether (40-70°C) followed by chromatography on columns of silica gel and Sephadex LH-20 we isolated seven pigments. Substance (IV) is a quinoid pigment of nonnaphthazarin structure with mp 72-74°C. Its structure is being determined. We did not detect in the plants from the Maritime Territory the β,β-dimethylacrylate of shikonin [R = $OCOCH{=}C(CH_3)_2$] found by Japanese workers in the roots of *L. erythrorhizon* [6]. The composition and physicochemical properties of the six naphthazarin pigments correspond to those given in the literature [6-9] and completely confirm the structures given. We have recorded the ^{13}C NMR spectra of these compounds for the first time (Table 1). It follows from an analysis of the spectrum that a change in the substituents at C_{11} does not substantially affect the chemical shifts of the carbon atoms of the naphthoquinone moiety and of the isopentenyl part of the chain. The assignment of the signals of the carbon atoms was performed by the method of selective decoupling from the protons.

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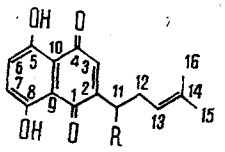

R = H (I)

R = OCOCH(CH$_3$)$_2$ (IIa)

R = OCOCH$_2$CH(CH$_3$)$_2$ (IIb)

R = OCOCH$_3$ (III)

R = OH (V) (shikonin)

R = OCOCH$_2$C(CH$_3$)$_2$OH (VI)

Compounds (IIa) and (IIb) were not separated chromatographically. Their structures were determined by alkaline hydrolysis, the products of which were shikonin (V) and a mixture of two acids — isobutyric and isovaleric. The mass spectrum of the mixture of (IIa) and (IIb) gave two molecular ions with M^+ 358 and M^+ 372 and showed fragmentation similar to that of substances (II), (III), and (VI). The formation of fragments with m/e 219, 220 with an intensity of 100% is characteristic of the mass spectra of substances having an oxygen atom at C_{11}. The absence of an oxygen atom at C_{11} (substance I) leads to the formation of a fragment with m/e 204 having an intensity of 100%.

Analysis of the ester substituents in compounds (II), (III), and (VI) was performed by GLC. A chromatogram was obtained which consisted of four peaks corresponding to acetic, isobutyric, isovaleric, and β-hydroxyisovaleric acids (Fig. 1).

EXPERIMENTAL

The UV and IR spectra were taken on Specord UV-VIS (ethanol) and UR-20 (CCl_4) instruments, respectively, and the NMR spectra on a Brüker HX-90E instrument with a working frequency of 22.63 MHz for ^{13}C nuclei and 90 MHz for protons in $CDCl_3$ solution. The characteristics of the NMR spectra are given in the δ scale, with TMS as internal standard. The following symbols are used: s) singlet; d) doublet; t) triplet; m) multiplet. The mass spectra were taken on an LKB-9000S mass spectrometer with the direct introduction of the sample at 70 eV and 20°C. The specific rotations of the optically active substances were determined in ethanol on a Perkin–Elmer 141 polarimeter. The melting points were measured on a Kofler block. The elementary analyses of all the compounds corresponded to the calculated figures. Gas–liquid chromatography was performed on a Shimadzu GC-5A chromatograph with PEG-20M as the stationary phase on Chromosorb W in a column 0.3 × 150 cm at 130°C.

The total pigment extract was separated on columns of acid silica gel in the petroleum ether (40-70°C)–diethyl ether (7:1) system and then with a gradient increase in the proportion of ether, and also on columns of Sephadex LH-20 in chloroform. The R_f values of the substances were determined by TLC on "Silufol" plates in the petroleum ether–diethyl ether (10:3.5) system.

TABLE 1. Chemical Shifts of the ^{13}C Carbon Atoms

Number of carbon atom	Compound					
	I	IIa	IIb	III	V	VI
1	183,1	178,4	178,4	178,3	180,3	177,2
2	151,6	148,6	148,6	148,2	151,5	147,6
3	134,6	131,4	131,4	131,5	131,9	131,4
4	183,1	177,0	177,0	176,9	179,7	175,7
5	163,2	167,2	167,2	167,2	165,9	168,4
6	131,3	132,6	132,6	132,7	132,4	133,2
7	131,0	132,3	132,3	132,5	132,4	133,0
8	163,5	166,7	166,7	166,6	165,3	167,8
9	111,5	111,6	111,6	111,5	111,4	111,6
10	111,7	111,9	111,9	111,8	111,6	111,8
11	26,7	69,1	69,1	69,5	68,5	69,8
12	29,8	33,1	33,1	32,9	35,8	32,9
13	122,5	118,0	118,0	117,9	118,7	117,8
14	133,7	135,8	135,8	136,0	137,0	136,3
15	17,9	17,9	17,9	17,9	18,1	17,9
16	25,8	25,7	25,7	25,7	25,9	25,7
1′		171,8	171,8	169,6		171,5
2′		34,1	43,4	20,9		46,6
3′		19,0	26,7			69,1
4′		19,0	22,4			29,1
5′			22,4			29,3

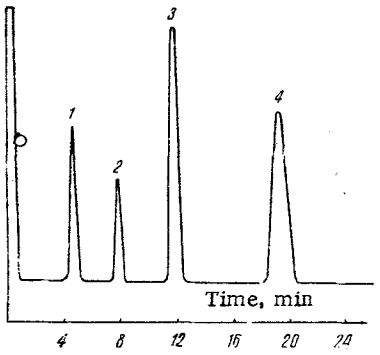

Fig. 1. GLC of the acids obtained by the alkaline hydrolysis of the pigments of a petroleum ether extract from *L. erythrorhizon*: 1) acetic; 2) isobutyric; 3) isovaleric; 4) β-hydroxyisovaleric.

Extraction and Chromatography of the Pigments. The air-dry roots of *Lithospermum erythrorhizon* (150 g) were ground to a powder and extracted in a Zaitsev extractor with 2 liters of petroleum ether (40-70°C) in an atmosphere of argon. Evaporation of the solvent under vacuum gave 3.60 g of a dark red syrup, which was chromatographed on a column containing 900 g of silica gel. Six bright red fractions were obtained: I) 50 mg; II) 1300 mg; III) 830 mg; IV) 150 mg; V) 40 mg; VI) 700 mg. Each fraction isolated (apart from IV) was evaporated and the residue was dissolved in methanol. The white precipitate that deposited, apparently consisting of plant waxes, was separated off, and the fractions were reevaporated and were then chromatographed on a column of Sephadex LH-20 in chloroform.

5,8-Dihydroxy-2-(4-methylpent-3-enyl)-1,4-naphthoquinone (I) (Deoxyshikonin). The chloroform eluate of fraction I was evaporated (12 mg) and crystallized from hexane. This gave 5 mg of red needles with mp 91-92°C (literature data: 92-93°C) [3], R_f 0.77. UV spectrum: λ_{max} 275, 493, 526, 568 nm (log ε 2.99; 2.81; 2.87; 2.48); IR spectrum (cm^{-1}): 2800-3000, 1658 (shoulder), 1612, 1579 (shoulder); M^+ 272; NMR spectrum: 1.60 (s, 3H, $3H_{15}$); 1.70 (s, 3H, $3H_{16}$); 2.10-2.50 (m, 2H, $2H_{12}$); 2.50-2.80 (m, 2H, $2H_{11}$); 5.14 (t, 1H, $1H_{13}$); 6.84 (d, 1H, $1H_3$); 7.19 (s, 2H, $1H_6$ and $1H_7$): 12.45 [s, 1H, O<u>H</u> (chelate)]; 12.61 [s, 1H, OH (chelate)].

5,8-Dihydroxy-2-[4-methyl-1-(2-methylpropanoyloxy)pent-3-enyl]-1,4-naphthoquinone (IIa) (Isobutyrylshikonin), and 5,8-Dihydroxy-2-[4-methyl-1-3-methylbutanoyloxy)pent-3-enyl]-1,4-naphthoquinone (IIb) (Isovalerylshikonin). The chloroform eluate of fraction II was evaporated and crystallized from hexane. It gave 305 mg of dark red low-melting crystals (mp < 40°C), R_f 0.62, $[\alpha]_D^{20}$ +560°. UV spectrum: λ_{max} 275, 493, 526, 568 nm (log ε 3.79; 3.70; 3.75; 3.48); IR spectrum, cm^{-1}: 2860-3000, 1658 (shoulder), 1612, 1579; M^+ 358 and 372; NMR spectrum: 0.89-1.21 [m, 6H, H_3', and H_4', (IIa), H_4', and H_5', (IIb)]; 1.59 (s, 3H, $3H_{15}$); 1.69 (s, 3H, $3H_{16}$); 2.10-2.70 :m, 3H, $3H_{12}$), 5.14 (t, 1H, $1H_{13}$); 6.04 (t, 1H, $1H_{11}$); 6.97 (s, 1H, $1H_3$); 7.14 (s, 2H, $1H_6$ and $1H_7$); 12.37 [s, 1H, O<u>H</u> (chelate)]; 12.54 [s, 1H, O<u>H</u> (chelate)].

5,8-Dihydroxy-2-(4-methyl-1-acetoxypent-3-enyl)-1,4-naphthoquinone (III) (Acetylshikonin). The chloroform eluate of fraction III was evaporated and crystallized from hexane. This gave 450 mg of red needles with mp 103-104°C (literature data: 104-105°C) [5]. R_f 0.42, $[\alpha]_D^{20}$ +440°.

UV spectrum: λ_{max} 275, 493, 526, 568 nm (log ε 3.80; 3.75; 3.80; 3.62). IR spectrum, cm^{-1}: 2800-3000, 1750, 1655 (shoulder), 1612, 1590 (shoulder). PMR spectrum: 1.58 (s, 3H, $3H_{15}$); 1.68 (s, 3H, $3H_{16}$); 2.14 (s, 3H, $COOH_3$); 2.20-2.70 (m, 2H, $2H_{12}$); 5.13 (t, 1H, $1H_{13}$); 6.02 (t, 1H, $1H_{11}$); 6.98 (d, 1H, $1H_3$); 7.14 (s, 2H, $1H_6$ and $1H_7$); 12.36 [s, 1H, O<u>H</u> (chelate)]; 12.52 [s, 1H, O<u>H</u> (chelate)].

5,8-Dihydroxy-2-(1-hydroxy-4-methylpent-3-enyl)-1,4-naphthoquinone (V) (shikonin). The chloroform eluate of fraction V was evaporated (50 mg) and crystallized from hexane. This gave 15 mg of brown prisms with mp 140-142°C (literature data: 148°C) [5]. R_f 0.23; $[\alpha]_D^{20}$ +140°.

UV spectrum: λ_{max} 275, 493, 526, 568 nm (log ε 3.86; 3.76; 3.87; 3.57); IR spectrum, cm^{-1}: 3615, 2800-3000, 1658 (shoulder), 1615, 1578 (shoulder); NMR spectrum: 1.65 (s, 3H, $3H_{15}$); 1.75 (s, 3H, $3H_{16}$); 2.20-2.80 (m, 2H, $2H_{12}$); 2.41 [s, 1H, OH (alcoholic)]; 4.91 (m, 1H, $1H_{13}$); 5.21 (t, 1H, $1H_{11}$); 7.14 (d, 1H, $1H_3$); 7.17 (s, 2H, $1H_6$ and $1H_7$); 12.45 [s, 1H, OH (chelate)]; 12.56 [s, 1H, OH (chelate)].

5,8-Dihydroxy-2-[1-(3-hydroxy-3-methylbutanoyloxy)-4-methylpent-3-enyl]-1,4-naphthoquinone (VI) (β-Hydroxyisovalerylshikonin). The chloroform eluate of fraction VI was evaporated, giving 460 mg of product which was crystallized from hexane. The 270 mg of dark red needles obtained had mp 82-84°C (literature data: 90-92°C) [7]. R_f 0.10; $[\alpha]_D^{20}$ +220°.

UV spectrum: λ_{max} 275, 493, 526, 568 nm (log ε 3.86; 3.71; 3.78; 3.57); IR spectrum, cm^{-1}: 3615, 3550, 2800-3000, 1732, 1655 (shoulder), 1613, 1520 (shoulder); NMR spectrum: 1.31 (s, 6H, $3H_{4'}$, and $3H_{5'}$); 1.59 (s, 3H, $3H_{15}$); 1.70 (s, 3H, $3H_{16}$); 2.60 (s, 2H, $2H_{2'}$); 2.40-2.70 (m, 2H, $2H_{12}$); 6.13 (t, 1H, $1H_{13}$); 6.09 (m, 1H, $1H_{11}$); 7.03 (d, 1H, $1H_3$); 7.16 (s, 2H, $1H_6$) and $1H_7$; 12.37 [s, 1H, OH (chelate)]; 12.56 [s, 1H, OH (chelate)].

Alkaline Hydrolysis of a Petroleum Extract of the Pigments. A mixture of 500 mg of the total extract and 300 ml of 2 N aqueous NaOH was stirred in an atmosphere of argon at 20°C for 24 h. The blue solution was rapidly filtered, poured onto ice, and acidified with dilute H_2SO_4. The red suspension was extracted with chloroform (3 × 150 ml), and the resulting extract was treated with saturated $NaHCO_3$ solution and was then acidified and extracted with ether. The ether was driven off and the residue was investigated by GLC. The GLC analysis showed the presence of the following acids in the hydrolysis products: 1) acetic; 2) isobutyric; 3) isovaleric; 4) β-hydroxyisovaleric (see Fig. 1). The phenolic hydrolysis product, shikonin, was obtained after evaporation of the chloroform solution and chromatography on Sephadex LH-20. mp 140-142°C (from hexane).

Alkaline Hydrolysis of Isobutyrylshikonin and Isovalerylshikonin. Fraction II was hydrolyzed by the method described above. GLC analysis showed the presence of hydrolysis products of isobutyric and isovaleric acids in approximately equal proportions.

SUMMARY

1. The roots of *Lithospermum erythrorhizon* Sieb. et Zucc. have yielded six naphthazarin pigments which have been characterized as shikonin itself and derivatives of it. A new quinoid pigment of nonnaphthazarin structure has also been isolated.

2. The pigments of the roots of *L. erythrorhizon* collected in the Maritime Territory have not been found to contain the β,β-dimethylacrylic ester of shikonin which has been reported for this species in the literature.

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