QUINOID PIGMENTS OF FAR EASTERN REPRESENTATIVES OF THE FAMILY BORAGINACEAE

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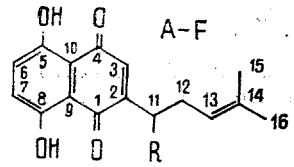
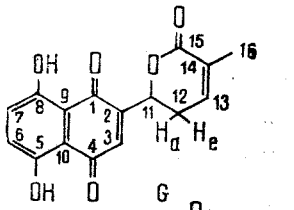
UDC 547.655.6+667.211+615.281

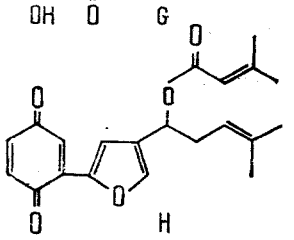
From nine species of plants of the family Boraginaceae we have isolated and identified 11 compounds, nine of which have proved to be previously known naphthoquinones — derivatives of shikonin. The structure of the previously unknown δ-lactone of 5-(5',8'-dihydroxy-1',4'-naphthoquinon-2'-yl)-5-hydroxy-2-methylpent-2-enoic acid has been established. The qualitative and quantitative compositions of the pigments of the species studied and of groups of plants of the Boraginaceae family have been determined.

Continuing the chemical study of plants of the family Boraginaceae, we have investigated the composition of the quinoid pigments present in hexane extracts of the roots of nine of their representatives. Preparative column chromatography of the extracts on silica gel followed by separation on Sephadex LH-20 led to the isolation and identification of the following substances [1-5]:



Pigment	R
A	H
B	OH
C	$OCOCH_3$
D_1	$OCOCH(CH_3)_2$
D_2	$OCOCH_2CH(CH_3)_2$
D_3	$OCOCH(CH_3)CH_2CH_3$
D_4	$OCOCH=C(CH_3)_2$
E	$OCOCH_2C(CH_3)_2OH$
F	$OCOCH_2C(CH_3)=C(CH_3)_2$





We were previously the first to describe pigment H [6]; subsequently, on the basis of a detailed investigation of ^1H and ^{13}C NMR spectra the positions of the substituents in the furan fragment of the molecule were refined [7]. Substance G is apparently a new, previously undescribed, compound.

The presence of three maxima in the visible region of the absorption spectrum (496, 526, 564 nm) made the assignment of pigment G to the group of naphthazarin derivatives probable. The IR spectrum of the pigment had the absorption bands of an ester carbonyl (1726 cm^{-1}), of an isolated double bond (1657 cm^{-1}), and of chelated quinoid carbonyls (1611 cm^{-1}). The assignment of the signals in the PMR spectrum of the pigment was made on the basis of an analy-

Pacific Ocean Institute of Bioorganic Chemistry of the Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 625-630, September-October, 1979. Original article submitted February 19, 1979.

 0009-3130/79/1505-0546$07.50

sis of the SSCCs for the H_3, H_{11}, H_{12a}, H_{12e}, H_{13}, and $3H_{16}$ protons in the first-order approximation. The values of the chemical shifts and SSCCs were refined by simulating fragments of the spectrum on a "Nicolet" B-N C-12 computer and by selecting parameters of the spectra to achieve satisfactory agreement of the frequencies of the lines in the epxerimental and theoretical spectra with maximum deviations of ±0.2 Hz.

As a result, the following refined parameters were obtained:

δH_3=7,38 ppm	J_{3-11}=—1,2 Hz
δH_6=7,17 ppm	J_{11-12a} =11,8 Hz
δH_7=7.17 ppm	J_{11-12e} =4,0 Hz
δH_{11}=5,68 ppm	J_{12-12} =—17,8 Hz
δH_{12a}=2.42 ppm	J_{13-12a} =2,4 Hz
δH_{12e}=2,90 ppm	J_{13-12e} =6,5 Hz
δH_{13}=6,69 ppm	J_{16-12a} =2,4 Hz
δH_{16}=2.00 ppm	J_{16-12e} =1,0 Hz
	J_{13-16} =—1,5 Hz

The SSCC of the protons at C_{12}, J_{12-12} = —17.8 Hz, is characteristic for protons located in the α-position to a double bond in a six-membered ring. The chemical shift of the H_{13} proton shows its β-position with respect to a carbonyl group conjugated with a double bond in a six-membered ring, and the H_{11} chemical shift confirms the presence of an oxygen atom at C_{11}.

It follows from these facts that the substituent in the naphthazarin fragment of the molecule of pigment G is an unsaturated δ-lactone.

The assignment of the signals in the ^{13}C NMR spectrum was done by the methods of off-resonance and selective decoupling from interaction with protons. The positions of the C_{13} and C_{14} signals in the spectrum confirmed the presence of a double bond conjugated with a carbonyl in a lactone ring. The molecular weight of the pigment, according to its high-resolution spectrum, M = 300.0633, corresponded to the empirical formula $C_{16}H_{12}O_6$ (M_{calc} = 300.0633). On the basis of the facts given, the structure of pigment G has been established as the δ-lactone of 5-(5',8'-dihydroxy-1',4'-naphthoquinon-2-yl)-5-hydroxy-2-methylpent-2-enoic acid.

Finally, the last pigment that we isolated, I, was characterized only by the results of mass spectrometry, and it can be ascribed the empirical formula $C_{17}H_{16}O_5$, M = 300.0997 (M_{calc} = 300.0997).

Table 1 shows that in the amount and composition of the pigments all the species studied from the family Boraginaceae can be divided into two groups. The first comprises species 1-3 of the Lithospermeae DC. branch, in the pigments of the roots of which shikonin and its acyl derivatives predominate. Characteristic of the second group of plants (4-8) is an extremely high relative amount of the cyclic lactone G. The total amount of quinones in the roots of this group of plants is low, and at the end of the vegetation period they have disappeared almost completely.

Plant 9, which grows only on sea coasts, represents a special case. The high moisture content complicates its drying, and in any method of drying a rapid blackening of the material is observed, apparently because of the presence of powerful enzyme systems. The very minute amount of quinones that it was possible to isolate from ethanolic extracts of the roots may be connected with this fact. It is not excluded that the high relative amount of deoxyshikonin (compound A) may, in part, be the consequence of the enzymatic deacylation of the native pigments of the plant. A number of additional features characteristic for the individual species were traced in the composition of the quinones of each group of plants. Only in plant 1 was a furan derivative detected, this apparently being a biogenetic precursor of the naphthazarin pigments:

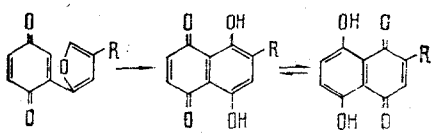
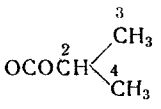
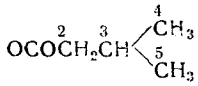
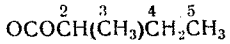
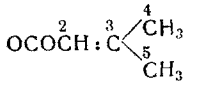


TABLE 1. Compositions of the Quinoid Pigments Isolated from Roots of Plants of the Family Boraginaceae

Species of plant	Relative amounts in the total quinoids, %									Amount of the total in the air-dry material, %
	A	B	C	D	E	G	F	H	I	
1. Lithospermum erythrorhison Sieb. et Zucc.	0,3	0,6	39,2	48,5	7,3	—	—	4,1	—	1,80
2. Macrotomia euchroma (Royle) Pauls.	1,4	1,4	24,8	57,7	6,2	—	8,6	—	—	1,50
3. Echium vulgare L.	Tr.	4,7	34,0	43,0	17,4	—	—	—	—	0,12
4. Lappula consanguinea Guerke	2,7	3,2	8,7	5,1	—	80,4	—	—	—	0,04
5. Lappula echinata Gilib.	6,9	11,4	6,6	Tr.	—	75,2	—	—	—	0,02
6. Eritrichium incanum DC.	6,9	13,5	9,1	—	—	70,4	—	—	—	0,05
7. Eritrichium sichotenze M. Pop.	2,2	3,4	1,3	—	—	92,3	—	—	—	0,05
8. Cynoglossum officinale L.	8,0	6,6	0,4	3,9	—	54,0	—	—	25,4	0,005
9. Mertensia maritima (L.) S. F. Gray	71,3	10,9	17,8	—	—	Tr.	—	—	—	0,0001*
R_f in system 1	0,50	0,17	0,41	0,46	0,10	0,10		0,41	0,05	
R_f in system 2	0,55	0,14	0,40	0,47	0,10	0,10		0,41	0,04	

*In the moist roots.

TABLE 2. Chemical Shifts of the Signals of the ^{13}C Nuclei of Acyl Substituents of Shikonin

Pigment	Acyl radical	Chemical shifts (ppm) of the C atoms			
		2	3	4	5
D_1		34,1	19,0	19,0	—
D_2		43,4	26,7	22,4	22,4
D_3		41,3	29,8	11,6	16,7
D_4		115,5	158,0	20,3	27,4

The teracrylic ester of shikonin was found only in the Central Asian species 2. Further interspecies differences have been established in the composition of the complex group of pigments D, which were usually isolated as a whole in the form of a monolithic chromatographic zone difficult to separate into its individual components. The ^{13}C NMR spectra of this combination of pigments showed a large number of signals in the strong-field region corresponding to various substituents at the carboxy group of esters of shikonin. The nature of these substituents was established from a comparison of the chemical shifts with the corresponding chemical shifts of the signals of the carbon atoms of standard acids (Table 2).

Such a comparison is justified, since we have shown previously that the chemical shifts of the signals of the ^{13}C nuclei of the naphthazarin fragment and of the isopentenyl moiety of the side chain in shikonin derivatives are independent of the nature of the acyl group at C_{11} [1].

Analysis of the spectral features shows that the compositions of the pigments of the plants of the first group are characterized by the following set of components:

plant 1, $D_1:D_2 = 1:2$;

plant 2, $D_1:D_2:D_3:D_4 = 2:2:1:1$;

plant 3, $D_2:D_3 = 1:1$.

The qualitative and quantitative compositions of these mixtures were also confirmed by GLC analysis of the products of their alkaline hydrolysis. Similar compositions of the pigments of plants 1 and 2 have been given previously in the literature [1-3, 5].

Thus, features established in the present work of the composition of the quinoid pigments of the Boraginaceae species studied may be considered as taxonomic characteristics of these plants in the flowering phase.

EXPERIMENTAL

Absorption spectra were recorded on a Specord UV-VIS instrument in chloroform and NMR spectra on a Brüker HX-90 E instrument with a working frequency of 22.63 MHz for ^{13}C nuclei and 90 MHz for protons in deuterochloroform (δ scale, 0 — TMS). Mass spectra were taken by the direct introduction of the substance into the ion source (70 eV, 20°C) on a LKB-9000 S instrument.

Extraction and Isolation of the Pigments. The plants were collected in the flowering phase in the Maritime Territory and Amur province.* The comminuted air-dry roots were extracted with hexane in a Zaitsev extractor. After elimination of the solvent, the residue was dissolved in methanol and the deposit of waxes was removed. The quinones were precipitated from the filtrate in the form of the copper complexes [8]. The washed complexes were decomposed with dilute hydrochloric acid, and the total quinones were extracted with ether.

Determination of the Yield and Preparative Separation of the Quinones. For the quantitative determination of the individual pigments, a weighed amount of the total quinones was deposited on a plate with a nonfixed layer of type L 5-40 μ silica gel of Czech manufacture previously treated with HCl and washed free from iron salts. The chromatogram was run in system 1) hexane—ethyl acetate—acetic acid (100:15:1) or system 2) hexane—acetone (20:1). The quinones formed red and yellow zones the colors of which changed to blue and violet on treatment with a 1% ethanolic solution of KOH or magnesium acetate.

The colored zones were removed from the plates (200 × 300 mm) and extracted with chloroform, and the optical densities of the extracts at 526 nm were determined. The concentrations of the pigments were found from calibration curves plotted for weighed samples of the purified quinones.

The preparative isolation of the quinones was carried out in columns of the same silica gel (100-160 μ) and also by preparative TLC in the solvent systems mentioned above. The fractions obtained were additionally purified by chromatography on columns of Sephadex LH-20 in chloroform.

Pigment G. We isolated 30 mg of a red-violet pigment from the roots of plants 4 and 5 (the pigments were concentrated in the bark of the roots) by the method described above; mp 160-162°C (CH_2Cl_2—hexane). Absorption spectrum (C_2H_5OH): λ_{max} 214, 281, 498, 526, 564 nm (log ε 3.92, 3.09, 3.71, 3.75, 3.55). IR spectrum, cm^{-1}: 3110, 3050, 3020, 2840, 1969, 1726, 1657, 1611. Mass spectrum [m/e (intensity, %)]: M^+ 300 (40), 282 (100), 254 (72), 239 (10), 227 (11), 203(36), 190(17), 175(19), 108(13), 82(40). PMR spectrum, ppm: 2.00 (m, J = 5.1 Hz, 3 H, $3H_{16}$); 2.20-2.60 (m, 1H, $1H_{12a}$); 2.75-3.10 (m, 1H, $1H_{12e}$); 5.58-5.78 (m, 1 H, $1H_{11}$); 6.60-6.80 (m, 1 H, $1H_{13}$); 7.17 (s, 2 H, H_6 and $1H_7$); 7.38 (d, J = 1.0 Hz, 1 H, $1H_3$); 12.39 (s, 1 H, OH_{chel}); 12.52 (s, 1H, OH_{chel}). ^{13}C NMR spectrum (a, b, and c — assignments of the signals not unambiguous).

C_1 — 176,0[a]	C_5 — 169,8[b]	C_9 — 111,6	C_{13} — 138,7
C_2 — 145,3	C_6 — 134,0[c]	C_{10} — 112,0	C_{14} — 128,7
C_3 — 132,0	C_7 — 133,4[c]	C_{11} — 73,2	C_{15} — 174,2[a]
C_4 — 175,8[a]	C_8 — 169,2[b]	C_{12} — 30,4	C_{16} — 17,0

*The roots of plant 2 (Table 1) were kindly given to us by B. S. Zakharov (Kazan' State University, Alma-Ata).

SUMMARY

1. From nine species of plants of the family Boraginaceae we have isolated and identified 11 compounds, nine of which have proved to be previously known naphthoquinones in the form of shikonin derivatives.

2. The structure of the previously unknown δ-lactone of 5-(5',8'-dihydroxy-1',4'-naphthoquinon-2'-yl)-5-hydroxy-2-methylpent-2-enoic acid has been established.

3. The qualitative and quantitative compositions of the pigments of the species and groups of plants of the family Boraginaceae studied have been determined.

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MIGRATION OF THE EXOCYCLIC DOUBLE BOND IN TERPENOID COUMARINS OF THE IRESANE SERIES

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UDC 547.9:582.80

In terpenoid coumarins of the iresane series with an exocyclic double bond, migration of the double bond into the ring with the retention of the configuration of the substituent in position 1 is observed in an acid medium. The reaction has been performed in CF_3COOH and has been monitored by the PMR method. Badrakemin has yielded conferol, badrakemone has yielded conferone, badrakemin acetate has yielded conferol acetate, colladonin has yielded moschatol, and farnesiferol A and gummosin have yielded the corresponding isomers with endocyclic double bonds. The rate of the reaction is affected by the nature of the substituent at C-6. The presence of a keto group increases the time of isomerization to 1.5 h as compared with the 5-10 min for compounds with an OH group at C-6. The increase in the time of the reaction leads to the formation of byproducts. The reaction does not take place in CH_3COOH.

The action of an acid on farnesiferol A and its stereoisomers (I) containing an exocyclic double bond leads to the migration of the double bond with the formation of the corresponding compounds (III) containing the double bond in the ring:

All-Union Scientific-Research Institute of Medicinal Plants, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 630-637, September-October, 1979. Original article submitted April 9, 1979.

 0009-3130/79/1505-0550$07.50