THE STRUCTURE OF OLIVOVARIN, A NEW NATURAL o-NAPHTHOQUINONE

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From the culture liquid of mutants 47 and 48 of Act. olivovariabilis [1, 2] we have isolated a yellow crystalline substance with the composition $C_{14}H_{12}O_7$ soluble in lower alcohols, acetic acid, and chloroform, and we have called it olivovarin (I).

The IR spectrum of (I) shows absorption bands that could be assigned to the stretching vibrations of hydroxy groups, of a carboxy group and of quinone carbonyls bound by hydrogen bonds.

The acetylation of olivovarin with acetic anhydride in pyridine formed a triacetyl derivative (II). The IR spectrum of the latter showed absorption bands indicating the aromatic and aliphatic nature of the acetylated hydroxyls. The NMR spectrum of (II) had three three-proton singlets, two of them (at 2.33 and 2.34 ppm) belonging to the protons of an acetoxy group in an aromatic nucleus and one (at 1.90 ppm) to the protons of an acetoxy group in a side chain.

Methylation with diazomethane gave the ethers (III and IV) the IR spectra of which showed the absorption band of the carbonyl of an ester grouping. The NMR spectra of these derivatives showed the presence in each of them of one methoxy carbonyl group (three-proton singlet at 4.08 ppm) and of aromatic methoxy groups: one in the ether (III) (three-proton singlet at 3.62 ppm) and two in the ether (IV) (three-proton singlets at 3.58 and 3.33 ppm).

The acetylation of (III) gave the diacetate of the dimethyl derivative of olivovarin (V); its IR spectrum lacked the absorption band of a hydroxy group. The NMR spectrum (V) showed, in addition to the signals of the protons of methoxy and methoxycarbonyl groups (singlets at 3.55 and 4.01 ppm) two three-proton singlets of acetoxy groups: in an aromatic nucleus at 2.32 ppm and in an aliphatic chain at 1.88 ppm. Thus, the olivovarin molecule contains one carboxy and three hydroxy groups two of which are phenolic.

The olivovarin molecule has three aromatic protons located on neighboring carbon atoms; they give characteristic one-proton signals in the NMR spectrum of (V): quartets at 7.23 ppm, $J_{7,6} = 8$ Hz, $J_{7,5} = 2$ Hz (H_7), and at 7.90 ppm, $J_{5,6} = 8$ Hz, $J_{5,7} = 2$ Hz (H_5), and also a triplet at 7.60 ppm, $J_{6,5} = J_{6,7} = 8$ Hz (H_6). The oxidation of olivovarin with an alkaline solution of potassium permanganate gave hemimellitic (benzene-1; 2,3-tricarboxylic) acid,* which is possible only if (I) contains an aromatic nucleus with three unsubstituted carbon atoms and three C–C substituents on adjacent carbon atoms.

Analysis of the structure of the signals of the aliphatic protons (two two-proton doublets at 2.54 and 2.85 ppm and a one-proton quintet at 5.40 ppm) show that the (V) molecule contains the fragments $-CH_2-CH(OAc)-CH_2-$ attached to carbon atoms not bearing protons.

Olivovarin reacts with o-phenylenediamine to form a quinoxaline derivative (VI), which shows the presence of an o-quinone grouping in it.

* The compound was identified by a comparison of the mass spectra of the methyl esters of the acid obtained and of an authentic sample of hemimellitic acid.

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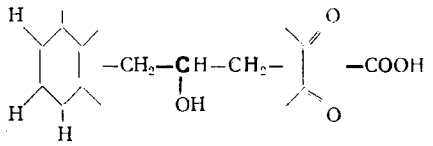
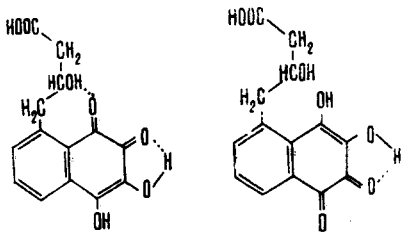
The facts given above show the presence of the following groupings in the olivovarin molecule:



a b c d

These groupings include 12 carbon atoms out of the 14 present in the molecule of (I). The other two carbon atoms are bound to the two phenolic hydroxyls and are included in an o-quinone system. The latter condition is satisfied by two possible structures: (Ia) and (Ib).



Ia Ib

In the NMR spectrum of the quinoxaline derivative (VI), the two protons at C_3 gave separate one-proton quartets at 3.16 ppm, ^2J = 12 Hz, $J_{H'_3, H_2}$ = 9 Hz (H'_3) and at 3.62 ppm, ^2J = 12 Hz, $J_{H''_3, H_2}$ = 4.8 Hz (H''_3), which is possible only if the methylene group and the quinoxaline nucleus are spatially close, i.e., the side chain and one of the quinone carbonyls (I) are in the peri position; this arrangement is found only in structural formula (Ia).

The correctness of this conclusion was confirmed by the IR spectra of olivovarin and its derivatives in the 1600-1700 cm^{-1} region (Fig. 1). In the molecule of (I), both quinone carbonyls are bound by hydrogen bonds, as is shown by the presence in the IR spectrum of olivovarin of only one absorption band, at 1625 cm^{-1}, and by the increase in the frequency of this band to 1675 cm^{-1} in compound (V) which lacks free hydroxy groups. In the trimethyl ether the OH group at C_2 remains unsubstituted, i.e., the formation of only one hydrogen bond, C_1=O...HO–, is possible, as is shown by the two absorption bands in the IR spectrum: of free (1650 cm^{-1}) and of bound (1610 cm^{-1}) quinone carbonyls. Similar changes can be found in a comparison of the spectra of olivovarin and its anhydro derivative (VII) which lacks an OH group at C_2. In this case, the spectrum of (VII) again has the absorption bands of free (C_1=O) and of hydrogen-bond-bound (C_2=O...HO–) quinone carbonyls.

It follows from a combination of the facts given that olivovarin is 8-(1'-carboxy-2'-hydroxypropyl)-3,4-dihydroxy-1,2-naphthoquinone. The results of the distillation of olivovarin with zinc dust agree with the structure proposed for it: In the reaction products we isolated and identified naphthalene, 1-methylnapthalene, and phenanthrene. The formation of phenanthrene may be represented as the result of the cyc-

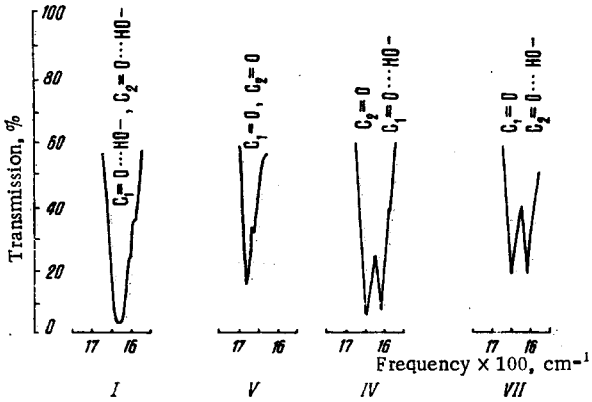
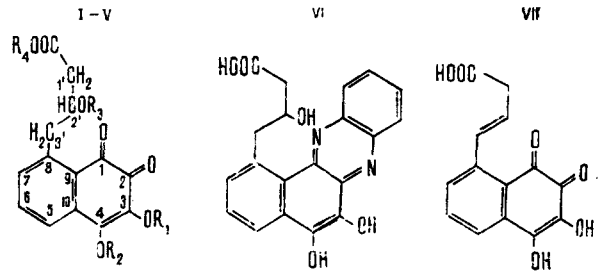


Fig. 1. IR spectra of olivovarin and its derivative (1600-1670 cm^{-1}).

lization of the side chain followed by dehydration and reduction reactions.



EXPERIMENTAL METHOD

The substances were purified and analyzed by column and thin-layer (in a nonfixed layer) chromatography on hydrated silicic acid, using the following mixtures of solvents: 1) benzene–acetone (1 : 2) (a), (4 : 1) (b), and (9 : 1) (c), and 2) chloroform–methanol (9 : 1). Gas–liquid chromatography (GLC) was performed on a Perkin–Elmer F-21 helium chromatograph (stationary phase 10% of ethylene adipate on Chromosorb A modified with 2% H_3PO_4, column 3 × 2000 mm, 190° C).

The UV, IR, NMR, and mass spectra were taken on a Hitachi recording spectrophotometer (in methanol), on a UR-10 spectrophotometer in tablets with KBr, on a Varian HA-100 spectrometer in $CDCl_3$, and on MKh-1309 and JMS-01 SG-2 (Jeol) mass spectrometers, respectively. The specific rotations were measured on a Jouan micropolarimeter in chloroform. The analyses of all the compounds corresponded to the calculated figures. The characteristics of the NMR spectra are given in the δ scale, ppm. The following abbreviations have been used: s) singlet; d) doublet; q) quartet; t) triplet; qu) quintet; and m) multiplet (HMDS was used as internal standard).

Preparation of Olivovarin (I). The ddep biosynthesis of olivovarin was effected by the cultivation of Act. olivovariabilis (strain 47 or 48) by a previously described method [3]. After the end of fermentation, the mycelium was filtered off, and the filtrate (~25 liters) was acidified with 10% HCl to pH 2 and extracted three times with ethyl acetate. The combined extract after washing with a saturated solution of NaCl and drying over Na_2SO_4 was evaporated to dryness in vacuum. The residue was chromatographed in mixture 2. The fractions were analyzed by thin-layer chromatography in benzene–acetone (2 : 1). The fractions containing the (I) were combined, evaporated, and rechromatographed in mixture 1b. The residue after the evaporation of the combined eluate containing olivarin was crystallized from acetone. This gave 2 g of (I), $C_{14}H_{12}O_7$, mp 178-179° C, $[\alpha]_D^{20}$ −22.5° (c 0.89; ethanol), M^+ 292. UV spectrum: λ_{max} 230, 230, 404 nm (log ε 4.2, 4.1, 3.7). IR spectrum, cm^{-1}: 3500, 1715, 1625, 1600, 1580. NMR spectrum: 2.42 (q, 2H, $2H_{1'}$); 2.78 (q, 2H, $2H_{3'}$); 4.3 (m, 3H, 20H, $H_{2'}$); 7.15 (q, 1H, H_7); 7.50 (q, 1H, H_5); 7.66 (t, 1H, H_6); 9.8 (s, 1H, COOH); 11.4 [s, 1H, $OH_{(chelate)}$].

Distillation of Olivovarin with Zn Dust. A mixture of 150 mg of (I) and 7.5 g of Zn dust was placed in a combustion boat which was introduced into a quartz tube. The latter was heated in a tube furnace to 500° C with the passage of N_2 through it. The reaction products were condensed in a receiver cooled with a mixture of CO_2 (dry) and acetone. The condensate obtained was analyzed by GLC. Three peaks were found corresponding in their retention times to naphthalene, 1-methylnaphthalene, and phenanthrene (4'05", 6'18", 12'20"). M^+ 128, 142, and 178, respectively.

Tri-O-acetylolivovarin (II). A solution of 292 mg of (I) in 1 ml of $(CH_3CO)_2O$ and 0.5 ml of pyridine was kept at ~20° C for 18 h and was then poured onto ice and extracted with chloroform. After being washed with 5% HCl and 5% $NaHCO_3$ solutions and with water and being dried over Na_2SO_4, the extract was evaporated to dryness. The residue was chromatographed in a thin layer in mixture 1c. The substance was eluted with methanol from the zone with R_f 0.3 and the extract was evaporated. This gave 60 mg of (II), $C_{20}H_{18}O_{10}$, mp 124-125° C (acetone) $[\alpha]_D^{20}$ +26° (c 1.07). UV spectrum: λ_{max} 244, 249, 263, 269, 343 nm (log ε 4.45, 4.46, 4.41, 4.39, 3.82). IR spectrum, cm^{-1}: 1775, 1745, 1715, 1675, 1655, 1600. NMR spectrum: 1.90 (s, 3H, CH_3CO); 2.32 (s, 3H, CH_3CO); 2.34 (s, 3H, CH_3CO); 2.6 (d, 2H, $2H_{1'}$); 2.86 (q, 2H, $2H_{3'}$); 5.34 (qu, 1H, $H_{2'}$); 7.30 (q, 1H, H_7); 7.63 (t, 1H, H_6); 8.02 (q, 1H, H_5); 9.34 (s, 1H, COOH).

Preparation of the Di- and Trimethyl Ethers of Olivovarin (III) and (IV), Respectively. An excess of an ethereal solution of diazomethane was added to a solution of 292 mg of (I) in 3 ml of chloroform–ether (2:1). After the mixture had been allowed to stand at room temperature for 18 h, the solvent was distilled off and the residue was chromatographed in a thin layer in mixture 1c. The substance was eluted with acetone from the zone with R_f 0.5, the eluate was evaporated, and the residue was crystallized. This gave 127 mg of (III), $C_{16}H_{16}O_7$, mp 83-84° C (ether–hexane), $[\alpha]_D^{20}$ – 18° (c 0.94), M^+ 320. UV spectrum: λ_{max} 250, 286, 417 nm (log ε 4.22, 4.17, 3.76). IR spectrum, cm^{-1}: 3500, 1740, 1635, 1615, 1580. NMR spectrum: 2.48 (d, 2H, $2H_{1'}$), 2.73 (q, 2H, $2H_{3'}$); 3.24 (m, 1H, OH); 3.62 (s, 3H, CH_3O); 4.08 (s, 3H, $COOCH_3$); 4.18 (m, 1H, $H_{2'}$); 7.09 (q, 1H, H_7); 7.40 (t, 1H, H_6); 7.45 (q, 1H, H_5); 11.66 [s, 1H, $OH_{(chelate)}$].

The substance was eluted with acetone from the zone with R_f 0.75. Evaporation yielded 50 mg of crystalline substance (IV), $C_{17}H_{18}O_7$, mp 70-71° C (acetone), $[\alpha]_D^{20}$ + 21° (c 0.88), M^+ 334. UV spectrum; λ_{max} 250, 286, 417 (log ε 4.22, 4.12, 3.73). IR spectrum, cm^{-1}: 3500, 1740, 1650, 1610, 1585. NMR spectrum: 2.45 (q, 2H, $2H_{1'}$); 2.82 (q, 2H, $2H_{3'}$); 3.33 (s, 3H, OCH_3); 3.58 (s, 3H, OCH_3); 3.83 (m, 1H, $H_{2'}$); 4.08 (s, 3H, $COOCH_3$); 7.10 (q, 1H, H_7); 7.48 (t, 1H, H_6); 7.52 (q, 1H, H_5); 11.71 [s, 1H, $H_{(chelate)}$].

Diacetate of the Dimethyl Ether of Olivovarin (V). A solution of 50 mg of (III) in 2 ml of a mixture of $(CH_3CO)_2O$ and pyridine (1:9) was kept at 20° C for 18 h, poured onto ice, and extracted with chloroform. After it had been worked up by the usual method, 54 mg was obtained of a substance $C_{20}H_{20}O_9$, mp 92-93° C (ether), $[\alpha]_D^{20}$ + 16° (c 0.89), M^+ 4.04. UV spectrum: λ_{max} 234, 269, 280, 310, 325, 339 nm (log ε 4.69, 4.02, 4.00, 3.81, 3.76, 3.75). IR spectrum, cm^{-1}: 1775, 1745, 1675, 1660, 1600, 1560. NMR spectrum: 1.88 (s, 3H, $COCH_3$); 2.32 (s, 3H, $COCH_3$); 2.54 (d, 2H, $2H_{1'}$); 2.85 (d, 2H, $2H_{3'}$); 3.55 (s, 3H, OCH_3); 4.01 (s, 3H, $COOCH_3$); 5.40 (qu, 1H, $H_{2'}$); 7.28 (q, 1H, H_7); 7.60 (t, 1H, H_6); and 7.90 (q, 1H, H_5).

Oxidation of Olivovarin. With constant stirring at ~95-100° C, 12 g of $KMnO_4$ was added in small portions to a solution of 1.3 g of (I) in 20 ml of 5% KOH until the color of the reaction mixture persisted. The mixture was kept at the same temperature for 1 h and was then cooled to ~20° C and was filtered from the sludge. The latter was washed twice with hot water, and the wash-waters were added to the filtrate. The combined solution was evaporated in vacuum to one tenth of its initial volume, acidified with a 20% solution of H_2SO_4 to pH_2, and extracted with ether. The ethereal extract, after being washed with water and dried over Na_2SO_4, was evaporated to dryness and the residue was crystallized from hot water. This gave 10 mg of hemimellitic acid with mp 180-182° C. When this substance was treated with an ethereal solution of diazomethane, the trimethyl ester of the acid was obtained. Mass spectrum: m/e 252 (M^+), 221 (M^+–31), 193 (M^+–59), 162 (M^+–59–31), 149 (M^+–59–30–14), 121 (149–28) and 105 (149–44).

Preparation of the Quinoxaline Derivative (VI). A mixture of a solution of 200 mg of (I) in 4 ml of glacial acetic acid and 130 mg of o-phenylenediamine in 2 ml of the same solvent was stirred at ~20° C for five days. The red-violet crystals of (VI) that deposited were filtered off, washed with ether, and dried in vacuum over P_2O_5. Yield 144 mg. The mother solution was evaporated to dryness and chromatographed on a column in mixture 1a. The fractions were analyzed by thin-layer chromatography in the same mixture. The fractions containing (VI) were combined and evaporated, the residue was crystallized. This gave another 20 mg of (VI) (total yield 164 mg) with the composition $C_{20}H_{16}N_2O_5$, mp > 220° C (acetic acid), $[\alpha]_D^{20}$ + 15° (c 0.33; dimethylformamide), M^+ 364. UV spectrum: λ_{max} 235, 278, 293, 438, 453, 524 (inflection) (log ε 4.50, 4.41, 4.02; 4.08, 4.10, 3.41). IR spectrum, cm^{-1}: 3500, 1715, 1620, 1573, 1535. NMR spectrum of the trimethylsilyl ether of (VII): 2.43 (d, 2H, $2H_{1'}$); 3.16 (q, 1H, $H'_{3'}$); 3.62 (q, 1H, $H''_{3'}$); 4.68 (m, 1H, $H_{2'}$); 7.04 (q, 1H, H_{arom}); 7.50 (m, 4H, H_{arom}); 0.02 (m, 2H, H_{arom}); 14.10 (s, 1H, COOH).

Anhydroolivovarin (VII). A solution of 50 mg of (I) in 1 ml of H_2SO_4 (92-85%) was allowed to stand for 4 h (~20° C), and was then poured onto ice and extracted with ethyl acetate. The extract was washed with a saturated solution of NaCl, dried over Na_2SO_4, and evaporated to dryness, and the residue was crystallized from acetone. This gave 37 mg of (VII), $C_{14}H_{16}O_6$, mp 189-200° C, $[\alpha]_D^{20}$ 0°, M^+ 274. UV spectrum: λ_{max} 234, 261, 230 (inflection), 414 nm (log ε 4.18, 4.25, 3.75, 3.63). IR spectrum, cm^{-1}: 3500, 1710, 1650, 1610, 1590, 1500.

SUMMARY

From the culture liquid of the mutant strains 47 and 48 of Act. olivovariabilis a new o-naphthoquinone–olivovarin – has been isolated. Structure (I) is proposed for it.

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