REACTIONS OF NITROSOPHENOLS. V.*
PREPARATION OF SOME DERIVATIVES
OF 1-HYDROXY-3-PHENOXAZONE

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1,7,9-Trihydroxy-3-phenoxazone (*I*) was found to represent the main component of florein. Compound *I* was identified as the triacetate *II*, the tri(methyl ether) *III*, and 1,3,7,9-tetraacetoxy-10-acetylphenoxazine (*IV*). Reaction of 4-nitrosophenol and resorcinol afforded a mixture of 1,7-dihydroxy-3-phenoxazone (*V*) and its tautomer, 1,3-dihydroxy-7-phenoxazone (*Va*). Isomeric diacetates *VI* and *VIa* (prepared by acetylation of the tautomeric mixture of *V* and *Va*) were separated by chromatography.

The dye obtained by Benedikt[1] by the action of fuming nitric acid on ethereal phloroglucinol was given the name florein on the basis of its similarity with the natural dyes hematein and brazilein. Benedikt suggested also constitutional relations between florein and both the Liebermann dyes (indophenols) and the pair resazurin–resorufin (7-hydroxy-3-phenoxazone 10-oxide and 7-hydroxy-3-phenoxazone). The constitution of these dyes was unknown at the time of Benedikt's investigations. For this reason and since no reinvestigations have been attempted, the constitution of florein has not been elucidated[2].

The formation of florein is markedly different from that of resazurin[3]. Thus, resazurin is formed in the cold while florein is obtained on heating the reaction mixture and after evaporation of the main portion of the solvent. The procedure of Benedict gives reproducible results and the properties of the product agree with those reported. The product, however, is not chromatographically homogeneous but represents a mixture of two dyes possessing very close R_F values. This fact seemingly supports the analogy with the pair resazurin–resorufin. The presence of an N-oxide of the phenoxazone type would not, however, correspond to the properties of florein. Firstly, the N-oxides of the above type are thermally labile while the forma-

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tion of florein requires heating and secondly, reduction of florein with zinc and hydrochloric acid leads to a leuco derivative the oxidation of which with air oxygen recovers a mixture of two dyes, identical with the starting mixture.
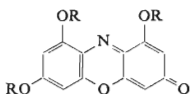

I, R = H
II. R = COCH$_3$
III, R = CH$_3$
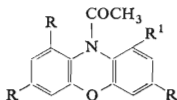
IV, R^1 = OCOCH$_3$
VIII, R^1 = H, R = OCOCH$_3$

By a small modification of the original Benedikt's procedure, a single compound was obtained, possessing the properties of a hydroxyphenoxazone. The assumed structure of 1,7,9-trihydroxy-3-phenoxazone (*I*) was confirmed by analysis and preparation of the triacetate *II*, the tri(methyl ether) *III*, and 1,3,7,9-tetraacetoxy-10-acetylphenoxazine (*IV*). Since compound *I* represents the principal component of florein, we propose to keep this name, especially with regard to the potential use of florein as analytical reagent.

Relatively pure *I* is also formed by the action of fuming nitric acid on a solution of phloro-glucinol in glacial acetic acid. An analogous use of nitrogen oxides afforded exclusively the nitro derivatives of phloroglucinol. Concerning the other component of the florein specimen prepared according to the procedure of Benedikt, no informations are available since we have not succeeded in isolating chromatographically a sufficient amount of the substance in a sufficient purity.

In connection with the isolation of compound *I* it was of interest to prepare some related substances which could be used as analytical reagents. Thus, condensation of 4-nitrosophenol with phloroglucinol and the subsequent chromatography afforded a small amount of 1,7-dihydroxy-3-phenoxazone (*V*). Compound *V* is also present (in addition to four other substances) in a mixture resulting in the condensation of 4-nitrosoresorcinol with phloroglucinol; this mixture is difficult to separate. In solutions, compound *V* might exist in a mixture with its tautomer, namely, 1,3-di-hydroxy-7-phenoxazone (*Va*). In fact, the acetylation affords a mixture of two isomeric diacetates which may be separated by chromatography. The predominant deep-orange diacetate was ascribed the formula *VIa* while the formula *VI* was allotted to the yellow-orange isomer of a negligibly lower R_F value. The allotment was made on the basis of ultraviolet and visible spectra of both isomers and their comparison with those of 7-acetoxy-3-phenoxazone (resorufin acetate) and 1,7,9-triacetoxy-3-phenoxazone (*II*). It was assumed that absorption maxima of the quinoid and an acetoxy group bearing system of compound *VI* will be located similarly as with the same system of compound *II*. On the other hand, the unsubstituted chromophoric system of compound *VIa* should be comparable with that of 7-acetoxy-3-phenoxazone.

Comparison of spectra of both pairs confirmed the above assumption; thus, the third absorption maximum differs by more than 12 nm (at 373 and 375 nm, resp., with the first pair and at 360 and 355 nm, resp., with the other).

V, R = H Va, R = H
VI, R = COCH$_3$ VIa, R = COCH$_3$
VII, R = CH$_3$ $VIIa$, R = CH$_3$
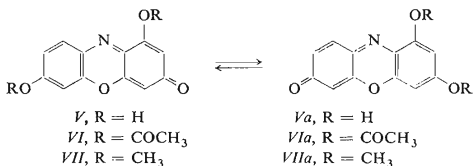
Treatment of the ethanolic solution of $V \rightleftarrows Va$ with diazomethane afforded a dimethyl derivative which behaved as a chromatographically homogeneous compound. The product may thus represent a mixture of two isomers of such a close R_F value that their separation was not possible, or a single isomer was formed (or, predominantly, one of the isomers). Some properties of the tautomeric mixture $V \rightleftarrows Va$ indicate that diazomethane could react preferentially with the form V: with aqueous sodium hydrogen carbonate, the mixture reacts instantaneously under the formation of a purple anion. On the other hand, the similar reaction with 7-hydroxy-3-phenoxazone (resorufin) occurs sluggishly and incompletely. For this reason, the acidity of the hydroxylic function at position 1 of the tautomer V must be almost as great as that of a carboxylic function in a strong carboxylic acid. The above hydroxylic group represents in combination with the carbonyl group at position 3 a vinylogue of the carboxylic function; the increased acidity of this system does not surprise[4]. The hydroxylic function at position 3 of compound Va also forms part of a vinylogous carboxyl but its acidity is considerably decreased by the presence of a chain of conjugated double bonds. The preferential reaction of diazomethane with compound V should lead to the dimethyl derivative VII the ultraviolet spectrum of which would be comparable with that of compound III; we did not, however, made any observation of this kind. Consequently, we may adopt the former alternative, namely, the formation of a mixture of VII and $VIIa$. This alternative is favoured also by the rather unsharp melting point value of the resulting di(methyl ether). Finally, the reductive acetylation of compound VIa afforded 1,3,7-triacetoxy-10-acetylphenoxazine ($VIII$).

The infrared spectra were investigated with all the above compounds, except for IV and $VIII$. Positions of absorption bands are in good agreement with the data for 3-phenoxazone and its derivatives reported by Musso and coworkers[5]. Consequently, allotments of most bands in the characteristic 1200—1700 cm^{-1} region were performed. The C=N band which is poorly intensive but clearly developed in the spectrum of 7-hydroxy-3-phenoxazone[6], appears as a shoulder with compound

I as well as the mixture $V \rightleftharpoons Va$. On the other hand, the corresponding derivatives exhibit a clearly developed band. The intensity of the quinoid double bond (at 1650 cm^{-1}) is considerably lower with *VI* than with *VIa*, probably due to the presence of an acetoxy group on the quinoid system of compound *VI*.

EXPERIMENTAL

Melting points (corrected) were taken on a heated microscope stage (Boetius block). Compounds were chromatographed on silica gel CH (Lachema, Neratovice, Czechoslovakia), purified on heating with chromic acid–sulfuric acid mixture, washed with water, and activated at 150°C for 20 hours. Ultraviolet and visible spectra were measured on a Beckman-DU apparatus in 96% ethanol (concentration, $2 \cdot 5 \cdot 10^{-5} \text{M}$). Infrared spectra were taken on an Infrascan (Hilger & Watts) apparatus with the use of potassium bromide.

1,7,9-Trihydroxy-3-phenoxazone (*I*)

a) Precooled solution of phloroglucinol (10 g) in ether (750 ml) was treated under stirring drop by drop with 4 ml of fuming nitric acid (nitric acid, d 1·25, was saturated at room temperature with gaseous nitrogen oxides, *cf.*[3]). The ether was slowly evaporated under stirring, the bath temperature being gradually raised up to 45°C. The last traces of ether were removed under diminished pressure and the residual purple thick liquid treated with water (200 ml). The resulting aqueous suspension of a red-brown precipitate was repeatedly washed with ether (to remove the main portion of the unreacted phloroglucinol and its nitro derivatives), decanted with water, collected with suction, washed with water, and dried for 10 hours over phosphorus pentoxide at 115°C/1 Torr; yield, 260 mg. Thin-layer chromatography in the solvent system 17 : 3 acetone–concentrated aqueous ammonia revealed a negligible contamination at the start line. With the use of the original procedure of Benedikt[1] (when, *inter alia*, the bath temperature was allowed to raise up to 80°C), the yields of florein were considerably higher, *e.g.*, 3·3 g of florein from 10 g of phloroglucinol, but the product was not homogeneous and consisted of two compounds which were difficult to separate (their ratio was approximately 1 : 1). When the decantation of the dye with water was not performed thoroughly enough, the product was light-coloured and contaminated with the starting phloroglucinol.

b) Pure *I*, although in a very poor yield, was also obtained by the action of fuming nitric acid (65% nitric acid presaturated at room temperature with gaseous nitrogen oxides) on a solution of phloroglucinol in glacial acetic acid. The dropwise addition of nitric acid should be performed in the temperature range of 35—39°C. The following ratio of components was the most suitable: 1 g of phloroglucinol, 5 ml of glacial acetic acid, 0·6 ml of fuming nitric acid. After the addition of fuming nitric acid, the stirring was continued for additional 15 min, the mixture poured into water and decanted.

Florein is a deep-red powder, sparingly soluble in polar solvents (orange solutions). Increasing content of moisture leads to a decreasing solubility of florein. Intensive purple-coloured solutions are obtained with aqueous hydroxides and carbonates of alkali metals (and in sodium hydrogen carbonate; instantaneously); by the action of acids, the colour changes through orange-yellow to red. Florein does not melt up to 350°C. The analytical sample was recrystallised from ethanol and then from xylene, and dried for 8 hours over phosphorus pentoxide at 140°C/0·5—1 Torr. Infrared spectrum: 1 657 (C=C quinoid), 1 618 (C=O), 1 578 (C=C aromatic), 1 505 sh (C=N), 1 490 sh, 1 475, 1 435, 1 372 (C—C), 1 250 (N—C_6H_5), 1 221, 1 167 cm^{-1} (C—O). For $C_{12}H_7NO_5$ (245·2) calculated: 58·78% C, 2·88% H, 5·71% N; found: 58·34% C, 3·00% H, 5·61% N.

1,7,9-Triacetoxy-3-phenoxazone (*II*)

A mixture of dry *I* (200 mg) and 1 : 1 pyridine–acetic anhydride (10 ml) was allowed to stand at room temperature for 4 hours and evaporated under diminished pressure at 40°C. The deep-orange residue was dissolved in 3 : 1 benzene–acetone and chromatographed. The eluate of the orange zone was evaporated and the residue recrystallised from benzene–cyclohexane to afford 115 mg of a powder, m.p. (rapid heating) 228—233°C (decompn.). The additional slower reddish band contained probably the partially acetylated *I*. The analytical sample was dried for 10 hours at 115°C/0·5—1·0 Torr. Ultraviolet and visible spectrum: 245 nm ($\varepsilon \cdot 10^{-3} = 18 \cdot 0$), 254 nm (18·7), 375 nm (13·1), 445 nm (11·2). Infrared spectrum: 1767 (CH_3CO), 1652 (C=C quinoid), 1622 (C=O), 1583 (C=C aromatic), 1517 (C=N); weak: 1472, 1430, and 1405; 1375 (C—C), 1321, 1285, 1256 (N—C_6H_5), 1195 cm^{-1} (C—O). For $C_{18}H_{13}NO_8$ (371·3) calculated: 58·22% C, 3·53% H, 3·77% N; found: 58·40% C, 3·58% H, 3·66% N.

1,7,9-Trimethoxy-3-phenoxazone (*III*)

A mixture of crude *I* (600 mg) and ethanol (600 ml) was refluxed for 30 min, cooled, and filtered. The filtrate was treated slowly drop by drop under stirring with 650 ml of ethereal diazomethane (prepared from 45·5 g of nitrosomethylurea), the reaction mixture allowed to stand at room temperature for 15 min, and evaporated under diminished pressure to dryness. The residue was dissolved in 3 : 1 benzene–acetone and chromatographed on a short column. The orange band of compound *III* was accompanied by a light-red slower band, probably of compound *I*. Yield, 250 mg of compound *III*, an orange powder, m.p. 295—297°C (ethanol). The analytical sample was dried for 10 hours over phosphorus pentoxide at 115°C/0·5—1·0 Torr. Ultraviolet and visible spectrum: 225 nm ($\varepsilon \cdot 10^{-3} = 27 \cdot 6$), 262 nm (17·6), 276 nm sh (11·0), 406 nm sh (14·0), 452 nm (23·1). Infrared spectrum: 1646 (C=C), 1617, 1608 (C=O), 1600, 1585, and 1563 (C=C aromatic), 1507 (C=N), 1486, 1450 (C=C aromatic), 1428, 1412, 1356 (C—C), 1305, 1267 sh, 1262 (N—C_6H_5), 1235, 1210, 1167 cm^{-1} (C—O). For $C_{15}H_{13}NO_5$ (287·3) calculated: 62·70% C, 4·56% H, 4·88% N; found: 62·71% C, 4·61% H, 5·07% N.

1,3,7,9-Tetraacetoxy-10-acetylphenoxazine (*IV*)

A mixture of compound *II* (50 mg), sodium acetate (100 mg), zinc powder (100 mg), and acetic anhydride (5 ml) was heated under stirring at 40°C for 20 min and then evaporated under diminished pressure to dryness at 40°C. The residue was extracted with three 20 ml portions of hot benzene, the extracts filtered and the filtrates concentrated until the crystals began to deposit. The concentrate was treated with a little light petroleum to afford 35 mg of needles, m.p. 157 to 160°C (benzene–light petroleum). As shown by chromatography in 3 : 1 benzene–acetone (detection with gaseous nitrogen oxides, bromine vapours, or ammonia), compound *IV* contained only a negligible amount of contaminations. For $C_{22}H_{19}NO_{10}$ (457·4) calculated: 57·77% C, 4·19% H, 3·06% N; found: 58·21% C, 4·01% H, 3·43% N.

1,7-Dihydroxy-3-phenoxazone (*V* ⇌ *Va*)

a) A solution consisting of phloroglucinol (2·6 g), 4-nitrosophenol (5·0 g), and ethanol (100 ml) was treated under stirring in the course of 10 minutes with concentrated sulfuric acid (10 ml). The stirring was continued for 30 minutes, the reaction mixture poured into 1 litre of water, and the precipitate collected by decantation. After two additional decantations (500 ml of water each), the precipitate was collected with suction and dried over phosphorus pentoxide at 110°C/ /0·5—1·0 Torr for 4 hours to afford 3·0 g a deep-brown product (this specimen was used in pre-

parations of the diacetate and the di(methyl ether)). Chromatography indicated the presence of a small amount of relatively pure $V \rightleftarrows Va$ along with strong contaminations at the start and front line. The product (400 mg) was extracted repeatedly with acetone (350 ml total); there was obtained 120 mg of the insoluble residue. The acetonic extracts were diluted with concentrated aqueous ammonia (3 parts of ammonia per 17 parts of acetone) and the whole was chromatographed in a 9×3.5 cm column with the use of the same solvent mixture. The purple eluates (about 2000 ml) were concentrated under diminished pressure at 45°C to the volume of 340 ml, the concentrate cooled, and precipitated by the addition of concentrated hydrochloric acid (6 ml). The precipitate was collected with suction, washed with water, and dried for 10 hours over phosphorus pentoxide at 140°C/0·5 — 1·0 Torr. Yield, 70 mg of crude compound V. In spite of two recrystallisations, the product was not entirely pure (traces of silica gel after combustion) but was chromatographically homogeneous. Infrared spectrum: 1737 weak, 1653 (C=C quinoid), 1608 (C=O), 1561 (C=C aromatic), 1505 sh (C=N), 1492, 1460 (C=C aromatic), 1365 (C—C), 1265 (N—C_6H_5), 1233 cm^{-1}. For $C_{12}H_7NO_4$ (229·2) calculated: 62·89% C, 3·08% H, 6·11% N; found: 61·54% C, 3·29% H, 5·72% N.

b) A solution consisting of 4-nitrosoresorcinol (0·1 g), phloroglucinol (0·1 g), and ethanol (2·5 ml) was treated with concentrated sulfuric acid (0·7 ml) and kept at room temperature under occasional shaking for 30 min. The resulting mixture was poured into water (50 ml) and the precipitate processed as given in paragraph *a*). The yield was not determined. As shown by chromatography in the solvent system 3 : 1 benzene–acetone with the use of an authentic specimen from the preceding preparation, the present product contained some $V \rightleftarrows Va$ along with some other light-yellow substance of the same R_F value, a purple substance at the start line, and additional two compounds near the front line of the chromatogram.

Acetylation. The predried unchromatographed product (800 mg) from paragraph *a*) was treated with a mixture (40 ml) of pyridine and acetic anhydride (1 : 1), the whole was allowed to stand at room temperature for 4 hours, and evaporated under diminished pressure (bath temperature 45°C). The solid residue was dried over potassium hydroxide pellets in an evacuated desiccator and repeatedly extracted with 3 : 1 benzene–acetone (170 ml total). The insoluble portion (340 mg) did not contain the diacetate. The extract was chromatographed on a 8×3.5 cm column. The eluate of the orange zone (consisting of a mixture of VI and VIa) was evaporated and the residue rechromatographed on a 4×40 cm column in 9 : 1 benzene–acetone to afford two zones located very closely to each other, namely, an orange and an orange-yellow zone (the separation was successful with absolutely dry solvents only). Elution of the orange zone, evaporation of the eluate, and recrystallisation of the residue from a mixture of benzene and light petroleum afforded 70 mg of an orange substance, the ultraviolet and visible spectrum of which was very similar to that of 7-acetoxy-3-phenoxazone[7]. For this reason, the substance was ascribed the structure VIa. Ultraviolet spectrum of VIa: 246 nm ($\varepsilon \cdot 10^{-3} = 17·0$), 252 nm (17·1), 360 nm (13·4), 450 nm (12·4); of 7-acetoxy-3-phenoxazone: 246 nm (17·6), 252 nm sh (16·0), 355 nm (11·8), 445 nm (12·7). Infrared spectrum: 1768 (CH$_3$CO), 1652 (C=C quinoid), 1614 (C=O), 1580 (C=C aromatic), 1518 (C=N), (weak: 1475, 1440, 1401), 1372 (C—C), 1327 weak, 1250 (N—C_6H_5), 1201, 1177 cm^{-1} (C—O). The analytical sample was dried for 3 hours over phosphorus pentoxide at 110°C/0·5—1·0 Torr. For $C_{16}H_{11}NO_6$ (313·3) calculated: 61·34% C, 3·54% H, 4·47% N; found: 61·40% C, 3·69% H, 4·62% N. Concerning the orange-yellow zone of a lower R_F value, a rechromatography was necessary. This operation, however, was accompanied by a considerable loss due to a partial hydrolysis on silica gel. Yield, 10 mg (after recrystallisation from benzene–light petroleum) of the substance which was ascribed the formula VI on the basis of spectral evidence (its content in the original mixture is almost the same as that of compound VIa). Ultraviolet and visible spectrum of VI: 246 nm ($\varepsilon \cdot 10^{-3} = 18·3$), 255 nm (17·1), 373 nm (12·6),

442 nm (13·1); of *II*: 245 nm (18·0), 254 nm (18·7), 375 nm (13·1), 445 nm (11·2). The structure *VI* is also favoured by a shift of vibrations of C=C quinoid and C=N bonds to lower values in contrast to the infrared spectrum of compound *VIa*. Infrared spectrum of *VI*: 1773 (CH_3CO), 1645 (C=C), 1617 (C=O), 1600 (C=C), 1571 (C=C aromatic), 1510 (C=N), 1468 (C=C aromatic), 1440 weak, 1372 (C—C), (weak: 1336, 1322, 1295), 1254 (N—C_6H_5), 1207, 1189 cm^{-1} (C—O). Compound *VI* (found: 61·69% C, 3·55% H, 4·46% N) is isomeric with *VIa*. Melting points of compounds *VI* and *VIa* are almost identical, considerably unsharp, and accompanied by decomposition: *VI*, m.p. 186—193°C, and *VIa*, m.p. 184—190°C (mixed melting point shows depression). As shown by chromatography, any of compounds *VI* and *VIa* is not contaminated by the other.

Methylation. The predried unchromatographed product $V \rightleftharpoons Va$ (400 mg) from paragraph *a*) was refluxed in ethanol (140 ml) for 30 minutes and the mixture was filtered (80 mg of insoluble material). The cooled filtrate was treated dropwise under stirring in the course of 5 minutes with 130 ml of ethereal diazomethane (from 9·5 g of nitrosomethylurea). The mixture was allowed to stand at room temperature for 10 minutes, evaporated under diminished pressure, the residue dissolved in 4 : 1 benzene–acetone, and the solution chromatographed to afford the following sequence of zones: two yellow zones at the front line, an orange zone of the di(methyl ether), a light-yellow zone near the start, and a strong brown zone at the start line. The orange zone afforded after recrystallisation from benzene–light petroleum 30 mg of an orange powder melting partially from 255°C with sublimation. The main portion and the sublimate melted (under browning) in the range of 280—286°C. The product representing obviously a mixture of isomers *VII* and *VIIa*, behaved as chromatographically homogeneous. Ultraviolet and visible spectrum: 224 nm ($\varepsilon . 10^{-3} = 23·1$), 258 nm (15·6), 460 nm (18·5); this spectrum was dissimilar to that of compound *III* or 7-methoxy-3-phenoxazone[7]. Infrared spectrum: 1648 (C=C), 1615 (C=O), 1596 (C=C), 1570 (C=C aromatic), 1510 (C=N), 1490, 1469 (C=C aromatic), (weak: 1412, 1360, 1340, 1332), 1305, 1280, 1268, 1260, 1235, 1217 cm^{-1}. For $C_{14}H_{11}NO_4$ (257·2) calculated: 65·36% C, 4·31% H, 5·45% N; found: 64·84% C, 4·43% H, 5·17% N.

1,3,7-Triacetoxy-10-acetylphenoxazine (*VIII*)

The title compound was prepared analogously to substance *IV*. Thus, 50 mg of compound *VIa* afforded 40 mg of *VIII*, needles, m.p. 150—152°C. As shown by chromatography, only a negligible contamination was present, probably a partially acetylated derivative. For $C_{20}H_{17}NO_8$ (399·3) calculated: 60·15% C, 4·29% H, 3·51% N; found: 60·84% C, 4·78% H, 3·63% N.

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