

The Constitution of Fluorescein Oxime and the Hydroquinonephthalein Oximes

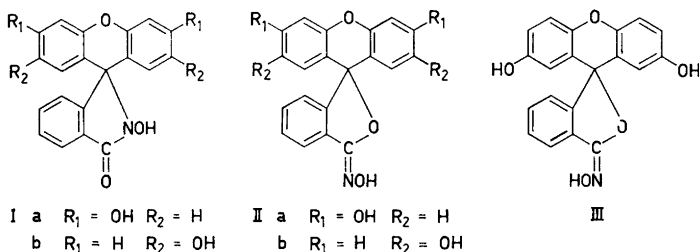
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The reaction product obtained from fluorescein and hydroxylamine in alkaline solution has been shown to be 3,7-dihydroxy-11-(2'-carboxyphenyl)-dibenz[b,f]oxazepine (VIII). Under similar conditions hydroquinonephthalein forms three products: Hydroquinonephthalein- α -oxime = spiro[(2-hydroxy-3-keto-2,3-dihydroisindole)-1,9'-(2',7'-dihydroxyxanthene)] (Ib), hydroquinonephthalein- β -oxime = 2,7-dihydroxy-9-(2'-carboxyphenyl)-acridine-*N*-oxide (XIb), and hydroquinonephthalein- γ -oxime = 2,7-dihydroxy-9-(2'-carboxyphenyl)-acridine (Xb).

Fluorescein reacts with hydroxylamine in alkaline solution and on acidifying with acetic acid a yellow compound A is obtained for which the structure Ia or IIa has been suggested.¹ Hydroquinonephthalein reacts with hydroxylamine under similar conditions and three compounds² have been isolated from the reaction, a colourless compound B, hydroquinonephthalein- α -oxime, to which the formula Ib was coined,² an orange product C, hydroquinonephthalein- β -oxime, and a yellow compound D, hydroquinonephthalein- γ -oxime for which the structures IIb and III, respectively, were proposed.³

As the reaction product obtained from phenolphthalein and hydroxylamine in alkaline solution, which for some time was thought to have a structure⁴ analogous to I, has been shown⁵ to be a *p*-hydroxyanil of *o*-(*p*-hydroxybenzoyl) benzoic acid a reinvestigation of the proposed structures I—III seemed warranted.



FLUORESCEIN OXIME

This yellow compound A, $C_{20}H_{13}NO_5$, is soluble in alkaline and mineral acid solution, but insoluble at intermediate pH-values. It is soluble in dimethylformamide and pyridine, but insoluble in most common organic solvents. It forms salts with strong acids, reacts with three moles of diazomethane to form a "trimethylether", $C_{23}H_{19}NO_5$, which can be hydrolysed in alkaline solution to a "dimethylether".

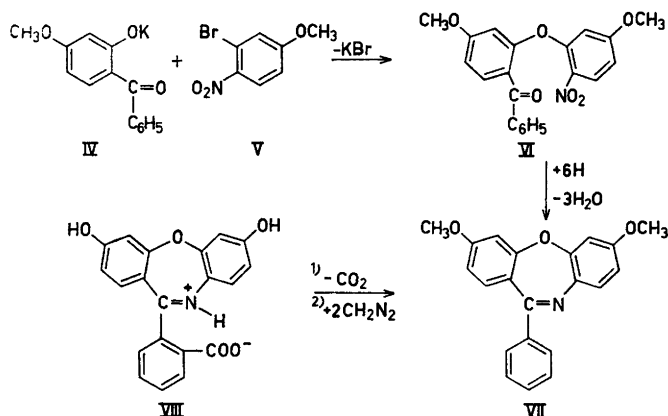
The fluorescein oxime is highly resistant towards hydrolysis. Boiling with 6 M sulfuric acid for two days leaves the compound unchanged, whereas treatment with 9 M sulfuric acid or 30 % hydrogen bromide at 150° under pressure cleaves the compound, but apart from phthalic acid no products were identified from the reaction mixture. In no cases fluorescein was detected.

The NMR-spectrum in $CDCl_3$ of the trimethylether contains besides other features three peaks at $\delta = 3.66$, 3.800, and 3.807 corresponding to three differently situated methyl groups. This excludes the proposed structures Ia and IIa.

The IR-spectrum of A contains a broad absorption band from 3600 cm^{-1} to 2200 cm^{-1} , but no bands between 1800 cm^{-1} and 1640 cm^{-1} . The IR-spectra of the salts of A contain an absorption peak at 1700 cm^{-1} corresponding to the presence of an aromatic carboxylic acid. The presence of a carboxyl group in A is supported by the IR-spectrum of the trimethylether which contains a peak at 1730 cm^{-1} probably caused by an aromatic ester group.

The IR-spectra of A, its hydrochloride and its trimethylether resemble those of phenolphthalein oxime and its corresponding derivatives. If a similar rearrangement as that occurring during the formation of phenolphthalein oxime takes place in the reaction between fluorescein and hydroxylamine the fluorescein oxime would be expected to be a derivative of phenyldibenzoxazepine.

A can be decarboxylated with copper in quinoline at 200°C , and the decarboxylated product forms a dimethylether VII, $C_{21}H_{17}NO_3$, which was shown to be 3,7-dimethoxy-11-phenyl-dibenz[b,f]oxazepine from the analysis, the



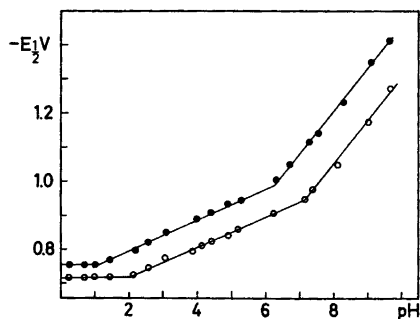


Fig. 1. Dependence on pH of the half-wave potentials (*vs.* S.C.E.) of fluorescein oxime ● and phenolphthalein oxime ○.

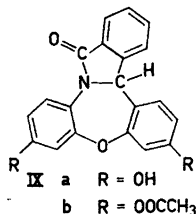
IR- and NMR-spectra, and the identity with the compound synthesised by the route described below.

The potassium salt of 2-hydroxy-4-methoxybenzophenone (IV) was condensed with 3-bromo-4-nitro-anisole (V) to 2-(2'-nitro-5'-methoxy-phenoxy)-4-methoxy-benzophenone (VI). When this compound was reduced with Raney nickel and hydrazine the product obtained, $C_{21}H_{17}NO_3$, proved to be identical with the dimethylether of the decarboxylated fluorescein oxime.

The formulation of fluorescein oxime as the zwitterion of 3,7-dihydroxy-11-(2'-carboxyphenyl)-dibenz[b,f]oxazepine (VIII) is consistent with all the known data of the compound, *e.g.* the solubility in acids and bases, the presence of a carboxyl group absorption in the IR-spectrum of the hydrochloride, but not in that of the parent compound, and the occurrence of three peaks in the NMR-spectrum of the trimethylether corresponding to three non-equivalent methyl groups.

Compound A is polarographically reducible and the dependence on pH of the half-wave potentials of A and of phenolphthalein oxime is depicted in Fig. 1. Controlled potential reduction of A consumes two electrons per molecule and yields a compound, E, $C_{20}H_{13}NO_4$, H_2O which also is obtained by reduction with zinc in alkaline solution.

The reduction product yields on treatment with acetic anhydride a diacetyl derivative, $C_{24}H_{17}NO_6$. The IR spectrum of this compound shows two absorption bands in the carbonyl region at 1750 cm^{-1} and 1685 cm^{-1} and no absorption in the N—H region at $3500\text{--}3200\text{ cm}^{-1}$. These facts suggest that the reduction product can be formulated analogously to the reduction product from phenolphthalein oxime, and the compound E would thus be 10,15*b*-dihydro-11-keto-3,7-dihydroxydibenzo [2,3:—6,7] [1,4] oxazepino [5,4-*a*]isoindole, IXa.



The singlet in the NMR-spectrum of the diacetyl derivative (IXb) at $\delta = 6.64$ is attributed to the hydrogen at carbon atom 15b. It is an unusual position for a hydrogen atom bonded to a saturated carbon atom, but as it is neighbour to a nitrogen atom and influenced by the ring currents from three phenyl groups held in a fixed position the δ -value seems not improbable. Furthermore, the corresponding hydrogen atom in 2,3-bis-(*p*-acetoxyphenyl)-phthalimidine gives a singlet at $\delta = 6.05$.

The proton at C-9 gives rise to a doublet centered at $\delta = 9.08$ ($J = 9.2$ cps). This position is explained by the influence from the 11-keto group. The corresponding proton in 2,3-bis(*p*-acetoxyphenyl)-phthalimidine is part of the multiplet at $\delta = 6.9-8.1$; the possibility of rotation of the phenyl groups in this compound diminishes the influence of the carbonyl group on the hydrogen atoms.

HYDROQUINONEPHTHALEIN- α -OXIME

For this compound the structure Ib has been suggested² and the available data are in accordance with this formulation as spiro[(2-hydroxy-3-keto-2,3-dihydroisindole)-1,9'-(2',7'-dihydroxyxanthene)]. The compound yields hydroquinonephthalein on acid hydrolysis and the IR-spectrum contains a carbonyl absorption at 1670 cm^{-1} which also is found in hydroquinonephthaleinimide and phenolphthaleinimide. The compound can be methylated to a trimethylether which can be hydrolysed to hydroquinonephthalein dimethylether. The NMR-spectrum of the trimethylether shows the presence of two equivalent methyl groups and one different from those two.

The compound does not rearrange on treatment with phosphorus pentachloride in ether.

HYDROQUINONEPHTHALEIN- β - AND γ -OXIMES

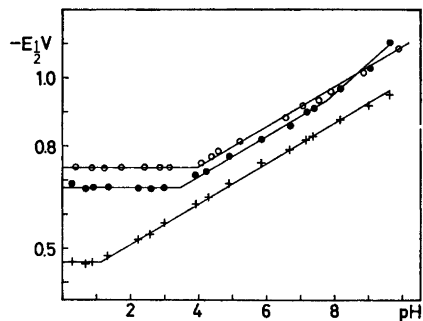
Meyer and Spengler state that these compounds are formed in varying yields, but that the total yield of the two compounds is more constant. They claim that both compounds can be hydrolysed on treatment with acid to hydroquinonephthalein; this statement, however, is not substantiated by the present investigation although conditions for the acid hydrolysis have ranged from mild to very drastic. Possibly impurities of the α -oxime in the β -oxime, which is difficult to purify without chromatographic separation, are responsible for Meyer and Spengler's results.

The compounds contain water of crystallisation which is very firmly held, and satisfactory analyses are difficult to obtain even after drying *in vacuo* over phosphorus pentoxide. When the phenol groups are methylated better analyses are obtained. Meyer and Spengler indicated analyses corresponding to $\text{C}_{20}\text{H}_{13}\text{NO}_5$ for both the β - and the γ -oxime.

These authors found that boiling of the β -oxime with methanolic sulfuric acid produced the γ -oxime and they formulated the compounds as *syn* and *anti* isomers with the γ -oxime being the more stable form III.

The β -oxime is soluble in alcohol, but the γ -oxime is insoluble in most organic solvents. Both are soluble in alkaline and acid solution; in acid solution the γ -oxime is more soluble than the β -oxime.

Fig. 2. Dependence on pH of the half-wave potentials (vs. S.C.E.) of 2,7-dimethoxy-9-phenylacridine \circ and hydroquinonephthalein- β -oxime; (1. wave +, 2. wave \bullet). The half-wave potentials of the γ -oxime are equal to those of the second wave of the β -oxime.



The β -oxime can be reduced with zinc in sodium hydroxide or electrolytically. When the reduction product is oxidised by bubbling air through the solution the γ -oxime is formed. Oxidation of the reduction product from the γ -oxime also yields the γ -oxime.

Both oximes form trimethylethers on treatment with diazomethane. The β -oxime forms two trimethylethers, one of them being the one obtained from the γ -oxime. Both trimethylethers can be hydrolysed to dimethylethers. The analyses of the trimethylethers of the β - and γ -oxime correspond to $C_{23}H_{19}NO_5$ and $C_{23}H_{19}NO_4$, respectively.

Both the β - and γ -oxime are polarographically reducible, (Fig. 2). In Fig. 2 are included the half-wave potentials of 2,7-dimethoxy-9-phenylacridine. Below pH 4 the β -oxime shows two well separated waves; the second wave has a half-wave potential equal to that of the γ -oxime. Above pH 4 the waves are poorly separated. Controlled potential reduction of the β -oxime at the potential corresponding to the first wave produces the γ -oxime in a reduction which consumes two electrons per molecule.

The IR-spectrum of the γ -oxime resembles that of fluorescein oxime and suggests a zwitterionic structure for the compound as it contains a series of broad bands from 3500 cm^{-1} to 2300 cm^{-1} , no absorption from 1800 cm^{-1} to 1640 cm^{-1} and broad bands from 1640 cm^{-1} to 1400 cm^{-1} . The IR-spectrum of the β -oxime contains a band of medium intensity at 1680 cm^{-1} and no bands corresponding to a carboxylate ion. The IR-spectra of the β - and γ -oximes suggest that they can not be *syn* and *anti* isomers.

The IR-spectra of the hydrochlorides of the two oximes are much more alike. They contain both a carbonyl band at 1700 cm^{-1} , probably caused by the presence of a carboxyl group. This is corroborated by the IR-spectra of the trimethylethers which both contain an absorption band at 1725 cm^{-1} corresponding to the presence of an aromatic ester group.

The NMR-spectra of the trimethylethers show peaks corresponding to two identical methyl groups and one different from the two.

Attempts to decarboxylate the β - and the γ -oximes were unsuccessful, but the dimethylether of the γ -oxime could be decarboxylated with cuprous oxide in quinoline and yields a compound F, $C_{21}H_{17}NO_2$; the formula indicated the loss of one oxygen compared with the corresponding dimethylether of the decarboxylated fluorescein oxime.

If a rearrangement analogous to that occurring during the formation of phenolphthalein oxime and fluorescein oxime were operating in the reaction between hydroquinonephthalein and hydroxylamine the compound F would presumably be either a fluorenone anil derivative or a phenylphenanthridine derivative depending of whether the phenyl group carrying the carboxyl group or one of those carrying a hydroxyl group had moved. Both types of derivatives were synthesised and were found to be different from F.

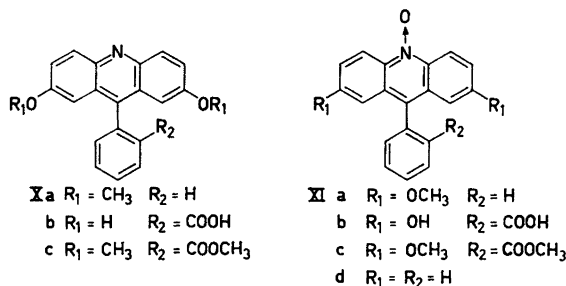
The NMR-spectrum (Table 1) of F contained a peak corresponding to two equivalent methyl groups. This and the empirical formula led to the formulation of F as 2,7-dimethoxy-9-phenylacridine (Xa) which was proved by its identity with authentic 2,7-dimethoxy-9-phenylacridine prepared from *p*-anisidine and benzaldehyde analogously to the preparation of 2,7-dimethyl-9-phenylacridine.⁶

Table 1. Chemical shifts (δ , ppm), number of hydrogen atoms in the signal(s) (ΣH), the number of peaks (m), and coupling constants ($J_{a,b}$; cps) in the NMR-spectra of 2,7-dimethoxy-9-phenylacridine (Xa), hydroquinonephthalein- γ -oxime (Xb), its trimethylether (Xc), 2,7-dimethoxy-9-phenylacridine-*N*-oxide (XIa), hydroquinonephthalein- β -oxime (XIb), and its trimethylether (XIc). Solvent: trifluoroacetic acid.

H	Xa	Xb	Xc	XIa	XIb	XIc
C-1 δ	7.30	7.08	6.97	7.27	7.05	6.97
+ C-8 ΣH	2	2	2	2	2	2
m	2	2	2	2	2	2
C-4 δ	8.31	8.26	8.29	8.71	8.70	8.75
+ C-5 ΣH	2	2	2	2	2	2
m	2	2	2	2	2	2
R ₁ δ	3.92		3.85	3.88		3.85
= ΣH	6		6	6		6
CH ₃ m	1		1	1		1
R ₂ δ			3.72			3.70
= ΣH			3			3
COOCH ₃ m			1			1
X δ	7.5–8.2	7.2–8.2	7.5–8.2	7.5–8.2	7.3–8.2	7.5–8.2
ΣH	7	6	6	7	6	6
m	multipl.	multipl.	multipl.	multipl.	multipl.	multipl.
$J_{1,3}$	2.1	2.1	2.7	2.8	2.6	2.9
$J_{3,4}$	9.1	9.5	9.1	9.5	9.6	9.3

The similarities in the UV-spectra and in the pattern of the NMR-spectra of the two oximes (in dimethylsulfoxide) and that of F suggested that no rearrangement occurred during the decarboxylation, and that the acridine nucleus was present in the two oximes.

The hydroquinonephthalein- γ -oxime is, therefore, suggested to be 2,7-dihydroxy-9-(2'-carboxyphenyl)acridine (Xb) in form of a zwitterion, and the hydroquinonephthalein- β -oxime should be the *N*-oxide of the γ -oxime (XIb).



These formulations are in accordance with the data obtained in this investigation. The empirical formula of the γ -oxime trimethylether contains one oxygen less than that of the β -oxime and suggests that the same is true for the oximes proper. The zwitterionic structure suggested by the IR-spectrum of the γ -oxime is easily explained, the absence of such a zwitterionic structure in the β -oxime is caused by the weaker basic properties of the acridine-*N*-oxide compared with those of the acridine. The similarities of the IR-spectra of the derivatives of the β - and γ -oximes are easily understood from the proposed formulations.

The UV-spectra of the compounds (Table 2) support the proposed structures. The UV-spectra of hydroquinonephthalein- β -oxime trimethylether (XIc) and of 2,7-dimethoxy-9-phenylacridine-*N*-oxide (XIa) are very much alike, and that of the β -oxime (XIb) and 9-phenylacridine-*N*-oxide (XI d) show only minor displacements of the bands compared with those of XIa and XIc. Especially the pattern above 300 $m\mu$, which is not found in the compounds not containing the *N*-oxide function, reveals the close structural relationship between the compounds.

The spectra of the γ -oxime (Xb) and its trimethylether (Xc) also disclose their kinship with that of 2,7-dimethoxy-9-phenylacridine (Xa). The spectra of hydroquinonephthalein and the α -oxime are consistent with their similar structure.

The NMR-spectra of some relevant compounds are given in Table 1. Again the similarities in chemical shifts and coupling constants support the structures Xb and XIb for the γ - and the β -oxime, respectively. The doublet of the hydrogen atoms at C-4 and C-5 are shifted from about $\delta = 8.3$ in Xa, Xb and Xc to δ about 8.75, in XIa, XIb and XIc, when the acridine derivative is converted into its *N*-oxide. In the spectrum of 9-phenylacridine-*N*-oxide the corresponding doublet is found at $\delta = 8.88$ ($J = 9.1$ cps). The position

Table 2. Extinction coefficients and wave lengths ($m\mu$) of the absorption maxima in the UV-spectra of fluorescein oxime (VIII), decarboxylated fluorescein oxime (VIII b), hydroquinonophthalin (XV), the α -oxime (Ib), the γ -oxime (Xb), the γ -oxime trimethyl ether (Xc), 2,7-dimethoxy-9-phenylacridine (Xa), hydroquinonophthalin- β -oxime (XIb), its trimethyl ether (XIc), 2,7-dimethoxy-9-phenylacridine-N-oxide (XIIa), and 9-phenylacridine-N-oxide (XIIb). Solvent 96 % alcohol.

	VIII	VIIIb	XV	Ib	Xb	Xc	Xa	Xb	XIc	XIIa	XIIb
λ $m\mu$	241	231	219	219(S)	229	229	229(S)	230(S)	229	229	232
ϵ 10^{-4}	1.99	7.86	3.46	4.18	4.86	2.26	1.85	4.56	2.50	1.02	1.05
λ $m\mu$		251	250(S)	250(S)	261	260	261	273	273	273	269
ϵ 10^{-4}		8.49	1.31	1.94	19.09	9.39	5.50	15.60	7.85	5.45	6.92
λ $m\mu$	282(S)	286(S)						303(S)	294(S)	294(S)	
ϵ 10^{-4}	0.61	0.94						1.00	1.71	1.80	
λ $m\mu$	347	354	322	320		358(S)	358(S)	317(S)	305(S)	306(S)	
ϵ 10^{-4}	0.52	0.69	0.65	0.64		0.96	1.03	0.68	1.23	1.19	
λ $m\mu$					380	375	376	375(S)	374(S)	374(S)	375
ϵ 10^{-4}					0.93	1.57	1.65	0.42	0.49	0.50	0.28
λ $m\mu$								395	392	392	406
ϵ 10^{-4}								0.89	0.97	1.08	0.60
λ $m\mu$					410			429	412	414	427
ϵ 10^{-4}					0.41			0.82	0.88	0.98	0.92
λ $m\mu$								455	438	438	452
ϵ 10^{-4}								0.96	1.00	1.14	0.76

S = shoulder

of the signals from the hydrogen atoms at C-1 and C-8 ($\delta \sim 7$) are not affected by the conversion.

The NMR-spectra of the trimethylethers of the β - and γ -oximes point to the presence of two equivalent methyl groups which is required by the above formulations but make structures analogous to that of fluorescein oxime unlikely.

The selective electrolytic reduction at controlled potential of the β -oxime to the γ -oxime consists in the reduction of the *N*-oxide function. The facile reoxidation of the reduction product from the γ -oxime, which would be 2,7-dihydroxy-9-(2'-carboxyphenyl)-acridan, is understandable as it is a derivative of *p*-aminophenol.

The β -oxime and its derivatives can lose the *N*-oxide function in several ways. During the preparation of the oximes varying amounts of the β -oxime is reduced to the γ -oxime, and during the methylation of the β -oxime or the purification of the products a certain amount of the corresponding derivative of the γ -oxime is formed.

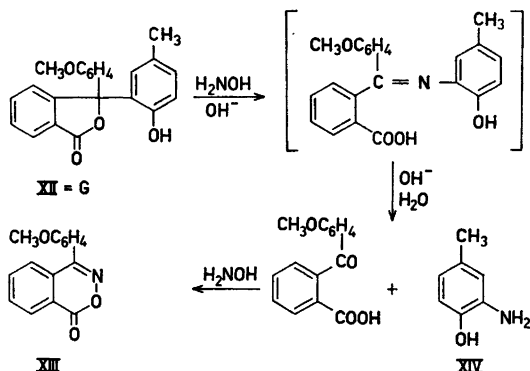
The suggestion of the β -oxime as an *N*-oxide of the γ -oxime is further substantiated by the fact that the trimethylether of the γ -oxime can be oxidised with *m*-chloroperbenzoic acid to the trimethylether of the β -oxime.

If the β -oxime is described as a derivative of hydroxylamine then the γ -oxime is the corresponding derivative of ammonia. Hydroquinonephthalein reacts with aqueous ammonia to form hydroquinonephthaleinimide, a derivative of phthalimidine, but when ammonia reacts with hydroquinonephthalein under the conditions where this compound with hydroxylamine forms the β -oxime another compound is formed which proved to be the γ -oxime.

Meyer and Kissin claim that the γ -oxime forms a tribenzoate and substantiate this with an analysis. However, their analyses fit better a dibenzoate of the new formulation of the γ -oxime which contains an oxygen less than that of Meyer and Kissin's. Furthermore, the NMR-spectrum of the benzoate points to the presence of two benzoyl groups.

Rearrangement of an *o*-hydroxyphthalein

During this investigation it was of interest to know whether a phthalein containing an *o*-hydroxyphenyl group would rearrange during a reaction with hydroxylamine. 3-(4'-Methoxyphenyl)-3-(2''-hydroxy-5''-methylphenyl)phthalide (XII) was treated with a warm, alkaline solution of hydroxylamine for 24 h. The isolated products were 4-(4'-methoxyphenyl)-2,3-benzoxazinone-1 (XIII) and 2-hydroxy-5-methylaniline (XIV). These products can be explained by a rearrangement similar to that occurring during the formation of phenolphthalein oxime and fluorescein oxime followed by hydrolysis of the formed anil to 2-hydroxy-5-methylaniline and 2-(4'-methoxybenzoyl)benzoic acid, which reacts with a second molecule hydroxylamine to form the isolated oxime anhydride.



DISCUSSION

The rearrangement at an electron deficient nitrogen atom occurring during the reaction between fluorescein or 3-(4'-methoxyphenyl)-3-(2''-hydroxy-5''-methylphenyl)phthalide are of the same kind as the one taking place during the formation of phenolphthalein oxime. The reaction starts with a nucleophilic attack by the hydroxylamine anion on the electron deficient center formed by the opening of the lactone ring of the phthalide. The occurrence of the rearrangement requires an activation of the moving phenyl group by a hydroxyl group *ortho* or *para* to the central carbon atom. In both phenolphthalein and fluorescein the hydroxyl groups are in the *para* position and in compound G the hydroxyl group is *ortho*. A hydroxyl group in the *ortho* position does not activate the phenyl group as much as one in the *para* position. A hydroxyl group *meta* to the central carbon atom does apparently not activate sufficiently to induce the rearrangement, as it does not occur during the reaction between hydroquinonephthalide and hydroxylamine, although a certain attack on the central carbon atom takes place which here leads to the formation of hydroquinonephthalide- α -oxime.

The role of the carboxyl group during the rearrangement is not clear. It has been suggested⁷ that an intermediate 2,3-benzoxazinone-1 derivative is formed during the reaction which on opening facilitated the removal of the oxygen with its bonding electrons as a carboxylate ion. However, in the strongly alkaline solution where the reaction takes place a removal of the oxygen as a hydroxyl ion should be possible. An attack by the carboxyl group on the hydroxylamine moiety would rather be expected to take place at the nitrogen atom with the formation of an isoindole derivative as, *e.g.*, hydroquinonephthalide- α -oxime than at the oxygen atom.

The reaction leading to hydroquinonephthalide- β -oxime is analogous to the reaction between fluorescein and aqueous ammonia at 180°–200° in which an acridine derivative is formed. Of the same type is also the well-known conversion of 2,6-dimethyl-4-methoxy-pyrylium salts to 2,6-dimethyl-4-methoxy-pyridine on treatment with ammonia.

EXPERIMENTAL

The NMR-spectra were recorded at 60 Mc/s on a Varian Associates A-60 spectrometer. The temperature of the 15–20 % solutions was $33^\circ \pm 2^\circ$. Tetramethylsilane (TMS) was used as internal standard and the chemical shifts are expressed in ppm (δ units) from TMS taken as 0.00. The IR-spectra were recorded on a Perkin-Elmer model 221 spectrophotometer or on a Perkin-Elmer Infracord. The polarograph was a Radiometer PO4d; the capillary delivered 2.2 mg of mercury per second at a corrected column height of 48.5 cm. The drop time was 4.2 sec (open circuit). The potentiostat was a Wadsworth Controlled Potential Electro-Depositor. Analyses were made by Dr. Weiler and Dr. Strauss, Oxford, and our Analytical Department.

Fluorescein oxime. Sodium fluoresceinate (38 g, 0.1 mole) was dissolved in a solution of 40 g of sodium hydroxide in 1 litre of water. 40 g of hydroxylammonium chloride dissolved in 100 ml of water were added and the mixture kept at 75° – 80° for 5 h. After cooling, the solution was made slightly acid with sulfuric acid and the precipitate of fluorescein oxime and unreacted fluorescein was collected. The mixture was suspended in acetone, which dissolved the fluorescein, and filtered. The fluorescein oxime was dissolved in hydrochloric acid and precipitated with sodium acetate. It was filtered, washed with water and acetone and dried. It was purified by dissolving it in a mixture of acetone and hydrochloric acid and precipitating the oxime with a solution of sodium acetate. It had no well-defined m.p. (Found: C 68.23; H 4.27; N 3.91. Calc. for $C_{20}H_{13}NO_5$, $\frac{1}{2} H_2O$: C 68.00; H 3.97; N 3.93). The oxime can be acetylated with acetic anhydride and pyridine to a colourless acetyl derivative, m.p. 144° (Found: C 67.58; H 4.06. Calc. for $C_{24}H_{17}NO_5$: C 66.82; H 3.97).

Methyl ester of fluorescein oxime. 5 g of fluorescein oxime in 100 ml of methanol containing 5 ml of concentrated sulfuric acid were refluxed for 10 h. After cooling the mixture was diluted with 300 ml of water and neutralised with sodium bicarbonate. The yellow precipitate, 4.5 g was filtered, washed and dried. The product was purified on a column of silica gel with methanol as eluent. The compound had no well-defined m.p. The IR-spectrum contained a band at 1715 cm^{-1} corresponding to an aromatic ester group, and the NMR-spectrum showed the presence of one methyl group bonded to oxygen. On alkaline hydrolysis fluorescein oxime was obtained.

Methylation of fluorescein oxime. In a mixture of ether and tetrahydrofuran 0.75 g of fluorescein oxime was treated with 0.5 g of diazomethane for 24 h. The ether was washed with dilute sodium hydroxide and water and evaporated after drying. The residue, 500 mg, was purified on a column of alumina with ether as eluent. Isolated were 400 mg of fluorescein oxime trimethylether. The compound was not crystalline and had no well-defined m.p. (Found: C 70.66; H 4.93. Calc. for $C_{23}H_{19}NO_5$: C 70.94; H 4.92). The NMR-spectrum contained signals at $\delta = 3.66$, $\Sigma H = 3$; $\delta = 3.800$, 3.807 , $\Sigma H = 6$; $\delta = 6.6$ – 8.0 , $\Sigma H = 10$. The same compound was obtained by treating the above mentioned methyl ester of fluorescein oxime with diazomethane.

Decarboxylation of fluorescein oxime. Fluorescein oxime (2.5 g) was dissolved in 25 ml of quinoline. 0.5 g of copper bronze was added and the mixture heated to 200°C for 24 h with stirring. After cooling, the reaction mixture was extracted with ether, filtered and the filtrate extracted with a dilute solution of sodium hydroxide. The alkaline extract was neutralised with hydrochloric acid and the product extracted with ether, which was dried and evaporated. The residue was purified on a silica gel column with a 1:1 mixture of ether and ligroin as eluent. The product, 3,7-dihydroxy-11-phenyl-dibenz[b,f]oxazepine, was recrystallized from chlorobenzene. (Found: C 72.28; H 4.34; N 4.45. Calc. for $C_{19}H_{13}NO_3$, $\frac{1}{2} H_2O$: C 72.06; H 4.52; N 4.48). The compound was methylated by adding a 100 % excess of diazomethane to an ethereal solution thereof. After 24 h the ether and the excess of diazomethane were evaporated and the residue chromatographed on a column of alumina using the same eluent as above. The methylated compound, 3,7-dimethoxy-11-phenyl-dibenz[b,f]oxazepine, which was obtained in a nearly quantitative yield, was recrystallised from ethanol, m.p. 132° . (Found: C 75.84; H 5.02; N 4.40. Calc. for $C_{21}H_{17}NO_5$: C 76.12; H 5.17; N 4.23). NMR-spectrum (solvent: trifluoroacetic acid): $\delta = 3.99$ (OCH_3), $\Sigma H = 3$, singlet; $\delta = 4.07$ (OCH_3), $\Sigma H = 3$, singlet; $\delta = 6.9$ – 8.0 , $\Sigma H = 11$, multiplet. The compound forms a hydrochloride, m.p. 157° .

4-Methoxy-2-(2'-nitro-5'-methoxy-phenoxy)-benzophenone. (VI). 100 ml of an ethanolic solution containing 10 g of 2-hydroxy-4-methoxybenzophenone⁸ were added to a solu-

tion of 2.5 g of potassium hydroxide in 25 ml of ethanol. The solvent was then evaporated *in vacuo* yielding a tan, hygroscopic potassium salt. 5 g of this salt were mixed with 4.35 g of 3-bromo-4-nitroanisole⁹ and 100 mg of copper bronze. The mixture was heated to 185°C for 24 h with stirring. After cooling, the reaction mixture was extracted with a mixture of ether and dilute sodium hydroxide. The ethereal layer was separated, filtered, dried, and evaporated. The residue was separated on a column of silica gel using a 1:1 mixture of ether and ligroin as eluent. The first fraction contained unreacted 3-bromo-4-nitroanisole. The second fraction, which crystallised on evaporation of the solvent, was recrystallised from ethanol, 2.75 g (39 %). The product, 4-methoxy-2-(2'-nitro-5'-methoxy-phenoxy)-benzophenone had m.p. 124.5°–125.5°. (Found: C 65.97; H 4.36; N 3.56. Calc. for C₂₁H₁₇NO₆: C 66.49; H 4.52; N 3.69).

3,7-Dimethoxy-11-phenyl-dibenz[b,f]oxazepine. 400 mg of the above mentioned nitro compound were dissolved in 10 ml of ethanol, and 0.1 ml of hydrazine hydrate was added. After heating on a steam bath Raney nickel was added, and the mixture was refluxed for half an hour. The ethanol was removed *in vacuo* and the residue extracted with ether and hydrochloric acid. The layers were separated, the aqueous layer was made alkaline and extracted with ether. The ethereal solution was dried, and on addition of dry hydrogen chloride a precipitate, 270 mg (70 %), was obtained, m.p. 157°. This hydrochloride was found to be identical with the above mentioned hydrochloride, m.p. 157°, obtained from the methylated, decarboxylated fluorescein oxime.

Hydroquinonephthalein- α -oxime was prepared according to Meyer and Spengler,² and the *trimethylether* according to Meyer and Kissin.³ NMR-spectrum of the trimethylether in trifluoroacetic acid: $\delta = 3.66$ (OCH₃), $\Sigma H = 3$, singlet; $\delta = 3.83$ (OCH₃), $\Sigma H = 6$, singlet; $\delta = 6.55$ (C-1' + C-8'), $\Sigma H = 2$, doublet, $J = 2.5$ cps; $\delta = 7.1-8.3$, $\Sigma H = 8$, multiplet.

Hydrolysis of hydroquinonephthalein- α -oxime trimethylether. 50 mg of the α -oxime trimethylether, m.p. 123°, were refluxed in a mixture of 15 ml of 30 % alcohol and 5 ml of conc. sulfuric acid for 4 h. The reaction mixture was neutralised with 33 % sodium hydroxide and the precipitate filtered off and dried. The product was shown to be hydroquinonephthalein dimethylether by the m.p. 197° and the IR-spectrum identical with that of the authentic compound.

The hydroquinonephthalein β - and γ -oximes were prepared according to Meyer and Spengler.² β -oxime: (Found: C 65.86; H 4.04. Calc. for C₂₀H₁₃NO₅·H₂O: C 65.75; H 4.14). γ -oxime: (Found: C 69.79; H 4.13. Calc. for C₂₀H₁₃NO₄½·H₂O: C 70.63; H 4.14).

Electrolytic reduction of γ -oxime. 0.5 g of the γ -oxime were reduced in 150 ml of 0.5 N sodium hydroxide at -1.65 V vs. S.C.E. The reduction consumed two electrons per molecule. During the reduction a compound yielding an anodic wave at -0.55 V vs. S.C.E. was formed. When the reduction was complete the product was oxidised anodically at -0.4 V vs. S.C.E. The oxidation consumed 2 electrons per molecule, and the formation of γ -oxime could be followed polarographically. On acidification of the reoxidised solution with acetic acid the γ -oxime precipitated, 0.35 g.

Electrolytic reduction of β -oxime in alkaline solution. 0.5 g of the β -oxime was reduced as described above for the γ -oxime. After the reduction which consumed four electrons per molecule the solution was acidified with acetic acid. Air was bubbled through the solution and a yellow precipitate gradually was formed. After 3 h the precipitate was filtered off, 0.35 g, which proved to be γ -oxime.

Electrolytic reduction of β -oxime in acid solution. 0.5 g of the β -oxime was suspended in 0.5 N hydrochloric acid containing 20 % alcohol and reduced at -0.60 V vs. S.C.E. corresponding to the reduction of the first wave. After the consumption of 1.9 electrons per molecule the first wave had disappeared and the second wave corresponding to the γ -oxime remained. The solution was neutralised with sodium acetate and the precipitate shown to be the γ -oxime from the IR-spectrum.

Methylation of hydroquinonephthalein- γ -oxime. 400 mg of the γ -oxime were dissolved in a mixture of ether and tetrahydrofuran and 0.2 g of diazomethane in ether was added. The mixture was kept at room temperature for 24 h and part of the solvent was removed by distillation. The remainder was washed with dilute sodium hydroxide to remove partly methylated products, and the organic solvent evaporated. The residue was purified on a column of alumina with benzene as eluent. Isolated were 300 mg of hydroquinonephthalein- γ -oxime trimethyl ether (2,7-dimethoxy-9-(2'-carbomethoxyphenyl)-acridine), m.p. 166°. (Found: C 74.03; H 5.17. Calc. for C₂₃H₁₉NO₄: C 73.98; H 5.13).

Methylation of hydroquinonephthalein- β -oxime. 4.0 g of the β oxime was methylated with 2 g of diazomethane as described above. After the chromatographic separation was isolated as the first fraction 0.5 g of the above mentioned trimethylether, m.p. 166°, and as the second fraction 2.5 g hydroquinonephthalein- β -oxime trimethyl ether (2,7-dimethoxy-9-(2'-carbomethoxyphenyl)-acridine-*N*-oxide), m.p. 206° (decomp.). (Found: C 70.83; H 5.02. Calc. for C₂₃H₁₉NO₅: C 70.94; H 4.92).

Decarboxylation of hydroquinonephthalein- β -oxime dimethyl ether. 0.4 g of this compound were dissolved in 10 ml of quinoline, 0.2 g of cuprous oxide was added and the mixture refluxed until the evolution of carbon dioxide ceased. The reaction mixture was cooled, diluted with acetone and filtered. The quinoline was removed by steam distillation and the residue dissolved in chloroform, which was washed with dilute sodium hydroxide. The residue from the chloroform layer was purified on a column of alumina with ether as eluent. Isolated were 120 mg of a yellow compound, m.p. 191°, shown to be 2,7-dimethoxy-9-phenylacridine. (Found: C 80.03; H 5.37; N 4.53. Calc. for C₂₁H₁₇NO₂: C 79.98; H 5.43; N 4.44).

Decarboxylation of hydroquinonephthalein- γ -oxime dimethyl ether. 0.4 g of this compound was decarboxylated as described above. Isolated were 150 mg of a product, m.p. 191°, identical with the compound obtained above.

2,7-Dimethoxy-9-phenylacridine. A mixture of 10 g of benzaldehyde, 16 g of *p*-anisidine and 16 g of its hydrochloride was heated to 220° for 1 h. After cooling, the reaction mixture was made alkaline with dilute sodium hydroxide and steam distilled, which removed the main part of unreacted benzaldehyde and *p*-anisidine. The residue was boiled with iron(III) chloride, which oxidised the acridane formed to the acridine. The mixture was purified on a column of alumina with ether as eluent. Obtained were 2.5 g of 2,7-dimethoxy-9-phenylacridine, m.p. 191°, identical with the decarboxylation products obtained above. NMR- and UV-spectra in Tables 1 and 2.

*2,7-Dimethoxy-9-phenylacridine-*N*-oxide.* 0.5 g of 2,7-dimethoxy-9-phenylacridine was dissolved in chloroform and treated with 0.5 g of *m*-chloroperbenzoic acid for 4 h at 0°. The reaction mixture was washed with dilute sodium hydroxide and water, the chloroform layer dried and evaporated. The residue was recrystallised from benzene, 400 mg, m.p. 228° (decomp.). (Found: C 76.15; H 5.16. Calc. for C₂₁H₁₇NO₃: C 76.12; H 5.17). NMR- and UV-spectra in Tables 1 and 2.

Oxidation of hydroquinonephthalein- γ -oxime trimethyl ether. 100 mg of γ -oxime trimethyl ether were dissolved in chloroform and treated with 100 mg of *m*-chloroperbenzoic acid for 4 h at 0°. The chloroform was washed with dilute sodium hydroxide, dried and evaporated. Obtained were 40 mg of a yellow compound, m.p. 206°, identical with the above mentioned hydroquinonephthalein- β -oxime trimethyl ether.

Reaction between hydroquinonephthalein and ammonia. 10 g of hydroquinonephthalein were dissolved in 100 ml of 2 N sodium hydroxide and treated with 50 ml of conc. aqueous ammonia at a steam bath for 1 h. After cooling, the mixture was neutralised with acetic acid, the precipitate filtered off and triturated with boiling acetone and methanol. The remaining insoluble compound, 1.85 g, proved to be hydroquinonephthalein- γ -oxime.

4,4'-Dimethoxy-2-nitrobiphenyl. 14.3 g of 3-nitro-4-bromoanisole¹⁰ and 14.5 *p*-iodoanisole were dissolved in 120 ml of nitrobenzene. 80 g of copper bronze were added and the mixture refluxed for 4 h with stirring. After cooling, the reaction mixture was diluted with chloroform, filtered and the organic solvents removed by steam distillation. The remanens was extracted with chloroform, which was dried and evaporated. The residue was chromatographed on a column of alumina with a 1:1 mixture of chloroform and ligroin as eluent. The yellow fraction was collected, the solvent removed and the 4,4'-dimethoxy-2-nitrobiphenyl recrystallised from methanol, m.p. 138°, 8.5 g. (Found: C 65.40; H 5.11. Calc. for C₁₄H₁₃NO₄: C 64.86; H 5.05).

4,4'-Dimethoxy-2-aminobiphenyl. 2 g of 4,4'-dimethoxy-2-nitrobiphenyl were dissolved in a mixture of 30 ml of toluene and 13 ml of ethanol. 2.5 ml of hydrazine hydrate and 100 mg of Raney nickel were added and the mixture heated on a steam bath. After the effervescence had ceased further 2.5 ml of hydrazine hydrate and 100 mg of Raney nickel were added and the reaction mixture boiled, until the vapours were neutral. The mixture was filtered and the solvent removed. The residue, 4,4'-dimethoxy-2-aminobiphenyl, was recrystallised from ethanol, m.p. 110°, yield 79%. (Found: C 73.13; H 6.31. Calc. for C₁₄H₁₅NO₂: C 73.34; H 6.59).

4,4'-Dimethoxy-2-benzamidobiphenyl. 0.5 g of 4,4'-dimethoxy-2-amino-biphenyl were dissolved in 3 ml of pyridine. 1 ml of benzoylchloride was added and stirred for 1½ h at room temperature. The mixture was poured into dilute sulfuric acid and extracted with ether. The ether was washed with dilute sulfuric acid, dilute sodium hydroxide, and water, dried and evaporated. The residue, 4,4'-dimethoxy-2-benzamidobiphenyl, was recrystallised from ethanol, m.p. 114°, yield 80 %. (Found: C 74.93; H 5.61. Calc. for C₂₁H₁₉NO₃: C 75.66; H 5.74).

3,8-Dimethoxy-6-phenylphenanthridine. 200 mg of 4,4'-dimethoxy-2-benzamidobiphenyl were refluxed with 5 ml of phosphorus oxychloride for 3 h. The mixture was poured on ice and made neutral. Isolated were 120 mg of 3,8-dimethoxy-6-phenylphenanthridine, m.p. 131° (methanol). (Found: C 79.62; H 5.38. Calc. for C₂₁H₁₇NO₂: C 79.98; H 5.43).

2,7-Dimethoxyfluorenone anil. 100 mg of 2,7-dimethoxyfluorenone¹¹ were dissolved in 1 ml of aniline and 20 mg of anhydrous zinc chloride were added. The mixture was heated with stirring to 165° for 1 h. The aniline was removed by steam distillation, the product taken up in ether and purified on a column of alumina with ether as eluent. Isolated were 50 mg of 2,7-dimethoxyfluorenone anil, m.p. 121°. (Found: C 79.52; H 5.44. Calc. for C₂₁H₁₇NO₂: C 79.98; H 5.43).

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