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Reaction of 1,2-Naphthoquinone-4-sulfonate with Aliphatic Amines: Structure of the Colored Products and Kinetics¹⁾

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Substitution of the 4-sulfonate in sodium 1,2-naphthoquinone-4-sulfonate (I) with many kinds of aliphatic amines (II) yields colored 4-substituted 1,2-naphthoquinones (Q): 4-morpholino-Q (IIIa), 4-piperidino-Q (IIIb), 4-pyrrolidino-Q (IIIc), 4-(1,2,4-triazol-1-yl)-Q (IIId), 4-(2-pyrrolyl)-Q (IIIe), 4-dimethylamino-Q (IIIf), 4-diethylamino-Q (IIIg), 4-isopropylamino-Q (IIIh), and 4-(4-amino-2-methyl-5-pyrimidylmethylamino)-Q (IIIi). The eliminated sulfite adds rapidly to the 4-position in I to form a colorless by-product, disodium 2-hydroxy-1-oxo-1,4-dihydro-4,4-naphthalenedisulfonate (IV). The rates of reactions were measured by polarography. The formations of IIIa and IV are successive second-order reactions. The pH profile of the rate constant, with a rounded peak at pH 10, suggests nucleophilic substitution of the free base (II) at the 4-carbon in the *o*-quinone form (I). The hydrolysis of IIIa to 2-hydroxy-1,4-naphthoquinone (V) is an acid-base-catalyzed pseudo-first-order reaction. The best conditions for photometric determination of morpholine (IIa) were found to be reaction of IIa with excess I at pH 8 and 25 °C for 30 min.

Keywords—1,2-naphthoquinone-4-sulfonate; aliphatic amine; substitution; kinetics; morpholine determination

The color reaction of sodium 1,2-naphthoquinone-4-sulfonate (I) with primary or secondary amines (II) is well known. It is reasonable to assume that the reaction products are 4-substituted 1,2-naphthoquinones (Q) (III) such as *N*-(1,2-naphthoquinon-4-yl)-amino-caproic acid²⁾ and 4-anilino-Q.³⁾ However, the proposed structures of 4-(2-piperidinyl)-Q⁴⁾ derived from piperidine and 4-(2-morpholinyl)-Q⁵⁾ derived from morpholine are not acceptable without further evidence regarding the 2-C-substitution.

We have previously investigated the colorimetric determination of a detergent, dodecyl-bis(aminoethyl)-glycine,⁶⁾ and morpholine in the course of studies on the stability of morsydomine.⁷⁾ However, the red substance derived from morpholine (IIa) and I seems to be unstable in the strongly alkaline solution proposed by Stevens.⁵⁾

These problems prompted us to study the reaction kinetics and the structure of the colored product. It was found in the present study that the sulfonate group in I is substituted by the nitrogen in IIa to give 4-morpholino-Q (IIIa). This reaction was studied kinetically by means of polarography, which offers an improved method for the determination of IIa.

It was shown in a separate paper⁸⁾ that the addition product of Q and sodium sulfite is disodium 2-hydroxy-1-oxo-1,4-dihydro-4,4-naphthalenedisulfonate (IV) and that 4-substituted Q decomposes to 4-hydroxy-Q, which converts to the tautomer, 2-hydroxy-1,4-naphthoquinone (V). The same by-products (IV, V) were also obtained in the reaction of I and IIa in this paper.

This paper also deals with the reaction of I and piperidine (IIb), pyrrolidine (IIc), 1,2,4-triazole (IIId), pyrrole (IIe), dimethylamine (IIIf), diethylamine (IIg), isopropylamine (IIIh), and

4-amino-5-aminomethyl-2-methylpyrimidine (IIIi). Most of the reaction products are 4-substituted amino-Q, *i.e.*, 4-piperidino-Q (IIIb), 4-pyrrolidino-Q (IIIc), 4-(1,2,4-triazol-1-yl)-Q (IIIId), 4-dimethylamino-Q (IIIIf), 4-diethylamino-Q (IIIg), 4-isopropylamino-Q (IIIh), and 4-(4-amino-2-methyl-5-pyrimidylmethylamino)-Q (IIIi). However, the reaction product of I and IIe was 4-(2-pyrrolyl)-Q (IIIe).

Most of these compounds (III) showed weak antibacterial activity in the concentration range of 15–125 $\mu\text{g/ml}$.

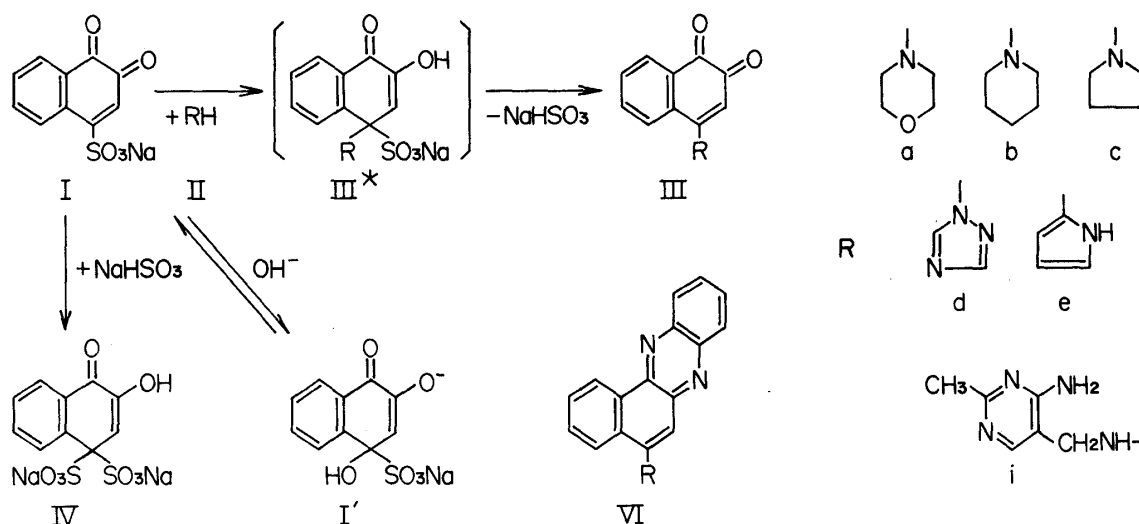


Chart 1

Experimental

Chemicals—The reagent (I) and amines (IIa, b, c, d, e, f, g, h) were from Wako Pure Chemicals, Ltd. Compound IIIi, an intermediate of thiamine, was provided by Takeda Chemical Industries, Ltd.

Instruments—Nuclear magnetic resonance (NMR) spectra were measured on Varian A-60A and Hitachi R-42FT spectrometers, ultraviolet (UV) absorption spectrum on a Perkin-Elmer 450 spectrometer, infrared (IR) absorption spectrum on a Perkin-Elmer 221 spectrometer, mass spectrum (MS) on a Hitachi MS-4 spectrometer, titration curve on Radiometer's Titrigraph, and pH on a Beckman G pH meter.

Polarography—A pen-recording polarograph (Yanaco PA2), a dropping mercury electrode with $m = 1.43 \text{ mg s}^{-1}$, $t = 5.03 \text{ s}$ and $k_c = 1.66$ and a normal calomel electrode as an external reference electrode were used. Polarograms were measured in a thermostat at $25 \pm 0.1^\circ\text{C}$ after bubbling nitrogen through the solution. Buffer solutions used were HCl (pH 0–2), 0.2 M NaOAc–HCl (pH 4), 0.1 M Na_2HPO_4 – KH_2PO_4 –NaOH (pH 6.2, 7.2, 8.0, 10.8 and 12.3), NaOH (pH 12–13) and Na_2CO_3 – NaHCO_3 (pH 9–11).

Kinetic Procedure—Slow reactions with half-lives of a few hours were followed by polarography at appropriate intervals. For example, 1 ml of 10 mM IIa was added to a mixture of 1 ml of 10 mM I and 8 ml of buffer solution at pH 6.43 after bubbling nitrogen through the mixture. Polarograms at every 20 min were recorded after stopping the nitrogen flow. Rapid reactions with half-lives of a few minutes were automatically recorded by measuring current–time curves at constant potential. For example, residual reagent (I) in a reaction mixture of 1 mM I and 1 mM IIa at pH 7.30 was measured at a potential of -0.2 V . The products (IIIa, V and IV) in the mixture were determined at potentials of -0.35 , -0.55 and -1.2 V , respectively.

Photometric Procedure—i) IIa (1–10 ppm). Phosphate buffer (0.1 M, pH 8, 10 ml) was added to 50 ml of test solution of IIa, then freshly prepared reagent (3% I, 1 ml) was added. After 30 min at $25 \pm 5^\circ\text{C}$, the absorbance (A_T) of the mixture at 480 nm was measured against the blank solution. A standard solution of 10 ppm IIa was treated in the same way and the absorbance (A_S) was measured. The concentration of IIa is given by $10 A_T/A_S$ (ppm).

ii) IIa (0.1–1 ppm). The reaction mixture (61 ml) mentioned in (i) was extracted with 10 ml of CHCl_3 , and the absorbance (A_T) of the extract at 450 nm was measured by reference to the blank solution. The absorbance (A_S) of the treated standard solution of 1 ppm IIa was measured. The concentration of IIa is given by A_T/A_S (ppm).

Preparation of Reaction Products—General Procedure: An aqueous solution (30 ml) of I (5 mmol) and II (2.5 mmol) at pH 8–10 was extracted 3 times with 20 ml of CHCl_3 . The extract was washed with 4 ml of H_2O , dried over Na_2SO_4 , and concentrated to a syrup, which was crystallized from C_6H_{12} to obtain III (2.5 mmol). The aqueous layer was acidified, washed with CHCl_3 , and evaporated to dryness to obtain IV (2.5 mmol).

4-Morpholino-Q (IIIa): A solution (1 l) of I (0.02 mol) and IIa (0.01 mol) at pH 8 was extracted twice with CHCl_3 (200 ml). The lower layer was washed with H_2O (50 ml) and evaporated to obtain red crystals (2.4 g, yield 99%), which were recrystallized from EtOH. mp 200 °C. *Anal.* Calcd for $\text{C}_{14}\text{H}_{13}\text{NO}_3$ (IIIa): C, 69.12; H, 6.39; N, 5.76; mol. wt., 243. Found: C, 69.10; H, 6.43; N, 5.76; mol. wt., 243 (MS). UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm (log ϵ): 244 (4.30), 275 (4.05), 325 (3.89), 487 (3.73). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1665, 1630 (C=O), 1590, 1540, 785 (arom.). NMR (in CDCl_3) δ : 3.36 (4H, m, CH_2NCH_2), 3.96 (4H, m, CH_2OCH_2), 5.98 (1H, 3-CH), 7.5–8.1 (4H, m, 5–8-CH); (in $\text{CF}_3\text{CO}_2\text{H}$) δ : 4.3 (CH_2OCH_2), 4.7 ($\text{CH}_2\text{N}^+\text{CH}_2$), 7.22 (3-CH), 8.0–8.4 (5–8-CH). MS m/e : 243 (M^+), 215 ($\text{M}^+ - \text{CO}$), 158 ($\text{M}^+ - \text{C}_4\text{H}_7\text{NO}$), 129 ($\text{M}^+ - \text{CO} - \text{C}_4\text{H}_8\text{NO}$), 102 (m/e 158–2CO), 85 ($\text{C}_4\text{H}_7\text{NO}^+$), 75 (C_6H_3^+), 55 ($\text{C}_3\text{H}_5\text{N}^+$).

4-Piperidino-Q (IIIb): NMR (in CDCl_3) δ : 1.8 (6H, m, β , γ - CH_2), 3.4 (4H, m, α - CH_2), 6.0 (1H, 3-CH), 7.6–8.1 (4H, m, 5–8-CH). This is compared with piperidine, NMR (in CDCl_3) δ : 1.5 (6H, m, β , γ - CH_2), 3.4 (4H, m, α - CH_2).

4-(1,2,4-Triazol-1-yl)-Q (IIIc): NMR (in CDCl_3) δ : 6.57 (1H, 3-CH), 8.26 (1H, 3'-CH), 8.57 (1H, 5'-CH), 7.7–8.3 (4H, m, 5–8-CH). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1665 (C=O), 1587, 1500 (ring), 778 (CH out-of-plane). This is compared with 1,2,4-triazole, NMR (in CDCl_3) δ : 8.24 (2H, 3,5-CH).

4-(2-Pyrrolyl)-Q (IIIe): UV $\lambda_{\text{max}}^{\text{pH}7}$ nm (log ϵ): 532 (3.91), 390 (3.83), 282 (3.71), 252 (4.32). NMR (in CDCl_3) δ : 1.6 (1H, NH), 6.56 (1H, 3-CH), 6.87 (1H, dd, $J_{34}=3.8$ Hz, $J_{35}=1.4$ Hz, 3'-CH), 6.46 (1H, dd, $J_{45}=2.7$ Hz, 4'-CH), 7.24 (1H, dd, 5'-CH), 7.5–8.2 (4H, m, 5–8-CH), NMR (in $(\text{CD}_3)_2\text{SO}$) δ : 6.20 (1H, 3-CH), 6.87 (1H, ddd, $J_{\text{NH}}=2.6$ Hz, 3'-CH), 6.38 (1H, ddd, $J_{\text{NH}}=2.4$ Hz, 4'-CH), 7.23 (1H, ddd, $J_{\text{NH}}=2.5$ Hz, 5'-CH), 7.5–8.3 (4H, m, 5–8-CH). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3300 (NH), 1690, 1640 (C=O), 1587, 1550 (ring), 845, 823, 800, 776 (CH out-of-plane).

4-(4-Amino-2-methyl-5-pyrimidinylmethylamino)-Q (IIIi): Reaction of I and IIi in 50% EtOH resulted in precipitation of IIIi (0.3 g). The residual solution was extracted with CHCl_3 to obtain IIIi (0.1 g). Yield: 53%. Thin layer chromatography (TLC) on Merck silica gel F_{254} , R_f (EtOH): 0.3, R_f (AcOH : BuOH : H_2O = 1 : 4 : 5) : 0.5. UV $\lambda_{\text{max}}^{\text{pH}7}$ nm (log ϵ): 460 (4.03), 340 (3.82), 300 (4.20), 274 (4.42), 264 (4.46), 236 (4.50). NMR (in $(\text{CD}_3)_2\text{SO}$) δ : 2.32 (3H, 2'-Me), 4.4 (2H, d, 5'- CH_2), 5.62 (1H, 3-CH), 6.76 (2H, 4'- NH_2), 7.6–8.2 (5H, m, 5–8-CH, 6'-CH), 8.5 (1H, 5-NH).

4-Dimethylamino-Q (IIIj): mp 153 °C. *Anal.* Calcd for $\text{C}_{12}\text{H}_{11}\text{NO}_2$ (IIIj): C, 71.64; H, 5.47; N, 6.97. Found: C, 71.39; H, 5.40; N, 6.73. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 218 (4.10), 243 (4.24), 278 (4.12), 470 (3.69). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1695, 1618 (C=O), 1587, 1543 (ring), 839, 798, 775 (CH out-of-plane). NMR (in CDCl_3) δ : 3.18 (6H, NMe_2), 5.86 (1H, 3-CH), 7.3–8.2 (4H, m, 5–8-CH). ^{13}C -NMR (in CDCl_3) δ : 43.3 (q, NMe_2), 105.7 (d, 3-CH), 127.9–133.8 (d, 5–8-CH), 132.2 (4a, 8a-C), 163.5 (4-C), 177.2, 181.6 (C=O). MS m/e : 201 (M^+), 186 ($\text{M}^+ - \text{Me}$), 173 ($\text{M}^+ - \text{CO}$), 158 ($\text{M}^+ - \text{NMe}_2 + \text{H}$), 102 (m/e 158–2CO).

5-Dimethylaminobenzo[*a*]phenazine (VI)—A solution of IIIj (1.5 mmol) and *o*-phenylenediamine (1.5 mmol) in 50 ml of EtOH and 0.5 ml of AcOH was heated at 50 °C for 6 h and poured into 750 ml of water to obtain yellow needles, which were recrystallized from EtOH– H_2O , mp 93 °C. *Anal.* Calcd for $\text{C}_{18}\text{H}_{15}\text{N}_3$ (VI): C, 79.09; H, 5.53; N, 15.37. Found: C, 78.83; H, 5.51; N, 14.98. MS m/e (i): 273 (100, M^+). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 228 (3.64), 275 (3.56), 300 (3.38), 424 (3.31). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1617 (C=N), 1589 (ring), 770, 756 (CH out-of-plane). NMR (in CDCl_3) δ : 3.05 (6H, NMe_2), 7.34 (1H, 6-CH), 7.6–9.5 (8H, m, aromatic CH). Fluorescence λ_{max} nm: 600 (emission), 430 (excitation).

Results and Discussion

Polarography

Naphthoquinones (I, III, V) gave a reduction wave. The half wave potentials ($E_{1/2}$) at pH

TABLE I. Characteristics of 4-Substituted 1,2-Naphthoquinones

No.	R	mp (°C)	Color ^{a)}	$E_{1/2}$ ^{b)}	$\delta_{3\text{CH}}$ ^{c)}	Solv. ^{d)}	pK_a (II)
I	Sulfonate		Y	0.09	7.0	D_2O	
IIIc	Triazolo	160	Y	0.10	6.57	CDCl_3	2.24
IIIe	Pyrrolyl	280	V	0.14	6.53	CDCl_3	0.4
IIIa	Morpholino	200	R	0.28	5.98	CDCl_3	8.33
IIIg	Et_2N -	137	R	0.30	5.99	CDCl_3	10.93
IIIb	Piperidino	137	R	0.35	6.00	CDCl_3	11.12
IIIc	Pyrrolidino	179	R	0.38	5.76	CDCl_3	11.3
IIIi	PyCH_2NH -	280	Or	0.40	5.62	$(\text{CD}_3)_2\text{SO}$	(9.6)
IIIh	Me_2CHNH -	200	R	0.43	5.87	CDCl_3	

a) Y, yellow; V, violet; R, red; Or, orange.

b) Half-wave potential: –V vs. N.C.E. at pH 7.

c) Chemical shift of 3-CH in the NMR spectrum.

d) Solvent for NMR.

7 were -0.09 V (I), -0.28 V (IIIa) and -0.44 V (V). The slopes of the $E_{1/2}$ -pH curves were close to -0.06 V/pH at pH 4–11. The diffusion current constants (k_D) were close to 3.0 ($\mu\text{A} \cdot \text{mm}^{-1} \cdot \text{mg}^{-2/3} \cdot \text{s}^{1/2}$). The reduction waves are ascribed to a 2-electron-2-proton reduction of the quinones to the hydroquinones. The correlation between the half-wave potentials and the substituents is shown in Table I. Electron-attracting groups such as sulfonate, triazole and pyrrole increase the electron affinity of the quinone to shift the reduction potential in a positive direction. Electron-releasing groups such as alkyl groups have the reverse effect.

Structures of the Colored Reaction Products (III)

The colored reaction product of I and dimethylamine is clearly 4-dimethylamino-1,2-naphthoquinone (III_f) because III_f combines with *o*-phenylenediamine to produce fluorescent 5-dimethylaminobenzo[*a*]phenazine and also because of the physicochemical properties. The products of reaction of I and the other amines are also considered to be 4-substituted 1,2-naphthoquinones (Q). Most of them (IIIa–i) other than III_e are considered to be coupled not at the carbon adjacent to the nitrogen but at the nitrogen on the basis of the NMR spectra. Contribution of the zwitter-ionic form (2-O^- , 4-N^+) to III seems to be small in neutral solution, in view of the hydrophobicity. Protonation on the 2-carbonyl oxygen of III in trifluoroacetic acid is assumed to occur on the basis of a large low-field shift (-1.3 ppm) of 3-CH and 4-NCH₂, and the structure may be stabilized by delocalization of the positive charge and π -electrons.

The reaction product of I and 1,2,4-triazole is not 4-(1,2,4-triazol-4-yl)-Q but 4-(1,2,4-triazol-1-yl)-Q (III_d), because the 3- and 5-protons of III_d are non-equivalent in the NMR and no NH band is observed in the IR, whereas the 3- and 5-protons of 1,2,4-triazole are equivalent in the NMR spectrum.

The reaction product of I and pyrrole is not 4-pyrrolo-Q but 4-(2-pyrrolyl)-Q (III_e), since it shows signals of 3 olefinic protons and an NH proton of the pyrrolyl group in the NMR and an NH band in the IR spectrum. This is the only product linked through carbon of the amine among many 4-substituted amino-Qs examined. The abnormal reactivity of pyrrole may be due to the low electron density on the nitrogen due to the quasi-aromatic ring. The quasi-aromatic substituents of III_d and III_e have a deshielding effect on the 3-CH of Q (Table I).

4-Amino-5-aminomethyl-2-methylpyrimidine, an intermediate of thiamine, reacts with I at the aliphatic amine to form III_i (confirmed by the CH₂NH coupling in the NMR). The reactivity of the 4-aminopyrimidine moiety seems to be lower than that of an aliphatic amine because of the low basicity ($\text{p}K_a$ 4.76), that is, the low electron density.

Recently Yano *et al.*⁹⁾ suggested on the basis of the spectroscopic data that 4-anilino-Q takes an *o*-quinone form, similar to that of 4-(*N*-methyl)anilino-Q, in neutral or acid solutions whereas it takes a *p*-quinoid 2-enolate form, similar to that of 2-methoxy-*N*-phenyl-1,4-naphthoquinone-imine, in a strongly alkaline solution. They also suggested that the compound is hydrolyzed quickly in an aqueous acid solution.

This is also the case for the 4-*sec*-amino-Q (III_h, i) studied in this report, whereas 4-*tert*-amino-Qs (IIIa–g) cannot take the *p*-quinoid 2-enolate form, and are stable in dry trifluoroacetic acid.

Kinetics of the Reaction of 1,2-Naphthoquinone-4-sulfonate (I) and Morpholine (IIa)

The sulfonate group in I is substituted by the amine (IIa) to produce 4-morpholino-1,2-naphthoquinone (IIIa) and sodium hydrogen sulfite (S), which reacts with I to give an addition product (IV). These compounds (I, III, IV) decompose to 2-hydroxy-1,4-naphthoquinone (V). The kinetics of these reactions are important not only to find the best conditions for the colorimetric determination but also in connection with the reaction mechanism. Therefore the rates of reaction were measured by polarography.

It is likely that the reactions of I with IIa (Eq. 1) and I with S (Eq. 2) are successive

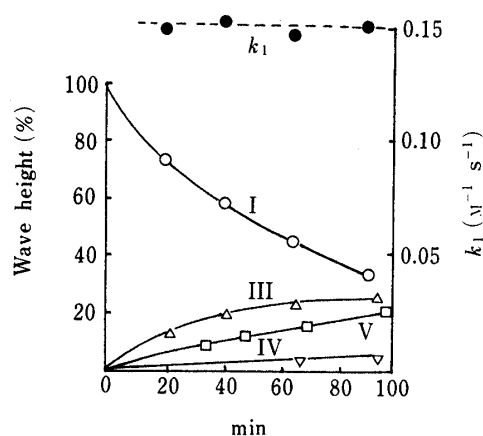


Fig. 1. Rate of the Reaction $2\text{I} + \text{IIa} \rightarrow \text{IIIa} + \text{IV}$ as Observed by Polarography

Initial concentrations of I and IIa were 1 mM, pH 6.43.

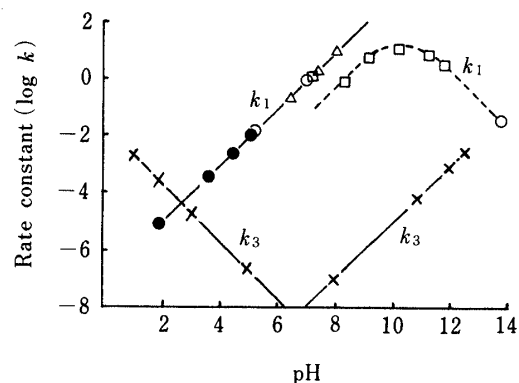


Fig. 2. pH Profiles of the Rate Constants at 25°C (—) and 0°C (---)

k_1 ($\text{M}^{-1} \cdot \text{s}^{-1}$) for $2\text{I} + \text{IIa} \rightarrow \text{IIIa} + \text{IV}$; $a = 1$ mM, $b = 100$ mM ($\bullet$), $a = 1$ mM, $b = 10$ mM ($\circ$), $a = b = 1$ mM ($\triangle$), $a = 2$, $b = 1$ mM ($\square$); k_3 (s^{-1}) for $\text{IIIa} \rightarrow \text{V}$, $a = 0.2$ mM.

second-order reactions.



The hydrolyses of I and IIIa are also expected to be pseudo-first-order reactions (Eqs. 3, 4).



Preliminary measurements showed that the rate constants were in the order $k_2 > k_1 > k_4 > k_3$ at pH 5–10. The rate constant k_1 was calculated by means of Eq. 5, where the initial concentration of I (a) was 1 mM, that of IIa (b) was 1–100 mM, and x is the concentration of IIIa and sulfite (S) after t s.

$$k_1 = \frac{2.3}{(2b-a)t} \log \frac{a(b-x)}{(a-2x)b} \quad (5)$$

Equation 6 was used under specific conditions ($a = 2b = 1$ mM).

$$k_1 = \frac{2x}{ta(a-2x)} \quad (6)$$

The predicted reaction kinetics (Eqs. 1 and 2) were in excellent agreement with the observations, as shown in Fig. 1 for example. The logarithmic rate constant ($\log k_1$) was proportional to pH in the range of pH 2–9 and to pOH in the range of pOH 1–3, showing a maximum at about pH 10 (Fig. 2). The pH profile can be interpreted in terms of an acid-base catalyzed reaction or a dissociation equilibrium of the ammonium salt ($\text{NH}^+ \rightarrow \text{N} + \text{H}^+$) prior to the rate-determining step. Since the acid dissociation constant ($\text{p}K_a$) is 9.3 at 0°C and 8.7 at 25°C , the free base [N] is predominant at $\text{pH} \gg \text{p}K_a$ and decreases at $\text{pH} \ll \text{p}K_a$ as shown by Eq. 7, where [II] is the total concentration of IIa.

$$[N] = \frac{[II]K_a}{K_a + [H^+]} \quad (7)$$

It was shown previously^{8b)} that the rate constants of the reactions of I and aromatic amines (pK_a 1—5) are independent of pH at $pH \gg pK_a$ and that the reaction is not acid-base-catalyzed. Therefore, the pH profile at pH 2—10 can be interpreted in terms of pH dependence of the fraction of free base.

The pH-profile in strongly alkaline media resembles those of the reactions of I with many kinds of amines and even with sulfite. It was reported elsewhere^{8a)} that hydroxide ion adds on the 4-position of I to form an enolate (pK_a 11) of 2,4-dihydroxy-1-oxo-1,4-dihydronaphthalene-4-sulfonate (I'). Therefore, the decreases of rate constants at $pH > pK_a$ 11 depend on the decrease of reactive I in equilibrium with inactive I'.

The reaction mechanism is considered to involve an initial nucleophilic attack of the free base (IIa) on the carbon adjacent to the sulfonate group of I to form an active intermediate; 2-hydroxy-4-morpholino-1-oxo-1,4-dihydronaphthalene-4-sulfonate (IIIa*) followed by elimination of sodium hydrogen sulfite to form IIIa.

Stability of 4-Morpholino-1,2-naphthoquinone (IIIa)

The rate of hydrolysis of IIIa to V was followed by polarography. The first-order rate constants (k_3) were calculated by means of Eq. 8, where a is the initial concentration of IIIa and $(a-x)$ is the concentration after t s.

$$k_3 = \frac{2.3}{t} \log \frac{a}{a-x} \quad (8)$$

The pH-profile of $\log k_3$ was V-shaped with a minimum at pH 6.7 as shown by Fig. 2 and Eq. 9.

$$\begin{aligned} k_3 &= 2.3 - pH \\ k_3 &= 1.0 - pOH \end{aligned} \quad (9)$$

Therefore the hydrolysis of IIIa to V in buffer solution is an acid-base-catalyzed pseudo-first-order reaction.

Photometric Determination of Morpholine

The kinetic study of the reaction of I and IIa mentioned above provides useful information for the determination of IIa. Under the conditions (pH 11.3) proposed by Stevens,⁵⁾ the formation of the colored substance (IIIa) is rapid, whereas IIIa and I⁸⁾ are unstable (Table II). At pH 7, the formation of IIIa is slow, although IIIa and I are stable. The optimum condition is pH 8, where the rate of formation of IIIa is sufficient, and IIIa and I are

TABLE II. Time to 99% Yield of IIIa ($t_{0.99}$), and Half-Lives of IIIa ($t_{0.5}$) and I ($t_{0.5}^I$) under Selected Conditions

	pH			
	7 ^{a)}	8 ^{a)}	9 ^{a)}	11.3 ^{b)}
$t_{0.99}$	2 h	12 min	74 s	2 s
$t_{0.5}$	1850 h	185 h	18.5 h	1 h
$t_{0.5}^I$	4.8 h	2.8 h	1.5 h	0.5 h

a) Initial concentration of I, 1 mM; that of IIa, 0.01 mM; at 25 °C.

b) Initial concentration of I, 0.4 mM; that of IIa, 0.1 mM; at 25 °C; proposed by Stevens.⁵⁾

fairly stable.

It was found that 2 mol of I and 1 mol of IIa react to form 1 mol each of IIIa and IV. The rate of the second-order reaction depends on the initial concentrations of I and IIa. Therefore an excess of concentrated reagent (I) should be used for the determination. As the red product (IIIa) is extracted with chloroform or ethyl acetate and is stable in these solvents, whereas the reagent (I) remains in the aqueous solution, it is preferable to measure the absorbance of the extract.

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References and Notes

- 1) Presented in the 28th Annual Meeting of the Kinki Branch, Pharmaceutical Society of Japan, Nishinomiya, Oct. 1978.
- 2) S. Belman and W. Troll, *J. Biol. Chem.*, **237**, 746 (1962).
- 3) S. Moritani, T. Momose, Y. Ohkura, and T. Momose, Meeting of the Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, June 1970.
- 4) F. Feigl, "Spot Tests in Organic Analysis," Sixth English Ed., Elsevier Publishing Co., Amsterdam, 1960, p. 314.
- 5) W. H. Stevens and K. Shov, *Analyst*, **90**, 182 (1965).
- 6) Y. Asahi and M. Hayashibara, *Takeda Kenkyusho-Ho*, **34**, 148 (1975).
- 7) Y. Asahi, *Chem. Pharm. Bull.*, **11**, 813 (1963).
- 8) a) Y. Asahi and M. Tanaka, Meeting of the Chugoku-Shikoku Branch, Pharmaceutical Society of Japan, Tokushima, Jan. 1979; b) Y. Asahi, M. Tanaka and K. Koyama, the 101st Annual Meeting of the Pharmaceutical Society of Japan, Kumamoto, April 1981.
- 9) H. Yano, M. Yamazaki, Y. Shimomura, M. Iwasaki, M. Ohta, Y. Furuno, K. Kouno, Y. Ono and Y. Ueda, *Chem. Pharm. Bull.*, **28**, 1207 (1980).