AN INTERMEDIATE OF REACTION OF 2-NAPHTHOL WITH ACTIVATED SLUDGE

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A redbrown intermediate has been isolated from a reaction mixture of 2-naphthol and the activated sludge, and its structure has been determined on the basis of its IR, MS, and especially 2D ^{1}H and ^{13}C NMR spectra. It corresponds to 4-(2-hydroxy-1-naphthyl)-6-hydroxy-1,2--naphthoquinone (I). In aqueous media I behaves as an acid-base indicator with the colour transition from yellow to redbrown. The concentration dissociation constants of I have been determined spectrophotometrically: $K_1 = 3\cdot47 \cdot 10^{-7}$, $K_2 = 3\cdot80 \cdot 10^{-10}$ for the standard state $c_0 = 1$ mol dm^{-3}, 298 K, 10^5 Pa.

The activated sludge represents a natural heterogeneous bacterial culture which can contain protozoa, fungi, moulds, and higher organisms. It is formed in a suspension form by long-term aeration of natural waste water containing biodegradable organic substances and sufficient amounts of inorganic nutrients containing especially phosphorus and nitrogen. The activated sludge has been used for years for decomposition of organic substances in both municipal and industrial waste waters. However, relatively little is known about the mechanism of this complex process. Therefore, in our Department[a] we have dealt with kinetics and mechanism of the reaction between the activated sludge and 2-naphthol as a model aromatic substrate which is often present in waste water from organic chemical industry. This biochemical reaction carried out in aqueous diammonium hydrogen phosphate (pH 7) produces a dark red intermediate (I) which later underoges another biochemical

I
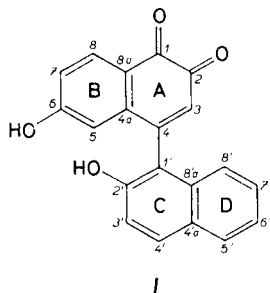
transformation to non-aromatic products. The rate of formation of I and its maximum amount are increased with increasing ratio of 2-naphthol to the activated sludge and with increasing temperature up to 40°C. The aim of this work is a study of structure and some physical and chemical properties of I.

EXPERIMENTAL

Preparation, isolation, and purification of I. A solution of 4 g 2-naphthol and 4·5 g diammonium hydrogen phosphate in 10 l distilled water was treated with 10 ml sedimented activated sludge, and the mixture was intensively aerated until reaching the deepest colour (two to three days; spectrophotometrical testing). Further biochemical processes were stopped by acidification with hydrochloric acid (pH about 1), the suspension of the activated sludge was filtered off, and the filtrate was concentrated on water bath to about one quarter of the original volume. Ten such concentrates were combined and extracted with diethyl ether, the extract was evaporated at normal pressure and room temperature until dry. The residue was reprecipitated from aqueous ammoniacal solution by acidification with hydrochloric acid, and the precipitate obtained was purified by TLC (silica gel, benzene-ethyl acetate (9 : 4), ascending arrangement). The yellow zone of compound I ($R_F = 0·23$) was removed (after spontaneous evaporation of the solvent system), mixed with ethanol, silica gel was filtered off, and the ethanolic solution of I was evaporated at 40°C in vacuum until dry. Yield: 0·2 g dark brown microcrystalline solid, i.e. 0·5% of the original amount of 2-naphthol. Its melting point was determined with an HMK apparatus (Franz Küstner, G.D.R.).

Spectral measurements. The infrared spectrum was measured with a Perkin–Elmer apparatus in KBr disc and in Nujol suspension. For the measurements of ultraviolet and visible spectra the compound I was dissolved (about 10^{-4} mol l^{-1}) in various Sörensen citrate, phosphate and borax buffers[1] of suitable pH values. These solutions were measured in 1 cm quartz cells with a Specord 40 (Zeiss Jena, G.D.R.) and the UV-VIS absorption spectra were recorded in the wavenumber region 50 000 – 12 000 cm^{-1} (200 – 833 nm).

The mass spectra were measured with a JMS 01 SG-2 (Jeol) apparatus at the acceleration voltage of 8 kV, ionisation energy of 75 eV, ionisation current of 200 μA, sample temperature 80 – 460°C, and resolution $R(10\%) = 1\ 000$. For the estimate of relative proportions of components we used the ionisation energy in the region of 18 – 20 eV and a temperature jump to 460°C.

The ^{1}H and ^{13}C NMR spectra were measured with a Bruker AM 400 apparatus at 400·13 and 100·61 MHz, respectively. For the measurements, 40 mg of compound I was dissolved in 0·5 ml hexadeuteriodimethyl sulphoxide, and the measurement was carried out in a 5 mm NMR test tube with application of a C/H dual probe at 300 K. The ^{1}H and ^{13}C chemical shifts are related to the solvent signal ($\delta(^1H) = 2·55$ and $\delta(^{13}C) = 39·60$) and transferred to the δ scale. The unidimensional NMR spectra were measured in the standard way with digital resolution about 0·1 Hz/point (^{1}H) and 1 Hz/point (^{13}C). The two-dimensional experiments were measured with application of the microprograms of pulse sequences supplied by the producer[2]: H, H-COSY (COSY): relaxation time 2 s; 90°(^{1}H) 10·5 μs; 90° mixing pulse; $F_1 = F_2 = 834·7$ Hz; data matrix 512 × 256; 8 scans during 128 time increments (zero filling in F_1); 2 dummy scans; sine-bell multiplication in both dimensions; the spectra were symmetrized. H,C-COSY (XHCORRC): relaxation time 2 s; 90°(^{1}H) 9·7 μs; 90°(^{13}C) = 8·5 μs; polarization time refocussing time 3·1 ms; F_1 834·7 Hz; F_2 1 784·6 Hz; 2 048 × 256; 64 scans during 128 time increments (zero filling in F_1); 2 dummy scans; Gaussian multiplication in t_1

shifted sine-bell multiplication in t_2. The unidimensional selective INEPT was optimized for the coupling constants $^3J(^{13}C, H)$ according to ref.[3] (the pulses number 400—2 000).

RESULTS AND DISCUSSION

The intermediate *I* is a dark brown microcrystalline solid with no sharp melting point: up to 146°C it remains unchanged, at higher temperatures (checked until 260°C) it is slowly decomposed (the originally dark crystals are disintegrated and turn light). Compound *I* is easily soluble in ether, ethanol, and methanol: the solutions are yellow to dark brown depending on the concentration. It is less soluble in aqueous alkalies to give brownred solution. In aqueous acids it dissolves very little to give yellow solutions which are stable in contrast to the aqueous alkaline solutions of *I*: the latter ones slowly turn brown. The colour change with pH in aqueous solutions is reversible. Hence the molecule of *I* must contain acidic protons and represents an acid-base indicator. According to chromatographic behaviour compound *I* is more polar than 2-naphthol.

The mass spectrum of *I* shows molecular ions of m/z 318, 316, and 314 in the ratio of 1 : 5 : 2. This mass is consistent with a compound composed of two naphthalene nuclei connected by a C—C bond and substituted with four hydroxy groups, or two hydroxy and two oxo groups, or four oxo groups, respectively. The presence of the two kinds of substituents in *I* was confirmed by IR spectroscopy. The IR spectrum of *I* contains the unambiguous characteristic C=O valence vibration at $1\,650\,cm^{-1}$. Another distinct absorption band at $1\,220\,cm^{-1}$ probably indicates the presence of C—O bonds.

The detailed structure of *I* was determined on the basis of two-dimensional ^1H and ^{13}C NMR spectra. The ^1H and ^{13}C chemical shifts of compound *I* are given in Table I. The attached proton test[4] differentiated ten quaternary carbon atoms and ten CH carbons. In accordance with this result and with those of MS and IR, the ^1H NMR spectrum shows signals corresponding to ten protons. Using the homonuclear correlated spectrum[5] (H, H-COSY) we determined the connectivity of protons as follows: singlet ($\delta = 6\cdot34$), AX system ($\delta = 7\cdot38$ and $7\cdot97$, $J = 8\cdot8$ Hz), AMX system ($\delta = 6\cdot13$, $J = 2\cdot4$, $\delta = 6\cdot85$, $J = 2\cdot4$ and $8\cdot5$, $\delta = 7\cdot99$, $J = 8\cdot5$) with the coupling constants characteristical of a 1,2,4-trisubstituted aromatic nucleus, and finally four multiplets ($7\cdot92 \rightarrow 7\cdot37 \rightarrow 7\cdot43 \rightarrow 7\cdot72$) corresponding to the protons of a 1,2-disubstituted nucleus. An unambiguous assignment of the corresponding carbons and protons was attained on the basis of analysis of the heteronuclear correlated spectrum[5] (H, C-COSY). The key problem consisted in an unambiguous assignment of the quaternary carbon atoms in relation to the individual protons and, hence, in determination of the way of mutual connection of the four substituted "benzene" nuclei. The ^{13}C chemical shifts of the quaternary carbon atoms were assigned with the help of the selective INEPT (ref.[3]). The selective INEPT spectra

were measured for all the isolated protons, and the analysis of the individual spectra and their mutual comparison made it possible to suggest the following structural formula *I*, i.e. 4-(2-hydroxy-1-naphthyl)-6-hydroxy-1,2-naphthoquinone. The two carbon signals at the lowest field ($\delta = 181 \cdot 23$ and $177 \cdot 43$) are characteristical of 1,2--naphthoquinone[6] (C^2HCl_3; $\delta = 180 \cdot 8$ and $178 \cdot 9$). As the compound *I* can be alkali titrated to two degrees, it is reasonable to presume that the other two oxygen atoms are bound as hydroxy groups (cf. the upfield shift of the ^1H chemical shifts ($\delta(^1H) <$ $7 \cdot 3$) and the ^{13}C chemical shifts ($\delta(^{13}C) > 151$)). The mutual interactions of individual carbon atoms with the protons ($^3J(^{13}C, H)$) are given in Table I. For the suggestion of structure *I* it was most important to determine the ring fusion of the "benzene" nuclei into two naphthalene nuclei and the connection of these naphthalene nuclei. The ring fusion of the "benzene" ring A containing two C=O groups with the ring B characterized by 1,2,4-trisubstitution follows from the interaction of C-1 and C-4a carbon atoms with the H-3 proton ($\delta = 6 \cdot 34$, singlet) and simultaneously with the H-8 proton ($\delta = 7 \cdot 99$, doublet, $J = 8 \cdot 5$). The ring fusion of the other two "benzene" nuclei and the attachment to 6-hydroxy-1,2-naphthoquinone follow from the interaction between C-1' carbon atom and H-3, H-3' protons ($\delta = 7 \cdot 38$) and with the H-8' proton ($\delta = 7 \cdot 72$). Hence the connection of the two naphthalene nuclei is realized at the positions C-4 and C-1'. These conclusions are confirmed by the fact that the ^{13}C chemical shifts of C-1' through C-8a' carbon atoms agree very well with $\delta(^{13}C)$ in 2-naphthol[5] (the greatest difference is observed with C-1', which is due to the substituent at this position) and, furthermore, by the fact that the substituent chemical shifts at the C-4 position (related to 1,2-naphtho-

TABLE I

The ^1H and ^{13}C NMR chemical shifts of compound *I* measured in hexadeuteriodimethyl sulphoxide at 300 K. X denotes the number of the proton which interacts with the respective carbon atom in the selective INEPT spectrum

H/C No.	$\delta(^1H)$	$\delta(^{13}C)$	X	H/C No	$\delta(^1H)$	$\delta(^{13}C)$	X
1	—	177·44	3, 8	1'	—	115·63	3, 3', 8'
2	—	181·33		2'	—	151·90	4'
3	6·34	130·62		3'	7·38	118·51	
4	—	152·21		4'	7·97	130·51	5'
4a	—	138·24	3, 8	4a'	—	127·88	6', 8'
5	6·13	115·74	7	5'	7·92	128·29	
6	—	164·92		6'	7·37	123·31	8'
7	6·85	117·08	5	7'	7·43	127·16	5'
8	7·99	132·71	5, 7	8'	7·72	124·13	
8a	—	123·72	5, 7	8a'	—	132·65	

quinone[6]) and C-1′ position (related to 2-naphthol[7]) are very similar: 7·0 and 6·2 ppm, respectively.

From the above-mentioned structure I it can be inferred that acid-base properties are connected with both hydroxy groups and, hence, compound I represents a dibasic acid. We determined both dissociation constants K_1 and K_2 by the spectrophotometric method, i.e. from the UV-VIS absorption spectra of aqueous solutions of I at various pH values. In the region from 50 000 to 41 000 cm^{-1} the spectrum of I is practically independent of pH, whereas in the remaining wavenumber region the spectral course is strongly pH-dependent. The whole set of the spectra measured can be divided into two parts: the spectra at pH $1-8\cdot3$ exhibit four isosbestic points and correspond to gradual dissociation of the first proton. The remainig spectra measured at pH $8\cdot3-13$ reflect the absorbance changes due to the dissociation of the second proton. From the UV-VIS spectral region of $41\,000-12\,000$ cm^{-1} we then selected suitable wavenumbers corresponding to the most distinct absorbance change with pH: for determination of K_1 the wavenumbers 1·75, 1·84, 1·86, 3·25, 3·27, 3·61, 3·63 . 10^3 cm^{-1} and for K_2 the wavenumbers 1·84, 1·90, 2·80, 2·90, and 3·375 . 10^3 cm^{-1}. The K_1 and K_2 values were obtained by finding the inflection points of the S-shaped dependences of the absorbances vs pH at a constant wavelength (where it is pH $=$ pK) and by rectification of these dependences according to Eq. (1) (ref.[8]):

$$\ln\left((A - A_{HA})/(A_A - A)\right) = n \cdot \text{pH} - \text{pK}, \tag{1}$$

where A_{HA} and A_A represent the limit absorbances of the solutions only containing the non-dissociated and the dissociated acid HA, respectively. From the results obtained it follows that the number n of the protons exchanged in both the first and the second dissociations is equal to one. The average values of the concentration dissociation constants of I to the first and to the second degrees are $K_1 = 3\cdot47 . 10^{-7}$ and $K_2 = 3\cdot80 . 10^{-10}$ for the standard state: the standard concentration $c_0 = 1$ mol dm^{-3}, 298 K, 10^5 Pa. From the information obtained so far it is impossible to determine which of the protons is dissociated first.

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