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SULPHONATION OF 2,6–DIMETHOXYNAPHTHALENE. REACTION MECHANISMS AND PRODUCTS

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Abstract: Protonation and sulphonation mechanism of 2,6–DMON is described. The products of their sulphonation, the sodium and barium salts of 2,6–DMON–4–monosulphonic acid and of 2,6–DMON–4,7–disulphonic acid have been prepared and characterized.

Keywords: 2,6–dimethoxynaphthalene, 2,6–dimethoxynaphthalene–4–monosulphonic acid salts, 2,6–dimethoxynaphthalene–4,7–disulphonic acid salts, protonation, sulphonation.

Within the naphthalenesulphonates system there are many derivatives with biological activity. Several salts of naphthalene sulponic acid derivatives have been found to exhibit antitussive and weak expectorant properties (1, 2). Suramin, the hexasulponic acid derivative, is well known as antiparastic, antiprotozoal (Trypanosoma) drug (3). It is the compound which was studied as the first non–nucleoside drug capable to inhibit human immunodeficiency virus type 1 (HIV–1) reverse transcriptase (RT) (4). Than many other napthalenesulphonic acid derivatives were evaluated for their inhibitory effect on two different functional domains of the HIV–1 RT, namely the ribonuclease H and DNA polymerase activities (5). Some naphtalene di– and trisulphonic acids have been evaluated for inhibitory potential against cytophathogenesis and purified recombinant HIV–1 and HIV–2 RT (6, 7). Those comounds were active at noncytotoxic doses, and demonstrated activities superior to suramin.

The arylsulphonates are clinically used in treatment of arthritis (8) or in veterinary as parenteral copper cupplement (9).

The other medical uses of naphthalenesulphonates are connected with their application as: diagnostic acid (10), fluoresent probe in protein conformation studies and visualisation reagent for proteins (11, 12) or biological stain (13).

As an extension of our earlier studies of sulphonation products of 2,7–dimethoxynaphthalene (14) we present now the results of our investigations in the sulphonation mechanism of 2,6–dimethoxynaphthalene together with their products of this reaction. Here sulphonation takes place at different ring positions than expected by the classical theory for unsubstituted naphthalene. These products may be used as intermediates for further syntheses or as final products with potential biological activity as analogues of bis–2,6–(1,1–dimethylethyl)–1–napthalenesulphonic acid sodium salt, the antitussive agent (1).

2,7–dimethoxynaphthalene (2,7–DMON) in aqueous solutions of increasing acidity is at first O–protonated at methoxy–oxygen atom, then a quinoid intermediate is formed (C–protonation), which at concentration above 80% H_2SO_4 is sulphonated to 2,7–DMON–3–monosulphonic acid (14). The latter is further sulphonated in 94–96% H_2SO_4 via another quinoid intermediate to a 2,7–DMON–3,6–disulphonic acid. A study of sulphonation of dimethoxynaphthalenes with neat SO_3 in aprotic solvent (CH_3NO_2) was described by Cerfontain et al. (15) but in such a medium other products were observed, indicating a sulphonation mechanisms different from that we observed in aqueous solutions (15).

The properties of the isomeric 2,6–DMON differ largely from that of 2,7–DMON what requires additional discussion. First, the monosulphonic derivative does not form the quinoid structure in concentrated H_2SO_4 and moreover, the sulphonation of 2,6–DMON takes place at different positions in the ring. also the UV–spectra of its mono–a and disulphonic derivatives are somewhat different, thus determinations of each individuum in the mixture is possible, although the principal features, similar in their spectra such as the conjugated K–bands and benzenoid B–bands are.

EXPERIMENTAL

All measurements and reactions were performed at room temperature, that is 22–24°C. 2,6–DMON was obtained from Aldrich 99% purity, mp.p. 153° and was used without further purification. Sulphuric acid of required concentration was prepared by addition of weighed amounts of Merck 98% H_2SO_4 (for determination of nitrogen grade) to weighed amounts of bidistilled water. The final concentration of H_2SO_4 was determined by acidimetric titration. $Ba(OH)_2 \cdot 8H_2O$ from Fluka, puriss. p.a. ≥ 98%. Diethyl ether „POCh", pure p.a. Ethyl alcohol: „POCh", absolute, spectrally pure. 72% $HClO_4$ p.a. from Apolda. Methyl alcohol absolute, p.a. „Odczynniki".

Preparation of the barium salt of 2,3–DMON–4–monosulphonic acid: 2 g samples of 2,3–DMON were dissolved in 20 ml of 81% or 82% H_2SO_4 and with constant strirring sulphonation proceeded for 20–22 hours.

Admixture of disulpho–derivatives was excluded by interruption of sulphonation before all DMON was used up. Between 20% and 30% of 2,3–DMON precipitated out after addition of 50 g of ice and was filtered off. After washing with water and drying m.p. 153°C. After separation of the unused substrate only a monosulphonic product was obtained as calculated by spectrophotometry absorbances.

After careful neutralisation with $Ba(OH)_2 \cdot 8H_2O$ and evaporation under vacuum the filtrate was extracted several times with diethyl ether until all 2,6–DMON was removed, as shown by the UV–spectroscopy of the diethyl ether extracts in which the salts of the sulphonic acids are insoluble. The neutral aqueous solution was filtered twice on a G4 sintered–glass crucible and after evaporation and drying the residue contained about 50% of the calculated amount of the monosulphonated product. The remained rest was adsorbed on $BaSO_4$ and rejected. Barium salt of 2,6–DMON–4–monosulphonic acid was obtained as a result of repeated extractions with ethyl alcohol. Purity was 93% according to elemental analysis; the remaining 7% was inorganic salts as determined by mineralisations with 72% $HClO_4$.

Preparation of disodium salt of 2,6–DMON–4,7–disulphonic acid: About 1 g of 2,3–DMON was dissolved in 10 ml of 94% H_2SO_4. The solution was stirred for 20 hrs. Than 80 g of ice was added and the solution was filtered to remove traces of suspension. 80% of H_2SO_4 was neutralised with barium hydroxide. $BaSO_4$ was filtered off and rejected. In the filtrate, ca 80% of the calculated amount of disulphonic acid was found by spectrophotometry. The filtrate was neutralised first with barium hydroxide then, when pH ca 1 was reached, $BaSO_4$ was filtered off and final neutralisation was made by dropwise addition of 0.5 M Na_2CO_3 solution. The neutral solution was evaporated to dryness. The residue (1.6 g) contained the crude product. After fractional crystallisations and drying 0.3 g (12% theoretical) of anhydrous product purity better than 95% was obtained. Melting points of sulphonic salts were above 300°C.

UV–spectra were recorded with a M–40 Zeiss or ATI Unicam UV4 spectrophotometer and the ^1H NMR with a Bruker AC200 spectrometer.

RESULTS

Spectrophotometric study of 2,6–DMON

The UV/VIS–spectra of 2,6–DMON in neat methanol contain one very strong K–band at ca 230 nm (absorptivity $\varepsilon_m = 7 \cdot 10^4$) due to $\pi \rightarrow \pi^*$ transitions and several weak B–bands extending from 250 to 350 nm ($\varepsilon_{max} = 5 \cdot 10^3$, Figure 1).

In aqueous sulphuric acid up to 76% (w/w) this spectrum remains virtually the same, only the intense band slightly decreases to $\varepsilon_m = 5 \cdot 10^4$. It has been noted however, that the very low solubility of 2,6–DMON in water and diluted H_2SO_4 rises sharply in more concentrated acid (Table 1).

Upon dilution a quantitative reprecipitation occurs indicating an acid–base equilibrium in which a soluble protonated form exists. As the UV/VIS spectrum does not change almost in the process, the proton must be added outside the naphthalene ring to a lone, non–bonding pair of electrons of one methoxy oxygen atom, without participation of the chromophore system of the molecule.

By application of a rearranged Krebs–Speakman equation (16, 17) and the H_o acidity scale (18) the apparent first protonation constant of 2,6–DMON, pK_I or $H_o^{1/2}$ [**I**] could be quantitatively determined.

By definition, this is the acidity value H_o, or concentration of H_2SO_4, at which one half of the base present in solution (here 2,6–DMON) is protonated, hence we prefer the designation $H_o^{1/2}$ to be used subsequently (19). A fairly constant $H_o^{1/2}$ was obtained at widely different acidities what confirms, that indeed a true acid–base equilibrium is present with a mean value of $H_o^{1/2}$ [**I**] ca – 5.2 in 66.3% H_2SO_4.

This equilibrium may be represented as:

Above 76% H_2SO_4 the spectra of fresh solutions (at time t_o) of 2,6–DMON change gradually with increasing acidity: the aromatic B–bands dec-

rease significantly and almost disappear in very concentrated H_2SO_4 and a new band appears at 370 nm (27 kK, cf. Figure 2). Its intensity rises with increasing acidity up to H_o ca. –9 (89% H_2SO_4) when its absorptivity ε_m attains about $1 \cdot 10^4$. Then its intensity levels off. Immediate dilution after dissolution causes reprecipitation of unchanged 2,6–DMON and the reappearance of its spectra characteristic for such diluted solutions indicating another rapid reversible process of protonation with the participation of the chromophore system of 2,6–DMON: the maximum of its long–wave band is shifted from 340 nm in 76% H_2SO_4 to 370 nm in 89% H_2SO_4 indicating an extension of the chromophore system equivalent to an addition of one double bond and formation of a quinoid cation in which a proton is directly added to a ring carbon atom. This might happen either by migration of a proton from the already protonated methoxy group, or by addition of another hydrogen cation in a second protonation process. A clue is provided by data collected in Table 2, where the absorptivities ε_o of 2,6–DMON at 370 nm (27 kK) are extrapolated to the initial time t_o and presented as a function of increasing acidity H_o of the solutions (cf. Figure 2, curve 1).

A sigmoidal curve characteristic of acid–base titration is obtained: its first derivative has a maximum at H_o= –7.3 in 82.7% H_2SO_4 characteristic for the inflection point of the titration curve at which the second neutralisation point ocurs. $H_o^{1/2}$ [**II**] = –7.3.

We consider this fact as an indication that in concentrated aqueous sulphuric acid a second reversible protonation takes place with formation of a dication of 2,6–DMON [**III**].

Spectrophotometric determination of sulphonation kinetics

Dication [**III**] is not stable – its spectra change with time: the quinoid band diminishes and a spectrum with aromatic bands reappears. The pattern of changes depends on the concentration of sulphuric acid. In the range 76–88% (w/w) H_2SO_4 the long–wave (quinoid) band at 370 nm decreases gradually and, simultaneosly, the B–band reappears around 345 nm overlapping with the rising side of the quinoid band (Figure 2). An isosbestic point at

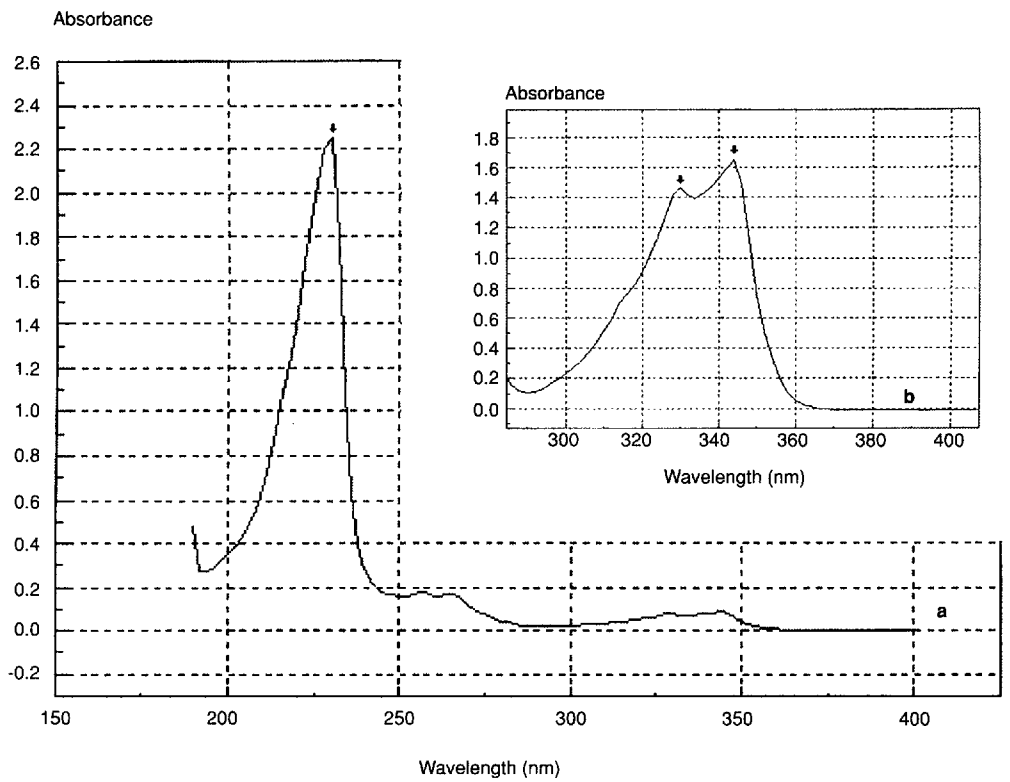


Figure 1. The UV/VIS–spectra of 2,6–DMON in methanol (l=1 cm)
a – $3.2 \cdot 10^{-5}$ M, b – $3.6 \cdot 10^{-4}$ M 2,6–DMON

λ 350 nm is observed. With increasing acidity the bands broaden somewhat and partly coalesce. When a steady – state is reached the spectra of solutions closely resemble those of the monosulpho–2,6–DMON.

This we consider as an indication, that at all concentrations above $1 \cdot 10^{-5}$ M of 2,6–DMON in 76–88% H_2SO_4 the same monosulphonic acid derivative is formed, although we retrieved it from more concentrated, 0.5 M 2,6–DMON solutions in H_2SO_4.

At higher concentrations of H_2SO_4 (88–97%) at first, just after the mixing of solutions the quinoid band appears at 370 nm and decreases in a pseudo–first order reaction in step with increasing acidity function h_o [where $H_o = -\log h_o$ (19)] with rate constants k_1 presented in Table 3.

We consider this primary process to be the same as the one observed in 76–88% H_2SO_4, that is the decomposition of the quinoid form with substitution of an SO_3 – group into the 2,6–DMON molecule.

But here the process does not stop, a further reaction takes place as evidenced by a gradual shift of the B–band maximum from 345 nm to 360 nm and a slow–down of the reaction rate (Figure 3). This indicates sulphonation to a disulpho–derivative. Indeed, the final spectra obtained after several hours are those of the disulphonic acid of 2,6–DMON prepared as described in the Experimental Part.

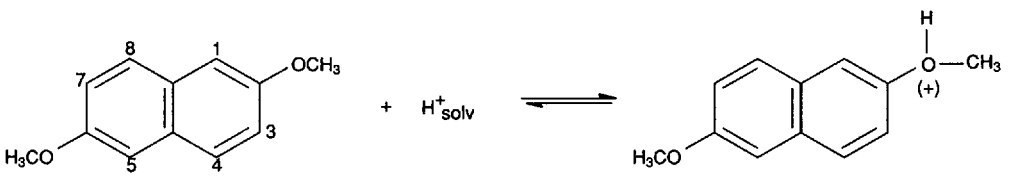
Further sulphonation was not observed up to 97% H_2SO_4.

Determination of the second sulphonation constant k_2 which describes the sulphonation of the monosulphonic derivative to the disulpho–2,6–DMON was more difficult, because the shift of the long–wave B–band is less expressed and very much slower. The B–band of the monosulpho–derivative has at time t_o a maximum at 345 nm in 97% H_2SO_4 with $\varepsilon_m = 5.4 \cdot 10^3$ while in the disulpho–derivative this band moves to 360 nm with $\varepsilon_m = 4.9 \cdot 10^3$. These differences, although small, are significant and enable the evaluation of k_2 both from the batochromic shift and the decrease of the maximum. Consistent values for k_2 in 97% H_2SO_4 have been obtained with $k_2 = 0.016 \pm 0.002$ min^{-1} (cf. Figure 3). In 90% H_2SO_4 k_2 ca. $1 \cdot 10^{-3}$. The very small value of k_2 may indicate that a different sulphonating agent operates, such as $H_2S_2O_7$, the

Table 1.

% H_2SO_4	H_O	S_H	$H_O^{1/2}$
12.38		$S_i = 0{,}9 \cdot 10^{-5}$	
21.37		$0{,}9 \cdot 10^{-5}$	
43.75		$0{,}9 \cdot 10^{-5}$	
61.56	−4.65	$1{,}37 \cdot 10^{-5}$	−4.94
68.35	−5.56	$3{,}01 \cdot 10^{-5}$	−5.19
71.13	−5.97	$4{,}80 \cdot 10^{-5}$	−5.33
74.79	−6.53	$12{,}56 \cdot 10^{-5}$	−5.41
76.03	−6.72	$36{,}00 \cdot 10^{-5}$	−5.13

$H_O^{1/2} = -5.20 \pm 0.2$



I II

Scheme 1.

concentration of which is low even in solutions of high acidity.

Salts of Monosulphonic and disulphonic acid of 2,6–DMON (mono–2,6–DMON and di–2, 6–DMON)

Barium and sodium salts have been prepared, but the former could be obtained with better yield and purity. Its solubility in aqueous neutral solutions was sufficient to obtain ^{1}H NMR spectra which permitted the identification of the principal product of sulphonation by comparison with the spectra of parent 2,6–DMON and with the spectra of the barium disulphonic salt of 2,6–DMON (discussed below).

The 2,6–DMON has a high symmetry which is reflected by a simple ^{1}H NMR–spectrum: In DMSO–d^6 there is a strong singlet at δ 3.82 ppm from equivalent OCH_3 groups, an asymmetric doublet of doublets centered at δ 7.12 ppm directed downfield, due to two equivalent protons in positions 3 and 7 which are strongly coupled (J=10 Hz) with those in vicinal 4, 8 positions which appear as an asymmetric doublet with J=10 Hz centered at δ 7.72, directed upfield) and weakly coupled (J ca 3 Hz) with two 1,5 protons (a close doublet at δ 7.25 ppm directed upfield).

There are three possible isomers of 2,6–DMON–monosulphonic acid with substitution in equivalent positions 1, 5 or 3, 7 and 4, 8, all of which are asymmetric, so their NMR spectra ought to be rather complicated, as seen in our product. A clue to its structure is provided by the position of the two closely spaced, strong methyl singlets of equal intensity at δ 3.78 and δ 3.85 (in D_2O) indicating a small downfield shift (Δ=0.07 ppm) of one methyl, clearly due to – SO_3^- placed at a distant position, that is at ring carbon 4 or 8. Then a group of signals due to (H7) appears ca; 7.08, singlet (H5) at δ 7.25, a singlet at δ 7.55 (H1) another close doublet at δ 7.65 (H8) and a singlet at δ 8.15 (H3). There are however other weak signals, in particular two at δ 3.96 and 3.94, which may be due to isomers in which the – SO_3 group is substituted in a position vicinal to one of the methoxy groups, shifting its signal downfield.

Although integration of these signal is not very reliable the total amount of these vicinal, or „ortho" isomers appears to be less than 10%, with ca 90% of the „para" (C4≡C8) monosulphonic 2,6–DMON isomer [IV] present in the products of sulphonation. After neutralisation with $Ba(OH)_2$ the barium salt of 2,6–DMON–4–monosulphonic acid, $[C_{10}H_5(OCH_3)_2SO_3]_2Ba$ was obtained.

^{1}H NMR–spectra in D_2O of the barium salt of 2,6–DMON–disulphonic acid, $C_{10}H_4(OCH_3)_2(SO_3)_2Ba$

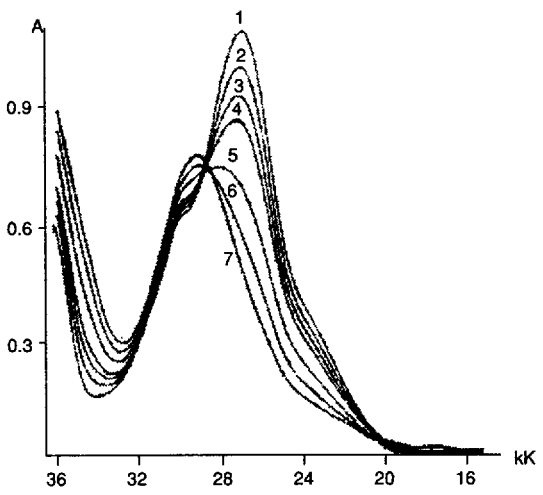
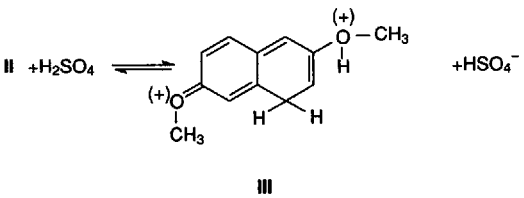


Figure 2. The UV/VIS–spectra of $2.67 \cdot 10^{-4}$ M 2,6–DMON in 82.35% H_2SO_4
1 – 1.08 min, 2 – 10 min, 3 – 20 min, 4 – 30 min, 5 – 60 min, 6 – 90 min, 7 – 120 min

Table 2.

% H_2SO_4	H_O	ε_O(370 nm)
76.66	–6.52	<100
77.44	–6.63	220
79.80	–6.94	540
81.93	–7.24	1450
82.35	–7.29	4040
83.75	–7.49	7480
88.56	–8.09	9770



Scheme 2.

Table 3.

% H_2SO_4	$-H_O$	$h_O \cdot 10^9$	k_1 [min^{-1}]	log k
88.56	8.09	0.12	0.015	-1.82
90.13	8.28	0.19	0.036	-1.43
93.46	8.68	0.48	0.135	-0.89
97.27	9.13	1.35	0.42	-0.38

present two widely separated strong singlets one at δ 3.76 ppm and another at δ 4.01 ppm clearly indicating the substitution of the second – SO_3–group in position C7 vicinal to the methoxy in position 6, shifting its signal downfield (to δ 4.01). There are two prominent signals at δ 8.22 and 8.18 ppm due to two ring protons near the SO_3^- groups, i.e. at C3 and C8. This is also an indication, that now both (benzene) rings of naphthalene are sulphonated. Other signals appear at δ 7.56 (H1) and between 8.15 and 8.25 (H3, H5 and H8).

Thus, the 2,6–DMON–4,7–disulphonic acid **[VI]** is the principal product (ca 80%) of sulphonation in 95% H_2SO_4 at room temperature.

The UV/VIS spectra of the mono– and disulphonic acids and salts of 2,6–DMON are rather similar to each other and also to the parent 2,6–DMON in water and in diluted aqueous solutions of sulphuric acid, but the quinoid band is absent even in very concentrated (97%) H_2SO_4.

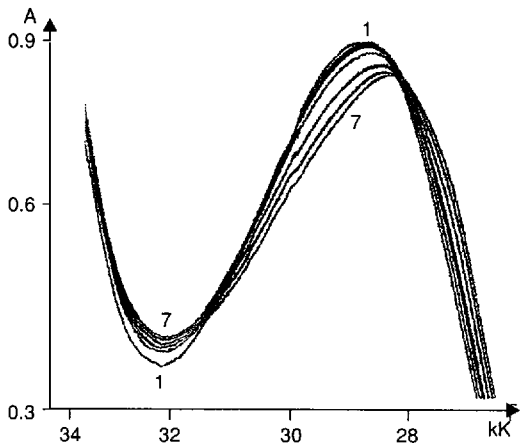


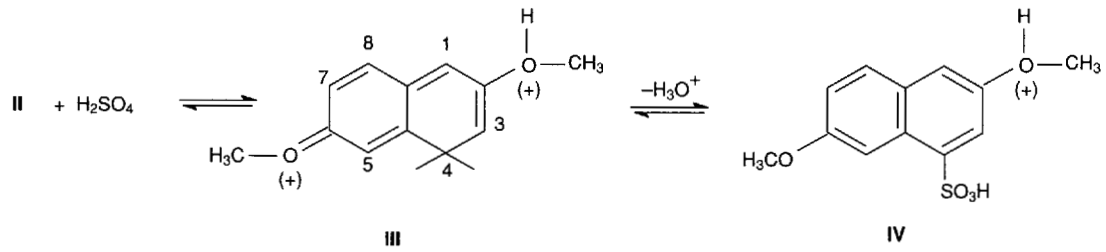
Figure 3. The UV/VIS–spectra of $1.6 \cdot 10^{-4}$ M 2,6–DMON–4–monosulphonic acid in 97% H_2SO_4
1 – 1.25 min, 2 – 10 min, 3 – 20 min, 4 – 30 min, 5 – 60 min, 6 – 90 min, 7 – 120 min

DISCUSSION

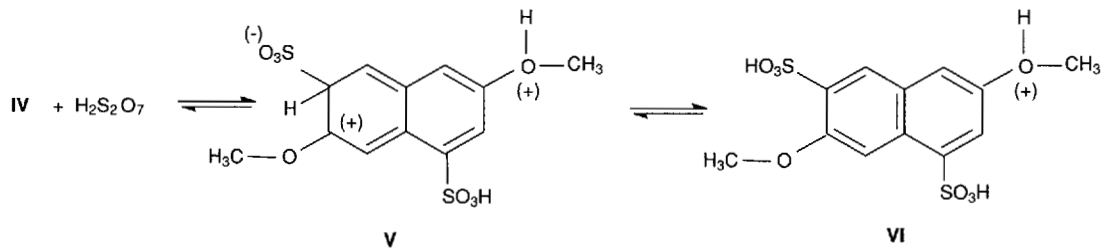
Stepwise protonation of 2,6–DMON takes place in conc. H_2SO_4 first on a methoxy–group (O–protonation, $H_0^{1/2}$ **[I]** = –5.2) then at a ring carbon atom (C–protonation, $H_0^{1/2}$ **[II]** = –7.3) with formation of a quinoid dication **[III]**. The real substrate in the sulphonation reaction which proceeds in aqueous sulphuric acid above 76 w/w %H_2SO_4 in two steps: first to a 2,6–DMON–4–monosulphonic acid **[IV]**; then to 2,6–DMON–4,7–disulphonic aid **[VI]** is the latter. Barium salts of both acids have been isolated and characterised. The sulphonations are rather specific yielding about 90% of the principal isomer. Only small amounts (less than 10%) of the 3–monosulphonic–2,6–DMON isomer have been detected in the isolated barium salt by ^1H NMR spectroscopy. These results clearly indicate, that the electronic structure of the protonated quinoid dication and not that of the neutral 2,6–DMON, which does not exist in concentrated aqueous sulphuric acid, determines the place of primary substitution of the SO_3–group.

Further sulphonation proceeds on the other ring of naphthalene with substitution at carbon C7 as evidenced by the NMR–spectra of the barium salt of 2,6–DMON–4,7–disulphonic acid discussed above. According to Kort and Cerfontain (20) in very concentrated aqueous sulphuric acid the principal sulphonating agent is $H_2S_2O_7$.

The results indicate, that 2,6–DMON in aqueous concentrated sulphuric acid is sulphonated via a mechanism different from the classical one, according to which substitution is expected to take place at position 1 (or 5) of the naphthalene ring (21). Indeed in aprotic solvents such as CH_3NO_2 where the protonated quinoid forms cannot be formed, sulphonation with SO_3 of 2,6–DMON pro-



Scheme 3.



Scheme 4.

ceeds at C(1), cf. ref. 15. We found that position 4 (or 8) is privileged in protic (or proton–releasing) solutions. One can argue, that there is less steric strain upon substitution in this position, but steric strain does not seem to be of primary importance as upon further sulphonation the second SO_3–group is substituted in a vicinal position 7, not 8.

Our results indicate, that this different mechanism is due to the fact, that here the substrate which undergoes sulphonation is not a neutral 2,6–DMON or monosulpho–2,6–DMON molecule but a protonated form, in which the electron densities in the naphtalene ring are different, as indicated by the structure [**III**].

It may be added that for the first time the salts of 2,6–DMON–4–monosulphonic acid and of 2,6–DMON–4,7–disulphonic acid have been prepared and isolated.

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