PREPARATION AND ACID-BASE PROPERTIES OF 5-HYDROXYBENZO[*a*]PHENAZINE-3-SULPHONIC ACID AND ITS 6-HALOGENO DERIVATIVES

J.KANDRNÁL and E.RUŽIČKA

Department of Analytical and Organic Chemistry, Palacký University, 771 46 Olomouc

Received July 27th, 1976

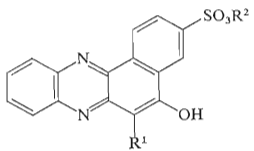
Potassium 5-hydroxybenzo[*a*]phenazine 3-sulphonate (*I*) and its 6-chloro (*II*) and 6-bromo derivatives (*III*) were prepared in analytically defined states. Their structure and acid-base properties are discussed.

A considerable drawback of 5-hydroxybenzo[*a*]phenazine and its 6-halogeno derivatives in analytical applications is their insolubility in water[1,2]. Therefore, the possibility of preparing sulpho acids of these phenazines, which would form salts readily soluble in water, was investigated. No substance of this type has been described in the literature.

Among several theoretically possible isomeric sulpho acids, derivatives of benzo[*a*]phenazine-3-sulphonic acid are most easily synthesized. For preparation of sulpho acid *Ia*, condensation of 2-hydroxy-1,4-naphthoquinone-6-sulphonic acid with 1,2-phenylenediamine was selected. The procedure for the preparation of the initial sulpho acid by hydrolysis of 1,2-naphthoquinone-4,6-disulphonic acid described in a patent[3] was not reproduced with a satisfactory yield. Much better results were obtained using hydrolysis under the conditions for the preparation of 2-hydroxy-1,4-naphthoquinone from 1,2-naphthoquinone-4-sulphonic acid[4,5]. Sulpho acid *Ia* is insoluble in water, while its salts are readily soluble and are difficult to salt-out from the solution. The potassium salt (*I*) is relatively least soluble. This compound was used as the initial substance for preparation of halogeno derivatives *II* and *III*. The dihalogeno derivatives prepared by chlorination or bromination of *I* were reduced to monohalogeno derivatives *II* and *III* by a procedure analogous to the preparation of the non-sulphonated substances[6].

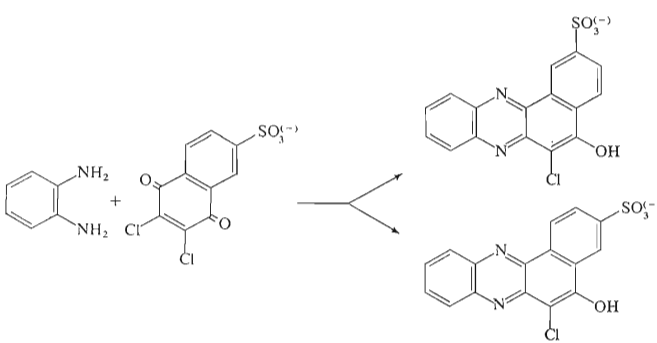
A substance chromatographically identical with chloro derivative *II* was prepared by condensation of relatively readily available[7] 2,3-dichloro-1,4-naphthoquinone 6-sulphonate with 1,2-phenylenediamine. The product obtained in this way appeared chromatographically pure and uniform, but, considering the reaction employed for its formation, the existence of two isomeric sulpho substances chromatographically

difficult to identify should be assumed. The UV and IR spectra also did not permit an unambiguous decision as to whether the substance was a mixture or an individual compound.



Ia, $R^1 = H$, $R^2 = H$
I, $R^1 = H$, $R^2 = K$

II, $R^1 = Cl$, $R^2 = K$
III, $R^1 = Br$, $R^2 = K$



EXPERIMENTAL

Samples for analysis were dried three days at laboratory temperature. The water of crystallization was determined by drying at 110°C over P_4O_{10} under a pressure of 10 Torr to a constant weight. In the elemental analysis of C, H, N, V_2O_5 was employed as a combustion catalyst. Potassium was determined by the Martin method[8], *i.e.* combustion in a stream of oxygen and sulphur dioxide in a platinum boat. The purity of the substances prepared was controlled chromatographically on Whatman No 4 paper, in a 2-propanol–water–ammonia 8 : 1 : 1 system. The UV spectra were measured in 96% ethanol and in ethanolic–aqueous buffered solutions on a Unicam SP 1800 instrument. The IR spectra were measured in KBr on a UR-20 spectrophotometer (Zeiss, Jena). The instrument was calibrated using polystyrene.

Potassium 2-Hydroxy-1,4-naphthoquinone 6-Sulphonate

5 g of potassium 1,2-naphthoquinone 4,6-disulphonate were transferred into 23 ml methanol and 2 ml H_2SO_4 (conc.), maintaining the temperature within 5—10°C. The reaction mixture, first solidified and then liquefied again. The temperature was gradually increased to the boiling point of the reaction mixture, 25 ml methanol were added and the mixture was heated for 15 minutes. After cooling, the precipitate was filtered off, washed with 2 × 15 ml methanol and 2 × 15 ml ether and dried at laboratory temperature. The product was obtained in an amount of 3·5 g. The amount obtained was introduced into 35 ml 1M-KOH and heated for 20 minutes at 60°C and then five minutes at 90°C. 5 ml 36% HCl and 6 g KCl were added and the mixture was left to crystallize freely. After cooling (10°C), the precipitate was filtered off. An amount of 2·0 g of a product with a yellow-orange colour was isolated.

5-Hydroxybenzo[*a*]phenazine-3-sulphonic Acid (*Ia*)

An amount of 0·5 g of potassium 2-hydroxy-1,4-naphthoquino 6-sulphonate was dissolved in 25 ml of water, 2 ml of acetic acid 0·5 g of 1,2-phenylenediamine hydrochloride were added. The mixture and was refluxed for 15 minutes and allowed to cool slowly. The yellow precipitate formed was filtered off, washed with 10 ml of 50% ethanol, 2 × 5 ml ethanol and 2 × 10 ml ether. An amount of 0·4 g of a substance was obtained, whose elemental analysis corresponded to the composition, $C_{16}H_{10}N_2O_4S.2\ H_2O$ (362·4). Calculated: 53·04% C, 3·89% H, 7·73% N, 9·94% H_2O; found: 52·64% C, 3·56% H, 7·25% N, 10·19% H_2O. R_F 0·58. IR spectrum: (cm^{-1}) 1 634 m, 1 618 m, 1 508 s, 1 536 m, 1 484 m, 1 460 m, 1 397 w, 1 333 m, 1 247 s, 1 239 s, 1 212 s, 1 185 s, 1 144 m, 1 127 m, 1 080 m, 1 038 m, 1 020 m, 1 008 m, 852 m, 774 m, 760 m, 715 m.

Potassium 5-Hydroxybenzo[*a*]phenazine 3-Sulphonate (*I*)

An amount of 0·5 g of *Ia* was dissolved in a mixture of 12 ml water, 0·15 g KOH and 0·2 ml acetic acid. The mixture was heated to 40°C, 8 ml saturated KCl solution were added and the solution was allowed to crystallize freely. It was cooled to 10°C after one hour and the red crystalline precipitate was filtered off and washed with 10 ml 50% ethanol, 2 × 15 ml ethanol and 2 × 15 ml ether. An amount of 0·35 g *I* was isolated. The substance for analysis was recrystallized from methanol. For $C_{16}H_9KN_2O_4S.3\ H_2O$ (418·5) it was calculated: 45·92% C, 3·61% H, 6·69% N, 9·35% K, 12·90% H_2O; found: 45·25% C, 3·02% H, 6·58% N, 9·71% K, 12·57% H_2O. R_F 0·58. UV spectrum: 239 nm (log ε 4·52), 282 (4·54), 303 (4·52), 366 (3·72), 418 (4·03), 433 (4·03). IR spectrum: (cm^{-1}) 1 634 m, 1 620 m, 1 577 s, 1 531 m, 1 495 w, 1 464 m, 1 429 m, 1 327 w, 1 249 m, 1 229 s, 1 146 m, 1 080 m, 1 039 s, 1 015 m, 883 w, 855 m, 910 w, 752 m, 717 m.

Potassium 5-Hydroxy-6-chlorobenzo[*a*]phenazine 3-Sulphonate (*II*)

An amount of 0·5 g of *I* was dissolved in 15 ml of water and saturated with chlorine to decolouration; 5 ml of saturated KCl solution were added and the yellowish precipitate was filtered off. An amount of 0·55 g of the product was obtained. It was dissolved in 20 ml water and 2 ml of a 5% SO_2 solution were gradually added at 40°C. After half an hour, 5 ml of saturated KCl solution were added and the precipitate formed was filtered off and washed with 2 × 20 ml ethanol and 2 × 10 ml ether. An amount of 0·35 g of dark-red chloro derivative *II* was obtained. For purification, 1 g of the raw product was dissolved in 40 ml 50% ethanol, heated to 45°C, 10 ml 25% KCl solution added and the product was allowed to crystallize freely. The dark-red crystalline precipitate was filtered off after one hour, washed with 10 ml 50% ethanol, 2 × 10 ml ethanol,

2 × 15 ml acetone and 2 × 20 ml ether. An amount of 0·85 g of the product was obtained. The sample for analysis was purified several times in this way. For $C_{16}H_8ClKN_2O_4.3\ H_2O$ (452·9) it was calculated: 42·43% C, 3·21% H, 6·18% N, 8·64% K, 11·93% H_2O; found: 42·25% C, 2·85% H, 5·93% N, 8·94% K, 11·56% H_2O. R_F 0·51. UV spectrum: 243 nm (log ε 4·48), 260 (4·48), 278 (4·48), 303 (4·49), 370 (3·80), 415 (3·82), 428 (3·84), 518 (3·56). IR spectrum: (cm^{-1}) 1 606 s, 1 582 s, 1 548 s, 1 538 s, 1 507 m, 1 455 m, 1 429 m, 1 370 m, 1 320 m, 1 220 s, 1 192 s, 1 125 m, 1 087 m, 1 038 s, 1 019 m, 903 w, 842 w, 766 m, 749 m.

Potassium 6-Bromo-5-hydroxybenzo[*a*]phenazine 3-Sulphonate (*III*)

The substance was prepared analogously as chloro derivative *II*, except that a hydrazine solution in acetic acid was employed for the reduction of the dibromo derivative. An amount of 0·5 g *I* yielded 0·40 g of raw product *III*. The sample for analysis was purified in the same manner as substance *II*. For $C_{16}H_8BrKN_2O_4.3\ H_2O$ (497·4) it was calculated: 38·64% C, 2·84% H, 5·63% N, 7·86% K, 10·87% H_2O; found: 38·69% C, 3·32% H, 5·52% N, 8·04% K, 10·42% H_2O. R_F 0·48. UV spectrum: 244 nm (log ε 4·48), 260 (4·41), 280 (4·45), 305 (4·47), 368 (3·82), 413 (3·77), 430 (3·78), 518 (3·56). IR spectrum (cm^{-1}): 1 630 m, 1 601 s, 1 580 s, 1 545 s, 1 505 w, 1 471 w, 1 456 w, 1 425 m, 1 402 w, 1 364 w, 1 319 m, 1 305 m, 1 220 m, 1 198 s, 1 162 m, 1 086 m, 1 039 s, 1 017 w, 855 m, 765 m, 750 m, 740 m.

Sodium 2,3-Dichloro-1,4-naphthoquinone 6-Sulphonate

75 g Flavianic acid (the sodium salt of 2,4-dinitro-1-naphthol-7-sulphonic acid) were dissolved in one litre of water, carboraffine was added and the solution filtered. To the filtrate were added 1 000 g 36% HCl, the mixture was cooled to 20°C and 70 g $NaClO_3$ added gradually over two hours. After six-hour standing, 30·7 g of a yellowish product were isolated. On saturating the filtrates with NaCl, another 3·5 g of a less pure substance were obtained. The overall yield (34·2 g) does not correspond to the literature[7] statement that the reaction is quantitative, but is close to the yield reported for the preparation of 2,3-dichloro-1,4-naphthoquinone from 1-naphthol[9], *i.e.* 50%. On evaporation of the reaction mixture as recommended by the original procedure[7], predominantly NaCl separates. For purification, 10 g of the raw product were dissolved in a mixture of 1 100 ml ethanol and 200 ml water, carboraffine was added and the mixture was filtered vnd cooled to 10°C. After one hour, 4·5 g of light-yellow needles were filtered off. On evaporating the filtrates to 150 ml, 2 g of the product of relatively good quality were further isolated.

Potassium 5-Hydroxy-6-chlorobenzo[*a*]phenazine Sulphonate

An amount of 2 g of sodium 2,3-dichloro-1,4-naphthoquinone 6-sulphonate was suspended in 50 ml water and 0·9 g 1,2-phenylenediamine were added gradually at 80°C. The mixture was refluxed for one hour, allowed to cool slowly and 50 ml ethanol and 25 ml 25% KCl solution were then added. The precipitate was filtered off after half an hour, washed with 10 ml 50% ethanol, 2 × 10 ml ethanol, 2 × 10 ml acetone and 2 × 10 ml ether. The substance was purified for analysis by the same procedure as *II*. For $C_{16}H_8ClKN_2O_4S.3\ H_2O$ (452·9) it was calculated: 42·43% C, 3·21% H, 6·18% N, 8·64% K, 11·93% H_2O; found: 42·45% C, 2·65% H, 6·10% N, 8·44% K, 11·47% H_2O. The UV and IR spectra and the R_F value are identical with the respective data for substance *II*.

The ionization constants were measured spectrophotometrically on a Specord UV VIS instrument (Zeiss, Jena) in 1 cm cuvettes. The pH was measured using a Beckman model G pH-meter. The constants were determined in aqueous and ethanolic–aqueous media of Britton–Robinson

buffers. The solution ionic strength was adjusted to 0·1 with $NaClO_4$. The ionization constant values are the averages of twenty experimental values.

RESULTS AND DISCUSSION

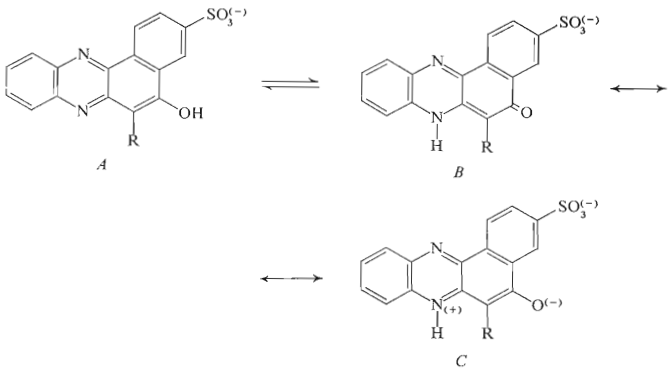
It has been shown[10,11] in the study of the tautomerism of 5-hydroxybenzo[*a*]phenazine and its derivatives that the absorption in the UV spectrum at 490–520 nm belongs to the lactam form, while the lactim tautomer absorbs in the region, 400 to 430 nm. Compared with non-sulphonated products (Table I), the content of lactam forms in derivatives *I–III* in 96% ethanol is somewhat lower. The tautomeric equilibrium, A ⇌ B, can, however, be influenced by increasing the solvent polarity; *e.g.*, in aqueous media the content of both forms of sulpho substance *I* is roughly the same, while in derivatives *II* and *III* the absorption maximum of the lactim form disappears and lactam tautomer B unambiguously predominates. On the basis of the previous work[10] it can be assumed that the two tautomeric forms have approximately the same molar absorption coefficients.

In the study of the UV spectra of 2- and 4-pyridones, the hypsochromic shift of the lactam form absorption maximum (5–10 nm) on replacement of ethanol by water as the solvent was ascribed to the existence of the betain mesomeric form. A similar solvent effect on the position of the absorption maxima of the lactam forms was also found in sulpho derivatives *I–III*. Although the shift is not as large as in pyridones, the existence of mesomeric form C can also be considered real here. The absorption maxima of the lactim forms exhibit only a slight bathochromic shift (2–3 nm) on increasing the solvent polarity (ethanol–water). The excited state here, in contrast to the lactam forms, is more polar than the ground state and hence is better stabilized by polar solvents.

TABLE I

The Last Long-Wave Maxima in the UV Spectra of 5-Hydroxybenzo[*a*]phenazine Derivatives (BF benzo[*a*]phenazine)

Substance	Ethanol nm, log ε		Water pH 4·52 nm, log ε	
I	433 (4·0)	—	434 (3·5)	511 (3·7)
II	428 (3·8)	518 (3·6)	—	516 (4·0)
III	430 (3·8)	518 (3·6)	—	516 (4·0)
5-Hydroxy-BF[10]	427 (4·0)	513 (3·0)	insoluble	
5-Hydroxy-6-chloro-BF[10]	429 (3·8)	517 (3·8)	insoluble	
6-Bromo-5-hydroxy-BF[10]	427 (3·7)	516 (3·8)	insoluble	



Ionization Constants

The spectrophotometrically determined ionization constants (Table II) are only apparent constants; in order to calculate the true ionization constants for the individual tautomeric forms, the value of the tautomeric constant would have to be known, which requires knowledge of the ionization constant of the O- or N-methyl derivative. Both these substances the in this case difficult to synthesize.

In the study of acid–base properties of 2-hydroxyphenazine, Perkampus and Rössel[13] found two ionization constants in the pH-function region (pK_1 2·59; pK_2 7·44; 10% methanol). The pK_2 value for sulpho substance *I* found here is very close to this value. The negative induction effect of a halogen in the 6-position increases the ionization constants of halogeno derivatives *II* and *III* by roughly one p*K* unit.

TABLE II

The Ionization Constants of 5-Hydroxybenzo[*a*]phenazine-3-sulphonic Acid Derivatives ($pK_2 \pm 2\bar{s}$)

% Ethanol	*I*, pK_2	*II*, pK_2	*III*, pK_2
0	7·40 ± 0·03	6·39 ± 0·02	6·33 ± 0·03
20	7·24 ± 0·03	6·09 ± 0·02	6·04 ± 0·03
40	7·58 ± 0·02	6·26 ± 0·03	6·21 ± 0·03

This relatively small increase also results from the effect of the substituent on the tautomeric equilibrium.

The presence of ethanol in water generally causes a decrease in the dissociation constants of substances of the HA^{-}-type[14]. However, the dissociation constants K_2 found here are somewhat anomalous. The values measured in 20% ethanol are higher than those in aqueous medium and only at 40% concentration does a decrease in the K_2 values appear. This anomaly can be caused by changes in the relative contents of the lactim and lactam or betain forms.

The first ionization constant of sulpho substance *I* could not be determined spectrophotometrically. In the region of the dye protonation (pH < 3) the substance quantitatively separates from the solution. The first ionization constants of halogeno derivatives *II* and *III* lie outside the pH-region studied; protonation occurs in 2–3N acid.

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Translated by M. Štuliková.