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Syntheses and Chelating Properties of Azothiopyrine Sulfonates

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New water-soluble chelating agents, azothiopyrine sulfonates, which possess mercapto and azo groups as a chelate-forming site and a sulfonic acid group as a functional group providing water-solubility, were prepared. Their acid dissociation constants and chelate formation with metal ions were determined by a spectrophotometric method.

Keywords——azothiopyrine sulfonates; water-soluble chelating agent; mercapto and azo groups; bidentate ligand; metal chelates; acid dissociation constant; spectrophotometry

In a previous paper,[1] we reported briefly that a new type of chelate-forming resin, which could be prepared easily by the modification of common anion exchange resin with azothiopyrine disulfonate (disodium 4,4′-(4-diazenediyl-5-mercapto-3-methyl-1,2-diazacyclopenta-2,4-dien-1-yl)dibenzenesulfonate), was extremely effective for the collection of mercuric ions present in liquids at extremely low concentrations. This paper presents full details of the syntheses and properties of azothiopyrine sulfonates as chelating agents.

5-Chloro-3-methyl-1-phenyl-4-phenyldiazenyl-1,2-diazacyclopenta-2,4-diene (I), an intermediate in the preparation of azothiopyrine,[2] was treated with 30% fuming sulfuric acid. The reaction mixture was neutralized with sodium carbonate, and two kinds of product sulfonates were separated from each other by paper chromatography (PPC)[3] with acetone, isoamyl alcohol and water (6: 5: 5) as a developing solvent system. The two sulfonates were inferred to be the monosulfonate (IIa, *Rf*=0.68) and the disulfonate (IIb, *Rf*=0.58). The optimum conditions for the preparation of IIa and IIb were investigated by varying the reaction conditions, namely the amount of I and that of fuming sulfuric acid, the reaction temperature and the reaction time. When I was sulfonated with about four times its weight of fuming sulfuric acid at 80° for two hours, the yields of IIa and IIb were found to be 65 and 35%, respectively. These reaction conditions were optimum for the preparation of IIa. The separation of IIa from IIb was easily achieved by making use of the difference in solubility in sodium chloride solution. When the sulfonation was carried out with about eight-fold excess of fuming sulfuric acid by weight at 110° for two hours, IIb was selectively obtained in almost quantitative yield. During sulfonation, the reaction product decomposed if the temperature went above 120°.

The occurrence of sulfonation at the 4 and 4′ positions of two phenylene groups was confirmed by analysis of the nuclear magnetic resonance (NMR) spectra. In the NMR spectrum of IIb, four doubletes (J=8 Hz, 2 A_2B_2) appeared in the region of 7.51—7.93 ppm, as shown in Fig. 1, due to eight protons of the 4- and 4′-substituted phenylene groups, indicating that sulfonic acid groups were introduced at the 4 and 4′ positions of the phenyl groups of I. The NMR spectrum of IIa exhibited five proton signals due to a phenyl group as a singlet at 7.24 ppm and four proton signals due to 4- or 4′-substituted phenylene group as two doublets (J=8 Hz, A_2B_2) at 7.54 and 7.79 ppm, as shown in Fig. 2, indicating that sulfonation occurred at either the 4 or 4′ position of the phenyl group of I. IIa and IIb were treated with sodium hydrosulfide to give the corresponding azothiopyrine sulfonates (IIIa, IIIb) in good yields, re-

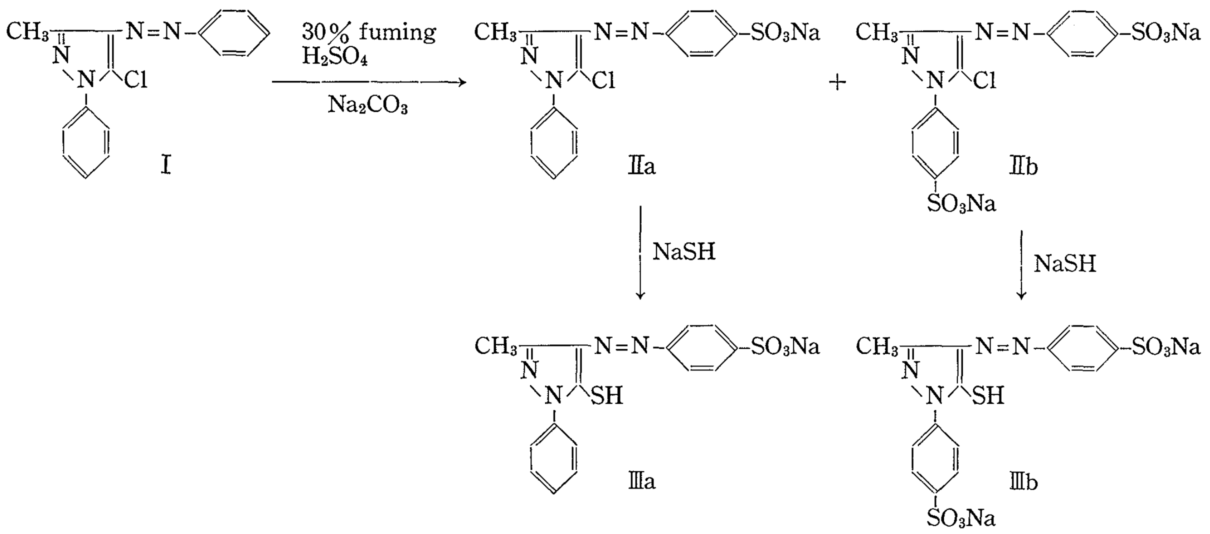


Chart 1

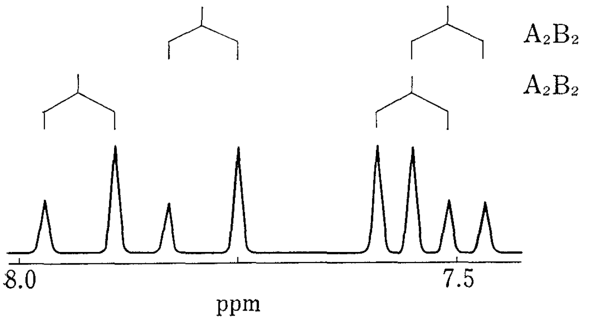


Fig. 1. NMR Spectrum of the Two Phenylene Groups of IIb in Deuterium Oxide

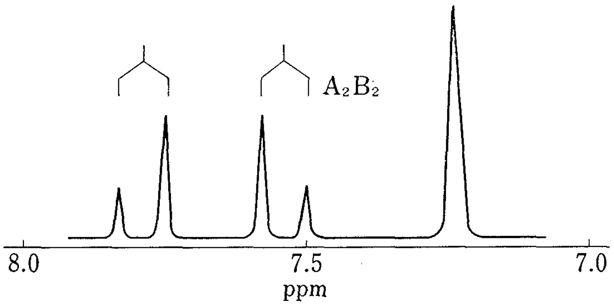


Fig. 2. NMR Spectrum of the Phenyl and Phenylene Groups of IIa in Deuterium Oxide

spectively. When azothiopyrine monosulfonate (IIIa) was treated with sodium hydrosulfite, sulfanilic acid (IV) and light yellow crystals (V) with a melting point of 76—78° were obtained as products of the reductive cleavage. The structure of V was confirmed to be 5,5′-dithio-bis-(4-amino-3-methyl-1-phenyl-1,2-diazacyclopenta-2,4-diene), based on the formula $C_{20}H_{20}N_6S_2$ obtained from the results of elemental analysis, the water-insolubility due to the absence of the sulfonic acid group, the presence of a molecular ion peak 408 *m/e* (M^+) in mass spectrum, and the presence of the characteristic amino stretching absorption bands at around 3310 and 3415 cm^{-1} in the infrared (IR) spectrum. The formation of V from IIIa may be explained in terms of the air oxidation of the reaction intermediate, 4-amino-5-mercapto-3-methyl-1-phenyl-1,2-diazacyclopenta-2,4-diene. From the results mentioned above, IIIa and IIIb were determined to be sodium 4-(4-diazenediyl-5-mercapto-3-methyl-1-phenyl-1,2-diazacyclopenta-2,4-diene)benzenesulfonate and disodium 4,4′-(4-diazenediyl-5-mercapto-3-methyl-1,2-diazacyclopenta-2,4-dien-1-yl)dibenzenesulfonate, respectively.

The NMR spectra of IIIa and IIIb in deuterium dimethyl sulfoxide exhibited one proton signal due to a thiol group as a singlet at low magnetic fields (16.11 and 16.14 ppm, respectively). This implies that an intramolecular hydrogen bond is formed between the mercapto group and the azo nitrogen atom adjacent to the phenylene group and results in a stable six-membered ring structure. A similar hydrogen bond was also found in azothiopyrine and methyl-azothiopyrine.[2)]

The color of aqueous solutions of azothiopyrine sulfonates was reddish-orange in acidic and neutral media, and yellow in an alkaline medium. In the spectral change of IIIb with the

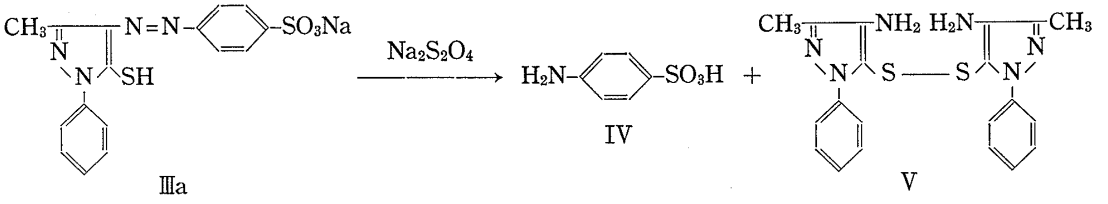
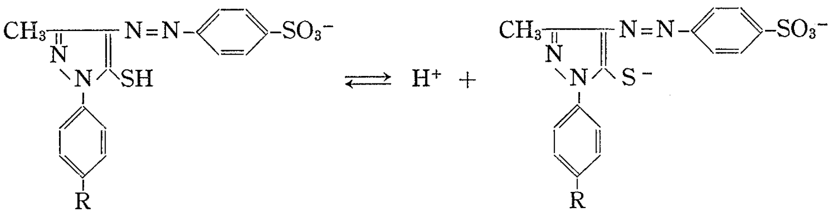


Chart 2

change of pH (shown in Fig. 3), three isosbestic points were observed at 273, 288 and 363 nm. This observation reflects the dissociation equilibrium of the thiol group shown in Chart 3. The acid dissociation constant was determined at an ionic strength of 0.1 by the spectrophotometric method. The values of pK_a were estimated to be 7.05 and 7.08 in IIIa and IIIb, respectively. The larger pK_a values of IIIa and IIIb than those of the thiol forms of 3-methyl-1-phenyl-5-thioxo-1,2-diazacyclopent-3-ene (3.17) and 3,4-dimethyl-1-phenyl-5-thioxo-1,2-diazacyclopent-3-ene (3.77)[4] may be a reflection of the presence of an intramolecular hydrogen bond between the mercapto and azo groups of azothiopyrine sulfonates, and the formation of a six-membered ring. IIIa and IIIb were found to be stable to air oxidation and in strongly acidic solution (even in 10 N hydrochloric acid solution).

Azothiopyrine sulfonates formed water-soluble metal chelates with a number of metal ions. Metal ions such as nickel (II), cobalt (II), zinc (II), cadmium (II) and lead (II) formed chelates in weakly alkaline media. These metal chelates had a color similar to that of IIIa and IIIb in alkaline solution, whereas metal ions such as palladium (II), mercury (II), copper



R=H or SO_3^-

Chart 3

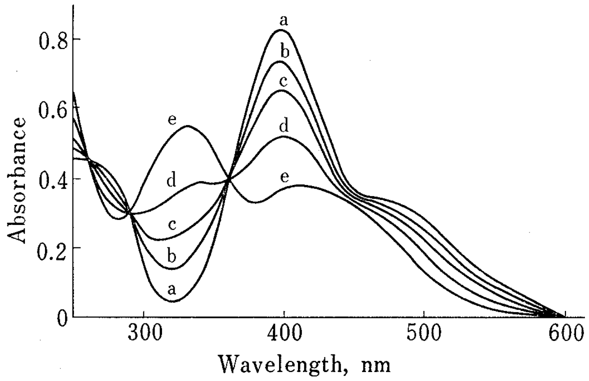


Fig. 3. Absorption Spectra of Azothiopyrine Disulfonate in Solutions at Various pH Values

Concentration of reagent, 4.83×10^{-5} M; a, pH 1.0—3.0; b, pH 6.4; c, pH 6.8; d, pH 7.3; e, pH 10.0—12.0.

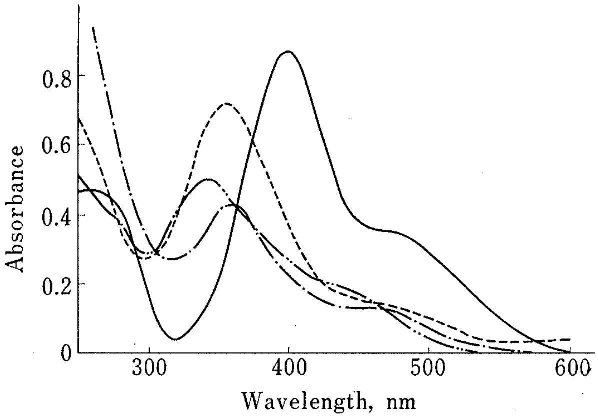


Fig. 4. Absorption Spectra of Azothiopyrine Disulfonate and Its Metal Chelates in Aqueous Solution

——, reagent; ------, Cu(II) chelate; —·—, Hg(II) chelate; —··—, Pd(II) chelate; pH, 3.5; concentration of reagent, 4.08×10^{-5} M. A 5-fold molar excess of metal ions over the reagent was added.

(II) and silver (I) formed chelates in acidic media with a clear color change from reddish-orange to yellow or yellowish-green. As shown in Fig. 4, the absorption maxima of the metal chelates were observed in a shorter wavelength region than those of IIIa and IIIb.

The high stability and selectivity of azothiopyrine sulfonates are advantageous for their application as reagents. They may be of some interest as metallochromic indicators.

Experimental

All melting points were determined in a capillary tube with a Laboratory Devices melting point apparatus, and are uncorrected. UV and visible spectra were measured with a Hitachi 200-10 spectrophotometer, IR spectra with a JASCO DS-701-G spectrometer and mass spectra with a JEOL JMS-D-300 spectrometer. NMR spectra were measured with a JEOL-PS-100 spectrometer, with tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. PPC was carried out by the ascending technique on Toyo filter paper No. 51A with acetone, isoamyl alcohol and water (6: 5: 5) as a solvent system.

5-Chloro-3-methyl-1-phenyl-4-phenyldiazenyl-1,2-diazacyclopenta-2,4-diene (I)——This compound was prepared by the reaction of 3-methyl-5-oxo-1-phenyl-4-phenyldiazenyl-1,2-diazacyclopent-3-ene with $POCl_3$, as reported previously.[2] Yellow needles. mp 109—110°. PPC: *Rf* 0.98.

Sodium 4-(5-Chloro-4-diazenediyl-3-methyl-1-phenyl-1,2-diazacyclopenta-2,4-diene)benzenesulfonate (IIa)——A mixture of 5 g of I and 20 g of commercial 30% fuming H_2SO_4 was heated in an oil bath for 2 hr at 75—80° (bath temperature). The reaction mixture was cautiously poured into 200 ml of ice water, and neutralized with Na_2CO_3. The solution was allowed to stand in an ice bath for 2 hr, and the resulting yellow precipitates were collected by filtration, washed with 10% NaCl solution and recrystallized from water to give IIa. Yield, 4.4 g (65%). Yellowish-orange granules. decomp. *ca.* 280°. *Anal.* Calcd for $C_{16}H_{12}ClN_4NaO_3S$: C, 48.19; H, 3.03; N, 14.05. Found: C, 47.92; H, 3.17; N, 14.28. PPC: *Rf* 0.68. UV $\lambda_{\max}^{H_2O}$ nm (log ε): 420 (3.27), 335 (4.37), 225 (4.16). NMR (D_2O) ppm: 2.20 (3H, s, CH_3), 7.24 (5H, s, C_6H_5), 7.54 and 7.79 (4H, d, $J=8$ Hz, C_6H_4). From the separated filtrate and washings, yellow precipitates were obtained by saturation with NaCl. These precipitates consist mainly of IIb with some IIa, and could be used for the preparation of IIb in a second run.

Disodium 4,4′-(5-Chloro-4-diazenediyl-3-methyl-1,2-diazacyclopenta-2,4-dien-1-yl)dibenzenesulfonate (IIb)——A mixture of 5 g of I and 40 g of commercial 30% fuming H_2SO_4 was heated in an oil bath for 2 hr at 105—110° (bath temperature). The reaction mixture was cautiously poured into 400 ml of ice water and neutralized with Na_2CO_3. This solution was saturated with NaCl, and the resulting yellow precipitates were collected by filtration, washed with a saturated NaCl solution until sulfate ion was no longer detected, and dried. The crude product, which weighed about 9.8 g and consisted of a mixture of IIb and NaCl (about 25%) was used for the preparation of IIIb. A part of this product was purified by repeated reprecipitation from water and 2-PrOH to give IIb free of NaCl. Yellow flakes. *Anal.* Calcd for $C_{16}H_{11}ClN_4Na_2O_6S_2$: C, 38.37; H, 2.21; N, 11.19. Found: C, 37.76; H, 2.55; N, 10.87. PPC: *Rf* 0.58. UV $\lambda_{\max}^{H_2O}$ nm (log ε): 425 (3.28), 335 (4.38), 230 (4.17). NMR (D_2O) ppm: 2.25 (3H, s, CH_3), 7.51 and 7.79 (4H, d, $J=8$ Hz, C_6H_4), 7.55 and 7.93 (4H, d, $J=8$ Hz, C_6H_4).

Sodium 4-(4-Diazenediyl-5-mercapto-3-methyl-1-phenyl-1,2-diazacyclopenta-2,4-diene)benzenesulfonate (IIIa)——An aqueous solution of 5 g of IIa (150 ml) was treated with 1.2 g of 70% (w/w) NaSH, and the mixture was heated on a steam bath for 15 min. The color of the solution changed from yellow to red during the reaction. Next, 10 g of NaCl was added to avoid the formation of gelatinous precipitates, and the reaction mixture was neutralized with dil. HCl. The resulting precipitates were collected by centrifugation, washed well with 2% NaCl solution and with some water, and dried. Recrystallization from MeOH gave dark red needles. Yield, 3.2 g (64%). mp *ca.* 270° (dec.). *Anal.* Calcd for $C_{16}H_{13}N_4NaO_3S_2$: C, 48.48; H, 3.31; N, 14.13. Found: C, 47.81; H, 3.71; N, 13.96. PPC: *Rf* 0.69. UV $\lambda_{\max}^{H_2O}$ nm (log ε): 465 (3.98), 397 (4.35), 256 (4.15), 228 (4.18). NMR (DMSO-d_6) ppm: 2.35 (3H, s, CH_3), 7.37—7.90 (9H, m, C_6H_4 and C_6H_5), 16.11 (1H, s, SH). The signal at 16.11 ppm disappeared when the DMSO-d_6 solution was shaken with D_2O.

Disodium 4,4′-(4-Diazenediyl-5-mercapto-3-methyl-1,2-diazacyclopenta-2,4-dien-1-yl)dibenzenesulfonate (IIIb)——An aqueous solution of 9.8 g of crude IIb (150 ml) was treated with 1.3 g of 70% (w/w) NaSH, and the mixture was heated on a steam bath for 15 min. The reaction mixture was cooled to room temperature, neutralized with dil. HCl and filtered. The dark reddish-violet filtrate was heated to 70° and treated with 30 g of NaCl. The solution was cooled to room temperature, and the salted-out precipitates were collected by filtration, washed well with 20% NaCl solution and with 75% aqueous 2-PrOH, and dried. The crude product, containing some NaCl, was recrystallized from 50% aqueous 2-PrOH to give pure IIIb as dark reddish-violet fine needles. Yield, 3.3 g (45%). *Anal.* Calcd for $C_{16}H_{12}N_4Na_2O_6S_3$: C, 38.55; H, 2.43; N, 11.24. Found: C, 37.94; H, 2.95; N, 10.94. PPC: *Rf* 0.58. UV $\lambda_{\max}^{H_2O}$ nm (log ε): 465 (3.98), 398 (4.35), 258 (4.13), 230 (4.24). NMR (DMSO-d_6) ppm: 2.37 (3H, s, CH_3), 7.47—8.07 (8H, m, $2C_6H_4$), 16.14

(1H, s, SH). The signal at 16.14 ppm disappeared when the DMSO-d_6 solution was shaken with D_2O.

Reaction of IIIa and Sodium Hydrosulfite——To 50 ml of a 50% aqueous MeOH solution of 1.0 g of IIIa, 1.1 g of 80% (w/w) $Na_2S_2O_4$ was slowly added with stirring. The initially reddish-orange solution became light yellow. The reaction mixture was extracted repeatedly with $CHCl_3$ unit the yellow color was no longer observed in the $CHCl_3$ phase. The remaining aqueous solution was concentrated to about 5 ml and made slightly acidic with HCl. The solution was allowed to stand in an ice-bath, then the deposited crystals were filtered off, washed with MeOH and recrystallized from water to give IV as colorless needles. Yield, 0.12 g (28%). decomp. *ca.* 280°. IV was identical with sulfanilic acid. On the other hand, the $CHCl_3$ extract was dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give yellow crystals. They were recrystallized from MeOH and H_2O (1: 2) to give V as yellow needles. Yield, 0.31 g (60%). mp 76—78°. *Anal.* Calcd for $C_{20}H_{20}N_6S_2$: C, 58.80; H, 4.93; N, 20.57. Found: C, 58.61; H, 5.20; N, 20.78. UV $\lambda_{\max}^{\mathrm{EtOH}}$ nm (log ε): 340 (3.88), 275 (4.14), 225 (4.21). IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3310 and 3415 (NH_2). MS *m/e*: 408 (M^+). NMR ($CDCl_3$) ppm: 2.15 (6H, s, $2CH_3$), 2.90 (4H, s, $2NH_2$), 7.29 (10H, s, $2C_6H_5$).

Spectrophotometric Determination of the Acid Dissociation Constants of Azothiopyrine Sulfonates——A stock solution was prepared by dissolving a known weight of azothiopyrine sulfonate (IIIa or IIIb) in water. An aliquot of this solution was diluted with water so that the final solution contained $4.0 \times 10^{-5}\,\mathrm{M}$ III. The pH was adjusted with a buffer solution, standardized by means of a pH meter, and the ionic strength was maintained at 0.1 by the addition of NaCl. The absorbance of the solution was measured at 398 nm, where the change of absorbance with change of pH of the solution was remarkable, as shown in Fig. 3. The pK_a was calculated by means of the following equation.

$$pK_a = pH_x + \log \{(A_x - A_i)/(A_u - A_x)\}$$

where A_x is the absorbance at pH_x, A_i is the absorbance of the thiol-ionized form obtained at pH 10—12, and A_u is that of the thiol-unionized form obtained at pH 1—3.

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References and Notes

1) M. Chikuma, M. Nakayama, T. Tanaka, and H. Tanaka, *Talanta*, **26**, 911 (1979).
2) T. Tanaka and K. Tanaka, *Chem. Pharm. Bull.*, in press.
3) S. Fujii, *Eisei Shikensho Hokoku*, **73**, 355 (1955); Pharmaceutical Society of Japan, "Standard Methods of Analysis for Hygienic Chemists with Commentary," Kanehara, Tokyo, 1973, pp. 375—377.
4) T. Tanaka, *Yakugaku Zasshi*, **91**, 338 (1971).