mutational changes involving changes in chromosome structure. The chromatograms of these two species showing the occurrence of lobinaline in the first species and its absence in the latter would suggest that these mutational changes have also resulted in changes in alkaloidal composition and in structural type in these two species. *L. syphilitica* and *L. puberula* have been shown to be cytogenetically related by Bowden. The chromatograms of these two species do not seem to show any close relationship. The *cardinalis* × *syphilitica* hybrids produce alkaloids from both the parent plants. These observations on *L. syphilitica* and the *cardinalis* × *syphilitica* hybrids support and extend the observations of Steinegger, *et al.* The plant 12-21-S1-3 (Fig. 3) was observed to be a diploid plant with mostly *cardinalis* genes but which might have been a hybrid. The chromatograms having spots due to lobinaline and lopheline and lophilacrine indicate that the sample was a hybrid involving *cardinalis* and *syphilitica*.

The chromatograms of *L. cardinalis* L. (Fig. 1 and Table I) show three spots: R_f 0.00, 0.48, and 0.80. Kaczmarek and Steinegger (16) obtained only two spots, R_f 0.45 and 0.86, with fresh extracts of the species. However these same authors observed an increased number of spots (5–8) with a 6-year-old petroleum ether extract.

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Bisulfite Reduction of *p*-Nitrobenzyl Alcohol

By LOUIS C. SCHROETER and TAKERU HIGUCHI

Bisulfite reduction of *p*-nitrobenzyl alcohol appears to follow a Piria mechanism leading to formation of α-hydroxy-6-sulfoamino-*m*-toluenesulfonic acid. Stoichiometry of the reaction involves one mole of nitro compound and three moles of bisulfite; pH profile of the initial rate of the stoichiometric reaction has been studied. Preliminary kinetic studies indicate an apparent heat of reaction of about 22 Kcal. mole^{-1}. This complicated sequential reduction reaction appears to involve two mechanistic pathways: a rate-determining initial S_N1 reaction dominates at pH 4, while at pH 7 the initial rate-determining reaction appears to possess typical S_N2 characteristics.

BISULFITE REACTIVITY toward the *p*-nitrobenzyl alcohol moiety of chloramphenicol was demonstrated during an investigation of the stability of the antibiotic in the presence of the thio compound (1). Complexity of the antibiotic molecule made evaluation of the kinetic results rather difficult and, for this reason, *p*-nitrobenzyl alcohol was employed as a model compound for the antibiotic so that certain mechanistic details of the reaction could be evaluated. Spectrophotometric and chromatographic evidence obtained during the kinetic study of both the antibiotic and the model compound indicated involvement of nitro reduction with formation of a very polar product; this served to differentiate the reaction from that which occurs between nitrobenzyl halides and sulfite (2). Nitro reduction plays only a minor role under reaction conditions which lead to near-quantitative yields of the nitrobenzyl sulfonic acid from the corresponding nitrobenzyl halides.

The reaction of either *p*-nitrotoluene or *p*-nitrobenzoic acid with sulfite in dilute aqueous solution under conditions similar to those employed with the *p*-nitrobenzyl alcohol leads to formation of both sulfoamino and sulfoaminosulfonic acid compounds (3, 4). This reduction-sulfonation reaction is known as the Piria reaction (3). The mechanism of the Piria reaction for *p*-nitrotoluene analogs of *p*-nitrobenzyl alcohol and other aromatic nitro compounds has been treated

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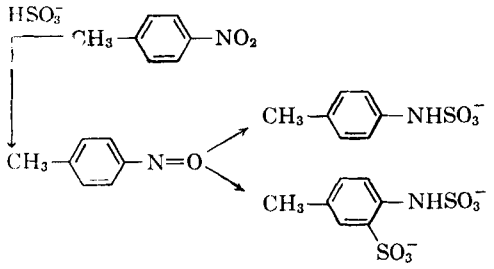
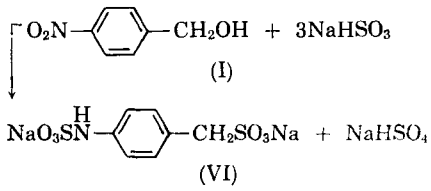
in detail (3–6), and the following pathway has been suggested (6) for the reaction of *p*-nitrotoluene with sulfite in aqueous systems

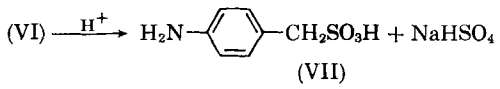


Hunter and Sprung (3) reported that the main product (72%) of the reaction was *p*-tolylsulfamic acid, while under similar conditions with *p*-nitrobenzoic acid as the reactant, the nuclear sulfonated product, 3-sulfo-4-sulfoaminobenzoic acid, was the main product (61%). The analogous mechanistic pathways for the *p*-nitrobenzyl alcohol-bisulfite reaction would be as follows

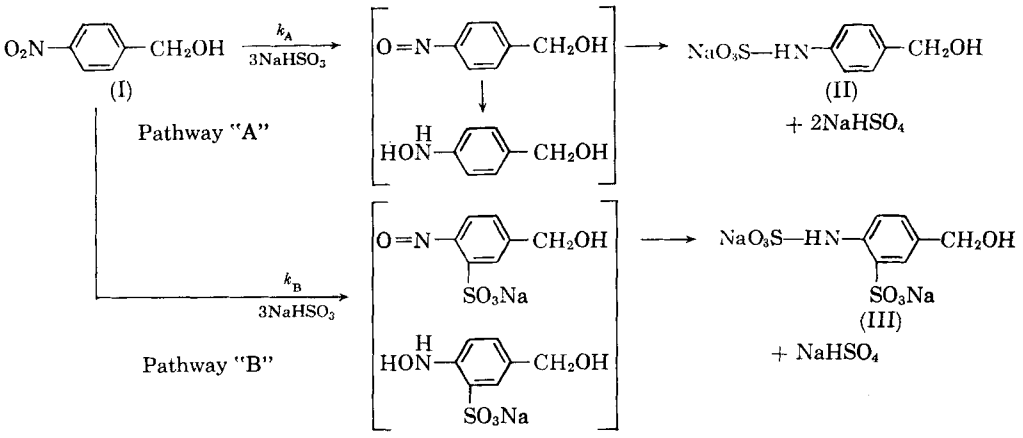
Isolation of product is of paramount significance in considering mechanisms because the following postulated reaction also consumes three moles of sulfite and produces a sulfoamino-sulfonic acid product



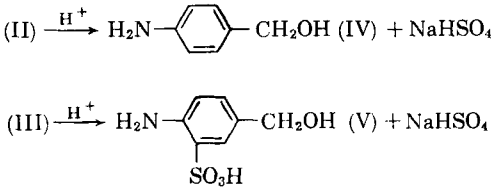
Acid Hydrolysis of Product:



Validity of this postulated mechanism could be tested simply by subjecting the product to acid hydrolysis; this would yield the slightly soluble zwitterionic *p*-aminobenzylsulfonic acid (VII). The highly polar nature of the products proposed



Acid Hydrolysis of Products:



Applicability of the above mechanism and determination of the main pathway (A or B) requires evaluation of several factors: (*a*) stoichiometry of reaction, i.e., total loss of bisulfite with respect to nitro compound; (*b*) molar yield of inorganic sulfate obtained by acid hydrolysis of reaction mixture; (*c*) product isolation.

in the above schemes makes isolation from contaminating inorganic sulfate very difficult. Repeated ethanolic extractions have been used with some success for the isolation of products from Piria reactions (3, 4). Although this technique is rather cumbersome, it serves to isolate all possible products under mild conditions. The procedure described by Nomine (7) for the isolation of a steroid sulfaminate offers a unique, rapid method in which the product is isolated as a water-insoluble quaternary ammonium salt. This latter procedure is generally applicable to the isolation of all postulated products from the reaction of sulfite with *p*-nitrobenzyl alcohol.

EXPERIMENTAL

Stoichiometry and Kinetic Studies

Solutions of *p*-nitrobenzyl alcohol and sodium bisulfite were prepared separately in various 0.3 *M* buffers. The nitro compound was dissolved in 150 ml. of buffer and the bisulfite in 50 ml. of nitrogen-flushed buffer. Ionic strength of the final combined solution (200 ml.) was maintained constant at 1.0 by addition of appropriate amounts of sodium chloride to the bisulfite solution. Solutions were equilibrated separately at the reaction temperature, then rapidly and simultaneously transferred to a reaction vessel immersed in a thermostat; this marked "zero time" for the reaction. Reaction solution was stirred at a fixed rate under nitrogen atmosphere during course of the reaction. Thermostat temperature was maintained ±0.1°. The pH of the reaction mixture was determined at the temperature of the study with a Beckman Zeromatic pH meter standardized against known buffers maintained at the same temperature. Sampling was performed by periodically withdrawing 5-ml. aliquots from the reaction mixture and quenching in an ice-water bath. Bisulfite content was determined by iodometric titration. Concentration of *p*-nitrobenzyl alcohol and of its reduction product was determined by ultraviolet spectrophotometry. Appropriate dilutions of the aliquots were made with pH 6.50, 0.01 *M* phosphate buffer; absorption was rapidly recorded from 350 to 220 mμ using a Cary model 11 recording spectrophotometer. Resultant spectra were evaluated by two component analysis using 235 and 277 mμ maxima.

Acid Hydrolysis and Sulfate Determination

Reduction Reaction.—Six millimoles (0.919 Gm.) of *p*-nitrobenzyl alcohol (Eastman WL recrystallized twice from water, m.p. 93°) were dissolved in 150 ml. of nitrogen-flushed water maintained at 55°. Sulfite solution was prepared to contain 12 mmoles (1.249 Gm.) of sodium bisulfite A.R. and 12 mmoles (1.543 Gm.) of sodium sulfite A.R. in 50 ml. of water at 55°. The solutions were simultaneously introduced into a reaction vessel fitted with a dropping funnel, nitrogen flushing tube, and a gas exit tube. The reaction was maintained at pH 6.5 ± 0.3 by appropriate additions of acid or base. The solution was thermostatted at 55° for 7 hours while water-saturated nitrogen bubbled through to mix the solution and to prevent autoxidation of sulfite.

Hydrolysis Reaction.—Subsequent to the reduction reaction, 30 ml. of concentrated hydrochloric acid was slowly added to the reaction solution through the dropping funnel. Flow of nitrogen through the solution was increased to expel sulfur dioxide; the exit tube was then connected to a sodium hydroxide absorption train. The acid hydrolysis reaction was maintained at 55° for 48 hours; nitrogen was constantly bubbled through the solution to expel sulfurous acid.

Sulfate Determination.—Barium chloride solution was added dropwise to the acidified hydrolysis reaction solution until no further precipitation of barium sulfate occurred. The collected precipitate was digested, washed, ignited, and weighed in the customary manner. The weight of barium sulfate from similarly treated blank containing no *p*-nitrobenzyl alcohol was subtracted from sample value to give net inorganic sulfate formed on hydrolysis: 12.32 mmoles $BaSO_4$ from sample minus 0.23 mmoles $BaSO_4$ from blank equals 12.09 mmoles $BaSO_4$ from sample hydrolysis. Ratio of sulfate obtained on hydrolysis to react nitro compound (PNBA): 12.09 sulfate/6.0 PNBA = 2.015 moles sulfate per mole PNBA.

Isolation of Acid Hydrolysis Product

Reduction reaction was carried out as described above. The following variations in hydrolysis conditions were employed

Added HCl, ml.	Hydrolysis—Time, hr.	Hydrolysis—Temp., ° C.	Product Analyses—Carbon, %	Product Analyses—Hydrogen, %
30	48	55	47.92	4.67
20	48	55	46.38	4.50
10	48	55	42.67	3.97
10	12	55	37.53	3.77
10	12	35	29.84	3.20
10	6	25	25.98	3.34

After cooling, the solutions were extracted with five 50-ml. portions of diethyl ether. The combined ethereal extracts were evaporated to dryness and weighed. Less than 10 mg. residue was obtained. The pH of the aqueous acid solution was adjusted to 2.5 by the addition of sodium carbonate. The yellow precipitate which formed was filtered from solution after 24 hours and dried under vacuum. Combustion analyses of products are tabulated above. Filtrate pH was adjusted to 9 with carbonate. The solution was extracted with five 50-ml. portions of chloroform. Chloroformic solutions evaporated to dryness yielded less than 10 mg. residue. Infrared spectra of products were compared with the known spectrum of *p*-aminobenzylsulfonic acid prepared as described below.

Product Isolation.—*A. Isolation of Sodium α-Hydroxy-6-sulfoamino-m-toluene Sulfonate (III) from a pH 6.5 Reduction Reaction.*—The reduction reaction between a fourfold molar excess of bisulfite with *p*-nitrobenzyl alcohol as described above was repeated at 55° with the pH maintained at 6.5.

One hundred milliliters of a reaction solution containing 3 mmoles of product, as shown by spectrophotometric analysis, was allowed to evaporate spontaneously to dryness at ambient temperatures. Care was taken in all subsequent operations to avoid temperatures higher than 25° so that product decomposition would be minimized. The dry residue was extracted with three 25-ml. portions of diisopropyl ether. The combined ethereal extracts, evaporated to dryness, yielded less than 5 mg. residue. The dried reaction residue was then extracted with four 50-ml. fractions of 65% ethanol. The combined ethanolic solutions were evaporated to dryness. The resultant residue was extracted with 200 ml. of 75% ethanol; the procedure was repeated using 85 and 95% ethanol. Residue from the 95% ethanol extraction was dissolved in 5 ml. of water and filtered. Residue from the aqueous filtrate was recrystallized four times from water-ethanol. Yield: 476 mg. (48.5%).

Anal.—Calcd. for $C_7H_7NNa_2O_7S_2$ (III): C, 25.69; H, 2.16; N, 4.28; S, 19.59. Found: C, 25.93; H, 4.08; N, 4.06; S, 16.97. Ultraviolet spectrum in water: ε 235.5 mμ = 10,850; ε 280 mμ = 1600. Infrared spectrum shows the following bands: –OH/–NH: 3550, 3425, 3300 cm.$^{-1}$; $-SO_3-$: 1300, 1250, 1230, 1170 cm.$^{-1}$; C–O: 1040.

B. Isolation of Sodium α-Hydroxy-6-sulfoamino-m-toluene Sulfonate (III) from a pH 4 Reduction Reaction.—The isolation was carried out exactly as described for the pH 6.5 product. Yield: 394 mg. (40.2%).

Anal.—Calcd. for $C_7H_7NNa_2O_7S_2$ (III): C, 25.69; H, 2.16; N, 4.28. Found: C, 25.15; H, 3.07; N, 4.26. The infrared spectrum was identical with that obtained from the pH 6.5 product.

C. Isolation of Potassium α-Hydroxy-6-sulfoamino-m-toluene Sulfonate (III) from a pH 6.5 Reduction Reaction.—An aliquot of a pH 6.5 reduction reaction was diluted to 100 ml., cooled to 25°, and extracted with six 50-ml. fractions of diisopropyl ether. The combined ethereal extracts evaporated to dryness yielded less than 10 mg. residue. A 150-ml. quantity of 5% benzethonium chloride solution was added slowly with stirring to the aqueous phase. The solution was allowed to stand 24 hours at 5° before pouring off the supernatant aqueous solution from the oily residue. The oily residue was dissolved in 200 ml. chloroform which was then washed with three 75-ml. portions of water and passed through a 2 × 20 cm. column containing anhydrous sodium sulfate. Eluate volume was adjusted to 200 ml. with chloroform rinsings from the column: eluate was evaporated to dryness under nitrogen at 25°. Residue was dissolved in 150 ml. absolute ethanol, and 50 ml. of 1 *M* ethanolic potassium acetate was added slowly with stirring. After 24 hours standing at 5°, the yellow precipitate was filtered from solution. Product recrystallized twice from ethanol-water.

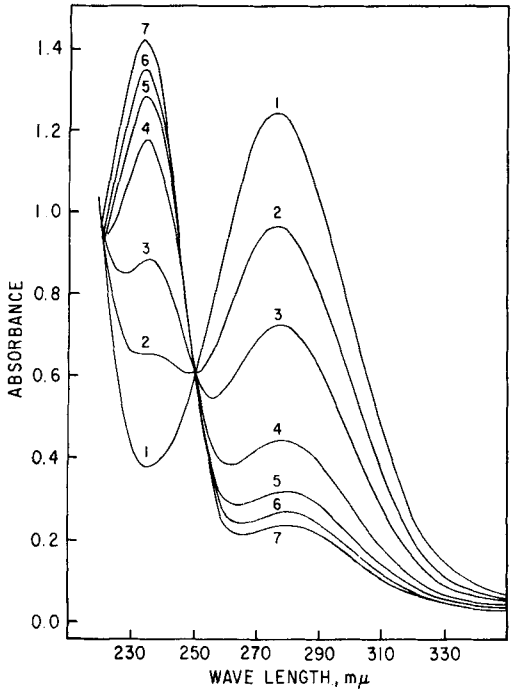


Fig. 1.—Ultraviolet spectra showing reduction of nitro compound (277 mμ) to sulfoamino product (235 mμ) in presence of excess bisulfite at 55°. Initial solution composition: 0.03 *M* *p*-nitrobenzyl alcohol and 0.12 *M* total sulfite in pH 6.50, 0.3 *M* buffer. Final ionic strength adjusted to 1.0. Figure redrawn from original spectrogram.

Anal.—Calcd. for $C_7H_7K_2NO_7S_2$: C, 23.39; H, 1.96; N, 3.90; S, 17.85. Found: C, 22.65; H, 1.91; N, 3.79; S, 16.82.

4-Aminobenzylsulfonic Acid (VII) Synthesis

Five mmoles (1.20 Gm.) of *p*-nitrobenzyl sulfonic acid, sodium salt synthesized by the procedure of Clutterbuck and Cohen (3), was dissolved in 50 ml. of water containing 1.4 Gm. finely powdered iron. The solution was acidified with acetic acid and warmed (70–80°) on a steam bath for 2 hours. After cooling, the solution was filtered. The filtrate was acidified with dilute hydrochloric acid and allowed to stand 24 hours at 5°, yielding light yellow crystals. The product was recrystallized three times from dilute hydrochloric acid. Yield: 465 mg. (49.6%).

Anal.—Calcd. for $C_7H_9NO_3S$ (VII): C, 44.90; H, 4.85; N, 7.48; S, 17.13. Found: C, 45.08; H, 4.91; N, 7.38; S, 16.94. The pKa determined in water was 4.56. Ultraviolet spectrum in water showed the following absorption maxima: ε 239.5 mμ = 4100; ε 283 mμ = 232; ε 325 mμ = 65. Infrared spectrum showed the following bands: –NH: 3180, 3040 cm.$^{-1}$; C=C/–NH deformation: 1628, 1582, 1540 cm.$^{-1}$; $-SO_3^-$/C–N: 1222, 1210, 1168, 1130, 1033, 1030 cm.$^{-1}$.

RESULTS AND DISCUSSION

Stoichiometry of the reaction was followed by iodometric titration of available sulfite and by two-component analysis of ultraviolet spectral curves such as Fig. 1. Rapid ultraviolet spectrophotometry of reduction reactions showed the presence of only two components and a single isosbestic point which suggested that the slow, rate-determining step in the sequential reaction was followed by more

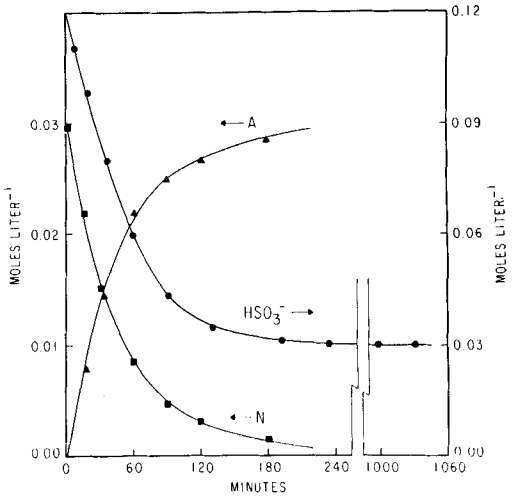


Fig. 2.—Reduction of nitro compound (*N*) with formation of product (*A*) and loss of bisulfite at 55° from solution containing excess bisulfite: 0.03 *M* *p*-nitrobenzyl alcohol and 0.12 *M* total sulfite in pH 6.50, 0.3 *M* buffer. Final ionic strength adjusted to 1.0. Molar concentration of *p*-nitrobenzyl alcohol (■) and its reduction product (▲) indicated by ordinate values to the left of the figure, while total molar concentration of sulfite (●) indicated by right ordinate values.

rapid steps so that the concentration of intermediates was always quite low. Excess bisulfite leads to total conversion of nitro compound with consumption of three moles of bisulfite for each mole of product formed, as shown in Fig. 2. Prolonged reaction times do not lead to further loss of bisulfite, thus establishing the ratio of three moles of bisulfite to one mole nitro compound; this was further verified by repeating studies at higher temperatures for prolonged periods, as shown in Table I. Equimolar concentrations of nitro compound and bisulfite lead to total consumption of bisulfite with formation of one-third molar concentration of product, as shown in Fig. 3.

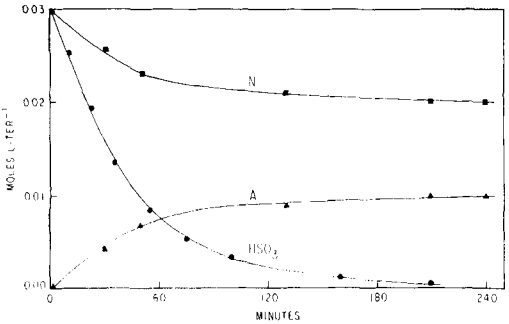


Fig. 3.—Reduction of nitro compound (N) with formation of product (A) and loss of bisulfite at 55° from solution containing excess nitro compound: 0.03 M p-nitrobenzyl alcohol and 0.03 M total sulfite in pH 6.50, 0.3 M buffer. Final ionic strength adjusted to 1.0.

TABLE I.—STOICHIOMETRY OF p-NITROBENZYL ALCOHOL BISULFITE REACTION AT pH 6.50 AND VARIOUS TEMPERATURES[a]

Temperature, °C.	—Bisulfite Concentration (moles L.⁻¹) at— 0 hr.	4 hr.	8 hr.	24 hr.
55	0.120	0.030	0.029	0.030
65	0.120	0.029	0.031	0.030
80	0.120	0.030	0.030	0.029

[a] Solution buffered with 0.30 M phosphate contained 0.03 M p-nitrobenzyl alcohol and 0.12 M sulfite. Ionic strength adjusted to 1.0 by addition of sodium chloride.

Total bisulfite consumption appears to be the same regardless of which mechanism is followed. Acidification of the reduction reaction followed by determination of inorganic sulfates is a powerful tool for establishing which mechanism predominates under given conditions: excess unreacted sulfite is driven off as volatile sulfurous acid, while total sulfate formed during reduction and acid hydrolysis is quantitatively recovered as the insoluble barium salt. Only the sulfoamino group is split under these conditions of acid hydrolysis; nuclear sulfonates are not affected. Isolation of approximately two moles of sulfate upon formation and subsequent hydrolysis of one mole of reaction product strongly supports pathway B of the Piria mechanism; nuclear sulfonation appears to predominate. Isolation of products from acid hydrolyses carried out under varied conditions yields products of variable composition. Under relatively mild conditions (6 hours at 25°), little or no sulfoamino hydrolysis takes place; this is confirmed by both infrared and combustion analysis. More rigorous conditions (30 ml. hydrochloric acid, 55° for 24 hours) lead to product decomposition as evidenced by infrared changes in bonded —NH and —OH. This is not too surprising since aryl sulfamates are known to be alkylating agents at higher temperatures (8). Optimal hydrolysis conditions appear to be at 55° for 48 hours; however, these and other conditions failed to yield infrared evidence for presence of either p-amino-benzylsulfonic acid or p-aminobenzyl alcohol hydrochloride. Reaction solutions made weakly basic, concentrated, and extracted with ether also failed to yield evidence for presence of p-aminobenzyl alcohol.

Product isolation under conditions of acid hydrolysis was complicated by observed tendency of the product to polymerize with heating. Also, strongly acidic conditions introduce the uncertainty factor of secondary reactions leading to artifacts. Isolation of the same product (III) from pH 4 and pH 6.5 reactions by two different procedures in which rigorous conditions were not employed strongly supports mechanism B. It is especially noteworthy that neither isolation procedure is especially selective, i.e., all possible sulfoamino products would be isolated. This is especially significant in considering possible mechanisms since 4-sulfoaminobenzyl alcohol (II) would be isolated under identical conditions if it were present in substantial amounts.

Dependence of the absolute initial rate of loss of bisulfite, $-d(\mathrm{Bi})/dt$, on pH is shown in Fig. 4, in which stoichiometric amounts of reactants (3 bisulfite:1 nitro compound) were reacted at 55°. The initial rate of reaction remains very nearly invariant from pH 3 to 6 exhibiting a rather complex relationship toward hydrogen ion concentration at values above 6. There appears to be a different sensitivity toward singly and doubly charged thio compound: the marked rate increase at pH 7 corresponds to higher initial concentrations of sulfite ion. Actually, considerable concentrations of pyrosulfite, $S_2O_5^{2-}$, ion may be present in concentrated bisulfite solutions (pH 4); however, this species exists in equilibrium with bisulfite ion (9). De-

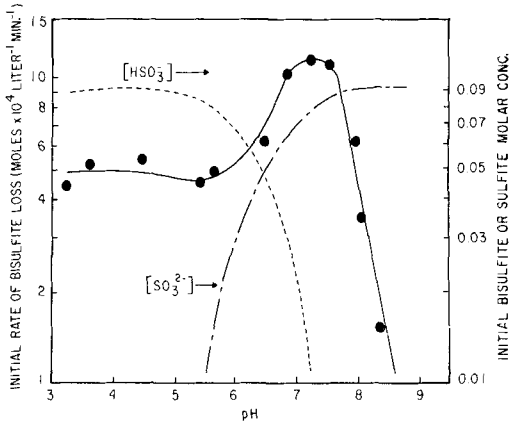


Fig. 4.—Semilog plot of absolute initial rate of bisulfite loss at 55° as a function of pH shown as solid circles joined by solid line. Initial solution composition: 0.03 M p-nitrobenzyl alcohol and 0.09 M total sulfite in 0.3 M buffer; total ionic strength adjusted to 1.0. Initial bisulfite or sulfite ion concentration at 55° in buffers (apparent second ionization constant of sulfurous acid, $pK'_2 = 6.5$) represented by dashed lines.

crease in reaction rate between pH 7.5 and 8 appears dependent on bisulfite ion concentration, but this effect may be more apparent than real inasmuch as this may reflect sensitivity toward increasing hydroxyl ion concentration.

The dependency of the initial rate of nitro loss on initial bisulfite or sulfite concentration is shown in Fig. 5. The initial reaction at pH 4.2 exhibited little dependence on bisulfite ion, and extrapolation of rate values to zero bisulfite concentration suggested that the slow, rate-determining step may involve a S_N1 mechanism. At higher pH values the intercept rate value, [Bi] → 0, decreases, becoming zero at pH values above 7. Plotting intercept rate values, [Bi] → 0, of the initial rates of nitro loss as a function of pH yields Fig. 6 which shows that the initial rate-determining reaction has S_N1 characteristics below about pH 4.6 while S_N2 characteristics predominate above pH 7. The following scheme appears consistent with observed results on the absolute initial rates of nitro loss from *p*-nitrobenzyl alcohol (PNBA) solutions in the presence of bisulfite or sulfite ion

$$\text{PNBA (I)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{(PNBA*)}$$

$$+ SO_3^{2-} \xrightarrow[k_3]{HSO_3^-} \text{Reduction Product (III)}$$

$$\downarrow k_4$$

$$\text{(III)}$$

$$-d(\text{PNBA})/dt = k_4(\text{PNBA})(SO_3^{2-}) + k_3(\text{PNBA*})(HSO_3^-) \quad \text{(Eq. 1)}$$

$$d(\text{PNBA*})/dt = k_1(\text{PNBA}) - k_2(\text{PNBA*}) - k_3(\text{PNBA*})(HSO_3^-) = 0 \quad \text{(Eq. 2)}$$

$$(\text{PNBA*}) = \frac{k_1(\text{PNBA})}{k_2 + k_3(HSO_3^-)} \quad \text{(Eq. 3)}$$

Substituting for (PNBA*) from Eq. 3 into Eq. 1, we have

$$-d(\text{PNBA})/dt = k_4(\text{PNBA})(SO_3^{2-}) + \frac{k_3 k_1(\text{PNBA})(HSO_3^-)}{k_2 + k_3(HSO_3^-)} \quad \text{(Eq. 4)}$$

At pH 4, the initial, rate-determining, reduction reaction may be approximately described by Eq. 5

$$-d(\text{PNBA})/dt = k_1(\text{PNBA})/k_2 \quad \text{(Eq. 5)}$$

which clearly indicates the proposed S_N1 characteristics and is in accord with observed results.

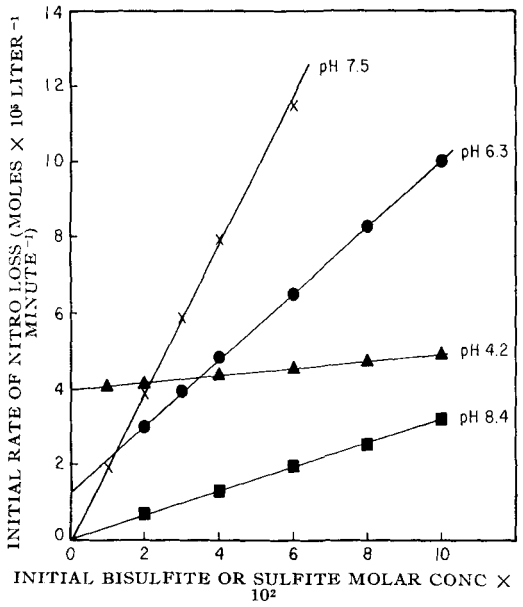


Fig. 5.—Initial rate of nitro loss, $-d(\text{Nitro})/dt$, as a function of initial bisulfite or sulfite ion concentration from buffered (0.3 *M*, μ1.0) solutions at 55°. Solutions initially contained 0.01 *M* *p*-nitrobenzyl alcohol.

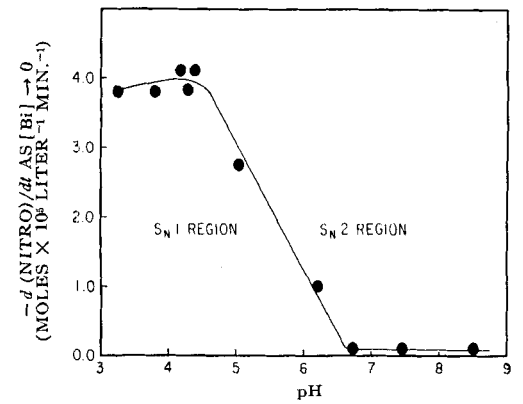


Fig. 6.—Initial rates of nitro loss, $-d(\text{Nitro})/dt$, from bisulfite-*p*-nitrobenzyl alcohol reactions extrapolated to zero bisulfite ion concentration, [Bi] → 0, (*cf.* Fig. 5) plotted as a function of pH. Buffered solutions (0.3 *M*, μ 1.0) initially contained 0.01 *M* *p*-nitrobenzyl alcohol and were thermostatted at 55°.

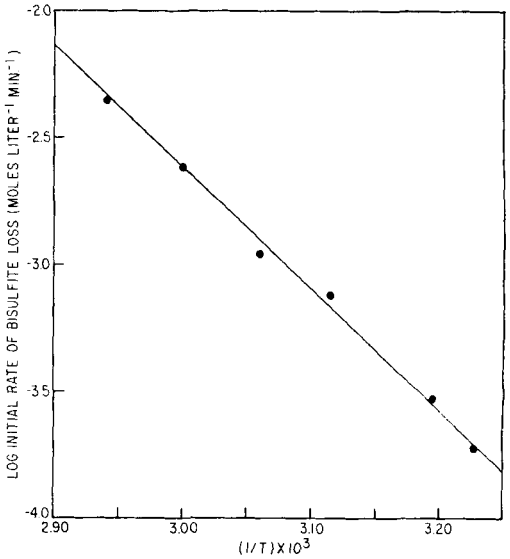


Fig. 7.—Log of initial rate of bisulfite loss from pH 7.0 solutions as a function of reciprocal absolute temperature. Initial solution composition: 0.03 *M* *p*-nitrobenzyl alcohol and 0.09 *M* total sulfite in 0.3 *M* buffer. Final ionic strength adjusted to 1.0.

While at pH 7, the initial, rate-determining, reduction reaction shows dependence on both the nitro and thio species, indicating that Eq. 4 may be simplified to

$$-d(\text{PNBA})/dt = k_4 (\text{PNBA}) (SO_3^{2-}) \quad \text{(Eq. 6)}$$

to describe the S_N2 initial reaction which dominates at higher pH values.

The apparent heat of activation of the initial reaction between stoichiometric amounts of reactants (3 bisulfite:1 nitro compound) determined at pH 7 where S_N2 characteristics dominate gave the value 22 Kcal. mole^{-1} (Fig. 7).

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Thioacetamide II

Substitute for Hydrogen Sulfide in the Quantitative Analysis of Medicinal Chemicals

By M. A. GHAFOOR and C. LEE HUYCK†

Assay procedures utilizing thioacetamide and hydrogen sulfide gas have been compared. The advantages of using thioacetamide are discussed.

THE PURPOSE of this study is to investigate the use of thioacetamide as a substitute for hydrogen sulfide in the analysis of medicinal chemicals. A survey of the literature concerning the use of thioacetamide as a substitute for hydrogen sulfide has been made by Jue and Huyck (1).

Swift and Butler (2) reported that since the rate of acid-catalyzed hydrolysis follows the equation

$$\frac{d\text{TA}}{dt} = -K\,\text{H}^+\,\text{TA}$$

(where TA = thioacetamide), the value of K is 0.21 L. mole^{-1} min.$^{-1}$ at 90° and 0.019 L. mole^{-1} min.$^{-1}$ at 60°. The activation energy is 19.1 Kcal. per mole. These figures mean that in a solution which is 1 M in hydronium ion, thioacetamide would be half hydrolyzed at 100° in 100 seconds, at 60° in 36 minutes, and at 25° in 21 hours. In solutions of pH 10 or more, hydrolysis is quite rapid and yields both thioacetate ions and acetamide.

The relation of hydronium ion concentration to the time of precipitation of arsenic in 0.1 M thioacetamide was studied by Butler and Swift (3). Two kinds of reactions with thioacetamide in sulfide precipitations were proposed: (*a*) hydrolysis controlled reaction in which hydrogen sulfide or hydrosulfide ions are formed as intermediates; (*b*) a direct reaction of metal ions with thioacetamide itself. Thioacetamide is a more rapidly acting reducing agent than hydrogen sulfide since arsenic acid is reduced much faster with thioacetamide than with hydrogen sulfide. The rate of precipitation of arsenic with valence of three by thioacetamide was measured in solutions from pH 1.0 to 3.8 and was found to follow quantitatively the calculated rate of hydrolysis of thioacetamide to hydrogen sulfide.

Molybdenum in titanium alloys was determined by precipitation as sulfide with thioacetamide by McNerney and Wagner (4). Stoner and Finston (5) clearly separated uranium from 200 times its weight of bismuth by precipitating bismuth sulfide from solution. Such a separation is impossible with gaseous hydrogen sulfide without a long digestion period.

The precipitation of metal sulfides is governed by the solubility product relation as shown by

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