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Pyrazine Chemistry. II. Derivatives of 3-Hydroxypyrazinoic Acid

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In a preceding paper[1] we described the preparation of a number of pyrazine derivatives starting with 3-aminopyrazinoic acid. We now wish to report a number of derivatives prepared from 3-hydroxypyrazinoic acid, the latter being readily available by the hydrolysis of lumazine.[2]

The route of synthesis of the simple pyrazine derivatives is shown in Chart 1. 3-Hydroxypyrazinamide (III) was converted to 3-aminopyrazinol (IV) by the Hofmann degradation. This is in contrast to the failure of the reaction[1] to degrade 3-aminopyrazinamide to 2,3-diaminopyrazine (VI). In the roundabout way illustrated in Chart 1 we have been able to prepare VI, the compound we have tried to make directly from 3-aminopyrazinamide.[1]

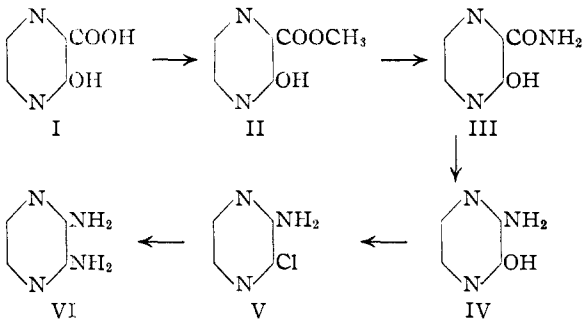

CHART 1

From these three new aminopyrazines (IV, V and VI) attempts were made to prepare sulfonamides. When 3-aminopyrazinol (IV) and acetylsulfanilyl chloride were mixed and heated in pyridine, condensation did not take place. Unreacted IV was recovered from the mixture. On dissolving IV in aqueous acetone containing one mole equivalent of sodium hydroxide and adding acetylsulfanilyl chloride, reaction occurred giving a yellow product which seemed to be purified easily. However, microscopic examination showed that the product is a mixture of two compounds, but elementary analysis of it for carbon, hydrogen, nitrogen and sulfur gave values in excellent agreement with those calculated for the desired sulfonamide as well as the expected sulfonate, 3-amino-2-pyrazinyl-*p*-acetamidobenzenesulfonate (VII). Both compounds have the same empirical formula. Evidently the product is a mixture of the sulfonamide and the sulfonate. This conclusion is substantiated by the fact that a fraction of the product is soluble in cold, dilute, aqueous alkali. On acid hydrolysis of the product we were able to isolate only 3-aminopyrazinol (IV).

(1) Ellingson, Henry and McDonald, THIS JOURNAL, **67**, 1711 (1945).

(2) Weijlard, Tishler and Erickson, *ibid.*, **67**, 802 (1945).

Analysis of the product obtained by interaction of 2-amino-3-chloropyrazine (V) and acetylsulfanilyl chloride in molar equivalents shows that two acetylsulfanilyl groups are present in the resulting compound. This fact is further substantiated by the analytical data obtained on the deacetylated compound, VIII (A or B). Unlike the pyrazine sulfonamides already described in the literature, this one and its diacetyl derivative are insoluble in dilute alkalies. Consequently the structure of the sulfonamide is either VIII-A or VIII-B.

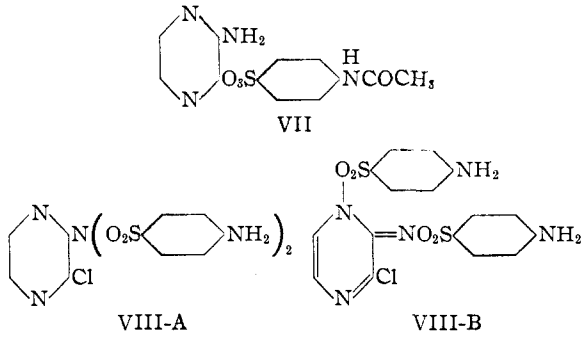

CHART 2

We have been unable to effect monocoupling of acetylsulfanilyl chloride with V by altering either the experimental conditions or the molar ratio of the reactants. Attempts to remove one sulfanilyl group from the dicoupled compound (VIII-A or B) were unsuccessful.

A similar instance of dicoupling has been reported in the case of 2-aminothiazoline.[3] The evidence[3b] indicates that in this compound one sulfanilyl group is linked to the imino nitrogen and the other to the ring nitrogen atom. The structure of the thiazoline derivative is similar to VIII-B. Nevertheless, we are not prepared to choose between VIII-A and VIII-B for the structure of our compound.

When 2,3-diaminopyrazine reacts with acetylsulfanilyl chloride, the product (IX) has two acetylsulfanilyl groups in the molecule. The fact that the product is soluble in dilute alkali, indicates that the groups are probably linked one to each amino group. This is substantiated by the fact that hydrolysis of IX splits it into sulfanilamide and 2,3-dihydroxypyrazine (X). Attempts to prepare the monocoupled compound from 2,3-diaminopyrazine (VI) and acetylsulfanilyl chloride were unsuccessful.

After we had submitted this paper for publication, a closely related instance of acid cleavage of

(3) (a) Sprague and Kissinger, *ibid.*, **63**, 578 (1941); (b) Raiziss and Clemence, *ibid.*, **63**, 3124 (1941); (c) Nathan, Hunter and Kolloff, *ibid.*, **65**, 949 (1943).

a heterocyclic sulfonamide into sulfanilamide and the hydroxyheterocycle has been reported.[4]

Since IX could not be deacetylated without cleaving the molecule at the pyrazine ring, VI was condensed with *p*-nitrobenzenesulfonyl chloride in the hope of obtaining 2,3-disulfanilamidopyrazine by subsequent reduction of the nitro groups. In this condensation three compounds were formed. The three were isolated by virtue of the fact that one (XIII) is more soluble in aqueous acetic acid than are the other two (XI and XII). The solubility of XI and the insolubility of XII in dilute aqueous alkali permitted their separation.

The solubility of XI in alkali, its elementary analysis and the structure of its analog, IX, indicate that the *p*-nitrobenzenesulfonyl groups are linked to the adjacent amino groups. Reduction of XI by means of iron in alkali or hydrogen over platinum black gave amorphous products which melted over a wide range and from which the only crystalline substance we were able to isolate proved to be sulfanilamide.

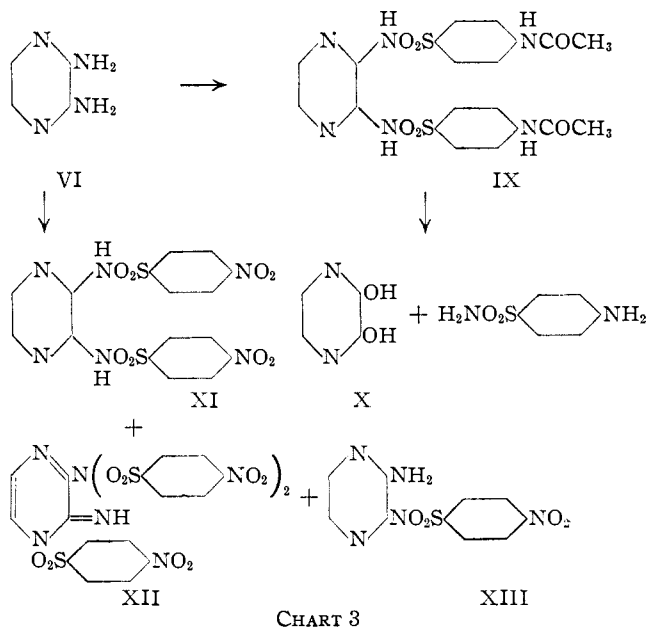

CHART 3

Analysis of compound XII shows that three *p*-nitrobenzenesulfonyl groups are in the molecule. The formula shown for XII in Chart 3 is mere speculation and is given as the structure we think probable because of its insolubility in aqueous alkali. Reduction of XII with iron in alkali or hydrogen over platinum black gave amorphous products which melted over a wide range and which we were unable to purify. A qualitative test showed the presence of amino groups, but evidently the product is a complex mixture.

Compound XIII isolated by concentration of the mother liquors from the more insoluble mixture of XI and XII proved to be 2-amino-3-*p*-nitrobenzenesulfonylaminopyrazine.

Of this group of new pyrazine derivatives the only one being studied for its antibacterial value as a sulfonamide is VIII (A or B). To give it a name we shall use the one corresponding to structure VIII-A, namely, 2-disulfanilylamino-3-chloropyrazine.

Experimental

3-Hydroxypyrazinoic Acid (I).—This pyrazine acid was prepared from lumazine as described by Weijlard, *et al.*[2]

Methyl-3-hydroxypyrazinoate (II).—To a suspension of 44.8 g. (0.32 mole) of crude 3-hydroxypyrazinoic acid (I) in 600 cc. of methanol was added 17 cc. of concentrated sulfuric acid. The mixture was stirred for forty-eight hours during which time it was boiled under reflux for ten hours. To the brown solution 200 cc. of water and sufficient sodium bicarbonate (60 g.) to neutralize the acid were added. The *p*H was then adjusted to 3 with hydrochloric acid. The solution was concentrated in vacuum on the steam-bath to about 150 cc. and extracted for forty-eight hours with ethyl acetate in a Kutscher–Steudel extractor. The hot ethyl acetate extract (1200 cc.) was decolorized with Darco, filtered and concentrated to about 300 cc. After cooling, the crystals were collected and dried. The yield was 38 g. (77.2%); yellow crystals which melted at 151°. For analysis[5] a sample was crystallized two times from ethyl acetate; m. p. 154° (cor.).

Anal. Calcd. for $C_6H_6N_2O_3$: C, 46.75; H, 3.92; N, 18.18. Found: C, 46.66, 46.71; H, 3.96, 3.93; N, 18.02, 18.15.

3-Hydroxypyrazinamide (III).—To a suspension of 89.6 g. (0.64 mole) of crude 3-hydroxypyrazinoic acid (I) in 1200 cc. of methanol was added 34 cc. of concentrated sulfuric acid. The mixture was stirred for forty-eight hours during which time it was boiled under reflux for ten hours. The brown solution which was filled with crystals was poured into 500 cc. of concentrated ammonium hydroxide and the resulting solution was heated, treated with Darco, filtered and cooled. The yellow crystals were collected and dissolved in 1000 cc. of hot water. After treatment with Darco the solution was filtered and made slightly acid by the addition of hydrochloric acid. The yellow crystals were collected and dried at 80°. The yield was 71 g. (79.9%); the compound melted at about 265° (dec.). For analysis a sample was crystallized twice from water. The slightly yellow needles melted at 263–265° (dec.).

Anal. Calcd. for $C_5H_5N_3O_2$: C, 43.17; H, 3.62; N, 30.21. Found: C, 42.46, 42.63; H, 3.91, 3.75; N, 29.73, 29.89.

3-Aminopyrazinol (IV).—To a solution of 32.9 g. of potassium hydroxide in 448 cc. of water in a one-liter three-neck flask fitted with a stirrer and thermometer were added 5.3 cc. of bromine and, after solution of the latter, 13.9 g. (0.10 mole) of 3-hydroxypyrazinamide (III). The temperature of the reaction mixture was raised to 85° and held there for one and one-half hours. At this temperature concentrate hydrochloric acid (24 cc.) was added gradually in order to acidify the solution. The deep red solution was cooled overnight. The tan crystals were collected and crystallized from 700 cc. of water, Darco being used for decolorizing. The small, almost colorless crystals were collected and dried at 80°. The yield was 9 g. (81%); m. p. 292–298° (dec.) on the Kofler micro hot stage. After another crystallization from water the compound was analyzed.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.24; H, 4.54; N,

(4) Stevens, Pfister and Wolf, *ibid.*, **68**, 1035 (1946).

(5) Dr. Carl Tiedcke, Laboratory of Microchemistry, New York, N. Y., performed all analyses reported in this paper.

37.82. Found: C, 43.10, 43.10; H, 4.74, 4.77; N, 37.86, 37.57.

2-Amino-3-chloropyrazine (V).—To 36 cc. of phosphorus oxychloride in a pressure bottle was added 22.2 g. (0.20 mole) of 3-aminopyrazinol (IV). The bottle was closed and heated in an oil-bath at 113° for six to eight hours. The brown, semi-solid contents were poured with stirring onto 500 g. of crushed ice. After the ice had melted, the brown, fluorescent solution was treated with Darco, filtered and neutralized with concentrated ammonium hydroxide (120 cc.). After thorough cooling the yellow crystals were collected and dissolved in 1000 cc. of boiling water. This solution was decolorized with Darco and filtered. The filtrate was cooled overnight; the crystals which separated were collected and dried at room temperature. The yield was 17.5 g. (67.6%) of slightly yellow crystals; m. p. 165–167°. For analysis a sample was crystallized from hot water. Long, almost colorless crystals which melted at 168° (cor.) were obtained.

Anal. Calcd. for $C_4H_4N_3Cl$: C, 37.08; H, 3.11; N, 32.44; Cl, 27.37. Found: C, 37.11, 37.29; H, 3.13, 3.13; N, 32.32, 32.46; Cl, 27.22, 27.32.

2,3-Diaminopyrazine (VI).—In a Carius tube were placed 6.45 g. (0.05 mole) of 2-amino-3-chloropyrazine (V), 20 cc. of concentrated ammonium hydroxide and a trace of copper powder. Four such tubes were prepared, sealed and heated at 123° for twenty-four hours. The contents of the four tubes were combined and the tubes rinsed with water so that the total volume of solution became about 400 cc. This was heated, decolorized with Darco, filtered and extracted for forty-eight hours with ethyl acetate in a Kutscher–Steudel extractor. During this time the ethyl acetate was changed four times; the total volume used was 1200 cc. The ethyl acetate extract was heated, decolorized with Darco, filtered and cooled. The light tan crystals were collected and air-dried. The yield was 10.5 g. (48%); m. p. 203°. For analysis the product was crystallized three times from ethyl acetate and finally from water. The colorless prisms melted at 205.6° (cor.).

Anal. Calcd. for $C_4H_6N_4$: C, 43.63; H, 5.49; N, 50.88. Found: C, 43.52, 43.45; H, 5.47, 5.30; N, 51.99, 51.71.

Reaction between IV and Acetylsulfanilyl Chloride.—To a solution of 8 g. of sodium hydroxide in 200 cc. of water in a two-liter, three-neck flask were added 22.2 g. (0.20 mole) of 3-aminopyrazinol (IV) and 500 cc. of acetone. To this solution was added a solution of 46.7 g. (0.20 mole) of acetylsulfanilyl chloride in 500 cc. of acetone. At room temperature the reaction mixture was stirred for two hours and then allowed to stand overnight. The orange solution was decolorized with Darco and after filtration the acetone was removed by distillation in vacuum, the removed acetone being replaced by water. The yellow solid was collected and dried at room temperature. The yield was 44 g. (71.4%); the crude product sintered at 187° and melted at 200° (dec.). This product was dissolved in 2400 cc. of hot 80% ethanol; the solution was decolorized with Darco and filtered. The yellow filtrate was diluted with 4000 cc. of hot water and the solution thoroughly cooled. The yellow crystals weighed 25.4 g., sintered at 197° and melted at 209° (dec.). After three additional crystallizations from 80% ethanol the crystals were almost colorless and melted at 215° (dec.). Microscopic examination of this product showed it to be a mixture of two compounds. The empirical formula for both the sulfonamide and sulfonate is $C_{12}H_{11}N_3O_4S$.

Anal. Calcd. for $C_{12}H_{11}N_3O_4S$: C, 46.75; H, 3.92; N, 18.17; S, 10.40. Found: C, 46.53, 46.56; H, 3.81, 3.80; N, 18.03, 18.19; S, 10.25, 10.30.

2-Disulfanilylamino-3-chloropyrazine (VIII-A or B).—To 150 cc. of dry pyridine in a one-liter, three-neck flask fitted with a stirrer and thermometer was added 25.8 g. (0.20 mole) of 2-amino-3-chloropyrazine (V). After solution was complete 102.8 g. (0.44 mole) of acetylsulfanilyl chloride was added. The reaction mixture was stirred at room temperature for sixteen hours, then at 60° for one hour. To the reaction mixture a solution of 18 g. of sodium hydroxide in 600 cc. of water was added. The pyridine was removed by distillation under reduced pressure. After about 500 cc. of distillate had been collected, the suspension remaining in the distilling flask was cooled, diluted with 500 cc. of ice water and filtered. The crude product (72.8 g.) was crystallized by solution in 2000 cc. of 75% dioxane (v/v). After treatment with Darco the solution was filtered and the filtrate was diluted with 2200 cc. of boiling water. After cooling the yellow crystals were collected and dried. The yield was 42.4 g. (41.6%); m. p. 217° (dec.). Before analysis the compound was crystallized twice from the dioxane–water solution. The yellow crystalline compound, 2-di-(N^4-acetylsulfanilyl)-amino-3-chloropyrazine, sintered at 218° and melted at 224° (dec.).

Anal. Calcd. for $C_{20}H_{18}N_5O_6S_2Cl$: C, 45.85; H, 3.46; N, 13.37; S, 12.22; Cl, 6.77. Found: C, 46.32, 46.00; H, 3.82, 3.75; N, 13.00, 13.28; S, 11.98, 12.06; Cl, 6.83, 6.60.

In order to deacetylate 2-di-(N^4-acetylsulfanilyl)-amino-3-chloropyrazine 25.8 g. (0.049 mole) of the compound was added to a solution of 250 cc. of 95% ethanol and 50 cc. of concentrated hydrochloric acid. The suspension was heated at 50–55° for four hours during which time the solid slowly dissolved. The brown fluorescent solution was neutralized by the addition of 45 cc. of concentrated ammonium hydroxide. After thorough cooling the solid was collected and suspended in 250 cc. of water to dissolve the major contaminant, ammonium chloride. The undissolved solid was collected and dried; 10.5 g. (48.5%) of small tan crystals; m. p. 203–205° (dec.). To purify the compound it was dissolved in 200 cc. of dioxane. The solution was decolorized with Darco, filtered, and the filtrate was diluted with 500 cc. of boiling water. The yield was 8.5 g. of small yellow crystals the melting point of which was unchanged. For analysis a sample was crystallized from 95% ethanol whereby small almost colorless crystals were obtained; m. p. 204–205° (dec.).

Anal. Calcd. for $C_{16}H_{14}N_5O_4S_2Cl$: C, 43.68; H, 3.21; N, 15.92; S, 14.58; Cl, 8.06. Found: C, 43.42, 43.30; H, 3.50, 3.46; N, 15.73, 15.79; S, 14.50, 14.64; Cl, 7.97, 8.03.

2,3-Di-(N^4-acetylsulfanilamido)-pyrazine (IX).—In 300 cc. of dry pyridine in a one-liter, three-neck flask fitted with a stirrer was suspended 22 g. (0.20 mole) of 2,3-diaminopyrazine (VI). To the suspension cooled in an ice-bath was added gradually 102.8 g. (0.44 mole) of acetylsulfanilyl chloride. After the addition was finished, the mixture was stirred at room temperature for fifteen hours. A solution of 18 g. of sodium hydroxide in 300 cc. of water was added to the brown viscous reaction mixture and the pyridine was removed by steam distillation under reduced pressure. The tan granular solid, after being dried, weighed 81 g. (80.4%); m. p. 229–231° (dec.). To purify the product it was dissolved in 1000 cc. of dilute ammonium hydroxide and the resulting solution was decolorized with Norite and filtered. On neutralization of the filtrate with acetic acid a yellow solid precipitated. This purification process was repeated four times after which the compound appeared as fine yellow needles which sintered at 245° and melted at 247° (dec.). For an analytical sample the compound was given a final crystallization from 50% acetic acid. The melting point of the fine, pale yellow needles remained unaltered.

Anal. Calcd. for $C_{20}H_{20}N_6S_2O_6$: C, 47.61; H, 4.00; N, 16.66; S, 12.71. Found: C, 47.15, 47.37; H, 4.16, 4.11; N, 16.43, 16.30; S, 12.80, 12.83.

Attempts to Deacetylate 2,3-Di-(N^4-acetylsulfanilamido)-pyrazine (IX).—A. A solution of 30 cc. of 4 *N* hydrochloric acid and 10 g. (0.0198 mole) of 2,3-di-(N^4-acetylsulfanilamido)-pyrazine (IX) was boiled under reflux for forty-five minutes. On cooling a small quantity of crystals separated from the brown solution. These were collected and dried; 0.8 g. This product was crystallized twice from water, first from 100 cc., then from

50 cc. The colorless crystals did not melt up to 350° and according to analysis are 2,3-dihydroxypyrazine (X).

Anal. Calcd. for $C_4H_4N_2O_2$: C, 42.85; H, 3.61; N, 24.99. Found: C, 43.13, 43.01; H, 3.46, 3.46; N, 24.70, 24.80.

B. A suspension of 10 g. of IX in a mixture of 100 cc. of ethanol and 20 cc. of concentrated hydrochloric acid was boiled under reflux for three hours. The brown solution was treated with Darco, filtered and neutralized by the addition of 44 cc. of a 20% sodium hydroxide solution. Nothing crystallized, so the solution was evaporated to dryness in vacuum and the residue was dissolved in a mixture of 200 cc. of water and 25 cc. of ethanol. This solution was decolorized with Darco, filtered, and cooled whereupon 0.5 g. of yellow crystals was obtained. The filtrate was concentrated in vacuum on the water-bath until crystals appeared. After thorough cooling these were collected and dried; 2.2 g. of yellow crystals. The combined crude products (2.7 g.) sintered about 125° and melted at 155°. On crystallization from 30 cc. of water colorless crystals were obtained; m. p. 164°. Mixed melting with an authentic sample of sulfanilamide (m. p. 165°) gave no depression. Evidently the product isolated in this experiment was sulfanilamide.

Reaction of 2,3-Diaminopyrazine (VI) with *p*-Nitrobenzenesulfonyl Chloride.—To a suspension of 11.0 g. (0.10 mole) of 2,3-diaminopyrazine (VI) in 150 cc. of cold dry pyridine was added with stirring 47 g. (0.21 mole) of crystalline *p*-nitrobenzenesulfonyl chloride at such a rate that the temperature did not exceed 20°. After the addition had been completed, the temperature was raised to 85° for ten minutes. The reaction mixture was allowed to stand at room temperature for fifteen hours and then a solution of 8.3 g. of sodium hydroxide in 200 cc. of water was added. The solution was diluted with water and then steam-distilled under reduced pressure to remove the pyridine. Finally the volume was concentrated to about 400 cc. and the yellow granular solid which precipitated was collected and dried. It weighed 40 g.

This product was crystallized from 2150 cc. of 63% acetic acid and gave 12.8 g. of tan crystals which melted at 195–202° and on microscopic examination proved to be a mixture of two kinds of crystals. Of this mixture 8.8 g. was crystallized from 80% acetic acid and gave 6.1 g. of a product which was still a mixture.

The mixture (6.1 g.) was suspended in 200 cc. of water containing 10 cc. of concentrated ammonium hydroxide. After two hours the insoluble fraction was collected and dried; it weighed 4.9 g. It was crystallized from 200 cc. of 80% acetic acid whereby 2.6 g. of colorless crystals were obtained. These melted at 204–205° (dec.) and were homogeneous on microscopic examination. Analysis of this compound shows that it has three *p*-nitrobenzenesulfonyl groups. Because of its low solubility in aqueous alkali we have represented the structure as XII in Chart 3.

Anal. Calcd. for $C_{22}H_{15}N_7O_{12}S_3$: C, 39.70; H, 2.27; N, 14.73; S, 14.45. Found: C, 39.58; H, 2.82; N, 14.85; S, 14.34.

On neutralization of the yellow filtrate with acetic acid crystals separated. This alkali soluble fraction (1.0 g.) was crystallized from 200 cc. of 80% acetic acid. The lemon yellow crystals melted at 202–203° (dec.). Analysis of the compound shows that two *p*-nitrobenzenesulfonyl groups are in the molecule; its structure is probably that represented by XI of Chart 3.

Anal. Calcd. for $C_{16}H_{12}N_6O_8S_2$: C, 40.00; H, 2.52; N, 17.49; S, 13.35. Found: C, 40.06; H, 2.89; N, 17.53; S, 13.28.

The acetic acid filtrate from the crystallization of the 40 g. of initially obtained crude product was concentrated under vacuum to a small volume. On cooling this deposited 20 g. of a yellow amorphous product. This crude material was suspended in 600 cc. of water containing 30 cc. of concentrated ammonium hydroxide. The insoluble fraction was collected and dried, 5.0 g.; m. p. 200–204° (dec.). It gave no depression on mixed melting with XII. The filtrate was neutralized with acetic acid and gave 14 g. of yellow crystals, m. p. 172° (dec.). This product was suspended in 400 cc. of water containing 30 cc. of ammonium hydroxide. This time 2.3 g. remained undissolved and on neutralization of the filtrate 11 g. of yellow crystals, melting at 174° (dec.), were obtained. The 11 g. were crystallized three times from 80% acetic acid. Small bright yellow needles were obtained; m. p. 178–179° (dec.). According to analysis and molecular weight determination the compound is 2-amino-3-*p*-nitrobenzenesulfonylaminopyrazine (XIII).

Anal. Calcd. for $C_{10}H_9N_5O_4S$: C, 40.67; H, 3.07; N, 23.72; S, 10.86; mol. wt., 295. Found: C, 40.78, 40.52; H, 3.28, 3.19; N, 23.60, 23.81; S, 11.16, 11.09; mol. wt., 318, 309.

Summary

1. The synthesis of five new simple pyrazine derivatives, methyl 3-hydroxypyrazinoate, 3-hydroxypyrazinamide, 3-aminopyrazinol, 2-amino-3-chloropyrazine and 2,3-diaminopyrazine, has been described.

2. From 3-aminopyrazinol a mixture of the sulfonamide and the sulfonate, 3-amino-2-pyrazinyl-*p*-acetamidobenzenesulfonate, was obtained.

3. From 2-amino-3-chloropyrazine a disubstituted sulfanilyl derivative was obtained.

4. Contrary to expectation, the usual conditions for deacetylation split 2,3-di-(N^4-acetylsulfanilamido)-pyrazine into 2,3-dihydroxypyrazine and sulfanilamide.

5. Interaction of 2,3-diaminopyrazine with two moles of *p*-nitrobenzenesulfonyl chloride gave a mixture of the mono-, di- and trisubstituted compounds instead of the expected disubstituted compound based on the analogous reaction between 2,3-diaminopyrazine and acetylsulfanilyl chloride.

6. Catalytic and iron reductions of the di- and tri-*p*-nitrobenzenesulfonyl derivatives of 2,3-diaminopyrazine were unsuccessful.

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