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THE PREPARATION OF 5-ARYLAZOPYRIMIDINES

by

Frederic Edwin Dutton

A Thesis Presented to the
Faculty of the School of Graduate
Studies in Partial Fulfillment
of the
Degree of Master of Arts

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INTRODUCTION

The synthesis of 5-arylazopyrimidines was undertaken in search of potential anticancer agents.

While many azo compounds have carcinogenic activity, it has been shown by several workers in this area that some 5-arylazopyrimidines have anticancer properties. S. Hibino(1) has shown that p-(2,4,6-triamino-5-pyrimidinylazo)benzene-sulfonic acid shows promise as an effective anticancer agent when used in conjunction with 6-mercaptopurine and adrenal steroidal hormones. When tested clinically, total remission of the leukemic process was achieved in 85.7% of the cases treated. In comparison, total remission was achieved in only 57.5% of the cases treated with a mixture of 6-MP and the hormone which did not include the azopyrimidine.

This paper discusses the synthesis of nine 5-arylazopyrimidines and includes the preliminary results of biological testing performed by Cancer Chemotherapy National Service Center.

HISTORICAL REVIEW

Diazotization

Diazonium salts are prepared from primary aromatic amines by dissolving the amine in an aqueous solution of a mineral acid and slowly adding a solution containing an excess of sodium nitrite while maintaining constant stirring at reduced temperatures.

Griess(2) who discovered the aromatic diazo compounds in 1858 used nitrous gases to diazotize amines and obtained satisfactory results. The current method, described above, which is much more convenient than that due to Griess, was introduced by Martius(3) in 1866.

The stoichiometric equation for diazotization is

$$Ar\text{-}NH_2 + 2HX + NaNO_2 \longrightarrow Ar\text{-}N_2X + NaX + 2H_2O$$

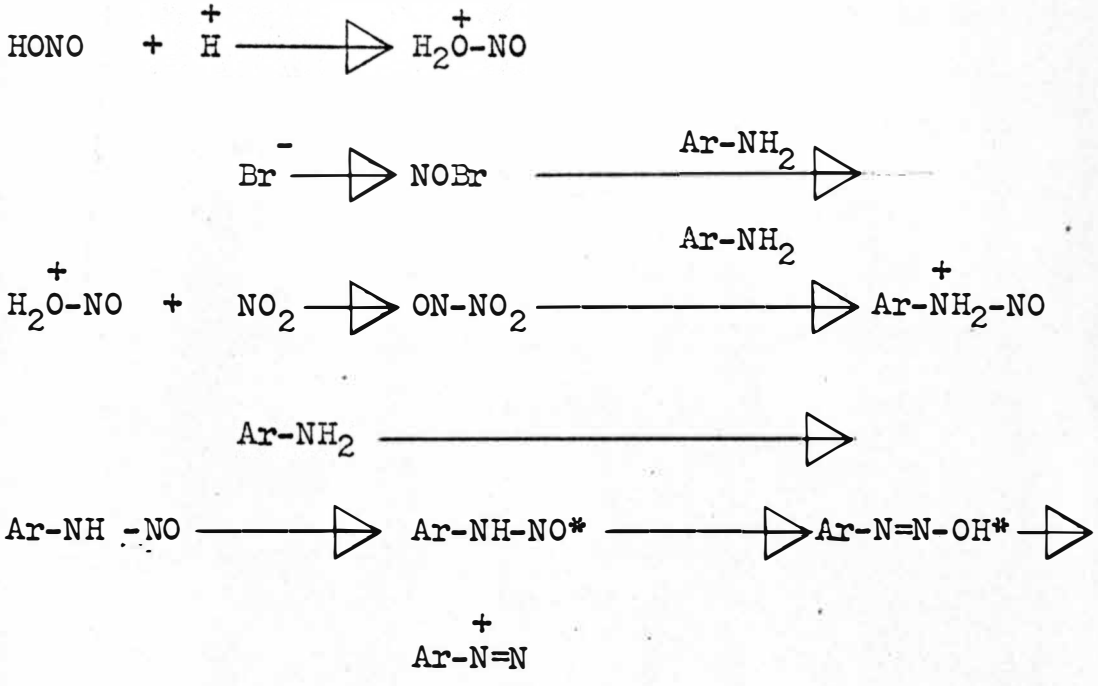
(X = Cl, Br, NO_3, HSO_4, etc.)

The studies carried out by Ridd, Schmid, Kenner and others have shown that the free amine, not the anilinium ion, is the species which is nitrosated. The actual derivative of nitrous acid which is responsible for the formation

of the arylnitrosamine varies with the pH and other conditions of the medium. In dilute acid solution the dinitrogen trioxide mechanism (as shown below) is the most important one and is believed to occur to a limited extent under other conditions.

If nitrosation of the amine were the only consideration, then the rate could be enhanced by decreasing the acidity. However, increasing the acidity enhances the formation of the nitrosating species ($NOBr$, $ON-NO_2$, $H_2\overset{+}{O}\_NO$) and thus it is not possible to make a comprehensive statement regarding the optimum conditions for diazotization.

The currently accepted mechanism is given in the following scheme(4):



*postulated as intermediates, but not proven experimentally

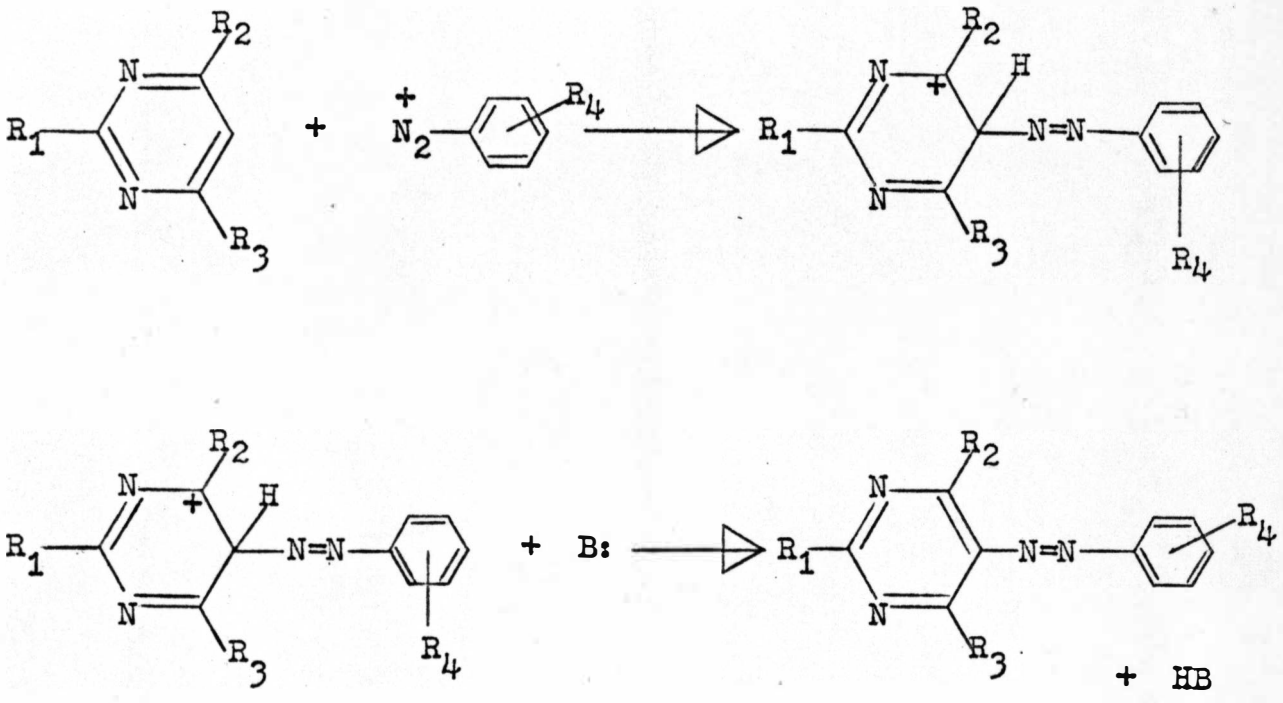
The Coupling Reaction

The diazo-coupling reaction involves an electrophilic substitution by a diazonium ion at either a saturated or unsaturated carbon atom which bears an active hydrogen atom. Such a reaction is often referred to as "C-coupling".

Coupling takes place between diazonium salts and (1) aromatic amines, (2) phenols and phenolic ethers, (3) compounds bearing an active methyl, methylene, or methine group, (4) certain aromatic hydrocarbons and some cyclic or open chain conjugated systems(5). Diazonium salts have also been coupled with aromatic heterocycles, such as pyrimidines(6), imidazoles(7), pyridines(8), pyrroles and indoles(9).

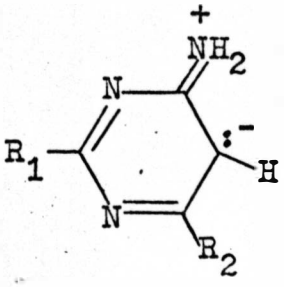
This investigation is concerned only with the coupling of diazonium salts to aromatic heterocycles, specifically pyrimidines, bearing amino and hydroxyl groups, and hence a discussion of this particular type of coupling follows.

The first coupling of a diazonium salt to a pyrimidine was done by Lythgoe(10) and coworkers in 1944. The mechanism has been shown to be a bimolecular electrophilic substitution process as illustrated below:

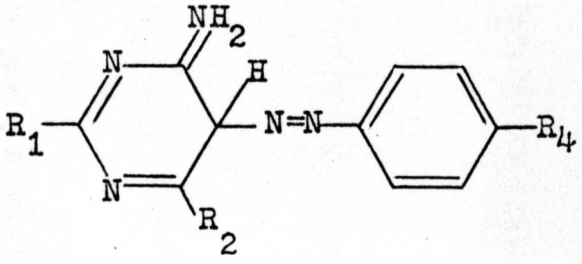


The properties of pyrimidines are governed to a large extent by the electron attracting properties of the two nitrogen atoms. Since they are separated by one carbon atom, each reinforces the electronic effect of the other in the 2-, 4-, and 6-positions and decreases the electron density at these sites. Therefore electrophilic attack is greatly retarded. The 5-position is affected only by the inductive effect of the nitrogens and not directly through resonance. Hence, all electrophilic substitution occurs here. In order for substitution to actually occur it is necessary that the pyrimidine have electron releasing groups in the 2- and 4-positions or the 4- and 6-positions(11).

Suitable groups are amino, hydroxy, thiol, and methylthio. These groups increase the electron density at the 5-position through resonance as shown below for an amino group:



Increased electron density at 5-position of the pyrimidine



Stabilization of the intermediate via resonance: several other resonance reference structures can also be written

EXPERIMENTAL

The analytical data for each compound can be found in the experimental section following the laboratory procedure for that compound and also in Table I. The ultraviolet and infrared spectral data can be found in Tables II and III respectively. Yields are based on the amount of material obtained after one recrystallization. The melting point data are uncorrected.

p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYLAZO)BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (I). p-Aminobenzenesulfonamide (sulfanilamide), 8.6 g. (0.05 mole), was dissolved in 100 ml. of 3 N hydrochloric acid and cooled to -10° to -5° at which time the hydrochloride of the sulfanilamide precipitated. This was diazotized by adding to the solution, slowly and with constant stirring, a solution prepared by dissolving sodium nitrite, 3.5 g. (0.05 mole), in 25 ml. of water and cooled to -10° to -5°. This operation was carried out in an ice-salt bath and the rate of addition was slow enough that the temperature of the reaction did not rise above 0°. The addition of the sodium nitrite solution caused the sulfanilamide hydrochloride to dissolve giving a pale yellow solution of its diazonium salt.

Next, 2,4-diamino-6-hydroxy-5-pyrimidine sulfate monohydrate, 12.1 g. (0.05 mole) was dissolved in 300 ml. of 3 N hydrochloric acid and cooled to about -5° causing a small amount of solid to form at the bottom of the beaker. To this was added, over a period of about 20 minutes, the diazonium salt solution prepared above. During this operation some coupling took place and a small amount of light yellow solid formed. This reaction was carried out in an ice-salt bath so that the temperature of the reaction mixture did not rise above 10°. After stirring one hour at these temperatures the reaction mixture was allowed to warm to room temperature. It was maintained at room temperature for twelve hours with the result that further reaction occurred as evidenced by the formation of a very thick slurry of bright yellow solid.

The azo compound (I) was collected by vacuum filtration and washed on the filter with several small portions of cold water, followed by 95% ethanol after which it was dried in a desiccator over anhydrous calcium chloride giving 17.2 g. of highly hydrated bright yellow powder. This was recrystallized by dissolving it in a boiling solvent mixture consisting of 75% (v/v) of 0.75 N hydrochloric acid and 25% (v/v) of 95% ethanol giving bright yellow needles. After drying for five days over P_2O_5 under vacuum

at 80° to remove the apparently large number of molecules of water of hydration an overall yield of 85% was obtained. Four additional recrystallizations were carried out in preparation of an analytical sample which was then dried as described above. Compound (I) decomposed slowly at 250°.

Anal. Calcd. for $C_{10}H_{11}N_7O_3S \cdot HCl \cdot \frac{1}{2}H_2O$: C, 33.86; H, 3.69; N, 27.64. Found: C, 34.00; H, 3.81; N, 27.32.

p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYLAZO)-N-2-PYRIMIDINYLBENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (II). N_1-(2-Pyrimidinyl)-4-aminobenzenesulfonamide (sulfadiazine), 12.5 g. (0.05 mole), was dissolved in 130 ml. of 3 N hydrochloric acid. This was cooled to -10° which caused the hydrochloride to precipitate. This was diazotized in the manner described for the preceding preparation in which sodium nitrite solution caused the sulfadiazine hydrochloride to dissolve giving a pale yellow solution of its diazonium salt.

2,4-Diamino-6-hydroxypyrimidine sulfate monohydrate, 12.1 g. (0.05 mole), was dissolved in 200 ml. of 3 N hydrochloric acid and cooled to about -10°. The diazonium salt solution prepared above was added to the pyrimidine solution over a time interval of about 20 minutes.

To prevent the temperature from rising above 10° the reaction mixture was placed in an ice-salt bath. When addition of the diazonium salt solution was complete, a

small amount of pale yellow solid began to separate from the solution. After stirring for one hour at about 0° the reaction mixture was placed in a refrigerator at that temperature for twelve hours. During this time a dark-yellow precipitate formed which was subsequently collected by filtration under vacuum, washed with several small portions of water followed by 30 ml. of 95% ethanol, and dried in a desiccator over anhydrous calcium chloride. A yield of 25.3 g. of crude material containing a large number of molecules of water of hydration was obtained. Compound (II) was recrystallized from a solvent mixture consisting of 75% (v/v) of 0.75 N hydrochloric acid and 25% (v/v) of 95% ethanol. This was accomplished by adding the compound to the boiling solvent in small portions. Dark yellow needles were obtained after one recrystallization. The highly hydrated material was dried in the manner used for compound (I) giving an overall yield of 83%. An analytical sample was prepared from material which had been recrystallized four times and dried as above. This melted with decomposition at 275°.

Anal. Calcd. for $C_{14}H_{13}N_9O_3S \cdot HCl \cdot \frac{1}{2}H_2O$: C, 38.85; H, 3.49; N, 29.12. Found: C, 39.04; H, 3.64; N, 29.01.

p-(4-AMINO-6-HYDROXY-2-METHYLTHIO-5-PYRIMIDINYLAZO)-BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (III). Sulfa-

nilamide, 8.6 g. (0.05 mole) was dissolved in 100 ml. of 3 N hydrochloric acid and diazotized with sodium nitrite in the usual manner. This solution, at -10° to -5°, was added over a 20 minute time interval to 4-amino-6-hydroxy-2-methylthiopyrimidine, 8.8 g. (0.05 mole), which had been previously suspended in 200 ml. of 3 N hydrochloric acid and cooled to below -5°. The reaction mixture was placed in an ice-salt bath in order to prevent the temperature from rising above 10°. A thick, yellow substance formed in the reaction mixture during addition of the diazonium salt solution. Stirring was maintained for one hour after addition at about 0° during which time the solid matter turned orange and settled to the bottom of the reaction flask leaving a dark green supernatant liquid. The orange solid was vacuum filtered, washed with water followed by 95% ethanol, and air dried. A yield of 13.9 g. of crude material was obtained. The product was recrystallized by adding it in small portions to boiling 95% ethanol followed by several drops of 37% hydrochloric acid until complete solution was obtained. This solution yielded bright orange needles of compound (III) in an overall yield of 75%. An analytical sample was prepared from material which had undergone two additional recrystallizations and dried for 24 hours in the manner described for compound (I). Compound (III) decomposed without melting at 215°.

Anal. Calcd. for $C_{11}H_{12}N_6O_3S_2 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 35.06; H, 3.74; N, 22.30. Found: C, 35.54; H, 3.75; N, 22.67.

p-(2,4,6-TRIAMINO-5-PYRIMIDINYLAZO)BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (IV). Sulfanilamide, 8.6 g. (0.05 mole), was dissolved in 100 ml. of 3 N hydrochloric acid and the temperature of the solution reduced to -10° to -5°. Diazotization of the sulfanilamide was carried out in the usual manner with a cold aqueous sodium nitrite solution. 2,4,6-Triaminopyrimidine, 6.2 g. (0.05 mole), was dissolved in 200 ml. of 3 N hydrochloric acid and cooled to less than -5°. The diazonium salt solution was added to the solution of the pyrimidine with constant mechanical stirring of the reaction mixture and at such a rate that the temperature of the reaction mixture did not rise above 10° at any time during the addition. The reaction mixture was stirred for an additional 15 minutes and then placed in a refrigerator at 0°. Precipitation began two hours later. Further coupling occurred during the 14 hours of refrigeration at 0° as indicated by the large bulk of yellow solid material which was deposited during that time interval.

The azo compound (IV) was removed from the supernatant liquid by vacuum filtration, washed with several small portions of water followed by 95% ethanol and air dried. A second smaller amount of the product was obtained by placing the filtrate in a refrigerator for an additional five hours.

In all 17.7 g. of crude material was obtained.

Azo compound (IV) was recrystallized from a solvent mixture consisting of 75% (v/v) of 0.75 N hydrochloric acid and 25% (v/v) of 95% ethanol yielding bright yellow needles for an overall yield of 88%. An analytical sample was prepared from material which had been recrystallized four additional times and dried for five days in the manner described for compound (I). Compound (IV) decomposed with melting at 315°.

Anal. Calcd. for $C_{10}H_{12}N_8O_2S \cdot HCl \cdot \frac{1}{2}H_2O$: C, 33.95; H, 3.99; N, 31.67. Found: C, 33.69; H, 4.10; N, 31.88.

p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYLAZO)BENZENEARSONIC ACID HEMIHYDRATE (V). p-Aminobenzenearsonic acid (arsanilic acid), 10.9 g. (0.05 mole), was dissolved in 100 ml. of 1 N hydrochloric acid to give the very soluble hydrochloride which could not be precipitated even at -7°. The arsanilic acid was diazotized by adding to its solution at -7° to -5° a solution of sodium nitrite, 3.5 g. (0.05 mole), which had been previously chilled to below -5°. This operation was carried out with constant mechanical stirring and at such a rate that the temperature of the reaction mixture was maintained below 0°. At the end of the addition the solution was pale yellow.

2,4-Diamino-6-hydroxypyrimidine sulfate monohydrate, 12.1 g. (0.05 mole), was dissolved in 200 ml. of 3 N hydro-

chloric acid and cooled to below 0°. This caused a small amount of the pyrimidine which had initially dissolved to reprecipitate. The diazonium salt solution was added to the pyrimidine solution at a carefully controlled rate and with constant mechanical stirring in order that the temperature of the reaction mixture remained below 10°. The reaction mixture was placed in a refrigerator at about 2° for twelve hours during which time a yellow-orange solid was deposited. The product was separated from the supernatant liquid by vacuum filtration. The product was then washed with several portions of water followed by 95% ethanol and air dried. A yield of 20.7 g. (97%) of crude compound was obtained.

Azo compound (V) was recrystallized by adding it in small portions to boiling water. The suspension which was initially formed was dissovled by adding a few drops of 6 N hydrochloric acid. More product could then be added and the process repeated until the solution was nearly saturated. On cooling, fine orange-brown needles were deposited. After one recrystallization an overall yield of 80% was obtained. In preparation of an analytical sample two additional recrystallizations were performed. The sample was dried for 163 hours according to the procedure described for compound (I). Compound (V) decomposed slowly without melting at 200° but does not melt below 330°.

Anal. Calc. for $C_{10}H_{11}N_6O_4As \cdot \frac{1}{2}H_2O$: C, 33.07; H, 3.33; N, 23.14; H_2O, 2.48; Cl, 0.00. Found: C, 33.27; H, 3.53; N, 22.76; H_2O, 1.56; Cl, 0.08.

p-(4,6-DIAMINO-2-(METHYLTHIO)-5-PYRIMIDINYLAZO)BENZENEARSONIC ACID (VI). p-Arsanilic acid, 10.9 g. (0.05 mole), was dissolved in 100 ml. of 3 N hydrochloric acid and the temperature lowered to -5°, but the hydrochloride did not precipitate. The p-aminoarsanilic acid was diazotized in the same manner as in the case of compound (V). 4,6-Diamino -2 methylthiopyrimidine, 7.8 g. (0.05 mole), was dissolved in 200 ml. of 3 N hydrochloric acid which precipitated as the hydrochloride on cooling to -5°.

The diazonium salt solution was added to the pyrimidine over a period of 15 minutes. Continuous mechanical stirring was maintained during the addition and the process was carried out in an ice-salt bath to prevent the temperature from rising above 10°. Stirring was continued for one hour at these reduced temperatures after which the reaction mixture was placed in a refrigerator for 72 hours during which time the solid turned a darker shade of yellow and much more solid was deposited. This was filtered under vacuum, washed with water followed by 95% ethanol and then air dried giving 8.2 g. (42%) of orange-yellow powder.

In the first recrystallization compound (VI) was added

in small portions to boiling 95% ethanol and a clear solution was obtained. Precipitation was induced by slowly adding approximately an equal volume of water and allowing the solution to cool to room temperature over a twelve hour period. Fine yellow-orange needles were obtained in an overall yield of 36%.

On further recrystallization, it was necessary to add 25% (v/v) water to the ethanol in order to affect solution; sometimes solution could be aided by adding a few drops of 37% hydrochloric acid. Again precipitation was obtained by allowing the solution to cool to room temperature over a period of several hours. In the preparation of an analytical sample, three such recrystallizations were carried out. The sample was dried for 142 hours under vacuum at 80° over P_2O_5. Compound (VI) melted with decomposition at 317°.

Anal. Calcd. for $C_{11}H_{13}N_6O_3SAs$: C, 34.38; H, 3.41; N, 21.87; H_2O, 0.00; Cl, 0.00. Found: C, 34.51; H, 3.60; N, 21.63; H_2O, 0.00; Cl, 0.18.

N_1-(2-THIAZOLYL)-p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYLAZO)BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (VII). N_1-(2-Thiazolyl)-p-aminobenzenesulfonamide (sulfathiazole), 12.8 g. (0.05 mole), was suspended in 150 ml. of water. Enough 37% hydrochloric acid (about 11 ml.) was added to

just bring about complete solution of the sulfathiazole. The solution was then cooled to -5° to 0° causing the hydrochloride to deposit. Since this was an extremely heavy precipitate, 50 ml. of water was added causing a large portion of the hydrochloride to redissolve. Sodium nitrite, 3.8 g. (0.055 mole), was dissolved in 30 ml. of water and cooled to -5°. This was added over a 20 minute period to the sulfathiazole which resulted in the simultaneous formation of the diazonium salt. The diazonium salt solution contained a very large amount of thick yellow solid at this point.

2,4-Diamino-6-hydroxypyrimidine, 12.1 g. (0.05 mole), was suspended in 200 ml. of 3 N hydrochloric acid and cooled to 0° to +5°. To this was added all at once the diazonium salt solution. Stirring was maintained until room temperature was reached and then allowed to stand at room temperature for twelve hours. The yellow solid material, which was only slightly darker in color than the solid obtained upon diazotization, was vacuum filtered, washed with two 20 ml. portions of water and four 20 ml. portions of ethanol and air dried, giving 24.6 g. of highly hydrated yellow powder.

Compound (VII) was recrystallized by adding it in small portions to boiling water. The solution was made clear by adding several drops of 37% hydrochloric acid after each portion of compound had been added. After one

recrystallization followed by the drying procedure used for compound (I) to remove the large number of molecules of water of hydration, an overall yield of 80% was obtained. Compound (VII) decomposed without melting over a range from 240° to 250°. Three additional recrystallizations were carried out in the preparation of an analytical sample.

Anal. Calcd. for $C_{13}H_{11}N_8O_3S_2 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 35.66; H, 3.22; N, 25.59; Cl, 8.10. Found: C, 35.59; H, 3.40; N, 25.46; Cl, 8.03.

N-(2-THIAZOLYL)-p-(4-AMINO-6-HYDROXY-2-METHYLTHIO-5-PYRIMIDINYLAZO)BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (VIII). Sulfathiazole, 12.8 g. (0.05 mole), was suspended in 150 ml. of water. Enough 37% hydrochloric acid (about 11 ml.) was added to bring about complete solution. Upon cooling to 0° the hydrochloride began depositing. Stirring for a very short time caused such extremely heavy precipitation that all the solvent was taken up by the salt. Fifty milliliters of water was then added and the mixture stirred until nearly homogeneous. It was then returned to the refrigerator and the temperature reduced to about 0°; more of the solid redissolved during this period. Sodium nitrite, 3.5 g. (0.05 mole), was dissolved in 25 ml. of water. After reducing the temperature to -5° this solution was added with constant

stirring to the sulfathiazole hydrochloride. The temperature was maintained at -5° to 0° throughout this operation. During the addition of the sodium nitrite solution a very dense yellow precipitate formed.

4-Amino-6-hydroxy-2-methylthiopyrimidine, 8.8 g. (0.05 mole), was suspended in 200 ml. of 3 N hydrochloric acid and cooled to about 0°. The diazonium salt mixture prepared above was slowly added to the pyrimidine with constant stirring. The reaction mixture was stirred for one hour at -5° to 0° after which it was allowed to warm to room temperature; stirring was then maintained for an additional hour. The reaction mixture was vacuum filtered, washed with five 25 ml. portions of water followed by one 25 ml. portion of ethanol and air dried giving 35 g. of orange-brown powder. Azo compound (VIII) was recrystallized by adding it in small portions to boiling ethanol followed by a few drops of 37% hydrochloric acid until complete solution was obtained. After one recrystallization followed by vigorous drying for 24 hours in the manner described for compound (I), an overall yield of 21.2 g. (49%) of red-orange needles was obtained.

Two additional recrystallizations were carried out in preparation of an analytical sample. Compound (VIII) melted with decomposition at 230°.

Anal. Calc. for $C_{14}H_{13}N_7O_3S_3 \cdot HCl \cdot \frac{1}{2}H_2O$: C, 35.85; H, 3.22; N, 20.91. Found: C, 36.13; H, 3.43; N, 20.80.

p-(4-AMINO-6-HYDROXY-2-(METHYLTHIO)-5-PYRIMIDINYL-AZO)-N_1-2-PYRIMIDINYLBENZENESULFONAMIDE HYDROCHLORIDE (IX). Sulfadiazine, 12.5 g. (0.05 mole), was dissolved in 150 ml. of 3 N hydrochloric acid and cooled to -10°. This caused the sulfadiazine hydrochloride to precipitate. To this was added an aqueous solution of sodium nitrite, 3.5 g. (0.05 mole), with the formation of the diazonium salt of sulfadiazine. 4-Amino-6-hydroxy-2-methylthiopyrimidine, 8.8 g. (0.05 mole), was suspended in 200 ml. of 3 N hydrochloric acid and the temperature lowered to -10°. The diazonium salt solution prepared above was slowly added to the pyrimidine with constant stirring, the temperature of the reaction mixture being maintained below 0° with the aid of an ice-salt bath. After stirring an additional hour at these temperatures, the reaction mixture was placed in a refrigerator at 0° for a twelve hour period. A yellow solid was formed very slowly and had a dark green supernatant liquid.

The yellow solid was removed from the liquid by vacuum filtration, washed with several small portions of water, followed by ethanol and air dried, giving 18.5 g. (88%) of orange powder. Compound (IX) decomposed without melting at 280°.

Anal. Calcd. for $C_{15}H_{14}N_8O_3S_2 \cdot HCl$: C, 39.60; H, 3.32; N, 24.63. Found: C, 39.61; H, 3.42; N, 24.23.

Attempted preparation of N_1-(p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYLAZO)PHENYLSULFONYL)ACETAMIDE (X). Sulfanilylacetamide, 10.7 g. (0.05 mole), was diazotized and added to 2,4-diamino-6-hydroxypyrimidine sulfate monohydrate, 12.1 g. (0.05 mole), using essentially the same procedure as for the other compounds. This resulted in the formation of 20.0 g. (air dried) of compound (I) from an unexpected hydrolysis of the N_1-acetyl group.

The product was recrystallized from a solvent mixture consisting of 50% (v/v) 1N hydrochloric acid, 25% (v/v) water, and 25% (v/v) of 95% ethanol. It was then dried for 240 hours according to the procedure used for compound (I). After one recrystallization, 12.5 g. (70%) of compound (I) was obtained. Four additional recrystallizations were performed in preparation of an analytical sample.

The identity of this compound was revealed by a comparison of the microanalytical data, visible-ultraviolet and infrared absorption data.

Anal. Calc. for $C_{12}H_{13}N_7O_4S \cdot HCl \cdot \frac{1}{2}H_2O$: C, 36.32; H, 3.81; N, 24.71. Found: C, 33.86; H, 3.66; N, 27.01. Calcd. for $C_{10}H_{11}N_7O_3S \cdot HCl \cdot \frac{1}{2}H_2O$: Compound (I): C, 33.86; H, 3.69; N, 27.64.

Degradation of p-(2,4-DIAMINO-6-HYDROXY-5-PYRIMIDINYL-AZO)BENZENESULFONAMIDE HYDROCHLORIDE HEMIHYDRATE (Compound (I)).

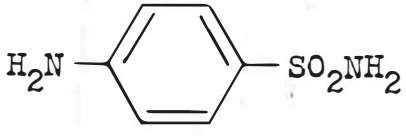
p-Aminobenzenesulfonamide (XI). p-(2,4-Diamino-6-hydroxy-5-pyrimidinylazo)benzenesulfonamide (I), 4.01 g. (0.05 mole), was partially dissolved in 100 ml. of boiling water contained in a 250 ml. Erlenemeyer flask. To this yellow suspension was added an excess of sodium dithionite, 10 g., in small portions followed by vigorous swirling of the reaction mixture after each addition until complete solution was obtained and no yellow colored solution could be detected. After boiling an additional five minutes the solution was cooled in an ice bath at 10° at which time a white precipitate was formed. This material was collected by vacuum filtration and washed on the filter with a few milliliters of cold water. The wet material was recrystallized from hot water, and air dried, giving 1.95 g. of white platelets which had a melting point range of 164° - 166°. This material was identified as sulfanilamide by a comparison of its infrared absorption spectrum with that of known sulfanilamide and by the fact that a mixture of the unknown material with an authentic sample of sulfanilamide gave no depression of the melting point on heating. A yield of 77% was obtained.

4-HYDROXY-2,5,6-TRIAMINOPYRIMIDINE SULFATE DIHYDRATE (XII). p-(2,4-Diamino-6-hydroxy-5-pyrimidinyl)azobenzene-

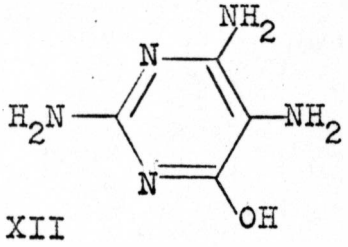
sulfonamide, 4.38 g. (0.012 moles), was treated in the same manner as described above. After boiling the clear solution for five minutes, it was allowed to cool to 40° at which time 100 ml. of 10% (v/v) sulfuric acid was added in one portion causing the formation of an off-white precipitate. The mixture was allowed to cool to room temperature during which time the crystals grew much larger. The mixture was vacuum filtered, washed with water on the filter. The wet crystals were recrystallized from 10% (v/v) sulfuric acid. After filtering and air drying, 2.46 g. of light yellow crystals were obtained. A comparison of the ultraviolet and infrared absorption data of this compound with that of known 4-hydroxy-2,5,6-triaminopyrimidine sulfate dihydrate showed them to be identical. An overall yield of 74% was obtained.

Structure Determination

In order to establish the structure of the above azo compounds a sodium dithionite reduction was performed on compound (I). The experimental details are given in the experimental section. The principal products of this reduction are 4-hydroxy-2,5,6-triaminopyrimidine sulfate dihydrate and sulfanilamide as shown below:



XI

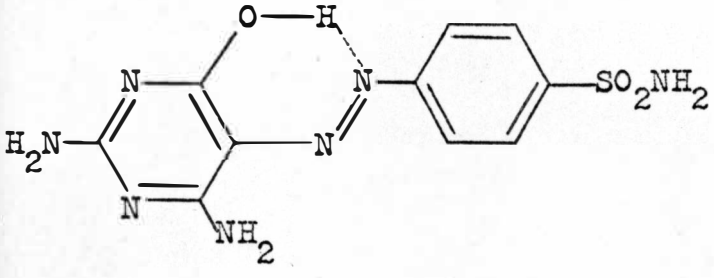


XII

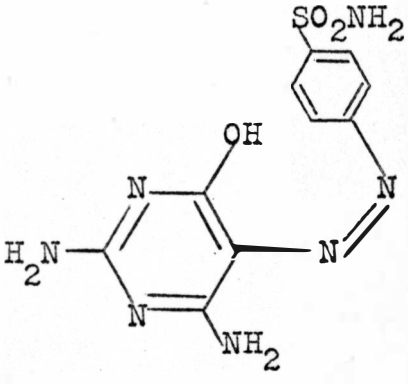
Product (XI) exhibited an identical infrared spectrum with known sulfanilamide. A mixture of sulfanilamide and compound (XI) caused no depression of the melting point. A comparison of the infrared and ultraviolet absorption data of product (XII) with that obtained on an authentic sample of 4-hydroxy-2,5,6-triaminopyrimidine sulfate dihydrate suggests that this product was the pyrimidine indicated above.

It is unreasonable that compound (I) could have a structure differing from the one assigned and lead to the products obtained on reductive cleavage. The structures assigned to compounds (I) - (X) can be found in Table II.

With regard to geometric isomers, it is believed that all the azo compounds discussed above exist wholly in the more stable trans form. According to Zollinger (38) the search for isomers of o-hydroxyazo and o-amino-azo compounds has been in vain. In addition to steric effects which make the trans isomer more stable than the cis isomer, hydrogen bonding between these substituents and the β—azo-nitrogen is thought to be a very important factor in the relative stability of the two isomers as shown below:



Trans isomer
Hydrogen bonding possible



Cis isomer
Cannot hydrogen bond

On this basis the trans form is suggested for azo compounds (I) through (IX).

The analytical data obtained on these compounds is summarized in Tables I - III

SUMMARY OF EXPERIMENTAL RESULTS

In the coupling reaction the solution of reactants were mixed below 10° because of the apparent instability of many of the diazonium salts used. Coupling took place at these low temperatures as evinced by the formation of yellow solid, although much more coupling occurred during the period of warming to room temperature.

An attempt to couple the diazonium salt of sulfanilamide to a pyrimidine bearing a 4-methyl substituent in place of the usual amino or hydroxy group failed. This failure emphasizes the necessity of having powerful electron releasing groups in the 4- and 6-positions when trying to couple under acidic conditions.

The experimental method used here gave yields which varied from 36% to 88%. These yields are based upon the weight of material obtained after one recrystallization followed by vigorous drying. It is believed that many of these reactions were nearly quantitative, since much material was lost on recrystallization. A summary of the yields obtained is given in Table IV.

Micro-analyses were performed by Galbraith Laboratories, Inc. (See Table I).

Visible-ultraviolet absorption data was obtained from

solutions prepared by dissolving the compound in 95% ethanol. The high intensity absorptions at about 400 mu is typical of highly conjugated aromatic systems. (See Table II).

Infrared absorption data were obtained from the Nujol mull of the compound. This was not found to very useful in characterizing arylazopyrimidines since the -N=N- band is of low intensity and probably arises in the 1600 cm^{-1} region where aromatic absorptions occur. (See Table III). The absence of a band in the 3700-3500 cm^{-1} region of the spectrum which would arise from the presence of a hydroxy group indicates that in the solid phase (mineral oil mull) these hydroxy pyrimidines probably exist in the tautomeric keto form. This is in agreement with the work done by Short and Thompson(13) and Brown(14).

The melting points with the exception of compound XI are uncorrected. All compounds decomposed on heating with the exception of XI. (See Table V).

All analytical data were obtained from material which had been recrystallized several times from an appropriate solvent and dried over phosphorous pentoxide at about 80° under vacuum for several hours.

BIOLOGICAL ACTIVITY

Compounds I - IX are currently being screened for biological activity by the Cancer Chemotherapy National Service Center. The following compounds have shown some presumptive anticancer activity:

1. p-(2,4-Diamino-6-hydroxy-5-pyrimidinylazo)-N-(2-pyrimidinyl)benzenesulfonamide hydrochloride hemihydrate (II).

   Active in P-1798 Lymphosarcoma.

2. p-(2,4-Diamino-6-hydroxy-5-pyrimidinylazo)benzenearsonic acid hemihydrate (V).

   Active in Walker-256.

3. N_1-(2-Thiazolyl)-p-(4-amino-6-hydroxy-2-methylthio-5-pyrimidinylazo)benzenesulfonamide hydrochloride hemihydrate (VIII).

   Active in Sarcoma-180.

4. N_1-(2-Pyrimidinyl)-p-(4-amino-6-hydroxy-2-methylthio-5-pyrimidinylazo)benzenesulfonamide hydrochloride hemihydrate (VIII).

   Active in Lewis Carcinoma.

The biological mode of action of these compounds is not understood.

TABLE I

MICROANALYSES

Compound	Formula	Analysis	% Carbon	% Hydrogen	% Nitrogen	% Chlorine	% Water
I	$C_{10}H_{11}N_7O_3S \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	33.86	3.69	27.64	-	-
		Found	34.00	3.81	27.32	-	-
II	$C_{14}H_{13}N_9O_3S \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	38.85	3.49	29.12	-	-
		Found	39.04	3.64	29.01	-	-
III	$C_{11}H_{12}N_6O_3S_2 \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	35.06	3.74	22.30	-	-
		Found	35.54	3.75	22.67	-	-
IV	$C_{10}H_{12}N_8O_2S \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	33.95	3.99	31.67	-	-
		Found	33.69	4.10	31.88	-	-
V	$C_{10}H_{11}N_6O_4As \cdot \frac{1}{2}H_2O$	Calcd.	33.07	3.33	23.14	0.00	2.48
		Found	33.27	3.53	22.76	0.08	1.56
VI	$C_{11}H_{13}N_6O_3SAs$	Calcd.	34.38	3.41	21.87	0.00	0.00
		Found	34.51	3.60	21.63	0.18	0.00
VII	$C_{13}H_{11}N_8O_3S_2 \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	35.66	3.22	25.59	8.10	-
		Found	35.59	3.40	25.46	8.03	-

TABLE I

Continued

Compound	Formula	Analysis	Carbon	Hydrogen	Nitrogen	Chlorine	Water
VIII	$C_{14}H_{13}N_7O_3S_3 \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	35.85	3.22	20.91	-	-
		Found	36.13	3.43	20.80	-	-
IX	$C_{15}H_{14}N_8O_3S_2 \cdot HCl$	Calcd.	39.60	3.32	24.63	-	-
		Found	39.61	3.42	24.23	-	-
X	$C_{10}H_{11}N_7O_3S \cdot HCl \cdot \frac{1}{2}H_2O$	Calcd.	36.32	3.81	24.71	-	-
		Found*	33.86	3.66	27.01	-	-

* Compare with the data for compound (I).

TABLE II
VISIBLE ULTRAVIOLET
SPECTRAL DATA

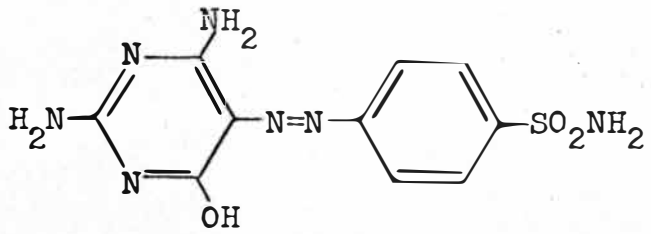
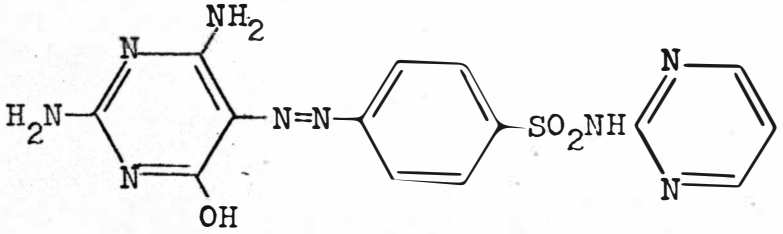
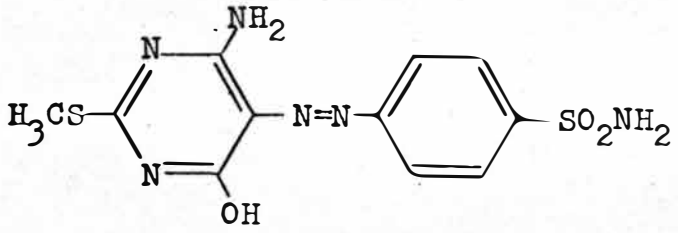
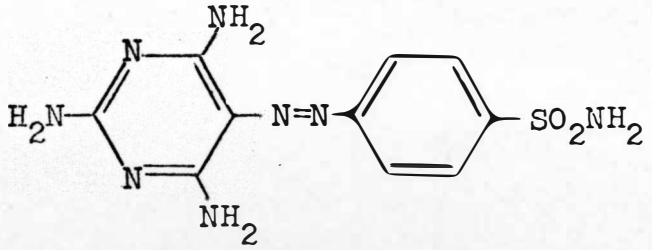
Compound	Structure	λ_{max}	ϵ_{Max}	λ_{min}	ϵ_{Min}
I		391 mu	25,200	293 mu	2,330
		240	12,180	221	7,720
		207	18,440	-	-
II		390	25,100	305	2,950
		242	19,100	231	18,000
		208	30,300	-	-
III		392	24,000	307	2,330
		240	14,800	222	10,800
IV		388	30,200	286	2,470
		251	16,900	228	9,140

TABLE II
Continued

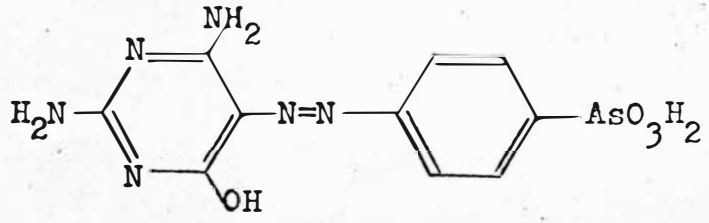
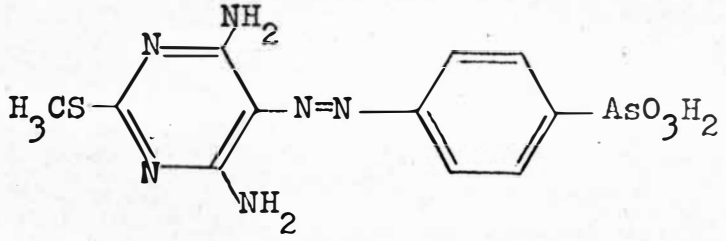
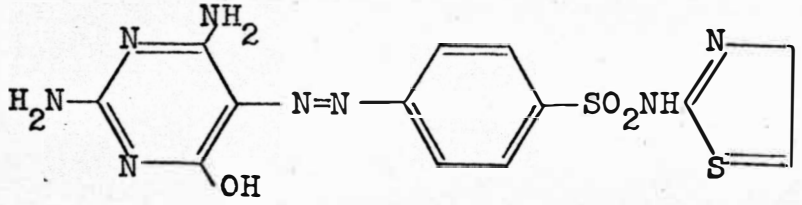
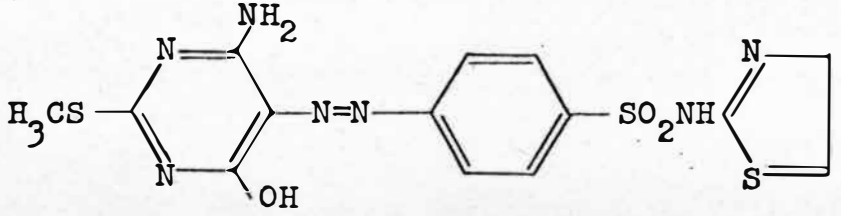
Compound	Structure	λ_{max}	ϵ_{Max}	λ_{min}	ϵ_{Min}
V		400 mu	40,700	286 mu	4,130
		212	12,600	202	10,600
VI		379	30,600	310	3,470
		284	4,950	-	-
		249	14,600	232	10,100
VII		401	32,200	311	5,440
		275	15,100	260	13,800
		241	14,700	223	11,300
VIII		393	26,800	318	2,400
		262	14,600	-	-
		243	17,000	226	11,200

TABLE II
Continued

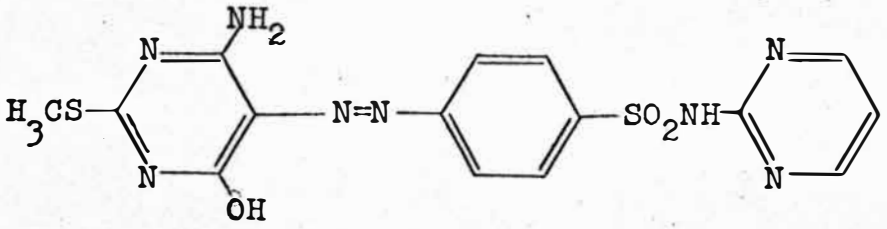
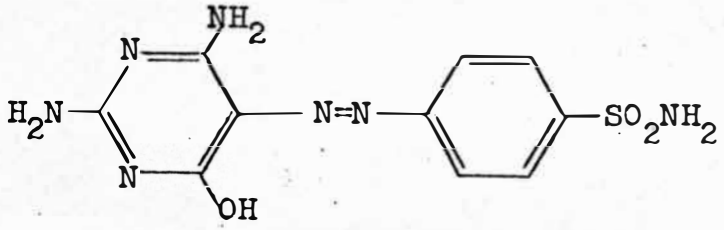
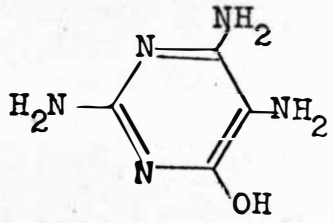
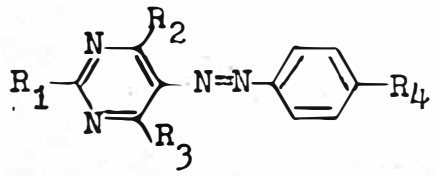
Compound	Structure	λ_{max}	ϵ_{Max}	λ_{min}	ϵ_{Min}
IX		389 mu	30,400	300 mu	2,370
		241	15,800	-	-
		218	19,900	-	-
X		391	25,100	293	2,280
		240	12,100	221	7,680
		207	18,350	-	-
XII		266	104	238	46
		208	222	-	-

TABLE III

Infrared Spectral Data



Compound	Substituents	Assignments (in $cm.^{-1}$) Amino or Lactam*	Sulfonamido
I	$R_1 = NH_2$, $R_2 = NH_2$	3100(S)*	1310(S)
	$R_3 = OH$, $R_4 = SO_2NH_2$		1145(S)
II	$R_1 = NH_2$, $R_2 = NH_2$	3300(W)	1320(M)
	$R_3 = OH$, $R_4 = SO_2NHC_4H_3N_2$	3100(M)*	1158(M)
III	$R_1 = CH_3S$, $R_2 = NH_2$	3200(M)*	1300(S)
	$R_3 = OH$, $R_4 = SO_2NH_2$		
IV	$R_1 = NH_2$, $R_2 = NH_2$	3300(M)	1300(S)
	$R_3 = NH_2$, $R_4 = SO_2NH_2$	3150(M)*	1147(S)

S = strong; M = medium; W = weak (Refer to relative intensity of the absorption)

Compound	Substituents	Assignments (in cm^{-1}) Amino or Lactam*	Sulfonamido
V	$R_1 = NH_2$, $R_2 = NH_2$	3380(M)	
	$R_3 = OH$, $R_4 = AsO_3H_2$		
VI	$R_1 = CH_3S$, $R_2 = NH_2$	3400(M)	
	$R_3 = NH_2$, $R_4 = AsO_3H_2$	3250(M)	
VII	$R_1 = NH_2$, $R_2 = NH_2$		1310(S)
	$R_3 = OH$, $R_4 = SO_2NHC_3H_2NS$		1130(S)
VIII	$R_1 = CH_3S$, $R_2 = NH_2$	3500(M)	1320(M)
	$R_3 = OH$, $R_4 = SO_2NHC_3H_2NS$	3200(M)*	1135(S)
IX	$R_1 = CH_3S$, $R_2 = NH_2$	3475(W)	1330(S)
	$R_3 = OH$, $R_4 = SO_2NHC_4H_3N_2$	3300(W)	1154(S)
X	$R_1 = NH_2$, $R_2 = NH_2$	3100(S)*	1310(S)
	$R_3 = OH$, $R_4 = SO_2NH_2$		1145(S)
XI	p-Aminobenzenesulfonamide	3350(M)	1300(S)
		3260(M)	1140(S)
			3480(M)

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VITA

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