action mixture was cooled until it solidified, then heated with a small amount of alcohol to remove any resinous materials. The yellowish reaction product was collected after cooling, washed several times with alcohol and crystallized from benzene as fluffy yellowish needles which exhibited strong fluorescence in solution.

With heliotropin the reaction was better carried out by fusing the reactants together in the direct flame for a few minutes. The reaction mixture was cooled, a small amount of alcohol was added, and the mixture was heated gently and cooled again. The precipitated yellowish material was collected and purified as described above.

The yields in all cases were almost quantitative, and the products were fairly soluble in acetone, glacial acetic acid and nitrobenzene from which they can also be crystallized. The solubility of the products in alcohol and petroleum ether was very limited. The analyses and some other properties are recorded in Table I.

Attempted Reactions of I with Aromatic Aldehydes in the Presence of Solvents and Basic Catalysts.—A pure sample of aminophenoxazone (I) was covered with the aldehyde and refluxed for 5 hours in excess alcohol containing a few drops of piperidine or dimethylaniline. The reaction mixture was cooled, the product was collected and crystallized from alcohol as red needles, m.p. and mixed m.p. with 3-aminophenoxazone-2 249°.

In the above experiment the alcohol could be replaced by benzene, decalin, dioxane, nitrobenzene or acetic acid with the same result.

Interaction of Aliphatic Aldehydes with I.—(a) A pure sample of the aminophenoxazone (I) was covered with acetaldehyde or propionaldehyde and allowed to reflux for one hour in an oil-bath. The reaction product was collected, washed with alcohol and crystallized also from alcohol as red needles, m.p. and mixed m.p. with 3-aminophenoxazone-2, 249°.

(b) Five-tenths gram of the aminophenoxazone (I) was suspended in 10 cc. of propionaldehyde in a sealed tube and placed in an electric furnace at 120° for 12 hours. The tube was cooled, opened and the contents were collected. The excess aldehyde was removed leaving a resinous material from which no pure substance could be obtained.

Action of Concd. Hydrochloric Acid on 2-Phenyl-5*H*-oxazolo[4,5-*b*]phenoxazine.—One gram of the substance was heated with 10 cc. of concd. hydrochloric acid for a few minutes. The yellow material changed readily to reddish-brown, then was collected and washed with few cc. of concd. hydrochloric acid. The product could not be crystallized as it was readily oxidized in the air. It was identified as 2 hydroxy-3-aminophenoxazone hydrochloride (III) since it dissolved readily in water, gave a positive Beilstein test for chlorine, precipitated silver chloride when treated with silver nitrate solution and on acetylation a colorless diacetate was given which was identical to that obtained by reductive acetylation of aminophenoxazone (I).

Preparation of 2-Phenyl-5*H*-oxazolo(4,5-*b*)phenoxazine from 2-Hydroxy-3-aminophenoxazine Hydrochloride and Benzaldehyde.—A sample of 2-hydroxy-3-aminophenoxazine hydrochloride obtained from the above experiment was covered with benzaldehyde and heated in the direct flame for a few minutes. The reaction product was collected, washed with alcohol and crystallized from benzene as golden yellow needles, m.p. 275°, undepressed when mixed with a pure sample obtained from 3-aminophenoxazone-2 and benzaldehyde as described before.

2-Phenyl-5-benzyloxazolo(4,5-*b*)phenoxazine (VIII).—(a) One gram of 3-aminophenoxazine-2 was covered with 6 g. of benzyl chloride and the mixture was refluxed on the direct flame for about 1.5 hours. The excess benzyl chloride was removed and the reaction mixture on cooling deposited a yellow solid which was collected, washed with a few drops of benzene and crystallized from the same solvent as light yellow needles of 2-phenyl-5-benzyl-oxazolo[4,5-*b*]phenoxazine, m.p. 246°. The substance exhibited a bluish-green fluorescence in benzene and a violet fluorescence in concd. sulfuric acid.

(b) 2-Phenyl-5*H*-oxazolo(4,5-*b*)phenoxazine (II, R = C_6H_5) was refluxed in a slight excess of benzyl chloride in the direct flame for 3 hours. The excess benzyl chloride was removed and the reaction mixture was cooled. The precipitated yellow product was collected, washed with a small amount of benzene and crystallized from the same solvent as light yellow needles, m.p. 246°, undepressed when mixed with a sample of 2-phenyl-5-benzyloxazolo[4,5-*b*]phenoxazine prepared in the previous experiment.

The N-benzyl derivatives of the 2-anisyl- and the 2-salicyloxazolo-phenoxazines were prepared similarly.

Analytical results of the N-benzyl derivatives and some other properties are summarized in Table II.

Attempted Reaction of 3-Aminophenoxazine-2 (I) with Benzyl Cyanide.—A pure sample of the aminophenoxazone (I) was covered with benzyl cyanide and the reaction was carried out as described for benzyl chloride. After isolating the reaction product, it proved to be unchanged material, m.p. and mixed m.p. 249°.

ASSIUT, EGYPT, U. A. R.

[CONTRIBUTION FROM THE ROLLIN H. STEVENS MEMORIAL LABORATORY OF THE DETROIT INSTITUTE OF CANCER RESEARCH]

5-Sulfanilamidotetrazole[1]

BY HANNA KOVACS NAGY,[2] ARTHUR J. TOMSON AND JEROME P. HORWITZ

RECEIVED AUGUST 24, 1959

The reaction of 5-aminotetrazole (I) and an arensulfonyl chloride (II) in either pyridine or aqueous sodium carbonate is shown to lead directly to a guanyl azide (IV) rather than the previously reported 5-arenesulfonamidotetrazole (V). Similarly, 1-amino-3-(*p*-acetamidobenzenesulfonyl)-guanidine affords the corresponding guanyl azide IVa on treatment with nitrous acid. The presence of an azide group in IV is established from infrared absorption measurements together with the fact that reduction of IV yields the corresponding guanidine VII. Furthermore, the cyclization of IV to a 5-arenesulfonamidotetrazole is readily effected with dilute base.

The successful application of several sulfanilamido heterocycles as chemotherapeutic agents for the treatment of bacterial infections prompted several independent attempts to prepare 5-sulfanilamidotetrazole (sulfatetrazole) (VI) between 1940 and 1952. The interaction of 5-aminotetrazole (I) and *p*-acetamidobenzenesulfonyl chloride (IIa) in either an aqueous suspension of calcium carbonate[3] or pyridine[4] yields a solid to which the structure N^1-(5-tetrazolyl)-N^4-acetylsulfanilamide (Va) was assigned. On the other hand, the same reactants, I and IIa, in aqueous sodium carbonate afford still another product, following *special treatment* with dilute sodium hydroxide, to which structure Va was also assigned.[5] These observa-

(1) This work was supported in part by research grants CY-2903 and CY-4519 from the National Cancer Institute, Public Health Service, and in part by an institutional grant from the United Foundation of Greater Detroit administered through the American Cancer Society, Southern Michigan Division.

(2) Research Fellow.

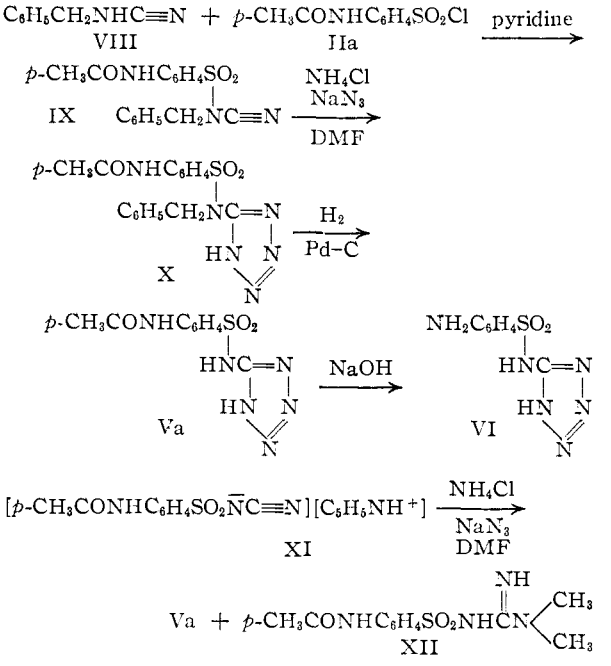
(3) G. Tappi and C. Migliardi, *Arch. sci. biol. Italy*, **27**, 164 (1941); *C. A.*, **36**, 1023 (1942).

(4) K. A. Jensen and O. Rosenlund Hansen, *Dansk. Tids. Farm.*, **15**, 229 (1941); *Chem. Zentr.*, **113** (I), 2528 (1942).

(5) H. Veldstra and P. W. Wiardi, *Rec. trav. chim.*, **61**, 627 (1942).

tions precipitated a controversy[6-8] which, to our knowledge, has never been resolved. A reinvestigation of the reaction between I and an arenesulfonyl chloride (II) showed (Chart I) that the product obtained from either pyridine or dilute sodium carbonate is the corresponding arenesulfonylguanyl azide IV. The latter, on treatment with either aqueous sodium hydroxide or sodium carbonate, is converted to the corresponding 5-arenesulfonamidotetrazole V.

Our studies began with a synthesis of Va by unequivocal methods. The requisite intermediate N^1-benzyl-N^1-cyano-N^4-acetylsulfanilamide (IX) was obtained from the reaction of benzylcyanamide (VIII) and IIa in pyridine. Treatment of IX with ammonium azide in dimethylformamide[9] (DMF) gave N^1-benzyl-N^1-(5-tetrazolyl)-N^4-acetylsulfanilamide (X). Debenzylation of X with hydrogen and palladized charcoal yielded a solid of the composition of Va whose melting point[10] was



in essential agreement with that recorded by Veldstra and Wiardi[5,8] for their product. Moreover, the synthetic product, on akaline hydrolysis, afforded a solid with properties in accord with those ascribed to sulfatetrazole (VI) by these same workers.[8]

The interaction of *p*-acetamidobenzenesulfonylcyanamide (XI) in the form of a pyridine salt and ammonium azide in DMF provided a more direct synthesis of Va. The method, however, leads to the formation of an additional product, 1-(*p*-acetamidobenzenesulfonyl)-3,3-dimethylguanidine (XII), at temperatures approaching the boiling point of the solvent (152–154°).

(6) G. Tappi, *Rec. trav. chim.*, **62**, 207 (1943).
(7) K. A. Jensen and O. Rosenlund Hansen, *ibid.*, **62**, 658 (1943).
(8) H. Veldstra and P. W. Wiardi, *ibid.*, **62**, 660 (1943).
(9) W. G. Finnegan, R. A. Henry and R. Lofquist, THIS JOURNAL, **80**, 3908 (1958).
(10) Many of the compounds prepared in this study melt with decomposition and the temperature of decomposition varies with the rate of heating.

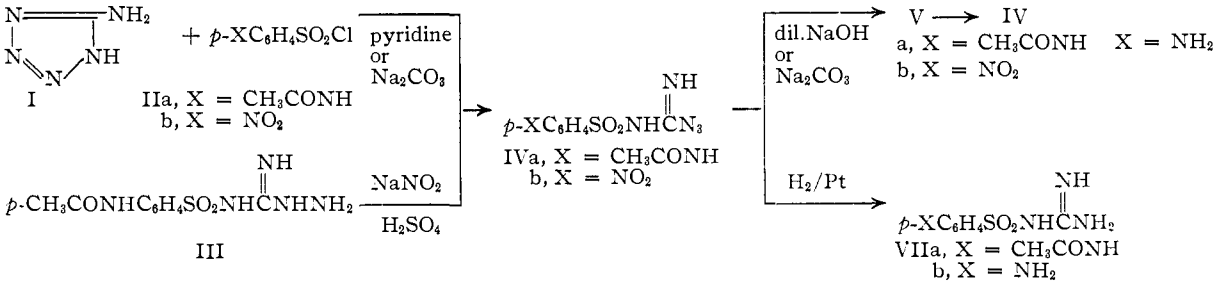
It has been pointed out that DMF decomposes slightly at its boiling point to give small amounts of dimethylamine and carbon monoxide.[11] However, the presence of either acidic or basic materials catalyzes this decomposition and a considerable amount of dimethylamine is produced at lower temperature. Accordingly, the isolation of XII in the conversion of XI to Va must be attributed to a competitive reaction due to the available dimethylamine.

A reinvestigation of the reaction between I and IIa in pyridine yielded a solid (IVa) with properties conforming to those previously recorded for this substance.[4,6] However, examination of the infrared spectrum (Nujol) of IVa revealed a relatively strong band at 4.66 μ which was attributed to an azide group[12] (4.67 μ). This observation appeared particularly significant since authentic samples of Va and VI, like many 5-substituted tetrazoles, are transparent in the region of 4–5 μ.[12] It was found, in accord with the spectral data, that catalytic hydrogenation of IVa gave *p*-acetamidobenzenesulfonylguanidine (VIIa) in 80% yield. Moreover, treatment of IVa with dilute sodium hydroxide produced a solid which afforded an infrared spectrum essentially superimposable with that of Va. Thus, the band at 4.66 μ present in IVa was no longer evident while a relatively intense band appeared at 9.54 μ. This vibration is within the region of the spectrum (9–10 μ) associated with the ring modes of 5-substituted tetrazoles.[12,13] Alkaline hydrolysis of this sample of Va gave a product identical with VI as prepared by the unequivocal synthesis.

Veldstra and Wiardi had earlier demonstrated that the product (IVa) derived from the action of IIa on I in pyridine is readily converted by 0.1 *N* sodium hydroxide to a product of the same composition and melting point as Va.[8] The latter had been obtained from the same reactants in an aqueous solution containing an equivalent quantity of sodium carbonate followed by reprecipitation of the crude product from dilute sodium hydroxide. Moreover, the isomerized product and that prepared in aqueous sodium carbonate both afforded VI on alkaline hydrolysis. Nevertheless, Veldstra and Wiardi rejected the possibility that the isomerized product and Va were identical. Rather, it was concluded that IVa, the isomerized product, and Va comprise the isolable tautomers of V.

The infrared spectrum of the product derived from the reaction of I and IIa in the presence of an equimolar quantity of sodium carbonate was indistinguishable from that of IVa. Furthermore, the so-called special treatment (*vide supra*) of this material with dilute sodium hydroxide yielded a solid which exhibited an infrared spectrum identical in every detail with Va. Alternatively, the same reaction effected in the presence of excess sodium carbonate afforded Va directly. Alkaline hydrolysis of Va prepared by either procedure yielded VI.

(11) A. B. Thomas and E. G. Rochow, THIS JOURNAL, **79**, 1843 (1957).
(12) E. Lieber, D. R. Levering and L. J. Patterson, *Anal. Chem.*, **23**, 1594 (1951).
(13) C. W. Roberts, G. F. Fanta and J. D. Martin, *J. Org. Chem.*, **24**, 654 (1959).



On the basis of the evidence accumulated in the present study it seems reasonable to assign the structure *p*-acetamidobenzenesulfonylguanyl azide (IVa) to the initial product of the reaction between I and IIa in either pyridine or aqueous sodium carbonate.[14] The conversion of IVa to Va is then in accord with the recognized facility with which guanyl azides undergo ring closure to tetrazole derivatives in the presence of base.[15]

The results of this phase of the study indicated the probable source of difficulty in the earliest recorded attempt to prepare sulfatetrazole (VI). Roblin, *et al.*,[16] obtained a solid, assumed to be 5-(*p*-nitrobenzenesulfonamido)-tetrazole (Vb), as the product of the reaction of I and *p*-nitrobenzenesulfonyl chloride (IIb) in pyridine. However, the attempted conversion of Vb to the *p*-amino analog VI with a variety of reducing agents led to apparent preferential cleavage of the tetrazole ring and the formation of sulfaguanidine (VIIb).

In accord with the accumulated evidence, it was found that the infrared spectrum of the presumed 5-(*p*-nitrobenzenesulfonamido)-tetrazole showed a relatively strong azide absorption band (4.66 μ). Furthermore, reduction of this material gave, in agreement with the earlier study, VIIb. However, treatment of this azide (IVb) with dilute sodium hydroxide gave a new product, Vb, of the same composition as IVb, but on reduction was smoothly converted to VI. Thus, the interaction of I and IIb in either pyridine or aqueous sodium carbonate yields *p*-nitrobenzene sulfonylguanyl azide (IVb) rather than the isomeric tetrazole derivative Vb. These observations explain the apparent ease of cleavage of the tetrazole ring.

In 1952, it was reported that 1-amino-3-(*p*-acetamidobenzenesulfonyl)-guanidine (III), on treatment with sodium nitrite in 4 *N* sulfuric acid, affords a solid which was also presumed to be Va.[17] Once again the product was observed to undergo apparent isomerization in dilute sodium hydroxide.

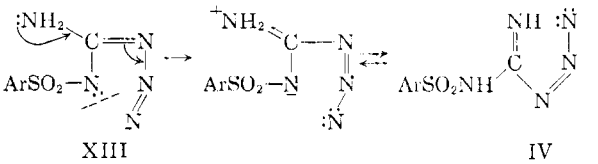
The conversion of III to a tetrazole derivative Va requires the intermediacy of a guanyl azide. Furthermore, ring closure of such azides is normally effected in either weakly acidic or alkaline media.[15] That this transformation should occur with apparent ease in strong mineral acid is contrary to precedent. Thus, guanyl azide hydrochloride, for example, is isolable from the action of sodium nitrite on aminoguanidine in hydrochloric acid.[18]

Repetition of the reaction between III and nitrous acid afforded a solid identical with IVa which was transformed (*cf.* Chart I) first to Va and finally to VI by the series of reactions described above.

It is tentatively assumed that the direct precursor of the guanyl azide derivatives IV, arising from the interaction of I and II, is the corresponding 1-substituted 5-aminotetrazole (XIII). This assumption is based on the considerations: (a) It has been shown that the course of reaction between arenesulfonyl chlorides and amino derivatives of nitrogen heterocyclic systems is consistent with the formation of a reactive ring-acylated intermediate[19]; the nature of the heterocyclic determines the subsequent behavior of this intermediate. (b) The alkylation of 5-aminotetrazole in basic aqueous medium leads to a mixture of isomers substituted in the 1- and 2-positions[20]; however, the 1-isomer generally predominates.

It is now established that the thermal isomerization of a 1-substituted 5-aminotetrazole and 5-substituted aminotetrazole involves the opening of the tetrazole ring to an activated guanyl azide.[21] The formation of the azide depends upon the transfer of electrons from the 5-amino group into the ring which facilitates the heterolysis of the nitrogen–nitrogen bond.[22] This electron shift, in turn, is enhanced by electronegative 1-substituents.

In a 1-arenesulfonyl-5-aminotetrazole (XIII), the strongly electronegative sulfur atom is attached to one of the nitrogen atoms involved in the heterolysis. Apparently, the influence of the sulfonyl moiety is such as to effect cleavage of the nitrogen–nitrogen bond at room temperature to give a guanyl azide IV.



Veldstra and Wiardi obtained a substance from treatment of what is now established as IVa with boiling alkali which was considered to be sulfacy-

(14) The product obtained from pyridine was reported to be identical with that isolated from calcium carbonate (see ref. 4). Accordingly, it is assumed that the three media promote the formation of IVa.

(15) E. Lieber and G. B. L. Smith, *Chem. Revs.*, **26**, 234 (1939).

(16) R. O. Roblin, J. H. Williams, P. S. Winnek and J. P. English, THIS JOURNAL, **62**, 2002 (1940).

(17) K. A. Jensen and O. Rosenlund Hansen, *Acta Chim. Scand.*, **6**, 195 (1952).

(18) J. Thiele, *Ann.*, **270**, 1 (1892).

(19) S. J. Angyal, *Australian J. Sci. Research*, **A5**, 374 (1952); *C. A.*, **47**, 4878 (1953).

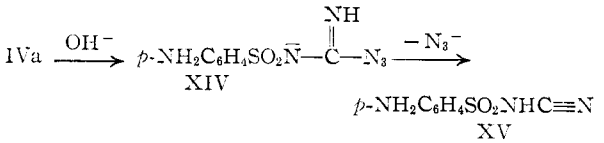
(20) R. A. Henry and W. G. Finnegan, THIS JOURNAL, **76**, 923 (1954).

(21) R. A. Henry, W. G. Finnegan and E. Lieber, *ibid.*, **76**, 88 (1954).

(22) R. A. Henry, W. G. Finnegan and E. Lieber, *ibid.*, **77**, 2264 (1955).

anamide (XV) on the basis of an elementary analysis.[8] An infrared spectrum of the alkaline degradation product, prepared by their procedure, showed, in accord with the original assignment, a prominent C≡N band at 4.62 μ.

The formation of XV requires the removal of a proton from IVa by hydroxide ion followed by elimination of azide ion from the resulting base XIV.[23] An essentially similar path has been



suggested to explain the alkaline decomposition of 1-phenyl-5-aminotetrazole.[22]

Experimental[24]

Benzylcyanamide (VIII).—A solution of 2.1 g. of cyanogen bromide (0.02 mole) in 14 ml. of anhydrous ether was added, dropwise with stirring, to a solution of 4.3 g. of benzylamine (0.04 mole), cooled externally by an ice–water-bath. The benzylamine hydrobromide was removed by filtration and the filtrate evaporated to dryness *in vacuo* at room temperature. The crude product, wt. 2.6 g. (95%), m.p. 42–44° (lit.[25] m.p. 43°), was used immediately.

N¹-Benzyl-N¹-cyano-N⁴-acetylsulfanilamide (IX).—To an ice-cold solution of 2.6 g. (0.02 mole) of VIII in 10 ml. of pyridine was added, with magnetic stirring and intermittent cooling, a solution of 4.7 g. (0.02 mole) of IIa in 40 ml. of pyridine. After stirring at room temperature for 68 hours, the mixture was concentrated *in vacuo* to a sirup which crystallized on trituration with several portions of ether. The solid was washed successively with dilute hydrochloric acid, aqueous sodium bicarbonate and water. The air-dried material was dissolved in methanol, treated with Norit and the solid that was deposited on cooling was collected; wt. 1.7 g. (26%), m.p. 133–134°.

Anal. Calcd. for $C_{16}H_{15}N_3O_3S$: C, 58.3; H, 4.6; N, 12.8. Found: C, 58.1; H, 4.8; N, 12.8.

N¹-Benzyl-N¹-(5-tetrazolyl)-N⁴-acetylsulfanilamide (X).—A solution of 6.6 g. of IX (0.02 mole) in 85 ml. of dimethylformamide containing 1.43 g. of sodium azide (0.022 mole) and 1.7 g. of ammonium chloride (0.031 mole) was heated to 125–130° for 22 hours. The inorganic salts were removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue was dissolved in water, the solution acidified with concentrated hydrochloric acid and the product was collected after cooling in an ice-bath for 1 hour. The solid was dissolved in aqueous sodium bicarbonate, treated with Norit and reprecipitated on acidification with concentrated hydrochloric acid; wt. 6.3 g. (85%), m.p. 194–195°. A single recrystallization from aqueous methanol provided an analytical sample, m.p. 195–196°.

Anal. Calcd. for $C_{16}H_{16}N_6O_3S$: C, 51.6; H, 4.3; N, 22.6. Found: C, 51.5; H, 4.3; N, 22.7.

N¹-(5-Tetrazolyl)-⁴-acetylsulfanilamide (Va).—A solution of 1.8 g. (4.8 mmoles) of X in 200 ml. of glacial acetic acid containing 0.5 g. of palladium–charcoal catalyst was shaken under 2 atm. of hydrogen for 3 hours. The catalyst was removed by filtration and the filtrate evaporated to dryness *in vacuo*. The residue crystallized from aqueous ethanol as a colorless solid, wt. 1.0 g. (77%), m.p. 215–217° dec.[10] (lit.[8] 207° dec.), λ_{max} 9.54 μ.

Anal. Calcd. for $C_9H_{10}N_6O_3S \cdot H_2O$: C, 36.0; H, 4.0; N, 28.0. Found: C, 36.1; H, 4.3; N, 27.9.

Pyridinium N⁴-acetylsulfanilylcyanamide (XI).—Acetic anhydride (5 ml.) was added to an ice-cold solution of 8.0 g. of sulfanilylcyanamide (0.04 mole)[26] in 55 ml. of pyridine and the reaction mixture was allowed to stand at room temperature for 24 hours. An oil separated on addition of absolute ether which solidified following trituration with several additional portions of ether. The product was recrystallized from a mixture of absolute ethanol and ether to give 11.0 g. (85%) of a colorless solid, m.p. 128–130°.

Anal. Calcd. for $C_9H_9N_3O_3 \cdot C_5H_5N$: C, 52.8; H, 4.4; N, 17.2. Found: C, 52.5; H, 4.5; N, 17.3.

1-N⁴-(*p*-Acetamidobenzenesulfonyl)-3,3-dimethylguanidine (XII) and Va.—A mixture of 1.43 g. of sodium azide (0.022 mole), 1.5 g. of ammonium chloride (0.028 mole) and 6.36 g. of XI (0.02 mole) in 50 ml. of technical DMF (b.p. 152–154°) was heated to 120–125° for 22 hours. The inorganic salts were removed by filtration and the filtrate evaporated to dryness *in vacuo*. The solid residue was suspended in 20 ml. of 1 *N* hydrochloric acid and collected. The air-dried material was suspended in a saturated solution of sodium bicarbonate and the insoluble material collected. A single recrystallization from methanol gave 0.6 g. (11%) of XII, m.p. 272–274°.

Anal. Calcd. for $C_{11}H_{16}N_4O_3S$: C, 46.5; H, 5.7; N, 19.7. Found: C, 46.7; H, 5.7; N, 19.8.

The alkali-soluble fraction was acidified with concentrated hydrochloric acid and the product collected, wt. 2.2 g. (36%), m.p. 219–220° dec. An infrared absorption spectrum derived from this material proved to be essentially superimposable with that of Va as obtained from X.

When the same reaction using the same quantities of reactants was repeated at a temperature of 65° for 16 hours, Va, obtained in 49% yield, was the only isolable product.

5-Sulfanilamidotetrazole (VI).—A solution of 1.0 g. (2.7 mmoles) of Va, obtained from X, in 20 ml. of 1 *N* sodium hydroxide was refluxed for 1 hour. The reaction mixture was cooled, and acidified with concentrated hydrochloric acid and evaporated to dryness in a stream of air at room temperature. The residue crystallized from water, following treatment with Norit, to give 0.6 g. (71%) of product, m.p. 202–203° dec. (lit.[5,8] 202–203° dec.), λ_{max} 9.58 μ.

Anal. Calcd. for $C_7H_8N_6O_6S$: C, 35.0; H, 3.4; N, 35.0. Found: C, 35.1; H, 3.6; N, 34.8.

***p*-Acetamidobenzenesulfonylguanyl Azide (IVa).**—**1. Pyridine Method.**[4,6,7]—To a solution of 1.0 g. (0.01 mole) of anhydrous 5-aminotetrazole in 12 ml. of pyridine, cooled externally by an ice–water-bath, was added, all at once, a cold solution of 2.3 g. (0.01 mole) of IIa in 10 ml. of pyridine. The reaction mixture was allowed to remain in the ice-bath for 1 hour, then poured into cold 4 *N* hydrochloric acid and the product collected, wt. 2.0 g. (71%), m.p. 162° dec. A single recrystallization from aqueous ethanol gave a colorless amorphous powder, m.p. 166° dec.[10] (lit.[4,6,7] 166° dec., 170° dec.), λ_{max} 4.54 and 4.65 μ.[27]

2. Sodium Carbonate Method.—To a solution of 1.8 g. (0.017 mole) of 5-aminotetrazole monohydrate in 18.5 ml. (0.018 mole) of 10% aqueous sodium carbonate was added, portionwise with stirring at room temperature, 4.9 g. (0.021 mole) of IIa. The mixture was stirred for 20 hours and the product collected; wt. 2.3 g. (46%), m.p. 162° dec., λ_{max} 4.54 and 4.64 μ.

3. From 1-Amino-3-(*p*-acetamidobenzenesulfonyl)-guanidine[17] (III).—A solution of 0.43 g. of sodium nitrite (6.2 mmoles) in 3 ml. of water was added dropwise with stirring to a solution of 1.0 g. of III (3.8 mmoles) in 21 ml. of water, cooled externally by an ice–salt mixture. The product was collected and recrystallized from water; wt. 0.7 g. (68%), m.p. 166–167° dec.[10] (lit.[17] 172–173° dec.), λ_{max} 4.53 and 4.64 μ. The infrared absorption spectrum of each of the samples of IVa are virtually identical.

***p*-Nitrobenzenesulfonylguanyl Azide (IVb).**—To a solution of 0.85 g. of I (0.01 mole) in 11 ml. of 10% aqueous sodium carbonate was added, portionwise, 2.21 g. (0.01 mole) of *p*-nitrobenzenesulfonyl chloride. The reaction mixture was

(23) The medium must also effect deacetylation at some stage of this sequence. However, this step is not relevant to the path by which XV is formed.

(24) All melting points are uncorrected. Analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Infrared absorption spectra were measured in Nujol using a Perkin–Elmer model 21 recording spectrophotometer.

(25) R. McKee, *Am. Chem. J.*, **36**, 211 (1906).

(26) L. C. Leitch, B. E. Baker and L. E. Brinkman, *Can. J. Research*, **23B**, 139 (1945).

(27) The azide group has a relatively strong absorption band in the region of 4.67 μ (see ref. 16). Each of the samples of IIIa, prepared according to the methods described in this section, exhibit an additional band of weaker intensity in the same region (4.52–4.54 μ). Since both of these bands disappear on conversion of III to the corresponding tetrazole derivative IV, it is assumed that the two vibrations are associated with the azide function.

stirred at room temperature for 4 hours, the yellow solid collected and washed several times with water; wt. 2.3 g. (85%), m.p. 185–186° dec. A single recrystallization from 95% ethanol provided an analytical sample, m.p. 187–189° dec. (lit.[16] 185–186° dec.), λ_{max} 4.54 and 4.66 μ.

Anal. Calcd. for $C_7H_6N_6O_4S$: C, 31.1; H, 2.2; N, 31.1. Found: C, 31.2; H, 2.5; N, 31.3.

Conversion of Arenesulfonylguanyl Azides (IV) to V.—(a) A suspension of 1.6 g. (5.6 mmoles) of IVa in 40 ml. of 10% sodium carbonate was heated on a water-bath until a clear solution had been obtained. The cooled reaction mixture was acidified with hydrochloric acid and the product collected; wt. 1.5 g. (93%), m.p. 207–212° dec.[10] (lit.[8] 207° dec.), λ_{max} 9.54 μ.

The same transformation, effected at room temperature in 0.2 *N* sodium hydroxide, gave Va in 75% yield. The infrared spectrum of Va as obtained by either of these procedures were indistinguishable from the corresponding spectra of samples of Va derived from X, XI or from the reaction of I and IIa in the presence of excess sodium carbonate.

(b) **5-(*p*-Nitrobenzenesulfonamido)-tetrazole (Vb).**—A suspension of 2.0 g. (7.7 mmoles) of the guanyl azide (IVb) 150 ml. of 1% sodium hydroxide was stirred at room temperature for *ca.* 5 minutes. The mixture was filtered, the in filtrate acidified with hydrochloric acid and the product collected; wt. 1.0 g. (50%), m.p. 203–204° dec. A single recrystallization from aqueous ethanol provided an analytical sample, m.p. 208–209° dec., λ_{max} 9.48 μ.

Anal. Calcd. for $C_7H_6N_6O_4S$: C, 31.1; H, 2.2; N, 31.1. Found: C, 31.1; H, 2.3; N, 31.2.

A sample (200 mg.) of Vb in 10 ml. of methanol containing 50 mg. of platinum oxide was shaken with hydrogen until the theoretical uptake was realized (20 minutes). The catalyst was removed and the filtrate evaporated to dryness and the residue crystallized from water; wt. 156 mg. (88%), m.p. 202–203° dec., λ_{max} 9.58 μ. An infrared spectrum of this product proved to be identical with VI.

Alternative Synthesis of Va.—To a solution of 2.06 g. (0.02 mole) of 5-aminotetrazole hydrate in 35 ml. of 10% sodium carbonate was added, portionwise with stirring, 4.9 g. (0.021 mole) of IIa. Sodium carbonate (3.5 g.) was added, portionwise, to the reaction mixture and the stirring was continued for 18 hours. The solution was then filtered, the clear solution acidified with hydrochloric acid and the solid collected; wt. 5.0 g. (88%), m.p. 202–207° dec. The crude product was dissolved in a saturated solution of sodium bicarbonate, treated with Norit, and the solid reprecipitated with hydrochloric acid; wt. 4.0 g., m.p. 207–210° dec. A single recrystallization from aqueous ethanol provided an analytical sample, m.p. 217–219° dec. (lit.[8] 207° dec.), λ_{max} 9.55 μ.

Anal. Calcd. for $C_9H_{10}N_6O_3S \cdot H_2O$: C, 36.0; H, 4.0; N, 28.0. Found: C, 36.2; H, 4.2; N, 27.5.

Reduction of IV. (a) *p*-Acetamidobenzenesulfonylguanidine (VIIa).—A solution of 2.0 g. (7 mmoles) of IVa in 150 ml. of methanol containing 0.3 g. of platinum oxide was shaken under a pressure of 3 atm. of hydrogen for two hours. The filtered solution was concentrated to a small volume and the product collected; wt. 1.4 g. (77%), m.p. 265–266° (lit.[28] 266°).

(b) **Sulfaguanidine (VIIb).**—A suspension of 0.6 g. of IVb (2.2 mmoles) in 125 ml. of methanol containing 0.2 g. of platinum oxide was shaken under 3 atm. of hydrogen for 2 hours. The filtered solution was concentrated to *ca.* 0.1 of the original volume and the product collected; 0.4 g. (84%), m.p. 180–183°. Three recrystallizations from water provided a colorless solid, m.p. 186–188° (lit.[16] 189–190°).

A sample (0.18 g.) of VIIb was treated with 0.1 ml. of acetic anhydride and 4 ml. of pyridine at 60° for 5 minutes and then allowed to stand at room temperature for 16 hours. The product that was deposited was collected and dried; wt. 0.15 g. (71%), m.p. 266–267° alone or when admixed with VIIa.

(28) K. Ganapathi, *Proc. Indian Acad. Sci.*, **13A**, 386 (1941); *C. A.*, **36**, 1022 (1942).

DETROIT 1, MICH.

[CONTRIBUTION FROM THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY, UNIVERSITY OF CALIFORNIA]

Products of γ-Irradiation of Cysteine and Cystine[1]

BY PERICLES MARKAKIS[2] AND A. L. TAPPEL

RECEIVED MAY 20, 1959

Cysteine and cystine in aqueous acidic solutions were exposed, in the absence and presence of oxygen, to γ-irradiation, at doses varying from 10^4 to 8×10^7 rad. Cystine, hydrogen sulfide, free sulfur, sulfate ion, ammonia and alanine were identified and determined among the irradiation products of cysteine; more than 80% of the sulfur and more than 90% of the nitrogen of cysteine could be accounted for. Qualitatively, the irradiated cystine solutions did not differ essentially from the irradiated cysteine solution; however, more free sulfur, sulfate ion and ammonia, less hydrogen sulfide, and very little cysteine were produced from cystine. Mechanisms for the radiolysis of these amino acids are discussed.

The sulfhydryl and disulfide groups which are present in amino acids, peptides, proteins and enzymes are known to play important biochemical and physiological roles.[3] The effect of ionizing radiations upon these groups is of primary concern in radiation biochemistry, and has been reviewed by Barron.[4] Malodorous sulfur compounds are produced from irradiated proteins, and have been associated with the undesirable flavors which develop during the radiation preservation of food, especially meat and fish.[5–7]

Cysteine and cystine have been the object of radiochemical investigations mainly because information from such studies will help in understanding the radiation chemistry of more complex sulfhydryl and disulfide compounds. In addition, cysteine is interesting because its injection into an animal provides some protection from lethal radiations.[8,9] Cysteine has also been used as a prototype in the search for more efficient protectors.[10]

Dale and Davies irradiated cysteine solutions with X-ray doses of up to 100,000 r., and determined the amount of H_2S liberated.[11] Swallow measured

(1) Supported in part by the U. S. Fish and Wildlife Service.

(2) Food Science Laboratory, Michigan State University, E. Lansing, Mich.

(3) E. S. G. Barron, *Advances in Enzymol.*, **11**, 201 (1951).

(4) E. S. G. Barron, *Ann. N. Y. Acad. Sci.*, **59**, 574 (1955).

(5) O. F. Batzer and D. M. Doty, *J. Agr. Food Chem.*, **3**, 64 (1955).

(6) E. P. Marbach and D. M. Doty, *ibid.*, **4**, 881 (1956).

(7) R. A. Sliwinski and D. M. Doty, *ibid.*, **6**, 41 (1958).

(8) H. M. Pratt, E. B. Tyree, R. L. Straube and D. E. Smith, *Science*, **110**, 213 (1949).

(9) D. E. Smith, *Rad. Research*, **10**, 335 (1959).

(10) Z. M. Bacq, A. Hevre, J. Lecompte, P. Fisher, J. Bavier, C. Dechamps, H. LeBihan and P. Rayet, *Arch. Intern. Physiol.*, **59**, 442 (1951).

(11) W. M. Dale and J. V. Davies, *Biochem. J.*, **48**, 129 (1951).