stored overnight in an ice box. The precipitate which formed was filtered and washed with ether to give 10.7 g. of crude product, m.p. 136.5–138°. Several recrystallizations from toluene gave 6.3 g. (21.8%) of VI, m.p. 144.5–145.5°.

Anal. Calcd. for $C_{18}H_{15}ON_3$: C, 74.7; H, 5.2; N, 14.5. Found: C, 74.9; H, 5.3; N, 14.7.

Further cooling of the mother liquor from the reaction mixture gave a second crop of material which when recrystallized from toluene gave 4.1 g. of a yellow solid, m.p. 246.5–247°.

Refluxing this latter product with tetralin for 3 hr. left it unchanged. The product was not further characterized.

7,8-Dihydrobenzimidazo[1,2-a]isoquino[3,2-c]pyrazinium bromide (I). Two and nine-tenths g. (0.01 mole) of VI was added to 30 ml. of 48% hydrobromic acid and the mixture refluxed for 4 hr. The resulting dark red solution was evaporated to dryness. Trituration of the light tan residue with acetone gave 5.0 g. of crude product. Recrystallization from water gave 2.1 g. (60%) of the yellow quaternary salt, m.p. 347–348° with decomposition. The analytical sample was recrystallized again from water, m.p. 355–356° with decomposition.

Anal. Calcd. for $C_{18}H_{14}N_3Br$: C, 61.4; H, 4.0; N, 11.9; Br, 22.7. Found: C, 61.1; H, 4.1; N, 11.7; Br, 22.8.

5,6,7,8-Tetrahydro-14bH-benzimidazo[1,2-a]isoquino[3,2-c]pyrazine (VII). Three and seven-tenths g. (0.01 mole) of I was suspended in 200 ml. of water at 55°, and hydrogenated over 50 mg. of platinum oxide at an initial pressure of 47 p.s.i. After 2 hr. the hot solution was filtered and the filtrate made basic with ammonium hydroxide. The precipitate was filtered and dried, yield 1.9 g., m.p. 211–216°. Repeated recrystallizations from ethyl acetate gave the pure product, m.p. 221–223°.

Anal. Calcd. for $C_{18}H_{17}N_3$: C, 78.5; H, 6.2; N, 15.3. Found: C, 78.6; H, 6.2; N, 15.5.

2-(3′-Isoquinolyl)benzimidazole. Thirty and nine-tenths g. (0.2 mole) of isoquinoline-3-carboxaldehyde[14] and 21.2 g. (0.2 mole) of *o*-phenylenediamine were added to 80 ml. of nitrobenzene and the mixture gradually heated during 40 min. to the boiling point of the nitrobenzene. This temperature was maintained for 10 min., then the reaction mixture was allowed to cool. After standing overnight in an ice box the tan solid which separated was filtered and washed with benzene, yield 28.7 g. Recrystallization of the crude product from toluene gave 20.7 g., m.p. 193–194°.

Anal. Calcd. for $C_{16}H_{11}N_3$: C, 78.3; H, 4.5; N, 17.1. Found: C, 78.3; H, 4.6; N, 17.2.

Attempted alkylations of 2-arylbenzimidazoles. Attempts to alkylate 2-(2′-pyridyl)benzimidazole[8] with ethylene chlorohydrin in boiling toluene, with ethyl bromoacetate in absolute ethanolic potassium hydroxide at room temperature, or with allyl bromide in hot, absolute ethanolic sodium ethoxide were unsuccessful. The pyridylbenzimidazole was recovered from the reaction mixtures. 2-(3′-Isoquinolyl)-benzimidazole failed to give identifiable alkylation products with ethylene chlorohydrin in dioxane or with ethyl bromoacetate in hot, absolute ethanolic potassium hydroxide.

Absorption spectra. Ultraviolet absorption spectra were determined using a Beckman recording spectrophotometer, Model DK-2.

EAST LANSING, MICH.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN STATE UNIVERSITY]

Tetrazole Analogs of Aminobenzoic Acid Derivatives[1]

J. M. McMANUS[2,3] AND ROBERT M. HERBST

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The three isomeric 5-nitrophenyltetrazoles and 5-(2′-hydroxy-4′-nitrophenyl)tetrazole have been prepared from the corresponding benzonitriles. Reduction of the 5-nitrophenyltetrazoles resulted in the formation of the corresponding 5-aminophenyltetrazoles which included the tetrazole analogs of *p*-aminobenzoic acid, *m*-aminobenzoic acid, and 2-hydroxy-4-aminobenzoic acid.

In recent years two aminobenzoic acid derivatives have been prominent in chemotherapy. *p*-Aminobenzoic acid plays a unique role in metabolism as a portion of folic acid, an essential material for the synthesis of nucleic acids. Evidence that certain drugs structurally related to *p*-aminobenzoic acid can inhibit its incorporation into folic acid[4] resulted in the application of the antimetabolite concept in chemotherapy as a means of combating bacterial invasion of the body. Those drugs which have been most effective in interfering with the utilization of *p*-aminobenzoic acid have been shown to be related to sulfanilamide[5] and have been used widely in the therapy of staphylococcal and pneumococcal infections. The second aminobenzoic acid derivative, important for its tuberculostatic activity,[6] is 2-hydroxy-4-aminobenzoic acid commonly referred to as *p*-aminosalicylic acid.

Because of the acidic nature of 5-substituted tetrazoles[7–9] the replacement of the carboxyl group of the aminobenzoic acids by the 5-tetrazolyl group should result in compounds of similar acidity and solubility. The possibility that the tetrazolyl analogs of the aminobenzoic acids might

(1) Based on a thesis submitted to Michigan State University in 1958 by James M. McManus in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) White Laboratories Fellow, 1955–1958.

(3) Present address: Chas. Pfizer & Co., Brooklyn, N. Y.

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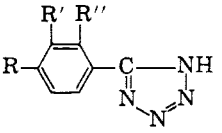
have useful therapeutic properties prompted investigation of their preparation.

Accordingly the preparation of the 5-nitrophenyltetrazoles necessary as intermediates was undertaken by two different techniques: first, the interaction of appropriate nitrobenzonitriles with hydrazoic acid in xylene solution; and second, interaction of the nitrobenzonitriles with sodium azide and acetic acid in refluxing *n*-butyl alcohol.[9] After completion of this work a procedure for the interaction of nitriles with ammonium or lithium azide in dimethylformamide appeared.[10] The new technique[10] permits much shorter reaction times.

Reduction of the nitrophenyltetrazoles to the corresponding aminobenzoic acid analogs was effected using either tin and hydrochloric acid as in the synthesis of 5-(3′-aminophenyl)- (I) and 5-(4′-aminophenyl)tetrazole (II) or hydrogen and platinum oxide as in the preparation of 5-(4′-aminophenyl)- and 5-(2′-hydroxy-4′-aminophenyl)tetrazole (III).



I. R, R″ = H; R′ = NH_2
II. R = NH_2; R′, R″ = H
III. R = NH_2; R′ = H; R″ = OH

The aminophenyltetrazoles possess amphoteric character; they are insoluble in cold water but readily soluble both in aqueous acids and alkalies. The acetyl derivatives used to characterize the aminophenyltetrazoles were prepared in glacial acetic acid solution with an equimolar amount of acetic anhydride.

Biological evaluation of the aminophenyltetrazoles is under way in the Research Laboratories of the Schering Corporation.

EXPERIMENTAL[11]

Preparation of Nitrophenyltetrazoles. 5-(3′-Nitrophenyl)-tetrazole. Procedure A. A mixture of 15.6 g. (0.11 mole) of *m*-nitrobenzonitrile and 50 ml. of xylene containing 6.8 g. of hydrazoic acid sealed in a Pyrex combustion tube was heated for 125 hr. at 140–145°. The contents of the tube were transferred to a flask and the tube washed with a small amount of warm benzene. Chilling the benzene-xylene mixture gave a crystalline product that was recrystallized from water using Norit to remove a trace of color, yield 8.6 g. (39%), m.p. 150.5–151.5°.

Anal. Calcd. for $C_7H_5N_5O_2$: C, 44.0; H, 2.6; N, 36.6. Found: C, 44.2; H, 2.7; N, 36.9.

Lossen[12] reported m.p. 145° for this compound.

Procedure B. A mixture of 32 g. (0.25 mole) of *m*-nitrobenzonitrile, 22 g. (0.34 mole) of sodium azide, and 20 g. (0.33 mole) of glacial acetic acid in 100 ml. of *n*-butyl alcohol was heated at reflux temperature for 4 days. An additional 5 g. of sodium azide and 10 g. of glacial acetic acid were added at this time and heating continued for 2 days. The reaciton mixture was diluted with 300 ml. of water and distilled until all the alcohol had been removed. Cooling and acidification of the residual solution caused separation of a pale yellow solid which was recrystallized from water using Norite to remove color. Yield 42 g. (88%) of product, m.p. 150.5–151.5°; mixture melting point with the material from procedure *A* was not depressed.

5-(2′-Nitrophenyl)tetrazole. Using procedure *A* the interaction of 25 g. (0.17 mole) of *o*-nitrobenzonitrile, 16.2 g. (0.25 mole) of sodium azide, and 15 g. (0.25 mole) of glacial acetic acid in 100 ml. of *n*-butyl alcohol gave 12.2 g. (38%) of 5-(2′-nitrophenyl)tetrazole. Recrystallization from water gave the pure product, m.p. 159.5–161°.

Anal. Calcd. for $C_7H_5N_5O_2$: C, 44.0; H, 2.6; N, 36.6. Found: C, 44.1; H, 2.8; N, 36.4.

5-(4′-Nitrophenyl)tetrazole. Following procedure *A* 15.6 g. (0.11 mole) of *p*-nitrobenzonitrile and 50 ml. of xylene containing 6.8 g. of hydrazoic acid gave 16.6 g. (77%) of product. The tetrazole was recrystallized from absolute ethanol, m.p. 218.5–219°. Pinner[13] reported m.p. 219° for this compound.

Anal. Calcd. for $C_7H_5N_5O_2$: C, 44.0; H, 2.6; N, 36.6. Found: C, 44.2; H, 2.7; N, 36.7.

Using procedure *B* 32 g. (0.25 mole) of *p*-nitrobenzonitrile, 22 g. (0.34 mole) of sodium azide and 22 g. (0.25 mole) of glacial acetic acid in 100 ml. of *n*-butyl alcohol gave 37 g. (78%) of 5-(4′-nitrophenyl)tetrazole. Recrystallization from water gave the pure product, m.p. 218.5–219°. A mixture melting point with the material from procedure *A* showed no depression.

5-(2′-Hydroxy-4′-nitrophenyl)tetrazole. A mixture of 41 g. (0 25 mole) of 2-hydroxy-4-nitrobenzonitrile,[14] 22 g. (0.34 mole) of sodium azide, and 22 g. (0.34 mole) of glacial acetic acid was heated at reflux temperature in 100 ml. of *n*-butyl alcohol for 6 days. The reaction mixture was diluted with 300 ml. of water and the mixture distilled until 250 ml. of distillate had collected. The solid which crystallized from the residual solution on cooling was recrystallized twice from water, yield 53.5 g. (94%). The analytical data indicate that this is the sodium salt of the desired tetrazole.

Anal. Calcd. for $C_7H_4N_5O_3Na$: N, 30.6. Found: N, 30.4.

The sodium salt was converted quantitatively into the free tetrazole by acidifying its hot aqueous solution to Congo red paper with concentrated hydrochloric acid. The free tetrazole was recrystallized from absolute ethanol, m.p. 283–283.5° with decomposition.

Anal. Calcd. for $C_7H_5N_5O_3$: C, 40.6; H, 2.4; N, 33.8. Found: C, 40.7; H, 2.8; N, 33.7.

Preparation of aminophenyltetrazoles. 5-(3′-Aminophenyl)-tetrazole (I). Concentrated hydrochloric acid (85 ml.) was added to a mixture of 17.2 g. of 5-(3′-nitrophenyl)tetrazole and 35 g. of granular tin in a 300 ml. 3-necked flask. The reaction started almost immediately and cooling was necessary. After stirring the mixture for an additional 15 min., the clear solution was decanted and the excess tin washed with a little water. The combined washings and acid solution were made basic with concentrated aqueous ammonia. The tin hydroxide was filtered and the filtrate acidified with glacial acetic acid until precipitation was complete. The colorless product, 11.5 g. (80%), was recrystallized from water, m.p. 199–200°.

Anal. Calcd. for $C_7H_7N_5$: C, 52.2; H, 4.4; N, 43.5. Found: C, 52.1; H, 4.5; N, 43.1.

5-(3′-Acetamidophenyl)tetrazole was obtained from I by interaction with acetic anhydride in glacial acetic acid. It separated from water as colorless crystals, m.p. 254–255° with decomposition.

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Anal. Calcd. for $C_9H_9N_5O$: C, 53.2; H, 4.5; N, 34.5. Found: C, 53.2; H, 4.6; N, 34.5.

5-(4'-Aminophenyl)tetrazole (II). (a) In a similar manner 17.2 g. of 5-(4'-nitrophenyl)tetrazole and 35 g. of granular tin were treated with 75 ml. of concentrated hydrochloric acid. II was isolated as in the preceeding preparation, yield 12.7 g. (88%), m.p. 267° with decomposition, after crystallization from aqueous ethanol.

(b) A suspension of 7.3 g. of 5-(4'-nitrophenyl)tetrazole in 150 ml. of glacial acetic acid was shaken with 150 mg. of platinum oxide catalyst at an initial hydrogen pressure of 49 p.s.i. After the theoretical amount of hydrogen had been absorbed, the chilled suspension of catalyst and product was filtered. The product was extracted from the mixture with hot ethanol and the solvent removed from the extract under reduced pressure. The residue was recrystallized from aqueous ethanol using Norit, yield 5.0 g. (82%), m.p. 267° with decomposition. Finnegan, Henry, and Lofquist[10] report m.p. 268–270° with decomposition.

Anal. Calcd. for $C_7H_7N_5$: C, 52.2; H, 4.4; N, 43.5. Found: C, 51.9; H, 4.3; N, 43.3.

5-(4'-Acetamidophenyl)tetrazole obtained from II with acetic anhydride in glacial acetic acid was recrystallized from glacial acetic acid with some difficulty. It separated as a colorless crystal powder, m.p. 278° with decomposition.

Anal. Calcd. for $C_9H_9N_5O$: C, 53.2; H, 4.5; N, 34.5. Found: C, 53.1; H, 4.7; N, 34.3.

5-(2'-Hydroxy-4'-aminophenyl)tetrazole (III). A suspension of 22.9 g. of the dry sodium salt of 5-(2'-hydroxy-4'-nitrophenyl)tetrazole in 150 ml. of water was shaken with 250 mg. of platinum oxide catalyst at an initial hydrogen pressure of 50 p.s.i. When hydrogen absorption was complete, the catalyst was filtered off and the filtrate warmed with a little sodium hydrosulfite to destroy a faint orange coloration. After treatment with Norit concentrated hydrochloric acid was added slowly to the cooled filtrate until no further precipitation occurred. The colorless, crystalline product was filtered off and dried, yield 13 g. (74%), m.p. 261–262° with decomposition.[15]

Anal. Calcd. for $C_7H_7N_5O$: C, 47.5; H, 4.0; N, 39.6. Found: C, 47.3; H, 4.2; N, 39.7.

5-(2'-Hydroxy-4'-acetamidophenyl)tetrazole was prepared from III by treatment with acetic anhydride in refluxing glacial acetic acid. It crystallized from water, in which it is difficultly soluble, as colorless needles, m.p. 281–282° with decomposition.

Anal. Calcd. for $C_9H_9N_5O_2$: C, 49.3; H, 4.1; N, 32 0. Found: C, 49.2; H, 4.3; N, 32.1.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN STATE UNIVERSITY]

Alkylation Studies with Aminotetrazoles[1]

KENNETH R. WILSON,[2] ROBERT M. HERBST, AND WILLARD J. HAAK[3]

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A group of 1,4-disubstituted 5-iminotetrazolines has been prepared by alkylation of 1-cyclohexyl-, 1-cyclohexylmethyl- and 1-β-cyclohexylethyl-5-aminotetrazole with benzyl chloride, substituted benzyl halides and β-phenylethyl and γ-phenylpropyl bromide. The products were characterized as hydrochlorides and as substituted thioureas formed by interaction with phenyl isothiocyanate. A brief summary of their activity in microbiological systems is included.

Recently it was shown that 1,4-dialkyl-5-iminotetrazolines with a benzyl or substituted benzyl group in one position and a moderately large alkyl group, *n*-octyl for instance, at the other position exert a marked inhibitory action on growth of bacteria, protozoa, and fungi.[4,5] The purpose of the present investigation was to prepare a variety of 1,4-dialkyl-5-iminotetrazolines in which cyclohexyl or cyclohexylalkyl groups replaced the *n*-alkyl group. The resulting compounds were submitted for screening of their activity in microbiological systems; a brief summary of these results is included.

The iminotetrazolines were prepared by heating a mixture of the appropriate 1-cyclohexyl- or 1-cyclohexylalkyl-5-aminotetrazole with a small excess of benzyl, substituted benzyl, β-phenylethyl or γ-phenylpropyl halide. The iminotetrazoline hydrohalide so formed was subjected to steam distillation to remove excess aralkyl halide. Liberation of the base and extraction of the base with ether or benzene served to separate the product from unused 5-aminotetrazole derivative. The bases were converted into hydrochlorides as which they were isolated and characterized (Table I). The hydrochlorides are only very slightly soluble in water, moderately soluble in the common alcohols, but show the unique characteristic of rather marked solubility in hot benzene, toluene, or chloroform. The bases can be liberated from the hydrochlorides by shaking a suspension of the latter in dilute aqueous alkali and extraction with ether or benzene. Continuous removal of the coating of insoluble base from the sparingly soluble hydrochloride is essential for the success of the process. Many of the bases are viscous liquids; a few are solids and can be crystallized from cyclohexane (Table II). The

(1) Based on material recorded in the doctoral thesis of Kenneth R. Wilson and in the master's thesis of Willard J. Haak.

(2) Parke, Davis Fellow, 1956–57. Present address: E. I. du Pont de Nemours & Co., Buffalo, N. Y.

(3) Present address: The Upjohn Company, Kalamazoo, Mich.

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