[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE WINTHROP CHEMICAL CO., INC.]

The Preparation of Sulfanilamidoindazoles[1]

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The high chemotherapeutic activity of the sulfanilamido derivatives of various aminoheterocycles (*e. g.*, sulfathiazole, sulfadiazine, sulfamethazine and sulfapyridine) has led to the preparation of many other sulfanilamido derivatives of similar structure. In these Laboratories it was thought desirable to prepare the various isomers of sulfanilamidoindazole in order to obtain information on the effect of substitution of the sulfanilamido group in different positions of the indazole ring. There was a twofold interest in the preparation of this series of compounds: derivatives could be prepared in which the sulfanilamide group would be on the benzene portion of the molecule (4-, 5-, 6-, and 7-sulfanilamidoindazole); further, 3-sulfanilamidoindazole has the sulfanilamido group substituted on the heterocyclic portion of the molecule and would have a closer analogy to sulfapyridine and sulfadiazine than would obtain for the other sulfanilamidoindazoles. In the course of this work 3-, 5-, 6-, and 7-sulfanilamidoindazoles were prepared. After most of our laboratory work had been completed we noted reference[2] to the preparation of 5- and 7-sulfanilamidoindazole. It is the purpose of this paper to present the preparation of the various sulfanilamido compounds we have made, and of the necessary intermediates for these compounds.

The aminoindazoles used in this work were first prepared by methods described in the literature However, during the course of the work several improvements were found which resulted in simplification of the procedure and in increased yields. The preparation of 5-, 6- and 7-aminoindazole was effected by reduction of the corresponding nitroindazoles, which in turn were prepared by the method of Porter and Peterson.[3] The reduction of nitroindazoles to aminoindazoles has been the object of attention of several investigators, most of whom have used a ferrous sulfate–ammonia reduction or some variation thereof. The method is efficient and the yields are fair; however, it was found that the reduction of nitroindazoles to aminoindazoles may advantageously be carried out catalytically. Experiments have shown that nitroindazoles in methanol are readily reduced to aminoindazoles by using Raney nickel as a catalyst, under a pressure of 30 to 40 atmospheres of hydrogen, and at a temperature of about 50°. The reduction is rapid, the isolation of aminoindazole is simple and the yield of desired product is relatively high. The aminoindazoles prepared by this method were usually pure enough to be used directly in the condensation reactions with *p*-acetaminobenzenesulfonyl chloride.

A variation in the synthesis of 3-aminoindazole by the method of Reissert and Grube[4] is described in the experimental part. *o*-Aminobenzonitrile, essential to the preparation of 3-aminoindazole, was obtained by stannous chloride reduction of *o*-nitrobenzonitrile. Confirming the experience of previous investigators, other methods of reduction of *o*-nitrobenzonitrile, *e. g.*, zinc dust and acetic acid, iron and acetic acid, yielded mainly *o*-aminobenzamide. Experiments also showed that catalytic reduction with Raney nickel invariably yielded *o*-aminobenzamide. No matter how mild the method of reduction, the water formed by the reduction of the nitro group would react with the cyano group to form the benzamide derivative. The simultaneous diazotization, reduction and ring closure of *o*-aminobenzonitrile to 3-aminoindazole was carried out by the method of Reissert and Grube.[4]

The acetylsulfanilamidoindazoles were secured by the usual procedures. The details of these methods are given in the experimental part of this paper. Table I is appended which presents pertinent data on these acetylsulfanilamidoindazoles.

The acetylsulfanilamidoindazoles were hydrolyzed by two methods. The usual method of refluxing these acetyl bodies with aqueous alkali or mineral acid for one-half to two hours was used and yields of about 75% were secured. The use of alcoholic hydrogen chloride for removal of acetyl groups[5] was successfully applied to the present group of compounds. The ease of removal of acetyl groups by this method recommends its use. Details for both methods are furnished in the experimental part of the paper.

(1) Presented before the Division of Organic Chemistry, A. C. S., Detroit, Michigan, April, 1943.

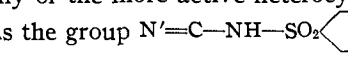
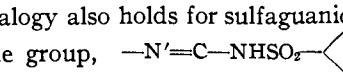
(2) Rajagopalan, *Current Science*, **11**, 146 (1942); *Chem. Abst.*, **36**, 6511[1] (1942).

(3) Porter and Peterson, "Organic Syntheses," **20**, 73 (1940).

(4) Reissert and Grube, *Ber.*, **42**, 3716 (1909).

(5) Kwartler and Lucas, THIS JOURNAL, **65**, 354 (1943).

The characteristics of the sulfanilamidoindazoles which have been prepared are listed in Table II.

Although the preparation of 3-sulfanilamidoindazole was relatively difficult, the structural relationship of 3-sulfanilamidoindazole to such heterocyclic sulfonamides as 2-sulfanilamidopyridine, induced us to prepare this compound. The common feature of 3-sulfanilamidoindazole and many of the more active heterocyclic sulfonamides is the group  , where N′ is the nitrogen of the heterocyclic ring. This linkage of atoms is common to sulfapyridine, sulfathiazole, sulfadiazine and sulfamethazine (4,6 dimethyl-2-sulfanilamidopyrimidine). The structural analogy also holds for sulfaguanidine in that the same group,  is found, but here the N′ is not in a heterocyclic ring system. It is significant that all of these compounds have proved to be so effective. Pharmacological work on these sulfanilamidoindazoles is still being carried out. Several of these compounds have been tested *in vitro*. It has been found that the sulfanilamidoindazoles have distinct bacteriostatic and bactericidal properties against a variety of organisms. The 5-sulfanilamidoindazole and 6-sulfanilamidoindazole are 2 times and 3 to 4 times, respectively, as active as sulfanilamide against streptococcus. The toxicity of these compounds appears to be low. Various other pharmacological data are being collected. The results of these chemotherapeutic studies will be reported elsewhere in detail.

Experimental

Preparation of Nitroindazoles.—5, 6 and 7-nitroindazoles were prepared by the method of Porter and Peterson.[3]

Preparation of 6-Aminoindazole.—A suspension of 41 g. (0.25 mole) of 6-nitroindazole and 20 g. of Raney nickel in 400 ml. of methanol was placed in an autoclave. Hydrogen was introduced until a pressure of 30 atmospheres was obtained; the temperature was raised to 50° and reduction proceeded at this temperature for eight hours. The mixture was filtered and the filtrate, after being reduced to one-third its volume, was added to 1500 ml. of water. The clear solution so obtained soon yielded crystals; the mixture was cooled to 10°, filtered and the crystals dried at 100°. There was obtained 27 g. of 6-aminoindazole, 81.2%, m. p. 204–205°. This material was sufficiently pure to be used in the subsequent condensation reactions. However, on recrystallization from alcohol–water the product had a melting point of 209–210°.

The preparation of 6-aminoindazole is also illustrative for the preparation of 5- and 7-aminoindazole.

Preparation of 3-Aminoindazole.—This preparation is a variation of the method of Reissert and Grube.[4]

To a mixture of 11.5 g. (0.10 mole) of *o*-aminobenzonitrile and 125 ml. of concentrated hydrochloric acid at 0° was added a solution of 7.55 g. of sodium nitrite in a minimum amount of water. The diazonium solution was added dropwise to a solution of 152 g. of stannous chloride in 69.5 ml. of concentrated hydrochloric acid kept at 0–5°. The tin double salt of 3-aminoindazole separated. It was filtered off and dissolved without washing in water; the aqueous solution was then boiled for ten minutes. The solution was clarified by treatment with charcoal and made strongly alkaline. The precipitated solid was filtered off and extracted with benzene. From the benzene solution there was obtained upon concentration 5.5 g. (42.5%) of 3-aminoindazole of melting point 154–156°.

Preparation of N^4-Acetylsulfanilamidoindazoles.—The preparations of 3- and 5-acetylsulfanilamidoindazoles are also illustrative of the formation of the other acetylsulfanilamidoindazoles described in this paper.

Preparation of 5-Acetylsulfanilamidoindazole.—A solution of 13 g. (0.056 mole) of *p*-acetaminobenzenesulfonyl chloride in 70 ml. of acetone was added dropwise to a mechanically stirred solution, kept at 0–5°, of 6.7 g. (0.05 mole) of 5-aminoindazole in 100 ml. of acetone. The reaction mixture was maintained slightly alkaline by the addition of dilute sodium hydroxide. After fifteen hours at room temperature, the acetone was removed by distillation, the volume being kept constant by the addition of water. The reaction mixture was cooled and neutralized with acetic acid. A gummy mass precipitated; this was dissolved in alkali, the alkaline solution was clarified and neutralized; 7.5 g. of 5-acetylsulfanilamidoindazole, m. p. 250–52°, separated.

Anal. Calcd. for $C_{15}H_{14}N_4O_3S$: N, 16.97. Found: N, 16.60.

Preparation of 3-Acetylsulfanilamidoindazole.—A solutions of 24.3 g. (0.104 mole) of *p*-acetaminobenzenesulfonyl chloride in 75 ml. of pyridine was added dropwise, during thirty minutes, to a solution of 12.5 g. (0.094 mole) of 3-aminoindazole in 75 ml. of pyridine. The temperature rose spontaneously to 40°, after which the reaction mixture was allowed to cool and remain at room temperature overnight. After heating at 60° for one hour, the mixture was cooled and poured into 750 ml. of water. A gum precipitated from the mixture and crystallized on rubbing; yield 23 g., m. p. 253–255°.

Anal. Calcd. for $C_{15}H_{14}N_4O_3S$: N, 16.97. Found: N, 17.11.

Preparation of Sulfanilamidoindazoles.—The preparations of 3- and 5-sulfanilamidoindazole are also illustrative of the formation of the other sulfanilamidoindazoles described in this paper.

Preparation of 5-Sulfanilamidoindazole.—A solution of 15 g. (0.045 mole) of 5-acetylsulfanilamidoindazole in 100 ml. of 6 *N* hydrochloric acid was refluxed for thirty minutes, treated with charcoal and filter-cel and then filtered. The acid solution on cooling deposited a hydrochloride which was filtered off and then dissolved in water and neutralized. The crude 5-sulfanilamidoindazole which separated was dissolved in dilute sodium hydroxide, the solution treated with charcoal and then neutralized with acetic acid to give 8.5 g. of 5-sulfanilamidoindazole; this

was crystallized from alcohol–water to give a material which melted at 247–248°.

Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: N, 19.45; S, 11.10. Found: N, 19.28; S, 11.21.

TABLE I

ACETYLSULFANILAMIDOINDAZOLES

Acetylsulfanilamidoindazole	M. p., °C. (uncor.)	Formula	N Analyses, %[6] Calcd.	Found
3-	253–255	$C_{15}H_{14}N_4O_3S$	16.97	17.11
5-	250–252	$C_{15}H_{14}N_4O_3S$	16.97	16.60
6-	245–246	$C_{15}H_{14}N_4O_3S$	16.97	16.72
7-	258–260	$C_{15}H_{14}N_4O_3S$	16.97	16.84

TABLE II

SULFANILAMIDOINDAZOLES

Sulfanilamidoindazole	M. p., °C. (uncor.)	Formula	N Analyses, % Calcd.	Found
3-	225–226	$C_{13}H_{12}N_4O_2S$	19.45	19.61
5-	247–248	$C_{13}H_{12}N_4O_2S$	19.45	19.28
6-	195–196	$C_{13}H_{12}N_4O_2S$	19.45	19.40
7-	254–256	$C_{13}H_{12}N_4O_2S$	19.45	19.64

(6) The authors are indebted to Miss E. A. Bass and Miss H. M. Hutchinson for the microanalyses.

Preparation of 3-Sulfanilamidoindazole.—A solution of 23 g. (0.07 mole) of 3-acetylsulfanilamidoindazole in 250 ml. of 20% alcoholic hydrogen chloride was shaken at room temperature for fifteen hours. During this time, the hydrochloride of 3-sulfanilamidoindazole separated; it was filtered off, dissolved in water and the aqueous solution neutralized in the cold to precipitate the crude product. This crude material was then crystallized from alcohol or from 50% acetic acid, to yield 11.5 g. (57.3%) of 3-sulfanilamidoindazole of melting point 225–226°.

Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: N, 19.45. Found: N, 19.61.

Summary

The synthesis of a series of acetylsulfanilamidoindazoles and sulfanilamidoindazoles has been described. *In vitro* tests indicate that the sulfanilamidoindazoles have a marked bacteriostatic and, in some cases, bactericidal action. Several of these compounds show promising activity against streptococcus hemolyticus and pneumococcus infections in mice.

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The Effect of *p*H Changes upon Some of the Properties of Sodium Thymonucleate Solutions[1]

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Introduction

The molecular sizes and shapes of several nucleic acids, calculated from their sedimentation and diffusion behavior, have been reported in a previous article.[4] The molecular cross-sectional diameters of the different nucleic acid preparations studied were calculated to be approximately the same, about 14 Å., whereas the molecular lengths varied from 40 to 5000 Å.

It seemed significant that the longest nucleic acid molecules were those which had been prepared by Hammarsten's method[5] in which only solutions neutral to litmus and temperatures close to 0° are used. The samples prepared by more vigorous methods had much lower weights. These differences suggested that the smaller nucleic acid molecules were fragments of long chains which had been degraded in the process of isolating them from their natural sources. A study of the effects produced on a nucleic acid of high molecular weight by *p*H changes from neutrality to acid and alkaline conditions has been made to test this hypothesis.

A few examples of the effect of acids and bases on the physical properties of nucleic acid solutions have been reported in the literature. Jones and Austrian[6] and Jones,[7] using the "α-salt" of thymonucleic acid prepared by Neumann's method,[8] found that the viscosity of a 4% solution was decreased by the addition of either acetic acid or sodium hydroxide. The solution could be changed back and forth from a gelatinous to a fluid state by the alternate addition of acid and alkali. The optical rotation was decreased by the addition of either reagent. Hammarsten,[5] using solutions of sodium thymonucleate prepared by his method,

(1) More complete details of this work are to be found in theses of the authors submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the Ph.D. degree in June, 1942.

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(4) H. G. Tennent and C. F. Vilbrandt, THIS JOURNAL, **65**, 424 (1943).

(5) E. Hammarsten, *Biochem. Z.*, **124**, 383 (1924).

(6) W. Jones and C. R. Austrian, *J. Biol. Chem.*, **3**, 1 (1907).

(7) W. Jones, *ibid.*, **5**, 1 (1908).

(8) A. Neumann, *Arch. f. (Anat. u.) Physiol.*, **22**, 374 (1898).