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Benzotriazines. I. A New Series of Compounds Having Antimalarial Activity

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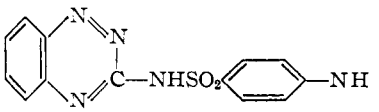
RECEIVED JANUARY 26, 1954

A series of substituted 1,2,4-benzotriazines has been prepared and tested for antimalarial activity. A high order of activity was observed with some of the compounds tested.

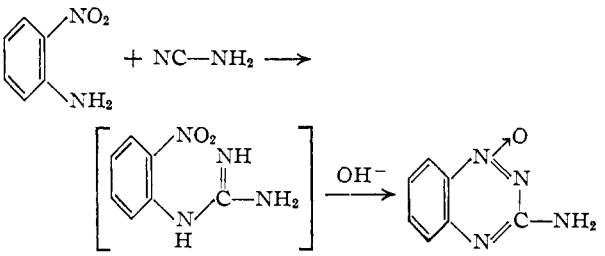
Although 2-sulfanilamidoquinoxaline possesses many desirable therapeutic properties and in contrast to many sulfonamides persists in the blood stream in high concentration, formation of the highly insoluble 3-hydroxy-2-sulfanilamidoquinoxaline[1] may cause formation of kidney stones in certain species when high levels of drug are administered. In an attempt to obtain compounds having the desirable therapeutic properties of sulfaquinoxaline, the preparation of a nitrogen isostere, 3-sulfanilamido-1,2,4-benzotriazine (I) was undertaken. Thus by eliminating the possibility



I

of hydroxylation by substituting a nitrogen for a carbon, one might obtain a more satisfactory sulfonamide.

The Arndt reaction[2] for the preparation of 3-amino-1,2,4-benzotriazine-1-oxide from cyanamide and *o*-nitroaniline offers a convenient method of obtaining the desired organic base and proved to be



equally applicable to most *o*-nitroaniline derivatives studied. In this method, the nitroaniline and cyanamide are melted and heated on a steam-bath and concentrated hydrochloric acid added. A violent exothermic reaction takes place which usually subsides after a few minutes. The resulting acidic solution of substituted guanidine is made alkaline and heated. The benzotriazine rapidly forms and precipitates from the solution. Yields vary from 20–60% depending on the amine used.

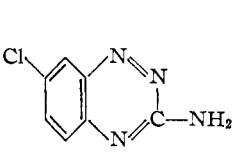
Although, in general, no difficulty in carrying out the reaction under these conditions was experienced, the violently exothermic nature of the condensation made it desirable to use a solvent for the preparation of larger quantities of the heterocyclic amine. Consequently, a procedure was developed which consisted of carrying out the condensation using acetic acid as solvent. This procedure gave generally higher yields and could be used for the preparation of larger quantities.

A third procedure was employed when those described above did not yield any product due to lack of formation of the substituted guanidine. In these cases, the condensation was carried out at somewhat higher temperature using cyanamide hydrochloride and the requisite amine. In the case of 2,4-dinitroaniline even this procedure did not yield any of the desired product, probably due to failure of condensation of the feebly basic amine with cyanamide.

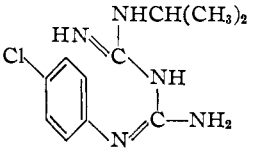
Difficulty was encountered in condensing the weakly basic amine of the N-oxide with either *p*-acetylaminobenzenesulfonyl chloride or *p*-nitrobenzenesulfonyl chloride. However, when the oxygen was removed the resulting 3-amino-1,2,4-benzotriazine could be condensed satisfactorily with *p*-nitrobenzenesulfonyl chloride and the condensation product was then reduced to the desired sulfonamide. The solubility in dilute alkali and ammonia shown by these sulfa compounds and their *p*-nitro precursors confirms the structure assignment by eliminating the possibility of direct attachment of sulfanilamido to one of the ring nitrogens.

In addition to the procedure described by Arndt which utilizes tin as the reducing agent, two procedures were used for the removal of the N-oxide. In both cases some dihydro product was formed which was oxidized to the desired triazine by adding ferric chloride. These are (1) reduction with phosphorus, iodine and water and (2) catalytic hydrogenation using Raney nickel. The low solubility of the materials in ordinary solvents limited the applicability of the latter method.

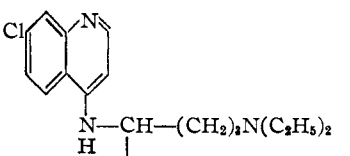
Since the antimalarial properties of the compounds were of interest, a series of corresponding chloro substituted compounds was also prepared in view of the ability of aromatically substituted chlorine to increase the effectiveness of known antimalarial agents. Although the sulfonamides were not effective in the treatment of avian malaria, the bases, 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide



II



Paludrine



Chloroquine

(1) J. R. Stevens, K. Pfister, 3rd, and F. J. Wolf, THIS JOURNAL, **67**, 1035 (1946).

(2) F. Arndt, *Ber.*, **46**, 3522 (1913).

TABLE I

SUBSTITUTED 1,2,4-BENZOTRIAZINES[a]

Substituent	M.p., °C.	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N	Antimalarial activity[b]
3-Amino	205–208	Not analyzed						0.4
3-Amino-1-oxide	267–268							N.A.
3-Sulfanilamido	216–217	51.8	3.7	23.3	52.0	3.9	23.1	.1
3-*p*-Nitrobenzosulfonamido	250–252	47.1	2.7	...	47.3	3.0	...	...
7-Chloro-3-amino	254–255	46.6	2.8	31.1	46.7	2.8	31.1	.01
7-Chloro-3-amino-1-oxide	302–305	42.8	2.6	28.5	43.3	2.5	28.8	.01
7-Chloro-3-sulfanilamido	219–220	46.5	3.0	20.9	46.8	3.2	21.0	.1
7-Bromo-3-amino	253	37.4	2.2	24.4	37.3	2.4	25.0	.01
7-Bromo-3-amino-1-oxide	294–295	34.8	2.1	23.2	35.0	2.1	23.2	.01
7-Iodo-3-amino-1-oxide	296–297	29.2	1.9	19.5	29.5	1.6	19.6	.02
7-Methyl-3-amino-1-oxide	271	54.4	4.6	31.8	54.5	4.7	32.6	N.A.
7-Methoxyl-3-amino-1-oxide	258–259	...	...	29.2	...	...	29.5	N.A.
6-Chloro-3-amino-1-oxide	293–295	42.8	2.6	...	42.7	3.0	...	N.A.
5-Chloro-3-amino-1-oxide	258–260	42.8	2.6	28.5	42.9	2.0	28.6	N.A.
5-Methyl-3-amino-1-oxide	260	54.4	4.6	31.8	54.7	4.4	32.2	N.A.
5,7-Dichloro-3-amino-1-oxide	287	36.4	1.7	24.2	36.4	1.8	24.0	0.05
7-Chloro-3-hydroxy-1-oxide	232–234	42.5	2.0	21.2	42.9	2.2	21.9	N.A.
7-Chloro-3-hydroxy	228–229	46.3	2.2	23.1	46.3	2.5	23.5	0.2
7-Chloro-3-acetylamino-1-oxide	256	45.3	3.0	...	45.3	3.1	...	.02
7-Chloro-3-succinylamino-1-oxide	250–254	44.3	3.1	18.8	44.6	3.1	19.2	.4

[a] The authors are indebted to Dr. A. O. Seeler and Miss C. Malanga of the Merck Institute for Therapeutic Research for performing these tests.

[b] Percentage in diet required to give protection. N.A. (not active) indicates failure at 0.4%, the highest level tested. This is test E-3 for blood-induced Gallinaceum malaria in the chick (*cf.* F. Y. Wiselogle, "A Survey of Antimalarial Drugs," 1941–1945, Vol. I, p. 476 (1946)).

and 7-chloro-3-amino-1,2,4-benzotriazine (II) were found to have excellent activity as suppressive agents in avian malaria. On a weight basis these compounds are about four times as potent as quinine and five times as potent as sulfadiazine, or about equivalent to sulfaquinoxaline. In the chick test administration once every 48 hours gives satisfactory results. The structural similarity of these compounds with Paludrine and Chloroquine is interesting.

Having observed that the 7-chloro derivative had antimalarial activity, a series of compounds was prepared to determine structural requisites for this activity. The study showed that effectiveness was limited to the series having a halogen in the 7-position and that maximum activity was obtained with an amine group in position 3. Activity could not be detected when the halogen was in other positions or when other groups were substituted in the 7-position. However, replacement of the amine group by hydroxyl or acylation of the amine group did not cause complete loss of the activity, although it was greatly diminished. The compounds tested are shown in Table I.

In addition to the compounds tabulated in Table I several substances were prepared by removal of the N-oxide with phosphorus and iodine. These products, which were not analyzed nor further characterized, are tabulated in Table II.

TABLE II

SUBSTITUTED 3-AMINO-1,2,4-BENZOTRIAZINES

Substituent	M.p., °C.
7-Methyl	217–218
7-Methoxyl	221–222
6-Chloro	250–251
5-Methyl	207–208

The *o*-nitroanilines used in this work were either available from commercial sources or could be obtained using methods described in the literature with the exception of 6-chloro-2-nitroaniline which was prepared by sulfonation, chlorination and desulfonation using a procedure similar to that described by Holleman[3] for the preparation of the corresponding bromo compound.

Acknowledgment.—We are indebted to Dr. Max Tishler for his interest and encouragement. Thanks are due also to Mr. R. N. Boos and his associates who supplied the microanalytical data.

Experimental

7-Chloro-3-amino-1,2,4-benzotriazine-1-oxide.—A mixture of 5 g. of 4-chloro-2-nitroaniline and 5 g. of cyanamide was placed in a 125-ml. erlenmeyer flask and heated on a steam-bath until a clear solution was obtained. Concentrated hydrochloric acid (15 ml.) was added. A violent reaction ensued which was cooled by immersion in a water-bath. Additional portions of cyanamide (1 g.) and concentrated hydrochloric acid (3 ml.) were added if required to yield a clear solution. The resultant mixture was cooled to room temperature, made strongly alkaline with 30% sodium hydroxide and warmed 3–5 minutes on a steam-bath until precipitation of the brilliant yellow product was complete. The brilliant yellow product was filtered and dried, yield 1.5 g., 26% yield based on 4-chloro-2-nitroaniline. Larger quantities were prepared using glacial acetic acid solvent as described below for the preparation of the bromo compound.

In a 2-liter flask fitted with a stirrer and heated by a Glas-col heater was placed 400 ml. of concentrated hydrochloric acid in 400 ml. of water. Then 20 g. of pulverized crude 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide was added. When the temperature reached 98–102° all of the 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide had dissolved. Then 1.0 g. of Darco G-60 is added and the mixture filtered through a line filter. The filtrate cooled to 10° with stirring in an ice-bath deposits beautiful brilliant yellow platelets of the hydrochloride. These were filtered and then slurried twice with vigorous stirring with 80–100 ml. of water to

(3) A. F. Holleman, *Rec. trav. chim.*, **27**, 153 (1908).

hydrolyze the unstable hydrochloride. The product becomes orange in color when the salt hydrolyzes.

The product must be dried *in vacuo* at about 100°. This method of purification either does not free the product entirely of 4-chloro-2-nitroaniline or hydrolyzes some of the 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide back to this material. During the high temperature drying the 4-chloro-2-nitroaniline sublimes out. Drying must be continued until no more sublimes. In addition if the washings have not been sufficient, HCl is evolved as the product dries; the yield is 75%.

7-Bromo-3-amino-1,2,4-benzotriazine-1-oxide.—A solution of 14 g. of 4-bromo-2-nitroaniline in 30 ml. of glacial acetic acid was heated to reflux, 21 g. of cyanamide and 30 ml. of concentrated hydrochloric acid were added dropwise and simultaneously at a rate sufficient to allow gentle reflux (about 10 minutes required). The mixture was then heated to maintain reflux for 15 minutes. After cooling to about 50° the mixture was made alkaline with 30% aqueous sodium hydroxide and heated to boiling for 10 minutes. The brilliant yellow product was separated by filtration, washed with ethanol and ether and dried, weight 7.25 g., yield 46.5% based on 4-bromo-2-nitroaniline. Recrystallization from pyridine yielded brilliant yellow needles, m.p. 294–295°.

5,7-Dichloro-3-amino-1,2,4-benzotriazine-1-oxide.—A mixture of 5 g. of 4,6-dichloro-2-nitroaniline and 5 g. of freshly prepared cyanamide dihydrochloride was heated in an oil-bath at 180–190° for 10 minutes. After the initial foaming subsided, 2 portions of 1 g. each of cyanamide dihydrochloride were added. The mixture was dissolved in 25 ml. of water, made alkaline with 30% sodium hydroxide and heated. The brilliant yellow product which separated was filtered and dried; yield 3.2 g., 64% of theory.

7-Chloro-3-amino-1,2,4-benzotriazine.—To a suspension of 1 g. of 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide in 350 ml. of ethanol was added 200 mg. of Raney nickel catalyst and the mixture shaken with hydrogen at 45 lb. pressure until 1 mole of hydrogen had been absorbed. The mixture of catalyst and some unchanged oxide was removed by filtration and discarded. The filtrate was concentrated to dryness *in vacuo* and the residue dissolved in hot ethanol and crystallized by cooling; yield 400 mg., 43% of theory.

7-Bromo-3-amino-1,2,4-benzotriazine.—A mixture of 20 g. of 7-bromo-3-amino-1,2,4-benzotriazine-1-oxide, 4.0 g. of red phosphorus and 1.5 g. of iodine in 300 ml. of glacial acetic acid was heated to reflux with stirring. After adding 5.0 ml. of water the mixture was refluxed for 18 hours. The mixture was filtered from excess phosphorus and the filtrate concentrated *in vacuo* to 50 ml. On pouring into 200 ml. of water, 14.6 g. of a greenish powder precipitated. The mother liquor was treated with ferric chloride until an immediate positive test was obtained with potassium iodide–starch test paper and again filtered yielding an additional 1.5 g. of product. The crude material, 16.1 g., was taken up in 500 ml. of boiling cellosolve, filtered and cooled yielding 9.0 g. of crystalline material, 49% yield.

7-Chloro-3-(*p*-nitrophenylsulfonamido)-1,2,4-benzotriazine.—A mixture of 10 g. of 7-chloro-3-amino-1,2,4-benzotriazine, 22 g. of *p*-nitrobenzenesulfonyl chloride and 125 ml. of pyridine was refluxed for four hours. At the end of this time the dark solution was concentrated to dryness *in vacuo*. The residue was extracted with 500 ml. of hot 1.25 *N* sodium hydroxide, and filtered. Upon cooling the filtrate, the sodium salt crystallized out in yellow platelets. This salt was filtered, dissolved in 100 ml. of water and acidified with glacial acetic acid. The yellow-green precipitate that formed was filtered and dried; wt. 6.6 g., 32.5% yield.

The crude product was further purified by dissolving in 6 *N* ammonium hydroxide, filtering and precipitating with glacial acetic acid, m.p. 240°.

Anal. Calcd. for $C_{13}H_8N_5O_4SCl$: C, 42.8; H, 3.2. Found: C, 43.2; H, 3.1.

7-Chloro-3-sulfanilamido-1,2,4-benzotriazine.—The reduction of 7-chloro-3-(*p*-nitrophenylsulfonamido)-1,2,4-benzotriazine was carried out by refluxing 2.4 g. of the material with 8 g. of "alcoholized" iron powder in 120 ml. of 95% ethanol and 0.5 ml. of concentrated hydrochloric acid for seven hours. The solution was made alkaline with 2.5 *N* sodium hydroxide and filtered using filter aid. The alcohol filtrate was concentrated to dryness *in vacuo* and the residue extracted with 3 *N* ammonium hydroxide and filtered. A bright yellow precipitate was obtained from the filtrate by acidifying with glacial acetic acid; wt. 1.25 g., 57% yield, m.p. 218–219°. The crude product was recrystallized from 36% aqueous acetic acid, m.p. 219–220°.

7-Chloro-3-acetylamino-1,2,4-benzotriazine-1-oxide.—A solution consisting of 19.6 g. of 7-chloro-3-amino-1,2,4-benzotriazine-1-oxide dissolved in 10.5 ml. of acetic anhydride and 200 ml. of pyridine was refluxed for eight hours. The solution was cooled and the precipitate filtered. A second crop was obtained by concentrating the filtrate *in vacuo* to a small volume yielding a total of 16.4 g. of greenish yellow product; m.p. 256°, 75% yield. The material may be recrystallized from cellosolve.

7-Chloro-3-hydroxy-1,2,4-benzotriazine.—Seven g. of 7-chloro-3-amino-1,2,4-benzotriazine was dissolved in 350 ml. of 50% sulfuric acid and cooled to 0°, when 2.8 g. of sodium nitrite was slowly added and the mixture allowed to stand for 15 minutes at room temperature. The solution was poured into water and the resulting precipitate filtered. The product was separated from unreacted amine by dissolving in strong alkali and filtering. The product obtained by acidification of the filtrate with acetic acid and filtering, weighed 3.5 g., 50% yield.

6-Chloro-2-nitroaniline.—A solution of 100 g. of *o*-nitroaniline in 100 ml. of concentrated sulfuric acid and 275 ml. of 20% oleum was warmed to 160°. After 5 minutes, the mixture was quenched in 2 kg. of ice and water. With good stirring a solution of 50 g. of chlorine in 700 ml. of glacial acetic acid was added. After standing 24 hours at room temperature, the mixture was filtered. The insoluble material, 2–8 g. was discarded and the filtrate steam distilled.

The steam distillation was carried out in a 5-liter three-necked flask equipped with a thermometer immersed into the liquid and heated by a Glas-Col heater. The mixture was heated gradually to 135°. When this temperature was reached, most of the acetic acid had distilled out and the product began to steam distil. Steam was introduced and the temperature gradually rose to 150°. The product solidified in the condenser and was melted out occasionally by shutting off the cooling water supply. Near the end of the steam distillation the mixture foamed badly and an antifoam agent such as tributyl phosphate was helpful. The distillate (approximately 5 liters) was filtered and the product recrystallized from petroleum naphtha (b.p. 60–70°). A total of 36.3 g. of material, m.p. 72–73°, was obtained (29% of theory).

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