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Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

II. Derivatives of 2-Halogeno-4-aminobenzoic Acid

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According to Johnson *et al.* [1] and Wyss *et al.* [2] 2-chloro-4-aminobenzoic acid is bacteriostatically active; this effect is neutralized by *p*-aminobenzoic acid and therefore seems to be of the same nature as the effect of sulfanilamide. As is well known the effect of sulfanilamide is greatly enhanced by the introduction of heterocyclic substituents in the amide group, and consequently it seemed possible that heterocyclic substituted amides of 2-chloro-4-aminobenzoic acid would show a higher activity than the free acid.

To investigate this problem we have synthesized some heterocyclic amides of 2-chloro-, 2-bromo- and 2-iodo-4-aminobenzoic acid. The compounds were prepared along conventional lines by reaction of the 2-halogeno-4-nitro-benzoylchlorides with the appropriate amine in pyridine solution, followed by catalytic reduction of the nitroamides thus formed.

The bacteriostatic activity of these heterocyclic derivatives, of 2-chloro- and 2-iodo-4-aminobenzamide and of 2-iodo-4-amino-benzoic acid was tested on the following bacteria:

Staphylococcus aureus, *Staphylococcus albus*, *Diplococcus pneumoniae* (type I), *Enterococcus*, *Proteus vulgaris*, *Escherichia coli*, *Eberthella typhosa*, *Salmonella paratyphi B*, *Shigella paradysenteriae*, *Shigella sonnei*, *Klebsiella pneumoniae* and *Corynebacterium diphteriae (gravis)*.

The bacteriostatic effect was tested in the following manner: the bacteria were inoculated in streaks on blood agar; strips of filter paper moistened with solutions or suspensions of the compounds were placed across the streaks of bacteria and the cultures were incubated at 37° for 24 hours. In case of a bacteriostatic effect the growth of the bacteria stops at a shorter or longer distance from the filter paper strips.

This method has been found to be very convenient for qualitative testing of the effect of bacteriostatic substances. All the compounds here described were found to be completely inactive. Most of the compounds are very slightly soluble even by addition of a little hydrochloric acid (the solutions investigated generally contained 10 mg/100 ml). The unsubstituted amides are somewhat more soluble and the sodium salt of 2-iodo-4-aminobenzoic acid is highly soluble; these compounds were however found to be inactive too.

On the other hand the last mentioned substances have been found by Lehmann to possess a pronounced bacteriostatic effect against *Mycobacterium tuberculosis* (these results are to be published elsewhere). In this respect these compounds resemble *p*-aminosalicylic acid, which similarly is only slightly active against bacteria other than *M. tuberculosis*.

EXPERIMENTAL

2-Chloro-4-nitrotoluene was prepared by chlorination of *p*-nitrotoluene with antimony trichloride as a catalyst [3]. After destillation a recrystallization from ethanol was necessary to obtain a melting point of 64—65°.

2-Chloro-4-nitrobenzoic acid was prepared by the method of Ullmann and Wagner [4] with some slight modifications: 21 g of 2-chloro-4-nitrotoluene and 200 ml of 0.1 *N* sodium hydroxide were heated to boiling in a 3 l roundbottomed flask provided with reflux condenser and separatory funnel. A solution of 31 g of potassium permanganate in 1.9 l of water was added at the same rate as it was reduced by the toluene. When all was added and the colour of the permanganate had disappeared the solution was filtered, cooled, acidified and extracted with ether (the solution may also be concentrated to a small volume and acidified; the chloro-nitrobenzoic acid then separate). The ether solution gave by evaporation a raw product melting at ca 138°; a recrystallization from water (lost 15 %) raised the melting point to 141°. Yield of 2-chloro-4-nitrobenzoic acid 7.2—8.3 g (30—34 %). From the manganese dioxide 7.4—9.5 g of the starting material could be recovered unchanged.

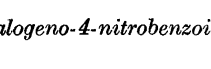
2-Bromo-4-nitrotoluene was prepared by bromination of *p*-nitrotoluene with iron as a catalyst.

2-Bromo-4-nitrobenzoic acid has previously been obtained by Frejka and Vitha [6] by oxidation of 2-bromo-4-nitrotoluene with nitric acid and potassium chlorate and mercury as catalysts. This method in our hands, however, gave only very small yields. Therefore we prepared the bromocompound in exactly the same way as the chloro compound and with about the same yield (32 %). Melting point of the raw product 161—164°, after recrystallization from water 166—167°.

2-Iodo-4-nitrobenzoic acid was prepared from 2-amino-4-nitrobenzoic acid by diazotation [7]. Yield 74 %, m. p. 144—145° after recrystallization from water.

These acids were converted into acid chlorides by the action of thionyl chloride. As a rule 4 g of the acid was heated with 8 ml of thionyl chloride until all had dissolved; after the excess of thionyl chloride had been removed *in vacuo*, the acid chloride was added, without further purification, to the equivalent amount of the heterocyclic amine dissolved in 10—20 ml of cold, anhydrous pyridine. After standing at room temperature

for some hours, the reaction mixture was poured into water and the amide which separated was filtered, dried, and recrystallized from acetic acid, pyridine or — in some cases — ethanol (most of the compounds are almost insoluble in ethanol). Yields of the crude products 75—85 % except for the derivative of 2-amino-4,6-dimethylpyrimidine, which could be obtained only in ca. 30 % yield. The compounds form white or pale yellow crystals which are only sparingly soluble in most solvents; melting points and analyses are presented in Table 1.

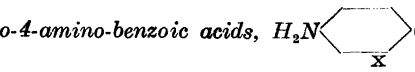
Table 1. Amides of 2-halogeno-4-nitrobenzoic acids, 

No.	X	R	Formula	M. p. °C	N % calc.	found
1	Cl	H	$C_7H_5O_2N_3Cl$	170—71	13.96	14.12
2	Cl	2-pyridyl	$C_{12}H_8O_3N_3Cl$	166—68	15.13	15.07
3	Cl	2-thiazolyl	$C_{10}H_6O_3N_3SCl$	262—64	14.81	14.61
4	Cl	2-(5-methyl)-thiadiazolyl	$C_{10}H_7O_3N_4SCl$	259—61	18.76	18.64
5	Cl	2-(4,6-dimethyl)-pyrimidyl	$C_{13}H_{11}O_3N_4Cl$	154—55	18.27	18.53
6	Br	2-pyridyl	$C_{12}H_8O_3N_3Br$	154—55	13.05	12.83
7	Br	2-thiazolyl	$C_{10}H_6O_3N_3SBr$	286—87	12.80	12.82
8	Br	2-(5-methyl)-thiadiazolyl	$C_{10}H_7O_3N_4SBr$	245—48	16.32	16.54
9	I	H	$C_7H_5O_3N_2I$	210—11	9.59	9.74
10	I	2-thiazolyl	$C_{10}H_6O_3N_3SI$	298—99 (d.)	11.20	11.41

Nos. 1, 5, 6, 8 and 9 were recrystallized from ethanol, nos. 2, 3 and 4 from acetic acid and nos. 7 and 10 from pyridine.

In addition to the heterocyclic amides the unsubstituted amides of 2-chloro- and 2-iodo-4-nitrobenzoic acid were prepared by addition of the acid chlorides to ice-cold, concentrated aqueous ammonia.

The amino derivatives were prepared from the nitro derivatives by catalytic hydrogenation; no splitting off of the halogen atoms was observed, even by prolonged action of hydrogen. The unsubstituted amides, the pyridine, and the pyrimidine derivatives were hydrogenated in alcohol solution (1—2 g of the nitro compound suspended in 25—50 ml of ethanol to which was added ca 0.1 g of PtO_2). The solid gradually went into solution; when the calculated amount of hydrogen had been absorbed (after 3—12 hours) the solution was filtered and concentrated *in vacuo;* upon the addition of water a precipitate separated which was filtered and recrystallized from concentrated or dilute alcohol. In case of the thiazole and thiadiazole derivatives the hydrogenation proceeded extremely slowly on account of the slight solubility of the nitro compounds in ethanol. When, however, the nitro compounds were dissolved in hot glacial acetic acid, the hydrogenation could be performed in the course of a few hours. After absorption of the calculated amount of hydrogen the solutions were diluted with water, neutralized, and filtered. These amino compounds were also recrystallized from ethanol. Yields 60—80 %. Melting points are

Table 2. Amides of 2-halogeno-4-amino-benzoic acids, 

X	R	Formula	M. p. °C	N % calc.	found
Cl	H	$C_7H_7O_2NCl$	162—64	16.35	16.29
Cl	2-pyridyl	$C_{12}H_{10}ON_3Cl$	171—72	16.96	16.79
Cl	2-thiazolyl	$C_{10}H_8ON_3SCl$	227—28	16.57	16.61
Cl	2-(5-methyl)-thiadiazolyl	$C_{10}H_9ON_4SCl$	261—62	20.85	20.82
Cl	2-(4,6-dimethyl)-pyrimidyl	$C_{13}H_{13}ON_4Cl$	201—202	20.25	20.34
Br	2-thiazolyl	$C_{10}H_8ON_3SBr$	183—85	14.09	14.19
Br	2-(5-methyl)-thiadiazolyl	$C_{10}H_9ON_4SBr$	215—17	17.88	18.04
I	H	$C_7H_7ON_2I$	174—75	10.70	10.82
I	2-thiazolyl	$C_{10}H_8ON_3SI$	200—01	12.17	12.19

presented in Table 2. The amino compounds are much more soluble in alcohol than the nitro derivatives. They can be dissolved in an excess of acid, separating again for the most part by diluting these solutions with water.

SUMMARY

Amides of 2-chloro , 2-bromo- and 2-iodo-4-aminobenzoic acid, unsubstituted and containing heterocyclic substituents in the amide group, were prepared. These compounds were found to be without any bacteriostatic effect on a great number of pathogenic bacteria, but some of them have some effect upon *Mycobacterium tuberculosis.*

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