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Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

I. Esters and Amides of 4-Aminosalicylic Acid

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Lehmann [1], in 1946, reported that 4-aminosalicylic acid possessed bacteriostatic properties against the tubercle bacillus. The compound is effective in the suppression of tuberculous infections of laboratory animals and has under the name PAS (*para*-amino-salicylic) been applied in the treatment of tuberculosis in man, which it seems to influence favourably.

On the basis of the observation by Bernheim [2], that salicylic acid and benzoic acid stimulate the respiration of the tubercle bacillus, Lehmann investigated several derivatives of salicylic acid and *p*-amino-benzoic acid in the hope of finding a compound acting as antagonist to salicylic acid. The most bacteriostatic active of these compounds was 4-aminosalicylic acid. It soon turned out, however, that its effect could not be considered as inhibitory towards salicylic acid. Considering the findings of Johnson *et al.*[3] and Wyss *et al.*[4], *viz.* that certain 2-substituted *p*-aminobenzoic acids act as competitors to *p*-aminobenzoic acid, it also seems more likely, that the effect of 4-aminobenzoic acid should be due to competition with the metabolite *p*-aminobenzoic acid.

As a matter of fact it could be shown that the inhibitory effect of *p*-aminosalicylic acid could be neutralized by addition of *p*-aminobenzoic acid. The bacteriostatic effect of *p*-aminosalicylic acid against bacteria, other than *Mycobacterium tuberculosis*, is very slight (*cf.* Sievers [5]); on pneumococci there was no inhibition until a concentration between 0.012 and 0.025 *M*; by addition of *p*-aminobenzoic acid making the solutions 0.0002 *M* as to this compound, there was no inhibition in the 0.025 *M* PAS-solution and only slight inhibition in a 0.05 *M* PAS-solution.*

* We thank Kai Schmith, M. D., for the performance of these experiments.

Although the competitive effect cannot be demonstrated so clearly as with the sulfonamides, due to the slight bacteriostatic effect of PAS against pneumococci, we think that these experiments prove that the effect of PAS is in fact inhibitory to *p*-aminobenzoic acid. Lehmann has obtained similar results from experiments with *M. tuberculosis* (private communication).

In order to investigate whether the bacteriostatic effect of 4-aminosalicylic acid could be enhanced by substitutions, we have prepared some of its esters and amides, derived from aliphatic amines. Amides with heterocylic substituents, which cannot be prepared in the same way as the aliphatic derivatives, will be described in a subsequent paper.

The bacteriostatic effect of these new compounds against *M. tuberculosis* has been determined by Lehmann, who will publish his results elsewhere. It may, however, be mentioned here that all the compounds inhibit the growth of tubercle bacilli, most of them having about the same bacteriostatic effect as the free acid.

EXPERIMENTAL

The esters of 4-aminosalicylic acid were prepared by catalytic hydrogenation of the corresponding nitroesters. 4-Nitrosalicylic acid was prepared from 4-nitrophthalimide [6], and the acid was esterified by refluxing with the appropriate alcohol in the presence of sulphuric acid:

20 g of *p*-nitrosalicylic acid and 8 ml of conc. sulphuric acid were dissolved in 160 ml of the alcohol and refluxed for 16—24 hours. The alcohol was removed *in vacuo*, water was added and the precipitate filtered, dried and crystallized from methanol. The nitroesters were recrystallized from the corresponding alcohol, decolourizing the solutions with a little charcoal (methanol or ethanol may also be used without any ester interchange). Yields varying from 40—60 %.

The esters form light yellow crystals, which are easily soluble in alcohol, benzene etc. and slightly soluble in water. Melting points and analyses are presented in Table 1. (The ethylester has previously been synthezised by Borsche [7], who describes it as white, it has, however, about the same colour as sulphur.)

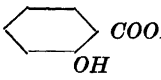
Table 1. Esters of 4-nitrosalicylic acid, O_2N  *COOR.*

R	Formula	M.p. °C	N analyses, % calc.	found
Ethyl	$C_9H_9O_5N$	90	6.63	6.61
n-Propyl	$C_{10}H_{11}O_5N$	33	6.23	6.25
iso-Propyl	$C_{10}H_{11}O_5N$	73	6.23	6.28
n-Butyl	$C_{11}H_{13}O_5N$	35	5.86	5.90
iso-Butyl	$C_{11}H_{13}O_5N$	62	5.86	5.79

The nitro-esters were hydrogenated in ethanolic solution (the use of higher alcohols is not convenient):

10 g of ethyl-4-nitrosalicylate were suspended in 50 ml of ethanol and 0,5 g PtO_2 was added. The suspension was shaken in an atmosphere of hydrogen at 4 atm.; the ester dissolves gradually under noticeable heat evolution. After two hours the calculated amount of hydrogen was absorbed, the solution was cold and a white crystalline product formed. The crystals were dissolved by heating, the solution filtered from platinum and concentrated *in vacuo*. By addition of water a white precipitate separated; it was filtered off and recrystallized from dilute alcohol or benzene. The hydrogenation of the other esters was carried out in smaller amounts (*e. g.* 1 g of ester, 20 ml of alcohol and 0.1 g of PtO_2).

The esters of 4-aminosalicylic acid form white crystals, which are very soluble in alcohol. They may conveniently be recrystallized from dilute alcohol or benzene. The yields amount to 70—80 %. Melting points and analyses are presented in Table 2.

Table 2. Esters of 4-aminosalicylic acid, 

R	Formula	M. p. °C	N analyses, % calc.	found
Ethyl	$C_9H_{11}O_3N$	115	7.73	7.51
n-Propyl	$C_{10}H_{13}O_3N$	102—03	7.17	7.11
iso-Propyl	$C_{10}H_{13}O_3N$	75—76	7.17	7.20
n-Butyl	$C_{11}H_{15}O_3N$	93—94	6.70	6.78
iso-Butyl	$C_{11}H_{15}O_3N$	84—85	6.70	6.73

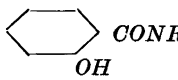
4-Nitrosalicylamide has been prepared by Borsche by reaction of ethyl 4-nitrosalicylate with alcoholic ammonia. It is, however, more convenient to use aqueous ammonia:

10 g of ethyl 4-nitrosalicylate and 50 ml of concentrated aqueous ammonia were heated at 100° for two hours in a closed vessel. The solution was turned to dryness in vacuo and the residue recrystallized from water, charcoal and a few drops of acetic acid being added.

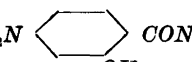
The methylamide was made in the same way. The other amides were prepared from the anhydrous amines:

Ethyl 4-nitrosalicylate (1—5 g) was placed in a thick walled glasstube and the same weight of the appropriate amine added under cooling. The tube was sealed and heated at 100° in water bath. At first a yellow crystalline solid (alkylammonium salt of the phenol) is formed, but gradually it deliquesces and is transformed into a syrup. The time

of heating varies from 1 hour (ethyl- and propylamides) to about 14 hours (diethyl- and dipropylamide). The reaction products were dissolved in 50 ml of ethanol; the solution was diluted to 250—300 ml with water, decolorized with carbon, filtered and the amides precipitated by addition of hydrochloric acid. The amides were recrystallized from dilute alcohol (1 : 1) containing about ½ % acetic acid. The amides of 4-nitrosalicylic acid (Table 3) form beautiful white or yellowish crystals, slightly soluble in water, very soluble in alcohol.

Table 3. Amides of 4-nitrosalicylic acid,  *CONRR'.*

R	R'	Formula	M. p. °C	N analyses, % calc.	found
H	H	$C_7H_6O_4N_2$	194	15.40	15.61
CH_3	H	$C_8H_8O_4N_2$	218	14.29	14.35
C_2H_5	H	$C_9H_{10}O_4N_2$	157	13.33	13.52
C_3H_5	H	$C_{10}H_{10}O_4N_2$	134	12.66	12.57
n-C_3H_7	H	$C_{10}H_{12}O_4N_2$	128	12.50	12.35
i-C_3H_7	H	$C_{10}H_{12}O_4N_2$	187	12.50	12.63
C_2H_5	C_2H_5	$C_{11}H_{14}O_4N_2$	183	11.76	11.75
n-C_4H_9	H	$C_{11}H_{14}O_4N_2$	122	11.76	11.82
i-C_4H_9	H	$C_{11}H_{14}O_4N_2$	167	11.76	11.86
n-C_3H_7	*n*-C_3H_7	$C_{13}H_{18}O_4N_2$	131	10.52	10.42
$C_6H_5CH_2$	H	$C_{14}H_{12}O_4N_2$	154	10.28	10.07

Table 4. Amides of 4-aminosalicylic acid,  *CONRR'.*

R	R'	Formula	M. p. °C	N analyses, % calc.	found
H	H	$C_7H_8O_2N_2$	162	18.42	18.40
CH_3	H	$C_8H_{10}O_2N_2$	158	16.86	16.93
C_2H_5	H	$C_9H_{12}O_2N_2$	138	15.55	15.73
C_3H_5	H	$C_{10}H_{12}O_2N_2$	134	14.58	14.54
n-C_3H_7	H	$C_{10}H_{14}O_2N_2$	142	14.43	14.27
i-C_3H_7	H	$C_{10}H_{14}O_2N_2$	161	14.43	14.58
C_2H_5	C_2H_5	$C_{11}H_{16}O_2N_2$	141	13.46	13.50
n-C_4H_9	H	$C_{11}H_{16}O_2N_2$	125	13.46	13.51
i-C_4H_9	H	$C_{11}H_{16}O_2N_2$	127	13.46	13.51
n-C_3H_7	*n*-C_3H_7	$C_{13}H_{20}O_2N_2$	121	11.86	11.98
$C_6H_5CH_2$	H	$C_{14}H_{14}O_2N_2$	129	11.57	11.48

The nitroamides were converted into the corresponding aminoderivatives by catalytic hydrogenation, 25 ml of ethanol and 0.1 g of PtO_2 being used for 1 g of the nitro-amide. After absorption of the calculated amount of hydrogen the solutions were filtered and evaporated almost to dryness *in vacuo;* the

residues were dissolved in boiling water, the solutions decolourized with carbon and filtered. By cooling the amides precipitate in good yields (70—80 %) in form of colourless crystals, which are more soluble in water than the corresponding nitro compounds.

By catalytic hydrogenation the allylamide of 4-nitrosalicylic acid is transformed into the *n*-propylamide of *p*-aminosalicylic acid. The allylamide of *p*-aminosalicylic acid was prepared by reduction of the corresponding nitro compound with zinc and hydrochloric acid in alcoholic solution.

4-Aminosalicylic amide has been synthesized also from methyl 4-aminosalicylate [8] and ammonia: A mixture of 8 g of methyl 4-aminosalicylate and 8 ml of conc. aqueous ammonia was heated in a closed vessel for two hours at 100°. The solution was evaporated almost to dryness *in vacuo*, and the crystals, which separated, were recrystallized from water. The compound was identified as the amide of 4-aminosalicylic acid. With *n*-butylamine and methyl 4-aminosalicylate the corresponding amide was isolated too. The higher esters on the contrary react very slowly with ammonia and amines, even at 140°, and only unchanged ester or oily products could be obtained.

SUMMARY

The bacteriostatic effect of 4-aminosalicylic acid is antagonized by *p*-aminobenzoic acid.

Some esters and amides of 4-aminosalicylic acid were prepared by catalytic hydrogenation of the corresponding nitro derivatives.

REFERENCES

1. Lehmann, J. *Lancet* **250** (1946) 15; *Svenska Läkartidn.* **43** (1946) 2029.
2. Bernheim, F. *Science* **92** (1940) 204; *J. Bact.* **41** (1941) 387.
3. Johnson, O. H., Green, D. E., and Pauli, R. *J. Biol. Chem.* **153** (1944) 37.
4. Wyss, O., Rubin, M., and Strandskov, F. B. *Proc. Soc. Exp. Biol. Med.* **52** (1943) 106.
5. Sievers, O. *Svenska Läkartidn.* **43** (1946) 2041.
6. Seidel, H., and Bittner, J. C. *Monatsh.* **23** (1902) 431;
   Seidel, H. *Ber.* **34** (1901) 4352.
7. Borsche, W. *Ann.* **390** (1912) 18.
8. Rosdahl, K.-G. *Svensk Kem. Tid.* **60** (1948) 12.

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