[CONTRIBUTION FROM THE KENT CHEMICAL LABORATORY OF THE UNIVERSITY OF CHICAGO.]

# ACETYLAMINOPHENYL SALICYLATE. THE PREPARATION OF SALOPHEN.

BY R. Q. BREWSTER.

Received April 30, 1918.

Difficulties in the obtaining of good yields of acetylaminophenyl salicylate, $CH_3CONHC_6H_4O_2CC_6H_4(OH)$, the drug known as salophen, were reported to Professor Stieglitz, the Chairman of the Committee on Synthetic Drugs of the National Research Council. At his request the investigation reported here was undertaken and the results are published for the benefit of all manufacturers interested in the products.

*p*-Nitrophenyl salicylate was supplied by the manufacturer primarily interested and was at first used without further purification. The first experiments were made with the aim of isolating the free base obtained from the reduction of the compound; in the later experiments, a continuous process was used for the preparation of salophen itself.

Experiments made by Miss Gladys Leavell in this laboratory on the reduction of *p*-nitrophenyl salicylate by the ordinary methods in aqueous acid solutions, with the aid of tin, stannous chloride, or zinc, showed that hydrolysis of the ester and its reduction product by the acids present formed the major difficulty in the carrying out of a successful reduction with satisfactory yields. Miss Leavell also showed that reduction by hydrogen chloride in ether solution with the aid of tin and reduction with sodium hydrosulfite gave no better results.

For the prevention of hydrolysis a non-aqueous solvent, glacial acetic acid, was finally employed and reduction accomplished by the addition of zinc dust.

## Preparation of *p*-Aminophenyl Salicylate.

After several runs had been made the following procedure was adopted:

The crude salicyl-*p*-nitrophenol (1 g.) was dissolved in 10 cc. of warm glacial acetic acid and this solution was dropped in the course of 10 or 15 minutes into a flask containing 8 g. zinc dust and 10 cc. glacial acetic acid. Reduction took place rapidly with the evolution of heat. After all the salicyl-*p*-nitrophenol solution had been added, and the mixture kept warm for 10 minutes, the liquid was filtered from the excess of zinc at the pump and the zinc residue washed with glacial acetic acid. The filtrate was distilled *in vacuo*, in a stream of carbondioxide, at a temperature of 40–50° C. The *p*-aminophenylsalicylate remains in the distilling flask as an oil containing some acetic acid and zinc acetate. This residue was made alkaline with ammonium hydroxide or sodium carbonate solution and extracted several times with ether as rapidly as possible. The ether extract was dried over anhydrous sodium sulfate, decanted,

and the ether distilled off. The free base was deposited as a light brown solid. The yield was 45% of the theoretical yield. When dry hydrogen chloride was passed into the dry ether solution of the base the hydrochloride was precipitated as a gray solid. The yield of the hydrochloride is nearly theoretical for the amount of base taken.

The free base (*p*-aminophenylsalicylate) was purified from 95% alcohol. Calc. for $C_{13}H_{11}O_3N$: C, 68.12; H, 4.80. Found: C, 68.06; H, 5.06.

**Preparation of Salophen from Crude Nitrophenyl Salicylate.**

During the course of the work it seemed that a better yield of the desired salophen could be obtained by the preparation of the acetyl derivative (salophen) directly, the isolation of the free base being thus avoided. Although the yield is not as good as desired this scheme is to be preferred.

The crude *p*-nitrophenylsalicylate (5 g.) was dissolved in about 20 cc. of glacial acetic acid and this solution added very slowly, and with constant shaking of the flask, to a mixture of 20 g. zinc dust and 15 cc. glacial acetic acid. Reduction was complete in about 20 minutes. The solution was then decanted from the excess of zinc and the zinc residue washed with hot glacial acetic acid. Since the acetic acid contained 0.5% water, sufficient acetic anhydride was added to render the acid anhydrous. The liquid was then boiled under a reflux condenser for 20 hours, at the end of which time it was allowed to cool and poured into 5 times its volume of water. Crude salophen was precipitated as a voluminous light brown solid. It was stirred well until the precipitate had collected, brought on a filter at the pump and dried in an oven at 90°. Yield, 55%. The melting point of the crude material is 175–180°. The filtrate contains a little salophen which may be recovered by distillation of the acid *in vacuo* from a 50° bath Sufficient salophen separated from the concentrated solution thus obtained to account in all for 60% of the theoretical yield.

For the purification of crude salophen the alcoholic solution was boiled with charcoal for the removal of the coloring matter, filtered while hot and the filtrate diluted carefully with water until a sample deposited crystals on cooling at the tap. The product thus obtained may be had quite pure by recrystallization from hot 50% ethyl alcohol. The pure product gave the m. p. 187°. When it was mixed with pure salophen (Bayer), no depression of the melting point could be detected. Very little material was lost in purification.

For the recovery of the acetic acid the acid liquors may be neutralized with lime and evaporated and the calcium acetate distilled with sulfuric acid.

**Preparation of *p*-Aminophenylsalicylate and of Salophen from Purified Nitrophenyl-salicylate.**

The *p*-nitrophenylsalicylate was purified by one recrystallization from alcohol and two from ligroin of high boiling point. The product is nearly

colorless and melts at 145–147° rather than 148°, the recorded melting point.

The experiments described above were repeated, the purified starting material being used. The yield of the free base was increased to 73% and of salophen to 80%.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY, NO. 313.]

## ACTION OF PANCREATIC ENZYMES UPON CASEIN.

BY H. C. SHERMAN AND DORA E. NEUN.

Received May 10, 1918.

The fact that our purified pancreatic amylase preparations show proteolytic activity also[1] has led us to extend this phase of our enzyme investigation to a comparative study of the hydrolysis of casein by various preparations derived from the pancreas.

The data summarized in Table I permit quantitative comparison of the proteolytic activities (1) of high grade commercial pancreatins, (2) of the 3 principal fractions recovered from the pancreatin in the course of making from it the pancreatic amylase preparations previously described,[1] (3) of the most active trypsin which we have found commercially available.

In each case the proteolytic activity was determined by allowing from 0.25 mg. to 2.0 mg. of the enzyme preparation to act upon 1 g. of casein (weighed air dry and containing 135 to 140 mg. of nitrogen) in a slightly alkaline solution: hydrogen ion concentration $= 1 \times 10^{-8.3}$; or $P_H = 8.3$ (Sörensen). The methods used in measuring the extent of hydrolysis were those described in our previous paper,[2] of which the ones chiefly employed in this investigation are, (1) the determination of the total nitrogen of digestion products which have passed through the early proteose stage, (2) the nitrogen converted to the amino form as determined by the Van Slyke method.

Pancreatins 6 and 7 were high grade commercial preparations representing practically the whole gland freed from water and fat. These formed the starting point for the laboratory preparations.

Residue 78 (N 14) was the material remaining when pancreatin was extracted once with 9 times its weight of 50% alcohol (as the first step in the process of preparing pancreatic amylase) the residue being dried by washing with alcohol and ether.

"Sac precipitate" is the material which settles out of the amylase solution during the dialysis in 50% alcohol which precedes the final precipitation of the amylase preparation as previously described.[3]

[1] Sherman and Schlesinger, THIS JOURNAL, **34**, 1110 (1912); **37**, 1306 (1915); Sherman and Neun, *Proc. Soc. Expt. Biol. Med.*, **15**, 55 (1918).

[2] Sherman and Neun, THIS JOURNAL, **38**, 2199 (1916).

[3] Sherman and Schlesinger, *Loc. cit.*