with air agitation to 1980 g. of approximately 80%[3] nitric acid (sp. gr. 1.438–1.455, 76–83%) which had been cooled to 0° and rendered water-white by air agitation and the use of 3 g. of urea. The temperature was maintained at −10 to +5° during the following additions and reaction times. Air agitation was continued for 15 minutes and then 1980 g. of approximately 80% sulfuric acid (sp. gr. 1.721–1.737, 79–81%) which had been cooled to −10° was added over a period of five minutes to the reaction mixture. The reaction was completed by using air agitation over a two-hour period.

The reaction mixture was then poured onto 500 g. of ice and filtered through glass cloth. After washing the precipitate with 4 l. of water, the precipitate was dissolved by heating at 50° in 2.5 l. of acetone containing 75 g. of ammonium carbonate. Ethanol and water were then added in sufficient quantities to the solution to form a solvent mixture containing 7 parts of acetone, 3 parts of water and two parts of ethanol. After the reaction mixture stood for one hour, the precipitated pentaaerythritol tetranitrate was filtered and washed with ethanol. The dried precipitate weighed 372 g. and had a m.p. of 132–135° (lit.[4] m.p. 141°). The combined filtrates were then poured into 7 l. of water and allowed to stand for 16 hours. After removal of the aqueous layer by decantation, the organic layer remaining was filtered. The precipitate of pentaerythritol tetranitrate was washed with ethanol and weighed 32 g. This combined with previously isolated material gave a total of 404 g. of pentaerythritol tetranitrate, a 36% yield. The combined filtrates were then shaken with one liter of water and the lower organic layer separated. The low-boiling material was removed from the organic layer by reduced pressure (2 mm.) distillation at 60° to give 450 g., a 47% yield of pentaerythritol trinitrate.

Anal. Calcd. for $C_5H_9O_{10}N_3$: N, 15.48. Found: N, 15.35–15.60.

The pentaerythritol trinitrate also was identified by preparation of the derivative pentaerythritol acetate trinitrate,[2] m.p. 86–88°. This material did not depress the melting point of an authentic sample of the acetate.

Limitations of this method and other attempted preparations are described below. On reduction of the concentration of sulfuric acid to 75% (sp. gr. 1.664–1.672) in two preparations, yields of 30 and 26% of pentaerythritol trinitrate and 20% of pentaerythritol tetranitrate were obtained. The mixed acid system 85% phosphoric–80% nitric acid using a similar procedure gave an average yield of 16% pentaerythritol trinitrate and a negligible quantity of pentaerythritol tetranitrate. Perhaps more concentrated solutions of phosphoric acid would have improved the yields by this method. By the use of 90% nitric acid nearly a quantitative yield of pentaerythritol tetranitrate was obtained while with 80% nitric acid an average yield of 21% of the lower nitrates and no pentaerythritol tetranitrate was obtained in two preparations.

Acknowledgment.—The authors wish to acknowledge the advice of Dr. L. G. Bonner during the course of this investigation. A number of the preparations of the pentaerythritol trinitrate were accomplished through the joint efforts of Mr. Richard Carter and Mr. Harold Frankhouser.

(3) The use of lower concentrations than 75% of nitric acid gave fume-offs during the final air agitation.

(4) P. Naoum, "Nitroglycerine and Nitroglycerine Explosives," Williams and Wilkins Co., Baltimore, Md., 1928, p. 245.

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Synthesis of 5-Hydroxymethylcytosine

BY CHARLES S. MILLER

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Wyatt and Cohen[1,2] isolated a new pyrimidine from formic acid hydrolysates of the desoxyribonucleic acid of the even-membered bacteriophages of *E. coli* and Wyatt and Cohen[1,2] and Weed and Courtenay[3] have shown that the pyrimidine exists in the bacteriophage nucleic acid as a nucleotide that yields the new base on suitable hydrolysis. This new pyrimidine base was shown to be 5-hydroxymethylcytosine by comparison with a synthetic sample prepared in this Laboratory.

This paper reports the synthesis of 5-hydroxymethylcytosine by two routes, (1) the lithium aluminum hydride reduction of 2-hydroxy-4-amino-5-carbethoxypyrimidine and (2) the lithium aluminum hydride reduction of 2-ethylthio-4-amino-5-carbethoxypyrimidine to the corresponding 5-hydroxymethyl derivative[4] followed by removal of the 2-ethylthio group by dilute hydrochloric acid hydrolysis.

Experimental

2-Ethylthio-4-amino-5-hydroxymethylpyrimidine.—A mixture of 500 ml. of dry ether and 35 ml. of lithium aluminum hydride solution (0.09 mole) in ether (approx. 0.1 g./ml.) was placed in a one-liter three-necked flask fitted with a condenser, soda lime tube and air-driven mercury seal stirrer. Finely pulverized 2-ethylthio-4-amino-5-carbethoxypyrimidine[5,6] (15.0 g., 0.066 mole) was added in small portions with stirring. After the addition was complete the mixture was allowed to stir for 0.5 hour at room temperature. The excess lithium aluminum hydride was decomposed by dropwise addition of 15 ml. of ethyl acetate. The product was then freed from the lithium-aluminum complex by dropwise addition of 9.3 ml. of water while good stirring was maintained. The solid was filtered and extracted repeatedly by suspension in acetone (about seven times). The ether filtrate and the combined acetone extracts were concentrated separately under reduced pressure to small volumes and the solid product collected on a filter. The crude yields were 80–85%, m.p. 147–151°. The product was purified by recrystalllization from ethyl or isopropyl alcohol; m.p. 151–152°, yield 75%.

Anal.[7] Calcd. for $C_7H_{11}ON_3S$: C, 45.38; H, 5.99; N, 22.68; S, 17.31. Found: C, 45.49; H, 6.14; N, 22.59; S, 17.13.

5-Hydroxymethylcytosine. (A).—A solution (50 ml., 0.13 mole) of lithium aluminum hydride in ether (approx. 0.1 g./ml.) was added to 325 ml. of pure dry N-ethylmorpholine contained in a 500-ml. three-necked flask fitted with an air-driven mercury seal stirrer and soda lime tube. Finely pulverized 2-hydroxy-4-amino-5-carbethoxypyrimidine[5] (4.6 g. 0.025 mole) was added in small portions to the N-ethylmorpholine solution and the mixture maintained at 45–50° with stirring for 2.5 hours. After cooling the excess lithium aluminum hydride was decomposed by dropwise addition of excess ethyl acetate. This was followed by dropwise addition of 10 ml. of water with good stirring. The solid phase was removed by filtration and washed with ether after which it was extracted four times by suspension in a minimum of water. The water extracts were combined and extracted eight times with ether. The water solution was neutralized with dilute sulfuric acid and the resulting precipitate removed by filtration and discarded. The filtrate was concentrated under reduced pressure to about 20 ml. and chilled overnight. The resulting white crystals with no melting point were filtered and washed with acetone (more crystals usually obtained by readjusting to *p*H 7 and further chilling). The yield varied from 18 to 35% after one recrystallization from hot water. Repeated recrystallization from hot water after treatment with charcoal gave a product which slowly decomposed above 200° without melting.

(1) G. R. Wyatt and S. S. Cohen, *Nature*, **170**, 1072 (1952).

(2) G. R. Wyatt and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953).

(3) L. L. Weed and T. A. Courtenay, *Federation Proc.*, **12**, 465 (1953).

(4) A. Dornow and G. Petsch, German Patent 870,260 (1953), *C. A.*, **48**, 2123 (1954).

(5) H. L. Wheeler and C. O. Johns, *Am. Chem. J.*, **38**, 601 (1907).

(6) T. B. Johnson, *ibid.*, **42**, 506 (1910).

(7) We are indebted to Joyce Pyett, G. M. Gustin, J. P. Laux and Kermit Streeter for the microanalyses.

Anal.[7] Calcd. for $C_8H_7O_2N_3 \cdot {}^1/_2H_2O$: C, 40.00; H, 5.37. Found after drying at 60°: C, 40.07; H, 5.55. Calcd. for $C_8H_7O_2N_3$: C, 42.55; H, 5.00; N, 29.78. Found after drying at 100° over P_2O_5 *in vacuo* for 50 hours: C, 42.61; H, 5.14; N, 29.72.

Ultraviolet absorption maximum on the anhydrous sample in water, 270 mμ (E_m 5970); in 0.1 *N* HCl, 279.5–280 mμ (E_m 9620); in 0.1 *N* NaOH, 283.5–284 mμ (E_m 7660).[8]

Infrared curves obtained on mineral oil mulls of the anhydrous material and a sample of Wyatt and Cohen's anhydrous material were identical.[9]

When diethyl Carbitol was used as the solvent in this procedure the ether extraction of the water extract was unnecessary and similar results were obtained.

(B).—A solution of 1.0 g. (0.005 mole) of 2-ethylthio-4-amino-5-hydroxymethylpyrimidine in 20 ml. of *N* hydrochloric acid was refluxed for 4 hours. After cooling, the solution was made alkaline with sodium hydroxide solution and chilled. Any precipitate at this point was removed and discarded. The solution was neutralized with hydrochloric acid, concentrated to a small volume under reduced pressure and chilled. The resulting crystals were filtered, and washed with acetone by suspension. After one recrystallization from water the yield was about 60%.

Anal. Found[7] after repeated recrystallization from water and drying at 100° for 60 hours *in vacuo* over P_2O_5: C, 42.56; H, 4.96; N, 29.75.

(8) We are indebted to W. R. McGaughran for the ultraviolet measurements.

(9) We are indebted to E. H. Unger for the infrared measurements.

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1-Formylpiperazine and Related Compounds

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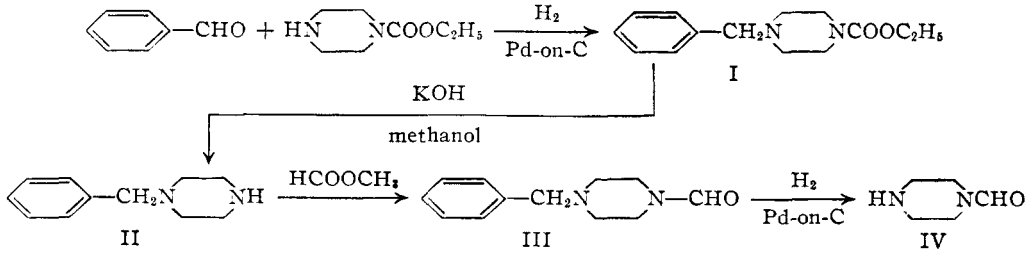
In general, reaction of piperazine with many reagents produces bis-substitution products or mixtures from which the mono-product is not readily separated. However, in this Laboratory we were able to obtain 1-formylpiperazine in 58–60% yield by heating equimolecular quantities of piperazine and methyl formate. In order to show how readily the 1,4-bis-formyl derivative was formed we mixed piperazine with two equivalents of methyl formate and distilled the reaction mixture after less than five minutes standing. A 74% yield of 1,4-bis-formylpiperazine was obtained.

1-Formylpiperazine also was prepared in good over-all yield by the procedure



Stewart and co-workers[1] described 1-benzyl-4 carbethoxypiperazine as an oil but gave no physical constants. 1-Benzylpiperazine (II) has been prepared by another method.[2] The excellent formylation procedure of Human and Mills[3] was used to obtain compound III.

The carbamyl, acetyl and benzenesulfonyl derivatives of 1-formylpiperazine were made by standard procedures for purposes of identification.

Experimental

1-Formylpiperazine (IV).—Thirty grams (0.5 mole) of methyl formate was added in one portion to 43 g. (0.5 mole) of anhydrous piperazine in a flask equipped with a reflux condenser. The temperature rose to 85° in one to two minutes. The mixture was then heated at 85° for five hours after which time it was distilled. The product boiled at 94–97° (0.5 mm.), $n^{25}D$ 1.5074, yield 33 g. (58%).

Anal. Calcd. for $C_5H_{10}N_2O$: C, 52.62; H, 8.83; N, 24.54. Found: C, 52.62; H, 8.74; N, 24.78.

Formylpiperazine is water soluble and forms a carbonate readily on exposure to air.

When the same quantities were heated in a stainless steel bomb for two hours at 100° a yield of 34.2 g. (60%) was obtained, b.p. 87–95° (0.3–0.4 mm.). Under the same autoclave conditions another experiment was carried out using a 1:3 ratio of methyl formate and piperazine. An 81% yield of IV was obtained, but the product was contaminated with piperazine.

1,4-Bis-formylpiperazine.—Sixty grams (1.0 mole) of methyl formate was added in one portion to 43 g. (0.5 mole) of anhydrous piperazine. The vigorous reaction which took place subsided in a few minutes. The material was distilled immediately. The product boiled at 151–159° (0.2–0.3 mm.) and solidified on standing, yield 52.5 g. (74%). After recrystallization from benzene it melted at 126–127°.

Anal. Calcd. for $C_6H_{10}N_2O_2$: N, 19.71. Found: N, 19.91.

1-Benzyl-4-carbethoxypiperazine (I).—Excess benzaldehyde (47.7 g., 0.45 mole) was mixed with 47.4 g. (0.3 mole) of carbethoxypiperazine. The mixture became warm and was allowed to stand 20 minutes when 100 cc. of absolute alcohol was added. Seven grams of 5% palladium-on-charcoal was added cautiously and the mixture hydrogenated under a pressure of 45 p.s.i. at 55–60° for one hour. After filtration and removal of solvent, distillation yielded 64 g. (86%) of a thick oil, b.p. 130–135° (0.15 mm.), $n^{25}D$ 1.5238.

Anal. Calcd. for $C_{14}H_{20}N_2O_2$: C, 67.71; H, 8.11; N, 11.28. Found: C, 67.81; H, 7.84; N, 11.32.

1-Benzylpiperazine (II).—A mixture of 63 g. (0.254 mole) of 1-benzyl-4-carbethoxypiperazine (I), 28.5 g. (0.508 mole) of potassium hydroxide and 170 cc. of methanol was heated to reflux and the methanol then distilled off slowly during a two-hour period. Heating was continued until the temperature of the residue was 130–135°. It was then cooled and treated with 75 cc. of benzene and 100 cc. of water. The aqueous portion was extracted with two 100-cc. portions of benzene. The benzene extract, after drying over anhydrous magnesium sulfate and removal of solvent, was distilled and 35.8 g. (80%) of 1-benzylpiperazine obtained, b.p. 89–96° (0.2 mm.), $n^{28}D$ 1.5430.

Anal. Calcd. for $C_{11}H_{26}N_2$: C, 74.95; H, 9.15; N, 15.90. Found: C, 75.10; H, 9.31; N, 15.70.

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