TABLE I

Compound Z = carbobenzoxy ME = methyl ester EE = ethyl ester	M.p. °C.	Crystn. solvent	Yield, % crude	pure	Molecular formula	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found
Z-L-Asparaginyl-L-serine ME	139–140[a]	MeOH–EtAc	30	22	$C_{16}H_{21}N_3O_7 \cdot H_2O$	49.86	49.9	6.01	5.8	10.90	10.8
Z-L-Asparaginyl-L-serylglycine EE (α- or β-)	139–140[a]	MeOH–EtAc	16	9	$C_{19}H_{26}N_4O_8 \cdot H_2O$	49.99	49.5	6.18	5.9	12.27	11.9
Z-L-Aspartyl-L-serine ME (α- or β-)	102	Water	54	31	$C_{16}H_{20}N_2O_8$	52.17	52.1	5.47	5.6	7.61	7.1
Z-L-Aspartyl-L-serylglycine ME (Probably β-)	144–145	Water	27	15	$C_{18}H_{22}N_3O_9 \cdot H_2O$	48.76	48.6	5.68	5.6	9.48	9.3
Z-L-Aspartyl-L-serylglycyl-L-glutamic acid di EE	182	Water	26	13	$C_{26}H_{36}N_4O_{12}$	52.34	52.2	6.08	6.0	9.39	9.1
Z-L-Serylglycine ME	105–106	EtAc	76		$C_{14}H_{18}N_2O_7$	54.19	54.2	5.85	5.7	9.03	8.7
Z-L-Serylglycyl-L-glutamic acid di EE	106–107	EtAc	25		$C_{22}H_{31}N_3O_9$	54.75	55.0	6.49	6.6	8.73	8.5

[a] Mixed melting point, 121–133°.

procedure described by Albertson and McKay.[9] Chloroform was the solvent, and ethyl chloroformate and triethylamine were used. The desired products separated from the chloroform solution, leaving triethylamine hydrochloride in solution. Recrystallization from methanol–ethyl acetate gave the pure peptide derivatives in 10–20% yields. The dipeptide derivative was recovered unchanged from a dioxane solution of hydrogen chloride, showing that it is the neutral (N-asparaginyl) isomer rather than the basic (O-asparaginyl) compound. The products are described in Table I.

Carbobenzoxy-L-aspartyl Compounds.—Carbobenzoxy-L-aspartic anhydride was prepared following the directions of Miller, Behrens and du Vigneaud.[10] The most convenient isolation method was to remove the excess acetic anhydride *in vacuo*, removing the last of the anhydride by distillation with dry dioxane. The product so obtained melted at 95–105°, and when treated with benzyl alcohol gave excellent yields of the α-benzyl ester, m.p. 85°. This crude anhydride therefore was used in the amino-ester couplings. For identification, it was recrystallized from acetone–petroleum ether to m.p. 108–110°; Le Quesne and Young[6] report 109–111°.

For the anhydride couplings the general directions of Le Quesne and Young were adapted to the L-serine series with chloroform as the reaction solvent. In the cases of the di- and tripeptide esters the crystalline products separated as gels, which were dried, dissolved in *n*-butyl alcohol, and fractionally extracted with sodium carbonate. Acidification of the aqueous extracts gave crystalline materials which proved to be essentially homogeneous. Evaporation of the chloroform solutions after removal of the crystalline fractions left acidic oils which remained oily when fractionated with sodium carbonate.

In the case of the tetrapeptide, both isomers remained in chloroform solution. The entire product was then transferred to ethyl acetate (for convenience) and extracted in seven portions with sodium carbonate. On acidification the first two extracts crystallized spontaneously, the third crystallized partly on seeding, and the remainder stayed oily even on seeding with the crystalline isomer. The properties of all the crystalline isomers are listed in Table I.

Carbobenzoxy-L-serylglycine methyl ester and carbobenzoxy-L-serylglycyl-L-glutamic acid diethyl ester were prepared by minor modifications of the procedure described previously[1] for L-serine peptides. Their properties are listed in Table I.

Carbobenzoxy-β- or α-L-aspartyl-L-serylglycine dimethyl ester was prepared by treatment of a solution of the monomethyl ester in chloroform–dioxane–ethyl acetate with excess diazomethane in ether. The product was recrystallized from methanol–ethyl acetate to a constant melting point of 160–161°.

Anal. Calcd. for $C_{19}H_{25}N_3O_9$: C, 51.93, H, 5.74; N, 9.56. Found: C, 51.6; H, 5.7; N, 9.4.

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(9) N. F. Albertson and F. C. McKay, THIS JOURNAL, **75**, 5323 (1953).

(10) G. L. Miller, O. K. Behrens and V. du Vigneaud, *J. Biol. Chem.*, **140**, 411 (1941).

A New Synthesis of Pentaerythritol Trinitrate

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A recent paper[2] described the preparation of pentaerythritol trinitrate through a three-step process, namely, the synthesis of pentaerythritol monoacetate, nitration of the acetate to the pentaerythritol acetate trinitrate and controlled saponification of this nitrated product to pentaerythritol trinitrate. The synthesis of this nitrato alcohol now has been accomplished by a simple one-step process involving controlled mixed acid nitration of pentaerythritol. The optimum concentration of the acids, 80% nitric acid and 80% sulfuric acid, gave in a large number of preparations 46–51% yields of pentaerythritol trinitrate with the accompanying formation of pentaerythritol tetranitrate in yields of 40–30%.

The yield of pentaerythritol trinitrate from this mixed acid nitration is very sensitive to slight changes in the concentration of either sulfuric or nitric acid. Reduction of the sulfuric acid concentration gave not only smaller quantities of the by-product, pentaerythritol tetranitrate, but also decreased the yield of pentaerythritol trinitrate obtained in the reaction.

Other nitrating systems gave less satisfactory yields. The use of 90% nitric acid gave almost quantitative yields of pentaerythritol tetranitrate while a slight reduction in concentration of nitric acid gave negligible quantities of both pentaerythritol tetranitrate and trinitrate. The mixed acid system, phosphoric and nitric acid, while giving better yields of pentaerythritol trinitrate than nitric acid alone was much less satisfactory under the conditions employed than sulfuric and nitric acid.

Experimental

Materials.—Pentaerythritol was nitration grade available from Trojan Powder Company.

Preparation of Pentaerythritol Trinitrate.—The equipment used was standard nitration apparatus with a 4-l. beaker as the nitration vessel surrounded by a lead-jacketed cooling bath with brine as the cooling medium. Pentaerythritol (480 g.) was added over a period of ten minutes

(1) This work was performed at Allegany Ballistics Laboratory, an establishment owned by the U. S. Navy and operated by Hercules Powder Company under Contract NOrd 10431.

(2) N. S. Marans, D. E. Elrick and R. F. Preckel, THIS JOURNAL, **76**, 1034 (1954).

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.

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(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

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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.