concentrated *in vacuo* and taken up in 20 ml. of ether. The ether solution was washed with aqueous potassium carbonate, with water, dried with anhydrous sodium sulfate, and evaporated to give 0.209 g. of a yellow oil with a smoky odor. About 10 mg. of the oil was dissolved in 0.1 ml. of ethanol, and a saturated ethanolic solution of picric acid added dropwise until precipitation was complete. The precipitate was centrifuged off and recrystallized from 4.0 ml. of ethanol to give quinaldine picrate, m.p.[5] and mixed m.p. 191–192°. Assuming the oil to have been pure quinaldine, the yield was 80%.

Preparation from Hydroquinone and Quinaldine.—Hydroquinone (0.200 g.) and quinaldine (0.70 ml.) were refluxed together in 10 ml. of ethyl acetate until solution was complete. A colorless crystalline precipitate was obtained on cooling and scratching. Recrystallization from ethyl acetate yielded material melting at 153–155°, mixed melting point with the material prepared from quinone and quinaldine 153–155°, yield 0.882 g. The ultraviolet and infrared absorption spectra of the substances prepared by the two methods were identical.

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Peptide Derivatives Containing Two Trifunctional Amino Acids. II

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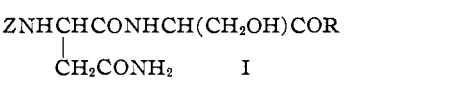
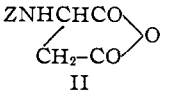
Our previous communication[1] was concerned largely with the preparation of peptides of L-histidine, L-serine and L-tyrosine. This paper deals with the synthesis of a number of new L-asparaginyl and L-aspartyl peptide derivatives of L-serine. Although very few L-asparaginyl peptides have been described in the literature,[2] du Vigneaud and associates[3] recently have prepared an L-asparaginyl peptide *via* the pyrophosphite route. Since the reagents for the closely related mixed carbonic anhydride coupling[4] are more readily available, this method was chosen, and carbobenzoxy-L-asparaginyl-L-serine methyl ester (I, R = OCH_3) and carbobenzoxy-L-asparaginyl-L-serylglycine ethyl ester (I, R = $NHCH_2CO_2C_2H_5$) have been obtained from carbobenzoxy-L-asparagine and the corresponding amino esters using ethyl chloroformate and triethylamine. Though the purified products were obtained in only 10–20% yield, the ease of the reaction makes it attractive. It is probable also that the yields can be improved considerably by minor changes in technique.



I

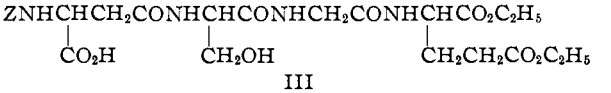
The preparation of pure L-aspartyl peptides presents more difficulty. Because of the nearly equal reactivity of groups attached to the two carboxyls of L-aspartic acid, it was considered undesirable to employ the mixed anhydride procedure.[5] Le Quesne and Young[6] have shown recently that the mixtures obtained by reaction of carbobenzoxy-L-aspartic anhydride(II) with amino acid esters can



II

be separated by fractional extraction, and therefore and also because of the mild conditions employed in the reaction, this route was chosen for coupling with L-serine derivatives.

Since the carboxyl group alpha to an amine is somewhat more acidic than one in the β-position, extractions with small amounts of weak base tend to remove the β-peptide derivatives in preference to the α-isomer. In our hands, reaction of II with L-serylglycyl-L-glutamic acid diethyl ester gave a mixture which was 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 when seeded with the crystalline compound. In this case, then, it is probable that the crystalline isomer is the β-derivative III.



III

Attempts to form crystalline derivatives of the oily α-isomer (amide, anilide, hydrazide, free acid) led only to gels or oils.

When the anhydride coupling was carried out with L-serine methyl ester or with L-serylglycine methyl ester, crystalline products separated from the reaction mixture, leaving about equal amounts of oily materials in solution. Therefore no direct comparison of the acid strengths of these compounds could be made. The crystalline isomers were shown to be homogeneous by fractional extraction, but again no crystalline derivatives of the oily materials could be obtained. Shortly after the preparation of the above compounds, our work in this field was terminated, and it was not possible for us to determine the configurations of the crystalline products, although, by analogy to III, they may be the β-isomers.

Several methods are available for determining these configurations. Sachs and Brand[7] have shown that both the amino and the peptide nitrogens of γ-glutamyl peptides react in the Van Slyke nitrogen determination, while only the amino nitrogen of the α-peptide is detected. This should also be applicable to the free aspartyl peptides. Another method would involve comparison of the diamides prepared from both the aspartyl and asparaginyl derivatives which were prepared earlier. To this end, the crystalline carbobenzoxy-L-aspartyl-L-serylglycine methyl ester was converted to the dimethyl ester, but termination of the work precluded carrying out the preparation of the amides.

Experimental[8]

Carbobenzoxy-L-asparaginyl Compounds.—These were prepared by minor modification of the mixed anhydride

(1) R. F. Fischer and R. R. Whetstone, THIS JOURNAL, **76**, 5076 (1954).

(2) E. Fischer and E. Koenigs, *Ber.*, **40**, 2048 (1907).

(3) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, THIS JOURNAL **76**, 3115 (1954).

(4) R. A. Boissonas and J. Schumann, *Helv. Chim. Acta*, **35**, 2237 (1952).

(5) After the completion of this work, Y. Liwschitz and A. Zilkha, THIS JOURNAL, **76**, 3698 (1954), reported the preferential synthesis of DL-α-aspartyl peptides using essentially this technique.

(6) W. J. Le Quesne and G. T. Young, *J. Chem. Soc.*, 24 (1952).

(7) H. Sachs and E. Brand, THIS JOURNAL, **75**, 4608 (1953).

(8) Melting points are corrected.

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

BY A. T. CAMP, N. S. MARANS, D. E. ELRICK AND R. F. PRECKEL

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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).