pension of the amino acid. When the resulting anhydride was polymerized, the polymer isolated was poly-β-cyano-L-alanine, as shown by infrared absorption at 2235 cm^{-1} (C≡N stretching).

In order to investigate this dehydration reaction further, we treated N^{α}-carbobenzoxy-L-asparagine with phosgene in dioxane. The product, which was isolated in 81% yield, was identical with authentic N^{α}-carbobenzoxy-β-cyano-L-alanine.[1,3] Hydrogenation of this material with palladium catalyst produced α,γ-diaminobutyric acid as confirmed by paper chromatography.

The dehydration reaction with L-glutamine and N^{α}-carbobenzoxy-L-glutamine gave lower yields. The resulting carbobenzoxy-γ-cyano-α-aminobutyric acid separated as an oil.[2] Hydrogenation led to ornithine and traces of proline which were identified by paper chromatography.

This convenient dehydration, which is free of side reactions, suggests various applications, for instance, for the synthesis of the neurotoxic amino acid β-cyano-L-alanine[5,6] and of asparaginyl and glutaminyl peptides.[7]

Experimental Section

All melting points are uncorrected. Prior to analysis the polymers were dried at 105° and the other compounds at 50° *in vacuo* over phosphorus pentoxide.

β-Cyano-N-carboxy-L-alanine Anhydride (I).—Dry phosgene was passed for 3 hr through a suspension of 13 g of asparagine (0.1 mole) in anhydrous dioxane at 60°. Excess phosgene was removed from the solution by a stream of dry nitrogen, and the solvent was distilled *in vacuo* at 45°; the crystalline residue was dissolved in ethyl acetate. The anhydride, which crystallized on the addition of petroleum ether (60–70°), was collected and washed with petroleum ether, and recrystallized from ethyl acetate–petroleum ether. The infrared spectrum showed an absorption peak for nitrile at 2235 cm^{-1}. The yield was 10.2 g (60%), mp 75° dec.

Anal. Calcd for $C_5H_4N_2O_3$: C, 42.86; H, 2.88; N, 20.00. Found: C, 42.59; H, 3.00; N, 19.81.

γ-Cyano-N-carboxy-L-butyric Anhydride.—This compound was prepared in the same manner by passing phosgene through a glutamine suspension and was recrystallized from ethyl acetate–petroleum ether. The infrared spectrum showed an absorption peak for nitrile at 2235 cm^{-1}. The yield was 42%, mp 90–92°.

Anal. Calcd for $C_6H_6N_2O_3$: C, 46.76; H, 3.92; N, 18.18. Found: C, 46.76; H, 3.96; N, 18.16.

Poly-β-cyano-L-alanine.—β-Cyano-N-carboxy-L-alanine anhydride (19 g) was dissolved in dry dioxane (500 ml) and triethylamine (0.1 ml) was added. Polymerization proceeded at room temperature (magnetic stirring) with evolution of carbon dioxide. After 48 hr the product was filtered and washed with dioxane and ether. The infrared spectrum showed an absorption peak for nitrile at 2235 cm^{-1}. The yield was 10 g. The polymer is insoluble in the common solvents, dimethylformamide, water, acetic acid, ethanol, and sodium bicarbonate.

Anal. Calcd for $(C_4H_4N_2O \cdot {}^1/_4H_2O)_n$: C, 47.76; H, 4.22; N, 27.86. Found: C, 48.18; H, 4.15; N, 27.22.

A sample of the poly-β-cyano-L-alanine was hydrolyzed in 6 *N* hydrochloric acid for 24 hr at 105°, and chromatographed in *n*-butyl alcohol–glacial acetic acid–water (25:6:25 v/v). Only one spot, identical with authentic aspartic acid, was detected with ninhydrin on the paper chromatogram.

N-Carbobenzoxy-β-cyano-L-alanine.—Dry phosgene was passed for 1 hr at room temperature through a solution of carbobenzoxy-L-asparagine (13.2 g) in anhydrous dioxane, and the reaction mixture left for 2 hr at room temperature. The excess of phosgene was removed by a stream of nitrogen and most of the solvent was distilled off *in vacuo* at 45°. Upon the addition of 1 *N* hydrochloric acid, the residue crystallized and was recrystallized from ethylene chloride. The infrared spectrum showed an absorption peak for nitrile at 2235 cm^{-1}. The yield was 10 g (81%), mp 131–134°, $[\alpha]^{25}D$ −19.0 (*c* 1.25 methanol) (lit.[6] mp 128°, $[\alpha]^{21}D$ −18.6 (*c* 1.28, methanol); lit.[4] mp 133–134°, $[\alpha]^{21}D$ −19 (*c* 1.26, methanol).

Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found: C, 58.15; H, 5.05; N, 11.29.

Hydrogenation of this compound with palladium on charcoal under acidic conditions produced α,γ-diaminobutyric acid, as indicated by paper chromatography with an authentic sample of α,γ-diaminobutyric acid.

N-Carbobenzoxy-γ-cyano-α-amino-L-butyric acid.—The reaction was carried out and the product treated as described for the preparation of N-carbobenzoxy-β-cyano-L-alanine. On acidification, a thick oil was obtained which was extracted in ethyl acetate, dried over sodium sulfate and concentrated to dryness under reduced pressure. The infrared spectrum of this oil showed an absorption peak for nitrile at 2235 cm^{-1}. The yield was 75%.

Anal. Calcd for $C_{13}H_{14}N_2O_4$: N, 10.68. Found: N, 10.35.

Hydrogenation of this compound with palladium on charcoal under acidic conditions led to ornithine and proline as established by paper chromatography with authentic samples of ornithine and proline.

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Registry No.—Asparagine, 70-47-3; glutamine, 56-85-9; phosgene, 75-44-5; I, 15275-68-0; γ-cyano-N-carboxy-L-butyric anhydride, 15231-19-3; N-carbobenzoxy-β-cyano-L-alanine, 3309-41-9; N-carbobenzoxy-γ-cyano-α-amino-L-butyric acid, 15231-21-7.

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Studies in the Xanthone Series. IX.[1] The Mechanism of the *para* Claisen Rearrangement of 1-(3,3-Dimethylallyloxy)-3,5,6-trimethoxyxanthone

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In a previous publication,[2] we reported that a Claisen rearrangement of 1-(3,3-dimethylallyloxy)-3,5,6-trimethoxyxanthone (**1**) gave four products, **2**, **3**, **4**, and **5**.[3] It was suggested[2] that the main product, **5**, formed by migration of the 3,3-dimethylallyl group to the *para* position, results from a Claisen rearrangement

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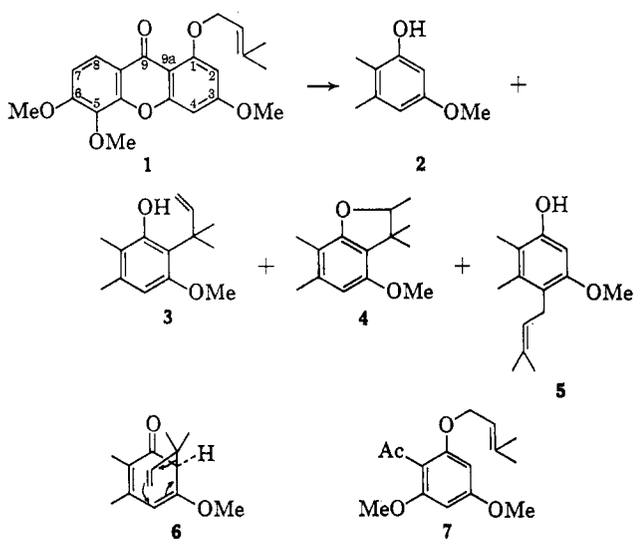
(3) 2,4-Di-(3,3-dimethylallyl)-1-hydroxy-3,5,6-trimethoxyxanthone was also isolated in one experiment.[2] It was suspected that this product arose from rearrangement of an impurity in **1** since analogous migrations gave no diallylation: see (a) H. D. Locksley, I. Moore, and F. Scheinmann, *J. Chem. Soc., Sect. C*, 2265 (1966); (b) F. Scheinmann and H. Suschitzky, *Tetrahedron*, **7**, 31 (1959); (c) reference 4. Thus if 1-hydroxy-3,5,6-trimethoxyxanthone **2** reacts as an ambident anion (N. Kornblum, P. J. Berrigan, and W. J. le Noble, *J. Amer. Chem. Soc.*, **85**, 1141 (1963)) allylation at C-2 and then oxygen will yield 2-(3,3-dimethylallyl)-1-(3,3-dimethylallyloxy)-3,5,6-trimethoxyxanthone as an impurity in **1**. Mass spectral examination showed that with some ether samples the impurity arising from concurrent O- and C-isoprenylation was indicated as a small peak at *m/e* 438 with respect to the molecular ion peak at *m/e* 370 due to the ether **1**. Both molecular ions lost a fragment of 55 mass units consistent with loss of C_4H_7 from a 3,3-dimethylallyl ether or side chain. The radioactive ether **1** used for the mechanism studies was free from this and other impurities as shown by mass spectral and chromatographic evidence.

to C-2 followed by Cope-type rearrangement to C-4. From the work of Schmid, *et al.*, it is clear that for a hindered *o*-dienone such as **6**, steric inhibition of enolization can allow a Cope-type rearrangement to become a competitive reaction,[4] but in our case experimental support was required for the intramolecular pathway.[5]

A crossing experiment was thus carried out with a mixture of the xanthone ether **1** containing a ^{14}C-labeled dimethylallyl group and inactive 2-(3,3-dimethylallyloxy)-4,6-dimethoxyacetophenone (**7**). Both ethers had previously been shown to give *para* rearrangement products[2] at similar rates by thin layer chromatography experiments.

A convenient radiochemical synthesis of the 3,3-dimethylallyl-3,3-dimethyl-$^{14}C_2$ bromide was devised starting from acetone, and then reaction with 1-hydroxy-



3,5,6-trimethoxyxanthone **2** gave the active 3,3-dimethylallyl-3,3-dimethyl-$^{14}C_2$ ether **1**. Acetone-1,3-$^{14}C_2$[6] was reacted with lithium acetylide–ethylenediamine complex in dimethyl sulfoxide to give 3-methyl-1-butyn-3-ol-4,3-methyl-$^{14}C_2$.[7] Reduction of this ethynyl carbinol with a Lindlar catalyst[8] gave 3-methyl-1-buten-3-ol-4,3-methyl-$^{14}C_2$ which was converted into 3,3-dimethylallyl-3,3-dimethyl-$^{14}C_2$ bromide by hydrogen bromide.[9] After rearrangement of the mixture (**7** and radioactive **1**), the products were separated by thin layer chromatography and the specific activities of the starting xanthone ether **1** and the *para* Claisen product **5** were compared. Both xanthones (**1** and **5**) had virtually the same specific activity thus demonstrating that the *para* Claisen rearrangement must proceed largely by an intramolecular pathway.

Experimental Section

Synthesis of Labeled 3,3-Dimethylallyl Bromide 3-Methyl-1-butyn-3-ol-4,3-methyl-$^{14}C_2$.[7]—Acetylene was passed into a suspension of lithium acetylide–ethylenediamine complex (9.2 g) in dimethyl sulfoxide (50 ml) with stirring at room temperature. The gas flow was stopped and acetone-1,3-$^{14}C_2$[6] (5.8 g) was added over 30 min, and then the mixture was cooled to 0°, acidified to pH 7 with 2 *N* hydrochloric acid, and extracted with ether. The ethereal layer was washed with water and dried and the solvent removed to yield a residue which was distilled to give radioactive 3-methyl-1-butyn-3-ol (2 g, 24%), bp 101–103° (1 atm) (lit.[10] bp 103–104°).

3-Methyl-1-buten-3-ol-4,3-methyl-$^{14}C_2$.—This compound was prepared in 64% yield by the method described for the inactive product,[8] bp 98° (1 atm) (lit.[8] bp 97–98° (750 mm), 87%).

3,3-Dimethylallyl-3,3-$^{14}C_2$ Bromide.—A solution of 45% w/v hydrogen bromide in acetic acid (12 ml) was added to the radioactive 2-methyl-1-buten-3-ol (5 g) with stirring at 10°. After standing for 15 min, the mixture was poured into water and then extracted with ether. The ethereal layer was washed with water and dried ($CaCl_2$) and the solvent removed to leave a residue which was fractionally distilled to give the radioactive 3,3-dimethylallyl bromide (4 g, 46%), bp 35° (12 mm) (lit.[11] bp 26–33° (12 mm)). The distillate was stored over anhydrous potassium carbonate.

The radioactive xanthone ether **1** and the phloroacetophenone ether **7** were prepared following the procedures described in an earlier paper.[2]

Counting Experiments.—The determination of the specific activity for the compounds **1** and **5** was carried out using a Nuclear Chicago liquid scintillation system 725. The instrument was calibrated by the balance point method to carry out channels ratio measurements on the isotope ^{14}C.[12] Additions of nonradioactive forms of **1** and **5** were made to ^{14}C-hexadecane standards in PPO/POPOP scintillator solutions. The quenching caused by these compounds could be interpreted by reference to a standard quench correction curve. This standard curve was obtained from a set of quenched standards as supplied by the Nuclear Chicago Corp. Accurately weighed samples of active **1** and **5** were dissolved in 1 ml of dioxane, and to these solutions was added 5 ml of PPO/POPOP scintillator. Small weights of sample (0.6 mg) were used to avoid color quenching. The activity of the scintillator solutions were determined and specific activities calculated. Duplicate results showed that the compounds **1** and **5** were of identical specific activity within the limits of experimental error (Table I).

TABLE I

	1	5
Measured activity, cpm	53.51	51.71
Background blank of 1 ml of dioxane and 5 ml of scintillator, cpm (with vial correction)	27.13	27.13
Corrected activity, cpm	26.48	24.58
Channels ratio	55.10	56.81
Efficiency (from quench correction curve), %	79	77.5
True count, dpm	33.52	31.72
Weight of compound $\times 10^{-4}$, g	6.4	6.0
Specific activity, dpm/g	52,380	52,870
% specific activity of **5**/specific activity of **1**	101%	
Estimated errors		
(1) Statistical counting error		1%
(2) Weighing errors		2%
(3) Interpolation error in quench correction curve (±1% variation in counting efficiency)		3%

The counting experiments were duplicated and the results were shown to be in agreement.

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Amino Acids and Peptides. XVI.[1] Synthesis of a Tetrapeptide Sequence (A_9–A_{12}) of Glucagon

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Protected peptides corresponding to the A_9–A_{12} sequence of the hyperglycemic hormone glucagon[2] have been elaborated recently by several investigators. The first synthesis gave the tripeptide N-benzyloxycarbonyl-L-asparaginyl-L-tyrosyl-L-serine methyl ester, prepared by a coupling between N-benzyloxycarbonyl-L-asparagine *p*-nitrophenyl ester and L-tyrosyl-L-serine methyl ester.[3] The second preparation yielded N-trifluoroacetyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-serine hydrazide, formed by joining N-trifluoroacetyl-β-*t*-butyl-L-aspartic acid with either O-benzyl-L-tyrosyl-O-benzyl-L-serine benzyloxycarbonylhydrazide or L-tyrosyl-O-benzyl-L-serine benzyloxycarbonylhydrazide.[4] Later, N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-serine methyl ester was made from N-benzyloxycarbonyl-β-*t*-butyl-L-aspartic acid and L-tyrosyl-L-serine methyl ester by a mixed anhydride procedure,[5] while the tetrapeptide under discussion was incorporated into a longer fragment by stepwise addition of individual amino acids from the carboxyl end.[6]

In continuation of earlier work, there is described here the formation of a protected A_9–A_{12} peptide and other related compounds. The procedure began with N^{ϵ}-benzylidene-L-lysine[7] (I), which on treatment with benzyl chloroformate and sodium hydroxide afforded both N^{ϵ}-benzyloxycarbonyl-L-lysine[8-11] (II) and N^{α}-benzyloxycarbonyl-L-lysine[7,10-12] (III). Reaction of compound III with *t*-butyl azidoformate[13] in the presence of dicyclohexylamine[14] led to N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine dicyclohexylammonium salt[12,15-17] (IV). Alternatively, III was esterified with thionyl chloride and methanol to obtain syrupy N-benzyloxycarbonyl-L-lysine methyl ester hydrochloride[7] (V), which was converted by *t*-butyl azidoformate[13] into N-benzyloxycarbonyl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester[16-19] (VI). Removal of the benzyloxycarbonyl group of compound VI by hydrogenation in acetic acid formed crystalline N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate[16,17] (VII). A 2-ethyl-5-phenylisoxazolium 3′-sulfonate[20,21] coupling between the amine VII and N-benzyloxycarbonyl-L-serine (VIII) yielded N-benzyloxycarbonyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (IX). The dipeptide IX on hydrogenation in acetic acid afforded the corresponding amine hydroacetate (X); addition of N-benzyloxycarbonyl-O-benzyl-L-tyrosine *p*-nitrophenyl ester[22] (XI) formed amorphous N-benzyloxycarbonyl-O-benzyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XII). The combination of N,O-dibenzyloxycarbonyl-L-tyrosine 2,4,5-trichlorophenyl ester[23] (XIII) and the amine X produced impure N,O-dibenzyloxycarbonyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XIV). A third approach to this tripeptide sequence involved an azide synthesis[24,25] between N-benzyloxycarbonyl-L-tyrosyl-L-serine hydrazide (XVI) and the amine X affording N-benzyloxycarbonyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XVIII). The hydrazide XVI was prepared from N-benzyloxycarbonyl-L-tyrosyl-L-serine methyl ester[26,27] (XV) in the usual manner, and from N,O-dibenzyloxycarbonyl-L-tyrosyl-L-serine methyl ester (XVII) by warming with excess hydrazine. The latter reaction is the second example of the removal of an O-benzyloxycarbonyl blocking group by hydrazine.[23]

Catalytic reduction in acetic acid of the tripeptide XVIII afforded L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester hydroacetate (XIX), which was coupled to N-benzyloxycarbonyl-β-*t*-butyl-L-aspartate α-2,4,5-trichlorophenyl ester[23] (XX) or the corresponding α-*p*-nitrophenyl ester[28-30] (XXI) to form the desired N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-lysine methyl ester (XXII). Reaction of compound XXII with hydrazine led to N-benzyloxycarbonyl-β-*t*-butyl-L-aspartyl-L-tyrosyl-L-seryl-N^{ϵ}-*t*-butyloxycarbonyl-L-

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