

Anal. Calcd. for $C_{30}H_{37}O_4N_3$: C, 63.47; H, 6.57; N, 7.40. Found: C, 63.47; H, 6.69; N, 7.27.

2,2',2''-(3,4',4'',5',5''-tetracarboethoxy-4,5',5''-trimethyl)tripyrrolymethene. A 0.3216 g. sample of 2,2',2''-(4,5',5''-trimethyl-3,4',4'',5-tetracarboethoxy)tripyrrolymethane in 20 ml. of acetone was oxidized with 0.06255 g. of potassium permanganate in 1.95 ml. of water. Crystallization of the reaction product from 95% ethyl alcohol yielded a mixture of red and yellow colored solids. After stirring the mixture twice with acetone and three times with 95% ethyl alcohol most of the yellow solid was dissolved. The red solid that remained and that obtained upon concentration of the combined extracts were combined and crystallized from 95% ethyl alcohol yielding 0.0210 g. of the red crystalline methene, m.p. 221.9–223.0° (dec.). Considering the recovered tripyrrolymethane (below) the yield of methene is 11%.

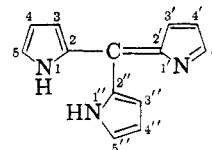
Anal. Calcd. for $C_{28}H_{35}O_8N_3$: C, 62.32; H, 6.16; N, 7.79. Found: C, 62.45; H, 6.37; N, 8.37.

The residue from the tripyrrolymethene mother liquors in 95% ethyl alcohol was applied to a column of Woelm's Alumina (non-alkaline, activity grade I) and the chromatogram was developed with ethyl ether. The lower, broad, tan colored zone was eluted with ether, the solution was evaporated, and the residue was crystallized from 95% ethyl alcohol yielding 0.1305 g. of orange-tan crystals of the methane, m.p. 197.0–198.3° (dec.). The product when crushed appeared as a white powder. The infrared spectrum of this compound is identical with that of the starting methane.

Anal. Calcd. for $C_{28}H_{35}O_8N_3$: C, 62.09; H, 6.51; N, 7.76. Found: C, 62.31; H, 6.55; N, 7.68.

Ultraviolet-Visible Absorption Spectra. Solutions of the tripyrrolymethenes in isopropyl alcohol were examined. Measurements were made with a Beckman Model DU or a Cary Model 11M Spectrophotometer. The results are presented in the following table.

TABLE II
ULTRAVIOLET-VISIBLE ABSORPTION SPECTRA
FOR 2,2',2''-TRIPYRROLYMETHENES



Substituents	Isopropyl Alcohol		Isopropyl alcohol plus $HClO_4^a$	
	λ_{max}	$\epsilon \times 10^{-3}$	λ_{max}	$\epsilon \times 10^{-3}$
4,4',4''-Tricarboethoxy-5,5',5''-trimethyl	490	43.1	487	115.7
	465–470	42.0		
	435–440 ^b	33.1		
	245–250 ^c	14.7		
3,3',3'',5,5',5''-Hexamethyl-4,4',4''-tricarboethoxy	220 ^d	38.3		
	486	38.3	497	82.1
	255 ^e	13.3		
3,4',4'',5-Tetracarboethoxy-3',3'',4,5',5''-pentamethyl	224	39.3		
	475	40.0	524	51.2
	264	23.1	486 ^f	26.1
3,4',4'',5-Tetracarboethoxy-4,5',5''-trimethyl	220	48.4		
	460–466	36.1	512	71.9
	262	20.2		
	220 ^d	39.8		

^a One milliliter of 10% aqueous perchloric acid per 100 ml. of solution. ^b Only long wave length maximum recorded. ^c Shoulder on ascending limb. ^d End absorption, not necessarily a maximum. ^e Inflection on ascending limb. ^f Inflection on descending limb.

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O-(Substituted)- α -amino- β -hydroxybutyric Acids

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The *O*-carbamyl derivatives of DL-threonine and DL-allothreonine, and *O*-carbazyl-DL-threonine were prepared from the corresponding carbobenzoxy-amino acid benzyl esters by condensation with phosgene followed by ammonia or *N*-carbobenzoxyhydrazine, and then hydrogenolysis, to produce the carbamyl- and carbazyl- derivatives, respectively. In contrast to the comparable serine derivatives, these compounds were not effective metabolic antagonists in several micro-biological assays.

Both *O*-carbamyl- and *O*-carbazyl- derivatives of DL-serine have been prepared and found to be competitive antagonists of glutamine in several microorganisms.^{1,2} The sulfur analogue of the former compound, *S*-carbamylcysteine,³ is also an inhibitory amino acid derivative; however, glutamine does not competitively reverse its toxicity, and in this respect it is similar to azaserine⁴ an

antitumor agent.⁵ The antitumor activity of several of these analogs⁶ prompted the synthesis and biological testing of a number of additional *O*-(substituted carbamyl)serine derivatives.⁷

In the present investigation, the *O*-carbamyl-derivatives of both threonine and allothreonine,

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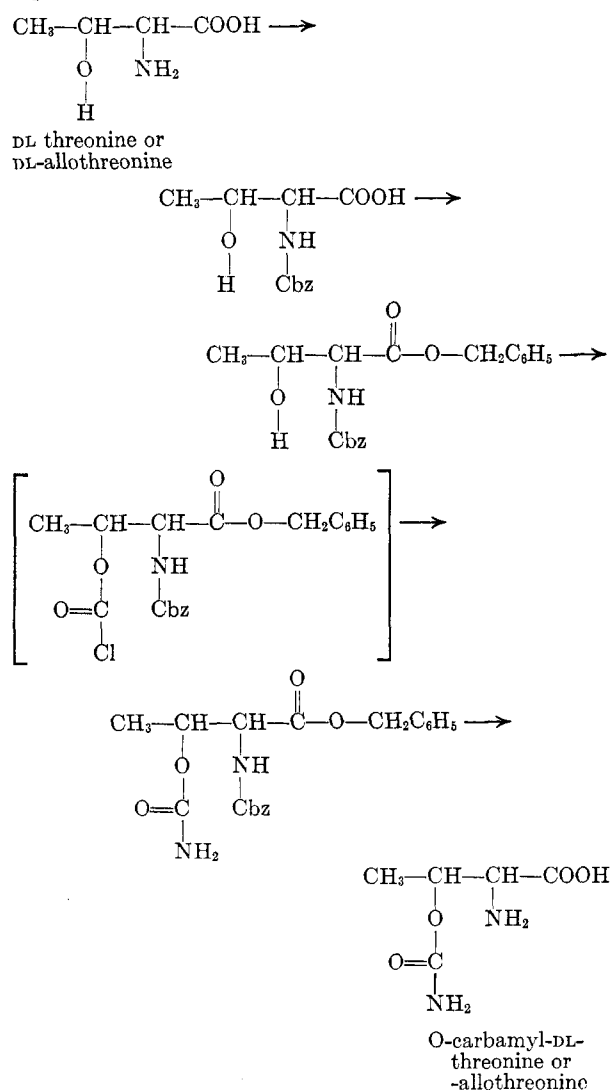
(1) C. G. Skinner, T. J. McCord, J. M. Ravel, and W. Shive, *J. Am. Chem. Soc.*, **78**, 2412 (1956).

(2) T. J. McCord, J. M. Ravel, C. G. Skinner, and W. Shive, *J. Am. Chem. Soc.*, **80**, 3762 (1958).

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and the *O*-carbazyl- derivative of threonine were prepared and their biological properties were examined. The two diastereoisomeric analogs, *O*-carbamyl-DL-threonine and *O*-carbamyl-DL-allothreonine, were prepared to determine whether either of the two derivatives exhibited a stereochemical specificity for the antagonism of glutamine.

The *O*-carbamyl- derivatives of threonine and allothreonine were synthesized using the same general procedure. The *N*-carboboxy amino acids were esterified with benzyl alcohol, and the isolated intermediates were then treated with phosgene, followed by treatment with ammonium hydroxide, to yield the corresponding *O*-carbamyl-*N*-carboboxy amino acid benzyl ester. The latter products on hydrogenolysis gave the desired amino acid analogue as indicated in the accompanying equations. *O*-Carbazyl-DL-threonine was prepared



by a comparable procedure except that the chloroformyl intermediate was condensed with *N*-carboboxyhydrazine instead of ammonia. The resulting intermediate was then hydrogenolyzed to

yield the desired *O*-carbazylthreonine. In contrast to the corresponding *O*-carbazylserine derivatives,² the threonine intermediates gave relatively poor yields, and the reaction mixtures crystallized with difficulty.

Neither *O*-carbamyl-threonine or -allothreonine were significantly inhibitory toward *Escherichia coli* 9723, *Lactobacillus arabinosus* 17-5, or *Streptococcus lactis* 8039 even at a level of 2 mg./ml. This is in contrast to *O*-carbamyl-DL-serine which is an effective competitive antagonist of glutamine.¹ The introduction of the additional methyl group in the derivatives apparently sterically hinders the analogs from interacting at appropriate enzyme sites. Similarly, the 3-methyl derivative of glutamic acid is not inhibitory to the growth of *E. coli*. It appears that, in a number of microorganisms, the substitution of methyl groups on the 3-carbon of glutamic acid (as well as on the 3-carbon of oxaloacetic acid) sterically prevents enzyme binding of the analogs at the place of attachment of the corresponding natural metabolite. The lack of biological activity of these *O*-carbamyl-threonine and allothreonine derivatives is apparently not due to the introduction of an oxygen for the 4-methylene group of glutamine, since the analog resulting from this single alteration is *O*-carbamylserine, an effective glutamine antagonist.

O-Carbazylthreonine is slightly inhibitory in these microbial systems, but it is still considerably less inhibitory on a weight basis than *O*-carbazylserine.² *O*-Carbazylthreonine inhibits the growth of either *E. coli*, *L. arabinosus*, or *S. lactis* at a level of concentration of about 200 γ /ml.; however, these toxicities are not reversed by glutamine. In view of the data observed above with the carbamyl derivative, this latter inhibition is probably due to the hydrazine portion of the molecule combining with active centers in the host, perhaps in a non-specific manner.

EXPERIMENTAL^{8,9}

Biological testing. The microbiological assays were carried out using previously reported procedures.¹ In all assays the inhibitors were dissolved in sterile water and added to sterile assay tubes without being heated.

N-Carboboxy-DL-threonine. Using the general procedure of Baer and Maurukas,¹⁰ 23.8 g. of DL-threonine was dissolved in 350 ml. of water in the presence of 150 ml. of ether and 25 g. of finely powdered magnesium oxide. The mixture was cooled in an ice bath, and 53 g. of carboboxy chloride was added dropwise over a 45 min. period with stirring. After stirring an additional 2 hr. at room temperature, the precipitate was filtered, the ether phase was recovered, and the aqueous phase was extracted with an additional 200

(8) We are indebted to Dr. J. M. Ravel and her staff at the Clayton Foundation Biochemical Institute for the microbiological assays.

(9) The chemical analyses were carried out by Mr. W. H. Orme-Johnson and Miss Judith Morehead. The melting points are uncorrected.

(10) E. Baer and J. Maurukas, *J. Biol. Chem.*, **212**, 31 (1955).

ml. of ether. The aqueous phase was then acidified to pH 3 with concentrated hydrochloric acid and extracted twice with ethyl acetate. After removal of the organic solvent *in vacuo*, the residue was crystallized from toluene-Skellysolve B to yield 32.0 g. of product, m.p. 75–78°.

Anal. Calcd. for $C_{12}H_{16}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.29; H, 5.79; N, 5.51.

N-Carbobenzoxy-DL-threonine benzyl ester. Using the general procedure of Ben-Ishai and Berger,¹¹ 15.0 g. of *N*-carbobenzoxythreonine, 10.0 g. of benzyl alcohol, and 1 g. of *p*-toluenesulfonic acid was dissolved in 125 ml. of benzene and heated to reflux for about 12 hr. The water formed in the reaction was removed by azeotropic distillation, and the benzene solution was washed twice with 5% potassium bicarbonate, and then dried over sodium sulfate. After removal of the solvent *in vacuo*, the residue was crystallized from toluene-*n*-hexane to yield 18 g. of product, m.p. 67–68°.

Anal. Calcd. for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.17; N, 4.08. Found: C, 66.28; H, 6.08; N, 4.19.

O-Carbamyl-N-carbobenzoxy-DL-threonine benzyl ester. Phosgene was bubbled through a cold solution of 6.0 g. of *N*-carbobenzoxythreonine benzyl ester in 75 ml. of toluene for about 2 hr., and then the reaction mixture was allowed to stand at room temperature overnight. The solvent was removed *in vacuo*, and the residue was freed of excess phosgene by repeated addition and evaporation *in vacuo* of small volumes of dioxane. The residual oil was finally dissolved in 50 ml. of dioxane, and added dropwise to a cold concentrated ammonium hydroxide solution with stirring. After reduction in volume *in vacuo*, the precipitate was separated and recrystallized from dioxane-water to yield 5 g. of product, m.p. 124–126°.

Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.16; H, 5.40; N, 7.38.

O-Carbamyl-DL-threonine. A solution of 2.0 g. of *O*-carbamyl-*N*-carbobenzoxy-DL-threonine benzyl ester in 100 ml. of 50% dioxane-water was hydrogenolyzed in the presence of 500 mg. of palladium black at room temperature and atmospheric pressure for about 5 hr. After removal of the catalyst, the filtrate was reduced to dryness *in vacuo*, and the residue was crystallized from ethanol-water to yield 1.0 g. of product, m.p. 189–191° (dec.).

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 37.03; H, 6.22; N, 17.28. Found: C, 37.32; H, 5.97; N, 17.16.

O-(N²-Carbenezoylcarbazyl)-N-carbobenzoxy-DL-threonine benzyl ester. Following a general procedure previously reported for the serine analogue,² 10 g. of the chloroformyl intermediate dissolved in 80 ml. of dioxane was added dropwise over a 1 hr. period to a well stirred ice-cold mixture of 6.5 g. of carbenezoylhydrazine and 1.1 g. of sodium carbonate in 100 ml. of 80% ethyl alcohol. The reaction mixture was allowed to remain at room temperature for 4 days, and it was then reduced to dryness, taken up in warm

alcohol, and the insoluble salt was filtered. Addition of water to the alcohol solution yielded a precipitate, m.p. 80–83°.

Anal. Calcd. for $C_{22}H_{23}N_3O_5$: C, 62.80; H, 5.46; N, 7.85. Found: C, 62.71; H, 5.38; N, 7.67.

The major portion of the reaction mixture crystallized so slowly that the oil residue was normally used directly for the following step without crystallizing.

O-(Carbazyl)-DL-threonine. A solution of 10 g. of *O*-(*N²*-carbenezoylcarbazyl)-*N*-carbobenzoxy-DL-threonine benzyl ester in 100 ml. of 50% dioxane-ethanol was hydrogenolyzed in the presence of 700 mg. of palladium black at room temperature and atmospheric pressure for about 3 hr. Throughout the period of hydrogenolysis, water was carefully added as needed to induce a slight turbidity in the reaction mixture. The catalyst was filtered, the filtrate was reduced to dryness *in vacuo*, and the residue was recrystallized from ethanol-water to yield 200 mg. of product, m.p. 172–173°.

Anal. Calcd. for $C_8H_{11}N_3O_4$: C, 33.90; H, 6.26; N, 23.72. Found: C, 34.19; H, 6.49; N, 23.64.

The residue contained additional product, as indicated by paper chromatography; however, it could not be readily separated from other ninhydrin positive contaminants.

N-Carbobenzoxy-DL-allothreonine. Using the procedure described above for the threonine analog, 12.5 g. of allothreonine and 13.0 g. of magnesium oxide, was suspended in 175 ml. of water and 100 ml. of ether. To this cold well-stirred mixture was added 28 g. of carbobenzoxy chloride. After the work-up procedure there was obtained 18 g. of product which was crystallized from toluene-Skellysolve G, m.p. 114–115°.

Anal. Calcd. for $C_{12}H_{15}NO_5$: N, 5.53. Found: N, 5.83.

N-Carbobenzoxy-DL-allothreonine benzyl ester. Using the procedure described above for the corresponding threonine derivative, a mixture of 8 g. of *N*-carbenezoylallothreonine, 5 g. of benzyl alcohol, and 0.5 g. of *p*-toluenesulfonic acid suspended in 100 ml. of benzene produced 7.9 g. of product, m.p. 74–75°.

Anal. Calcd. for $C_{19}H_{21}NO_5$: N, 4.08. Found: N, 4.25.

O-Carbamyl-N-carbobenzoxy-DL-allothreonine benzyl ester. Using the technique as previously described for the threonine analog, 5.1 g. of *N*-carbenezoylallothreonine benzyl ester yielded, after treatment with phosgene followed by ammonium hydroxide, 3.6 g. of product, m.p. 81–82°.

Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.16; H, 5.74; N, 7.25. Found: C, 62.25; H, 5.59; N, 7.25.

O-Carbamyl-DL-allothreonine. An alcohol-water solution containing 1.1 g. of *O*-carbamyl-*N*-carbenezoylallothreonine benzyl ester in the presence of 200 mg. of palladium black was hydrogenolyzed for about 4 hr. at room temperature and atmospheric pressure. After removal of the catalyst, the filtrate was reduced to dryness *in vacuo*, and the residue was recrystallized from alcohol-water to yield 118 mg. of product, m.p. 205–210° (dec.).

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 37.03; H, 6.22; N, 17.28. Found: C, 37.10; H, 6.33; N, 17.55.

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