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Some *O*-(substituted carbamyl)serines

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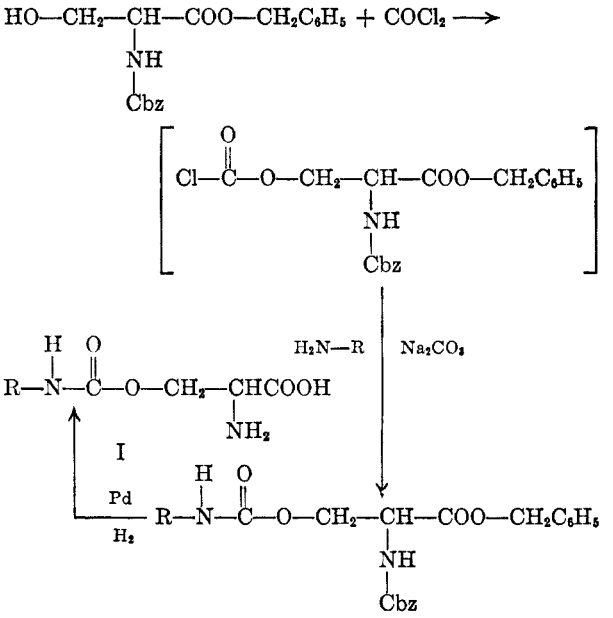
N-Carbobenzoxy-DL-serine benzyl ester was converted to 16 new *O*-(substituted carbamyl)-*N*-carbobenzoxyserine benzyl esters either by condensation with phosgene followed by aminolysis of the chloroformyl intermediate, or by direct interaction with an appropriately substituted isocyanate derivative. The resulting carbamyl derivatives were then hydrogenolyzed to yield the corresponding *O*-(substituted carbamyl)-DL-serines. Using *Lactobacillus arabinosus*, *Streptococcus lactis*, and *Escherichia coli* as the test organisms, all of these *O*-(substituted carbamyl)serines were essentially nontoxic at the highest level which could be tested (200–500 γ/ml.) with the exception of *O*-methylcarbamylserine. Glutamine competitively reverses the toxicity of the latter compound.

O-Carbamyl-L-serine has been found to be a competitive antagonist of glutamine in several microbiological systems.[1] Structurally this inhibitor is somewhat analogous to azaserine[2]; however, the toxicity of the latter compound is not competitively reversed by glutamine, and thus it has greater potential as a possible chemotherapeutic agent.[3] Structural analogs of *O*-carbamyl-DL-serine (the DL isomer of *O*-carbamylserine is one-half as active on a weight basis as the L-form[1]) have been prepared and studied in these laboratories in an effort to produce potent glutamine antagonists. Preferably, the effects of these inhibitory analogs should not be reversed by physiological concentrations of glutamine. *S*-Carbamyl-L-cysteine, the sulfur analog corresponding to the oxygen derivative (*O*-carbamyl-L-serine) of glutamine, has been found to inhibit the growth of microorganisms. The inhibition involves essential biological functions in which glutamine has a role, but its toxicity is not competitively reversed by glutamine.[4] Further, *O*-carbazyl-DL-serine, (I, R = NH_2) an analog of glutamine in which two different groups were modified, was prepared and found to inhibit microbial growth. The inhibitory effects of the carbazyl derivative are reversed competitively by glutamine.[5] Such a dual modification of the structure of glutamine is present in *N*-substituted amides of *O*-carbamyl-DL-serine, and in the present investigation the synthesis and determination of microbiological activity of these substituted amides was undertaken.

N-Substituted amides of *O*-carbamyl-DL-serine were conveniently prepared from *N*-carbobenzoxy-DL-serine benzyl ester. Using Method A (Table I) the ester was treated with phosgene to form the *O*-chloroformyl derivative which was not isolated but added in dioxane solution to an alcohol solution containing the appropriate amine in the presence of anhydrous sodium carbonate. Attempts to replace the sodium carbonate as the alkaline condensing agent by using an excess of the amine resulted in aminolysis of the ester grouping. Hydrogenolysis of the condensation product in the presence of palladium black produced the desired *N*-substituted amide of *O*-carbamyl-DL-serine as indicated in the accompanying equations.



An alternate preparative procedure was also used (Method B, Table I) which involved reacting the free alcohol group in *N*-carbobenzoxyserine benzyl ester with the appropriate alkyl or aryl isocyanate to form the intermediate *O*-(substituted carbamyl)-*N*-carbobenzoxyserine esters. In several instances a solid formed at this stage which was filtered and recrystallized; however, when the intermediate failed to precipitate, the reaction mixture was reduced to dryness *in vacuo* and the residue was then crystallized from organic sol-

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

(2) J. A. Moore, J. R. Dice, E. D. Nicolaides, R. D. Westland, and E. L. Wittle, *J. Am. Chem. Soc.*, **76**, 2884 (1954).

(3) C. C. Stock, H. C. Reilly, S. M. Buckley, D. A. Clarke, and C. P. Rhoads, *Nature*, **173**, 71 (1954).

(4) J. M. Ravel, T. J. McCord, C. G. Skinner, and W. Shive, *J. Biol. Chem.*, **232**, 159 (1958).

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

TABLE I

O-(SUBSTITUTED CARBAMYL)-*N*-CARBOBENZOXY-DL-SERINE BENZYL ESTERS

R—NHCOOCH$_2$CHCOOCH$_2$C$_6$H$_5$
|
NH
|
Cbz

R	M.P., °C.	Yield, %	Empirical Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
			Method A						
CH_3—	92–93	77	$C_{20}H_{22}N_2O_6$	62.16	62.05	5.74	5.72	7.25	7.26
$C_4H_3OCH_2$—[a]	87–90	88	$C_{24}H_{24}N_2O_7$	63.73	64.06	5.35	5.49	6.19	6.24
$C_5H_4NCH_2$—[b]	75–78	61	$C_{25}H_{25}N_3O_6$	64.78	64.53	5.44	5.28	9.07	9.16
$C_6H_5CH_2$—	90–93	60	$C_{26}H_{26}N_2O_6$	67.51	68.05	5.67	5.94	6.06	6.29
$C_6H_5CH_2CH_2$—	99–102	85	$C_{27}H_{28}N_2O_6$	68.08	68.26	5.93	6.23	5.88	6.04
			Method B						
C_2H_5—	88–91	73	$C_{21}H_{24}N_2O_6$	62.98	63.09	6.04	6.35	7.00	7.30
C_6H_5—	110–113	65	$C_{25}H_{24}N_2O_6$	66.95	67.12	5.40	5.29	6.28	6.22
p-BrC_6H_4—	103–105	75	$C_{25}H_{23}BrN_2O_6$	56.93	57.27	4.40	3.97	5.31	5.46
p-$CH_3C_6H_4$—	87–88	74	$C_{26}H_{26}N_2O_6$					6.06	6.27
p-$CH_3OC_6H_4$—	108–110	75	$C_{26}H_{26}N_2O_7$	65.26	65.28	5.48	5.25	5.86	5.78
o-$CH_3OC_6H_4$—	89–90	80	$C_{26}H_{26}N_2O_7$	65.26	64.92	5.48	5.73	5.86	6.10
p-$C_2H_5OC_6H_4$—	115–116	80	$C_{27}H_{28}N_2O_7$	65.84	65.94	5.73	5.53	5.69	5.70
o-$C_2H_5OC_6H_4$—	119–121	89	$C_{27}H_{28}N_2O_7$	65.84	66.06	5.73	5.80	5.69	5.73
α-$C_{10}H_7$—[c]	103–106	84	$C_{29}H_{26}N_2O_6$	69.86	70.12	5.26	5.08	5.62	5.85
β-$C_{10}H_7$—[c]	110–113	96	$C_{29}H_{26}N_2O_6$	69.86	70.10	5.26	5.48	5.62	5.63
o-$C_{12}H_9$—[d]	48–50	65	$C_{31}H_{28}N_2O_6$	70.97	70.84	5.38	5.39	5.34	5.39

[a] Furfuryl-. [b] α-Pyridylmethyl-. [c] Naphthyl-. [d] Biphenyl-.

vents. Hydrogenolysis of the *O*-(substituted carbamyl)-*N*-carbobenzoxyserine ester was accomplished by the procedure previously described above, to yield *O*-(substituted carbamyl)-DL-serine derivatives (Table II) as indicated in the accompanying equations.

R—NCO + HOCH$_2$—CH—COOCH$_2$C$_6$H$_5$ ⟶
|
NH
|
Cbz

H O
| ‖
R—N—C—O—CH$_2$—CH—COO—CH$_2$C$_6$H$_5$
|
NH
|
Cbz

Pd, H$_2$ ↓

H O
| ‖
R—N—C—O—CH$_2$—CH—COOH
|
NH$_2$

The *O*-(substituted carbamyl)-DL-serine derivatives were chromatographically pure α-amino acids as determined in three solvent systems using the ascending technique, followed by development with ninhydrin reagent. Since many of these derivatives are only slightly soluble in water, the concentration range for microbiological testing was limited in several instances. The microorganisms used included *Lactobacillus arabinosus* 17-5, *Streptococcus lactis* 8039, *Escherichia coli* 9723, and the biological testing procedures have previously been indicated.[1] All of these *O*-(substituted carbamyl)-DL-serine analogs were essentially inactive at the highest level which could be tested (200–500 γ/ml.) in all three organisms with the exception of the *O*-(methylcarbamyl)-DL-serine derivative which was inhibitory for *L. arabinosus*, *S. lactis*, and *E. coli* at levels of about 50, 100, and 500 γ/ml., respectively. Further studies with *S. lactis* showed that glutamine competitively reverses the toxicity of the latter compound; one part of glutamine is inhibited by about 500 parts of the analog.

In view of the general lack of biological activity found with these glutamine analogs containing the larger substituted groups, it appears that such a modification of the carbamylserine nucleus prevents binding of the molecule with essential enzyme sites normally utilizing glutamine.

EXPERIMENTAL[6,7]

*O-(Substituted carbamyl)-N-carbobenzoxy-*DL*-serine benzyl esters. Method A, Table I.* Several of these compounds were prepared by the same general technique, and a description of only the *O*-(methylcarbamyl)serine benzyl ester will be described. The physical characteristics and analytical data of these derivatives are summarized in Table I.

(6) We are indebted to Dr. J. M. Ravel and her staff at the Clayton Foundation Biochemical Institute, The University of Texas, for the microbiological assays. Some of the chemical analyses were carried out in the authors' laboratories by Mr. D. L. Ross, the others were obtained through Drs. G. Weiler and F. B. Strauss, Oxford, England. The melting points are uncorrected.

(7) A resume of the biological testing procedures used in this study is given in reference 1.

TABLE II

O-(SUBSTITUTED CARBAMYL)-DL-SERINES

R—NHCOOCH$_2$CHCOOH
|
NH$_2$

R	M.P., °C.	Yield, %	Empirical Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
CH_3—	203–205	71	$C_5H_{10}N_2O_4$	37.03	37.39	6.22	6.11	17.28	17.15
C_2H_5—	191–193	61	$C_6H_{12}N_2O_4$	40.90	40.75	6.87	6.91	15.90	15.92
C_6H_5—	191–192	60	$C_{10}H_{12}N_2O_4$	53.56	53.94	5.40	5.31	12.50	12.39
$C_6H_5CH_2$—	204–205	62	$C_{11}H_{14}N_2O_4$	55.45	55.83	5.92	5.65	11.76	11.70
$C_6H_5CH_2CH_2$—	190–192	72	$C_{12}H_{16}N_2O_4$	57.13	57.43	6.39	6.28	11.11	11.02
p-$CH_3C_6H_4$—	201–202	61	$C_{11}H_{14}N_2O_4$	55.45	55.26	5.92	5.96	11.76	11.68
p-$CH_3OC_6H_4$—	195–197	56	$C_{11}H_{14}N_2O_5$	51.96	52.14	5.55	5.35	11.02	10.80
o-$CH_3OC_6H_4$—	184–185	55	$C_{11}H_{14}N_2O_5$	51.96	52.07	5.55	5.69	11.02	10.90
o-$C_2H_5OC_6H_4$—	135–137	42	$C_{12}H_{16}N_2O_5.H_2O$	50.34	50.59	6.34	6.64	9.79	9.94
p-$C_2H_5OC_6H_4$—	194–195	43	$C_{12}H_{16}N_2O_5$	53.72	53.79	6.01	6.17	10.44	10.30
β-$C_{10}H_7$—[a]	214–216	54	$C_{14}H_{14}N_2O_4$					10.22	10.12
o-$C_{12}H_9$—[b]	141–143	63	$C_{16}H_{16}N_2O_4.H_2O$	60.36	60.45	5.70	5.72	8.80	8.59
α-C_4H_7O—CH_2—[c]	180–182	85	$C_9H_{16}N_2O_5$	46.54	46.62	6.95	6.64	12.06	12.10
α-C_5H_4N—CH_2—[d]	193–194	54	$C_{10}H_{13}N_3O_4$	50.20	50.40	5.48	5.44	17.57	17.51

[a] Naphthyl-. [b] Biphenyl-. [c] Tetrahydrofurfuryl-. [d] Pyridylmethyl-.

N-Carbobenzoxy-DL-serine benzyl ester[8] (5.5 g.) was suspended in 60 ml. of toluene containing a large molar excess of phosgene. The reaction mixture was kept in a tightly closed system at room temperature for about 16 hr. to effect complete solution. After removal of the solvent, a pale yellow oil resulted which was freed of residual phosgene by the repeated addition and evaporation of benzene *in vacuo*. A solution of this oil in 25 ml. of dioxane was added slowly to a well stirred mixture containing 2 ml. of 40% methylamine and 1.0 g. of anhydrous sodium carbonate in 50 ml. of ethanol-water (1:1) maintained at 5 to 10°. After stirring an additional 3 hr. at room temperature, the reaction mixture was taken to dryness *in vacuo* to remove the solvents and excess amine. The residue was extracted with hot benzene, and the inorganic salts were removed by filtration. The resulting benzene solution was treated with Skellysolve C and placed in a deep freeze. There was obtained 3.1 g. of product, m.p. 72–75°, and an additional 1.8 g. of material was recovered from the mother liquor and washings. A sample was recrystallized from benzene–Skellysolve C and dried *in vacuo* over paraffin for analysis, m.p. 92–93°.

With two amines, phenylethylamine and furfurylamine, the condensation product precipitated directly from the reaction mixture and was recovered without evaporation of the solvent.

Method B, Table II. Using the same general procedure for all of the *O*-(substituted carbamyl)serine benzyl ester derivatives, prepared by Method B, a mixture of 1.65 g. (0.05 mole) of *N*-carbobenzoxy-DL-serine benzyl ester and two molar equivalents of the corresponding isocyanate in 25 ml. of toluene was heated under reflux for 8 to 10 hr. The reaction mixture was then cooled, and in several instances a precipitate formed at this stage which was filtered, washed with Skellysolve G, and dried *in vacuo*. When an oil formed the reaction mixture was taken to dryness under reduced pressure with warming. The residue was crystallized from benzene–Skellysolve G to yield the desired product. All of the *O*-(substituted carbamyl)-*N*-carbobenzoxyserine benzy esters were recrystallized from benzene-hexane and dried over paraffin *in vacuo* for elemental analysis; however, the initially isolated material was sufficiently pure to hydrogenolyze directly to the corresponding *O*-(substituted carbamyl)serine without further purification.

O-(Methylcarbamyl)-N-carbobenzoxy-DL-serine methylamide. In a separate experiment in which an attempt was made to use an excess of the amine as the condensing agent instead of sodium carbonate as per Method A, Table I, the chloroformyl derivative from 5.0 g. of *N*-carbobenzoxyserine benzyl ester was added to an ice cold solution of 200 ml. of 40% methylamine. After the reaction solution was reduced to dryness *in vacuo* there was recovered 3.0 g. of amide from the reaction mixture, m.p. 140–141°.

Anal. Calcd. for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.59. Found: C, 54.41; H, 6.18; N, 13.34.

O-(Substituted carbamyl)-DL-serines (Table II). All of these derivatives were prepared by the same general procedure. To a solution of the corresponding *O*-(substituted carbamyl)-*N*-carbobenzoxy-DL-serine benzyl ester dissolved in dioxane-ethanol (1:1) water was added until a slight turbidity persisted. The resulting mixture was stirred under hydrogen gas at atmospheric pressure and room temperature in the presence of palladium black for 6–8 hr. After hydrogenolysis was essentially complete, as evidenced by a spot test with ninhydrin reagent, the reaction mixture was heated to effect solution of the precipitated product. In most instances it was necessary to add additional ethanol or dioxane to effect complete solution. The hot solution was then filtered to remove the catalyst, and in most instances crystallization of the desired product occurred when the combined filtrates were placed in the refrigerator overnight. When the product failed to crystallize, the solvents were removed *in vacuo* with warming, and the residue was recrystallized from ethanol-water. The *O*-(substituted carbamyl)-DL-serines were finally filtered and dried over phosphorus pentoxide *in vacuo*.

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(8) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).