Journal of Medicinal and Pharmaceutical Chemistry

VOL. 3, No. 3 (1961)

S-(Alkyl- and Arylcarbamoyl)-L-cysteines

DONALD L. ROSS,* CHARLES G. SKINNER and WILLIAM SHIVE,
Clayton Foundation Biochemical Institute and *The Department of Chemistry, The University of Texas, Austin, Texas*

Introduction

S-Carbamoylcysteine has been prepared and found to be a non-competitive antagonist of glutamine in several microbial systems;[1] in addition, it acts as an antitumour agent for implanted RC mammary adenocarcinoma tissue in mice.[2] These properties are similar to those reported for azaserine,[3] a previously described antitumour agent.[4] The reactive groups in these analogues apparently interact chemically with the enzyme after the analogue associates with the site of glutamine utilization. In an effort to obtain more efficient pharmacological agents, the preparation of additional derivatives of this type was considered since non-competitive antagonists can be effective *in vivo* under conditions such that physiological concentrations of a metabolite would reverse the effects of a competitive antagonist.

Substitutions in the amide portion of glutamine have resulted in competitive glutamine antagonists for micro-organisms,[5-7] and similar results were obtained upon substitution of the amide portion of the competitive glutamine antagonist, *O*-carbamoyl-serine.[8] Accordingly, a series of *S*-(substituted-carbamoyl)-cysteines was prepared, and some of these, particularly the alkyl substituted derivatives, were found to have unusual inhibitory properties.

Experimental†

Microbial assays. The method of obtaining *Streptococcus lactis* 8039 cells for inoculation of growth assays has been described.[9]

* Predoctoral Fellow (CF-10,027), National Cancer Institute, United States Public Health Service.

† All melting points are uncorrected. The microanalyses were performed by

The assay procedure and medium used for the survey of toxicities of the *S*-(substituted-carbamoyl)-L-cysteines were modifications of previously reported techniques; the modified medium does not contain adenine, guanine or uracil, the concentrations of glutamic and aspartic acids are increased, and it contains ornithine in place of arginine.[1] For the specific assays studying the properties of *S*-ethylcarbamoyl-L-cysteine, the original basal medium was used[10] except that the concentration of glutamic acid was increased to 100 μg/ml and calcium pantothenate (0·2 μg/ml) was added.

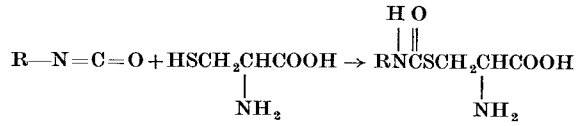
S-(*Substituted-carbamoyl*)-L-*cysteines* (Table I). Commercial L-cysteine hydrochloride monohydrate was dried *in vacuo* over phosphorus pentoxide for about one day. The anhydrous L-cysteine hydrochloride (1·0 g) was dissolved in dimethylformamide (15 ml), and a 10 per cent molar excess of the appropriate isocyanate was added with stirring. In most instances, the cysteine solution was cooled before the addition of the isocyanate since the condensation reaction was exothermic. The reaction flask was then stoppered and allowed to remain at room temperature for about 24 h, after which the reaction mixture was evaporated *in vacuo* to a viscous, light yellow syrup. The resulting residue was taken up in 30 ml of ether to dissolve the unreacted isocyanate, and then 30 ml of water was added.

For the *alkyl derivatives*, the ether phase was separated and the aqueous phase was washed once with an equal volume of ether, and then carefully neutralized with 1 N sodium hydroxide solution. After evaporation of the neutral aqueous phase to about one-half of the original volume, a precipitate formed. The product was crystallized by carefully warming the mixture to redissolve the solid followed by addition of ethanol with external cooling. The derivatives were finally recrystallized from water and ethanol.

With the *aryl derivatives*, in most instances the addition of water to the ether reaction mixture caused the condensation product to precipitate; however, when this did not occur, neutralization of the resulting aqueous phase with 1 N sodium hydroxide

Alfred Bernhardt, Germany, and in the authors' laboratories by Mr. C. Hedgcoth. The authors are indebted to Dr. J. M. Ravel and Mrs. Jean Humphreys for assistance with the microbial assays. The three aliphatic isocyanates used were kindly furnished by the Ott Chemical Company and the Carwin Company; the other intermediates were obtained through normal commercial sources.

Table I. *S*-(Substituted-carbamoyl)-L-cysteines



R	m.p., °C	Yield, %	Empirical formula	Analyses					
				Calcd.			Found		
				C	H	N	C	H	N
CH_3CH_2—	182–183	74	$C_6H_{12}N_2O_3S$[a]	37·5	6·3	14·6	37·8	6·3	14·8
$CH_3CH_2CH_2$—	179–180	62	$C_7H_{14}N_2O_3S$			13·6			13·6
$CH_3CH_2CH_2CH_2$—	181–182	50	$C_8H_{16}N_2O_3S$			12·7			12·5
$CH_3CH_2OCOCH_2$—	174–175	57	$C_8H_{14}N_2O_5S$	38·4	5·6	11·2	38·3	5·5	11·0
C_6H_5—	181–182	54	$C_{10}H_{12}N_2O_3S$	50·0	5·0	11·7	49·9	5·0	11·5
o-$CH_3C_6H_4$—	166–167	69	$C_{11}H_{14}N_2O_3S$	52·0	5·7	11·0	52·3	5·5	11·0
m-$CH_3C_6H_4$—	178–179	56	$C_{11}H_{14}N_2O_3S$			11·0			10·8
p-$CH_3C_6H_4$—	190–191	69	$C_{11}H_{14}N_2O_3S$[b]	52·0	5·7	11·0	52·3	5·6	10·8
o-ClC_6H_4—	161–162	75	$C_{10}H_{11}N_2O_3S$			10·2			10·0
m-ClC_6H_4—	187–188	75	$C_{10}H_{11}N_2O_3S$			10·2			10·4
p-ClC_6H_4—	187–188	57	$C_{10}H_{11}N_2O_3S$	43·7	4·0	10·2	44·2	4·2	10·2
o-$NO_2C_6H_4$—	152–153	78	$C_{10}H_{11}N_3O_5S$			14·7			14·4
m-$NO_2C_6H_4$—	156–157	61	$C_{10}H_{11}N_3O_5S$			14·7			14·5
o-$CH_3OC_6H_4$—	151–154	31	$C_{11}H_{14}N_2O_4S$			10·4			10·2
p-$CH_3OC_6H_4$—	185–186	75	$C_{11}H_{14}N_2O_4S$	48·9	5·2	10·4	48·7	5·1	10·3
o-$CH_3CH_2OC_6H_4$—	151–152	67	$C_{12}H_{16}N_2O_4S$			9·9			9·8
p-$CH_3CH_2OC_6H_4$—	174–175	79	$C_{12}H_{16}N_2O_4S$			9·9			9·8
o-C_6H_5—C_6H_4—	138–140	50	$C_{16}H_{16}N_2O_3S$			8·9			8·7
β-$C_{10}H_7$—	192–196	71	$C_{14}H_{14}N_2O_3S$			9·7			9·5

[a] *Anal.* S: calcd., 16·7; found, 16·6. [b] *Anal.* S: calcd., 12·6; found, 12·3.

induced the derivative to precipitate. The reaction product was washed with ether, then water, and finally air dried. These derivatives were recrystallized by dissolving them in hot 5 per cent ethyl alcohol at pH 1, followed by adding dropwise 1 N sodium hydroxide to induce turbidity and finally cooling.

Results and Discussion

The previously reported technique[1] of direct condensation of cysteine with potassium cyanate suggested that the corresponding alkyl and aryl substituted derivatives could be prepared in the same fashion. The reactivity of the mercapto grouping is sufficiently greater than the α-amino group in its ionic state to result in a preferential reaction at the sulphur atom. Initial attempts to condense various substituted isocyanates directly with cysteine hydrochloride monohydrate resulted in a mixture of products; however, when the cysteine was dried *in vacuo* over phosphorus pentoxide, the resulting anhydrous material readily condensed with the appropriate isocyanate using dimethylformamide as a solvent. The solvent was then removed *in vacuo,* and the residue was recrystallized to yield the desired derivatives which are summarized in Table I. These compounds are unstable in alkaline solutions, but are relatively stable under mildly acidic conditions.

The inhibitory properties of these derivatives were determined in *S. lactis* by the assay procedure previously used for studying the inhibitory properties of *S*-carbamoylcysteine.[1] Of the various compounds listed in Table I, *S*-(ethyl, propyl- and butylcarbamoyl)-L-cysteines were the most effective inhibitors giving complete inhibition at about 50 μg/ml. *S*-(*m*-Tolyl-, *m*-chlorophenyl-, *p*-chlorophenyl- and *p*-methoxycarbamoyl)-L-cysteines gave comparable inhibition of growth at a concentration of 200 μg/ml. The remaining analogues were either not inhibitory at this concentration, or were so insoluble that this concentration could not be attained in the assay media.

S-Ethylcarbamoyl-L-cysteine was chosen as a representative of the more active derivatives for preliminary biological studies of these substituted derivatives. As anticipated from the properties of *S*-carbamoylcysteine, glutamine has only a negligible

effect in reversing the inhibition of the ethyl derivative upon growth of *S. lactis;* however, that a combination of uracil, purines and citrulline, which increases about tenfold the amount of *S*-carbamoylcysteine necessary for inhibition of growth,[1] would have no appreciable effect upon the toxicity of the ethyl derivative was unexpected. On the other hand, it was found that a mixture of proline and asparagine or, more effectively, a mixture of glycylproline and glycylasparagine, exerted a reversing effect which required more than a tenfold increase in the concentration of the ethyl analogue to inhibit growth. These results suggest that the ethyl analogue inhibits a role of glutamine or a glutamyl derivative in the biosynthesis of both asparagine and proline. In view of the unusual activities of these *S*-alkylcarbamoylcysteines, a more extensive study of their biological properties is warranted.

Summary. Nineteen new *S*-(alkyl- and arylcarbamoyl)-L-cysteine derivatives were prepared through interaction of the appropriate isocyanate and L-cysteine using dimethylformamide as a solvent. *S*-(Ethyl-, propyl- and butylcarbamoyl)-cysteines were inhibitory to the growth of *Streptococcus lactis* at about 50 μg/ml; whereas the other derivatives were either less toxic or so insoluble in the assay media that they could not be examined at sufficiently high concentrations to indicate an inhibition. Some biological results using the derivative suggest that it inhibits a role of glutamine (or a glutamyl derivative) in the biosynthesis of both asparagine and proline.

(*Received 31 October, 1960*)

References

[1] Ravel, J. M., McCord, T. J., Skinner, C. G. and Shive, W. *J. biol. Chem.*, **232,** 159 (1958)

[2] Skinner, C. G., McKenna, G. F., McCord, T. J. and Shive, W. *Tex. Rep. Biol. Med.*, **16,** 493 (1958)

[3] Moore, J. A., Dice, J. R., Nicolaides, E. D., Westland, R. D. and Wittle, E. L. *J. Amer. chem. Soc.*, **76,** 2884 (1954)

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

[5] Sakato, Y., Hashizume, T. and Kishimoto, Y. *J. agric. chem. Soc. Japan*, **18,** 269 (1942); through *Chem. Abstr.*, **45,** 3528 (1951)

[6] Schilling, E. D. and Strong, F. M. *J. Amer. chem. Soc.*, **77,** 2843 (1955)

[7] Edelson, J., Skinner, C. G. and Shive, W. This Journal, **1,** 165 (1959)
[8] McCord, T. J., Skinner, C. G. and Shive, W. *J. org. Chem.*, **23,** 1963 (1958)
[9] Ravel, J. M., Estes, J. M., Mollenhauer, B. F. and Shive, W. *J. biol. Chem.*, **229,** 93 (1957)
[10] Ravel, J. M., Woods, L., Felsing, B. and Shive, W. *J. biol. Chem.*, **206,** 391 (1954)