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Synthesis and Biological Activity of Some N-(Substituted) Glutamines

JEROME EDELSON, CHARLES G. SKINNER and WILLIAM SHIVE

Clayton Foundation Biochemical Institute and the Department of Chemistry, The University of Texas, Austin, Texas, U.S.A.

The search for amino acid antagonists which might serve as efficient anti-tumor agents has been a subject of study for many years. Recently, two glutamine analogues, azaserine (*O*-diazoacetyl-L-serine)[1] and DON (6-diazo-5-oxo-L-norleucine),[2] have been reported to possess some anti-cancer activity;[3, 4] but, in contrast, an efficient but competitive glutamine antagonist, *O*-carbamyl-L-serine,[5] did not possess significant anti-tumor activity.* Attempts have been made to increase the biological effectiveness of *O*-carbamylserine by altering the structure of the molecule by introducing various groups on the amide nitrogen.[6] These *O*-(substituted carbamyl)serine derivatives were essentially biologically inactive with the exception of the *O*-(methylcarbamyl)-analogue. The latter compounds are analogues of *N*-(substituted)glutamines with an oxygen atom in lieu of a methylene group in the parent amino acid chain. Several *N*-(substituted)-glutamines have been isolated from natural sources[7, 8] and others have been synthesized and found to inhibit the growth of microorganisms.[9,10] For the purpose of comparison with the corresponding *O*-(substituted carbamyl)serine derivatives, additional *N*-(substituted)glutamines were synthesized and their biological properties were examined.

Experimental Methods†

Biological Assays

For *Streptococcus lactis* 8039 and *Lactobacillus arabinosus* 17–5 a modification[5] of a previously reported[11] amino acid medium

* Unpublished data, these laboratories.

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was employed, and for *Escherichia coli* a previously described[12] inorganic salts–glucose medium was used. Water solutions of the compounds were added aseptically to the previously autoclaved assay tubes.

Organic Syntheses

Aminolysis of N-*carbobenzoxyglutamic anhydride* (Table I). The interaction of benzylamine and *N*-carbobenzoxyglutamic anhydride will be described as a typical preparative procedure for all the amines used; the others are summarized in Table I. A mixture of 0·02 mole of *N*-carbobenzoxyglutamic anhydride and 0·02 mole of benzylamine dissolved in a 1 : 1 mixture of ethanol–benzene was stirred overnight at room temperature. The solvent was removed *in vacuo*, the residue was taken up in ethanol, and 4·7 g of material was recovered by the addition of Skellysolve B. If the reaction product failed to crystallize readily, the residue from above was taken up in a dilute sodium hydroxide solution and extracted with ether to remove the excess amine, after which the remaining alkaline aqueous phase was made acid and extracted with ether. Addition of Skellysolve B to the latter ether solution precipitated the reaction product.

Hydrogenolysis of N - (*substituted*) - N^2 - *carbobenzoxyglutamines* (Table II). Hydrogenolysis of all of the amide derivatives described above, and summarized in Table II, to form the corresponding glutamic amides were essentially identical. A solution of 2·5 g of the benzylamine condensation product dissolved in 50 ml of alcohol–water containing about 200 mg of palladium black was hydrogenated at atmospheric pressure for about 4 h. The reaction mixture was warmed to effect complete solution, filtered to remove the catalyst, and part of the solvent was removed *in vacuo* to yield 1·5 g of reaction product, which was recrystallized from ethanol–water.

The recrystallized mixture products were then analysed for carbon, hydrogen, and nitrogen to confirm their empirical formula, after which, a quantitative ninhydrin reaction[13] was carried out to indicate the position of the amino substituent. Since this assay is specific for only α-amino acid groupings, the percentage of carbon dioxide liberated is a direct measure of the percentage of γ-substitution.

Table I. Preparation of some amide derivatives of glutamic acid.
Reaction product of amine and *N*-carbobenzoxyglutamic anhydrides

Amine used	Yield, %	M.p., °C	Empirical formula	Analysis, %					
				calc.			found		
				C	H	N	C	H	N
Methyl	71	62–64	$C_{14}H_{18}N_2O_5$			9·52			9·68
n-Hexyl-	94	69–70	$C_{19}H_{28}N_2O_5$			7·69			7·71
Phenyl-	55	156–157	$C_{19}H_{20}N_2O_5$	64·03	5·66	7·86	64·36	5·69	7·96
Benzyl-	64	146–147	$C_{20}H_{22}N_2O_5$			7·56			7·33
Phenethyl-	29	144–145	$C_{21}H_{24}N_2O_5$	65·62	6·30	7·29	66·13	6·51	7·46
Furfuryl-	53	122–125	$C_{18}H_{20}N_2O_6$	60·00	5·60		59·90	5·89	
β-Pyridylmethyl-	94	113–114	$C_{19}H_{21}N_3O_5$	61·44	5·70	11·32	61·49	5·98	11·06

Table II. Preparation of some glutamic amides.
Hydrogenolysis product from reaction mixtures Table I

Amide derivative	Yield, %	M.p., °C (dec.)	Empirical formula	Analysis, %						α-Amino acid content, %*
				calc.			found			
				C	H	N	C	H	N	
Methyl-	72	203–205†	$C_6H_{12}N_2O_3$	44·99	7·55	17·49	45·23	7·27	17·39	101·9
n-Hexyl-	53	150–151	$C_{11}H_{22}N_2O_3$	57·36	9·63	12·17	57·45	9·60	12·29	35·0
Phenyl-	17	197–199	$C_{11}H_{14}N_2O_3$	59·44	6·35	12·61	59·82	6·02	12·67	101·2
Benzyl-	85	225–226	$C_{12}H_{16}N_2O_3$	61·00	6·83	11·86	61·14	6·76	11·90	98·8
Phenethyl-	46	230–231	$C_{13}H_{18}N_2O_3$	62·38	7·25	11·19	62·67	7·34	11·09	94·2
Furfuryl-	63	225–226	$C_{10}H_{14}N_2O_4$	53·09	6·24	12·39	52·96	6·58	12·26	89·2
β-Pyridyl-methyl-	47	194–196	$C_{11}H_{15}N_3O_3$	55·68	6·37	17·71	55·81	6·64	17·80	60·6

* Analyses based on the quantitative ninhydrin reaction.
† Lichtenstein, N., *J. Amer. chem. Soc.*, **64**, 1021 (1942), reported m.p.. 192° for this derivative prepared through a different procedure.

Results and Discussion

The most convenient method of preparing these compounds involved an aminolysis of *N*-carbobenzoxyglutamic anhydride followed by hydrogenolysis of the carbobenzoxy group to yield the amino acid; even though this technique frequently results in a mixture of the two possible isomers.[14,15] Both the intermediate *N*-carbobenzoxyglutamic amide and the hydrogenolysis product gave the anticipated elemental analysis. Since the initial question involved in this study was the biological activity of the *N*-(substituted)glutamine derivatives, no extensive effort was made to separate α and γ amides except by the usual recrystallization procedures. The quantity of the desired γ-isomer which was present in the isolated product was determined using the quantitative ninhydrin procedure which is specific for the α-amino acid grouping.[13] Unexpectedly, except in only two cases, the product isolated was exclusively the γ-isomer as determined by the quantitative ninhydrin reaction, and indicated in Table II. The materials isolated from the hydrogenolysed *N*-carbobenzoxyglutamylamide derived from *n*-hexylamine and β-pyridylmethylamine when analysed by the quantitative ninhydrin procedure constituted 35·0 and 60·6 per cent, respectively, of the γ-isomer.

Paper chromatographs of the amide derivatives (Table II) using the ascending technique followed by development with ninhydrin reagent confirmed the above data. The products isolated from the interaction of *N*-carbobenzoxyglutamic anhydride with furfurylamine, benzylamine, aniline, methylamine and phenethylamine, followed by hydrogenolysis, were chromatographically pure α-amino acids. Further, only one ninhydrin active material could be found when these latter samples were examined by electrophoretic techniques.

In view of the low yields obtained in some of the examples cited in Table II, it is possible that the corresponding α-isomer was formed in the reaction mixture to some extent, but that the work-up procedure separated the materials. This would be anticipated since two isomers were observed in two of the examples.

In contrast to the biological inactivity of the *O*-(substituted carbamyl)serine analogues,[6] *N*-benzylglutamine was inhibitory toward *S. lactis* at a level of about 20 γ/ml, and was competitively

reversed over an eightfold range of concentrations by the natural amino acid (Table III). The latter analogue was not inhibitory

Table III. Reversal of N-benzylglutamine toxicity by glutamine
Test organism: *S. lactis* 8039, incubated 22 hours at 30°

N-Benzylglutamine, γ/ml	L-Glutamine, γ/ml				
	0	0·25	0·5	1·0	2·0
	galvanometer readings*				
0	46	44	36	43	39
2	41				
5	37	44			
10	41	43	40		
20	12	38	40	43	45
50	0	9	22	30	39
100		0	12	33	37
200			1	8	31
400					25

* A measure of culture turbidity; distilled water reads 0, an opaque object 100.

toward *Lb. arabinosus* or *E. coli* at concentration levels sufficiently low to determine a glutamine reversing effect. The corresponding oxa analogue, *O*-(benzyl-carbamyl)serine, was inhibitory at a measurable level (300 γ/ml) against *S. lactis*; however, its limited solubility in the assay medium prevented a study of any competitive relationships with glutamine. The other glutamine derivatives described in this paper were essentially inactive biologically in all three of the microbiological systems studied, with the exception of the previously reported *N*-methylglutamine which was inhibitory to *S. lactis* at a level of about 100 γ/ml. It is interesting to note that neither the phenyl- nor the phenethylglutamine derivatives possess significant toxicity in these test systems; thus, in the aromatic series the structure associated with *N*-benzylglutamine is unique in this respect.

Summary. The interaction of *N*-carbobenzoxyglutamic anhydride with various amines, followed by hydrogenolysis of the reaction product, has led to the synthesis of six new glutamyl amides. The inhibitory properties

of these compounds were studied in three organisms; however, only the *N* benzylglutamine was found to be effective. The corresponding phenyl- and phenethyl-analogues were inactive in the test systems studied. *N*-Benzylglutamine was inhibitory toward *Streptococcus lactis* at a level of about 20 γ/ml, and was competitively reversed over an eightfold range of concentrations by glutamine.

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