The Synthesis of Anthglutin and Its Analogues

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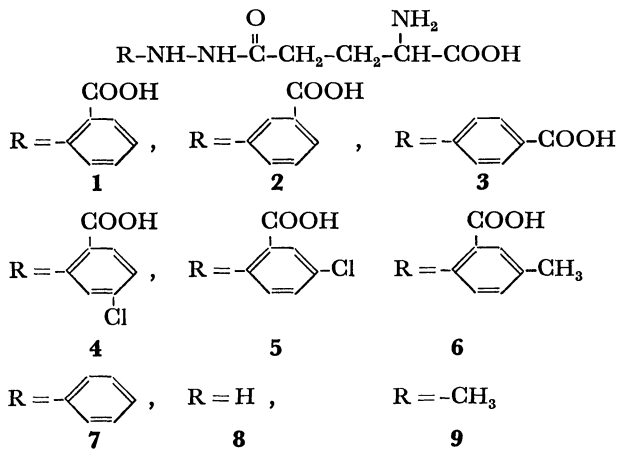
Synopsis. Anthglutin (1-γ-L-glutamyl-2-(2-carboxyphenyl)hydrazine) and its analogues were synthesized. Their inhibitory activity on γ-glutamyl transpeptidase was measured. 2-Carboxyphenyl derivatives, including anthglutin, showed inhibitory activity, while the other derivatives did not. The 2-carboxyl group seems to be essential for the inhibitory activity.

In previous papers,[1,2] we reported that anthglutin, an inhibitor of γ-glutamyl transpeptidase, was isolated from a cultured medium of Penicillium oxalicum, and Structure (**1**), 1-γ-L-glutamyl-2-(2-carboxyphenyl)hydrazine, was proposed. We have confirmed the structure of anthglutin by the synthesis of 1-γ-glutamyl-2-(2-carboxyphenyl)hydrazine (**1**).



Several compounds related to anthglutin were synthesized and measured for their inhibitory activity on γ-glutamyl transpeptidase (Tables 1 and 2). 2-Carboxyphenyl hydrazine derivatives (**4**—**6**) inhibited an enzyme activity, showing that the 2-carboxyl group of anthglutin is a functional group for the inhibitory activity. As has been shown in a previous paper,[1] the α-carboxyl and α-amino groups of the glutamyl moiety of anthglutin also seem to be essential for forming a complex between the inhibitor and the enzyme.

Experimental

Materials. Commercially available aminobenzoic acid derivatives were used. The *N-t*-BOC-L-glutamic acid α-benzyl ester was obtained from the Sigma Chemical Company.

γ-Glutamyl transpeptidase was partially purified by the method of Orlowski and Meister;[3] it was thereby purified about 140-fold from the whole homogenate of the hog kidney.

Synthesis. Carboxyphenylhydrazine compounds were synthesized, according to the method described by Stephenson,[4] from the appropriate aminobenzoic acid compounds. 1-γ-L-Glutamyl-carboxyphenylhydrazine compounds were prepared by condensation between the N-*t*-BOC-L-glutamic acid benzyl ester and the appropriate free base form of the hydrazine, according to the method described by F. Hoffmann-La Roche & Co.[5]

1-γ-L-Glutamyl-2-(2-carboxyphenyl) hydrazine (1). 2-Carboxyphenylhydrazine (0.96 g, 6.31 mmol), the *N-t*-BOC-L-glutamic acid α-benzyl ester (1.05 g, 3.11 mmol), and triethylamine (0.6 ml) were dissolved in dichloromethane (10 ml). To the solution we then added dicyclohexylcarbodiimide (0.88 g, 4.53 mmol) dissolved in dichloromethane (1.0 ml), and the mixture was stirred for 3 h at room temperature. After removing the precipitate thus obtained by filtration, the filtrate was subjected to column chromatography on silica gel, eluting with chloroform–methanol–acetic acid (400:10:1). After the solvent had been removed *in vacuo* at 30 °C, the residue was dissolved in ethanol (20 ml) containing cyclohexene (1.0 ml). To the solution we added palladium–charcoal (5%, 400 mg), after which the mixture was refluxed for 1 h with stirring. The catalyst was removed

Table 1. Yields, melting points, and analytical data

Compound	Yield/%	Mp/°C	Molecular formula	Found (Calcd)(%) C	H	N
2	18.3	187	$C_{12}H_{15}N_3O_5$	51.27 (51.24	5.31 5.38	14.84 14.94)
3	14.1	175	$C_{12}H_{15}N_3O_5$	51.44 (51.24	5.41 5.38	14.70 14.94)
4	24.4	165	$C_{12}H_{14}N_3O_5Cl$	45.75 (45.65	4.31 4.47	13.18 13.31)
5	16.8	172	$C_{12}H_{14}N_3O_5Cl$	45.90 (45.65	4.51 4.47	13.11 13.31)
6	25.4	165	$C_{13}H_{17}N_3O_5$	52.61 (52.88	5.97 5.80	14.08 14.23)
7	26.3	202	$C_{11}H_{15}N_3O_3$	55.57 (55.68	6.48 6.47	17.68 17.71)
8	24.6	164	$C_5H_{11}N_3O_3$	37.26 (37.26	6.87 6.88	26.10 26.08)
9	22.7	152	$C_6H_{13}N_3O_3$	41.26 (41.14	7.41 7.43	24.03 24.00)

Table 2. Inhibition of γ-glutamyl transpeptidase anthglutin analogues

Compounds (R–)	$K_i/\mu M$
Anthglutin (P. oxalicum)	5.7
2-Carboxyphenyl-(**1**)	5.9
3-Carboxyphenyl-(**2**)	200.0
4-Carboxyphenyl-(**3**)	>1000.0
2-Carboxy-5-chlorophenyl-(**4**)	7.5
2-Carboxy-4-chlorophenyl-(**5**)	11.6
2-Carboxy-4-methylphenyl-(**6**)	8.7
Phenyl-(**7**)	>1000.0
N-Unsubstituted (**8**)	Not inhibit
Methyl-(**9**)	Not inhibit

In the presence of the analogues (6.5 μM), inhibition was determined according to a method described previously.[1] Substrate (γ-glutamyl-*p*-nitroanilide), 0.42—4.21 mM; acceptor(glycylglycine), 47.9 mM; enzyme, hog kidney γ-glutamyl transpeptidase; incubation, pH 8.5, 37 °C, 15 min.

by filtration, and the filtrate was evaporated *in vacuo* at 30 °C to dryness to give a white powder. The powder thus obtained was treated with trifluoroacetic acid (1.0 ml) and anisole (0.25 ml) for 1 h in an ice bath.[6] The reaction product was thus precipitated by the addition of ether (25 ml). The precipitate thus obtained was purified on a column of Dowex 2×8 (formate form) according to the method described previously.[1] The product thus obtained (210 mg) was identical with a sample of natural anthglutin (yield, 24%; mp 170—171.5 °C; UV (water): 243 and 322 nm (pH 2.0), and 240 and 307 nm (pH 7.0); $[\alpha]_D^{20}$: +22.6° (*c*, 0.9, 0.05 mol dm^{-3} HCl)), as established by paper chrcmatography and the inhibitory activity (Table 2). Found: C, 51.21; H, 5.32; N, 14.88%. Calcd for $C_{12}H_{15}N_3O_5$: C, 51.24, H, 5.38; N, 14.94%.

Analogues Anthglutin. Analogues of anthglutin (**2**—**6**) were synthesized by the procedure described above using the appropriate hydrazine compound.

The 1-γ-L-glutamyl hydrazine compounds (**7**—**9**) were prepared by condensation between L-pyroglutamic acid and the appropriate free-base form of the hydrazine compound, under reflux overnight in 85% ethanol, according to the method described by Yale *et al.*[7] The results thus obtained are shown in Table 1.

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