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The Synthesis of γ-Alkylamides of L-Glutamic Acid. The Reactions of Metallic Salts of L-Pyrrolidonecarboxylic Acid with Primary Alkylamines

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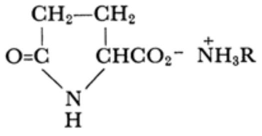
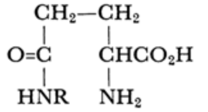
L-Theanine, a principal component of flavors contained in green teas, and its homologs have been synthesized by the reactions of several metallic salts of L-pyrrolidonecarboxylic acid with primary alkylamines. Hitherto, γ-alkylamides of L-glutamic acid had been prepared in low yields by the reactions of L-pyrrolidonecarboxylic acid with anhydrous alkylamines. The use of metallic salts, especially cupric, zinc, and manganese salts, increased the yields of the γ-amides remarkably.

L-Theanine is a form of *N*-(γ-L-glutamyl)ethylamine known to be a principal component of the flavors contained in green teas, especially those of a higher grade.[1] Several procedures are available for the synthesis of L-theanine, e. g., the treatment of γ-ethyl *N*-benzyloxycarbonyl-L-glutamate with anhydrous ethylamine,[2] and that of L-pyrrolidonecarboxylic acid with aqueous[3] or anhydrous[4] ethylamine. The ring-opening reactions of pyrrolidonecarboxylic acid with primary alkylamines are equilibrium reactions and are remarkably affected by the solubilities of the products, γ-alkylamides of glutamic acid, in the reactant alkylamines.





$\mathrm{O{=}C(HNR)CH_2CH_2CH(NH_2)CO_2H}$

The yield of optically active L-theanine from L-pyrrolidonecarboxylic acid is compared with that of DL-theanine from DL-pyrrolidonecarboxylic acid in Fig. 1.

As a rule, the γ-alkylamides of racemic glutamic acid are less soluble in alkylamine than those of the optically active isomer. Consequently, if the L-amides produced could be made insoluble in the corresponding alkylamines by some means, the yields of the γ-amides might be increased. The present studies were initiated in search of a method which could displace the equilibrium and which would lead to complete conversion.

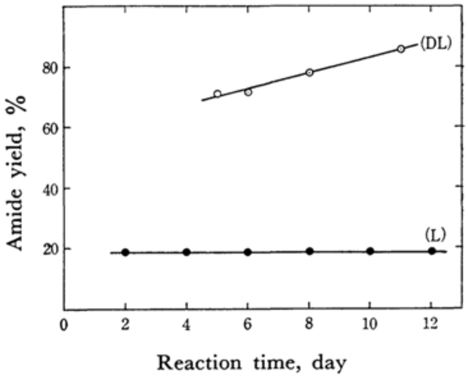


Fig. 1. The comparison of the yields of L- and DL-theanines. (Ethylamine and the respective acids were heated at 60°C in the molar ratio of 4 : 1.)

Experimental

Cupric L-Pyrrolidonecarboxylate.—Into a solution of 12.9 g. (0.1 mol.) of L-pyrrolidonecarboxylic acid in 100 ml. of water, basic cupric carbonate was added with stirring under a slight warming until the evolution of carbon dioxide ceased. After the mixture had then been filtered, the filtrate was concentrated under reduced pressure. The crystalline mass was filtered and then dried over sulfuric acid under reduced pressure. The yield of the cupric salt was 35.6 g. (91%); m. p. 190—210°C (decomp.).

Found: C, 30.50; H, 5.37; N, 7.42; Cu, 15.88. Calcd. for $C_{10}H_{12}O_6N_2Cu\cdot4H_2O$: C, 30.65; H, 5.14; N, 7.15; Cu, 16.21%.

Zinc L-Pyrrolidonecarboxylate.—The zinc salt was prepared according to the procedure for the cupric salt described above.

Found: C, 33.62; H, 4.77; N, 7.61. Calcd. for $C_{10}H_{12}O_6N_2Zn\cdot2H_2O$: C, 33.58; H, 4.51; N, 7.84%.

Manganese L-Pyrrolidonecarboxylate.—This was prepared in a similar manner, but attempts to crystallize the resultant syrup were unsuccessful.

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1) Y. Sakato, *Nippon Nogeikagaku Zasshi,* **23**, 262 (1950).

2) Y. Sakato, T. Hashizume, Y. Kishimoto, ibid., **23**, 269 (1950).

3) N. Lichtenstein, *J. Am. Chem. Soc.,* **64**, 1021 (1942).

4) Y. Tuchiya, Japanese Pat. 197059.

L-Theanine, *N*-(γ-L-Glutamyl)ethylamine.— Thirty-two grams of anhydrous cupric L-pyrrolidonecarboxylate dried at 100°C under reduced pressure were treated with 51 ml. of anhydrous ethylamine for seven days at 70°C in a sealed glass tube. At the end of this time, the solution was concentrated to dryness under reduced pressure to yield the violet crystalline mass. The residue was suspended in 300 ml. of water, and then the cupric ions were removed by the usual method using hydrogen sulfide. The solution, now free from cupric ions, was decolorized with activated charcoal, concentrated under reduced pressure, and cooled. The colorless crystalline precipitate was collected and dried over concentrated sulfuric acid under reduced pressure.

Yield: 30.3 g. (87%); m. p. 214—215°C (decomp.).

Found: C, 48.06; H, 8.11; N, 16.05.

Calcd. for $C_7H_{14}O_3N_2$: C, 48.26; H, 8.10; N, 16.08%.

Cupric complexes of L-theanine prepared by the method described above were recrystallized from hot water before analysis.

Found: C, 40.79; H, 6.54; N, 13.31; Cu, 15.61. Calcd. for $C_{14}H_{26}O_6N_4Cu$: C, 41.01; H, 6.39; N, 13.66; Cu, 15.50%.

TABLE I. THE REACTIONS OF VARIOUS SALTS OF L-PYRROLIDONECARBOXYLIC ACID WITH ANHYDROUS ETHYLAMINE

Run[a)]	Metallic Ion	Molar ratio[b]	Yield[c)]	Solubility[d)]
1	Ca^{2+}	10.0	0	Insol.
2	Cr^{2+}	5.0	0	Sol.
3	Mn^{2+}	7.8	80	Sol.
4	Co^{2+}	6.0	trace[e)]	Sol.
5	Ni^{2+}	6.0	trace[e)]	Sol.
6	Cu^{2+}	7.8	88	Sol.
7	Zn^{2+}	7.8	60	Sol.
8	Cd^{2+}	5.0	0	Sol.
9	Hg^{2+}	7.8	0[f)]	Sol.
10	Pd^{2+}	10.0	0	Insol.

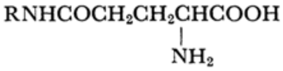
a) Experiments at 70°C for seven days.
b) Molar ratio of ethylamine to the metallic salt of L-pyrrolidonecarboxylic acid.
c) Yield of the metallic salt of L-theanine.
d) Solubility of the metallic salt of L-pyrrolidonecarboxylic acid in ethylamine.
e) A syrup was obtained from this run.
f) Metallic mercury was isolated from this run.

Results

The preliminary experiments have revealed that cupric, zinc and manganese salts of L-pyrrolidonecarboxylic acid are soluble in alkylamines, but the corresponding salts of the γ-alkylamides are insoluble. Thus, the use of these salts provides the best method for preparing γ-alkylamides of L-glutamic acid.

The experimental results of the reactions of various salts of L-pyrrolidonecarboxylic acid with anhydrous ethylamine are summarized in Table I. Similarly, the results of the reactions of zinc L-pyrrolidonecarboxylate with several primary alkylamines are given in Table II. The physical properties of the γ-alkylamides prepared are shown in Table III.

TABLE III. PHYSICAL PROPERTIES OF THE γ-ALKYLAMIDES OF L-GLUTAMIC ACID



$$RNHCOCH_2CH_2\underset{\displaystyle NH_2}{\underset{|}{C}}HCOOH$$

R	M. p., °C	$[\alpha]_D^{30}$ [a)]	Taste
H	185—186	+6.1	nice and sweet
CH_3CH_2-	214—215	+6.1	↓
$CH_3CH_2CH_2-$	222—223	+5.8	
$CH_3(CH_2)_2CH_2-$	222—223	+5.7	
$CH_3(CH_2)_3CH_2$	207—208	+5.7	bitter

a) Specific rotation was measured in a neutral aqueous solution. (*c* 2, H_2O)

The L-Theanine thus prepared was identified by a comparison of its melting point, paper chromatographic behavior and infrared spectrum with those of an authentic sample prepared by the aminolysis of γ-ethyl *N*-benzyloxycarbonyl-L-glutamate with ethylamine, followed by hydrogenolysis.[2)] Accordingly, it is evident that the alkylamino group is located in the γ-position of the L-glutamic acid residue.

We wish to express our deep thanks to Dr. Haruomi Oeda for his kind encouragement and valuable suggestions.

TABLE II. REACTIONS OF ZINC L-PYRROLIDONECARBOXYLATE WITH SEVERAL ALKYLAMINES

Run	Amine	Molar ratio	Temp. °C	Time day	Yield %	Analysis, % Calcd.	Analysis, % Found
1	NH_3	12	50	1	trace[a)]		
2	Ethylamine	7.8	70	6	59	C 48.26 H 8.10 N 16.08	C 48.06 H 8.11 N 16.05
3	*n*-Propylamine	8	70	6	36	C 51.08 H 8.57 N 14.87	C 51.11 H 8.86 N 14.84
4	*n*-Butylamine	8	70	6	24	C 53.44 H 8.96 N 13.85	C 53.44 H 8.96 N 13.76
5	*n*-Amylamine	9	70	6	20	C 55.53 H 9.32 N 12.94	C 55.51 H 9.27 N 12.95

a) Identified by paper chromatography