BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 39 1701—1705 (1966)

A Molecular Compound between Glutamic Acid and Pyroglutamic Acid*

By Yoshihisa SUZUKI and Nobuya NAGASHIMA

Central Research Laboratories, Ajinomoto Co., Inc., Suzuki-cho, Kawasaki

(Received January 13, 1966)

A new molecular compound, which consists of L-glutamic acid, L-pyroglutamic acid and water of crystallization in a 1 : 1 : 1 mole ratio, has been discovered. In its crystal, the unit-cell is monoclinic with $a=5.10$, $b=7.20$, $c=18.38$ Å, and $\beta=96.6°$, and the space group has been determined to be $P2_1$. Equilibrium measurements in the system of L-glutamic acid, L-pyroglutamic acid and water at 20°C, 30°C, and 50°C, showed that the transition temperature from the molecular compound to L-pyroglutamic acid and α-form L-glutamic acid is 35.5°C, while that to L-pyroglutamic acid and β-form L-glutamic acid is 20.5°C. Thus, the molecular compound is stable only below this temperature. This molecular compound has an incongruent solubility. When it is placed in water, a crystallization of L-glutamic acid takes place as the molecular compound is dissolved. A similar molecular compound consisting of D-glutamic acid and D-pyroglutamic acid is also obtained, but no molecular compound of the L-glutamic acid and D-pyroglutamic acid, or vice versa, is obtainable. On the basis of these facts, an optical resolution is actually possible for glutamic acid. When this molecular compound is heated, it is converted into another molecular compound at about 70°C due to the loss of the water of crystallization; then it is decomposed to L-glutamic acid (β-form) and L-pyroglutamic acid at about 90°C.

A new molecular compound, which consists of L-glutamic acid, L-pyroglutamic acid and water of crystallization in a 1 : 1 : 1 mole ratio, was discovered, although complex of these two acids in solution had been assumed without any confirmation by Kergle.[1] It is prepared[2] in the following way: 10 g. of L-glutamic acid and 60 g. of L-pyroglutamic acid are dissolved in 100 g. of hot water, and then the solution is cooled to 20°C. After several hours, crystals with a shape of rhombic plate are obtained. The chemical analysis shows that these crystals are the 1 : 1 : 1 molecular compound.

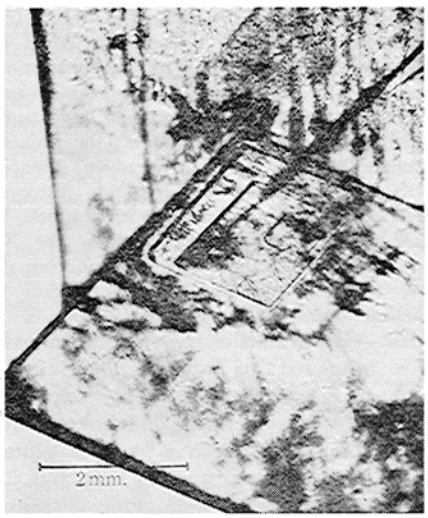

Fig. 1. A large spiral layer on the crystal surface of the molecular compound, which consists of L-glutamic acid, L-pyrroglutamic acid, and water of crystallization.

Found: C, 40.99; H, 6.30; N, 9.54; Amino-N, 4.81 (Van Slyke); L-glutamic acid, 50.8 (enzymatic analysis). Calcd. for L-glutamic acid - L-pyroglutamic acid - water: C, 40.81; H, 6.17; N, 9.52; Amino-N, 4.77; L-glutamic acid, 50.0%.

On the surface of one of these molecular compound crystals, a large spiral layer was found (Fig. 1).

Crystallographic Examination

X-Ray diffraction studies were carried out of the crystals obtained with CuK_α radiation. The oscillation and Weissenberg methods were used for single crystals, and diffractometer techniques, for powder specimens. The unit-cell is monoclinic with $a=5.10$, $b=7.20$, $c=18.38$ Å, and $\beta=96.6°$.

For reference, the cell dimensions of L-glutamic acid and L-pyroglutamic acid are shown below:

L-glutamic acid:

(α-form)[3] $a=7.06$, $b=10.3$, $c=8.75$kX

(β-form)[4] $a=5.17$, $b=17.34$, $c=6.95$Å

L-pyroglutamic acid:

* Presented in part at 14 th Annual Meeting of the Chemical Society of Japan, Tokyo, April, 1961.

1) E. Kergle, "Glutaminsäure," Wissenschaftliche Verlagsgesellschaft M. B. H., Stuttgart (1954), p. 12.

2) Japanese Pat. 414586 (Japanese Publ. 11140/1963).

3) J. D. Bernal, *Z. Krist.*, **78**, 363 (1931).

4) S. Hirokawa, *Acta Cryst.*, **8**, 637 (1955).

$a=14.6,\ b=8.99,\ c=13.5$Å

The Weissenberg pattern shows a systematic absence of $(0k0)$ reflections with $k \neq 2n$. This indicates that the space group is either $P2_1$ or $P2_1/m$. The density measurements ($d_0=1.454$, $d_c=1.456$ g. cm^{-3}) show that there are two molecular compounds (L-glutamic acid - L-pyroglutamic acid - water) in the unit cell. Accordingly, the possibility of $P2_1/m$ is eliminated, and the space group is determined to be $P2_1$. The X-ray powder diffraction data are shown in Table I. The intensities reported in Table I are peak heights above the background, so scaled that the most intense line is given a value of 100.

TABLE I. X-RAY POWDER DIFFRACTION DATA FOR MOLECULAR COMPOUNDS

	L-Glutamic acid, L-pyroglutamic acid and water		L-Glutamic acid and L-Pyroglutamic acid	
hkl	*d*, Å	I/I_0	*d*, Å	I/I_0
001	18.4	60	16.8	15
002	9.2	5	7.02	15
011	6.7	15	5.52	10
003	6.15	25	5.43	5
012	5.68	10	5.12	5
100	5.11	10	4.67	5
013	4.69	10	4.38	5
004	4.60	10	4.21	100
110	4.15	100	4.13	20
112	3.98	60	4.07	10
014	3.88	10	3.93	10
005	3.68	20	3.74	3
104 } 020 }	3.60	50	3.59	40
			3.41	5
021	3.53	10	3.34	5
022	3.36	15	3.27	10
015	3.28	15	3.15	5
105	3.16	3		
023	3.11	10		
120	2.94	30		

The Determination of the Conditions of the Molecular Compound Formation

In an attempt to determine the conditions under which the molecular compound is formed, solubility measurements of various saturated solutions of L-glutamic acid, L-pyroglutamic acid and water have been made. For each set of solubility measurements, an examination was also made of the constitution of the solid phase which is in equilibrium with the saturated solution in question.

Material.—*α-Form L-Glutamic Acid.*—The pH of a solution of 300 g. of commercial mono-sodium L-glutamate mono-hydrate dissolved in 10 l. of water was brought to about 3.2 by promptly adding 35% hydrochloric acid at room temperature. The crystals thus formed were filtered, washed with water, and dried in vacuo. The results of the analysis of this L-glutamic acid were as follows:

Found: N, 9.53%; $[\alpha]_D^{20}$ + 31.7 (*c* 8, 2N HCl).
Calcd.: N, 9.52%.

These crystals were found by the X-ray diffraction method[5] to be α-form, with a 98% purity.

β-Form L-Glutamic Acid.—First, a glutamate solution was prepared by dissolving 300 g. of mono-sodium L-glutamate monohydrate in 1.5 l. of water. Into this solution 35% hydrochloric acid was slowly stirred at 80°C. After 1 hour, the pH of the solution reached about 3.2; then the solution was cooled to room temperature. The crystals formed were filtered, washed with water, and dried in vacuo. The results of the analysis of this L-glutamic acid were as follows:

Found: N, 9.52%; $[\alpha]_D^{20}$ +31.8 (*c* 8, 2N HCl).

These crystals were found by the X-ray method to be 100% pure β-form L-glutamic acid.

L-Pyroglutamic Acid.—L-Glutamic acid was suspended in five times as much water and kept at 120°C for about 20 hr. The solution was then decolorized by active charcoal and passed through a column of the cation exchange resin (Diaion SK in H^+-form) in order to remove the unreacted glutamic acid. The effluent was concentrated in vacuo, the crystals first deposited were removed (DL-pyroglutamic acid), and then the resultant solution was cooled. The crystals formed in this stage were filtered, washed with cold water, and dried in vacuo. The results of the analysis of these crystals were as follows:

Found: N, 10.82%; $[\alpha]_D^{20}$, −11.3 (*c* 4, water). Calcd.: N, 10.85%.

Procedure.—L-Glutamic acid, L-pyroglutamic acid and water were mixed in a glass tube with a spiral stirrer. The tube was placed for at least six hours in a water bath regulated at 20°C, 30°C, or 50°C. The temperature fluctuation of the bath was within ±0.1°C. An aliquot was taken with a cotton-stoppered pipet (warmed when necessary), and the concentration of L-glutamic acid in the filtrate was determined by the Warburg method with a decarboxylase from squash. The L-pyroglutamic acid content was determined as the difference between the total nitrogen determined by the Kjeldahl method and the amino nitrogen as calculated from the L-glutamic acid content. The solid-phase constitution was examined by the X-ray powder diffraction method. The equilibrium was sometimes reached from the under-saturation side, and sometimes from the supersaturation side.

Results.—The results of the solubility measurements are tabulated in Table II and are given graphically in Figs. 2, 3 and 4. The solubility of L-glutamic acid is somewhat different according to whether the solid phase is α-form glutamic acid or β-form glutamic acid.[6] It has been reported by Sakata[7] that L-glutamic acid in the α-form as a solid phase in a saturated aqueous solution gradually transforms into β-form, and that some amino acids have an inhibitory action on this transition. The present experiment showed

5) H. Takahashi, T. Takenishi and N. Nagashima, This Bulletin, **35**, 923 (1962).
6) Y. Sakata, *Agr. Biol. Chem.*, **25**, 835 (1961).
7) Y. Sakata, ibid., **25**, 829 (1961).

that L-glutamic acid tends to be crystallized in the α-form from the supersaturated solution in the presence of L-pyroglutamic acid, and that such an α-form crystal does not readily transform into the β-form in the solution.

As may be seen in Figs. 2, 3, and 4, the isotherm at 20°C shows a clear stable zone of the molecular compound, only a short stable zone at 30°C, and no stable zone, only a metastable zone, at 50°C. In the metastable zone, however, the molecular compound is easily crystallized by the cooling of the saturated solution at an elevated temperature.

The molecular compound was obtained only from the supersaturated side in the temperature range examined.

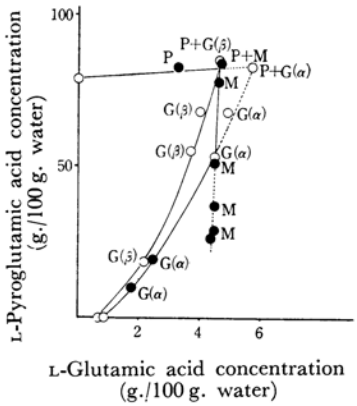

Fig. 2. Composition of the liquid phase in the system of L-glutamic acid, L-pyroglutamic acid and water at 20°C.
○ means the data from unsaturation side, and ● from supersaturation side. For each point observed, the constitution of the solid phase in equilibrium with the liquid phase in question is shown by P, M, G(α), and (β). The notations are the same as in Table II.

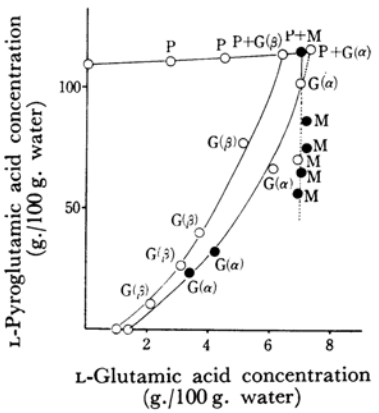

Fig. 3. Composition of the liquid phase and the constitution of the solid phase in the system of L-glutamic acid, L-pyroglutamic acid and water at 30°C. The notations are the same as in Table II.

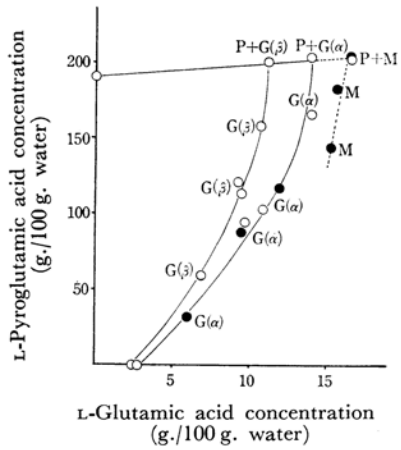

Fig. 4. Composition of the liquid phase and the constitution of the solid phase in the system of L-glutamic acid, L-pyroglutamic acid and water at 50°C. The notations are the same as in Table II.

Since this molecular compound has an incongruent solubility, it is decomposed by water, and at the same time a precipitation of L-glutamic acid takes place.

At each temperature three invariant points can be determined: at one of them, the solid phase is composed of both L-pyroglutamic acid and α-form L-glutamic acid; at the second point, the solid phase is composed of both L-pyroglutamic acid and β-form L-glutamic acid; and at the third point, the solid phase is composed of L-pyroglutamic acid and the molecular compound. The logarithm of the L-glutamic acid concentrations at the invariant points are plotted against the temperature in Fig. 5. Here three straight lines are obtained. The intersecting points of these lines give the transition temperatures from the molecular compound to the mixtures of the two acids. Thus the transition temperature from the molecular compound to L-glutamic acid in the α-form and L-pyroglutamic acid is about

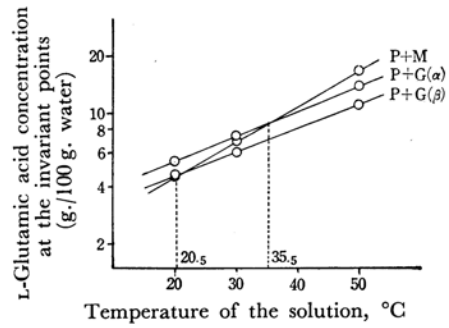

Fig. 5. Estimation of the transition points by three different invariant points.
The notations of the solid phase are the same as in Table II.

Table II. Equilibrium in the system of L-glutamic acid, L-pyroglutamic acid and water

U: from unsaturated side S: from supersaturated side
P: L-pyroglutamic acid M: the molecular compound
G(α): α-form L-glutamic acid G(β): β-form L-glutamic acid

Temp. °C	Method	Liquid phase composition (g. per 100 g. water) P	G	Solid phase composition
20	U	79	0	P
	U	82	2.8	P
	S	83	3.3	P
	S	84	4.7	P+M
	U	83	5.7	P+G(α), metastable
	S	78	4.6	M
	S	51	4.5	M, metastable
	S	37	4.5	M, metastable
	S	29	4.5	M, metastable
	S	26	4.4	M, metastable
	U	68	4.9	G(α), metastable
	U	53	4.5	G(α)
	S	19	2.5	G(α)
	S	10	1.8	G(α)
	U	0	0.94	G(α)
	U	85	4.6	P+G(β)
	U	68	4.0	G(β)
	U	55	3.7	G(β)
	U	18	2.2	G(β)
	U	0	0.71	G(β)
30	U	109	0	P
	U	111	2.7	P
	U	112	4.5	P
	S	115	7.0	P+M
	U	116	7.3	P+G(α), metastable
	S	86	7.2	M, metastable
	S	74	7.2	M, metastable
	U	70	6.9	M, metastable
	S	65	7.0	M, metastable
	S	56	6.9	M, metastable
	U	102	7.0	G(α), metastable
	U	66	6.1	G(α)
	S	32	4.2	G(α)
	S	23	3.4	G(α)
	U	0	1.4	G(α)
	U	114	6.4	P+G(β)
	U	77	5.1	G(β)
	U	40	3.7	G(β)
	U	26	3.1	G(β)
	U	9.9	2.1	G(β)
	U	0	1.0	G(β)
50	U	191	0	P
	U	204	14.0	P+G(α)
	S	203	16.5	P+M, metastable
	U	220	16.5	P+M, metastable
	S	183	15.7	M, metastable
	S	144	15.3	M, metastable
	U	165	14.0	G(α)
	S	117	11.9	G(α)
	U	103	10.9	G(α)
	U	94	9.7	G(α)
	S	88	9.5	G(α)
	S	31	6.0	G(α)
	U	0	2.8	G(α)
	U	201	11.2	P+G(β)
	U	159	10.7	G(β)
	U	121	9.3	G(β)
	U	113	9.5	G(β)
	U	59	6.9	G(β)
	U	0	2.4	G(β)

35.5°C, and the transition temperature from the molecular compound to L-glutamic acid in the β-form and L-pyroglutamic acid is about 20.5°C. The molecular compound is stable below this temperature.

The Optical Resolution of DL-Glutamic Acid by the Use of the Molecular Compound[8]

A similar molecular compound which consists of D-glutamic acid, D-pyroglutamic acid and water was also ascertained to be formed. It was found, however, that no molecular compound which consists of L-glutamic acid and D-pyroglutamic acid or vice versa can be obtained. It may be expected that, taking advantage of these facts, one can resolve the racemic form of glutamic acid or pyroglutamic acid. Such an optical resolution is actually possible for glutamic acid, but it is not for pyroglutamic acid.

Materials.—The racemic glutamic acid monohydrate and racemic pyroglutamic acid were presented by Dr. T. Akashi.* The results of the analysis of these two materials were as follows:

DL-glutamic acid monohydrate:
Found: N, 8.49%; $[\alpha]_D^{20}$, ±0.00 (*c* 8, 2 N HCl). Calcd.: N, 8.48%.

DL-pyroglutamic acid:
Found: N, 10.84%; $[\alpha]_D^{20}$, ±0.00 (*c* 4, water). Calcd.: N, 10.85%.

8) Japanese Pat. 412634 (Japanese Publ. 8311/1963).

* Chief Chemist in the Central Research Laboratories of Ajinomoto Co., Inc.

Procedure and Results.—I) A mixture of 13.6 g. of DL-glutamic acid mono-hydrate and 54.5 g. of L-pyroglutamic acid in 60 g. of water was heated until it dissolved at 80°C; then the solution was cooled at 20°C under stirring, with a slight seeding of the molecular compound between L-glutamic acid and L-pyroglutamic acid. After 4 hr. the precipitated crystals were filtered out. In the analysis of the crystals and the mother liquor, the method of amino-nitrogen determination in the ninhydrin method was adapted, as well as the two methods of analysis in the solubility measurements described above. The total concentration of optically active and inactive glutamic acid was determined by the amino-nitrogen measurements. The concentration of L-glutamic acid was determined by the enzymatic method.

The crystals were weighed (8.6 g.) and were found to be 68% the molecular compound (L-glutamic acid, L-pyroglutamic acid and water), 17% DL-glutamic acid monohydrate, and 10% L-pyroglutamic acid. The mother liquor was also weighed (103 g.); its concentration was as follows; DL-glutamic acid 4.0%, D-glutamic acid 2.6%, L-pyroglutamic acid 44%.

II) Into a mixture of 10.1 g. of DL-glutamic acid mono-hydrate and 24 g. of L-pyroglutamic acid in 31 g. of 5% hydrochloric acid, about 6 g. of 15% caustic soda, including 1.8 g. of L-pyroglutamic acid, was stirred. By cooling at 20°C with a slight seeding of the molecular compound (L-glutamic acid, L-pyroglutamic acid and water), 2 g. of the crystals were obtained after 1 hr. The crystals were 90% the molecular compound (L-glutamic acid, L-pyroglutamic acid and water) and 10% L-pyroglutamic acid. L-Glutamic acid was obtained from the decomposition of these crystals by water.

III) A mixture of 10 g. of DL-pyroglutamic acid and 2.5 g. of L-glutamic acid in 100 g. of water was cooled in an ice box. After 5 days the crystals precipitated were examined by the X-ray powder diffraction method; they were thus shown to be a mixture of the two acids, with none of the molecular compound present.

An Anhydrous Molecular Compound

A thermal analysis of the above-described molecular compound has been made. It was shwon that the compound is converted into another molecular compound of L-glutamic acid and L-pyroglutamic acid (1 : 1) at about 70°C; then it is decomposed to L-glutamic acid (β-form) and L-pyroglutamic acid at about 90°C. This transformation was shown in the differential thermal analysis curve, and in the X-ray diffraction patterns and the IR spectra of the specimens of all the stages. The results of the elementary analysis of the latter molecular compound were:

Found: C, 43.67; H, 5.95; N, 10.18. Calcd.: C, 43.4; H, 5.84; N, 10.14%.

This anhydrous molecular compound could not be obtained from the aqueous solution. The X-ray powder diffraction data are shown in Table I.

We wish to express our thanks to Professor Masamichi Tsuboi of the University of Tokyo for reading the manuscript of this paper, and also to Drs. Haruomi Oeda, Shinichi Motozaki, Mei Ohno and Tadao Takenishi in the Central Research Laboratories, Ajinomoto Co., Inc., for their encouragement.