Journal of Thermal Analysis, Vol. 48 (1997) 1385-1392

POLYMORPHISM OF (+)N-TOSYL-*L*-GLUTAMIC ACID

H. Hajmowicz, L. Synoradzki, K. Bujnowski and J. Zachara

Warsaw University of Technology, Faculty of Chemistry, Laboratory of Technological Processes, ul. Noakowskiago 3, 00–664 Warsaw, Poland

(Received September 29, 1995; in revised form April 2, 1996)

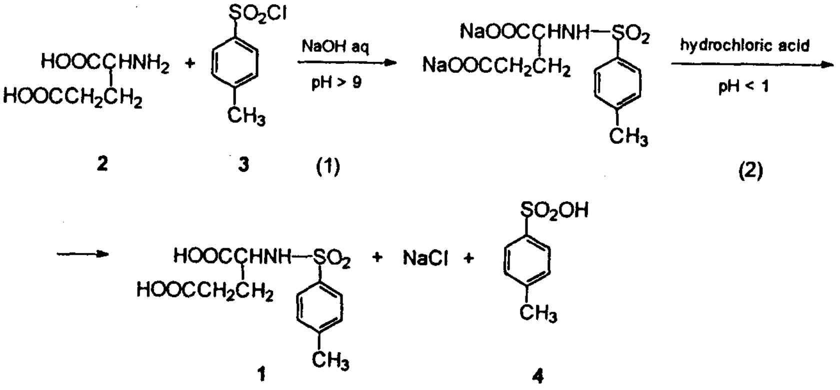
Abstract

It has been shown that polymorphism is the reason for the occurrence of (+)N-tosyl-*L*-glutamic acid **1** with various melting points. **1** occurs in two crystalline forms: α and β. Form α-**1** (prisms) having a melting point of 145–147°C is chemically pure and stable. Form β-**1**, however, is unstable and is formed as a result of the stabilizing effect of an organic solvent not introduced into the structure of the crystal. At about 125°C the β forms is transformed to the α form. The melting point of the β form depends on the amount and type of solvent contained in the crystal, which, during measurement cannot leave the system.

Keywords: crystallization, melting point, N-tosyl-*L*-glutamic acid, polymorphism, thermogravimetric analysis, X-ray powder diffraction

Introduction

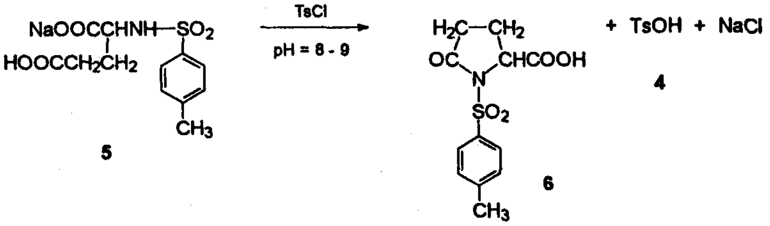
1 is used for the separation of racemates into enantiomers. Its synthesis has been known for a long time as a step in the preparation of *L*-glutamine and is carried out in order to protect the amino group with the relatively easily leaving tosyl group. *L*-Glutamic acid **2** reacts in aqueous alkali solution with *p*-toulenesulfonic chloride **3** applied in reaction (1) in the form of a solution in an organic solvent. After acidification of the reaction mixture with hydrochloric acid **1** crystallizes from the reaction (2) medium.



0368–4466/97/ $ 5.00

John Wiley & Sons, Limited
Chichester

In the literature different forms and melting points are reported for **1**: 115–117°C needles (water) [1], 124–126°C [2], 127.5–128.5°C [3], 130–132°C [4], 131°C prisms (ethyl acetate) [5], 134°C [6], 135°C needles (alcohol+water) [7], 143–145°C [8], 145–146°C (water) [9]. The differences in melting points are explained as resulting from various impurities present in the products [9]. Insufficient acidification (*pH*>1) during the isolation of **1** (2) causes co-precipitation of monosodium N-tosylglutaminate hydrate **5** (*mp*=115–117°C). Insufficient removal of the by-product formed – *p*-toluenesulfonic acid **4**, and eventually of products of other side reactions, may be another source of impurities. The sodium salt **5** can react with **3** involving water abstraction and cyclization to γ-lactam of N-tosyl-2-pyrrolidone-5-carboxylic acid **6** (3). **6** crystallizes from an aqueous medium in the form of the monohydrate (*mp*=67–70°C).



Reaction (1) has been studied by controlling in the reaction mixture the contents of substrates **2** and **3**, possible intermediates **4** and **6**, and the main product **1** (TLC, test with ninhydrin). In a correctly performed process (*pH*=9–10) no **6** was observed (at *pH*>9 it undergoes hydrolysis to **1**). Under optimum conditions, **1** was obtained in ca. 98% purity (TLC), but with different melting points.

Experimental

Thermal analyses were performed with a derivatograph produced by MOM, Budapest. Samples of salts (about 0.15 g) were put in platinum crucibles and placed in a quartz envelope-flushed with a constant flow of purified nitrogen. The T, TG, DTG and DTA curves were recorded at a heating rate of 9°C min^{-1}. X-ray analyses were performed by means of the powder method using a HZG-4 bicircular diffractometer and CuK$_\alpha$ radiation. The infrared spectra of samples in KBr pellets were recorded with a Perkin-Elmer 577 spectrophotometer over the 4000–200 cm^{-1} range. The organic solvent was identified using a Hewlett Packard GC/MS apparatus. Melting points were determined on a capillary and microscopic plate (Böthius) apparatus and are uncorrected.

Crystallization of α*-1*

Crude **1** (containing ca. 5% NaCl) was dissolved in water, and then the rest of the solvent was distilled off under reduced pressure. The aqueous solution (10%) was filtered through a sintered plate at 60°C. At 50°C a crystallization seed of α-**1** was added, and then the mixture was slowly cooled (5°C h^{-1}) to 0°C with occasional stirring. A white macrocrystalline precipitate was isolated and dried at 20°C in air.

Crystallization of β-1

1 (containing ca. 5% NaCl) was dissolved in water, and then the rest of the solvent was distilled off under reduced pressure. The aqueous solution (10%) was filtered through a sintered plate at 60°C. At 50°C toluene was added (5% with respect to water), and then the mixture was quickly cooled (25°C h^{-1}) to 0°C. A white fine crystalline precipitate was isolated and dried at 20°C in air. The same procedure was applied in experiments with addition of other solvents.

Results and discussion

It was found that **1** can be obtained in the form of prisms or needles depending on the crystallization and drying conditions. The importance of using mild conditions in drying the crystals obtained from aqueous crystallization systems should be noted. Even at a small (10%) water content in the precipitate, already at a small rise in temperature (40°C) the precipitate deliquesces. This phenomenon is connected

Table 1 *X*-ray powder diffraction patterns of forms α- and β-**1**

α-**1**				β-**1**			
d/Å	I/I_0	d/Å	I/I_0	d/Å	I/I_0	d/Å	I/I_0
8.99	12	3.773	3	6.97	10	3.132	4
7.98	2	3.705	5	6.93	49	3.060	10
7.68	18	3.587	20	6.14	11	3.000	4
7.42	56	3.546	2	5.82	3	2.920	4
6.45	12	3.520	1	5.27	24	2.800	3
6.03	17	3.469	2	5.07	100	2.787	5
5.91	8	3.445	<1	4.740	15	2.732	5
5.138	4	3.366	3	4.328	24		
5.126	4	3.225	8	4.206	12		
4.896	4	3.169	12	4.183	12		
4.827	24	3.123	3	3.979	4		
4.666	5	3.083	5	3.684	36		
4.466	49	2.998	14	3.654	13		
4.332	3	2.995	1	3.523	4		
4.250	6	2.921	1	3.480	4		
4.122	22	2.877	2	3.448	6		
4.047	100	2.847	14	3.302	8		
3.965	4	2.809	2	3.200	3		
3.931	47	2.800	4	3.182	3		

with the solubility of **1** in water, which increases rapidly with rising temperature. When, in the final drying period of drying of **1**, the temperature was increased and the pressure decreased too rapidly, a mixture of prisms and needles was often obtained in a ratio depending on the crystallization and drying conditions.

X-ray powder diffraction analysis of both crystalline forms of **1** (prisms and needles) carried out by means of CuK_α radiation revealed that they are indeed two different crystalline forms (α and β) with different crystalline structures of relatively low symmetry (Table 1).

The optimum conditions of crystallization from water of the α and β phases have been determined (Table 2). Samples of **1** were dried after crystallization in air at room temperature for 24 h. Near infrared spectra (NIR in ethyl acetate solution) have shown that the α- and β-forms dried in this manner do not contain water.

Table 2 Conditions of crystallization from water of the two crystalline forms of **1** (concentration 10%, temperature 50–0°C)

	α-**1** (prisms)	β-**1** (needles)
Crystallization system	water	water and organic solvent[a]
Stirring	periodic	continuous
Cooling rate	$5°C\ h^{-1}$	$25°C\ h^{-1}$
Crystallization time	8–15 h	0.5–2 h

[a] The solvents used (toluene, benzene, ethyl acetate, chloroform, ethyl ether) do not mix with water. 1 does not dissolve in these solvents or dissolves slightly

The α form crystallizes from aqueous solution practically only after inoculation and very slowly. Large, well shaped crystals (*mp* 145–147°C) are obtained, the structure of which was studied by X-ray analysis and will be described separately. If an organic solvent (toluene, benzene, ethyl acetate, chloroform, ethyl ether) is added to an aqueous solution of **1** in an amount of 0.1 wt.%, then the β form immediately precipitates as a very fine solid substance. The degree of crystallite development which was found on the basis of the half-width of reflexions in the powder diffraction patterns depends on the type of solvent added.

Crystals of β-**1** exhibit very strong anisotropy. Despite the changes in the crystallization conditions the thickness of the needles of individual monocrystals does not exceed 0.001 mm at a length reaching a few cm. Attempts to grow a crystal suitable for X-ray studies failed.

Thermogravimetric analysis of α-**1** and β-**1** was carried out in the temperature range 20–150°C (heating rate $3°C\ min^{-1}$, nitrogen atmosphere) (Fig. 1). α-**1** does not show any change in mass when heated to the melting point. The only thermal effect (endothermic) occurs at the melting point. During the heating of β-**1** slight mass losses are observed, occurring in different temperature ranges, depending on the nature of the organic solvent used as an additive in the crystallization. At ca. 125°C a small endothermic effect is observed and then a considerable effect at 145–147°C corresponding to melting. It has been proved that the effect at ca. 125°C

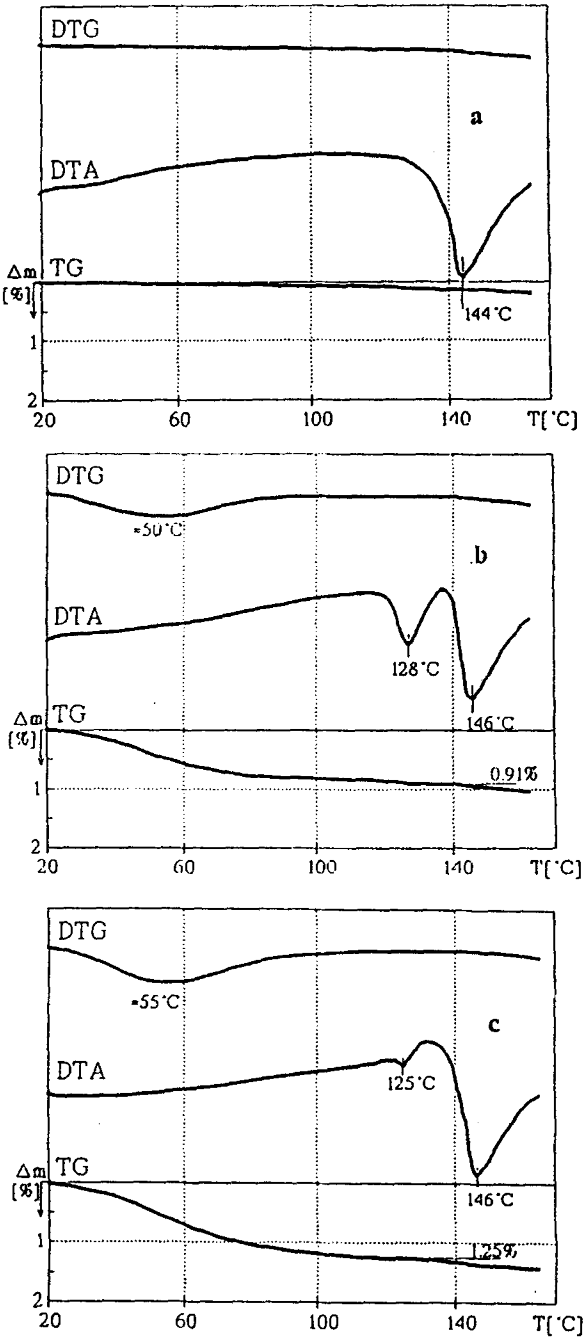


Fig. 1 TG, DTG and DTA curves of (+)N-tosyl-*L*-glutamic acid **1**. 1a) α-**1**, 1b) β-**1** (ethyl acetate), 1c) β-**1** (ethyl ether), 1d) β-**1** (toluene) – which corresponds to samples no. 1, 4, 6, 2, respectively, in Table 3

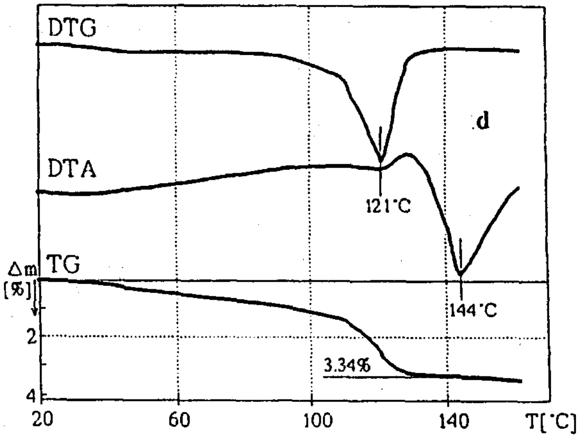


Fig. 1 Continued

results from the β-1 to α-1 phase transformation. The X-ray powder diffraction pattern of sample β-1 (ethyl ether) subjected to drying at 80°C under 50 hPa (and thus completely free of ether – TG) (Table 3) did not exhibit changes with respect to the sample prior to drying. However, after raising the temperature of drying to 120–125°C a change in crystalline form was observed. A weakly developed form α-1 was then obtained. In the TG curve of sample β-1 (toluene) a maximum in the rate of mass loss was observed at ca. 125°C and then a broad thermal effect, resulting probably from phase transformation and melting. The X-ray powder diffraction pattern of a β-1 sample (toluene) dried within the range 110–120°C under 50 hPa and completely free of toluene is characteristic of the weakly developed form α-1. The endothermic effects observed in the DTA curves at 125°C correspond to the β-1 to α-1 polymorphic transformation.

The presence of the organic solvent used as an additive in the crystallization process was confirmed by heating the β-1 samples and determining the solvent content in the air above the precipitate or by washing with another solvent, in which **1** does not dissolve, and determining their content in the washing solvent (GC/MS) (Table 3). The solvent content in β-1 varies (0.7–3%) (Table 3). The ratio of the number of moles contained in the crystals does not exhibit regularities. These facts permit the suggestion that the solvent plays only the role of a surface stabilizer and is not an element of the structure.

The melting point of β-1 depends on the nature and amount of the organic solvent contained in the sample. Depending on the volatility of the solvent and strength of interactions with the surface of the crystal, melting may proceed in a broad or narrow temperature range which may be 20°C lower than of α-1 (Table 3). Thus, **1** has only one melting point, corresponding to form α-1 145–147°C.

The properties of the compound occurring in both crystalline forms have been studied. It was found that the compound:

– is chromatographically pure (TLC) and exhibits the same retention time,

– has an identical absorption infrared spectrum in solution (THF, one intense band of the carbonyl group at 1725 cm^{-1}),

– has a specific rotation $[\alpha]^{20} = +14 \pm 0.5^\circ$ ($c=5$, acetone),

– has the same chemical composition close to theoretical ($C_{12}H_{15}NO_6S$):
calculated: 47.83% C, 5.01% H, 4.65% N, 10.64% S
α-**1**: 48.20% C, 5.22% H, 4.66% N, 10.88% S
β-**1**: 48.08% C, 5.23% H, 4.34% N, 10.46% S

In the infrared spectra of α-**1** and β-**1** crystals (IR in KBr) differences are observed in the absorption bands characteristic of the carboxylic group. In the spectrum of α-**1** the O–H absorption band (stretching) occurs at 3300 cm^{-1}, and two bands (asymmetric doublet) corresponding to C=O vibrations (stretching) occur at 1720 cm^{-1} and that of greater intensity at 1700 cm^{-1}. However, in the spectrum of β-**1** the O–H absorption band occurs, depending on the solvent, at 1720–1710 cm^{-1}. These shifts indicate the occurrence of various intermolecular interactions resulting from the different surroundings of molecules in the crystalline structures of both forms (Table 3).

Table 3 Properties of crystalline forms

No.	Preparation method[a]	X-ray form of **1**	*mp*/°C	IR (KBr)/cm^{-1}		Mass loss/		Approximate range of mass loss/oC
				O–H	C=O	Identification GC/MS	% TG	
1	Water	α	145–147	3300	1720 1700	no mass loss	–	
2	Water + toluene	β	128–130	3340	1720	toluene	3.80	45–125
3	Water + benzene	β	130–132 143–146	3310	1710	benzene	0.72	35–75
4	Water + ethyl acetate	β	129–130	3315	1710	ethyl acetate[b]	0.84	30–90
5	Water + chloroform	β	144–146	3315	1710	chloroform	3.51	30–100
6	Water + ethyl ether	β	131 146–147,	3310	1715	ethyl ether	1.30	30–100
7	β-KTG (toluene)[c]	α	145–147	3280	1710 1690			
8	β-KTG (ethyl ether)[d]	β	145–147					
9	β-KTG (ethyl ether)[e]	α	145–147					

[a] Samples no. 1–7: crystallization, [b] washed with toluene (without dissolution of **1**), since it was not detected in the air above the precipitate, as in the case of the other samples, [c] dried at 120°C and 50 hPa, [d] dried at 80°C and 50 hPa, [e] dried at 120°C and 50 hPa

Conclusions

It has been proved that the existence of 1 in two polymorphic forms is the reason for the different melting points measured. Only form α-**1** of *mp* 145–147°C is chemically pure and stable. Form β-**1**, however, is surface stabilized with an organic solvent. The melting point of this crystalline form depends on the type of the organic solvent. Depending on its amount, volatility and strength of interactions with the surface of the crystal, the melting point can vary from 120 to 147°C.

References

1 P. Bergell, Z. Physiol. Chem., 104 (1994) 182.
2 K. Sakota, K. Koine and O. Okita, Nippon Kagaku Zasshi, 90 (1969) 77.
3 F. Goodman, Biochemistry, 3 (1964) 1529.
4 P. Badyopadhyay, Indian J. Chem. Sect. A, 19 (1980) 10, 920.
5 C. R. Harington and R. C. Moggridge, J. Chem. Soc., (1940) 706.
6 N. M. Nandi, J. Choudhury and S. Tarat, Indian J. Chem. Sect. A, 29 (1990) 3, 288.
7 E. Knoop and H. Oesterlin, Z. Physiol. Chem., 170 (1927) 205.
8 J. Rudinger and K. Poduska, Collect. Czech. Chem. Commun., 24 (1959) 2013.
9 J. Rudinger, Collect. Czech. Chem. Commun., 19 (1954) 366.