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Intramolecular dehydration of L-glutamic acid

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A number of various thermal dehydrations of L-glutamic acid were performed in order to obtain crude L-pyroglutamic acid. Some old procedures and some new ones were investigated. Also, a great deal of work was done on the purfication of crude L-pyroglutamic acid. We can say that among other very good procedures for synthesis and purification we can count our own, modified synthesis in aqueous solution of L-glutamic acid (acid: water = 1:3) and recrystallization from ethanol. The most efficient purification was carried out on ion-exchange resins (Dowex, Amberlite). Some new systems for TLC of L-glutamic acid and L-pyroglutamic acid were also examined.

Pyroglutamic acid (PGA), also termed pyrrolidon carboxylic acid or 5-oxo--L-proline. It can be formed enzymatically (as an intermediate in amino acid metabolic and transport pathways) or during protein biosynthe sis (where *it* becomes the amino-terminal residue of many biologically significant peptides and proteins). It can also be obtained by heating glutamic acid (Glu) by the process of dehydration. Structurally, it may by considered to be internally cyclized Glu which will be utilized here. In addition, the presence of the internal amide bond which forms between nitrogen-I and carbon-5, produces unique properties for this common amino: acid, and chemically defines PGA as 2-carboxy-y-butyrolactam.

Scheme 1. The dehydration of Glu into PGA

The internal linkage is neutral and functionally acts as an amide. The basic electron pair of nitrogen-I is in resonance, attracted toward carbon-5 by the presence of the double-bonded oxygen. Scheme I presents the reaction of dehy dration Glu into PGA.

The main procedure for producing a large amount of PGA even in the 19th century was to heat Glu in an aqueous solution. Haitinger2 was the first to obtain PGA in 1882, but the real report on it came from Anderlini 3 in 1889. Menozzi4 showed the structure of PGA in 1892. After them, many workers came with their reports: Abderhalden and Kauttzsch5 in 1910, Skola6 in 1920, Okinaka? in 1921 and Foremans in 1928. Wilson and Cannan9 showed that the dehydration reaction increased in extreme pH and that balance was going right, producing more PGA. PGA was also synthesized by P.M. Hardyi o by heating Glu and then introducing the solution to ion-exchange resins. Ohme and Berger !ı were also able to produce PGA in large quantities by heating a concentrated solution of Glu for 42 h. Besides that PGA was obtained by heating an aqueous solution of Glu at 95 *C C* for 168 h. 12

What should be noticed about all these procedures is that they do not include the use of any kind of catalyst and that many of them give a high percentage of PGA.

In addition, we can mention some procedures without water or with the use of various catalysts. PGA was obtained by heating pure solid Glu at 190-197 °cu or from an aqueous solution with $Me_2N\,CH_2CH_20H14$ as a catalyst. Some other authors have used metals {Al, Mg).I:

Conversion can be successfuly achieved even in organic solvents,16 For example, Blade-Fontl 7 has produced PGA in toluene. Other reportsl S-23 can also be mentioned.

PGA is also produced by some insects which can be used to obtain pharmaceutically pure PGA.

PGA may by its presence as an amino terminal acid on a protein or peptide enhance or be responsible for the biological function or activity of the same. An example is the tripeptide thyrotropin releasing factor which has the sequence PGA--His-Pro.1 Using PGA for pharmaceutical purposes is necessary not only to obtain it in high percentages but also to get it pure enough for such use.

Accordingly, due to the shortcomings of the aforementioned procedure, a new procedure is needed to synthesize PGA at high yield and lower cost.

An investigation of various procedures for the thermal intramolecular dehydration of Glu as well as the purification of the obtained crude PGA, was undertaken in order to obtain higher purity. The present paper compares some previously known procedures and our new method.

EXPERIMENTAL

All the chemicals used were of p. a. purity grades.

TR spectra were recorded on a Perkin-Elmer infrared spectrophotometer, model 580 in the form of KBr pellets.

Mass spectra were obtained on a Varian Matt 311 A mass spectrometer, using a direct probe and 70 eV ionizing energy.

Melting points were taken on an Electrothermal melting point apparatus and were not corrected.

All the synthetized compounds had very high purity (TLC) and satisfactory C, H, N-analyses.

Thin layer chromatography 24 (TLC, *Rr* values) was used for the detection of row and purified products. Eight different systems for elu ation were analyzed and two of them were choosen for use. TLC was performed on glass plates coated with Silica C (size 2.5 x 10.5 cm and thickness I mm, and 6 x 20 cm, 0.25 *mm).* The best solvent systems were 11-propanol : water (7 :3J and 11-butanol: acetic acid :water (4 :I ; 1). Ninhydrin and an iodine-starch solution were used for the detection of spots. The observed *Rr* v1lues in given systems for Glu and PGA are given in Table Ґ.

TABLE I. Rr values for Glu and PGA in given systems

o.	System	Developing time/distance (minute) (cm)	*Rr* Glu	Rr PGA
I.	11-Propanol:water (7:3)	180/15	0.37	0.58
2.	11-Butanol:aceticacid: :water (4:1:I)	180/15	0.37	0.61

RESULTS AND DJSCUSSION

As mentioned above, dehydration and purification were performed by various methods. Methods and results are given in Table IT.

TABLE II. Methods and row products characteristics24

Meth-od	Weight of Glu (grams)	Volume of water (cm3)	Roaction time (hours)	Woight of	Yield (%)	Melting point (°C)	Rr* PGA
1.	5	6.1	30	3.9	78	132-135	0.55
2.	a 8	32	15	5.6	70	142-144	0.62
	b 15	60	15	11.25	75	145-148	0.65
3.	a 10	40	15	7.3	73	142-146	0.65
	b 15	60	15	10.65	71	140-142	0.66
4.	16+ 12.5	25	42	24.9	87.4	135-140	0.62
5.	10	30	18	8.15	81.5	145-150	0.70

* System 11-butanol: acetic acid : water (4:1:1)

Method 1. Our modified process of the known Okinaka7 procedure: Glu and water (1 : 10) were heated under reflux with stirring in an oil bath (t=l 50±5 °C, *n=300* min-1) for 30 hours. 20 cm3 of water was then added and heating continued for 2 hours. After cooling, the solution was run through a column filled with Dowex 50 WX 8 (20/50 mesh) resin and evaporated under vacuum at 50 °C.

Methods 2 and 3 represent the procedure by Hardy. They folllowed the Ref. JO, except for the resins. In method 2 we used Dowex 50 WX 8 (20/50 mesh) and in method 3 the resin was Amberlyte 15.

The procedure of method 4 proceeded in the same manner as in the previously mentioned patent by Ohme and Berger. ll

Method 5. This is our process.24 Glu and water (10 g in 30 rm3) were heated under reflux for 18 h. After heating, 2/3 of the added water was removed by distillation and crystallization was performed.

The presented data indicate that the desjred PGA was obtained in the greatest yield using the 4th method. Moreover the advantage of this method is the simplicity of the experimental proredures and the very good purity of the product.

After these very good results in yield and unsatisfactory in purity, different purification procedures were used to obtain PGA of higher purity. These results are shown in Table III.

TABLE HI. Cham teri ti of the purified PGAH

Crude PGA (method)	Purification	YieJdb (%)	Melting point ("C)	RrC PGA	[IX] 2od
2a.	E	53.7	156-158	0.59	-11.56
2b.	E+ P	34.3	156-157	0.65	-11.56
3a.	E	40.5	157	0.63	-11.25
Jb.	E+P	38.0	156	0.66	-I1.56
4.	E	51.0	158	0.68	-t1.56
5.	E	56.25	157-158	0.65	-11.56

a) The letters indicate solvent (E=95% ethanol, P= petrolether).
b) Calculated on Glu from Table II.
c) System: 11-butanol-acetic acid-water (4:1 :1).
d) c= 20; 1= 20 mm, water.

As can be seen from Tables II and JU, very good results in yield were obtained. The best one was in method 4, then in our modified method 5. After purification, which was carried out in ethanol, our method gave the best yield with also good other characteristics.

We also tried to examine the kinetics of this dehydration reaction using colorimetric and polarimetric methods. For the colorimetric method we used the reaction of amides wit h p-d imethyl-amino-benzaldehyde but some problems appeared. First, the color was unstable, and second, we could not be sure that the concentration we had in the samples was the same as in the reaction vessel because the solubilities of Glu and PGA were very different in water.26

The colorimetric procedure2 7 is characteristic for amino acids with pirolidon moiety. Concentration posed a problem in the polarimetric method, too. The problem was for sure in the equipment and maybe in the solubility.

Our results fit the equation for the first order rate lo' but we cannot prove for sure. Further investigation is needed.

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