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THERMAL ISOMERIZATION OF *N*-OXALYL DERIVATIVES OF DIAMINO ACIDS

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Key Word Index—*Lathyrus sativus*; α- and β-*N*-oxalyl-α,β-diaminopropionic acids; α-,γ-*N*-oxalyl-α,γ-diaminobutyric acids; δ-*N*-oxalylornithine; ε-*N*-oxalyllysine; neurotoxins; thermal isomerization.

Abstract—The neurotoxic constituent of the legume *Lathyrus sativus*, β-*N*-oxalyl-α,β-diaminopropionic acid, was thermally isomerized to an equilibrium mixture (60/40) containing the non-toxic α-*N*-oxalyl-α,β-diaminopropionic acid. The same equilibrium mixture was established by starting with the α-isomer but required longer time. α- and γ-*N*-Oxalyl-α,γ-diaminobutyric acids also underwent thermally induced isomerization with α-γ, or γ-α migration of the oxalyl group. δ-*N*-Oxalylornithine and ε-*N*-oxalyllysine did not isomerize under these conditions. The observation that the higher homologues do not undergo isomerization suggests the intramolecular nature of the reaction.

INTRODUCTION

The human neurological disorder resulting from excess consumption of the seeds of grass pea (*Lathyrus sativus*) is believed to be caused by the non-protein amino acid β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP, also called β-*N*-oxalylamino-L-alanine, BOAA, **1**) [1]. β-ODAP was first identified in the legume simultaneously by two different groups [2, 3] in 1964. γ-*N*-Oxalyl-α,γ-diaminobutyric acid (**2**) is the *N*-oxalyl derivative of the neurotoxic amino acid, α,γ-diaminobutyric acid (DAB), found in *L. sylvestris* and *L. latifolius* [4]. As early as 1966, Bell and O'Donovan reported that in ethanolic solution β-ODAP slowly equilibrates with the α-isomer (**5**), and that these substances interconvert more readily when heated [5]. Subsequently it was shown that the α-isomer is not acutely toxic to one-day-old chicks or to neonatal mice [6, 7] or to rat spinal cord *in vivo* [8]. The compound does not interact with receptors mediating excitatory glutamate responses in the central nervous system [9], nor is it a glial toxin as is the β-isomer [10]. The non-toxic nature of the α-isomer, and the observation that the β-isomer which is present in the seed may be isomerized to the non-toxic form, opens opportunities to explore various processing and cooking methods as a means of thermal detoxification of the legume. In 1990 it was reported that the thermal isomerization of β-ODAP, in deutrated water, to a mixture of the two isomers, can be followed conveniently by NMR spectroscopy [11]. We have now conducted more investigations of the isomerization of α- and β-ODAP (**5** and **1**), α-, and γ-oxalyl α,γ-diaminobutyric acid (α- and γ-ODAB) (**6** and **2**), and the oxalyl derivatives of ornithine (δ-OORN, **3**) and lysine (ε-OLYS, **4**).

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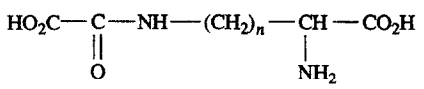
RESULTS AND DISCUSSION

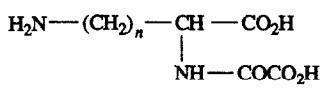
Isomerization of α- and β-ODAP

β-ODAP underwent a smooth transformation to an equilibrium mixture of β-ODAP and α-ODAP (ratio = 3:2) in *ca* 30 hr. The spectrum recorded immediately after heat assisted dissolution of β-ODAP in D_2O was free of α-ODAP, which indicated that the formation of the α-isomer is a fairly slow process. However, after 8 hr at 55° the ^1H NMR spectrum indicated the presence of *ca* 20% of the α-isomer.

Approximately 20–30 hr were needed before the equilibrium was established; the ratio of the two isomers did not change up to 60 hr. Pure α-ODAP in D_2O isomerized to a mixture of the two isomers under the same conditions, but the reaction took a much longer time (up to 100 hr) to reach the same equilibrium ratio. At 55° isomerization of β-ODAP was further accompanied by the formation of small amounts of α,β-diaminopropionic acid (DAP), presumably resulting from hydrolysis of the oxalyl compound, which was not unexpected at pH 2.3. However, maintaining an aqueous solution of β-ODAP at room temperature (approx 22°) for one week led to the formation of a mixture containing only 29% α (71% β) and no trace of α,β-diaminopropionic acid was detected. Heating a solution of the sodium salt of β-ODAP (pH 6.6) at 55° gradually led to the formation of the α-isomer. After 40 hr the α-isomer was only *ca* 10%, and after 96 hr



1 $n=1$ β-ODAP
2 $n=2$ γ-ODAB
3 $n=3$ δ-OORN
4 $n=4$ ε-OLYS



5 $n=1$ α-ODAP
6 $n=2$ α-ODAB

it amounted to only 18%. There was also no indication of any hydrolysis product at the higher pH value that prevailed during the isomerization process. This observation led us to conclude that the isomerization reaction at pH 6.6 was considerably slower. The reason for the reduced rate of isomerization may be the existence of a less preferred rotamer that may dominate at the pH value near neutrality and different from that which would prevail at pH 2.3. Additionally, the mechanism may involve the reaction of a protonated oxalyl carboxyl group, which would be more evident at pH 2.3 than at higher pH values as the pK_a of this group in β-ODAP is 1.85 [12]. Hydrolysis of the equilibrium mixture of α and β-ODAP with dil. DCl proceeded smoothly, but slowly, at 60° to furnish DAP. The content of DAP was 43% after 5 hr, *ca* 68% after 12 hr and after 24 hr the hydrolysis was essentially complete, the reaction mixture containing 95% DAP.

Isomerization of α-, and γ-oxalyl-α,γ-diaminobutyric acid

The isomerization of γ-ODAB (**2**) was carried out in D_2O solution (pH 2.3) as well as in a phosphate buffer made using D_2O (pH 6.1). A solution of α-ODAB (**6**) in D_2O underwent isomerization at 55° to the γ-isomer, at a rate that was considerably slower than that of α-ODAP. After 40 hr at this temperature only about 16% of the γ-isomer was obtained. The isomerization of the γ-isomer to α-ODAB was relatively faster since 21% of the α-isomer was obtained in half the time (20 hr). Interestingly, it was found that for ODAB, the equilibrium mixture contained greater amounts of the α-isomer (*ca* 60% α and 40% γ). This result is to be contrasted with ODAP which at equilibrium yielded 40% α- and 60% β-isomers. Isomerization of γ-ODAB at pH 6.1 using phosphate buffer was observed to proceed considerably more slowly, leading to a mixture containing 31% α and 69% γ after 100 hr. Thus the isomerization proceeded at lower rates at higher pH as was observed for β-ODAP.

δ-N-Oxalyl-L-ornithine (**3**) *and ε-N-oxalyl-L-lysine* (**4**)

Neither compound at pH 2.3 yielded an isomeric product when their solutions in D_2O were heated at 55° for 44 hr indicating that, under these experimental conditions, there was no migration of the oxalyl group. Failure to observe any migration of oxalyl group in these two higher homologues may be due to the longer distance between the two nitrogens in each of these two compounds making the formation of a cyclic intermediate (or transition state) unfavourable.

CONCLUSIONS

It is worth noting that the conversion of α-ODAP (**1**) to the 3:2 ratio of the β/α mixture is faster than that of the β-isomer (**5**). This rather unexpected observation may suggest the involvement of an intermediate, probably a diketopiperazine type as proposed by Bell and O'Donovan [5]. The isomerization of the α- and β-isomers of ODAP (**1** and **5**) and also of ODAB (α- and γ, **2** and **6**) but not of the higher homologues δ-*N*-oxalylornithine (**3**) and ε-*N*-oxalyllysine (**4**) indicate that a cyclic intermediate (or transition state) may be short lived but is essential for the isomerization. It can also be concluded from this observation that the rearrangement is intramolecular, since an intermolecular mechanism would also permit isomerization of the higher homologues **3** and **4**. It is conceivable that the rate of conversion of the α-ODAP to the intermediate is slower than that of the β-isomer.

EXPERIMENTAL

N-Oxalyl derivatives. L-β-ODAP, L-α-, and γ-*N*-oxalyl-α,δ-diaminobutyric acids [7], L-Na-β-ODAP·2H_2O [13], δ-*N*-oxalyl-α,δ-diaminovaleric acid (δ-*N*-oxalylornithine) and ε-*N*-oxalyl-α,ε-diaminocaproic acid (ε-*N*-oxalyllysine) [14] were prepared by previously published methods. Phosphate buffer (pH 6.1) was prepared using Sorensen's method [15].

General isomerization procedure. The *N*-oxalyldiamino acid (*ca* 3–5 mg) was dissolved in *ca* 1–1.5 ml D_2O in a NMR tube and kept in a water bath thermostated at 55°. The pH of the soln was *ca* 2.3. ^{1}H NMR spectra were recorded periodically at room temp. using either a Brüker WB250 or a Brüker AM360 spectrometer. The progress of the reaction was determined by observing the increase in intensity of the signals attributable to the non-equivalent protons at C-3 or α-ODAP at δ3.52 and 3.33 (see Table 1). The increase in intensity of these signals is accompanied by a decrease in intensity of the corresponding signals of the protons of the β-isomer at δ3.83 and 3.72.

Hydrolysis of α-ODAP⇌β-ODAP equilibrium mixture. A soln of β-ODAP (4.9 mg in 1.5 ml D_2O) in a 5 mm NMR tube was heated in a water bath for 60 hr. The formation of a 3/2 mixture of the β/α isomers was established by ^{1}H NMR spectroscopy. Three drops of *ca* 5% DCl were added and the mixture kept in the water bath at 55°.

pH of seed extracts. In order to make the pH of the isomerization medium comparable to physiological

Table 1. ^{1}H NMR spectral data of oxalyl derivatives of diamino acids [360 MHz, relative to D_2O (δ_{HOD} = 4.75 ppm), pH 2.3]

H	α-ODAP	β-ODAP	α-ODAB	γ-ODAB	δ-OORN	ε-OLYS
2	4.65 *dd* (5.4, 7.9)	4.09 *dd* (4.1, 6.9)	4.43 (4.9, 9.1)	3.84 (7.4, 6.02)	3.86 (6.2, 6.1)	3.83 (6.2, 6.2)
3a	3.52 (5.4, 13.3)	3.83 (4.1, 14.9)				
			2.30–2.21 *m*	2.25–2.03 *m*	1.85 *m*	1.85 *m*
3b	3.33 (7.9, 13.2)	3.72 (6.9, 14.9)				
4	—	—	3.01 (7.8)	3.40 *m*	1.60 *m*	1.53 *m*
5	—	—	—	—	3.23 (6.8)	1.38
6	—	—	—	—	—	3.19 (7.1, 7.1)

J (Hz) in parentheses.

conditions, the pH of an aq. slurry of powdered *L. sativus*, which contained approximately the same concentration of β-ODAP as the test soln used for the isomerization experiments, was found to be 6.1. The pH of an aq. soln of the sodium salt of β-ODAP was 6.6. This pH value was considered close enough to physiological pH and was used without adjustment.

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