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THE MECHANISM OF THE REARRANGEMENT OF THE NEUROTOXIN β-ODAP TO α-ODAP*

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Abstract—The diketopiperazine suggested to be the intermediate during the spontaneous isomerization of β-ODAP and α-ODAP was synthesized. Its behaviour was studied at selected pH values and provided evidence that its natural occurrence is unlikely. 2-Hydroxy-imidazolidine-2,4-dicarboxylic acid (for the rearrangement β-ODAP↔α-ODAP) or 2-hydroxy-pyrimidine-2,4-dicarboxylic acid (for the rearrangement γ-ODAB ↔α-ODAB) are suggested to be the unstable intermediates.

INTRODUCTION

The neurotoxin β-ODAP (3-*N*-oxalyl-2,3-diaminopropanoic acid) occurs as a free non-protein amino acid in the seeds of grass-pea (*Lathyrus sativus*), a hardy crop in regions in Africa and Asia. The seeds are a common food for several hundred million people, but, in the case of drought-triggered famine, overconsumption of seeds containing β-ODAP can give rise to irreversible spastic paraparesis, called lathyrism, in epidemic proportions.

Bell and O'Donovan [1] have shown that in mild acidic solution β-ODAP undergoes a rearrangement to the non-toxic 2-*N*-oxalyl isomer (α-ODAP) until a pH-dependent equilibrium between both compounds is established. The same equilibrium position is reached from α-ODAP (Fig. 1).

An α,β-diketopiperazine (DKP) was proposed to be the intermediate in this isomerization. Bell and O'Donovan [1] also studied the rearrangement of γ-ODAB (4-*N*-oxalyl-2,4-diamino-butanoic acid) to its α-isomer. Abegaz *et al.* [2] have studied similar rearrangements in a series of higher homologues of β-ODAP, namely 4-*N*-oxalyl-2,4-diamino-butanoic acid, δ-*N*-oxalylornithine and ε-oxalyl-lysine. They suggested an intramolecular rearrangement, probably involving a cyclic intermediate. From these data we can conclude that the equilibrium occurs easily when a five-membered or six-membered ring can be established as in the case of ODAP. It is less easy when only a six-membered ring can be the intermediate, as in the case of ODAB. A seven-membered ring is not likely and no rearrangement occurs when a larger ring would be postulated as the intermediate. This means that for the rearrangement of γ-ODAB it is unlikely that the seven-membered homologue of DKP should be formed if the DKP is indeed the intermediate for the rearrangement of β-ODAP. In this case it is not expected that the rearrangement of γ-ODAB should occur. The experimental results indicate that rearrangement of γ-ODAB occurs easily and consequently a seven-membered intermediate for this rearrangement can be excluded (Fig. 2).

On the basis of its chemical properties, the DKP is expected to be a stable compound. In a review article Eggum and Sørensen [3] mention a series of examples of amino acid derivatives which in aqueous solution show ring formation. The formation of lactam pyroglutamic acid from glutamic acid is the best known example. The yield depends on both temperature and pH. At elevated temperature in acid circumstances only 0.1% rearrange into the lactam (in this case the amino group is protonated and an attack of the free electrons of the nitrogen is prohibited), 98% between pH 4 and 10, but 9% at pH 12. In comparison with this rearrangement of glutamate we followed the isomerization of β-ODAP with ^{1}H NMR, but found no evidence for the occurrence of a DKP at some selected pH values [4].

In order to understand the mechanism of this rearrangement more fully, we have synthesized the DKP and studied its behaviour in solution at physiological, acidic and alkaline pH values. It was concluded that in mild acidic conditions and at physiological pH (where the experiments of Bell and O'Donovan [1] were performed) the natural occurrence of a DKP is unlikely. Therefore other intermediates for the rearrangement of β-ODAP to

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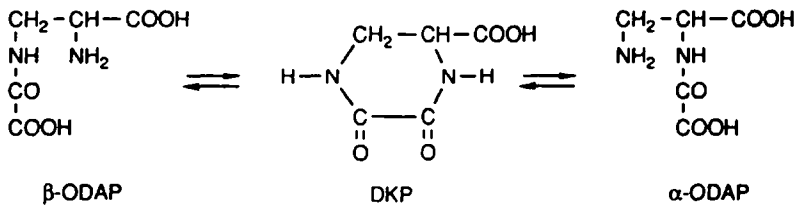

Fig. 1.

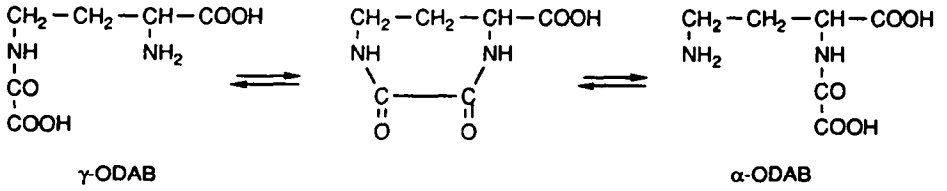

Fig. 2.

α-ODAP and for the rearrangement of γ-ODAB to its α-isomer are proposed.

RESULTS AND DISCUSSION

Previously the rearrangement of β-ODAP to α-ODAP was studied after dissolving β-ODAP in water, i.e. in a mild acidic medium [1]. An equilibrium of about 7:3 β-ODAP:α-ODAP was found. No trace of an intermediate was detected by NMR analysis. The thermal rearrangement at 55° was studied by Abegaz *et al.* [5]. When the solution was acidified with a few drops of DCl it was found that the mixture of α-ODAP and β-ODAP slowly hydrolysed to 2,3-diamino-propanoic acid (DAPRO). The latter suggests a competition between the rearrangement and the hydrolysis of β- and α-ODAP to DAPRO. Again, no trace of an intermediate was found.

The ^{1}H NMR spectra of the compounds of interest, β-ODAP, α-ODAP, DAPRO and the DKP all showed a comparable ABX spin system, which, moreover, was very pH-dependent [4]. In order to be certain that the assignments of the compounds were correct, we have previously [4] reported the NMR data of β-ODAP, α-ODAP and DAPRO at different pH values. In order to consider the occurrence of the DKP its NMR parameters must be known also. Therefore we have synthesized DKP and measured its NMR parameters at the same pH values. The results are given in Table 1.

When DKP was dissolved in DCl (pH 0.3), rearrangements into α-ODAP and β-ODAP occurred. In a ^{1}H NMR spectrum run a few hours after dissolving the DKP in DCl the proportions found for DKP:α-ODAP:β-ODAP were 56:28:16, respectively. There was more α-ODAP than β-ODAP, in contrast to the spontaneous chemical equilibrium. After two days, resonances related to a fourth compound appeared, which was identified as DAPRO. After 15 days the DKP had completely disappeared while the resonances for DAPRO predominated. There was now a proportion DAPRO:α-ODAP:β-ODAP of 53:32:15. After one month there was 99% DAPRO and the DKP had completely disappeared but the proportion α-ODAP:β-ODAP remained the same, the DAPRO proportion grew while the DKP proportion diminished.

In contrast with β-ODAP in DCl, where the hydrolysis was so fast that no rearrangement was observed, in dilute DCl the DKP first hydrolysed to α-ODAP and β-ODAP and, after their formation the individual compounds further hydrolysed to DAPRO. Thus, in this (strong) acidic medium the hydrolysis of β-ODAP to DAPRO was so fast that the DKP, or any other potential intermediate during the rearrangement of β-ODAP to α-ODAP, was not formed since neither the DKP nor α-ODAP were detected.

When the DKP was examined in solution at pH 2.5 and at a physiological pH (7.1), even after three weeks only minor amounts of α- and β-ODAP were seen (less than 1%). Even though most of the rearrangements reported in the literature were studied at these pH values, the stability of the DKP was expected because the amide bond is very stable. It has been reported [1] that after one week at these pH values β-ODAP hydrolysed to about 30% of the α-isomer. The α-form must be the result of hydrolysis of the intermediate. Since almost no hydrolysis of the DKP was seen under these conditions it cannot be the intermediate at neutral or physiological pH.

At pH 12.1 the hydrolysis of the DKP to α- and β-ODAP starts immediately (the resonances of these compounds are identical with the data given by De Bruyn *et al.* [4]). After 24 hr an equilibrium α-ODAP:β-ODAP was established at 35:65. After one week the proportions of α-ODAP:β-ODAP became 22:78. Consequently in (strong) basic circumstances no further hydrolysis to DAPRO occurred at room temperature.

With this information, the proposal that DKP is the cyclic intermediate for the isomerization of ODAP becomes unlikely. We can raise the following arguments

Table 1. ^{1}H NMR data of α-ODAP, β-ODAP, DAPRO, and DKP at some strategic pH values

	Chemical shifts			Coupling constants		
	H_X	H_A	H_B	J_{AB}	J_{AX}	J_{BX}
pH 0.3						
DKP	4.52	3.96	3.80	−13.62	4.85	3.54
α-ODAP	4.90	3.64	3.43	−13.43	5.39	8.39
β-ODAP	4.39	3.97	3.88	−14.80	4.54	6.03
DAPRO	4.53	3.68	3.60	−13.76	8.18	5.25
pH 2.5						
DKP	4.41	3.91	3.75	−13.53	4.82	3.97
α-ODAP	—	—	—	—	—	—
β-ODAP	4.17	3.88	3.78	−14.89	4.09	6.90
DAPRO	3.49	3.49	4.05	—*	—*	—*
pH 7.0						
DKP	4.19	3.84	3.70	−13.23	4.79	4.99
α-ODAP	4.48	3.48	3.30	−13.19	5.23	8.12
β-ODAP	3.96	3.83	3.69	−14.83	3.76	7.86
DAPRO	3.82	3.32	3.28	−13.25	7.26	6.21
pH 12.1						
DKP	4.17	3.84	3.68	−13.22	4.67	4.93
α-ODAP	4.26	3.07	2.95	−13.54	4.33	7.15
β-ODAP	3.44	3.52	3.37	−13.15	4.70	7.38
DAPRO	3.27	2.82	2.73	−12.90	3.10	6.39

*Degeneration of the spin system.

against the occurrence of the DKP as intermediate. (1) The DKP is expected to be a stable compound. When the isomerization of β-ODAP was studied at some selected pH values we did not find any trace of the intermediate in the NMR spectrum. A stable compound like DKP should be found. From this information it is expected that the real intermediate must be very unstable. (2) At physiological pH and in mild acidic solutions the equilibrium β-ODAP:α-ODAP is reached after three days. In the same circumstances after three weeks the DKP remains unchanged at ≈99%. It is not possible that such a stable compound can be the intermediate when the equilibrium is reached in three days. (3) When a parallel study was performed for the higher homologue γ-ODAB, the equilibrium was achieved more slowly, but the equilibrium position was similar. If the intermediate for ODAP isomerization was a DKP, then a seven-membered higher homologue must be the intermediate in the isomerization of ODAB. From the chemical point of view this is unlikely, as pointed out before.

Experimental data that might have been misleading can now be correctly evaluated. During the synthesis procedure according to Harrison [6], DAPRO was dissolved in water and a mixture of dimethyloxalate dissolved in methanol was added. After work-up some DKP was isolated by paper electrophoresis at pH 2 as shown from a NMR spectrum. The question arose if the origin of this DKP resulted from the rearrangement, or arose as an artifact during this reaction. With the information that we have now, the origin of the DKP must be found in the reaction circumstances, as e.g. a further cyclization reaction with the methyl ester of α-ODAP or β-ODAP.

Taking into consideration the studies of Abegaz *et al.* [2] on the homologues where it seems that the rearrangement is very fast when a five-membered intermediate can be formed, we propose the unstable 2-hydroxy-imidazolidine-2,4-dicarboxylic acid (Fig. 3).

The rearrangement is so fast or the proposed intermediate is so unstable that no traces are found in the ^{1}H NMR spectrum. We propose the higher homologue of the imidazolidine derivative, namely 2-hydroxy-pyrimidine-2,4-dicarboxylic acid as the intermediate in the isomerization of ODAB, which, as a six-membered ring, is expected to be more stable but its concentration is so small that it cannot be detected either (Fig. 4).

Unfortunately, because these compounds are not stable, we could not detect them. So far a direct proof has not been possible.

The further homologues δ-*N*-oxalylornithine and ε-*N*-oxalyllysine do not isomerize under the usual conditions. However, from a synthetic point of view one important question arises. For the formation of the imidazolidine there should be an attack of the free amino group on the carbonyl of a usually little reactive amide carbonyl. In textbooks of organic chemistry we find several examples of reactions called 'transaminations' and 'acetylation of amines by amides' [7]. In the latter case salts of the amine are used. The amine can also act as nucleophile as in transamidation reactions (Fig. 5).

Moreover, in certain circumstances, the reason for the relative inertness of the amide bond can be suspended as proposed here by a hydrogen bridge. In this case a hydrogen bridge can be formed between the proton on the amide nitrogen and the carbonyl of the free acid. In

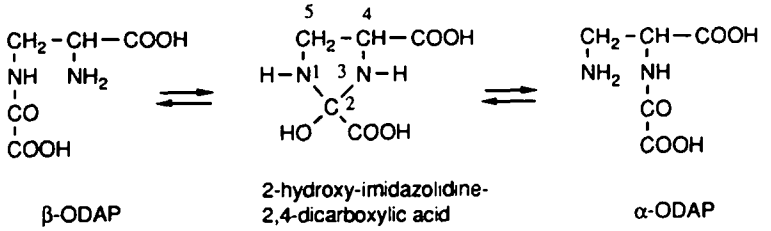

Fig. 3.

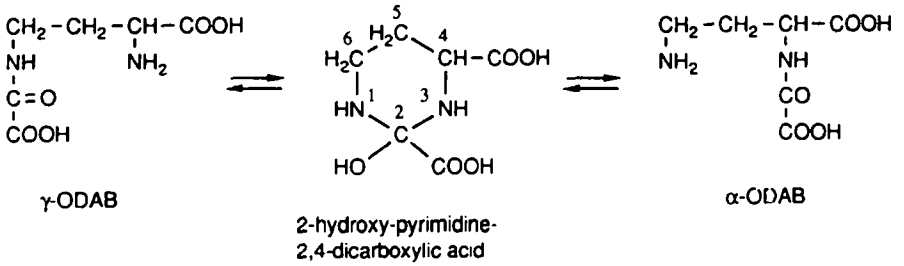

Fig. 4.

Fig. 5.

this case we obtain a quasi five-membered ring with one double bond from the free acid carbonyl and an exo double bond from the amide carbonyl. The cause of the inertness of the amide bond, an amide–imidol tautomery does not occur. In the literature there are some examples of similar reactions in relatively neutral circumstances [8, 9] (Fig. 6).

Finally, it was also found that the IR spectrum of the synthesized DKP is different from that of the hypothetical DKP as given by Bell and O'Donovan [1]. Characteristic absorptions were ascribed as follows: 3325 cm^{-1} (CONH stretch), 1630–1660 cm^{-1} (CONH stretch) and 1590 cm^{-1} (CO_2^-). We find a sharp absorption at 3600 cm^{-1}, a broad absorption at 3450 cm^{-1} while we find only a small sharp absorption peak (1650 cm^{-1}) in the region 1500–1700 cm^{-1}. These findings are in agreement with a six-membered lactam (1640–1670 cm^{-1}) where the second amide band is missing. In a cyclic amide a band at 3440 cm^{-1} is to be expected, while the band at 3550 cm^{-1} can be ascribed to the acid. Finally, we have also measured the ^{13}C NMR data of the DKP at some selected pH values. The data are given in Table 2.

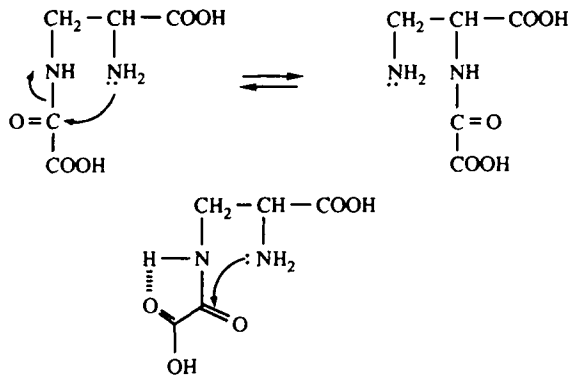

Fig. 6.

EXPERIMENTAL

NMR measurements of ODAP and DAPRO. Depending on the pH required, the compounds were dissolved in DCl (pH 0.3), D_2O (pH 2.5) or in a NaPi-buffer (pH 7.1 and 12.1).

Synthesis and characterization of DKP. The α,β-diketopiperazine was synthesized following a procedure developed by one of the authors [10]. Finely powdered DAPRO, acetic acid salt (200 mg, 1 mmol) was dissolved in 2 ml TMSCN (trimethyl silylcyanide) with stirring for 30 min at 60°. The solvent was evapd under vacuum and the residue dissolved in 5 ml CH_2Cl_2. Then 95 μl (1.1 mmol) oxalyl chloride was slowly added with stirring at −20°. The reaction mixt. was warmed up to room temp. and evapd to dryness.

The α,β-diketopiperazine was purified by prep. chromatography, using a 300 × 7.8 mm column of phenominex C18 (10 μ). Elution solvent was H_2O – TFA (99:1). Flow: 2 ml min^{-1}. The absorption was measured at 254 nm (the carbonyl band was at 214 nm) pointing to an enolized system in these circumstances. Mp ≈ 210° (decomp.). MS *m/z*; 158 $[M]^+$, 140 $[M-H_2O]^+$, 130 $[M-CO]^+$, 112 $[M-H_2O-CO]^+$. The ^1H NMR spectrum in DMSO-d_6 shows 2 labile proton resonances (δ_A

Table 2. ^{13}C NMR chemical shift of DKP at some strategic pH values

pH	CH_2	CH	COOH (DAPRO)	CO (Oxalyl)	CO (Oxalyl)
0.3	42.4	53.2	173.3	—	—*
2.0	42.5	53.1	189.2	160.6	160.1
7.1	45.4	56.9	178.3	162.5	162.4
12.1†	—	—	—	—	—

*Could not be distinguished from the resonances for α- and β-ODAP.

†The rearrangement/hydrolysis is so fast that no ^{13}C NMR spectrum of DKP could be run with our facilities.

$=3.71$; $\delta_B=3.42$; $\delta_X=4.13$, $J_{AX}=4.62$ Hz, $J_{BX}=?$, $J_{AB}=-13.08$ Hz. The resonances for H-A and H-X are enlarged because of their coupling constant with the labile protons. Besides this ABX spin system there are two further doublets at $\delta=8.71$ and $\delta=8.74$, respectively, of labile protons, with $J=4.37$ Hz and 4.56 Hz, pointing to $^3J_{(CH-NH)}$ couplings (in DMSO soln).

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