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DIMORPHISM OF β-*N*-OXALYL-L-α,β-DIAMINOPROPIONIC ACID

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Key Word Index—β-*N*-oxalyl-L-α,β-diaminopropionic acid; non-protein amino acid; structural dimorphism.

Abstract—The neurotoxic non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) exists in two crystalline modifications; a metastable monohydrate and a thermodynamically stable anhydrous form. Reproducible methods for preparing each polymorph are described and their structures are discussed on the basis of their general physical characteristics and their IR spectra and X-ray powder patterns. The ionic properties of the amino acid were studied in solution and revised pK_a values for the two carboxyl functions are reported.

INTRODUCTION

The non-protein amino acid, β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) was first isolated from seeds of *Lathyrus sativus* [1, 2] and subsequently identified in a large number of species of *Lathyrus*, *Crotalaria* and *Acacia* [3–5]. Apart from the use of the amino acid as a taxonomic marker, the compound is of considerable interest because of its being implicated in the crippling human neurological disease neurolathyrism [6], and because of its remarkable neuroexcitatory properties in the central nervous system of animals [7], where it acts at the same receptor as kainic acid [8].

β-ODAP has been isolated previously either in the anhydrous state [2, 9, 10], as the hemihydrate [1], or as the monohydrate [11–13]. Recently, we obtained different batches of β-ODAP, prepared by precipitation at pH 2 [13], that exhibited significantly different IR spectra and variable elemental analyses, even though the preparations were electrophoretically homogeneous. To resolve these difficulties β-ODAP was investigated in the solid state. IR spectra and X-ray powder pattern studies revealed that the compound existed in two different crystalline modifications which other physical methods showed were either anhydrous or monohydrated. Methods were developed for obtaining each form unambiguously.

RESULTS

The method of crystallization of β-ODAP was critical in determining the structural modification that was isolated. Thus, rapid recrystallization from water produced the metastable monohydrate (**1**). Alternatively, slow recrystallization from water, or more rapidly from aqueous ethanol, produced the thermodynamically stable anhydrous form (**2**). Form **1** was readily converted to form **2** by shaking the solid under a saturated solution of β-ODAP.

The purity and state of hydration of the two morphological forms were established by a number of physical techniques. The state of hydration was determined directly by thermogravimetric analysis, and these data were confirmed by titration, molar extinction coefficient determination and elemental analysis (Table 1). The results show good agreement with theoretical values for both forms and the decomposition temperatures were identical (217°).

There were marked differences in the IR spectra of the two modifications (Table 2). Many typical group frequencies were similar in both modifications [14], e.g. both spectra contained an obvious –CONH stretch at *ca* 1690 cm^{-1}. The hydrated form **1** showed a typical amino acid type NH– stretching region and bands typical of the $-NH_3^+$ group (2690, 2930 and 3240 cm^{-1} [14]) were observed. Probably this form is essentially zwitterionic in the solid state. In marked contrast the anhydrous form **2** showed a much simpler NH-stretching region; only frequencies assignable to –NH and $-NH_2$ groups (e.g. 3330 and 3120 cm^{-1}) were observed.

The crystallinity of both modifications was established using X-ray powder photography; the main *d* spacings are summarized in Table 3, where only the strongest lines are reported. The two modifications were obviously crystalline and of very different structure.

In the course of determining the MWs of the two forms by titrimetry, results were obtained that were inconsistent with previously published pK_a values [2]. (These values had been determined directly from

Table 1. Some physical properties of the two modifications of β-ODAP

Compound	% C	% H	% N	% H_2O*	MW†	E_m‡
β-ODAP§	34.09	4.57	15.91	—	176.1	—
Form (2)‖	33.71	4.67	15.43	—	173.5	6.87×10^3
β-ODAP. H_2O§	30.93	5.19	14.43	9.27	194.1	—
Form (1)¶	30.96	5.08	14.33	9.20	195.2	6.87×10^3

*Thermogravimetrically at 5°/min under N_2.
†By pH titration.
‡In H_2O at 20° at 195 nm (λ_{max} for both forms).
§Theoretical values.
‖Recrystallized during 17 hr at *ca* 20° from H_2O.
¶Recrystallized rapidly from H_2O.

Table 2. IR spectra of the two modifications of β-ODAP

Form 1 H_2O	Form 2 anhydrous
3440 *m*	3330 *m*
3390 *s*	3120 *w*
3240 *m*	3070 *s b*
3130 *w*	2980 *s b sh*
3010 *w sh*	2700 *m b sh*
2930 *w b*	2510
2860 *w b sh*	
2690 *m b*	
2640 *w sh*	
2580 *w*	
2550 *w*	
2500 *w*	1750 *s b sh*
1750 *s b*	1693 *m*
1690 *s b*	1670 *m sh*
1615 *m*	1617 *m*
1605 *w sh*	1587 *w*
1565 *m*	1547 *w sh*
1530 *m*	1515 *m b*
1510 *m sh*	1421 *m*
1432 *m*	1380 *w sh*
1387 *m*	1353 *m*
1365 *w sh*	1305 *w*
1330 *m sh*	1275 *w*
1310 *m b*	1225 *w*
1280 *m sh*	1165 *m*
1250 *w sh*	1130 *m*
1190 *w*	1083 *w*
1150 *w*	1052 *w*
1090 *w*	997 *w*
1075 *w sh*	938 *m*
1000 *w*	843 *w*
932 *m b*	
898 *w*	829 *w*
842 *w*	770 *w*
834 *m*	740 *w sh*
760 *m*	720 *m*
750 *w*	660 *w*
725 *w*	
700 *w*	
635 *w*	

w–Weak; *m*–medium; *s*–strong; *sh*–shoulder; *b*–broad.

Table 3. Major *d* spacings from X-ray powder photographs of the two modifications

Form 1 (monohydrate) (*d*Å)	Form 2 (anhydrous) (*d*Å)
8.846	5.556
6.535	4.997
5.810	4.172
4.133	3.414
3.427	3.204
3.038	2.806
2.823	2.327

For simplicity only the strongest lines are reported in each case.

the titration curve, a method that is known to be inaccurate for overlapping ionizable groups [15].) Using a more accurate method [16] the following pK_a values were obtained, with those of **2** in parenthesis: pK_{a1} 1.85 (1.95); pK_{a2} 2.20 (2.95); pK_{a3} 9.20 (9.25).

DISCUSSION

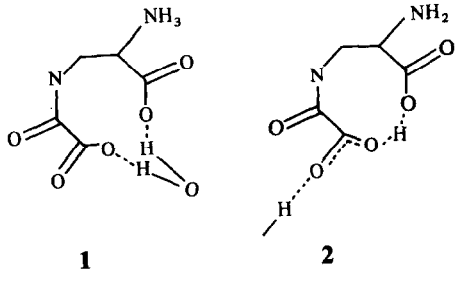
Previously reported syntheses of β-ODAP where the anhydrous product was recovered used either precipitation at pH 2.1–2.2 from an undefined K^+ or Li^+ salt [9] or recrystallization from hot water [2, 10]. The monohydrate has been recovered following freeze-drying and washing with cold water and acetone [11], by recrystallization from hot water [12], or by precipitation at pH 2 with acetone from the pyridinium salt [13]. Our results show that the method of crystallization must be carefully controlled in order to obtain a sample of predictable morphology. As the final (thermodynamically stable) form is the anhydrous modification, rapid recrystallization, i.e. induced nucleation and low temperature favours the production of the metastable monohydrate (a product of kinetic control). This situation is analogous to the dimorphism of HgI_2, where, by varying the conditions of recrystallization, either red (thermodynamically stable) or yellow (metastable) forms are obtained [17].

We found no evidence for the existence of a hemihydrate [1]. It may be significant that the conditions used to crystallize this hemihydrate (50% v/v ethanol–water) are precisely those used to crystallize an equimolar mixture of β-ODAP and its α-oxalyl isomer, which also gave a hemihydrate on elemental analysis [18]. Thus, the original isolation of β-ODAP by Murti *et al.* [1] may have been such an isomeric mixture, a supposition that is reinforced by the specific rotation value quoted ($[\alpha]_D - 28.1°$, c 1.99, 5 M HCl), which is *ca* half way between the values given for the pure α- and β-isomers [11, 13] under similar conditions. Paradoxically, we found that when pure β-ODAP was recrystallized from 50% v/v ethanol–water, the anhydrous form was recovered.

An obvious carboxylate stretch at 1400 cm^{-1} was not found in the IR spectra of either form; hence it is probable that hydrogen bonding leads to cyclization of both modifications. Two possible structures are consistent with these observations (Fig. 1). In the hydrated form **1** the water molecule bridges the two carboxylates; in form **2** the proton (which in **1** resides in the $-NH_2$ group) bridges the carboxylates. In form **2** hydrogen bonding to the next molecule in the crystal is indicated. Model building indicates that neither ring structure is unduly strained.

As the IR spectra of the anhydrous and hydrated forms of β-ODAP are very distinctive (Table 2), earlier reports of identical IR spectra seem unreliable [11, 13]. Previously we reported the IR spectrum of a sample of hydrated β-ODAP [13], but we now believe that this spectrum was of the anhydrous form. This spectrum was obtained by a service laboratory; it seems likely that the β-ODAP hydrate was inadvertently dehydrated while being dried prior to IR spectroscopy. However, attempts to reproduce this result by dehydrating a sample of β-ODAP hydrate by heating at 120° have failed to give exactly the spectrum expected of the anhydrous modification. Whether inadvertent dehydration explains the discrepancy between references [11, 13] and [2] is therefore uncertain.

The results presented in this paper demonstrate unequivocally the hydrate isomerism of β-ODAP. Failure to appreciate the conditions under which the two forms of β-ODAP may be crystallized has lead to confusion in earlier reports, particularly when IR spectra were considered. As the anhydrous form is thermodynamically stable, this form is probably the more suitable compound to isolate. Our previous difficulties with β-ODAP were probably due to the inadvertent production of mixtures of the two forms.



1 **2**

Fig. 1. Suggested structures of the two modifications of β-ODAP in the solid state. Form **1**, monohydrate; form **2** anhydrous. Charges have been omitted.

EXPERIMENTAL

β-ODAP was prepared as previously described [13]; reproducible methods for crystallizing the two modifications were as follows.

Hydrated form **1**. H_2O at *ca* 70° was satd with β-ODAP, the hot soln was quickly filtered and the filtrate rapidly cooled in ice, crystn being encouraged by vigorous scratching. The hydrate was recovered by filtration, washed with ice-cold H_2O and dried over P_2O_5 or $CaCl_2$ *in vacuo*. The H_2O of crystn was not lost when dried over these desiccants at *ca* 20°, but was removed by heating in the absence of a desiccant at *ca* 78°.

Anhydrous form **2**. H_2O at *ca* 70° was satd with β-ODAP and rapidly filtered. The filtrate was allowed to crystallize for 17 hr at *ca* 20°. Alternatively H_2O–EtOH (1:1) was satd with β-ODAP at *ca* 65° and crystn occurred during slow cooling over 3–4 hr at *ca* 20°. A more rapid method was to saturate H_2O at 65° with β-ODAP, whereupon EtOH was added to the point of precipitation; cooling in ice then crystallized the anhydrous form. The solid was recovered by filtration, washed with ice-cold H_2O–EtOH (1:1), EtOH and Et_2O and dried over P_2O_5 or $CaCl_2$ *in vacuo*

Conversion of **1** *to* **2**. Form **1** was suspended in H_2O and shaken for 17 hr at *ca* 20°. The resulting solid material was removed by filtration, washed with ice-cold H_2O and dried over P_2O_5 *in vacuo*.

High voltage electrophoresis. The electrophoretic purity of these products was confirmed at pHs 1.9 and 3.0 [13].

IR spectra were recorded in Nujol or hexachlorobutadiene mulls between KBr or CsI plates.

X-ray powder photographs were recorded by the Debye–Scherrer method using Cu–K_α radiation (1.5418 Å).

Thermogravimetric analysis was carried out under N_2 at 5° (for H_2O content) or 10° (thermal decomposition)/min, with *ca* 5 mg samples.

pK_a *values*. Titrations were performed under N_2, adding 0.1 or 0.01 M NaOH to the soln of β-ODAP dissolved in acid. In the dilute solns used (β-ODAP $< 10^{-2}$ M) no corrections were made for changes in ionic strength. pK_a values were calculated as described by Rossotti [14], from plots of

$$\frac{J-1}{J}|H^+| \text{ vs } \frac{2-J}{J}|H^+|^2.$$

MWs were determined by acidimetric titration, end points being estimated by plots of ΔpH vs vol.

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