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THE SYSTEM D-MALIC ACID–L-CITRULLINE

R. CEOLIN

Laboratoire de Chimie Physique, Faculté de Pharmacie, 2 bis, boulevard Tonnellé,
F-37042 Tours Cedex (France)

P. KHODADAD

Laboratoire de Chimie Minérale 1, Faculté de Pharmacie, 5, rue J.B. Clément,
F-92296 Chatenay-Malabry Cedex (France)

F. LEPAGE

Centre de Recherche, Laboratoires BIOCODEX, Chemin d'Armancourt,
ZAC de Mercières F-60200 Compiegne (France)

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ABSTRACT

The system D-malic acid–L-citrulline has been studied by differential thermal analysis and X-ray powder diffractometry. The 1 : 1 stoichiometry of the spontaneously forming binary compound was established by means of the Tammann method. This compound melts peritectically at 363 K ($\Delta_{\pi}H = 26$ kJ mol^{-1}). It was characterized by means of single-crystal 3-dimensional X-ray diffraction analysis: monoclinic, $P2_1$, $a = 10.22$ Å, $b = 5.615$ Å, $c = 12.43$ Å, $\beta = 90.0°$, $V = 713$ Å^3, $Z = 2$, $D_{\text{calc}} = 1.44$ g cm^{-3}, $M = 309.8$.

INTRODUCTION

Malic acid and citrulline are known for their detoxificative and hepatoprotective properties in human therapeutics. The present study is part of a general one undertaken to check whether the synergy observed when both compounds are used together could be related to the emergence of well-defined molecular or ionic associations.

THERMAL STUDIES

Samples were prepared by mixing D-malic acid (DM) and L-citrulline (LC) in different molar ratios. Grinding the solid mixtures for about half an hour led to heterogeneous (solid LC + liquid) mixtures in the LC-rich range of the system and to an homogeneous liquid in its DM-rich range.

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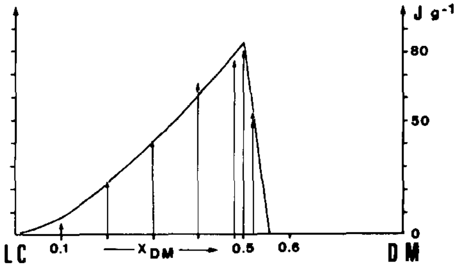

Fig. 1. Tammann curve for the endothermic effect at 363 K for D-malic acid–L-citrulline.

Starting with the α-form of L-citrulline [1–3], a new compound forms spontaneously in about one week, as demonstrated by X-ray powder diffraction.

To follow this transformation, samples were taken from time to time and studied by differential thermal analysis (DTA). This procedure was ended when no further change in the enthalpies of the thermal effects was observed.

A Mettler FP800 thermal analyzer connected to an Epson HX-20 computer was used. The samples were weighed using a microbalance sensitive to 0.01 mg.

With a heating rate of 10 K min^{-1}, an endothermic effect was observed at 363 K (onset of the peak) within the molar fraction range $0 < x_{\mathrm{DM}} < 0.6$. As shown in Fig. 1, this effect is maximum for $x_{\mathrm{DM}} = 0.5$, steeply decreases for $x_{\mathrm{DM}} > 0.5$ and becomes zero for $x_{\mathrm{DM}} = 0.55$.

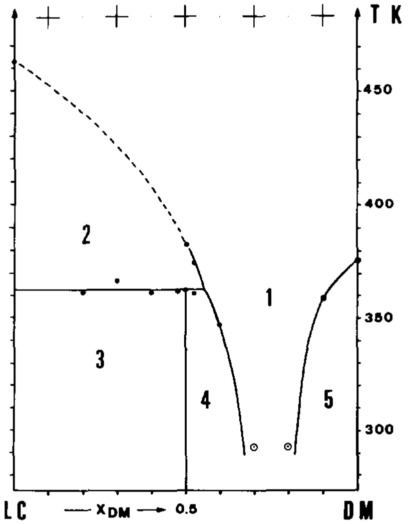

Fig. 2. Phase diagram of the D-malic acid–L-citrulline system: (⊙), visual observation. L-citrulline melting at 463 K, from ref. 3. Phase regions: 1, liquid; 2, LC + liquid; 3, LC + DM–LC; 4, DM–LC + liquid; 5, DM + liquid.

Attempts to obtain the complete phase diagram failed for two reasons: (1) in the DM-rich part, the samples corresponding to $x_{DM} = 0.7$ and 0.8, stored in closed aluminium caps at room temperature for two years, remain in the liquid state; (2) in the LC-rich part, the closed sample containers broke at ca. 400–410 K because the portion of L-citrulline which was transferred into the liquid phase decomposed.

However, the experimental results given in Fig. 2 shows that the 1 : 1 compound DM–LC melts uncongruently. At 363 K, the following peritectic reaction occurs

$$\text{solid DM–LC} \rightleftharpoons \text{solid L-citrulline} + \text{liquid } \pi \qquad [x_{DM}(\pi) \simeq 0.55]$$

It is to be noted that the spontaneous formation of solid DM–LC occurs only if α-L-citrulline is used. Its formation could not be observed even after several months when the δ-form was used. Thus, it may be assumed that the stable equilibria are those involving the α-form of L-citrulline.

TABLE 1

X-ray diffraction powder pattern for D-malic acid–L-citrulline 1 : 1 binary compound. Intensities I_{obs} were estimated visually and expressed using the code m = medium, v = very, w = weak, s = strong, ? = dubious

d_{obs} (Å)	d_{calc} (Å)	I_{obs}	*hkl*	d_{obs} (Å)	d_{calc} (Å)	I_{obs}	*hkl*
12.35	12.43	m	011	2.790	2.792	ms	213
10.10	10.22	w	100	2.761	2.736	ms	021
7.89	7.89	m	101	2.712	2.718	ms	014
5.24	5.31	mw	102		2.637		312
5.11	5.11	m	011	2.631	2.631	w	303
			200		2.627		114
4.910	4.918	mw	110	2.456	2.459	vw	220
4.667	4.726	m	201	2.412	2.412	w	221
4.560	4.573	vs	111	2.378	2.382	ms	313
4.158	4.143	ms	003	2.357	2.363	vw	402
3.940	3.947	ms	202	2.325	2.325	vw	410
3.855	3.856	w	112	2.305	2.296	vw	304
3.831	3.840	vw	103	2.266	2.265	ms	123
3.763	3.778	s	210	2.205	2.219	m	115
3.608	3.614	vs	211	2.169	2.175		403
3.411	3.407	?	300		2.165		320
3.330	3.333	w	013	2.152	2.133	w	321
3.270	3.286	m	301	2.079	2.082	m	024
2.246	3.228	m	212		2.077		215
3.218	3.218	m	203		2.072	vw	006
3.173	3.169	m	113		2.045		322
2.976	2.987	w	302		2.044		500
2.942	2.973	vw	104	2.041	2.040	vw	124
2.896	2.912	mw	310		2.030		106
2.824	2.835		311	2.023	2.028	m	413
	2.805		020				

CRYSTALLOGRAPHIC STUDIES

Plates of about 50 μm × 50 μm × 10 μm were slowly (1 year) grown at room temperature from a heterogeneous sample ($x_{DM} = 0.6$) consisting of an excess of solid DM–LC and a DM-rich syrupy liquid. The crystals were washed with ethanol.

Using an Enraf–Nonius Weissenberg camera (oscillation and Weissenberg methods) and Cu $K\alpha$ X-radiation ($\lambda = 1.5418$ Å), the unit cell constants were found to be: $a = 10.22 \pm 0.01$ Å, $b = 5.615 \pm 0.005$ Å, $c = 12.43 \pm 0.01$ Å. Although the α, β and γ angles were found to be equal to 90.0°, the symmetry is that of a primitive monoclinic network, because significant differences between the intensities I_{hkl} and $I_{\bar{h}kl}$ were observed.

The same results were found using the precession method and Mo $K\alpha$ radiation ($\lambda = 0.7107$ Å). The $0k0$ reflections were observed only for $k = 2n$, thus supporting space group $P2_1$ or $P2_1/m$ [4].

The space group was assumed to be the non-centro-symmetric one ($P2_1$) since the components of the crystal are optically active (α_D measurements on a 0.2% aqueous solution at 22° C yield the value +2.28°).

The X-ray diffraction spectrum of DM–LC was obtained by means of an Enraf-Nonius FR 552 camera (50 kV, 20 mA, λ Cu $K\alpha_1 = 1.5405$ Å, 1 h exposure).

The observed interplanar distances, d_{obs} (Å), are in good agreement with the calculated ones, d_{calc}. Both sets of data are given in Table 1. The corresponding intensities were estimated visually on X-ray powder diffraction films and also on single-crystal Weissenberg photographs.

DISCUSSION AND CONCLUSIONS

The 1 : 1 incongruently melting compound (DM–LC) of the system D-malic acid–L-citrulline forms spontaneously at room temperature starting with the α-form of L-citrulline rather than its δ-form, the latter leading to a metastable eutectic equilibrium between the initial components. The homologous compound, LM–LC of the system L-malic acid–L-citrulline, was found to be a molecular one, since no proton transfer was observed [5].

The present X-ray study reveals a significant difference between the volumes of both unit cells: 713 Å^3 and 689 Å^3 for DM–LC and LM–LC, respectively. In other words, the denser packing is observed for LM–LC, which is molecular. Thus, DM–LC could also be a molecular compound.

However, the different packings of the two networks could also be due to ionization of DM–LC, accompanied with conformational changes of L-citrulline, due to the influence of chirality on reticular aggregation.

Such changes have been observed in similar compounds: L-arginine L-aspartate [6] and D-arginine L-aspartate salts [7] for which the unit cell volumes are not significantly different (703 $Å^3$ and 698 $Å^3$ for the L–L and D–L salts, respectively).

Unfortunately, structural comparisons between LM–LC and DM–LC were not possible because the DM–LC crystals were too small for the crystalline structure to be determined.

Nevertheless, thermal studies show that LM–LC and DM–LC behave very similarly: they both melt uncongruently at ca. 370 K, leading to peritectic liquids of similar compositions ($x_{DM} \simeq 0.55$). It could thus be thought that DM–LC is also a molecular compound.

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