



Thermal Reactions of Malic Acid Benzylamine Salts

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Malic acid, a component present in fruit, is known to be an important precursor for the prebiotic formation of polyaspartic acid. Malic acid is a dicarboxylic acid, possessing one hydroxyl group. It yields many types of crystalline salts with amino compounds. Although the thermal reactions of the amino salts have been reported, their dehydration reaction pathway has not been studied. This paper describes the dehydration process using thermal gravimetry and differential thermal analysis. The results show that the malic acid monobenzylamine and the dibenzylamine salts release water molecules during an endothermic reaction to afford malic acid benzylimide and malic acid dibenzylamide, respectively. The latter compound is stable up to 230 °C.

Key Words: Thermal analysis, Malic acid, Benzylamine salt.

INTRODUCTION

Malic acid is known as a component in wine and in extracts of grape and other fruits. This bifunctional organic compound, possessing one hydroxyl and two carboxyl groups, forms the monoammonium salt¹⁻³ and other N-substituted ammonium salts⁴. Upon heating, the monoammonium salt affords polyaspartic acid^{3,5,6}. This has been suggested as an important pathway to the prebiotic polyaspartic acid^{3,5,6}. Microwave activation of ammonium malate can also afford polyaspartic acid⁷⁻⁹. One of the proposed mechanisms for the thermal peptide formation is as follows. Dehydration from the C2 and C3 positions proceeds first to afford maleic acid and fumaric acid monoammonium salts, which themselves react to afford their monoamides (Fig. 1). Monoammonium salt and monoamide with α -carboxylate are supported as the superior structure rather than those with β -carboxylate due to stronger acidity of α -carboxylate. The monoamide nitrogen attacks the C=C double bond to afford polyaspartic acid. Another possible mechanism involves first (similarly) the formation of the monoamide, followed by the nucleophilic substitution of the ammonium nitrogen to the C2 position to afford polyaspartic acid.

Although thermal reactions using N-substituted ammonium salts of malic acid have been reported⁴, their dehydration mechanism has not been reported and N-substituted polyaspartic acids have not been obtained.

This paper describes the amide formation mechanism using N-benzyl-ammonium salts (**3** and **4** in Fig. 2) of malic acid (**1**). Thermal analysis of the resulting dibenzylamides showed unexpected thermal stability at elevated temperatures. We found some similarity and some difference between the ammonium salts and N-benzyl-ammonium salts of malic acid in terms of their thermal reactions.

EXPERIMENTAL

The thermal analyzer used was a Shimadzu DT-40 (Shimadzu, Kyoto, Japan). Infrared spectra were recorded using a Hitachi Model 260-50 infrared spectrophotometer (Hitachi, Tokyo, Japan). ¹H NMR spectra were measured with a JOEL 400MHz ¹H NMR instrument (Joel, Tokyo, Japan).

DL-Malic acid (**1**) was supplied by Fuso Chemical Co., Ltd. (Osaka, Japan). Benzylamine (**2**) was purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan).

General procedure of heating reaction: DL-Malic acid (1.34 g, 10 mmol) and benzylamine (1.07 g, 10 mmol or 2.14 g, 20 mmol) were mixed in a test tube (20 cm × 2 cm ID) and stood for several minutes under nitrogen flow until the test tube cools down to room temperature. The test tube was immersed into an oil bath controlled at 160 °C and the reaction was carried out for 4 h under a nitrogen flow.

Sample preparation for thermal analysis

DL-Malic acid mono-(N-benzyl-ammonium) salt (3**):** DL-Malic acid (13.4 g, 100 mmol) was dissolved in ethanol

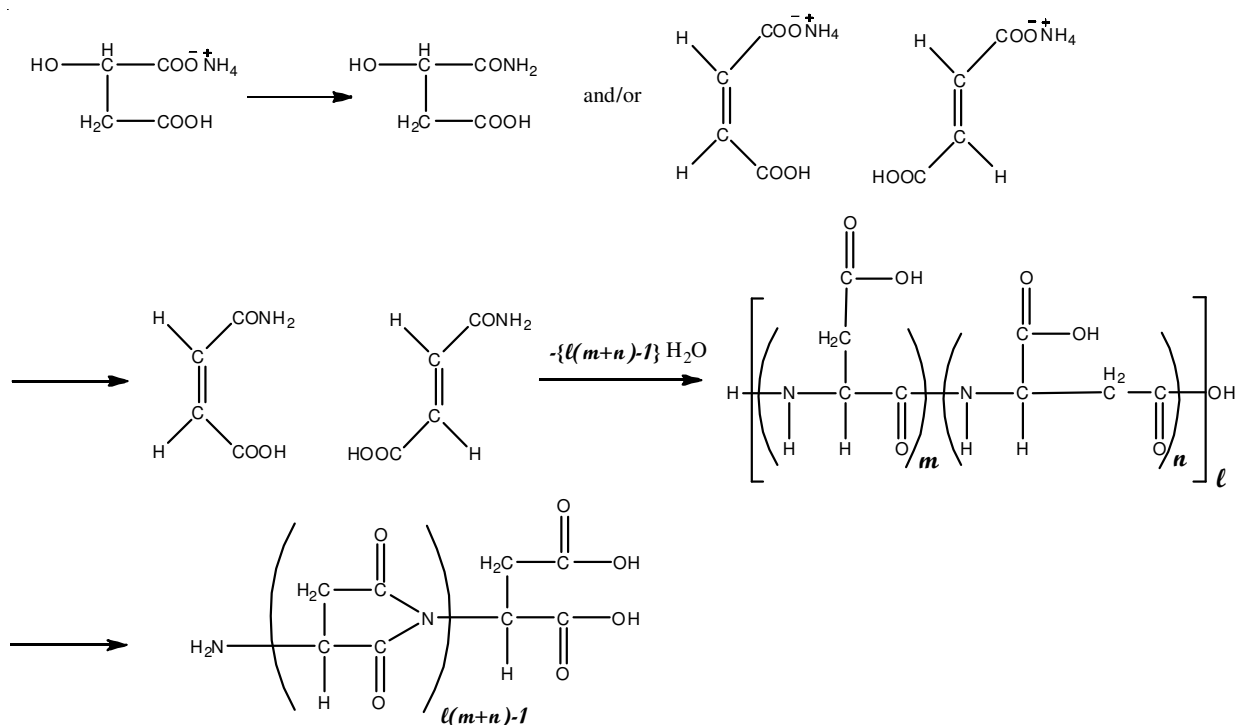


Fig. 1. Formation of aspartic acid by heating DL-malic acid mono ammonium salt

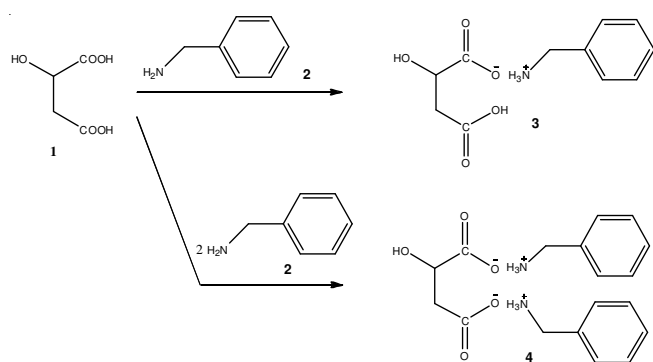


Fig. 2. Preparation of DL-malic acid N-benzyl-ammonium salts (3, 4)

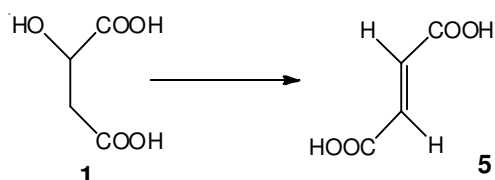
(50 mL) and the resulting solution was cooled in an ice bath. Benzylamine (10.7 g, 100 mmol) was added dropwise to the cooled solution to yield a precipitate (21 g, 87 %). The precipitate (5 g) was recrystallized to yield 2.2 g (44 %; m.p. 145–146 °C). Elemental analysis of the resulting crystals was consistent with the calculated value. Calcd. (%) for $\text{C}_{11}\text{H}_{15}\text{NO}_5$: C, 54.76; H, 6.26; N, 5.80. Found (%): C, 54.75; H, 6.27; N, 5.80. The higher acidity of the carboxylic group at the C2 position (α) (α : $\text{pK}_a = 3.4$; β : $\text{pK}_a = 5.13$) suggests that the α -carboxyl group preferentially forms a salt with benzylamine.

DL-Malic acid di-(N-benzyl-ammonium) salt (4): DL-Malic acid (13.4 g, 100 mmol) was dissolved in ethanol (50 mL) and the resulting solution was cooled in an ice bath. Benzylamine (21.4 g, 200 mmol) was added dropwise to the cooled solution to yield a precipitate (30.4 g, 87 %). The precipitate (5 g) of the total amount was recrystallized to yield 3.6 g (72 %; m.p. 140–142 °C). Elemental analysis of the resulting crystals was consistent with the calculated value, as the half hydrate. Calcd. (%) for $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_5 \cdot 1/2\text{H}_2\text{O}$: C, 60.49; H, 7.05; N, 7.84. Found (%): C, 60.22; H, 6.78; N, 7.49.

Procedure of thermal analysis: Samples of DL-malic acid dibenzylamine salt (8–13 mg, weighed to within 0.1 mg) were placed in the aluminum sample pans of the differential thermal analyzer. The α -aluminum reference disk was placed in the center of the second aluminum pan. The temperature of the heating chamber was programmed from 27–400 °C using a heating rate of 10 °C/min, under a nitrogen flow (50 mL/min).

RESULTS AND DISCUSSION

Thermal reaction of DL-malic acid (1): DL-Malic acid (1.34 g, 10 mmol) in a test tube (20 cm $\times$ 2 cm ID) was heated under a nitrogen flow for 4 h at 160 °C and at 180 °C to afford fumaric acid (5) (Scheme-I). The yields were 81 and 88 %, respectively. Analytical data were as follows. ^1H NMR (CD_3OD): $\delta = 6.93$ (2H, s). IR (KBr, ν_{max} , cm^{-1}): 3050–3100 ($-\text{COOH}$), 1670 ($\text{C}=\text{C}$), 1000 ($\text{C}=\text{C}$), 900 ($-\text{COOH}$). Calcd. (%) for $\text{C}_4\text{H}_4\text{O}_4$: C, 41.39; H, 3.47. Found (%): C, 41.91; H, 3.65.



Scheme-I

Thermal analysis of DL-malic acid (Fig. 3) shows the dehydration. The DTA curve shows a sharp endothermic peak, which corresponds to the melting point of DL-malic acid, because the sample weight did not reduce during the endothermic peak revealed in DTA curve.

Thermal reaction of DL-malic acid mono-(N-benzyl-ammonium) salt (3): DL-Malic acid (1) (1.34 g, 10 mmol)

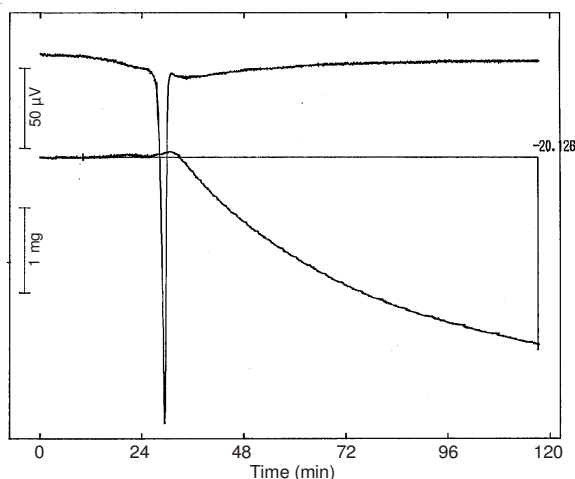
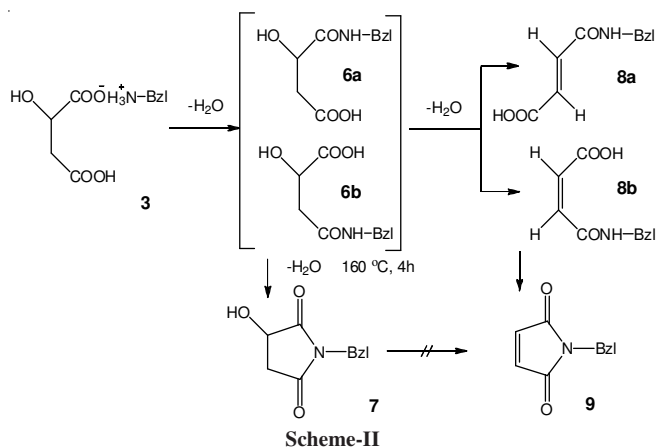


Fig. 3. Thermal analysis of DL-malic acid (**1**). Temperature was programed from 27–160 °C at 10 °C/min under a nitrogen flow (50 mL/min). Until 40 min after start of heating, the temperature seems to reach 160 °C

and benzylamine (**2**) (1.07 g, 10 mmol) were mixed and then heated under a nitrogen flow at 160 °C for 4 h (**Scheme-II**). The resulting reaction mixture showed several spots on a thin layer chromatogram, run using chloroform (CHCl₃): ethyl acetate (EtOAc) (3:1) as developing solvent ($R_f = 0.81, 0.35$ and other values). After the extraction of a chloroform solution of the reaction mixture with 0.1 M HCl, followed by drying with anhydrous magnesium sulfate, the solution was evaporated to dryness and then the resulting residue was loaded onto a silica gel column, for chromatographic separation (developing solvent, CHCl₃-AcOEt (3:2)). N-Benzyl-maleimide (**6**) (57 %) and N-benzyl-maleimide (**9**) (8 %) were isolated.



Analytical data were as follows. N-Benzyl-maleimide (**6**): m.p. 116–117 °C. $R_f = 0.35$ (CHCl₃:AcOEt (3:1)). ¹H NMR (CD₃Cl): $\delta = 7.40$ (5H, m), 4.68 (2H, s), 3.48 (1H, s), 2.85 (2H, dd, $J = 6.0, 7.9$ Hz). Calcd. (%) for C₁₁H₁₁NO₃: C, 64.38; H, 5.40; N, 6.81. Found (%): C, 64.29; H, 5.36; N, 6.81. N-Benzyl-maleimide (**9**): m.p. 60–62 °C, $R_f = 0.81$ (CHCl₃:AcOEt (3:1)). ¹H NMR (CD₃Cl): $\delta = 7.32$ (5H, m), 6.67 (2H, s), 4.65 (2H, s).

Fig. 4 shows the thermal analysis curve of the mono-(N-benzyl-ammonium) salt (**3**) of DL-malic acid. The DTA curve shows two larger and two smaller endothermic peaks. The first, larger, sharp peak corresponds to the endothermal dehydration

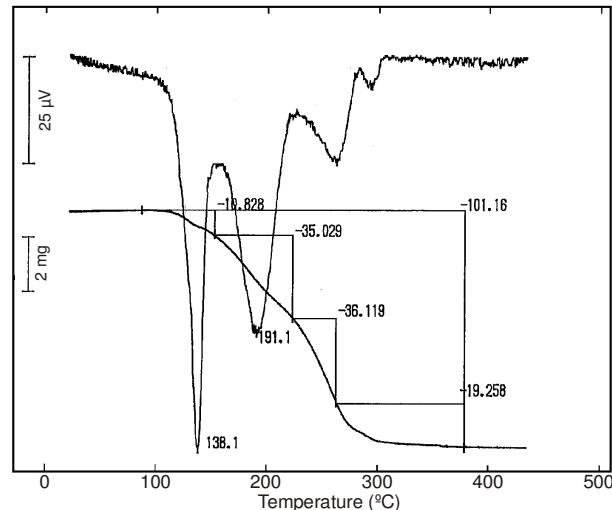


Fig. 4. Thermal analysis of DL-malic acid mono-benzylamine salt (**3**). Temperature was programed from 27–440 °C at 10 °C/min under a nitrogen flow (50 mL/min)

occurring at the same time as the melting. When the endothermic peak revealed, the weight loss reduced to 10.8 % of the sample weight. The value (10.8 %) is due to a little larger value than the theoretical weight loss by dehydration of one molecule (7.46 %).

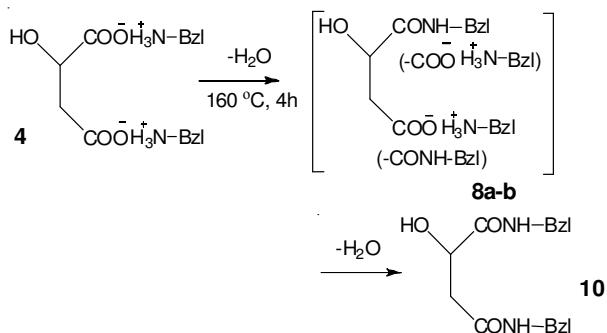
The first dehydration can be explained by an amidation between benzylamine and one carboxyl group of malic acid. Another possible explanation is dehydration from the hydroxyl group at the C2 position of malic acid and hydrogen at the C3 position. However, the dominant formation of N-benzyl-maleimide (**7**) (57 %) compared with N-benzyl-maleimide (**9**) (8 %) explains the preferential amide formation rather than dehydration from the C2 and C3 positions.

The second larger peak emerges after the first peak. The sequential dehydration reaction is suggested to proceed until the second peak emerges. However, until the end of the second endothermic peak, a much greater weight loss (35 %) than the theoretical value (7.46 %) is observed. The second larger peak may include the second dehydration accompanied by degradation to show 35 % weight loss. In the second dehydration step, it is proposed that dehydration takes place with the formation of N-benzyl-maleimide (**7**) by cyclization of malic acid mono-N-benzylamide (**6a, 6b**) as shown in **Scheme-II**. N-Benzyl-maleimide (**7**) seems to be formed by dehydration from the C2 and C3 positions of malic acid mono-N-benzylamide (**8a, 8b**). The remaining carboxyl group may catalyze the dehydration. The later emerging, two smaller peaks in Fig. 4 may indicate a thermal degradation.

Thermal stability of N-benzyl-maleimide (7): The thermal reaction of isolated N-benzyl-maleimide (**7**) was carried out at 160 and 200 °C for 4 h and 240 °C for 1 h, as shown in **Scheme-II**. N-Benzyl-maleimide (**9**) did not form by dehydration from N-benzyl-maleimide (**7**).

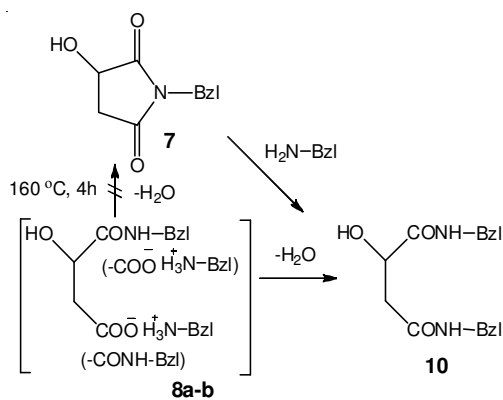
Thermal reaction of DL-malic acid di-(N-benzyl-ammonium) salt (4): DL-Malic acid (**1**) (1.34 g, 10 mmol) and benzylamine (**2**) (2.25 g, 21 mmol) were mixed and heated at 160 °C for 4 h under nitrogen flow. The resulting residue was recrystallized from chloroform to afford malic acid di-N-benzylamide (**10**) (1.52 g, 48 %), as shown in **Scheme-III**

(m.p. 147-148 °C). ^1H NMR (CD_3Cl): δ = 7.62 (2H, br), 7.35 (10H, br), 5.22 (1H, br), 4.38 (6H, m), 2.80 (2H, m); ^1H NMR ($\text{CD}_3\text{Cl}/\text{D}_2\text{O}$): δ = 7.35 (10H, br), 4.38 (5H, m), 2.80 (2H, dd, J = 4.0, 7.0, 15Hz). IR (KBr, ν_{max} , cm^{-1}): 3300 (NH), 1650 (amide I), 1550 (amide II), 730, 710 (Ph). Calcd. (%) for $\text{C}_{18}\text{H}_{24}\text{N}_2\text{O}_5$: C, 69.21; H, 6.45; N, 8.96. Found (%): C, 69.14; H, 6.43; N, 8.91.



Scheme-III

Fig. 5 shows the thermal analysis of the DL-malic acid di-(N-benzyl- ammonium) salt. The first peak is a sharp peak, followed by two broad peaks. The former almost corresponds to the melting of the DL-malic acid dibenzylamine salt. However, a weight loss that commenced before the end of the first peak means that dehydration began during the melt of the crystal. The dehydration corresponds to the first amide formation (Scheme-IV).



Scheme-IV

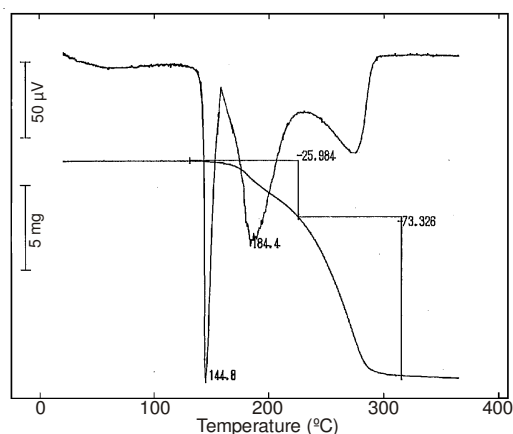


Fig. 5. Thermal analysis of malic acid di-benzylamine salt (**4**). Temperature was programed from 27-370 °C at 10 °C/min under a nitrogen flow (50 mL/min)

Thermal analysis of the DL-malic acid dibenzylamine salt (**4**) was carried out under conditions similar to those used in the thermal reaction (temperature programmed 27-160 °C at 5 °C/min). Results are shown in Figs. 6 and 7. Fig. 6 shows a sharp peak and a following broad endothermic absorption on the DTA curve and fast weight loss from the starting point of melting on the TG curve. Fig. 7 shows a magnified range of Fig. 6. Two endothermic peaks, which seem to be due to melting and dehydration, are evident. The weight loss until this point (15.2 %) almost corresponds to the dehydration of 2.5 molecules (12.6 %).

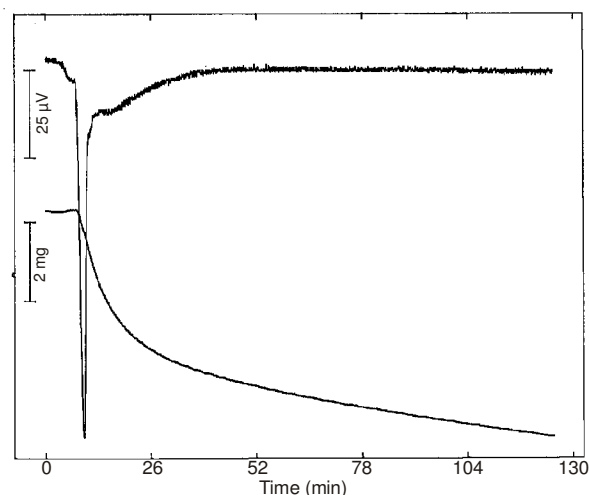


Fig. 6. Thermal analysis of malic acid di-benzylamine salt (**4**). Temperature was programed from 27-160 °C at 50 °C/min under a nitrogen flow (50 mL/min)

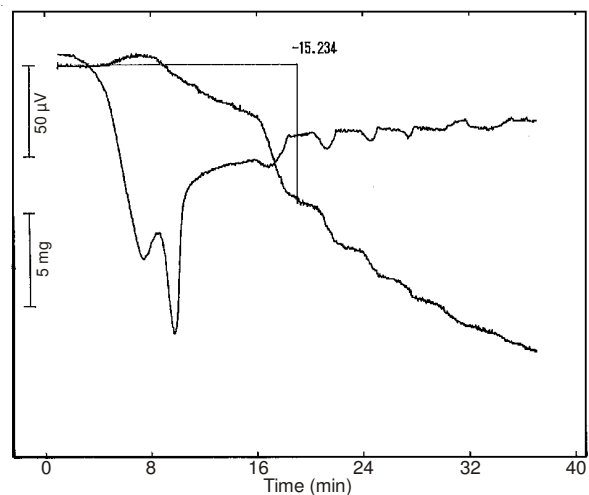


Fig. 7. Thermal analysis of malic acid di-benzylamine salt (**4**). Temperature was programed from 27-160 °C at 50 °C/min under a nitrogen flow (50 mL/min)

Thermal reaction of N-benzyl-malimide (7) and benzylamine (2): N-Benzyl-malimide (**7**) (1.03 g, 5.0 mmol) and benzylamine (**2**) (0.47 g, 5.0 mmol) were mixed and heated at 160 °C for 4 h under nitrogen flow to afford malic acid dibenzylamide (**10**) (1.66 g, 70 %; m.p. 147 °C). Results show that the ring of the N-benzyl-imide (**7**) was opened by nucleophilic attack of benzylamine.

Thermal stability of DL-malic acid di-N-benzyl-amide (10): Fig. 8 shows the thermal analysis of a standard sample of DL-malic acid di-N-benzyl-amide (**10**). Two sharp peaks

TABLE-1
RESULTS OF THERMAL REACTIONS OF DL-MALIC ACID (1), BENZYLAMINE (2) AND RELATED COMPOUNDS

Substrate	Benzylamine (equivalent)	Temperature (°C)	Reaction time (h)	Product	Yield (%)	m.p. (°C)
Malic acid (1)	Non	160	4	Fumaric acid (5)	80	—
Malic acid (1)	Non	180	4	Fumaric acid (5)	88	—
Malic acid (1)	1	160	4	N-Benzyl-malimide (7)	57	116-117
Malic acid (1)	1	160	4	N-Benzyl-maleimide (9)	8	60-62
Malic acid (1)	2	160	4	Malic acid di-N-benzylamide (10)	48	147-148
Malic acid (1)	3	160	4	Malic acid di-N-benzylamide (10)	79	147-148
N-Benzyl-malimide (7)	1	160	4	Malic acid di-N-benzylamide (10)	70	147
N-Benzyl-malimide (7)	Non	160	4	Non	0	—
N-Benzyl-malimide (7)	Non	200	4	Non	0	—
N-Benzyl-malimide (7)	Non	240	4	Non	0	—
Malic acid di-N-benzylamide (10)	1	160	4	Non	0	—
Malic acid di-N-benzylamide (10)	Non	160	12	Non	0	—
Malic acid di-N-benzylamide (10)	Non	190	4	Non	0	—

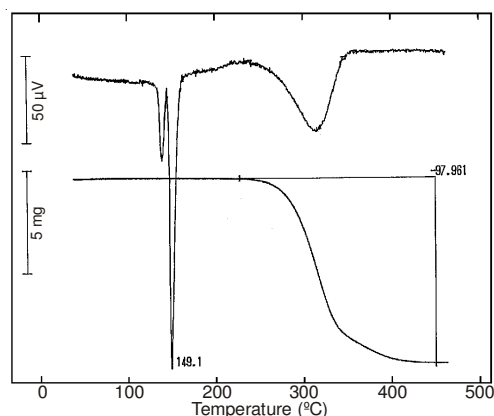


Fig. 8. Thermal analysis of malic acid di-N-benzylamide (10). Temperature was programmed from 27-460 °C at 10 °C/min under a nitrogen flow (50 mL/min)

for the melting of DL-malic acid di-N-benzyl-amide (10) appear on the DTA curve. This compound (10) is stable, with no weight loss up to *ca.* 230 °C.

This research has demonstrated that thermal reactions of DL-malic acid N-benzyl-ammonium salts (3, 4) pass through direct amidation between a carboxyl group and benzylamine (2). Results of the thermal reaction are summarized in Table-1. The reaction pathway demonstrated by this research is shown in Fig. 9. The reaction pathway is supported by thermal analysis data of starting materials and predicted intermediates.

Conclusion

Thermal reactions of DL-malic acid mono-N-benzylamine and di-N-benzylamine salts (3, 4) were carried out to afford the imide and di-N-benzylamide (10), respectively. Once the

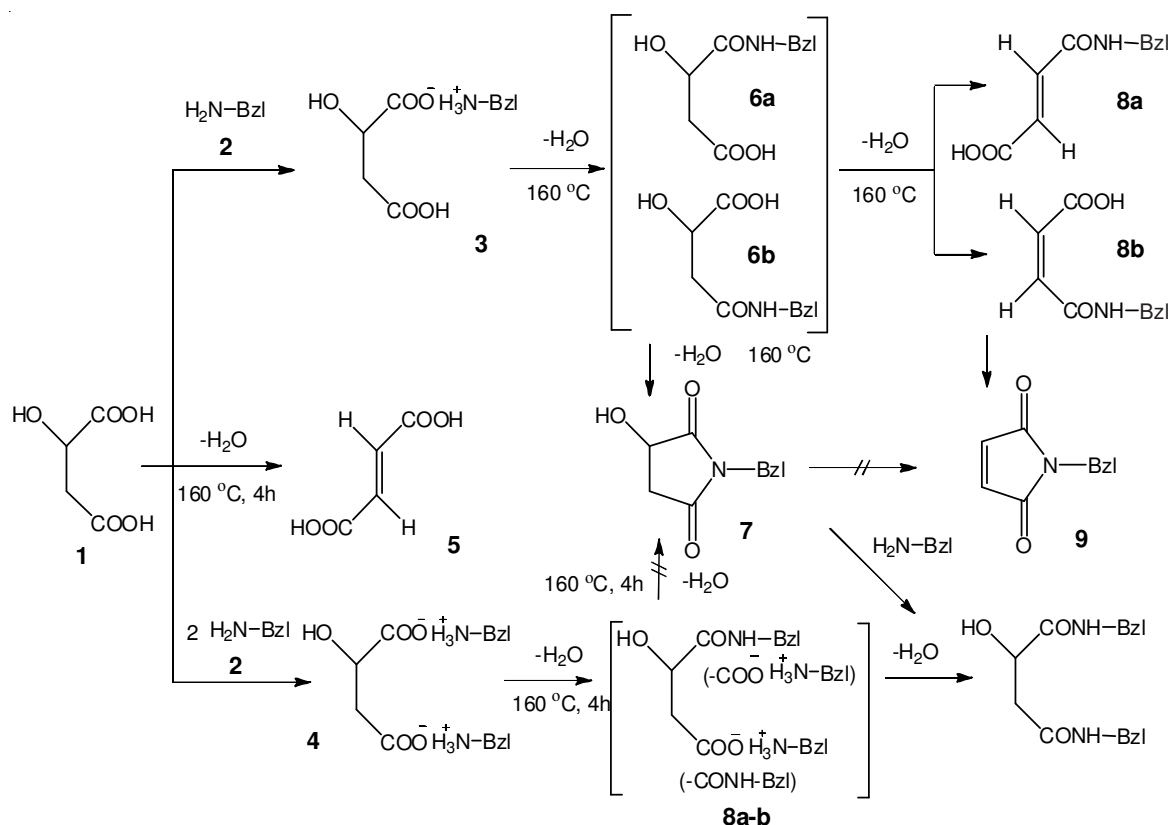


Fig. 9. Summary of plausible pathways in thermal reactions of DL-malic acid (1), benzylamine (2) and related compounds

two carboxyl groups of malic acid are lost upon thermal reaction with benzylamine, dehydration from malic acid does not occur. This was confirmed by the thermal stability of the imide (**7**) and dibenzylamide (**10**). The approach to studying thermal reactions by using thermal analysis will be useful for explaining many organic reaction pathways.

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