HPLC Studies on the Organic Subset of the Oscillatory BZ Reaction. 2. Two Different Types of Malonyl Radicals in the Ce^{4+}−Malonic Acid Reaction

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Applying combined HPLC and NMR techniques, it was found that, besides the already known 1,1,2,2-ethanetetracarboxylic acid (ETA), monomalonyl malonate (MAMA) is also a product of the Ce^{4+}−malonic acid reaction. This is indirect evidence that two different types of organic radicals are formed in the reaction: the alkyl and the carboxylato malonyl radicals. While ETA is a recombination product of two alkyl radicals, MAMA is formed in the recombination of one alkyl and one carboxylato radical.

Introduction

In the first paper of this series Gao et al.[1] reported HPLC studies on the products of the Ce^{4+}−malonic acid reaction. Even before their studies it was known from ESR measurements[2,3] that the very first but highly unstable intermediate of the reaction is an alkyl malonyl radical. Nevertheless, there were no direct experimental data available prior to Gao's work on further reactions of these radicals. It was generally assumed (see e.g. Barkin et al.,[4] Györgyi et al.[5]) that the malonyl radicals react with each other in a disproportionation reaction to form tartronic and malonic acids. Gao et al. have shown that there is no tartronic acid among the first molecular intermediates of the Ce^{4+}−malonic acid reaction; thus, the radical−radical reaction cannot be a disproportionation. Two product peaks were found in the chromatogram, the second of which was identified as 1,1,2,2-ethanetetracarboxylic acid (ETA) a recombination product of the alkyl malonyl radicals. The first peak was not identified, however. The aim of the present work is to identify this unknown product to obtain more information about the mechanism of the reaction.

Experiments

Materials. *Malonic acid* (Fluka puriss) was recrystallized from acetone and acetone/chloroform following the procedures proposed by Noszticzius et al.[6,7] All organic solvents applied in our experiments were of reagent grade. *Tartronic acid* (Hereaus, purum) was purified by extracting its crystals with ethyl acetate; e.g., 2 g of tartronic acid was mixed with 20 mL of ethyl acetate, and the mixture was stirred for 3−4 h. Then the tartronic acid crystals were filtered, washed with chloroform, and dried. The procedure was repeated if the product was not pure enough. The purity of the compounds was checked by HPLC. The *potassium salt* of ETA was produced by the hydrolysis of its ethyl ester. The method of Horri et al.[8] applied previously[1] was slightly modified. To facilitate hydrolysis, Horri et al. boiled the ester in a methanol/KOH mixture. We found, however, that ETA decarboxylates at higher temperatures rather easily. To avoid this, we changed the solvent from methanol to water after the first step of the hydrolysis, and this way we could carry out the process at 40 °C only. (The first step produces a mixture of potassium salts of different half esters, which are not soluble in methanol and are consequently difficult to hydrolyze further in methanol but readily dissolve and hydrolyze in water.) The solid potassium salt of ETA was precipitated from its aqueous solution with methanol. The product can be purified if necessary by dissolving it in water and reprecipitating with methanol. *Free ETA acid in crystalline form* was produced from its potassium salt. One gram of the potassium salt was dissolved in 5 mL of 1 M sulfuric acid saturated with $NaHSO_4$. This mixture was extracted with 20 mL of ether three times. The ether phases were combined, and the ether was distilled. The remaining solid acid was dried in vacuum at room temperature for 10 min to remove the last traces of ether. The free ETA acid readily dissolves in acetone-d_6, which was necessary to study its H-NMR spectrum in that solvent. *The potassium salt of 1,1,2-ethanetricarboxylic acid (ETRA)* was prepared by hydrolyzing triethyl 1,1,2-ethanetricarboxylate (Aldrich 99%) with KOH in a similar way to the case of ETA. A mixture of *monomalonyl malonate (MAMA)* and malonic acid was prepared by forming the half ester of malonic acid with tartronic acid (the OH group of which reacts like an alcohol), applying an excess of malonic acid. In this reaction a twofold excess of malonic acid was applied to avoid the formation of different byproducts. First 150 mL of ether was stirred with 5 g of P_2O_5 for 5 min to remove any water present in the ether. As P_2O_5 gets sticky when it absorbs water, the procedure was performed in a 250 mL Erlenmeyer flask equipped with a large magnetic stirrer bar. The flask was covered with a watch glass to prevent large losses of ether due to evaporation. (The whole procedure was carried out under the hood.) The water free ether then was poured into another Erlenmeyer flask containing 3 g of malonic acid and 0.5 g of tartronic acid in solid form. When these acids were dissolved in the ether, 5 g of P_2O_5 was added and the stirring was continued at laboratory temperature for 30 min. Then the ether phase was transferred again to a new Erlenmeyer flask containing 0.5 g of tartronic acid in solid form. When most of the tartronic acid dissolved, another 5 g of P_2O_5 was added and the stirring was continued again for 30 min. The same procedure

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TABLE 1: Retention Times and H-NMR Shifts for Some Organic Acids[a]

acid	retention time (s)	H-NMR shift (ppm) CH	CH_2	CH_3
injection peak (strong inorganic acids)	480			
monomalonyl malonate (MAMA)	570	5.57	3.60	
1,1,2,2-ethanetetracarboxylic acid (ETA)[b]	600	4.10		
oxalic acid (OA)	600			
mesoxalic acid (MOA)	640			
tartronic acid (TA)	800	4.73		
1,1,2-ethanetricarboxylic acid (ETRA)[b]	840	3.77, 3.80, 3.84	3.65, 3.72	
glyoxylic acid (GOA)[b]	965	5.18		
malonic acid (MA)	1040		3.38	
succinic acid (SA)	1240		2.58	
formic acid (FA)	1385	8.11		
acetic acid (HOAc)	1500			1.98

[a] Retention times were measured under the HPLC conditions applied in this work. H-NMR shifts were measured in acetone-d_6 with TMS as standard. [b] These acids take part in a slow reversible reaction in acetone-d_6, and the characteristic NMR peaks are decreasing in time as a consequence. Thus NMR measurements should be made within 1−2 h after the preparation of the sample. The reaction is reversible because after the acetone-d_6 is evaporated and the NMR sample is dissolved in 0.01 M H_2SO_4, the HPLC shows no chemical change.

was then repeated with the last 0.5 g portion of tartronic acid. (Thus the total amount of tartronic acid used in the synthesis was 1.5 g. The stepwise addition of the tartronic acid was necessary because of its low solubility in ether.) After 30 min of stirring, the ether phase was separated and extracted twice in a separatory funnel with 5 mL of 1 M H_2SO_4 saturated with $NaHSO_4$ to remove all phosphoric acid, most of the tartronic acid, and some of the contaminating byproducts. The ether phase was distilled, and the remaining solid mixture of MAMA and malonic acid was dried in vacuum to remove traces of ether. As the solid product is very hygroscopic, sample solutions for HPLC and NMR were prepared from the solid in the distilling flask on the spot.

H_2SO_4 (Merck 95%), $Ce(SO_4)_2 \cdot 4H_2O$ (Riedel de Haen), and other chemicals used were of reagent grade and used without further purification. All aqueous solutions were prepared with doubly distilled water. The Ce^{4+}−malonic acid reaction was carried out excluding oxygen (by bubbling nitrogen through the reactants and the reaction mixture before and during the reaction).

Analytical Techniques. *H-NMR measurements* were made on a Bruker ARX-200 automatic NMR spectrometer. Deuterated acetone was used as a solvent. Another choice would have been D_2O, but we wanted to avoid the isotope exchange of the mobile hydrogens via enolization, which occurs in D_2O.[9]

HPLC experiments were performed with a Merck Polyspher OA KC column (length 30 cm, diameter 7.8 mm) at 45 °C. Shimadzu HPLC equipment (LC-10AS pump, CTO-10A column oven, SPD-10A dual-wavelength UV detector working at 220 nm) was used. The output signals from the detector were measured by two Keithley 155 microvoltmeters simultaneously. The microvoltmeters were set to different sensitivities. One channel was used with high sensitivity to detect small peaks, and the other was used with low sensitivity to measure the HPLC peaks of the main components. The outputs of the microvoltmeters were connected to an AT 286 computer equipped with a 12 bit AD converter. The sample was injected using a Rheodyne 7010 injector with a 20 μL sample loop. The 0.01 M H_2SO_4 eluent was filtered with a membrane filter (Sartorius, cellulose nitrate, pore size 0.2 μm) and degassed by applying vacuum.

We started our work by repeating the experiments of Gao et al. but under somewhat different conditions (see Figure 1). The main difference was that a more dilute, 0.01 M, sulfuric acid was used as an eluent with a lower flow rate of 0.4 mL/min. (Previously these parameters were 0.02 M and 0.5 mL/min, respectively.) The eluent concentration and the flow rate were changed to get a better column stability and peak resolution. (Earlier it was a problem that the hydrodynamic impedance of the column was increasing continuously using the more concentrated eluent.) Thus the retention times are slightly different from that of the previous work, and the two peaks (1 = unknown, 2 = ETA) are better resolved. In Table 1 retention times of some organic acids are given for the new conditions.

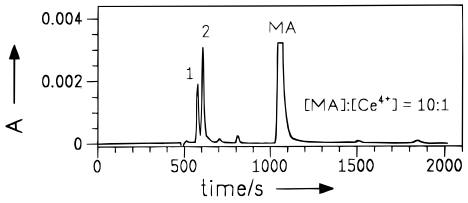


Figure 1. Chromatogram of the products of the Ce^{4+}−MA reaction. Initial conditions: $[Ce^{4+}]_0 = 0.02$ M, $[MA]_0 = 0.2$ M, $[H_2SO_4]_0 = 1$ M. When the reaction was over, the mixture was diluted with water by a factor of 100 and 20 μL of this sample was injected onto the column. *A* is the absorbance.

Preparative HPLC and Concentration of the Samples. To identify an unknown peak in the HPLC chromatogram, the separation of this component on a small scale for further experiments was sometimes necessary. As it is known, HPLC itself can be used to collect relatively pure but small samples of the separated components especially if the original sample injected onto the column is concentrated enough. To obtain more concentrated samples, we extracted with ether a relatively large reaction volume containing the products of the Ce^{4+}−malonic acid reaction and accumulated them into a much smaller volume by evaporating the ether phase. One hundred milliliters of 0.1 M Ce^{4+} (in 1 M H_2SO_4) was added dropwise to 100 mL of 1 M malonic acid (also in 1 M H_2SO_4) in a nitrogen atmosphere. When the reaction was complete $NaHSO_4$ was added to the solution to make it nearly saturated with $NaHSO_4$. Then it was extracted four times with 200 mL of ether. After the first extraction the 200 mL ether phase was poured into the flask of a Büchi rotavapor-R containing 5 mL of 0.01 M H_2SO_4 eluent as an aqueous phase. The ether was distilled, and then the ether phase of the next extraction step was added. After the distillation of the last fraction of the ether, the remaining aqueous phase containing the concentrated sample was used for HPLC experiments. The last traces of ether were removed from the concentrated sample by bubbling nitrogen through it. The same technique was used to transfer organic acids from the aqueous phase to deuterated acetone for NMR measurements. The only difference in the procedure was that the ether phase

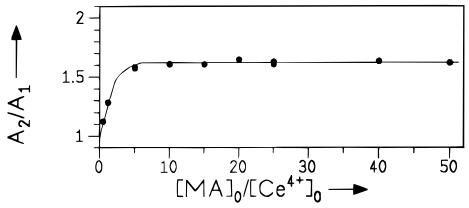


Figure 2. Ratio A_2/A_1 as a function of the ratio $[MA]_0/[Ce^{4+}]_0$. See text for explanation of the symbols.

was evaporated to dryness without adding anything to it. Traces of ether were removed in vacuum.

Results and Discussion

As a first step it was necessary to check whether or not the unknown peak was really a first molecular intermediate produced in the Ce^{4+}−malonic acid reaction simultaneously with ETA. Another possibility would have been that the unknown compound was formed in a further oxidation of ETA by Ce^{4+}, that is in a consecutive reaction. To answer this question, we measured the peak height of the unknown peak 1 (denoted by A_1) and of that for ETA (A_2), as a function of the initial malonic acid concentration while keeping all other initial reagent concentrations constant. If the unknown peak were a product of the Ce^{4+}−ETA reaction, then starting the Ce^{4+}−malonic acid reaction with a higher malonic acid concentration should decrease the yield of the unknown product (this is because most of the Ce^{4+} would react with the excess malonic acid to form ETA and not with the ETA to form the unknown product). Our experiments (Figure 2), however, prove that this is not the case.

As Figure 2 shows, the ratio A_2/A_1 is independent of the malonic acid concentration if the latter is in a great enough excess compared to Ce^{4+}. If the [MA]/[Ce^{4+}] ratio is above 25, then even the absolute values of the peaks themselves are constant within the experimental error. Consequently the unknown intermediate should be produced simultaneously with ETA in a parallel process. This parallel process could be an unknown route of recombination of the known alkyl malonyl radicals or a recombination of some other type of radicals. Anyhow, to solve this problem a chemical identification of peak 1 is necessary. This task turned out to be more difficult than the identification of peak 2. As we know, the second peak was identified as ETA by Gao et al. In that case, however, to assume a recombination between the already known alkyl malonyl radicals was a straightforward hypothesis. In addition ETA was easy to produce from its commercially available ethyl ester. Now, in the absence of a good working hypothesis and without having a synthetic reference standard, it was more difficult to solve the problem. We tried to obtain more information about the unknown compound by heating it and trying to identify its decomposition products with the HPLC technique again. As a first step, a more concentrated solution of the unknown compound was necessary. To this end a larger amount of the reaction products was extracted with ether and accumulated into a small volume of 0.01 M sulfuric acid solution. (The details of the extraction−accumulation method are given in the experimental part.) Twenty microliters of the concentrated mixture was analyzed by HPLC. The chromatogram is shown in Figure 3a.

As can be seen in Figure 3a the peak heights of the unknown product and of ETA were increased by nearly two orders of magnitude compared to those in Figure 1. The ratio of the two peaks was also changed; compared to the ETA the relative amount of the unknown product was increased. This indicates

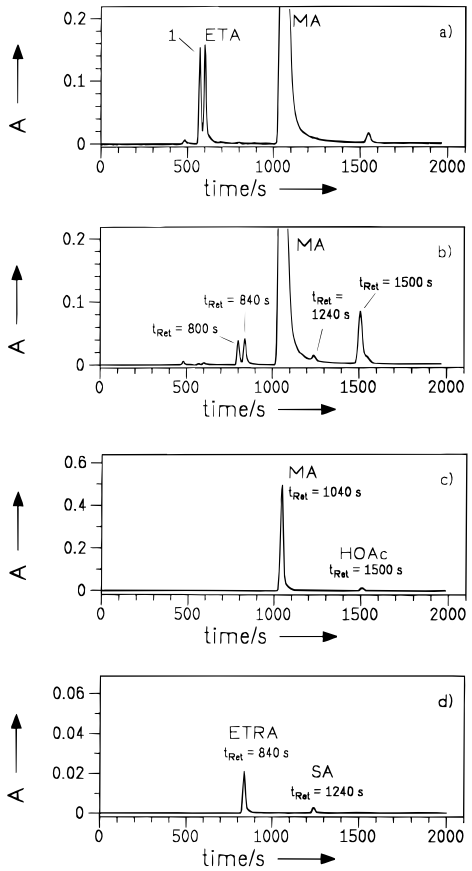


Figure 3. (a) Chromatogram of the products of the Ce^{4+}−MA reaction enriched with the extraction−accumulation techniques as described in the experimental part. (b) The chromatogram of the same products after heating the sample. (c) Decomposition products of malonic acid, $[MA]_0 = 0.049$ M, and (d) of ETA, $[ETA]_0 = 4.8 \times 10^{-4}$ M after the same heating procedure.

that the latter is more soluble in ether than ETA. In a next step a small part of the concentrated sample was heated to 70 °C and was kept at this temperature for 15 min. Then a 20 μL sample was analyzed again by HPLC. The result can be seen in Figure 3b. As the figure shows, peak 1 (the unknown product) and peak 2 (ETA) have disappeared and four new peaks at 800, 840, 1240, and 1500 s appeared instead. To decide which decomposition product is coming from which initial component, we applied the same heating procedure to pure malonic acid and ETA under similar conditions. HPLC chromatograms of the decomposition products are given in Figure 3c (starting material: malonic acid) and in Figure 3d (starting material: ETA). From these experiments and using the retention times of some selected organic acids listed in Table 1, we can draw the following conclusions: (i) The peak at 1500 s can be identified as acetic acid, which is a decomposition product of malonic acid:

$$CH_2(COOH)_2 \rightarrow CH_3COOH + CO_2$$

(ii) The peak at 840 s is due to 1,1,2-ethanetricarboxylic acid (ETRA), which is a decarboxylation product of ETA:

$$(HOOC)_2CHCH(COOH)_2 \rightarrow (HOOC)_2CHCH_2COOH + CO_2$$

(iii) The peak at 1240 s is succinic acid, which appears in a further decarboxylation now from ETRA:

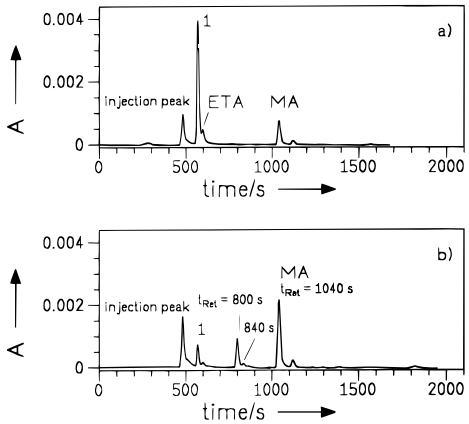


Figure 4. (a) Chromatogram of a fraction separated by the column and collected after the HPLC detector between 550 and 580 s retention time. As can be seen, this fraction contains the first peak mainly. (b) Chromatogram of the same sample after the heating procedure.

$$(HOOC)_2CHCH_2COOH \rightarrow HOOCCH_2CH_2CH_2COOH + CO_2$$

(iv) As we could see the new peaks at 840, 1240, and 1500 s discussed above were decomposition products of components which were already known before. The peak is 800 s is an exception, however; thus, it should be a decomposition product of the unknown compound. According to Table 1 this decomposition product is most probably tartronic acid.[10]

At this point of the investigation we still had no idea about the chemical identity of peak 1, but we had a strong hint that at least one of its decomposition products is tartronic acid. We could not be sure, however, that this is the only decomposition product, as some other could hide under the huge peak of malonic acid (see Figure 3b). Thus we decided to use the HPLC as a preparative device to separate the unknown compound and get rid of most of the malonic acid. Such a separation, however, causes dilution, which means a loss in sensitivity. To avoid such problems, we used again the concentrated sample for this separation. Two hundred microliters of the eluent containing the first peak was collected right after the detector of the HPLC apparatus. To check the purity of the collected sample, 20 μL of it was reinjected onto the column. The resulting chromatogram in Figure 4a shows that most of the ETA and the malonic acid were really removed and the main component remaining in the sample was the unknown compound. In the next step such a separated sample was heated with the same method as before to observe its decomposition products. The chromatogram of these products is shown in Figure 4b. It is an important feature of the chromatogram that, besides the expected appearance of tartronic acid, the originally small peak of malonic acid was increased considerably. Assuming that the products are really tartronic and malonic acids, we could calculate from the peak heights the ratio of these decomposition products. This ratio was 1:1 within the experimental error.

At this point of our research enough data have been accumulated to make some working hypotheses. First, we can assume that one molecule of the unknown compound gives one molecule of malonic acid and one molecule of tartronic acid. Another piece of information is that the unknown compound should be produced in a radical−radical recombination process. In addition it is known from the work of Gao et al. that 1 mol of Ce^{4+} consumes 1 mol of malonic acid. A working hypothesis compatible with all this information is that the unknown compound is formed in a recombination reaction between two different types of malonyl radicals. While one is the already known alkyl malonyl radical, the other is a carboxylato malonyl radical. Thus

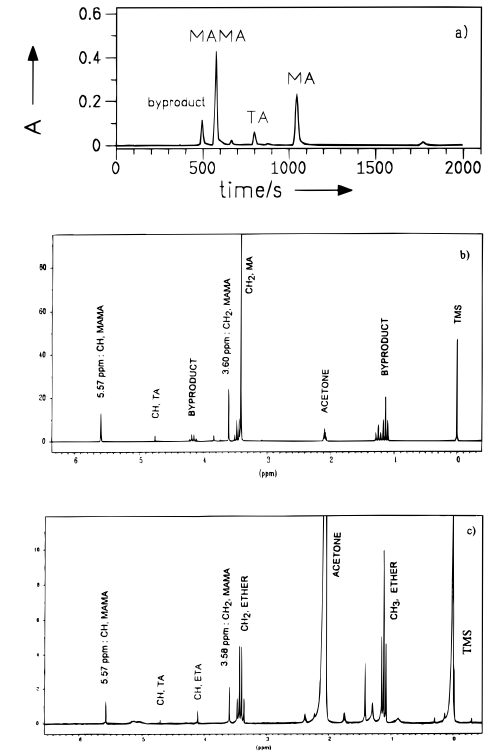
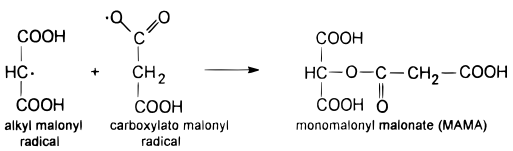
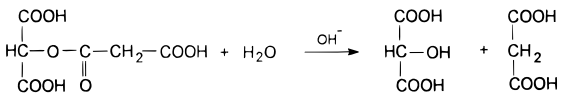


Figure 5. (a) Chromatogram of a mixture containing the synthesized MAMA, malonic acid, tartronic acid, and an unknown byproduct. Preparation of the sample: after the synthesis (see the experimental part) and evaporation of the ether, about 10 mg of the dry mixture was dissolved in 5 mL of 0.01 M H_2SO_4 and 20 μL was injected onto the column. (b) Proton NMR of the same mixture. Preparation of the sample: about 10 mL of the ether phase containing the mixture after the synthesis was evaporated to dryness and was dissolved in 1 mL of acetone-d_6. (c) Proton NMR of the first unknown peak. Preparation of the sample: 5 HPLC fractions between 550 and 580 s were collected (the total volume was about 1 mL). This volume was extracted with 20 mL of ether four times. Before the extraction the aqueous phase was made 1 M for H_2SO_4 and saturated for $NaHSO_4$. The ether phase was evaporated to dryness and was dissolved in 1 mL of acetone-d_6.



Consequently, if our working hypothesis holds, the unknown compound should be monomalonyl malonate (MAMA). MAMA is a half ester of malonic acid, where tartronic acid plays the role of an alcohol. When heated, a hydrolysis of 1 mol MAMA should give 1 mol of TA and 1 mol of MA, just as expected. Without heating, a hydrolysis with KOH at room temperature gave the same products:



The next step was to prove our hypothesis. To this end we had to synthesize the ester MAMA. (As we were not able to find any reference to MAMA in the chemical literature, there was no other possibility. Our method of synthesis is given in the experimental part.) A mixture containing MAMA and malonic acid as main components was produced. The HPLC

chromatogram of this mixture is shown in Figure 5a. The retention time of this synthetic MAMA was the same within experimental error as the retention time of the unknown product found in the Ce^{4+}−malonic acid reaction. To obtain a final piece of evidence, a H-NMR spectrum of the above mixture (Figure 5b) was compared to the H-NMR spectrum of the unknown peak separated by preparative HPLC (Figure 5c). From these spectra it is straightforward that the unknown peak can be really identified as MAMA. In the H-NMR spectrum of MAMA the peak at 3.60 ppm close to the 3.43 ppm peak of the malonic acid can be assigned to the CH_2 protons of MAMA, while the peak at 5.57 ppm is due to the CH proton[11] of MAMA. The intensity ratio of the CH_2 and CH proton peaks is very close to the theoretical 2:1 ratio. (Peaks due to some known and unknown contaminants are also marked in the NMR spectra.)

Conclusion

According to the experimental evidence presented here, the first molecular intermediates of the Ce^{4+}−malonic acid reaction are recombination products of two different types of malonyl radicals. Our HPLC and NMR studies on the reaction products show that two alkyl malonyl radicals form one molecule of ETA and that one alkyl and one carboxylato malonyl radical form one molecule of MAMA. An important consequence of these results is that, besides the already known alkyl malonyl radicals, carboxylato type malonyl radicals are also produced in the Ce^{4+}−malonic acid reaction. We estimate that in the present experiments about 15% of the malonyl radicals were carboxylato type while the rest were alkyl radicals. The importance of these findings to the whole BZ mechanism, however, is not clear presently. The ratio of alkyl and carboxylato type radicals for different variants of the BZ reaction is not known either. Further research is planned to answer these questions.

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(10) The measured retention times were very reliable and reproducible within a few seconds or sometimes less; thus, they could be used for chemical identification rather well. Nevertheless, a retention time alone is usually not enough to identify an unknown compound with absolute certainty; e.g., as Table 1 shows, retention times for ETA and oxalic acid are the same within experimental error. Therefore some chemical reactions of the unknown compounds, like the decarboxylation reactions applied here, or the spectrophotometric measurements applied by Gao et al. can be very helpful to provide additional information in critical cases. In addition to the hydrolysis and decarboxylation reactions, we used H-NMR for this purpose.

(11) *High Resolution NMR Spectra Catalog*; Bhacca, N. S., Hollins, D. P., Johnson, L. F., Eds.; Varian Assoc. and National Press: Palo Alto, CA, 1963; Vol. 2, spectrum 546. This is a H-NMR spectrum of the diethyl ester of the tartronic acid, where the alcoholic hydroxyl group of the tartronic acid is esterified by acetic acid. Thus the CH proton in this compound is in a very similar environment to that of the CH proton in MAMA. The assignment for this proton according to the reference book is 5.52 ppm in $CDCl_3$, while for the CH group of MAMA this is 5.57 ppm in acetone-d_6 according to our measurements.

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