

The Chemistry of Dicarboxylic Acids

I. A New Method for Quantitative Determination of Malonic Acid

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Malonic acid interacts with acetic acid anhydride to form a coloured compound on which it has proved possible to base a method for quantitative determination of the acid. A description is given of an analytical method depending on the absorption exhibited at 345 m μ by the compound produced. The influence of the presence of oxalic acid, succinic acid and citric acid on the reaction has been examined. In addition the ultraviolet and infrared spectra of the coloured compound formed are given.

Malonic acid can be determined quantitatively by direct titration; gravimetrically after precipitation of its barium salt¹; by oxidation in sulphuric acid solution by means of potassium permanganate², sodium dichromate³ or ceric sulphate⁴; and by measuring the areas of the coloured spots resulting from paper chromatography either of the acid or its salts.

The object of the present investigation is to develop a spectrophotometric method — as far as possible a method specific to malonic acid and enabling quantitative determination of the acid even when present in relatively small quantities. The interest in determining malonic acid arises in connection with ozonolysis of unsaturated fatty acid methyl esters. As in natural fatty acids the double bonds are often placed with a distance of three carbon atoms the ozonolysis gives rise to formation of malonic acid as an end product in the oxidation processes. The amount of malonic acid produced corresponds to the content of fatty acids with this configuration. A suitable chemical reaction on which to base the analytical method was found in the interaction between malonic acid and acetic acid anhydride which results in the appearance of a yellowish-green to yellowish-brown colour which — especially on dilution with glacial acetic acid — shows strong fluorescence⁵.

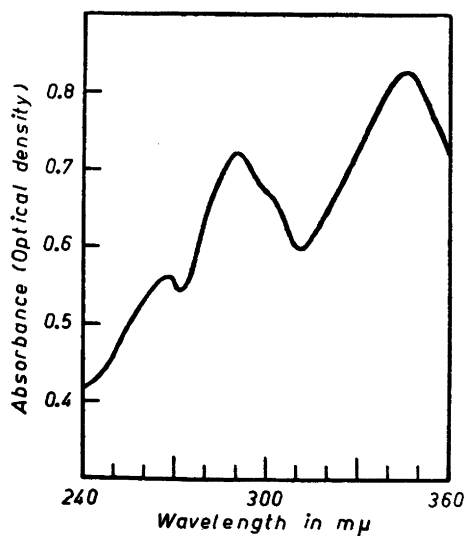


Fig. 1. The ultraviolet spectrum of "malonic colour".

PRELIMINARY INVESTIGATIONS

The ultraviolet spectrum of the coloured compound formed when crystalline malonic acid is heated with acetic acid anhydride shows a distinct absorption maximum at 290 $m\mu$ and likewise at 345 $m\mu$ (Fig. 1). The peak at 345 $m\mu$ is the highest and also the most stable, for which reason it was chosen as the basis of the present work. If D is the optical density measured in a 1 cm layer and c the corresponding concentration in mg/ml of the malonic acid used for the experiment, the reaction coefficient, α , is defined by the equation

$$\alpha = \frac{D}{c} \quad (1)$$

In order to examine the influence of the reaction time on α , a series of eight experiments at different but constant temperatures was made. The temperatures and quantities of malonic acid used appear from Table 1.

The reaction vessel used was a 250 ml round-bottom flask with a stoppered opening for drawing samples and with a reflux condenser, through which a thermometer was passed into the flask. The flask was completely immersed in a thermostat consisting of a 5 l beaker with oil and a contact thermometer with relay, heating element (150 W) and magnetic stirrer; the beaker was insulated by means of a 1 cm layer of cotton fabric.

To start the experiment 50 ml acetic anhydride (analytical grade, Merck) was transferred by means of a pipette to the preheated reaction flask. After a period of 20 min (15–20 min were necessary to establish constant temperature) the sample of crystalline malonic acid (analytical grade, Merck) was introduced by means of a 1 ml weighing cup, and the flask was swirled slightly to effect rapid solution of the sample. One minute after the introduction of the sample the thermometer reading was 0.5°C below the reaction

Table 1. Summary of the experiments at eight different temperatures (cf. Fig. 2).

Experiment	Temperature, °C	Malonic acid, mg
A	69.9	78.5
B	79.7	58.5
C	89.2	43.1
D	99.5	33.9
E	111.6	20.3
F	120.2	25.0
G	129.3	16.4
H	138.3	12.2

temperature, and after altogether 10 min the temperature had again reached the desired level.

During the individual experiments about 20 samples were drawn and, after dilution with glacial acetic acid (analytical grade, Merck), measured against corresponding mixtures of acetic acid anhydride and glacial acetic acid in a Beckman spectrophotometer model DU.

The reaction coefficient was calculated for the individual samples, and the results of the eight experiments are represented graphically in Fig. 2. It appears from the figure that the intensity of the absorption at 345 $m\mu$ increases with increasing temperature and reaction time. The only exception from this observation is graph H at which the temperature is 138.3°C. The reason is

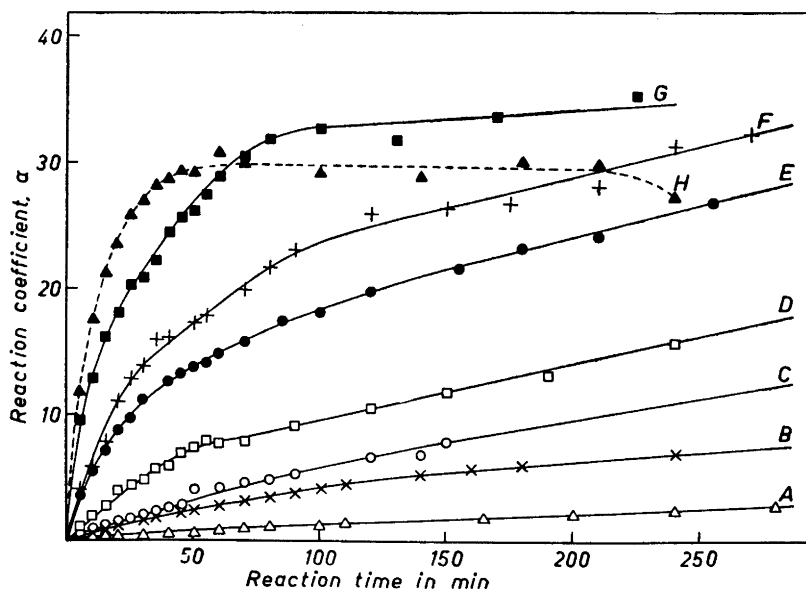


Fig. 2. The influence of the reaction time on the reaction coefficient at different temperatures (cf. Table 1).

that malonic acid decomposes at high temperatures to form acetic acid and carbon dioxide. The pure acid decomposes when heated to its melting point (135.6°C), while in solution the reaction often occurs at a lower temperature.

DEVELOPMENT OF ANALYTICAL METHOD

The object of the first experiments was to ascertain whether reproducible reaction coefficient values could be obtained at 100.0°C, 110.0°C, and 125.0°C.

The thermostat consisted of an enamelled tank, thoroughly insulated with glass wool and containing about 17 litres of oil. It was fitted with a stirrer, a contact thermometer with relay and an adjustable heating element. The heating element operated for about two thirds of the time, and the temperature was kept constant within $\pm 0.1^\circ\text{C}$. The thermostat was arranged to hold four reaction vessels (test tubes).

By means of a pipette 5 ml acetic acid anhydride was transferred to each of the tubes, and after a preheating period of 20 min malonic acid was introduced by means of a little weighing cup into three of the vessels, the fourth serving as a blank. The reaction was allowed to proceed for 60 min and was then stopped by transferring the reaction vessels to a beaker with ice water. The four samples were now transferred quantitatively into 100 ml volumetric flasks by means of glacial acetic acid (the samples which had been kept at 125.0°C were further diluted five times) and measured in a Beckman spectrophotometer model DU (wavelength: 345 $m\mu$, slit width: 0.18 mm with hydrogen lamp, and a 1 cm cuvette).

According to this procedure each experimental series provides three analytical results, and in Table 2 three series of this kind are given for each of the temperatures chosen. It will be seen that reproducibility is best at 110.0°C, the individual experimental series resulting in identical reaction coefficient values, although the thermostat used was not sufficiently stable to ensure that results obtained from day to day were in complete agreement. At 125.0°C the tendency of malonic acid to decarboxylation seems to be so pronounced that it is impossible to obtain reliable results, even though the relatively high value of the reaction coefficient, α , would enable a greater accuracy when

Table 2. Values of the reaction coefficient α at 100.0°C, 110.0°C and 125.0°C.

No.	100.0°		110.0°		125.0°	
	Malonic acid mg	α	Malonic acid mg	α	Malonic acid mg	α
1	10.5	3.77	11.6	6.43	13.1	18.6
2	14.2	3.95	13.2	6.39	10.8	18.9
3	11.3	4.15	8.6	6.38	10.2	18.0
4	12.3	3.97	14.6	6.73	11.3	17.0
5	11.5	4.02	11.1	6.77	12.9	17.5
6	11.4	3.96	12.7	6.69	12.1	19.0
7	11.4	3.66	13.6	6.54	6.8	17.7
8	12.6	3.66	11.4	6.60	11.9	17.3
9	13.0	4.22	11.6	6.57	10.5	18.7

working with small quantities of malonic acid. The lack of reproducibility of the results obtained at 100.0°C is due to the fact that at this temperature the viscosity of the thermostat oil is so high as to prevent sufficiently uniform transmission of heat to the four reaction vessels — this resulted in highly varying α values even within the same experimental series.

The conclusion to be drawn from this is that it is possible to perform the analysis at 110°C but that in case the thermostat available is not an extremely good one, it is necessary to include a control sample with malonic acid in each experimental series.

The influence of other compounds on the reaction

It is necessary to know whether other compounds which may be present in the sample to be analyzed have any influence on the reaction producing the coloured compound. It was examined whether the presence of oxalic acid and succinic acid, impurities which occur frequently together with malonic acid (for instance after chromatographic separation) will result in variations in the reaction coefficient values found. Table 3 shows that small quantities of these compounds (analytical grade, Merck) do not interfere in the malonic acid determination.

Table 3. The influence of the presence of oxalic acid and succinic acid on the malonic acid determination.

No.	Oxalic acid mg	Malonic acid mg	Succinic acid mg	α
1	2.9	11.2	1.55	7.14
2	—	10.3	—	7.10
3	5.1	9.7	4.4	7.01

In another experiment 11.7 mg citric acid monohydrate (analytical grade, Merck) was treated according to the described analytical method. This gave rise to a uniform absorption ($D =$ about 0.1) between 240 $m\mu$ and 360 $m\mu$, while a mixture of equal parts of citric acid and malonic acid resulted in a smaller absorption throughout the range 240—360 $m\mu$ than that produced by malonic acid alone. It is thus impossible to determine malonic acid in mixtures of malonic acid and citric acid without a preliminary separation, and samples to be analyzed must therefore in each individual case be examined to find out whether such a separation is necessary prior to the malonic acid analysis. The control sample should as far as possible be of the same composition as the mixture in which the content of malonic acid is to be determined.

THE RECOMMENDED ANALYTICAL METHOD

Use a thermostat with vigorous stirring and containing a liquid with good heat transmission characteristics. Adjust the temperature at 110°C and maintain this temperature within $\pm 0.1^\circ\text{C}$. As reaction vessels use three or

Table 4. Two series of malonic acid determinations. (Samples diluted to 100 ml before the measurement.)

Malonic acid mg	Optical density, D	α	Malonic acid found, mg
11.0	0.814	—	10.9
11.1	0.828	7.46	—
10.4	0.777	—	10.4
10.9	0.791	—	10.9
11.0	0.797	7.25	—
9.7	0.698	—	9.6

more absolutely identical test tubes (length 12 cm, external diameter 2 cm, Pyrex glass), and keep the test tubes half-way immersed in the thermostat liquid during the process.

By means of a pipette transfer 5 ml acetic acid anhydride, analytical grade, to each test tube. Place the test tubes in the thermostat at 0.5 min intervals. After a preheating period of 20 min transfer, by means of small weighing cups, the weighed samples (plus one standard sample with a known content of malonic acid) to the test tubes. One test tube is used for a blank determination, and in this test tube a weighing cup without malonic acid is placed. Exactly 60 min after the introduction of the samples into the reaction vessels remove the latter from the thermostat and place in a beaker with ice water. Allow the solutions to cool down to room temperature, and then dilute with sufficient glacial acetic acid, analytical grade, to produce solutions suitable for measurement in a spectrophotometer at 345 $m\mu$. On the basis of the known content of malonic acid in the standard sample determine the reaction coefficient, α , using this coefficient and (1) for the subsequent calculation of the malonic acid content in the samples examined (*cf.* Table 4).

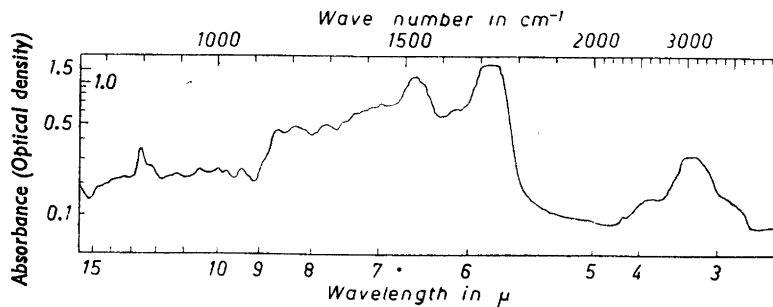


Fig. 3. The infrared spectrum of "malonic colour".

SPECTRA OF THE "MALONIC COLOUR"

The ultraviolet spectrum (Fig. 1) was obtained by means of a recording spectrophotometer (Beckman, DK-2, scanning time: 6—7 minutes); the material used being 12.2 mg malonic acid treated according to the above analytical method. After the peak at 345 $m\mu$ the absorption decreases uniformly with increasing wavelength ($D_{400\ m\mu} = 0.10$, $D_{450\ m\mu} = 0.02$, and $D_{500\ m\mu} = 0.00$). When the coloured solution is allowed to stand (after dilution with glacial acetic acid) the peak at 345 $m\mu$ becomes gradually smaller, but the change produced in the course of 24 h is hardly measurable; the reaction is not materially influenced by daylight.

The infrared spectrum (Fig. 3) was obtained from 1.1 mg "malonic colour" in 300 mg potassium bromide (Perkin-Elmer spectrophotometer, model 21).

The "malonic colour" was prepared by treating 1 g malonic acid with 50 ml acetic acid anhydride for 60 min at 110°C. The acetic acid anhydride was distilled off in vacuum, and the residue was dissolved in methyl acetate, filtered and evaporated to dryness in vacuum at a temperature not higher than 70°C, the treatment of the residue being repeated once.

The spectrum shows that the compound in question is a polymer with a considerable number of C=O bonds; it seems moreover to contain acid and ester groups, while the presence of more or less modified aromatic rings is likely.

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