On the Detection of Malic Acid by means of Brucine.

By

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When on an object glass some grains of solid brucine are added to a drop of an aqueous solution of malic acid — either *l* or *dl* — we observe through the microscope the formation of a swarm of quite typical crystals, which, in our opinion, may be useful for the identification of malic acid.

The reaction product is the normal l-brucine l-malate, $(C_{23}H_{26}N_2O_4)_2HOOC \cdot CH(OH) \cdot CH_2COOH$. The acid salt does not crystallize very readily, so it is essential that an excess of brucine, at least about 7 parts of brucine to one part of malic acid, be used. The normal salt of l-brucine and d-malic acid hardly crystallizes at all; so when we start from a racemic malic acid, only the l-fraction is active in this way.

As a rule the crystals obtained are large, very clearly cut, trapeziform, thin plates as shown by fig. 1. Both angles at the base are 49°. Sometimes they are doubled, forming hexagons with four angles of 131° and two angles of 98°, at other times a parallelogram will appear with angles of 49° and 131°. When their growth is disturbed, such as at the border of the drop, twins are formed. The crystals are very highly birefringent, always showing an extinction at right angles to the longest dimension. They can attain quite a considerable size; we often found crystals as large as 0,5 mm. Even when prepared without any special care, crystals of 50—100 μ will appear.

When the amount of malic acid is rather small, it may be advisable to heat the preparation on the object glass to a just beginning crystallization at the border of the drop and then to inoculate the middle of the drop with the border crystals in order to obtain a free growth. However this heating should always be carried out with the utmost precaution in order to prevent the decomposition of malic acid into fumaric acid or maleic anhydride.

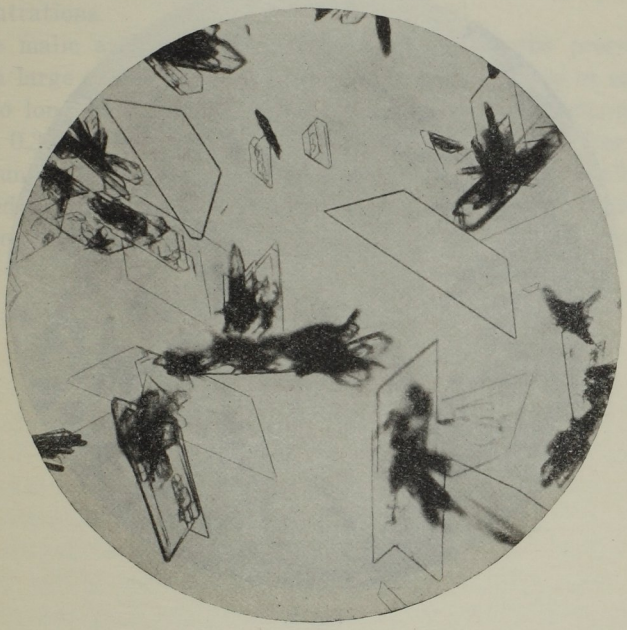

Fig. 1.

Fig. 2 shows a border crystallization and some crystals obtained in this way. They can be easily distinguished from the recrystallization product of the brucine, which appears as a mossy growth on the original grains.

When l-malic acid is present alone, the typical trapeziform crystals will appear — without any heating or increase of concentration on the object glass — when the concentration of the aqueous solution exceeds about 0,3% (1 : 300). So when working

with a micro-drop of 0,010 cc, the sensitivity of the reaction may be said to be 30 γ [1] or in FEIGL's formula:

$$30\ (M)^{0,010}$$

When the concentration of the malic acid is below 0,3%, it is advisable to concentrate it beforehand by a very careful evaporation.

Fig. 2.

Up to now we found no other acids, which give quite the same reaction. Fumaric acid and oxalic acid also give typical crystallizations with brucine, which are in some respects like that of malic acid. However, the crystals obtained from fumaric acid are smaller, they show other angles, are never trapeziform and do not show an extinction at right angles but a decidedly oblique one. In the same way oxalic acid gives small hexagons, never

[1] We wish to point out that this is by no means the smallest amount, which can be detected in this way.

trapeziform crystals. Maleic acid gives rise to the formation of prisms, tartaric and malonic acid very fine needles or prisms, citric acid small crystals of a rather indefinite shape; succinic acid and the lower fatty acids give hardly any reaction at all. Lactic acid, which is of special importance because it will come together with malic acid in the course of STAUDINGER's systematic organic analysis, gives a crystallization product consisting of very fine and small needles and that only at relatively high concentrations.

The malic acid reaction is not disturbed by the presence of even a large excess of lactic acid or of sugars, glucose or saccharose, so long as the concentration of the malic acid remains over about 0,3%. Free mineral acids always interfere, also free acetic acid, unless it is neutralized by adding an excess of brucine. It is evident that the alkali salts of malic acid cannot give the reaction unless decomposed beforehand by means of acetic acid.