

linkages and our problem is now to determine what nitrogen linkages it does contain.

The general results of this paper are as follows:

1. Eight generalizations have been given which cover pretty well the conditions under which organic nitrogen does or does not add hydrogen chloride stoichiometrically.

2. About seventy per cent of the total nitrogen in gliadin, eight per cent in edestin and one hundred per cent in zein does not add hydrogen chloride stoichiometrically.

3. All nitrogens having the peptide linkage, —CONH—, should add hydrogen chloride stoichiometrically.

4. There are apparently no peptide linkages in zein.

\* This work is part of the programme now being carried out at Cornell University under a grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University.

### THE TAUTOMERIC FORM OF MALIC ACID\*

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The specific rotations of the plane of polarized light of solutions of the so-called laevo-rotary malic acid and its sodium salt change with increasing dilution from being dextro-rotary to being laevo-rotary, as may be seen from the data of Woringer<sup>1</sup> and of Thomsen<sup>2</sup> in table 1.

TABLE 1

MALIC ACID			SODIUM MALATE		
<i>C</i>	<i>M</i>	( <i>a</i> ) <sub>D</sub>	<i>C</i>	<i>M</i>	( <i>a</i> ) <sub>D</sub>
63.2	4.71	+1.788°	87.76	4.93	+3.82°
45.68	3.41	+0.600	83.94	4.71	+3.35
36.00	2.68	+0.003	76.08	4.27	+2.37
26.73	1.99	-0.589	65.34	3.68	+0.68
20.92	1.56	-1.050	57.14	3.21	-0.78
15.87	1.18	-1.228	46.78	2.62	-2.54
10.65	0.79	-2.346	36.51	2.05	-4.24
7.52	0.56	-2.479	16.56	0.93	-6.94

In this table *C* is number of grams of solute per 100 cc. of solution, *M* is the number of mols of solute in 1000 cc. of solution and (*a*)<sub>D</sub> is the specific rotation calculated from the equation

$$(\alpha)_D = \frac{a_D \times V}{l \times C}$$

where  $a_D$  is the observed rotation of the plane of polarized sodium light,  $l$  is the length of the tube in decimeters, and  $C$  is the number of grams of solute contained in the volume  $V$  of the solution.

Addition of salts, acids and bases tend to make the laevo-rotary acid more dextro-rotary. With rising temperature, the pure acid and also the solutions become more laevo-rotary. These changes cannot be due to electrolytic dissociation, because the effect of hydrochloric acid is quite marked up to relatively high concentrations and it would take relatively little acid to force back the dissociation of malic acid to a negligible value. Another reason is that we get a similar change with the concentration with malic ester in alcoholic solution.

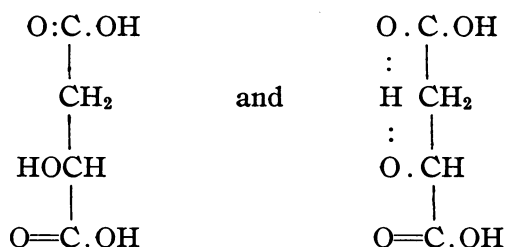
The changes cannot be due to changing polymerization, because freezing-point determinations show no signs of polymerization even at relatively high concentrations. More important are the observations by Lowry<sup>3</sup> on the tartrates that there is no direct relation between the degree of polymerization of a solute and its optical rotatory power. Lowry found that there are solutions of ethyl tartrate in various solvents in which the ester is polymerized increasingly with concentration and the specific rotation changes but little with change in concentration; while in other solvents the rotations change markedly with dilution although there is little or no evidence of polymerization.

The effects cannot be due to hydrates because we get them in alcohol and in acetone as well as in water and with the ester just as well as with the free acid. Rising temperature has the same qualitative effect as decreasing the concentration. Since rising temperature must decompose hydrates, we should have to conclude that there are fewer hydrates in dilute solutions than in more concentrated ones, which does not seem probable. Further, we can get the temperature effect with the anhydrous acid, where there can be no hydrates.

The change with the concentration cannot be due to the reversible formation and decomposition of a lactone of the ordinary type because we get the effect with ethyl malate as well as with malic acid. The change cannot be due to a reversible conversion of laevo-malic acid into dextro-malic acid, because then a solution of equivalent amounts of dextro- and laevo-malic acids would become optically active on addition of salts, acids and bases. Hydrochloric acid or sodium hydroxide imparts no activity to a solution of *dl*-malic acid. The changes on adding electrolytes to a solution of dextro-malic acid are equal and opposite in sign to the changes in laevo-malic acid under the same conditions.

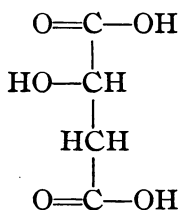
We have thus eliminated ionization, polymerization, hydrate formation,  $\beta$ -lactone formation and formation of *d*-malic acid from *l*-malic acid as playing important parts in the change of optical rotation of malic acid with concentration, though some or most of these may play a minor part.

This leaves only a tautomeric change as a possibility and this is the view taken by Arndtsen, Armstrong and Walker, Lowry, de Malleman, Longchambon, Darmois and others. There is no question but that the changing rotation is due to the hydrogen of the hydroxyl group in the malic acid. If we replace it by a methyl group, the optical rotation is practically independent of the concentration. If we form a uranyl complex, we also eliminate the change of the rotation with the concentration pretty completely. Lowry has reached the same conclusion. Lowry would make the two formulas:

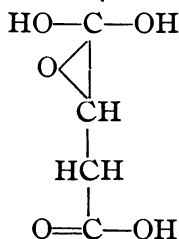


In the second formula the hydroxylic hydrogen is coördinated internally with the carbonyl group, the hydrogen being bivalent. We rather prefer to retain normal valences, and we, therefore, postulate that, in the case of laevo-malic acid, we have the following two formulas

Form I (Laevo)



Form II (Dextro)



Form I is the dilute solution form while Form II will predominate in concentrated solutions. In order to account for anomalous dispersion, we postulate that the two forms have different rotatory dispersion. There is, of course, no way of distinguishing between Form II and the Lowry type, because the two are merely different ways of saying the same thing.

Our Form II has two uncommon features. In the first place it contains two hydroxyl groups attached to the same carbon; but we have that in chloral hydrate. In the second place there is an ethylene oxide oxygen linkage. This might be called an alpha lactone with the water not split off. This formation of a ring structure is believed to account for the reversal of the sign of rotation. It is well known that the formation of the lactide from lactic acid, while not a lactone formation in the same

sense, does involve a change from a chain to a ring structure and does show the lactide to possess an opposite and remarkably enhanced rotation when compared with the original lactic acid. For many years Holmberg has been developing evidence to show that the hydrolysis of *l*-chloro-succinic acid to *l*-malic acid involves an intermediate dextro-rotatory lactone, the three substances being of the same relative configuration. That such a dextro-rotatory lactone of malic acid should yield a laevo-rotatory malic acid on addition of water makes it seem reasonable that the reverse change might take place, causing the *l*-malic acid to become rotatory when a ring structure was produced.

The general results of this paper are as follows:

1. The changes in optical rotation and the anomalous dispersion of solutions of *l*-malic acid in solution are due to the presence of two tautomeric forms in dynamic equilibrium.
2. The laevo-rotatory form is the ordinary malic acid while the dextro-rotatory form is an ethylene oxide resulting from the migration of the hydrogen of the hydroxyl on the asymmetric carbon to the carbonyl oxygen of the carboxyl group.
3. It is immaterial whether one postulates the ethylene oxide formula or the coördinated form preferred by Lowry. The ethylene oxide formula has the merit of only requiring normal valences.
4. If the hydrogen of the hydroxyl on the asymmetric carbon is replaced by a methyl group which would be not expected to migrate readily, the optical rotation with changing concentration becomes negligible.
5. Since the phenomena occur just as well with the ester as with the free acid, the migrating hydrogen cannot be the carboxyl hydrogen.
6. The uranyl complex with malic acid does not show changing rotation with changing rotation.
7. The abnormal rotation and the anomalous dispersion cannot be accounted for satisfactorily on the basis of polymerization, electrolytic dissociation hydrates, lactones of the ordinary type, or of a structural reversal of the asymmetric carbon atom.

\* This work is part of the programme now being carried out at Cornell University under a grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University.

<sup>1</sup> Woringer, *Z. physik. Chem.*, **36**, 636 (1901).

<sup>2</sup> Thomsen, *J. prakt. Chem.*, **143**, 753 (1887).

<sup>3</sup> Lowry and Cutter, *J. Chem. Soc.*, **125**, 1465 (1924).