were recovered, and a small amount of what appeared to be aniline hydrochloride.

4. Six hours at 250°. Complete decomposition of the quinazolone into aniline, ammonia and carbon dioxide. Crystals of ammonium chloride separated in the tube.

2-Methyl-4-quinazolone and Benzoyl Chloride.—Ellinger and Riesser[1] found that, by the action of benzoyl chloride upon 4-hydroxyquinoline, 4-chlorquinoline could be obtained. It therefore seemed worth trying this reagent upon 2-methyl-4-hydroxyquinazoline (2-methyl-4-quinazolone), in the hope that here too the hydroxyl group might be replaced by chlorine, since all other methods so far used to get this chlorquinazoline have failed. Experiments directed to this end, however, proved unsuccessful. When a mixture of five grams of the quinazolone and 50 cc. benzoyl chloride were heated together, the quinazolone slowly dissolved, and in three hours a clear solution was obtained, but no chlorquinazoline could be recovered from the dark liquid.

NEW YORK CITY.

[FROM THE LABORATORY OF BIOLOGICAL CHEMISTRY OF WASHINGTON UNIVERSITY, ST. LOUIS.]

STUDIES ON MALIC ACID. I. THE TRANSFORMATION OF MALIC ACID TO SUGAR BY THE TISSUES OF THE MAPLE (ACER SACCHARINUM).

BY W. R. BLOOR.

Received February 2, 1912.

Although malic acid is one of the most widely distributed plant acids, very little is known definitely of its chemical relations to the other organic plant substances, or of its function in the plant organism. Together with tartaric and citric acids, malic acid is generally regarded as a product of "intramolecular" respiration and, like them, is most closely related to glucose. In certain members of the Crassulaceae—thick-leaved desert plants which have adapted themselves to life in places where moisture and carbon dioxide are scarce—it has been demonstrated by Kraus[2] that there is an accumulation of malic acid at the expense of the sugar during the night, and a transformation of malic acid into sugar during the day. By this process of molecular rearrangement the plant is supplied with energy during the night while the precious carbon dioxide is preserved for use during the succeeding day. These same plants use malic acid also as a form of reserve material, the calcium malate deposited often amounting to half the dry weight of the leaf. The disappearance of malic acid accompanied by an increase of sugar is well known

[1] *Ber.*, 42, 3336 (1909).

[2] Kraus, *Abhandl. Naturforsch. Gesellsch. Halle*, 16, 393 (1886).

to take place during the ripening of fruits which contain malic acid, *e. g.*, the apple. In neither case is the transformation quantitative, considerably more malic acid disappearing than can be accounted for by the sugar formed. It became of interest to know whether the ability to transform malic acid to sugar is common to all plants which produce malic acid.

Maple sap contains malic acid in the form of neutral calcium malate to the extent of about 1% of the sugar content, and since a supply of this material—the so-called "sugar sand"—became available[1] it was decided to determin whether the tissues of the maple could effect the above transformation. Presumably the power to bring about the change would be most marked in those parts of the tree which were showing signs of life at the time of the sap flow—the swelling buds and the shoots which carried them—and these tissues were accordingly used for the experiment.

The malic acid preparations used were:

1. Neutral calcium malate obtained by twice recrystallizing the "sugar sand."

2. Acid calcium malate, obtained by precipitating half the calcium from the neutral malate by means of oxalic acid, and recrystallizing the product.

3. Malic acid—by removal of all the calcium from the crude neutral malate by oxalic acid. This product contained a small amount of cane sugar as impurity.

4. Pure malic acid—by precipitation of all the calcium from the calcium malate of preparation 1.

Well developed, healthy shoots of *Acer Saccharinum*, of the previous year's growth, were collected at the time when the leaf buds first showed signs of swelling and later when the buds had opened and before much growth had taken place. The buds were separated from the shoots and both treated as follows: After a preliminary washing with cold water, the tissues were chopped as fine as possible, ground in a meat mill till well disintegrated, and used at once for the experiments, which were carried out as follows.

Five to six grams of the fresh tissue were weighed into a 350 cc. Erlenmeyer flask, 200 cc. of boiled water added and then enough of the malic acid preparation to make a 0.5% solution was measured in with a pipet. Ten cubic centimeters of xylene were added as a preservative, the mixture well shaken, loosely stoppered and placed in a south window. A mixture containing all the substances except the malic acid was similarly treated and used as a blank. After exposing to sunlight for a suitable time, the acidity and the sugar content of each solution were determined in the following way:

[1] Through the kindness of Dr. W. H. Warren, formerly of this school.

Acidity.—Determined by titration with standard alkali and phenolphthalein. Correction was made for initial acidity (due to the malic acid preparation) and for the acidity of the blank.

Sugar.—To the neutral mixture from above was added 15 cc. of 10% neutral lead acetate, the solution shaken, filtered, and the precipitated lead malate washed twice with cold water. To the filtrate was added excess of sulfuric acid, the precipitated lead sulfate filtered off and the filtrate and washings boiled to hydrolyze any disaccharides present. After neutralization with sodium hydroxide and filtering, sugar was determined by Allihn's method. Correction was made for reducing substance in the blank and when necessary, in the malic acid preparations.

MAPLE SHOOTS.

SERIES I.—SUNLIGHT.

0.5% solutions of neutral calcium malate, acid calcium malate, and malic acid.

	Change in acidity. cc. 0.1 *N*.	Change in reduction. Mg. Cu_2O.
Experiment I.—2 days' sunlight.		
Neutral Ca malate	Loss, 0.7	No change
Acid Ca malate	Loss, 2.1	Gain, 47.0
Malic acid	Loss, 9.7	Gain, 131.2
Experiment II.—4½ days' sunlight.		
Neutral Ca malate	No change	Gain, 34.0
Acid Ca malate	Loss, 8.4	Gain, 136.2
Malic acid	Loss, 18.2	Gain, 92.0
Experiment III.—2 days' sunlight, 1 dull day.		
Acid Ca malate	Loss, 0.75	Loss, 8.0
Pure malic acid	Loss, 7.5	Loss, 16.0
Malic acid (containing sugar, prep. 3)	Loss, 5.25	Gain, 4.6
Pure malic acid (1% solution)	Loss, 17.3	Gain, 16.0
Malic acid (1% solution)	Loss, 1.5	Gain, 14.0
Experiment IV.—10 days' sunlight, 2 dull days.		
Acid Ca malate	Loss, 3.0	Gain, 54.0
Pure malic acid	Loss, 14.3	Gain, 42.0
Malic acid (containing sugar)	Loss, 11.2	Gain, 61.0
Pure malic acid (1% solution)	Loss, 5.8	Gain, 82.0
Malic acid (1% solution)	Loss, 22.3	Gain, 99.0

The tissue of maple shoots in solutions of malic acid or malates is here shown to produce, in nearly all cases, an increase in the reducing power together with a decrease in the acidity of the solutions. There is no constant relation between the loss of acidity and the gain in reducing power.

MAPLE SHOOTS.

Series II. Incubator.

In order to determin whether light was necessary for the transformation, some experiments were carried on in an incubator at a temperature of 38°. The solutions as before contained 0.5% of the malic acid compound.

	Change in acidity. cc. 0.1 *N*.	Change in reduction. Mg. Cu_2O.
Experiment I.—3 days.		
Acid Ca malate	Loss, 4.47	Gain, 7.0
Pure malic acid	Loss, 5.7	Loss, 33.0
Malic acid (with sugar)	Loss, 5.7	Gain, 8.0
Experiment II.—4 days.		
Acid Ca malate	Loss, 1.49	Gain, 24.0
Pure malic acid	Loss, 6.50	Gain, 63.2
Malic acid (with sugar)	No change	Loss, 5.0
Experiment III.—8 days.		
Acid Ca malate	Loss, 2.8	Gain, 7.7
Malic acid (with sugar)	Loss, 8.94	Loss, 35.7

In this series there is a lack of constancy of results, which is probably due to the much more favorable conditions for bacterial growth. The net results, however, indicate that the changes noted in Series I may be produced by warmth alone and that therefore light is not necessary for the transformation. There is then a strong probability of enzyme action.

MAPLE SHOOTS.

Series III. Tissue boiled for five minutes.

These experiments were made to determin whether the changes noted were caused by enzymes; also to exclude the possibility of an increase in reducing power due to hydrolysis of sugar-producing substances in the tissue by the malic acid. The solutions containing the maple tissue were boiled for five minutes.

	Change in acidity, cc. 0.1 *N*.	Change in reduction, Mg. Cu_2O.
Experiment I.—3 days' sunlight.		
Acid Ca malate	Loss, 1.5	Slight gain
Pure malic acid	Loss, 7.5	Gain, 7.6
Malic acid (with sugar)	Loss, 7.5	Gain, 4.1
Experiment II.—6 days (4 cloudy, 2 sunlight).		
Acid Ca malate	Loss, 2.2	Loss, 17.0
Pure malic acid	Loss, 12.6	Loss, 5.5
Malic acid (containing sugar)	Loss, 7.5	Gain, 11.0
Experiment III.—13 days (10 sunlight).		
Acid Ca malate	Gain, 1.5	Gain, 4.0
Pure malic acid	Loss, 3.0	No change
Malic acid (with sugar)	Gain, 1.1	Loss, 20.7

Boiling prevents any appreciable increase in reducing power, indicating the probable presence of an enzyme in the tissues, and excluding the

possibility of hydrolysis due to the acidity of the solutions. An attempt was made to extract the enzyme with water. About 9 g. of the finely divided shoots were allowed to stand in water over night, the extract filtered off, mixed with the malic acid preparations and diluted with water to the same volume as before.

MAPLE SHOOTS.

Series IV. Water Extract.

	Change in acidity, cc. 0.1 *N*.	Change in reduction, mg. Cu_2O.
Experiment I.—4 sunny, 2 cloudy days.		
Acid Ca malate	Gain, 1.52	Gain, 7.8
Malic acid, pure	Gain, 5.2	Gain, 15.2
Malic acid, impure (containing sugar)	No change	Gain, 42.6
Experiment II.—4½ days' bright sunshine.		
Acid Ca malate	Loss, 2.22	No change
Malic acid, pure	Loss, 4.44	Gain, 20.0
Malic acid (containing sugar)	No change	Loss, 25.0

The results indicate that the active principle is somewhat soluble in water.

MAPLE BUDS (LEAF BUDS).

0.5% solutions of neutral calcium malate, acid calcium malate, and malic acid.

	Change in acidity, cc. 0.1 *N*.	Change in reduction, mg. Cu_2O.
Experiment I.—Buds swelling. 2 days' sunlight.		
Neutral Ca malate	Gain, 6.25	Loss, 48.0
Acid Ca malate	No change	Loss, 29.0
Malic acid (containing sugar)	Loss, 11.9	Loss, 40.0
Experiment II.—Buds swelling. 4½ days' sunlight.		
Neutral Ca malate	No change	No change
Acid Ca malate	Loss, 2.23	No change
Malic acid (containing sugar)	Loss, 11.9	Loss, 37.0
Experiment III.—Buds opening. 2 days' sunlight.		
Acid Ca malate	Gain, 15.6	Loss, 94.5
Malic acid	Gain, 52.5	Loss, 43.0
Experiment IV.—Buds opening. 10 days' sunlight.		
Acid Ca malate	Gain, 15.6	....
Malic acid	Gain, 47.6	Loss, 39.4

The tissues of the swelling buds mixed with solutions of malic acid or its salts cause a decrease in the reducing power of the solutions, which is generally accompanied by an increase of acidity. The change is more marked in the more rapidly growing (opening) buds and probably means a breaking down of the sugar by the growing tissues.

The experiments reported above were carried out in the spring of 1911 and were limited to the time of opening of the leaf buds. For obvious reasons the work is incomplete, but as circumstances prevent its con-

tinuance for some time, the foregoing results are offered with the expectation of supplementing them later as opportunity permits.

In conclusion, I wish to express my appreciation of the kindness of Prof. G. T. Moore, of the Missouri Botanical Gardens, in placing at my disposal the resources of the gardens.

Conclusions.

The tissue of maple shoots, when mixed with solutions of malic acid or malates and exposed to sunlight, cause an increase in reducing power and a decrease of acidity in the solutions—which may be interpreted to mean a transformation of the malic acid into sugar.

A less pronounced change of the same kind is produced in darkness at 38°.

The active principle which produces the change is somewhat soluble in water, is destroyed by boiling and is therefore probably of enzyme nature.

The tissue of maple buds similarly treated brings about a decrease of reducing substance and an increase of acidity in solutions of malic acid or its salts.

[CONTRIBUTION FROM THE FOOD RESEARCH LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

OSMOTIC ACTIVITY IN THE EGG OF THE COMMON FOWL.[1]

BY A. D. GREENLEE.

Received January 26, 1912.

Introduction.

Eggs, when fresh, contain a large percentage of moisture, and like all other highly aqueous substances they lose moisture on standing by evaporation to the external atmosphere. Chemical analyses of eggs by various investigators are fairly numerous, but little has been done to correlate the change in moisture content with the age or condition of the egg. König[2] reports an analysis of eggs by Bostock as early as 1855. Langworthy[3] determined the percentage in the whole egg with and without the shell, and on the white and yolk separate, and on boiled eggs. Lebbin[4] found the relative percentage of yolk, white, and shell. Cook[5] made a more extensive study of eggs, and, together with other changes, found that "eggs in storage for one year show a loss of weight equivalent to 10% of the total weight, which loss is largely water from the whites." He also found that "when fresh eggs are boiled a loss in weight

[1] Preliminary paper.

[2] *Chemie der Menschlichen Nahrungs und Genussmittel*, 1, 98.

[3] U. S. Dept. Agr., *Farmers' Bulletin* 128.

[4] *Z. öffent. Chemie*, 6, 148 (1900).

[5] U. S. Dept. Agr., Bureau of Chemistry, *Bull.* 115.