Botany. — *"The action of the respiratory enzymes of Sauromatum venosum Schott"*, By Dr. TH. WEEVERS (Communicated by Prof. F. A. F. C. WENT).

(Communicated in the meeting of September 30, 1911).

The production of heat in the spadix of Aroideae has been long known; it was first observed by LAMARCK in 1777. Since it has been repeatedly investigated [1]) and was found to be a process involving absorption of oxygen. KRAUS [2]) showed that in the tissue starch and sugar are used up, whilst CO_2 and sometimes organic acids are produced.

In 1901 HAHN [3]), in a short paper, stated that in the press juice of *Arum maculatum* an enzyme is present, which decomposes glucose with formation of carbon dioxide. After removal of the CO_2 by boiling, the liquid still had an acid reaction. He observed neither formation of alcohol in the air, nor in a hydrogen atmosphere, although in the latter case also the glucose was decomposed and CO_2 was formed.

This short note was not followed by any detailed communication, so that it seemed to me desirable to investigate whether the interesting results of HAHN's inquiry were confirmed with other objects.

Sauromatum venosum Schott appeared to me to be suitable for investigation on account of its size and the ease with which it can be obtained.

For the purpose of orientation, I rubbed up in a mortar the fertile as well as the sterile part of a spadix, and made the pulp up to 250 c.c. with water. One half of this was at once boiled for 5 minutes; to the other half a few drops of toluene were added and after the flask had been closed by a plug of cotton-wool it remained for 48 hours at 16° C. Its contents were then boiled, a certain amount was filtered off from both halves and the reducing sugar as well as the organic acids were determined in the filtrate. (Indicator phenolphtaleïne, which gave a sharp endpoint.)

In the flask in which autolysis had taken place were found 10 mg. glucose and 8,0 c.c. 0,1 N. acid, in the control flask 80 mg. glucose and 0,5 c.c. 0,1 N. acid.

[1]) SAUSSURE, Ann. sc. nat. 1822.
VROLIK and DE VRIESE, Ann. Sc. nat. 1836.
GARREAU, Ann. Sc. nat. 1851.
[2]) G. KRAUS, Abh. naturf. Ges. Halle, 1882. Ann. Jard. bot. 1896.
[3]) M. HAHN, Ber. chem. Ges. Bd. 33, 1900.

So this preliminary experiment showed, that in the autolysis sugar was decomposed and organic acid formed. In further experiments however a different plan was adopted. After the tissue had been rubbed fine in a mortar, I strained the pulp through the finest plankton gauze; the fluid thus obtained, which was almost perfectly clear, was heated with a threefold quantity of 95% alcohol, the precipitate filtered, washed out with alcohol and dried in the air in the dark till all the alcohol had evaporated (about 12 hours).

The powder then obtained i.e. a crude enzyme or a mixture of enzymes was used for further experiments and was found to have a strong glucoclastic action; similarly the pressed-out, dried mass had this action, even after treatment with acetone.

The former result was unexpected because HAHN, with a crude enzyme prepared in the same way from *Arum maculatum*, only obtained a very weak action and because more investigators in this field hold the view, that destruction of cell-structure and treatment with alcohol weakens the action of the respiratory enzymes in tissues rich in water, and even destroys it.

ZALESKI [1]) says: "Ueberhaupt kann man sagen, dass die Zerstörung der Struktur am stärksten die Atmung derjenigen Objekte vermindert, die im Zustande tätigen Lebens sich befinden, oder wasserreich sind. Noch stärker wird die Kohlensäureausscheidung zerriebener Objekte nach dem Behandeln derselben mit organischen Lösungsmitteln wie Aceton und Alkohol vermindert, und manchmal hört diese ganz auf."

This cannot therefore be generally valid, at least not for the object here investigated, which is rich in water and is in condition of active life.

In exactly the same way I have been able to obtain from the fresh leaves of *Sauromatum* a crude enzyme, that decomposes glucose while forming CO_2 and organic acid. The action was however weaker.

The dried preparation of the enzymes was generally used at once for the experiments, after some days the activity diminished, although it did not completely disappear.

If I made the preparations by treating the press juice with acetone, I obtained a precipitate that was similarly active after removal of the acetone.

The experiments were made as follows.

The enzyme preparation was mixed in a flask of 750 cc. capacity, with 1% glucose solution sterilised by boiling, and with some

[1]) W. ZALESKI Atmungsenzyme der Pflanzen. Bioch. Zeitsch. Bd. 31. 1911.

drops of toluene. In experiments with access of air, a regulated current of air sterilised [1]) and free from carbonic acid came into the closed flask, and the issuing current of air passed first through a calcium chloride apparatus and then through a potash-bulb (with $CaCl_2$ tube). The increase of weight of the potash bulb indicated the amount of carbonic acid formed, whilst in some experiments this was also shown qualitatively with baryta water.

For the experiment in a hydrogen-atmosphere the flask was first filled with hydrogen that had been purified with dilute-potash, after which, with the necessary precautions the enzyme, a solution of sugar and a few drops of toluene were introduced. Then during the experiment a purified current of hydrogen passed through the flask and carried the CO_2, which had been produced, into the potash bulbs; at the end air free from CO_2 passed through, in order to be able to determine the increase in weight of the bulbs.

The experiments always lasted 48 hours [2]), only once did I determine the CO_2 produced in successive periods of 3 and 6 hours, but found no great differences. Indeed the method was not suitable for successive determinations with small intervals, since after terminating the experiment by heating the liquid in the flask above 70° C., one ought to pass the current through for a short time longer in order to get all the carbonic acid into the potash bulbs [3]).

The liquid in the flask was always boiled for a moment at the end of the experiment and the first distillate was tested by means of the iodoform reaction for alcohol, the result was always negative, both in the aerobic experiments and in those in a hydrogenatmosphere [4]).

Then the liquid was cooled, made up to a definite volume and filtered. I determined the quantity of organic acid in the filtrate, equally that of the glucose, the latter one before and after boiling with dilute hydrochloric acid [5]). Control experiments, in which the liquid was boiled immediately after mixing, gave the total quantities

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[1]) For this purpose the air passed through strong caustic potash, to which some toluene had been added.

[2]) The experiment with a preparation from the spadices took place in daylight; since organic acids in aqueous solutions are gradually decomposed by light, the quantities found are somewhat too small.

[3]) If traces of CO_2 should remain behind, the result would naturally be too low.

[4]) Only a few drops were distilled over. Generally the odour indicated traces of butyric acid.

[5]) The glucose determinations were made according to N. Schoorl's method. Ned. Tijdschrift voor Pharmacie 1899. The quantity after boiling with HCl was generally as great as before boiling, the former values are given.

of reducing sugar and organic acid at the beginning of the experiment; the latter in the experiments with the alcohol precipitate from the spadices amounted to 0,1 cc 0,1 N at most.

Below are given some of the experiments.

I. Experiment in CO_2 free air.
400 mg. crude enzyme, acetonic preparation.
Decréase of glucose from 437 to 360 mg. = 77 mg.
Increase of the potash bulbs by CO_2 production 34 mg.
Increase of organic acids 9 c.c. 0.1 N.

II. Experiment in CO_2 free air.
750 mg. pressed and dried tissue powder, that had been treated with acetone.
Decrease of glucose from 457 to 217 mg. = 240 mg.
Increase of potash bulbs by CO_2 production 140 mg.
Increase of organic acids 25 c.c. 0.1 N.

The glucose can be more strongly decomposed, so that after boiling with HCl only traces of reducing sugars remain; in these experiments K_2HPO_4 was generally added, which according to the investigations of ZALESKI and REINHARDT [1]) stimulates the action of the enzymes. This made a determination of organic acid difficult, because the potassium phosphate had an acid reaction and the quantity added perhaps partly entered into combination during the experiments [2])

III. Experiment in H atmosphere, potassium phosphate added.
800 mg. crude enzyme (alcoholic preparation).
Decrease of glucose from 437 mg. to 2 mg. = 435 mg.
Increase of potash bulbs by CO_2 production 165 mg.

IV. Experiment in air free from CO_2, potassium phosphate added.
1.500 gr. pressed out material, which had first been dried at 60° C.; then acetone had been poured over it and it had again been dried at 60° C.
Decrease of the glucose from 485 mg. tot 5 mg. = 480 mg.
Increase of potash bulbs by CO_2 production 187 mg.

Finally two more experiments with the crude enzyme from the leaves; no potassium phosphate was added.

[1]) W. ZALESKI and H. REINHARDT Biochem. Zeitschr. Bd 27. 1910.

[2]) Compare the investigations of A. HARDEN and W. J. YOUNG. Biochem. Zeitschr. 1911.

V. Experiment in air free from CO_2.
1.200 gr. crude enzyme (alcoholic preparation).
Decrease of glucose from 375 mg. to 340 mg. = 35 mg.
Increase of potash bulbs by CO_2 production 27 mg.
Increase of organic acid from 1 c.c. to 3.2 c.c. = 2.2 c.c. 0.1 N.

VI. Experiment in hydrogen atmosphere.
1.200 gr. crude enzyme (alcoholic preparation).
Decrease of glucose from 375 mg. to 343 mg. = 32 mg.
Increase of potash bulbs by CO_2 production 14 mg.
Increase of organic acid from 1 c.c. to 3.5 c.c. = 2.5 c.c. 0.1 N.

Decomposition of glucose has taken place in all my experiments, in those in the air, as well as in those in a hydrogen atmosphere, carbonic acid and organic acids are always formed, but no alcohol, an enzyme identical with zymase cannot therefore have acted in this case, the more so because in the experiments of KOSTYTSCHEW and PALLADIN [1]) the production of organic acid never occurred at all.

In the anaerobiosis of Agaricinae KOSTYTSCHEW [2]) also found production of CO_2 without formation of alcohol; PALLADIN and KOSTYTSCHEW [3]) pointed out, that in the absence of carbohydrates carbonic acid is similarly formed by old etiolated shoots of Vicia Faba, but without alcohol.

Indeed in all these experiments, which were for the most part conducted with intact parts, killed by freezing, formation of acids never took place, at least it is never mentioned. Only in the much discussed experiments of STOKLASA c.s. [4]) is there any question of the production of acid. In these the press juices of sugar-beet, barley, peas and lupin seedlings were treated with alcohol and the precipitate was used for the experiments. On addition of glucose the latter was decomposed and there resulted CO_2 and organic acids, but also alcohol. The organic acids were chiefly lactic acid, then acetic acid and formic acid, whereas it will be shown below, that these acids were absent in my own experiments.

The above described action of the respiratory enzymes of Sauromatum seems therefore to be very specific and shows much agreement with the action of the press juice of Arum maculatum only; the considerable formation of acid reminds us of the metabolic processes

1) PALLADIN & KOSTYTSCHEW. Ber. d. d. bot. Ges. 1906.
2) KOSTYTSCHEW. Ber. d. d. bot. Ges. 1907.
3) PALLADIN & KOSTYTSCHEW. Ber. d. d. bot. Ges. 1907.
4) J. STOKLASA, ADOLF ERNEST, KARL CHOCENSKY. Ber. d. d. bot. Ges. 1907.

of Fungi in which so often acids are formed and of the nocturnal production of acids in Crassulaceae, in which according to more recent investigations the formation of acid may be connected with the decomposition of carbohydrates [1]. The question now is, what acids are formed in the decomposition of glucose by the enzyme of Sauromatum venosum?

The quantity of liquid which was not required for the other experiments was evaporated, extracted with ether and the ethereal extract used for further investigations. The small quantity available was not sufficient for macrochemical investigation, I therefore made the analysis in about the same way as that described by H. BEHRENS [2].

The ether was distilled off and water added. First of all I tried, whether the acid was volatile with steam, this turned out not to be the case, only a minute quantity passed over, too small for further investigation. Moreover the liquid had already boiled for a few minutes as described above, and the odour then indicated traces of butyric acid.

The acid reaction is therefore not caused by formic, acetic or propionic acid, nor by higher fatty acids.

By sublimation on a microscopic slide a sublimate was only obtained at a fairly high temperature, when the substance was coloured brown. The sublimate was not crystalline even when breathed on, so that it cannot be oxalic or succinic acid. The test with lead acetate for malonic acid yielded an equally negative result.

On the other hand the aqueous solution gave distinctly BERG's test [3] i. e. a yellow colouration with a solution of two drops $FeCl_3$ 45° B and two drops HCl 22° B in 100 c.c. water. This reaction is peculiar to organic acids with one or more CHOH groups, oxyacids therefore of which the best known are lactic, tartaric, malic and citric acid.

The test for cobaltolead lactate as well as for potassium and silverbitartrate gave a negative result, the two first mentioned acids are absent. On the other hand, testing with $AgNO_3$, there was proof of the presence of citric acid, as shown by the crystals of silver

[1] HUGO DE VRIES. Verh. Konink Akad. v. Wet. 1884.
G. KRAUS. Abhandl. naturf. Ges. Halle 1884.
O. WARBURG. Unters. bot. Inst. Tübingen 1886.
A. MAYER. Landw. Versuchsst. 1887.
K. PURIEWITSCH. Bot. Centr. 1894.

[2] H. BEHRENS. Anl. zur mikrochem. Analyse 4e Heft 1897.

[3] BERG et GERBER. Bull. soc. chim. 1896.

citrate (see BEHRENS). Citraconic acid must therefore be present in the sublimate and after adding NH_3, evaporating and redissolving in water, crystals of the thallous salt of citraconic acid were actually obtained on adding thallous nitrate.

Once I obtained crystals of silver malate, after the addition of $AgNO_3$, it might therefore be possible, that malic acid was here formed as well as citric acid.

Both acids are known to be final or intermediate products in the decomposition of sugar, I have already mentioned malic acid [1]) in this connexion and WEHMER [2]) has shown that Citromyces spec, Penicillium luteum and Mucor pyriformis, when grown in sugar solutions produce citric acid; this acid also frequently occurs in Phanerogams (comp. CZAPEK. Biochemie der Pflanzen).

Whether in addition to citric and perhaps malic acid, other less known acids are also formed by the enzyme in the decomposition of glucose is of course still an open question.

If we calculate the quantities of acid found as citric acid [$C_3H_4OH(COOH)_3 + H_2O$, mol. weight 210] we find:

I	experim.	for	77	mg.	glucose	34	mg.	CO_2	and	63	mg.	citric acid
II	,,	,,	240	,,	,,	140	,,	,,	,,	175	,,	,,
V	,,	,,	35	,,	,,	27	,,	,,	,,	15.5	,,	,,
VI	,,	,,	32	,,	,,	14	,,	,,	,,	17.5	,,	,,

It is my intention by using more material to determine the quantity of citric acid formed, for only then can it be ascertained whether the decomposition of sugar to CO_2 and citric acid is complete. It seems natural at the same time to investigate what enzymes are present in the crude enzyme, whether oxygen is absorbed in the process and how the object behaves after freezing by PALLADIN's method, questions which the method explained above leaves unanswered.

The results of this investigation can be summarised as follows.

By pressing out and precipitating the press juice with alcohol or acetone, there can be obtained from the spadix of Sauromatum venosum Schott. a crude enzyme, that decomposes glucose with the

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[1]) A preleminary experiment was carried out in order to obtain from the leaves of Echeveria spec. in the same way as here from Sauromatum a crude enzyme that produced CO_2 and malic acid from glucose, but with negative result.

The malic acid of Crassulaceae, which has been investigated by J. H. ABERSON (Ber. chem. Ges. 1898) yields in sublimation little or no fumaric and maleic acid, but gives an amorphous silver salt.

[2]) C. WEHMER. Ber. d.d. bot. Ges. 1893.

formation of carbonic and organic acids, but without any production of alcohol neither in the air nor in a hydrogen atmosphere.

Destruction of the cellular structure and treatment with alcohol or acetone do not therefore inactivate the respiratory enzymes in the present case, their power of decomposing sugar remains very marked.

In the same way a crude enzyme is obtained from the leaves of Sauromatum, which is similar, but has a weaker action.

In the ether extract of the acid liquid citric acid was demonstrated, which acid very probably must be formed by the respiratory enzymes at the expense of the glucose.

Amersfoort, September 1911.

Zoology. — "*Pleistophora gigantea Thélohan a parasite of Crenilabrus melops.*" By N. H. SWELLENGREBEL. (Communicated by Prof. MAX WEBER.)

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Among the neosporidia the microsporidia distinguish themselves by their spores, which are smaller than those of the allied myxosporidia and do not possess such distinct polar capsule and polar filament as the spores of the latter group.

According to MINCHIN (1903) the microsporidia are divided into:

1. *Polysporogenea*; the trophozoite (i. e. the vegetative generation) forms many pansporoblasts, each of which contains many spores.

2. *Oligosporogenea*; the trophozoite transforms itself entirely or partly into one single pansporoblast. Each pansporoblast contains 4, 8 or many spores.

The parasite that I wish specially to describe here has the following life-history. Trophozoites with one or more nuclei are found in the connective tissue of the skin and in the mesenterium of *Crenilabrus melops*. After encystment the trophozoites form by successive division an unequal number of sporoblasts, each containing two nuclei. These sporoblasts become spores by the formation of a thick membrane. The spores have one or two nuclei, whilst there is nothing that points to the existence of a polar capsule with polar filament.

THÉLOHAN (1895) described a microsporidium, *Glugea gigantea*, found in the abdomen of *Crenilabrus melops*. He has not been able to investigate the development of this parasite, but only states that