On the Nature of Oxidases.

By

K. Asō, Tokyo.

In a former article[1]) I had shown that the liberation of iodine from potassium iodid by certain plantjuices does not go parallel with the blue guaiac reaction, as Bach had supposed and that in one case at least, it was positively shown that the liberation of iodine was due to traces of nitrous acid. That article had induced Chodat and Bach[2]) to some remarks which led me to make some further trials. These show that the view of Chodat and Bach cannot be generally sustained.

In the first place I must adhere to my view that there is no parallelism between the iodine liberation and the guaiac reaction.[3]) In the second place I am convinced contrary to the opinion of Chodat and Bach, that the guaiac reaction upon peroxides is not so sensitive as the liberation of iodine by peroxids.

In order to prove this latter assertion the following observation was made. The common para-aldehyde of commerce has generally an acid reaction and yields with potassiumiodid-starch very soon an intense blue reaction due to the liberation of iodine. I entertained the supposition that this reaction is not caused by the pure para-aldehyde, but by an admixture of an organic peroxid, very probably by the acetylhydroperoxid.[4]) Similar peroxids have been observed as a result of autoxidation of other aldehydes and also the common ether forms after long contact with the air, an organic peroxid, which sometimes even caused explosions in distilling such an old ether to the last drop.

I shook therefore about 20 c. c. of commercial para-aldehyde, with an equal volume of 10 % sodium carbonate solution

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[1]) Beihefte. Bot. Centr.-Blt. XI. Heft 1. „Which compound in certain plantjuices can liberate iodine from potassium iodid?“

[2]) Berichte d. d. chem. Ges. 1994. Heft 1.

[3]) This view is also entertained by Raudnitz. (Monatschrift für Kinderheilkunde. II. Heft 8.)

[4]) Page, R. H. (Amer. Pat.) mentioned in the Chemiker-Ztg. Benzaldehyde (commercial) produced also the iodine reaction in traces.

and after washing until the alkaline reaction disappeared, a portion of that para-aldehyde was distilled off. There was now observed that the iodine reaction above-mentioned did not take place neither at once nor within 15 minutes, but only an exceedingly weak reaction slowly appeared later on, which however was not intensified by the addition of some acetic acid. The original para-aldehyde, however, gave an intense reaction within a few minutes. This difference was sufficient to prove that it is not the para-aldehyde itself, which causes the iodine reaction, but some impurity, which can only have been the peroxid above-mentioned, to judge from analogy. Now it was interesting to observe that the original para-aldehyde which produced such an intense iodine reaction had no reaction whatever on tincture of guaiacum, not even on addition of some hydrogen peroxid. These mixtures were still colorless even after half an hour. Therefore I can not agree with Bach and Chodat[1]) when they believed „daß die Guajakreaktion auf Peroxyde bei weitem empfindlicher ist als die Jodkalium-Stärke-Reaktion".

Also in regard to nitrites, both reagents were compared as follows: The test was made with 0.01 %, 0,001 %, 0,0005 % and 0,0001 % solution of potassium nitrite with the following result:

Concentration of Nitrite-solution	Potassium iodid-starch reaction.[1])	Guaiac. reaction
0,01 %	Distinct immediately	Distinct immediately
0,001 %	„	No reaction at once, but very faint blue after 15 minutes
0,0005 %	„	No reaction at once, only trace after $^1/_2$ hour
0,0001 %	No reaction at once, very faint after 15 minutes.	No reaction at all

From this table, it is quite clear that the guaiacum reaction is less delicate than the potassium iodid-reaction. Most of plant-juices produce very strong guaiac reaction, but no potassium iodid reaction. Hence the substance which produces the guaiac reaction must be quite different from that which produces po-

[1]) Ber. D. Chem. Ges. 37. p. 38.
[2]) Of course, it is necessary to add some acetic acid for this test.

tassium iodid starch reaction that is, the former is caused by oxidase very frequent in plantjuices. and the latter by nitrite which is present in certain plantjuices, as I had positively proved in one case (l. c. page 212).

But if the iodine liberation by certain plantjuices would be due always to traces of nitrite and not to enzyms, how is the fact to be explained that this property is lost in most cases[1]) on heating? The probable answer is here that plantjuices are often slightly acid and contain at the same time small quantities of amido-compounds. Under this condition traces of nitrites must dissapear on warming. while after addition of some alkali, the reaktion will probably be maintained after boiling.

About 15 c. c. of 0,001 % potassium nitrite solution were mixed with an equal volume of 1 % asparagine solution (aqueous) and divided into three equal parts. To one part, I added a drop of dilute acetic acid and to another, a drop of dilute caustic potash free from nitrite while the third served as control. These solutions were heated to boiling for three minutes then about 1 c. c. of potassium iodid-starch solution was added and to the alkaline as well as the control solutions also, some dilute acetic acid to render the reaction slightly acid; the result obtained was:

Control	Alkaline solution	Acid solution
Distinctly and immediately	Distinctly and immediately	No reaction at all.

This test was repeated: 30 c. c. of 0,001 % potassium nitrite solution were mixed with 30 c. c. of 1 % asparagine solution and divided into three parts. To one was added a drop of dilute acetic acid, to the other a drop of dilute caustic potash solution while the third served as control. These solutions were kept boiling for five minutes and tested with potassium iodid-starch as above-mentioned with the following result:

Control	Alkaline solution	Acid solution
The reaction appeared, but slower and weaker than in the alkaline liquid	Distinctly and immediately	no reaction at all after several hours.

[1]) Once, I ground four buds of Sagittaria with 20 c. c. water. The pressed juice which produced strong reaction with guaiac tincture. Griess reagent as well as potassium iodid-starch was divided into two halves and one was boiled for one minute while the other served as control. Both

From the above results, I became convinced that amido-compounds decompose nitrite in a very faint acid solution and it is necessary to make the solution alkaline to preserve the nitrite. Hence I made analogous experiments with plantjuices.

Experiment with Sagittaria.

18 grams of the buds of Sagittaria were crushed, extracted with 100 c. c. water and divided into three equal parts. To one a few drops of acetic acid, to the other a few drops of caustic potash were added while the third served as control. Each solution was heated to boiling for five minutes and tested as mentioned before:

	Control	Alkaline solution	Acid solution
Potassiumiodid-starch reaction	Distinct	Distinct	No reaction even after several hours
Griess reaction	„	„	faint
Guaiac reaction	No reaction at all	No reaction at all	No reaction at all.

Quite similar a test was repeated, but in this case, the solutions were heated to 95 ⁰ C for 10 minutes and filtered after acidification with acetic acid, which had produced some precipitate:

	Control	Alkaline solution	Acid solution
Potassiumiodid-starch reaction	No reaction at first, but after 10 min. appeared gradually	Distinctly at once	No reaction at first, but after 10 min.. a reaction appeared although weaker than in the control case
Griess reaction	Distinctly	Distinctly	Distincly
Guaiac reaction	No reaction at all	No reaction at all	No reaction at all.

solutions were filtered and the filtrates were tested with the reagents above-mentioned. Hereupon Griess' and iodine reaction appeared very distinctly, though the latter was a little weaker in boiled liquid than in the control; but no blue reaction of guaiac at all appeared. Similar facts were observed repeatedly.

23 grams of the buds of Sagittaria were crushed and extracted with 150 c. c. water. The filtrate was divided into three equal parts. To one, a drop of dilute acetic acid, to the other a drop of dilute caustic potash was added while the third served as control. These solutions were kept at 98 ° C for half an hour and tested with potassiumiodid-starch as above-mentioned after filtering. After a few hours, the following was observed:

Control	Alkaline solution	Acid solution
Distinct	Distinct	No reaction.

Experiment with Potato.

20 grams of potato buds (2—5 cm long) which had developed in darkness were crushed in a mortar. The pressed juice was mixed with some concentrated solution of basic lead acetate. To the filtrate therefrom, some sulphanilic acid and sulphuric acid were added and again filtered. The filtrate gave a very faint Griess reaction upon an addition of α-naphthylamine hydrochloride, but no iodine reaction[1]). Also the color reactions for oxidizing enzyms were obtained very intensely with the original juice.

In order to separate the substance which produces the guaiac reaction from that which yields the reaction of Griess, the following experiments were made: 10 buds of Sagittaria (about 10 grams) were crushed with 10 c. c. water; the filtrate showed a decided reaction of Griess, but only a very feeble reaction with potassium-iodid-starch. The guaiac reaction was however very strong. This filtered juice was mixed with about three times of its volume of alcohol (90 %) and the precipitate washed with alcohol. The filtrate showed a distinct reaction of Griess, but no reaction for oxidizing enzyms nor a reaction with potassium-iodid-starch[2]), while the solution of the precipitate showed very strong reactions for oxidizing enzyms, but no Griess reaction.

In the next experiment, 35 buds of Sagittaria (about 33 grams) were crushed with 50 c. c. water. To 60 c. c. of the pressed juice which yielded a very strong reaction with potassium-iodid-starch, 200 c. c. of strong alkohol (90 %) were added. The mixture was left for twenty four hours and filtered. The filtrate was evaporated on a waterbath and the residue was dissolved in 20 c. c. water and filtered. The filtered liquid gave

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[1]) Of course, the iodine reaction is not so delicate as the reaction of Griess.

[2]) Perhaps the quantity of nitrite was too small.

a strong Griess reaction as well as the iodine reaction very decidedly, but not the guaiac reaction while the aqueous solution of the well-washed precipitate gave in the contrary not the Griess reaction nor the iodine reaction, but a strong guaiac reaction. This result proved positively that the substance which gives the guaiac reaction is not the same that liberates iodine from potassium-iodid.

Bach and Chodat[1]) have mentioned that when a freshly cut surface of Sagittaria bulb with paper moistened with potassium-iodid-starch, is touched a bluish violet ring will appear along the peripheral tissue after a short time, further a blue ring with a paper moistened with guaiac tincture, and further with m-phenylendiamine, along the same lines. I have repeated these experiments and made a similar observation. Hence I took off the peripheral part of twenty one bulbs and crushed it with 30 c. c. water. The pressed juice gave a strong guaiac reaction and a moderate Griess reaction, but the iodine reaction only slightly. To 30 c. c. of the juice about 100 c. c. absolute alcohol were added and the alcoholic filtrate was evaporated to dryness. The residue was dissolved iu 10 c. c. water and tested with the following result:

Guaiac reaction	Griess' reaction	Jodine reaction
No reaction at all	moderately	slightly.

In the case of a feeble iodine reaction, the addition of a drop of dilute sulphuric acid is preferable to accelerate the reaction.

The aqueous solution of the precipitate produced a very strong guaiac reaction, but no Griess nor iodine reaction. Moreover, I carried out several tests with the juice of skinned bulbs according to Bach and Chodats method, but neither Griess nor iodine reaction was obtained while the guaiac reaction appeared very strong. Also, on application of guaiac tincture on the scratched surface of the freshly cut bulbs the blue color appeared at once, while there was no reaction obtained with potassium-iodid-starch. This result convinced me that the bulb of Sagittaria (excluding the skin) contain common oxidase, but no nitrite.

40 grams of Potato buds (2—5 cm long) were crushed and the juice was pressed out. This juice did neither yield the potassium-iodid-starch reaction nor the Griess reaction. which however appeared very weak after purification. About 5 c. c. of the juice was mixed with some concentrated solution of basic lead acetate and filtered. The filtrate was mixed with sulphuric acid and sulphanilic acid, then with α-naphthylamine hydro-

[1]) Berichte der D. Chem. Ges. XXXVII. 1904. Heft 1. p. 39.

chloride. The Griess reaction appeared, but only in traces. The main part of the juice (about 25 c. c.) was mixed with three times of its volume of alcohol (90 %) and filtered. The filtrate showed a very faint Griess reaction, but no reaction for oxidizing enzyms and also with potassium-iodid-starch, while the aqueous solution of the well-washed precipitate gave very strong reactions for oxidizing enzyms, but not the Griess reaction and also no iodine reaction.

Experiment with Pea.

4 grams of the root of a full grown peaplant were crushed and extracted with 30 c. c. wather. The juice obtained had almost a neutral reaction and gave strong reactions for oxidizing enzyms, but neither liberation of jodine nor the Griess reaction. 20 grams of the green parts[1]) of the pea plants were crushed and extracted with 30 c. c. water, and tested in a similar way as above with the same results. 4 grams of the root-tubercles were now crushed, and the pressed juice of a faint acid reaction gave strong reactions for oxidizing enzyms, but no liberation of iodine nor the diphenylamine reaction. Also Fehlings solution was not reduced. Further 5 grams of the white part of the stem of pea-shoots (5—10 cm. long) were crushed and extracted with 2 c. c. water. After filtering and adding a few drops of concentrated solution of basic lead acetate, the filtrate was mixed with some sulphanilic acid and sulphuric acid and filtered. To the filtrate, a few drops of α-naphthylamine hydrochloride were added, whereupon the Griess reaction appeared feebly, but distinctly. Again, 10 grams of the white part of the pea-stem were crushed and the juice was pressed out. The juice produced strong color reactions for oxidizing enzyms and diphenylamine reaction for nitrate, but neither Griess nor potassium-iodid-starch reaction for nitrite. The juice was now treated with a few drops of concentrated solution of basic lead acetate and filtered. To one part of the filtrate, some sulphanilic acid and sulphuric acid were added and again filtered. The filtrate showed here again a distinct Griess reaction upon the addition af α-naphthylamine hydrochloride. With 10 grams of the green part of the same shoots, were, after the same treatment, tested in the samer manner. But neither the diphenylamine reaction nor the Griess reaction was here obtained.

16 grams of Sagittaria shoots (of green color) were crushed with 50 c. c. water and filtered. The filtrate showed very strong reactions for several oxidizing enzyms, but a very weak potassium-iodid-starch reaction.[2]) This filtrate was diluted with

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[1]) In tis case, it is necessary to add much guaiac tincture; otherwise the blue color soon fades out.

[2]) After boliing and filtering, the filtrate showed a weak iodine reaction, but no reaction for oxidizing enzyms.

10 times of its volume of water and tested again. Hereupon very strong color reactions for oxidizing enzyms were still obtained and a faint Griess reaction, but the liberation of iodine was not more observed, what is a further proof that the substance producing the guaiac reaction is not identical with that which produces the jodine reaction.

Conclusion.

1. The guaiac reaction for peroxids is not so sensitive as the potassium-jodid-starch reaction.

2. The guaiac reaction for nitrites is much weaker than the iodine reaction for nitrites.

3. The reason why certain plantjuices which can liberate iodine loose that property on heating is very probably due to the acidity of the juice and the presence of traces of amido-compounds, which is a very favorable condition for the decomposition of nitrites.

4. It was positively shown that the substance which gives the guaiac reaction is not the same as that which liberates iodine.

5. While the white underground stem of the peaplant gave traces of nitrite reaction, this was never obtained with the green parts of these plants, nor with the root.