RESEARCHES ON GLUCOSIDES. THE PHYSIOLOGICAL FUNCTION OF SALICIN

by

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§ 1. INTRODUCTION.

In the year 1934 there was published in "Planta" a paper by G. KERSTAN (4) in which a method is worked out for quantitative estimation of glucosides besides sugars in plantextracts. KERSTAN makes use of the fact that in aqueous solutions glucosides are almost completely adsorbed by medicinal carbon, whilst monoses and sucrose are only adsorbed to a very slight degree. In a second paper, published in the same volume of "Planta", the author uses this method for researches on the function of aesculin in the metabolism of *Aesculus* (5).

In the introduction to the latter KERSTAN mentions former communications on this subject; his judgment of them is however neither accurate, nor just. My own work on glucosides (12, 13) e.g. is criticized in the following sentences: "their results (Jahrb. f. wiss. Botanik 1903) are much injured by the insufficient method. More noteworthy seems a later communication of the same author (Rec. d. Travaux bot. Néerl. 1910)".

A peculiar light is thrown on the question, whether this judgment may be called trustworthy, by the fact that the quantitative method used in both papers was the same, at least as regards salicin, the glucoside, which I have studied in detail and the only one treated in both papers. KERSTAN on the contrary has studied the physiological function of salicin only in passing and had paid particular attention to aesculin.

Nevertheless the above mentioned remark of KERSTAN induced me to subject the methods used to a critical revision, especially with regard to salicin in order to prove whether my former conclusions were justified.

§ 2. METHODS.

The method I used was based on the following: an aqueous solution of salicin gives no reduction of Fehling solution. Emulsin however hydrolyses this solution of salicin almost completely into

glucose and saligenin (ortho-oxybenzylalcohol) and of these products only the former gives a reduction of the Fehling solution. Making use of plantspecies which contain no other glucosides hydrolysable by emulsin, we can say that the quantity of salicin is to be deduced from the increase of glucose caused by addition of the enzyme.

The species which was studied, *Salix purpurea*, contains besides salicin the glucoside populin. As a solution of the latter gives no reduction of Fehling and is not hydrolysed by emulsin, the method may be used without inconvenience.

Another question is the activity of the preparations of emulsin, which activity appeared to vary considerably. A preparation obtained in the year 1934 from SCHUCHARDT, even after having been kept for some years, almost completely hydrolysed a solution of salicin. In 24 hours 89 % and in 48 hours 98 % of the solution was hydrolysed. A preparation obtained from the same firm in the year 1938 gave less favorable results: after 24 hours 88 % was hydrolysed but this limit was not exceeded; nor did a preparation from the British Drughouses give results which enabled us to use it for quantitative salicin estimations. The cause of the difference is unknown; always a quantity of thymol was added, sufficient to prevent possible action of bacteria.

In the experiments of 1934—1938 which will be described later on the emulsin preparation of SCHUCHARDT 1934 was always used.

Leaving undecided whether KERSTAN's adsorption method generally speaking has definite advantages or not, it may at any rate be maintained, that the above mentioned method of salicin estimation is sufficiently exact for physiological purposes; at least provided that the solutions containing salicin are subjected to the influence of active emulsin for 48 hours.

With his adsorption method KERSTAN has made only very few experiments concerning salicin; a few about the quantity of this substance in leaves at night and in the morning, but not a single one concerning changes in the quantity of salicin in the bark during sprouting.

Besides the exactitude of methods the question whether there is comparable material may be said to be of the greatest importance in such physiological experiments. KERSTAN made use of the old method of percentage values of fresh weight, a method which has been criticized over and over again, e.g. by CORNELIA A. GOUWENTAK (3). In my opinion the method of KERSTAN is altogether impracticable in experiments as these, in which the quantity of reserve-material before and after sprouting is to be compared. Not only

may the percentage of water in the bark (salicin is exclusively present in the bark and not in the wood) strongly vary during this long period, but the starch reserve might also lessen so much that the salicin percentage of fresh weight might increase, whilst the absolute quantity of the glucoside had decreased.

It is therefore necessary to use other methods, methods by which the total quantities of salicin are estimated in parts of the plant which are comparable as to form, size and structure.

In studying the changes in the salicin percentage of leaves, the method of using the halves of leaves may be employed. If the object has symmetrical leaves a hundred of right halves may well be compared with a hundred left ones. It is possible to cut off the left halves of e.g. 100 isolated leaves and to use these as a control, whilst the right halves with the midrib are submitted to the conditions of the experiment; e.g. they may be put in tapwater in the dark for 12 hours and then be cut off from the midrib and analyzed.

In the case of branches the question is not so simple. With *Salix purpurea* the difficulty was solved in the following manner. By making sprouts of one motherplant a clone of c. 20 willows was obtained and from each object of this clone a heavy branch was cut off near the soil. Quite near this cut 4 to 5 side-branches of the same diameter sprouted. Next spring a piece of the same length was cut off from each of these sidebranches. In this way 4 or 5 lots of c. 20 pieces of branches were obtained, sufficiently corresponding in structure, size and weight to be comparable.

As a rule two of the lots were immediately analyzed after cutting, also before the buds sprouted. One lot of pieces was put in tapwater in the dark, another one in daylight, both at c. 15° C. The latter were analyzed 5—6 weeks later, when the buds had sprouted.

A comparison of the two lots analyzed before sprouting showed that they were indeed comparable entities, as to the dry weight and the quantity of reserve material. The average value obtained from these two lots was used as a control. to be compared with the values from the lots sprouted in tapwater.

In order to get a good insight into the question whether a definite substance e.g. a glucoside has the function of reserve material or not, it appeared necessary to study not only possible changes in the quantity of this compound itself, but to compare these changes e.g. in case of sprouting with those of other compounds.

Especially substances, whose function as a reserve, is generally accepted e.g. starch or proteins, must be taken into account.

In my former studies of *Salix purpurea* this requirement was only incompletely satisfied. Besides salicin, no other substance than

monoses and sucrose had been studied; it was therefore necessary to fill up this gap.

At the same time it is evident, that in this work besides the reservematerials of the bark those of the wood of the branches must also be taken into account. For during sprouting the reservematerial is mobilized, so that transport along the modullary rays may take place.

In my previous papers on *Salix purpurea* I had arrived at the conclusion, that whilst the buds are sprouting, salicin is hydrolyzed by the enzyme salicase, belonging to the emulsincomplex. The glucose formed is used in metabolism, whilst the aglucon saligenin (ortho-oxybenzylalcohol) is transmuted into pyrocatechol (ortho-dioxybenzol). This is proved by the fact that the quantity of the latter compound increased during sprouting whilst saligenin is only an occasionally observed transitory product. In the case of branches which unfolded their buds when put in the dark in tapwater, the increase of pyrocatechol was estimated to be in molecular proportion to the decrease of salicin. From this was concluded that nearly the whole quantity of the aglucon of salicin was transmuted into pyrocatechol.

With regard to pyrocatechol the quantitative method, used in previous papers was, however, not wholly reliable, so that it seemed desirable to repeat the experiments with another method. Prof. Dr. P. KARRER from Zürich was so kind as to draw my attention to a paper by GARDNER and HODGSON (2) in which pyrocatechol is estimated by means of a standard solution of iodine. A standard aqueous solution of the phenol is prepared and to a known volume of this solution a quantity of 0,1 N. iodine solution is added in excess. A few drops of starch solution (Amylum solubile) are than added and afterwards aqueous sodiumhydroxyde is run in drop by drop until the colour, due to the iodine disappears. By this means excess of alkali, which has an injurious effect on the accuracy of the process is avoided. Dilute sulphuric acid is added in sufficient excess to separate the unabsolved iodine, the amount of which is estimated by 0.1 N. thiosulphate. In the case of pyrocatechol 0.01 g. required 3,65 cc of 0,1 N. iodine.

In the experiments of this paper the estimation of the quantity of pyrocatechol took place in the following way: An aqueous extract of the bark or the leaves of *Salix purpurea* was obtained by a thrice repeated extraction; the extracts were combined and by evaporation concentrated to a definite volume. The latter is shaken up several times with a sufficient quantity of ether till all the pyrocatechol is dissolved into this liquid. Then the ether is evaporated and the rest dissolved in water, which aqueous solution is treated with basic

leadacetate in excess. Pyrocatechol is precipitated, the precipitate washed out and treated with dilute sulphuric acid in excess, so that all the Pb. is precipitated as sulphate and the pyrocatechol is liberated. Finally the filtrate which contains the pyrocatechol is estimated in the way described above.

A description of the manner in which the objects were treated may be given in the following words. First wood and bark were separated; the wood was cut to pieces and quickly dried in an air-oven at 100° C. Then an aliquot part of the wood of each piece of branch was pulverized and this portion used for estimation of:
1°. the quantity of proteins.
2°. the quantity of total carbohydrates.

Ad 1: in a part of this pulverized wood which was extracted with boiling water to remove the soluble N-compounds the proteins were estimated by means of the Kjeldahl method.

Ad 2: for estimation of the total quantity of carbohydrates 3 portions of c. 1 g. finely pulverized wood were treated in an autoclave for c. 4 hours and at c. 5 atmospheres. Then it was filtered through asbestos and the filtrate boiled, after adding dilute HCl, for 3 hours in a recipient with a spiral condensor. Afterwards the solution was quickly cooled, and almost neutralized with aqueous sodium hydroxyde. Estimation of the total quantity of reducing sugars took place by the method of SCHOORL (8).

The difference between the percentage of total carbohydrates (average of 3 portions) obtained in this manner and the percentage of carbohydrates which could be extracted with warm water, was calculated as starch. Control experiments with the takadiastase enzyme showed that the quantity of maltose was negligeable.
With regard to the bark the "modus operandi" must be another one: 1°. because the glucoside salicin is present here, 2°. because pyrocatechol must be estimated in the manner already described. A portion of the material immediately dried in an air-oven at 100° C. was treated in the same manner as was described for the wood, in order to estimate the total of carbohydrates and proteins. By far the greater part of the fresh bark was however repeatedly extracted with boiling water; the extracts were combined and by evaporation on a waterbath concentrated to a definite volume. The clear brown solution was repeatedly shaken up with ether, at least if an estimation of pyrocatechol was desired. After removal of the ether, dissolved in the water, an aqueous solution of basic Pb-acetate was added and filtered hot. In the filtrate the excess of lead was removed with a solution of Na_2HPO_4, so that after filtering a clear and slightly yellow solution was obtained. The latter was concentrated to a

definite volume and used for estimation in the following manner:

Estimated were:

1°. the directly reducing sugars (p %);

2°. the sugars obtained after treatment with emulsin, and hydrolysing of salicin. (q %);

3°. the sugars obtained after treatment with invertase (r %);

q—p is the percentage of sugar derived from salicin, so that the quantity of the latter may be calculated by multiplication with the quotient of the corresponding molecular weights 286 and 180.

r—p is the percentage of reducing sugars derived from sucrose.

The percentage of total carbohydrates obtained in the above described way, must be diminished by the percentage of monoses, sucrose and salicinglucose; the rest can be taken into account as starch, because here too, the quantity of maltose is negligeable.

§ 3. THE PHYSIOLOGICAL FUNCTION OF SALICIN DURING SPROUTING.

In the above described manner experiments with sprouting branches of *Salix purpurea* were made in the years 1934—1938.

Experiments of 1935.

Of the above described clone 4 portions of 20 branches each were made; the total fresh weight of these lots was almost equal.

Lot A and B, used as controls were analyzed in March, immediately after being cut off and before the sprouting.

The differences between A and B were insignificant: dryweight of the wood 380 and 390 g (percent. difference from the average, 385 g, is 1,3). Dryweight of the bark is resp. 131 and 135 g (percent. difference from the average, 133 g, is 1,5). The bark contained resp. 4,2 and 4,0 % of salicin (percent. difference from the average 4,1 % is 2,4). For sucrose these values were 2,2 and 2,4 % (percent. difference from the average 4,3); for monoses these values were: 2,25 and 1,95 % (difference from the average 7,1 %).

Control. Average of lot A and B.

wood, dry weight 385 g		
starch	11,8 %	45,430 g
monoses + sucrose	0,1 %	0,385 g
proteins	2,55 %	10,241 g
bark dry weight 133 g		
salicin	4,1 %	5,453 g
starch	17,5 %	23,275 g
monoses	2,1 %	2,793 g
sucrose	2,4 %	3,192 g
proteins	8,44 %	11,225 g

wood, dry weight 345 g

starch	8,7 %	30,015 g
monoses	1,2 %	4,140 g
sucrose	1,0 %	3,456 g
proteins	1,81 %	6,245 g

bark, dry weight 111,3 g

salicin	2,0 %	2,225 g
starch	14,9 %	16,584 g
monoses	1,4 %	1,558 g
sucrose	0,3 %	0,334 g
proteins	6,0 %	6,679 g

The roots contain traces of monoses, 0,060 g sucrose, no salicin no starch.

green shoots, dry weight 31,7 g

salicin	2,3 %	0,729 g
starch	12,7 %	4,020 g
monoses	0,9 %	0,285 g
sucrose	0,4 %	0,127 g
proteins	16,25 %	5,151 g

lot D branches, sprouted in the dark
wood dry weight 343 g

starch	6,7 %	22,981 g
monoses	0,9 %	3,087 g
sucrose	0,7 %	2,401 g
proteins	1,75 %	6,003 g

bark, dry weight 115,3 g

salicin	1,8 %	2,075 g
starch	8,3 %	9,570 g
monoses	1,4 %	1,614 g
sucrose	0,3 %	0,346 g
proteins	7,5 %	8,648 g

etiolated shoots, dry weight 11,8 g

salicin	2,6 %	0,307 g
starch	—	—
monoses	2,9 %	0,342 g
sucrose	2,0 %	0,236 g
proteins	10,62 %	1,253 g

The roots contain traces of monoses and sucrose, no starch, no salicin.

To answer the question, whether salicin has the function of a reserve substance, it is important to ascertain how much of the different reservesubstances remains in bark and wood after sprouting. Comparison of the sprouted branches with the controls is therefore indispensable and as a continual exchange of substances may take place between wood and bark, through the medullary rays, it has no sense to compare them apart. The different carbohydrates are also combined because an intensive conversion of insoluble into soluble ones takes place in spring, so that the quantity of sugars may even increase during this period at the expense of the starch.

Control, average of lot A and B

salicin		5,453 g
monose	2,985 g	75,075 g
sucrose	3,385 g	
starch	68,705 g	
proteins		21,405 g

Branches after sprouting in daylight, lot C
bark and wood without the shoots.

salicin		2,226 g c. 41 % of the average,	decrease 59 %
monose	5,698 g	56,081 g c. 75 % „ „ „ „	25 %
sucrose	2,784 g		
starch	46,599 g		
proteins		12,923 g c. 60 % „ „ „ „	40 %

Branches after sprouting in the dark, lot D
bark and wood without the shoots.

salicin		2,075 g c. 38 % of the average,	decrease 62 %
monoses	4,701 g	39.999 g c. 53 % „ „ „ „	47 %
sucrose	2.747 g		
starch	32,551 g		
proteins		14,651 g c. 68 % „ „ „ „	32%

IN THE LIGHT AS WELL AS IN THE DARK THE PROPORTIONAL DECREASE OF SALICIN EXCEEDS THAT OF THE PROTEINS AND TOTAL CARBOHYDRATES. FROM THIS FACT ONE MAY CERTAINLY CONCLUDE, THAT IN SALIX PURPUREA THE GLUCOSIDE SALICIN HAS THE FUNCTION OF A RESERVE SUBSTANCE.

Even if we add the salicin in the young shoots, in the supposition that the glucoside might have been transported, the proportional decrease of salicin still exceeds that of the proteins and total carbohydrates. This appears from the following data:

In the experiment in daylight 2,945 g or c. 54 % of the salicin remains, if we add the quantity in the shoots; in the experiment in the dark these values are 2,382 g or 44 %. Moreover it will appear later on that this supposition is out of the question; the glucoside in the young shoots is formed by synthesis.

In the dark the growth in length of the etiolated shoots is indeed considerable, but the dry weight is much less, only 37 % of that of the green shoots. The explanation of the fact, that sprouting in the dark causes a 32 % decrease of the proteins, whilst that in daylight is 40 % may be given in this way. That the percentage decrease of carbohydrates is in the dark much greater than in daylight (47 % in the first case, 25 % in the latter) is of course a consequence of the carbon assimilation in daylight.

Experiments in the year 1936.

Similar experiments were made in the year 1936, in which however it was tried to make the lots of branches still more equal; a comparison with the controls A and B of the former experiments will prove that I succeeded in this purpose.

Lots A′ and B′, used as controls, were analyzed before the buds sprouted; lot C′ sprouted in tapwater in daylight, lot D′ in tapwater in the dark; each lot consisted of 12 pieces.

The dry weight of the wood was resp. 155 and 159 g (average 157 g), that of the bark resp. 50,75 and 50,3 g (average 50,525 g). The difference between these values and the average weight was in the case of the wood 1,3 %, in that of the bark 0,4 % of the average. In the year 1935 these differences with the average were resp. 1,3 % and 1,5 %.

With regard to the quantity of salicin monose and sucrose the differences from the average were in 1935 resp. 2,4 %, 7,1 % and 4,4 %, whilst those in 1936 were only 1,3 %, 3,4 % and 5,6 %. The four lots are also comparable entities.

Control, average lot A′ and B′.

wood, dry weight 157 g			
starch	12 %	18,840 g	
monoses and sucrose	—	—	(traces)
proteins	2,5 %	3,925 g	
bark dry weight 50,525 g			
salicin	8,11 %	4,095 g	
starch	10,77 %	5,442 g	
monoses	1,45 %	0,733 g	
sucrose	3,18 %	1,607 g	
proteins	7,5 %	3,787 g	

Branches, sprouted in daylight lot C'

wood, dryweight 147 g		
starch and sugars	9,6 %	14,112 g
proteins	2,44 %	3,587 g
bark, dry weight 41,7 g		
salicin	5,7 %	2,377 g
starch	9,0 %	3,753 g
monoses	2,7 %	1,126 g
sucrose	0,8 %	0,334 g
proteins	4,38 %	1,826 g
green shoots, dry weight 8,9 g		
salicin	0,27 %	0,024 g
starch	6,6 %	0,587 g
monoses	0,5 %	0,045 g
sucrose	0,13 %	0,012 g
proteins	19,37 %	1,724 g

The roots contained 0,040 g of sucrose and monose, no salicin, no starch.

branches sprouted in the dark, lot D'

wood, dry weight 148 g		
starch and sugars	8,6 %	12,728 g
proteins	2,51 %	3,715 g
bark, dry weight 41,3 g		
salicin	5,23 %	2,160 g
starch	9,19 %	3,795 g
monoses	1,72 %	0,710 g
sucrose	0,30 %	0,124 g
proteins	4,12 %	1,702 g
etiolated shoots, dry weight 4,90 g		
salicin	1,43 %	0,070 g
starch	—	—
monoses	2,96 %	0,145 g
sucrose	1,80 %	0,088 g
proteins	10,1 %	0,495 g

The roots contained 0,010 g of monoses and sucrose, no salicin, no starch.

In the same way as in the year 1935 it was examined, how much of the different reserve substances remained after sprouting. No difference was made between wood and bark; all the carbohydrates were taken together.

Control, average A′ and B′						
salicin	4,095 g					
total carbohydrates	26,622 g					
proteins	7,712 g					
branches after sprouting in daylight, lot C′						
salicin	2,377 g	c. 58 %	of the	average,	decrease	42 %
total carbohydrates	19,325 g	c. 73 %	„	„	„	27 %
proteins	5,413 g	c. 70 %	„	„	„	30 %
branches after sprouting in the dark, lot D′						
salicin	2,160 g	c. 53 %	„	„	„	47 %
total carbohydrates	17,357 g	c. 65 %	„	„	„	35 %
proteins	5,417 g	c. 70 %	„	„	„	30 %

The result of the experiment of the year 1936 differs only in accidental circumstances from that of the previous year. The percentage of salicin in the bark was higher, that of the starch lower, whilst the percentage of the latter in the wood was nearly equal to that of 1935.

In 1936 the dryweight of the branches was less than half that of the year 1935, whilst the experiment lasted shorter. In consequence of these facts the young etiolated shoots, as well as the green ones had a much smaller weight and accordingly the decrease of the reserve substances in wood and bark was much less in 1936.

In the later year salicin, carbohydrates and proteins diminished in the dark resp. 47 %, 35 % and 30 %. In the year 1935 these values were resp. 62 %, 47 % and 32 %.

FOR ALL THAT THE PERCENTAGE DECREASE IS IN BOTH CASES GREATER WITH REGARD TO SALICIN THAN TO PROTEINS AND CARBOHYDRATES. This fact is in my opinion an irrefutable proof of the reserve-function of salicin in *Salix purpurea*.

The decrease of salicin in absolute measure is however much smaller than that of the carbohydrates and the significance as a reserve substance may be said to be in proportion to it.

Reviewing the experiments with branches, which after being cut off sprouted in daylight the results are the following:

In the year 1936 the decrease as to salicin, carbohydrates and proteins was resp. 59 %, 26 % and 40 %; the decrease of salicin is again the strongest, which corroborates the results of the preceding

experiments. That the percentage decrease of the carbohydrates was almost the same in both years may be caused by transport of the product of carbon assimilation from the green shoots to the branches.

Be that as it may, it has been made clear by this series of experiments, that at all events the salicin in *Salix purpurea* has the function of a reserve substance. In my former papers I proved the same for the glucoside arbutin in *Vaccinium Vitis-idaea* and *Pirus communis* (13), and for the glucoside gaultherin in *Gaultheria procumbens* (11). STEKELENBURG (10) has demonstrated it for different glucosides whose aglucon gives hydrocyanic acid.

One should however by no means conclude that all glucosides have the same function. In the opinion of KLEIN and LINSER (6) aesculin is a reserve substance in the bark of *Aesculus Hippocastanum*; but KERSTAN l.c. maintains the contrary. As was already made clear in the introduction there are very serious difficulties with regard to the quantitative method of KERSTAN because the latter used percent. values of fresh weight. This applies however especially to absolute values and the experiments of KERSTAN showed that the behaviour of aesculin is quite different from that of the carbohydrates. Whilst the glucosidesugar keeps almost the same value during sprouting (calculated pro 1 g. of fresh weight), the monoses and sucrose greatly diminish, also the total of carbohydrates. The contrast between glucoside and carbohydrates is striking and this fact gives strong support to the assertion of KERSTAN, concerning the physiological behaviour of aesculin.

With salicin KERSTAN has made no physiologicale xperiments, at least not with regard to the behaviour of this glucoside during sprouting. To the behaviour of salicin in the leaves I will revert later on.

As was already asserted in the discussion of methods, it was desirable to repeat once more and with a better quantitative method the experiments as to the relation of salicin and pyrocatechol. In these experiments the decrease of salicin and the increase of pyrocatechol during sprouting ought to be compared. This was accomplished in the years 1937 and 1938 in the way already described. Of two lots of comparable branches one was analyzed before sprouting whilst the other one was put in tapwater in the dark and analyzed after sprouting.

12 control branches before sprouting: salicin 2,023 g, pyrocatechol 0,174 g;
12 branches 5 weeks in the dark: salicin 1,765 g, pyrocatechol 0,270 g.

The increase of pyrocatechol is 0,096 g, the decrease of salicin 0,258 g, which values stand in the proportion of 97: 260. As the molecular weights are in the proportion of 110: 286 or 100: 260,

we can say that practically the changes are in the proportion of the molecular weights. In other words the aglucon saligenin is almost completely transmuted into pyrocatechol.

The same result was obtained in the year 1938 with better developed etiolated shoots.

control branches before sprouting salicin 2,419 g, pyrocatechol 0,166 g;

branches after sprouting in the dark salicin 1,938 g, pyrocatechol 0,360 g.

Increase of pyrocatechol 0,166 g, decrease of salicin 0,481 g, which values are in the proportion of 99 : 260.

These quantitative experiments completely confirm the results obtained in my former papers by means of a somewhat dubious method of pyrocatechol estimation and prove the transmutation of the aglucon saligenin into pyrocatechol.

In making similar experiments with cut-off branches put in tap-water in daylight we get however an altogether different result. In the year 1938 the following data were obtained: the decrease of salicin with regard to the control-branches was 0,219 g and the increase of pyrocatechol was 0,048 g. The proportion of these values is as 59: 260; in other words the quantity of pyrocatechol formed in this experiment is much smaller. This fact might be explained by supposing that saligenin is not wholly transformed into pyrocatechol. In former experiments it has been ascertained that during sprouting the enzyme salicase, which causes the hydrolyzing of salicin into saligenin and glucose and which was isolated from *Salix spec.* in 1909 by SIGMUND (9), was present in the bark of *Salix purpurea.* In these circumstances a small quantity of saligenin was sometimes present. Whether this explanation of the fact that less pyrocatechol is formed in branches sprouting in daylight, is the right one, seems however doubtful.

§ 4. THE PHYSIOLOGICAL FUNCTION OF SALICIN IN THE LEAVES.

The same transformation of the aglucon of salicin into pyrocatechol was ascertained in my former papers in full grown leaves of *Salix purpurea.* In order to explore whether salicin here too plays the part of a reserve substance the quantity of this glucoside was studied in full grown leaves at night and by day. It appeared that during the day salicin increases if the leaves had the possibility of carbon assimilation. On the contrary the quantity of salicin decreased during the night, especially if transport of the leaves into the branches was possible.

It appeared moreover that the quantity of pyrocatechol differs

in the morning and in the evening; the latter substance decreases during the day and increases during the night; in other words the changes of pyrocatechol are here too quite contrary to those of salicin.

On account of the doubt arisen about the reliability of the quantitative method of pyrocatechol estimation these experiments were repeated whilst pyrocatechol was estimated according to the method of GARDNER and HODGSON.

Per 100 halves of the leaves of *Salix purpurea* there was present:
in the morning 8h 0,134 g salicin and 0,013 g pyrocatechol.
„ „ evening 20h 0,156 g „ „ 0,008 g „

The decrease of pyrocatechol is 0,008 g, the increase of salicin 0,022 g, which values are in the proportion of 95: 260. a proportion which agrees with my former results.

What is the conclusion of the facts mentioned above? During the day salicin is synthesized at the expense of the glucose formed by assimilation and by using the pyrocatechol already present in the leaves. On the other hand hydrolyzing of salicin by means of salicase predominates during the night; the quantity of glucose originated by this process is transported to the bark, whilst the aglucon saligenin is transmuted into pyrocatechol.

A proof of the latter is given by the following experiment in which per 100 halves of leaves the increase of pyrocatechol during the night was 0,060 g, the decrease of salicin 0,194 g. The proportion of these values is 80 : 260, a result which is not so good as in the former experiment.

There is no question of a transport function of salicin; KERSTAN pretends that such is my opinion but without any cause. On the contrary the function of salicin is in my opinion that of a reserve-substance; it is a compound in which glucose is fixed and temporarily accumulated but not transported. The supposition of PFEFFER in his "Pflanzenphysiologie" (7) is at least valid for this glucoside.

KERSTAN made some experiments with leaves of *Salix fragilis* by means of the adsorptionmethod. His calculation was alas again made according to the fresh weight, a value which especially in leaves is extremely variable. Moreover *Salix fragilis* is a less suitable object because the percentage of salicin is much smaller than in *Salix purpurea*. Yet in isolated leaves put in the dark he notes a decrease of salicin; this is even more the case with leaves which remained in connection with the branches during the night. During the day he notes in leaves left in connection with the plant an increase of salicin, both observations which wholly agree with mine.

From the above one may by no means conclude that salicin has

always and exclusively this function of a reservematerial.

1°. it is doubtful whether this glucoside or its aglucon has perhaps still another function in metabolism;

2°. it is doubtful whether substances as aromatic compounds and alkaloids or glucosides always have a definite function; the possibility exists that they have no function at all in metabolism.

With regard to the first it must be remarked, that in later years pyrocatechol has played a part in considerations about the respiration of different higher plants. From recent papers I will cite that of BOSWELL and WHITING (1), entitled "A study of the polyphenol oxidase system in potato tubers", in which pyrocatechol seems to be an important part of an oxidase system.

With regard to the second I may refer to my own paper "The relation between taxonomy and chemistry of plants" (14). In this paper I defended the thesis that each genus, often each species of the higher plants has its own specific combination of chemical compounds: the production of these compounds must be considered as a characteristic of the species as much as its morphological or anatomical pecularities. And a definite significance for the existence of the species in question can no more be assigned to each of these chemical compounds than to all these morphological and anatomical characteristics.

In the case of salicin, the combination of this glucoside with populin (benzoylsalicin) is wholly characteristic for the genera Salix and Populus of the Salicaceae.

SUMMARY.

The glucoside salicin, present in *Salix purpurea* and the greater part of the other Salix species, has the function of a reserve substance, which is hydrolyzed during sprouting by the enzyme salicase belonging to the emulsine complex. The two products are glucose and saligenin (ortho-oxybenzylalcohol); the former of which is used in metabolism, whilst the aglucon saligenin is quantitatively transmuted into pyrocatechol (orthodioxybenzol). This is proved by experiments with cut-off branches put in tapwater, which sprout in the dark. The decrease of salicin and the increase of pyrocatechol are in proportion of their molecular weights.

The decrease of the glucoside salicin expressed in the quantity of this compound present before sprouting is always greater than the relative decrease of the total quantities of carbohydrates and proteins. This fact is an irrefutable proof of the reserve function.

In absolute measure the decrease of the glucoside is however much smaller than that of the carbohydrates and the siginificance

as to a reserve material may be said to be in proportion to it.

In the leaves of *Salix purpurea* the quantity of salicin increases in assimilating leaves, in which synthesis the pyrocatechol is used as well as the products of carbonassimilation. During the night, when transport of the products formed by assimilation is easily ascertainable, salicin decreases in the leaves, whilst pyrocatechol increases. The conclusion is, that during the night salicin is hydrolyzed by salicase; the formed glucose is transported whilst the aglucon is transmuted into pyrocatechol. Salicin has therefore not the function of a transport material, but may be regarded as a condensation product of glucose and the polyphenol pyrocatechol, which product by its non-diosmobility is a suitable accumulation product.

The transmutation of saligenin into pyrocatechol forms a compound which according to several workers in this field of science sometimes together with a dehydrase and a polyphenolase forms an important oxidizing system.

It is, in fact a question whether the production of different compounds as glucosides, alkaloides and benzolderivatives in the higher plants must always be of importance for the metabolism of the species, which contain them. Lately I maintained, that each genus of the higher plants has its own specific combination of these substances. The production of these compounds is to be regarded as a characteristic of the species or genus quite in the same way as its morphological or anatomical pecularities.

A special significance for the metabolism or the struggle for existence of the species in question can no more be assigned to each of the chemical compounds than to each of these morphological or anatomical characteristics.

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