Although hydrogen sulphide is volatile and most of it escapes during the boiling, the increase of the number of molecules formed during that action (however small this may be) is large enough to account for the observed difference. The fact that the deviation has been found larger in the case of toluene than with *m*-xylene as solvent is also in agreement with the observed fact that more hydrogen sulphide is evolved in the first than in the second case.

Our research on the action of sulphur on *p*-xylene was not conducted merely with the idea of confirming the researches of ARONSTEIN and MEIHUIZEN (we were not quite sure whether the *m*-xylene then used had been completely free from *p*-xylene) but also to throw more light on the mechanism of the process and particularly on the question of the primary formation of stilbene and the secondary formation of dibenzyl.

Chemical Laboratory of the Polytechnical School.

DELFT, September 1902.

Physiology of Plants. — *"Investigations of Glucosides in connection with the Internal Mutation of plants,"* by mr. TH. WEEVERS. (Communicated by Prof. C. A. LOBRY DE BRUYN).

(Communicated in the Meeting of 27 September 1902.)

The purpose I had in view in this investigation was to trace for some plants, whether the amount of glucosides remains unchanged during the development or not; and to investigate in the latter case by what conditions these changes are determined.

At the same time the manner in which those changes took place formed another subject for study: whether glucosides were transported as such, or whether a decomposition could be stated, and in the latter case what were the components in which this took place.

Salix species and Aesculus hippocastanum L. were especially used for the investigations; Gaultheria procumbens L. and Fagus sylvatica were also submitted to a prefatory study.

The glucosides to be mentioned here are salicine for the Salix species, gaultherine for Gaultheria and Fagus, aesculine and moreover some glucosides not yet chemically determined for Aesculus hippocastanum.

As for salicine the quantitative valuations were made as follows. The salicine was entirely extracted by boiling water from the parts to be examined and the extract treated with basic lead acetate. The

surplus was removed by dinatriumphosphate and the liquid then obtained reduced to a definite volume. In this two estimations of sugar were made, one before, the other after allowing emulsine to work in upon it for 48 hours. Prefatory experiments with pure salicine had proved that in this way it was completely decomposed: the increase of the reduction after inversion was to be attributed only to the glucose formed of salicine. [1]).

From this increase of the glucose the quality of the salicine could then be calculated.

This same method was followed in order to state the salicine in various parts of the plant; then however, after inversion the liquid was extracted with ether, so that saligenine might enter into it. This substance is easily recognised by the physic qualities of its crystals and by the substitute of bromine obtainable with brominewater and moreover by its salt of copper. The efforts to point out salicine in the tissue itself were unsuccessful; the method formerly used by THEORIN [2]), namely that of adding concentrated sulphuric acid, proved impracticable, as it during the produced erroneous results.

For the above mentioned Salix species salicine is found in the bark of the branches, but not in the wood; young buds are rich in it, likewise the assimilating leaves. It appears in young ovaries but disappears during the process of ripening.

Although an inverting enzyme was not to be extracted, it proved necessary to kill the parts immediately in boiling water, otherwise considerable alterations in the quantity of salicine presented themselves. Thus e.g. after slow drying 25 pCt. disappeared out of the bark.

The following series of determinations for the purpose of investigating the quantity of salicine during the budding period, was made with *one* specimen to exclude individual differences.

The total quantity in various successive stages was calculated by taking a branch with a definite number of sidebuds as object. The weight of the different parts of this branch together with the procentic values of the quantity of salicine in corresponding parts of the same object in the successive stages gave the total quantity of salicine of this branch in those stages [3]).

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[1]) Before inversion a solution of salicine does not reduce even with boiling; neither does saligenine formed by means of inversion at the same time as glucose.

[2]) See Theorin Öfversigt af Kongl Vetenskaps. Akademiens Förhandlingen 1884. No. 5. Concentrated H_2SO_4 gives with salicine a coloring of red.

[3]) In corresponding parts of *one* object was an equal quantity.

Under observation were only branches without genitals; those with catkins gave a different result [1]).

Branches of $1^1/_2$—4 mM. diameter (wood and bark together).

March 24th 3.2 pCt. [2])
April 17th 2 "
May 21st 0.4 "

Branch of 4—8 mM. diameter (only bark; hence the quantity is higher).

March 24th 4.1 pCt.
April 17th 2.8 "
May 21st 2.1 "

For Salix Helix L. the figures for the bark of branches were

March 24th 4.4 pCt.
April 17th 2.7 "

The quantity of glucose is a little variable; however, it does not rise above 0.5 pCt.; the quantity of fecula diminishes when budding from 9.5 pCt. to 6 pCt.

In the young buds of Salix purpurea there is before the budding 4.4 pCt. and of Salix Helix 6.2 pCt. During the budding this quantity decreases greatly, disappears even for S. purpurea entirely (17 April) but rises again quickly, when assimilation begins, to 3.7 pCt in leaves and 3 pCt. in young shoots (21 May).

Of the absolute quantity of salicine in a branch with 300 buds

± 36 pCt. disappeared from 24 March—17 April
± 18 " " " 17 March—21 May,

the assimilation, begun already before May 21, having given rise to new salicine.

Experiments with branches placed in the dark in water [3]) showed the following:

After the roots have been formed, a number of long etiolated shoots bud forth, consuming by their development besides the fecula also a great quantity of the salicine in the bark (± 70 pCt.).

At first the young shoots contain a great quantity 7.2 pCt., this, however, keeps on decreasing; the absolute quantity calculated for 100 young shoots also diminishes:

for 100 young shoots long 18 mM. there is 28 mG. salicine
" " " " " 125 " " " 15 " "

[1]) The quantity of salicine is at the same instant lower in branches with catkins than in those without; the salicine diminishes more quickly.

[2]) These procentic values are calculated for dry weight.

[3]) These were branches of 6—10 m.M. diameter, the young shoots coming from sleeping buds.

These quantities are small compared to the entire quantity consumed ± 330 mG. for 100 young shoots.

When the young buds were budding forth saligenine was found in them, the branches were immediately killed in boiling water, the extract after cooling down extracted with ether; so all influence of enzyme could be excluded. It becomes very probable that the salicine is analysed before the consumption, on account of saligenine being found; the quantity, however, is so small that if really the analysis of salicine were to take place as indicated, and a decomposition to precede the consumption, saligenine can only be an intermediate stage. Either the aromatic half disappears as such, or another aromatic substance must be the definite product of the decomposition.

In the young leaves developing normally, salicine soon makes its appearance again after having disappeared for a moment; we can expect that this increase is connected with and due to the assimilation, as etiolated shoots do not show it. In order to state whether the leaves were really the place of a new formation and the light really had a part in it, the quantity of salicine before and after darkening was compared.

The quantity in the leaves was determined in the evening after sunset and in the morning before sunrise (*one* specimen). Likewise in the evening leaves were halved, one half with midrib left on the plant, the other half analysed. The following morning the remaining half was cut off from the midrib and also analysed[1]). Provided that a sufficient number, 100 or 200 leaves were halved, a comparison could very well be made.

For a small-leafed specimen a 100 leaves

8 P. M. 7 Aug. 47.5 mG. glucose 87.2 mG. salicine
4 A. M. 8 " 27.4 " " 60.2 " "

For a big-leafed specimen a 100 leaves

8 P. M. 7 Aug. 80 mG. glucose 177.7 mG. salicine.
4 A. M. 8 " 31.9 " " 142.7 " "

So in both cases we see a decrease during an 8 hours' summer night of respectively 30 and 20 % of the salicine in the leaf in the evening.

For experiments with entire leaves of *one* specimen:

8 P. M. 7 Aug. 4.6 % salicine.
4 A. M. 8 " 3.2 % "
8 A. M. 8 " 4.6 % "

[1]) See Lotsy. Mededeelingen 's Lands Plantentuin XXXVI.

Thus here too a decrease of 30 % during the night followed up by an equal increase on the following day. If branches on the plant are enveloped in black waxed paper the decrease amounts after 48 hours only to 35 %, no great difference with that of 8 hours; increase, however, did not take place, so light proves to be a necessary factor. The experiments of etiolating told the same.

If this quantity of salicine disappearing from the leaves was removed to the bark, an increase would have to be observed there. This was indeed the case, for branches rich in leaves the increase of the quantity of salicine of the bark amounted in *one* night to 2.5 %; for branches with few leaves to 1.1 %.

From the etherextract prepared in the above described manner, of the parts of Salix purpurea still another substance could be isolated by means of subliming. According to the micro-chemic qualities this was a substance resembling phenol and qualified by its compound of lead and of lime, besides reaction with tetrachloorchinon as an ortho-derivate [1]). The substance did not show Aldehydreactions. The further micro-chemical qualities corresponded to those of the simplest ortho-phenol, catechol. After a repeated crystallisation out of benzol the melting-point proved to be 104°. Elementary analysis and determination of molecular weight confirmed the fact, that it was catechol.

As the material which furnished the substance was quickly killed both in boiling water and in boiling alcohol and the etherextract already showed the crystals before sublimation, influence of enzym [2]) is not probable and formation out of resin is not possible.

Treatment with ferrichloride followed by additon of natrium hydro-carbonate also furnished in the tissue the reaction of catechol. The red colour was clearly visible in the unopened cells of the sections of the bark, young etiolated shoots showed them faintly, older ones more. Catechol is like salicine only to be found in the bark [3]).

The supposition was aroused that catechol might be the aromatic substance, remaining there as definite product of decomposition of the salicine. In order to test the accuracy of this supposition, an investigation had to be made whether the quantity of catechol of the parts of Salix purpurea were varying.

For a quantitative determination of the catechol the method of

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[1]) According to an investigation of Prof. H. Behrens which will shortly appear, communicated to me by Miss Grutterink.

[2]) The black colour of the dying leaves is caused by the influence of a "tyrosinase" on catechol.

[3]) Catechol was also obtained out of Salix Helix L., S. babylonica L., S vitellina L, Populus alba L., P. monilifera Ait, sometimes only very little.

DEGENER (Journal f. Prakt. Chemie 1879) could not be used on account of a flavon-like colouring matter not closer examined, and also precipitated by a basic leadacetate. So the method of Prof. BEHRENS to determine Indigo was followed. The sublimate of a solution of catechol of a known strength in absolute alcohol was compared with that of the alcoholic solution of the remainder of the ether evaporated dry. Now it was examined how much this liquid had to be diluted to obtain an equivalent sublimate. The sublimation was performed by means of the brass table described by Prof. WIJSMAN. Under certain precautions the determination could be accurately made to milligrammes.

The quantity of catechol of the leaves was in the evening 0.6 pCt. } with *one*
" " " " " " " " " " morning0.1 " } specimen

" " " " " " bark " " " evening0.6 " } with the
" " " " " " " " " " morning0.4 " } same specimen.

So the quantity of the catechol here proved to change in reverse order as that of the salicine. In the leaves the salicine diminishes in the night, the catechol increases, and in the bark the catechol diminishes and the salicine increases. Is there any connection between the extent of these changes?

For that purpose for one and the same object catechol was determined as well as salicine.

200 halves of leaves 8 P. M. 225 mGr. salicine (4.5%) ± 32 mG. catechol (0.65%)
" " " " 4 A. M. 162 " " (3.3%) ± 52 " " (1.05%)

So 63 mG. salicine less, 20 mG. catechol more.

The proportion of these values, given the degree of accuracy of the determination of catechol, pretty well agrees with the proportion of the molecular weights.

A comparison was also made of the change in salicine with that in catechol for leaves budding forth in the dark.

17 Gr. bark before budding 351 mGr. salicine 36 mGr. catechol
17 " " after " 232 " " 55 " "
budding etiolated shoots 55 " " 4 " "

(a great increase in the bark, in the young shoots only a small part of the catechol thus formed to be found) 64 mG. salicine was used, 23 mGr. catechol was formed.

These two values stand in the ratio of 36 to 100, the molecular weights in that of 38 to 100.

So it is very natural to assume here a decomposition of the salicine into sugar and catechol with saligenine as intermediate stage (see

above). For this then a CH_2 group out of the lateral chain would have to be decomposed, as saligenine is orthooxybenzylalcohol and catechol is the orthodiphenol.

Corresponding to this the quantity of catechol of the bark is large in May(1.1 pCt.), a greater part of the salicine then having disappeared, much lower in July (0.3 pCt.) when the loss has been repaired [1]). Where now has the decomposition taken place?

PFEFFER says Kap. VIII, Pflanzenphysiologie 2) Auflage: „vielleicht dienen die esterartigen Verbindungen der Kohlenhydrate mit Phenolkörpern zur Herstellung von schwer diosmirende Verbindungen bei deren Zerspaltung im allgemeinen der Phenolkörper in der Zelle intact verbleibt, um fernerhin wieder zur Bindung von Zucker benutzt zu werden."

The facts are excellently explained in the following way:

The decomposition of the salicine takes place in every cell, the glucose is conveyed in the direction of the green parts, the catechol remains in the cell and binds glucose, coming from cells situated closer to the bark, to salicine.

Glucose is transportmatter and salicine is transitory reservematter.

The glucose being comsumed in young parts in greater quantities than its supply is, catechol must be found, but only so much as corresponds to the decrease of the absolute quantity of salicine.

100 young shoots 18 m.M. long 28 m.G. salicine, traces of catechol.
100 „ „ 85 m.M. „ 21,6 m.G. „ 2 m.G. „

6.4 m.G. salicine corresponds when calculated to 2,5 m.G. catechol, when observed to 2 m.G.

This correspondence adds great strength to the hypothesis. [2])

In the bark the loss of consumed glucose is not repaired, so catachol increases greatly.

As for Aesculus, here it was especially the germination which was studied. The glucosides found in the ripe seedlings being not yet chemically determined, it was only necessary to base the method of the quantitative definition on the quantity of sugar formed by inversion. I had to trace whether the quantity of sugar bound in glucoside decreased during the germination.

To this end the seedlings were ground and extracted with methylalcohol, of this extract the alcohol was evaporated, and the watery liquid

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[1]) I here mean the quantity in the bark of thicker leafless branches where no difference between night and day is observed.

[2]) Also the facts observed at the change of night and day can be excellently explained in this way.

extracted with ether to get rid of oil and resin. The extracted liquid served as definition of the reduction *before* and *after* inversion by boiling it for 2 hours with HCl [1]).

From the difference of this reduction the quantity of reducing sugar originating from the glucoside could be calculated; it amounted to 13 pCt.

During the germination this quantity decreased in cotyledons by 60 or 70 pCt. Fecula and albumen by 70 or 80 pCt. The germinating plants contained only 1 or 2 pCt. of glucose bound in the shape of glucoside, the consumption of the glucosided sugar during the germination could be regarded as proved by the 70 pCt. decrease of the absolute quantity.

The localisation of aesculine was observed by fluorescence of its watery solution, to be seen when there are not too few sections. Aesculine was to be found in ungerminated seeds only sporadically in the plumule; when germinating it appears in greater quantity in the stalks of cotyledons, not in the cotyledons themselves. Stalk and hypocotyledon internodium contain aesculine when germinating in the dark as well as in the light, so light is not necessary for the formation.

The stalks of the leaves show the aesculine only when developing in the light and not in the dark; this seems to point to the fact, that the aesculine of the normal germinating plant originates from two sources: that it is formed for the greater part by reforming of substances out of the cotyledons and side by side with this, that it is prepared independently in the stalks of the leaves from substances assimilated by the leaves. Experiments with full-grown plants, in the light and in the dark, with coloured and with normal leaves made this the more propable, but full certainty can only be given by means of later quantitative definitions.

Studies on Gaultheria procumbens showed what changes took place in the quantity of the gaultherine, the investigations have however not yet been brought to an end. The method of quantitative definitions was founded on the observation of the quantity of methylsalicylate which could be formed out of it. This was redistilled with vapour out of the parts, caught in alcoholic potash and saponificated with it. The kaliumsalicylate formed in this way was determined according to the method of MESSINGER and VORTMANN [2]). For smaller quantities the colorimetric method of determination was used with $Fe Cl_3$.

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[1]) After inversion and neutralisation the liquid was treated with leadacetate.

[2]) MESSINGER and VORTMAN, Zeitschrift f. Anal. Chem. 38 bl. 292.

Ber. d. deutschen chem. Gesellschaft. Berlin. Bd. 22. 2313.

With Fagus sylvatica where TAILLEUR [1]) found methylsalicylate only in the germinating plant, the latter method showed that it was also present in the full-grown plant. Methylsalicylate is to be found sporadically in the buds of the beech shortly before budding, during that process it is found in the young leaves and shoots as well as in the branches of the preceding year. Young long branches are richest in it, 0.02 pCt. As soon as the leaves have unfolded, this substance begins to disappear again and is nowhere to be found in a week's time.

Further particulars to be looked for in the dissertation to appear shortly.

Physics. — *"Some observations on the course of the molecular transformation."* By Prof. J. D. VAN DER WAALS.

As is well known, acetic acid may be considered as a mixture of simple and double molecules and we find a decreasing number of double molecules when we investigate the saturated vapour of this substance at increasing temperature. The same applies also to NO_2. We are apt to conclude from these two best known instances of molecular transformation that this course is the only one that is possible. We may, however, easily convince ourselves that also the opposite course may occur, and it appears to me that we may conclude from figure (1) of the communication of Prof. H. W. BAKHUIS ROOZEBOOM in the Proceedings of the previous session, that for the transformation of acetaldehyde and paraldehyde this opposite course perhaps occurs.

Let us take the equation for the molecular transformation, as it occurs Cont. II, pag. 29, namely:

$$log\,\frac{(v-b)x}{(1-x)^2} = \frac{2(E_1-E_2)}{R_1T} + 1 - \frac{2(H_1-H_2)}{R_1}$$

The quantity $1-x$ of this equation represents the quantity of the substance expressed in grams which occurs in the form of simple molecules, x therefore that which occurs in the form of double molecules. If molecules were formed consisting of n simple molecules, the equation would be modified into the following one:

$$log\,\frac{(v-b)^{n-1}x}{(1-x)^n} = \frac{A}{T} + B.$$

It is true that we only find the equation in this simple shape if

[1]) TAILLEUR, Comptes Rendus A. Sc. Tome 132 p. 1235.