ON PHOTOTROPIC CURVATURES IN AVENA, CAUSED BY PHOTOCHEMICAL INACTIVATION OF AUXIN-A VIA ITS LACTONE

by

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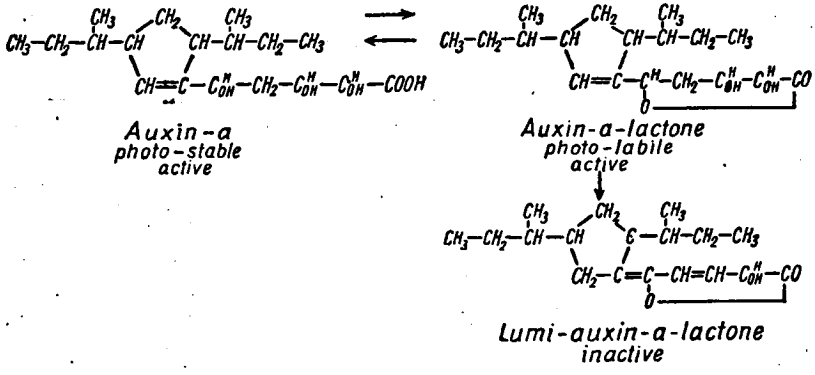
In their study on the auto-inactivation of auxin-a and of auxin-b, F. KÖGL, C. KONINGSBERGER and HANNI ERXLEBEN (1936) mention the photochemical inactivation of the auxin-a-lactone, "a phenomenon which possibly will be of the highest interest for the explanation of the phototropic curvatures" (p. 274). The spectral phenomena associated with this inactivation particularly have been studied in the thesis of C. KONINGSBERGER (1936). He stated that the absorption bands immediately found with solutions of auxin-a-lactone in the ultraviolet spectrum are not due to the lactone itself but to the ready conversion, by the radiation, of the lactone into a physiologically inactive substance, called "lumi-auxin-a-lactone". KÖGL (1936) gave as a preliminary equation:



Originally these transformations were thought to be only of purely chemical interest, since it was generally believed that

only auxin-a itself occurred in the plant. It then seemed to KÖGL, however, that possibly the equilibrium stated in solutions in vitro between auxin-a and the auxin-a-lactone also would occur in the vegetable cell. According to KÖGL (1937, p. 470) in that case the photo-inactivation of the auxin-a-lactone could also play a part in the physiological phenomena of phototropism and of photo-growthreactions.

WENT (1928) originally explained the phototropical curvature in *Avena* by a partial (± 20%) inactivation of the auxin by the light and by a transversal shifting of the basipetal transport of the auxin ("redistribution"). Later especially the latter factor, the "redistribution" of auxin caused by illumination, has been stressed, until recently VAN OVERBEEK (1936, a, b, c) found a quite different behaviour against light in decapitated coleoptiles of *Avena* provided with auxin and with β-indole-acetic acid. When illuminated allsidedly during the time wanted for their growth substance curvatures, these curvatures with auxin proved to be much smaller in the light than in the dark. With hetero-auxin the difference was much smaller or almost zero. When illuminated between the first and the second decapitation, applied agar blocks both with auxin and with hetero-auxin induced larger curvatures than in non illuminated plants. Although VAN OVERBEEK, at that moment, could not know the results obtained by KÖGL and his collaborators, refered to above, he explained his results by a destruction by the light of the auxin, whilst hetero-auxin would almost not be affected by the light.

BURKHOLDER and JOHNSTON (1937), repeating WENT's experiments and adding some new ones, stated a marked decrease in growth promoting properties of irradiated tissues normally containing growth substance and of agar blocks containing growth substance (from tobacco and corn). The light source being rich in ultraviolet and the light intensity being very high (cf. DU BUY, 1933), their results cannot be compared with those of VAN OVERBEEK; further unfortunately their experiments on auxin inactivation in agar blocks are scanty.

The effect of illumination previous to the application of growth substance was explained by VAN OVERBEEK by a destruction by the light of regenerated auxin and of auxin still present in the stump. Conclusive proof of a partial inactivation of auxin-a by light in the plant, therefore, only can be obtained with coleoptiles which do not regenerate their own auxin and which are as poor in residual auxin as possible. SKOOG (1937) claims that such

coleoptiles can be obtained by deseeding them ± 18 hours ahead and by duly decapitating them twice. In fact, VAN OVERBEEK (1936, b) did some experiments with deseeded coleoptiles with very clear results, but in most of his experiments "normal" coleoptiles were used.

We decided to adopt SKOOG's method since in (unpublished) experiments by Miss G. J. H. AMSHOFF, who repeated those of VAN OVERBEEK, irregular effects of illumination were also found in hetero-auxin series. We ascribed these to auxin still present or newly regenerated in "normal" coleoptiles. Also the fact that in VAN OVERBEEK's (1936, b) graphs 2 and 3 the curvatures are not proportional to the concentration of the growth substance in the agar block doubtlessly must be ascribed to residual auxin, present in the coleoptiles and not only to regeneration as VAN OVERBEEK does.

All experiments have been done by the second author with deseeded plants, treated after SKOOG's method and cultivated in an air-conditioned dark room (at 23° C and ± 95% air humidity) till they had to be illuminated. This was done in a large thermostat with glass door at the same temperature and air humidity as in the dark room.

Apart from the high sensitivity to auxin of the deseeded coleoptiles and the lack of regeneration, they proved to have another important advantage. During the 8 weeks of our experiments nothing could be found of the irregular daily fluctuations which in this laboratory "normal" plants use to show in their reactivity to growth substance. While, in testing a standard solution in the same dark room, normal plants showed a capricious variability on subsequent days, the deseeded plants always gave constant curvatures. This experience can only mean that the variability of "normal" plants is due to variations either

1) in the transport or in the action of a food factor or
2) in the transport or in the amount of the precursor (SKOOG, 1937) or in its conversion into auxin.

Since the conversion of the precursor into auxin seems to depend upon the state of the redox-system (SKOOG, 1937; VAN RAALTE, 1937), this may be the most evident cause of the variability, but for the moment it is not justified to discriminate between the different possibilities.

First VAN OVERBEEK's (1936, b) experiments with deseeded coleoptiles were repeated. In the experiments four series of plants were used. In two series the coleoptiles were provided unilaterally with agar blocks containing hetero-auxin, in the

two others with agar blocks soaked in an extract (prepared according to VAN RAALTE's method, 1937) of ground tips of *Avena* coleoptiles. Immediately after placing the agar blocks the plants were treated as follows:

series I: hetero-auxin; during 5 hours in the dark

series II: hetero-auxin; during 5 hours illuminated with $1000/\pi$ MC

series III: auxin extract of *Avena;* during 5 hours in the dark

series IV: auxin extract of *Avena;* during 5 hours illuminated with $1000/\pi$ MC.

The illuminated series had to be transported into the thermostat, where they were placed on the vertically rotating axis of a clinostat. The rotating plants were illuminated through the glass door with an intensity of about 1000 MC. After 5 hours the plants were photographed in the usual way. The results are represented in table I. As in VAN OVERBEEK's experiments with deseeded plants the hetero-auxin series show exactly the same curvatures in the dark and in the light, but a distinct and al-

TABLE I

Influence of light on the growth substance curvatures of deseeded coleoptiles of *Avena*. Light intensity $1000/\pi$ MC; tray with water for cooling between bulb and thermostat. Hetero-auxin concentration in all experiments 5 x 10^{-7}. Photographed after 5 hours.

date	hetero-auxin 5. 10^{-7}				auxin extract from Avena				auxin curvature light/dark × 100
	Number of plants	dark	Number of plants	light	Number of plants	dark	Number of plants	light	
29/10	30	10.3±0.3	32	10.6±0.5	—	—	—	—	—
2/11	17	10.4±0.5	17	10.4±0.6	9	10.8±1.0	17	6 3±0.9	58
3/11	12	10.0±1.1	16	10.2±0.6	15	15.6±1.0	21	9.9±0.7	63
5/11	20	10.2±0.4	21	10.7±0.5	19	11.1±0.7	24	7.5±0.6	67
9/11	22	10.6±0.4	18	10.5±0.5	16	11.9±0.5	21	7.6±0.5	63
12/11	21	10.7±0.5	15	10.8±0.8	23	13.1±0.7	21	7.9±0.7	61
16/11	—	—	—	—	19	10.3±0.4	23	6.3±0.4	61
19/11	—	—	—	—	22	8.2±0.6	21	5.3±0.4	63
					highly purified auxin preparation from urine				
26/11	17	10.3±0.6	19	10.6±0.6	24	15.5±0.6	18	10.2±0.5	65
30/11	23	10.7±0.6	11	10.4±1.1	20	10.9±0.4	22	7.0±0.5	64
1/12	20	10.3±0.4	19	10.4±0.5	17	10.7±.06	19	6.8±0.6	63
								Average	63

most constant difference occurs in the case, where extracts from *Avena* coleoptiles (of unknown chemical concentrations) were applied. Since, according to SKOOG, practically all the native auxin in the deseeded coleoptiles has disappeared, the different behaviour must be ascribed to a partial inactivation by the light of the growth substance in the *Avena* extract.

One may argue, however, that possibly some photochemically active agent may have been present in these extracts, to which the inactivation of the auxin has to be ascribed. For that reason the same experiments have been repeated with a highly purified preparation of auxin, courteously supplied by Prof. KÖGL. With an adequate dilution of this preparation exactly the same result was obtained (lower half of table I). Since the evidence that the growth substance in the *Avena* coleoptile is really identical with the auxin-a as isolated by KÖGL and his cooperators from urine, malt and maize-oil is based only on strong direct indications, VAN OVERBEEK's and our experiments strongly endorse this identity by proving a photochemically identical behaviour.

VAN OVERBEEK (1936, c) also measured the rate of curvature of coleoptiles unilaterally provided with auxin. Calculating the differences of the rates of the curvatures in the light and in the dark, he found a close resemblance with the base growth response of the intact seedling upon illumination. This part of the auxin curvature in light (as compared to that in the dark) and of the base light growth reaction is ascribed by VAN OVERBEEK to "oxidative inactivation of auxin-a" by the light. The decrease in the rate of curvature in light, however, is followed by an increase, but only if very low auxin concentrations are applied (1936, c, fig. 1, p. 422). This is explained (1936, b, p. 308) by "an increase in response of the plant to growth hormone which is independent of the type of hormone". Since VAN OVERBEEK did not use deseeded plants in these experiments this "increase in response" may be explained also in terms of inactivation of residual auxin and inhibition of regeneration of auxin by light (see VAN OVERBEEK, 1936, b, p. 297 and p. 303).

The method of deseeded plants opens a way to discriminate directly on the influence of photo-inactivation on the phototropic base response. Such deseeded and decapitated plants are practically free from residual auxin and cannot regenerate it. When allsidedly provided with growth substance and subsequently illuminated unilaterally, they must behave differently when supplied with β-indole acetic acid or with auxin-a. In the first case they should not curve at all, with auxin-a they must show

a phototropic response. This really proves to be the case.

In these experiments the agar blocks were placed horizontally on the cut surface. Always three or four series ran parallel. When the first positive results were obtained, they were checked with a solution of crystalline auxin-a, also kindly supplied to us by Prof. KÖGL. After adjusting the agar blocks on the stumps the plants were left for half an hour in the dark room in order to let the growth substance diffuse into the stumps. Then they were placed in the thermostat and unilaterally illuminated during 5 hours with 100 MC. After that time they were photographed in the usual way. The results are represented in table II.

In series I, III and IV the primary leaf was pulled loose after the first decapitation; at the second decapitation the cut surface was made horizontal as precisely as possible; then the agar block was applied on it. Some times the primary leaf, apparently sticking to the inner wall of the coleoptile at the base, continued

TABLE II

Phototropic curvatures of deseeded coleoptiles after 5 hours of unilateral illumination with 100 MC. Agar blocks horizontal on the cut surface. In series III and IV b the concentrations of the growth substances are physiologically the same.

Series	treatment	Data	Number of plants	positive phototropic curvature
I	blank control: plain agar blocks without growth substance on the stumps	7/12	37	0.7 ± 0.4
		10/12	33	1.0 ± 0.4
II	coleoptiles *not* decapitated but ± 5 mm of the tip covered with a tin foil cap	7/12	9	10.9 ± 0.8
III	agar blocks with hetero-auxin; 5. 10^{-7}	7/12	29	0.4 ± 0.5
		10/12	32	0.9 ± 0.5
IVa	agar blocks with purified auxin preparation	7/12	33	8.1 ± 0.4
b	agar blocks with pure auxin; 2.5 .10^{-7}	10/12	34	7.0 ± 0.3

to grow a little. In that cases it lifted the agar block from the cut surface of the coleoptile. Such plants, of course, have been left out of consideration. Figure 1 gives some representative plants of the series.

It is clear that phototropical curvatures only occur if auxin-a is supplied either from the coleoptile tip (II) or from an agar block (IV). The minute curvatures measured in the blank control and in the hetero-auxin series may safely — if they are real at all — be ascribed to the last traces of auxin-a still

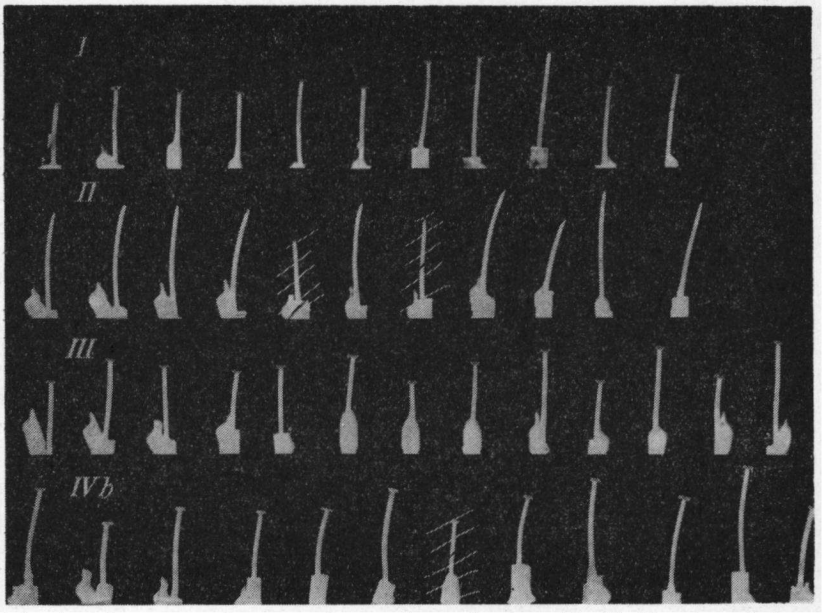


Fig. 1. The roman figures refer to the series in table II. Illuminated from the right side. Before photographing the tin foil caps in series II have been removed.

present in the coleoptiles (I and III). The total lack of curvatures with hetero-auxin shows that in these stumps no "redistribution" (shifting of the descending hetero- auxin to the shade side) of the hetero-auxin is caused by the light.

We still tried to discriminate whether in this respect auxin-a

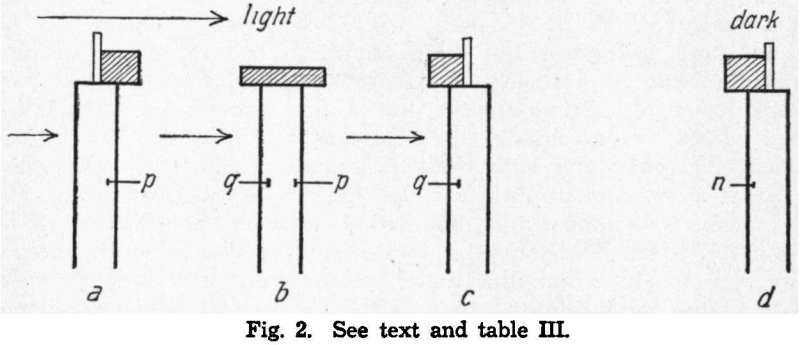


Fig. 2. See text and table III.

behaves in a different way. To that purpose the experiments were taken, as indicated in fig. 2. The results are given in table III.

If there were any lateral shifting in the transport of the auxin-a nothing had to be redistributed in the plants with the agar blocks at the shade side (a), since all auxin was already at the shade side; these plants then should show the same curvatures as the dark controls (d). In fact this does not hold true: the curvatures in series a are — though slightly — smaller than those of the dark plants. The difference is small but seems to be real and can only be ascribed to a partial photo-inactivation of the auxin-a at the shade side, into which still some light of low intensity penetrated. If the agar block is applied at the light side of the coleoptile (series c), the curvature is practically reduced to zero; the very weak curvature of 0.8° having little probability to be real.

TABLE III

Agar blocks with 2. 10^{-7} pure auxin-a, light intensity 100 MC, photographed after 5 hours.

Series	treatment	Number of plants	curvature (respective the light)
a	agar blocks unilateral at the shade side	35	+ 10.8 ± 0.5
b	agar blocks horizontal; auxin supply allsided	25	+ 10.0 ± 0.4
c	agar blocks unilateral at the light side	22	— 0.8 ± 0.5
d	agar blocks unilateral; dark control plants	33	13.2 ± 0.5

Apparently at the applied light intensity at the light side practically all the auxin-a is inactivated, this intensity at the shade side being too low to inactivate considerable amounts of auxin. These experiments therefore shall be repeated within a wide range of light intensities, but there is little doubt that the next consideration holds true. Putting at these low concentrations of auxin-a and with these practically auxin free coleoptiles growth (auxin supplied allsidedly) (:) curvature (:) amount of auxin, we can assume that in the dark an amount of auxin = n (see fig. 2) is delivered to the side of the coleoptile below the agar block. When illuminated this amount n will be reduced to q at the light side and to p at the shade side. The curvatures obtained must therefore be proportional in series a to p, in

series b to (p — q) and in series c to q. The difference of the curvatures in series a and c must therefore be p—q, that means equal the curvature of series b. Curiously enough the obtained values exactly — perhaps more by incident then in reality — agree with this theoretical postulate:

series	a:	p = 10.8	
„	c:	q = 0.8	
		p—q = 10.0	series b: p—q = 10.0

Assuming that the rate of inactivation of the auxin-a is a linear function of the light intensity, one could also calculate from the obtained data the light intensities in the shade side (n—p) as compared to that at the light side of the coleoptile (n—q). Many more data, however, will be wanted to ascertain the ratio 1 : 30 as photographically determined by VAN DILLEWIJN (1927, p. 323).

We believe to have given definite proof that the "phototropism" in the reported experiments is merely due to a photochemical process: the inactivation of the auxin-a via its lactone.

In vitro a photochemical inactivation of pure auxin-a solutions by the conversion of auxin-a-lactone into lumi-auxin-a-lactone was only stated in quartz tubes and in ultraviolet light [1]). There was little evidence that ultraviolet rays, in our case, could reach the plants since the light (1000 MC) had to pass three layers of thick glass (two of the water tray and one in the door of the thermostat). To ensure this, however, auxin-a solutions themselves were used as indicators for ultraviolet light. In the illuminated thermostat, close to the plants, we placed a small tube with a solution of auxin-a and another tube was kept in the dark room. After illumination during 5 hours the contents of both tubes were tested on (deseeded) *Avena* coleoptiles on the auxin activity of the solutions. In another experiment blocks of agar containing auxin-a were put in two small corked tubes to prevent them to desiccate and one tube was illuminated for 5 hours in the thermostat, the other one kept in the dark room. After the illumination both sets of agar blocks were tested on *Avena*. The results, given in table IV, show that the illumination has not affected at all the auxin activity.

This proves that the inactivation of the auxin by light in the

[1]) According to a private communication by Prof. KÖGL he and his co-workers are now studying the influence of longer wave lengths.

TABLE IV

	dark		illuminated	
	Number of plants	curvature	Number of plants	curvature
tubes with auxin solution ..	20	8.2 ± 0.6	22	8.1 ± 0.4
auxin containing agar blocks	19	8.1 ± 0.5	19	8.4 ± 0.5

coleoptiles is due to the visible part of the spectrum, a fact which only can be explained by assuming the presence of a sensibiliser in the growing cells of the coleoptile. SKOOG (1935, p. 237) stated already that auxin in dilute eosin solutions is photochemically inactivated by the visible light [1]), eosin acting catalytically.

It is still impossible as yet to ascertain the nature of the sensibiliser in the coleoptile. According to BLAAUW (1909), V. J. KONINGSBERGER (1922) and others the blue-violet part of the spectrum (4900—3700 Å) has the greatest effect in phototropism and photo-growthreactions. This part of the spectrum, however, is not absorbed by auxin (C. KONINGSBERGER, 1936) but greatly by carotinoids, also present in coleoptiles (WALD and DU BUY, 1937; BÜNNING, 1937). By this fact evidence is given that carotinoids may be the sensibiliser for the photochemical inactivation of the auxin-a-lactone. If carotinoids actually would be the sensibiliser, the discrepancy between the auxin-theory and BÜNNING's carotene-theory of phototropism would be eliminated. BÜNNING, who believes that only carotene containing cells are sensitive to light, however, did not find carotene in the basal parts of the coleoptile, a fact which perhaps can be ascribed to the applied method.

It seems improbable that a photo-inactivation of auxin-a-lactone only would occur in the base of the coleoptile, in which it has been shown to occur by our experiments. Although the evidence that the auxin in the plant can be "redistributed" by unilateral light — as postulated by the CHOLODNY-WENT-theory of phototropism — cannot be denied, it is improbable that the photo-

[1]) WENT and THIMANN (1937, p. 163), quoting SKOOG's paper, say that SKOOG showed „that traces of eosin cause rapid photodynamic inactivation of solutions of indole-acetic acid". SKOOG himself, however, speaks of „auxin". In the literature the term „auxin" is often used for growth substances in general. In future it will be advisable to mention distinctly the kind of growth substance applied in experiments.

inactivation of auxin-a-lactone would not occur in the tip of the coleoptile and thus play an important part in phototropism in general. It seems, therefore, worth while to study phototropism again from the point of view of the inactivation of auxin-a-lactone by the light.

The curvatures obtained in our experiments with unilateral illumination were defined as "phototropism". In fact they only may be compared with the so called basal reaction. But still there is a difference, due to a time-factor. In "normal" coleoptiles, at illumination of their bases, the auxin already present in the reacting cells will be inactivated almost immediately; this is followed by an inactivation of auxin-a gradually moving downward from the tip. In our case at the beginning of the illumination no auxin is present in the radiated cells so that only auxin-a can be inactivated during of after its downward transport. To a certain degree our tin-foil capped plants can be compared with "normal" plants. The complete similarity of the curvatures in fig. 1 of series II and IV shows that the time factor has no effect on the final shape of the curvature, i.e. on the total growth retardation in the basal photo-growthreaction. That means that this reaction in continuous illumination does not depend on the light *quantity* but is mastered by the light *intensity,* the latter determining the rate at which lumi-auxin-a-lactone is formed according to the equation:

active auxin-a ⇄ active auxin-a-lactone → inactive lumi-auxin-a-lactone.

If one assumes that auxin-a behaves in its transport quite differently from hetero-auxin, our results also could be explained by assuming that the light hampers the transport of auxin-a (cf. Du Buy, 1933). This explanation, however, is very unlikely as compared to that offered by the investigations of Kögl and his co- workers on the conversion by light of the active auxin-a-lactone into the inactive lumi-auxin-a-lactone. We believe to have shown indeed that the base response of the *Avena*-coleoptile is to be ascribed to this photochemical process. There is therefore no reason to assume that the base response "appears to be more of the nature of a shock reaction or typical "stimulus" ", as recently has been done by Went and Thimann (1937, p. 174).

SUMMARY.

1. The method of SKOOG (1937) of determining the activity of auxin preparations on deseeded and decapitated coleoptiles of *Avena* is not only accurate and sensitive but gives also very constant results.

2. VAN OVERBEEK's statement (1936, a, b, c) that the growth substance curvatures are affected by illumination if auxin-a is applied has been confirmed. With β-indole acetic acid no influence of light was stated. The decrease of the curvature with auxin-a by light must be ascribed to the photo-inactivation of the auxin-a-lactone.

3. In the plant auxin-a apparently can be transformed into its lactone.

4. Deseeded and decapitated coleoptiles show phototropic curvatures (base response) if auxin-a is applied as growth substance, and not with β-indole acetic acid.

5. The base response in decapitated coleoptiles is to be ascribed only to the partial photo-inactivation of the auxin-a; no evidence of a "redistribution" (lateral transport) of auxin-a in the base of the coleoptile, as postulated by the CHOLODNY-WENT theory, could be obtained.

6. Since this photo-inactivation in our experiments did not occur in vitro a sensibiliser must be present in the reacting cells. In the literature evidence is given that carotinoids may act as sensibiliser.

The authors wish to tender their sincere thanks to Prof. KÖGL, who supplied them with the hardly accessible auxin preparations.

Botanical Laboratory,
Utrecht, December, 1937.

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