RESEARCHES ON PLANT GROWTH REGULATORS

XVI. THE EFFECT OF PLANT GROWTH SUBSTANCES ON COACERVATES

by

H. L. BOOIJ AND H. VELDSTRA

Research Laboratory, Combinatie N.V. en Amsterdamsche, Bandoengsche en Nederlandsche Kinine-fabriek, Amsterdam (Netherlands)

I. INTRODUCTION

On the whole one can form two different ideas concerning the mode of action of ergons in general and therefore also concerning compounds active as growth substances, which we will now consider more closely.

First of all we think of a function as a "carrier" in an enzymatically regulated process, as in the case of a number of vitamins of the B-group, which act as hydrogen-transporting coenzymes in oxidation-reduction chains, including a chemical reaction with the substrate.

As an alternative there is the possibility of an effect in which the part of the cell with which the interaction takes place ("receptor"), is influenced in a strictly physico-chemical sense and in which case there is no question of an ordinary chemical reaction (for a detailed discussion of these questions cf. VELDSTRA, 1947).

In preceding investigations concerning plant growth substances (VELDSTRA, 1944) the last-mentioned idea has been chosen as a starting hypothesis. This was done especially on account of the particular structure and configuration essential for growth substance activity in the compounds concerned. The physico-chemical influence was supposed to be exerted upon the protoplasmic membrane in such a manner that the growth substances, in physiologically active concentrations, would exert a turgescent effect on this membrance and that as a result of this the possibilities for transport of water and substances dissolved therein would increase. By this means the cell-elongation might then also be influenced.

The work of THIMANN AND SCHNEIDER (1938) and that of BUNGENBERG DE JONG *et al.* (1938) was considered to contain an important argument in favour of this conception. The former investigators studied the influence of neutral salts on the growth of Avena coleoptiles at a maximal active auxin concentration.

Growth is stimulated and the cations exert their action in the order: Ca < Li < Na < K. This is the same series as that in which these cations appear in the ionic spectrum of lecithin (TEUNISSEN, 1936; BUNGENBERG DE JONG AND TEUNISSEN, 1938). Since phosphatides play an important part in the protoplasmic membrane it is not very surprising that the influence of these cations on the permeability shows the same order again (BOOIJ, 1940). This hypothesis, concerning the activity of growth substances, can

References p. 277.

also give a plausible explanation for some known properties of plant growth substances and related compounds.

1. The effect of plant growth substances is confined to certain concentrations. A stimulating effect is observed at lower concentrations, whereas higher concentrations can have an inhibiting effect. It has already been mentioned that in this case at low concentrations a turgescent effect on the protoplasmic membrane may be considered to occur which would then imply a condensing effect for the higher concentrations. Although the situation is exactly the other way about for oleate coacervates — models of the protoplasmic membrane — this effect can very occasionally be observed in lecithin-coacervates. In such a case the added substance influences the electrical charge of the coacervate.

2. In certain cases the inhibiting effect caused by an excess of growth substance can be eliminated with the aid of a little ethylene chlorohydrin. In low concentrations this compound has a condensing effect on oleate coacervates, whereas it has a turgescent effect at higher concentrations. It may be possible that ethylene chlorohydrin also has a turgescent effect on the plant cell that has been condensed by an excess of growth substance.

3. The difference between stem- and root-cells regarding their response to growth substances might be explained by the differences in nature and quantity of the sensitisers in the membrane, or by the differences in nature of the fatty acids that occur in the form of esters inside the lipoid components of the membrane.

4. It is quite possible that the protoplasmic membrane of young cells has properties different from those of older tissue. This entails the possibility that the same growth substances may have a turgescent action in one case and a condensing one in the other.

5. An overdose of growth substance (see experiments on potato tubers, VELDSTRA, 1944, p. 155) not only inhibits the growth of the sprouts but also inhibits other biological processes. This may be understood if a general condensation of the protoplasmic membrane allows practically no transportation of compounds, necessary for these processes.

6. The increase in the quantity of auxin that can be extracted after treating plants with indole acetic acid (VON GUTTENBERG, 1942) can easily be explained by means of a "displacement-mechanism". It is not necessary to regard indole acetic acid as an activator of the natural auxins.

The task we now set ourselves was to see whether we could verify the hypothesis mentioned above by experiments on model systems for the protoplasmic membrane.

The question we have to face is whether the effect of plant growth substances and related compounds on oleate coacervates (models for the protoplasmic membrane — see preceding paper) will show a similar relation with their chemical structure as was found for that between structure and physiological activity. An affirmative answer would be a considerable support for the hypothesis.

The first experiments on this subject, performed by BUNGENBERG DE JONG, were encouraging. A coacervate, formed by adding a concentrated KCl solution to an oleate solution, disappeared under the influence of α-naphthalene acetic acid. Quantitative experiments confirmed this observation. They were carried out as is described in the preceding paper (BOOIJ AND BUNGENBERG DE JONG, 1947). The method consists in the determination of the KCl concentration necessary to obtain acertain degree of coacervation before and after addition of the substance to be examined. The shifting is a measure for the turgescent or condensing effect (see Fig. 1).

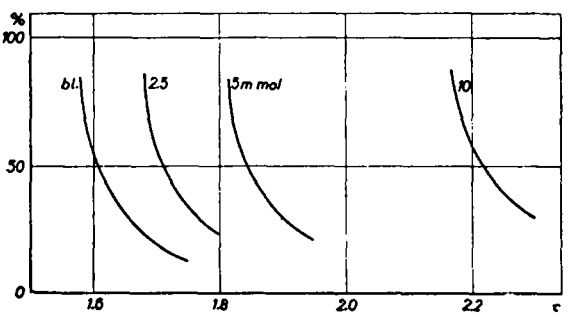


Fig. 1. Turgescent effect of naphthalene acetic acid on the coacervation of oleate by means of KCl (bl = blank, abscissa: concentration of KCl in mol/l, ordinate: volume of the coacervate layer in %).

It must be pointed out once more

References p. 277.

that in our experiments the p_H is much higher than in the biological experiment. Thus in our case we study the activity of the anion, while on the other hand the biological data suggest that the non-dissociated molecule would be the active principle.

Besides growth substances plant growth inhibitors are also known, as *e.g.* compounds which check the germination of seeds (blastocholines). Especially unsaturated lactones appear to possess this property (cf. VELDSTRA AND HAVINGA, 1943, 1945). A trial attempt was made to study the effect of these blastocholines (coumarin serving as a model) on oleate coacervates. Difficulties are encountered because the medium has to be alkaline, resulting in an opening of the lactone ring. The o-oxy-*cis*-cinnamic acid formed has, in low concentrations at least, no influence. Therefore a lower p_H must be chosen. By using potassium acetate as a separating agent and omitting the alkali, a slight condensing effect of coumarin could be observed (Fig. 2).

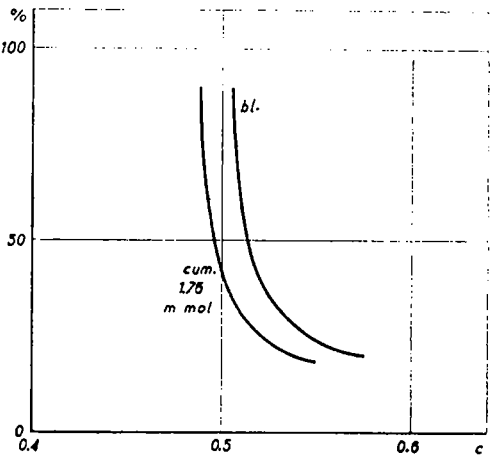


Fig. 2. Condensing effect of coumarin (abscissa: concentration of potassium acetate in mol/l).

It must be taken into account that also in this medium coumarin is slowly transformed. This transformation can readily be verified by irradiating the solution with ultraviolet light. A solution of coumarin in distilled water shows practically no fluorescence (slightly purple). On adding alkali a strong green fluorescene rapidly appears. The same happens in the presence of potassium acetate, but here the green colour is slower in appearing. The coacervate is formed while unchanged coumarin is still present. It is of course clear that this experiment only has a qualitative value. The conclusion may be drawn that coumarin has a condensing effect, but this experiment does not disclose anything about the degree of this effect. One can, however, ascertain that in this model system the qualitative relations are as they might be expected to be according to the hypothesis.

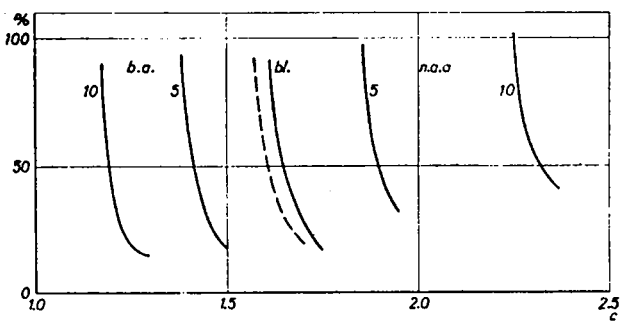


Fig. 3. Influence of benzylalcohol (b.a.) and naphthalene acetic acid (n.a.a.). A mixture of 5 m mol of benzylalcohol and 5 m mol of naphthalene acetic acid (dotted line) lies close to the blank test (bl.). Abscissa: KCl in mol/l.

From previous experiments on oleate coacervates it was known that many substances have a condensing effect on these coacervates. As an example of such a compound with a benzene nucleus benzylalcohol may be mentioned (BUNGENBERG DE JONG, SAUBERT, BOOIJ, 1938). This compound also proved to be active in the blastocholine test, which must mean that in a sense benzylalcohol may be considered to be an "inhibiting substance". In this connection it is interesting to note

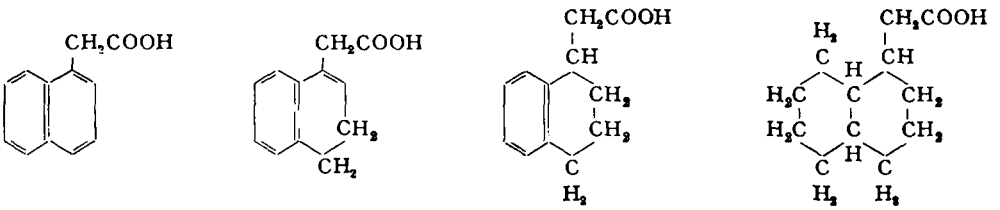
References p. 277.

that the effect of benzylalcohol on oleate coacervates is clearly opposed to that of naphthalene acetic acid (Fig. 3).

After these preliminary experiments it seemed reasonable to examine whether a correlation exists between the turgescent effect of growth substances and related compounds on oleate coacervates and the physiological effect exerted on plant cells by these compounds.

II. THE EFFECT OF GROWTH SUBSTANCES AND RELATED COMPOUNDS ON COACERVATES, IN PARTICULAR ON OLEATE COACERVATES

1. Polarographic experiments (VELDSTRA, 1944) seem to indicate that the function of the double bonds in the ring system of growth substances is not due to their reducibility (To be discussed in detail in the following paper). They rather seem to be partly responsible for the (polarographically measured) boundary-activity.



I II III IV

The boundary-activity decreases in the series naphthalene acetic acid, dihydro-, tetrahydro-, and decahydro-naphthalene acetic acid (I–IV) (half suppression values 9, 12, 24 and 40 micro-mol/l respectively). The physiological activity decreases parallel herewith.

The turgescent effect of these compounds on oleate coacervates, however, appears to increase in the direction already mentioned (Fig. 4).

In this case there is evidently no parallelism with the physiological effect. In a sense there is conformity with the results of the investigation on the condensing effect of non-electrolytes on oleate coacervates (BUNGENBERG DE JONG, SAUBERT, BOOIJ, 1938) in which it was demonstrated that, *i.e.*, cyclohexanol with a saturated ring system has a stronger effect than the aromatic benzylalcohol, although the latter contains an additional carbon atom.

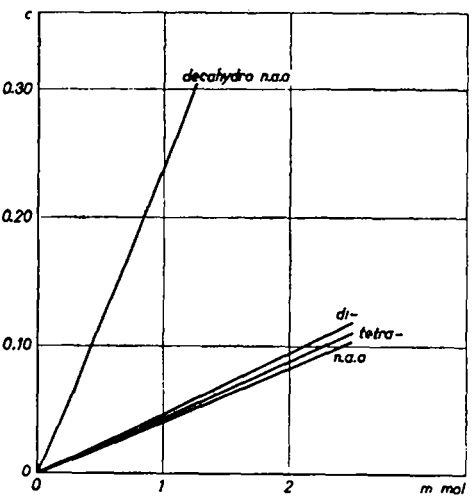
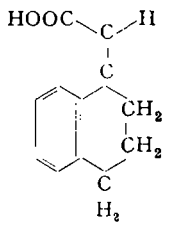


Fig. 4. Shifting of the KCl-curve in mol/l (ordinate) under influence of different hydrogenated derivatives of naphthalene acetic acid (abscissa: concentration of the added compounds).

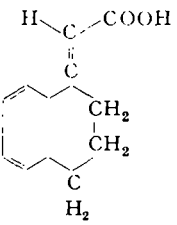
2. The investigation on structure and activity showed that the position of the carboxyl-group in regard to the ring system is very important for the physiological activity. The ideal position is found when the

References p. 277.

direction of the dipole is perpendicular to the plane of the ringsystem. The difference in physiological activity between *cis*- and *trans*-cinnamic acid was explained on this base and the two forms of 1, 2, 3, 4-tetrahydronaphthylideneacetic acid (of mp 92° and 163° respectively) were considered to be an analogous pair of *cis*- and *trans*-compounds. Because of the fact that the substance of mp 92° is active in the pea-test, whereas that of mp 163° doesn't show any activity, it was deemed very probable that the former compound possesses *cis*- (V), the latter one *trans*-configuration (VI). The only difference between these acids is formed by the position of the carboxyl-group with regard to the ringsystem: this being "favourable" for the appearance of physiological activity with the *cis*-compound, "unfavourable" in this respect for the *trans*-isomer.



V



VI

Experiments with the oleate coacervates show a different picture (Fig. 5). Here the higher melting isomer has a stronger effect than the lower melting one (which is slightly more active than naphthalene (1) acetic acid.

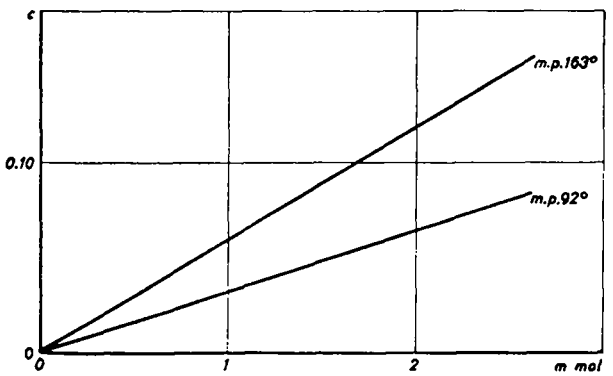


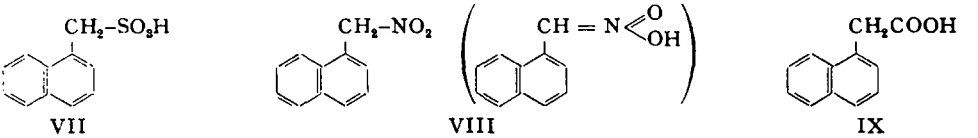
Fig. 5. Influence of the 1, 2, 3, 4,-tetrahydronaphthylidene (1) acetic acids of mp 92° and 163° on the shifting of the KCl-curve (ordinate).

A comparison of the two structures for these compounds immediately shows that the length (distance between the –COOH group and the remotest part of the ringsystem) of the *trans*-compound (VI) is greater than that of the *cis*-isomer. As previous investigations revealed that a lengthening of the chain generally results in a stronger activity in the coacervate (observed for the condensing effect of organic non-electrolytes and the turgescent effect of normal fatty acids) we must conclude that the compound of mp 163° — because of its higher activity in the oleate-coacervate — as compared to that of mp 92° is the "longer" one and thus possesses *trans*-configuration. The lower melting compound then must be the *cis*-isomer.

So on the one side in a quantitative sense the activities in the model system are reversed to those found with the biological object, on the other side these relations can be considered to constitute an elegant proof for the *cis*- and *trans*-structures, ascribed to the compounds of mp 92° and 163° respectively on account of their physiological activities.

References p. 277.

3. An important question, which was examined in the earlier investigations, was whether compounds containing other groups than COOH might be active as growth substances (see VELDSTRA, 1944). For that purpose three compounds were compared, naphthalene (1) methane sulphonic acid (VII), naphthalene (1) nitromethane (VIII) and naphthalene (1) acetic acid (IX).



VII VIII IX

In the pea-test these acids proved to have an activity of 0; 4 and 100 respectively.

Such large differences are not observed in the oleate coacervates (Fig. 6) and moreover, they are exactly inversed as compared to those for the physiological effect.

4. In the homologous series of acids derived from naphthalene curious differences in activity were found in the pea-test. Naphthalene (1) acetic acid (X) proved to have a much stronger effect than β-naphthalene (1) propionic acid(XI) (which has a very slight activity), whereas γ-naphthalene (1) butyric acid (XII) again has a slight effect.

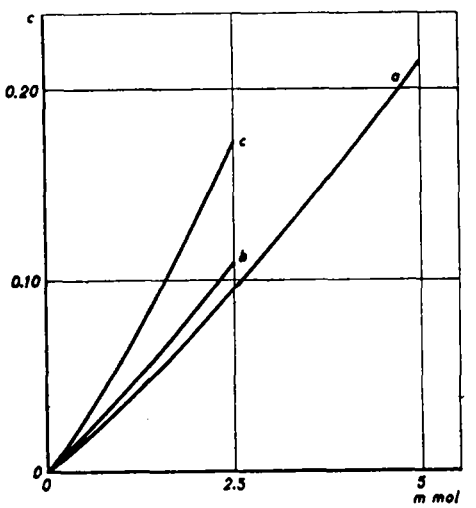


Fig. 6. Comparison of the effect of naphthalene (1) methanesulphonic acid (c), naphthalene (1) nitromethane (b) and naphthalene (1) acetic acid (a). Ordinate: shifting of the KCl-curve in mol/l.

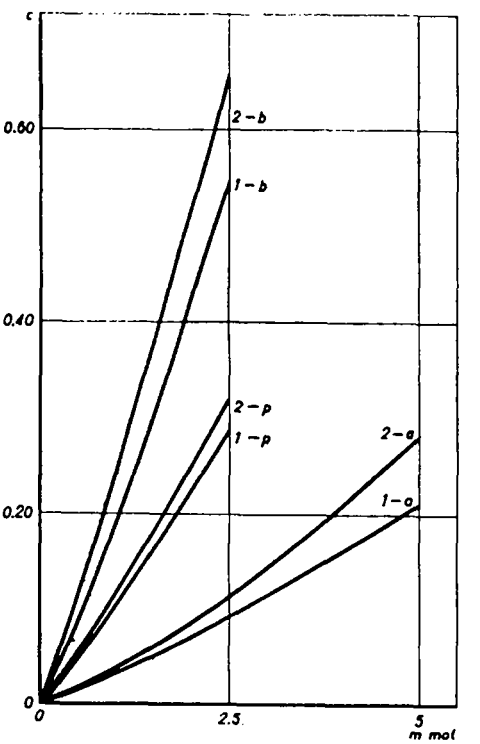
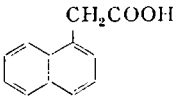


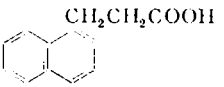
Fig. 7. Shifting of the KCl-curve (ordinate) under influence of naphthalene (1) acetic acid (1-a), naphthalene (1) propionic acid (1-p), naphthalene (1) butyric acid (1-b), naphthalene (2) acetic acid (2-a), naphthalene (2) propionic acid (2-p) and naphthalene (2) butyric acid (2-b).

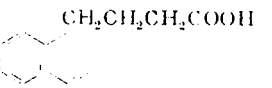
So there is evidently a certain oscillation in this homologous series. Naphthalene (1) acetic acid proved to exert a slightly stronger effect than naphthalene (2) acetic acid (XIII), but, on the other hand, β-naphthalene (2) propionic acid (XIV) is slightly more active than β-naphthalene (1) propionic acid.

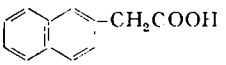
References p. 277.

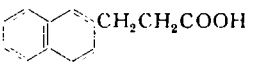
The properties with regard to the oleate coacervates are much simpler (Fig. 7). Napthalene (1) derivatives are somewhat shorter than naphthalene (2) derivatives and evidently this should be brought into relation with the decrease in activity (compare: II, 2).

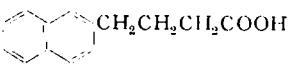

X


XI

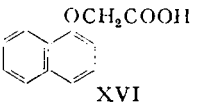

XII

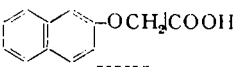

XIII


XIV


XV

Lengthening of the carbon chain of the non-polar part generally results in an increase of activity. In this way a naphthoxygroup also proved to have a stronger effect than a naphthyl-group in the corresponding position.


XVI


XVII

We compared naphthoxy (1) acetic acid (XVI) with naphthalene (1) acetic acid and naphthalene (1) propionic acid; also naphthoxy (2) acetic acid (XVII) with naphthalene (2) acetic acid and naphthyl (2) propionic acid.

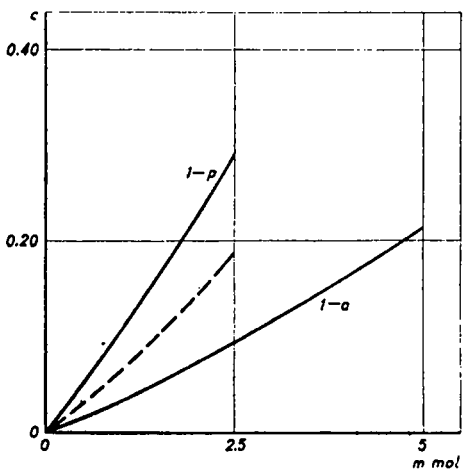


Fig. 8a. Effect of naphthoxy (1) acetic acid (dotted line) as compared with naphthalene(1) acetic acid (1-a) and naphthalene (1) propionic acid (1-p).

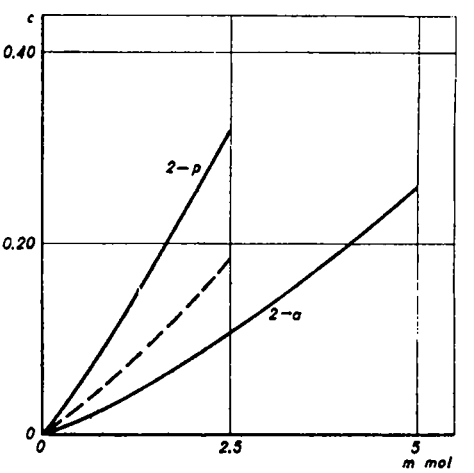
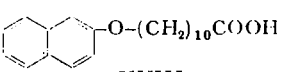


Fig. 8b. Effect of naphthoxy (2) acetic acid (dotted line) as compared with naphthalene (2) acetic acid (2-a) and naphthalene (2) propionic acid (2-p).

The result of the experiment is obvious: the length of the molecule is decisive for the degree of activity.


XVIII

We found a remarkable result with ω-naphthoxy (2) undecanoic acid (XVIII). Considering the great length of the molecule (about twice that of naphthoxy (2) acetic

References p. 277.

acid) the effect is surprisingly low. We must, however, note that this acid has no biological activity. A comparison with the effect of the homologous series of fatty acid anions, however, can immediately explain the relatively low activity. Naphthoxy (2) acetic acid has a stronger effect than octanoic acid but not so strong as nonanoic acid. Therefore it may be compared with a normal fatty acid with 8 ½ C-atoms.

ω-Naphtoxy (2) undecanoic acid contains 9 C-atoms more and would therefore have to be compared with an acid of 17 ½ C-atoms. Practically speaking we would have to compare this compound with stearic acid. And our experiments indeed proved that stearic acid has a comparatively slight effect (see BOOIJ AND BUNGENBERG DE JONG, 1949, Fig. 7).

5. The ideal shape of a growth substance molecule, as deduced from earlier experiments, was supposed to be a ring system with the carboxyl-group in a peripheral position. Considerations of this kind led to experiments with trichloroacetic acid. In this acid the carboxyl-group is permanently in an "ideal" position, with regard to the lipophilic "attaching", system, consisting of the $-C(Cl)_3$-group. And indeed there is a very weak growth substance activity with trichloroacetic acid (in relatively high concentrations) (VELDSTRA, 1947).

It is also worth while to compare trichloroacetic acid, as regards its effect on oleate coacervates, with related compounds. The activity of acetic acid has already been mentioned in the previous paper. In low concentrations it has no effect but in very high concentrations it has a condensing influence. Trichloroacetic acid does not show any activity in low concentrations either. Therefore again — as was done with acetic acid — the method must be changed to enable measurement of the influence at high concentrations. This method has already been described in the previous paper and it makes use of KOH instead of KCl as a means of obtaining coacervation of the oleate solutions. Then we observe (Fig. 9) that trichloroacetic acid (XIX) and trimethylacetic acid (XX) have a turgescent effect.

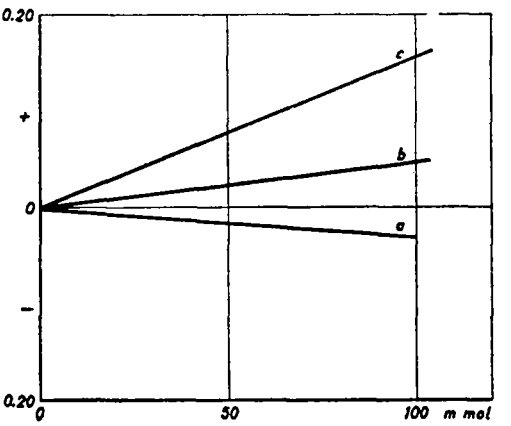
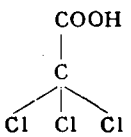
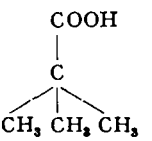


Fig. 9. Influence of acetic acid (a), trimethylacetic acid (b), and trichloroacetic acid (c) on the coacervation of oleate. Acetic acid has a condensing effect (−), the other two compounds a turgescent effect (+). Owing to the large quantities of added acids, KOH, instead of KCl, was used to induce coacervation. As usual the influence of the anions is measured in these experiments.



XIX



XX

If a chlorine atom is introduced into each methyl-group of the latter mentioned acid the turgescent effet is decidedly stronger, as appears from the lower concentrations which now cause a comparable effect, so that the normal KCl-method can be used. This has been ascertained for $(ClCH_2)_3$–C–COOH and also for $(ClCH_2)_3$–C–CH_2OSO_3H, the activity of which approximates that of naphthalene (1) acetic acid (Fig. 10).

Although, as expected, all compounds active as growth substances show a tur-

References p. 277.

gescent effect on this model system of the protoplasmic membrane, little is to be seen of a more quantitative connection between the degree of this effect and the physiological activity of the compound. Only the experiment with trichloroacetic acid revealed a certain conformity.

The basis of the explanation given by BOOIJ AND BUNGENBERG DE JONG for their experiments on the influence of fatty acid anions on oleate coacervates, is formed by considerations regarding the difference and resemblance between the "substrate" and the "agent". The effect is very small if the resemblance between the acting molecule and the substrate is very great. In the extreme case — the agent is identical with the substrate, *e.g.*, the effect of the added stearate on a stearate coacervate — the effect is practically nihil. On the other hand we have to face the possibility that a total lack of conformity between "agent" and "substrate" might also produce a minimal effect. Viewed from this angle it is possible that the relations growth substance / coacervate and growth substance / protoplasmic membrane are not sufficiently comparable, in other words, that the oleate coacervate is not yet complete enough a model. One has to bear in mind for example, that here a protein-component of the membrane is not taken into consideration. Therefore model experiments with other model systems ("substrates") are very desirable from a theoretical point of view.

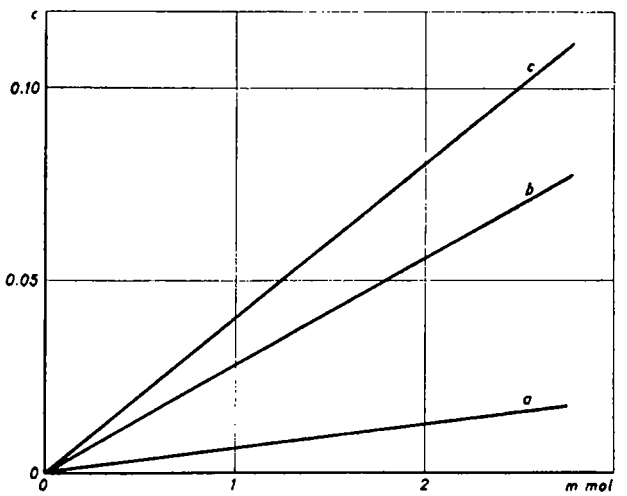


Fig. 10. Influence of (tris-chloromethyl-) acetic acid (a)*, (tris-chloromethyl-) ethane sulphuric acid (b) and naphthalene (1) acetic acid (c) on the coacervation of oleate.

* Its real activity very probably is higher, because it appeared that in alkaline solution this compound rather rapidly decomposes.

In our model experiments attention is immediately drawn to the difference between the strong effect of several normal fatty acids and the weak activity of *e.g.*, naphthalene (1) acetic acid. With the biological object this relation is exactly reversed (compare: VELDSTRA, 1947 and the following paper).

Furthermore, in colloid-chemical experiments the growth substances only show their turgescent effect in comparatively high concentrations, in contrast with the very low active concentration for the biological object.

In searching a better "substrate" one may be guided by these facts: the desired model is one with which the relation between the effect of naphthalene (1) acetic acid and *e.g.*, undecanoic acid is different from that in an oleate coacervate, while the former compound must preferably be active in low concentrations.

First of all we turn our attention to the stearate coacervate. In view of this model's close resemblance to the oleate coacervate it already seems probable that in this case there are few possibilities for a totally different behaviour. This surmise is confirmed by an experiment with naphthalene (1) acetic acid. It shows quite the same reactions

References p. 277.

TABLE I

Shifting	of the KCl-curve (in mol/l) with the oleate coacervate under the influence of 1.25 m.mol anion of		of the temperature of separation (in ° C) of a desoxycholate coacervate under the influence of 1 m.mol anion of	Shifting
0.39	decanoic acid	.....	decanoic acid	17.5°
0.33	γ-naphthalene (2) butyric acid			
0.305	decahydro-naphthalene acetic acid			
0.275	γ-naphthalene (1) butyric acid		γ-naphthalene (2) butyric acid	8.5
0.23	β-naphthoyl (2) propionic acid		β-naphthoyl (2) propionic acid	7.5
0.185	ω-naphthoxy (2) undecanoic acid			
0.175	nonanoic acid	.....	nonanoic acid	7.0
0.16	β-naphthalene (2) propionic acid			
0.145	β-naphthalene (1) propionic acid		γ-naphthalene (1) butyric acid	5.2
0.14	β-naphthoyl (1) propionic acid		β-naphthalene (2) propionic acid	5.0
0.115	α-naphthoxy (1) propionic acid		β-naphthoyl (1) propionic acid	4.0
0.085	naphthoxy (2) acetic acid		β-naphthalene (1) propionic acid	3.8
0.085	naphthoxy (1) acetic acid		α-naphthoxy (1) propionic acid	3.7
0.075	naphthalene (1) methane sulphonic acid		naphthoxy (2) acetic acid	3.3
0.07	*trans*-tetrahydro-naphthylidene acetic acid		naphthoxy (1) acetic acid	3.2
0.06	*cis*-tetrahydro-naphthylidene acetic acid		naphthalene (2) acetic acid	3.0
0.055	naphthalene (2) acetic acid			
0.055	octanoic acid	.....	octanoic acid	2.7
0.055	dihydro naphthalene (1) acetic acid		naphthalene (1) methane sulphonic acid	2.5
0.05	naphthalene (1) acetic acid		naphthalene (1) acetic acid	2.5
0.05	tetrahydro-naphthalene (1) acetic acid		β-naphthalene (2) iso succinic acid	2.2
0.05	γ-indole (3) butyric acid		*trans*-tetrahydro naphthylidene acetic acid	2.0
0.02	phenyl butyric acid		tetrahydro naphthalene (1) acetic acid	2.0
0.015	naphtoic acid		*cis*-tetrahydro naphthylidene acetic acid	1.8
0.01	β-naphthalene (2) isosuccinic acid		dihydro naphthalene (1) acetic acid	1.8
0.01	indole (3) acetic acid		β-naphthalene (1) iso succinic acid	1.6
0.01	naphthyl (1) sulphuric acid		phenyl butyric scid	1.4
0.005	β-naphthalene (1) iso succinic acid			
0.005	hexanoic acid	.....	hexanoic acid	0.8
0.005	traumatic acid		naphtoic acid	0.7
			naphthyl (1) sulphuric acid	0.7
			γ-indole (3) butyric acid	0.1
			indole (3) acetic acid	0.0
			traumatic acid	0.0

References p. 277.

as in oleate coacervates. Therefore the stearate coacervate does not seem a very interesting object for starting extensive experiments.

Experiments with desoxycholate coacervates promised to be of greater importance theoretically. The technique of measurement with these coacervates has already been mentioned in the preceding paper. A mixture of sodium desoxycholate (10%) and a certain solution of KOH has a well-defined temperature of separation. This point is shifted by the addition of other compounds (in case of a turgescent effect in the direction of a lower temperature) and the degree of shifting is a measure for the effect.

The influence of a large number of compounds on a desoxycholate coacervate has been examined. See Table I, in which the shifting (in °C) caused by 1 m mol (final concentration) of the added substance may be found. In this table the degree of the turgescent effect of the same compound on oleate coacervate is also given (*viz.*, the shifting of the KCl-curve in N KCl, caused by 1.25 m mol of added substance).

Of course the effects — expressed in different units — cannot be compared mutually and only the sequence of the compounds can be noted. For the greater part the sequence is similar for both models. The differences are of minor importance and, what is most important, with desoxycholate the physiologically active compounds do not occupy an exceptional position either.

The same rules as those already discussed in connection with the experiments on oleate coacervates also hold for desoxycholate. Here too the substitution of the acid group by another one is of little consequence — compare naphthalene (1) acetic acid with naphthalene (1) methanesulphonic acid — and the effect increases with a lengthening of the carbon chain — see *e.g.*, naphthalene (1) acetic acid, naphthalene (1) propionic acid and naphthalene (1) butyric acid. Therefore it cannot be said that these two "substrates" are different as far as our experiments are concerned.

Only one compound has a much stronger effect with desoxycholate than with oleate. This is ω-naphtoxy (2) undecanoic acid. It has already been deduced that because of the length of the molecule this substance more or less resembles stearic acid. There is a great difference between desoxycholate and oleate as to the effect of the series of homologous fatty acids (BOOIJ AND BUNGENBERG DE JONG, 1949). With the oleate coacervate a minimum is observed in the neighbourhood of pentadecanoic acid, but this minimum is not found with the desoxycholate coacervate. Stearic acid has a strong turgescent effect on this model in nice agreement with the already mentioned fact that ω-naphthoxy (2) undecanoic acid shows a great activity.

So evidently these trials with a "substrate" of a different structure were not satisfactory. Some efforts were made to "enforce" a different character on an oleate coacervate by adding certain compounds. Since most of the growth substances contain a naphthalene nucleus, we first of all tried to confer a different character upon the oleate coacervate by adding naphthalene.

Naphthalene is not easily soluble in a 2% oleate solution. The best results were obtained by adding an excess of naphthalene to the oleate solution, boiling for a short time, and putting the solution in a refrigerator. The next morning the solution is separated from crystallised naphthalene by filtration and used for the coacervation experiments.

The concentration of KCl, now required for coacervation, is considerably less than that required for an untreated oleate solution. This is due to the fact that naphthalene isa condensing substance. The influence of naphthalene (1) acetic acid and of undecanoic acid (Fig. 11) is now determined.

References p. 277.

There is no question of a typical change in character of the coacervate. The difference between the two acids remains quite as obvious as before the treatment. In principle a condensing substance (benzylalcohol) also retains the same effect on the oleate treated with naphthalene.

Similar experiments were made on an oleate solution to which cholesterol had been added. The result of these experiments is again comparable with that obtained with untreated oleate. Neither did efforts with m- and o-cresol amount to anything. Therefore it must be concluded that it is impossible to enforce a different character on the oleate coacervate by adding condensing substances.

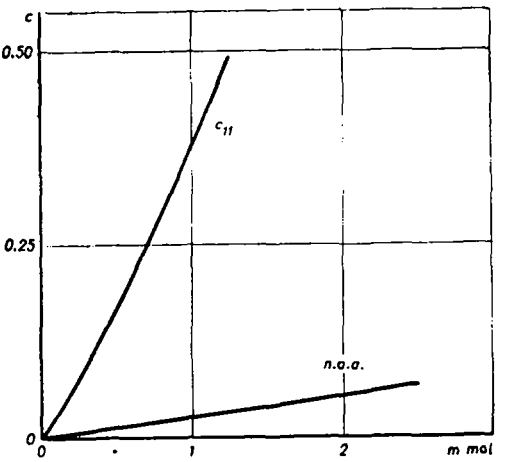


Fig. 11. Influence of naphthalene (1) acetic acid (n.a.a.) and undecanoic acid (c 11) on a coacervate condensed with naphthalene.

III. DISCUSSION

If the results of this investigation are compared with the conclusions set down in the previous article then naphthalene (1) acetic acid appears to have a comparatively weak turgescent effect, which is about equal to the activity of octanoic acid. Of the latter acid it is known that the low activity is a result of the fact that so little is adsorbed into the soap micelles. The equilibrium concentration (C_E) is much greater than the quantity adsorbed into the micelles –(C_M)– (see BOOIJ AND BUNGENBERG DE JONG, 1947, Fig. 13).

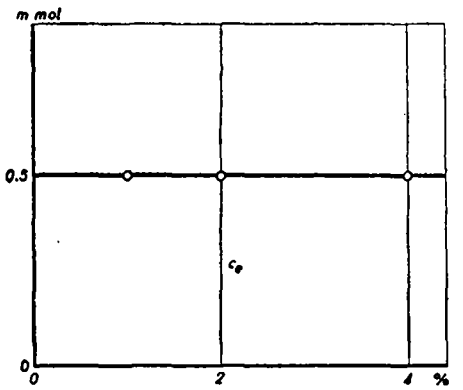


Fig. 12. The quantity of naphthalene (1) acetic acid required to cause a shifting of the KCl-curve of 0.06 mol/l in different concentrations of the oleate solution (standard 1, 2, 4%) is always the same. This means that practically no naphthalene (1) acetic acid is adsorbed into the micelles.

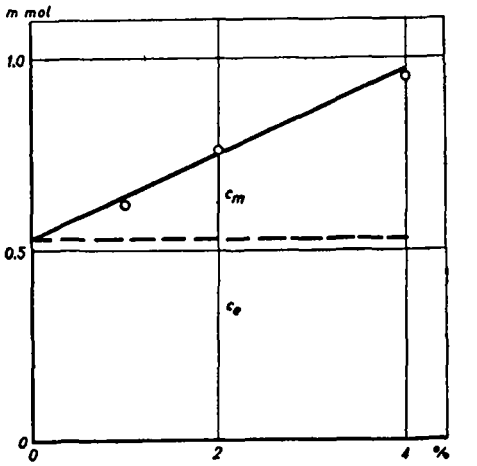


Fig. 13. For naphthalene (2) butyric acid the situation is different. Here the curve crosses the ordinate at 0.52 m mol, which must be the equilibrium concentration of this compound.

For nonanoate C_M becomes slightly larger and in the homologous series of fatty acids C_M fairly rapidly becomes practically 100%.

References p. 277.

Of course the question arises whether the weak turgescent effect of naphthalene (1) acetic acid must also be ascribed to the low value of C_M, *i.e.*, to the small quantity of substance adsorbed into the micelles. The answer to this question can be obtained by comparing the effect for different oleate concentrations (see previous article for the method used). In Fig. 12 we find the result of this experiment: it is evident that the quantity of naphthalene (1) acetic acid adsorbed into the micelles is extremely small indeed.

Therefore we may conclude that the slight effect is due to the small quantity adsorbed.

If we regard a longer molecule, *e.g.*, γ-naphthalene (2) butyric acid, we see that the effect increases. An analysis of the quantity of adsorbed substance (Fig. 13) shows that here C_M is rather large. As to its effect naphthalene (2) butyric acid occupies a position between nonanoic and decanoic acid and therefore is comparable to a fatty acid containing 9½ C-atoms (see Fig. 7 of BOOIJ AND BUNGENBERG DE JONG, 1948). It has been calculated (see previous paper) that under certain conditions —.a shifting of log C_{KCl} by 0.04 and a concentration of the standard oleate solution of 2% — for nonanoate C_M is equal to 19% of the added quantity. For naphthalene (2) butyric acid C_M amounts to 31% under comparable conditions. As for a fatty acid of "9½ C-atoms" a comparable quantity would indeed be adsorbed into the micells (see Fig. 13 of the previous paper) the important conclusion must be drawn, that for the extent of the effect the distribution of the added substance between soap micelles and medium is significant in the first place.

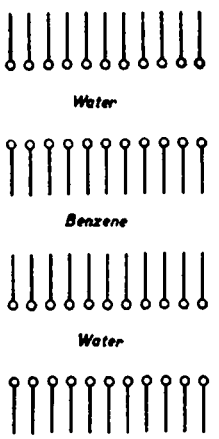


Fig. 14. Diagram of the micelles in an oleate solution to which benzene has been added (acc. to HESS, KIESSIG AND PHILIPPOFF, 1941).

Efforts to enforce a different character on the oleate coacervate by adding naphthalene, cholesterol, etc. have failed. Perhaps this must be ascribed to the fact that in the soap-micelles these substances are adsorbed between the terminal methyl-groups of the parallelly arranged soap molecules. This only results in the micelles becoming thicker while the lateral distance between the soap molecules remains unchanged. HESS *et al.* (1941) made this course of events seem very probable in an investigation on the addition of benzene to a soap solution. In that case Röntgen-diagrams show that upon adsorption of benzene (see Fig. 14) the micelle only becomes thicker.

In our experiments on the addition of naphthalene, cholesterol, etc. we might also suppose that something similar happens.

It has been made probable, however, that the structure-disturbing factor acts in a direction parallel to the soap molecule. It would then be comprehensible why no influence is found upon addition of naphthalene etc.; the turgescent effect of naphthalene (1) acetic acid and of undecanoic acid remains unchanged in principle.

Another possibility is that the condensing and the turgescent effects *do* take place parallel to the soap molecules but that they are additive. In this case addition of naphthalene only results in less KCl being required for coacervation. No influence on the turgescent effect of *e.g.*, undecanoic acid will be observed. Experiments on the effects of a mixture of naphthalene acetic acid and benzylalcohol (Fig. 3) render this last conception probable.

References p. 277.

In general, the result of our investigation is that for growth substances a turgescent effect on the models is observed, but that this effect is not limited to these substances and that it is therefore not specific for these physiologically active compounds. Moreover the differences in physiological activity within the group of growth substances derived from naphthalene are not reflected in the model experiments.

In the first place the relations in these experiments appear to be much simpler than in experiments on the biological activity. Quite the most important rule which may be deduced from our experiments is: the longer the non-polar part of the molecule, the stronger the activity; quite in harmony with the rules found for the turgescent effect of the fatty acids and for the condensing effect of organic non-electrolytes in previous experiments (exceptions to this rule are those molecules that closely resemble the size of the "substrate" molecules as regards their length).

Furthermore it must be noted that the growth substances must be present in a much higher concentration in the model experiments than in the biological tests in order to be efficient. Perhaps this draw-back is not so very important; for as we know, the equilibrium-concentration (C_E) is not so much what matters in these experiments but rather the amount of substance absorbed (C_M). In this case the relation between the mass of the "substrate" and that of the agent plays an important part. In the model experiments the relative quantity of substrate is much greater than in experiments on plants.

It is, however, very important that in experiments with coacervates the normal fatty acids have a much stronger effect than the compounds possessing growth substance activity, this in contrast to the physiological experiments. A hypothesis on the mechanism of growth substance activity will in any case have to take this fact into account.

Attention must be drawn to the fact that all these experiments with coacervates took place at a high p_H. Only in that case the turgescent effect is noticeable. This means that this effect must be ascribed to the dissociated acids, i.o.w. to the anions. The non-dissociated molecule of all sorts of acids (see BUNGENBERG DE JONG, BOOIJ, SAUBERT, 1938) always has the same — condensing — effect. This holds for mineral and organic acids and is ascribed to the release of oleic acid from the oleate coacervate. Hence this effect is non-specific; only the concentration, but not the nature of the acid is of importance. This could also be confirmed for naphthalene (1) acetic acid. It is of course evident that the dissociation constant of the acid should not be too low, but if this condition is fulfilled the effects of all acids are completely identical.

Consequently we can conclude from our investigations on model systems that the anions are the active principles, completely different from the impression gained from biological investigations concerning growth-substance activity.

If the influence of the p_H on this activity is examined then this effect appears to be very closely connected with the dissociation-curve of the compound concerned (BONNER, 1938, cf. STRUGGER, 1932, VAN SANTEN, 1938, 1940). And the conclusion is drawn that only the non-dissociated molecule would be active, no activity being found at a p_H for which all molecules are dissociated. This would also explain why *e.g.*, naphthalene (1) methanesulphonic acid shows no growth substance activity. The dissociation constant of this substance is high, so at the p_H of the experimental conditions anions are exclusively present.

Now the problem does not become simpler. On the one hand it is improbable that the growth substances would act as co-ferments in chemical reactions (VELDSTRA, 1944;

References p. 277.

see also the following paper), while on the other hand it is difficult to imagine a more physico-chemical effect of the non-dissociated molecule.

Then the remark will be made that may be our model of the protoplasmic membrane (the oleate coacervate) gives a wrong impression. It is of course very well possible that the phosphatides of the protoplasmic membrane react in another manner with growth substances and fatty acids. However, it was shown that a coacervate of desoxycholate — apart from small differences — is influenced in the same way as an oleate coacervate. This is really very remarkable if the great differences between the oleate and the desoxycholate molecules are considered.

Probably the solution of this problem will be found in the fact that the driving forces which cause the absorption of the compound into the micelles are not in the first place the London-Van der Waals forces between the non-polar parts of "agent" and "substrate", but more probably the mutual forces between the water molecules, an effect which drives a non-polar compound out of the solution. With a lengthening of the carbon chain (or, in general: if the non-polar part of the molecule becomes larger) the "solubility" in water of course decreases. The relation between "agent" and "substrate" will then only be of secondary importance. It is evident that these reflections only hold for "fatty" (in the sense of: sparingly soluble in water) agents and substrates. It may be expected that for watersoluble substances other factors play a part.

For the physiological experiments this reflection has the following consequences. If the activity of a series of compounds resides on the outside of the cell, it is very improbable that we should find rules different from the simple ones known for oleate coacervates. Here *e.g.*, reference may be made to the rule of TRAUBE, which FÜHNER AND NEUBAUER (1906) find for the hemolytic influence of the normal alcohols on erythrocytes, to the influences exerted by the length of the carbon chain in hemolysis of erythrocytes with the aid of normal fatty acids (MEYER BODANSKY, 1928), and to the fact that in the germination of sweet-pea pollen under influence of organic non-electrolytes practically the same series is found again as for the effect of these compounds on oleate coacervates (BOOIJ, 1940). If the growth substances reacted only on the outside of the protoplasm then the introduction of a group with stronger acidity (as *e.g.*, in naphthalene (1) methanesulphonic acid) would scarcely have any influence on the activity (as compared with that of naphthalene acetic acid).

These facts point out that there are objections against considering the physiological effect of the growth substances to be one that exclusively influences the permeability. An important part of it most probably is localised inside the cell. The observation that non-dissociated molecules are exclusively "active" might then be explained by the fact that only uncharged molecules can easily pass the negatively charged protoplasmic membrane.

This, however, does not necessarily mean that the undissociated form of these molecules will also be the "active" one inside the cell. On the contrary, physico-chemical considerations make it far more probable that the anions will be active. Besides, a great number of the molecules will dissociate at the p_H existing in the plant cell. From this point of view the connection between the degree of dissociation of the acid active as growth substance and the degree of the activity would not be of primary character but only secondary, *viz.*, of importance for the transport of the growth substance to the place of action.

A further analysis of the growth substance activity in connection with the results

References p. 277.

of a comparative investigation on models and biological objects will be given in the next publication.

SUMMARY

1. In order to test the hypothesis that the action of synthetic compounds active as plant growth substances is localized largely in the protoplasmic membrane (influencing permeability), the influence of this type of compounds upon coacervates was studied.

In case of the hypothesis being valid, a turgescent action upon the coacervate would be expected, running parallel with the physiological activity.

2. *a.* In the series of naphthalene (1) acetic acid, di-, tetra- and decahydronaphthalene (1) acetic acid there is an increase of turgescent action upon oleate coacervates.

In botanical objects, however, physiological activity decreases in the same sequence.

b. The turgescent action upon an oleate coacervate in *trans*-, 1, 2, 3, 4-tetrahydronaphthylidene (1) acetic acid is stronger than in its *cis*-isomer, whereas, in contrast with the *cis*-form, the *trans*-isomer exerts no growth promoting action.

c. Replacement of the carboxyl-group by a group of stronger acidic character causes a decrease of growth substance activity; with the oleate coacervate the reverse is observed.

d. In the homologous series of naphthalene (1)-acetic acid, -propionic acid and -butyric acid the influence upon the oleate coacervate increases (due to the fact that — in this sequence — the added substance is increasingly adsorbed by the soap micelles). The physiological activity in these series shows a decline, accompanied by a certain oscillation.

e. With the oleate coacervate the relative activities of acetic acid and trichloroacetic acid are in accordance with the expectations based on the hypothesis.

3. In general the action of the anions of acids with growth substance activity and related compounds on the oleate coacervate can be explained by the rules given previously (BUNGENBERG DE JONG).

4. All the compounds with growth substance activity show the expected turgescent effect in coacervate systems (serving as a model for the protoplasmic membrane); in a quantitative measure, however, the course is not parallel to the physiological activity, but just the reverse.

5. This difference will have to be explained either by the fact that the relations growth substance/coacervate and growth substance/protoplasmic membrane are not sufficiently comparable (in other words, that the oleate coacervate as a model lacks completeness) or by the circumstance that an essential part of the growth substance activity — in contrast with the hypothesis — is not localized in the membrane.

On the strength of several arguments given the latter explanation must be considered to be the likeliest one.

6. It seems probable that the growth substance action unfolds itself largely in the protoplasm and that therefore the growth substances will have to pass the membrane.

In that case the influence of the p_H on growth substance activity becomes plausible, as the non-dissociated molecules of the acids permeate much more easily than their anions.

7. A more detailed analysis of growth substance action on the basis of results of comparative investigations on models and biological objects is presented in the next paper.

RÉSUMÉ

1. Pour vérifier l'hypothèse que l'action des substances synthétiques agissant en tant qu'hormones végétales de croissance, est localisée essentiellement dans la membrane protoplasmique (modifiant ainsi la perméabilité), l'influence de ces substances sur des coacervats a été étudiée. Au cas où l'hypothèse serait juste, on devrait s'attendre à une action turgescente vis à vis du coacervat, et qui serait parallèle à l'activité physiologique.

2. *a.* Dans la série de l'acide 1-naphthalène-acétique, et des acides di-, tétra- et déca-hydronaphtalène-1-acétique, on trouve un accroissement de l'action turgescente sur les coacervats d'oléate. Toutefois, l'activité physiologique vis à vis de matériel végétal décroît dans le même ordre.

b. L'action turgescente sur le coacervat d'oléate de l'acide trans- 1, 2, 3, 4-tétrahydronaphthylidène-1-acétique est plus forte que celle de son isomère *cis*, alors que, en contraste avec la forme *cis*, l'isomère *trans* n'exerce aucune action de croissance.

c. Le remplacement du groupement carboxyle par un groupement à caractère plus acide, provoque une diminution de l'activité comme substance de croissance; c'est l'inverse qui se produit dans l'action sur un coacervat d'oléate.

d. Dans la série homologue des acides naphthalène-1-acétique, naphthalène-1-propionique et naphtalène-1-butyrique, l'influence sur un coacervat d'oléate croît (ce qui est dû au fait que, dans

References p. 277.

cet ordre, les substances sont de plus en plus adsorbées par les micelles de savon. L'activité physiologique dans cette série diminue, accompagnée d'une certaine oscillation.

e. Les activités relatives de l'acide acétique et de l'acide trichloroacétique sur le coacervat d'oléate sont en accord avec l'hypothèse initiale.

3. En général, l'action, sur le coacervat d'oléate, des anions des acides doués d'une activité de substance de croissance, peut être expliquée par les règles données précédemment (BUNGENBERG DE JONG).

4. Tous les composés doués d'une activité de substance de croissance manifestent un effet de turgescence vis à vis des coacervats (lesquels constituent un modèle de la membrane protoplasmique); quantitativement néanmoins, cet effet n'est pas parallèle à l'activité physiologique, mais lui est inverse.

5. Cette différence devrait être expliquée soit par le fait que les relations entre les substances de croissance et le coacervat d'une part, et les substances de croissance et la membrane protoplasmique d'autre part, ne sont pas suffisamment comparables, (en d'autres termes, que le coacervat d'oléate n'est pas un modèle suffisant) ou par le fait qu'une partie essentielle de l'activité de la substance de croissance, contrairement à l'hypothèse émise, n'est pas localisée dans la membrane. C'est cette dernière explication qui semble la plus plausible.

6. Il semble probable que l'action de la substance de croissance s'exerce surtout dans le protoplasma et que, par conséquent, la substance de croissance doit franchir la membrane. Dans ce cas, l'influence du p_H sur l'activité de la substance de croissance devient compréhensible, car les molécules non dissociées des acides traversent la membrane beaucoup plus facilement que leurs anions.

7. Une anlyse plus détaillée de l'action des substances de croissance, analyse basée sur les résultats d'études comparatives sur des modèles et sur du matériel biologique, est donnée dans le mémoire suivant.

ZUSAMMENFASSUNG

1. Um die Hypothese, dass die Wirkung synthetischer Verbindungen, die als Pflanzenwuchsstoffe aktiv sind, hauptsächlich in der Protoplasmamembran lokalisiert ist (also die Permeabilität beeinflusst), zu prüfen, wurde der Einfluss dieser Art von Verbindungen auf Koazervate untersucht.

Im Falle der Gültigkeit der Hypothese müsste eine Turgeszenzwirkung auf die Koazervate auftreten, die erwartungsgemäss parallel mit der physiologischen Aktivität verlaufen sollte.

2. *a.* In der Reihe Naphtalin(1)essigsäure, Di-, Tetra- und Dekahydronaphtalin(1)essigsäure tritt eine Zunahme der Turgeszenzwirkung auf Oleatkoazervate auf.

Bei botanischen Objekten nimmt die physiologische Aktivität jedoch in derselben Reihenfolge ab.

b. Die Turgeszenzwirkung auf Oleatkoazervat von *trans*-, 1, 2, 3, 4, tetrahydronaphtyliden(1) essigsäuren ist stärker als die des *cis*-Isomeren, während — im Gegensatz zur *cis*-Form — das *trans*-Isomere keine wachstumsfördernde Wirkung hat.

c. Ersetzen der Carboxylgruppe durch eine Gruppe mit stärker saurem Charakter verursacht eine Abnahme der Wuchsstoffwirkung; beim Oleatkoazervat wird die entgegengesetzte Wirkung beobachtet.

d. In der homologen Reihe Naphtalin(1)essigsäure, -propionsäure, und -buttersäure nimmt der Einfluss auf das Oleatkoazervat zu (diese Zunahme beruht darauf, dass die zugefügte Substanz in dieser Reihenfolge stärker von den Seifenmicellen adsorbiert wird). Die physiologische Aktivität in dieser Reihe zeigt eine Abnahme, die von einer gewissen Oszillation begleitet ist.

e. Beim Oleatkoazervat sind die relativen Aktivitäten von Essigsäure und Trichloressigsäure in Übereinstimmung mit den Erwartungen auf Grund der Hypothese.

3. Im allgemeinen kann die Wirkung von Anionen der Säuren mit Wuchsstoffaktivität und von verwandten Verbindungen auf das Oleatkoazervat mit den Regeln, die bereits früher angegeben wurden (BUNGENBERG DE JONG), erklärt werden.

4. Alle Verbindungen mit Wuchsstoffaktivität zeigen den erwarteten Turgeszenzeffekt bei Koazervatsystemen (die als Modell für die Protoplasmamembran dienen); quantitativ betrachtet verläuft die Aktivität jedoch nicht parallel mit der physiologischen Aktivität, sondern gerade umgekehrt.

5. Dieser Unterschied muss entweder dadurch erklärt werden, dass die Beziehung Wuchsstoff/Koazervat und die Beziehung Wuchsstoff/Protoplasmamembran nicht genügend vergleichbar sind (mit anderen Worten, dass das Oleatkoazervat als Modell nicht vollständig ist), oder dadurch, dass ein bedeutender Teil der Wuchsstoffaktivität — im Gegensatz zur Hypothese — nicht in der Membran lokalisiert ist.

Auf Grund der Beweiskraft verschiedener angegebener Argumente muss die letztere Erklärung als die wahrscheinlichere betrachtet werden.

6. Es scheint wahrscheinlich zu sein, dass die Wuchsstoffaktivität sich hauptsächlich im Protoplasma entfaltet, und dass deshalb der Wuchsstoff erst die Membran passieren muss.

References p. 277.

In diesem Falle wird der Einfluss des pH auf die Wuchsstoffaktivität plausibel, da die undissoziierten Säuremoleküle sehr viel schneller diffundieren als ihre Anionen.

7. Eine ausführlichere Analyse der Wuchsstoffaktivität auf der Grundlage der Ergebnisse vergleichender Untersuchungen an Modellen und biologischen Objekten wird in der folgenden Arbeit gegeben.

REFERENCES

M. BODANSKY, *J. Biol. Chem.*, 79 (1928) 241.
D. M. BONNER, *Botan. Gaz.*, 100 (1938) 200.
H. L. BOOIJ, *Thesis*, Leyden 1940, *cf. Rec. trav. botan. néerland.*, 37 (1940) 1.
H. L. BOOIJ, H. G. BUNGENBERG DE JONG, *Biochim. et Biophys. Acta*, 242
H. G. BUNGENBERG DE JONG, P. H. TEUNISSEN, *Kolloid.-Beihefte* 47 (1937) 263.
H. G. BUNGENBERG DE JONG, H. L. BOOIJ, G. G. P. SAUBERT, *Protoplasma* 29 (1938) 526.
H. G. BUNGENBERG DE JONG, G. G. P. SAUBERT, H. L. BOOIJ, *Protoplasma*, 30 (1938) 1.
H. FÜHNER, E. NEUBAUER, *Arch. exp. Path. Pharmakol.*, 56 (1906) 333.
H. v. GUTTENBERG, *Naturwissenschaften*, 30 (1942) 109; *Planta* 33 (1943) 576; H. VON GUTTENBERG, R. BÜCHSEL, *Planta*, 34 (1944) 49; cf. CHR. DETTWEILER, *Planta*, 33(1943) 258.
K. HESS, H. KIESSIG, W. PHILLIPPOFF, *Fette und Seifen*, 48 (1941) 377.
A. M. A. VAN SANTEN, *Proc. Kon. Ak. Wetensch.*, Amsterdam 41 (1938) 513; *Thesis* Utrecht 1940.
S. STRUGGER, *Ber. deut. botan. Ges.*, 50 (1932) 77.
P. H. TEUNISSEN, *Thesis* Leyden 1936.
K. V. THIMANN, C. L. SCHNEIDER, *Am. J. Botany*, 25 (1938) 270, cf. H. BORRISS, *Jahrb. wiss. Botan.*, 85 (937) 732; K. WUHRMANN, *Protoplasma*, 29 (1938) 361.
H. VELDSTRA, *Enzymologia*, 11 (1944) 97, 137.
H. VELDSTRA, *Biochim. et Biophys. Acta*, 1 (1947) 364.
H. VELDSTRA, E. HAVINGA, *Rec. trav. chim.*, 62 (1943) 841.
H. VELDSTRA, E. HAVINGA, *Enzymologia*, 11 (1945) 373.

Received April 10th, 1948

(Publication postponed on authers' request.)