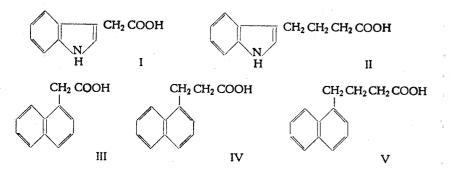
Botany. — Researches on plant growth regulators. XII. Comparative investigation of a number of homologous and isomeric synthetic growth substances in the rooting of cuttings. By W. KRUYT and H. VELDSTRA. (Communicated by Prof. V. J. KONINGSBERGER.)

(Communicated at the meeting of September 27, 1947.)

Introduction.

If one compares the effect in root formation between plant growth substances derived from indole and from naphthalene respectively, it may be noticed that e.g. indole acetic acid (I) and indole butyric acid (II) are on the whole equally active (in certain cases indole butyric acid is even more active). When, however, the side-chain is lengthened, starting from a-naphthalene acetic acid (III) the activity clearly decreases and thus a-naphthalene propionic acid (IV) and a-naphthalene butyric acid (V) are practically never used.



The difference between derivatives of indole and naphthalene is all the more outstanding in the primary growth reaction as determined in the pea-test, in which at a similar activity of indole acetic acid, indole butyric acid and α -naphthalene acetic acid, the activity of α -naphthalene butyric acid is but very small.

In the studying of the relation between structure and activity (VELDSTRA (1)) as yet no explanation has been proved for this remarkable fact. None of the comparative methods used shows a differentiation running parallel to the mentioned differences. As we suspected an important detail of the connection between structure and activity to be hidden in these relations, we thought it advisable to compare once more with certain cuttings all the named acids, especially the naphthalene derivatives, as to their activity in root formation, in order that we might verify the results obtained by other investigators partly on heterogeneous material (compare GRACE (2)).

Beside the already mentioned homologous series, isomeric β -substituted naphthalene-derivatives were also tested.

Material and methods.

Our experiments on cuttings were carried out in the hothouses of "De Proeftuin" at Boskoop ¹). The material was collected in the morning in fair weather and used as soon as possible. As testing subject we used \times *Viburnum Burkwoodii* Burkw. From the branches measuring 17–28 cm the part directly under the soft top was taken. The cuttings all had 4 leaves and consisted of two internodes, cut off directly above the node. Finally the length was 11–18 cm and the diameter $\pm 2\frac{1}{2}$ mm. The cuttings from the various sources were always distributed equally among the groups.

In the course of the afternoon the bundled cuttings were put 2 to 3 cm deep in glass dishes with fresh growth substance solutions (prepared with distilled water). Beside an entirely untreated group another lot was always placed in pure water only as another control. A dish filled with water and without cuttings was present during the treatment to allow a determination of the evaporation in the end so that the uptake could be more or less accurately calculated and thus the dose of growth substance for each cutting.

During the experiment maximum and minimum temperature were registered.

After 24 hours of soaking the lower ends of the cuttings were washed in rain-water and placed in so-called Rhododendron-pots (inside measurements: top diam. 9 cm, bottom diam. 5 cm and depth 8 cm). In so doing 5 cuttings came to be placed along the rim.

The rooting medium (peat/sand = 1:2) had been prepared beforehand in sufficient quantity to eliminate possible differences in composition between the various lots. The amounts of fresh, sifted peat and washed coarse sand were measured with a large flowerpot so that the proportion peat/sand was known by volume. An investigation by KRUYT (3-6) in sequence of the work done by CHADWICK (7); ESPER and ROOF (8); HITCHCOCK and ZIMMERMAN (9-10); HUBERT, RAPPAPORT and BEKE (11); LAURIE (12); LAURIE and CHADWICK (13); LONG (14); SMITH (15) etc., had proved that by a right choice in the composition of the medium one can exert an important influence on the root formation. In this case probably the $p_{\rm H}$ plays the most important part. The $p_{\rm H}$ of a mixture peat/sand = 1:2 shortly after mixing is 5.3 whereas this value increases to 5.7 after a full month.

After carefully mixing the peat and the sand the material was watered now and then and mixed again until the right humidity had been obtained. The pots which had previously sucked up plenty of water were then filled with the mixture. After placing the cuttings in previously prepared holes in the medium it was pressed firmly.

The pots were placed in a hothouse under double glass and dug in into humid peat. We have strived to distribute the influence of the position as much as possible among the various groups. Therefore the groups were not kept separately but the pots were placed in the breadth of the hotbed or hothouse, behind each other, in sequence of the groups of which the rooting experiment consists. When the row is full a new one is placed next to it till one pot of very group has been dug in. Then one starts again with the first group and so on. Of course one must see to it that the number of pots in one row does not happen to correspond with the number of groups because then there would be no shifting. In that case an empty pot is occasionally placed in between the filled ones (so as to get a shifting of position).

As soon as everything was dug in, it was watered thoroughly with rain-water after which the windows were closed. The tending took place in the usual way (5). Soil and air temperatures were registered nearly daily during the root formation.

At the end of the experiment the most important result for us, viz. the number of roots on each cutting, was determined and the manner in which they were attached to the cuttings was also noted. The measuring of all the roots was practically impossible because of the large number of cuttings and the extensive root formation (in some cases more than an average of 40 per cutting !). It would have damaged too much the material which had

¹) We owe many thanks to the Board and Director of "De Proeftuin" for the hospitality granted.

to serve again in further experiments. Therefore measuring was confined to the longest root of each cutting.

We owe many thanks to Messrs. H. BOSCH and P. DE VOGEL, chief assistants of the "Tuinbouwvoorlichtingsdienst" at Boskoop for their assistance in the collecting and preparing of the cuttings and for the careful attending of the experiments.

Discussion of the results.

The first experiment which lasted from July 26 till August 24 1944 consisted of 21 groups containing 30 cuttings each. Beside the two control groups (untreated and water) we examined the reaction of α - and β -naphthalene acetic acid, α - and β -naphthalene propionic acid, α - and β -naphthalene butyric acid (in the form of their potassium salts), each in three concentrations (6, 3 and 1.5×10^{-4} mol./1 respectively) and besides these β -indole acetic acid (as the free acid) in a concentration of 25 mg/1²).

During the treatment the minimal temperature was 17.4° C, the maximal temperature 31.3° C, the average 24.4° C; temperature of the soil during the process of root formation: minimal 18.8° , maximal 28.8° , average 23.4° C; air temperature minimal 17.1° , maximal 31.7° , average 23.4° C. No soil heat was supplied.

Table I shows the situation at the ending of the experiment.

As a result of the uptake of water we already note a rise in the percentage of root formation; the average number of roots for each rooted cutting does not change however. The concentration of n.a.a., n.p.a. and n.b.a. was always 6×10^{-4} mol./1 at the start; corresponding with 111.6, 120 and 128.4 mg of acid per litre. From the data obtained we may deduce the following.

a-N.a.a. is without doubt the most active substance. Of its homologues a-n.b.a. still has a moderate effect, but a-n.p.a. has only a very weak effect. So the oscillation in the homologous series, already noticed by GRACE (2), evidently also occurs in this case. The order a-n.a.a. > a-n.b.a. > a-n.p.a. is also to be seen in the type of the root formation. With the first-mentioned acid no basal rooting takes place, a-n.b.a. at 3×10^{-4} mol./1 gives two specimens with basal roots, while in the groups of a-n.p.a. a total of 25 such cases occur.

The β -isomers are weaker than the *a*-substituted naphthalene-derivatives, which shows up strongly especially with n.a.a. For the remainder the order β -n.a.a. > β -n.b.a. > β -n.p.a. also holds in this case. β -N.a.a. and β -n.b.a. have only a slightly stronger effect than water.

The effect of β -i.a.a. (free acid) here lies between that of α -n.a.a. and α -n.b.a.

²) We use the following abbreviations:

n.a.a. = naphthalene acetic acid

n.p.a. = naphthalene propionic acid

n.b.a. = naphthalene butyric acid

i.a.a. = indole acetic acid

i.b.a. = indole butyric acid

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			ated	a. 111.6 mg/1 55.8 ,, 27.9 ,,	111.6 mg/l 55.8 " 27.9 "	120 mg/1 60 <i>"</i> 30 <i>"</i>	o.a. 120 mg/l 60 " 30 "	128.4 mg/1 64.2 32.1	128.4 mg/1 64.2 " 32.1 "	.a. 25 mg/l
			untreated water	α-n.a.a. ,,	β-n.a.a. ,,	a-n.p.a. "	β-n.p.a. ,,	α-n.b.a.	β-n.b.a. ,,	i.a.a.
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On account of the number of dead specimens and the occurrence of basal decay with the rooted as well as with the non-rooted cuttings, the concentration of 6×10^{-4} mol./1 is in most cases too high. It is remarkable that the most active compound (*a*-n.a.a.) evidently gives an increase in root length (in the next experiment this phenomenon is not so prominent). β -I.a.a. also has a favourable influence on the root length. This conclusion, however, could only have been drawn definitely if the length of all the roots had been determined and thus the average root length. For this the measuring of only the longest root of every cutting is very likely insufficient.

On the whole the rooting was normal; the roots were coloured white and had a tip tinged with yellow. In the groups 3 and 15 (with the highest concentrations of α -n.a.a. and α -n.b.a. respectively) the roots were slightly thicker than normal.

The second, more complete, experiment in which also the potassium salts of β -i.a.a. and β -i.b.a. in three different concentrations were included, followed on September 20 1944.

During the treatment with the growth substance solutions the minimal temperature was 12.4° C, the maximal temperature 21.7° C, the average therefore 17.1° C. The number of ml sucked up by each cutting varied between 0.53 and 0.80; the dose calculated in μ g per cutting may be found in the fifth column of table II.

During the proces of rooting from Sept. 27 '44 up to and including Sept. 28 '44; from Oct. 3 '44 up to and including Oct. 8 '44 and from Oct. 16 '44 onwards soll heating was applied. The temperature of the bed was not read at the place of experiment itself in consequence of lack of thermometers, but in another place in the hothouse. The soil temperature was minimal 10.4° C and maximal 19.9° C (with an average of 15.3°) whereas these values for the air temperature were 9.7° C and 24.3° C respectively (with an average of 16.5°). In the place at which these temperatures were taken there was no soil heating, so that very possibly during the period that the hothouse was heated the soil and air temperatures around the cuttings were higher than is stated above.

The results of this experiment are collected in table II and also recorded in photographs made on Oct. 30 and Oct. 31 1944, that is some days after the ending of the experiment. During this time the cuttings were dug in in boxes with the cutting-medium and so placed in the hothouse bed. By doing this the roots had the opportunity to increase in length during these few days and even the number of roots had increased. Evidently the transferring into loose medium stimulates the elongation of the roots. Therefore the photographs of the second experiment do not show an exact picture of the situation at the end on October 26 and 27, but can only be used for mutual comparison. We may suppose that the growth in length was approximately of the same proportion in all groups. The figures in table II give a reliable summary.

The starting concentrations of n.a.a., n.p.a., n.b.a., i.a.a. and i.b.a. were again 6×10^{-4} mol./1, that is 111.6, 120, 128.4, 105 and 121.8 mg of

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TABLE II

acid per litre respectively. On account of the number of dead specimens and the occurrence of basal decay this concentration here also proves to be too high in most cases for the rooted as well as for the non-rooted cuttings. This was even more noticeable in the first experiment, in which case the material was slightly less ripened and therefore softer.

It is remarkable that in this case the water-control had a smaller percentage of rooting than the totally untreated cuttings.

From the table and from the photographs it may be concluded that β -i.a.a. is the most active, so that with the highest concentration of 6×10^{-4} mol./1 even an average of 43 roots per cutting may be counted. It is followed by β -i.b.a. and α -n.a.a.; β -n.a.a. is clearly weaker than the a-isomer, whereas α -n.p.a. and α -n.b.a. only differ slightly (probably n.p.a. is slightly more active, see photographs) and find their place at the end of the series. β -N.p.a. and β -n.b.a. are practically inactive. (It may be noticed that here β -n.a.a. has much less influence than in the previous experiment; possibly the hardyness of the cuttings plays a part in this). Of course the sequence of activity of the compounds examined here need not be exclusively the result of their structure and activity but may perhaps also be due to a specific sensibility of the object tested in these experies ments. Therefore it might be advisable to make such trials on one or more other plants too.

Just as in the first experiment we again notice that by β -i.a.a. the longitudinal growth of the roots is considerably stimulated. The effect of α -n.a.a. in this respect is weaker than was noted in the first experiment.

The following remarks can be made about the rooting process in the following groups:

1: normal rooting, roots white with yellowish tips. aroup

- 2: weaker rooting, as a whole shorter than in group 1; roots normal.
- 3: thick, short roots; more heavier rooting than group 1.
- 4: also rather thick roots; somewhat longer than in group 3.
- 5: roots normal; slightly longer than the roots of group 4, approximately as in group 1 (though of course more abundant).
- 6: roots normal; somewhat heavier rooting than in group 1.
- 7: roots normal; amount of rooting slightly less than in group 1.
- 8: roots normal; rooting as in group 7. ••
- 9: roots normal; rooting as in group 2.
- 10: roots normal, amount of rooting as in group 1; probably slightly shorter,
- 11: roots normal, approximately as in group 9; roots shorter than in group 1.
- 12: roots normal; weaker rooting than in group 2.
- 13: roots normal; still weaker than in group 12.
- 14: roots normal; as in group 13.
- 15: roots slightly thickened; amount of rooting sometimes as in group 1; as a whole less than group 2.
- 16: roots slightly thickened; rooting as in group 15.
- 17: roots normal; very weak rooting.
- 18: roots normal; rooting as in group 2.
- 19: roots normal; weaker rooting than in nr 18.

- group 20: roots normal; stronger rooting than groups 18 and 19; approximately as in group 1.
 - 21: roots thinner than in group 1; heavy rooting, length of roots as in group 1. Too high a concentration shows itself very occasionally by the appearance of aerial roots at the top of the cutting (see fig. 1).
 - 22: roots still thin; heavy rooting, roots longer than in group 21.
 - roots normal; heavy rooting, slightly longer than in group 22. 23:
 - 24: roots normal; heavy rooting, shorter than in group 1.
 - 25: roots normal; heavy rooting, as in group 24.
 - 26: roots normal; heavy rooting; roots longer than in group 25, approximately as in group 1.

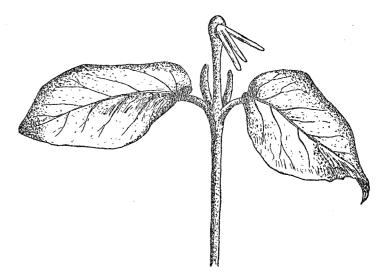


Fig. 1. Formation of aerial roots at the top of a cutting due to treatment with indole acetic acid (105 mg/l).

Summary.

On cuttings of \times Viburnum Burkwoodii Burkw. the activity of the potassium salts of α - and β -naphthalene acetic acid, α - and β -naphthalene propionic acid and α - and β -naphthalene butyric acid has been compared with that of β -indole acetic acid and β -indole butyric acid. The starting concentration was always 6×10^{-4} mol./1 whilst beside this two dilutions (3 and 1.5×10^{-4} mol./1) have been examined.

The highest concentration proved to be too high in most cases, resulting in dead specimens and basal decay. β -I.a.a. is the most active substance after which come β -i.b.a. and α -n.a.a.; β -n.a.a. has a definitely weaker activity than the α -isomer; α -n.p.a. and α -n.b.a. do not differ greatly, their activity is very weak and weak respectively. Their β -isomers are practically inactive.

Thus a typical difference between indole- and naphthalene-derivatives could be established. In the former case lengthening of the side-chain did not greatly decrease the activity; however, in the latter case it did. We could establish, as did the other investigators that the β -substituted naphthalene-derivatives are less active than the α -isomers.

Of the most active compounds β -i.a.a. and α -n.a.a. also stimulate the longitudinal growth of the roots.

One should take into account a possible specific sensibility of a testing subject in comparing the activity of synthetic growth substances so that a repetition with cuttings from other plants would be advisable.

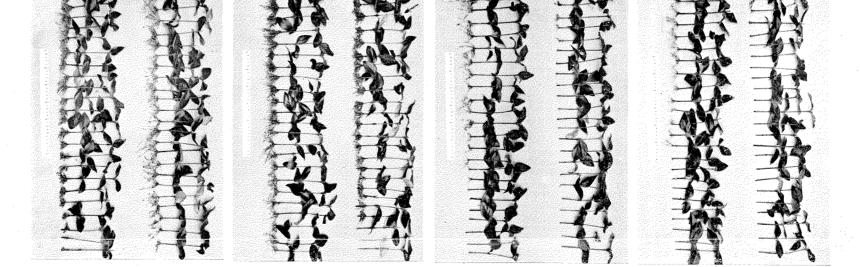
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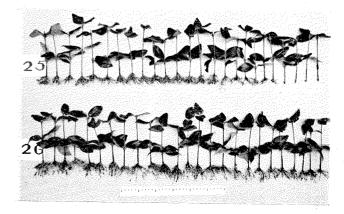
= untreated

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3. 4. 5 = α -n.a.a., resp. 111.6, 55.8, 27.9 mg/l 6, 7, 8 = β -n.a.a., resp. 111.6, 55.8, 27.9 mg/l 9, 10, 11 = α -n.p.a., resp. 120 , 60 , 30 mg/l 12, 13, 14 = β -n.p.a., resp. 120 , 60 , 30 mg/l 15, 16, 17 = α -n.b.a., resp. 128.4, 64.2, 32.1 mg/l 18, 19, 20 = β -n.b.a., resp. 128.4, 64.2, 32.1 mg/l 21, 22, 23 = β -i.a.a., resp. 105 , 52.5, 26.25 mg/l 24, 25, 26 = β -i.b.a., resp. 121.8, 60.9, 30.45 $\mathrm{mg/l}$



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One should take into account a possible specific sensibility of a testing subject in comparing the activity of synthetic growth substances so that a repetition with cuttings from other plants would be advisable.

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