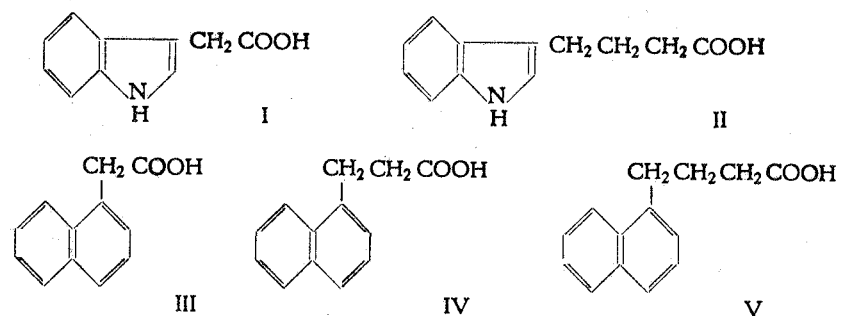


Botany. — *Researches on plant growth regulators. XII. Comparative investigation of a number of homologous and isomeric synthetic growth substances in the rooting of cuttings.* By W. KRUYT and H. VELDSTRA. (Communicated by Prof. V. J. KONINGSBERGER.)

(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 α -naphthalene acetic acid (III) the activity clearly decreases and thus α -naphthalene propionic acid (IV) and α -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 Boskoop¹⁾. 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 flower-pot 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_{H} plays the most important part. The p_{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 every 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.

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./l 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 α -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 hardiness 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 experiments. 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:

- group 1: normal rooting, roots white with yellowish tips,
- „ 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.
- „ 23: roots normal; heavy rooting, slightly longer than in group 22.
- „ 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.

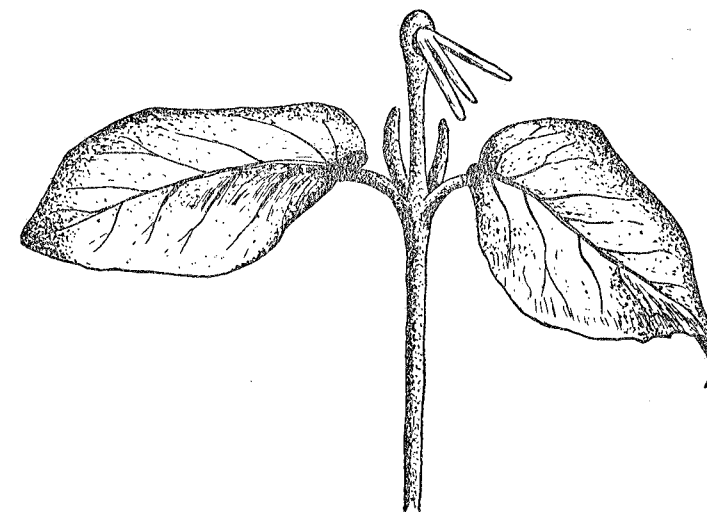


Fig. 1. Formation of aerial roots at the top of a cutting due to treatment with indole acetic acid (105 mg/l).

Summary.

On cuttings of *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./l whilst beside this two dilutions (3 and 1.5×10^{-4} mol./l) 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.

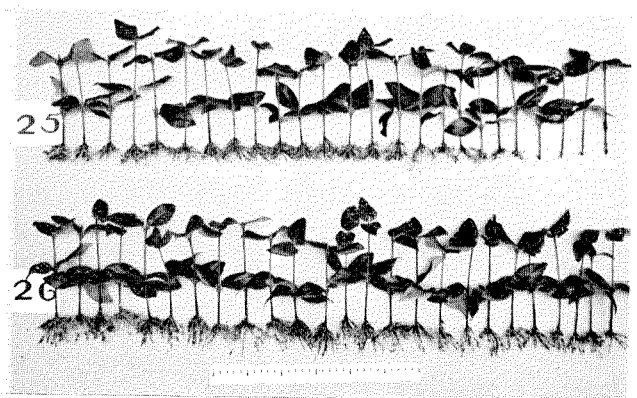
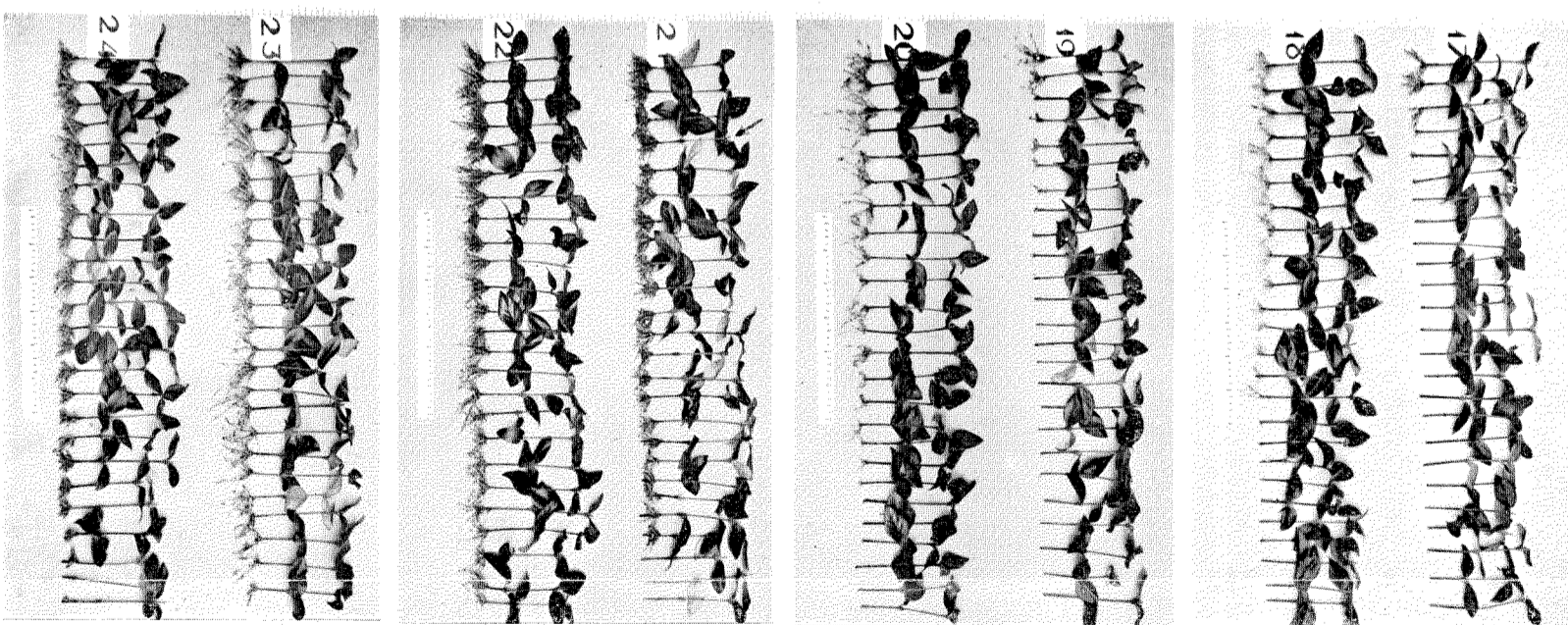
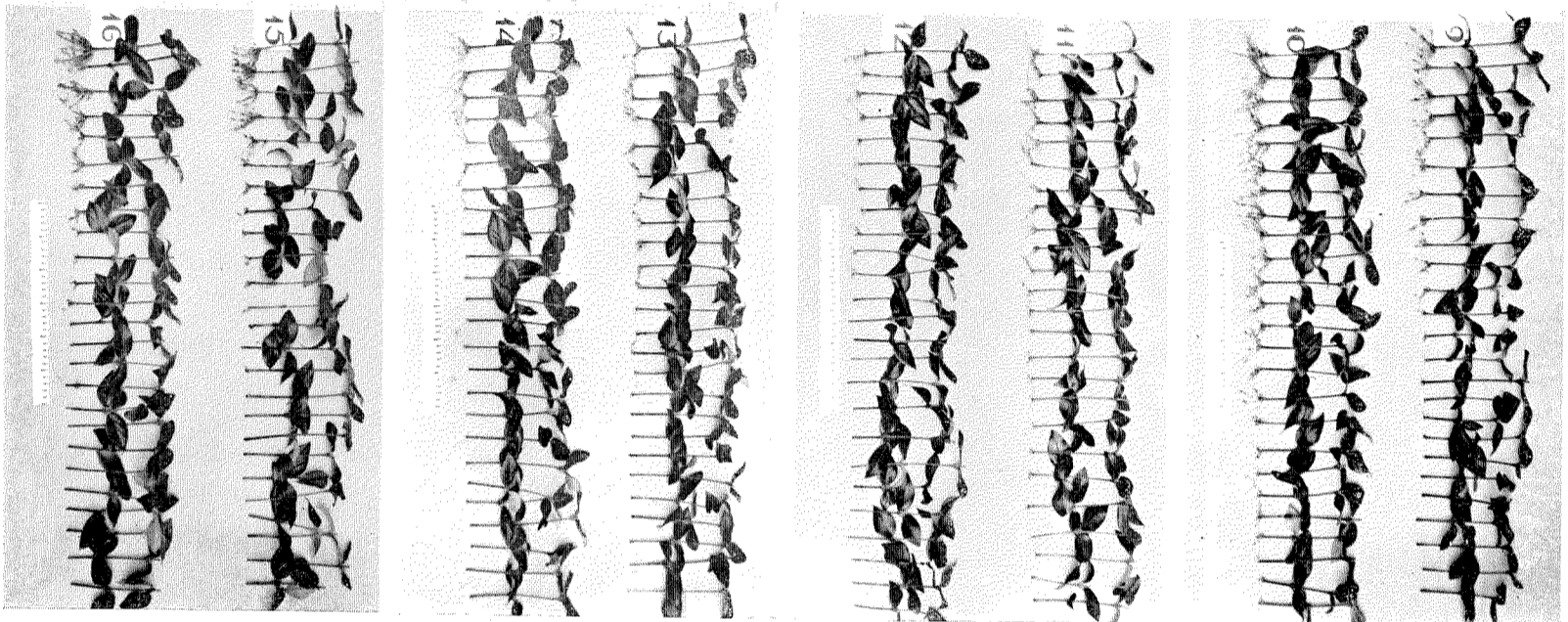
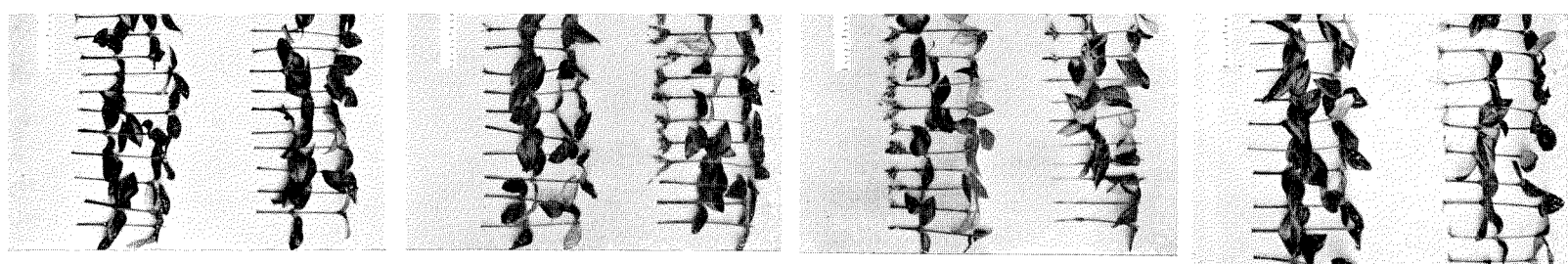
Thus a typical difference between indole- and naphthalene-derivatives could be established. In the former case lengthening of the side-chain did not greatly decrease the activity; however, in the latter case it did. We could establish, as did the other investigators that the β -substituted naphthalene-derivatives 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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- 1 = untreated
- 2 = water
- 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 mg/l

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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