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Studies on the Phytohormone Activity of Naphthoic Compounds, I. On the Derivatives of Naphthoic Acid-1.

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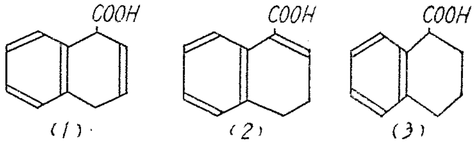
From the list of growth-promoting substances, Koepfli, Thimann and Went (2) concluded that the primary growth-promoting activity is connected with the presence of : 1) a ring system as nucleus, 2) a double bond in this ring, 3) a side chain, 4) a carboxyl group (or a structure readily converted to a carboxyl) on this side chain at least one carbon atom removed from the ring, and 5) a particular space relationship between the ring and the carboxyl group. Veldstra (9, 10) restated that the substance must have the ring system of high surface activity and need the definite spatial relationship between the carboxyl group (or its dipole) and the ring system. And Went (11) claimed also that the side chain composed of at least two carbon atoms should be attached adjacent to a double bond of the ring.

In contrast to the above principle substances with their carboxyls attached directly to the rings were found growth promoting, namely 2 - bromo - 3 - nitrobenzoic acid by Zimmerman and Hitchcock (12), and derivatives of naphthoic acid - 1 by Mitsui (5). In both of these cases, however, the tomato test was used with green tomato plants which contained natural auxin. So the epinastic response caused by a substance cannot necessarily be attributed to the primary growth-promoting activity of the substance, as Haagen-Smit and Went (1) and Thimann (7) have pointed out. So it is desirable to make standard auxin tests with such substances. This report deals with the auxin activity of 1, 4 - dihydro - [1], 3, 4 - dihydro - [2] and 1, 2, 3, 4 - tetrahydro-naphthoic acid - 1 [3], which were found by Mitsui active in the tomato test.



(1) (2) (3)

(4) In the following, " naphthoic acid-1 " will be represented by NcA

Mitsui (5) also found with the tomato test that the *d*-isomer of 1, 4-dihydro-NcA was about 100 times more active than its *l*-isomer, and the *l*-isomer of 1, 2, 3, 4-tetrahydro-NcA was 10 times more active than its antipode. As regards the difference in activity between optical isomers, there is only a report on alpha-indole-3-propionic acid. Hence it is important to see the differential activity of those isomers in the auxin tests.

Experimental

The growth activity was measured with the standard *Avena* method and the pea test. And the formative activities as callus formation, bud inhibition and root formation were also observed. NcA-derivatives and indole-3-acetic acid were used as free acid.

1. *Avena curvature.*——Each *Avena* seedling was grown in a glass tube which was 1.8 cm. in the internal diameter and 5 cm. high, stuffed with well-washed moist saw dust. Substances to be tested were applied with the agar block on the twice decapitated coleoptile, as usual.

As shown in Table 1, the most active were *d*- and *r*-1, 4-dihydro-NcA. Less active were *l*- and *r*-1, 2, 3, 4-tetrahydro-NcA, while *l*-1, 4-dihydro-, 3,4-dihydro- and *d*-1, 2, 3, 4-tetrahydro-NcA did not induce any curvature even at the concentration of 1000 mg./1. It is to be noted that the *l*-isomer of the dihydro-compound is inactive while that of the tetrahydro-compound is active.

Table 1. The lowest concentration inducing the *Avena* curvature.

Substance	Concentration in mg./1.
d-1, 4-Dihydro-NcA	5
l-1, 4-Dihydro-NcA	Inactive, 1000
r-1, 4-Dihydro-NcA	5
3, 4-Dihydro-NcA	Inactive, 1000
d-1,2,3,4-Tetrahydro-NcA	Inactive, 1000
l-1,2,3,4-Tetrahydro-NcA	600
r-1,2,3,4-Tetrahydro-NcA	600

2. *Pea curvature.* —— Peas (var. Alaska) were grown on the moist sand in a dark room at the constant temperature of 25° C. At the age of 7 days, the top was cut off at 3.5-5 mm. below the terminal bud and the stem was split lengthwise and cut off. Split sections were immersed in the solutions of NcA-derivatives for 24 hours, and then photographed.

Table 2. Range of concentration effective on the pea curvature.

Substance	Concentration Range mg./l.
d-1, 4-Dihydro-NcA	0.01 - 50
l- 1, 4-Dihydro-NcA	0.5 - 50
r-1, 4-Dihydro-NcA	0.01 - 50
3, 4-Dihydro-NcA	50 - 100
d-1,2,3,4,-Tetrahydro-NcA	5 - 50
l- 1,2,3,4-Tetrahydro-NcA	0.5 - 50
r- 1,2,3,4-Tetrahydro-NcA	0.5 - 50

Table 2 shows the range of effective concentration, the higher values indicating the limit above which the pea stem was injured. Just as in the case of the *Avena* test, *d* - and *r* - 1, 4-dihydro-NcA are more active than *l* - and *r* - 1, 2, 3, 4, - tetrahydro - NcA. The order of the activity among the three least active substances are *l* - 1, 4 - dihydro - , *d* - 1, 2, 3, 4 - tetrahydro - and 3, 4 - dihydro - NcA.

3. *Callus formation.* —— *Vicia Faba* was grown in a dark room at 25° C. and relative humidity of 85 - 90 %. When the seedling was 20 - 25 cm. high, the etiolated stem was decapitated at beneath the second node. The cut-surface was smeared with lanolin paste of the substance to be tested, and the callus formation at the cut-surface was observed 7 days after the application.

Fig. 1 shows the effect of 0.1 % paste. More effective are *l* - and *r* - 1, 2, 3, 4 - tetrahydro - NcA than the rest of the substances tested, including indole - 3 - acetic acid. With weaker paste of 0.01 %, callus was formed only by these two most active substances. When the stem was treated with the 1 % paste, the treated part turned dark brown due to injury, followed by swelling of the zone just below the injured part. In this case, too, the swelling was conspicuous with *l* - and *r* - 1, 2, 3, 4 - tetrahydro - NcA, and very slight or hardly noticeable with other substances.

4. *Bud inhibition.* —— Etiolated stem of the pea seedling, grown for 7 days in a dark room at 25° C., was smeared with lanolin paste at a zone 1 or 3 cm. above a lateral bud. And after the treated seedling was kept 8 days in the same dark room, the growth of the bud was measured.

Table 3 and Fig. 2 show that the bud-inhibiting effect is remarkable with both of the optical antipodes of 1,4-dihydro-NcA, although the *l*-isomer was inferior to the *d*-isomer in the standard *Avena* method and the pea test. While *l*-1,2,3,4-tetrahydro-NcA inhibits the bud if applied 1 cm. apart from this, it does not if the site of application is 3 cm. apart. This might be due either to the weakness of the inhibiting action, or to the difficulty in the transport. Scarcely effective is the *d*-1,2,3,4-tetrahydro-NcA, as well as 3,4-dihydro-NcA.

Table 3. Inhibition of bud growth by application of lanolin paste on the etiolated pea stem at 1 and 3 cm. above the lateral bud.

Substance	Concentration mg. per g. of lanolin	Length of bud after 8 days (cm.)	
		1 cm.	3 cm.
d-1,4-Dihydro-NcA	10	0	0
l-1,4-Dihydro-NcA	10	0	0
r-1,4-Dihydro-NcA	10	0	0
3,4-Dihydro-NcA	10	14.8	14.1
d-1,2,3,4-Tetrahydro-NcA	10	12.6	13.8
l-1,2,3,4-Tetrahydro-NcA	10	0	11.6
r-1,2,3,4-Tetrahydro-NcA	10	0	12.8
Indole-3-acetic acid	10	0	0
Control	–	16.6	16.7

5. *Root formation.* —— The root-forming activity was tested with the substances in question by Went's method. Etiolated pea cuttings were washed with distilled water for 4 hours, and then the tip or the base of the cutting was treated with solutions of the substances for 15 hours. The basal parts were then immersed in 2 % sucrose solution for 6 days followed by 7 days in distilled water, and the number of roots grown out was counted. All of the substances showed the root-forming activity at their optimum concentrations, they being toxic at higher concentrations. The optimum is lower with *d*-1, 4-dihydro-NcA, and higher with 3, 4-dihydro-NcA than with other substances (Table 4). As to the tetrahydro-derivatives, the number of roots formed is less with *d*-form than with *l*-form.

When the tip of the cutting was treated with the solution of optimum concentration callus was formed there, and roots grew out mostly from this swollen apical part. And in case the concentration of tip treatment was lower no callus formation occurred, small number of roots growing only from the basal part. Whereas, the optimal concentration of indole-3-acetic acid for tip treatment is too high for the basal treatment, this does not seem to be the case with NcA-derivatives.

Table 4. Number of roots per ten plants after treatment by optimal concentration of solution at the tip or base of etiolated pea cutting.

Substance	Concentration mg./l.	Site of treatment	
		Tip	Base
d-1,4-Dihydro-NcA	5	80	110
l-1,4-Dihydro-NcA	10	63	103
r-1,4-Dihydro-NcA	5	85	105
3,4-Dihydro-NcA	50	58	77
d-1,2,3,4-Tetrahydro-NcA	20	62	69
l-1,2,3,4-Tetrahydro-NcA	20	96	93
r-1,2,3,4-Tetrahydro-NcA	20	98	90
Indole-3-acetic acid	10	102	20
Control	0	43	45

Discussion

Naphthoic acid-1 derivatives, in which the carboxyl group is directly attached to the ring system, were tested for the auxin activities in order to confirm Mitsui's observation which was made with green tomato plants. The relative activity of these substances and their optical isomers here studied, expressed as percentage of the activity of indole-3-acetic acid, are summarized in Table 5. It is to be noted that all of the five tests were made with etiolated plants to avoid the indirect effect on the naturally occurring auxin.

Table 5. Relative activities of naphthoic acid-1 derivatives, expressed as % of the activity of indole-3-acetic acid.

Substance	Avena test	Pea test	Callus formation	Bud inhibition	Root formation*
d-1,4-Dihydro-NcA	3	100	ca. 100	100	150
l-1,4-Dihydro-NcA	Inactive	50	ca. 100	100	60
r-1,4-Dihydro-NcA	3	100	ca. 100	100	150
3,4-Dihydro-NcA	Inactive	6	<100	10	10
d-1,2,3,4-Tetrahydro-NcA	Inactive	30	<100	20	30
l-1,2,3,4-Tetrahydro-NcA	0.02	80	>100	60	50
r-1,2,3,4-Tetrahydro-NcA	0.02	80	>100	60	50

* Tip treatment

Generally speaking, *d*-(and *r*-) 1, 4-dihydro-NcA has the strongest auxin activity, *l*-(and *r*-) 1, 2, 3, 4 - tetrahydro-NcA follow this and 3, 4-dihydro-NcA is the weakest through all kinds of test. *d*-1, 4-Dihydro-NcA shows roughly the same activity of indole-3-acetic acid, except the *Avena* test. This fact seems to present an exception to Koepfli, Thimann and Went's rule which claims the necessity of at least one carbon atom between the ring system and the carboxyl group, and also to Went's requirement that the side chain is to be attached adjacent to a double bond in the ring.

For the minimal structural requirement of plant growth-promoting substance Veldstra (9, 10) proposed the high surface activity of the ring system and larger angle between the carboxyl group and the ring. Table 6 and Fig. 3 show the surface activity and the angle in question. The surface activity lowers as the number of double bonds of the ring system decreases. And there is no relation between the auxin activity and the surface activity.

Table 6. Half suppression value of naphthoic acid-1 derivatives, after Mitsui (6).

Substance	HSV (in 10^{-6} mol./l.)
Naphthoic acid-1	2.5
d-1, 4-Dihydro-NcA	4.4
l- 1, 4-Dihydro-NcA	3.1
r-1, 4-Dihydro-NcA	4.0
3, 4-Dihydro-NcA	5.3
d-1, 2, 3, 4-Tetrahydro-NcA	6.0
l- 1, 2, 3, 4-Tetradydro-NcA	7.8
r-1, 2, 3, 4-Tetrahydro-NcA	7.5
Indole-3-acetic acid	3.6

Of 1, 4-dihydro-NcA the angle between the carboxyl group and the ring is larger in *d*-isomer than in *l*-isomer, and of 1,2,3,4-tetrahydro-NcA, it is larger in *l*- isomer than in *d*-isomer. So there is parallelism between the auxin activity and the angle of carboxyl group. Inactivity of 3,4-dihydro-NcA the surface activity of which is higher than 1,2,3,4-tetrahydro-NcA can be accounted for rather by the small angle between the carboxyl group and the ring system.

Relative activities of the substances are different according to the reactions, for example both of the isomers of 1,4-dihydro-NcA show the same activity in callus formation and bud inhibition, and *l*-1,2,3,4-tetrahydro-NcA is more active than indole-3-acetic acid in callus formation. It may be possible that the results of reactions of a few to 24 hours, as *Avena* and pea tests, are different from those to be observed after several days.

Summary

Hydrogenated naphthoic acids which have the carboxyl group attached directly to the ring system are shown to be active in the standard *Avena* method, the pea test, callus formation, bud inhibition and root formation, etiolated plants being used in any case. Hence Went's structural requirement for auxin that the side chain composed of at least two carbon atoms should be attached adjacent to a double bond in the ring have to be revised.

Generally speaking, *d*- and *r*-1,4-dihydronaphthoic acid-1, and next to it *l*- and *r*-1,2,3,4-tetrahydronaphthoic acid-1, are strongly active while 3,4-dihydronaphthoic acid-1 is almost inactive.

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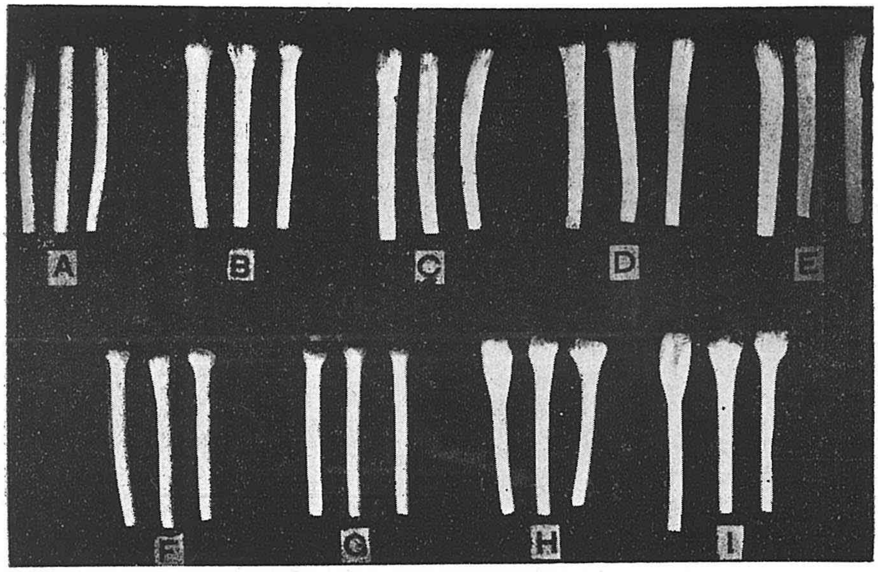

Figure 1. Callus formation 7 days after treatment at the decapitated surface with 0.1 % lanolin paste. (A) Control. (B) Indole-3-acetic acid. (C) *d*-1,4-Dihydro-NcA. (D) *l*-1,4-Dihydro-NcA. (E) *r*-1,4 Dihydro-NcA. (F) 3,4-Dihydro-NcA. (G) *d*-1,2,3,4-Tetrahydro-NcA. (H) *l*-1,2,3,4-Tetrahydro-NcA. (I) *r*-1,2,3,4-Tetrahydro-NcA.

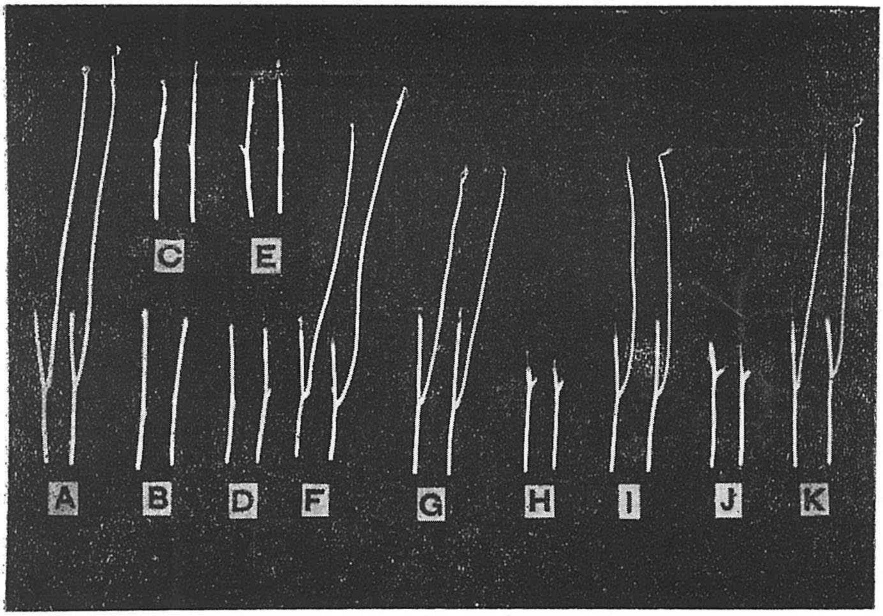

Figure 2. Bud inhibition in etiolated pea stem, 8 days after smearing of 1% lanolin paste containing derivative of naphthoic acid-1 at 3 (A,B,C,D,E,F,G,I,K) or 1 (H,J) cm. above the lateral bud. (A) Control. (B) Indole-3-acetic acid. (C) *d*-1,4-Dihydro-NcA. (D) *l*-1,4-Dihydro-NcA. (E) *r*-1,4-Dihydro-NcA. (F) 3,4-Dihydro-NcA. (G) *d*-1,2,3,4-Tetrahydro-NcA. (H,I) *l*-1,2,3,4,-Tetrahydro-NcA. (J,K) *r*-1,2,3,4-Tetrahydro-NcA.

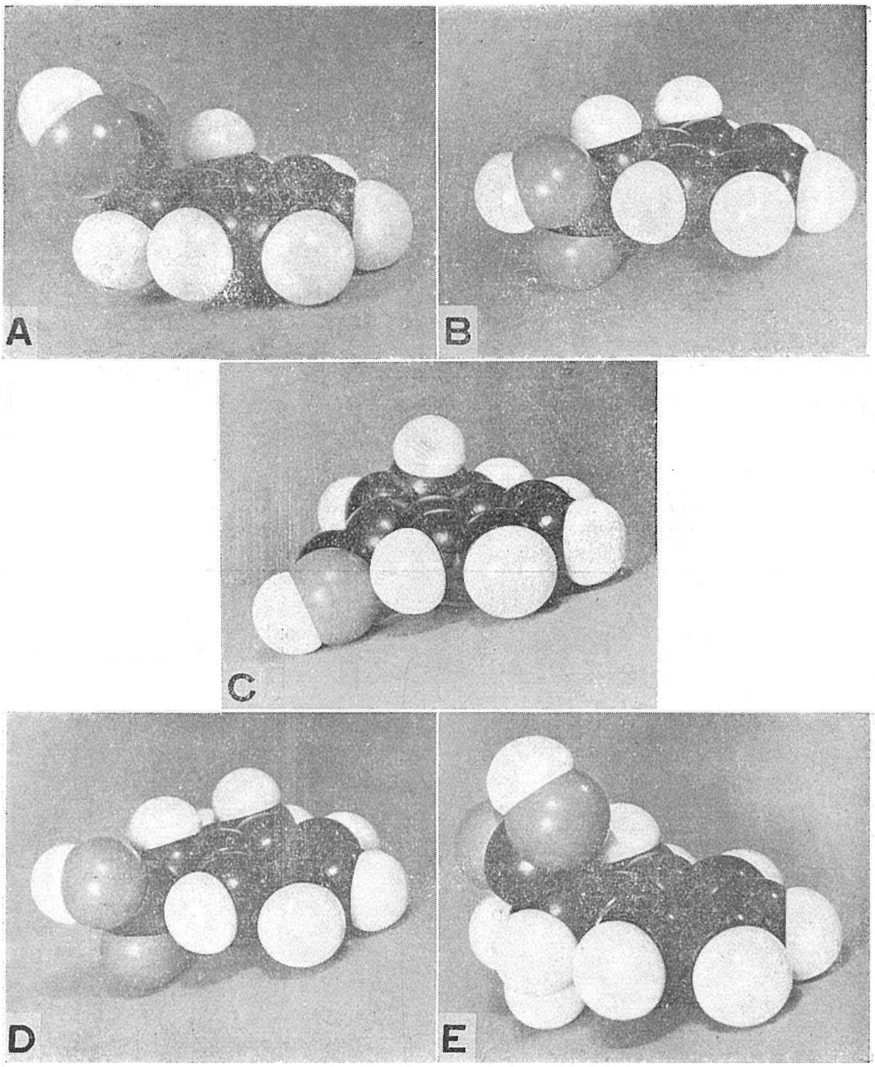

FIGURE 3. Models of naphthoic acid-1 derivatives, according to Mitsui (6). (A) *d*-1,4-Dihydro-NcA. (B) *l*-1,4-Dihydro-NcA. (C) 3,4-Dihydro-NcA. (D) *d*-1,2,3,4-Tetrahydro-NcA. (E) *l*-1,2,3,4-Tetrahydro-NcA.