Studies on the Plant Growth Activity of Substituted Naphthoic Acids

By

Koichi KOSHIMIZU

Studies on the Plant Growth Activity of Substituted Naphthoic Acids

By

Koichi KOSHIMIZU

Acknowledgement

I have to record my sincere thank to Professor T. Mitsui, Department of Agricultural Chemistry, Kyoto University, for his constant encouragement and earnest guidance during the course of this work.

I wish to acknowledge Professor S. Takei, Director of Chemical Research Institute, Professor J. Ashida, Department of Botany, Professor M. Nakajima and Assistant Professor T. Hashizume, Department of Agricultural Chemistry, Kyoto University, for constant encouragement and helpful support.

I must acknowledge the earnestness of Mr. T. Fujita, who gave seriously supported to me from the beginning of my research carrier. I have also to acknowledge Dr. C. Nagata, Department of Fuel Chemistry for his constant guidance in the calculation and suggestive discussion, Mr. J. Kato, Department of Botany for the bioassay of the plant growth activity, and Mr. H. Sato and Misses. Y. Oishi, T. Oyabu and C. Furuki in our laboratory for the microanalysis.

I have to thank members of the Laboratory of Chemical Technology of Agricultural Products and the Laboratory of Agricultural Chemicals in this Department.

Contents

Studies on the Plant Growth Activity of Substituted Naphthoic Acids.

By

Koichi KOSHIMIZU

I. Introduction

Ever since the discovery of indole-3-acetic acid as a native auxin (1), numerous workers have attempted to find other new substances which would modify growth either in the same manner as auxins or in some other manner, and many efforts have been made to correlate the physiological activity with certain structural features of those compounds.

In an early study on the auxin activity of a number of substances, Koepfli, Thimann and Went (2) stated the five requirements which were necessary for auxin activity. In subsequent years a more number of compounds were screened for activity, and the results obtained have led to restatement of these requirements in different forms (3-5).

One of the most striking developments was the demonstration that 2-bromo-3-nitrobenzoic acid was active for cell-elongation (6). Thus, one of the requirements as formulated by Koepfli and his coworkers, a carboxyl group on the side chain removed from the ring at least one carbon atom, has been completely abandoned. This led to the investigation of

many substituted benzoic acids (7-10) and several naphthoic acids (11-13). The structural features of physiologically active benzoic acid derivatives essential for plant growth activity have been analysed in order to obtain a insight into the mode of action. However, it has not been studied so extensively and systematically on substituted 1-naphthoic acid derivatives as benzoic acid derivatives.

It is reasonably expected that these two series of compounds, in which a carboxyl group is bound directly to the aromatic ring, are more or less similar to each other in their physical and chemical properties, 1-(or 2-) naphthoic acids corresponding to 2,3-(or 3,4-) dialk(en)yl benzoic acids. Hence, studies of substituted naphthoic acids may contribute to the understanding of the structural requirement for the growth promoting activity. From this viewpoint 1-naphthoic acid derivatives substituted with chlorine, bromine, methyl or nitro group in various positions were prepared and their growth activity was measured by means of the pea straight growth and the callus formation tests.

In this paper, from the results thus obtained, the deduction of the mode of growth promoting action has been attempted with considerations of the true molecular form and a reactivity index.

This work was presented at the annual meeting of agricultural Chemical Society of Japan, Tokyo, April 10, 1959, and at the monthly meeting of Kansai Branch of the Agricultural Chemical Society of Japan, Osaka, July 19, 1958, and Kyoto, September 19, 1959, respectively, and a part of this work has been contributed to the Bulletin of the Agricultural Chemical Society of Japan.

II. Results and Discussion

Activities of halogen (chlorine and bromine)substituted 1-naphthoic acids on the straight growth of pea stem section are shown in Figs. 1 and 2. The growth activity is intensified by the substitution at 2 or 8 position, while it is reduced by substitution at one of the other position, 3- and 5-halogeno-acids being almost inactive. Essentially the same effects of halogen substitutions were found also in the callus forming activity (Table I).

The order of methyl substituted 1-naphthoic acids with respect to their growth activities corresponds well with that of the halogeno-acid series except that, unlike 2-chloro acid, 2-methyl acid is less active than the parent acid. (Fig. 3 and Table I) All the nitro acids are less active than the parent acid, as shown in Fig. 4 and Table I.

Veldstra and his coworkers (14-16) have affirmed that the interaction of the growth substances is determined by an adsorption phenomenon at the cellular interface by several weak bonds, and that the essential requirements for a growth promoting substance are as follows; (a) the HL-balance between the basal lipophilic ring system and the hydrophilic substituent such as carboxyl group, (b) definite spatial position of the hydrophilic substituent in relation to the ring system. From

Table I. Callus forming activity of 1 per cent lanolin paste of substituted 1-naphthoic acid derivatives and of indole-3-acetic acid, decapitated epicotyle of Vicia Faba L. being the test object.

Substances	Activity *	Substances	Activity *
1-Naphthoic acid	+	3-Nitro-	Inactive
2-Chloro-	+++	4-Nitro-	±
3-Chloro-	Inactive	5-Nitro-	Inactive
4-Chloro-	±	8-Nitro-	±
5-Chloro-	Inactive	2-Methyl-	+
6-Chloro-	+	3-Methyl-	Inactive
8-Chloro-	+++	4-Methyl-	Inactive
3-Bromo-	Inactive	6-Methyl-	Inactive
4-Bromo-	±	8-Methyl-	++
5-Bromo-	Inactive	Indole-3-acetic acid	++
6-Bromo-	+		
8-Bromo	+++		

* Order of activity: +++, ++, +, ±.

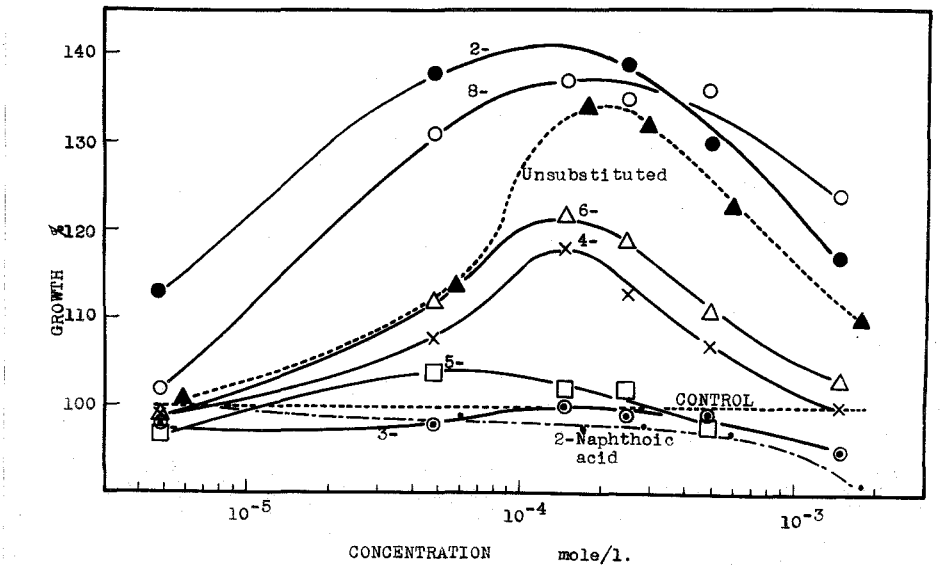


Fig. 1. Activity of chloro-1-naphthoic acids and 2-naphthoic acid in the straight growth of pea stem section. Each point in Figs. 1-4 is the average of two runs of experiments, 10 section pieces being used for each lot in each run.

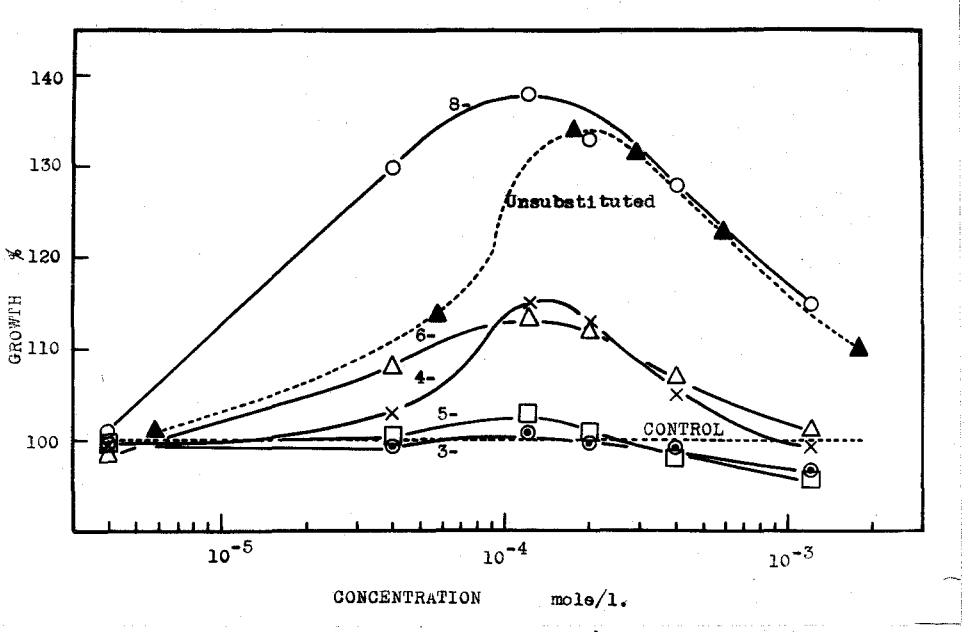


Fig. 2. Activity of bromo-1-naphthoic acids in the straight growth of pea stem section.

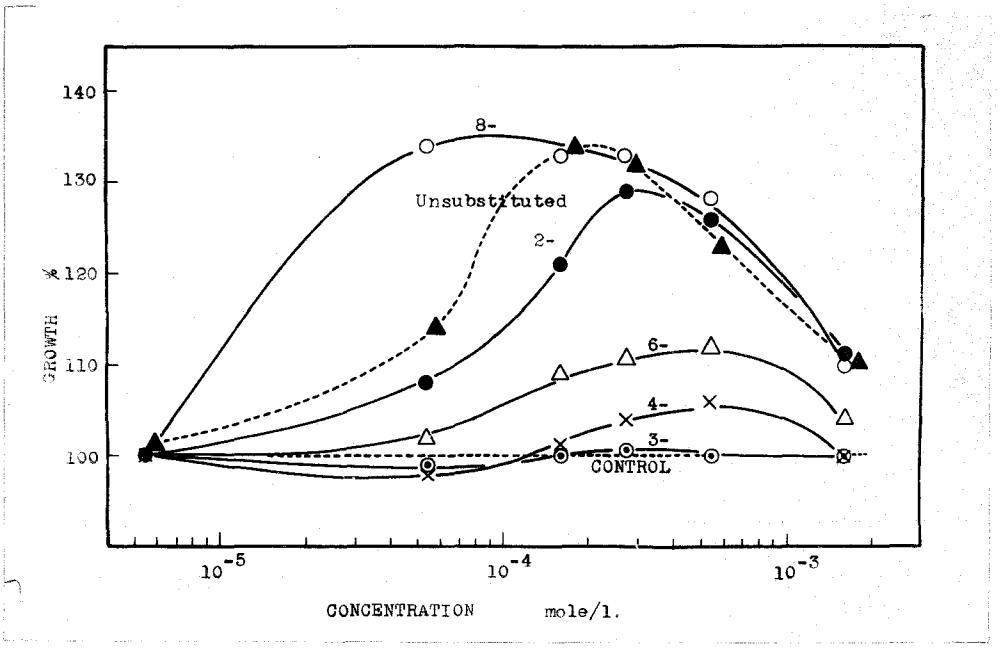


Fig. 3. Activity of methyl-1-naphthoic acids in the straight growth of pea stem section.

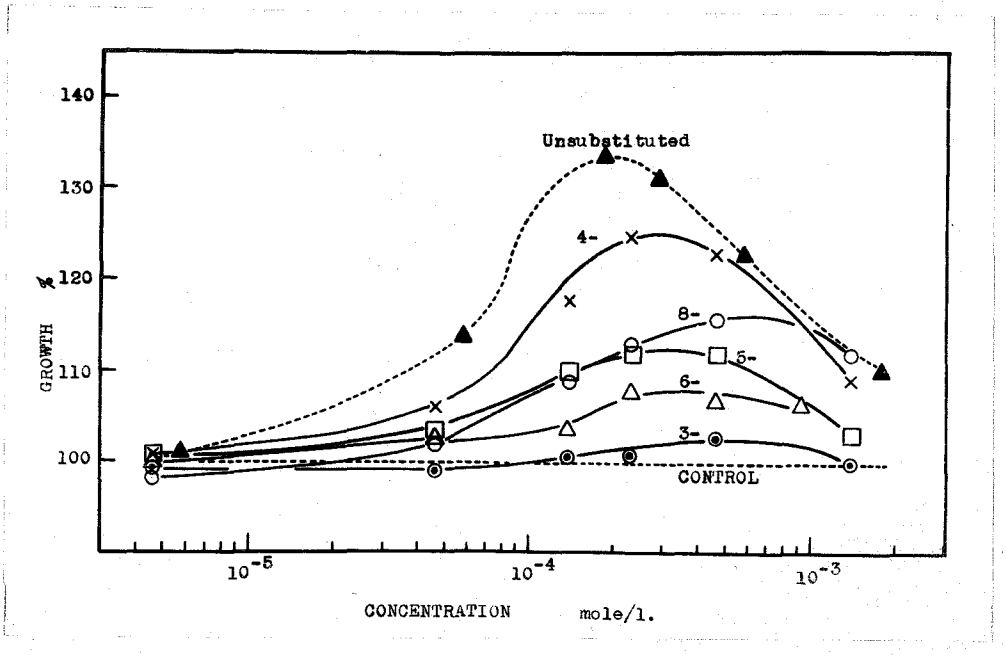


Fig. 4. Activity of nitro-1-naphthoic acids in the straight growth of pea stem section.

this viewpoint Veldstra (17,18) has stated that the activities of substituted benzoic acids are dependent on a non-flat structure caused by the ortho substituent of such a size that the carboxyl group is not masked. On the same bases he has considered that the weak activity of 1-naphthoic acid is attributable to a structure in which the carboxyl group is hindered slightly by the spatial influence from the peri position, and that the enhanced activities of 2-chloro-, 8-halogeno- (chloro-, bromo-and iodo-) and 8-methyl acids result from the carboxyl group being twisted more strongly by the influence of the neighboring bulky substituent (18).

Recently, Fujita and his co-workers have studied the molecular structures of these 1-naphthoic acids by measuring their dipole moments (19) and U.V. (19) and I.R. spectra (20), and concluded that 1-naphthoic acid and its 3-, 4-, 5- and 6-substituted derivatives are equilibrium mixtures of two coplanar (or almost coplanar) rotational isomers in which the direction of the carboxyl group is different from each other, and that the 2- and 8-substituted compounds are in non-coplanar structure in which the carboxyl group is twisted considerably from the ring plane by strong sterical effect of the neighboring bulky substituent, the angle of twist being about 60°.

The present results concerning the activities of 2-chloro-,

8-halogeno- and 8-methyl-acids are in good agreement with the experimental results by Veldstra in spite of the different test methods. The fact that the 3-, 4-, 5- and 6-substituted halogeno- and methyl-acids are less active than the parent 1-naphthoic acid may be explained by shifts in the HL-balance, and the enhancement of the activity by the 2- and 8-substitutions may be due to the strong steric effect overcoming the suppressive effect of the shift in the HL-balance. It is, however, difficult to explain by his hypothesis what determines the order of activities of 3-, 4-, 5- and 6-substituted acids which all have the carboxyl group in a same spatial relation to the ring system as 1-naphthoic acid. It can be expected that nitro substitution gives effects different from halogen and methyl substitutions, because, by the first named, the molecule becomes more lipophilic so as to make the HL-balance improper, and the carboxyl group may be twisted less in the 8-nitro acid than in the 8-halogeno- and the 8-methyl acids owing to the twist of nitro group itself.

On the other hand, Muir and Hansch (21-23) have advocated a hypothesis of a definite chemical reaction between the auxin molecule and a cellular proteinous material. According to the hypothesis, at least one of the positions on the aromatic ring ortho to the carboxyl group must be capable of reacting

with an electron rich plant substrate. Therefore, the ortho position must have a proper electron density and the group or atom at this position must be prone to be displaced under the conditions of reaction. Recently, Fukui and his coworkers (24-27) have presented the frontier electron theory, and found a distinct parallelism between the reactivity indices derived from their theory and the experimental chemical reactivity of π-electron systems. They have applied this theory to a number of benzoic acid derivatives (28), and concluded that the chemical reactivity of benzoic acid derivatives at the ortho position with a nucleophilic group of the plant substrate is the most important factor determining the plant growth activity.

Thus, it is expected that the order of activity of the substituted 1-naphthoic acids may be determined by an order of the chemical reactivity at a definite position of naphthalene ring. In the calculation of the reactivity indices, however, it is necessary to consider molecular structures of these acids, because, when the carboxyl group is twisted from the naphthalene ring plane by sterical effect of the neighboring substituents, the actual reactivity would be different from that of coplanar structure.

The reactivity indices of these 1-naphthoic acids have been carried out with the above mentioned consideration of the

sterical configuration.

It has been shown that, in frontier electron theory, "Superdelocalizability (Sr)" is a good index for expressing the effect of aromatic substitutions intermolecularly (24,25). However, it has been emphasized that the "Approximate Superdelocalizability (Sr')", the contribution of the frontier electrons to Sr, is a better index than Sr in some in vivo reactions (28, 29). This approximate superdelocalizability is defined as (28)

(a) for an electrophilic reaction

$$Sr'^{(E)} = 2\frac{Cr^{(m)2}}{\lambda_m}$$

(b) for a radical reaction

$$Sr'^{(R)} = \frac{Cr^{(m)2}}{\lambda_m} + \frac{Cr^{(m+1)2}}{-\lambda_{m+1}}$$

(c) for a nucleophilic reaction

$$Sr'^{(N)} = 2\frac{Cr^{(m+1)2}}{-\lambda_{m+1}}$$

where $Cr^{(m)}$ or $Cr^{(m+1)}$ is the coefficient of the r-th atomic π-orbital in the following molecular orbitals: the highest occupied denoted by m and the lowest unoccupied by m+1, and λ_m or λ_{m+1} is the coefficient of the resonance integral when the energy of a molecular orbital is expressed in the form $\alpha+\lambda\beta$.

Recently, it has been indicated (30) that Sr^{1} is, depending on the kind of reacting groups, not only an index for the chemical reactivity, but also for the ability of molecular complex formation. Therefore, even if a good parallelism between Sr^{1} and physiological activity should be established with in vivo reaction, it does not allow one to decide on whether the physiological action was due to the substitution reaction or to the molecular complex formation, since one is not yet equipped with an unequivocal knowledge of the type or the nature of the reacting group on the substrate molecule in living cell.

The results of the calculation of reactivity indices are indicated in Tables II and III. As is clearly seen, the growth activities show a good parallelism with the values of $Sr^{1}(N)$ or $Sr^{1}(R)$ at the 8 position, the parallelism with $Sr^{1}(R)$ being a little better than that with $Sr^{1}(N)$, while $Sr^{1}(E)$ does not show any parallelism. No correlation exists at the other positions, including the 2 position which corresponds to the ortho position of the benzoic acid derivatives (Table III.) As to the benzoic acid derivatives, Fukui and his coworkers (28) concluded a parallelism only with $Sr^{1}(N)$ at the ortho position, but the growth activity varies parallel also with $Sr^{1}(R)$ somewhat worse than with $Sr^{1}(N)$. With these

Table II

Relation between Reactivity Indices and Plant Growth Activity of 1-Naphthoic Acid Derivatives.

	$Sr^1(R)$	$Sr^1(N)$	$Sr^1(E)$	Pea straight growth*	Callus formation*
	at 8 position				
5-NO_2-	0.9058	1.3843	0.4273	±	Inactive
4-NO_2-	0.6460	0.6994	0.5926	+	±
2-Cl-	0.4542	0.3305	0.5778	+++	+++
8-Cl-	0.4538	0.3592	0.5484	+++	+++
8-Br-	0.4519	0.3454	0.5584	+++	+++
2-CH_3-	0.4336	0.2543	0.6129	+	+
8-CH_3-	0.4305	0.2128	0.6482	+++	++
5-Br-	0.4286	0.2793	0.5780	Inactive	Inactive
5-Cl-	0.4187	0.2778	0.5597	Inactive	Inactive
4-Cl-	0.4113	0.2441	0.5785	+	±
1-Naphthoic	0.4081	0.2108	0.6054	++	+
4-Br-	0.4036	0.2342	0.5729	+	±
6-Cl-	0.4015	0.2156	0.5873	+	+
3-Cl-	0.4013	0.2121	0.5904	Inactive	Inactive
6-Br-	0.3990	0.2144	0.5837	±	+
3-Br-	0.3977	0.2117	0.5836	Inactive	Inactive
8-NO_2-	0.3639	0.2171	0.5108	±	±
6-NO_2-	0.3618	0.1393	0.5842	Inactive	.....
3-NO_2-	0.3584	0.1097	0.6071	Inactive	Inactive
6-CH_3-	0.3402	0.2014	0.4790	Inactive	Inactive
4-CH_3-	0.3325	0.1475	0.5174	Inactive	Inactive
3-CH_3-	0.3196	0.2065	0.4328	Inactive	Inactive
2-Naphthoic**	0.8467	1.2657	0.4276	Inactive	.....

* Order of activity: +++, ++, +, ±, see Fig. 1-4 and Table 1.
** The value at 1 position.

Table III

Relation between Reactivity Indices and Plant Growth Activity of 1-Naphthoic Acid Derivatives.

	$Sr^1(N)$ at 2 position	Pea straight growth*	Callus formation*
4-NO_2-	1.7313	+	±
3-CH_3-	1.1397	Inactive	Inactive
4-Cl-	1.0817	+	±
4-Br-	1.0605	+	±
6-Cl-	1.0498	+	+
6-Br-	1.0415	±	+
1-Naphthoic	1.0179	++	+
5-Br-	1.0167	Inactive	Inactive
6-NO_2-	0.9682	Inactive	.....
3-Br-	0.9607	Inactive	Inactive
6-CH_3-	0.9598	Inactive	Inactive
3-Cl-	0.9421	Inactive	Inactive
5-Cl-	0.9269	Inactive	Inactive
4-CH_3-	0.8217	Inactive	Inactive
5-NO_2-	0.6906	±	Inactive
2-Cl-	0.4882	+++	+++
8-Cl-	0.4509	+++	+++
8-Br-	0.4440	+++	+++
8-CH_3-	0.3934	+++	++
2-CH_3-	0.3120	+	+
8-NO_2-	0.2533	±	±
3-NO_2-	0.0144	Inactive	Inactive
2-Naphthoic	1.2657**	Inactive	.....

* Order of activity: +++, ++, +, ±,
** The value at 1 position.

MO-treatments alone, therefore, one is not yet in the position to decide on which of the two types of group, nucleophilic or radical group of the plant substrate, is more apt to be involved in the interaction. However, it seems to be more probable to consider that an electron rich aromatic component or a negative ion in the substrate protein is involved in the interaction. It is quite inconsistent to conclude the existence of a true chemical reaction, such as a nucleophilic substitution, between the growth substance and the plant substrate, because the 8-methyl acid, which is unable to undergo any substitution at the 8 position, is highly active. Therefore, it is reasonable to conclude that the molecular complex forming ability of 1-naphthoic acid derivatives at the 8 position with an electron rich plant substrate is a very important factor determining their growth activity.

Thus, the Muir and Hansch's hypothesis seems to be revised as following; the interaction between the growth substance and the plant substrate are not true chemical reaction but presumably a molecular complex formation, and the position at which the interaction occurs varies depending on the kind of series of compounds, that is, it is not always the ortho position to the carboxyl group (31). From this viewpoint, the activity found in some 2,6-dimethyl substituted benzoic acid

derivatives (18) also may be well explained.

The Veldstra's hypothesis is also to be modified so that the absorptive bonding between the growth substance and the plant substrate is determined predominantly by the ability to form the molecular complex at a definite position, and that the structural requirement, such as non-flat configuration, is included in that for a strong molecular complex forming ability.

Thus, from the above discussions, a combination of Veldstra's and Muir-Hansch's hypotheses seems to be required. The concept of the molecular complex formation is well consistent with the reversibility in the earlier stage of the growth substance action (32).

There are, of course, some other important factors, for example, dissociation constant or HL-balance which seems to affect solubility, permeability and diffusibility into the tissues and cells. Steric circumstance of the molecule should also be considered.

The last named may probably explain the fact that the 5-substituted and some of the 4-substituted are inactive or weakly active in spite of their relatively large indices (Table II); in another word, there would be a severe restriction to the length of the molecule in transverse direction,

in order for the 1-naphthoic acid derivatives to reach the site of action or to fill the gap which has been proposed to exist (33) in the plant substrate (receptor protein). This hypothesis seems to give a satisfactory explanation why the correlation between the growth activity and the theoretical index is close at the 8 position only, though these acids in vitro shall be attacked by a reagent at the 2- or 4-position far more easily than at the 8 position.

In the benzoic acid derivatives, Veldstra (18) found that all 4-substituents larger than fluorine are incompatible with activity, and Fukui and his coworkers (28) showed that some of the p-chlorobenzoic acids have large theoretical indices at the ortho position in spite of their inactiveness. Although the molecular geometry of the benzoic acid derivatives is different from that of the 1-naphthoic acid derivatives, the situation in vivo may be similar to each other. From the same bases the inactiveness of 2-naphthoic acid* (13) could be explained.

---

* In the case of 2-naphthoic acid, the one of the two non-equivalent ortho positions with the greater reactivity indices, the 1 position, seems to be an appropriate position for the interaction with the substrate.

The above conclusion that an interaction with the plant substrate should occur at the 8 position neighboring to the carboxyl group leads to the suggestion, similar to the case of benzoic acid derivatives (34), that the role of the carboxyl group would be to allow the molecule to approach to the substrate so that the neighboring position may easily be subjected to an interaction with another site of the plant substrate.

When the discussions in this paper are combined with previous observations on the structure-activity relationships, it appears justified at present to reformulate the requirements for appreciable auxin activity in plant growth substances as:

1) In the earlier stage of the growth action, the ability of the compounds to form molecular complex at a definite position with plant substrate is a very important factor determining the plant growth activity.
2) In addition to the ability to form molecular complex, the physiological activity is decided by such a size and form of the molecule that is able to fit in the site of action in the plant substrate.

III. Calculation

The calculation of S_r^1 is carried out by using the simple LCAO-MO treatment, solving the secular equation. The parameters used in the calculation are the same as those by Fukui and his coworkers (28) as shown in Table IV. The coulomb integral of the substituent X, that of the C atom attached to X, and the resonance integral of the C-X bond, are written as $\alpha+a_x\beta$, $\alpha+a_Y\beta$ and $l\beta$, respectively. The coulomb integral of the two O atoms in the carboxyl group is taken to be equal with their value $\alpha+2\beta$, in order to simplify the calculation. As the resonance integral between the carboxyl-C and C_1, 0.5β (35) is used in the 2- and 8-substituted acids, while β is used in the other cases. In 8-nitro acid, in which both carboxyl and nitro groups are twisted from the plane, the resonance integral of the C-N bond is also taken as 0.5β.

Table IV.

Parameters used in the calculation

Substituent X	a_x	a_r	l
Cl	2	0.5	0.8
Br	1.8	0.4	0.7
CH_3	2	0	1
NO_2	$\begin{cases} a_N = 1 \\ a_O = 1 \end{cases}$	0.2	1

IV. Experimental

i. Chemical Section*

3- and 6-Nitro-1-naphthoic acid

Naphthalic acid anhydride (36): Twenty five grams of commercial acenaphthene was dissolved in 300 ml. of glacial acetic acid by warming, and 180 g. of coarsely pulverized sodium dichromate was added to the solution in portions, maintaining the reaction temperature below 85°. After adding, the reaction mixture was heated with stirring on a boiling water bath for two hours, and was allowed to stand overnight at room temperature, and finally three hours at 120° on an oil bath being stirred. The reaction mixture was poured into four liter of hot water and stirred. The precipitate which formed was collected on a filter, and washed well with hot water. Recrystallization from glacial acetic acid gave 27 g. of anhydride in long needles, m.p. 269-270° (reported: 270-271°(36)).

3-Nitronaphthalic acid anhydride (37): To a solution of 27 g. of naphthalic acid anhydride in 200 g. of concentrated sulfuric acid was added 14 g. of potassium nitrate in portions, being stirred and externally cooled with ice-bath so that

---

* All melting points are uncorrected.

the reaction temperature was maintained at about 10°. After about ten minutes on a boiling water bath, the reaction mixture was cooled and poured onto ice. The crystalline precipitate was filtered and washed with cold water. Recrystallization from glacial acetic acid gave 23 g. of 3-nitro anhydride of scaly crystalline, m.p. 240° (reported: 247° (37)).

The mercuration of 3-nitronaphthalic acid anhydride (38): A filtered solution of 20 g. of 3-nitro acid anhydride in 400 ml. of water and 10 g. of sodium hydroxide was mixed with a solution of 18 g. of mercuric oxide in 60 ml. of water and 15 ml. of glacial acetic acid, and the mixture was refluxed for about thirty hours. No more carbon dioxide was evolved and a test sample of the mixture dissolved in sodium hydroxide. The solution gave no metallic mercury when tested with a clean copper wire. The precipitate which formed was filtered, and washed with water. It was dried in air bath at 100°. The yield of a mixture of anhydro-3-nitro-8-hydroxymercuri-1-naphthoic acid and the corresponding 6-nitro compound was 33.9 g.

3-Nitro-1-naphthoic acid (38): To the mixture of mercury compounds was added 200 ml. of conc. hydrochloric acid and it was refluxed on an oil bath for 3 hours. The preceipitate

was filtered, and dissolved in 1N-sodium hydroxide solution. Insoluble solid was filtered off and the filtrate was acidified with hydrochloric acid. The resulting gluely precipitate which was a mixture of 3- and 6-nitro naphthoic acid, was filtered, and dried. The mixed acids were dissolved in 190 ml. of hot glacial acetic acid, filtered from a slight insoluble residue and cooled. Fine needle crystals separated, m.p. 260-263°. This was nearly pure 3-nitro-1-naphthoic acid. Repeated crystallization from alcohol gave 9.3 g. of 3-nitro acid in needles, m.p. 262-264° (reported: 270.5-271.5° (38)).

Anal. Calc'd. for $C_{11}H_7O_4N$: C, 60.83; H, 3.25.

Found: C, 60.93; H, 3.53.

6-Nitro-1-naphthoic acid (38): The mother liquors from the crystallization of the mixed acids were evaporated to dryness on a boiling water bath under reduced pressure. The residual solid was treated with 70 ml. of absolute alcohol, saturated with dry hydrogen chloride, refluxed for a short time, allowed to stand for several hours, cooled and filtered. This was fairly pure ethyl 6-nitro-1-naphthoate. Further crystallization from ethyl alcohol raised the melting point to 111-112°.

To 19 g. of the ester was added 150 ml. of 46% hydrobromic

acid and the mixture was refluxed for an hour. After cooling, the precipitate was filtered, and washed with water. Recrystallization from alcohol gave 16 g. of 6-nitro acid in needles, m.p. 219-220° (reported: 227-227.5°(38)).

Anal. Calc'd for $C_{11}H_7O_4N$: C, 60.83; H, 3.25.

Found: C, 60.54; H, 3.43.

4-Nitro-1-naphthoic acid

Nitroacenaphthene (37): To a solution of 50 g. of acenaphthene in 500 ml. of hot glacial acetic acid was gradually added 140 ml. of concentrated nitric acid (S.G. 1.38), being stirred and occasionally cooled with water so that the reaction temperature was maintained at 50° to 65°. The mixture was then cooled to room temperature, and crystalline was filtered and washed with small amount of glacial acetic acid. Recrystallization from alcohol gave 50 g. of nitroacenaphthene, m.p. 106° (reported: 106° (37)).

4-Nitronaphthalic acid anhydride (37): To a solution of 20 g. of nitro acenaphthene in 240 g. of glacial acetic acid was added 140 g. of coarsely pulverized sodium dichromate in portions, maintaining the reaction temperature below 80°. Thereafter, the reaction mixture was heated at 100° with stirring for 5 hours, and then poured into water. The reddish-orange colored crystalline precipitate was filtered, and

washed well with hot water. The precipitate was dissolved as completely as possible in 10% sodium bicarbonate solution with external heating, and filtered. The filtrate was acidified with hydrochloric acid, and the precipitate which formed was recrystallized from glacial acetic acid to give a yield of 16 g. of yellow needles, m.p. 228-229° (reported: 230° (37)).

The mercuration of 4-nitronaphthalic acid anhydride (38): The anhydride was mercurated in the same way as described in the previous section. The time of refluxing was fifty hours. From 10 g. of the anhydride was obtained 15 g. of mercurated product, mainly anhydro-4-nitro-8-hydroxymercuri-1-naphthoic acid and a little of the 5-nitro compound.

4-Nitro-1-naphthoic acid (38): Treatment of this mixture with hydrochloric acid gave a 92% yield of mixed acids, m.p. 205-216°. Twelve grams of the mixed acids on several crystallizations from glacial acetic acid gave 6.8 g. of 4-nitro-1-naphthoic acid, m.p. 223-224° (reported: 225-226° (38)).

Anal. Calc'd for $C_{11}H_7O_4N$: C, 60.83; H, 3.25

Found: C, 60.71; H, 3.27

5- and 8-Nitro-1-naphthoic acid

5-Nitro-1-naphthoic acid (39): A gruely mixture of 120 g. of 1-naphthoic acid in 380 g. of nitric acid (S.G. 1.38) was gradually heated on a water bath with stirring. When the

temperature of the reaction mixture rose up to about 70°, the reaction occured vigorously with evolution of brownish-red fumes. The reaction mixture became massive. After about 30 minutes on a boiling water bath, the reaction mixture was added to a large amount of water. The crystalline mass was collected by filtration and dissolved in 1N sodium hydroxide solution. Insoluble solid was filtered off and the filtrate was acidified with hydrochloric acid. The resulting precipitate was the mixture of 5-nitro-1-naphthoic acid and 8-nitro acid, 195 g., m.p. 189-198°. The mixed acids were dissolved in hot alcohol, filtered from a slight residue and cooled. Fine light yellow colored needles were separated, m.p.235-237°. This was nearly pure 5-nitro-1-naphthoic acid. Repeated crystallization from alcohol yielded 40 g. of 5-nitro acid, m.p. 239°(reported: 239°(39)).

Anal. Calc'd for $C_{11}H_7O_4N$: C, 60.83; H, 3.25

Found: C, 60.73; H, 3.20

8-Nitro-1-naphthoic acid (39): The mother liquors from the recrystallization of the mixture of 5- and 8-nitro acids were evaporated to dryness on a boiling water bath under reduced pressure. Repeated fractional crystallization of the residual mixture gave 35 g. of 8-nitro acid as yellow rhombic crystals, m.p. 217°(reported: 215°(39)).

Anal. Calc'd for $C_{11}H_7O_4N$: C, 60.83; H, 3.25

Found: C, 60.57; H, 3.18.

2-Chloro-1-naphthoic acid

2-Hydroxy-1-naphthoic acid (40): A mixture of 11.6 g. of 2-naphthol in 40 g. of pyridine and 3.2 g. of sodium hydroxide was stirred at room temperature for two hours. After the sodium hydroxide dissolved completely, 20 g. of pyridine was added a fresh, and the solution was distilled to remove the moisture in the solution until the boiling point reach 115°. Dry carbon dioxide was then bubbled through an entry tube at 80° for three hours. After the completion of the reaction, the pyridine was removed under reduced presure. The residual was acidified with dilute hydrochloric acid, and then extracted with two 50 ml. portions of ether. The combined ether extracts were shaked with three 50 ml. portions of 10% sodium bicarbonate solution. Each alkaline layer was washed successively with a 50 ml. portion of ether, and the combined alkaline solutions were acidified with hydrochloric acid. The crude 2-hydroxy-1-naphthoic acid was collected by filtration, washed with water, and dried. Recrystallization from alcohol gave 7.3 g. of 2-hydroxy acid, m.p. 156°.

2-Chloro-1-naphthoic acid (41): Two grams of 2-hydroxy-1-naphthoic acid and 8 g. of phosphorus pentachloride were

mixed in a glass tube fitted with calcium tube at the top. The reaction took place at once with evolution of hydrogen chloride. The tube was shaken occasionally. The reaction mixture became viscous yellowish-brown coloured liquid. After completing preliminary reaction, the tube was sealed and heated at 190-200° for eight hours. The dark brown coloured oil with fluorescence which was obtained, was exposed to atmospheric moisture for a few days. A small amount of water was then added to the resulting reaction mixture, and the crystalline was collected by filtration. Recrystalization from benzene and carbon tetrachloride gave 1.3 g. of 2-chloro acid in colourless needles, m.p. 155-156°(reported: 152-153°(41)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.41

Found: C, 64.17; H, 3.69.

3-, 4-, 5- and 6-Chloro-1-naphthoic acids

3-, 4-, 5- and 6-Amino-1-naphthoic acids (39): To a solution of 10 g. of a nitro acid in 125 ml. of concentrated aqueous ammonia was gradually added a solution of 80 g. of ferric sulfate in 125 ml. of water at room temperature with vigorous stirring. After completing the addition, the reaction mixture was filtered and the filtrate was condensed to a half under reduced pressure. The condensed solution

was neutrallized with acetic acid to give the corresponding crude amino-1-naphthoic acid, which was used for the following Sandmyer-reaction without purification.

3-Chloro-1-naphthoic acid: A solution of 3.2 g. of 3-amino-1-naphthoic acid, 10 ml. of 12% sodium hydroxide solution, 1.2 g. of sodium nitrite and 50 ml. of water, was gradually added to 60 ml. of concentrated hydrochloric acid, being stirred and externally cooled with ice-bath so that the reaction temperature was maintained at 0 to 5°. The diazonium solution which was filtered from a slight residue, was added slowly with mechanical stirring at 0-5° by external cooling to the cuprous chloride solution, which was prepared by adding 25% sodium bisulfite solution to a solution of 12 g. of crystalline cupric sulfate and 18 g. of sodium chloride in 60 ml. of water until disappearing the colour of cupric ion on a boliling water bath. The addition of the diazonium solution took about thirty minutes, then additional an hour of stirring with ice bath, two hours in room temperature being stirred, and finally heated on a water bath for thirty minutes at about 60°. The resulting precipitate was filtered, washed with water, and then dissolved in 1N-sodium hydroxide solution. Insoluble solid was filtered off and the filtrate was acidified with hydrochloric acid to give the crude 3-

chloro acid. Sublimation under reduced pressure (3mm.) at 160-170° and recrystallization from alcohol gave 1.3 g. of 3-chloro acid as colourless needles, m.p. 215-216.5°(reported: 223°(42)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.41; Cl, 17.17
Found: C, 63.76; H, 3.66; Cl, 17.08.

4-Chloro-1-naphthoic acid was prepared in the same way as described above. The resulting precipitate was sublimed under reduced pressure(3mm.) at 160-170° and recrystallized from alcohol and glacial acetic acid. From 2 g. of 4-amino acid was obtained 0.8 g. of 4-chloro acid as needles, m.p. 220-221°(reported: 225°(42)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.41; Cl, 17.17
Found: C, 64.07; H, 3.64; Cl, 16.96.

5-Chloro-1-naphthoic acid (39) was similarly prepared from 5-amino acid. From 5 g. of the amino acid was obtained 1.4 g. of 5-chloro acid in needles, m.p. 247°(reported: 245°(39)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.41; Cl, 17.17
Found: C, 64.09; H, 3.40; Cl, 17.04.

6-Chloro-1-naphthoic acid: Similar treatment starting with 1.7 g. of 6-amino acid gave 0.8 g. of 6-chloro acid in needles, m.p.213°(reported: 215.8-216.2°(43)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.41

Found: C, 63.89; H, 3.64.

3-, 4-, 5- and 6-Bromo-1-naphthoic acids

3-Bromo-1-naphthoic acid: A diazonium solution prepared from 1.8 g. of 3-amino acid was added with stirring at 0-5° by external cooling to a hydrobromic acid solution of cuprous bromide, which was prepared by adding 19.5 g. of sodium bisulfite to a solution of 33. 5 g. of crystalline cupric sulfate and 35 g. of sodium bromide in 110 ml. of water. The reaction mixture was then treated with the same procedure described above. Recrystallization from alcohol gave 0.8 g. of 3-bromo acid as long needles, m.p. 231.5-232.5°(reported; 236°(42)).

Anal. Calc'd for $C_{11}H_7O_2Br$: C, 52.62; H, 2.81; Br, 31.83

Found: C, 52.56; H, 2.82; Br, 32.18.

4-Bromo-1-naphthoic acid: Similar treatment starting with 1.7 g. of 4-amino acid gave 1.4 g. of 4-bromo acid as needles, m.p. 220-221°(reported: 221°(42)).

Anal. Calc'd for $C_{11}H_7O_2Br$: C, 52.62; H, 2.81

Found: C, 52.70; H, 3.03.

5-Bromo-1-naphthoic acid, similarly prepared from 1.8 g. of 5-amino acid, was obtained in 1 g. of needles, m.p. 256-257°(reported: 246°(39), 262°(43)).

Anal. Calc'd for $C_{11}H_7O_2Br$: C, 52.62; H, 2.81

Found: C, 52.55; H, 3.07.

6-Bromo-1-naphthoic acid: The precipitate which was similarly prepared from 0.25 g. of 6-amino acid was sublimed under reduced pressure and recrystallized from alcohol to give 0.1 g. of 6-bromo acid as needles, m.p. 183-184°.

Anal. Calc'd for $C_{11}H_7O_2Br$: C, 52.62; H, 2.81

Found: C, 52.43; H, 2.83.

8-Chloro- and bromo-1-naphthoic acids

Anhydro-8-hydroxymercuri-1-naphthoic acid(44): A solution of 21.8 g. of naphthalic acid in 520 ml. of water and 13.5 g. of sodium hydroxide was mixed with a solution of 23.8 g. of mercuric oxide in 65 ml. of water and 18 ml. of glacial acetic acid. A slight excess of acetic acid was added and the mixture was refluxed for ninety-eight hours. The precipitate was collected on a filter, washed with water and dried at 100°. The yield was 34.5 g.

8-Chloro-1-naphthoic acid(44): An alkaline solution of 13.3 g. of anhydro-8-hydroxymercuri-1-naphthoic acid was treated with 2 g. of sodium chloride, boiled for a few minutes, barely acidified with hydrochloric acid and filtered. The resulting chloromercuri-compound was stirred with 40 ml. of glacial acetic acid and treated at room temperature with a glacial acetic acid solution of 2.4 g. of chlorine. Reaction took place at once and most of the material went into

solution. The mixture was heated to boiling and filtered from a slight residue. The filtrate on cooling and on concentration gave a crude 8-chloro-1-naphthoic acid. Recrystallization from benzene gave 11g. of 8-chloro acid, m.p. 171-172°(reported: 170-170.5°(44)).

Anal. Calc'd for $C_{11}H_7O_2Cl$: C, 63.94; H, 3.42; Cl, 17.17

Found: C, 63.63; H, 3.70; Cl, 17.28.

8-Bromo-1-naphthoic acid(44): The chloromercuri-compound, preparing from 10 g. of anhydro-8-hydroxymercuri-1-naphthoic acid in the same way described above, was suspended in 135 ml. of glacial acetic acid. To this suspension was added portionwise 4.7 g. of bromine dissolved in 20 ml. of 40% sodium bromide solution. After adding the solution became clear on boiling, but the colour of bromine persisted. This colour was removed by treatment with a little sodium bisulfite. After boiling with charcoal and filtering, the solution was cooled. Crystals which formed were recrystallized from benzene to give 4 g. of 8-bromo acid, m.p. 176-178° (reported: 176-179°(44)).

Anal. Calc'd for $C_{11}H_7O_2Br$: C, 52.62; H, 2.81; Br, 31.83

Found: C, 52.83; H, 2.85; Br, 32.08.

2-Methyl-1-naphthoic acid

1-Bromo-2-methylnaphthalene (45): To a solution of 25 g.

of 1-methylnaphthalene (b.p. 115°/13mm.) in 20 ml. of carbon disulfide was gradually added with stirring in the dark a solution of 28.1 g. of bromine in 10 ml. carbon disulfide. After adding, the reaction mixture was heated on a boiling water bath for an hour. Excess of bromine was removed by treatments with 2N-sodium hydroxide and with water. Solvent was removed under reduced pressure and the residual liquid was distilled in vacuo. A fraction of 155-163°/12mm. was collected. Redistillation gave 34.9 g. of 1-bromo-2-methyl-naphthalene, b.p. 158-160°/12mm.(reported: b.p. 165-170°/13mm. (45)).

2-Methyl-1-naphthoic acid (45): 2.4 g. of Magnesium was covered with 10 ml. of anhydrous ether, and therein 1.6 g. of 1-bromo-2-methylnaphthalene and a crystal or two of iodine were added. The mixture was heated on a water bath at 45° or higher until the reaction started. The stirrer was started and a solution 20.6 g. of 2-methyl-1-bromonaphthalene in 50 ml. of anhydrous ether was added at such a rate that the reaction was vigorous but not violent. The addition required about an hour. After addition, refluxing was continued for an hour with stirring. The Grignard reagent which formed as a yellowish brown coloured oil was dissolved by the addition of 53 ml. of dry benzene, and cooled by an ice-salt mixture.

Then, dry carbon dioxide was bubbled with stirring to the Grignard reagent, regulating the rate of flow of the carbon dioxide so that the temperature did not rise above -2°. The time required for the completion of the reaction was about half an hour. When the reaction was completed the temperature falled below -7°. To the cooled reaction mixture was added slowly and cautiously 25% sulfuric acid solution with stirring until no further reaction took place and all of the excess magnesium had dissolved. The oily layer was separated, and the water layer was extracted with ether. The combined ether-benzene extracts were shaken with three 20 ml. portions of 25% sodium hydroxide. Each alkaline layer was extracted successively with a 20 ml. portion of ether, and the combined alkaline extracts were then heated to 100° to drive off volatile impurities. The solution was cooled and acidified with hydrochloric acid. The precipitate which formed was filtered, washed with water and then recrystallized from acetic acid and benzene to give 12 g. of 2-methyl acid, m.p. 125-126° in pillars (reported: 126-127°(45)).

Anal. Calc'd for $C_{11}H_{10}O_2$: C, 77.40; H, 5.41

Found: C, 77.50; H, 5.61.

4-Methyl-1-naphthoic acid

1-Methyl-4-bromo-naphthalene (46): 7.6 ml. of Bromine in 40 ml. of carbon tetrachloride was added during two hours at -7° to a stirred solution of 20 g. of 1-methylnaphthalene (b.p. 115-116°/13mm.) in 50 ml. carbon tetrachloride containing small amounts of iron powder and iodine. Stirring was continued for a further an hour at -7° and the mixture was then left for two hours as the temperature rose. Excess of bromine was removed by treatments with 2N-sodium hydroxide solution and with water. The solvent was removed under reduced pressure and a fraction, b.p. 160-165°/13mm., was collected. Redistillation gave 28.5 g. of 1-methyl-4-bromonaphthalene, b.p. 160-163°/13mm.(reported: 162-163°/13mm.(46)).

4-Methyl-1-naphthoic acid (45), was prepared in the same way as described in the preceeding section from 1-methyl-4-bromonaphthalene. Sublimed under reduced pressure and recrystallized from glacial acetic acid. From 22.2 g. of 4-bromo-1-methylnaphthalene was obtained 17 g. of 4-methyl acid in needles, m.p. 175-176°(reported: 175°(45)).

Anal. Calc'd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41

Found: C, 77.17; H, 5.65.

3-Methyl-1-naphthoic acid

1-Nitro-2-methylnaphthalene (47): 100 g. of

2-Methylnaphthalene was heated to fuse, and to the melt was gradually added 71 ml. of concentrated nitric acid, being stirred and controlling the rate of addition of the nitric acid so that the reaction temperature was maintained at 70° to 75°. The reaction mixture was then heated on a water bath at 70-75° being stirred for six hours, and cooled. To the reaction mixture was added 1 liter of water and the crystalline was separated from oil by filtration. Recrystallization from alcohol gave 50 g. of 1-nitro-2-methylnaphthalene in yellow coloured long needles, m.p. 81°(reported: 81°(47)).

1-Amino-2-methylnaphthalene (47): A mixture of 62.5 g. of iron powder in 31.5 ml. of water and 2.5 ml. of concentrated hydrochloric acid was heated at 50°, and to the mixture was added 45.5 g. of 1-nitro-2-methylnaphthalene in portions, being stirred and externally heated on a water bath so that the reaction temperature was maintained at 70 to 75°. An amount of water which was equal to the evaporated, was occasionally added to the reaction mixture. The addition required about two hours, and then the reaction mixture was heated on a boiling water bath for twelve hours. After cooling, it was made alkaline with 1N-sodium hydroxide solution. The water was distilled off, and the residual was extracted with three 50 ml. portions of benzene. The combined

benzene extracts were filtered and the filtrate was dried over anhydrous potassium carbonate. The benzene solution was saturated with dry hydrogen chloride. The precipitated amine hydrochloride which was collected by filtration, was dissolved in 25 ml. of 35% sodium hydroxide solution. The solution was heated on a boiling water bath for thirty minutes. After cooling the mixture was extracted with three 50 ml. portions of benzene, dried with anhydrous potassium carbonate. The benzene was removed under reduced pressure and the residual liquid was distilled in vacuo to give 35.3 g. of 1-amino-2-methylnaphthalene, yellowish brown coloured oil, b.p. 123°/1mm.(reported: 165°/12mm.(47)).

2-Methyl-4-bromo-1-naphthylamine (42): 8 ml. of Bromine in 40 ml. of carbon tetrachloride was added with stirring at -5° to a solution of 23 g. of 2-methyl-1-naphthylamine in 300 ml. of carbon tetrachloride containing iodine and iron powder. After ten minutes' stirring the precipitated amine hydrobromide was filtered off and dried under vacuum. When it was dissolved in hot ethanol and poured into aqueous sodium hydrogen carbonate, crude 4-bromo-2-methyl-1-naphthylamine was obtained. Three recrystallisations from n-hexane gave 9 g. of 2-methyl-4-bromo-1-naphthylamine, m.p. 78°(decomp.) (reported: 78°(42)).

1-Bromo-3-methylnaphthalene (42): Seventeen grams of the foregoing amine, dissolved in 116 ml. of acetic acid, was added to a stirred, freshly prepared solution of 6.5 g. of sodium nitrite in 42 ml. of concentrated sulphuric acid below 20°. Stirring was continued for thirty minutes, and the mixture was then added during thirty minutes to a stirred suspension of 19.5 g. of cuprous oxide in 167 ml. of ethanol. After evolution of nitrogen ceased, the mixture was steam-distilled. After ether extraction of the distillate in vacuo. to yield 7 g. of 1-bromo-3-methylnaphthalene, b.p. 118-119°/1mm., yellow coloured oil (reported: 127°/3.5mm.(42)).

3-Methyl-1-naphthoic acid: 1-Bromo-3-methylnaphthalene was converted to 3-methyl acid by the same method described for 1-bromo-2-methylnaphthalene. The resulting precipitate was sublimed and recrystallized from aqueous alcohol. From 6.7 g. of bromide was obtained 1 g. of 3-methyl acid as colourless needles, m.p. 171-172°(reported: 171-172°(42)).

Anal. Calc'd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41

Found: C, 77.34; H, 5.58.

6-Methyl-1-naphthoic acid (48)

A suspension of 12 g. of pure furoic acid in 100 ml. of thiophene-free toluene was cooled in an ice-bath and 30 g. of aluminum chloride was added in portions with stirring.

After the addition was complete, the mixture was warmed overnight on a water bath at about 60°. The dark red viscous reaction mixture was poured into cold aqueous hydrochloric acid and stirred vigorously at 60°until the tan cake formed on first contact with the water was entirely dissolved. The clear red toluene layer was separated and washed once with water. Thorough extraction with two 50 ml. portions of 10% sodium bicarbonate solution yielded, on acidification, 2 g. of crude acid, which was recrystallized from benzene giving 1.2 g. of 6-methyl acid in colourless plates, m.p. 176-177°(reported: 176.5-177°(48)).

Anal. Calc'd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41

Found: C, 77.66; H, 5.43.

8-Methyl-1-naphthoic acid

Acenaphthenequinone(49): A mixture of 30 g. of acenaphthene in 210 ml. of glacial acetic acid was heated to about 95° with stirring. The flame was then removed, and 130 g. of coarsely pulverized sodium dichromate was added to the solution over a period of 15 to 30 minutes. The addition was made as fast as possible without causing vigorous boiling. After all the dichromate had been added, the mixture refluxed for 15 minutes, during which time it became very viscous. 700 ml. of Hot water was then added, and the mixture was stirred for 10 minutes.

The red precipitate was filtered and washed with hot water to remove chromium salt. A suspension of the solid in 220 ml. of a 10% sodium carbonate solution was heated on a boiling water bath for an hour and filtered immediately. To the residual red solid was added 300 ml. of a 40% sodium bisulfite solution, and the mixture was refluxed with stirring for 45 minutes, whereupon 500 ml. of water was added and refluxing was continued for 10 minutes. The hot solution was filtered, and then the filtrate was heated to boiling and acidified by the slow cautious addition of 110 ml. of concentrated sulfuric acid. The mixture was digested for 15 minutes over a low flame and then filtered. The yellow precipitate was washed thoroughly with 200 ml. of water and 100 ml. of dilute ammonia, and dried to give a yield of 16 g., m.p. 243-245°(reported: 243-245°(49)).

1,8-Naphthaldehydic acid (50): Twenty grams of acenaphthenequinone and 140 ml. of 30% potassium hydroxide solution were heated with constant stirring at 150° for ten minutes. The reaction mixture was then diluted with water and filtered. The filtrate was acidified with hydrochloric acid, and the precipitate which formed was separated by filtration and dissolved as completely as possible in sodium bicarbonate solution. The solution was treated with

charcoal, and acidified to reprecipitate the product. The resulting 1,8-naphthaldehydic acid was recrystallized from toluene to give a yield of 14.5 g., m.p. 167°(reported: m.p. 167° (50)).

8-Methyl-1-naphthoic acid(50): A mixture of 33 g. of amalgamated mossy zinc, 54 ml. of glacial acetic acid, 54 ml. of concentrated hydrochloric acid and 6 g. of 1,8-naphthaldehydic acid dissolved in 50 ml. of toluene was heated under reflux for thirty hours. Three 15 ml. portions of concentrated hydrochloric acid were added during this time at six- to eight-hour intervals. The toluene layer was washed with several portions of water and extracted with sodium bicarbonate solution. The sodium bicarbonate solution was acidified, allowed to stand overnight, and filtered. When recrystallized from n-hexane and benzene, the residue yielded 0.4 g. of 8-methyl-1-naphthoic acid melting at 153°(reported: 153°(50)).

Anal. Calc'd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41

Found: C, 77.50; H, 5.77.

ii. Biological Section

Pea straight growth test(51)

The sections were cut off from the third internode of etiolated pea seedling which had been cultured in the darkroom at 25° for 7 days. The internode was about 15-20 mm. long, and the section was 5.25± 0.017 mm. in the average length. Sections were floated in test solutions for 24 hours, and then their length was measured again. The measurement was made with a stereoscopic microscope, placing the section on a rule of 0.01 mm. scale. This was appropriate for testing weakly active growth substances such as 1-naphthoic acid derivatives.

Callus formation test(52)

Vicia Faba was grown in a dark room at 25° 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.

V. Summary

1-Naphthoic acid derivatives substituted with chlorine, bromine, methyl or nitro group in various positions of the ring were prepared, and their growth activities were measured in the pea straight growth test and in the callus formation. (Figs. 1-4, and Table I) It is difficult to explain by spatial structure hypothesis of Veldstra what determines the order of activities of 1-naphthoic, 3-, 4-, 5- and 6-substituted acids which have the carboxyl group in coplanar (or almost coplanar) spatial relation to the ring system, and to elucidate by the ortho reaction hypothesis of Hansch and Muir why 2-methyl acid is physiological active. These discussions led to the conception that their activity may be determined by a chemical reactivity at a definite position of naphthalene ring. Thus, the reactivity indices of these 1-naphthoic acids are calculated, according to the frontier electron theory, with the consideration of the sterical configuration of the carboxyl and nitro groups. From the relationship between the activity and the reactivity indices of these acids(Table II), a conclusion that the ability of substituted 1-naphthoic acids to form molecular complex at 8 position with plant substrate is a very important factor determining the plant growth activity has been reasoned out. From the fact that the 5-substituted

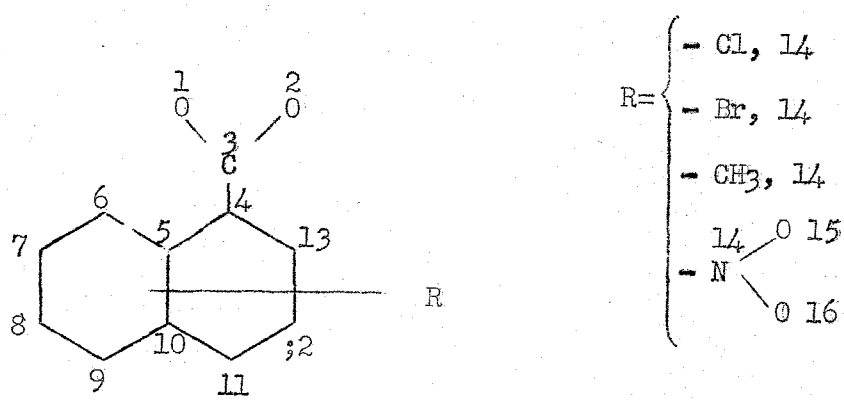
and some of the 4-substituted are inactive or weakly active in spite of their relatively large indices(Table II), it is suggested that there would be severe restriction to the lenghth of the molecule in transverse direction, in order for the 1-naphthoic acid derivatives to fit in the site of action in the plant substrate.

When the discussions in this paper are combined with previous observations on the structure-activity relationships, it appears justified at present to reformulate the requirements for appreciable auxin activity in the plant growth substances as:

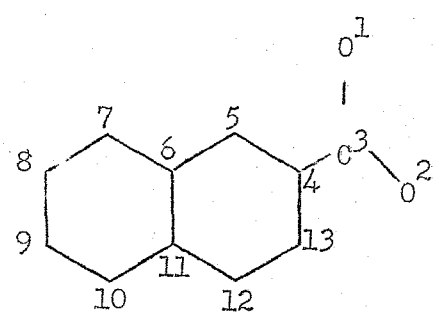
1) In the earlier stage of the growth action, the ability of the compounds to form molecular complex at a definite position with plant substrate is a very important factor determining the plant growth activity.
2) The plant growth activity is decided by such a size and form of the molecule that is able to fit in the site of action in the plant substrate.

VI. Appendix

In this chapter the frontier electron density of 1-naphthoic acid derivatives and 2-naphthoic acid, which was obtained by the simple LCAO-MO treatment, is summarized. The atoms in the molecule were numbered in order, beginning with oxygen in the carboxyl group, as following:



1-Naphthoic acid and its derivatives



2-Naphthoic acid

r	$C_r(m)2$	$C_r(m+1)2$
1-Naphthoic acid		
1	0.03531	0.09088
2	.03531	.09088
3	.06012	.49286
4	.02984	.13845
5	.01112	.06045
6	.42087	.06930
7	.11940	.11062
8	.16689	.02371
9	.39633	.14686
10	.00085	.00078
11	.25383	.40743
12	.14394	.03308
13	.05763	.33467
2-Cl-1-naphthoic acid		
1	0.01019	0.08335
2	.01019	.08335
3	.01882	.50237
4	.33586	.25992
5	.00055	.01322
6	.37032	.15038
7	.13279	.08498
8	.14061	.06510
9	.36571	.16614
10	.00016	.00486
11	.32613	.30734
12	.14334	.03333
13	.10787	.22213
14	.03737	.02359
3-Cl-1-naphthoic acid		
1	0.03441	0.08830
2	.03441	.08830
3	.05852	.47731
4	.29086	.13679
5	.01133	.06648
6	.41086	.06890
7	.11532	.11773
8	.16376	.02281
9	.38588	.15381
10	.00076	.00056
11	.24550	.43254
12	.13869	.03614
13	.05752	.30609
14	.05219	.00428

r	$C_r(m)2$	$C_r(m+1)2$
4-Cl-1-naphthoic acid		
1	0.03352	0.08864
2	.03352	.08864
3	.05697	.46255
4	.28346	.16163
5	.01068	.05753
6	.40286	.06941
7	.11469	.09908
8	.15911	.03026
9	.37998	.13268
10	.00092	.00495
11	.24202	.38949
12	.13577	.05969
13	.05540	.30760
14	.09114	.04777
5-Cl-1-naphthoic acid		
1	0.03296	0.16797
2	.03296	.16797
3	.05597	.42081
4	.27879	.12880
5	.01025	.04970
6	.39007	.08662
7	.11156	.09905
8	.15348	.03848
9	.36851	.14130
10	.00067	.00002
11	.23783	.35676
12	.13384	.03657
13	.05415	.28908
14	.13890	.01692
6-Cl-1-naphthoic acid		
1	0.03432	0.08883
2	.03432	.08883
3	.05836	.48058
4	.29014	.13697
5	.01030	.05238
6	.40877	.07028
7	.11797	.09941
8	.16025	.02635
9	.38239	.16378
10	.00090	.00092
11	.24597	.41407
12	.14080	.03218
13	.05513	.34223
14	.06031	.00312

r	$C_r(m)2$	$C_r(m+1)2$	r	$C_r(m)2$	$C_r(m+1)2$
8-Cl-1-naphthoic acid			5-Br-1-naphthoic acid		
1	0.01003	0.07132	1	0.03226	0.08745
2	.01003	.07132	2	.03226	.08745
3	.01842	.43226	3	.05559	.46910
4	.33121	.21238	4	.27168	.14057
5	.00047	.00726	5	.01073	.05593
6	.35346	.16593	6	.39719	.08828
7	.11930	.13776	7	.10859	.10918
8	.13824	.05565	8	.16304	.03712
9	.34228	.23052	9	.36846	.15314
10	.00003	.00020	10	.00143	.00001
11	.31413	.32704	11	.22795	.39538
12	.13433	.06251	12	.13391	.03830
13	.10513	.20829	13	.05106	.32140
14	.12308	.01752	14	.14579	.01676
3-Br-1-naphthoic acid			6-Br-1-naphthoic acid		
1	0.03419	0.08894	1	0.03376	0.08935
2	.03419	.08894	2	.03376	.08935
3	.05848	.48115	3	.05779	.48369
4	.28855	.13719	4	.28489	.13734
5	.00864	.06493	5	.01313	.05439
6	.40392	.06899	6	.40367	.07003
7	.12034	.11588	7	.10550	.10220
8	.15639	.02302	8	.16868	.02566
9	.38514	.15199	9	.39206	.15935
10	.00116	.00061	10	.00049	.00089
11	.25409	.42603	11	.24611	.41235
12	.14662	.03536	12	.13355	.03241
13	.04968	.31310	13	.05922	.34027
14	.05853	.00383	14	.06728	.00278
4-Br-1-naphthoic acid			8-Br-1-naphthoic acid		
1	0.03443	0.08882	1	0.00977	0.07430
2	.03443	.08882	2	.00977	.07430
3	.05909	.46792	3	.01820	.45201
4	.29032	.15539	4	.32133	.21433
5	.01034	.05805	5	.00107	.00941
6	.39529	.06912	6	.35479	.16115
7	.11028	.10146	7	.12824	.12823
8	.15968	.02853	8	.13551	.05492
9	.36940	.13570	9	.35044	.21849
10	.00039	.00362	10	.00006	.00026
11	.24253	.39322	11	.30714	.32724
12	.14433	.05247	12	.12969	.06281
13	.05306	.31299	13	.10588	.20715
14	.09644	.04389	14	.12817	.01537

r	$C_r(m)2$	$C_r(m+1)2$	r	$C_r(m)2$	$C_r(m+1)2$
2-CH_3-1-naphthoic acid			6-CH_3-1-naphthoic acid.		
1	0.01483	0.08136	1	0.02325	0.09368
2	.01483	.08136	2	.02325	.09368
3	.02963	.51548	3	.04480	.51006
4	.47502	.15868	4	.18871	.13990
5	.00987	.02669	5	.08439	.07464
6	.35965	.13149	6	.29301	.06716
7	.06378	.12313	7	.00165	.12932
8	.20386	.03281	8	.26673	.01939
9	.26780	.19765	9	.53078	.11996
10	.02185	.00238	10	.00501	.00057
11	.25489	.33959	11	.23171	.39375
12	.02203	.12258	12	.05001	.03438
13	.17453	.16135	13	.11872	.31999
14	.08739	.02547	14	.13839	.00356
3-CH_3-1-naphthoic acid			8-CH_3-naphthoic acid		
1	0.02748	0.09442	1	0.00685	0.10134
2	.02748	.09442	2	.00685	.10134
3	.05238	.51495	3	.01516	.63643
4	.22401	.13984	4	.20922	.21716
5	.01239	.05053	5	.02891	.03994
6	.26806	.06924	6	.33225	.10766
7	.18665	.09798	7	.26313	.05520
8	.06257	.02502	8	.09826	.04378
9	.34454	.13400	9	.45379	.11616
10	.01286	.00125	10	.00101	.00135
11	.39446	.36327	11	.23745	.31279
12	.25244	.02778	12	.07931	.06063
13	.00221	.38216	13	.11760	.18895
14	.13244	.00509	14	.15018	.01714
4-CH_3-1-naphthoic acid			3-NO_2-1-naphthoic acid		
1	0.04649	0.08464	1	0.03436	0.02337
2	.04649	.08464	2	.03436	.02337
3	.09056	.49261	3	.05667	.11798
4	.37655	.08546	4	.29282	.04888
5	.00799	.05771	5	.03247	.11461
6	.31316	.06085	6	.43458	.02704
7	.06214	.11695	7	.08508	.14371
8	.16707	.01115	8	.20290	.00504
9	.24667	.14864	9	.37713	.15728
10	.01169	.00285	10	.00012	.00071
11	.22345	.36449	11	.19510	.53075
12	.26713	.00272	12	.09321	.04261
13	.02556	.33896	13	.10854	.00354
14	.11486	.14832	14	.00205	.33280

r	$C_r(m)2$	$C_r(m+1)2$
15	.02532	.21417
16	.02532	.21417
4-NO_2-1-naphthoic acid		
1	0.02667	0.05902
2	.02667	.05902
3	.04256	.25462
4	.22903	.19978
5	.01288	.03018
6	.43658	.05392
7	.13932	.03672
8	.14882	.04727
9	.43223	.04343
10	.00971	.04055
11	.22216	.15811
12	.08249	.17539
13	.06747	.13348
14	.00413	.26008
15	.05962	.22418
16	.05962	.22418
5-NO_2-1-naphthoic acid		
1	0.03624	0.03727
2	.03624	.03727
3	.05591	.17544
4	.31348	.09927
5	.00658	.01691
6	.32382	.23481
7	.12260	.04504
8	.09222	.20122
9	.33675	.08312
10	.00274	.03670
11	.29034	.14888
12	.12676	.06606
13	.07236	.11714
14	.00524	.28471
15	.08935	.20812
16	.08935	.20812
6-NO_2-1-naphthoic acid		
1	0.03607	0.02085
2	.03607	.02085
3	.05918	.10582
4	.30783	.04269
5	.00059	.01372
6	.42009	.03521
7	.19505	.00486
8	.10925	.04213
9	.31913	.38454
10	.00573	.00236
11	.23619	.26549
12	.18082	.00666
13	.03247	.24465
14	.00233	.35630
15	.02960	.22705
16	.02960	.22705
8-NO_2-1-naphthoic acid		
1	0.01144	0.00765
2	.01144	.00765
3	.01982	.04151
4	.38495	.04664
5	.00095	.01339
6	.34930	.07138
7	.08541	.16342
8	.15300	.01803
9	.31333	.20106
10	.00007	.00017
11	.35556	.10783
12	.15957	.01467
13	.10439	.08326
14	.00242	.57359
15	.02419	.32487
16	.02419	.32487
2-Naphthoic acid		
1	0.01084	0.10235
2	.01084	.10235
3	.01991	.58164
4	.08951	.12049
5	.27572	.48583
6	.00155	.00634
7	.34026	.18040
8	.17264	.00696
9	.09949	.15422
10	.38300	.05484
11	.00699	.09170
12	.36524	.07334
13	.22401	.03955

Compounds	λ_m	λ_{m+1}	Compounds	λ_m	λ_{m+1}
1-Naphthoic Acid	0.69520	-0.32880	8-Br-	0.63536	-0.46655
2-Cl-	.64090	.45501	2-CH_3-	.58682	.51714
3-Cl-	.69587	.32491	3-CH_3-	.61940	.33531
4-Cl-	.69635	.28438	4-CH_3-	.60523	.41249
5-Cl-	.69696	.31186	5-CH_3-	.33340	.35755
6-Cl-	.69597	.32599	8-CH_3-	.51260	.50601
8-Cl-	.64453	.46191	3-NO_3-	.71579	.24656
3-Br-	.69209	.32590	4-NO_2-	.73668	.07710
4-Br-	.68994	.29514	5-NO_2-	.75787	.16962
5-Br-	.68720	.31611	6-NO_2-	.71915	.25269
6-Br-	.69161	.32671	8-NO_2-	.68390	.32877

If the carboxyl group in the 2- and 8-chloro, bromo and methyl substituted acids, or the carboxyl and nitro groups in the 8-nitro substituted acid are coplanar with the ring plane, these frontier electron densities are as follows.

r	$C_r(m)2$	$C_r(m+1)2$	r	$C_r(m)2$	$C_r(m+1)2$
2-Cl-1-naphthoic acid			7	.10897	.13286
1	0.03481	0.09288	8	.15519	.02461
2	.03481	.09288	9	.36568	.17206
3	.05923	.48754	10	.00076	.00057
4	.29419	.16510	11	.23629	.40676
5	.01080	.05410	12	.13425	.03265
6	.41618	.07135	13	.05321	.33621
7	.11883	.09631	14	.14682	.00891
8	.16433	.03126	8-Br-1-naphthoic acid		
9	.39268	.13090	1	0.03193	0.08663
10	.00093	.00511	2	.03193	.08663
11	.25155	.37847	3	.05507	.46736
12	.14382	.01157	4	.26889	.13542
13	.05655	.34095	5	.01230	.04973
14	.02126	.04157	6	.39043	.07353
8-Cl-1-naphthoic acid			7	.11783	.12702
1	0.03286	0.08525	8	.15141	.02438
2	.03286	.08525	9	.37269	.16548
3	.05579	.45910	10	.00091	.00062
4	.27793	.13443	11	.22932	.40681
5	.00988	.04637	12	.12886	.03276
6	.38944	.07494	13	.05399	.33570

r	$C_r(m)2$	$C_r(m+1)2$
14	.15436	.00799
2-CH_3-1-naphthoic acid		
1	0.04699	0.07995
2	.04699	.07995
3	.08376	.45823
4	.39189	.08928
5	.03789	.07103
6	.40613	.06183
7	.05249	.13286
8	.23517	.01103
9	.30423	.16475
10	.01396	.00301
11	.18965	.42350
12	.02938	.09691
13	.10336	.27891
14	.05799	.04866
8-CH_3-1-naphthoic acid		
1	0.02231	0.09890
2	.02231	.09890
3	.04688	.54264
4	.17471	.14193
5	.05985	.08720
6	.36315	.05786
7	.25293	.07562
8	.10609	.02143
9	.46548	.10570
10	.00249	.00123
11	.16819	.39998
12	.07604	.03293
13	.06671	.32524
14	.17287	.01065
8-NO_2-1-naphthoic acid		
1	0.00530	0.02259
2	.00530	.02259
3	.00824	.11100
4	.04592	.05218
5	.00007	.01262
6	.04982	.06812
7	.00799	.36888
8	.02409	.01675
9	.04303	.40373
10	.00001	.00007
11	.03887	.27171
12	.02329	.01468
13	.00657	.24503
14	.00076	.37327
15	.87033	.00839
16	.87033	.00839

Compounds	λ_m	λ_{m+1}	Compounds	λ_m	λ_{m+1}
2-Cl-	0.69547	-0.29104	2-CH_3-	0.66496	-0.39402
8-Cl	.69706	.32069	8-CH_3-	.55060	.34240
8-Br-	.68673	.32275	8-NO_2-	.76075	.21665

VII. References

1. F. Kögl, A. J. Haagen-Smit and H. Erxleben; Z. physiol. Chem., 228, 90(1934).
2. J. B. Koepfli, K. V. Thimann, and F. W. Went; J. Biol. Chem., 122, 763 (1938).
3. L. J. Audus; Biol. Revs. Cambridge Phil. Soc., 24, 51(1949).
4. H. Linser; Verh. Zool. Botan. Ges. Wien, 92, 199(1951).
5. K. V. Thimann, "Plant Growth Substances", F. Skoog, Ed., Madison, Wisc., 1951, p.21.
6. P. W. Zimmerman, and A. E. Hitchkock; Contrib. Boyce Thompson Inst., 12, 321, 491, 497(1942).
7. J. A. Bentley; Nature, 165, 449(1950).
8. R. M. Muir and C. Hansch; Plant Physiol., 26, 369(1951).
9. H. Veldstra and C. van de Westeringh; Rec. trav. chim., 71, 318(1952).
10. P. W. Zimmerman, A. E. Hitchcock and E. A. Prill; Contribs. Boyce Thompson Inst., 16, 419(1952).
11. H. Veldstra, and C. van de Westeringh; Rec. trav. chim., 70, 1113, (1951).
12. H. Veldstra; ibid., 71, 15(1952).
13. T. Mitsui and A. Tamura; J. Agr. Chem. Soc. of Japan, 25, 17(1951).
14. H. Veldstra, Enzymologia, 11, 97, 137(1944).

15. H. Veldstra and H. L. Booij, Biochim. Biophys. Acta, 3, 278(1949).

16. H. Veldstra, Ann. Rev. plant Physiol., 4, 151(1953).

17. H. Veldstra, Rec. trav. chem., 71, 15(1952).

18. H. Veldstra, "The chemistry and mode of action of plant growth substances", Butterworths Scientific Publication, London, 1955, p.117.

19. T. Fujita, K. Koshimizu and T. Mitsui, unpublished.

20. T. Fujita, Z. Kumazawa and K. Koshimizu, unpublished.

21. R. M. Muir and C. Hansch, Plant Physiol., 26, 369(1951).

22. C. Hansch, R. M. Muir and R. L. Metzenberg, ibid., 26, 812(1951).

23. R. M. Muir and C. Hansch, ibid., 28, 218(1953).

24. K. Fukui, T. Yonezawa and H. Shingu, J. Chem. Phys., 20, 722(1952).

25. K. Fukui, T. Yonezawa, C. Nagata and H. Shingu, ibid., 22, 1433(1954).

26. K. Fukui, T. Yonezawa, C. Nagata, Bull. Chem. Soc. Japan, 27, 423(1954).

27. K. Fukui, T. Yonezawa and C. Nagata, J. Chem. Phys., 26, 831(1957).

28. K. Fukui, C. Nagata and T. Yonezawa, J. Am. Chem. Soc., 80, 2267(1958).

29. C. Nagata, K. Fukui, T. Yonezawa and Y. Tagashira, Cancer Research 15, 233(1955).

30. K. Fukui, T. Yonezawa, and A. Imamura, Abstract of the Symposium on the Electronic Structure of Molecule (Chemical Society of Japan, 1959). p.71. cf. R. S. Mulliken, J. Am. Chem. Soc., 74, 811(1952).

31. In the phenoxyacetic and S-phenylthioglycolic acid derivatives, Fukui et al.suggested an interaction at the meta position. K. Fukui, C. Nagata and T. Yonezawa, Abstract of the Symposium on π-electrons (Chemical Society of Japan, 1956) p.24.

32. R. J. Foster, D. H. McRae and J. Bonner, Plant Physiol., 30, 323(1955).

33. H. Linser, "The Chemistry and Mode of Action of Plant Growth Substance", Butterworths Scientific Publications, London, 1955, p.141.

34. K. Fukui, C. Nagata and T. Yonezawa, Gann, 49, 43(1958).

35. The resonance integral is given approximately as $\beta \cos\theta$, when the angle of twist of the carboxyl group is θ . cf. C. A. Coulson, "Steric Effects in Conjugated Systems", Butterworths Scientific Publications, London, 1958, p.10.

36. C. Graebe and E. Gfeller, Ber., 25, 652(1892).

37. C. Graebe and N. Briones, Ann., 327, 77(1903).

38. G. J. Leuck, R. P. Perkins and F. C. Whitmore, J. Am. Chem. Soc., 51, 1831(1929).

39. A. G. Eckstrand, J. prakt. Chem., (2), 38, 155, 241(1888).

40. Am. Pat. 2,132,357; Chem. Zentr., 1938, II, 4312.

41. H. Rabe, Ber., 22, 392(1889).

42. A. Fischer, W. J. Mitchell, G. S. Ogilvie, J. Packer, J. E. Packer and J. Vaughan, J. Chem. Soc., 1426(1958).

43. T. L. Jacobs, S. Winstein, R. B. Henderson, J. Bond, J. W. Ralls, D. Seymour and W. H. Florsheim, J. Org. Chem., 11, 229(1946).

44. F. C. Whitmore and A. L. Fox, J. Am. Chem. Soc., 51, 3363(1929).

45. F. Mayer and A. Sieglitz,Ber., 55, 1835(1922).

46. R. D. Topsom and J. Vaughan, J. Chem. Soc., 2842(1957).

47. H. E. Fierz-David and E. Mannhart, Helv. Chim. Acta, 20, 1024(1937).

48. C. C. Price, E. C. Chapin, A. Goldman, E. Krebs and H. M. Shafer, J. Am. Chem. Soc., 63, 1857(1941).

49. Org. Syntheses, 24, 1(1944).

50. B. C. Fuson and G. Munn, J. Am. Chem. Soc., 71, 1870(1949).

51. J. Kato, Physiol. Plant., 11, 200(1958).

52. J. Kato, Mem. Coll. Sci. Univ. Kyoto, B20, 32(1951).