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Studies on the Relation between Auxin Activity and Chemical Structure

I. On phenyl- and naphthyl-thioglycolic acid derivatives and related compounds

By

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S-Phenylthioglycolic and *S*-naphthylthioglycolic acids correspond, respectively, to phenoxyacetic and naphthoxyacetic acids, the benzene or naphthalene nucleus and the side chain being connected with each other by sulfur in the former case, and by oxygen in the latter case. Hence it is worth while for those who are interested in the relation of chemical structure and auxin activity to see the activity of derivatives of phenyl- and naphthyl-thioglycolic acids and to compare them with those of well known phenoxy- and naphthoxy-compounds. Among the substances tested by Thompson *et al.* (4), thirty-nine derivatives of thioglycolic acid were reported to inhibit or accelerate growth. Muir *et al.* (2) found that *S*-phenylthioglycolic acid was nearly as active as phenoxyacetic acid in the elongation of *Avena* coleoptile cylinder. For the inhibiting action on the oat root, Wilske and Burström (5) determined that 2,4-dichlorothiophenoxyacetic acid was one third as active as 2,4-dichlorophenoxyacetic acid (2,4-D).

In the course of his synthetic studies of phenyl- and naphthyl-sulfon compounds, Sugii (3) synthesized many derivatives of phenyl- and naphthyl-thioglycolic acids and tested for the effect causing epinasty in tomato and *Ambrosia elatior* L. (3). He then kindly furnished the present author with those compounds for his use.

This paper reports the results of testing these compounds for growth promoting, callus forming and bud inhibiting activities.

Methods and Materials

Used were twenty-three *S*-phenylthioglycolic, two *S*-benzylthioglycolic, eight

S-naphthylthioglycolic, two dithioresorcin-*S.S*-diacetic, three phenyl- and naphthyl-sulfon acetic, and five phenyl- and naphthyl-sulfon-ε-aminocapronic compounds, each in the form of sodium salt.

The growth activity was determined by using the pea test. Growing regions of stems of Alaska peas, grown for 7 days in the darkroom at 25°C., were split and washed with distilled water for 2–4 hours. They were immersed in solutions of various concentrations of the compounds, adjusted to pH 5,6–5,8 with 0,1 N HCl. They were photographed after 24 hours. Determined were the molar concentration of each compound which induced the same reaction as $5{,}7 \times 10^{-5}$ M solution of indole-3-acetic acid (IAA). The latter concentration expressed in percentage to the former was named as the relative activity. For five compounds of low activity, by which the curvature corresponding to that induced by $5{,}7 \times 10^{-5}$ M of IAA could not be caused, the relative activity was represented by the threshold concentration of IAA divided by that of each compound and multiplied by 100. Those compounds which did not show positive reaction at 300 mg./l. were deemed to be inactive.

For the formative activity, callus formation on the etiolated epicotyl of *Vicia Faba* was observed. The epicotyl of a seven-day-old seedling of *Vicia Faba* grown in a dark room at 25° C. was decapitated just under the terminal bud. And the cut surface was smeared with 1 % lanolin paste of a compound to be tested. For comparison, pure lanolin, 1 % paste of sodium salt of IAA and 0,01 % paste of sodium salt of 2,4-D were used. The treated materials were kept in the darkroom for a week, and the callus formation near and at the cut surface was observed.

The activity of substances to inhibit the growth of axillary buds was tested as follows: The etiolated pea seedling grown in a darkroom at 25° C. was decapitated at 1 cm. above the first lateral bud. And the cut surface of stem was smeared with 1 % lanolin paste of a compound to be tested, pure lanolin being used for the control. The average growth in length of the lateral bud, fifteen samples for each substance, was determined after 8 days. And the degree of inhibition was expressed in percentage of the control.

Results

GROWTH ACTIVITY. — The growth activities of 45 substances as tested with peas are summarized in the table. The epinasty-inducing activity, determined by Sugii (3), are reproduced in the same table for comparison. The results of the two independent determinations are in accord with each other, except for a few trivial differences.

In the *S*-phenylthioglycolic acid group, halogen and methyl substituted substances (from *2* to *17**) were active, and nitro, amino and carboxyl substituted

* Italicized are the numbers of substances listed in the table.

ones (from *18* to *25*) were inactive. *S*-(2-Nitro-4-chlorophenyl)-thioglycolic acid (*21*) was only very slightly active. *S*-Benzylthioglycolic acid (*26*) and its derivative (*27*) were completely inactive. Both the alpha and beta forms of *S*-naphthylthioglycolic acid (*28*, *29*) were active, but their derivatives (from *30* to *34*), except *S*-(beta-tetrahydronaphthyl)-thioglycolic acid (*35*), were inactive. Phenyl- and naphthyl-sulfon acetic acids (*38*, *39*, *40*) and sulfon-ε-aminocapronic acids (from *41* to *45*) were also inactive.

Strongly active were *p*-chloro-; 2,4-, 3,4- and 2,5-dichloro-; 2,4,5-trichloro-; *p*-bromo-; 2,5-dibromo- and 3-methyl-4-chloro-substitutions of *S*-phenylthioglycolic acid. Some were even more active than IAA.

FORMATIVE ACTIVITY.—In general, compounds strongly active in the growth test were also powerful in the callus formation, except minor irregularities.

BUD INHIBITING ACTIVITY. — Of all the tested compounds, only six showed some activity in inhibiting the growth of the lateral bud. They were *S*-(2,4-dichlorophenyl)-, *S*-(3,4-dichlorophenyl)-, *S*-(2,5-dichlorophenyl)-, *S*-(2,4,5-trichlorophenyl)-, *S*-(2,4-dimethylphenyl)- and *S*-(3-methyl-4-chlorophenyl)-thioglycolic acids (*5*, *6*, *7*, *8*, *15*, *16*). They belong to the group of substances that are strongly active in the tests for growth activity and callus formation. But some of the very active substances in these tests, namely *p*-chloro-, *p*-bromo-, and 2,5-dibromo-compounds (*4*, *9*, *10*), failed to inhibit the lateral bud growth. It is also to be noticed that even the six substances active in the bud inhibition are much less active than IAA in this effect, while they are nearly as active as, or even more active than the latter in the growth promotion.

The substances (*3-9*, *15*, *16*) which were found active in the callus formation in *Vicia Faba*, also induced callus and roots in the present test plant, pea, at the treated part.

Discussion

The growth regulating activity of forty-five derivatives of phenyl- and naphthyl-thioglycolic acid and related compounds was determined by curvature of a split pea stem, callus formation in *Vicia Faba*, and bud inhibition in pea seedling. The activities of the most effective compounds were in the order comparable to IAA, with respect to the pea curvature and the callus formation. However, they were very low in the bud inhibiting activity. The low bud inhibiting effect of these substances may be ascribed to the limited transportation in the plant tissue, since, for example, *S*-(*p*-chlorophenyl)- and *S*-(3-methyl-4-chlorophenyl)- thioglycolic acids which are strongly active in the pea test induced restricted curving of *Avena* coleoptile at the apical part, when applied at the decapitated tip.

S-(*p*-Chlorophenyl)-thioglycolic acid in the table showed even higher activity than IAA in the pea test. This substance has been reported by Thompson *et al.* (4) to have the strongest inhibiting effect among phenylthioglycolic compounds in the corn-germination test and the kidney-bean tests. His results may be concordant with the present ones, since auxins inhibit the growth of root at the concentration suitable for the promotion of stem growth and also inhibit the stem growth at the high concentrations as used in the kidney-bean tests.

According to Zimmerman (6), chloro-substituting phenoxy compounds have stronger auxin activity than the corresponding bromo- and iodo-substituting ones, the para-compound being the strongest among the mono-substituted ones. Roughly the same tendency was found in the *S*-phenylthioglycolic acid derivatives. It was reported by Leaper and Bishop (1) that a 2,5-dichloro-compound showed the strongest activity among dichlorophenoxyacetic acid derivatives in the tomato test. The relative activity of *S*-(beta-naphthyl)-thioglycolic acid was 9 while that of beta-naphthoxyacetic acid was 20. Thus, with respect to the change of auxin activities due to substitutions, phenyl- and naphthyl-thioglycolic acid derivatives are similar to phenoxy-and naphthoxy-compounds in some cases, but different in others. Such a pattern of activities may depend on how the physical and chemical nature of the molecule will change owing to the replacement of oxygen atom by sulfur atom. Hence, studies in the auxin activities of phenyl- and naphthyl-thioglycolic acid derivatives will contribute to the problem of the relation of auxin activity to the structure of substance. Discussions concerning this problem will be made in a later paper.

Summary

Of the thirty-five phenyl- and naphthyl-thioglycolic acid derivatives and ten related compounds tested, sixteen were found significantly active in the pea test and in the callus formation test. They showed weak or no activity in the inhibition of lateral bud growth.

In conclusion, the author wishes to thank Professor Joji ASHIDA for his cordial guidance, Dr. Michiyasu SUGII for his kind offer of the compounds on which the present research depends, and Professor Tetsuo MITSUI for giving advice on chemical problems.

Literature cited

1) Leaper, J. M. F. and J. R. Bishop. 1950. Relation of halogen position to physiological properties in the mono-, di- and trichlorophenoxyacetic acids. Bot. Gaz, **112**: 250–258.

2) Muir, R. M., C. H. Hansch and A. H. Gallup. 1949. Growth regulation by organic compounds. Plant Physiol. **24**: 359–366.

3) Sugii, M., and A. Sugii. 1953. Studies on the phytohormones. I. On the growth promoting activity for plants of aryl thioglycolic acid derivatives. Bull. Inst. Chem. Res. Kyoto Univ. **31**: 27–33.

4) Thompson, H. E., C. P. Swanson and A. G. Norman. 1945. New growth-regulating com-

pounds. I. Summary of growth-inhibitory activities of some organic compounds as determined by three tests. Bot. Gaz., **107**: 476-507.
5) Wilske, C. and H. Burström. 1950. The growth-inhibiting action of thiophenoxyacetic acids. Physiol. Plant., **3**: 58-67.
6) Zimmerman, P. W. 1943. Present status of "plant hormones". Ind. and Engi. Chem., **35**: 596-601.

Table. Summary of the results of the growth promoting, the callus forming and the bud inhibiting activities of the phenyl- and naphthyl-thioglycolic and related compounds, with supplementary representation of Sugii's results on epinasty in tomato.

+++ : very active; ++ : active; + : slightly active.

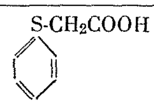
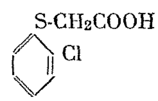
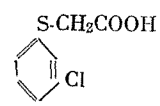
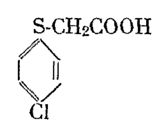
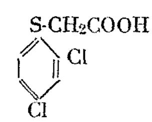
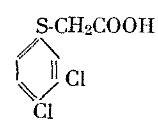
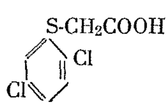
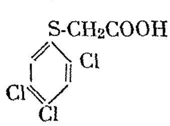
No.	Compound	Formula	Pea test: Relative activity	Pea test: Effective conc. range mg./l.	Epinasty: Threshold conc. mg./g.	Callus formation (*Vicia*)	Bud inhibition (pea) %
1	*S* Phenylthioglycolic acid		0.07[1]	30–200	Inactive	+	0
2	*S*-(*o*-Chlorophenyl)-thioglycolic acid		12	30–200	3.0	+	0
3	*S*-(*m*-Chlorophenyl)-thioglycolic acid		20	10–100	1.0	+	0[4]
4	*S*-(*p*-Chlorophenyl)-thioglycolic acid		130	1–100	1.0	+++	0[4]
5	*S*-(2,4-Dichlorophenyl)-thioglycolic acid		74	1–50	0.25	+++	20[4]
6	*S*-(3,4-Dichlorophenyl)-thioglycolic acid		74	1–50	0.5	+++	15[4]
7	*S*-(2,5-Dichlorophenyl)-thioglycolic acid		74	5–50	0.25	+++	24[4]
8	*S*-(2,4,5-Trichlorophenyl)-thioglycolic acid		84	1–30	1.0	+++	30[4]

Table Continued

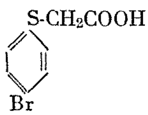
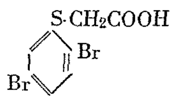
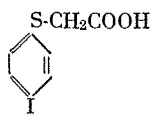
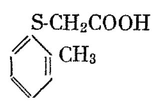
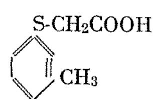
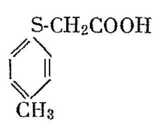
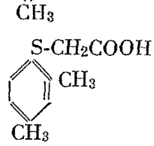
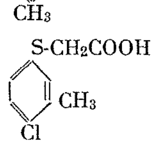
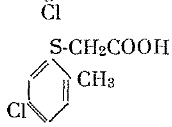
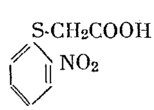
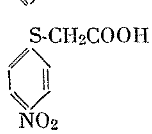
No.	Compound	Formula	Pea test		Epinasty	Callus	Bud
9	*S*-(*p*-Bromophenyl)-thioglycolic acid		70	5–100	10.0	++	0[4]
10	*S*-(2,5-Dibromophenyl)-thioglycolic acid		100	5–50	0.5	+++[3]	0
11	*S*-(*p*-Iodophenyl)-thioglycolic acid		0.2[1]	20–50	Inactive	+	0
12	*S*-(*o*-Methylphenyl)-thioglycolic acid		10	10–200	2.5	++	0
13	*S*-(*m*-Methylphenyl)-thioglycolic acid		10	10–100	2.5	++	0
14	*S*-(*p*-Methylphenyl)-thioglycolic acid		0.5	10–300	5.0	++	0
15	*S*-(2,4-Dimethylphenyl)-thioglycolic acid		20	5–50	1.0	++	24[4]
16	*S*-(3-Methyl-4-chlorophenyl)-thioglycolic acid		140	0.5–50	1.0	++	24[4]
17	*S*-(2-Methyl-5-chlorophenyl)-thioglycolic acid		27	5–100	5.0	++	0
18	*S*-(*o*-Nitrophenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
19	*S*-(*p*-Nitrophenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive[3]	0

Table Continued

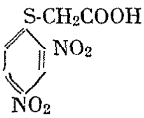
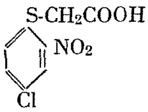
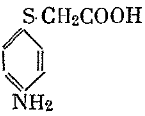
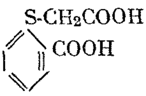
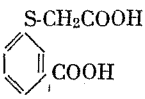
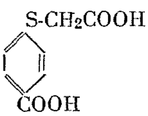
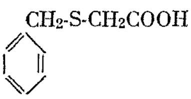
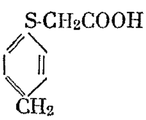
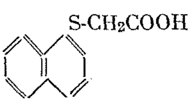
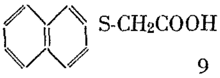
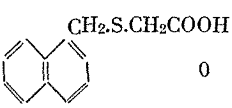
No.	Compound	Formula	Pea test		Epinasty	Callus	Bud
20	S-(2,4-Dinitrophenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
21	S-(2-Nitro-4-chlorophenyl)-thioglycolic acid		0.03[1]	100–200	Inactive	Inactive	0
22	S-(p-Aminophenyl)-thioglycolic acid		0	Inactive	15.0	Inactive	0
23	S-(o-Carboxylphenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
24	S-(m-Carboxylphenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
25	S-(p-Carboxylphenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
26	S-Benzylthioglycolic acid		0	Inactive	Inactive	Inactive	
27	S-(p-Benzylphenyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
28	S-(alpha-Naphthyl)-thioglycolic acid		6	10–200	Inactive	+	0
29	S-(beta-Naphthyl)-thioglycolic acid		9	10–50	5.0	+	0
30	S-(alpha-Menaphthyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0

Table Continued

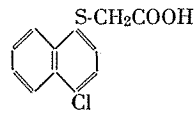
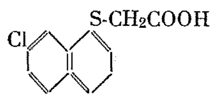
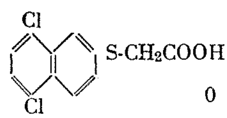
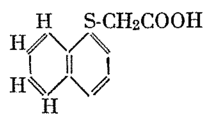
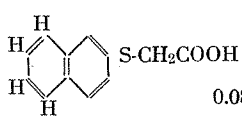
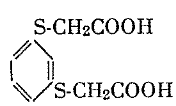
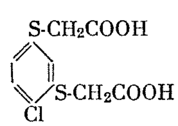
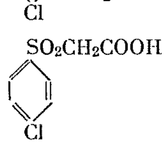
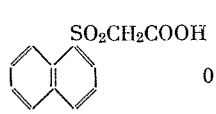
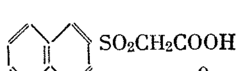
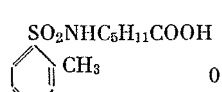
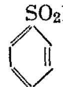
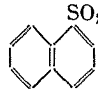
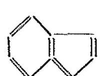
No.	Compound	Formula	Pea test		Epinasty	Callus	Bud
31	*S*-(4-Chloro-1-naphthyl)-thioglycolic acid		0	Inactive	Inactive	+	0
32	*S*-(7-Chloro-1-naphthyl)-thioglycolic acid		0	Inactive	Inactive	+	0
33	*S*-(5,8-Dichloro-2-naphthyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
34	*S*-(alpha-Terahydronaphthyl)-thioglycolic acid		0	Inactive	Inactive	Inactive	0
35	*S*-(beta-Terahydronaphthyl)-thioglycolic acid		0.08[1]	30–50	15.0	Inactive	0
36	Dithioresorcin-*S.S*-diacetic acid		0.01[1]	300[2]	Inactive	Inactive	0
37	4-Chlorodithioresorcin-*S.S*-diacetic acid		0	Inactive	Inactive	Inactive	0
38	*p*-Chlorophenylsulfon acetic acid		0	Inactive	Inactive	Inactive	0
39	alpha-Naphthylsulfon acetic acid		0	Inactive	Inactive	Inactive	0
40	beta-Naphthylsulfon acetic acid		0	Inactive	Inactive	Inactive	0
41	*o*-Methylphenylsulfon-ε-aminocapronic acid		0	Inactive	Inactive	Inactive	0

Table Continued

No.	Compound	Formula	Pea test		Epinasty	Callus	Bud
42	*p*-Methylphenylsulfon-ε-aminocapronic acid	$SO_2NHC_5H_{11}COOH$  CH_3	0	Inactive	Inactive	Inactive	0
43	*p*-Chlorophenylsulfon-ε-aminocapronic acid	$SO_2NHC_5H_{11}COOH$  Cl	0	Inactive	Inactive	Inactive	0
44	alpha-Naphthylsulfon-ε-aminocapronic acid	$SO_2NHC_5H_{11}COOH$ 	0	Inactive	Inactive	Inactive	0
45	beta-Naphthylsulfon-ε-aminocapronic acid	 $SO_2NHC_5H_{11}COOH$	0	Inactive	Inactive	Inactive	0
46	Indole-3-acetic acid	 COOH N H	100	—	—	++	100
47	Control		—	Inactive	—	Inactive	0

1) $\frac{\text{Threshold molar concentration of IAA}}{\text{Threshold molar concentration of the compound}} \times 100$

2) This compound was toxic above the threshold concentration for the pea test.

3) Tested with 0,1% paste, as 1% paste was toxic.

4) These compounds induced the formation of adventitious roots.