Inhibition of Plant Growth by Halogenated Benzoic Acids

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Thirty-four substituted benzoic acid derivatives were applied to tomato and Pinto bean foliage. Among the group of trihalogen-substituted derivatives evaluated, the 2,3,5-substituted members— 2,3,5-triiodo (TIBA), 2-bromo-3,5-diiodo, 2-chloro-3,5-diiodo, 2-iodo-3,5-dichloro, 2,3,5-tribromo, 2-bromo-3,5-chloro, 2-iodo-3,5-dibromo, and 2,3,5-trichloro—were more effective in inhibiting growth and more phytotoxic than derivatives substituted in other positions. In general, growth inhibition was correlated with formative, epinastic, and other abnormal morphological changes. The di- and monohalogenated derivatives were relatively in-

active. Eight derivatives of TIBA were also evaluated, but, with the exception of the methyl ester, were considerably less active than the parent acid. The 2,3,5-halogenated derivatives were more effective also in inhibiting growth of soybeans than the other substituted acids evaluated. Abnormal morphological changes were somewhat less striking on soybean than on Pinto bean. TIBA reduces polar auxin transport to a greater extent than any halogenated benzoic acid tested thus far. Therefore, these related acids probably will not prove better than TIBA for inhibiting vegetative growth and increasing bean yields.

Organic chemicals as plant growth regulators will probably head the list of factors to increase crop yields and lower production costs for the farmer. At present, herbicides are the most important commercial plant growth regulators, but interest in plant growth retardants, inhibitors, and dwarfing chemicals is mounting. Several chemicals which inhibit the growth of grass, tobacco suckers, and flowers are now on the market. Halogenated benzoic acids have marked effects on plants, are growth inhibitors rather than retardants, and in a few cases are used as selective weed killers.

Plant growth inhibitors may produce several beneficial effects (Cathey, 1964b). Five characteristic plant changes cited by Cathey (1964a) are: Staminate flower production is inhibited and pistillate flower formation is greatly increased in cucumber and spinach plants; treated plants are less susceptible to water stress and take up less water than untreated ones; leaves of treated bean and petunia plants are protected from photochemical air pollutants while the ones on untreated plants are severely damaged; frost resistance of treated herbaceous plants is increased; and resistance to high salt content and pH change in the soil is increased. Later reports claim increased yields of soybeans (Anderson, 1966, 1967), grapes (Coombe, 1965), cherries (Tukey, 1965), and apples (Stebbins, 1965) from plants treated with growth-inhibiting chemicals. Improved control of some insects (Tahori et al., 1962) and plant diseases (Crossan and Fieldhouse, 1964) are secondary benefits. A recent development is the antilodging effect of varied plant growth inhibitors. Soybean (Army, 1966) and small grain (Beatty, 1965) producers will soon have new chemicals to help control lodging and facilitate harvesting.

One plant growth regulator which reduces soybean height is 2,3,5-triiodobenzoic acid (TIBA). This chemical has intrigued plant biochemists for many years. Its major

effect is an inhibition of 3-indolylacetic acid (IAA) transport from the apex of growing plants to other plant parts. TIBA was shown (Keitt and Baker, 1966) to be more effective than any of the 14 halogenated benzoic acids tested. This decrease in auxin transport causes changes in plant growth and flowering. Treatment of indeterminate soybean plants (Greer and Anderson, 1965) with TIBA at the start of flowering caused the plants to change from vegetative to seed development more rapidly than normal. The soybean plant structure was benefited by decreasing plant height, decreasing top weight, and providing earlier ground cover through increased lower branch growth. These effects gave increased bean yields. Maximum yields were obtained when the soybeans were grown in narrow rows with high plant populations (Anderson, 1966, 1967). Since TIBA reduces vegetative growth, this new soybean production system provides greater photosynthetic efficiency as measured by an increased bean yield of about 20%. It will make an important contribution to the economical production of needed protein.

The chlorinated benzoic acids sold as herbicides have undesirable toxic and formative effects on beans and, therefore, are not useful for retarding growth. One compound, 3-amino-2,5-dichlorobenzoic acid (Amiben), has achieved success as a pre-emergence weed control chemical for soybeans and other crops. As expected, soybean growth was reduced when the beans were grown in a nutrient solution containing small quantities of various substituted benzoic acids (Sutherland et al., 1960).

The greenhouse data in this report compare the plant growth inhibition produced by foliar application of TIBA with the inhibition caused by other halogenated benzoic acids. The desired result is maximum growth inhibition at low dosage rates and minimum phytotoxicity. TIBA meets this requirement better than any other chemical tested.

MATERIALS AND METHODS

Synthesis (at International Minerals and Chemical Corp., Libertyville, Ill.). Chemical intermediates were purchased from supply houses such as the Aldrich Chemical Co. or

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Eastman Kodak and used as is. Melting points are uncorrected. Analyses were made by Micro-Tech Laboratories, Skokie, Ill., or by L. Ferrara and his associates at International Minerals and Chemical Corp., Libertyville, Ill.

3,5-DIIODO-2-FORMAMIDOBENZOIC ACID (No. 18). Acetic anhydride (50 ml.) and formic acid (25 ml.) were combined in a 3-necked 500-ml. flask (Huffman, 1958). This solution was cooled and 3,5-diiodoanthranilic acid (50 grams, 0.125 mole) and 100 ml. of ether were added. The mixture was stirred for 20 hours at room temperature. The product was filtered from the mixture and reslurried several times with ether. The yield was 47 grams (88%), m.p. 195–196.5° C.

Analysis Calcd. for $C_8H_5I_2NO_3$: N, 3.34. Found: N, 3.16.

3,5-DIIODO-2-METHOXYBENZOIC ACID (No. 22). 2,3,5-Triiodobenzoic acid (50 grams, 0.1 mole) was added to a freshly prepared solution of sodium methoxide (7.5 grams of Na, 0.35 gram atom) in 700 ml. of methanol. The solution was refluxed overnight, poured into 2 liters of water, and acidified with hydrochloric acid. The product was filtered from the cooled mixture; yield 40 grams (~100%). Recrystallization from methanol gave 35 grams of product sintering at 200° C., m.p. 204–5° C. Analysis by gas chromatography demonstrated 99.9+% purity.

Analysis Calcd. for $C_8H_6I_2O_3$: C, 23.8; H, 1.50; I, 62.73. Found: C, 24.3; H, 1.52; I, 62.73.

2-CHLORO-5-IODOBENZOIC ACID (No. 23). A solution of sodium nitrite (27.9 grams, 0.42 mole) in 150 ml. of sulfuric acid was added slowly, at 0° to 5° C., to a solution of 5-iodoanthranilic acid (99 grams, 0.375 mole). The solution was stirred an additional hour and poured over 4.5 kg. of ice. The solution was filtered and divided into three equal parts. Sodium chloride (150 grams) was added to one portion of the solution, followed by a solution of cuprous chloride (13 grams, 0.13 equivalent) in 75 ml. of concentrated hydrochloric acid. The mixture was heated on the steam bath for 2 hours to complete the decomposition of the diazonium salt. The precipitate was filtered from the cooled solution, washed with water, and recrystallized from dilute ethanol. The yield of product melting at 155.5–6° C. was 16.5 grams (47%).

Analysis Calcd. for $C_7H_4ClIO_2$: C, 29.76; H, 1.43. Found: C, 29.77; H, 1.64.

3,5-DIBROMO-2-FORMAMIDOBENZOIC ACID (No. 27). Using the above formylation procedure, 3,5-dibromoanthranilic acid (27 grams, 0.092 mole) was allowed to react with 100 ml. of acetic anhydride and 50 ml. of formic acid. The product was recrystallized from acetone; yield 12 grams (41%), m.p. 185–7° C.

Analysis Calcd. for $C_8H_5Br_2NO_3$: N, 4.78. Found: N, 4.59.

3,5-DICHLORO-2-FORMAMIDOBENZOIC ACID (No. 28). Using the above formylation procedure, 3,5-dichloroanthranilic acid (43.2 grams, 0.21 mole) was allowed to react with 100 ml. of acetic anhydride and 50 ml. of formic acid. The product was recrystallized from acetone; yield 14 grams (29%), m.p. 240° C.

Analysis Calcd. for $C_8H_5Cl_2NO_3$: N, 6.00. Found: N, 5.99.

n-DECYL-2,3,5-TRIIODOBENZOATE (No. 36). 2,3,5-Triiodobenzoyl chloride (50 grams, 0.1 mole) (Klemme et al., 1940) was dissolved in 150 ml. of benzene and 500 ml. of 1-decanol. The solution was allowed to stand at room temperature overnight, then refluxed on the steam bath for 2 hours. The volatile material was removed by distillation at reduced pressure. The product was recrystallized twice from acetone; yield 55 grams (88%), m.p. 38–40° C. An analytical sample recrystallized from methanol had m.p. 42–2.5° C.

Analysis Calcd. for $C_{17}H_{23}I_3O_2$: C, 31.9; H, 3.60; I, 59.5. Found: C, 31.8; H, 3.59; I, 58.7.

N-METHYL-2,3,5-TRIIODOBENZAMIDE (No. 38). A solution of 2,3,5-triiodobenzoyl chloride (25 grams, 0.125 mole) in 100 ml. of tetrahydrofuran was added slowly to a cold 500-ml. solution of 40% methylamine in water. Stirring was continued for 3 hours. The product was filtered from the mixture and recrystallized from acetone. The yield was 42.5 grams of material melting at 235–43° C. The product was recrystallized twice from tetrahydrofuran–ethyl acetate; yield 30 grams (49%), m.p. 243–5° C.

Analysis Calcd. for $C_8H_6I_3NO$: N, 2.8. Found: N, 2.7.

N,N-DIMETHYL-2,3,5-TRIIODOBENZAMIDE (No. 39). Using the above procedure, 2,3,5-triiodobenzoyl chloride (176 grams, 0.34 mole) was allowed to react with dimethylamine (600 ml. of 25% aqueous solution). The product was filtered from the mixture and recrystallized from hot water; yield 134 grams (73%), m.p. 125–7° C.

Analysis Calcd. for $C_9H_8I_3NO$: N, 2.66. Found: N, 2.70.

2,3,5-TRIIODOBENZOIC-2',2'-DIMETHYLHYDRAZIDE (No. 40). The above procedure, using 2,3,5-triiodobenzoyl chloride (51.8 grams, 0.1 mole) and dimethylhydrazine (13.5 grams, 0.2 mole) gave 34 grams (63%) of crude product. Recrystallization from ethanol yielded 18 grams (33%), m.p. 197.5–8° C.

Analysis Calcd. for $C_9H_9I_3N_2O$: C, 19.95; H, 1.67; I, 70.30; N, 5.17. Found: C, 20.10; H, 1.68; I, 70.05; N, 4.83.

2,3,5-TRIIODO-N-(2'-HYDROXYETHYL)BENZAMIDE (No. 41). Methyl 2,3,5-triiodobenzoate (50 grams, 0.0975 mole) was added to 300 ml. (excess) of ethanolamine. A thick slurry was formed which turned into a clear solution within half an hour. The solution was stirred overnight. The product was isolated by pouring the ethanolamine solution into ~2.5 liters of water. The precipitate was allowed to stand for several hours before filtration. The yield of air-dried material was 54 grams (~equal to theoretical). The melting point, after three recrystallizations from ethanol, was 207–9° C. The infrared absorption spectrum of this compound, λ_{max} 1640 cm.$^{-1}$, is characteristic of amides.

Analysis Calcd. for $C_9H_8I_3NO_2$: C, 19.91; H, 1.49; I, 70.13; N, 2.58. Found: C, 19.89; H, 1.50; I, 70.60; N, 2.47.

2,3,5-TRIIODO-N-(2'-AMINOETHYL)BENZAMIDE (No. 42). Methyl 2,3,5-triiodobenzoate (50 grams, 0.097 mole) was added to 300 ml. of ethylenediamine with stirring. The solution was stirred for $1/2$ hour, poured into 1 liter of water, and the product filtered from the solution. The yield of crude material was 50 grams (92%). Recrystal-

Table I. Phytotoxicity and Growth-Regulating Effects of Substituted Benzoic Acids

COOH

Compound No.	Source of Reference	2	3	4	5	6	Rate, P.P.M.	Phytotoxicity Rating[a] and Other Effects[b] Tomato	Pinto bean	Growth Inhibition Rating[c] Tomato	Pinto bean
Control, maleic hydrazide	Buy						5000	3	2	4	4
							1000	2	2bi	4	4
							500	1	1r	2	3
							100	1	1r	1	2
							20	1	1	1	2
Three halogens											
1 (TIBA)	Buy	I	I		I		5000	2s	4s	3	4
							1000	1s	3s, r	3	4
							500	1s	1s, r	3	4
							100	1s	1s, r	..	3
							20	1s	1r	2	3
2	Buy		I	I	I		5000	1s	3s	1	3
							1000	1sf	1s	1	1
3	Goldberg et al. (1946)	I		I		I	5000	5	3	..	4
							1000	1	1	1	4
							500	1	1	1	4
							100	1	1	1	2
							20	1	1	1	1
4	Buy	I	NH₂	I		I	5000	1	2	2	2
							1000	1	2	1	2
5	Buy	I	NHCOCH₃	I		I	5000	1	1	1	1
6	Weintraub et al. (1952)	Br	I		I		5000	3s, e	4	5	..
							1000	2s, e	2bi, lc, f	3	5
							500	1s, e	2s, bi, r, lc	3	5
							100	1s, se	1f, r, bi	3	4
7	Weintraub et al. (1952)	Cl	I		I		5000	4se	4	5	..
							1000	1s, se	1bi, f	4	5
							500	1s, e	1s, r, bi	3	4
							100	1s, se	1s, r	3	3
8	Weintraub et al. (1952)	I	Cl		Cl		5000	1f	5	1	..
							1000	1f	1f	1	4
9	Weintraub et al. (1952)	Br	Br		Br		5000	5	2f, lc	..	5
							1000	1e, s, lc	1f	2	4
							500	1s, e, lc	1s, r	2	4
							100	1s, e, lc	1s, r	2	3
10	Buy	Br	NH₂	Br		Br	5000	1	1	1	1
11	Weintraub et al. (1952)	Br	Cl		Cl		5000	5	5	..	..
							1000	1f	1f, bi	2	4
12	Weintraub et al. (1952)	I	Br		Br		5000	5	2sp, bi, f	..	5
							1000	1s, e, lc	1f, r, bi	2	4
							500	1s, e, lc	1f, r	2	4
							100	1s, e	1f	1	3
13	Weintraub et al. (1952)	Cl	Cl		Cl		5000	5	5	..	..
							1000	1s	1r, sp, f	2	4
							500	1f	1r, sp	1	4
							100	1f	1f, r	1	3
Two halogens											
14	Wheeler and Johns (1910)	I			I		5000	1	2	1	4
							1000	1	2	1	3
15	Wheeler and Liddle (1908)		I		I		5000	5	3	..	3
							1000	1	1	1	1
16	Buy	NH₂	I		I		5000	1	3	1	3
							1000	1	1	1	1
17	Buy		I	NH₂	I		5000	1	1	1	1
18	New	NHCHO	I		I		5000	1	1	1	1
19	Knoefel et al. (1961)	NHCOCH₃	I		I		5000	1	1	1	3
							1000	1	1f	1	1
20	Buy	OH	I		I		5000	5	1	..	1
							1000	1	1	1	1

Table I. Continued

No.	Source					Rate	Tomato	Pinto bean	Tomato	Pinto bean
21	Buy		I	OH	I	5000	1	1	1	1
22	New	OCH$_3$	I		I	5000	1f	4	3	..
						1000	1	1	1	1
23	New	Cl			I	5000	1f	5	3	..
						1000	1f	2s	1	3
24	Buy	Br			Br	5000	1s	5	1	..
						1000	1f	2s, e	1	3
25	Buy		Br		Br	5000	1	1	1	1
26	Rosanoff and Prager (1908)	NH$_2$	Br		Br	5000	1	1	1	1
27	New	NHCHO	Br		Br	5000	1	1	1	1
28	New	NHCHO	Cl		Cl	5000	1	1	1	1
29	Wheeler and Oates (1910)	NHCOCH$_3$	Br		Br	5000	1	1	1	2
30	Buy	OH	Br		Br	5000	1	1	1	1
31	New	NHCOCH$_3$	Cl		Cl	5000	1	1	1	2
						1000	1	1	1	3
One halogen										
32	Buy	NH$_2$			I	5000	1	4	1	..
						1000	1	1	1	1
33	Buy	NH$_2$			Cl	5000	1	2	1	1
						1000	1	2	1	1
34	Buy	Cl			NH$_2$	5000	1	1	1	1

[a] Phytotoxicity rating
1 = no injury or normal stand
2 = slight injury or slight reduction in stand
3 = moderate injury or moderate reduction in stand
4 = severe injury or severe reduction in stand
5 = all plants killed or no stand
[b] Formative effects

bi	= bud inhibition	s	= severe
e	= epinasty	se	= severe epinasty
f	= formative	sf	= slight formative
lc	= leaf curl	sl	= strap leaf
r	= rosette	sp	= stem proliferation

[c] Growth inhibition rating
1 = equal to the control
2 = slight to $^1\!/_4$ inhibition of growth
3 = $^1\!/_4$ to $^1\!/_2$ inhibition of growth
4 = $^1\!/_2$ to $^3\!/_4$ inhibition of growth
5 = $^3\!/_4$ to complete inhibition of growth

Table II. Phytotoxicity and Growth Regulating Effects of TIBA Derivatives[a]

COR

Compound No.	Source	R	Rate, P.P.M.	Phytotoxicity Rating and Other Effects		Growth Inhibition Rating	
				Tomato	Pinto bean	Tomato	Pinto bean
35	Marth and Mitchell (1964)	OCH$_3$	5000	2s, se	2f, se	5	5
			1000	2s, e	2f, bi	5	5
			500	2s, e	2f, bi	4	4
			100	1s, e	1f, bi	3	4
36	New	OC$_{10}$H$_{23}$(n)	5000	1	1	1	1
37	Thompson et al. (1946)	NH$_2$	5000	1	1f	1	3
			1000	1	1	1	1
38	New	NHCH$_3$	5000	1	1	1	3
			1000	1	1	1	1
39	New	N(CH$_3$)$_2$	5000	1	1	1	1
40	New	NHN(CH$_3$)$_2$	5000	1f	2f	4	4
			1000	1f	2f	4	3
41	New	NHCH$_2$CH$_2$OH	5000	1	1	1	1
42	New	NHCH$_2$CH$_2$NH$_2$	5000	1	1	1	1

[a] See Table I footnotes.

Table III. Response of Pinto Bean and Soybean to Substituted Benzoic Acids[a]

Compound No.	Benzoic Acids	Rate, P.P.M.	Phytotoxicity Rating and Other Effects		Growth Inhibition Rating	
			Pinto bean	Soybean	Pinto bean	Soybean
Control (maleic hydrazide)	. . .	1000	2	2bi	5	5
		500	2	2	4	4
		100	1	1	3	2
		20	1	1	3	1
		4	1	1	1	1
1 (TIBA)	2,3,5-I	1000	2	2s, r	5	5
		500	2	2f	4	4
		100	1	1f	3	4
		20	1	1	3	4
		4	1	1	1	3
3	2,4,6-I	500	1	1	4	3
		100	1	1	2	2
		20	1	1	1	1
4	3-NH₂-2,4,6-I	500	2	1	1	1
6	2-Br, 3,5-I	1000	2	2	5	5
		500	2	1r	5	4
		100	1	1	1	1
7	2-Cl, 3,5-I	1000	2	2	5	4
		500	2	1	4	4
		100	2	1	3	3
		20	1r	1	2	2
8	2-I, 3,5-Cl	500	3	3bi	5	5
		100	2	2f	3	3
		20	1r	1	2	2
		4	1r	1	2	2
9	2,3,5-Br	1000	2	2	5	5
		500	2	2s, r	4	4
		100	2	1f. se	3	3
		20	1f	1f	3	3
11	2-Br, 3,5-Cl	500	3	3bi	5	5
		100	2	2sl	3	3
		20	1r	1	3	2
		4	1r	1	3	2
12	2-I, 3,5-Br	1000	2	2r	5	5
		500	2	1r	4	4
		100	1	1f	3	4
		20	1f	1sl	3	3
13	2,3,5-Cl	1000	2	2f, sl	5	5
		500	2	2f, sl	4	4
		100	2	1sl	3	3
		20	1	1sl	2	2
18	2-NHCHO, 3,5-I	500	1	1	1	2
		100	1	1	1	1
19	2-NHCOCH₃, 3,5-I	1000	2	2	3	2
		500	1	1	1	2
		100	1	1	1	1
		20	1	1	1	1
24	2,5-Br	500	2	4	2	. .
		100	1	1f	2	2
		20	1	1	1	1
29	2-NHCOCH₃, 3,5-Br	1000	1	1	1	1
35	1-COOCH₃, 2,3,5-I (an ester)	1000	2	2f, r	4	4
		500	2	2f, r	4	4
		100	1	1f, r	3	3
		20	1	1	3	3
36	1-COOC₁₀H₂₃(n), 2,3,5-I (an ester)	500	2	2	2	2
		100	1	1	1	1

[a] See Table I footnotes.

lization from methanol yielded 35 grams of product, m.p. 186-8° C.

Analysis Calcd. for $C_9H_9I_3N_2O$: C, 19.95; H, 1.67; N, 5.17. Found: C, 19.74; H, 1.63; N, 5.10.

Biological Evaluations (at Boyce Thompson Institute, Yonkers, N. Y.). Pinto bean (*Phaseolus vulgaris* var. Pinto.),

soybean (*Glycine max* var. Lee), and tomato (*Lycoperisicum esculentum* var. Bonny Best) plants were grown in 4-inch clay pots. When the Pinto bean and soybean plants had their primary leaves nearly fully expanded and the tomatoes were 4 to 5 inches high, a pot containing one or more plants of each test species was treated by placing on a

revolving turntable in a hood and spraying with 100 ml. of the desired concentration of the test chemical. Each chemical was formulated with 100 p.p.m. of Triton X-100 (McCallan, 1948). A small amount of the chemical reached the soil in the pots. The plants were then maintained in the greenhouse for approximately 4 weeks, at the end of which time the plant growth responses obtained were recorded.

A code for plant effects is given at the end of Table I. Phytotoxicity and growth inhibition data for substituted benzoic acids applied to Pinto bean and tomato are recorded in Table I. This table has three sections which list trihalogenated, dihalogenated, and monohalogenated benzoic acids in order. Table II lists results for derivatives of TIBA and Table III shows comparative results with Pinto bean and soybean. Some variation in test results with the same chemical will be noted in comparing Tables I and III, since these were separate experiments. Some of the 2,3,5-trihalogenated compounds approach TIBA in effectiveness. However, Table III shows that undesirable phytotoxicity is greater and growth inhibition is less with compounds such as 2-chloro-3,5-dibromobenzoic acid (No. 7) or 2-iodo-3,5-dibromobenzoic acid (No. 12).

ACKNOWLEDGMENT

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