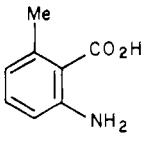
Herbicidal Activity of 6-Methylanthranilic Acid and Analogues

Gareth J. Thomas

Analogues of 6-methylanthranilic acid (1) have been synthesized and their herbicidal activities determined in order to define structure–activity relationships. Isomers of 1 in which carboxyl and amino functions are not ortho are inactive, and anthranilic acids that lack a 6-substituent are considerably less active than 1. For optimum activity the 6-substituent should have a lipophilicity parameter $\pi = 0.7$ and should be electron donating. Analogues of 1 with a modified carboxyl function are considerably less active, whereas high activity is retained in a series of N-alkyl and N-acyl derivatives. Introduction of additional substituents gives analogues of 1 with greatly reduced activity.

A variety of substituted benzoic acids exhibit plant growth regulating and herbicidal activity. Substituents that are most effective in enhancing these activities are halogen and methyl (Audus, 1972). James and Wain (1968) have tested a series of anthranilic acids, including 6-methylanthranilic acid (1), as plant growth regulators



1

and found that only halogen-substituted derivatives are active. It was therefore interesting to find that 6-methylanthranilic acid possesses considerable plant growth regulating and herbicidal activity. It increases the sugar content of sugar cane, selectively retards the growth of grasses, stimulates fruit ripening, increases fruit set and frost resistance, and shows herbicidal activity against a variety of species (de Silva et al., 1979). Analogues of 6-methylanthranilic acid have now been synthesized and their herbicidal activities determined in order to define structure–activity relationships in this series.

MATERIALS AND METHODS

Compounds **2–4** and **8–10** were obtained from commercial sources, and analogues 11 (Baker et al., 1952a), **19** and **20** (Piper and Stevens, 1962), **25** and **47** (Gabriel and Thieme, 1919), **28** and **29** (Courtin, 1975), **48**, **50** and **51** (Baker et al., 1952b), and **53–55** (Theilacker and Hopp, 1963) were synthesized by literature procedures. Catalytic hydrogenation of the corresponding nitro derivatives gave the amines **5–7**, **23**, **26**, and **49**. The anthranilic acids **13**, **14**, and **56**, were obtained through oxidation of 4-*n*-propylisatin (Baker et al., 1952a), 4-isopropylisatin [derived from *m-iso*propylaniline (Kovacic et al., 1966)], and 7-methoxy-4-methylisatin (Bullock and Johnson, 1957), respectively. **15** was obtained through hydrolysis of 6-methoxyanthranilamide (Farkas et al., 1974), and a similar procedure was employed for the homologue **16**. Catalytic hydrogenation of 2,6-dinitrobenzoic acid gave **18**, while catalytic hydrogen transfer from cyclohexene gave **22**, which was further converted to **17** through N-benzoylation and catalytic hydrogenation and to **21** by Sandmeyer reaction to give 2-bromo-6-nitrobenzoic acid followed by reduction of the corresponding ammonium salt with sodium dithionite. The amide **24** resulted from an unusual transformation (Moll et al., 1963) on catalytic hydrogenation of 2-methyl-6-nitrobenzonitrile (Gabriel and Thieme, 1919). Reduction of **47** with sodium borohydride in the presence of aluminium chloride gave 2-methyl-6-nitrobenzyl alcohol, which was hydrogenated to give **27** and was treated with phosphorus tribromide to give 2-methyl-6-nitrobenzyl bromide, which, on reaction with potassium cyanide followed by acid hydrolysis and catalytic hydrogenation, gave the phenylacetic acid **30**. The *N*-methyl derivative **31** was prepared by treatment of 1 with dimethyl sulfate, while the N,N-dialkyl derivatives **32** and **33** were obtained through reductive alkylation. 1 was converted to the N-acyl derivatives **34–39** under conventional Schotten-Baumann conditions and also served as a precursor of **42–46** through Sandmeyer reactions and of **52** by chlorination. **40** was synthesized through condensation of **26** with 2-methyl-6-nitrobenzoyl chloride, saponification, and catalytic hydrogenation. All compounds gave satisfactory spectral (NMR, IR, MS) and microanalytical data. Melting points of new compounds are given in Table I.

The 2% solutions of test compounds in acetone were diluted immediately before use with an equal volume of water containing, in the case of postemergence application, 0.1% of the wetting agent Etalfix R (Dr. R. Maag, Ltd.), a nonionic wetting agent containing 25% isooctylphenol–(6 to 7)-ethoxylate, which is not phytotoxic at the indicated rate. Solutions were applied at a rate of 10 kg of a.i./ha, either 1 day after sowing seeds in a sterilized loam soil (preemergence) or when plants had reached a suitable growth stage (postemergence). Test plants were *Sorghum halpense*, *Echinochloa crus-galli*, *Alopecurus pratensis*, *Avena fatua*, *Fagopyrum vulgare*, *Stellaria media*, *Datura stramonium*, and *Chrysanthemum segetum*. Four types of herbicidal effects were assessed as a percentage of the maximum possible for both pre- and postemergence application on all eight species, and herbicidal activity was assessed by calculating a phytotoxicity index I_t. Greater weighting was given to necrosis on the basis that, whereas good herbicidal activity results eventually in 80–100% necrosis, slow-acting herbicides or sublethal doses may cause different effects during the standard observation period. Thus

$$I_t = \%\ \text{necrosis} + 0.5(\%\ \text{chlorosis} + \%\ \text{growth retardation} + \%\ \text{deformation})$$

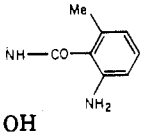
RESULTS AND DISCUSSION

3-, 4-, and 5-methylanthranilic acids were considerably less active than 6-methylanthranilic acid, and isomers **5–9** were virtually inactive, indicating the necessity of an ortho arrangement of carboxyl and amino functions. The inactivity of anthranilic acid **10** itself demonstrated the importance of a 6-substituent, and Hansch analysis of 6-substituted anthranilic acids 1, 11, **13–19**, and **21–23** sug-

Roche Products Limited, Welwyn Garden City, Hertfordshire AL7 3AY, England.

0021-8561/84/1432-0747$01.50/0

Table I. Structures and Herbicidal Activities

	substituents (except H)							
compd	1	2	3	4	5	6	I_t	mp °C
1	CO_2H	NH_2				Me	795	
2	CO_2H	NH_2			Me		185	
3	CO_2H	NH_2		Me			170	
4	CO_2H	NH_2	Me				35	
5	CO_2H		NH_2			Me	15	
6	CO_2H			NH_2		Me	0	
7	CO_2H				NH_2	Me	40	
8	CO_2H			NH_2	Me		5	
9	CO_2H		NH_2	Me			20	
10	CO_2H	NH_2					20	
11	CO_2H	NH_2				Et	665	
12	CO_2H	$NHCOCH_3$				Et	560	144
13	CO_2H	NH_2				n-C_3H_7	10	124–125
14	CO_2H	NH_2				i-C_3H_7	25	135–136
15	CO_2H	NH_2				OMe	0	
16	CO_2H	NH_2				OEt	35	75
17	CO_2H	NH_2				$NHCOC_6H_5$	55	195
18	CO_2H	NH_2				NH_2·HCl	0	
19	CO_2H	NH_2				Cl	715	
20	CO_2H	$NHCOCH_3$				Cl	420	
21	CO_2H	NH_2				Br	185	
22	CO_2H	NH_2				NO_2	0	
23	CO_2H	NH_2·HCl				CO_2H	30	
24	$CONH_2$	NH_2				Me	175	
25	CN	NH_2				Me	25	
26	CO_2Me	NH_2				Me	35	
27	CH_2OH	NH_2				Me	310	
28	SO_3H	NH_2				Me	20	
29	SO_2NH_2	NH_2				Me	20	
30	CH_2CO_2H	NH_2				Me	140	201–203
31	CO_2H	NHMe				Me	500	129.5–130
32	CO_2H	NMe_2				Me	165	107–111
33	CO_2H	NEt_2				Me	370	129–130
34	CO_2H	NHCHO				Me	695	
35	CO_2H	$NHCOCH_3$				Me	820	
36	CO_2H	$NHCOCH_2CH_3$				Me	690	156–157
37	CO_2H	$NHCOCH_2CH_2CH_3$				Me	535	129
38	CO_2H	$NHCOCH_2CH_2CH_2CH_2CH_3$				Me	545	93.5–94.5
39	CO_2H	$NHCOC_6H_5$				Me	400	
40	CO_2H					Me	20	162.3–163.5
41	CO_2H	OH				Me	85	
42	CO_2H	SH				Me	80	
43	CO_2H	$S-)_2$				Me	10	
44	CO_2H	Cl				Me	515	
45	CO_2H	Br				Me	445	
46	CO_2H	I				Me	170	
47	CO_2H	NO_2				Me	125	
48	CO_2H	NH_2	Me			Me	25	
49	CO_2H	NH_2		Me		Me	25	
50	CO_2H	NH_2			Me	Me	45	
51	CO_2H	NH_2				$-(CH_2)_4-$	50	
52	CO_2H	NH_2	Cl		Cl	Me	70	186–187
53	CO_2H	NH_2	NO_2			Me	55	
54	CO_2H	NH_2			NO_2	Me	225	
55	CO_2H	$NHCOCH_3$			NO_2	Me	130	
56	CO_2H	NH_2	OMe			Me	0	126–126.5

gested that for optimum activity this substituent should have a lipophilicity parameter $\pi = 0.7$ and should be electron donating. Analogues **24–30** in which the carboxyl function of **1** was replaced by other groups were considerably less active than **1**.

N-Alkylation or N-acylation of **1** gave derivatives **31–40**, which all possessed significant herbicidal activity, but the relative insensitivity of the activity to the nature of the alkyl or acyl residue suggested that there is little scope for improvement of the herbicidal activity of **1** through functionalization of the amino group. Analogues **41–43**, **46**, and **47** in which the amino function of **1** was replaced by other groups had only moderate activity. The high herbicidal activity of chloro- (**44**) and bromotoluic acid (**45**) is consistent with the observation that the plant growth inhibiting activity of benzoic acids is directly related to the electron acceptor ability of the molecule (Stom and Khutoryanskii, 1972). However, in view of the low level of activity of other analogues lacking an amino group, **44** and **45** may well have a different mode of action from the anthranilic acid derivatives. Analogues **48–56**, which contain three substituents of **1** together with additional substituents, were considerably less active than **1**.

This study has facilitated the definition of structure–activity relationships among analogues of 6-methylanthranilic acid (**1**), but their mode of action remains

unknown. The fact that 1 and the 6-chloro and 6-bromo analogues 19 and 21 were either inactive or had only slight activity in the wheat cylinder, pea segment, and pea curvature tests (James and Wain, 1968) suggests that these compounds do not possess auxin activity. Additional studies will be required to elucidate the mode of action of 6-methylanthranilic acid and analogues.

ACKNOWLEDGMENT

I am grateful to W. H. de Silva, P. F. Bocion, and P. Eggenberg for carrying out biological evaluations and to A. Krohn for Hansch analysis.

Registry No. 1, 4389-50-8; 2, 2941-78-8; 3, 2305-36-4; 4, 4389-45-1; 5, 2840-04-2; 6, 2486-75-1; 7, 52130-17-3; 8, 2486-70-6; 9, 2458-12-0; 10, 118-92-3; 11, 66232-56-2; 12, 66232-47-1; 13, 66232-53-9; 14, 66232-54-0; 15, 53600-33-2; 16, 90321-28-1; 17, 90321-29-2; 18, 90321-30-5; 19, 2148-56-3; 20, 19407-42-2; 21, 20776-48-1; 22, 50573-74-5; 23, 6946-22-1; 24, 1885-31-0; 25, 56043-01-7; 26, 18595-13-6; 27, 65658-16-4; 28, 39967-87-8; 29, 90321-31-6; 30, 37777-66-5; 31, 66232-39-1; 32, 66232-55-1; 33, 90321-32-7; 34, 66232-45-9; 35, 66232-41-5; 36, 66232-48-2; 37, 66232-49-3; 38, 66232-50-6; 39, 66232-44-8; 40, 66232-60-8; 41, 567-61-3; 42, 17839-53-1; 43, 18239-19-5; 44, 21327-86-6; 45, 90259-31-7; 46, 54811-50-6; 47, 13506-76-8; 48, 15540-91-7; 49, 90321-33-8; 50, 5628-48-8; 51, 90321-34-9; 52, 90321-35-0; 53, 89977-14-0; 54, 89977-13-9; 55, 90321-36-1; 56, 90321-37-2.

LITERATURE CITED

Audus, L. J. "Plant Growth Substances"; Leonard Hill: London, 1972; Vol. 1, p 87.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952a**, *17*, 164.
Baker, B. R.; Schaub, R. E.; Joseph, J. P.; McEvoy, F. J.; Williams, J. H. *J. Org. Chem.* **1952b**, *17*, 149.
Bullock, E.; Johnson, A. W. *J. Chem. Soc.* **1957**, 1602.
Courtin, A. *Chimia* **1975**, *29*, 260.
de Silva, W. H.; Bocion, P. F.; Eggenberg, P.; de Mur, A. *J. Plant Dis. Prot.* **1979**, *86*, 546.
Farkas, L.; Soti, F.; Incze, M.; Nogradi, M. *Chem. Ber.* **1974**, *107*, 3874.
Gabriel, S.; Thieme, A. *Chem. Ber.* **1919**, *52*, 1079.
James, C. S.; Wain, R. L. *Ann. Appl. Biol.* **1968**, *61*, 295.
Kovacic, P.; Levisky, J. A.; Goralski, C. T. *J. Am. Chem. Soc.* **1966**, *88*, 100.
Moll, H.; Musso, H.; Schroder, H. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 212.
Piper, J. R.; Stevens, F. J. *J. Org. Chem.* **1962**, *27*, 3134.
Stom, D. I.; Khutoryanskii, V. A. *Fiziol. Biokhim. Kul't. Rast.* **1972**, *4*, 183; *Chem. Abstr.* **1972**, *77*, 97678c.
Theilacker, W.; Hopp, R. *Justus Liebigs Ann. Chem.* **1963**, *669*, 85.

Received for review October 12, 1983. Accepted March 21, 1984.

Metribuzin Metabolites in Mammals and Liver Microsomal Oxidase Systems: Identification, Synthesis, and Reactions

Marian Saeman Bleeke and John E. Casida*

Mercapturic acid derivatives are the major urinary metabolites of metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] in intraperitoneally treated mice and orally treated rats, accounting for ~20% of the dose. These mercapturates of metribuzin and deaminometribuzin, in which the methylthio substituent is replaced by an *N*-acetylcysteinyl moiety, are also the major products on incubation of mouse and rat liver microsomes with metribuzin in the presence of *N*-acetylcysteine and NADPH. Other NADPH-dependent metabolites are deaminometribuzin and protein-bound material, the latter formed in large amounts only when *N*-acetylcysteine is not added. Deamination appears to be more important in rat than in mouse metabolism, both in vivo and in vitro. These findings suggest the formation of metribuzin sulfoxide and deaminometribuzin sulfoxide as activated intermediates. Oxidation of metribuzin and deaminometribuzin with *m*-chloroperbenzoic acid yields the corresponding sulfoxides, which react readily with *N*-acetylcysteine or protein in neutral aqueous solutions. The *N*-amino group is also cleaved on peracid oxidation, but *S*-methyl sulfoxidation occurs more rapidly.

Metribuzin or Sencor [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4*H*)-one] is a potent photosystem II inhibitor used as a pre- and postemergence herbicide against a variety of broadleaf and grass weeds in potatoes, soybeans, and other tolerant crops (Draber et al., 1968; Eue, 1972). Acid hydrolyzes the SMe substituent from the metribuzin ring to give a diketo derivative (Frear et al., 1983a), and metal-catalyzed oxidation with *tert*-butyl hydroperoxide cleaves either the C–SMe or N–NH_2 group depending on the catalyst to form the diketo or deamino derivative, respectively (Nakayama et al., 1982). The metabolic fate of metribuzin is reported in several plant systems but not in animals. Soybean, sugarcane, and/or tomato form metribuzin *N*-glucoside and malonyl *N*-glucoside, the homoglutathione conjugate, and the deamino-, diketo-, and deaminodiketo derivatives (Hilton et al., 1974; Mangeot et al., 1979; Frear et al., 1983a,b). Metribuzin sulfoxide is a possible intermediate in metabolic formation of the homoglutathione conjugate (Frear et al., 1983b).

This investigation considers the metabolism of metribuzin in rats and mice and their liver microsomal oxidase systems. It also develops a chemical model for the observed reactions with emphasis on the importance of metribuzin sulfoxide as an activated intermediate.

Pesticide Chemistry and Toxicology Laboratory, Department of Entomological Sciences, University of California, Berkeley, California 94720.

MATERIALS AND METHODS

Chromatography and Analysis. Thin-layer chromatography (TLC) utilized precoated silica gel 60 F-254 20

0021-8561/84/1432-0749$01.50/0