Structure–Activity Relationships in a Group of N-Substituted Tribromoimidazoles

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Sixteen N-substituted derivatives of 2,4,5-tribromoimidazole were synthesized by reaction of the sodium salt of the parent heterocycle with the appropriate carbonyl, thiocarbonyl, or carbamyl chloride. These compounds were then tested for biological activity in terms of weed control and crop safety. The agricultural screening data obtained were analyzed and several structure–activity correlations proposed. The herbicidal activities of these materials can be related to steric and/or lipophilicity considerations. While steric bulk appears to control pre- vs. postemergent weed control, crop tolerance is dependent on the lipophilic characteristics of the compounds.

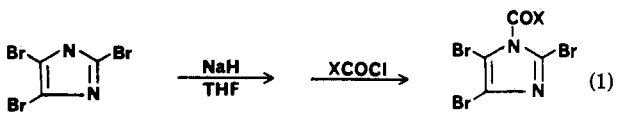
The herbicidal properties of various trihaloimidazoles have been known for a number of years (Brookes et al., 1967a; Draper et al., 1970; Rutz and Gubler, 1968; Roechling and Buechel, 1970). These compounds are classified as broad-spectrum, postemergent herbicides with limited crop selectivity. In this respect, as well as in their accepted mode of action as photosynthetic inhibitors, the trihaloimidazoles are very similar to their benzimidazole analogues (Buechel, 1972).

Both trichloro- and tribromoimidazoles are weakly phytotoxic (Brookes et at., 1967b). N-Substituted derivatives of these compounds have greatly enhanced herbicidal activity when compared to the unsubstituted materials. In fact, it is possible to obtain both insecticidal activity (Pissiotas, 1976a) and herbicidal activity by derivatizing the ring nitrogen atoms of tribromoimidazole.

Our interest in the tribromoimidazoles developed as an outgrowth of our agricultural synthesis program. In order to explore more fully the phytotoxic activity of this class of compounds, we chose to prepare a series of N-substituted tribromoimidazoles. Biological testing of these materials as herbicides revealed interesting structure–activity trends and relationships.

EXPERIMENTAL SECTION

Synthetic Methods. The N-substituted tribromoimidazoles were synthesized in one step from 2,4,5-tribromoimidazole (Balaban and Pyman, 1922) by a modification of the method of Brookes et at. (1967). That is, treatment of 2,4,5-tribromoimidazole with sodium hydride in tetrahydrofuran followed by the addition of an appropriate acid chloride (X = OR, SR, NR_2) gave the desired materials in good yields (eq 1). The compounds made and

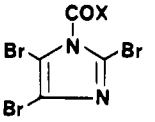


(1)

tested are listed in Table I. The IR and H NMR spectra of these samples were consistent with the proposed structures for each of the compounds prepared. As an example we have included the experimental procedure for the synthesis of compound 1. All of the remaining tribromoimidazole derivatives were produced by the same method.

1-(*N*,*N*-Dimethylcarbamyl)-2,4,5-tribromoimidazole (1). To a suspension of 417 mg (17.4 mmol) of sodium hydride in 25 mL of tetrahydrofuran was added, in portions, 5 g (15.8 mmol) of 2,4,5-tribromoimidazole at such a rate as to control the evolution of hydrogen. The resulting suspension was cooled to 0 °C, and 1.7 g (15.8 mmol) of *N*,*N*-dimethylcarbamyl chloride was added by drop. The reaction mixture was allowed to come to room temperature and stirred overnight. The precipitated sodium chloride was removed by filtration, and concentration of the filtrate in vacuo gave 5.9 g (96%) of 1 as a yellow oil.

Table I. Tribromoimidazoles Prepared and Tested



compd	X	log *P*	compd	X	log *P*
1	NMe_2	1.45	9	SMe	3.24
2	NEt_2	2.47	10	SEt	3.70
3	pyrrolidinyl	2.47	11	S-*n*-Pr	4.23
4	N-*n*-Pr_2	3.49	12	S-*i*-Pr	4.21
5	*N*-diallyl	3.49	13	OMe	2.49
6	N-*i*-Bu_2	4.51	14	OEt	3.01
7	NMePh	2.39	15	*O*-allyl	3.52
8	N(OMe)Me	0.96	16	*O*-*n*-Bu	4.05

Biological Testing. Each of the tribromoimidazoles prepared was tested independently for both pre- and postemergent activity on a variety of grassy and broadleaf weeds. The more active compounds were further tested at lower application rates on a representative selection of both crops and weeds. All of these testing procedures were conducted in the same manner. Thus, experimental detail are given for only one set of pre- and postemergent tests.

Preemergence Herbicide Test. On the day preceding treatment, seeds of eight different weed species are planted in loamy sand soil in individual rows. One species per row is planted across the width of the flat. The seeds used are green foxtail (FT) (*Setaria viridis*), watergrass (WG) (*Echinochloa crus-galli*), wild oat (WO) (*Avena fatua*), annual morningglory (AMG) (*Ipomoea lacunosa*), velvetleaf (VL) (*Abutilon theophrasti*), Indian mustard (MD) (*Brassica juncea*), curly dock (CD) (*Rumex crispus*), and yellow nutsedge (YNS) (*Cyperus esculentus*). Ample seeds are planted to yield about 20–40 seedlings per row. The number of seedlings in each row is determined by the size of the plants.

By use of an analytical balance, 600 mg of the compound to be tested is weighed and placed in a 60-mL wide-mouthed clear bottle. The compound is then dissolved in 45 mL of acetone or some other suitable solvent. Eighteen milliliters of this solution is transferred to a 60-mL

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Table II. Preemergence Herbicidal Activity of Tribromoimidazoles 1–16 at 4 lb/acre[a]

compd	FT	WG	WO	AMG	VL	MD	CD	YNS	tolerant crops
1	50	40	70	100	85	100	0	0	
2	0	0	0	100	50	100	40	N	
3	90	25	25	80	80	100	80	0	
4	0	0	0	0	0	0	0	0	
5	100	100	60	100	100	100	95	0	corn, milo
6	0	0	0	10	0	0	0	0	
7	0	0	0	0	0	0	0	N	
8	50	25	0	35	25	75	35	0	
9	20	25	20	65	10	75	75	0	
10	50	50	25	25	75	60	50	N	
11	85	60	0	0	0	100	85	0	rice
12	40	25	0	20	0	80	90	0	rice
13	80	65	10	65	50	85	100	25	
14	0	0	0	85	80	80	75	N	
15	0	0	0	20	0	0	85	0	
16	0	0	0	20	0	20	0	0	

[a] The letter N indicates not tested. Abbreviations for species tested: foxtail (FT), watergrass (WG), wild oats (WO), annual morningglory (AMG), velvetleaf (VL), mustard (MD), curlydock (CD), and yellow nut sedge (YNS).

wide-mouthed clear bottle and diluted with 22 mL of a water and acetone mixture (19:1) containing enough polyoxyethylene sorbitan monolaurate emulsifier to give a final solution of 0.5% by volume. The solution is then sprayed on a seeded flat by means of a spray application device calibrated to deliver 80 gal/acre. The resulting application rate is 4 lb/acre.

After treatment, the flats are placed in a greenhouse having a controlled temperature of 70–80 °F and watered by sprinkling. Two weeks after treatment, the degree of plant injury or control is determined by comparison with untreated check plants of the same age. An injury rating from 0 to 100% is recorded for each species, with 0% representing no injury and 100% representing complete control. Thus, a value of 60% indicates that 60% of the species in question did not survive. The results of these tests are given in Table II.

Postemergence Herbicide Test. This test is conducted in a manner almost identical with the testing procedure for the preemergence herbicide test. The only difference is that the seeds of the eight different weed species are planted 10–12 days before treatment and allowed to germinate. Also, watering of the treated flats is confined to the soil surface. The foliage of the sprouted plants is not watered. The results of these tests are given in Table III.

Calculation of log *P*. The water–octanol partition coefficients, log *P*, for these tribromoimidazoles were calculated from a base value of 2.50 for the parent tribromoimidazole. This base value was determined in an additive fashion by use of published fragment constants (Nys and Rekker, 1973, 1974, 1975) and the observed log *P* for imidazole of −0.08 (Leo et al., 1971). Thus, the addition of three bromine atoms, each having a fragment value of 0.86, to imidazole, brings the base log *P* up to 2.50. All of the subsequent compounds were subjected to the same treatment; that is, substituent constant values were added to or subtracted from the base tribromoimidazole log *P* of 2.50. The calculated log *P* values are reported in Table I.

Calculation of Molar Refractivity (MR). MR values were calculated by the addition method. The figures for each individual substituent were taken from Hansch et al. (1973). The MR values determined for the substituent combinations in compounds 1–16 are listed in Table IV.

Table III. Postemergence Herbicidal Activity of Tribromoimidazoles 1–16 at 4 lb/acre[a]

compd	FT	WG	WO	AMG	VL	MD	CD	YNS	tolerant crops
1	100	45	30	100	100	100	80	0	
2	100	40	95	100	100	100	90	0	corn, cotton, milo
3	100	0	95	100	100	100	100	N	milo, soy, corn, cotton
4	90	75	35	100	100	100	50	N	
5	0	0	50	100	100	100	60	0	corn, milo
6	95	25	35	100	85	100	45	0	
7	100	0	25	100	100	100	90	N	
8	100	85	60	100	100	100	60	30	
9	100	25	50	100	100	100	75	0	milo
10	100	50	75	100	100	100	50	0	wheat, rice
11	100	95	40	100	95	100	50	0	rice
12	100	100	50	100	85	100	70	0	rice
13	100	90	60	100	95	100	35	0	
14	100	40	75	100	100	100	75	0	
15	95	100	70	100	100	100	35	0	
16	100	75	70	100	100	100	100	0	

[a] The letter N indicates not tested. Abbreviations for species tested: foxtail (FT), watergrass (WG), wild oats (WO), annual morningglory (AMG), velvetleaf (VL), mustard (MD), curlydock (CD), and yellow nut sedge (YNS).

Table IV. Molar Refractivity Values for Tribromoimidazole Substituent Combinations

subst combin	MR	subst combin	MR
dimethyl	11.3	diallyl	29.0
methoxymethyl	13.5	di-*n*-propyl	30.0
diethyl	20.6	methylphenyl	31.1
pyrrolidinyl	22.0	diisobutyl	39.2

RESULTS AND DISCUSSION

A cursory glance at the data presented in Tables II and III indicates that the herbicidal activities of the substituted tribromoimidazoles 1–16 are, in a general sense, broad spectrum and postemergent. The phytotoxic effects appear to be evenly distributed throughout the grass and broadleaf weed species tested. As mentioned earlier, this type of herbicidal activity has been previously reported for pesticides in the trihaloimidazole family. However, closer examination of the data in Tables II and III reveals some interesting structure–activity relationships.

Consider the phytotoxic characteristics of compounds 1–18. Tribromoimidazoles **1**, **3**, **5**, and **8** are active herbicides by both pre- and postemergent application. In contrast, **2**, **4**, **6**, and **7** show good postemergent weed control, but they have little or no preemergent activity. All of these materials have the same general structural features, that is an *N*,*N*-dialkylcarbamyl group covalently bonded to one of the nitrogen atoms in a tribromoimidazole ring. In spite of this fact, they show reasonably different herbicidal activities. Thus, it is necessary to think about these molecular structures in more detail in order to explain this unusual phytotoxicity.

To understand these data, we considered three potential sources of molecular dissimilarity among compounds 1–8. Each material was assessed in terms of its lipophilicity and electronic and steric character. Our analysis of the herbicidal data was then focused on these three criteria.

The lipophilicities of compounds 1–8 are described by the log *P* values calculated for each molecule. These figures are reported in Table I. The numbers range from 0.96 for **8** to 4.51 for **6** in evenly spaced increments. The pre- and postemergent active materials **1**, **3**, **5**, and **8**, have log

P values scattered throughout this range as do the only postemergent active compounds **2**, **4**, **6**, and **7**. Therefore, it is not reasonable to attempt to correlate the observed herbicidal activity differences between these two groups of compounds with their lipophilicity properties.

The electronic characteristics of compounds **5**, **7**, and **8** immediately stand out from the group of molecules **1–8**. Compounds **1–4** and **6** are electronically very similar as they all have simple alkyl group substituents at the exocyclic nitrogen. By contrast, **5** is diallyl substituted, **7** bears an aromatic moiety, and **8** is substituted by the heteroatomic group methoxy. These three compounds all share a common electronic feature in that they are all π excessive. This group distinction still offers no explanation for the anomalous phytotoxicity of the compounds in question. If the herbicidal activities of **1–8** were related to molecular electronics, then **5**, **7**, and **8** would be expected to be similar in their weed control behavior. Likewise, one would predict parallel phytotoxic responses to **1–4** and **6**. Inspection of the data shows that **5**, **7**, and **8** are not alike in their herbicidal activities, nor are **1–4** and **6**. Therefore, the observed variation in herbicidal activity cannot be due to differences in electronic properties alone.

Another molecular feature considered in comparison of these molecules is the steric factor. Organic chemistry has long established that the series methyl, methoxy, ethyl, allyl, propyl, butyl, and phenyl are ordered from smallest to largest in terms of space occupied. It is also generally agreed that cyclopentane imparts less steric hindrance to a molecule than its ring-opened analogue diethyl (Hansch et al., 1973). By analogy one can infer that compound **3** occupies less space than its complement **2**.

For a more quantitative representation of molecular size, one can consider the physicochemical parameter of molar refractivity (MR). This term is derived from a mathematical relationship based on molecular weight, density, and index of refraction (Hansch et al., 1973). As steric bulk increases, the MR value also increases. Calculated MR values for the substituents on compounds **1–16** are contained in Table IV. For a previous use of molar refractivity in biological structure–activity correlations see Hansch and Coats (1970) and Craig (1971).

With this information the steric character of compounds **1–8** can be correlated with the observed herbicidal activities. Of the group of compounds numbered **1–8**, compounds **4**, **6**, and **7** are the most sterically encumbered. All three show postemergent phytotoxicity exclusively. Compound **2** was also classified as active postemergent only. It is smaller than **4**, **6**, and **7**. Closer examination of the data shows some weak preemergence weed control by **2**. However, to be consistent from the perspective of steric bulk, one should compare the large size of the *N*,*N*-diethyl moiety of **2** with the analogous, but smaller, tetramethyleneamine substituent of **3**. The agricultural testing data for these two materials clearly show that the sterically less hindered molecule **3** has a broader scope of phytotoxicity. The larger dialkyl system **2** exhibits severly reduced preemergent herbicidal activity. In a similar fashion, compounds **1**, **5**, and **8** also correlate smaller molecular size with enhanced preemergent weed control. Thus, the biological data strongly support our hypothesis that N-carbamylated tribromoimidazoles sacrifice preemergent weed control as the steric bulk of the carbamyl group is increased.

This trend is also evident in the N-thiocarbamylated tribromoimidazoles **9–12** as well as in the N-carbamylated tribromoimidazoles **13–16**. A sharp decline in the preemergent phytotoxicity with a concurrent increase in molecular size is exhibited by compounds **13–16**. This tendency is present, but less obvious for the thiocarbamylated materials **9–12**.

As an addendum to our steric analysis of the herbidical activities of **1–8**, we feel that it is important to draw attention to the phytotoxic characteristics of compounds **4** and **5**. The activity of **4** is strictly postemergent while that of **5** is strongly preemergent. The overwhelming structural similarity between **4** and **5** does not allow for much speculation about possible reasons for their contrasting herbicidal activities. The only obvious differences between these two materials is in the π-excessive nature of **5** as compared to **4**. This feature also plays an important role in the conformational flexibility of their respective carbamyl moieties. That is, the allyl side chains of **5** are most likely aligned to provide maximum overlap of all the π electrons in the system. Possibly this preferential molecular conformation is a fortuitous fit into the active site of the photosynthetic process. The nonrestricted, more mobile nature of **4** may not be suited to such an enzyme fit. Alternatively, **5** could exhibit enhanced binding to an enzyme active site simply due to its π-excessive character. Whatever the cause for the difference in herbicidal activity between **4** and **5**, the observation is certainly intriguing.

In Tables II and III we have included information on the crop tolerances demonstrated by our tribromoimidazoles **1–16**. Inspection of these data allows us to recognize a trend in the observed activity patterns for crop safety. The direction of the structure–activity relationship here is much more obvious than it was for the weed control correlations. In this case, a reasonable conclusion points to lipophilicity considerations when reviewed with respect to the experimentally determined crop tolerance information. In general, the tribromoimidazoles having log P values of 3.00 or less show safety to both grass and broadleaf crops, while compounds whose calculated log P is greater than 3.00 are safe only on grass crops. For example, consider **2** (log P = 2.47) and **3** (log P = 2.47). Both of these compounds cause little or no injury to cotton and soy (broadleaves) or to corn and milo (grasses). Moving up the log P scale we see that **5** (log P = 3.49), **9** (log P = 3.24), **10** (log P = 3.70), **4** (log P = 4.23), and **12** (log P = 4.21) are safe only on the grass crops milo, rice, and wheat.

In summary, we have studied the herbicidal properties of a series of N-substituted tribromoimidazoles. These compounds show intriguing activity patterns that were analyzed and correlated to differences in molecular characteristics. The seemingly inconsistent weed control abilities of these materials were explained in terms of relative steric bulk. A relationship was also established between the lipophilicities of the compounds and the crop tolerances that they exhibit. This study provides an instructive example of the importance of physicochemical parameters in influencing the herbicidal activities within a class of analogous chemical compounds.

Registry No. **1**, 94847-66-2; **2**, 102306-47-8; **3**, 102306-48-9; **4**, 102306-49-0; **5**, 102306-50-3; **6**, 102306-51-4; **7**, 102306-52-5; **8**, 102342-06-3; **9**, 102306-53-6; **10**, 15327-29-4; **11**, 102306-54-7; **12**, 102306-55-8; **13**, 15287-47-5; **14**, 7682-42-0; **15**, 15287-53-3; **16**, 15287-51-1; Me_2NCOCl, 79-44-7; 2,4,5-tribromoimidazole, 2034-22-2; 3-Me-4-$NO_2C_6H_3OH$, 2581-34-2; fenitrooxon, 2255-17-6; fenitrothion, 122-14-5.

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Determination of Adulterated Natural Ethyl Butyrate by Carbon Isotopes

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Ethyl butyrate, a flavor chemical found in many foods, was isolated from microbial sources and orange juice. The two authentic "natural" samples and one synthetic sample were analyzed for ^{14}C content. The natural samples yielded values consistent with their natural origin (ca. 125% of modern), while the synthetic sample was devoid of ^{14}C, as expected for a petrochemical material. From the carbon stable isotope ratio, it was possible to differentiate between the two types of natural ethyl butyrate, but this technique proved less useful in distinguishing between "natural" and "artificial" material.

The use of ^{14}C analysis in the food industry for determining sources of ethanol was first suggested by Faltings (1952). It has since been applied to fermented spirits (Martin et al., 1981; McWeeney and Bates, 1980), vinegar (Krueger and Krueger, 1985), caffeine (Allen, 1961), cinnamon (Hoffman and Salb, 1980), citric acid (Volpe et al., 1982), and other flavor chemicals (Devron et al., 1980; Bricout and Koziet, 1978).

Ethyl butyrate is an important flavor chemical found in many foods. It is most abundant in fruit juices such as orange, apple, and strawberry. Foods are often formulated to taste like the above flavors by the addition of "natural" and/or "artificial" flavors, with a corresponding claim on the product label. Artificial flavors are usually made from inexpensive and abundant petrochemical sources, while natural flavors are generally expensive and in limited supply. With the current consumer trend toward natural foods, there is a possibility for fraud by adulterating natural flavorings with inexpensive artificial flavoring materials.

Research in our lab has centered on obtaining ethyl butyrate from sources that comply with the Code of Federal Regulations (21 CFR 101.22.a.3) for natural flavors. In light of the above legal and economic aspects, it was important to have an analytical method that would distinguish between natural and artificial ethyl butyrate. We report in this paper the use of ^{14}C analysis as a means to identify ethyl butyrate obtained from petrochemical sources.

MATERIALS AND METHODS

Samples of authentic natural ethyl butyrate were prepared by standard methods of extraction and distillation (Kesterson and Braddock, 1976) to obtain Food Chemical Codex quality product. Artificial ethyl butyrate was purchased from Fritzsche Dodge & Olcott Inc. The samples were coded and submitted blind to the analysts.

^{14}C Analysis. Approximately 5 g of sample was placed in a 2-L combustion bomb and the bomb charged with 100 psi of O_2. The sample was ignited electrically. When the pressure surge subsided and the bomb cooled, the resultant gases were passed through traps of dry ice/trichloroethylene and liquid O_2. *Caution*! Proper precautions must be observed in the handling of liquid O_2, which is used instead of liquid N_2 to avoid O_2 condensation in the gas line. The liquid O_2 trap was isolated and any residual noncondensible gas evacuated. An aliquot of the CO_2 was taken for ^{13}C analysis and the remaining CO_2 converted to methane with tritium-free H_2 over 0.5% ruthenium on alumina at 475 °C. The methane was purified by passing through a trap of dry ice/trichloroethylene, trapping on silica gel at liquid N_2 temperature, and evacuating excess H_2. Methane was released by warming the silica gel trap and freezing the evolved methane in a liquid N_2 trap and expanded into a storage flask to await ^{14}C counting. The ^{14}C activity of methane was determined by counting for 1000 min in a low-level proportional gas counter with anticoincidence circuitry, which was calibrated using the NBS oxalic acid standard. Counter background was measured using a 300-million-year-old marble with no ^{14}C activity. ^{14}C activities were corrected for isotope fractionation by normalizing to $^{13}C = -25.0‰$ using

$$^{14}C_{norm} = {}^{14}C_{meas}\left[1 - \frac{2[25 + \delta(^{13}C)]}{1000}\right]$$

^{13}C Analysis. CO_2 was analyzed to determine its $^{13}C/^{12}C$ ratio by using a dual collecting mass spectrometer according to AOAC (13th ed., 31.153, 1980).

RESULTS AND DISCUSSION

^{14}C Analysis. Carbon-14, present in the atmosphere mainly as $^{14}CO_2$, is produced in nature by cosmic radiation (Friedlander and Kennedy, 1962). The relative abundance

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