THE SYNTHESIS OF SOME ACID ANALOGS OF 2-THIOBENZIMIDAZOLE AND BIOLOGICAL ASSAY AS INHIBITORS OF THE GROWTH OF PLANTS

By

THEODORE LYNN REBSTOCK

AN ABSTRACT

Submitted to the School of Advanced Graduate Studies of Michigan
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Approved____

A study was made of the effect of chemical structure on the inhibitory activity toward growth and on herbicidal action on cranberry bean plants and root development in cucumber seedlings of a number of acid analogs of 2-thiobenzimidazole. Different chemical groups were substituted in the benzene ring portion of the benzimidazole nucleus and the thioether acid side chains were varied.

The following acid analogs of 2-thiobenzimidazole were synthesized and characterized: (2-benzimidazolylthio)acetic, α(2-benzimidazolylthio)-propionic, β(2-benzimidazolylthio)propionic, (5-chloro-2-benzimidazolylthio)acetic, β(5-chloro-2-benzimidazolylthio)propionic, α(5-chloro-2-benzimidazolylthio)propionic, (4,6-dichloro-2-benzimidazolylthio)acetic, (5,6-dichloro-2-benzimidazolylthio)acetic, α(5,6-dichloro-2-benzimidazolylthio)propionic, (4,5,6-trichloro-2-benzimidazolylthio)acetic, α(4,5,6-trichloro-2-benzimidazolylthio)propionic, (5-bromo-2-benzimidazolylthio)-acetic, α(5-bromo-2-benzimidazolylthio)propionic, (5-nitro-2-benzimidazolylthio)acetic, α(5-nitro-2-benzimidazolylthio)propionic, (5-methoxy-2-benzimidazolylthio)acetic, α(5-methoxy-2-benzimidazolylthio)propionic, (5,6-dimethoxy-2-benzimidazolylthio)acetic, (5-methyl-2-benzimidazolylthio)-acetic, α(5-methyl-2-benzimidazolylthio)propionic, (4,6-dimethyl-2-benzimidazolylthio)acetic, α(4,6-dimethyl-2-benzimidazolylthio)propionic, (5,6-dimethyl-2-benzimidazolylthio)acetic, α(5,6-dimethyl-2-benzimidazolylthio)propionic, (5-phenyl-2-benzimidazolylthio)acetic and α(5-phenyl-2-benzimidazolylthio)propionic acids.

The acids were prepared by employing a Williamson type synthesis with appropriately substituted 2-thiobenzimidazoles and monochloroacetic,

α-bromopropionic, or β-bromopropionic acids as the reactants. The 2-thiobenzimidazoles were prepared from suitably substituted o-phenylenediamines by the method described by Van Allan and Deacon (1) using a mixture of potassium hydroxide dissolved in aqueous ethanol and carbon disulfide. The o-phenylenediamines were prepared from the appropriately substituted o-nitroaniline or dinitrobenzene derivatives by either reducing the nitro-compound with stannous chloride or mossy tin in concentrated hydrochloric acid.

Although none of the compounds were herbicidal when tested at a concentration of 0.005 molar, variations in the structure of the benzimidazoles did influence the inhibitory activity. The β-propionic acids were slightly better inhibitors than the corresponding acetic acid derivatives whereas the α-propionic acids were generally the poorest inhibitors.

The nature of the substituent in the aromatic nucleus also affected the inhibitory activity of the 2-thiobenzimidazole analogs, and these substituents had a greater influence than did the variations of the acid side chains. A chlorine or a bromine atom in the 5-position of the benzimidazole nucleus greatly increased the inhibitory activity with a chlorine atom slightly more effective than a bromine atom. The substitution of a second chlorine atom in the 6-position also resulted in a compound which was a much better inhibitor than the unsubstituted compound, although this derivative was not quite as effective as the 5-monochlorobenzimidazole. When the chlorine atoms were substituted in the 4- and 6-positions, the inhibitory power was reduced still further; however, this compound was still a better inhibitor than the parent compound, (2-benzimidazolylthio)acetic acid. Three chlorines in positions 4,5 and 6 almost completely removed the inhibitory activity of the benzimidazole compound.

Nitro-, phenyl-, methyl- and methoxyl-groups greatly decreased the inhibitory activity and the substitution of two methyl groups in either the 5- and 6- or the 4- and 6-positions almost completely destroyed the ability of these compounds to inhibit the growth of the test plants.

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1. Van Allan, J. A. and B. D. Deacon, 2-Mercaptobenzimidazole, Org. Syn., 30, 56-57 (1950).

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INTRODUCTION

INTRODUCTION

In the few years since the identification of 3-indoleacetic acid as a growth hormone present in corn by Kögl, Haagen-Smit and Erxleben (26), interest in the field of plant-growth regulators has rapidly increased. Not only has academic interest in these substances been greatly stimulated, but the manufacture of chemicals having growth-regulating properties has grown into a multimillion dollar industry.

Leopold (32) defines growth-regulators as organic compounds, other than nutrients, small amounts of which are capable of modifying growth. Substances which either stimulate, inhibit or otherwise alter growth are included in this definition.

The uses of these compounds in agriculture are numerous. Among the applications are the stimulation of root formation on cuttings, prevention of preharvest drop of fruit, increase in fruit set, production of seedless fruit, prolongation of dormancy in nursery stock, delay in blossoming of fruit trees, improvement in regulation of flowering in pineapples, defoliation, thinning of fruits, hastening of fruit maturity, hastening the coloring of fruit, reducing water loss in fresh vegetables, ripening fruit artificially, and destruction of weeds and fibrous plants.

Methods of Biological Assay

Several biological tests have been devised in order to establish that a compound has growth-regulating properties. The tests used for the determination of the biological activity of these substances are usually ones which utilize either stimulation or inhibition of cell elongation. One of the tests frequently employed is the *Avena* test in which the chemical is incorporated in a block of agar. The agar block is then placed on one side of the coleoptile of an oat seedling from which the tip has been removed. If the substance is active there is a difference in growth rate between the side of the coleoptile to which the substance is applied and the side to which none is applied resulting in a curvature of the coleoptile. After a given length of time the curvatures are measured and the degree of curvature is a measure of the growth promoting or inhibiting properties of the compound.

Another test is the split pea test. The basis for this test lies in the differential growth of the epidermal cells of etiolated pea stems in response to the growth-regulating compound. Peas are grown seven days in the dark. After the shoot is ten centimeters in length, the tip is removed and the stem split lengthwise down the middle. The two halves are placed in the test solution and after a given length of time the curvature is measured. As in the *Avena* test, the degree of curvature is a measure of the potency of the test solution. The *Avena* and split pea tests are dependent upon the polar transport of the growth-regulating substance.

A group of tests which are not dependent upon the polar transport of the test substance are the straight-growth tests. In these tests either oats or pea seedlings are selected and cut into uniform sections. The sections are placed in Petri dishes containing the test solution and cultured under controlled conditions for a given period of time. The sections are then measured and the length is a measure of the activity of the test solution.

An additional test is the root-growth test in which sensitive seeds, such as cucumber, are grown under controlled conditions. The amount of inhibition of root development is used to measure the potency of the test substance.

A further useful and simple test is the bean leaf test. Young red kidney bean plants are grown until the first two heart-shaped leaves are formed. The test solution is either placed on the upper surface of the leaf blade at its base, or the leaves are dipped in the test solution. The effect is to suppress the growth of the shoot, and the weight of the shoot after a given number of days is a measure of the potency of the test material.

Relation of Chemical Structure to Growth-Regulating Properties

A large number of chemical compounds have been found to have growth-regulating properties. Several derivatives of indoleacetic acid have been isolated from plant sources and shown to possess such properties. Indoleacetic acid was first isolated from corn by Kögl et al. (26), and more recently the ethyl ester of this acid was isolated and characterized from immature corn kernels by Redemann, Wittwer and Sell (45). Another derivative, 3-indoleacetonitrile, has been found to be present in cabbage by a group of English workers (22).

A number of derivatives of indoleacetic acid have been prepared in the laboratory in which alterations were made in the pyrrole nucleus, the benzene ring or the side chain. Such groupings as methyl, methoxyl or halogen were substituted in the molecule (13, 19, 27, 51). When the pea test was employed, substitution in the pyrrole nucleus markedly decreased the activity, whereas substitution in the benzene ring as with 4-, 5-, and 6-chloro or fluoro decreased the activity only slightly or even enhanced the activity (19).

Phenylacetic acid, cinnamic acid and related compounds have been quite extensively studied. Not only does the nature of the substituent, but also the location have an appreciable effect on the biological activity of this group of compounds (24, 37, 55, 61). Fusion of an additional six-carbon ring to the phenyl nucleus as in naphthylene and phenanthrene results in a considerable decrease in activity (60).

Since 2,4-dichlorophenoxyacetic acid (2,4-D) is one of the most frequently used chemicals for herbicidal purposes, interest in compounds related to it has been extensive; and a large number of selected compounds have been synthesized (56, 63). Muir, Hansch, and Gallup (35) investigated in a systematic manner the effects of nuclear substitution in phenoxyacetic acids on cell elongation. Two-, 3-, and 4-chloro- or bromo-phenoxyacetic acids proved to be more active than the parent compound with the effect being greatest in the 3- and 4-positions. Trihalogen substitution with the 2- and 6-positions occupied resulted in inactive compounds.

The effect of all mono-, di-, and trichlorophenoxyacetic acids on tomato plants and as inhibitors of the growth of Lupinus albus seedlings was compared by Leaper and Bishop (30). These workers observed that the greatest physiological activity of chlorophenoxyacetic acids was associated with the presence of two unsubstituted positions in the benzene ring para to each other. The possibility of the formation of compounds in the plant having quinoid structures was suggested as being perhaps connected with their maximum herbicidal potency. Veldstra (59) has reported that of the three mono-nitrophenoxyacetic acids, only the 3-nitro derivative is active in the pea test.

The effect of substitution in the side chain of aryloxyacetic acid was studied by Osborne and Wain (42). In different tests (straight growth and pea tests) these workers found that substitution of an alkyl group in the acetic acid side chain had little effect upon activity in most cases. However, α,α-disubstituted compounds were inactive in straight growth, but some of them were slightly active in the pea test. In the light of these results, it was suggested that a chemical reaction involving a hydrogen atom on the carbon adjacent to the carboxyl group operates in the growth response.

Another group of compounds displaying some activity are the substituted benzoic acids. Zimmerman and Hitchcock (68) first reported a substituted benzoic acid, 2-bromo-3-nitrobenzoic acid, with mild activity for cell elongation. Bentley (3) found 2,3,6-trichlorobenzoic acid to be highly active in straight growth. As a result of numerous studies with substituted benzoic acids, one may conclude that hydrophilic groups (OH, NH_2) do not cause the resulting benzoic acids to become active whereas lipophilic ones (Cl, Br, I, CH_3) may do so. A nitro group is effective mainly in the 3-position. Ortho substitution starts to activate benzoic acid in straight growth with the activation being pronounced with di-ortho substitution provided that the substituent is not larger than chlorine or methyl. The activation is greatest for 2,3,6-derivatives. The 4-position apparently has to remain free for activity (60).

Optical isomerism is also associated with growth activity. Kögl and Verkaaik (25, 28) found (+)α-indole-3-propionic acid to be thirty times as active in the Avena test as the (-) isomer. More recently Wain (48, 62) reported the (+) form of α-naphthoxy-2-propionic acid to be very active in six different types of tests, whereas the (-) form had only slight activity. Wain also found that the activity in straight growth and pea tests of the racemates of α(2,4-dichlorophenoxy) propionic and α(2,4,5-trichlorophenoxy) propionic acids was due primarily to the (+) acids (49). Veldstra and van de Westeringh (61) were able to resolve the physiologically active racemates of 1,2,3,4-tetrahydro-1-naphthoic acid and α-alkyl-phenylacetic acid and found the (-) form of the former and the (+) form of the latter highly active in the pea test.

Applying the pea test and using a large number of active compounds, Koepfli, Thimann, and Went (24) formulated the following five structural requirements for cell elongation: (1) a ring system nucleus, (2) a double bond in the ring, (3) a side chain, (4) a carboxyl group or a structure readily converted to a carboxyl on the side chain at least one carbon removed from the ring, and (5) a particular space relationship between the ring and the carboxyl group. More recently Veldstra (61) has condensed the five requirements into two: (1) a basal ring system (nonpolar part) with high interface activity and (2) a carboxyl group (polar part) in such a spatial position with respect to the ring system that on adsorption of the active molecule to a boundary, this functional group will be situated as peripherally as possible.

Theories of the Mechanism of Action of Plant Growth-Regulators

A number of theories have been advanced to explain at least in part the mechanism of action of plant growth-regulators. As early as 1942 Skoog et al. (47) postulated that a growth-regulating compound may act as a sort of coenzyme by serving as a point of attachment for some substrate onto an enzyme controlling growth. The configuration and reactivity of the compound would affect activity through altering the "fit" and functioning of the points of attachment.

Veldstra (60) has found that the degree of fat solubility as influenced by the ring structure and the water solubility as influenced by the side chain structure could be correlated with growth-regulating activity. He concluded that activity was greatest when the lipophilic and the hydrophilic properties were balanced. Thus, he thought of the auxin action as being something of a physical bonding of some lipoidal material to some non-aqueous phase.

A third suggestion by Muir et al. (36) was that plant-growth regulators may react by a nucleophilic substitution at a position on the ring ortho to the carboxyl group or the side chain carrying this group and with the carboxyl group itself. The cysteinyl unit of a protein was considered to be the most likely substrate for the two-point ortho reaction. Kinetic evidence has been set forth by Foster et al. (14) in support of a theory of two-point attachment.

In recent studies of the disappearance of indoleacetic acid in pea brei, Siegel and Galston (46) observed that some of the acid was bound to protein in the brei and also that in the presence of adenosine triphosphate the binding reaction was facilitated. This suggests that energy might be involved in this binding reaction.

Leopold and Guernsey (33) after studying the reaction of several acids having growth-regulating activity with coenzyme A (CoA) in the presence of tomato mitochondria, found that the presence of several of these compounds could bring about the enzymatic disappearance of the free sulfhydryl group of CoA. It was further found that the most active compounds were the most effective in this reaction. Adenosine triphosphate also facilitated the reaction. These workers suggested that the growth-regulating compounds may form a thiol ether with CoA. The theory is further supported by the finding that CoA and adenosine triphosphate increase auxin-induced growth in the pea straight-growth test, and that the greatest enzymatic activity for the reaction appears to be associated with tissue in the most rapid state of growth. However, Price and Leopold (43) were unable to repeat the experiment and have discounted the theory.

Several theories based on the effects of auxin on enzymes have been postulated. Northen (41) after observing that auxin causes a decrease in cytoplasmic viscosity, suggested that these compounds bring about dissociation of the protein constituents of the cytoplasm. Such a dissociation might increase water permeability, increase the osmotic value of the cytoplasm, and possibly bring about increased enzymatic activity. Hund (18) has shown that mild protein dissociation can sometimes activate enzymes. Such dissociation might increase the availability of substrates for the enzyme and consequently, increase respiratory activity and growth might follow. Northen points out further that if dissociation activities are carried to the extreme, the stimulation effects upon enzymes in respiration would be reversed by dissociation of essential constituents for enzymes and he proposed that this might be the nature of inhibition of growth by auxin and the concomitant inhibition of respiration.

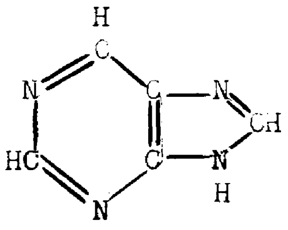
Thimann (54) has proposed another theory of auxin action. He suggests that auxins may act not as enzyme-activating agents, but as agents protecting growth enzymes from inactivation. On this basis, the structural requirements for an active auxin may be explained on the basis of structural specificity to antagonize enzymatic inhibitors.

A suggestion by Bonner and Bandurski (7) is that auxin may serve in some way to couple or mesh together the respiratory processes with the growth processes. Auxin would act in some role which would make the energy formed in respiration available to the growth process. The ineffectiveness of auxin in the presence of agents such as arsenate and dinitrophenol which uncouple phosphorylation and the evidence that phosphorylation reactions commonly limit growth were pointed out as indications suggesting the participation of auxin in phosphorylation and energy transfer reactions. However, the suggestion lacks support of direct experimental evidence.

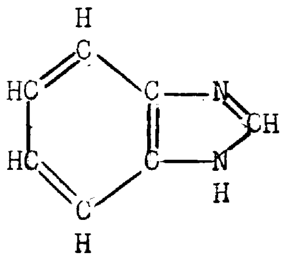
Statement of Problem

Although a very large number of compounds have been tested for growth-regulating properties, only a relatively small number of these were found to possess such activity. In many cases, the search appears to have been a trial and error process and not too systematic. However, the selection and testing of compounds structurally related to substances naturally occurring in plants which are thought to be involved in the natural growth processes of plants would seem to be a more logical approach to the problem. Moreover, such a systematic approach might lead to a valuable clue in the more complete understanding of the phenomena of plant growth. This problem has been explored extensively but is still far from being understood.

Several substances structurally related to benzimidazole are known to occur naturally in biological systems. A derivative of this compound, 5,6-dimethylbenzimidazole, makes up a portion of the vitamin B_{12} molecule. Purines, which are an important component of nucleic acid structures and nucleotides, also possess a structural similarity to benzimidazole. Since nucleic acids are thought to play an important role in the synthesis of protein by living material and growth is also associated with the synthesis of proteins, it would not seem too unlogical to assume that substances which might act as antagonists to portions of nucleic acids would also influence the growth of plants.



Purine



Benzimidazole

Several workers have presented evidence which indicates that benzimidazole is an antagonist of purine compounds. Woolley (66) has found that benzimidazole inhibited the growth of several yeasts and bacteria and that the inhibition could be completely removed by aminopurines. Klotz and Mellody (23) have reported that yeast nucleic acid caused a marked reversal of the inhibitory effect of benzimidazole on the growth of the bacterium, Escherichia coli. Recently Gillespie et al. (17) have shown that 4-methoxy-6-methylbenzimidazole was an effective growth inhibitor of Tetrahymena gelii, a quanine-requiring protozoan and of developing embryos of Rana pipiens. By using peas as the test material, Galston et al. (16) have found that benzimidazole is a metabolic antagonist of adenine and caused an inhibition of cell elongation. Thus, there is little doubt that benzimidazoles are biologically active compounds.

Since benzimidazole derivatives have been found to have an inhibitory effect on bacteria and yeast cells as well as on cell elongation of pea seedlings, a number of analogs of benzimidazole were synthesized in the present work and tested on plant material in an attempt to increase the inhibitory activity of the benzimidazole and if possible to introduce herbicidal properties. As a trial compound, (2-benzimidazolylthio)acetic acid was synthesized and tested on cranberry bean plants. This compound was found to have an appreciable inhibitory effect on the test plants. Different acid side chains were then attached to the benzimidazole nucleus through a thio ether linkage at the 2-position and different substituents were placed in the benzene ring portion of the molecule. The resulting acids fulfill the structural requirements for compounds having physiological action in cell elongation as postulated by Koepfli et al. (24). These substances were tested on plant material for growth-regulation activity by (1) the bean leaf test and (2) cucumber root inhibition test.

EXPERIMENTAL

EXPERIMENTAL

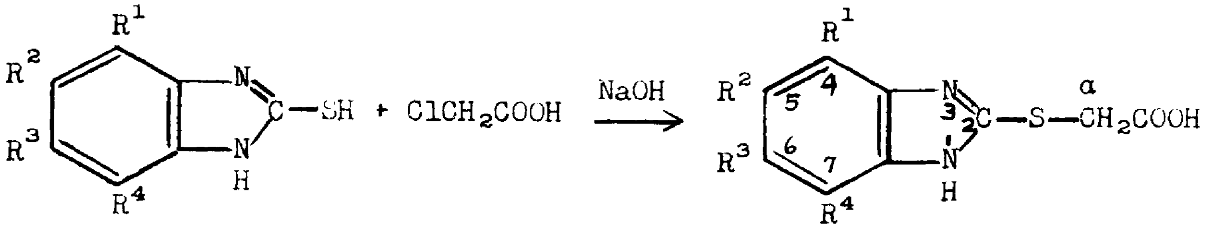
Synthesis of Compounds

The acids were prepared by employing a Williamson type synthesis with appropriately substituted 2-thiobenzimidazoles and monochloroacetic, α-bromopropionic or β-bromopropionic acids as the reactants. The 2-thiobenzimidazoles were prepared from suitably substituted o-phenylenediamines by the method described by Van Allan and Deacon (58) using a mixture of potassium hydroxide dissolved in aqueous ethanol and carbon disulfide. In the instances where the o-phenylenediamines were not available, these compounds were prepared from the corresponding o-nitroaniline or o-dinitrobenzene derivative by either reducing the nitro compound with stannous chloride or tin in concentrated hydrochloric acid.

The 2-thiobenzimidazole was furnished by the Monsanto Chemical Company. The 3,4-dimethylaniline was purchased from the Aldrich Chemical Company and guaiacol from Mallinckrodt Chemical Works. All of the other chemicals employed as starting material for these syntheses were secured from Distillation Products Industries.

The following is a summary of the reactions utilized in the synthesis of these compounds. Only the functional groups taking part in the reactions are indicated in the structural formulas of the reactants.

Preparation of Acids



R^1 = H, CH_3 or Cl

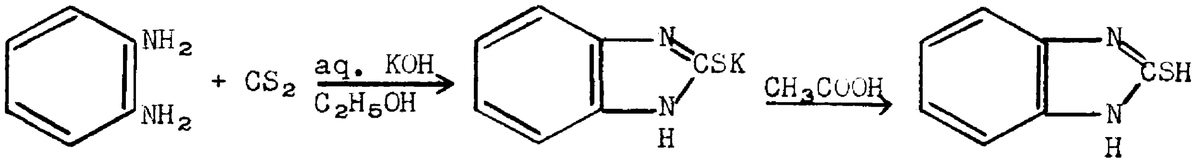
R^2 = H, Cl, Br, CH_3, CH_3O or phenyl

R^3 = H, CH_3, CH_3O or Cl

R^4 = H

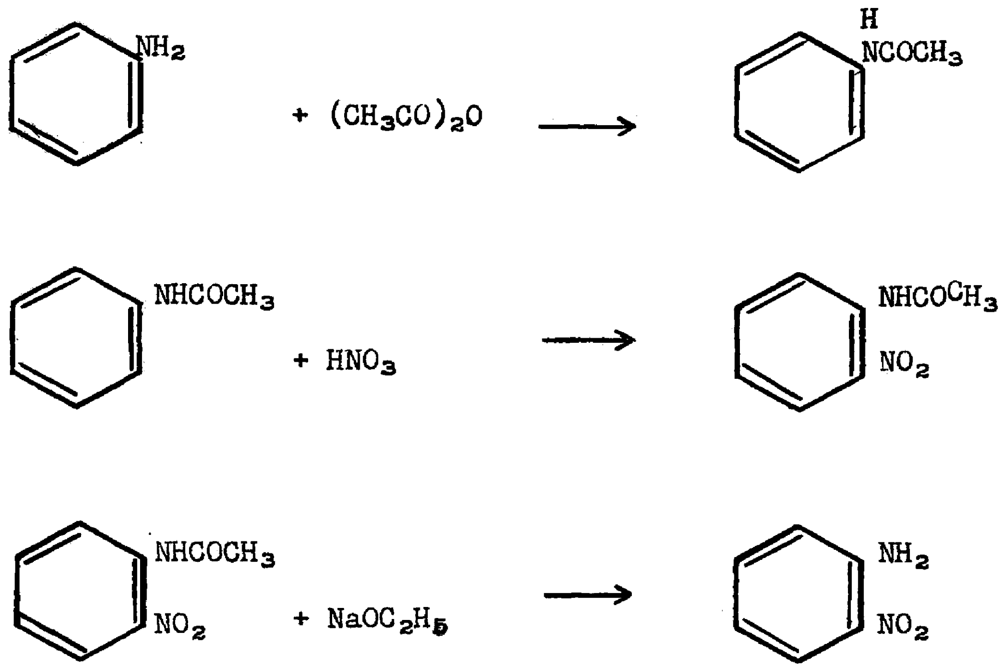
The β-propionic and α-propionic acid derivatives were prepared from the reaction of the 2-thiobenzimidazole with either β-bromopropionic acid or α-bromopropionic acid.

Preparation of 2-thiobenzimidazoles

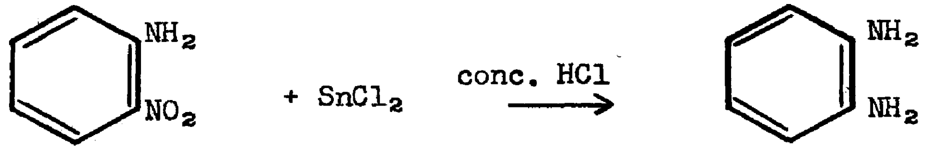


Preparation of o-phenylenediamines

4-Chloro- and 4-nitro-o-phenylenediamine were secured from Distillation Products industries. 1,3-Dichloro-4,5-diaminobenzene, 4-bromo-1,2-diaminobenzene and 4,5-dimethyl-1,2-diaminobenzene were prepared from 2,4-dichloroaniline, m-bromoaniline and 3,4-dimethylaniline, respectively.

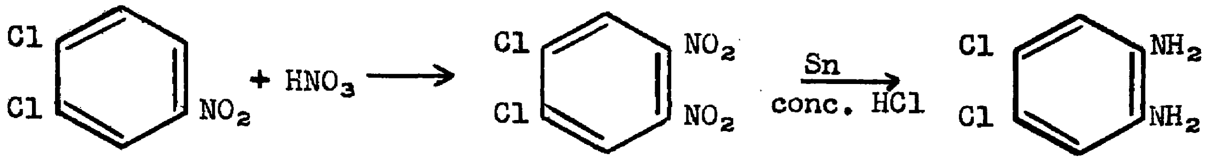


4-Methoxy-1,2-diaminobenzene, 4-phenyl-1,2-diaminobenzene, 4-methyl-1,2-diaminobenzene and 3,5-dimethyl-1,2-diaminobenzene were prepared from 2-nitro-4-methoxyaniline, 4-amino-3-nitrobiphenyl, 4-methyl-2-nitroaniline and 2,4-dimethyl-6-nitroaniline, respectively, as the starting material.

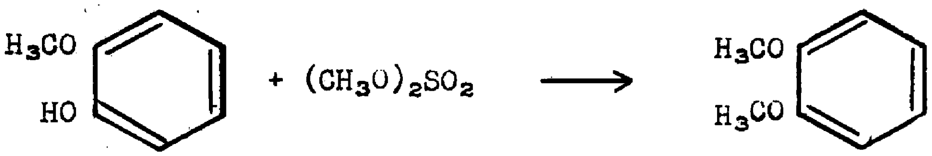


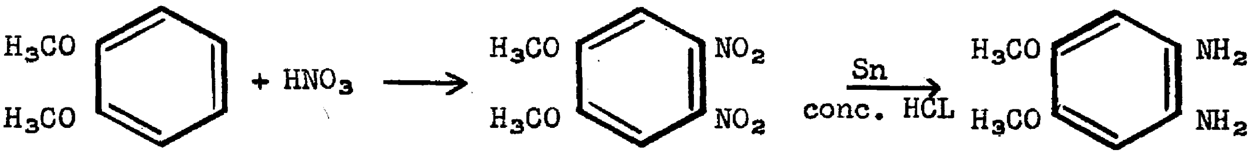
1,2-Dichloro-4,5-diaminobenzene and 1,2-dimethoxy-4,5-diaminobenzene were synthesized by starting with 1,2-dichloro-4-nitrobenzene and 2-methoxyphenol (guaiacol), respectively.

1,2-Dichloro-4,5-diaminobenzene

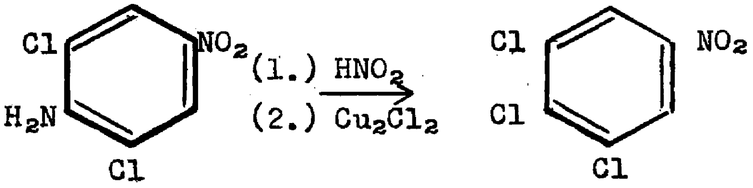


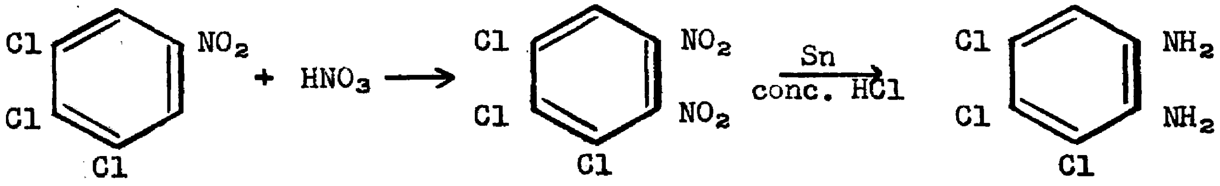
1,2-Dimethoxy-4,5-diaminobenzene





1,2,3-Trichloro-5,6-diaminobenzene was prepared from 2,6-dichloro-4-nitroaniline.





(2-Benzimidazolylthio)acetic acid

To a solution of 3.0 g. (0.02 mole) of 2-thiobenzimidazole in 20 ml. of 2 N sodium hydroxide was added 1.9 g. (0.02 mole) of monochloroacetic acid dissolved in 10 ml. of water. After gentle refluxing for one hour, the mixture was cooled and filtered. The filtrate was carefully acidified with dilute hydrochloric acid until the solution was acid to Congo red and placed in the refrigerator for crystallization. The material was collected on a filter and recrystallized from hot water. Two and one-half grams of fine needles having a melting point of 215° C. were obtained.[1]

Anal. Calcd. for $C_9H_8O_2N_2S$: C, 51.91; H, 3.87; N, 13.45. Found: C, 51.76; H, 3.62; N. 13.58.

β(2-Benzimidazolylthio)propionic acid

To a solution of 3 g. (0.02 mole) of 2-thiobenzimidazole in 20 ml. of 2 N sodium hydroxide was added 3.1 g. (0.02 mole) of β-bromopropionic acid. After refluxing for one hour, the solution was cooled and filtered. The clear filtrate was acidified with dilute hydrochloric acid until acid to Congo red, cooled and filtered. The precipitate on the filter was taken up in a small volume of hot ethanol and treated with Norite. Following filtration, an equal volume of distilled water was added; and after standing in the refrigerator, 0.9 g. of white needles with a melting point of 178-179° C. was obtained.[2]

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[1] Everett (12) gives 190° C. as the melting point of this compound whereas Stephan and Wilson (50) report 215° C.

[2] Stephan and Wilson (50) report 179° C.

Anal. Calcd. for $C_{10}H_{10}O_2N_2S$: C, 54.04; H, 4.54; N, 12.60. Found: C, 54.11; H, 4.83; N, 12.67.

α(2-Benzimidazolylthio)propionic acid

To a solution of 3 g. (0.02 mole) of 2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 3.1 g. (0.02 mole) of α-bromopropionic acid. After refluxing for one hour, the solution was cooled and filtered. The clear filtrate was made acid to Congo red with dilute hydrochloric acid, cooled and filtered. The precipitate was taken up in a minimum volume of hot ethanol and treated with Norite. After filtering, distilled water was added until a cloudiness persisted and crystallization allowed to proceed in the refrigerator. A yield of 1.8 g. of white needles was obtained which melted at 181-182° C.

Anal. Calcd. for $C_{10}H_{10}O_2N_2S$: C, 54.04; H, 4.54; N, 12.60. Found: C, 53.87; H, 4.48; N, 12.72.

(5-Chloro-2-benzimidazolylthio)acetic acid

5-Chloro-2-thiobenzimidazole. Twenty-one grams (0.15 mole) of 4-chloro-o-phenylenediamine was dissolved in 150 ml. of 95% ethanol containing 9.5 g. of potassium hydroxide dissolved in 25 ml. of water, and 11 ml. of carbon disulfide was added. After refluxing the mixture for three hours on the steam bath, 6 g. of Norite was cautiously added and the refluxing continued an additional 10 minutes. The hot mixture was filtered and diluted with 150 ml. of water heated to 70° C. The solution was acidified with 33% acetic acid and placed in the refrigerator for crystallization. The material was collected on a filter and recrystallized from aqueous ethanol. The yield of material melting at 295-297° C.[1] was 18 g.

(5-Chloro-2-benzimidazolylthio)acetic acid. A solution of 3.7 g. (0.02 mole) of 5-chloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and 1.9 g. (0.02 mole) of monochloroacetic acid in 10 ml. of water was boiled gently for one hour. After cooling, the solution was filtered and the filtrate made acid to Congo red with dilute hydrochloric acid. After standing in the refrigerator, the precipitate was removed by filtration and recrystallized from hot aqueous ethanol. Three and and three-tenth grams of material with a melting point of 193-194° C. was obtained.

Anal. Calcd. for $C_9H_7O_2N_2SCl$: C, 44.54; H, 2.91; N, 11.54. Found: C, 44.67; H, 3.25; N, 11.23.

α(5-Chloro-2-benzimidazolylthio)propionic acid

To a solution of 3.7 g. (0.02 mole) of 5-chloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 3.1 g. (0.02 mole) of α-bromopropionic acid. After refluxing for one hour, the mixture was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid and cooled in the refrigerator. After collecting the precipitate on a filter, it was dissolved in a small volume of hot ethanol, treated with Norite and filtered. Water was added to the hot alcohol solution until a permanent cloudiness was obtained and crystallization

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[1] Bywater *et al.* (8) give 295-297° C. as the melting point.

occurred upon standing in the refrigerator. The yield of white crystals melting at 167° C. was 2.0 g.

Anal. Calcd. for $C_{10}H_9O_2N_2SCl$: C, 46.78; H, 3.53; N, 10.91. Found: C, 46.55; H, 3.43; N, 10.92.

β(5-Chloro-2-benzimidazolylthio)propionic acid

To a solution of 3.7 g. (0.02 mole) of 5-chloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 3.1 g. (0.02 mole) of β-bromopropionic acid. After refluxing for one hour, the mixture was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid and placed in the refrigerator. The precipitate was filtered off and dissolved in a small volume of hot ethanol after which the ethanol solution was decolorized by boiling with a small amount of Norite. After filtering, distilled water was added to the hot alcohol solution until a permanent cloudiness was obtained and the solution placed in the refrigerator. Three and one-tenth grams of material was obtained which melted with decomposition at 103-105° C.

Anal. Calcd. for $C_{10}H_9O_2N_2SCl$: C, 46.78; H, 3.53; N, 10.91. Found: C, 46.58; H, 3.73; N, 11.23

(4,6-Dichloro-2-benzimidazolylthio)acetic acid

2,4-Dichloroacetantilide. To a mixture of 40.8 g. (0.4 mole) of acetic anhydride and 40.8 g. of glacial acetic acid was slowly added 48.6 g. (0.3 mole) of 2,4-dichloroaniline. After refluxing the mixture for one hour, it was poured into 200 ml. of water. Upon cooling the precipitate was removed by filtration and recrystallized by dissolving in a minimum volume of boiling methanol, filtering and adding water until a permanent cloudiness persisted. After standing in the refrigerator, 47 g. of crystals were obtained which melted at 143° C.[1]

4-Amino-5-nitro-1,3-dichlorobenzene. In a mixture of 46 ml. of concentrated sulfuric acid and 16.2 ml. of glacial acetic acid was suspended with stirring 47 g. of 2,4-dichloroacetanilide. After cooling to 20° C., a mixture containing equal volumes (16.2 ml. of each) of concentrated nitric and concentrated sulfuric acids was added dropwise over a period of 15 minutes. During this period the temperature was allowed to rise to 40° C. and the mixture was maintained at this temperature with occasional cooling as long as the temperature had a tendency to rise. After two hours the yellow solution was poured into cracked ice and placed in the refrigerator overnight. The precipitate was collected on a filter and recrystallized from hot ethanol yielding 20 g. of crystals. The nitro compound was further purified by vacuum sublimination. The sublimed product appeared as yellow needles which melted at 100-101° C.[2]

4,5-Diamino-1,3-dichlorobenzene. Four and seventy-six hundredths g. of 4-amino-5-nitro-1,3-dichlorobenzene were added in small portions with stirring to 24 g. of stannous chloride dissolved in 30 ml. of concentrated hydrochloric acid heated on the steam bath. At the beginning the material dissolved and was decolorized; but after about two-thirds of the nitroamine had been added, a white precipitate appeared. The stirring was continued an additional hour, after which time the mixture was cooled

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[1] Blas and Arimany (6) give a melting point of 143° C.

[2] Datta and Mitter report the melting point as 100° C.

and made alkaline with 6 N sodium hydroxide. The mixture was extracted with three 50 ml. portions of diethyl ether and the combined ether extracts were dried over anhydrous sodium sulfate. The ether was removed from the drying agent by means of filtration and most of the solvent removed by distillation. Normal-hexane was added to the concentrated ether solution and the diamine crystallized out upon cooling. The yield of diamine melting at 56-7° C. was three grams[1].

4,6-Dichloro-2-thiobenzimidazole. Seven g. of 4,5-diamino-1,3-dichlorobenzene was dissolved in 45 ml. of 95% ethanol to which was added 2.9 g. of potassium hydroxide dissolved in 7 ml. of water. Three and two-tenths ml. of carbon disulfide was cautiously added and the mixture refluxed for two hours on the steam bath. After slight cooling, Norite was carefully added and the refluxing was resumed for an additional ten minutes. The Norite was removed by filtration and the hot filtrate added to 50 ml. of warm distilled water (60-70° C.). The solution was acidified with dilute acetic acid and allowed to stand overnight in the refrigerator. Seven grams of material was obtained which did not melt below 300° C.

(4,6-Dichloro-2-benzimidazolylthio)acetic acid. Two and two-tenths g. (0.01 mole) of 4,6-dichloro-2-thiobenzimidazole was suspended in 10 ml. of 2 N sodium hydroxide to which was added 1 g. of monochloroacetic acid dissolved in 5 ml. of water. After gentle refluxing for one hour, the solution was cooled and filtered. The clear solution was made acid to Congo red with dilute hydrochloric acid and placed in the refrigerator overnight. The precipitate was collected on a filter and dissolved in a

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[1] Witt (65) gives 60.5° C. as the melting point.

small volume of boiling ethanol. After treating with Norite, water was added to the filtered solution until a cloudiness persisted, and the solution placed in the refrigerator for crystallization. The crystallization from aqueous ethanol was repeated a second time yielding 0.7 g. of purified material melting with decomposition at 222-224° C.

Anal. Calcd. for $C_9H_6O_2N_2SCl_2$: C, 39.00; H, 2.18; N, 10.11. Found: C, 39.34; H, 2.36; N, 10.09.

(5,6-Dichloro-2-benzimidazolylthio)acetic acid

1,2-Dichloro-4,5-dinitrobenzene (29). To a mixture of 160 ml. of concentrated sulfuric acid and 106 ml. of fuming nitric acid (sp. gr. 1.5) was carefully added 40 g. of 1,2-dichloro-4-nitrobenzene and the mixture stirred and heated for six hours at 110° C. After cooling by the addition of 200 g. of cracked ice, the precipitate which formed was collected on a filter and washed with cold water until the washings were no longer acid to litmus paper. Forty-five g. of crude material was obtained. After recrystallization first from ethanol solution and then twice from glacial acetic acid, the 1,2-dichloro-4,5-dinitrobenzene melted at 95-97° C.

1,2-Dichloro-4,5-diaminobenzene (67). Thirty g. of 1,2-dichloro-4,5-dinitrobenzene was suspended in 200 ml. of concentrated hydrochloric acid and heated with stirring on the steam bath while 50 g. of mossy tin was added in small portions. After all of the tin had been added, the mixture was heated an additional thirty minutes. Following the addition of 350 ml. of water and 5 g. of Norite, the mixture was filtered while still hot and the filtrate concentrated under reduced pressure until crystallization began to occur (150 ml.). After cooling and saturating the mixture with hydrogen chloride gas, the precipitate was collected on a filter and washed with cold concentrated hydrochloric acid. The amine hydrochloride was dissolved in water and an excess of 3 N sodium hydroxide added to free the base. The free diamine was extracted from the mixture with three 100 ml. portions of diethyl ether. After drying the combined ether extracts with anhydrous sodium sulfate and removing the drying agent by filtration, the ether was removed by distillation. The diamine was recrystallized from hot water yielding 10 g. of material which melted with decomposition at 158-160° C.

5,6-Dichloro-2-thiobenzimidazole. A mixture of 9 g. of 1,2-dichloro-4,5-diaminobenzene, 50 ml. of 95% ethanol, 3.1 g. of potassium hydroxide dissolved in 10 ml. of water, and 4 ml. of carbon disulfide was refluxed 3 hours on the steam bath. Norite was then cautiously added and the refluxing continued 10 minutes longer. Following filtration, the clear solution was added to 60 ml. of hot water and acidified with dilute acetic acid. After standing overnight in the refrigerator, 9 g. of material was collected. The material was recrystallized from hot aqueous ethanol and yielded 9 g. of 5,6-dichloro-2-thiobenzimidazole which did not melt below 300° C.

(5,6-Dichloro-2-benzimidazolylthio)acetic acid. To a solution of 2.2 g. (0.01 mole) of 5,6-dichloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1 g. of monochloroacetic acid dissolved in 5 ml. of water. After refluxing for 1 hour, the solution was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid and placed in the refrigerator. The precipitate was collected on a filter and dissolved in a small volume of hot methanol. After treating with Norite and filtering, distilled water was added to the hot filtrate until a permanent cloudiness was obtained. Crystallization occurred upon standing in the refrigerator. Two and two-tenths gram of material which melted with decomposition at 219-221° C. was obtained.

Anal. Calcd. for $C_9H_6O_2N_2SCl_2$: C, 39.00; H, 2.18; N, 10.11. Found: C, 39.38; H, 2.41; N, 10.08.

α(5,6-Dichloro-2-benzimidazolylthio)propionic acid

To a solution of 2.2 g. (0.01 mole) of 5,6-dichloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1.7 g. of α-bromopropionic acid dissolved in 5 ml. of water. The solution was refluxed for 1 hour after which time it was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid, cooled in the refrigerator and the precipitate recrystallized from hot aqueous methanol. The yield of recrystallized α(5,6-dichloro-2-benzimidazolylthio)propionic acid decomposing at 230-231° C. was 2.0 g.

Anal. Calcd. for $C_{10}H_8O_2N_2SCl_2$: C, 41.25; H, 2.77; N, 9.62. Found: C, 41.28; H, 3.02; N, 9.69.

(4,5,6-Trichloro-2-benzimidazolylthio)acetic acid

<u>1,2,3-Trichloro-5-nitrobenzene</u>. Twenty and seven-tenth g. (0.1 mole) of 2,6-dichloro-4-nitroaniline was dissolved in 250 ml. of hot glacial acetic acid and the solution cooled rapidly during which time the material began to precipitate. The mixture was gradually added with stirring into a

cooled solution of 7.6 g. of sodium nitrite dissolved in 53 ml. of concentrated sulfuric acid maintaining the temperature of the mixture below 20° C. during the addition. The sodium nitrite solution was prepared by slowly adding pulverized sodium nitrite to cooled and vigorously stirred concentrated sulfuric acid. After two hours the cooled diazonium compound was stirred into a solution of cuprous chloride dissolved in concentrated hydrochloric acid. The mixture was heated at 90° C. on the steam bath for two hours and after cooling the crystals of 1,2,3-trichloro-5-nitrobenzene were removed by filtration. The material was further purified by recrystallization from aqueous acetic acid. The yield of material melting at 65° C.[1] was 13 g.

The cuprous chloride was prepared by adding 11 g. of sodium bisulfite and 7 g. of sodium hydroxide dissolved in 80 ml. of water to a stirred solution of 50 g. of cupric sulfate pentahydrate and 13 g. of sodium chloride in 160 ml. of warm water. After cooling to room temperature, the liquid was decanted from the precipitated cuprous chloride and the solid washed several times by suspension in water and removing the water by decantation. The cuprous chloride was obtained as a white powder and was dissolved in 400 ml. of concentrated hydrochloric acid.

<u>1,2,3-Trichloro-5,6-dinitrobenzene (20)</u>. To a stirred mixture of 65 g. of nitric acid (sp. gr. 1.52) and 65 g. of concentrated sulfuric acid was added in small portions 13 g. of 1,2,3-trichloro-5-nitrobenzene. The stirring was continued while the mixture was heated an additional hour on the steam bath. After cooling, the nitrating mixture was poured onto

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[1] Bezzubete and Rozina (4) give 68-69° C. for the melting point of 1,2,3-trichloro-5-nitrobenzene.

400 g. of crushed ice and allowed to stand in the cold overnight. The nitration product was filtered off and washed thoroughly with cold water. After several recrystallizations from hot 95% ethanol, 13 g. of 1,2,3-trichloro-5,6-dinitrobenzene was obtained which melted at 105-106° C.

1,2,3-Trichloro-5,6-diaminobenzene. Twenty-six and eight-tenth g. (0.1 mole) of 1,2,3-trichloro-5,6-dinitrobenzene was suspended in 155 ml. of concentrated hydrochloric acid. While the mixture was stirred and heated on the steam bath, 47.6 g. (0.4 mole) of mossy tin was cautiously added in small portions. After all of the tin had been added, the mixture was heated an additional hour on the steam bath during which time the suspension turned brown in color. After cooling, the mixture was made basic to litmus paper with 6 N sodium hydroxide and the crude diamine extracted with two 250 ml. portions of diethyl ether. The ether extract was washed with distilled water and dried over anhydrous sodium sulfate. After removing the sodium sulfate by filtration, the ether was distilled under reduced pressure. The yield of crude diamine was 15 g. After recrystallization from hot water, 5 g. of purified material melting at 105-106° C. was obtained.

Anal. Calcd. for $C_6H_5N_2Cl_3$: N, 13.24. Found: N, 13.16.

4,5,6-Trichloro-2-thiobenzimidazole. Five g. of 1,2,3-trichloro-5,6-diaminobenzene was dissolved in 75 ml. of 95% ethanol along with 2 g. of potassium hydroxide dissolved in 10 ml. of water. After the careful addition of 4 ml. of carbon disulfide, the mixture was refluxed for three hours on the steam bath. One gram of Norite was added to the slightly cooled solution and the refluxing resumed for an additional 10 minutes. Following filtration, the hot filtrate was added to an equal volume of warm water and made acid with dilute acetic acid. After standing overnight in the refrigerator, 6.5 grams of product which did not melt below 300° C. was collected.

4,5,6-Trichloro-2-benzimidazolylthio)acetic acid. A mixture of 2.53 g. (0.01 mole) of 4,5,6-trichloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and 1 g. of monochloroacetic acid was refluxed one hour. After cooling and filtering, the solution was made acid to Congo red with dilute hydrochloric acid. The crude acid which separated after standing in the cold was filtered off and recrystallized from hot aqueous methanol. The yield of purified product was 1.3 g. of compound which melted with decomposition at 205-207° C.

Anal. Calcd. for $C_9H_6O_2N_2SCl_3$: C, 34.69; H, 1.62; N, 8.99. Found: C, 34.75; H, 1.89; N, 9.33.

α(4,5,6-Trichloro-2-benzimidazolylthio)propionic acid

Two and fifty-three hundreth g. (0.01 mole) of 4,5,6-trichloro-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and 1.7 g. of α-bromopropionic acid was refluxed for one hour. After the solution was cooled and filtered, the filtrate was made acid to Congo red with dilute hydrochloric acid. The mixture was placed in the refrigerator and the following day the precipitated acid was removed by filtration. After recrystallization from hot aqueous methanol, 2.6 g. of material was obtained. The purified acid melted with decomposition at 222-224° C.

Anal. Calcd. for $C_{10}H_7N_2O_2SCl_3$: C, 36.88; H, 2.17; N, 8.60. Found: C, 37.09; H, 2.11; N, 8.84.

(5-Nitro-2-benzimidazolylthio)acetic acid

5-Nitro-2-thiobenzimidazole. To a solution of 23 g. (0.15 mole) of 4-nitro-o-phenylenediamine in 250 ml. of 95% ethanol and 9.5 g. of potassium hydroxide in 25 ml. of water was added 11 ml. of carbon disulfide. After refluxing for 3 hours, 6 g. of Norite was cautiously added and the mixture refluxed an additional 10 minutes. The solution was filtered and the hot filtrate added to 150 ml. of warm water (60-70° C.). The solution was then acidified with dilute acetic acid and placed in the refrigerator for crystallization to occur. The orange precipitate was collected on a filter and washed with cold distilled water. After recrystallization from hot aqueous ethanol, the yield of 5-nitro-2-thiobenzimidazole was 16.5 g. This material did not melt below 300° C.

(5-Nitro-2-benzimidazolylthio)acetic acid. To a solution of 3.5 g. of 5-nitro-2-thiobenzimidazole dissolved in 25 ml. of 3 N sodium hydroxide was added 3.1 g. of monochloroacetic acid dissolved in 10 ml. of water. After refluxing for 2 hours, the solution was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid and after standing in the refrigerator, the bright yellow precipitate was collected on a filter. The precipitate was recrystallized from hot aqueous ethanol yielding 3.5 g. of crystals which softened at 92-94° C., resolidified at a somewhat higher temperature, and finally melted with decomposition at 191-193° C.

Anal. Calcd. for $C_9H_7O_4N_4S$: C, 42.68; H, 2.79; N, 16.59. Found: C, 42.54; H, 2.96; N, 16.34.

α(5-Nitro-2-benzimidazolylthio)propionic acid

To a solution of 3.5 g. of 5-nitro-2-thiobenzimidazole dissolved in 25 ml. of 3 N sodium hydroxide was added 3.1 g. of α-bromopropionic acid dissolved in 15 ml. of distilled water. After refluxing for 2 hours, the solution was cooled and filtered. The filtrate was made acid to Congo red with dilute hydrochloric acid and the resulting mixture placed in the refrigerator to cool. The yellow precipitate was removed by filtration, washed with cold water and recrystallized from hot aqueous ethanol. The yield of purified α(5-nitro-2-benzimidazolylthio)propionic acid which melted with decomposition at 186-188° C. was 2.8 g.

Anal. Calcd. for $C_{10}H_9O_4N_3S$: C, 44.94; H, 3.39; N, 15.72. Found: C, 45.03; H, 3.15; N, 15.99.

(5-Methoxy-2-benzimidazolylthio)acetic acid

4-Methoxy-o-phenylenediamine (9). To a solution of 225 g. of stannous chloride dissolved in 450 ml. of concentrated hydrochloric acid was gradually added with stirring 42.9 g. of 2-nitro-4-methoxyaniline while maintaining the temperature of the reaction mixture below 20° C. When the addition was completed, the mixture was stirred at 20° C. for an additional 2 hours. Then the solution was made alkaline to litmus paper with 30% sodium hydroxide being careful to keep the temperature below 40° C. The alkaline mixture was extracted with three 100 ml. portions of benzene, the combined benzene extracts washed with water and finally dried over anhydrous sodium sulfate. The dried and filtered benzene solution was evaporated to dryness under reduced pressure and 15 g. of a deep purple oil was obtained. The crude diamine was not further purified.

5-Methoxy-2-thiobenzimidazole. Fifteen g. of the crude 4-methoxy-o-phenylenediamine was dissolved in 100 ml. of 95% ethanol along with 6.6 g. of potassium hydroxide dissolved in 20 ml. of water. Eight ml. of carbon disulfide was cautiously added and the mixture refluxed for 3 hours. After slight cooling, 2 to 3 g. of Norite was carefully added and the refluxing resumed for 10 minutes. The hot mixture was filtered and the filtrate added to 100 ml. of hot water (60-70° C.). Following the acidification of the mixture with dilute acetic acid, it was placed in the refrigerator for crystallization. Ten grams of material melting at 266° C. was collected on a filter.[1]

(5-Methoxy-2-benzimidazolylthio)acetic acid. To a solution of 1.8 g. (0.01 mole) of 5-methoxy-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1 g. of monochloroacetic acid dissolved in 10 ml. of water and the resulting mixture was refluxed for 1 hour. The solution was then cooled, filtered, and made acid to Congo red with 6 N hydrochloric acid. After standing in the cold, the precipitate was filtered off and washed with cold water. The yield of (5-methoxy-2-benzimidazolylthio)acetic acid recrystallized from aqueous ethanol and melting with decomposition at 194-196° C. was 1.2 g.

Anal. Calcd. for $C_{10}H_{10}O_3N_2S$: C, 50.41; H, 4.23; N, 11.76. Found: C, 50.79; H, 4.21; N, 11.77.

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[1] Bywater *et al.* (8) gives the melting point of 5-methoxy-2-mercaptobenzimidazole as 261-263° C.

α(5-Methoxy-2-benzimidazolylthio)propionic acid

One and eight-tenth g. (0.01 mole) of 5-methoxy-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and 1.8 g. of α-bromopropionic acid were refluxed for 1 hour. After cooling and filtering, the solution was made acid to Congo red with 6 N hydrochloric acid and placed in the refrigerator. The precipitate was collected on a filter and recrystallized from aqueous alcohol. The yield of α(5-methoxy-2-benzimidazolylthio)propionic acid which melted with decomposition at 151-152° C. was 1.8 g.

Anal. Calcd. for $C_{11}H_{12}O_3N_3S$: C, 52.36; H, 4.79; N, 11.10. Found: C, 52.63; H, 4.85; N, 11.07.

(5,6-Dimethoxy-2-benzimidazolylthio)acetic acid

1,2-Dimethoxybenzene (veratrole) (1). Forty-one g. of potassium hydroxide dissolved in 60 ml. of water was added at the rate of 2 drops per second to 62 g. of rapidly stirred 2-methoxyphenol (guaiacol). Twenty seconds after the addition had started, 80 g. of methyl sulfate (redistilled and neutralized with potassium carbonate) was added at the same rate. The reaction mixture turned a reddish-brown in color. As soon as all of the reactants had been added, the mixture was poured into a beaker and diluted with 200 ml. of distilled water. The oil which separated was extracted with two 100 ml. portions of diethyl ether. The ether was washed several times with water and dried over anhydrous sodium sulfate. After removing the sodium sulfate by filtration, the ether was removed by distillation and the residue distilled at atmosphere pressure. That fraction which boiled at 205-207° C. was collected and consisted of 70 g. of veratrole.

1,2-Dinitro-4,5-Dimethoxybenzene (11). Sixty-five g. of veratrole was added dropwise with stirring over a period of 80 minutes to 192 ml. of concentrated nitric acid (sp. gr. 1.42) cooled to 0 to -3° C. After continuing the stirring for another 5 minutes, the temperature was permitted to rise to 3-5° C. and 105 ml. of concentrated sulfuric acid was run in slowly over a period of 1 hour while maintaining this temperature. After stirring an additional 15 minutes, the mixture was warmed up gradually (20 minutes time) to 54-55° C., kept at this temperature for 10 minutes, and then the temperature increased over a period of 5 minutes to 58-60° C. and held at that point for 10 minutes. A canary yellow slurry resulted which in 5 minutes was cooled to 23-25° C., then slowly poured into a mixture of 200 g. of crushed ice and 200 ml. of water and the mixture diluted with water to a volume of 2500 ml. The product was a pale yellow liquid containinga deposit of dull yellow crystals. After occasional stirring for 30 minutes, the solid was removed by filtration and washed repeatedly with cold water until the last traces of acid had been removed and finally dried at 50-52° C. overnight in a vacuum oven. A yield of 97 g. of crude material was obtained. The 4,5-dimethoxy-1,2-dinitrobenzene recrystallized from alcohol melted at 130-132° C.

4,5-Dimethoxy-1,2-diaminobenzene (15). Twenty-nine g. of 4,5-dimethoxy-1,2-dinitrobenzene was suspended in 200 ml. of concentrated hydrochloric acid and heated on the steam bath. With occasional vigorous shaking, 50 g. of mossy tin was added in small portions. After all of the tin had been added, the dark reddish-brown mixture was heated an additional hour during which time the solution was complete. Three hundred and fifty ml. of water and 5 g. of Norite were added and the hot mixture filtered. The solution was concentrated under reduced pressure to a volume of 150 ml., cooled and saturated with hydrogen chloride gas. After additional cooling, the precipitate was filtered off and washed with cold concentrated hydrochloric acid. The material was dissolved in 200 ml. of water and carefully neutralized with sodium carbonate to free the organic base. The alkaline mixture was placed in a continuous liquid-liquid extractor and extracted with chloroform until extraction of the diamine was complete. The chloroform extract was dried with anhydrous sodium sulfate and the drying agent removed by filtration. Upon distillation of the chloroform under reduced pressure using nitrogen gas for agitation, a dark reddish-brown solid remained. The yield of the crude 4,5-dimethoxy-1,2-diaminobenzene was 10 g. and melted at 115-120° C. The material was not further purified. Frisch and Bogert (15) report a melting point of 131° C. for the purified diamine.

<u>4,5-Dimethoxy-2-thiobenzimidazole</u>. Ten g. (0.06 mole) of crude 4,5-dimethoxy-1,2-diaminobenzene was dissolved in 60 ml. of 95% ethanol to which had been added 4 g. of potassium hydroxide dissolved in 10 ml. of distilled water. Four ml. of carbon disulfide was cautiously added and the mixture was refluxed 3 hours on the steam bath. After cooling slightly, 2 g. of Norite was added and the mixture refluxed an additional 10 minutes. Following the filtration of the hot mixture, the solution was added to 60 ml. of hot water (60-70° C.) and acidified with dilute acetic acid. After cooling in the refrigerator, 8 g. of 4,5-dimethoxy-2-thiobenzimidazole was collected on a filter. The material which was recrystallized from aqueous ethanol melted with decomposition at 285-287° C.

(5,6-Dimethoxy-2-benzimidazolylthio)acetic acid

To a solution of 2.1 g. (0.01 mole) of 5,6-dimethoxy-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1 g. of monochloroacetic acid dissolved in 10 ml. of distilled water. The solution was refluxed for 1 hour, cooled and filtered. After making acid to Congo red with 6 N hydrochloric acid, the mixture was cooled overnight in the refrigerator. The precipitate was collected on a filter, dissolved in a minimum volume of hot ethanol, treated with Norite, and filtered. Distilled water was added to the hot alcohol solution until a permanent cloudiness was obtained, and the mixture placed in the refrigerator for crystallization. The yield of recrystallized (5,6-dimethoxy-2-benzimidazolylthio)acetic acid melting at 241-243° C. with decomposition was 0.6 g.

Anal. Calcd. for $C_{11}H_{12}O_4N_2S$: C, 49.24; H, 4.51; N, 10.44. Found: C, 49.24; H, 4.69; N, 10.48.

(5-Phenyl-2-benzimidazolylthio)acetic acid

3,4-Diaminobiphenyl (2). Sixty-eight g. of stannous chloride was suspended in 125 ml. of concentrated hydrochloric acid. The solution was cooled and with vigorous stirring, 21.4 g. of 4-amino-3-nitrobiphenyl was added in small quantities at such a rate that the temperature did not rise above 25° C. The stirring was continued an additional 2 hours during which time the red suspension became white. The mixture was poured slowly into 300 ml. of 6 N sodium hydroxide being careful to keep the temperature below 40° C. The free diamine was extracted from the mixture with three

200 ml. portions of diethyl ether. The ether was washed with distilled water and dried over anhydrous sodium sulfate. After filtering, the ether was removed under reduced pressure leaving 15 g. of the crude diamine. The melting point of the 3,4-diaminobiphenyl recrystallized from ethanol was 103° C.

5-Phenyl-2-thiobenzimidazole. To a solution of 15 g. of 3,4-diaminobiphenyl dissolved in 175 ml. of 95% ethanol containing 5.2 g. of potassium hydroxide dissolved in 10 ml. of water was added 7 ml. of carbon disulfide. The mixture was refluxed 3 hours on the steam bath, Norite added to the slightly cooled solution, and the refluxing resumed an additional 10 minutes. After filtering the hot solution, it was added to 75 ml. of hot water and made acid with dilute acetic acid. Following standing overnight in the refrigerator, the precipitate was collected on a filter and washed with cold water. The yield of crude 5-phenyl-2-thiobenzimidazole was 18 g. The product recrystallized from aqueous ethanol did not melt below 300° C.

(5-Phenyl-2-benzimidazolylthio)acetic acid. Two and three-tenth g. (0.01 mole) of 5-phenyl-2-thiobenzimidazole was dissolved in 30 ml. of 2 N sodium hydroxide to which was added 1 g. of monochloroacetic acid dissolved in 10 ml. of water. After refluxing the solution for 1 hour, it was cooled, filtered, acidified to Congo red with 6 N hydrochloric acid, and placed in the refrigerator. The precipitate was collected on a filter and washed with cold water. The yield of (5-phenyl-2-benzimidazolylthio)acetic acid recrystallized from boiling aqueous methanol was 2.1 g. This compound melted with decomposition at 220-221° C.

Anal. Calcd. for $C_{15}H_{12}O_2N_2S$: C, 63.36; H, 4.25; N, 9.62. Found: C, 63.40; H, 4.21; N, 9.79.

α(5-Phenyl-2-benzimidazolylthio)propionic acid

To 2.3 g. (0.01 mole) of 5-phenyl-2-thiobenzimidazole dissolved in 30 ml. of 2 N sodium hydroxide was added 1.7 g. of α-bromopropionic acid dissolved in 10 ml. of water. After refluxing for 1 hour, the cooled solution was filtered and made acid to Congo red with 6 N hydrochloric acid. The mixture was cooled in the refrigerator and the precipitate collected on a filter. After recrystallization from hot aqueous methanol, 2.6 g. of α(5-phenyl-2-benzimidazolylthio)propionic acid was obtained which melted with decomposition at 205-207° C.

Anal. Calcd. for $C_{16}H_{14}O_2N_2S$: C, 64.41; H, 4.73; N, 9.39. Found: C, 64.16; H, 4.52; N, 9.35.

(5-Bromo-2-benzimidazolylthio)acetic acid

m-Bromoacetanilide. To a mixture of 81 g. of acetic anhydride and 81 ml. of glacial acetic acid was added slowly 100 g. (0.58 mole) of m-bromoaniline. The mixture was refluxed one hour and after cooling was poured into 500 ml. of water. Crystallization occurred upon standing in the refrigerator. The yield of the acetanilide recrystallized from methanol-water was 64.5 g. Meta-bromoacetanilide had a melting point of 87° C.[1]

2-Nitro-5-bromoacetanilide (34). A solution of 64.5 g. of m-bromoacetanilide in a mixture of 60 g. of acetic anhydride and 27 g. of glacial

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[1] Blanksma (5) reports the melting point of m-bromoacetanilide as 87.5° C.

acetic acid was cooled to 0° C. The temperature was held between 0° and -5° C. while a mixture of 27 g. of glacial acetic acid and 30 g. of fuming nitric acid (sp. gr. 1.5) was slowly added. The mixture was allowed to stand overnight at room temperature after which time it was poured into 1000 g. of crushed ice. The precipitate was filtered off and washed thoroughly with cold water. The yield of the crude nitration product was 64 g. This material was stirred with two 250 ml. portions of benzene and the insoluble material filtered off. The benzene was removed by distillation and the residue recrystallized from 95% ethanol. The yield of 2-nitro-5-bromoacetanilide which melted at 135° C. was 15 g.

2-Nitro-5-bromoaniline (65). A solution of 0.1 g. of metallic sodium in 240 ml. of absolute methanol was added to 24 g. of 2-nitro-5-bromoacetanilide and the solution boiled under reflux for 3 hours. The solvent was removed under reduced pressure and the residue recrystallized from methanol. The yield of bright yellow needles recrystallized once from methanol was 15 g. The 2-nitro-5-bromoaniline melted at 149-150° C.

4-Bromo-1,2-diaminobenzene. Sixty-eight grams (0.3 mole) of stannous chloride was dissolved in 125 ml. of concentrated hydrochloric acid. The mixture was cooled in an ice bath while 21 g. (0.1 mole) of 2-nitro-5-bromoaniline was added in small portions at such a rate that the temperature did not rise above 20° C. After an additional 2 hours of stirring, the mixture became colorless. The suspension was slowly added to a stirred solution of 250 ml. of 30% sodium hydroxide with cooling at such a rate that the temperature did not exceed 40° C. The alkaline mixture was extracted with three 200 ml. portions of diethyl ether, the combined ether extracts washed with water, and dried over anhydrous sodium sulfate. After removing the drying agent by means of filtration, the ether was removed under reduced pressure leaving a yellowish-brown oil which solidified upon standing in the refrigerator. The material was not further purified and consisted of 17 g. of melting point 60° C.[1]

5-Bromo-2-thiobenzimidazole. Fourteen g. of the crude 4-bromo-1,2-diaminobenzene was dissolved in 30 ml. of 95% ethanol containing 5 g. of potassium hydroxide dissolved in 15 ml. of distilled water. To this solution was carefully added 6.5 ml. of carbon disulfide and the mixture was refluxed for 3 hours on the steam bath. After slight cooling, 2 g. of Norite was cautiously added and the refluxing resumed an additional 5 minutes. The hot mixture was filtered and the filtrate added to 100 ml. of hot distilled water. After making acid with dilute acetic acid, the mixture was placed in the refrigerator for crystallization. The yield of crude 5-bromo-2-thiobenzimidazole was 13 g. The compound recrystallized from aqueous ethanol did not melt below 300° C.[2]

(5-Bromo-2-benzimidazolylthio)acetic acid. Two and three-tenth g. (0.01 mole) of 5-bromo-2-thiobenzimidazole was dissolved in 30 ml. of 2 N sodium hydroxide. One g. of monochloroacetic acid dissolved in 10 ml. of water was added and the mixture refluxed 1 hour. After cooling, the mixture was filtered and the filtrate made acid to Congo red with 6 N hydrochloric acid. Following cooling in the refrigerator, the precipitate was collected on a filter and recrystallized from aqueous methanol. The yield of recrystallized (5-bromo-2-benzimidazolylthio)acetic

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[1] Hubner (21) reports the melting point of 4-bromo-1,2-diaminobenzene as 63° C.

[2] Bywater *et al.* (8) give the melting point for this compound as 300-301° C.

acid was 2.0 g. This material melted with decomposition at 194-196° C.

Anal. Calcd. for $C_9H_7O_2N_2SBr$: C, 37.64; H, 2.46; N, 9.76. Found: C, 37.86; H, 2.68; N, 9.84.

α(5-Bromo-2-benzimidazolylthio)propionic acid

Two and three-tenth g. (0.01 mole) of 5-bromo-2-thiobenzimidazole was dissolved in 30 ml. of 2 N sodium hydroxide. To this solution was added 1.7 g. (0.01 mole) of α-bromopropionic acid and the mixture refluxed 1 hour. After cooling, the reaction mixture was filtered and the filtrate made acid to Congo red with 6 N hydrochloric acid. Following cooling in the refrigerator, the precipitate was collected on a filter and recrystallized from hot aqueous methanol. The yield of α(5-bromo-2-benzimidazolylthio)propionic acid was 1.3 g. The pure compound melted with decomposition at 184-185° C.

Anal. Calcd. for $C_{10}H_9O_2N_2SBr$: C, 39.88; H, 3.01; N, 9.30. Found: C, 39.43; H, 2.89; N, 9.45.

(5-Methyl-2-benzimidazolylthio)acetic acid

4-Methyl-1,2-diaminobenzene. Crystalline 4-methyl-2-nitroaniline (38.6 g.) was gradually added with stirring to a solution of 225 g. of stannous chloride dissolved in 450 ml. of concentrated hydrochloric acid. The temperature of the reaction mixture was kept below 20° C. during the addition. After all of the material had been added, the mixture was stirred an additional 2 hours and then slowly added to 1000 ml. of 30% sodium hydroxide while maintaining the temperature below 40° C. during the addition. The alkaline mixture was extracted with three 150 ml.

portions of benzene and the combined benzene extracts washed with distilled water. After drying the benzene solution over anhydrous sodium sulfate, the drying agent was removed by filtration and the benzene removed by distillation under reduced pressure. There remained a residue of 21 g. of crude 4-methyl-1,2-diaminobenzene. The crude material melted at 80° C.[1]

5-Methyl-2-thiobenzimidazole. A mixture of 21 g. (0.173 mole) of crude 4-methyl-1,2-diaminobenzene dissolved in 150 ml. of 95% ethanol, 9.5 g. of potassium hydroxide in 25 ml. of water, and 11 ml. of carbon disulfide was refluxed 3 hours on the steam bath. After cooling slightly, 5 g. of Norite was cautiously added and the refluxing resumed for an additional 10 minutes. The hot mixture was filtered and the filtrate added to 200 ml. of warm water. Following neutralization with dilute acetic acid, the mixture was placed in the refrigerator for crystallization. The precipitate was collected on a filter and washed with cold water. The yield of 5-methyl-2-thiobenzimidazole recrystallized from aqueous ethanol was 20 g. The melting point of the compound was 287° C.[2]

(5-Methyl-2-benzimidazolylthio)acetic acid. Three and three-tenth g. (0.02 mole) of 5-methyl-2-thiobenzimidazole was dissolved in 40 ml. of 2 N sodium hydroxide. A solution of 1.9 g. of monochloroacetic acid dissolved in 10 ml. of water was added and the mixture refluxed 1 hour. After cooling, the mixture was filtered and the filtrate made acid to

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[1] Noelting and Stoecklin (39) give 88.5° for the melting point of pure 4-methyl-1,2-diaminobenzene.

[2] Lellmann (31) gives for the melting point of 5-methyl-2-thiobenzimidazole, 284-285° C.

Congo red with 6 N hydrochloric acid. The mixture was cooled in the refrigerator and the precipitate collected on a filter. Following recrystallization from aqueous ethanol, the yield of (5-methyl-2-benzimidazolylthio)acetic acid was 2.1 g. This compound melted with decomposition at 197-200° C.

Anal. Calcd. for $C_{10}H_{10}O_2N_2S$: C, 54.04; H, 4.54; N, 12.60. Found: C, 54.00; H, 4.62; N, 12.61.

α(5-Methyl-2-benzimidazolylthio)acetic acid

One and six-tenth g. (0.01 mole) of 5-methyl-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and 1.7 g. of α-bromopropionic acid was refluxed 1 hour. After cooling, the reaction mixture was filtered and the filtrate made acid to Congo red with 6 N hydrochloric acid. The mixture was cooled overnight in the refrigerator and the precipitate collected on a filter. The yield of α(5-methyl-2-benzimidazolylthio)-propionic acid recrystallized from aqueous methanol was 1.3 g. This material melted with decomposition at 162-164° C.

Anal. Calcd. for $C_{11}H_{12}O_2N_2S$: C, 55.91; H, 5.12; N, 11.86. Found: C, 56.09; H, 5.47; N, 11.71.

(4,6-Dimethyl-2-benzimidazolylthio)acetic acid

3,5-Dimethyl-1,2-diaminobenzene. To a stirred and cooled solution of 136 g. (0.6 mole) of stannous chloride dissolved in 250 ml. of concentrated hydrochloric acid was added 33.2 g. (0.2 mole) of 2,4-dimethyl-6-nitroaniline at such a rate that the temperature did not exceed 20° C. After the addition was completed, the mixture was stirred an additional 4 hours during which time a light brown solution was obtained. The reaction mixture was gradually added to 560 ml. of 30% sodium hydroxide at such a rate that the temperature remained below 40° C. The alkaline mixture was extracted with three 250 ml. portions of diethyl ether, the ether extract washed with water, and dried over anhydrous sodium sulfate. After filtering the solution to remove the drying agent, the ether was removed under reduced pressure leaving 22 g. of crude 3,5-dimethyl-1,2-diaminobenzene. After recrystallization from hot water the diamine melted at 77° C.[1]

4,6-Dimethyl-2-thiobenzimidazole. Thirteen and six-tenth g. of 3,5-dimethyl-1,2-diaminobenzene was dissolved in 150 ml. of 95% ethanol containing 6.3 g. of potassium hydroxide dissolved in 15 ml. of water. To this solution was added 7 ml. of carbon disulfide following which the mixture was refluxed on the steam bath for 3 hours. After slight cooling, 2 g. of Norite was cautiously added and the refluxing resumed for an additional 10 minutes. The hot solution was filtered and the filtrate acidified with dilute acetic acid. After standing overnight in the refrigerator, the precipitate was collected on a filter. The yield of crude 4,6-dimethyl-2-thiobenzimidazole was 12.2 g. The compound recrystallized from aqueous ethanol did not melt below 300° C.

(4,6-Dimethyl-2-benzimidazolylthio)acetic acid. To a solution of 1.8 g. (0.01 mole) of 4,6-dimethyl-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1 g. of monochloroacetic acid dissolved in 10 ml. of water. The solution was refluxed an hour, after which time

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[1] Noelting and Thesmar (40) report the melting point of 3,5-dimethyl-1,2-diaminobenzene as 77-78° C.

it was cooled and filtered. The filtrate was made acid to Congo red with 6 N hydrochloric acid and placed in the refrigerator. The precipitate was collected on a filter, washed with cold water and recrystallized from aqueous ethanol. The yield of purified (4,6-dimethyl-2-benzimidazolylthio)acetic acid was 1.3 g. This material melted with decomposition at 247-249° C.

Anal. Calcd. for $C_{11}H_{12}O_2N_2S$: C, 55.91; H, 5.12; N, 11.86. Found: C, 56.01; H, 4.92; N, 11.85.

α(4,6-Dimethyl-2-benzimidazolylthio)propionic acid

One and eight-tenth g. (0.01 mole) of 4,6-dimethyl-2-thiobenzimidazole was dissolved in 20 ml. of 2 N sodium hydroxide. A solution of 1.7 g. of α-bromopropionic acid dissolved in 10 ml. of water was added and the mixture refluxed an hour. After cooling, the mixture was filtered and the filtrate made acid to Congo red with 6 N hydrochloric acid. The acid mixture was cooled in the refrigerator, the precipitate collected on a filter and washed with distilled water. The α(4,6-dimethyl-2-benzimidazolylthio)propionic acid was recrystallized from aqueous ethanol giving 1.6 g. of material which melted with decomposition at 160-161° C.

Anal. Calcd. for $C_{12}H_{14}O_2N_2S$: C, 57.58; H, 5.64; N, 11.19. Found C, 57.68; H, 5.99; N, 10.98.

(5,6-Dimethyl-2-benzimidazolylthio)acetic acid

3,4-Dimethylacetanilide. Seventy-three g. (0.6 mole) of 3,4-dimethylaniline was slowly added to a mixture of 82 g. (0.8 mole) of acetic anhydride and 82 g. of glacial acetic acid and the solution refluxed one hour. After cooling, the solution was added to 400 ml. of water and the mixture placed in the refrigerator overnight. The crude acetanilide was collected on a filter and washed with cold water yielding 80 g. of material. The compound was recrystallized by dissolving in a small volume of hot ethanol, adding water until a permanent cloudiness remained, and then cooling in the refrigerator while crystallization occurred. The recrystallized 3,4-dimethylacetanilide melted at 86° C.

3,4-Dimethyl-6-nitroacetanilide. A mixture of 45 ml. of concentrated sulfuric acid and 125 ml. of concentrated nitric acid (sp. gr. 1.42) was cooled in an ice-salt bath to -10° C. To the rapidly stirred mixture was slowly added 38 g. of 3,4-dimethylacetanilide while maintaining the temperature below -5° C. during the addition. Fifteen minutes after the addition was completed, the viscous mixture was poured into 600 g. of crushed ice and placed in the refrigerator overnight. The precipitate was collected on a filter and washed with cold water until the washings were no longer acid to litmus. The yield of nitrated product was 35 g. This material was recrystallized by dissolving in a small volume of hot ethanol, adding water until a permanent cloudiness remained, and cooling in the refrigerator. The melting point of the recrystallized 3,4-dimethyl-6-nitroacetanilide was 107° C.[1]

3,4-Dimethyl-6-nitroaniline. Five-tenth g. of metallic sodium was dissolved in 250 ml. of absolute methanol. Thirty-four g. of 3,4-dimethyl-6-nitroacetanilide was added and the mixture refluxed for 3 hours. Upon

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[1] Noelting *et al*. (38) give 107° C. as the melting point of 3,4-dimethyl-6-nitroacetanilide.

cooling crystallization occurred and the orange crystals were collected on a filter. The yield was 22.8 g. of 3,4-dimethyl-6-nitroaniline with a melting point of 142-143° C.[1]

1,2-Diamino-4,5-dimethylbenzene. To a stirred and cooled solution of 90.5 g. (0.4 mole) of stannous chloride dissolved in 170 ml. of concentrated hydrochloric acid was added 22 g. (0.13 mole) of 3,4-dimethyl-6-nitroaniline at such a rate that the temperature did not rise above 20° C. After the addition had been completed, the mixture was stirred for an additional 3 hours during which time the mixture turned a light yellow color. The reaction mixture was slowly added to 325 ml. of 30% sodium hydroxide being careful to maintain the temperature below 40° C. The yellow mixture was extracted with two 250 ml. portions of diethyl ether, the combined ether extracts washed with water, and dried over anhydrous sodium sulfate. After removing the drying agent by means of filtration, the ether was evaporated leaving a residue of 15 g. of the crude diamine. After recrystallization from hot water, 10 g. of 1,2-dimethyl-4,5-diaminobenzene was obtained with a melting point of 125° C.[2]

5,6-Dimethyl-2-thiobenzimidazole. To a solution of 10 g. of 1,2-diamino-4,5-dimethylbenzene dissolved in 150 ml. of 95% ethanol containing 10 g. of potassium hydroxide dissolved in 25 ml. of water was carefully added 5.3 ml. of carbon disulfide. The mixture was refluxed 3 hours on the steam bath after which time it was cooled slightly while 2 g. of Norite

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[1] The melting point of 3,4-dimethyl-6-nitroaniline was reported by Noelting *et al.* (38) as 139-140° C.

[2] Noelting *et al.* (38) give 125-126° C. as the melting point for 1,2-diamino-4,5-dimethylbenzene.

was cautiously added, and the refluxing resumed for an additional 10 minutes. The hot mixture was filtered and the filtrate acidified with dilute acetic acid. Two-hundred ml. of water was added and the mixture placed in the refrigerator. Twelve grams of crude 5,6-dimethyl-2-thiobenzimidazole was collected. The compound recrystallized from aqueous ethanol did not melt below 300° C.

(5,6-Dimethyl-2-benzimidazolylthio)acetic acid. To a solution of 1.8 g. (0.01 mole) of 5,6-dimethyl-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide was added 1 g. of monochloroacetic acid dissolved in 10 ml. of water. After refluxing 1 hour, the solution was cooled and filtered. The filtrate was made acid to Congo red with 6 N hydrochloric acid and placed in the refrigerator. The yield of (5,6-dimethyl-2-benzimidazolylthio)acetic acid was 2.0 g. After recrystallization from aqueous methanol, the compound melted with decomposition at 207-209° C.

Anal. Calcd. for $C_{11}H_{12}O_2N_2S$: C, 55.91; H, 5.12; N, 11.86. Found: C, 55.74; H, 5.33; N, 12.11.

α(5,6-Dimethyl-2-benzimidazolylthio)propionic acid

A solution of 1.7 g. of α-bromopropionic acid dissolved in 10 ml. of water was added to a solution of 1.8 g. (0.01 mole) of 5,6-dimethyl-2-thiobenzimidazole dissolved in 20 ml. of 2 N sodium hydroxide and the mixture refluxed for 1 hour. After cooling, the solution was filtered and the filtrate made acid to Congo red with 6 N hydrochloric acid. The acidified mixture was placed in the refrigerator and the following day 1.6 g. of material was collected on a filter. The α(5,6-dimethyl-2-benzimidazolylthio)propionic acid recrystallized from aqueous methanol melted with decomposition at 208-210° C.

Anal. Calcd. for $C_{12}H_{14}O_2N_2S$: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.97; H, 5.91; N, 11.32.

Analysis of Compounds

Analysis of these compounds for carbon and hydrogen was done by a micro-combustion method according to the directions described by Steyermark (52) employing a Sargent Automatic Micro-Combustion Apparatus. The nitrogen was determined by means of a semi-micro Kjeldahl method employing the procedure and distillation apparatus as described by Redemann (44). The digestion was carried on for a period of eight hours using a mixture of anhydrous cupric sulfate and potassium sulfate as the catalysis. In the two cases where the compounds contained nitro groups, this grouping was first reduced with hydriodic acid by the method of Friedrick as described by Steyermark (53); and after the reduction, the conventional semi-micro Kjeldahl procedure was utilized.

The melting points of the compounds were determined on a Fisher-John's Melting Point Block and corrected for calibration of the instrument. Several of the 2-thiobenzimidazoles did not melt below 300° C. Since the apparatus does not record temperatures above 300° C., the melting points of these compounds were reported as not melting below 300° C.

In Table I are tabulated the melting points and analyses for carbon, hydrogen and nitrogen together with the calculated values for these elements for each of the acid analogs of benzimidazole.

The neutralization equivalents of these compounds were also determined experimentally as further evidence for their structure. The acid analogs of 2-thiobenzimidazole were dissolved in a known excess of 0.01 N sodium hydroxide solution. A preliminary titration using a Beckmann pH meter

TABLE I

PROPERTIES OF 2-THIOBENZIMIDAZOLE ANALOGS

Acid analog of 2-thiobenzimidazole	M.p. °C	Carbon		Hydrogen		Nitrogen	
		Calcd.	Found	Calcd.	Found	Calcd.	Found
(2-Benzimidazolylthio)acetic	214-215	51.91	51.76	3.87	3.62	13.45	13.58
β(2-Benzimidazolylthio)propionic	178-179	54.04	54.11	4.54	4.83	12.60	12.67
α(2-Benzimidazolylthio)propionic	181-182	54.04	53.87	4.54	4.48	12.60	12.72
(5-Chloro-2-benzimidazolylthio)acetic	193-194	44.54	44.67	2.91	3.25	11.54	11.23
β(5-Chloro-2-benzimidazolylthio)propionic	103-106	46.78	46.58	3.53	3.73	10.91	10.88
α(5-Chloro-2-benzimidazolylthio)propionic	166-167	46.78	46.55	3.53	3.43	10.91	10.92
(4,6-Dichloro-2-benzimidazolylthio)acetic	222-224	39.00	39.34	2.18	2.36	10.11	10.09
(5,6-Dichloro-2-benzimidazolylthio)acetic	219-221	39.00	39.38	2.18	2.41	10.11	10.08
α(5,6-Dichloro-2-benzimidazolylthio)propionic	230-231	41.25	41.28	2.77	3.02	9.62	9.69
(4,5,6-Trichloro-2-benzimidazolylthio)acetic	205-207	34.69	34.75	1.62	1.89	8.99	9.33
α(4,5,6-Trichloro-2-benzimidazolylthio)propionic	222-224	36.88	37.09	2.17	2.11	8.60	8.84
(5-Bromo-2-benzimidazolylthio)acetic	194-196	37.64	37.86	2.46	2.68	9.76	9.84

α(5-Bromo-2-benzimidazolylthio)propionic	180-181	39.88	39.43	3.01	2.89	9.30	9.45
(5-Nitro-2-benzimidazolylthio)acetic	191-193[1]	42.68	42.54	2.79	2.96	16.59	16.34
α(5-Nitro-2-benzimidazolylthio)propionic	186-188	44.94	45.03	3.39	3.15	15.72	15.99
(5-Methoxy-2-benzimidazolylthio)acetic	194-196	50.41	50.79	4.23	4.21	11.76	11.77
α(5-Methoxy-2-benzimidazolylthio)propionic	151-152	52.36	52.63	4.79	4.85	11.10	11.07
(5-Methyl-2-benzimidazolylthio)acetic	197-200	54.04	54.00	4.54	4.62	12.60	12.61
α(5-Methyl-2-benzimidazolylthio)propionic	160-162	55.91	56.09	5.12	5.47	11.86	11.71
(4,6-Dimethyl-2-benzimidazolylthio)acetic	247-249	55.91	56.01	5.12	4.92	11.86	11.85
α(4,6-Dimethyl-2-benzimidazolylthio)propionic	160-161	57.58	57.68	5.64	5.99	11.19	10.98
(5,6-Dimethyl-2-benzimidazolylthio)acetic	207-209	55.91	55.74	5.12	5.33	11.86	12.11
α(5,6-Dimethyl-2-benzimidazolylthio)propionic	208-210	57.58	57.97	5.64	5.91	11.19	11.32
(5-Phenyl-2-benzimidazolylthio)acetic	215-216	63.36	63.40	4.25	4.21	9.85	9.79
α(5-Phenyl-2-benzimidazolylthio)propionic	200-202	64.41	64.16	4.73	4.52	9.39	9.35
(5,6-Dimethoxy-2-benzimidazolylthio)acetic	241-243	49.24	49.24	4.51	4.69	10.44	10.48

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[1] This compound softened at 92-94° C., resolidified at a somewhat higher temperature and finally melted with decomposition at 191-193° C.

indicated that these salts could be titrated satisfactorily to a methyl red endpoint. The excess sodium hydroxide was titrated with 0.01 N hydrochloric acid to a methyl red endpoint. The results of these determinations are summarized in Table II.

TABLE II

NEUTRALIZATION EQUIVALENTS OF SOME ACID ANALOGS OF 2-THIOBENZIMIDAZOLE

Acid	Formula	Neut. Equiv.	
		Calcd.	Found
(2-Benzimidazolylthio)acetic	$C_9H_8O_2N_2S$	208.2	208.7
α(Benzimidazolylthio)propionic	$C_{10}H_{10}O_2N_2S$	222.3	218.8
β(2-Benzimidazolylthio)propionic	$C_{10}H_{10}O_2N_2S$	222.3	220.0
(5-Chloro-2-benzimidazolylthio)acetic	$C_9H_7O_2N_2SCl$	242.7	245.5
α(5-Chloro-2-benzimidazolylthio)propionic	$C_{10}H_9O_2N_2SCl$	256.7	253.0
β(5-Chloro-2-benzimidazolylthio)propionic	$C_{10}H_9O_2N_2SCl$	256.7	259.0
(4,6-Dichloro-2-benzimidazolylthio)acetic	$C_9H_6O_2N_2SCl_2$	277.1	277.3
(5,6-Dichloro-2-benzimidazolylthio)acetic	$C_9H_6O_2N_2SCl_2$	277.1	274.1
α(5,6-Dichloro-2-benzimidazolylthio)propionic	$C_{10}H_8O_2N_2SCl_2$	291.2	292.3
(4,5,6-Trichloro-2-benzimidazolylthio)acetic	$C_9H_5O_2N_2SCl_3$	311.6	310.2
α(4,5,6-Trichloro-2-benzimidazolylthio)propionic	$C_{10}H_7O_2N_2SCl_3$	325.6	324.4
(5-Bromo-2-benzimidazolylthio)acetic	$C_9H_7O_2N_2SBr$	287.1	288.1

α(5-Bromo-2-benzimidazolylthio)propionic	$C_{10}H_9O_2N_2SBr$	301.2	302.4
(5-Nitro-2-benzimidazolylthio)acetic	$C_9H_7O_4N_3S$	253.2	250.5
α(5-Nitro-2-benzimidazolylthio)propionic	$C_{10}H_9O_4N_3S$	267.3	271.0
(5-Methoxy-2-benzimidazolylthio)acetic	$C_{10}H_{10}O_3N_2S$	238.3	235.7
α(5-Methoxy-2-benzimidazolylthio)propionic	$C_{11}H_{12}O_3N_2S$	252.3	255.1
(5-Methyl-2-benzimidazolylthio)acetic	$C_{10}H_{10}O_2N_2S$	222.3	226.4
α(5-Methyl-2-benzimidazolylthio)propionic	$C_{11}H_{12}O_2N_2S$	236.3	240.3
(4,6-Dimethyl-2-benzimidazolylthio)acetic	$C_{11}H_{12}O_2N_2S$	236.3	231.7
α(4,6-Dimethyl-2-benzimidazolylthio)propionic	$C_{12}H_{14}O_2N_2S$	250.3	248.2
(5,6-Dimethyl-2-benzimidazolylthio)acetic	$C_{11}H_{12}O_2N_2S$	236.3	234.9
α(5,6-Dimethyl-2-benzimidazolylthio)propionic	$C_{12}H_{14}O_2N_2S$	250.3	253.7
(5-Phenyl-2-benzimidazolylthio)acetic	$C_{15}H_{12}O_2N_2S$	284.3	282.8
α(5-Phenyl-2-benzimidazolylthio)propionic	$C_{16}H_{14}O_2N_2S$	298.4	299.4

Biological Assay

Bean Leaf Test

The test solutions of the benzimidazole analogs were prepared by suspending enough of the benzimidazole compound to give an 0.005 M solution in 10 ml. of distilled water to which had been added 2 ml. of a saturated solution of sodium bicarbonate. The mixture was stirred until solution was complete and then the pH of the solution adjusted to 7.0-7.1 by the dropwise addition of an aqueous 25% acetic acid solution, following which the final volume was made to 100 ml. with distilled water.

Seeds of the bean, Phaseolus vulgaris, variety cranberry, were selected for uniformity of size and planted in four-inch pots in the greenhouse. After germination, all but the most uniform seedlings were removed from each pot. Six to seven days after planting when the primary leaves of the seedlings were expanding, they were treated with solutions of the benzimidazole analogs. Biological assay consisted of dipping the expanding primary leaves of the bean seedlings into a 0.005 M solution of the test compound. The primary leaves of control plants were dipped into a solution prepared in the same manner as previously described but containing only sodium bicarbonate and acetic acid at pH 7.0-7.1.

Visual observations were taken as to the relative amount of inhibition brought about by each of the compounds as compared to control plants at definite time intervals after the treatment.

In one of the experiments, a portion of the plants were treated a second time. For this experiment, groups of twelve uniform plants were treated with each of the compounds. The plants were contained in six pots, each containing two plants. Four days after the initial treatment, six of the plants from each group were treated a second time by dipping the leaves into a solution of the same compound used in the initial treatment. Fifteen days after the initial treatment, the plants were clipped at the first internode. That portion of the plant above the first internode was weighed and this weight was recorded as the fresh weight of the plant. After drying for sixteen hours at 65° C. in a forced draft oven, the plants were again weighed and this weight recorded as the dry weight of the plant. The plants were weighed in groups and an average weight was calculated for each plant.

Root Inhibition Test

A filter paper was placed in the bottom of a Petri dish and ten cucumber seeds (variety Marketer) were spread evenly over the filter paper. Five milliliters of the test solution was pipetted into each dish and the cover placed on the dish. The seeds were allowed to germinate at room temperature (approximately 25° C.) under laboratory conditions of alternating light and darkness. After eight days the longest root radical was measured and the length of this radical used as a measure of the inhibitory power of the test compound as compared to cucumber seeds treated with a neutral solution of sodium bicarbonate and acetic acid. The solutions of the test compounds for this test were prepared in the same manner as described for the bean leaf test.

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

When the bean leaf test was employed as the method of biological assay, the day following the treatment the first effects of the compounds were clearly visible. The leaves of the treated plants had acquired a deeper green color than those of the control plants and there was a marked downward curling and wrinkling of the leaves which gave the appearance that all portions of the leaf were not growing at the same rate. Two to three days after the dipping, some of the compounds caused the leaves of the plants to become chlorotic. The response of the plants to each of the compounds was not the same. Some of the benzimidazole analogs caused a much greater inhibition of growth than did others, whereas a few appeared to inhibit growth only very slightly or not at all. The nature of the acid side chain and also the nature and location of the groups substituted in the aromatic portion of the compound greatly influenced the degree of inhibition.

After four to five days some of the plants appeared to have grown only very slightly whereas others began to show signs of recovery from the treatment depending upon the compound used. Figure 1 shows the appearance of typical plants five days after treatment with the benzimidazole analogs. The pot at the right of each row contains control plants of the same age and grown under identical greenhouse conditions. Each of the other pots contains plants treated with a different benzimidazole compound. The figure clearly shows that the compounds which caused the greatest inhibition of growth were the chlorine substituted ones with the 5-chloro-compounds being the most inhibitory. Many of the most active

Figure 1. The appearance of bean plants five days after treatment of the leaves with acid analogs of 2-thiobenzimidazole.

Row I. (Top left to right)
α(5-phenyl-2-benzimidazolylthio)propionic acid,
(5-phenyl-2-benzimidazolylthio)acetic acid,
(4,5,6-trichloro-2-benzimidazolylthio)acetic acid,
α(5,6-dimethyl-2-benzimidazolylthio)propionic acid,
(5,6-dimethoxy-2-benzimidazolylthio)acetic acid,
control.

Row II.
(5-methoxy-2-benzimidazolylthio)acetic acid,
(5-methyl-2-benzimidazolylthio)acetic acid,
α(4,5,6-trichloro-2-benzimidazolylthio)propionic acid,
(2-benzimidazolylthio)acetic acid,
α(5-nitro-2-benzimidazolylthio)propionic acid,
control.

Row III.
(5-bromo-2-benzimidazolylthio)acetic acid,
(5,6-dichloro-2-benzimidazolylthio)acetic acid,
β(2-benzimidazolylthio)propionic acid,
α(5-bromo-2-benzimidazolylthio)propionic acid,
(4,6-dichloro-2-benzimidazolylthio)acetic acid,
control.

Row IV.
β(5-chloro-2-benzimidazolylthio)propionic acid,
(5-chloro-2-benzimidazolylthio)acetic acid,
α(2-benzimidazolylthio)propionic acid,
α(5,6-dichloro-2-benzimidazolylthio)propionic acid,
α(5-chloro-2-benzimidazolylthio)propionic acid,
control.

Figure 1. The appearance of bean plants five days after treatment of the leaves with acid analogs of 2-thiobenzimidazole.

compounds also caused the leaves to become chlorotic. The introduction of a second chlorine into the ring at a position ortho to the first, such as in (5,6-dichloro-2-benzimidazolylthio)acetic acid, resulted in a compound which was slightly less inhibitory than the mono-chloro-compound but still caused a greater inhibition of growth than did the corresponding unsubstituted benzimidazole derivative. However, when the two chlorine substituents were in positions meta to each other as in (4,6-dichloro-2-benzimidazolylthio)acetic acid, the inhibitory activity was only slightly greater than that caused by (2-benzimidazolylthio)acetic acid. A bromine atom substituted in the 5-position of the aromatic ring also enhanced the inhibitory activity although not to quite as great an extent as did a chlorine atom in the same position. The introduction of a third chlorine atom in the ring, such as in (4,5,6-trichloro-2-benzimidazolylthio)acetic acid, almost completely destroyed the ability of the compound to inhibit growth. Other substituents such as methyl-, methoxyl-, nitro-, and phenyl-radicals decreased the inhibitory activity of the benzimidazole analogs.

The nature of the acid side chain also had an effect on the inhibitory activity, although not to as great an extent as did the substituents in the aromatic ring portion of the molecule. In most instances the β-propionic acid derivative was slightly more active than the compound with an acetic acid side chain, whereas the α-propionic acid analog was the least active.

Figures 2, 3, and 4 show a closer view of plants treated with some of the most active compounds. In all of the Figures the pot on the right contains the control plants. Figure 2 contains in the left pot plants treated five days previously with β(5-chloro-2-benzimidazolylthio)propionic

Figure 2. The appearance of bean plants five days after treatment of the leaves with acid analogs of 2-thiobenzimidazole. The left plants were treated with β(5-chloro-2-benzimidazolylthio)propionic acid, the center with (5-chloro-2-benzimidazolylthio) acetic acid, and the plants on the right are controls.

Figure 3. The appearance of bean plants five days after treatment of the leaves with acid analogs of 2-thiobenzimidazole. The plants on the left were treated with α(5,6-dichloro-2-benzimidazolylthio)propionic acid, the center plants with (5-bromo-2-benzimidazolylthio)acetic acid, and the ones on the right are controls.

Figure 4. The appearance of bean plants five days after treatment of the leaves with acid analogs of 2-thiobenzimidazole. The plants on the left were treated with (5,6-dichloro-2-benzimidazolylthio)acetic acid, the center plants with α(2-benzimidazolylthio)propionic acid, and the ones on the right are controls.

acid. These plants had practically no additional growth after the treatment. The leaves which had not fallen from the plant were twisted downward into a very peculiar shape and appeared to have decreased in size. The plants in the middle pot were treated with (5-chloro-2-benzimidazolylthio)acetic acid. Although the inhibition caused by this compound was not quite as severe as that caused by the previous chlorine substituted compound, it was still appreciable. Both of the compounds caused the leaves to become very chlorotic.

In Figure 3 the plants in the pot on the left were treated with α(5,6-dichloro-2-benzimidazolylthio)propionic acid and those in the center pot treated with (5-bromo-2-benzimidazolylthio)acetic acid. These compounds also greatly inhibited the growth of the plants. In general, the plants treated with the α-propionic acid derivatives did not show as severe a chlorosis as did the plants treated with the other acid derivatives. The plants in Figure 4 were treated with (5,6-dichloro-2-benzimidazolylthio)-acetic acid and α(2-benzimidazolylthio)propionic acid. All of the plants show the characteristic downward curling as well as the wrinkled appearance of the treated leaves.

After six to seven days some of the plants appeared to be recovering from the initial treatment. Therefore, another experiment was undertaken in which a portion of the plants were treated a second time in order to determine if an additional treatment would enhance the inhibition or perhaps have a herbicidal effect on the plant. The previous experiment showed that the maximum inhibitory effect was manifest after about four days. For this experiment, twelve pots each containing two uniform plants were separated into groups and each group was treated with a different compound. After four days, the plants in six of the pots from each group were dipped a second time with the same compound as was used for the first treatment. Both the fresh and the dry weights of the plants as well as visual observations were used as a measure of the inhibition of growth. Fifteen days after the first treatment the plants were clipped at the first internode and that portion of the plant above the internode was weighed. After recording the fresh weights of the plants, they were dried at 65° C. in a forced air oven and the dry weights recorded.

In general, the plants treated a second time were inhibited to a greater extent than were those dipped only once. In no instance were the treatments clearly herbicidal at an 0.005 M concentration of the test compound.

Eight days after the initial treatment additional observations were noted. The plants treated a second time with (2-benzimidazolylthio)acetic acid had very little new growth beyond the primary leaves. The primary leaves appeared almost like those of the control plants although they were a deeper green in color and these plants were slightly smaller in size than were those receiving only one treatment. Plants treated only once with this compound showed some growth beyond the primary leaves although not as much as the non-treated plants. Those bean plants treated with α(2-benzimidazolylthio)propionic acid appeared to be recovering from the effects of this compound; however, they were nevertheless slightly smaller in size than were the control plants and those treated twice showed less growth above the primary leaves. The plants treated only once with β(2-benzimidazolylthio)propionic acid also appeared to be overcoming the effects of this treatment but the leaves of those treated a second time were either dying or had already fallen from the plant.

As in the previous experiment the 5-chloro-substituted compounds caused the greatest amount of inhibition. The plants treated twice with (5-chloro-2-benzimidazolylthio)acetic acid were approximately the same size as they were when initially treated. Many of the leaves had fallen from the plant and new growth beyond the primary leaves was very limited. Those receiving only one treatment were still greatly inhibited although there was a small amount of new growth beyond the primary leaves. Plants treated with α(5-chloro-2-benzimidazolylthio)propionic acid were not inhibited as greatly as with the acetic acid derivative. The plants treated a second time showed less growth above the primary leaves whereas those treated only once appeared to be recovering. Beta(5-chloro-2-benzimidazolylthio)propionic acid was the most active of the 5-chloro-substituted compounds. The leaves of these plants were very chlorotic and were severely twisted downward. Those treated twice showed little new growth and some of the leaves had fallen from the plant.

The 5,6-dichloro-derivatives were almost as active as the 5-chloro-compounds. The plants whose leaves were dipped a second time into a solution of (5,6-dichloro-2-benzimidazolylthio)acetic acid were about the same size as when first treated and had scarcely any new growth above the primary leaves. However, the growth above the primary leaves appeared to be almost as abundant as that of the control plants when only one treatment had been applied. The leaves of both groups of plants were a deeper green in color and curled downward. Alpha(5,6-dichloro-2-benzimidazolylthio)propionic acid caused about the same effect but this effect was not quite as severe. The (4,6-dichloro-2-benzimidazolylthio)-acetic acid was not quite as active as the preceeding 5,6-dichloro-compound.

Evidently the 5- and 6-positions are the most important in the benzimidazole nucleus for growth inhibition, and substitution in the 4-position appears to hinder the inhibitory reaction. Plants treated either once or twice with this compound had practically no new growth beyond the treated primary leaves. The primary leaves of these plants were almost as large as those of the control plants but were a deeper green in color. Plants treated either once or twice with (4,5,6-trichloro-2-benzimidazolylthio)acetic acid and α(4,5,6-trichloro-2-benzimidazolylthio)propionic acid were only slightly inhibited eight days after the initial treatment.

The (5-bromo-2-benzimidazolylthio)acetic acid compound inhibited the growth of plants almost as much as did the corresponding 5-chloro-derivative. Plants treated twice with this compound were about the same size as when initially treated and did not show any additional growth above the treated primary leaves whereas those treated only once were still appreciably inhibited but did show evidence of a moderate amount of growth above the primary leaves. The leaves of both groups of plants had chlorotic spots, curled downward, and were a deeper green color. As was true in the previous cases, α(5-bromo-2-benzimidazolylthio)propionic acid did not inhibit growth as strongly as did the acetic acid derivative. However, the leaves of these plants were also chlorotic and the leaves of the plants receiving two dippings were affected more than those treated only once.

A nitro group in the 5-position of the benzimidazole ring almost completely destroyed the inhibitory activity of these compounds. The (5-nitro-2-benzimidazolylthio)acetic acid caused almost no visible effects on the bean plants but α(5-nitro-2-benzimidazolylthio)propionic acid did have a very slight inhibitory action.

When plants were treated twice with (5-methoxy-2-benzimidazolylthio)-acetic acid, eight days after the initial dipping there was practically no new growth above the primary leaves. However, the remainder of the plant was inhibited only slightly and those receiving only one treatment appeared to be almost normal except for some chlorotic spots on the leaves. Alpha(5-methoxy-2-benzimidazolylthio)propionic acid caused practically no visible effect on the plants eight days after treatment.

Those plants treated with 5-methyl-, 5,6-dimethyl-, and 4,6-dimethyl-2-thiobenzimidazole analogs were only slightly inhibited. In general, the acetic acid derivatives were slightly more inhibitory than the propionic acid derivatives. The 5-phenyl-substituted benzimidazoles were also very poor inhibitors of the growth of bean plants. A second treatment with these compounds enhanced the visible inhibitory effects of the substances only very slightly if at all. In Table III the compounds are rated as to their relative inhibitory power as determined by means of visual observations.

Fifteen days after the initial treatments many of the plants were still markedly affected. Plants treated once with (2-benzimidazolylthio)-acetic acid and β(2-benzimidazolylthio)propionic acid were still somewhat inhibited after this period of time. The stems of the plants which had been treated twice with (2-benzimidazolylthio)acetic acid had grown very little since the initial treatment; however, numerous small leaves appeared at the tip of the stem, but these leaves were bunched together in a cluster which gave the plants a much different appearance from the control plants. Many of the leaves and several of the plants treated twice with β(2-benzimidazolylthio)propionic acid had died; however, the cause of death may

TABLE III

RELATIVE INHIBITION OF CRANBERRY BEAN PLANTS 8 DAYS AFTER TREATMENT WITH AN 0.005 MOLAR SOLUTION OF 2-THIOBENZIMIDAZOLE ANALOGS

Acid analog of 2-thiobenzimidazole	Relative inhibition[1]
(2-Benzimidazolylthio)acetic	+++
α(2-Benzimidazolylthio)propionic	+
β(2-Benzimidazolylthio)propionic	+++
(5-Chloro-2-benzimidazolylthio)acetic	+++++
α(5-Chloro-2-benzimidazolylthio)propionic	++
β(5-Chloro-2-benzimidazolylthio)propionic	++++++
(5,6-Dichloro-2-benzimidazolylthio)acetic	+++++
α(5,6-Dichloro-2-benzimidazolylthio)propionic	++
(4,6-Dichloro-2-benzimidazolylthio)acetic	++++
(4,5,6-Trichloro-2-benzimidazolylthio)acetic	0
α(4,5,6-Trichloro-2-benzimidazolylthio)propionic	+
(5-Bromo-2-benzimidazolylthio)acetic	+++++
α(5-Bromo-2-benzimidazolylthio)propionic	++
(5-Methyl-2-benzimidazolylthio)acetic	+
α(5-Methyl-2-benzimidazolylthio)propionic	0
(5,6-Dimethyl-2-benzimidazolylthio)acetic	0
α(5,6-Dimethyl-2-benzimidazolylthio)propionic	0
(4,6-Dimethyl-2-benzimidazolylthio)acetic	+
α(4,6-Dimethyl-2-benzimidazolylthio)propionic	+
(5-Methoxy-2-benzimidazolylthio)acetic	+
α(5,Methoxy-2-benzimidazolylthio)propionic	0

TABLE III (Concluded)

Acid analog of 2-thiobenzimidazole	Relative inhibition[1]
(5-Nitro-2-benzimidazolylthio)acetic	0
α(5-Nitro-2-benzimidazolylthio)propionic	+
(5-Phenyl-2-benzimidazolylthio)acetic	+
α(5-Phenyl-2-benzimidazolylthio)propionic	+
Control	0

[1] The number of plus signs indicate the relative magnitude of inhibition of new leaf growth as compared to control plants.

have been due to "damping off." The plants treated with α(2-benzimidazolylthio)propionic acid appeared to have completely recovered from the treatment.

As was true at the end of eight days, the substitution of a chlorine atom in the 5-position resulted in compounds that still caused the greatest inhibition of growth even after fifteen days. Those plants treated twice with (5-chloro-2-benzimidazolylthio)acetic acid had many small leaves in a cluster at the top of the plant and the stems appeared not to have grown in length since the initial treatment. The stems of these plants were also swollen in size. The stems of plants treated with β(5-chloro-2-benzimidazolylthio)propionic acid were also swollen in size and many of the primary leaves had dropped from the plants. Those plants treated with α(5-chloro-2-benzimidazolylthio)propionic acid were beginning to recover from the treatment but the primary leaves were still chlorotic.

The effect of the treatment of plants with (4,6-dichloro-2-benzimidazolylthio)acetic acid appeared to be more severe after fifteen days than it was at the end of eight days. The stems of these plants were swollen and the new leaves which had formed were in a cluster at the tip of the stem. The plants treated only once were inhibited almost as severely as the plants treated a second time.

Plants treated with (5,6-dichloro-2-benzimidazolylthio)acetic acid had about the same appearance as the plants treated with the corresponding 5-chloro-derivative although the effect was not quite as intensive. Those plants treated with α(5,6-dichloro-2-benzimidazolylthio)propionic acid were still slightly smaller than the control plants but they appeared to have overcome the initial inhibition. The plants treated with the

4,5,6-trichloro-derivatives appeared to be almost the same as the control plants fifteen days after the initial treatment.

The substitution of a bromine atom in the benzimidazole nucleus resulted in a compound which was almost as good an inhibitor as the chlorine compound. The stems of the plants treated with (5-bromo-2-benzimidazolylthio)acetic acid were swollen and had grown very little in length since the initial treatment. Many small leaves appeared in a cluster at the tip of the plants. Those treated only once had an appearance similar to those treated twice but were somewhat larger. Alpha(5-bromo-2-benzimidazolylthio)propionic acid treated plants were showing signs of recovering from the inhibition after fifteen days although the primary leaves were still chlorotic.

Plants treated twice with 5-nitro-, 5-methoxyl-, and 5-methyl-derivatives were still slightly inhibited although the inhibition was not nearly as severe as that caused by the unsubstituted benzimidazole derivative whereas those treated only once had almost completely recovered after fifteen days. The 5-phenyl-, 4,6-dimethyl-, and 5,6-dimethyl-compounds had only a very slight inhibitory effect visable fifteen days after treatment.

Table IV lists the average fresh and dry weights of plants fifteen days after the initial treatment. The fresh and dry weights of those plants treated twice with the compounds were usually less than those receiving only a single treatment. In general, the weights of the plants are in agreement with the observations made on the living plants in respect to the relative amount of inhibition.

TABLE IV

FRESH AND DRY WEIGHTS OF CRANBERRY BEAN PLANTS 15 DAYS AFTER TREATMENT WITH ANALOGS OF 2-THIOBENZIMIDAZOLE[1]

Acid analog of 2-thiobenzimidazole	One treatment Av. wt. per plant		Two treatments[2] Av. wt. per plant	
	Fresh wt. G.	Dry wt. G.	Fresh wt. G.	Dry wt. G.
(2-Benzimidazolylthio)acetic	3.7	0.5	3.2	0.4
α(2-benzimidazolylthio)propionic	3.8	0.5	3.9	0.5
β(2-Benzimidazolylthio)propionic	3.1	0.4	0.9	0.2
(5-Chloro-2-benzimidazolylthio)acetic	3.3	0.4	2.4	0.3
α(5-Chloro-2-benzimidazolylthio)propionic	4.2	0.6	3.1	0.4
β(5-Chloro-2-benzimidazolylthio)propionic	2.9	0.5	1.3	0.2
(4,6-Dichloro-2-benzimidazolylthio)acetic	4.6	0.7	3.8	0.5
(5,6-Dichloro-2-benzimidazolylthio)acetic	2.0	0.3	2.4	0.4
α(5,6-Dichloro-2-benzimidazolylthio)propionic	3.8	0.6	3.5	0.5
(4,5,6-Trichloro-2-benzimidazolylthio)acetic	4.5	0.6	4.6	0.6
α(4,5,6-Trichloro-2-benzimidazolylthio)propionic	4.8	0.7	3.8	0.6
(5-Bromo-2-benzimidazolylthio)acetic	2.8	0.4	1.7	0.3

α(5-Bromo-2-benzimidazolylthio)propionic	3.6	0.5	2.8	0.4
(5-Nitro-2-benzimidazolylthio)acetic	4.9	0.8	5.3	0.8
α(5-Nitro-2-benzimidazolylthio)propionic	4.3	0.6	4.5	0.6
(5-Methoxy-2-benzimidazolylthio)acetic	4.8	0.6	3.5	0.5
α(5-Methoxy-2-benzimidazolylthio)propionic	5.0	0.9	5.0	0.7
(5-Methyl-2-benzimidazolylthio)acetic	4.9	0.5	4.1	0.5
α(5-Methyl-2-benzimidazolylthio)propionic	4.6	0.7	3.9	0.5
(4,6-Dimethyl-2-benzimidazolylthio)acetic	4.4	0.7	3.6	0.6
α(4,6-Dimethyl-2-benzimidazolylthio)propionic	3.1	0.5	3.6	0.5
(5,6-Dimethyl-2-benzimidazolylthio)acetic	3.6	0.6	3.8	0.6
α(5,6-Dimethyl-2-benzimidazolylthio)propionic	4.1	0.6	4.6	0.5
(5-Phenyl-2-benzimidazolylthio)acetic	3.7	0.6	3.7	0.6
α(5-Phenyl-2-benzimidazolylthio)propionic	3.6	0.7	3.5	0.6
Control	5.3	0.8	5.3	0.8

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[1] The weight represents that portion of the plant above the first internode.

[2] These plants were dipped a second time four days after the initial treatment.

The results of the cucumber root assay are shown in Table V. The influence of two different concentrations, namely 0.005 M and 0.0005 M, are given as the percentage of the root length of the controls.

As a rule, the compounds which caused the greatest inhibition of growth of the bean plants also caused the greatest inhibition of root growth of the cucumber seeds. The root radicals which did emerge from the treated seeds were almost devoid of root hairs, whereas the radicals of the control seeds had numerous root hairs. In almost all cases, the amount of inhibition was greater when the seeds were treated with the 0.005 M solution than it was with the 0.0005 M solution. However, with a few exceptions, the ten fold dilution of the test solution did not reduce the inhibitory activity of the α-propionic acid derivatives as greatly as it did the acetic acid derivatives, although at the higher concentration the acetic acid derivatives were usually better inhibitors than the corresponding α-propionic acid compound. The β-propionic acid derivatives were slightly more inhibitory than the derivatives of the other acids.

As was observed with the bean leaf test, the substitution of a halogen in the benzene nucleus of the benzimidazole gave rise to an increase in inhibitory activity. The 5-chloro- and 5-bromo-compounds caused about the same degree of inhibition when tested at an 0.005 M concentration but at the greater dilution, the 5-chloro-compounds were slightly more inhibitory. The 5,6-dichloro-compounds were almost as effective as the corresponding mono-halogen compounds, whereas the 4,6-dichloro-derivative was slightly less effective and had about the same effect as did the acetic acid derivative of the 4,5,6-trichloro-compound.

TABLE V

RELATIVE ROOT LENGTH OF CUCUMBER SEEDLINGS 8 DAYS AFTER TREATMENT OF THE SEEDS WITH SOLUTIONS OF ANALOGS OF 2-THIOBENZIMIDAZOLE[1]

Acid analog of 2-thiobenzimidazole	Concentration 0.005 Molar	 0.0005 Molar
	%	%
(2-benzimidazolylthio)acetic	74	69
α(2-benzimidazolylthio)propionic	59	64
β(2-benzimidazolylthio)propionic	51	55
(5-chloro-2-benzimidazolylthio)acetic	46	55
α(5-chloro-2-benzimidazolylthio)propionic	64	68
β(5-chloro-2-benzimidazolylthio)propionic	28	48
(4,6-dichloro-2-benzimidazolylthio)acetic	62	84
(5-nitro-2-benzimidazolylthio)acetic	59	66
α(5-nitro-2-benzimidazolylthio)propionic	67	90
(5-methoxy-2-benzimidazolylthio)acetic	64	84
α(5-methoxy-2-benzimidazolylthio)propionic	73	68

(5-methyl-2-benzimidazolylthio)acetic	49	79
α(5-methyl-2-benzimidazolylthio)propionic	64	157
(5,6-dichloro-2-benzimidazolylthio)acetic	41	52
α(5,6-dichloro-2-benzimidazolylthio)propionic	59	66
(5-phenyl-2-benzimidazolylthio)acetic	31	71
α(5-phenyl-2-benzimidazolylthio)propionic	44	77
(5-bromo-2-benzimidazolylthio)acetic	46	75
α(5-bromo-2-benzimidazolylthio)propionic	51	53
(4,6-dimethyl-2-benzimidazolylthio)acetic	46	88
α(4,6-dimethyl-2-benzimidazolylthio)propionic	33	96
(5,6-dimethyl-2-benzimidazolylthio)acetic	72	106
α(5,6-dimethyl-2-benzimidazolylthio)propionic	59	106
(4,5,6-trichloro-2-benzimidazolylthio)acetic	54	88
α(4,5,6-trichloro-2-benzimidazolylthio)propionic	62	64
Control	100	100

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[1] Results are expressed as the percentage of the root length of controls.

The α-propionic acid derivative of the 4,5,6-trichloro-benzimidazole was slightly more inhibitory than was the acetic acid compound.

The methyl substituted compounds markedly inhibited root formation at a concentration of 0.005 M, but at the lower concentration the inhibition was practically non-existant and in some cases, root formation may even have been stimulated. This was evident not only as an increase in root length but also in the appearance of numerous root hairs.

The 5-nitro-, 5-phenyl-, and 5-methoxyl-compounds caused only a slight amount of inhibition in the bean leaf test but with the root test the results were slightly different. The methoxyl- and nitro-compounds were still among the poorest of the inhibitors tested, but the 5-phenyl-derivatives caused an appreciable inhibition at a concentration of 0.005 M and the inhibition, although reduced, was nevertheless still marked at a concentration of 0.0005 M.

From the results of these two methods of assay several conclusions can be drawn with respect to the relationship of the structure of these compounds to inhibitory activity.

(1) The nature of the acid side chain has an effect on the activity of the compound. The β-propionic acids were slightly better inhibitors than the corresponding acetic acid derivatives whereas the α-propionic acid compounds were generally the poorest inhibitors.

(2) The nature and location of the substituents in the aromatic nucleus also affects the inhibitory activity of the 2-thiobenzimidazole compounds, and these substituents have a greater influence than do variations of the acid side chains. A chlorine or a bromine atom in the 5-position of the benzimidazole ring greatly increases the inhibitory

activity with a chlorine atom slightly more effective than a bromine atom. In these compounds the 5- and 6-positions are equivalent. The substitution of a second chlorine atom in a position ortho to the first, as for example in the 5- and 6-positions, also results in a compound which is a much better inhibitor than the unsubstituted compound, although this derivative is not quite as effective as the 5-monochlorobenzimidazole.

(3) A substituent in the 4-position appears to interfere in some manner with the inhibitory reaction in the plant. When the chlorine atoms are substituted in the 4- and 6-positions, the inhibitory activity is reduced, but this compound is still a better inhibitor than the parent compound. Further evidence for this is to be found in the observation that three chlorine atoms in positions 4,5 and 6 almost completely removes the inhibitory activity of the benzimidazole compound.

Several possible explanations could be postulated for this reduction in inhibitory activity. (a) A constituent in the plant may undergo some sort of a reaction with the 4-position of the benzimidazole and this reaction is at least, in part, hindered when a substituent other than hydrogen is present in this position. (b) Another explanation would be that a substituent in the 4-position causes a steric effect which prevents, at least in part, the combination of the benzimidazole with a constituent of the plant.

(4) Nitro-, phenyl-, methyl- and methoxyl-groups greatly decrease the inhibitory activity and the substitution of two methyl groups in either the 5- and 6- or the 4- and 6-positions almost completely destroyed the ability of these compounds to inhibit the growth of the test plants.

(5) The thio ether linkage also contributes to the inhibitory activity of these compounds. When an acetic acid side chain was connected directly to the benzimidazole nucleus in the 2-position, this compound, 2-benzimidazoleacetic acid, caused no visable inhibition of growth when tested on bean plants.

Maleic hydrazide is a compound which shows considerable promise for use as a growth inhibitor. This compound appears to produce effects opposite to those generally associated to the plant growth hormone, indoleacetic acid. For example, maleic hydrazide inhibits terminal growth and stem elongation, destroys apical dominance, stimulates lateral bud development, induces leaf and fruit abscission, hastens the production of seedstalk and flowers when applied during the time the flower primordia are being formed and delays flowering when applied during the later reproductive stages (57). The cranberry bean plants treated with an 0.005 M solution of (5-chloro-2-benzimidazolylthio)acetic acid or (5-bromo-2-benzimidazolylthio)acetic acid have an appearance similar to bean plants treated with a 5000 ppm solution (approximately 0.045 M) of maleic hydrazide.[1] Thus, (5-chloro-2-benzimidazolylthio)acetic acid and (5-bromo-2-benzimidazolylthio)acetic acid caused about the same observable inhibition of bean plants at approximately a tenth of the molar concentration as did maleic hydrazide.

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[1] Observation of Dr. C. L. Hamner, Department of Horticulture, Michigan State University.

SUMMARY

A study was made of the effect of chemical structure on the inhibitory activity toward growth and herbicidal action on cranberry bean plants and root development in cucumber seedlings of a number of derivatives of (2-benzimidazolylthio)acetic acid. Different chemical groups were substituted in the benzene ring portion of the benzimidazole and the acid side chains were varied.

The following acid analogs of 2-thiobenzimidazole were synthesized: (2-benzimidazolylthio)acetic, β(2-benzimidazolylthio)propionic, α(benzimidazolylthio)propionic, (5-chloro-2-benzimidazolylthio)acetic, β(5-chloro-2-benzimidazolylthio)propionic, α(5-chloro-2-benzimidazolylthio)-propionic, (4,6-dichloro-2-benzimidazolylthio)acetic, (5,6-dichloro-2-benzimidazolylthio)acetic, α(5,6-dichloro-2-benzimidazolylthio)propionic, (4,5,6-trichloro-2-benzimidazolylthio)acetic, α(4,5,6-trichloro-2-benzimidazolylthio)propionic, (5-bromo-2-benzimidazolylthio)acetic, α(5-bromo-2-benzimidazolylthio)propionic, (5-nitro-2-benzimidazolylthio)-acetic, α(5-nitro-2-benzimidazolylthio)propionic, (5-methoxy-2-benzimidazolylthio)acetic, α(5-methoxy-2-benzimidazolylthio)propionic, (5,6-dimethoxy-2-benzimidazolylthio)acetic, (5-methyl-2-benzimidazolylthio)-acetic, α(5-methyl-2-benzimidazolylthio)propionic, (4,6-dimethyl-2-benzimidazolylthio)acetic, α(4,6-dimethyl-2-benzimidazolylthio)propionic, (5,6-dimethyl-2-benzimidazolylthio)acetic, α(5,6-dimethyl-2-benzimidazolylthio)propionic, (5-phenyl-2-benzimidazolylthio)acetic and α(5-phenyl-2-benzimidazolylthio)propionic acids.

Although none of the compounds was herbicidal when tested at a concentration of 0.005 M, variations in the structure of the benzimidazoles did influence the inhibitory activity. The β-propionic acids were slightly better inhibitors than the corresponding acetic acid derivatives whereas the α-propionic acids were generally the poorest inhibitors.

The nature of the substituent in the aromatic nucleus also affected the inhibitory activity of the 2-thiobenzimidazole analogs, and these substituents had a greater influence than did variations of the acid side chain. A chlorine or a bromine atom in the 5-position of the benzimidazole nucleus greatly increased the inhibitory activity with a chlorine atom slightly more effective than a bromine atom. The substitution of a second chlorine atom in the 6-position also resulted in a compound which was a much better inhibitor than the unsubstituted compound, although this derivative was not quite as effective as the 5-monochlorobenzimidazole. When the chlorine atoms were substituted in the 4- and 6-positions, the inhibitory power was reduced still further; however, this compound was still a better inhibitor than the parent compound. Three chlorines in positions 4,5 and 6 almost completely removed the inhibitory activity of the benzimidazole compound.

Nitro-, phenyl-, methyl- and methoxyl-groups greatly decreased the inhibitory activity and the substitution of two methyl groups in either the 5- and 6- or the 4- and 6-positions almost completely destroyed the ability of these compounds to inhibit the growth of the test plants.

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