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SYNTHESIS OF BENZOTHIAZOLES: PHAEOMELANIN MODEL COMPOUNDS

The Ohio State University PH.D. 1980

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SYNTHESIS OF BENZOTHIAZOLES:
PHAEOMELANIN MODEL COMPOUNDS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Ibrahim Ahmed Ismail, B.A., M.S.

* * * * *

The Ohio State University
1980

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ACKNOWLEDGMENTS

I would like to thank The Ohio State University chemistry department for financing my graduate education. Many thanks and appreciation to my advisor Dr. Miles Chedekel for all the help he has given me over the years. I would also like to thank Dr. David Hart for his help, friendship and understanding in the completion of this project.
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Chapter 1
INTRODUCTION AND RATIONALE

Photobiology of Melanins:

"MELANINS" are of interest because of their widespread occurrence in biological systems. Amongst their many functions, they afford protection against the damaging effect of sunlight and are responsible for the color of human skin. Current evidence indicates that the distribution pattern, size and type of melanosomes or melanin granules in the epidermis are responsible for skin color.

It has been suggested that exposure to sunlight is frequently responsible for the development of skin cancer in humans. Observations which support this suggestion are:

(1) the more frequent occurrence of skin cancer on sun exposed relative to unexposed areas of the body; (2) the greater incidence of disease in regions of the earth that receive the greatest sunlight. (3) the more common occurrence of cutaneous cancer in whites who sunburn readily than in people with more heavily pigmented skin and (4) the greater prevalence of skin cancer among persons who work outdoors than those indoors. Epidermal melanins apparently are photoprotective in nature. Indeed, Pathak and Fitzpatrick have proposed four mechanisms to account for this photoprotective nature:
(a) melanin acts as a neutral density filter, (b) melanin absorbs the radiant energy and dissipates it as heat, (c) melanin can also utilize the absorbed energy and undergo immediate oxidation through the generation of semiquinoid free radicals in the polymer, (d) melanin as a stable free radical has a capacity to act as a biological electron exchange polymer.

Chemical Nature of Melanins

The term eumelanin refers to nearly all black and brown pigments that possess a high molecular weight. Its insolubility in nearly all known solvents, makes purification difficult. Acid hydrolysis, which could cause extensive degradation, has been the method used to isolate eumelanins from cellular material. Difficulties, however, have precluded the isolation of a single chemical compound of definite composition.

Irrespective of the fact that melanin has not been isolated as a single chemical compound of definite composition, the use of sophisticated biochemical and biophysical techniques have shed some light on its structure and composition. The results so far indicate that the pigment is a copolymerize of compounds biosynthetically derived from tyrosine, in particular DOPA. The chromatophore of melanin is linked to a protein and the whole unit is called a melano-protein. In practice however, the term melanin may refer to either the chromatophore alone and/or the melano-protein.
Much of what is known about the nature of eumelanins is based on their synthesis in vitro. However, there is no proof that these synthetic eumelanins are identical in structure to natural eumelanins since physiological conditions in animal tissues cannot be simulated in vitro. The work of Nicolaus and coworkers, using chemical and modern biochemical techniques, has demonstrated that the chromophore portion of the eumelanin molecule of animals is an irregular polymer made up of indole-5,6-quinone units, which can be present in different stages of oxidation. This fact seems consistent with classical concepts as well as with more recent work showing that synthetic eumelanin consists of a number of different monomer units linked in various ways.

The generally accepted pathway (figure 1) for the biosynthesis of eumelanin involves tyrosinase, which catalyzes the oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and DOPA to DOPA-quinone, and is the only enzyme involved in this pathway.
Figure 1; Biosynthetic pathway of eumelahin
This view has been challenged and it has been suggested that at least one other enzyme must be present for the conversion of tyrosine to eumelanin. Thus it has been suggested that a synergistic action of peroxidase and dopa oxidase is involved.\(^{12}\)

Because it has not been possible to isolate the melano-protein in an unaltered and pure state, not much is known about the protein portion of natural eumelanin or its effect on the molecule. The protein is apparently linked to eumelanin by sulfur containing amino acids, in particular cysteine, and possibly by direct peptide linkages.\(^{13}\)

**Phaeomelanin:**

The biochemistry of phaeomelanin is not as well known as that of eumelanin. Both chemical and morphological differences between the pigments have been attributed to the degree of polymerization of the tyrosine oxidation products or their states of oxidation.\(^{14}\) In this discussion the term "phaeomelanin" will refer to the non-black melanin-like epidermal pigments wherein both the color and origin are specified.

Unlike eumelanin, phaeomelanin is soluble in alkali and exhibits fluorescence under ultra-violet light.\(^{15}\) The pigment has been shown to be photolabile under physiologically relevant conditions.\(^{16}\) It has also been demonstrated that irradiation of phaeomelanin produces highly reactive oxygen based radicals, such as superoxide and hydroxyl, as well as mutagenic compounds.\(^{17}\)
Prota and Thompson have reviewed various characterizations of phaeomelanin and have also determined some of the chemical properties of these pigments.\textsuperscript{18} The pioneering work of Prota and others demonstrated that oxidizing a mixture of DOPA and cysteine with alkaline potassium ferricyanide produced pigments which were identical with those obtained from red chicken feathers. They indicated that 5'-S-cysteinyldopa (1), is an important intermediate in the biosynthesis of phaeomelanin.\textsuperscript{19}

These experiments suggested a direct linkage in the biosynthetic pathways of both eumelanin and phaeomelanin as indicated in figure 2.

Phaeomelanins were found to be intractable and not "usefully studied by the usual spectroscopic methods."\textsuperscript{20} Both infrared and nuclear magnetic resonance spectra exhibit only broad bands of little diagnostic value and the ultraviolet spectrum is reported to show no definite maxima. On treatment with boiling hydriodic acid, phaeomelanin affords several isolable hydrolysis products 2-7.\textsuperscript{21}
Figure 2; Biosynthetic pathway of both eumelanin and phaeomelanin
(8)

(9a) $R = \text{OH}$
(9b) $R = \text{NH}_2$

(10a) $R = \text{OH}$
(10b) $R = \text{NH}_2$

(11)

(12)
Permanganate oxidation yields 8-11. Under the reaction conditions, 8 is produced from 9a. In addition, 9a and 10a arise from 9b and 10b. The largest product of the oxidative degradation, isolated in low yield is 12. It was concluded that all degradation products could be accommodated by the sequence of reactions outlined in figure 3. Compound 14 is therefore believed to be the source of the pyridine carboxylic acid and its degradation products. Quinoline 14, was itself isolated in very low yield. In a follow-up report by Fattoruso and others, 7-amino-3-carboxy-6-hydroxyisoquinoline (14) and 7-amino-3-carboxy-6-hydroxy-1-methylisoquinoline (17) were isolated from the degradation products of phaeomelanin in hot hydriodic acid.

![Chemical Structure](image)

Based on an analysis of the forementioned degradation studies and knowledge of the biosynthesis of phaeomelanin, the general structure 15 has been proposed for the phaeomelanin chromophore. This structure does not however, explain the spectroscopic data especially the UV/VIS spectra, obtained on the natural pigment.
Figure 3; Proposed degradative pathway of phaeomelanin
(15)
Thus oxidation of some of the phenolic groups to the quinoid form, resulting in extended conjugation, was proposed to account for the intense visible absorption spectra of the pigment. Structure 16 therefore seems to represent a model better suited to explain the chemical and spectroscopic data available on phaeomelanin.

As pointed out already, phaeomelanin has several types of functionalities. However, in light of the biosynthetic pathway presented and a study of the degradation products, it has been suggested that the chromophore which most probably absorbs the light responsible for the photodegradation of phaeomelanin, is the benzothiazole moiety (e.g. 5). The chain of reactions responsible for the photodegradation of phaeomelanin is now believed to be initiated by the oxidation of the phenol moiety 15 to the quinone 16.

As already indicated, degradation of the protein-free chromophore of phaeomelanin afforded the two amino acids 5 and 7 as major products. These were isolated as their methyl esters and characterized using spectroscopic techniques. Thus, the validity of current speculation about the structure of phaeomelanin depends, in part, on the correct interpretation of spectral data obtained on these amino acids. Based solely on the published spectral data structures such as 18 and 19 could not be ruled out as alternative structures for those degradation products assigned structures 5 and 7 respectively.
(16)
In order to place speculation about the structure of phaeomelanin on firmer ground, it was felt that unequivocal proof of the structure of these key amino acids should be sought. This thesis presents our efforts to understanding the chemistry of benzothiazoles believed to be involved in phaeomelanin biosynthesis. Specifically, the identity of 5 as a phaeomelanin degradation product has been confirmed by a total synthesis to be described herein. Before presenting the details of this work, pertinent developments in benzothiazole chemistry will be reviewed to provide the reader with an overview of the field.
Chapter 2

HISTORICAL

Nomenclature, Properties and Occurrence of Benzothiazoles:

The fusion of a benzene ring to a thiazole system produces a class of heterocycles called benzothiazoles whose properties are different from those of both benzene and thiazole. The more important are the bicyclic systems wherein the benzene ring is fused to the 4,5-positions of the thiazole. Benzothiazole (20), and the related naphthothiazoles 21, 22 and 23 are shown below.

![Diagram of benzothiazoles and naphthothiazoles]

\[ \text{α-naphthothiazole} \quad \text{β-naphthothiazole} \quad \text{β,β-naphthothiazole} \]
It should be pointed out that in the early literature, in particular the French and Japanese, the numbering system designated sulfur as 2, with the adjacent non-benzenoid carbon as 1. This inconsistency in numbering must be recognized when one reads the older literature on these heterocycles. The currently accepted numbering system is that indicated for compound 20.

The history of benzothiazoles started with the discovery of the thiazole nucleus by Hantzsch in 1887. Hofmann then prepared the first derivatives of benzothiazoles in 1897, namely 2-chlorobenzothiazole and 2-phenylbenzothiazole. In the following years, many examples of various derivatives of the basic nucleus 20 have been reported. This has been a result of the interesting physiological properties of these systems which include antimalarial and sedative properties. For example, the following substituted benzothiazole 24, was recently synthesized and tested for physiological properties. It was reported to possess sedative, anticonvulsant, antihistaminic, analgesic and muscle relaxing properties.

\[
\begin{align*}
\text{R} & = \text{OCH}_3 \\
\text{R}^1 & = \text{R}^2 = \text{CH}_3 \\
\text{R}^3 & = \text{H}
\end{align*}
\]
In addition, derivatives such as 25 possessing a pyrazolone moiety are also reported to exhibit powerful anti-inflammatory activity and low toxicity.\textsuperscript{31}

25

Industrial applications\textsuperscript{32} of benzothiazoles include the use of carbocyanine dyes as photographic sensitizers\textsuperscript{33} and azo dyes for various color applications on Dacron, Nylon and Cellulose acetates.\textsuperscript{34} In addition the parent 2-mercaptobenzothiazole (26) and its derivatives are known to accelerate rubber vulcanization.\textsuperscript{35}

26

Studies on firefly bioluminescence have indicated that a small molecular substrate called luciferin undergoes an enzyme-catalyzed oxidation that results in light emission.\textsuperscript{36} The unique structure of this molecule has triggered an interest in its biosynthesis. The structure of firefly luciferin (27) contains a benzothiazole moiety.
This structure is postulated to arise from p-benzoquinone and cysteine via a series of enzyme-catalyzed condensations and dehydrations.\textsuperscript{37} To the author's knowledge, with the exception of phaeomelanin pigments already discussed, this is the only other reported case of a benzo-thiazole ring system appearing in nature.

\textbf{Uses of Benzothiazoles in Synthesis:}

The ability of benzothiazoles to stabilize carbanions at and adjacent to the 2-position has been exploited to a certain extent in organic synthesis. For example, in biological systems, carbonyl groups are often formed by oxidation of amines. A large variety of oxidizing agents have been applied to accomplish this transformation in the laboratory.\textsuperscript{38} In a biomimetic approach, the conversion of a primary amine 28 into a carbonyl 34 has been accomplished by base catalyzed prototropic rearrangement of the Schiff base 31, as illustrated in scheme -1.\textsuperscript{39}
The reactions were practically quantitative and isomerization was spontaneous. Ready isolation of products was accomplished following acid hydrolysis of 33 to give ketone 34 and the respective 2-methylaminobenzothiazole 35.
The crucial factor in such "transaminations", is the ability of the benzothiazole residue, such as 29 and 30, to delocalize efficiently the negative charge produced in the intermediate 32 after deprotonation of the Schiff base 31.

In an excellent series of papers by Corey and Boger, the usefulness of benzothiazoles as carbonyl anion and cation equivalents was demonstrated. The reactions outlined in Scheme -2 were devised to accomplish the transformation of a 2-vinyl benzothiazole moiety 38 to an α,β-unsaturated aldehyde 37 or ketone 41 in excellent yield. Other transformations using the vinyl benzothiazole 38 are outlined in equations 1 - 3.

\[
\begin{align*}
\text{eq. -1} & \\
38 & \xrightarrow{\text{BT}} \left[ \begin{array}{c}
\text{BT} \\
N
\end{array} \right] \xrightarrow{\text{PhCH}_2X} 44
\end{align*}
\]

\[
\begin{align*}
\text{eq. -2} & \\
43 & \xrightarrow{\text{BT}} \left[ \begin{array}{c}
\text{BT} \\
\text{Ph}
\end{array} \right] \xrightarrow{\text{PhCH}_2X} 44
\end{align*}
\]

\[
\begin{align*}
\text{eq. -3} & \\
43 & \xrightarrow{\text{BT}} \left[ \begin{array}{c}
\text{BT} \\
\text{Ph}
\end{array} \right] \xrightarrow{\text{PhCH}_2X} 44
\end{align*}
\]
Scheme 2
Equations 1 and 2 show the problems of α-alkylation, dialkylation, and self-condensation that usually plague the direct α-alkylation of α,β-unsaturated aldehydes were circumvented by using the allylic anions generated from 38 and 43. Equation 3 illustrates the use of vinyl benzothiazole 38 in the stereocontrolled synthesis of α,β-unsaturated ketones 47.

When 38 was treated with the α-lithioderivative of acetone dimethylhydrazone, followed by quenching with methanol or methyl iodide, the corresponding ketones 45 were obtained after hydrazone hydrolysis. After unmasking the benzothiazole, acid catalyzed cyclization provided the octalones 47 in good yield. In a similar annelation scheme treatment of 38 with methyl lithium followed by propargyl bromide afforded the benzothiazole 48 which was converted to 49 by Hg(II) catalyzed hydration of the terminal triple bond and benzothiazole hydrolysis.
The spiro-enone 50 was subsequently obtained in 78% overall yield by base catalyzed cyclization. It should be recognized that the property exploited in all of the above cases is the ability of the benzothiazole to delocalize a negative charge into its pi framework. The high yields, mild conditions and stereochemical results obtained, are an added testimony to the usefulness of these heterocycles in organic chemistry.

Synthesis of Benzothiazoles:

1. Via 2-Aminothiophenols

Various synthetic approaches have been developed for the construction of benzothiazoles with different substituents on both the benzene and the thiazole rings. The great number of approaches developed so far suggests that no general method for producing all kinds of benzothiazoles has been developed. A close examination of existing approaches indicates that the technique or methodology to be employed is strongly dictated by the substituent desired in the 2-position of the final product.
The availability of 2-substituted benzothiazoles is strongly dependent on routes in which the fused thiazole ring is constructed from an acyclic precursor. One of the earliest and most versatile approaches to 2-substituted benzothiazoles is outlined in equation -4. It involves the reaction between an o-aminothiophenol (51) and a carboxylic acid or an appropriate derivative.

\[
\begin{align*}
\text{NH}_2 & \quad + \quad \text{RCOX} \\
\text{SH} & \quad \rightarrow \\
\text{NH-C-X} & \quad \rightarrow \\
\text{R} & \quad \text{S} \\
51 & \quad 52 & \quad 53
\end{align*}
\]

(eq. -4)

This approach seems to work best when carboxylic acid chlorides and anhydrides are used. Acids, esters and imino esters have also been used successfully. The o-acyl amino derivatives 52 which are intermediates in this reaction, have been shown to cyclize readily. The o-acyl aminophenyl disulfides 54 on reduction with stannous chloride, tin and hydrochloric acid are also known to yield benzothiazoles.
A recent report recommended the use of a 1:10 (W:W) mixture of \( \text{P}_2\text{O}_5/\text{CH}_3\text{SO}_3\text{H} \) for effecting the construction of 2-substituted benzothiazoles from \( \text{o-} \)aminothiophenols and carboxylic acids. Even this reagent mixture which is reported to give very good yields of products, does not seem to solve the problems associated with other procedures. Besides the difficulty in handling the reagent, no report of any benzothiazoles with other than 2-substituents was recorded. Moreover, the method was not recommended for \( \alpha,\beta \)-unsaturated carboxylic acids. Other reports have included the use of alkyl selenoesters in ethanol to afford good yields of benzothiazoles that possess isopropyl, n-propyl and n-butyl substituents in the 2-position as shown in equation -5.49

\[
\text{NH}_2 \quad \text{SH} \quad \text{Se} \quad \text{C}_2\text{H}_5\text{OH} \quad \text{R}
\]

R= isopropyl; n-propyl; n-butyl

(eq. -5)

Again in this sequence only commercially available \( \text{o-} \)aminothiophenols were used. Whether good yields could be obtained with substituents on the benzene ring is unknown. Moreover, no report of \( \text{R} = \text{CH}_3 \) was mentioned. The reader should be reminded that our interest in benzothiazoles was limited to those that possess a methyl group or a hydrogen in the 2-position as well as substituents on the benzene ring. Though in principle this scheme looks attractive, it is beset by serious problems.
The difficulties encountered with condensations of 51 with acid derivatives include long times, elevated temperatures and drastic acid catalysis which often leads to poor yields or total destruction of both reactants and products. However, the biggest drawback of this method is unavailability of appropriately substituted o-aminothiophenols 51. Only o-aminothiophenol itself is commercially available. Most of the reported methods for preparing substituted o-aminothiophenols are ambiguous and in some cases the products have not been characterized adequately. For example, thiol 55 has been reported as a thick oil 50 as needles with unspecified melting point 51 and as a hydrochloride 52.

![Chemical Structure](image)

The construction of a substituted benzothiazole, followed by thiazole cleavage has been used to prepare o-aminothiophenols of the type required in equation 4. (Scheme -3) 53. A recent report outlines a strategy for the preparation of disulfides derived from previously unavailable 4,6 disubstituted o-aminothiophenols 54 (Scheme -4).
Scheme 3

1. diazotize
2. H₃PO₄
3. H₂N-NH₂·H₂O

Scheme 4
Because there are a number of methods for reductively cleaving disulfides\textsuperscript{55}, these readily isolable products are useful precursors of complex \(\text{o-aminothiophenols}\).

II. \textit{Via} Substituted Anilines

Ring closures of the type shown in equation -6 are applicable to the synthesis of a wide variety of benzothiazoles that possess substituents in the 2-position.

\[
\begin{array}{c}
\text{R} \\
\text{X}
\end{array}
\xrightarrow{\text{K}_2\text{Fe(CN)}_6 \text{NaOH}}
\begin{array}{c}
\text{56} \\
\text{57}
\end{array}
\]

(eq. -6)

These kinds of reactions can be divided into two classes which are implemented according to the nature of \(R\) desired in \textit{57}. When \(R\) is an alkyl group, an alkoxy or a carboxy group or derivative, the cyclization is usually carried out in alkaline potassium ferricyanide\textsuperscript{56}. This procedure is known as the Jacobson synthesis. The problem with this synthesis is the strong dependence of ring closure on the nature and location of substituents on the ring. Some examples are outlined below. When an electron donating or withdrawing group is present in the 4-position, the yields reported are not good. For example, 2-methyl-4-methoxybenzothiazole (58) and 2-methyl-4-chlorobenzothiazole (59) are prepared in only 15 and 10\% yields respectively by this method.
Although, 2-methyl-6-methoxybenzothiazole (60) was formed in 75-6% yield by the Jacobson reaction, the synthesis of 2,6-dimethyl-4-methoxybenzothiazole (62) was not very successful. Thus, when 61 was treated with alkaline potassium ferricyanide in 17% NaOH, only about 15% of the desired 62 was obtained. The rest of the reaction mixture consisted of unreacted 61 and the starting amide 63.

Adding more potassium ferricyanide did not convert unreacted 61 to 62. Only more of 63 was obtained.
When various thioacetanilides possessing electron withdrawing groups, such as NO₂, CN, and SO₃H were subjected to the Jacobson conditions, tautomerization took place to give 65.

\[
\text{S} \quad \begin{array}{c}
\text{N} \\
\text{NH-C-CH₃}
\end{array}
\]

\[
\text{O₂N} \quad \begin{array}{c}
\text{N} \\
\text{NH-C-CH₃}
\end{array}
\]

When 4-nitrothioacetanilide (64) was treated under the above conditions, only a 33% yield of the benzothiazole was obtained. The 3-nitroanalogue 66 gave only 2-methyl-7-nitrobenzothiazole (62) and no 2-methyl-5-nitrobenzothiazole (68).

\[
\text{O₂N} \quad \begin{array}{c}
\text{N} \\
\text{NH-C-CH₃}
\end{array}
\]

\[
\text{N} \quad \begin{array}{c}
\text{N} \\
\text{CH₃}
\end{array}
\]

\[
\text{O₂N} \quad \begin{array}{c}
\text{N} \\
\text{CH₃}
\end{array}
\]

In recent years, many different modifications of this ring closure process have been developed. However, they all seem to have limitations as are described.
Bunnett and Hrutfiord developed a ring closure process dependent on an aryne intermediate. The principle is illustrated in equation 7.

(eq. -7)

\[ X = \text{Halogen atom ortho or meta to side chain} \]

\[ Y_H = \text{Functional group which, upon loss of proton, forms a nucleophilic group.} \]

Treatment of thiobenz(o-bromo)-anilide (69) with potassium amide in liquid ammonia gave a 90% yield of 2-phenylbenzothiazole (71), as illustrated in scheme -5.
The formation of 71 from both 72 and 69 led these workers to propose an aryne mechanism. It was, however, later reported by Spitulnik that compound 73 gave a 69% yield of 6-chloro-2-methyl benzothiazole (74) upon treatment with one mole of sodium methoxide.因此

Spitulnik suggested that an aryne mechanism was not operating since it was felt that sodium methoxide would not be able to dehydrohalogenate 73. Further work in this area has suggested that these reactions may be light mediated and that they proceed by an $S_{RN}$ radical mechanism as shown in scheme -6. The use of light for effecting ring closure to give 2-methyl substituted benzothiazoles was recently reported (equation -8). The yields were good and various dihaloderivatives of 75 were used.
1. $\left[ \begin{array}{c} \text{NH} \\
\text{X} \\
\text{S} \\
\text{R} \end{array} \right]$ $\rightarrow$ $\text{NH} \cdot \text{S} + \text{x}^-$

$X = \text{Br; Cl; I}$

$R = \text{alkyl or aryl}$

2. $\text{NH} \cdot \text{S} \leftrightarrow \text{NH} \cdot \text{S} + \text{H}^+$

3. $\text{NH} \cdot \text{S} \rightarrow \left[ \begin{array}{c} \text{S} \\
\text{N} \\
\text{R} \end{array} \right]$ $-$

4. $\left[ \begin{array}{c} \text{S} \\
\text{N} \\
\text{R} \\
\text{X} \end{array} \right] + \text{R}^1 - \text{X} \rightarrow \text{S} \cdot \text{R} + \left[ \begin{array}{c} \text{R}^3 \text{X} \end{array} \right]^{-}$

Scheme -6
When there was competition between Br and Cl in the substitution, as in 77, only 4,6-dichloro-2-methylbenzothiazole (78) was produced.

Another versatile route to benzothiazoles has been developed by Hugerschoff. He found that treatment of an appropriately substituted N-aryl thiourea 79 with bromine in an inert solvent such as chloroform or carbon tetrachloride gave the hydrobromide 80 of the 2-amino-benzothiazole as shown in scheme -7.
Advantages of the Hugerschoff synthesis include the ready availability of the aryl thioureas \(79\) from commercially available anilines. The possibility of starting with disubstitution was demonstrated and the conditions for ring closure were mild. The method was shown to be general for both the benzothiazole and napthothiazole series. In this way, 1-naphthyl-thiourea (82) gave 2-aminonaphtho[1,2]-thiazole (83) and 2-naphthylthiourea (84) gave 2-aminonaphtho[1,2]thiazole (85) in good yields.\(^{66}\)
N-Alkyl and unalkylated aryl thioureas reacted readily while N,N-dialkyl-N'-aryl thioureas were far less reactive.

When an alkyl group was attached to the same nitrogen that held the aryl group, as in 87 and 88, cyclization produced the 2-iminothiazolines and respectively.
When N, N'-diaryl thioureas such as 91 are used, ring closure could take place to give either 92 or 93.

In such cases, however, the reaction usually follows only one path. Ring closure occurs on the aryl group that is unsubstituted. When both aryl groups are substituted in the para position, the group that holds the least inactivating substituent is involved in the ring closure.

Although bromine has found widespread use in the Huggerschoff synthesis, other reagents can be employed satisfactorily. Cyclizations with sulfuryl chloride, sulfur monochloride and chlorine have been implemented.

Another widely used method for construction of benzothiazoles with substituents is thiocyanation. Thiocyanation involves the replacement of a hydrogen atom by a thiocyanato group through the use of thiocyanogen. The thiocyanation reaction (equation -9) is limited to aromatic amines and phenols.
When there is competition between a phenolic group and an amino group, substitution is usually directed by the amino group. For example, o-aminophenol (24) gave 4-thiocyano-2-hydroxyaniline (25) in good yield.

Due to instability and water sensitivity (eq. -10) it is always preferable to generate thiocyanogen in situ before use.

(\text{SCN})_2 + \text{H}_2\text{O} \rightarrow \text{HSCN} + \text{HOSCN} 
(eq. -10)

Thiocyanogen has been generated in various ways including electrolysis. However, it is most easily generated from salts by chemical reagents and this method of generation has found widespread use in benzothiazole synthesis. For example, cupric thiocyanate generates thiocyanogen merely by dissociation of the cupric to the cuprous salt as shown in equation -1.

2\text{Cu(SCN)}_2 \rightarrow 2\text{CuSCN} + (\text{SCN})_2 
(eq. -1)
It is best to prepare the cupric thiocyanate by mixing equal amounts of copper sulfate and sodium or potassium thiocyanate. This mixture is easy to prepare and handle.

A variation of this method which has also found widespread use in benzothiazole synthesis, involves the generation of thiocyanogen in methanol or acetic acid by treating potassium or sodium thiocyanate with bromine in the cold. The thiocyanogen is usually generated in the presence of the substituted aniline and the product in this case is the hydrobromide of the 2-amino benzothiazole. The free base can readily be generated by digesting the hydrobromide salt in warm alkali. The procedure provides ready accessibility to benzothiazoles with both electron withdrawing or donating substituents. These advantages, coupled with easy, clean work-ups and moderate to good yields, makes thiocyanation the most attractive route to 2-aminobenzothiazoles and their derivatives. The thiocyano group is readily introduced into aromatic amines. It attacks a free para position if available, otherwise, o-substitution occurs. When 2,4-xylidine was treated with thiocyanogen, 2-amino-4,6-dimethylbenzothiazole (96) was obtained in 79% yield (equation -12). Thus, when the para position is substituted, benzothiazoles are obtained directly.

\[
\begin{align*}
\text{CH}_3 & \quad \text{NH}_2 \\
\text{CH}_3 & \quad \text{SCN}_2 \\
\text{CH}_3 & \quad \text{NH}_2
\end{align*}
\]

(eq. -12)
2-Amino-6-methylbenzothiazole (97) was prepared in 80% yield and 2-amino-6-chloro-4,7-dimethylbenzothiazole (98) in 65% yield using the thiocyanation procedure.

Even the electron withdrawing $-\text{CO}_2$ group did not deter ring closure which was accomplished in 67% yield from p-aminobenzoic acid to give 2-amino-6-carboxy benzothiazole (99).

Electrophilic Aromatic Substitution Reactions of Benzothiazoles

Substitution patterns of benzothiazoles in electrophilic aromatic substitution reactions are not as easy to predict as in the benzene series. The difficulty arises from the fact that substituents on both the benzene and thiazole rings produce varying effects on the substitution patterns observed. Resonance structures 100 - 103 clearly indicate electrophilic aromatic substitution should be favored at positions 4 and 6.
Quantum mechanical calculations of the electron distribution in benzothiazole are in agreement with such forms and with experimental observations.\textsuperscript{80}

In addition, a recent \textsuperscript{13}C-NMR study established that the 6-position of benzothiazole (20) was the site of highest electron density.\textsuperscript{81} Care must be exercised, however, when applying these results to other than benzothiazole itself. Substituents in the 2-position appear to direct electrophilic substitution only to the 6-position. Thus, 2-substituted benzothiazoles behave much like benzothiazole itself. Nitration of either benzothiazole (20) or 2-methylbenzothiazole (104) gives over 80\% of the 6-nitro analogue 105.\textsuperscript{82}
It has been reported that nitration of 2-amino-4-nitrobenzothiazole (106) gave 83% of 2-amino-4,6-dinitrobenzothiazole (107). When an electron withdrawing group is present on the benzene ring, it seems to retard reactivity especially if it is in the 6-position. Attempts to nitrate 6-nitro-2-methylbenzothiazole (108) with fuming nitric acid did not give any of the 4,6-dinitro-2-methyl benzothiazole (109).
In addition, attempts to nitrate 6-cyano-2-methyl-benzothiazole (110) with fuming nitric acid and concentrated sulfuric acid did not give any of the expected 6-cyano-2-methyl-4-nitro benzothiazole (111).

\[ \text{110} \quad \text{111} \]

In summary, 2-substituted benzothiazoles favor electrophilic aromatic substitution at the 6-position. Minor amounts of other isomers are also always reported although certainty of their positions is not clear. Whether the 7-position or the 5-position is favored, when the 6-position is substituted, has yet to be determined with certainty.

**Pictet-Spengler and Bischler-Napieralski Reactions:**

A subgoal of this research was to examine methods for converting benzothiazole amino acids such as 5 to isoquinoline derivatives believed to be related to the phaeomelanin chromophore (eq. 15). We intended to accomplish this goal by use of the Pictet-Spengler \(^{86,87}\) and Bischler-Napieralski reactions. \(^{88,89}\) A brief review of these reactions will be presented here. The Pictet-Spengler reaction involves the condensation of a \(\beta\)-aryl ethylamine 112, with an aldehyde (or its equivalent) to give a Schiff base 113.
Subsequent electrophilic attack usually affords ring closure under acidic conditions to give 114 (see scheme -8). Various catalysts, including concentrated hydrochloric and sulfuric acids have been used to effect ring closure. The reaction has also been adapted to the so called "Physiological Conditions" of PH, temperature and concentration. However, reaction times are long and few, highly reactive aldehydes (eq. formaldehyde) have been observed to react under these conditions.

\[ RCHO \]

\[ R = H, \text{alkyl or alkoxy} \]
\[ R^1 = \text{alkyl or aryl} \]

Scheme -8
Tryptophan (115), reacts with formaldehyde at PH = 6.5 and 38° for 15 hours to give an 80% yield of the adduct 116. There are, however, no reported cases in the literature, of benzothiazole amino acids such as 5, undergoing the Pictet-Spengler reaction.

As a result, the reaction would provide valuable information about ring closure orientation as well as reactivity of the benzothiazole amino acids.

In the Bischler-Napieralski reaction, the N-acyl derivative 117 of a β-phenethylamine is treated with a dehydrating agent under reflux, in an inert solvent such as toluene or xylene. Cyclodehydration usually follows ring closure to give dihydroisoquinolines 118.

R = alkyl or aryl
Chapter 3

RESULTS AND DISCUSSION

In considering synthetic pathways that would be amenable to large scale preparation of 5, two strategies seemed promising. The first of these involved the construction of a properly substituted benzothiazole to which the required α-aminopropionic side chain could be built. The second called for the construction of a suitably substituted aniline followed by annelation of the benzothiazole ring system by one of the methods outlined above. This approach requires that the aniline carry an amino acid synthon that would survive the reaction conditions necessary for ring closure and could be converted to the amino acid without benzothiazole ring destruction.

![Chemical structure](image)

Previous work\textsuperscript{91} from this laboratory in pursuit of the first option had culminated in the synthesis of 124 outlined in scheme-9. Unfortunately attempts to functionalize the benzylic position of 122 with N-bromo-succinimide (NBS; 1 eq.) only led to ring bromination yielding 123 and none of the desired 125. An extra equivalent of NBS was required to produce the dibromo compound 124. It was apparent that the methyl group of the 6-position of 122 could not be selectively functionalized by bromination.
The absence of any of the 5-bromoderivative 126, showed that the 7-position in 122 was however, quite susceptible to electrophilic aromatic substitution. Although this was an unexpected result, it did provide valuable information in light of further studies anticipated at that time. The importance of this result will become apparent to the reader at a later time. In an attempt to slow down the rate of substitution at position 7, 2-acetamido-4-methoxy-6-methyl benzothiazole (127) was prepared from 121 by treatment with hot acetic anhydride.

\[ \text{OCH}_3 \]
\[ \text{NHCOCCH}_3 \]

126. 127

This was subjected to allylic bromination using either N-bromosuccinimide or N,N-dibromohydantoin. Unfortunately, these attempts to brominate the 6-methyl group were unsuccessful. In all cases tried with different free radical initiators, only starting material was recovered. The acetamido group was apparently too deactivating for any reaction to occur. Consequently this method of functionalizing the C-6-methyl group was abandoned.
In pursuing a properly functionalized benzothiazole from which a side chain could be constructed, 6-carbethoxy-4-methoxy benzothiazole (133) was prepared as shown in scheme -10. Oxidation of 3-methoxy-4-nitrotoluene (128) with basic permanganate solution, furnished 129 in quantitative yield. This acid was readily esterified and the resulting nitro ester 130 was reduced to the amine 131 by hydrogenation over PtO₂. Platinum or palladium on carbon as catalyst, did not effect the reduction. Although small scale hydrogenation of 130 was successful, it was not practical on a large scale. As an alternative, nitro acid 129 was reduced with tin and hydrochloric acid to give 4-amino-3-methoxybenzoic acid (136) as colorless needles. This acid was esterified with refluxing ethanol saturated with HCl gas to give 131 in an overall yield of about 70% from 129.

\[
\begin{align*}
\text{OCH}_3 & \quad \text{NH}_2 \\
\text{HO}_2C &
\end{align*}
\]

Conversion of 131 to 2-amino-6-carbethoxy-4-methoxy benzothiazole (132) was tried in two different ways. The first of these involved the preparation of thiourea 137 using ammonium thiocyanate in refluxing chlorobenzene.
Scheme -10
Although the yields of the thiourea 137 were moderate, attempts to convert 137 to 132 using bromine in chloroform did not proceed in acceptable yields. However, when 131 was treated with a mixture of CuSO₄ and KSCN in refluxing methanol, excellent yields of 132 were obtained. It was preferable to mix the cupric sulfate and potassium thiocyanate before adding the mixture in portions to the methanolic solution of 131. With 2-aminobenzothiazole 132 in hand, removal of the 2-amino group was attempted using diazotization techniques.

The difficulty of diazotization of 2-aminobenzothiazoles stems from their less basic character as compared to aryl amines. Consequently, vigorous conditions were required to replace the 2-amino group with hydrogen. The use of ethanol⁹² and/or hypophosphorous⁹³ acid proceed in only moderate yields. Only a few cases have been reported⁹⁴ for making the tetrafluoroborate salts when the parent 2-aminobenzothiazole is soluble in fluoroboric acid. Due to the insolubility of 132 in most solvents, diazotization of this amine had to be carried out in 85% phosphoric acid.
Compound 132 was even insoluble in 48% HBF₄ after heating vigorously on the steam bath for long periods of time. This insolubility in fluoroboric acid precluded any attempts of making the tetrafluoroborate salt. Diazotization of 132 with sodium nitrite gave a maroon colored solution which was rapidly treated with 50% hypophosphorous acid. Dilution of the resulting viscous, yellow solution with water followed by repeated extraction with chloroform furnished the ester 133 and the acid 138.

After esterfying 138, a moderate yield of the ester 133 was obtained.

At this point conversion of ester 133 to aldehyde 134, for use in homologation to the propionic acid side chain was investigated. This turned out to be very problematic. Treatment of ester 133 with lithium aluminum hydride at -78° in ether gave at least five products recognizable on TLC. Unfortunately proton magnetic resonance analysis of the crude mixture showed no signal for an aldehyde proton. The use of diisobutyl aluminum hydride (DIBAL-H) in ether at -78° also showed a multitude of products after work-up.
Although aldehyde functionality was detected by both NMR (10.26) and IR (1715 cm\(^{-1}\)), such small amounts were present that the procedure was not deemed useful for large synthetic applications. It had been reported that treatment of 6-carbomethoxy-2-methyl benzothiazole (139), with LAH at -78° furnished the 6-hydroxy-methyl derivative (140). This was the only reported case of treating a benzothiazole derivative with LAH.

![Chemical structures](image)

It is suspected that the 2-methyl group in 139 blocks reduction of the thiazole ring. In our case the ring is very susceptible to hydride reduction and ring opening may have been a serious problem. At the time this work was in progress, a report appeared in which it was stated that benzothiazoles are readily cleaved to o-aminothiophenol derivatives upon treatment with diborane. Since it appeared that reduction of 132 might be difficult to achieve without cleaving the labile thiazole ring, the second strategy of building a substituted aniline was pursued at this point.
Scheme -11
Preparation of the required aniline 143, was accomplished as outlined in scheme -11. NBS bromination of 5-methyl-2-nitroanisole (128) in refluxing carbon tetrachloride with benzoyl peroxide as the radical initiator produced a 60% yield of the bromide 141. Starting material could be recovered and recycled to give 141 in an overall yield of 88%. Displacement of the benzylic bromide of 141 with sodio-diethylacetamidomalonate proceeded smoothly to afford 142 in good yield. The malonate anion was generated using NaH (50% oil dispersion) in dry dimethyl formamide (DMF). Quenching the purple colored solution with cold water afforded white crystals in 94% yield after recrystallization. Reduction of 142 was accomplished by hydrogenation over PtO₂ at room temperature. However, the reaction was slow and large quantities of 143 could not be obtained by this method. Fortunately, large scale reduction of 142 was accomplished using stannous chloride in methanol saturated with HCl gas. The reaction mixture was neutralized with NH₄OH and the voluminous tin oxide salts were filtered off. Evaporation of solvent afforded a brown oil which solidified on standing. This material could successfully be used in the next step without further purification. Thiocyanation was accomplished by adding bromine to a cooled solution of 143 and potassium thiocyanate in acetic acid. Concentration of the reaction mixture, after filtration, gave a brown residue which was poured into water to give yellow plates of the hydrobromide of 144.
The free base was liberated by dissolution of the salt in warm sodium bicarbonate solution. Repeated recrystallization from ethanol provided 144 as colorless needles. It was not necessary to generate the free base for diazotization to give 145. The crude hydrobromide from the thiocyanation of 143 was merely dried en vacuo to a fine brown powder before dissolution in warm 85% H₃PO₄. After cooling to -10° in an ice-salt bath, the brown viscous solution was diazotized slowly with NaNO₂ dissolved in the minimum amount of water. The resulting reddish brown solution was then added dropwise to a large excess of 50% H₃PO₂ with vigorous stirring until no more gas evolution was observed. Repeated extraction of the brown solution with methylene chloride afforded the malonyl derivative 145. This was easily hydrolyzed to amino acid 146 in refluxing 3N or 6N hydrochloric acid under a nitrogen atmosphere.

Finally, treatment of amino acid 146 with 48% hydriodic acid under reflux gave the target benzothiazole 5 as its HI salt.
Unfortunately, compound 5 was found to be unstable, difficult to purify and very hygroscopic. It was apparent why Fattoruso and others had not characterized amino acid 5 in its native state\textsuperscript{97}. They only reported data for the corresponding methyl ester 147 and its bis-2,4-dinitrophenyl derivative 148. The methyl ester 147 was subsequently prepared by heating 5 in refluxing methanol saturated with HCl gas and the derivative 148 was prepared by the standard procedures using 2,4-dinitrofluorobenzene\textsuperscript{98}.

Since the Italian group\textsuperscript{99} provided no melting point for compound 147, it was prepared as the hydrochloride and characterized as such. Spectral data (nmr, ir, ms) of 147 and the melting point of its bis-2,4-dinitrophenyl derivative 148 agreed with those reported for the amino acid isolated from acid hydrolysis of phaeomelanin. Since this amino acid would be the important intermediate in a study of phaeomelanin biosynthesis, it was important to fully characterize it and prove that assigned structure 5 was correct.
This has been achieved in an unambiguous nine step synthesis of 147 that is readily amenable to large scale preparations of this amino acid. The stage was now set for further synthetic studies directed toward the monomers suspected to comprise the phaeomelanin chromophore.

As a model compound, 3-methoxyphenylalanine (149), was chosen to study the Pictet-Spengler and Bischler-Napieralski reactions. Literature reports indicate that 149 can be prepared from m-methoxybenzaldehyde\(^{100}\). With 143 at hand, however, it was considered reasonable that its conversion to 149 should be readily achieved.

When 143 was diazotized with aqueous NaNO\(_2\) in 40% H\(_3\)PO\(_4\), the malonyl derivative 150 was obtained in 68% yield after treatment with 50% H\(_3\)PO\(_4\).
Hydrolysis of 150 with refluxing 3N hydrochloric acid provided 149 which was purified as its white hydrochloride salt.

With 149 in hand, a study of its Pictet-Spengler reactions was undertaken. Contrary to a previous report, treatment of 149 with methylal under reflux gave only intractable materials. However, treatment of 149 with a 37% solution of formaldehyde under nitrogen, with 10% HCl as solvent, did provide tetrahydroisoquinoline 151 in more than 90% yield. None of the isomer 152 was observed. Structural assignment was based on 13C chemical shift data that indicated unsubstituted ortho positions to a methoxy group (133.410 ppm).

The reader should be reminded (see scheme -9) of the unexpected result that showed the high reactivity of the 7 position in benzothiazole 122 under electrophilic aromatic substitution conditions. This result was significant in helping to predict the isomer expected from 146 in the Pictet-Spengler reaction.
Treatment of the hydrochloride of 146 with a 37% solution of formaldehyde in 10% HCl as solvent, provided 153 after 6 hours of stirring at room temperature or warming at 60° for 2 hours.

As in the methoxyalanine model system, no other isomer was detected. Regiochemical assignment was based on the disappearance of the proton at the 7-position of 146. This is a distinct singlet (pmr) for 146 appearing at 7.66. This result demonstrates the high reactivity of the 7-position of 146 toward electrophiles.

The mechanism of the Pictet-Spengler reaction does not seem unlike other examples of aromatic substitution by electrophilic attack. The reaction itself, is a special case of the well known Mannich reaction. In this reaction, a compound containing an active hydrogen atom is treated with formaldehyde and ammonia or a primary or secondary amine. The active hydrogen is replaced by an amino methyl group in an overall one carbon homologation as shown in equation 13.

\[
\begin{align*}
&\ce{C-H + CH2O + HNR2 -> O-CH2NR2 + H2O} \\
&\text{(eq. -13)}
\end{align*}
\]
As shown (see scheme -12) for benzothiazole amino acid 146, the mechanism involves the formation of the Schiff base 155. The Schiff base 155, can then undergo electrophilic aromatic substitution followed by loss of proton to give the expected product. Evidence for this mechanism comes from the isolation of many Schiff bases prior to ring closure by acid catalysis. The Pictet-Spengler adducts 151 and 153 were far less soluble in water and alcoholic solvents than their amino acid precursors. The mass spectral data for both 151 and 153 indicated very minor amounts of the N-CH₃ derivatives. These could not however be separated by MPLC or detected by NMR. Evidence for the competition between the Clarke-Eschweiler¹⁰' and Pictet-Spengler reactions is widely documented in the literature. For example, Teitel and others found N-methylation to occur readily on dopa (157), when subjected to Pictet-Spengler conditions¹⁰⁵. The hydride producing source is apparently the small amount of formic acid present in formalin solutions.

![Chemical Structure](image-url)
Scheme 12

1. 

2. 

3. 

4. 

Scheme -12
The next model compound of interest and the one that best resembles the suspected phaeomelanin chromophore was tetrahydroisoquinoline 158.

Condensation of 146 with benzothiazole-2-carboxaldehyde (159), should in principle provide 158 under Pictet-Spengler conditions. Aldehyde 159 had previously been reported with conflicting properties. The first report indicated a compound with a melting point of 36°C. Two subsequent reports listed it as a yellow solid but with melting points of 71° and 75°C. It was therefore necessary to prepare and fully characterize this aldehyde. Oxidation of 2-methylbenzothiazole (160) with SeO₂ in refluxing dioxane solution provided a 28% yield of 159 after chromatography. This material was a yellow solid with a melting point of 71-72°C.
The aldehyde proton appearing at 10.26 (CDCl₃) as a distinct singlet and the IR (KBr) showed a carbonyl stretch at 1690 cm⁻¹. The mass spectrum indicated no peaks above the molecular ion.

With the cheap, commercially available 160, the yield of 28% was acceptable for the purpose at hand.

Before describing our attempts to prepare imine 161, the proposed Pictet-Spengler substrate leading to tetrahydroisoquinoline 158, a discussion of a closely related study is appropriate. Fattorusso and others reported the preparation of the Schiff base 163 from 4-hydroxy-2-benzothiazole carboxaldehyde (162) and dopa (157). Moreover, they claimed to have made the Pictet-Spengler adduct 164 by acid catalyzed (2N HCl) ring closure of 163. Unfortunately, neither yields nor spectral data for 164 were reported.
Besides their spectral data for 163 were suspect. The assigned chemical shift data for 163 were 5.57δ (Ha) as a singlet and 3.90δ (Hb) as a multiplet. The benzylic protons (Hc) were assigned a chemical shift of 2.91δ and broad. This was surprising since our experience with substituted phenyl alanines in both the benzothiazole and phenyl alanine systems had always shown the benzylic protons to be a doublet in DMSO-d₆.
Apart from the lack of data on 164, it was claimed to be subsequently hydrolyzed with 2N HCl to give 165 and 166 which were characterized. The ambiguity of their claims led us to believe that 164 was never actually realized but merely assumed.

One could easily argue that hydrolysis of 163 was occurring and none of 164 was actually formed (Scheme-13).

Various attempts to prepare imine 161 under the aqueous acidic conditions employed by Fattorusso, proved fruitless. A recent report indicated that Pictet-Spengler reactions using tryptophan methyl ester derivatives with various aldehydes proceeded well under
aprotic, non-acidic conditions. It was decided to attempt the
synthesis of amino ester 167 from methyl ester 169 and aldehyde 159
under these conditions.
The ester 169 was prepared from acid 149 in refluxing methanolic HCl and purified as its hydrochloride salt. The free base, generated with triethylamine, was treated with aldehyde 159 in dry benzene and the mixture was subjected to reflux with water removal (via Dean-Stark trap). This procedure gave imine 168. None of the Pictet-Spengler adduct 167 was detected. The structure of imine 168 was supported by its IR, NMR and mass spectra. Our original suspicions of the spectral data presented for 163 by Fattorusso and others were confirmed by the appearance of the imine proton (Ha) of 168 as a distinct singlet at δ 8.2, while the benzylic protons (Hb) appeared as a multiplet at δ 3.3. Comparison of these values with those presented by Fattorusso, leads us to doubt whether compounds 163 and 164 were ever realized.

In an attempt to effect ring closure, imine 168 was warmed in refluxing toluene. Unfortunately, 168 was recovered unchanged. An attempt to trigger ring closure with BF3Et2O, led only to hydrolysis of 168 to give back the ester 169. The aldehyde portion could not be retrieved from the reaction mixture after hydrolysis and work-up. Whether hydrolysis was caused by acidic impurities in boron trifluoride-etherate was not determined.

At this stage, it was felt that a much stronger bond might be required, to couple the two components that comprise 167, and also to withstand the acidic conditions that might be needed to effect ring closure.
The amide linkage was a logical choice and consequently the N-acyl derivative 170 was prepared from 169 and 2-benzothiazolyl chloride (171).

Although 171 has been reported\textsuperscript{112}, no spectral data were recorded. Consequently, it was necessary to prepare 171 and confirm its structure. Lithiation of benzothiazole (20) in ether at -78° with n-butyllithium provided a yellow solution of 2-lithiobenzothiazole (172). Quenching with a slush of dry-ice in ether,

afforded benzothiazole-2-carboxylic acid (173) as a yellow solid. This was converted to 174 with KOH in methanol, which was treated with oxalyl chloride to give 171 in good yield.
The infrared spectrum of 171 showed a carbonyl stretch at 1740 cm\(^{-1}\). Since this was low for an acid chloride, it was decided to examine a standard reaction of acid chlorides with this material.

Upon warming 171 with anhydrous methanol for 30 minutes, the methyl ester 175 was obtained. Nuclear magnetic resonance indicated a singlet at \(\delta 4.1\) for the \(-OCH_3\) and the infrared spectrum showed the carbonyl stretch at 1735 cm\(^{-1}\).

\[
\begin{align*}
\text{175} \\
\text{COOCH}_3
\end{align*}
\]

With 171 fully characterized, it was now used to prepare amide 170.

Treatment of the ester 169 with 171 in dry methylene chloride, using triethylamine as catalyst, provided 170 as a colorless, viscous oil. When amide 170 was subjected to the Bischler-Napieralski reaction conditions (POCl\(_3\), xylene), only starting material was recovered. Phosphorous pentoxide in refluxing toluene however, provided compound 176 on the basis of its IR, NMR, mass and \(^{13}\text{C}\) spectral data.

\[
\begin{align*}
\text{176} \\
\text{OCH}_3 \\
\text{CH}_3\text{O}_2\text{C}
\end{align*}
\]
Having observed the behavior of the model systems under both Pictet-Spengler and Bischler-Napieralski reaction conditions, it was time to see how the benzothiazole systems would behave under the same reaction conditions.

The amino acid ester 177, was prepared from 146 in the same fashion that was used to prepare 149.

\[
\begin{align*}
\text{OCH}_3 \\
\text{CH}_3\text{O}_2\text{C}\text{-NH}_2 \\
\text{177}
\end{align*}
\]

When a mixture of 177 and 159 was warmed under reflux in dry benzene, with water removal (Dean-Stark) a mixture of compounds was obtained. One of these was the imine 178 which was characterized by its nmr.

\[
\begin{align*}
\text{OCH}_3 \\
\text{CH}_3\text{O}_2\text{C} \\
\text{178}
\end{align*}
\]

As observed in the methoxy alanine system, 178 showed a singlet for the imine proton (Ha) at δ 8.25. Spectral data were recorded on three other compounds isolated by HPLC. It was however, impossible to make any structural assignments based on the complexity of these spectra.
Preparation of the amide 179 was therefore undertaken to gain an insight into its behavior under Bischler-Napieralski reaction conditions. The methyl ester 177, generated in situ with triethylamine, readily condensed with 171 to give 179 as a yellow solid after chromatography. The amide 179 exhibited IR, NMR and mass spectral data consistent with the assigned structure.

When 179, dissolved in dry xylene, was warmed to reflux with POCl₃, no cyclization was observed. The use of P₂O₅ as catalyst, provided a mixture of two compounds readily observed by NMR. Various attempts to separate these were unsuccessful. The components possess the same Rf values in various solvent combinations. From the spectral (NMR) data obtained, it was clear that the mixture was made up of two components in a ratio of 1:0.5. Difficulties encountered in trying to separate these components may be due to their similarity in constitution.
Chapter 4

CONCLUSION AND SUGGESTIONS

During the course of these studies, the structure of one of the key amino acids isolated from the degradation of phaeomelanin was confirmed by an unambiguous eight-step total synthesis starting from 5-methyl-2-nitrophenol. In addition, a study of the Pictet-Spengler and Bischler-Napieralski reactions of this amino acid led to the preparation of some phaeomelanin model compounds. Although some attempts to prepare model compounds met with failure, the author strongly believes that efforts should be made to improve the forementioned Pictet-Spengler and Bischler-Napieralski reactions. The use of different solvents and catalysts should, in time, provide conditions that will lead to the desired tetrahydroisoquinolines.

Since the structure of phaeomelanin has not been determined, these experiments can only pave the way to an understanding of the proposed structure of the chromophore of this important pigment. The determination of the structure of phaeomelanin will require new methods of isolation that will provide the intact melano-protein as a single entity of definite composition. Synthetic studies of the type described in this thesis, may however, contribute to an understanding of the structure of the phaeomelanin chromophore in the following manner. Once a synthesis of a portion of the presumed chromophore is accomplished, it will be important to subject this synthetic material and the natural pigment to identical degradation conditions.
If there is any relationship between the synthetic and natural substances, similar degradation products should be obtained. If different products are obtained from such a procedure, then the proposed structure of phaeomelanin may not be correct.

Further information about the constitution of phaeomelanin could be obtained by using isotopically labelled cysteine and/or dopa as precursors to labelled phaeomelanin under enzyme catalyzed conditions. It could be determined whether the radioactivity is incorporated in a specific or random manner. Random incorporation will indicate extensive degradation and/or rearrangement of the initially labelled cysteine and dopa before they are incorporated into the pigment.

The structure of phaeomelanin is certainly complex. It is hoped that the studies described herein will provide some chemical tools which will be useful as pursuit of the structure and properties of the phaeomelanin chromophore continues.
Chapter 5

EXPERIMENTAL

INSTRUMENTATION: All melting points were taken on a Fisher-Johns meltingpoint apparatus and are uncorrected. Infrared spectra were recorded on a Beckman 4250 and Perkin-Elmer 457 grating spectrophotometers. NMR spectra were recorded on Varian EM 360 or 390 instruments and are reported in parts per million downfield from internal tetramethylsilane (TMS) using the $\delta$ scale as follows: Chemical shift (multiplicity, integration). Mass spectra were taken by Dick Weissenberger on an AE1 MS-9 spectrometer at 70 eV.

ANALYSES: Combustion analyses were performed by Atlantic Microlab Inc., Atlanta, Georgia 30366.

The author thanks Dr. Miles Chedekel and Mr. Dale Sharp for preparing analytically pure samples of 142, 143, 144, 145 and 147.

MATERIALS: 5-Methyl-2-nitrophenol and dimethylacetamidomalonate were obtained from the Aldrich Chemical Company and used as purchased without further purification. N-Bromosuccinimide (NBS) was recrystallized from water at all times and dried in a vacuum desiccator over $\text{P}_2\text{O}_5$ before use. Except where indicated, solvents were used as obtained from commercial sources. Benzothiazole and 2-methylbenzothiazole were obtained from Aldrich.
Dimethylformamide was warmed under reflux over CaH₂ and distilled prior to use. Benzene was dried with sodium wire and toluene was distilled from CaH₂. The preparations of 3-methoxy-4-nitrotoluene and 2-amino-4-methoxy-6-methylbenzothiazole have previously been reported.

2-Acetamido-4-methoxy-6-methylbenzothiazole (127). In 5 ml of acetic anhydride, 500 mg (2.58 mol) of 2-Amino-4-methoxy-6-methylbenzothiazole (121) was dissolved. The mixture was stirred at room temperature for thirty minutes and heated on a steam bath for another thirty minutes. The solution was cooled and the resulting white voluminous precipitate was filtered, washed with copious amounts of water, and dried in a vacuum dessicator. The crude product was recrystallized from 95% ethanol to give 600 mg (98%) of 127 as white fluffy crystals: mp 250-2° (dec.); ir(KBr) 3200, 1660, 1540 cm⁻¹; nmr(CDCl₃) 2.2(S,3H), 2.5(S,3H), 3.9(S,3H), 6.7(S,1H), 7.2(S,1H).

Exact mass calcd: for C₉H₁₂N₂O₂S: 236.2944. Found: 236.2951.

Ethyl, 3-methoxy-4-nitrobenzoate (130). To a solution of 51.3 g (307.0 mmol) of 128, in 3.5 l of boiling water containing 25.0 g of Na₂CO₃, was added 200 g (1.27 mol) of finely ground KMnO₄ in portions. The solution was warmed under reflux for 1½ hours, filtered through diatomaceous earth and cooled to 0°. The
filtrate was neutralized with concentrated H$_2$SO$_4$. The resulting white precipitate was filtered, dried in a vacuum dessicator, and recrystallized from 95% ethanol to give 60.5 g (100%) of 129 as colorless plates: mp 230-3° (lit. 233°); ir(KBr) 2990, 1690, 1600, 5300, 1245 cm$^{-1}$.

A solution containing 27.0 g (0.137 mol) of 129, 200 ml of absolute ethanol, 150 ml of benzene (dried over Na) and 5 ml of concentrated H$_2$SO$_4$, was warmed under reflux for 24 hours using a Dean-Stark trap for water removal. The solution was allowed to cool and washed with 100 ml of 10% Na$_2$CO$_3$ solution. The organic layer was dried (Na$_2$SO$_4$) and concentrated en vacuo to give 29.6 g of 130 as a yellow residue. The crude 130 was recrystallized from benzene/petroleum ether (50:50) to give 25.4 g (82%) of pure 130 as yellow needles: mp 93-4° (lit. 93°); ir(KBr) 3100, 2890, 1720, 1620, 1510 cm$^{-1}$; nmr(CDCl$_3$) 1.3 (t,3H), 3.9 (S,3H), 4.3 (q,2H), 6.7 (d,1H), 7.7 (m,2H).

Exact mass calcd. for C$_{10}$H$_{11}$NO$_3$: 225.0637. Found: 225.0640.

Ethyl-4-amino-3-methoxybenzoate (131). Nitroester 130 (25.4 g, 0.113 mol) was dissolved in 300 ml of ethyl acetate and 0.3 g of PtO$_2$ was added. The mixture was hydrogenated in a Paar apparatus under 65 psi of hydrogen for 3 days. The solution was filtered and solvent was removed en vacuo to afford a colorless solid.
which was recrystallized from ligroine to give 16.8 g (73%) of amine 131 as colorless needles: mp 81-83°, ir(KBr) 3400, 3000, 2600, 1730, 1640 cm⁻¹; nmr (CDCl₃) 1.3 (t,3H,J=6HZ), 3.8 (S,3H), 4.2 (S,2H), 4.3 (q,2H,J=6HZ), 6.5 (d,1H,J=6HZ), 7.4 (m,2H).


1-(2-Methoxy-4-carbethoxy)phenyl-2-thiourea (137). The amine 131 (2.00 g, 9.80 mmol) was dissolved in 20 ml of freshly distilled chlorobenzene and 0.6 g (3.40 mmol) of concentrated sulfuric acid was added dropwise over a 15 minute period. To the mixture was added 1.66 g (21.8 mmol) of ammonium thiocyanate followed by heating at 120° for 3 hours. The mixture was cooled and the resulting crystals were collected, washed with cold water, dried in a vacuum dessicator, and recrystallized from ethanol to afford 1.0 g (40%) of thiourea 137 as plates: mp 146-151°; ir(KBr) 3040, 3080, 3200, 17.0, 1680 cm⁻¹; nmr (Acetone-d₆) 1.3 (t,3H, J=6HZ), 2.8 (S,2H), 4.0 (S,3H), 4.3 (q,2H,J=6HZ), 7.7 (m,3H), 8.7 (d,1H).


2-Amino-6-carbethoxy-4-methoxybenzothiazole (132). In 135 ml of anyhydrous methanol was dissolved 10.0 g (0.049 mol) of amine 131. To the solution was added a mixture of 50.0 g (0.514 mmol) of KSCN and 4.0 g (0.251 mol) of CuSO₄.
The resulting mixture was heated under reflux for 2 hours, cooled and filtered. The filtrate was diluted with water and heated to boiling. Ethanol was added dropwise to the hot solution and the solution was cooled rapidly to provide 132 as a yellow solid. The crude 132 was recrystallized from 95% ethanol to provide 11.6 g (94%) of benzothiazole 132 as slightly yellow crystals; mp 256–267°, ir(KBr) 3400, 3280, 1680, 1660, 1560 cm⁻¹; nmr (DMSO-d₆) 1.3 (t, 3H, J=6Hz), 3.9 (s, 3H), 4.3 (q, 2H, J=6Hz), 7.2 (s, 1H), 7.6 (s, 1H), 7.9 (s, 1H).


6-Carbethoxy-4-methoxybenzothiazole (133). Aminobenzothiazole 133 (24.8 g, 0.11 mmol) was dissolved in hot 85% H₃PO₄ (100 ml) and cooled to -5° in an ice-salt bath. To the mixture was added 40 ml of concentrated HNO₃ followed by dropwise addition of a solution of 16.0 g (0.232 mol) of NaNO₂ in 10 ml of water with vigorous stirring. The resulting red solution was then added dropwise to a stirred solution of 50% H₂PO₄ (150 ml). After gas evolution ceased, the yellow solution was extracted with 2 x 10 ml ether and the combined extracts were dried (Na₂SO₄) and concentrated to a brown oil, which was dissolved in 10% HCl (50 ml) and refluxed for 4 hours. The solution was cooled, the precipitate filtered and recrystallized
from 95% ethanol to give 12.5 g (54%) of the acid 138; mp 210–212°; 
ir(KBr) 3300, 1690, 1580 cm⁻¹; nmr (DMSO-d₆) 4.1 (S,3H), 7.5 (S,1H), 8.4 (S,1H).


The acid 138 (12.5 g, 0.060 mol) was dissolved in a mixture of 
100 ml of absolute ethanol, 150 ml of dry benzene and 3 ml of 
concentrated sulfuric acid. The solution was warmed under reflux 
for 24 hours with water removal using a Dean-Stark trap. The 
mixture was washed with 5% NaHCO₃. The organic phase was dried 
over Na₂SO₄ and evaporated to give a brown residue which was 
crystallized from hexane to afford 12.8 (92%) of 133 as yellow 
needles; mp 86–87°; ir(KBr) 3100, 2950, 2890, 1725, 1575, 1450 cm⁻¹; 
nmr (CDCl₃) 1.4 (t,3H), 4.1 (S,3H), 4.4 (q,2H), 7.6 (S,1H), 8.2 
(S,1H), 9.0 (S,1H).


α-Bromo-3-methoxy-4-nitrotoluene (141). A mixture of 100 g 
(0.60 mol) of 128, 106.6 g (0.60 mol) of N-bromosuccinimide and 2 g 
of dibenzoyl peroxide in 3 ℓ of carbon-
tetrachloride was warmed under reflux 
for 3 days. Suspended succinimide was 
filtered and the filtrate concentrated 
to give a brown oil which was kept in 
the refrigerator overnight. The re-
sulting yellow precipitate was collected
and recrystallized from carbontetrachloride to give 80 g (50%) of 144 as long, yellow needles: mp 100-101°C; ir(KBr) 3480, 3340, 2960, 1700, 1640 cm⁻¹; nmr (CDCl₃) 4.0 (s,3H), 4.5 (s,2H), 7.1 (m,2H) 7.8 (d,1H). Exact mass calcd for C₂₀H₁₇N₀₃Br: 244.9688. Found: 244.9693.

Diethyl-1-acetamido-2-(3-methoxy-4-nitrophenyl)-1,1-ethane dicarboxylate (142). To a suspension of 4.6 g (0.192 mol) of NaH in 100 ml of freshly distilled dimethylformamide (DMF) was added in portions, 21.1 g (0.099 mol) of diethylacetamidomalonate. The mixture was stirred until gas evolution ceased and was filtered into a solution of DMF (100 ml) containing 20.0 g (0.099 mol) of 141. The mixture was stirred for 2 hours. The brown solution was poured into 2 l of cold water and the resulting precipitate was collected, dried en vacuo, and recrystallized from ethyl acetate to give 29.2 g (77%) of 142 as colorless crystals: mp 158-160°C; ir(KBr)3480, 3340, 2960, 1700 1640 cm⁻¹; nmr (DMSO-δ₆) 1.3 (t,6H), 2.1 (s,3H), 3.6 (s,2H), 4.0 (s,3H), 4.2 (q,4H), 6.8 (t,2H), 7.8 (d,1H), 8.3 (s,1H). Exact mass calcd for C₁₇H₂₂N₂O₆: 382.1376. Found: 382.1382.

Diethyl-1-acetamido-2-(3-methoxy-4-aminophenyl)-1,1-ethane dicarboxylate (143). Method A. A mixture of 5.0 (0.0131 mol) of 142 and 0.1 g of PtO₂ in 200 ml of ethyl acetate was hydrogenated in a Paar apparatus.
under 70 psi of hydrogen for 4 days. The solution was filtered and concentrated en vacuo. The brown residue was recrystallized from ligroine to give 4.1 g (89%) of 143 as long, colorless needles: mp 93-94°; ir (KBr) 3460, 3340, 2980, 2920, 1740, 1700, 1660, 1520 cm⁻¹; nmr (CDCl₃) 1.3 (t, 6H), 2.0 (S, 3H), 3.2 (b, 2H), 3.6 (S, 2H), 3.8 (S, 3H), 4.3 (q, 4H), 6.5 (m, 3H), 7.3 (S, 1H).


Method B. To 25 ml of anhydrous methanol saturated with HCl gas (1.2 g) was dissolved 3.0 g (0.013 mol) of stannous chloride dihydrate. To the solution was added 1.0 g (2.6 mmol) of 142 in portions, followed by careful hydrolysis with concentrated NH₄OH. The white suspension was filtered, extracted with 2 x 100 ml of chloroform, and the extracts were dried over Na₂SO₄. The solution was concentrated to give a brown oil which crystallized en standing. The crude product was recrystallized from ether/ligroine to provide 0.81 g (89%) of 143.

Diethyl-1-acetamido-2-(6-benzothiazole-2-amino-4-methoxy)-1,1-ethane dicarboxylate (144). To a mixture of 5.0 g (14.2 mmol) of 143 and 3.8 g (28.0 mmol) of KSCN in 30 ml of glacial acetic acid cooled to 10° was added dropwise a solution of 4.5g
acetic acid. The resulting dark solution was filtered. The filtrate was concentrated to give a yellow residue which was washed with large amounts of water, filtered and dried en vacuo. The resulting solid presumably the hydrobromide salt of 144, was digested in warm Na₂CO₃ to give yellow crystals of 144, which was subsequently recrystallized from 50% aqueous ethanol to afford 4.1 g (71%) of 144 as colorless needles: mp 251-253°; ir(KBr) 3410, 3390, 2980, 2940, 1740, 1660 cm⁻¹; nmr (DMSO-d₆) 1.3 (t,6H), 2.1 (S,3H), 3.5 (S,2H), 3.9 (S,3H), 4.3 (q,4H), 6.5 (S,1H), 6.9 (S,1H), 7.4 (S,2H), 8.2 (S,1H).


Diethyl-1-acetamido-2-(6-benzothiazole-4-methoxy)-1,1-ethane dicarboxylate (145). The hydrobromide of 144 (13.1 g, 0.027 mol) was dissolved in hot 85% H₃PO₄ (200 ml) and cooled to -15° in an ice-salt bath. To the cold solution was added 9.2 g (0.134 mol) of NaNO₂ in 10 ml of water. The resulting solution was added to 200 ml of 50% H₃PO₂ with vigorous stirring. After gas evolution ceased, the mixture was diluted with 200 ml of cold water.
and extracted with 3 x 10 ml CH₂Cl₂. The combined extracts were
dried (Na₂SO₄) and concentrated to give a yellow residue which
was chromatographed over silica gel using EtOAc: CHCl₃ (30:70) as
eluant. Appropriate fractions were concentrated and recrystallized
from CH₂Cl₂/Hexane to provide 5.0 g (48%) of 145 as white crystals:
mp 122-123°; ir(KBr) 3255, 1745, 1739, 1639, 1565 cm⁻¹; nmr (DMSO-d₆)
1.2 (t, 6H), 2.0 (S, 3H), 3.5 (S, 2H), 2.9 (S, 3H), 4.2 (q, 4H), 6.7 (S, 1H),
7.3 (S, 1H), 8.2 (S, 1H), 9.2 (S, 1H).

α-Amino-β(6-benzothiazole-4-methoxy) propionic acid (146).
Malonyl derivative 145 (300 mg, 0.76 mmol) was dissolved in 15 ml
of 3 N hydrochloric acid was warmed under reflux under a nitrogen
atmosphere for 12 hours. The brown, acidic solution was briefly treat-
ed with decolorizing carbon, filtered
and concentrated to give a slightly
brown solid. The crude solid was re-
crystallized from 95% ethanol to
afford 180 mg (82%) of 146 as the
hydrochloride salt: mp 255-257° (dec)
; ir(KBr) 3400, 3000, 1740, 1490 cm⁻¹
nmr (DMSO-d₆) 3.3 (d, 2H), 4.0 (S, 3H),
4.75 (m, 1H), 7.1 (S, 1H), 7.6 (S, 1H), 7.6 (S, 1H), 8.6 (b, 3H), 9.3 (S, 1H).
Methyl-α-amino-2(6-benzothiazole-4-hydroxy)propionate (147). Amino acid 146 (200 mg, 0.693 mol) was dissolved in HCl-saturated methanol and the resultant solution was warmed under reflux under a nitrogen atmosphere for 3 hours. After it had been cooled, the solvent was removed en vacuo and the residue crystallized from acetone to afford 160 mg (80.0%) of the hydrochloride salt of 147; mp 198-202°; ir(KBr) 3490, 3300, 1740, 1490 cm⁻¹; nmr (DMSO-d₆) 3.2 (d,2H), 3.6 (S,1H), 3.75 (S,3H), 4.2 (m,1H), 6.7 (s,1H), 7.3 (S,1H), 8.7 (b,3H), 9.1 (S,1H).

Methyl-α-amino-2(6-benzothiazole-4-methoxy)propionate (177). Amino acid 146 HCl (1.5 g, 5.20 mmol) was dissolved in 75 ml of HCl saturated methanol and warmed under reflux for 12 hours under a nitrogen atmosphere. The mixture was cooled, solvent was removed en vacuo, and the yellow residue was crystallized from absolute ethanol to give 1.32 g (84%) of 169 as the white hydrochloride salt: mp 198-200° (dec); ir(KBr) 3400, 2850, 1740, 1600, 1250 cm⁻¹, nmr(DMSO-d₆) 3.3 (d,2H), 3.6 (S,3H), 4.0 (S,3H), 4.3 (m,1H), 7.0 (S,1H), 7.4 (S,1H), 9.7 (b,3H), 9.2 (S,1H), 9.2 (S,1H).
Exact mass calcd for C_{12}H_{14}N_{2}O_{5}S: 266.0725. Found: 266.0731.

Preparation of free base (177). Into 3 ml of dichloromethane (dried over activated Alumina) was suspended 200 mg (6.61 mmol) of the hydrochloride of 146. To the mixture was added with stirring, 0.7 g (6.61 mmol) of triethyl amine. The mixture was washed with 2 x 5 ml of 1N HCl and the organic layer separated, dried (Na_{2}SO_{4}) and concentrated to give 136 mg (77%) of 177 as a brown oil: nmr (CDCl_{3}) 1.7 (b,2H), 3.1 (m,2H), 3.7 (S,3H), 3.8 (m,1H), 4.0 (S,3H), 6.7 (S,1H), 7.3 (S,1H), 8.8 (S,1H).

Diethyl,1-acetamido-2(3-methoxyphenyl)1,1-ethane dicarboxylate (150).

Amine 143 (10.0 g, 0.028 mol) was dissolved in 100 ml of 40% H_{3}PO_{4} and cooled to -15°. To the mechanically stirred solution was added dropwise a solution of NaNO_{2} (5.8 g, 0.085 mol) dissolved in the minimum amount of water. The resulting solution was added dropwise to 100 ml of 50% H_{3}PO_{4} stirred overnight, and diluted with 200 ml of cold water. The resulting precipitate was collected and recrystallized from ligroine to afford 6.5 g (68%) of 150 as yellow needles: mp 80-81°; ir (KBr) 3250, 1750, 1640, 1270 cm^{-1}; nmr (DMSO-d_{6}) 8.1 (S,1H), 6.9 (m,4H), 4.2 (q,4H,J=HZ), 3.7 (S,3H), 3.4 (S,2H), 1.9 (S,3H), 1.2 (t,6H,J=HZ).

Exact mass calcd for C_{17}H_{23}N_{2}O_{8}: 337.1525. Found: 337.1532.
3-Methoxyphenylalanine (149). The malonyl derivative 150 (1.5 g, 4.45 mmol) was added to 200 ml of 3 N hydrochloric acid and warmed under reflux for 18 hours under a blanket of nitrogen. The hot acidic solution was briefly treated with de-colorizing carbon, filtered and concentrated to give a slightly yellow solid. The crude solid was recrystallized from 95% EtOH to afford 0.8 g (78%) of amino acid 149 as the white hydrochloride salt: mp 162-5°; ir (KBr) 3300, 1740, 1600, 1490 cm⁻¹; nmr (DMSO-d₆) 3.2 (d, 2H, J=Hz), 3.7 (S, 3H), 4.1 (t, 1H, J=Hz), 7.1 (m, 4H), 8.5 (b, 3H).

Methyl-α-amino-2(3-methoxyphenyl) propionate (169). The hydrochloride of 149 (1.1 g, 4.75 mmol) was dissolved in 25 ml of anhydrous methanol saturated with HCl gas and was warmed under reflux under a nitrogen atmosphere for 12 hours. Solvent was removed en vacuo to provide a slightly yellow colored solid which was recrystallized from absolute ethanol to give 800 mg (69%) of 169 as its white hydrochloride salt: mp 280-282°; ir (KBr) 3400, 2000, 1750, 1600, 1320 cm⁻¹; nmr (DMSO-d₆) 3.2 (d, 2H), 2.7 (S, 3H), 3.8 (S, 3H), 4.2 (t, 1H), 7.1 (m, 4H), 8.8 (b, 3H); nmr (CDCl₃, free base) 1.9 (b, 2H), 2.9 (m, 3H),
3.7 (S,3H), 3.8 (S,3H), 6.7 (m,3H), 7.2 (m,1H).

Exact mass calcd for C_{11}H_{15}NO_{3}: 209.1052. Found: 209.1055.

3-Carboxy-6-methoxy 1,2,3,4-tetrahydroisoquinoline (151). A mixture of 2 ml of 10% HCl, 100 mg (0.43 mol) of the hydrochloride of 149 and 0.5 ml (6.63 mmol) of 37% formaldehyde solution was stirred under a resulting blanket of nitrogen for 12 hours. The resulting white, crystalline precipitate was collected, dried, recrystallized from absolute ethanol to give 101 mg (96%) of the white hydrochloride salt of 151: mp 258-260° (dec); ir(KBr) 2940, 2820, 1750, 1620, 1550 cm⁻¹; nmr (DMSO-d_6) 3.2 (d,2H), 3.7 (S,3H), 4.2 (S,2H), 4.4 (t,1H), 6.8 (m,2H), 7.2 (m,1H).

Exact mass calcd for C_{11}H_{15}NO_{3}: 207.0895. Found: 207.0905.

Anal. Calcd. for C_{11}H_{15}NO_{3}: C, 54.22; H, 5.79. Found: C, 54.43; H, 5.92.

Thiazolo[4,5-h] isoquinoline, 6,7,8,9-tetrahydro-4-methoxy-7-carboxylic acid (153). A mixture of 0.5 g (0.35 mmol) of the hydrochloride salt of 146, 2.0 ml of 10% aqueous HCl, and 0.5 ml of 37% formaldehyde solution was stirred under nitrogen for 8 hours. The mixture was then warmed at 65° for 1 hour and concentrated en vacuo. The resulting white residue was recrystallized from absolute ethanol to give 100 mg (95%) of 153 as the hydrochloride:
mp 240-253° (dec.); ir (KBr) 3400, 2920, 1740, 1590 cm⁻¹; nmr (DMSO-d₆) 3.4 (d, 2H), 4.0 (s, 3H), 4.3 (s, 2H), 4.5 (t, 1H), 7.1 (s, 1H), 9.3 (s, 1H).

Exact mass calc'd for C₁₂H₁₂N₂O₃S; 264.0569. Found: 264.0576.

Benzothiazole-2-carboxaldehyde (159). A mixture of 5.0 g (0.034 mol) of 2-methylbenzothiazole and 5.7 g (0.058 mol) of selenium dioxide in 50 ml of dioxane was warmed under reflux for 24 hours. The hot solution was filtered and concentrated en vacuo. The resulting yellow residue (3.2 g) was chromatographed over silica gel (250 g) and eluted with EtOAc: Hexane (20:80) to give 1.5 g (28%) of aldehyde 159 as yellow needles: mp 71-72°; ir (KBr) 3080, 2990, 2820, 1690, 1480 cm⁻¹ (CDCl₃) 7.5 (m, 2H), 7.9 (m, 1H), 8.2 (m, 1H), 10.1 (s, 1H).

Exact mass calc'd for C₆H₅NOS: 163.0092. Found: 163.0096.

N-(2-benzothiazoly1 methylene)-3-methoxyphenyl-alanine methyl ester (168).

To a solution of amino acid methyl ester 169 (100 mg, 0.48 mmol) in dry benzene (20 ml) was added benzothiazole-2-carboxaldehyde (159; 78.0 mg, 0.48 mmol). The mixture was warmed under reflux for 12 hours under nitrogen, with water separation via a Dean-Stark trap. The solvent was removed under reduced pressure to give
146 mg of a brown residue which was chromatographed over silica gel (10 g) with EtOAc: Hexane (20:80) as eluant. The orange oil obtained was crystallized from hexane to provide 83 mg (49%) of imine 168 as yellow needles. mp 61-62°; ir (KBr) 2980, 2840, 1740, 1730, 1580, 1440 cm⁻¹; nmr (CDCl₃) 3.3 (m,2H), 3.7 (S,3H), 3.75 (S,3H), 4.3 (dd,1H), 6.6-7.9 (m,8H), 8.2 (S,1H).

2-Carboxybenzothiazole (173). Freshly distilled benzothiazole (20) (5.3 g, 0.039 mol) was added to an ethereal solution (150 ml) of 0.039 mol of n-butyllithium at -78°. Immediately after the addition, the clear, yellow solution was added to a slush of dry ice and ether, followed by hydrolysis with 50 ml of 0.2 N H₂SO₄. The resulting precipitate was filtered and dried in a vacuum dessicator to give 5.2 g (75%) of 173 as yellow crystals: mp 115-117° (lit. 107°); ir(KBr) 2900, 1710, 1500, 1270 cm⁻¹; nmr (DMSO-d₆) 7.6 (m,2H), 8.2 (m,2H).

2-Benzothiazolyl chloride (171). Into 20 ml of anhydrous methanol was dissolved 1.0 g (5.59 mmol) of 2-carboxybenzothiazole(173). A solution of 0.31 g (5.59 mmol) of KOH in 5 ml of methanol was added dropwise. The precipitate was collected and
dried en vacuo to give 1.1 g (91%) of the white, crystalline potassium salt 174: ir (KBr) 1650 cm\(^{-1}\).

Oxalyl chloride (0.59 g, 4.61 mmol) was added dropwise to a cooled (10°) suspension of the salt 174 (1.0 g, 4.61 mmol) in 30 ml of dry benzene. The mixture was warmed under reflux for 5 minutes. The hot solution was filtered and the filtrate concentrated to provide a yellow residue, which was recrystallized from ligroine to give 600 mg (66%) of 171 as yellow needles; mp 116-117° (lit, 115°); ir (KBr) 3060, 1740, 1480, 1320 cm\(^{-1}\); nmr (CDCl\(_3\)) 7.6 (ra, 2H), 7.9 (m, 1H), 8.3 (m, 1H).

Exact mass calcd for C\(_{9}\)H\(_{7}\)N02SCl\(_3\)^{55}: 196.9702. Found: 196.9697.

Methyl-2-benzothiazole carboxylate (175). Acid chloride 171 (100 mg, was dissolved in 5 ml of anhydrous methanol followed by warming under reflux on a steam bath for 1 hour. The solution was cooled, washed with 10 ml of water and extracted with 2 x 10 ml chloroform. The organic layer was separated, dried (Na\(_2\)SO\(_4\)) and concentrated. The yellow residue was recrystallized from benzene to give 85 mg (87%) of 175 as white plates: mp 90-91° (lit. 92); ir(KBr) 2950, 1740, 1450 cm\(^{-1}\); nmr (CDCl\(_3\)) 4.1 (S, 3H), 7.5 (m, 2H), 7.9 (m, 1H), 8.2 (m, 1H).

Exact mass calcd for C\(_{9}\)H\(_{7}\)NO\(_2\)S: 193.0197. Found: 193.0194.
Methyl,α-N(2-benzothiazolyl)-β-(3-methoxyphenyl) propionate (170).

To 5 ml of methylene chloride (dried over activated Alumina-1) was added 425.9 mg (2.04 mmol) of 169 HCl and 717 mg (7.10 mmol) of triethyl amine.

To the stirred mixture was added a solution of 935 mg (4.07 mmol) of 171 dissolved in 3 ml of CH₂Cl₂ followed by warming under reflux for 25 minutes.

The mixture was cooled, washed with 2 x 10 ml of 1 N HCl and the organic layer separated and dried over anhydrous Na₂SO₄. Evaporation en vacuo afforded a brown residue (1.1 g) which was chromatographed over silica gel (100 g) with EtOAc: Hexane (20:80) as eluant to provide 650 mg (86%) of 170 as a colorless, viscous oil exhibiting the following properties: ir (CDCl₃) 3400, 2920, 1740, 1670 cm⁻¹; nmr (CDCl₃) 3.2 (d, 2H), 3.7 (s, 3H), 2.75 (s, 3H), 5.1 (m, 1H), 6.7 (m, 2H), 7.3 (m, 4H), 7.9 (m, 2H).


Methyl,α-N(2-benzothiazolyl)-β-(6-benzothiazole-4-methoxy) propionate (179). Amino acid ester 177 HCl (1.14 g, 3.77 mmol) was suspended in 10 ml of dry dichloromethane and 1.0 ml of triethylamine was added dropwise.

After complete dissolution had occurred, a solution of 1.12 g (5.69 mmol) of 171 in 5 ml of dichloromethane was added
dropwise. The mixture was warmed under reflux for 30 minutes under
a nitrogen atmosphere. The solution was cooled, washed with 20 ml
of water, dried (Na$_2$SO$_4$) and concentrated. The resulting yellow
residue (1.82 g) was chromatographed over silica gel (200 g) with
EtOAc: Hexane (40:60) as eluant a material obtained from appropriate
fractions was recrystallized from hexane to give 581 mg (39%) of 179
as white, fluffy crystals: mp 97-98°; ir (KBr) 3400, 3090, 2960,
1740, 1670, 1530 cm$^{-1}$; nmr (CDCl$_3$) 3.3 (d,2H), 3.7 (S,3H), 3.9 (S,3H),
5.1 (m,1H), 6.8 (S,1H), 7.3 (S,1H), 7.4 (m,2H), 7.9 (m,2H), 8.8 (S,1H).
Exact mass calcd. for C$_{20}$H$_{17}$N$_3$O$_3$S$_2$: 427.0770. Found: 427.0670.

Anal. Calcd. for C$_{20}$H$_{17}$N$_3$O$_3$S$_2$: C, 56.19; H, 4.01. Found: C, 56.47;
H, 4.44.

1-(2-benzothiazolyl)-3-carbomethoxy-6-methoxyisoquinoline (176).

To a solution of amide 170 (200 mg, 0.54 mmol)
in 20 ml of dry toluene was added 800 mg
(0.56 mmol) of P$_2$O$_5$ and the mixture was
warmed under reflux for 3 hours under a
nitrogen atmosphere. The mixture was cooled,
made basic with NH$_4$OH and extracted with
3 x 10 ml of ether. The ether extracts were
dried (Na$_2$SO$_4$) and evaporated en vacuo to a brown residue (1.1 g)
which was chromatographed over silica gel (100 g) with EtOAc: Hexane
(15:85) as eluant. The crude material obtained (72 mg) was recrystall-
ized four times from EtOAc: Hexane (15:85) to give 176 mg (23%) of 176 as colorless needles: mp 101-102°; ir (KBr) 3420, 3060, 1720, 1610 cm⁻¹; nmr (CDCl₃) 3.9 (S,3H), 4.0 (S,3H), 7.2-8.4 (m,6H), 8.6 (S,1H), 9.8 (d,1H).

Figure 4; NMR spectrum compound 62
Figure 5; NMR spectrum of compound 127
Figure 6; NMR spectrum of compound 131
Figure 7; NMR spectrum of compound 132
Figure 8; NMR spectrum of compound 138
Figure 9; NMR spectrum of compound 133
Figure 10; NMR spectrum of compound 142
Figure 11; NMR spectrum of compound 143
Figure 12: NMR spectrum of compound 144
Figure 13; NMR spectrum of compound 145
Figure 14; NMR spectrum of compound 146.HCl
Figure 15; NMR spectrum of compound 147
Figure 16; NMR spectrum of compound 177.HCl
Figure 17; NMR spectrum of compound 177
Figure 18; NMR spectrum of compound 150
Figure 19; NMR spectrum of compound 149
Figure 20; NMR spectrum of compound 169.HCl
Figure 21: NMR spectrum of compound 169
Figure 22; NMR spectrum of compound 151.HCl
Figure 23; NMR spectrum of compound 153.HCl
Figure 24; NMR spectrum of compound 159
Figure 25; NMR spectrum of compound 168
Figure 26; NMR spectrum of compound 173.
Figure 27; NMR spectrum of compound 171
Figure 28; NMR spectrum of compound 175
Figure 29; NMR spectrum of compound 170
Figure 30; NMR spectrum of compound 179
Figure 31; NMR spectrum of compound 176
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