AMINOPHENYLALANINE AND DERIVATIVES

By

Ramanbhai Chaturbhai Amin

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1948
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INTRODUCTION

The concept that substances chemically related to a metabolite may interfere with the normal function of that metabolite in living cells is attracting wide-spread interest among chemists and biologists. For the organic chemists this concept frequently provides a unique starting point for the synthesis of biologically active compounds and the study of the relation between chemical structure and activity. The stimulus for many of the investigations under review came from the discovery of the striking relationship between p-aminobenzoic acid and sulfanilamide type compounds. Not all important antagonists are structurally related to the affected metabolite, however, and the value of specific antagonisms in elucidating the functions of metabolites and explaining the action of drugs has been recognised for sometime.

Also many types of antagonisms are known. They may be classified according to their effects, namely, into direct chemical or physicochemical effects, which frequently obey the laws of mass action, and indirect or physiological antagonisms due to opposite but independent actions. Although there is no sharp line of demarcation, in this review the emphasis is placed on antagonists having a direct, reversible effect on the synthesis or utilization of specific metabolites. In general, naturally occurring antagonisms (i.e., the antagonist may also be a metabolite taking part in normal cell processes) and the numerous antagonisms between pairs of drugs have not been included.

In 1940 Woods (1) isolated in a crude form from yeast cells a substance which inhibited the anticyacterial action of sulfanilimide and sulfapyridine. Stamp (2) and Green (3) had obtained similar extracts from
other organisms. After concentrating his extract, Woods concluded from an examination of its chemical properties that the substance might be structurally related to sulfanilamide. The behavior of the material, resembling the competitive inhibition of enzyme reactions by compounds structurally related to the substrate or product, pointed to the same conclusion. Accordingly p-aminobenzoic acid (PABA) was tried and found to be highly active. Both the unsubstituted amino group and the carboxyl group para to it proved to be necessary for antisulfanilamide activity. Woods considered that the results provided strong circumstantial evidence for the identity of the yeast factor, and suggested that PABA is an essential metabolite for the growth of micro-organisms.

Many organisms are unable to synthesise one or more of the known amino acids. These essential metabolites are the building blocks of the cell proteins, and they are usually required in larger quantities than many of the growth factors. Proteins are not only the basic units of cell structure but also, in conjunction with active prosthetic groups, assume according to Mitchell (4), the role of enzymes to catalyse many metabolic reactions. Finally some of the amino acids probably serve as precursors of other essential metabolites Dixon (5).

In studying the production of indole from tryptophan, Fildes (6) noted that the growth of E. coli was inhibited by indoleacrylic acid. Following up this earlier observation, he (7) found that M/8000 β-indoleacrylic acid

\[
\text{CH}_2=\text{CH-CONH}_2
\]

\(\beta\text{-Indoleacrylic acid}
\]

\[
\text{CH}_2=\text{CH-CONH}_2
\]

\(\text{Indole}
\]

\[
\text{CH}_2=\text{CH-CONH}_2
\]

\(\text{Tryptophan}
\]

prevented growth, while a number of related substances, such as indoleacetic and propionic acids, produced very slight or no growth inhibition. Since indoleacrylic acid is closely related to tryptophan in several respects,
Fildes reasoned that indoleacrylic acid might interfere with an enzyme system concerned with the metabolism of tryptophan. He demonstrated experimentally that, although not itself a growth stimulent, the latter counteracted the inhibition produced by the former. Although the growth was proportional to the amount of tryptophan added, there was no quantitative relation between the antagonistic effect of these two compounds. Unlike the sulfonamide–PABA relationship, the response of the organisms was a function of the tryptophan concentration only, regardless of the amount of indoleacrylic acid present. These results, which were confirmed on B. typhosum, led to the suggestion that indoleacrylic acid interferes with the formation of tryptophan rather than its utilisation. Under these conditions no quantitative relationship between the two would be expected and minimum amount of tryptophan necessary for growth should reverse any reasonable amount of indoleacrylic acid. However, there should be a quantitative relationship between the acrylic acid and a precursor of tryptophan. Indole is a precursor for B. typhosum, but no interference with the action of indoleacrylic acid by this substance was found. To explain the lack of any reversing action of indole, Fildes assumed that either the blocking effect of indoleacrylic acid on tryptophan synthesis is not reversible or, because of the growth inhibition produced by indole itself at higher concentration, the effect would not be demonstrated.

Block and Erlenmeyer (8) reported that 1-naphthylacrylic acid and styrylacetic acid resembled indoleacrylic acid in their behavior.
These substances were prepared to study the antagonistic effect to the growth stimulating action of tryptophan. (Growth stimulation usually indicated that the metabolite was a limiting factor for the growth of the particular organism, rather than a growth factor as defined by Fildes. Thus the synthetic ability of the organism for the metabolite is limited, and the addition of the performed metabolite provides a more favorable medium for growth. Under these conditions, any growth inhibition is likely to be less effective regardless of whether it is a specific antagonist for the metabolite in question. Consequently, specific antagonisms are frequently more difficult to establish when the metabolite is a growth stimulant.)

While trans-cinnamic acid gave similar results, the authors concluded that tryptophan metabolism was involved in this case. Dihydrocinnamic, benzoic, and fumaric acids produced no effect.

With several species of lactic acid bacteria, Snell (9) was able to substitute anthranilic acid for tryptophan. Since anthranilic acid is a position

\[
\begin{align*}
\text{Anthranilic acid} & \quad \text{Orhanilic acid} & \quad 2\text{-Orhanilamidopyridine} \\
\text{isomer of } \text{p-aminobenzoic acid (PABA)} & \text{he studied the corresponding isomer of sulfanilamide (orthanilamide) as well as orthanilic acid and 2-orthanil-} \\
& \text{amidopyridine. None of these substances inhibited growth promoted by anthra-} \\
& \text{nilic acid. Snell concluded that, if it existed at all, the antibacterial power of orthanilamide in relation to anthranilic acid was of a much lower} \\
& \text{order of magnitude than in the case of the corresponding sulfanilamide -PABA} \\
& \text{relationship. Anderson (10) found that the bacteriostatic action of DL-5-}
\end{align*}
\]
methyltryptophan on *E. coli* in simple media was reversed by L-tryptophan, the inhibition ratio being approximately 1000:1 over a considerable range of concentrations.

![Methyltryptophan](image)

5-Methyltryptophan

On the other hand they also demonstrated that the tryptophan requirements of certain *E. coli* bacteriophages could be met by the 5-methyl derivative. Earlier, Gordon and Jackson (11) had shown that the latter compound was toxic to rats on a tryptophan-deficient diet, but had no effect on animals receiving an adequate diet.

Gladstone (12) investigating the amino acid requirements of *E. anthracis* observed a number of curious interrelationships among groups of chemically related amino acids. When leucine or valine was eliminated from the synthetic medium, the organism failed to grow, and the removal of isoleucine resulted in delayed and incomplete growth. The absence of all three amino acids, surprisingly enough, allowed growth to occur, but the addition of any one of these amino acids to a mixture in which all three were originally absent completely inhibited growth. Isoleucine was the most effective, preventing growth at a concentration of M/312,500 under these conditions. The presence of the unnatural isomers did not account for the results, since the same effects were obtained with both purified isomers. Further study revealed several other similar relationships, which were summarised as follows by Gladstone. The toxicity of leucine, \((\text{CH}_3)_2\cdot\text{CH} \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{(NH}_2)_2 \cdot \text{COCH},\)
threonine, CH₃CHOH.CH(NH₂).COOH, and α-aminobutyric acid, CH₃.CH₂.CH(NH₂).COOH, is counteracted by valine, (CH₃)₂.CH.CH(NH₂).COOH, and vice versa.

The toxicity of isoleucine, CH₃.CH₂.CH(CH₂).CH(NH₂).COOH, norleucine, CH₃.CH₂.CH₂.CH₂.CH(NH₂).COOH and serine, CH₂OH.CH(NH₂).COOH, is counteracted by a combination of valine, (CH₃)₂.CH.CH(NH₂).COOH, and leucine, (CH₃)₂.CH.CH₂.CH(NH₂).COOH. The toxicity of serine, CH₂OH.CH.CH(NH₂).COOH, is counteracted by threonine, CH₃CHOH.CH(NH₂).COOH, and vice versa.

On the basis of the similarity in chemical structure of the growth inhibitors and their antagonists, Gladstone suggested that some common action necessary for growth might be involved. Thus, an excess of one amino acid might block the enzymes necessary either for the synthesis of another chemically related, or for building it when synthesised into more complex substances. Numerous other instances in which one amino acid may interfere with the utilisation of another have been reported. The results of these studies are summarised in Table I. It is usually assumed in these cases that the antagonist interferes with an essential metabolite; an excess of one (the antagonist) blocks the function of the other (the metabolite) because of the structural similarity between them. Such effects are most frequently observed in relatively simple culture media.
Table I
Antagonistic Relationship Between Amino Acids

<table>
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<th>Antagonist</th>
<th>Metabolite</th>
<th>Organism</th>
<th>Other Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{H}_2\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot$</td>
<td>$\text{H}_2\text{N}\cdot\text{CO}\cdot\text{CH}_2\cdot$</td>
<td>Yeast</td>
<td>$\beta$-Alanine acts as growth stimulant only in presence of asparagine or aspartic acid.</td>
<td>(15)</td>
</tr>
<tr>
<td>$\text{COOH}$</td>
<td>$\text{CH}(\text{NH}_2)\cdot\text{COOH}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-Alanine</td>
<td>Asparagine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}_2\cdot$</td>
<td>$\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot$</td>
<td>Escherichie coli</td>
<td>Norvaline also an antagonistic; inhibition ratio</td>
<td>(16)</td>
</tr>
<tr>
<td>$\text{CH}_2\cdot\text{CH}(\text{NH}_2)\cdot$</td>
<td>$\text{CH}(\text{NH}_2)\cdot\text{COOH}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COOH</td>
<td>Methionine</td>
<td></td>
<td>Norleucine : Methionine 1000 : 1 approximately</td>
<td></td>
</tr>
<tr>
<td>Norleucine</td>
<td>Methionine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{COOH}\cdot\text{CH}_2\cdot\text{CH}(-\text{NH}_2)\cdot\text{COOH}$</td>
<td>$\text{H}_2\text{N}\cdot\text{CO}\cdot\text{CH}_2\cdot\text{CH}_2\cdot$</td>
<td>Lactobacillus casei</td>
<td>Asparagine and glutamic acid also effective in preventing growth inhibition caused by aspartic acid.</td>
<td>(17)</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>Glutamine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{CH}_2(\text{NH}_2)\cdot\text{COOH}$</td>
<td>$\text{CH}_3\cdot\text{CH}(\text{NH}_2)\cdot\text{COOH}$</td>
<td>Streptococcus fecalis R</td>
<td>Bacteriostatic action of glycine also lowered by pyridoxine.</td>
<td>(18)</td>
</tr>
<tr>
<td>Glycine</td>
<td>$\alpha$-Alanine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{(CH}_3)_2\cdot\text{CH}_2\cdot$</td>
<td>$\text{CH}_3\cdot\text{CH}_2\cdot\text{CH}(\text{CH}_3)\cdot$</td>
<td>Pasteurilla</td>
<td>Valine also reverses antagonistic action of leucine; not due to growth promoting action.</td>
<td>(19)</td>
</tr>
<tr>
<td>$\text{CH}(\text{NH}_2)\cdot\text{COOH}$</td>
<td>$\text{CH}(\text{NH}_2)\cdot\text{COOH}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>Isoleucine</td>
<td></td>
<td></td>
<td></td>
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### Table I (cont'd)

<table>
<thead>
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<th>Metabolite</th>
<th>Organism</th>
<th>Other Observations</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine,</td>
<td>Isoleucine and</td>
<td>Neurospora crassa</td>
<td>Antagonistic effect limited to mutant strain, requiring</td>
<td>(20)</td>
</tr>
<tr>
<td>or Norleucine,</td>
<td>Valine</td>
<td></td>
<td>preformed isoleucine and valine.</td>
<td></td>
</tr>
<tr>
<td>or Norvaline.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH₂C(NH).NH⁻</td>
<td>NH₂(CH₂)(CH₂)₅⁻</td>
<td>Neurospora crassa</td>
<td>Only natural forms of amino acids showed effects limited</td>
<td>(21)</td>
</tr>
<tr>
<td>(CH₂)₃CH(NH₂)⁻</td>
<td>CH(NH₂).COOH</td>
<td></td>
<td>to mutant strain; inhibition ratio approximately 2 : 1</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>Lysine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Norleucine</td>
<td>Methionine</td>
<td>Protens morganii</td>
<td>Norvaline and allothreonicine also antagonistic but</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>less specific. Ratio Norleucine : Methionine 1000 : 1</td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>β-Alanine</td>
<td>Yeast</td>
<td>Growth stimulated by pantothenic acid but unaffected by</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>asparagine.</td>
<td></td>
</tr>
</tbody>
</table>
Several \( \alpha \)-amino sulfonic acids structurally related to the natural \( \alpha \)-amino carboxylic acids were studied by McIlwain (13,14). The sulfonic acid analogs of amino acids such as glycine, alanine, leucine and valine were prepared by treating the corresponding aldehyde bisulfite derivatives with aqueous ammonia. It was found that in a chemically defined medium organisms which required preformed amino acids were inhibited by \( \alpha \)-amino-sulfonic acids. There appeared to be considerable overlapping in the reverse-effect of the amino acids.

Thus, the growth inhibition produced by \( \alpha \)-aminoisobutanesulfonic acid was partly reversed by glycine, or alanine, more effectively by valine, but not at all by leucine.

\[
\begin{align*}
\text{\( \alpha \)-Aminoisobutanesulfonic acid} & \quad \text{Valine} \\
\left(\text{CH}_3\right)_2\text{CH}_-\text{CHSO}_2\text{H} & \quad \left(\text{CH}_3\right)_2\text{CH}_-\text{COOH}
\end{align*}
\]

However, of the various metabolites tested, only the amino acids were capable of preventing the growth inhibition of the \( \alpha \)-aminosulfonic acids. In general, bacteria which synthesise their own amino acids were not susceptible. McIlwain also investigated the action of cysteic acid, taurine and tauremid, the respective analogs of aspartic acid and \( \beta \)-alanine. These sulfonic acids were not inhibitory, and sometimes stimulated suboptimal growth.

One of the early experiments indicative of a metabolic antagonism between an essential amino acid and related compound was reported by Dyer, (24) who initiated biochemical studies on the relationship between methionine and ethionine. She observed that ethionine was toxic to young rats maintained on a methionine-deficient diet. This toxicity was offset by
the addition of methionine to the diet. Later the "antimethionine" effect of ethionine was shown also for *E. coli* by Harris and Kohn, (16) who reported that the inhibition of bacterial growth by ethionine was completely overcome by methionine.

\[
\begin{align*}
\text{C}_{6}\text{H}_{5}\cdot\text{S.}\text{CH}_{2}\text{CH}_{2}\text{CH.}\text{COOH} & \quad \text{CH}_{3}\text{SCH}_{2}\text{CH}_{2}\text{CH.}\text{COOH} \\
\text{NH}_{2} & \quad \text{NH}_{2}
\end{align*}
\]

Ethionine \hspace{2cm} Methionine

Roblin and his co-workers (25) synthesised methoxinine and studied its action on *E. coli* and *Staph. aureus*. The growth inhibitory effect of this compound was prevented by L-methionine but not by the D-isomer. One mole of DL-methionine reversed the antibacterial action of 500 to 1000 moles of DL-methoxinine or DL-ethionine.

Histamine is present in appreciable quantities in most body fluids and tissues. It is a substance of multiferous actions, minute amounts exerting a powerful vasodilator effect while contracting other smooth muscle. To account for the absence of these effects under normal conditions, histamine is generally assumed to occur in a bound form in vitro. Although its role has not been completely elucidated, the liberation of histamine appears to be an important factor in anaphylactic shock (26) and many allergic conditions such as asthma and hay fever (27). Most vasoconstrictor substances counteract the vasodilator effect of histamine, and spasmolytic agents such as atropine, neutralise the coun-
teracting action of histamine on smooth muscle. However, other agents have been found which, at least in some cases, appear to be more direct and specific histamine antagonists. Edlbacher and his co-workers (28) first demonstrated that arginine, histidine, and cysteine inhibit the effect of histamine on isolated strips of guinea pigs.

Similarly, methylation of the imino group, as 1-(3)-methyl histidine and anserine, destroyed the antagonistic action of the parent compound.

Nielsen (29) observed that the growth stimulating action of \( \beta \)-alanine on yeast was antagonised by \( \beta \)-aminobutyric acid.

\[
\begin{align*}
H_2NCH_2-CH_2COOH & \quad H_2NCH_2-CH_2COOH \\
n\text{\( \beta \)-Alanine} & \quad \text{\( \beta \)-Aminobutyric acid} \\
& \quad H_2NCH_2CH_2SO_3H
\end{align*}
\]
In the absence of $\beta$-alanine this acid had neither growth-promoting nor
growth-inhibiting action. Taurine, $\beta$-hydroxypropionic acid and
$\beta$-alanylglycine were inert. Nielsen and Johansen (30) concluded that
$\beta$-aminobutyric acid interfered with the synthesis of pantothenic acid
from $\beta$-alanine by yeast cells. They found isoserine, but not N-methyl-
$\beta$-alanine, to have a similar action and suggested that the antagonists
might actually be converted to inert compounds containing $\beta$-aminobutyric
acid or isoserine instead of $\beta$-alanine.

The similarity in structure of $\beta$-alanine and propionic acid
suggested that propionates might owe their bacteriostatic and fungistic
activity to a competitive interference with the synthesis or utilisation
of $\beta$-alanine or pantothenic acid. Wright and Skeggs (31) studied the
comparative bacteriostatic activity of sodium salts of acetic, propionic
acids. The results indicated that the bacteriostatic activity of sodium
propionate for E. coli was counteracted to a considerable degree by small
amounts of $\beta$-alanine. $\beta$-Alanine was specific among the amino acids
and growth factors studied in showing this effect. Adenine, and to a
lesser extent biotin, functioned synergistically with sodium propionate
in inhibiting the growth of E. coli.

The effect of $\beta$-2-thieny1alanine (32,33), an isoster of phenyl-
alanine, upon the growth of Saccharomyces cerevisiae, E. coli and certain
other micro-organisms has been reported by du Vigneaud and his co-workers
(34,35) and by Beerstecher and Shive (36).
Phenylalanine  

$\beta$-2-Thienylalanine

Thienylalanine was inhibitory toward the growth of these organisms, and the inhibition was counteracted by phenylalanine. These investigators found that as the concentration of phenylalanine in the medium was increased large amounts of thienylalanine were required to produce inhibition growth. Thus, thienylalanine was shown to act as an "antiphenylalanine" for these organisms. Other amino acids, including tyrosine, were ineffective in preventing the growth-inhibitory action.

Beerstecher and Shive (36) studied the competitive inhibition of phenylalanine utilisation in \textit{E. coli} with $\beta$-hydroxyphenylalanine (37) and $\beta$-2-thienylalanine, and the effect of phenylalanine and tryptophan on this inhibition. The results indicate that tryptophan may be a precursor of phenylalanine. $\beta$-Hydroxyphenylalanine also inhibits competitively the functioning of phenylalanine in \textit{Lactobacillus arabinosus 17-5} and in \textit{Streptococcus fecalis R}, both of which require an outside source of phenylalanine for maximum rate of growth. In an attempt to determine how tryptophan and phenylalanine were metabolically related, $\beta$-phenylethylamine, phenylsulfuric acid, and phenylpyruvic acid were tested and found to show no effect on the toxicity of thienylalanine or $\beta$-hydroxyphenylalanine either in the presence or absence of tryptophan. Competitive
inhibition of growth of a micro-organism results from the competition of the analogue with a metabolite for specific enzyme, that the analogue-enzyme complex is incapable of performing the normal function of the metabolite, and that the growth of the micro-organism is a function of some product normally formed by the blocked enzyme, when this system becomes a limiting factor (38). According to this concept, β-hydroxyphenylalanine and β-2-thienylalanine block the utilisation of phenylalanine in a process essential to the growth of the organism. Yuan and Li (39), pointed out that examination of the formula of thienylalanine reveals a structural similarity to the sulfur-amino acid, methionine. They suggested that thienylalanine might serve as a dietary source of methionine.

\[
\text{\begin{align*}
\text{H}_3\text{C} & \text{CH}_2\text{CH}_2\text{NH}_2 \\
\text{S} & \text{CH}-\text{COOH} \hfill \text{HC} & \text{CH} \hfill \text{HC} & \text{CH}_2\text{CH}-\text{COOH} \\
\text{S} & \text{HC} & \text{C} & \text{NH}_2
\end{align*}}
\]

\text{Methionine} \quad \beta-2\text{-Thienylalanine}

Since furan compounds are isosteric with analogous thiophene compounds (40) Clark and Dittmar (41) studied the effect of β-furylalanine on the growth of micro-organisms and compared its activity with that of β-2-thienylalanine.

\[
\text{\begin{align*}
\text{HC} & \text{CH} \hfill \text{HC} & \text{CH} & \text{NH}_2 \\
\text{HC} & \text{C} & \text{CH}_2\text{CH}-\text{COOH} \hfill \text{HC} & \text{CH} & \text{NH} & \text{CH}_2\text{CH}-\text{COOH} \\
\text{O} & \text{HC} & \text{C} & \text{NH}_2
\end{align*}}
\]

\text{β-2-Furylalanine} \quad \text{β-2-Pyrrolessalanine}
They found that phenylalanine was the most active amino acid for the reversal of the furylalanine inhibition of both *S. cerevisiae* and *E. coli* growth. This would indicate that furylalanine, like thienylalanine is an "antiphenylalanine" for these organisms. However leucine, isoleucine, and tryptophan also have an appreciable effect on the inhibition of yeast growth by furylalanine. For the reversal of the inhibition of growth of *E. coli*, tyrosine and tryptophan had a high degree of activity. This is similar to the effect that these amino acids have on the toxicity of thienylalanine. Just in what way the amino acids which showed an appreciable activity in counteracting the "antiphenylalanine" properties of furylalanine, are related to phenylalanine remains to be studied. Since thienylalanine and furylalanine have been shown to inhibit microbial growth presumably due to their structural similarity to phenylalanine.

The corresponding nitrogen analog \( \beta \)-2-pyrrolealanine was synthesised quite recently by Herz, Dittmer and Cristol (42) to correlate the structure of amino acids with their biological activity. These workers were not able to isolate \( \beta \)-2-pyrrolealanine in a pure form. The crude product was tested and found to inhibit the growth of *S. cerevisiae* and *E. coli*. The inhibition was reversed by phenylalanine.

The mechanism of action of all drugs cannot be explained on the basis of a direct antagonism. Nevertheless, it is apparent that simple enzymatic reactions, the growth of micro-organisms, and the function or response of various tissues may be antagonised by mechanisms which are strikingly similar. Consequently, it may not be unreasonable to assume that this resemblance is more than superficial and that, for example, the inhibitory action of malonic acid or succinic acid dehydrogenase, the antibacterial action of pantoyltaurine, and the antagonistic effect of
trimethyloctylammonium chloride toward the action of acetylcholine on smooth muscles are closely related phenomena. In each case the antagonist appears to compete with the metabolite for an active center, or receptor and in so doing blocks the normal reaction, whether it be the oxidation of succinic acid, the growth of micro-organisms dependent on the pantothenic acid, or the contraction of a muscle under the stimulus of acetylcholine. Since in many respects this is a relatively new and rapidly developing field, it is not possible to assess all implications inherent in the broad concept of metabolic antagonists. However, as an approach to the mechanism of action of a number of drugs, as a guide in the synthesis of new therapeutic agents, and as a means of evaluating the normal mode of synthesis and function of metabolites in living cells, the concept appears to offer many possibilities as yet unexplored.
RESEARCH AIM

Most of the metabolic antagonists reported in the review show that relatively slight structural changes in many biologically essential amino acids result in the formation of new compounds which may be metabolically antagonistic to those from which they are structurally derived. This gives encouragement to the idea that possibly better and more effective and useful antagonists are still undiscovered, and that the search for such products is a worthy endeavor.

Womack and Rose (43) have demonstrated in a convincing manner that phenylalanine is essential to growth in rats. They have also shown that good growth is possible with a very limited intake of tyrosine. Tyrosine cannot replace phenylalanine, but whether phenylalanine can be converted to tyrosine in the normal intact organisms remains to be determined. p-Aminophenylalanine has not been found in nature; as an ammuno analogue of tyrosine it is not inconceivable that it may be metabolised in a manner similar to that of tyrosine and hence may be expected to be physiologically active. Mattocks (44) synthesised in this laboratory p-aminophenylalanine ethyl ester. Dr. Porter (45) made a preliminary examination of this compound and observed that it stimulated acid production by Strep. fecalis, and that it would replace phenylalanine and tyrosine (to a limited degree) with this organism but not with L. delbrückii. Through the courtesy of Dr. Porter, the results of microbiological examination of p-aminophenylalanine ethyl ester were given in Table II.

This observation encouraged the suggestion that all the isomeric aminophenylalanines be synthesised so that their biological properties may be compared.
TABLE NO II

Effect of p-Aminophenylalanine ethyl ester on Lactobacillus delbrückii and Streptococcus fecalis (45):

<table>
<thead>
<tr>
<th>Medium</th>
<th>L. delbrückii</th>
<th>Strept. fecalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Basal medium</td>
<td>III** 12</td>
<td>III 11.6</td>
</tr>
<tr>
<td>(2) &quot; compound (5mg/ml final amount)</td>
<td>III 11.7</td>
<td>III 15.5</td>
</tr>
<tr>
<td>(3) &quot; (no phenylalanine) compound</td>
<td>±? 0.9</td>
<td>-III 11.4</td>
</tr>
<tr>
<td>(4) &quot; (no tyrosine) compound</td>
<td>±? 1.3</td>
<td>± 5.95</td>
</tr>
<tr>
<td>(5) &quot; (no phenylalanine or tyrosine)</td>
<td>±? 1.2</td>
<td>±? 2.5</td>
</tr>
<tr>
<td>Basal medium (not inoculated, control)</td>
<td>- 0.9</td>
<td>- 2.0</td>
</tr>
</tbody>
</table>

*Medium and technique employed was that described by Stokes and his co-workers (46,47).

**Degree of growth as measured by turbidity

±?Questionable growth

With L. delbrückii it looks like p-aminophenylalanine ethyl ester will not replace phenylalanine or tyrosine column (3,5), but is not inhibitory column (2). However with Strept. fecalis it may actually
stimulate activity some see column (2) in the complete medium, replace phenylalanine (3) completely and tyrosine to a limited degree.

Takeoki Sasaki (48) was able to synthesise DL-phenylalanine by allowing glycine anhydride to condense with aldehydes to yield 3,6-dialkylidene-2,5-diketopiperazine, which was converted by hydrolytic cleavage into the acid.

\[
\begin{align*}
\text{H-COOH} & \xrightarrow{\text{Si-gl-COOC}GH} \text{R} \cdot \text{CH} = \text{C} \cdot \text{CO} \cdot \text{NH-CR} \xrightarrow{\text{HI+P}} 2\text{R} \cdot \text{CH}_2 \cdot \text{CH-COOH} \\
& \text{NH}_2
\end{align*}
\]

Hidenosuke Uede (49) undertook the synthesis of the three aminophenylalanine by the Sasaki method. The preliminary condensation product of the glycine anhydride with o-nitrobenzaldehyde yielded no free o-aminophenylalanine on reduction. While in the case of m- and p-nitrobenzaldehyde, he was able to get the corresponding aminophenylalanes.

Rissert (50) found that both o- and p-nitrotoluene, but not m-nitrotoluene take part in a Claisen-type reaction with ethyl oxalate, forming the corresponding nitrophenylpyruvic acid. The synthesis of o- and p-aminophenylalanine was carried out by the following sequence of reactions:

\[
\begin{align*}
\text{NO}_2 \cdot \text{CH}_3 & \xrightarrow{\text{COOC}_2\text{H}_5} \text{NO}_2 \cdot \text{CH}_2 \cdot \text{C} \cdot \text{COOC}_2\text{H}_5 \\
& \xrightarrow{\text{HCHO}} \text{CH}_2 \cdot \text{C} \cdot \text{COOH} \xrightarrow{\text{H}_2\text{NCOH}} \text{CH}_2 \cdot \text{C} \cdot \text{COOH} \\
& \xrightarrow{\text{H}_2/\text{Pd}} \text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH-COOH}
\end{align*}
\]
Possible methods for the synthesis of m-Aminophenylalanine are indicated as follows:

\[
\text{CHO} \xrightarrow{\text{HNO}_3, \text{H}_2\text{SO}_4} \text{CHO} \xrightarrow{\text{Al(O-isoC}_3\text{H}_7)} \text{CH}_2\text{OH}
\]

\[
\text{CH}_2\text{Cl} \xrightarrow{\text{SOCl}_2} \text{CH}_2\text{NO}_2 \xrightarrow{\text{CH}_2\text{(COOC}_2\text{H}_5)} \text{CH}_2\text{-CH(COOCH}_2\text{H}_5)}
\]

\[
m\text{-Nitrobenzylmalonic ester is the common starting point for any-one of the three routes indicated below:}
\]

\[
\text{A} \xrightarrow{\text{CH}_2\text{(COOC}_2\text{H}_5)} \xrightarrow{\text{NaOCH}_2\text{H}_5} \xrightarrow{\text{RONO}} \xrightarrow{\text{HOH}} \xrightarrow{\text{H}_2, \text{Pd}} \text{NH}_2
\]
The evidence of successful results of Berry (51) in the nitration of substituted melonic esters with sodium ethoxide and butyl nitrite; and also in the nitration of substituted melonic acids with hydrogen chloride and butyl nitrite suggested that \( \alpha \)-oximino-\( \beta \)-(m-nitrophenyl)-propionic acid might be conveniently expected by methods A and B, respectfully. Experiments in these directions were performed, but the products obtained showed that no reaction had taken place. Similar results were obtained in the attempts to prepare \( \alpha \)-oximino-\( \beta \)-(p-nitrophenyl)-propionic acid. It was assumed that nitrosation failed in the cases of m- and p-nitrobenzylmelonic acids or esters due to the presence of nitro groups; and these reactions were not further studied.

Lepercq (52,53) in 1893, reported that the reaction of esters of \( \alpha \)-halogen acids with sodium nitrite yields esters of \( \alpha \)-oximino acids. Hamlin (54) was able to get 65% yield of ethyl\( \alpha \)-oximinoceproste from ethyl\( \alpha \)-bromoceproste with sodium nitrite. This observation encouraged the synthesis of m-aminophenylelenine ethyl ester by undergoing the reactions as outlined under C.

Weerman (55) prepared o-nitrophenyleceteldehyde from o-nitrocinnamide by the Hofmann degradational reaction; and reduction of this compound yielded indole. It was thought to prepare an indole derivative such as a lower homolog of tryptophen to study its physiological activity by the following sequence of reactions.
Condensation of ethyl orthoformate with o-nitrotoluene was not accomplished; the condensation of ethyl formate was tried but no reaction took place. It was thought the following type of reaction might take place.

\[
\begin{align*}
\text{Condensation} & \quad \text{Ethyl orthoformate} \\
\text{Ethyl formate} & \quad \text{No reaction}
\end{align*}
\]
The condensation failed from the beginning.

Janny (56) was successful in preparing the benzylximino grouping by allowing to react benzylchloride acetoxime in presence of sodium ethoxide to form benzylximinosacetone. Waters (57) was able to get 56% yield of β-phenyl-α-benzylximino-propionic acid by condensing β-phenyl-α-ximinopropionic acid with benzyl chloride in sodium hydroxide solution. Weaver (58) used sodium ethoxide solution as the condensing agent, and got 66% yield. It was found to try the condensation of α-ximinopropionic acid with benzyl chloride by the above two procedures to get the desired compound β-indolyl glycine.
Benzylation of \( \alpha\)-oximino-\( \beta\)-(o-nitrophenyl)-propionic acid was not obtained; so it was thought to carry out benzylation of \( \alpha\)-oximino-\( \beta\)-(p-nitrophenyl)-propionic acid ester to study whether the nitro group on the phenyl nucleus was a hindrance. The same type of negative result was obtained with p-isomer. Time did not permit further attempts at the condensation of o-nitrophenylpyruvic acid with ethyl formate and ethyl orthoformate, and the reaction of benzyl chloride with \( \alpha\)-oximino-\( \beta\)-(o-nitrophenyl)-propionic acid.

The reduction of p-nitrophenylpyruvic acid ethyl ester formed the expected p-aminophenyllactic acid ethyl ester. The catalytic reduction of o-nitrophenylpyruvic acid itself gives interesting results. In the presence of acid, the product is salt of o-aminophenyllactic acid. In neutral solvent the product is \( \alpha\)-indolecarboxylic acid, undoubtedly resulting from internal anil formation as indicated.
Indole reacts with ethyl oxalate to form ethyl $\beta$-indolylglyoxalate (59). It was expected that this $\alpha$-ketonic acid ester would form an oxime, the reduction of which would give the hitherto elusive $\beta$-indolylglycine ester. The customary conditions for oxime formation were tried but failed to yield the desired product, and insufficient intermediate did not permit further studies along this line. The reduction of ethyl $\beta$-indolylglyoxalate itself resulted in the formation of the ester of $\beta$-indolylglycolic acid.

During the course of this investigation a study of the reaction of ethyloxalate with $p$-tolylarsenic acid, $\alpha$-end $\gamma$-picoline was carried out under varying conditions, with the hope of obtaining intermediates leading to the corresponding alanine derivatives. The ethyl oxalate condensation with these compounds to get the corres-
ponding pyruvic acids was not successful.

It was thought interesting to prepare ethyl nitromalonate, and to study its reaction with benzyl chloride, and compounds like o-, m-, and p-nitrobenzyl chloride to get intermediates for the corresponding phenylaleneine derivatives.

\[
\begin{align*}
\text{CH}_2(\text{COOC}_2\text{H}_5)_2 & \xrightarrow{\text{HNO}} \text{NO}_2\text{-CH(COOCC}_2\text{H}_5)_2 & \text{C}_6\text{H}_5\text{CHCl} & \xrightarrow{\text{NO}} \text{NO}_2\text{-C-CH}_2\text{C}_6\text{H}_5 \\
\text{HOH} & \xrightarrow{\text{CO}_{2}} \text{C}_6\text{H}_5\text{CH}_2\text{C}_{\text{COOH}} & \text{H}_2 & \xrightarrow{\text{NH}_2} \text{C}_6\text{H}_5\text{CH}_2\text{C}_{\text{COOH}}
\end{align*}
\]

Ethyl nitromalonate was obtained in encouraging yields, but the reaction of its sodio derivative with benzyl chloride did not give pure ethyl benzynitromalonate. Regretably because of lack of sufficient time, this interesting and promising approach was not carried to a satisfactory conclusion.
Meyer and Belle (60) first prepared ethyl o-nitrophenylpyruvic acid but did not mention the yield. Elkes et al (59) followed this method, and found that it was time consuming, and some of the product destroyed during isolation. DiCarlo (61) showed that ethyl o-nitrophenylpyruvate which was formed during the condensation of ethyl oxalate with o-nitrotoluene should be hydrolysed completely, and then excess of o-nitrotoluene removed by distillation with steam, to get pure compound in a very good yield. His method was followed in early experiments during this study and found also unsatisfactory. After personal correspondence with DiCarlo and following his suggestions and undergoing various modifications the following method was found satisfactory:
In a one-liter 3-neck flask fitted with reflux condenser, calcium chloride tube, mechanical stirrer and dropping funnel, was placed 160 ml. absolute ethanol, in which was dissolved completely 13.8 g. (0.6 mole) of sodium. Then with the contents of flask kept at 0-5°C by means of an ice-salt bath, was added over a period of thirty minutes a mixture of 82.2 g. (0.6 mole) of o-nitrotoluene, and 86.6 g. (0.6 mole) of ethyl oxalate. The reaction mixture was stirred at that temperature for 3 hours more and was then allowed to stand at room temperature for about 15 additional hours. The reaction mixture was then refluxed on a water bath for 30 minutes, and to it was added 350 ml. water, and the whole refluxed for 4 hours, then allowed to stand overnight. During this period the solution changed color from dark brown to faint yellow, and separated into two layers. The solution was distilled with steam; the recovered o-nitrotoluene weighed 13 g. The flask was stoppered and placed in an ice-salt bath, and to the contents was added very carefully and with vigorous shaking cold conc. HCl. As the acid was added, oil began to separate and then to crystallise; the light yellow crystalline product separated was chilled in the ice-box till the supernatent liquid was clear. The product was transferred to sintered glass funnel, leaving in the flask the dark brown decomposed mass. The crystalline yellow product was washed with water thoroughly, and then dried. To this was added hot toluene to remove contaminating impurities. The product thus obtained weighed 88 g. and melted at 117°C. It was taken up in hot 40% ethanol, purified with charcoal, the solution filtered and the product allowed to crystallise. The product weighed 84 g., yield 79.6% based on o-nitrotoluene not recovered, and melted at 121°C. The reported melting point for o-nitrophenylpyruvic acid is 119-121 (61).
A modification of preparing oximes of $\alpha'$-keto acids outlined by Shemin and Herbst (62) was adopted. In 600 ml. beaker 16.72 g. (0.08 mole) of o-nitrophenylpyruvic acid was dissolved in 80 ml. of 50% alcohol. To this was added a solution of 16.98 g. (0.12 mole) of sodium acetate and 8.34 g. (0.12 mole) hydroxylammonium chloride dissolved in 80 ml. of 50% alcohol. The slightly alkaline solution was warmed on steam bath for about 10 minutes and then was kept at room temperature over a period of 20 hours. The reaction mixture was now cooled at 0-5° and made acid to Congo red with dilute HCl. The solution was then diluted with excess of water to force out the oxime and was kept in the ice box overnight. The beautiful white needles were precipitated at the bottom. The product was filtered, washed with water till free from chloride, and dried, the yield was 15.8 g. melting at 159.5°. The product after recrystallisation from 30% ethanol and decolorization with charcoal, weighed 14.0 g., 78.12% of that theoretically calculated. The recrystallised product melted at 161°. This value agreed with that found by Rissert (63) while Neber and Huh (64) reported 171° m. p. Neutrel equivalent; calculated for C$_9$H$_8$O$_5$N$_2$ 224; found 225.6 and 225.3.

Preparation of palladium charcoal catalyst: The procedure of Hertung (65)
was followed. To 3 g. of nuchar was added 0.3 g. of palladium chloride crystals. This mixture was placed in a bottle and 100 ml. of 50% alcohol was added. The bottle was fitted to the Parr hydrogenator (Parr Instrument Co. Inc.). The air was removed by evacuating the bottle, filling it with hydrogen and repeating the process at least four times; then left an atmosphere of practically pure hydrogen keeping a pressure of 30 lbs/sq. in.

\[
PdCl_2 + H_2 \rightarrow Pd + 2 HCl
\]

When the uptake of hydrogen had ceased the bottle was removed, and the contents filtered on a suction funnel. The palladinized charcoal was washed thoroughly with 95% alcohol, and then with ether. Care must be taken to avoid spontaneous ignition of the material. The catalyst when nearly dried was placed in a vacuum desiccator over concentrated sulfuric acid. After standing overnight it was ready for use.

**o-Aminophenylethylamine dihydrochloride:**

\[
\begin{align*}
\text{CH}_2\text{C-COOH} & \quad \text{H}_2 \quad \overset{\text{Pd}}{\rightarrow} \\
\text{NO}_2 & \quad \text{NH}_3\text{HCl}
\end{align*}
\]

To 2.24 g. (0.01 mole) of \(\alpha\)-oximino-\(\beta\)-(o-nitrophenyl)-propionic acid dissolved in 100 ml. of 50% alcohol was added 5 ml. of 36% hydrochloric acid and the previously prepared palladium-charcoal catalyst. The reduction was carried out in the Parr hydrogenator at a pressure of 50 lbs/sq. in. until the theoretical quantity of hydrogen 1120 ml. was taken up during a period of 4\(\frac{1}{2}\) hours. The rate of hydrogen absorption was followed by observing the fall in pressure on a gauge previously calibrated 4 lbs \(\equiv\) 1000 ml. The catalyst was filtered off, the filtrate was con-
centred under water pump. As the solvent was removed, the white crystalline product was left suspended in water. When all the alcohol was removed, the product in water suspension was chilled in the ice box. The flocculent white precipitates were settled down at the bottom. The product was filtered and dried, 1.8 g. (71.1%) of slightly pinkish white crystals of \( \alpha \)-amino-\( \beta \)-(o-aminophenyl)-propionic acid dihydrochloride was obtained. The compound melted at 204° with decomposition and gave xentropetric reaction test. Nitrogen: calculated for \( \text{C}_9\text{H}_4\text{O}_2\text{N}_2\text{Cl}_2 \) 11.06% found, 10.87 and 10.91%.

\( \alpha \)-Aminophenyllactic acid hydrochloride:

\[
\begin{align*}
\text{CH}_2\text{C}_6\text{COOH} & \quad \text{Pd} \\
\text{NO}_2 & \quad \text{H}_2 (\text{H}^+) \\
\text{NH}_2\text{HCl} & \quad \text{CH}_2\text{CH}_2\text{COOH}
\end{align*}
\]

It was thought that during reduction of \( \alpha \)-nitrophenylpyruvic acid, if the amino group as formed by reduction of the nitro group was protected as amine hydrochloride salt, then cyclisation would not take place. This reaction was carried out in the presence of hydrochloric acid solution, and was found true.

To a solution of 2.09 g. (0.01 mole) of \( \alpha \)-nitrophenylpyruvic acid in 100 ml. of 50% alcohol was added 1.5 ml. of 36% hydrochloric acid and 3 g. of 10% palladium charcoal catalyst. The calculated quantity of hydrogen was taken up by the compound within a period of 3½ hours. The reaction mixture was filtered to remove the catalyst. The alcohol was removed under reduced pressure. The yield of \( \alpha \)-aminophenyllactic acid hydrochloride, purple color crystalline product, was 1.5 g. (69.1% theoretical). The product melted at 180° with decomposition. Nitrogen: calculated for \( \text{C}_9\text{H}_11\text{O}_3\text{N}_4\text{HCl} \) 6.43%; found 6.58% and 6.53%. 
\( \alpha \)-Indolecarboxylic acid:

\[
\begin{align*}
\text{O-Nitrophenylpyruvic acid} & \xrightarrow{H_2/Pd} \alpha\text{-Indolecarboxylic acid} \\
\end{align*}
\]

\( \alpha \)-Nitrophenylpyruvic acid has been converted into \( \alpha \)-indolecarboxylic acid by chemical procedures \((59,66,67,68,69,70)\) but not by catalytic methods. It was thought that \( \alpha \)-nitrophenylpyruvic acid would cyclise and give \( \alpha \)-indolecarboxylic if shaken in neutral solution in an atmosphere of hydrogen with the catalyst. To a solution of 2.09 g. (0.01 mole) of \( \alpha \)-nitrophenylpyruvic acid in 100 ml. of 50% alcohol was added 3 g. of 10% palladium charcoal catalyst. The hydrogenation went smoothly to the calculated end point within 3 hours. Treatment of the reduction product in the ordinary manner yielded 1.2 g. (73%) \( \alpha \)-indolecarboxylic acid. The product melted at \( 204^\circ \) with decomposition. This compound is reported to melt at \( 202-204^\circ \) \((70)\).

Indole:

\[
\begin{align*}
\text{Indole} & \xrightarrow{-\text{CO}_2} \text{Indolecarboxylic acid} \\
\end{align*}
\]

In the literature various methods have been described for the preparation of indole directly or indirectly; these will not be reviewed here. According to the method of Elkes et al \((59)\) 16.4 g. (0.1 mole) of pure
$\alpha$-indolecarboxylic acid was placed in a 250 ml. round bottom ground glass flask fitted with a long air condenser. The product was heated on a sand bath keeping temp. 240-250° for a period of 5 hours, till decarboxylation was complete. The dark colored liquid left was then cooled, and distilled under reduced pressure from a modified Claisen flask. The colorless liquid distilled at 140-143° at 8 mm. pressure. The distillate collected weighed 7.4 g. (63.2% theoretically calculated), and solidified on cooling. The product melted at 51° and possessed an intense fecal odor.

Ethyl $\beta$-indolylglutarate:

\[
\begin{align*}
\text{H} & \quad \text{H} \\
\text{N} & \quad \text{N} \\
\text{C} & \quad \text{C} \\
\text{OOC}_2\text{H}_5 & \quad \text{OOC}_2\text{H}_5 \\
& \quad \text{NaO}_2\text{H}_5
\end{align*}
\]

According to the method of Elkes (59), in a 500 ml. 3 neck flask fitted with reflux condenser, calcium chloride tube, stirrer and dropping funnel, was placed 50 ml. of absolute ethanol, in which was dissolved 4 g. (0.174 mole) of sodium. The contents of the flask were cooled by ice-salt bath, and to them were added over a period of 20 minutes 10 ml. of pyridine (dried over BeS) and a mixture of 11.7 g. (0.1 mole) indole dissolved in 10 ml. absolute ethanol and 14.6 g. (0.1 mole) of ethyl oxalate. The solution was stirred for one hour on cold bath with exclusion of moisture and then refluxed for four hours on a water bath, cautiously at first because of frothing. After the reaction was complete, alcohol was removed under reduced pressure and then the residue was neutralised while stirring with cold 2 normal HCl. A brown crystalline product precipitated, was
filtered, and washed with water, and then with ether to remove the tarry impurities. The product was dried in a vacuum desiccator over conc. \( \text{H}_2\text{SO}_4 \). On recrystallisation from alcohol the product obtained weighed 9.0 g. (41.4% theoretically calculated) and melted at 178° the value agreed with that of Elkes (59).

**Ethyl \( \beta \)-indolyglycocate:**

\[
\begin{align*}
\text{Fe} & \xrightarrow{\text{H}_2} \text{H} & \\
\end{align*}
\]

To 2.17 g. (0.01 mole) of ethyl \( \beta \)-indolyglyoxalate in 100 ml. 70% ethanol, was added 3 g. of palladium charcoal catalyst. This mixture was shaken in an atmosphere of hydrogen at a pressure of 50 lbs/sq. in. for 2 hours; there was found no absorption of hydrogen; the catalyst was filtered, and to the filtrate was added 0.1 g. of Adams's platinum oxide catalyst (American Platinum Works) and was shaken in the atmosphere of hydrogen. The theoretical quantity of hydrogen was taken up in 3 hours. The catalyst was filtered, alcohol was removed under reduced pressure. The product was found oily suspended in water, on cooling it was crystallised. The product was extracted with ether, ether was evaporated and brown crystalline product 1.84 (83.8%) was obtained.

**Nitrogen:** calculated for \( \text{C}_{12} \text{H}_{13} \text{O}_\text{N} \) 6.46%; found 6.29% and 6.33%.
Attemped preparation of oxime of ethyl $\beta$-indolyglyoxelate:

In a 250 ml. beaker 4.34 g. (0.02 mole) of ethyl $\beta$-indolyglyoxelate was dissolved in 20 ml. of 75% ethanol. To this was added a solution of 4.34 g. (0.02 mole) of sodium acetate and 1.39 g. (0.02 mole) of hydroxyl ammonium chloride in 20 ml. water. The resulting homogeneous mixture was kept at room temperature over a period of 20 hours. Then the reaction mixture was cooled at 0-5° and acidified with dilute HCl. The white crystalline product precipitated out at the bottom, which was filtered and dried. The product did not melt and proved to be an inorganic salt. The filtrate was concentrated, and extracted with ether. The ether was removed, and the product thus obtained, melted at 176° showing ethyl $\beta$-indolyglyoxelate, and no oxime formation took place. The reaction was repeated using sodium hydroxide instead of sodium acetate, but the results were not encouraging. The lack of sufficient material did not permit adequate study of the desired oximation reaction.
m-Aminophenylalanine

The successful synthesis of m-aminophenylalanine largely depends on the preparation of the intermediate oxime. The case of nitrosation of malonic acids and esters pointed m-nitrobenzylmalonic acid and ester as a source of the desired oxime and the synthesis by these routes was undertaken.

Preparation of m-nitrobenzaldehyde: Following the procedure of Heyman (71), concentrated 700 ml. sulfuric acid was taken in a two liter 3 neck flask fitted with reflux condenser, stirrer and separatory funnel, and surrounded by ice-salt bath. To the cooled acid was added very carefully 300 ml. fuming nitric acid, the temperature during the addition was kept 5-10°. Then 212 g. (2 moles) of benzaldehyde was added very carefully over a period of 2 hours with continuous stirring to the nitration mixture keeping the temperature 10-15°. After the addition of benzaldehyde was complete, the stirring was continued for one hour more, and then the reaction was transferred to the crushed ice in a 5-liter beaker. As the ice was melted, more ice was supplied. The granular yellow product precipitated was chilled for about 1 hour till completely settled down at the bottom. The product was filtered through a sintered glass funnel and washed thoroughly to remove the unreacted benzaldehyde. The product was sucked dry, and was taken in 95% alcohol, and treated with charcoal. The hot alcoholic filtrate was chilled overnight, yellow crystalline product was obtained, the yield was 165 g. From the filtrate on further cooling, more product was obtained. The total yield was 190 g. (62.9%), melted at 58.5°, the value agreed to that of Lippmann (72).
Preparation of aluminium isopropoxide: According to the directions of Wilds (73), 27 g. of aluminium previously cleaned with emery paper, to it was added 300 ml. of isopropyl alcohol (dried over calcium chloride and distilled at 83°) and 0.5 g. mercuric chloride. The flask was fitted with spiral Hinsburg condenser, with calcium chloride tube. The solution was allowed to reflux on the hot plate, after 1/2 hour, added 4 ml. of carbon tetrachloride through the condenser. The aluminium required 7 hours to go completely in solution, then the solution was refluxed for 2 hours more. The hot solution was transferred to the Cleisen flask. First alcohol was distilled under reduced pressure. The distillate was collected at 140-142° at 8 mm. pressure. The yield was 162 g. (73.8% theoretically calculated). The molten aluminium isopropoxide was transferred into wide mouth amber colored bottle, and the bottle was sealed with paraffin to exclude moisture.

Preparation of m-nitrobenzyl alcohol: Becker (74) and Thorp & Wildman (75) prepared m-nitrobenzyl alcohol from corresponding aldehyde by Cannizaro reaction; but the yield was low due to equal amount of formation of alcohol and corresponding acid.

\[
\text{2} \quad \text{CHO} \quad \text{KOH} \quad \text{CH}_2\text{OH} \quad + \quad \text{COOK} \quad + \quad \text{H}_2\text{O}
\]

Reduction with aluminium isopropoxide appeared to be the versatile reaction for the preparation of m-nitrobenzyl alcohol in fairly large yield. In 200 ml. of isopropyl alcohol, 60.4 g. (0.4 mole) of m-nitrobenzylaldehyde was dissolved, and the solution was transferred in a 2-liter 3-neck flask fitted with Allihn condenser and with a stirrer. The solution was refluxed on the oil bath, and to it
was added 14 g. (0.068 mole) of aluminium isopropoxide and 300 ml. of isopropyl alcohol. The solution was allowed to reflux for 8 hours, keeping the temperature of the oil bath at 110°. During this period acetone as formed, was distilled off and more isopropyl alcohol was supplied. The refluxing was continued for 2 hours more till the distillate did not give the test for acetone with 2-4 dinitrophenyl-hydrazine reagent. Most of the remaining alcohol was removed under reduced pressure, the residue was cooled and hydrolysed with cold dilute HCl. The oily layer separated was extracted with ether, then the ether extracts were mixed and treated with 10% sodium bisulfite solution to remove unreacted aldehyde. The ether extract was washed with water, and dried over anhydrous sodium sulfate. Ether was removed, and the residue was distilled under reduced pressure. The colorless distillate was collected at 181-183° at 6 mm. pressure, and it solidified on cooling. m-Nitrobenzyl alcohol thus obtained was 42 g. (68.6%), and melted at 27° which agreed with the melting point 27°, reported by Becker (74).

Preparation of m-nitrobenzyl chloride: In a 500 ml. two-neck flask fitted with a dropping funnel, and reflux condenser attached with a gas absorbing trap, was placed 55 g. (0.36 mole) of m-nitrobenzyl alcohol, this was heated on a water bath and during the course of ½ hour, 59.5 g. (0.5 mole) of thionyl chloride was added drop by drop. The solution was then refluxed for 3 hours, till the reaction was complete. Excess of thionyl chloride was distilled, and the dark liquid residue crystallised on cooling. The product was dissolved in 95% alcohol and treated with charcoal. The clear filtrate was poured in water, which crystallised on cooling. The dark, shining, crys-
talline product was filtered, and dried in a desiccator over conc. 
H₂SO₄. The product was irritating to the skin, it weighed 51.0 g.
(82.6%), and melted at 47 °, which agreed with the melting point
45-47 °, reported by Norris (76).

**m-Nitrobenzylmalonic ester:**

\[
\text{CH}_2\text{Cl} \quad \xrightarrow{\text{Na-CH(COOC}_2\text{H}_5)_2} \quad \text{CH}_2\text{CH(COOC}_2\text{H}_5)_2
\]

Gulland (77) obtained a mixture of ethyl m-nitrobenzylmalonate
and ethylbis-(m-nitrobenzyl)-malonate by allowing m-nitrobenzyl chloride to react with sodio-malonic ester. It was thought by using highly
diluted reagents and a large excess of sodio-malonic ester only the
mono-substituted product might be obtained, and this method was used.
In a 500 ml. 3-neck flask fitted with reflux condenser attached with
calcium chloride tube, stirrer and separatory funnel was placed 60 ml.
of absolute ethanol, in which was dissolved 2.3 g. (0.1 mole) of sodi­
um. To the boiling sodium ethoxide solution were added over a period
of one hour 48 g. (0.3 mole) of ethyl malonate (distilling 97-99 ° at
6 mm.) and 17.15 g. (0.1 mole) of m-nitrobenzyl chloride dissolved
in 15 ml. absolute ethanol. The solution was refluxed for 4 hours,
excess of alcohol was removed, and the residue was acidified with
dilute HCl, the oily layer which separated was extracted with chloro­
form, chloroform was removed on steam bath, and the residue was dis­
tilled under reduced pressure. The forerun consisted of chloroform
and ethylmalonate, and the final distillate consisted of a pale yellow liquid which was collected at 140-141° at mm. pressure.

The yield was 17.0 g. (57.6% of the theoretical amount). Nitrogen: calculated for C_{14}H_{17}O_{6}N 4.74%; found 4.66% and 4.69%.

m-Nitrobenzylmalonic acid:

\[
\begin{align*}
\text{CH}_2\text{CH(COOCH}_3)\text{H}_2 & \\
\text{NO}_2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{CH(COOCH}_3)\text{H}_2 & \\
\text{NO}_2
\end{align*}
\]

In 80 ml. of 50% ethanol were dissolved 15 g. (0.051 mole) m-nitrobenzylmalonic ester and 7.5 g. of potassium hydroxide. The solution was refluxed on water bath for 3 hours. Alcohol was removed, and the dark aqueous solution remained which was acidified at 0°, with conc. HCl and then extracted with ether. The ethereal extract was dried over anhydrous sodium sulfate, and then ether was removed, leaving behind the residual m-nitrobenzylmalonic acid. The product was recrystallised from 30% ethanol by treating with charcoal. The colorless crystalline m-nitrobenzylmalonic acid thus obtained, weighed 8.5 g. (71%), and melted at 170° with decomposition. The value agreed with that reported by Gulland (77).

Attempted nitrosation of m-nitrobenzylmalonic ester: According to the directions of Barry (51), in a 500 ml. 3-neck flask equipped with stirrer, reflux condenser and dropping funnel was placed 50ml. of absolute ethanol, in which was dissolved 0.78 g. (0.034 mole) sodium.
The flask was cooled by ice-salt bath, and to the sodium ethoxide solution was added with stirring 10 g. (0.034 mole) m-nitrobenzylmalonic ester over 20 minutes; the color changed to cherry red. To this reaction mixture was added 5.2 g. (0.051 mole) of butyl nitrite (78), and the solution was stirred for \( \frac{1}{2} \) hour more, and then the water bath was heated to 75\(^\circ\) and stirring continued for 15 minutes more. Alcohol was removed, and the solution was cooled and acidified with cold dilute \( \text{H}_2\text{SO}_4 \). The oily layer separated was extracted with ether; the ethereal extract was shaken with 10% NaOH solution to extract the expected oximino compound. The alkaline extract was heated for \( \frac{1}{2} \) hour to hydrolyse any free ester remaining. Now the solution was acidified with cold dilute \( \text{H}_2\text{SO}_4 \), the dark brown tarry mass was isolated which failed to give any definite crystalline product, and was not further characterised. The experiment was repeated using isopropyl nitrite instead of butyl nitrite, but the results were no more encouraging.

**Attempted nitrosation of m-nitrobenzylmalonic acid:** Again, following the directions of Berry (51), in a 250 ml. beaker surrounded by ice-salt bath, 4.78 g. (.02 mole) of m-nitrobenzylmalonic acid was dissolved in 20 ml. ether. To it was added 4.5 g. (.04 mole) of n-butyl nitrite with stirring and dry HCl gas was slowly passed beneath the solution over a period of 20 minutes. The reaction mixture became deep brown in color, ether was removed, and a dark brown product was obtained. It was recrystallised from alcohol and the product melted at 149\(^\circ\). This compound contained 8.3% nitrogen on analysis. Calculated nitrogen for m-nitrobenzylmalonic acid, \( \text{C}_{10}\text{H}_9\text{O}_5\text{N} \), 5.91%. Calculated nitrogen for m-nitrophenylpropionic acid, \( \text{C}_{10}\text{H}_9\text{O}_4\text{N} \), 7.26%. This sug-
gests that nitrosetion, if it took place at all, proceeded only partially. This reaction was not further investigated.

**m-Nitrophenylpropionic acid:**

\[
\begin{align*}
\text{CH}_2\text{CH(COOC}_2\text{H}_5)_2 \xrightarrow{\text{HOH}} \text{CH}_2\text{CH}_2\text{COOH}
\end{align*}
\]

In one liter 3-neck flask equipped as usual, was placed 20 g. of KOH dissolved in 100 ml. of 80% alcohol, the flask was heated on the water bath. To the hot alkaline solution was added gradually 56.2 g. (0.2 mole) of m-nitrobenzylmalonic ester over a period of 40 minutes by continuous stirring. The solution was refluxed for 16 hours, water was added to the mixture during this period as required to prevent solidification. Alcohol was removed under reduced pressure. Then the aqueous solution was heated for 3 hours on the oil bath at the temperature of 170-180 °. The solution was cooled, and was transferred in a beaker surrounded by an ice-salt bath, about 200 g. of crushed ice was added to the mixture, and cold conc. HCl was added drop by drop with continuous stirring. During the course of addition of the acid, some potassium salt of the acid was separated, but redissolved on further addition of the acid, then the mixture was made acidic to Congo red. The oily layer which separated was extracted with ether. The ether extract was dried, the ether was evaporated, leaving a syrupy liquid which was dried overnight in the vacuum desiccator. The product was recrystallised from alcohol and treated with charcoal. Beautiful white crystalline
-m-nitrophenylpropionic acid thus obtained, weighed 22.5 g. (62%) and melted at 118°, this value agreed to that reported by Grabiel and Steudemann (79).

\[ \text{CH}_2\text{CH}-\text{COBr} \]

\[ \text{Br} \rightarrow \text{Br} \]

\[ \text{CH}_2\text{CH}-\text{CO}_2\text{H}_5 \]

The procedure of Zelinsky (80) and adopted by Hamlin (54) was followed, 18.1 g. (0.2 mole) of -m-nitrophenylpropionic acid was dissolved in 20 ml. dry benzene. The solution was placed with 0.93 g. (0.03 mole) of phosphorus in 500 ml. three neck flask provided with a stirrer, reflux condenser attached with a gas trap, and separatory funnel. To the reaction mixture was added stirring very carefully, 10 g. (0.2 mole) of bromine. The reaction was carried out by heating on a water bath at 70-80° for 4 hours, till no more bromine vapour came off. The acylbromide thus formed was cooled, and to it was added 30 ml. of absolute ethanol over a period of 15 minutes. After the addition of alcohol was complete, the reaction mixture was warmed on a water bath for half an hour. Then the mixture was transferred to the separatory funnel, the ester layer separated was washed three times with water, and dried over anhydrous sodium sulfite. The solution
was distilled under reduced pressure. The clear distillate of \( \alpha \)-bromo-
\( \beta \)-(m-nitrophenyl)-propionic acid ethyl ester was collected at 129-132°/12 mm. The yield was 19.2 g. (63.4% theoretically calculated). Bromine:
calculated for \( \text{C}_{11}\text{H}_{12}\text{O}_4\text{BrN} \) 27.77%, found 26.98% and 26.94%.

\( \alpha \)-Oximino-\( \beta \)-(m-nitrophenyl)-propionic acid ethyl ester:

\[
\begin{align*}
\text{CH}_2=\text{CH}-\text{COOC}_2\text{H}_5 & \quad \xrightarrow{2\text{NaNO}_2} \quad \text{CH}_2=\text{C}\text{OOCC}_2\text{H}_5 \\
\text{NO}_2 & \quad \text{NO}_2 \\
\end{align*}
\]

According to the directions of Hamlin (54) in 500 ml. Erlenmeyer flask, was placed a solution of 17.28 g. (0.051 mole) of \( \alpha \)-bromo-\( \beta \)-(m-nitrophenyl)-propionic acid ethyl ester in 70 ml. of 95% ethyl alcohol. To this was added with stirring a solution of 24.8 g. (0.36 mole) of sodium nitrite dissolved in 80 ml. water. The resulting homogeneous mixture was allowed to stand at room temperature for 8 days with occasional shaking. The white crystalline product was seen precipitating out, allowed it to stand for 4 days more. The crystalline product obtained was filtered, and dried, the yield was 2.4 g. Recrystallisation from alcohol, the product obtained, weighed 1.9 g. (15.6%) and melted at 92°. Nitrogen: calculated for \( \text{C}_{11}\text{H}_{12}\text{O}_5\text{N}_2 \) 11.11% found 10.94% and 10.91%.

\( m \)-Aminophenylalanine ethyl ester dihydrochloride:

\[
\begin{align*}
\text{CH}_2=\text{CH}-\text{COOC}_2\text{H}_5 & \quad \xrightarrow{\text{H}_2/Fd} \quad \text{CH}_2=\text{CH}-\text{COOC}_2\text{H}_5 \\
\text{NO}_2 & \quad \text{NH}_2\text{HCl} \\
\end{align*}
\]

To 1.26 g. (0.005 mole) of \( \alpha \)-oximino-\( \beta \)-(m-nitrophenyl)-propionic
acid ethyl ester dissolved in 80 ml. of 70% alcohol was added 2.5 ml. of
36% HCl and 3 g. palladium-charcoal catalyst. The mixture was shaken in
hydrogen at a pressure of 50 lbs/sq. in. and theoretical quantity of
hydrogen was absorbed in 3 hours. The mixture was filtered to remove the
catalyst, and the clear filtrate was concentrated under reduced pressure,
leaving a deep yellow color solution about, 3 ml., to the hot residual
solution was added isopropyl ether, and a yellow crystalline product was
obtained on cooling. The product was filtered and dried, 0.9 g. (64.1%)
of the ethyl ester of m-aminophenylalanine dihydrochloride was obtained.
The crystals were yellow, and melted at 227° with decomposition. Nitrogen:
calculated for C_{11}H_{16}O_{2}N_{2}·2HCl 9.96%; found 9.74% and 9.77%. 
p-AMINOPHENYLALANINE

Erlenmeyer and Lipp (81) nitrat ed phenylalanine and obtained the p-nitro compound in rather low yields. Reduction of this compound gave p-aminophenylalanine. Friedlender (82) obtained p-aminophenylalanine by reduction of p-nitrophenyl-α-nitroacrylic acid.

p-Nitrophenylpyruvic acid ethyl ester: Wislicenus and Schulz (83) were able to get 79% yield of p-nitrophenylpyruvic acid ethyl ester by allowing p-nitrotoluene to condense with ethyl oxalate in the presence of sodium methoxide. Mattocks (44) followed their method and duplicated their results. However, since these previous workers did not obtain the stable ester, the method is given in detail.

In a one liter-3-neck flask fitted with mercury sealed stirrer, dropping funnel and reflux condenser to which is attached a calcium chloride tube, was placed 100 ml. of methanol, in which 6.9 g. (0.3 mole) sodium was dissolved. The flask was kept in ice-salt bath at 0.5. To the sodium ethoxide solution were added over a period of half an hour 43.8 g. (0.3 mole) of ethyl oxalate and 41.1 g. (0.3 mole) of powdered p-nitrotoluene. The reaction mixture began to change color to bright red after 2 hours stirring, and the stirring was continued for 3 hours more; it was then allowed to stand overnight at room temperature. The reaction mixture was turned to dark red and a red crystalline product had settled out. The entire mixture was transferred to the sintered glass funnel, and the crystals sucked dry, and washed on the funnel with absolute ether to remove unreacted material. The product was dried in a desiccator over calcium chloride. This dark red product was the sodium salt of the
Quinoid form of ethyl p-nitrophenylpyruvate and the yield was 69 g. Twenty five g. of the sodium salt was added with stirring to boiling 30 ml. glacial acetic acid. The clear red solution thus obtained was transferred into 500 ml. water, whereupon beautiful flocculent yellow crystals precipitated out. The product was filtered washed with water and dried; the yield was 20.5 g. and the crystals melted at 104°. On recrystallisation from alcohol, the product weighed 18.0 g. (71.2%) and melted at 106°. Previously recorded melting point 106° (44). Contrary to the observations of Mettocks, this keto ester was found stable at least half a year without any visible change in physical properties.

**p-Aminophenylactic acid ethyl ester hydrochloride:**

![Chemical structure]

In 80 ml. 95% ethanol containing 1.1 g. 36% hydrochloric acid, was dissolved 2.37 g. (0.1 mole) of p-nitrophenylpyruvic acid ethyl ester, and to the clear solution was added 3 g. of 10% palladium-charcoal catalyst. The reaction mixture was reduced as usual. The calculated quantity of hydrogen was taken within 3 hours. The catalyst was filtered, alcohol was removed from the filtrate, leaving only 5 ml. of solution. On the addition of isopropyl ether to this hot solution a brown crystalline product precipitated, which filtered and dried giving 1.8 g. (73.3%) of ethyl ester of p-aminophenylactic acid hydrochloride. This melted at 142° with decomposition. Nitrogen: calculated for C\textsubscript{10}H\textsubscript{15}O\textsubscript{3}N.HCl 5.72%; found 5.63% and 5.65%
In 20 ml. of 20% ethanolic KOH solution was dissolved 4.9 g. (0.02 mole) of p-aminophenyllactic acid ethyl ester hydrochloride, and the solution refluxed for 4 hours, till hydrolysis was complete. The solution was cooled, and acidified with cold dilute HCl, a beautiful faintly yellow product was obtained. Recrystallisation from 70% alcohol gave 2.8 g. (76.5%) of p-aminophenyllactic acid, which melted at 188°C. This value agrees with that reported by Wislicenus and Schulz (83).

α-Oximino-ß-(p-nitrophenyl)-propionic acid ethyl ester:

In the preparation of oxime various modifications under different conditions were tried, and it was found that oxime could be obtained in good yield from the sodium salt of the quinoid form of p-nitrophenylpyruvic acid ethyl ester instead of from the free ester if necessary precautions are observed. In 400 ml. beaker, 23.1 g. (0.1 mole) of the sodium salt of the quinoid form of p-nitrophenylpyruvic acid ethyl ester was dissolved in 50 ml. methanol, and to the solution, warmed on the water bath, was added 20 ml. glacial acetic acid. The resulting clear, deep red colored solution was mixed with stirring with 13.8 g. (0.2 mole) hydroxylamine hydrochloride and 27.2 g. (0.2 mole) sodium acetate dissolved in 100 ml. water. The reaction mixture was transferred to a one-liter Erlenmeyer flask, if any oily liquid was left it was dissolved
by adding sufficient quantity of methanol. The solution was then allowed to stand with occasional shaking, at room temperature for 3 hours and was then placed in the ice box overnight, when yellow needles precipitated. The product was filtered, washed with water and dried. On recrystallisation from alcohol 20.4 g. (80.9%) yellow crystalline \( \alpha \)-oximino-\( \beta \)-(p-nitrophenyl)-propionic acid ethyl ester was obtained, which melted at 163\(^\circ\)

\( \alpha \)-Oximino-\( \beta \)-(p-nitrophenyl)-propionic acid:

\[
\begin{align*}
\text{CH}_2\text{C-COOCH}_2\text{H}_5 \\
\text{NOH} \\
\text{NO}_2
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2\text{C-CHOH} \\
\text{NOH} \\
\text{NO}_2
\end{align*}
\]

In 15 ml. of 95% ethanol was dissolved 7.56 g. (0.03 mole) \( \alpha \)-oximino-\( \beta \)-(p-nitrophenyl)-propionic acid ethyl ester, and to it was added 25 ml. of 10% sodium hydroxide. The solution was refluxed for 4 hours, and then the dark red solution was cooled and acidified with cold conc. \( \text{H}_2\text{SO}_4 \). A yellow crystalline product precipitated, was filtered and dried. On recrystallisation from alcohol by treating with charcoal, yellow crystals of \( \alpha \)-oximino-\( \beta \)-(p-nitrophenyl)-propionic acid were obtained weighing 4.5 g. (67%) and melting at 127\(^\circ\). The neutral equivalent of the acid was obtained by dissolving in 60% hydro-alcoholic solution and using phenolphthalein as an indicator. Neutral equivalent: calculated for \( \text{C}_9\text{H}_8\text{O}_5\text{N}_2\text{O}_2 \); found 222.5 and 222.8.

Reduction of \( \alpha \)-oximino-\( \beta \)-(p-nitrophenyl)-propionic acid and of the ethyl ester was carried out with palladium charcoal catalyst, but found the rate of absorption of hydrogen was very slow, and as the
reaction became sluggish, extra palladium chloride was added but without effect. It was obvious that a different catalyst was needed for the reduction of these compounds, so a mixed catalyst platinum-palladium was prepared according to the directions of Iwamoto and Hartung (84).

**Platinum-palladium mixed catalyst:** In 250 ml. of normal sodium acetate solution, 0.1 g. of palladium chloride, 0.05 g. of chloroplatinic acid and 3 g. of nuchar were suspended. The mixture was shaken in an atmosphere of hydrogen until absorption was complete. The catalyst was filtered off, washed with alcohol, and then with ether, and then dried over conc. sulfuric acid in desiccator overnight before use.

**p-Aminophenylalanine dihydrochloride:**

\[
\begin{align*}
\text{CH}_2\text{-C}-\text{COOH} & \quad \text{NOH} \\
\text{NO}_2 & \quad \frac{\text{H}_2}{\text{Pd-Ft}} \\
\end{align*}
\]

To 2.24 g. (0.01 mole) \(\alpha\)-oximino-\(\beta\)-(p-nitrophenyl)-propionic acid dissolved in 100 ml. of 70% alcohol, was added 5 ml. of 36% HCl and 3 g. of the palladium-platinum catalyst described above. The reduction mixture was shaken at a pressure of 60 lbs/sq. in. The calculated quantity of hydrogen was taken up in 6 hours. The catalyst was filtered off, and the solvent was removed under reduced pressure. After evaporation of solvents there remained a residue of faint brown crystals. This was redissolved in absolute ethanol, and was reprecipitated by the addition of isopropyl ether, filtered and dried over conc. \(\text{H}_2\text{SO}_4\) in a desiccator. The crystals were
brown in color and very hygroscopic, the yield was 1.96 g. (77.4%) melting at 194° with the evolution of gas. Nitrogen: calculated for C₉H₁₂O₂N₂·2HCl 11.06; found 11.19% and 11.22%.

p-Aminophenylalanine ethyl ester dihydrochloride:

\[
\text{NH}_2\text{Cl} \quad \text{NH}_2\text{HCl}
\]

To 2.52 g. (0.01 mole) of α-oximino-β-(p-nitrophenyl)-propionic acid ethyl ester dissolved in 100ml. of 70% ethanol, was added 5 ml. of 36% HCl and 3 g. of the palladium-platinum catalyst. The reduction mixture was shaken at a pressure of 50 lbs/sq. in. The calculated quantity of hydrogen, 1120 ml. was taken up in 5 hours. The catalyst was filtered off, the solvent was removed under reduced pressure, the residue of yellow crystalline product was dissolved in absolute ethanol and reprecipitated by addition of isopropyl ether. The product weighing 2.2 g. (78.2%) was p-aminophenyl ethyl ester dihydrochloride, melting at 97° with decomposition. This observation agreed with the melting point reported by Mattocks (44).

p-Nitrobenzylmalonic ester:

\[
\text{CH}_2\text{Cl} \quad \text{NaCH(COO}_2\text{H}_5\text{)} \quad \text{CH}_2\text{-CH(COO}_2\text{H}_5\text{)}
\]
Lellman and Schleich (85) reported that alkylation of malonic ester with p-nitrobenzyl chloride gave low yields due to formation of much bis compound. Mattocks, in this laboratory, was able to get 7.3% of p-nitrobenzylmalonic ester. It appeared in order to study different conditions with a view of increasing the yield of mono substituted product, and avoiding the formation of bis compound. In one liter-3-neck flask, 120 ml. absolute ethanol was placed, in which was dissolved 4.6 g. (0.2 mole) sodium. To the hot sodium ethoxide solution was added over a period of 20 minutes 96 g. (0.6 mole) of ethyl malonate and the solution was refluxed for half an hour. To this was added a hot solution of p-nitrobenzyl chloride 34.3 g. (0.2 mole) dissolved in 30 ml. of absolute ethanol. The reaction mixture was refluxed for 3.5 hours; alcohol was removed. The thick viscous mess was taken up in 50 ml. water, and transferred with stirring to a beaker containing 300 g. ice and 30 ml. of glacial acetic acid. A white shining flocculent product precipitated on further chilling, leaving the unreacted ethyl malonate at the bottom. The product was filtered, washed with water and dried. The filtrate was concentrated and cooled, but no further crystalline product was obtained. The product was recrystallised from alcohol, and weighed 24.5 g. (40.6%) melting at 60°. The value agreed with that reported for p-nitrobenzylmalonic ester (85). Thus, it appears that if a large excess of ethyl malonate is employed, it is possible to obtain an appreciable yield of mono-p-nitrobenzylmalonic ester.
**p-Nitrobenzylmalonic acid:**

\[
\text{CH}_2-\text{CH(COOCH}_2\text{H}_5) \quad \xrightarrow{\text{HOH}} \quad \text{CH}_2-\text{CH(COOH)}_2
\]

In 60 ml. of 70% alcohol were dissolved 14.75 g. (0.05 mole) of p-nitrobenzylmalonic ester and 8 g. KCH; the solution was refluxed for 3 hours. Alcohol was distilled off, and the aqueous solution was acidified, and chilled overnight in the ice box. The dark yellow product was recrystallised from 70% alcohol by treating with charcoal. The faint yellow product weighed 9.8 g. (82%), and did not melt but charred at 238°. Ressert (86) reported that this acid charred at 240°.

**Attempted alkaline nitrosation of p-nitrobenzylmalonic ester:**

Following the directions of Berry (51), to a solution of 0.39 g. (0.017 mole) of sodium in 40 ml. absolute ethanol, was added 5 g. (0.017 mole) of p-nitrobenzylmalonic ester, and the mixture was stirred for 20 minutes, the color changed to purple. The solution was cooled to 5°; and to it was added 3.5 g. (0.034 mole) of n-butyl nitrite, and then the reaction mixture was heated on the water bath for 20 minutes at 60°; alcohol was removed under reduced pressure; the residue was acidified, and extracted with ether. The ether was evaporated off, and dark gummy product was obtained. A repetition of this reaction with isopropyl nitrite gave similar results, and no further attempt was done to identify this product.
Attempted nitrosation of p-nitrobenzylmalonic acid:

As p-nitrobenzylmalonic acid did not dissolve in ether, the compound was suspended in ether and treated with isopropyl nitrite and dry hydrogen chloride, but it was found that no reaction had taken place. In 15 ml. of dioxane 4.78 g. (.02 mole) of p-nitrobenzylmalonic acid was dissolved, and to it was added 3.6 g. (.04 mole) of isopropyl nitrite with stirring and dry HCl gas was slowly passed beneath the solution for 15 minutes. The reaction mixture became reddish brown, dioxane was removed, and a dark gummy product was left, which failed to give any crystalline product, and was not further characterised.
Attempted condensation of ethyl orthoformate with o-nitrotoluene:

\[ \text{CH}_3 \text{C} \left( \text{OC}_2\text{H}_5 \right) + \text{HC} \left( \text{OC}_2\text{H}_5 \right) \xrightarrow{\text{NaOC}_2\text{H}_5} \text{CH}_2\text{C} \left( \text{OC}_2\text{H}_5 \right) \]

\[ \rightarrow \text{CH}_2\text{CHO} \]

In a 250 ml. three neck flask fitted as usual, was placed 30 ml. absolute ethanol, in it was dissolved 2.3 g. (0.1 mole) sodium, and to it was added with stirring a mixture of 13.7 g. (0.1 mole) o-nitrotoluene and 14.8 g. (0.1 mole) of ethyl orthoformate, the temperature of the reaction being maintained at 0-5°. The reaction mixture was stirred for 3 hours more and kept overnight, the color of the solution changed to dark brown. The reaction mixture was refluxed for 1½ hours on a water bath, alcohol was removed under reduced pressure, the residue was taken in water and treated with acetic acid keeping slightly alkaline, and then was extracted with ether. From the extract the ether was removed, and the residual liquid was distilled under reduced pressure. The distillate was collected at 155-175°/6 mm. and was dark red. Nitrogen: calculated for C_{12}H_{17}O_N 5.8% found 12.04%. Now it was thought that during refluxing the reaction mixture, the product might have decomposed; so reaction was carried out in cold with the same amount of reactants without refluxing the resulting
reaction mixture, but kept in ice box for 3 days, the solution changed color to dark red. Then the solution was treated with acetic acid, and extracted with ether. Ether was removed, and the residual liquid was distilled under reduced pressure. The first distillate collected at 80-85°/6 mm. was o-nitrotoluene, and the other collected at 85-90°/6 mm. was ethyl orthoformate.

**Attempted condensation of ethyl formate with o-nitrotoluene:**

\[
\begin{align*}
\text{CH}_3 \text{NO}_2 + \text{HCOC}_2\text{H}_5 & \xrightarrow{\text{NaOC}_2\text{H}_5} \text{CH}_3\text{CHO} \\
& \text{C}_6\text{H}_5\text{NO}_2
\end{align*}
\]

Wislicenus (87) was able to get 90% yield of ethyl formylphenylacetate from ethyl phenyleacetate by treating with ethyl formate using sodium ethoxide the condensing agent. Pechmann (88) prepared ethyl formylacetate in 70% from ethyl acetate and Yourtee (89) prepared ethyl \(\alpha\)-\(\alpha\)-diformylsuccinate from ethyl succinate. In a 250 ml. three-neck flask fitted as usual, was placed 100 ml. of absolute ethanol, in it was dissolved 6.9 g. (0.3 mole) of sodium. To the sodium ethoxide solution was added a mixture of 41.1 g. (0.3 mole) of o-nitrotoluene and 18.6 g. (0.3 mole) of ethyl formate. The reaction mixture was stirred for 6 hours at 5-10°, the color changed to dark red; it was allowed to stand for 40 hours at room temperature. Alcohol was distilled off, and the cold residue was acidified with dilute HCl and extracted with ether. The ether was removed, leaving reddish oil. The product was at once subjected to catalytic reduction, 15 ml. of this product was dissolved in 60 ml. alcohol, and to it was added palladium charcoal catalyst.
The mixture was shaken in an atmosphere of hydrogen. During the course of 3 hours 2000 ml. of hydrogen was taken up. The catalyst was filtered, the filtrate was distilled under reduced pressure; the dark crystalline mass left was treated with alcoholic HCl; and white crystalline product was obtained. Benzenesulfonyl derivative of this product melted at 124° and was identified the derivative as o-toluidine. The result indicated that no condensation had taken place.

Attempted condensation of ethyl orthoformate with o-nitrophenylpyruvic acid:

\[
\begin{align*}
\text{CH}_2\text{C} & \text{C-OH} \\
\text{NO}_2 & \text{+ HC(OC}_2\text{H}_5\text{)}_3 \text{NaO}_2\text{H}_5 \longrightarrow \text{CH}_2\text{C} & \text{C-OH} \\
\text{CH(OC}_2\text{H}_5\text{)}_2 & \text{NO}_2
\end{align*}
\]

To a solution of 2.3 g. (0.1 mole) of sodium in 40 ml. absolute ethanol, was added a mixture of 20.9 g. (0.1 mole) of o-nitrophenylpyruvic acid dissolved in 10 ml. alcohol and 14.8 g. (0.1 mole) of ethyl orthoformate. The reaction mixture was stirred for 4 hours at 5-10°, and allowed to stand overnight. Then the solution was refluxed for one hour, alcohol was removed. The residual liquid was treated with HCl. A dark brown tar resulted, from which no product could be isolated. However, further investigation of this reaction was not attempted.
Attempted preparation of \( \beta-(p\text{-nitrophenyl})-\alpha\text{-benzyl oximino propionic acid ethyl ester} \):

\[
\begin{align*}
\text{CH}_2\text{C-CCOC}_2\text{H}_5 & \quad \text{NOH} \\
\text{NO}_2 & \quad \text{C}_6\text{H}_5\text{CH}_2\text{Cl} \\
\end{align*}
\]

In a 500 ml. 3-neck flask, fitted with a reflux condenser bearing a calcium chloride tube, stirrer and separatory funnel, was placed 90 ml. of absolute ethanol, in it was dissolved 1.32 g. (0.06 mole) of sodium. To this was added 7.56 g. (0.03 mole) of finely powdered \( \alpha\text{-oximino-}\beta-(p\text{-nitrophenyl})\text{-propionic acid ethyl ester} \), and stirred well, a homogeneous red color solution resulted. To the hot solution was added 7.6 g. (0.06 mole) of benzyl chloride; the mixture was refluxed for 3 hours till neutral to litmus. To this reaction mixture was added 20 ml. of 20% alcoholic KOH solution, and refluxed for 2 hours. Alcohol was distilled under reduced pressure. The residue was acidified with cold dilute HCl and extracted with ether. The ether extract was dried, ether was removed, and the dark red product obtained was recrystallised from alcohol by treating with charcoel. The red product weighed 3.5 g. charred at 148\(^\circ\). The product was dissolved in 10% NaHCO\(_3\) solution, and was extracted with ether to remove the impurities. The aqueous extract was acidified, and tarry red product was obtained. The reaction was repeated three times under different conditions, but there was no evidence that formation of the desired product had taken place.
Attempted preparation of \( \beta - (o\text{-nitrophenyl})\alpha\text{-benzyloximino propionic acid:} \\

In a 250 ml. 3 neck flask, as usual a solution of 0.92 g. (0.04 mole) of sodium in 60 ml. absolute ethanol was prepared. To this was added 4.48 g. (0.02 mole) of \( \alpha\text{-oximino}\beta - (o\text{-nitrophenyl})\text{-propionic acid}, \) and then 5.06 g. of benzylchloride was added to the hot solution. The mixture was refluxed for 3 hours till neutral to litmus. To the mixture was added 20 ml. of 20% ethanolic KOH, and refluxed for 2 hours. Alcohol was removed, and the residue was acidified with cold dilute HCl; and extracted with ether. On evaporation of the ether, 2.8 g. of yellow solid, melting at 176°C was obtained. Due to deep yellow color, no neutral equivalent could be obtained. Nitrogen: calculated for C\(_{16}\)H\(_4\)O\(_5\)N\(_2\) 12.89% found 6.49%-6.45%.

Waters' method was tried to prepare \( \alpha\text{-ethoximino acid} \) by using diethyl sulfate, but no reaction was observed. Every evidence showed that nitro group in the phenyl nucleus hinders the preparation of benzyloximino and ethoximino compounds.

Study on ethyloxalate condensation with p-tolylarsonic acid:

\[
\begin{align*}
\text{CH}_3 & + \text{COOC}_2\text{H}_5 & \xrightarrow{\text{NaOCH}_3} & \text{CH}_2\text{-O-} \text{COOC}_2\text{H}_5 \\
\text{AS}_3\text{H}_2 & & & \text{AS}_3\text{H}_2
\end{align*}
\]

The p-tolylarsonic acid was prepared according to the method described by Ruddy (90). In a 250 ml. 3 neck flask was placed 25 ml. methyl alcohol in which 1.15 g. (0.05 mole) of sodium was dissolved, to it were added in the cold 7.30 g. (0.05 mole) of ethyl oxalate and 10.8 g. (0.05 mole) of
p-tolylarsonic acid. The reaction mixture was stirred for 2 hours, and allowed to stand overnight. The colloidal white mixture was centrifuged, and white crystalline product was obtained. Oxime of this product prepared, melted at 335°C, with decomposition, while p-tolylarsonic acid melted at 330°C. The arsenic content of the compound was determined by the modified method of Waters (91). Calculated for oxime, C_{11}H_{14}O_6NAs, As 22.6%. Found 32.4%. Calculated for p-tolylarsonic acid, C_7H_9O_3As, 34.7%. This indicated that reaction was not accomplished. The reaction was repeated with three equivalents of sodium methoxide, but found the same product. This showed that AsO_3H_2 group might interfere in the Claisen type condensation.

Further study on ethyl oxalate condensation with \( \alpha \)-picoline:

\[
\begin{align*}
\text{CH}_3 & + \text{COOC}_2\text{H}_5 & \text{NaOC}_2\text{H}_5 & \rightarrow \\
\text{CH}_2\text{C}-\text{O}-\text{COOC}_2\text{H}_5
\end{align*}
\]

In a 250 ml. 3-neck flask was placed 60 ml. of absolute ethanol, in which was dissolved 4.6 g. (0.2 mole) of sodium; to it was added over a period of 30 minutes, a mixture of 18.62 g. (0.2 mole) of \( \alpha \)-picoline and 27.4 g. (0.2 mole) of ethyl oxalate. The reaction mixture was stirred for 4 hours and then allowed to stand overnight. The reaction mixture was refluxed for 20 minutes and then to it was added 120 ml. of water, and again refluxed for 3 hours. The reaction mixture was distilled with steam till free from \( \alpha \)-picoline. Then the solution was acidified in the cold. A black brown tar resulted from which it was impossible to extract with any of the usual organic solvents any well defined product. It was thought
the reaction might be sensitive to heat, so it was carried out without refluxing in the cold. Then the reaction mixture was acidified with alcoholic HCl, white crystalline product precipitated, it was filtered and dried. On analysis it was found to be the inorganic compound NaCl. Without success an effort was made to identify the filtrate by preparation of an oxime. This proved that condensation was not accomplished. Similar negative results were obtained in the case of γ-picoline. However, further investigation of this reaction was not studied.

**Ethyl nitromalonate:** The literature (91, 92, 93,) relating to the preparation of ethyl nitromalonate is very unsatisfactory. Recently Canonico (94) found that specially prepared MnO₂ oxidised ethyl oximinomalonate to ethyl nitromalonate in glacial acetic acid at room temperature. The method outlined by Arndt and Rose (95) for the preparation of methyl nitromalonate was followed. In a one-liter 3-neck flask, fitted with a stirrer, separatory funnel, and condenser, was placed 100 ml. of ethyl malonate. To it was added very carefully over a period of 2 hours, keeping the temperature 0-5°, 300 ml. of fuming nitric acid (density 1.5). Then the mixture was stirred for 1 hour more, and then removed from the cooling bath, allowing the temperature to rise slowly to 30°; the reaction mixture was then kept for ½ hour at 30-35°. Again it was placed in the cooling bath at 0-5°; and stirred for 2 hours. Then the reaction mixture was poured on crushed ice in a 3-liter beaker with stirring. The oil separated, was extracted with ether. The ether extract was shaken with 20% Na₂CO₃ solution with successive volumes till the alkaline extract was no longer red. The ether extract was washed with water, and then dried over anhydrous sodium sulfate. Ether was evaporated off on the steam bath; the residual oily liquid was distilled under reduced pressure. The distillate first
collected at 110°/8mm was unreacted ethyl malonate, and then the distillate collected at 135-138°/8mm was ethyl nitromalonate. The yield was 32 g.;

| D | 1.4449. |

**Ethyl benzylnitromalonate:**

\[
\text{NO}_2\text{CH(COOC}_{2}\text{H}_5\text{)}_2 + \text{CH}_2\text{Cl} \overset{\text{NaOC}_{2}\text{H}_5}{\longrightarrow} \text{CH}_2\text{-(COOC}_{2}\text{H}_5\text{)}_2\text{NO}_2
\]

In a one-liter 3-neck flask fitted as usual, was placed 50 ml. of absolute ethanol, in it was dissolved 2.3 g. (0.1 mole) of sodium. To the sodium ethoxide solution was added over a period of \( \frac{1}{2} \) hour with stirring, 20.5 g. (0.1 mole) of ethyl nitromalonate and 12.65 g. (0.1 mole) of benzylchloride. The reaction mixture was refluxed on water bath for 3 hours, till it was slightly alkaline to litmus. Alcohol was distilled under reduced pressure and the residue was acidified with cold dilute HCl and then extracted with ether. The ether extract was washed and dried. Ether was evaporated on water bath; and the residual liquid was distilled under reduced pressure. The distillate was collected at 65-67°C/8mm; the yield was 8.5 g. (28.9% theoretically calculated) \( \gamma \text{D} \text{26.5} \text{1.5212. Nitrogen: calculated for C}_{14}\text{H}_{17}\text{O}_{6}\text{N-4.7%, Found 4.75%}.}
MICROBIOLOGICAL ACTIVITY

Dr. Porter's results of preliminary testing of the ethyl ester of p-aminophenylalanine (as hydrochloride) on bacteria have already been described. (vide supra page 18). Through the courtesy of Messrs. Warren Weaver and Joseph P. La Rocca, nine of the compounds prepared during the course of this investigation have been submitted for testing their effect on fungi. The results of these tests, while only of a preliminary nature and as yet unverified, are summarised in Table III. It is surprising that compounds numbered 2, 3 and 4 show complete inhibition on A. niger under the conditions tried. Although it is too early to put too much reliance on these results, they do offer some justifications for undertaking the program of synthesis described in this thesis. These findings encourage the hope that some of the products here described for the first time may prove useful.
TABLE NO III
Fungicidal Activity

Organism: *A. niger*  
Concentration: 1000 parts per million

<table>
<thead>
<tr>
<th>NOS.</th>
<th>Compound</th>
<th>Growth</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hours</td>
<td>Millimeters</td>
</tr>
<tr>
<td>1</td>
<td>$\alpha$-Oximino-$\beta$-(o-nitrophenyl)-propionic acid</td>
<td>169</td>
<td>51</td>
</tr>
<tr>
<td>2</td>
<td>o-Aminophenylactic acid</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>o-Aminophenylalanine</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>$\alpha$-Indolecarboxylic acid</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl $\beta$-indolylglyoxylate</td>
<td>&quot;</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>$\alpha$-Oximino-$\beta$-(p-nitrophenyl)-propionic acid</td>
<td>&quot;</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>$\alpha$-Oximino-$\beta$-(p-nitrophenyl)-propionic acid ethyl ester</td>
<td>92</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>p-Aminophenylactic acid ethyl ester hydrochloride</td>
<td>169</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>p-Aminophenylalanine</td>
<td>&quot;</td>
<td>39</td>
</tr>
</tbody>
</table>

D. A. Control  
Plain Control
DISCUSSION AND SUMMARY OF RESULTS

The objectives of the research here described may be considered
under the following broad headings:

A. The condensation of ethyl oxalate with an active methyl group, e.g.,

\[ \text{X-CH}_3 + \text{COOC}_2\text{H}_5 \rightarrow \text{X-CH}_2\text{-COOC}_2\text{H}_5 \]

It was found that with o- and p-nitrotoluene this reaction proceeded to
the extent of 70 to 80 per cent. Neither of the esters, nor of the cor-
responding acids obtained by hydrolysis of the esters, is new. However,
it was found that ethyl p-nitrophenylpyruvate, contrary to earlier de-
scriptions, is stable over at least half a year if appropriate precautions
are taken in its isolation, as described under "experimental". The method
of isolation of o-nitrophenylpyruvic acid was improved and better yields
were obtained. Ethyl oxalate treated under similar conditions, with
\(\alpha\) - and \(\gamma\)-picoline, showed evidence of reaction, but the corresponding
derivatives of pyruvic acid were not isolated. p-Tolylarsonic acid did not
react with ethyl oxalate.

The condensation of o-nitrotoluene, or o-nitrophenylpyruvic acid,
with ethyl formate or ethyl orthoformate was also attempted. It is ex-
pected that the desired formyl derivative will prove useful intermediate
for the synthesis of indole compounds, e.g.,
It is regretted that the desired aldehydes were not obtained, and it is hoped that further intensive studies of this aspect of the general problem will overcome the difficulties.

B The conversion of the pyruvic acid compounds into the corresponding \( \alpha \)-ketoximino acids, and the reduction of these to the corresponding alanine derivatives, thus:

\[
\begin{align*}
\text{NO}_2\cdot \text{C}_6\text{H}_4\text{CH}_2\text{C-COOH} \ (\text{or C}_2\text{H}_5) & \rightarrow \text{NO}_2\cdot \text{C}_6\text{H}_4\text{CH}_2\text{C-NCOOH} \ (\text{or C}_2\text{H}_5) \\
\text{NH}_2\cdot \text{C}_6\text{H}_4\text{CH}_2\text{C-COOH} \ (\text{or C}_2\text{H}_5) & \rightarrow \text{NH}_2\cdot \text{C}_6\text{H}_4\text{CH}_2\text{C-NH}_2
\end{align*}
\]

By this procedure both the o- and p-aminophenylalanines were prepared.

The o-isomer is described here for the first time; it was obtained as the dihydrochloride. As already mentioned, this compound showed 100 per cent inhibition, under the conditions employed in screening tests, to the growth of \( \text{A. niger} \).

The p-isomer was previously described by Erlenmeyer and Lipp (81), who obtained it via the nitration of phenylalanine, and by Takaoki Sasaki (48), who employed glycine anhydride and p-nitrobenzaldehyde as starting materials. It is believed that the procedure described in this thesis is superior to either of those methods. The method employed here is essentially that described by Mattocks (44). He, however, limited his investigations to the preparation and hydrogenation of the oxime of
the ethyl ester of p-nitrophenylpyruvic acid. In the current studies this work was repeated and extended to the oxime of the free acid as well. That is, the oxime of the ester was hydrolysed to the oxime of the free acid, and the oximino acid was reduced, in the presence of hydrogen chloride, to p-aminophenylalanine, isolated as its dihydrochloride. The oximino acid and the dihydrochloride are here described for the first time.

The conversion of the oximino acids into their corresponding benzyl-oximino acids, according to the procedures of Waters (57) and of Weaver (58) was unsuccessful, in the limited number of experiments undertaken, and the investigation was not pursued further.

C. The synthesis of m-aminophenylalanine:
Since m-nitrotoluene cannot be used, as may its isomers, for the synthesis of an intermediate leading to m-aminophenylalanine, resort was had to other procedures. The most promising approach appeared to be through m-nitrobenzylmalonic acid (or ester). Accordingly the available m-nitrobenzaldehyde was reduced, by means of aluminium isopropanoxide, to the alcohol, which was, in turn, converted into m-nitrobenzyl chloride. By allowing this chloride to react with a large excess of malonic ester, a yield of 57.6 per cent of mono-m-nitrobenzylmalonic ester was obtained.

Berry (51), in his review, reported that o'-oximino acids may be conveniently obtained in good yields from either the mono-substituted malonic acids or their esters. In the current study both m-nitrobenzylmalonic acid and its ester, were treated with nitrosating agent according to the directions given by Barry. But in neither instance did the product prove to be the desired oxime of m-nitrophenylpyruvic acid (or ester). There was reaction, but the products were not identified. That this behavior of m-nitrobenzylmalonic acid is not an isolated phenomenon was
demonstrated by similar results when p-nitrobenzylmalonic acid or ester were similarly treated.

Previous investigators reported that the reaction of p-nitrobenzyl chloride with ethyl sodiomalonate produced predominantly the disubstituted derivative. Mattocks (44) obtained a yield of only 7 per cent of the mono derivative. It has now been found that if 1 mole p-nitrobenzyl chloride is employed for 3 moles ethyl malonate and 1 mole sodium ethoxide, then yields up to 40.6 per cent of mono-p-nitrobenzylmalonic ester are obtained.

In view of these negative results another procedure was tried, namely the reaction of the α-bromo ester with alkali nitrite, a reaction first reported by Lepercq (52,53) and also studied by Hemlin (54). Accordingly the m-nitrobenzylmalonic acid was converted, by steps already described, into ethyl α-bromo-β-(m-nitrophenyl)-propionate. This ester, allowed to stand in ethanolic solution with sodium nitrite, formed a low yield of the desired ester of α-oximino-β-(m-nitrophenyl)-propionic acid. Since the quantity of product was limited only the ester was hydrogenated and the dihydrochloride of m-aminophenylalanine ester obtained. However, no difficulty would be anticipated in converting the intermediate oximino ester into the corresponding oximino acid, and thus, a synthesis of m-aminophenylalanine appears possible.

In the course this study on m-aminophenylalanine the following compounds were prepared for the first time;

α-β-Bromo-(m-nitrophenyl)-propionic acid ethyl ester.
α-β-Oximino-(m-nitrophenyl)-propionic acid ethyl ester.
m-Aminophenylalanine ethyl ester dihydrochloride.
D  The preparation of aminophenylactic acids (or esters):
The mere availability of the appropriate intermediates suggested their
collection into \(-\) and \(p\)-aminophenylactic acids.

\[
\begin{align*}
\text{X-CO(OH)}(\text{or C}_2\text{H}_5) & \quad \rightarrow \quad \text{X-COOH(or C}_2\text{H}_5) \\
\end{align*}
\]

By this procedure \(-\) and \(p\)-aminophenylactic acids and \(p\)-aminophenylactic ethyl ester were prepared. The \(\alpha\)-isomer and the ethyl ester of
the \(p\)-isomer are not been reported in the available literature. They
were isolated as hydrochloride. \(\alpha\)-Aminophenylactic acid showed 100 per cent inhibition, while the ethyl ester of the \(p\)-isomer showed 3 per cent
inhibition to the growth of \(A.\ niger\).

It was anticipated that \(\alpha\)-nitrophenylpyruvic acid if shaken
in neutral solution in an atmosphere of hydrogen with the catalyst,
would cyclise and give \(\alpha\)-(indolecarboxylic acid).

This result is consistent with the observations of previous workers
\((50, 59, 66, 68, 69, 70)\), who reduced \(\alpha\)-nitrophenylpyruvic acid with
ammonia and ferrous sulfate, and sodium hydrosulfite in aqueous sodi­
um hydroxide solution in the usual manner. \(\alpha\)-(Indolecarboxylic acid
showed 100 per cent inhibition to the growth of \(A.\ niger\).

In order to obtain \(\alpha\)-aminophenylactic acid it was necessary
to avoid the spontaneous cyclization into the indole heterocycle.
This was accomplished by carrying out the hydrogenation in the
presence of inorganic acid. This suggests that if the aromatic amin
group is converted into the ammonium salt as it forms, it is no longer available for anil reaction which precedes the formation of the indole nucleus.

**Indole derivatives:**

As mentioned above, one aim was to prepare the compound of structure I,

![Structure I](image1)

and a hypothetically desirable intermediate for the synthesis of β-indolylglycine, II, a lower homolog of tryptophan. Compound I was not obtained, however, by the methods tried. Hence a new route to compound II was sought through ethyl β-indolylglyoxalate, as follows:

![Chemical Reaction](image2)
Indole was obtained in yields up to 63 per cent by decarboxylation of L-indolecarboxylic acid, whose preparation is discussed under "experimental". The chemical reduction employed by Rissert (50,67) is recommended by Tyson (96) for the preparation of indole; but now the catalytic reduction method employed in this investigation may be better and more convenient; and the overall yield of indole is 32 per cent. The reaction of indole with ethyl oxalate was described by Elkes, Ellicot and Hems (59); their results were substantially duplicated. The attempts to convert this α-keto ester into the α-oximino ester were unsuccessful, and the lack of sufficient material did not permit adequate study of the desired oximation reaction. It was possible, however, by means of Adams's platinum oxide catalyst, to reduce the ketonic ester to the corresponding ethyl β-indolyglycolate; which is reported here for the first time.

Ethyl nitromalonate as an intermediate:

From a single experiment, in which benzyl chloride was allowed to react with ethyl nitromalonate in the presence of sodium ethoxide, the product was probably impure benzlnitromalonic ester. This type of reaction is analogous to the alkylation of nitrocetic acid, described by Lyttle and Weisb lut (97), and therefore merits further study.
BIBLIOGRAPHY

2. Stamps, Lancet 2, 10 (1939).
   67, 290 (1945).
34. DuVigneaud, McKennis, Simmonds, Dittmer and Brown, J. Biol.
    Chem. 159, 385 (1945).
35. Dittmer, McKennis and DuVigneaud, Ibid. 164, 761 (1946).
43. Womack and Rose, J. Biol. Chem. 107, 449 (1934).
44. Mattocks, Ph. D. Thesis, University of Maryland, (1945)
45. Dr. Porter, Private communication.
48. Takeoki Sasaki, Ber. 54B, 163 (1921).
49. Hidenosuke Ueda, Ber. 61B, 146 (1928).
50. Rissert, Ibid. 30, 1030 (1897).
56. Janny, Ber. 16, 170 (1883).
60. Meyer and Belle, Ann. 403, 1188 (1914).
62. Shemin and Herbst, Ibid. 60, 1951 (1938).
63. Rissert, Ber. 41, 3813 (1908).
66. Fischer, Ann. 236, 142 (1866).
67. Rissert and Heller, Ber. 37, 4364 (1904).
72. Lippmann and Hawliczeck, Ber. 9, 1463 (1876).
74. Becker, Ber. 15, 2090 (1882).
79. Grabiel and Steudemann, Ber. 15, 846 (1882).
80. Zelinsky, Ibid. 20, 2026 (1887).
81. Erlenmeyer and Lipp, Ber. 15, 1544 (1882).
82. Friedlander, Ann. 229, 227 (1885).
83. Wislicenus and Schultz, Ibid. 436, 45 (1924).
84. Iwamoto and Hartung, J. Org. Chem. 9, 514 (1944).
85. Lellman and Schleich, Ber. 20, 435 (1887).
86. Rissert, Ibid. 22, 635 (1896).
87. Wislicenus, Ibid. 20, 2930 (1887).
88. Pechmann, Ibid. 25, 1047 (1892).
91. Kloblie, Rec. trav. chim. 8, 283 (1889).
92. Retz, Monatsh 25, 60 (1904).
93. Willstatter and Hottenroth, Ber. 37, 1775 (1904).
94. Canonica, Gazz. chim. ital. 77, 92 (1947); C.A. 42, 1885 (1948).