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A STUDY OF THE ESTERS
OF
L-TYROSINE AND ITS DERIVATIVES

A dissertation submitted to the
Graduate School of Arts and Sciences
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by

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The author wants to express his sincere gratitude to Dr. Hoke S. Greene for his counsel and guidance during this work. The author also wishes to denote his gratitude to Dr. I. R. MacGregor for the many impromptu discussions which proved so valuable.

DEDICATION

This thesis is affectionately dedicated to my Wife, Dorothy, for her unremitting assistance, constant understanding, ceaseless inspiration and sustaining spiritual strength, all of which contributed immeasurably to the completion of this prolonged task.

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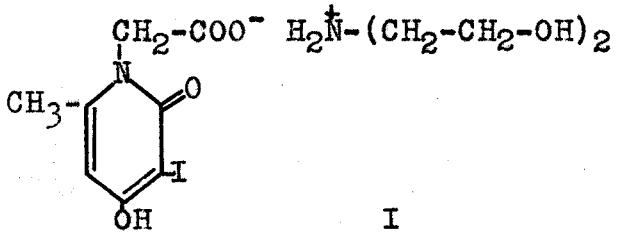
HISTORICAL

I. Iodinated Organic Compounds as X-ray Contrast Media.

The use of X-rays as a means of diagnosing disorders of the body had its inception very shortly after their discovery by Roentgen in 1895. In order to increase the usefulness of this tool in exploring the various organs, cavities and hidden recesses, it was recognized quite early that such parts of the body should be made more opaque to X-rays in order that they cast a deeply contrasting shadow. Various substances have been introduced into the body and their opacity to X-rays determined. One of the first materials used to opacify the gastro-intestinal tract was bismuth, introduced in 1903. At present, a barium sulfate suspension is the accepted material for this purpose.

Binz in 1935 (1) first discovered that iodinated organic compounds could serve as diagnostic agents for X-ray visualization. One of the iodinated pyridone derivatives which he studied is still one of the most successful radiopaques (Diodrast, III). As more iodinated compounds were synthesized and tested, the requirements for a satisfactory radiopaque compound became more clearly defined. Among such requirements are the following (2): 1), ability to produce adequate opaqueness; 2), rapid excretion; 3), lack of systemic absorption; and 4), low toxicity. A fifth desirable trait could be listed as high water solubility, if the compound is to be administered orally.

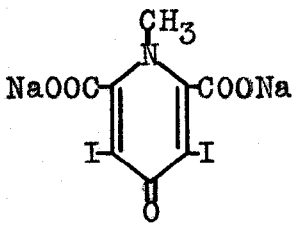
In order to cast deeply contrasting shadows, a compound must have a high percentage of iodine, preferably over 50%. Since at least two iodine atoms are usually required to achieve this percentage, the molecular weight of the iodinated compounds is generally above 300. Such high molecular weight materials cannot be expected to have a high solubility in neutral aqueous media; and yet the roentgenologist prefers a solubility of at least 40%. The alkali salts of iodinated organic acids in many cases do not approach such a solubility. One of the most significant contributions to this field was made by Schinder (3), who showed that the iodinated acids could be rendered much more water soluble by using diethanolamine to form the salt. In this way the compound known as Rayopake (I) was prepared and is a very valuable agent for the observation of the urinary bladder or urethra (Cystourethrography).



I

In addition to their inherent opacity to X-rays, some of the iodinated compounds possess a selective affinity for certain organs and tissues. By proper choice, a high concentration of the contrasting agent can be obtained in the organ or tissue under examination. One of the most successful

applications of such a principle is in the X-ray examination of the kidneys and ureters (Urography) (4). Among the more successful agents for this purpose are the salt of 1-methyl-3, 5-diiodochelidamic acid (Neo-Ipax, Uroselectan B, II), diethanolammonium 3,5-diiodo-4-pyridone-1-acetate (Diodrast, III), sodium iodomethanesulfonate (Skiodan, IV), sodium o-iodohippurate (Hippuran, V), and sodium 3-acetamido-2,4,6-triiodobenzoate (Urokon, VI).



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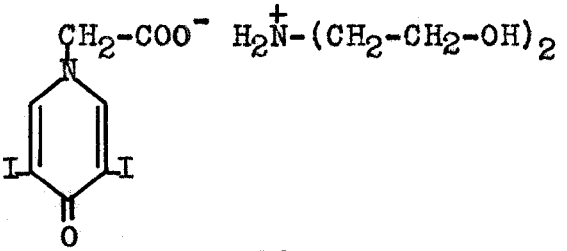

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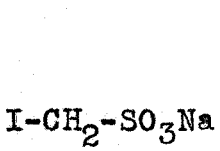

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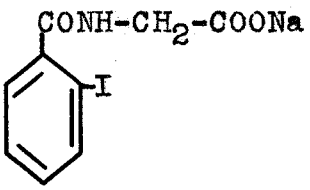

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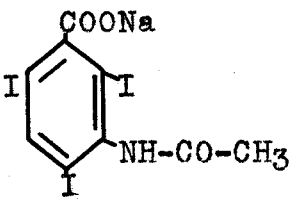

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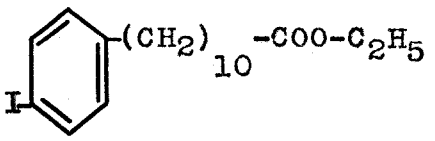
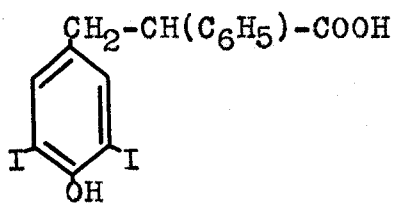
One of the well tolerated aromatic derivatives is ethyl 11-(p-iodophenyl) undecanoate (Pantopaque, VII), which is extensively used for the visualization of the spinal cord and subarachnoid spaces (Myelography).



VII

Several of the above mentioned agents have been used in the visualization of the lungs and gall bladder. However, in the examination of the gall bladder (Cholecystography) other substances that can be administered orally are preferred: α-phenyl-β-(3,5-diiodo-4-hydroxyphenyl)-propionic acid (Priodax, VIII) (5); α-ethyl-β-(2,4,6-triiodo-3-aminophenyl) propionic acid (Telepaque, IX) (6); 2-(3,5-diiodo-4-hydroxybenzyl) cyclohexanecarboxylic acid (Monophen, X) (7); and the sodium salt of tetraiodophenolphthalein (Iodikon, XI).



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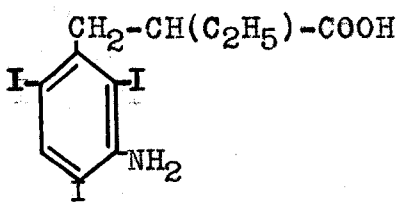

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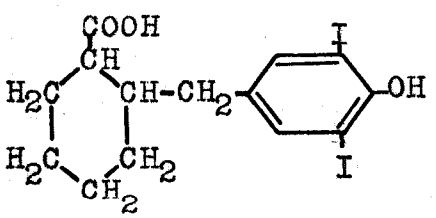

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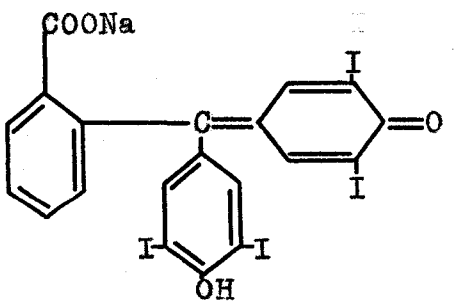


XI

Many additional iodinated compounds have been synthesized and tested in recent years; Archer and coworkers (8,9,10,11,12,13) and Papa, Schwenk and coworkers (14,15,16,17,18) have been particularly active in this field.

II. The use of silver salts in the synthesis of esters.

Esters are probably the most important derivatives of an organic acid. They are most conveniently prepared by the direct interaction of an alcohol and acid in the presence of a catalytic amount of a mineral acid. This procedure was introduced by Emil Fischer in 1895 (19) and consequently is known as the Fischer method of esterification. However, long before Fischer introduced this simple and effective procedure, esters were quite well known. The majority of such compounds had been prepared by the so-called silver-salt method; this method involved the interaction at an elevated temperature of an alkyl halide (usually the iodide) and the silver salt of the acid whose ester was desired. After a sufficient reaction time, the silver halide was removed by filtration and the ester obtained by distillation. Friedel (20) was one of the first to report the use of this method. He prepared iso-propyl acetate from iso-propyl iodide and silver acetate. In 1871, Lieben and Rossi (21) reported the preparation of n-butyl acetate by reacting silver acetate with n-butyl halides; when n-butyl iodide and the silver salt were heated at 108° for 6.5 hours, they obtained a 93.5% yield of the ester; and when n-butyl

bromide and the silver salt were heated at 100-130° for 20 hours, the yield was 87.5%. Linnemann also in 1871 (22) reported the use of the silver-salt method to prepare the ethyl esters of benzoic, acetic, propionic, and n-butyric acids in yields of 74%, 85%, 91% and 43%, respectively. Gartenmeister (23) made a study of the boiling points and specific volumes of 26 esters of the normal fatty acids; he prepared the esters by the silver-salt method. Eckstrand (24) was able to adapt the method to preparation of the solid esters of substituted naphthoic acids. For example, he synthesized ethyl 5-chloro-1-naphthoate by heating together in a sealed tube the silver salt of 5-chloro-1-naphthoic acid and ethyl iodide in a boiling water bath.

With the introduction of the Fischer method, the importance of the silver-salt method decreased; it is at present used rather infrequently. This is mainly due to two reasons: the silver-salt method is a time consuming process, very often requiring 24 hours or longer to obtain a satisfactory yield of ester; and the silver salts and alkyl iodides are rather expensive chemicals. However, there are at least two circumstances where this method will give a satisfactory yield of the ester when the Fischer method fails. The yields of methyl esters obtained from 2,6-dibromobenzoic acid, 2,6-dinitrobenzoic acid, and 2,4,6-trimethylbenzoic acid by the Fischer method are practically nil. However, the three esters can be obtained by the silver-salt method (25, 26). Likewise, the reaction of the silver salt of 2,4,6-trihydroxybenzoic acid and methyl

iodide resulted in a nearly quantitative yield of the methyl ester (27). The reaction of a silver salt with an alkyl halide takes place at the oxygen atom of the carboxyl group, whereas the reaction of the free acid and alcohol in the presence of an acid catalyst occurs at the carboxyl carbon atom. The silver-salt method yields the desired ester where a steric effect operates against the Fischer method.

The Fischer method also fails whenever there is the possibility of an acid catalyzed isomerization. When 3-methyl-3-butenoic acid was directly esterified with methyl alcohol, there was obtained methyl 3-methyl-2-butenoate.

$$CH_2{=}\underset{\displaystyle CH_3}{\overset{|}{C}}{-}CH_2{-}COOH + CH_3OH \xrightarrow{H} CH_3{-}\underset{\displaystyle CH_3}{\overset{|}{C}}{=}CH_2{-}COOCH_3$$

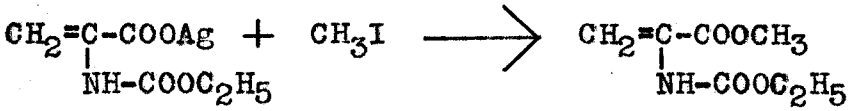
However, the interaction of the silver salt of 3-methyl-3-butenoic acid and methyl iodide for 72 hours gave a 47% yield of methyl 3-methyl-3-butenoate (28).

$$CH_2{=}\underset{\displaystyle CH_3}{\overset{|}{C}}{-}CH_2{-}COOAg + CH_3I \longrightarrow CH_2{=}\underset{\displaystyle CH_3}{\overset{|}{C}}{-}CH_2{-}COOCH_3$$

Jeffrey and Vogel (29) likewise used the silver-salt method to prepare a number of esters of the unsaturated acids, vinylacetic, maleic, fumaric and crotonic.

Dickey and Coover (30) have employed the silver-salt method to prepare esters of unsaturated acids which are capable

of easy polymerization. For example, the interaction of the silver salt of α-carbethoxyamino acrylic acid and methyl iodide resulted in a 23% yield of the corresponding methyl ester.

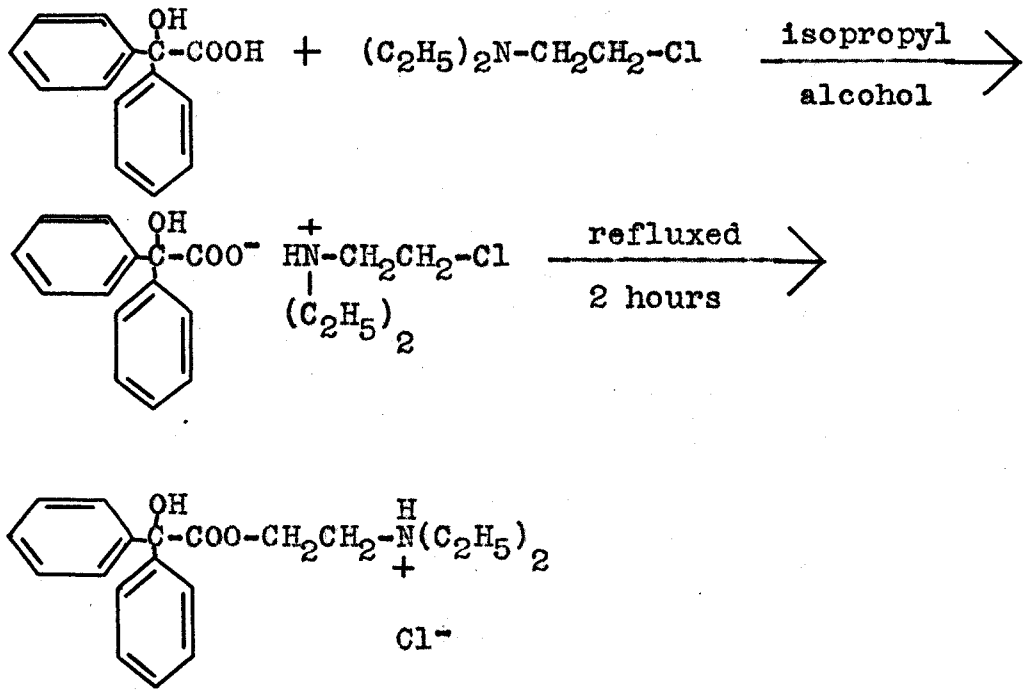


In like manner, the silver salt of the corresponding isopropyl derivative was converted into its methyl ester in a 31% yield. Hauser, Saperstein and Shivers (31) refluxed silver benzoate and triphenylmethyl chloride in benzene for six hours to give a 50% yield of triphenylmethyl benzoate. Fieser and Heymann (32) reacted silver acetate and 1,3-dimethyl-10-bromoanthrone-9 in the presence of acetic acid to give a 76% yield of the corresponding acetoxy derivative.

III. The direct synthesis of basic esters from carboxylic acids and chloroalkyl amines.

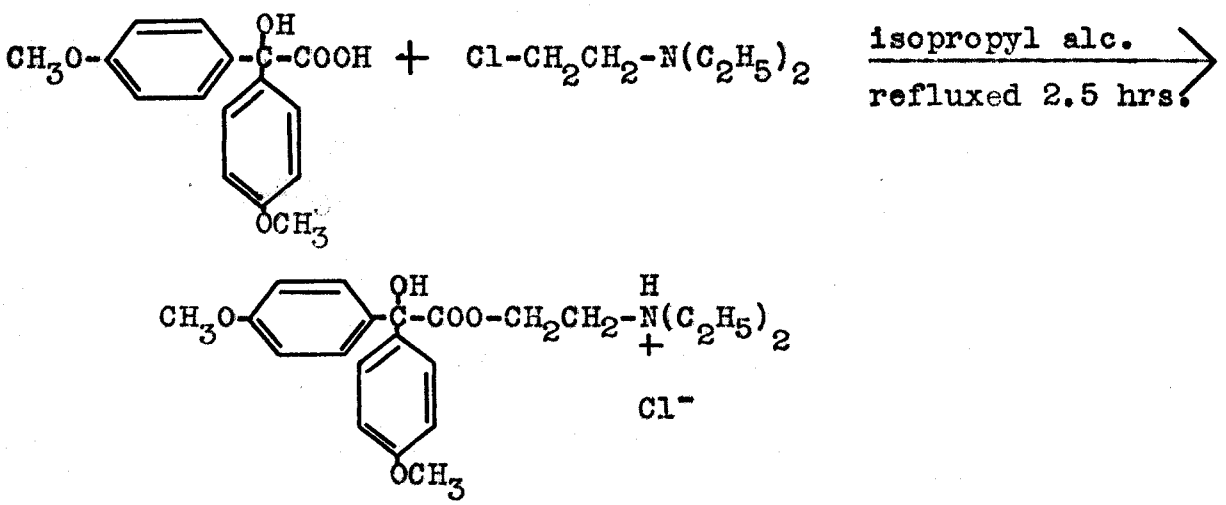
A carboxylic acid can usually be converted into the ester of a dialkylamino alcohol by reacting the carboxylic acid chloride with the amino alcohol. However, this method is not successful where the acid chloride is unstable or because of the presence of reactive functional groups, as in benzilic and tropic acids. Horenstein and Pahlicke (33) were the first to employ a rather novel method for the preparation

of such basic esters. For example, they dissolved 0.05 mole of benzilic acid in 40 cc. of dry isopropyl alcohol and neutralized the acid with 0.05 mole of β-diethylaminoethyl chloride. The resulting solution was refluxed for two hours; on cooling, there was deposited β-diethylaminoethyl benzilate hydrochloride, which amounted to an 82.7% yield after recrystallization from isopropyl alcohol.



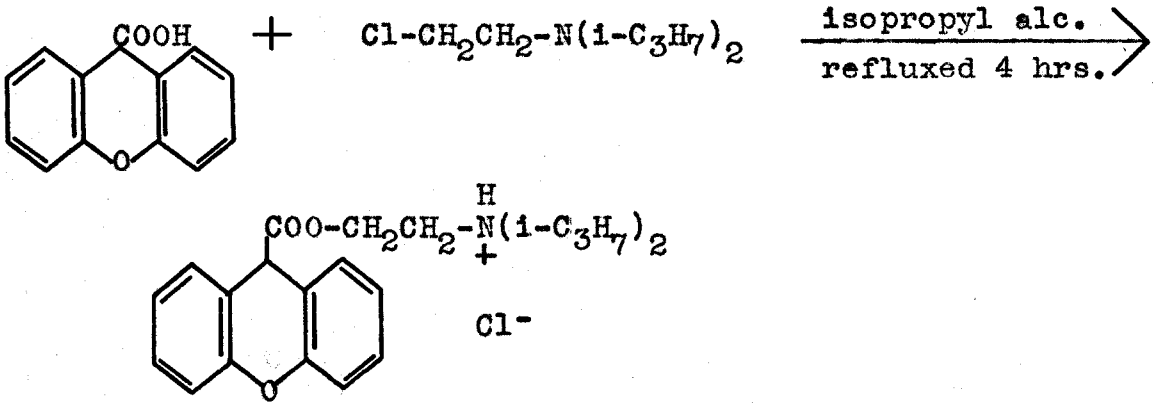
As can be readily recognized, this process involves two consecutive steps: the conversion of the acid to its anion, followed by a nucleophilic displacement of the chlorine atom by this anion, giving the ester hydrochloride directly. Horenstein and Pahlicke also prepared the β-diethylaminoethyl esters of salicylic and mandelic acids by a similar procedure.

Burtner and Cusic employed this modification in the preparation of numerous basic esters which were evaluated for apasmolytic action. They prepared 39 basic esters of various arylacetic acids (34) by one of two methods: the conventional reaction of the acid chloride with the dialkylamino alcohol, and the method of Horenstein and Pahlicke. They reported that this latter method appeared to be quite generally applicable and that the yields were high and the products satisfactorily pure. They also prepared 21 basic esters of polynuclear carboxylic acids by this latter method (35). They reported a typical synthesis as follows: 0.062 mole of anisilic acid and 0.062 mole of β-diethylaminoethyl chloride were refluxed in 60 cc. of isopropyl alcohol for 2.5 hours. On cooling, there was deposited 21.5 g. (81.5%) of pure β-diethylaminoethyl anisilate hydrochloride.



Robinson and Cusic (36) prepared 34 basic esters of 9-xanthene carboxylic acid and 9 basic esters of tropic acid

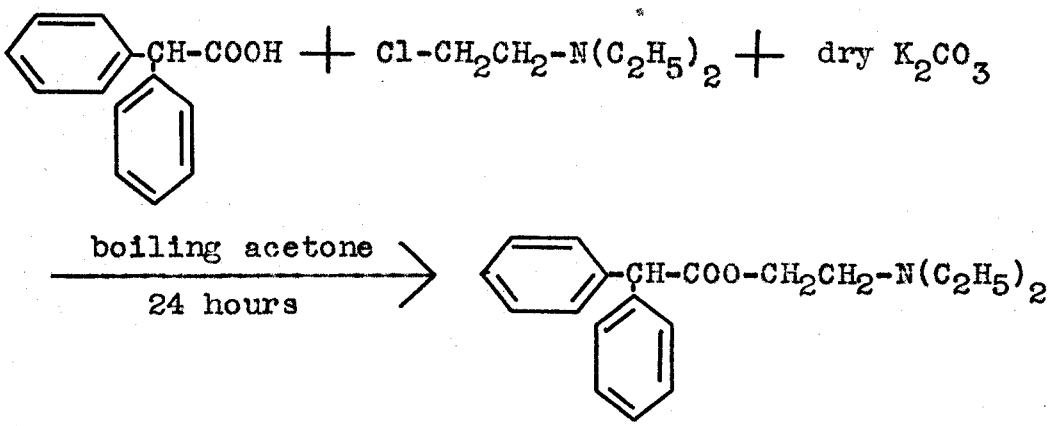
by this method; such esters were evaluated as sympathetic ganglion blocking agents and for neurotropic spasmolytic action. They reported an 84% yield of β-diisopropylaminoethyl-9-xanthene carboxylate by refluxing equimolar amounts of 9-xanthene carboxylic acid and β-diisopropylaminoethyl chloride in isopropyl alcohol for four hours.



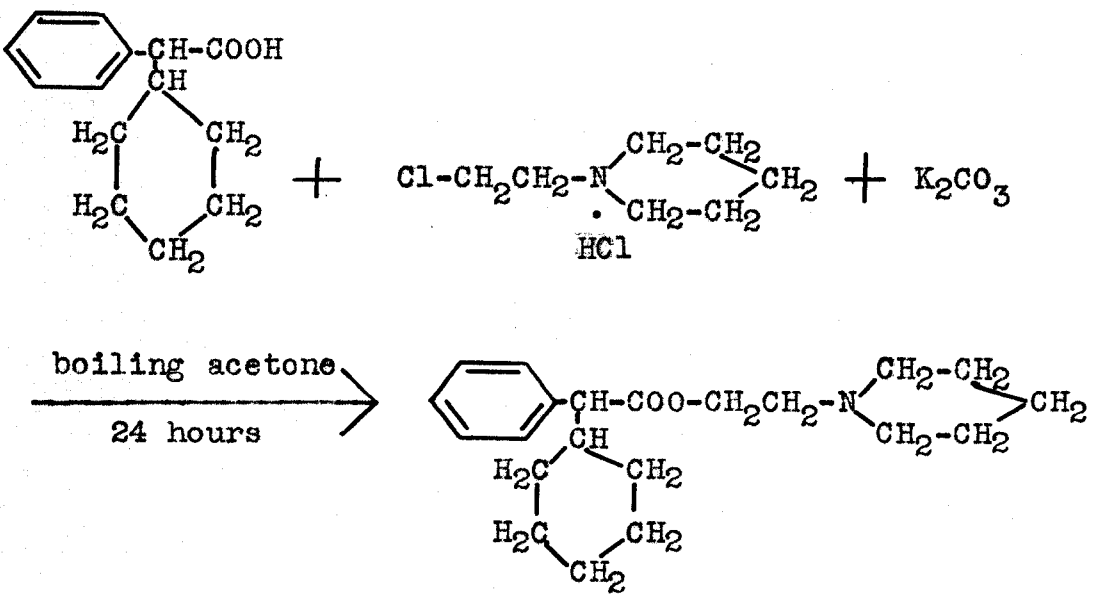
Two rather recent patents (37) used this method for the preparation of a number of basic esters. An 85% yield of β-diethylaminoethyl-α-(1-naphthyl) butyrate was obtained by refluxing for 14 hours in isopropyl alcohol equimolar quantities of β-diethylaminoethyl chloride and α-(1-naphthyl) butyric acid. Use of γ-diethylaminopropyl chloride gave the corresponding γ-diethylaminopropyl ester.

Burtner (38) has employed this method for the synthesis of some basic esters of substituted benzoyl aliphatic acids. For example, the β-diethylaminoethyl ester of β-(2-methoxy-5-cyclohexylbenzoyl) propionic acid was prepared from the chloroalkyl amine and the acid in isopropyl alcohol.

There have been reported several modifications of the original method of Horenstein and Pahlicke. These have involved the use of the alkali salt of the acid in place of the free acid. Miescher, Hoffmann and Panizzon (39) reacted diphenylacetic acid, β-diethylaminoethyl chloride and dry potassium carbonate in dry acetone for 24 hours. After removing the precipitated potassium chloride by filtration, the addition of dry hydrogen chloride gave the pure ester hydrochloride.

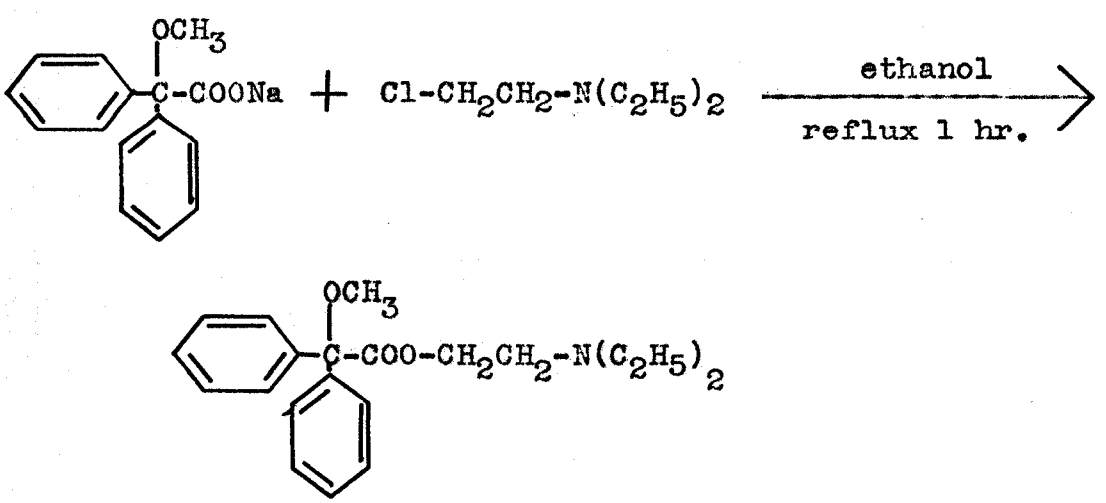


In a similar fashion (40) there was obtained a nearly quantitative yield of aminoester when α-cyclohexyl-phenylacetic acid, β-(1-piperidyl) ethyl chloride hydrochloride, and potassium carbonate were refluxed in dry acetone for 24 hours.



Geigy (41) reacted α,α-dipropylpropionic acid and β-diethylaminoethyl chloride in the presence of potassium hydroxide and ethyl acetate to obtain the amino ester.

Wander (42) added the methyl ether of benzilic acid to a solution of sodium ethoxide in absolute ethanol to obtain the sodium salt of the acid. The addition of an equivalent molar amount of β-diethylaminoethyl chloride, followed by a reflux of one hour, gave the ester.



DISCUSSION OF RESULTS

I. Esters of 3.5-diiodo-l-tyrosine.

Since iodinated organic compounds containing more than 50% iodine have a potential use as radiographic diagnostic agents, a study of the synthesis of some simple esters of 3,5-diiodo-l-tyrosine was undertaken. 3,5-diiodo-l-tyrosine itself contains 58.6% iodine; but it is too rapidly absorbed by the body to be of any practical use as a diagnostic agent. Snapper and Grunbaum (43) have shown that inorganic iodide ion can be demonstrated in the saliva and urine 50 minutes after oral or subcutaneous administration of 3,5-diiodo-l-tyrosine. This is about as rapidly as iodide ion appears in these two body excretions after the ingestion of sodium or potassium iodide. However, the N-benzoyl derivative appeared unaltered in the urine under the same conditions. This rapid elimination of the iodine atoms of 3,5,-diiodo-l-tyrosine by the body prevents its use for diagnosis purposes: the opacity due to the iodine atoms is too rapidly lost for practical purposes; and the rapid increase in body iodine could result in iodism.

The first step towards the elimination of an ester from an animal body is its hydrolysis by means of enzymes, the esterases. By converting 3,5-diiodo-l-tyrosine into a simple ester, it was hoped that this preliminary hydrolysis would slow down the over-all rate at which iodide ions are

liberated into the body, prolonging the usefulness of the iodinated esters as radiopaques, as well as lowering toxicity due to excess iodide ion.

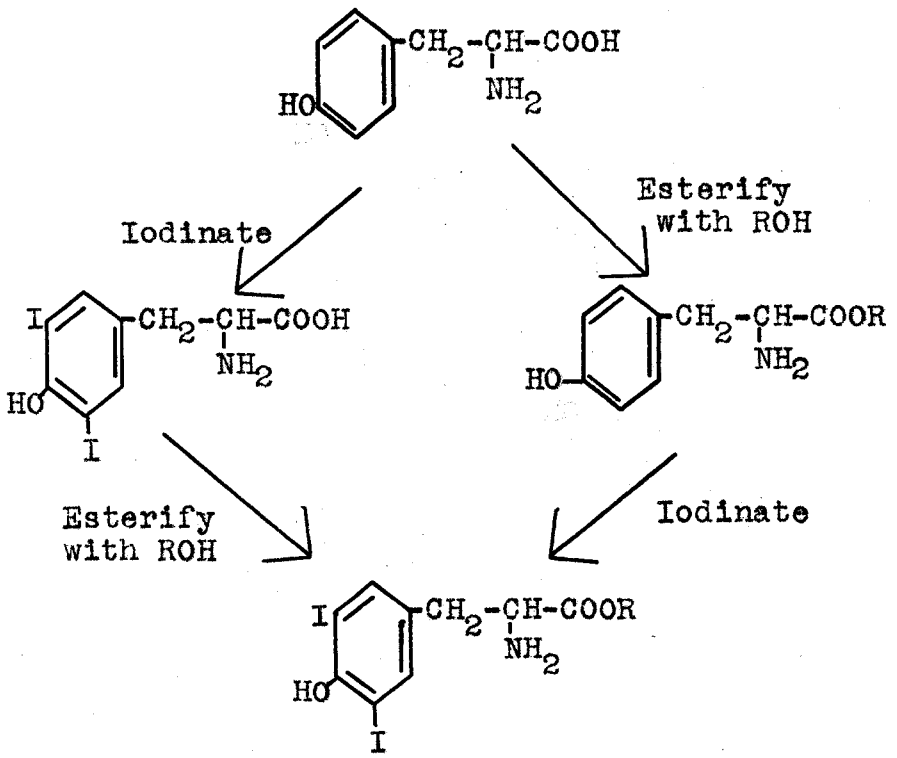
There are at the present time a number of iodinated organic compounds in use as diagnostic agents. Some of them require a rather complicated and prolonged synthetic sequence. l-Tyrosine is a naturally occurring amino acid, readily available from casein. Its conversion to the desired iodinated ester would be merely a two step process.

A. Possible synthetic methods.

There are two possible methods for the preparation of the simple esters of 3,5-diiodo-l-tyrosine.

Method I: Iodinate l-tyrosine to 3,5-diiodo-l-tyrosine and then esterify.

Method II: Esterify l-tyrosine and then iodinate to the 3,5-diiodo-l-tyrosine derivative.



Method I Method II

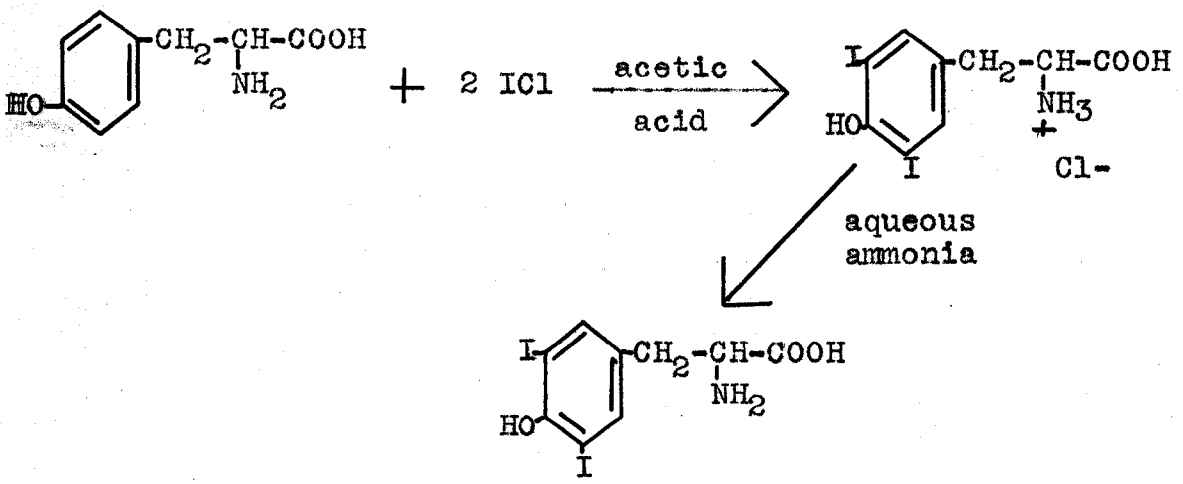
Bauer and Strauss (44) have reported the synthesis of the methyl and ethyl esters of 3,5-diiodo-l-tyrosine. They prepared l-tyrosine methyl ester by direct esterification; reaction with iodine in alkaline solution gave the desired 3,5-diiodo-l-tyrosine methyl ester in 39% yield (iodination step only). Similarly, the ethyl ester was prepared in 78% yield. These syntheses are examples of Method II above.

Obviously, if a number of esters are desired, Method I would be preferred. The direct esterification of an amino acid is best done by the Fischer method (45), which dissolves the amino acid in the alcohol by means of an excess of dry hydrogen chloride. Abderhalden and Guggenheim (46)

first reported the synthesis of 3,5-diiodo-l-tyrosine methyl ester by this method, without reporting a yield. Dickinson and Trikojus (47) made a study of the stability of the iodine atoms in 3,5-diiodo-l-tyrosine at various hydrogen ion concentrations. They found that at a pH of 10.50, there was a 12.9-14.2% decomposition in one hour at an average temperature of 82°; at a pH of 8.85, a 19.0-25.1% decomposition; and at a pH of 6.50, a 96.0-100.0% decomposition. Harington and Rivers (48) likewise observed that 3,5-diiodo-l-tyrosine was extremely unstable to heat between pH 4 and 9. Since Method I would prepare the esters in strongly acid media, it was decided to critically examine this method.

B. Preparation of 3,5,-diiodo-l-tyrosine.

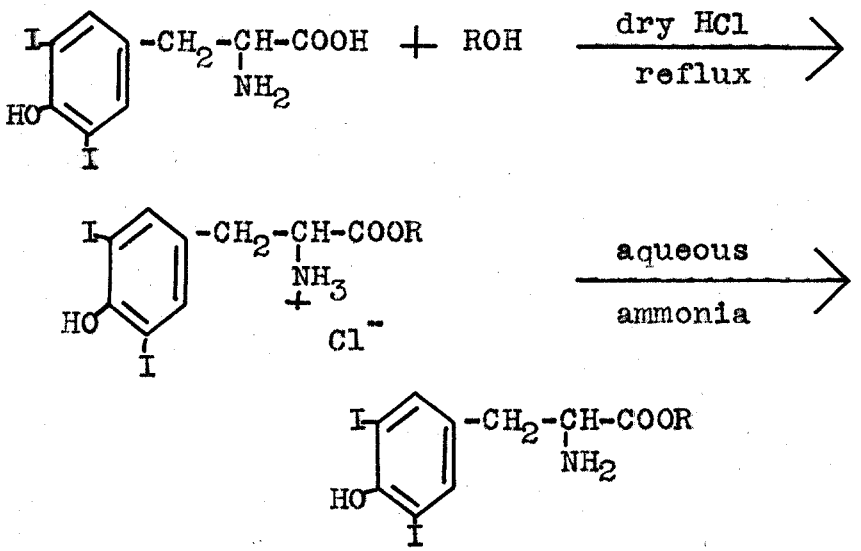
3,5-diiodo-l-tyrosine has been prepared by a number of modifications of two main methods. One of these is the direct interaction of iodine and l-tyrosine in an alkaline medium. Savitskii (49) reported an 88% yield by this procedure. The second method utilizes iodine monochloride in an acid medium. Block and Powell (50) have reported an 80-85% yield of pure white product by using iodine monochloride in glacial acetic acid.



Due to its simplicity, the method of Block and Powell was used in the preparation of the 3,5-diiodo-l-tyrosine required in this work.

C. Prepared esters.

Seven simple esters of 3,5-diiodo-l-tyrosine were prepared by the Fischer method, according to the following scheme:



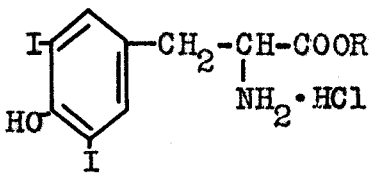
The intermediate, crude ester hydrochlorides were directly neutralized to yield the crude free ester. With alcohols whose boiling points were above 100°, discoloration due to the liberation of iodine occurred if the reaction was carried out at the boiling point. However, in all cases, a colorless ester was readily obtained on recrystallization.

Table I lists the various ester hydrochlorides prepared, percent yield of the crude material, recrystallizing solvent, percent yield of purified material, decomposition temperature when heated at the rate of 10° per minute, and the chloride analysis.

Table II gives a list of the corresponding esters obtained by neutralizing an aqueous methyl alcohol solution of the crude ester hydrochloride with aqueous ammonia. Also included are the percent yields of the crude esters, recrystallizing solvents, percent yield of the purified ester, overall percent yield from 3,5-diiodo-l-tyrosine (DIT), decomposition temperature when heated at the rate of 10° per minute, and the iodine analysis.

TABLE I

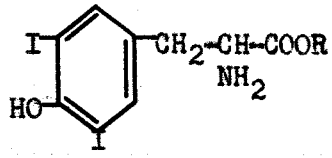
Ester Hydrochlorides of 3,5-Diiodo-l-Tyrosine



R	% yield crude	Recryst. solvent	% yield purified from crude	Decompn. point @ 10°/min.	% Cl Theory	% Cl Found
CH_3	89.7	2 N HCl	72	202-203°	7.34	7.35
C_2H_5	81.0	1.5 N HCl	73.5	212-214°	7.13	7.11
n-C_3H_7	79.3	1 N HCl	84	211-213°	6.93	6.89
i-C_3H_7	81.3	0.25N HCl	78.3	226-228°	6.93	6.90
n-C_4H_9	67.2	0.25N HCl	73.5	188-190°	6.74	6.70
i-C_4H_9	76.2	0.25N HCl	77.2	205-206°	6.74	6.75
n-C_5H_{11}	74.9	0.25N HCl	78.3	190-191°	6.57	6.53

TABLE II

Esters of 3,5-Diiodo-l-Tyrosine



R	% Yield Crude	Recryst. Solvent	% Yield Purified From Crude	Overall % Yield From DIT	Decompn. Point @ 10°/min.	% I Theory	% I Found
CH_3	96.8	aqueous methyl cellosolve	72	64.5	187-188°	56.75	56.92
C_2H_5	96.7	methyl cellosolve	85	66.5	174.5-175.5°	55.01	54.92
n-C_3H_7	91.7	ethylene dichloride	90	65.5	151-152°	53.39	53.69
i-C_3H_7	79.8	ethanol	76	49.5	156-157°	53.39	53.40
n-C_4H_9	95.3	ethanol	95	60.7	139-141°	51.94	51.76
i-C_4H_9	82.3	ethanol	90.5	56.6	151-153°	51.94	51.94
n-C_5H_{11}	85.5	methyl cellosolve	82.5	52.8	113-116°	50.40	50.52

II. Unsuccessful attempts to prepare a β-amino ester of an α-amino acid.

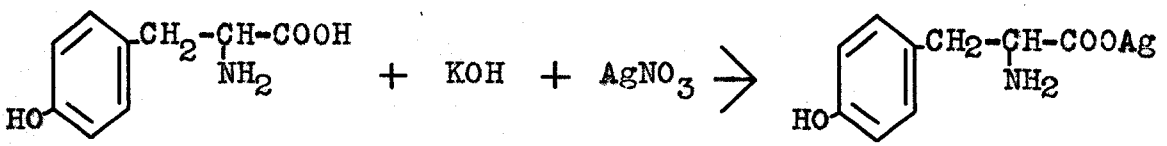
One part of this thesis was the preparation of such amino alkyl esters of l-tyrosine as might be expected to have local anesthetic properties. The enormous amount of work which has been done in the field of local anesthetics has fairly well defined the anesthesiophore group as consisting of an aromatic acid esterified with a tertiary amino alcohol (51). The extensively used Procaine is the most important example of a synthetic local anesthetic containing a β-amino ester group. Consequently, the purpose of this part of the thesis was to synthesize β-amino esters of l-tyrosine. Hopefully, such molecules should have local anesthetic properties due to the β-amino ester moiety, and low toxicity due to the l-tyrosine portion, a naturally occurring α-amino acid.

As originally proposed, such syntheses were to be performed by reacting the silver salt of l-tyrosine with an amino bromide dissolved in an inert solvent. However, this method was unsuccessful in the present case. Seven other methods were also studied. Five of these methods proved to be valueless as a means of obtaining a β-amino ester of l-tyrosine. A sixth showed some promise but was not studied sufficiently to determine its efficacy for the synthesis of a β-amino ester. However, the seventh additional method was successful, with β-diethylaminoethyl-l-tyrosinate being obtained in good yield.

A. The silver salt method.

1. Use of an α-amino acid.

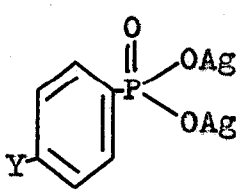
The silver salt of l-tyrosine was prepared according to the procedure in Organic Syntheses (52).



The pure white product analyzed 35.20% silver (theory---37.45%); this discrepancy was due to unreacted l-tyrosine. This slightly impure silver salt was employed as such.

A few trial runs were done using this silver salt and β-diethylaminoethyl bromide. The insoluble silver salt and an isopropyl ether solution of the bromoalkyl bromide were stirred for 24 hours at 40-50°. The mixture darkened considerably. On filtering, there was obtained a reddish-brown filtrate, which failed to yield any product.

Bost, Quin and Roe have reported the use of the silver salt method to prepare some amino esters of aromatic phosphonic acids (53). Using 0.03 mole of silver phosphonates of the general structure



0.06 mole of freshly distilled β-dimethylaminoethyl chloride and 100 cc. of anhydrous ethanol, they stirred the slurry at

50° for 6 hours. The amino esters were recovered in yields of 30-40%. This suggested the use of absolute ethanol as a solvent. Consequently, silver-l-tyrosinate, freshly distilled β-diethylaminoethyl chloride, and absolute ethanol were stirred at 50° for 6 hours. The initially colorless slurry became dark brown; no product could be recovered from the reddish-brown filtrate.

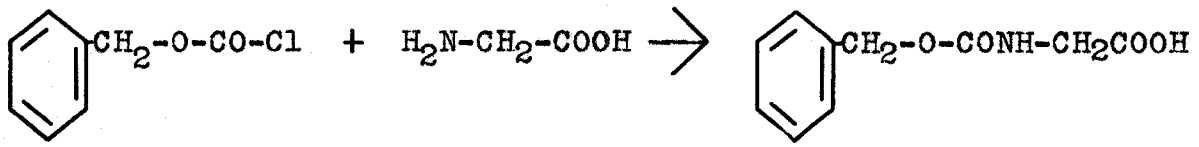
The discoloration of the reaction mass was considered as due to the phenolic group in silver-l-tyrosinate; and the apparent lack of reactivity of the silver could conceivably be due to complexes with the primary amino group in the silver salt. Consequently, it was decided to attempt the synthesis of the well-known ethyl ester of l-tyrosine by the silver salt method. This ester is easily prepared by the Fischer method of esterification and can be readily recrystallized from ethyl acetate to yield colorless crystals, m.p. 108-9°. In order to use the most favorable conditions for ester formation, a solution of redistilled ethyl iodide in purified methyl isobutyl ketone was reacted with insoluble silver-l-tyrosinate; the slurry was stirred in a Morton flask in subdued light at 35° for 19 hours. The resultant yellow colored slurry was filtered. Analysis of the residue for soluble silver ion showed that only 28% of the silver salt had been converted into insoluble silver iodide. Since ethyl-l-tyrosinate is quite soluble in methyl isobutyl ketone, any such product

should be in the filtrate. However, concentration of the filtrate *in vacuo*, with the eventual use of ethyl acetate as a crystallizing solvent, failed to yield any ethyl ester of l-tyrosine.

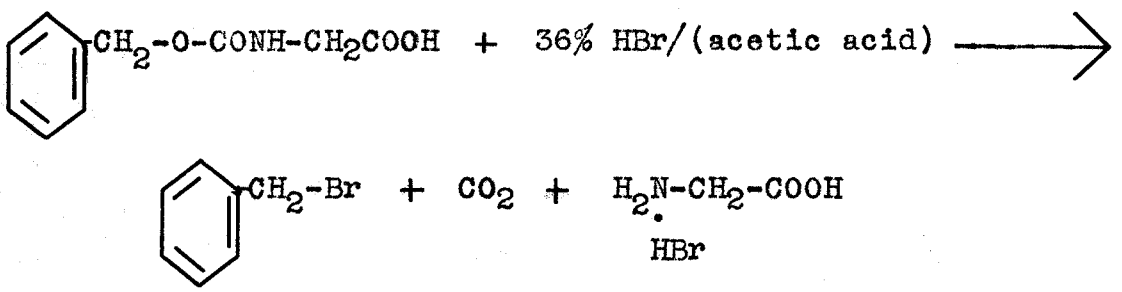
2. Use of a "protected" α-amino acid.

The silver salt method of preparing esters is successful with acids and halides which possess no interfering groups. For example, Vogel (54) gives directions for the preparation of diethyl maleate in 58% yield by reacting a slurry of silver maleate and a benzene solution of ethyl iodide. It was considered that the use of a less complex α-amino acid than l-tyrosine might shed some light on a silver salt procedure. The silver and phenolic groups in silver-l-tyrosinate certainly are reactive to one another; this is evidenced by the discoloration of a wet sample even when being dried in a darkened vacuum desiccator. Even the free amino group might be complexing with the silver to prevent its availability to react with the halide. To eliminate these possibilities and attempt to find out whether a silver salt of an α-amino acid could be used to react with a halide to yield the corresponding ester, the simplest α-amino acid, glycine, was studied. There were a number of reasons for studying glycine in some detail. First, it is readily available and inexpensive. Second, the amino group can be "protected" very readily by means of

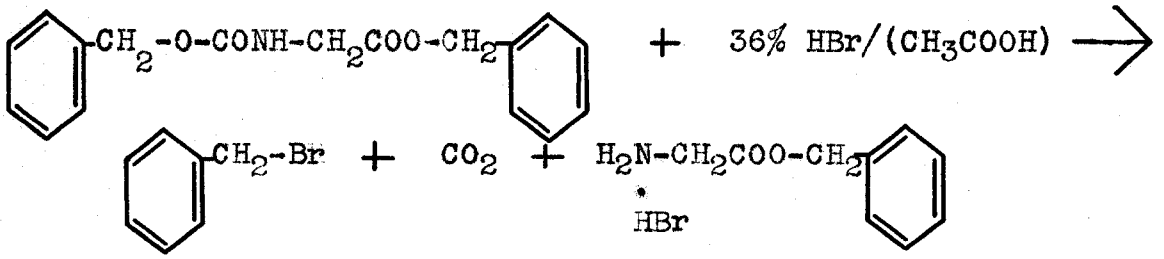
benzyl chloroformate. Farthing (55) has recently reported an improved method of preparing the required benzyl chloroformate and pure, crystalline carbobenzoxyglycine in a 70% yield.



Third, this carbobenzoxy "protecting" group can be removed under very mild conditions. Ben-Ishai and Berger (56) have shown that a saturated solution of hydrogen bromide in glacial acetic acid will cleave the carbobenzoxy group at room temperature, resulting in the formation of glycine hydrobromide in a 96% yield.



These same authors also showed that a benzyl ester group is unaffected under these conditions. When the benzyl ester of carbobenzoxyglycine was reacted with the hydrobromic acid reagent at room temperature, they obtained a 92% yield of benzyl glycinate hydrobromide.

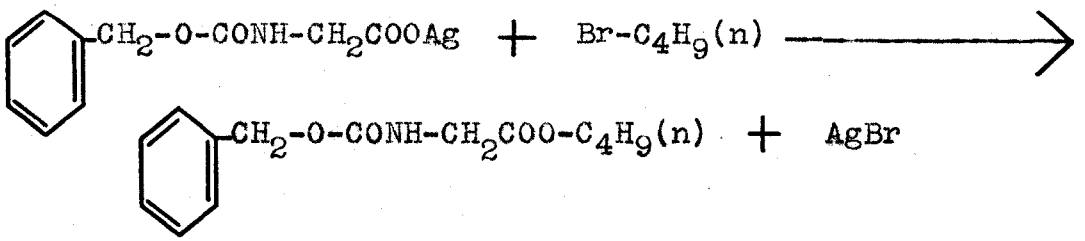


Albertson and MacKay (57) have employed nitromethane as the solvent for the anhydrous hydrobromic acid cleavage of peptides. The ammonium bromide formed from the reaction of hydrogen bromide with nitromethane caused no difficulty in purification.

Fruton and Bergmann (58) have also shown that the carbobenzoxy group can be removed without removal of an ethyl ester group. Hydrogenation of carbobenzoxy-l-glutamyl-l-tyrosine ethyl ester in the usual way resulted in formation of l-glutamyl-l-tyrosine ethyl ester in a 97% yield.

It was thought that an amino ester group would likewise be unaffected under these conditions.

Pure, crystalline carbobenzoxyglycine was converted into its silver salt by the usual method. The colorless salt analyzed 33.96% silver (theory---34.13%) and showed a decomposition point of about 200°. The reaction of silver carbobenzoxyglycinate and n-butyl bromide was studied in detail.

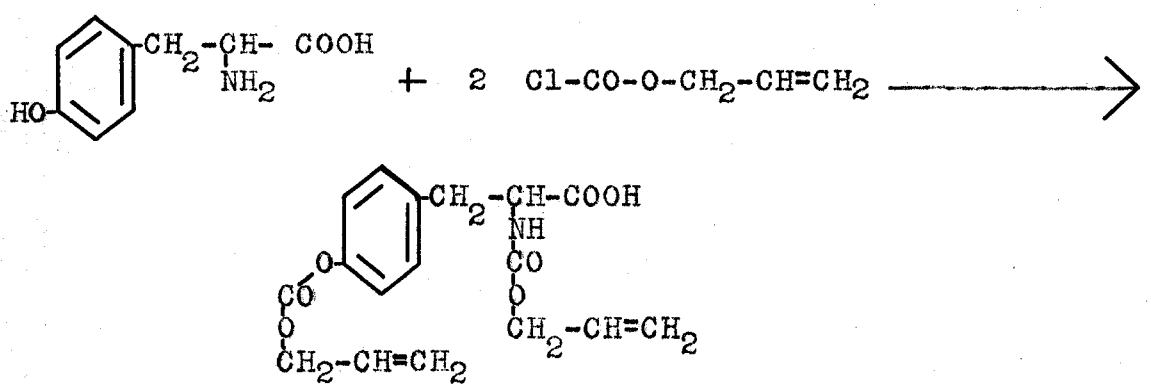


0.10 mole of white silver carbobenzoxyglycinate was covered with a solution of 0.125 mole of redistilled n-butyl bromide in 400 cc. of absolute ethanol. The colorless suspension was refluxed and stirred in a Morton flask for 8 hours and the resulting light-tan suspension filtered. Analysis of the residue for soluble silver ion showed that only 38.3% of the original silver carbobenzoxyglycinate had reacted. There was also recovered from this residue 9.4 g. (0.045 mole) of carbobenzoxyglycine.

When the filtrate was concentrated in vacuo and finally distilled at 0.5-1.0 mm, there was obtained 2.4 g. of an oil collected at 65-180°, and 1.9 g. of a colorless oil at 180-185°. An elemental analysis of this latter oil proved it to be the desired n-butyl ester of carbobenzoxyglycine. The boiling range was consistent with the value of 147-151° @ 0.5-1.0 mm for the ethyl ester, as reported by Barkdoll and Ross (59). The 1.9 g. of colorless oil represented a yield of only 7.2% from the original silver salt, or 18.7% when based on the amount of the silver salt which had actually reacted.

It was evident that the silver-salt method gave very poor results when used with either the free α-amino acid or the "protected" α-amino acid. Based on these results, the silver-salt method was discarded as a means of obtaining esters of α-amino acids.

Because of the unfavorable results with silver carbobenzoxyglycine, the silver salt of "protected" l-tyrosine was never used in an attempt to prepare an ester. However, some preliminary work was done in an attempt to obtain such a silver salt. O,N-Dicarbobenzoxy-l-tyrosine was prepared according to the procedure of Abderhalden and Bahn (60). However, it was not possible to obtain a pure product by recrystallization. Gish and Carpenter (61) have recently reported the preparation of O,N-di-p-nitro-benzyloxycarbonyl-D, L-tyrosine; however, repeated crystallizations yielded a product melting at 154-162°. Stevens and Watanabe (62) first reported the preparation of recrystallized, pure O,N-dicarboallyloxy-l-tyrosine, melting at 105-106°.



Consequently, this derivative was prepared and converted to its silver salt. The preparation of this silver salt was rather difficult as it had a strong tendency to form a gum and absorbed silver ions, becoming quite discolored. It was finally possible to get a small amount of a white product which gave an analysis for silver of 23.33% (theory---23.65%). This material melted with decomposition at 133-135°.

B. Alcoholysis of l-tyrosine methyl ester.

One of the common methods of preparation of esters is by the method of alcoholysis, or ester interchange, as exemplified by the equation

$$R\text{-}COOR' \quad R''OH \rightleftharpoons R\text{-}COOR'' \quad R'OH$$

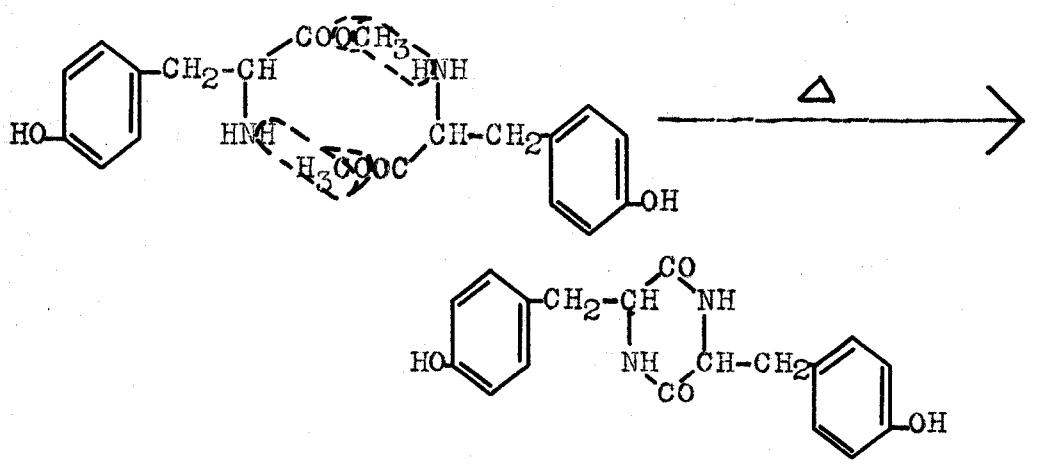
The interchange is catalyzed by either acidic or basic catalysts. This process has been studied quite extensively and there is a rather voluminous literature covering the subject (63). Since the simple methyl ester of l-tyrosine is readily obtained in good yield, attempts were made to prepare amino esters by the alcoholysis of the methyl ester. Both β-dimethylaminoethyl alcohol and β-diethylaminoethyl alcohol were used. There were three variations tried:

1. Methyl-l-tyrosinate was dissolved by warming in a large molar excess of the amino alcohol, and the solution refluxed under vacuum for various periods of time. It was thought that the amino alcohol itself might serve as the catalyst.
2. Similar to 1, except a small amount of sodium dissolved in the reaction mixture. Sodium alcoholates are recognized as the best catalysts for alcoholysis, sodium ethylate being about 1000 times more effective than the equivalent amount of hydrochloric acid (63). It was recognized that the sodium phenoxide formed by

this procedure would be much less effective, but it might aid in catalyzing the reaction.

3. l-Tyrosine methyl ester hydrochloride was dissolved by warming in a large molar excess of the amino alcohol, and the solution refluxed under vacuum for various periods of time.

By none of these variations was it possible to isolate any of the desired amino ester; in all procedures, one product always isolated was l-tyrosine anhydride, 3,6-bis(p-hydroxybenzyl)-2,5-piperazinedione. One careful study of variation 1 resulted in isolation of a 25% yield of l-tyrosine anhydride. Fischer and Schrauth (45) first reported the preparation of this diketopiperazine. By heating l-tyrosine methyl ester in a methyl alcohol solution at 135-140° for one-half hour, they obtained a 35% yield of l-tyrosine anhydride.



When variation 3 was used, there was always recovered as the first product the hydrochloride of the amino alcohol; based on the amount of methyl ester hydrochloride used, this

amounted to a nearly quantitative recovery of hydrogen chloride.

It was evident from these results that alcoholysis of a tyrosine ester would not yield the desired amino ester; the formation of a diketopiperazine accounted for the major reaction product that could be recovered.

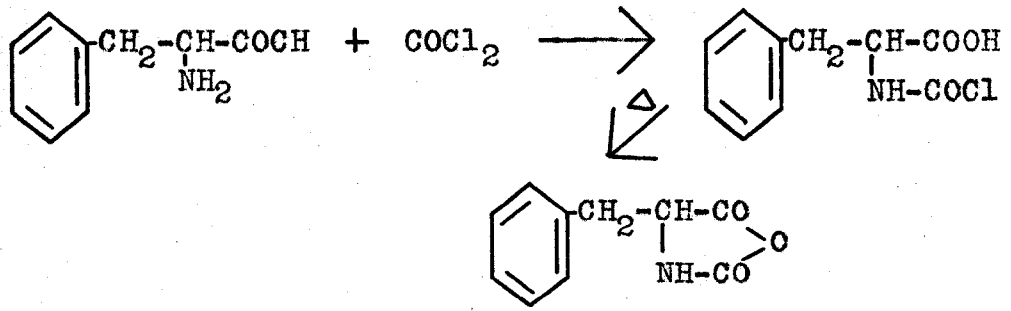
C. Attempted preparation and use of l-tyrosine-N-carboxy anhydride.

Leuchs (64) was the first to show the existence of N-carboxy anhydrides of α-amino acids. By heating carbethoxy amino acid chlorides, ethyl chloride was eliminated with the formation of the internal anhydride. Thus, carbethoxy glycyl chloride gave rise to glycine N-carboxy anhydride:

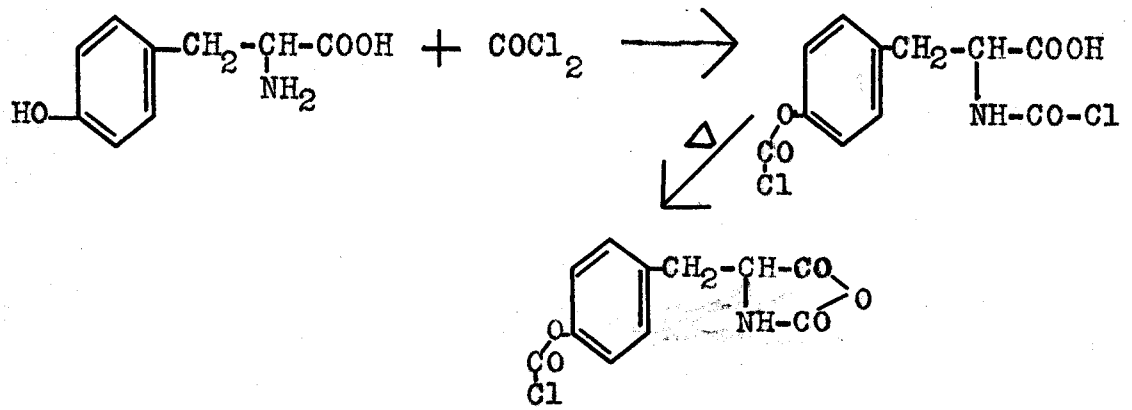
$$C_2H_5\text{-}O\text{-}CONH\text{-}CH_2CO\text{-}Cl \xrightarrow{\Delta} CO\text{-}NH\text{-}CH_2\text{-}CO\ (\text{-}O\text{-}) + C_2H_5Cl$$

In the presence of water, this anhydride, and those of other amino acids (65), rapidly open with the loss of carbon dioxide and regeneration of the amino acid. Reaction with alcohols results in formation of the amino acid esters; reaction with amino acids yields a peptide. These simple reactions are accompanied by a polycondensation to yield a polymer of the amino acid. Under proper conditions (66), this latter reaction becomes predominant. Recently, much work has been done towards improving methods for synthesizing these anhydrides and using them to prepare various peptides and their derivatives (67,

68, 69, 70). Farthing and Reynolds (68) successfully prepared the N-carboxy anhydride of DL-phenylalanine by passing gaseous phosgene into a stirred suspension of the amino acid in dioxane at 30-40° until the amino acid dissolved. Removal of the solvent and excess phosgene by heating <u>in vacuo</u> at 40° then afforded the desired N-carboxy anhydride



The same procedure applied to l-tyrosine gave a product which showed a positive chloride test when placed in contact with water. Treatment of this material with β-diethylaminoethyl alcohol in dioxane resulted in the precipitation of the amino alcohol hydrochloride along with the formation of a gum. Bailey (70) has reported the successful use of O-acetyl-N-carbobenzoxy-l-tyrosine anhydride in the preparation of simple peptides. However, the presence of the free phenolic group in l-tyrosine could result in the formation of a chloroformic acid ester, according to the following scheme:

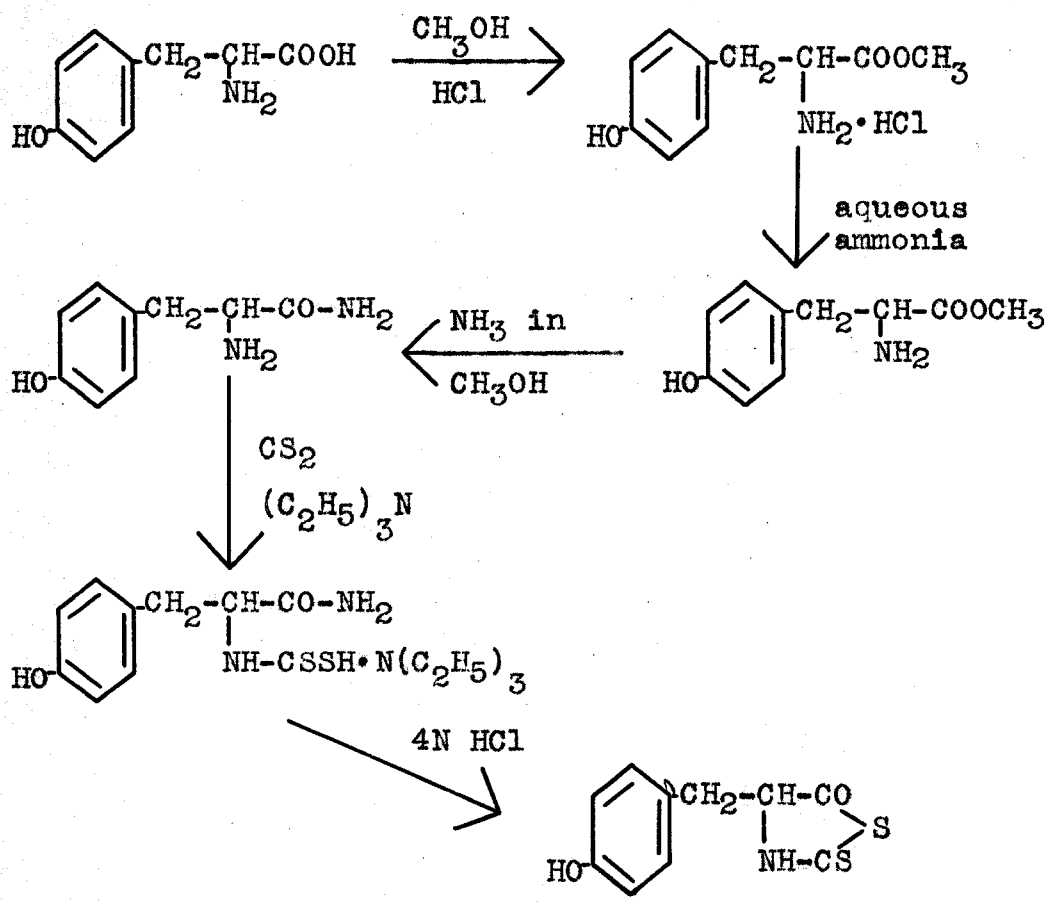


This intermediate would account for the observed results when treated with the amino alcohol.

This preliminary work done with l-tyrosine and its reaction product with phosgene indicated that further study of this method for the preparation of the desired amino esters would be of little value. Consequently, this phase of the work was abandoned.

D. Attempted use of l-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone.

Davis and Levy (71) were the first to report the preparation of this derivative of l-tyrosine by the following sequence of reactions:

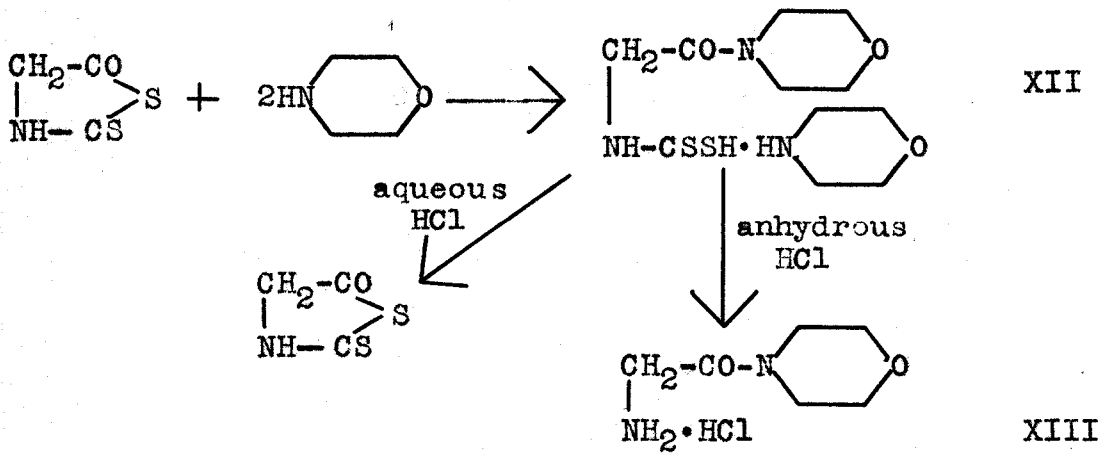


This sequence was repeated as a part of this work and the overall yield of the thio-thazolidone from l-tyrosine was 59%.

As can be observed, this thio-thiazolidone is a sulfur analog of the unknown N-carboxy anhydride of l-tyrosine. Cook, Heilbron and Levy were among the first to study such sulfur derivatives of α-amino acids (72). They observed that these sulfur analogs are much more stable than the corresponding N-carboxy anhydrides, and have done much work to explore their use as intermediates for the synthesis of peptides. They studied the properties of 2-thio-5-thiazolidone, the corresponding derivative of glycine, and showed that an

alcoholic solution of hydrogen chloride would convert this derivative into the amino acid ester hydrochloride in 80% yield.

Cook and Levy (73) studied the action of bases on 2-thio-5-thiazolidone. Using morpholine as a model secondary amine, they observed the following sequence of reactions:



XII

XIII

The morpholinium salt of N-dithiocarboxyglycine morpholide (XII) was obtained in 87% yield by performing the reaction at 0° in acetone. Treatment of this morpholinium salt with concentrated hydrochloric acid at 0° regenerated the original 2-thio-5-thiazolidone in an 84% yield. However, passing dry hydrogen chloride into a chloroform solution decomposed the dithiocarbamate salt to yield glycine morpholide hydrochloride (XIII).

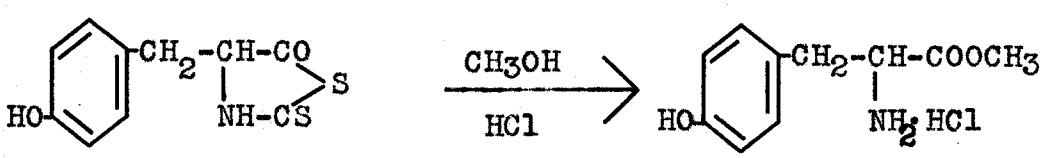
These results with the thio-thiazolidone related to glycine suggested the possibility of using the thio-

thiazolidone related to l-tyrosine to obtain the desired amino esters. There appeared to be two possible methods:

Method 1: Allow the thio-thiazolidone and amino alcohol hydrochloride to react in an inert solvent containing excess acid; this should yield the amino ester dihydrochloride directly.

Method 2: Allow the thio-thiazolidone and the amino alcohol to react in an inert solvent to form the amino ester dithiocarbamate salt of the amino alcohol; then decompose this salt with dry hydrogen chloride in chloroform.

Trial runs were first performed. In the case of Method 1, this was done by allowing the thio-thiazolidone to react overnight with a methanolic solution of hydrogen chloride at room temperature.

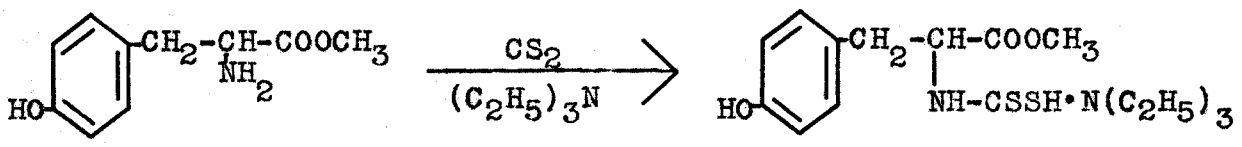


On neutralizing the reaction mass the next day and recrystallizing from ethyl acetate, colorless crystals melting at 111-126° were obtained. Repeated recrystallization failed to alter this melting range. Davis and Levy (71) reported that

the thio-thiazolidone as initially obtained from l-tyrosine was optically active, "but racemized during 2-3 hours in methanol solution. However, in the presence of 0.1 equivalent of hydrogen chloride, or in ethanol solution, it was considerably more stable, and little or no racemization was apparent in ethyl acetate. In the presence of bases, optical rotation was rapidly lost". As shown by the measured specific rotation, this material was partly racemized l-tyrosine methyl ester.

Attempts to react β-diethylaminoethyl alcohol hydrochloride and the thio-thiazolidone in dioxane/HCl solvent were not successful, and this method was discarded.

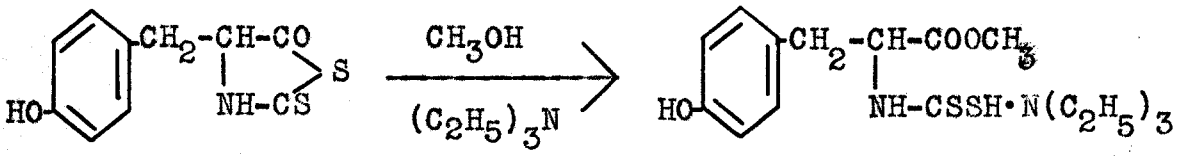
In the case of Method 2, preliminary work once again consisted in performing various reactions with l-tyrosine methyl ester. When the methyl ester was treated with carbon bisulfide and triethyl amine in alcohol solution, there was obtained an 84% yield of the triethylammonium dithiocarbamate derivative (XIV):



XIV

When XIV was treated with dry hydrogen chloride in chloroform, methyl-l-tyrosinate was recovered. However, attempts to prepare the dithiocarbamate derivative by reacting the

thio-thiazolidone, methanol and triethylamine, were unsuccessful:

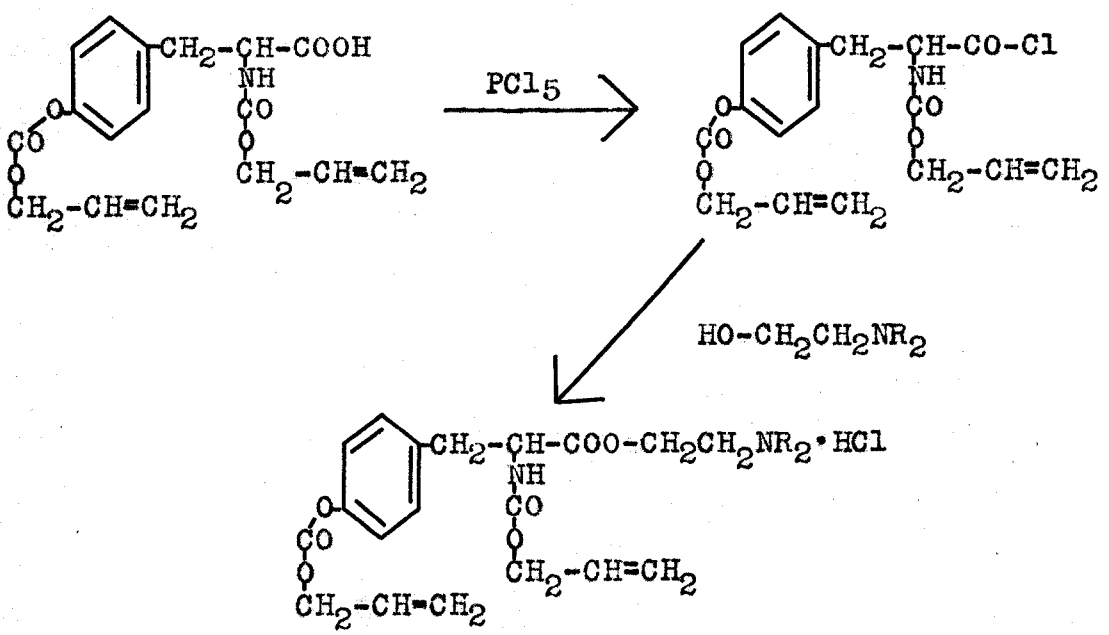


Similarly, the use of β-diethylaminoethyl alcohol in this same type of reaction gave unsuccessful results.

The series of negative results obtained with the thio-thiazolidone derived from l-tyrosine caused this phase of the work to be abandoned. It seemed quite evident that the desired amino esters would not be obtained through this intermediate.

E. Attempted preparation and use of O,N-dicarboallyloxy-l-tyrosine acid chloride.

The acid chlorides of "protected" amino acids have been used as intermediates for the preparation of peptides. Abderhalden and Bahn (60) reported the preparation of O,N-dicarbobenzoxy-l-tyrosine acid chloride as an intermediate in the synthesis of l-tyrosylglycine. Obviously, these acid chlorides could be used to prepare esters as well as peptides. Since O,N-dicarboallyloxy-l-tyrosine could be obtained as a pure intermediate, the next attempted procedure was to convert this acid into its acid chloride and react it with the amino alcohol.



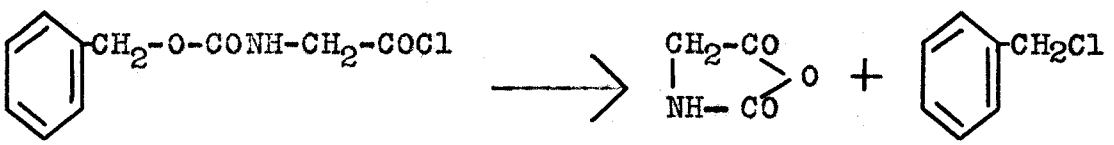
The resultant amino ester then could be treated with the previously described HBr/acetic acid reagent to remove the carboallyloxy groups.

O,N-Dicarboallyloxy-l-tyrosine and phosphorus pentachloride were reacted in anhydrous ether solution, according to the method of Karrer and Heynemann (74). The addition of ligroin with chilling resulted in a crystalline product, which was recrystallized from ether-ligroin. The product so obtained melted at 35-45°; repeated recrystallizations failed to improve this property.

All attempts to use this acid chloride failed due to its instability and seemingly lack of purity. One sample was placed in a refrigerated vacuum desiccator for 44 hours; there was no visible change at the end of this time. However, when the desiccator and its contents were allowed to warm to room temperature over a three hour period, the colorless

crystals decomposed, with gas evolution, and there remained a colorless oil.

Bergmann and Zervas (75) prepared the acid chloride of carbobenzoxyglycine and reported that it melted at 43° with decomposition into glycine-N-carboxy anhydride and benzyl chloride:



No doubt, a similar decomposition of O,N-dicarboallyloxy-l-tyrosine acid chloride had occurred; the gas evolution could have been carbon dioxide resulting from polymerization.

The attempted preparation and use of O,N-dicarboallyloxy-l-tyrosine acid chloride proved to be quite unsuccessful.

F. Attempted direct esterification of l-tyrosine hydrochloride.

Simple esters of l-tyrosine are easily prepared by the Fischer method. From the inception of this work, the application of this method for the preparation of amino esters had been considered. It was recognized that a problem of solubility had to be surmounted. l-Tyrosine hydrochloride is a crystalline solid, only soluble in polar solvents which can not be used in esterifications, e.g., water and alcohol. The amino alcohol hydrochlorides are likewise solids, only slightly more soluble in inert solvents. Unless the reactants

could be brought together in the liquid state, it was obvious that no esterification could occur.

Due to the failure of the previously described attempts to synthesize an amino ester of l-tyrosine, a further study was made of solvents for l-tyrosine hydrochloride and the amino alcohol hydrochloride. One of the more recently described industrial solvents is dimethylformamide; its solvent powers are quite unique. This uniqueness was further proven when l-tyrosine hydrochloride dissolved quite freely; likewise, the amino alcohol hydrochloride was readily soluble. Here, finally, was a solvent for the reactants; whether it would be inert in the present instance remained to be proven.

Initially, a dimethylformamide solution of β-diethylaminoethyl alcohol hydrochloride (0.1 mole) and l-tyrosine hydrochloride (0.05 mole) was heated at 65° for 18 hours. Analysis of the reaction mass showed that only a 20% reaction had occurred. It was evident that the equilibrium, rate, or both were unfavorable. The various factors which shift equilibrium and increase the rate of an esterification were then incorporated.

The following procedure proved effective: The dimethylformamide solution of reactants plus a catalytic amount of concentrated sulfuric acid was partly refluxed at 100° under a vacuum of 100 mm. The distillate was returned to the boiling reaction mixture through an anhydrous calcium sulfate column. After so heating for about 65 hours, no

residual l-tyrosine could be detected.

Since the reaction mass always turned deep reddish-brown when sulfuric acid was used as catalyst, this acid was omitted in subsequent reactions. The presence of a free phenolic group in l-tyrosine is conducive to unwanted products in the presence of concentrated sulfuric acid. In place of the concentrated sulfuric acid, the warm solution of reactants was saturated with dry hydrogen chloride. The reaction was catalyzed as effectively; however, when the reaction mass was cooled, a considerable quantity of dimethylamine hydrochloride was always precipitated.

All attempts to recover any product from this procedure proved fruitless. There was obtained a small amount of l-tyrosine anhydride as the only recognizable material. The difficulty was in removing the solvent dimethylformamide; even the use of prolonged vacuum distillation was not completely satisfactory. There was also considerable decomposition, as evidenced by the red-brown color of the reaction mixture.

Apparently the use of "unprotected" l-tyrosine at temperatures of 100° for times of 65 hours resulted in unwanted changes. This suggested the use of a "protected" amino acid in a similar procedure.

G. Direct esterification of carbobenzoxyglycine.

Due to the difficulties encountered in the attempted

direct esterification of l-tyrosine hydrochloride in dimethylformamide solution, it was decided to attempt a direct esterification of a "protected" amino acid. Once again, carbobenzoxyglycine was initially studied. Preliminary solubility tests showed that carbobenzoxyglycine was readily soluble in hot chloroform or ethylene dichloride. Such tests likewise showed that the salts of β-diethylaminoethyl alcohol formed with either hydrogen chloride or p-toluene sulfonic acid were soluble in the same two solvents. p-Toluene sulfonic acid was chosen as the acid to be used for both salt formation and catalysis.

Equal molar quantities of carbobenzoxyglycine, β-diethylaminoethyl alcohol and p-toluene sulfonic acid, plus an additional catalytic amount of p-toluene sulfonic acid, were partly refluxed in ethylene dichloride solution for 5 hours. During this period, the distillate was returned to the reaction pot through an anhydrous calcium sulfate column. Extraction of the ethylene dichloride solution with carbonate solution and subsequent acidification of the aqueous layer, resulted in a 26% recovery of unreacted carbobenzoxyglycine.

In view of the anticipated difficulty in separating the expected amino ester from any unreacted amino alcohol, the procedure was modified by using a 2:1 molar ratio of carbobenzoxyglycine to β-diethylaminoethyl alcohol and a heating period of 17 hours. This molar excess of carbobenzoxyglycine was readily recovered in 95-100% yields at the end

of the reaction. Working up of the reaction mixture gave a good yield of a deep yellow oil. A small quantity of this oil was vacuum distilled at 0.5-1.0 mm, and a yellow oil collected at 195-203°. This oil was water insoluble, soluble in dilute acids, and when treated with HBr/acetic acid reagent evolved a copious quantity of gas.

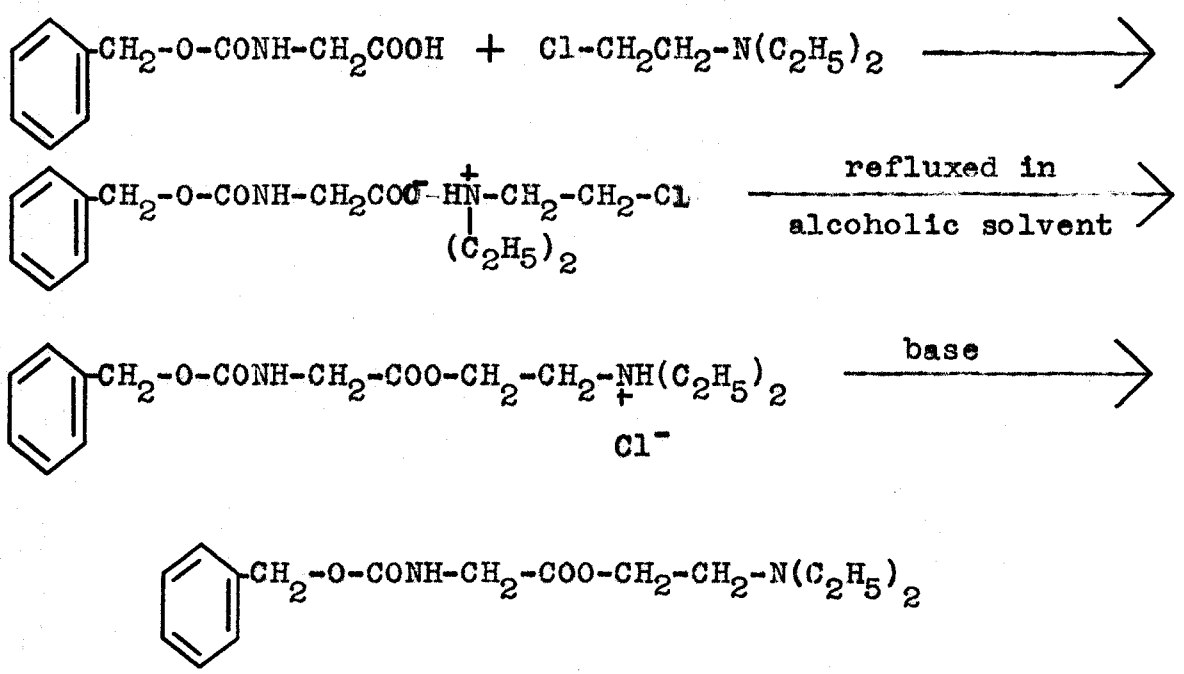
These results were considered as showing the presence of the desired amino ester as an impure oil. This oil was not further purified and studied, due to the highly favorable results obtained by the succeeding procedure to be discussed. However, it seems quite likely that further study of the conditions of this reaction, and particularly the recovery and purification of the reaction product, would prove this to be a satisfactory method of synthesis.

It should be mentioned that a trial reaction was performed using O,N-dicarboallyloxy-l-tyrosine, which is quite soluble in ethylene dichloride. The preliminary results were the same as for carbobenzoxyglycine.

III. Successful preparation of a β-amino ester of an α-amino acid.

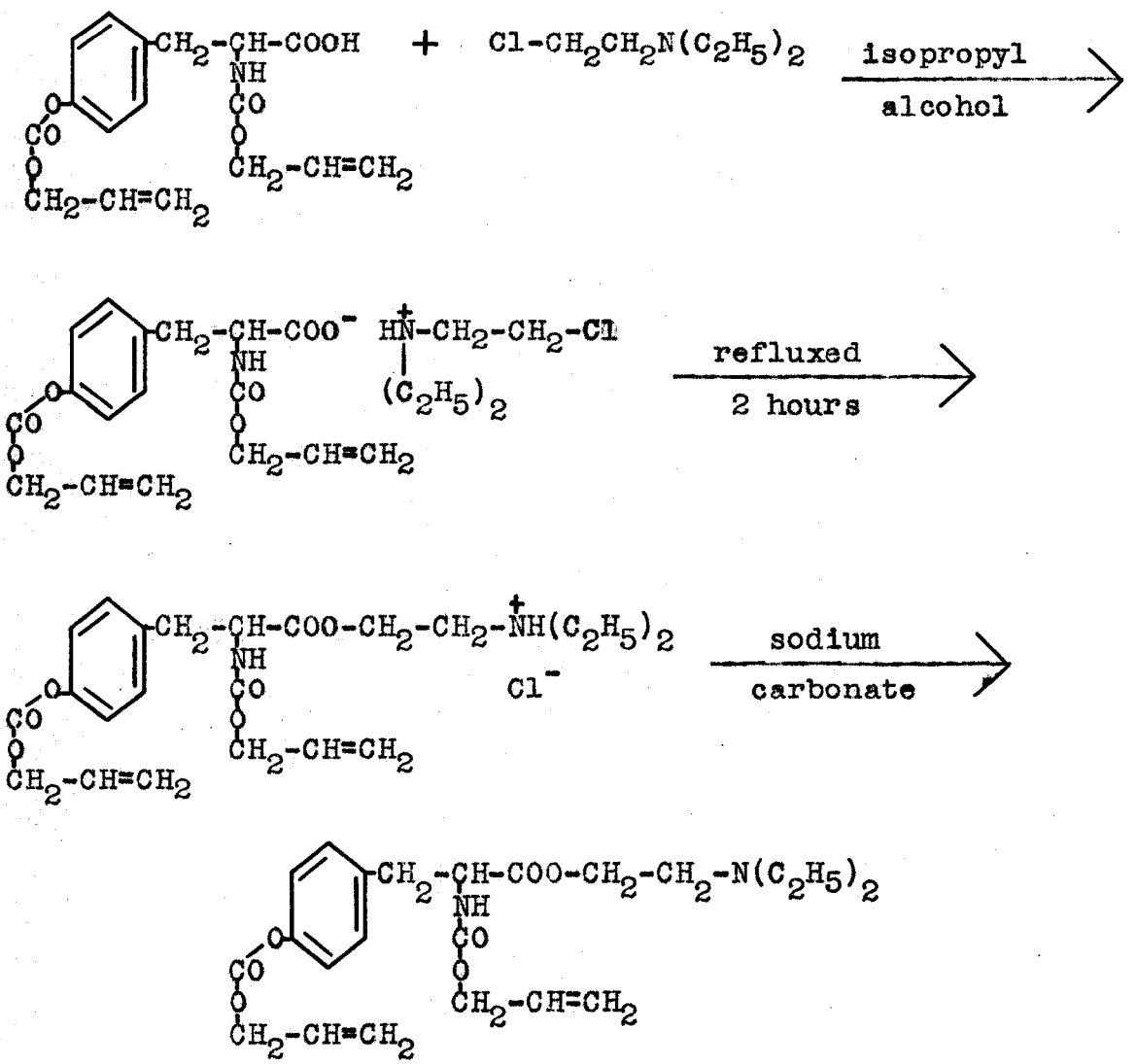
Horenstein and Pahlicke (33) were the first to use the reaction of a β-chloroalkyl amine and an acid to yield a β-amino ester. The excellent yields of amino esters reported for this type of reaction (34, 35, 36) suggested its application to a "protected" amino acid, carbobenzoxyglycine.

Equimolar quantities of carbobenzoxyglycine and freshly vacuum distilled β-diethylaminoethyl chloride were refluxed in an anhydrous alcoholic solvent. When the reaction mixture was worked up, there was obtained a nearly colorless oil, whose properties and elemental analysis proved it to be the desired β-diethylaminoethyl carbobenzoxyglycinate.

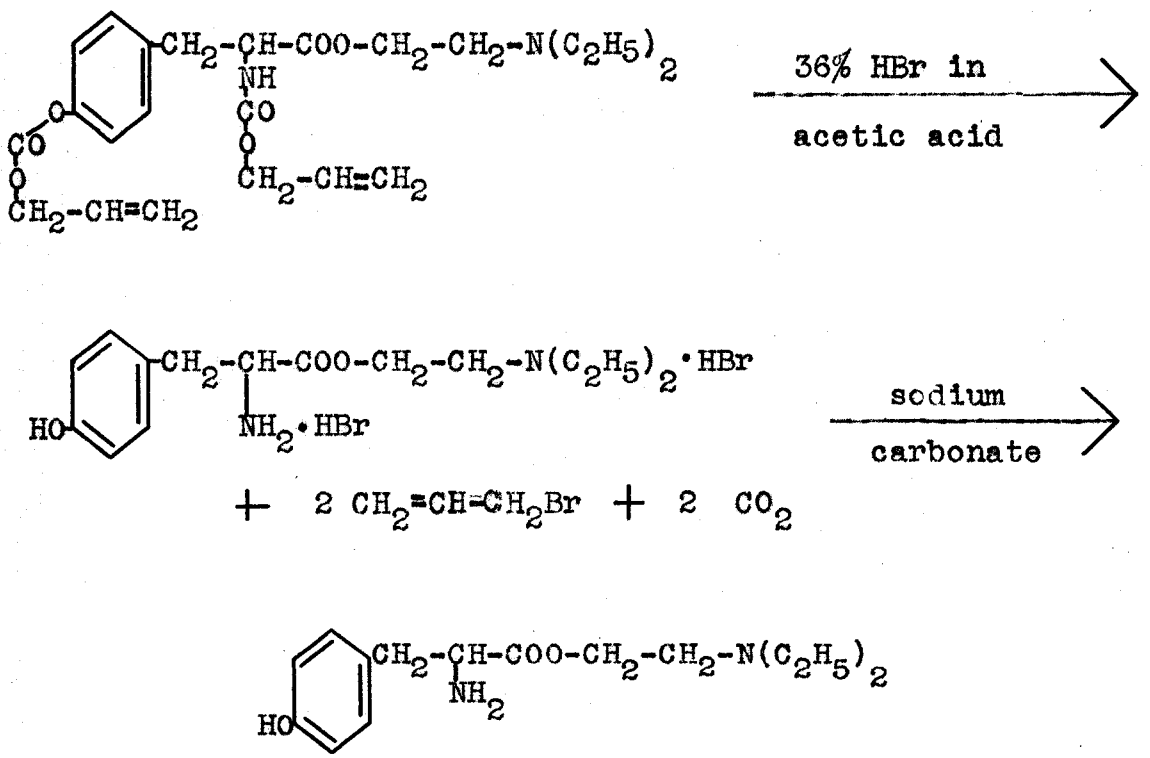


The yield of the amino ester was partly dependent on the alcohol used as a solvent, as alcoholysis was a competing reaction. When the reactants were refluxed for 1.5 hours in anhydrous ethanol, 18% of unreacted carbobenzoxyglycine was recovered. Based on the reacted carbobenzoxyglycine, there was obtained a 48% yield of the amino ester and a 19% yield of the ethyl ester. However, refluxing for two hours in isopropyl alcohol left only 5% of the carbobenzoxyglycine unreacted. And based on the reacted carbobenzoxyglycine, there was obtained a 70% yield of vacuum distilled amino ester and only a 2.4% yield of the isopropyl ester.

These results with carbobenzoxyglycine showed this to be an effective method of obtaining a β-amino ester of a "protected" α-amino acid. Consequently, O,N-dicarboallyloxy-l-tyrosine was next used as the reactant. Equimolar amounts of this l-tyrosine derivative and freshly vacuum distilled β-diethylaminoethyl chloride were refluxed for two hours in absolute isopropyl alcohol. There was obtained an 80% yield of a colorless, very viscous, non-distillable oil. Elemental analysis proved this to be the β-diethylaminoethyl ester of O,N-dicarboallyloxy-l-tyrosine.



This "protected" amino ester was reacted with saturated HBr/acetic acid reagent. On working up the reaction products, there was obtained a 50% yield of β-diethylamino-ethyl-l-tyrosinate.



EXPERIMENTAL

I. Purification of l-tyrosine.

The l-tyrosine used throughout this work was obtained from the Eastman Kodak Company, a product of the hydrolysis of casein. This crude l-tyrosine was purified by recrystallizing from 1-2% hydrochloric acid according to the procedure of Tsuchiya (76). Analysis for nitrogen by the method of Kjeldahl (77) gave 7.78% (theory: 7.73%).

II. Preparation of 3.5-diiodo-l-tyrosine.

All of the required 3,5-diiodo-l-tyrosine was prepared from purified l-tyrosine by the method of Block and Powell (50). It was purified by dissolving in 2N hydrochloric acid, clarifying the solution, and precipitating the dihydrate by the dropwise addition of concentated ammonia solution to a pH of 4.3 (isoelectic point). The colorless material decomposed at 199-200° when heated at the rate of 10° per minute.

III. Preparation of 3,5-diiodo-l-tyrosine methyl ester hydrochloride.

8.7g of 3,5-diiodo-l-tyrosine (0.02 mole) was covered with 48 cc. (1.2 mole) of dry methyl alcohol. The suspension was saturated with dry hydrogen chloride to yield a hot clear solution. The hydrogen chloride was continued to

be passed in slowly until needles began forming. The solution was allowed to cool with final cooling in an ice-bath, and the colorless needles collected. The filtrate was treated with 24 cc. of concentrated hydrochloric acid and chilled again to yield a second crop of crystals. Both crops were combined to yield 8.7 g (89.7%) of the crude hydrochloride, decomposing at 202-204° when heated at the rate of 10°/minute.

This hydrochloride could be recrystallized from 2N hydrochloric acid at the rate of 20cc/gram in a 72% yield. The colorless crystals did not show a sharp melting point, but decomposed at 202-203° when the bath was heated at the rate of 10° per minute.

Analysis (78)	% Cl
Found:	7.35
Calc'd for $C_{10}H_{12}NO_3I_2Cl$:	7.34

IV. Preparation of 3,5-diiodo-l-tyrosine methyl ester.

17.0 g of the crude hydrochloride was dissolved in 90% methyl alcohol at the rate of 25cc/gram. While the solution was swirled, concentrated ammonia was added dropwise to precipitate the free crystalline ester. The slightly ammoniacal mixture was cooled well in an ice-bath to yield 15.2 g of the crude ester (96.8%). This decomposed at 177-178° when heated at 10°/minute.

The crude ester was recrystallized from wet methyl cellosolve. The mixed solvent was prepared by adding 4cc. of

water to each 30cc. of pure methyl cellosolve. This mixed solvent was used at the rate of 38cc. per gram of crude methyl ester. In the recrystallization of the esters of 3,5-diiodo-l-tyrosine, the elevated temperature required for solution in the solvent very often resulted in discoloration due to liberation of iodine. In the case of the methyl ester, the hot, clarified solution was cooled rapidly to 50-60°, allowed to cool slowly to room temperature and then placed in the refrigerator overnight. The resultant fine crystals (72% yield) were just faintly off-white in color. The over-all yield of recrystallized methyl ester from 3,5-diiodo-l-tyrosine was 62.5%. Decomposed at 187-188° when heated at 10°/minute.

Analysis (x)	% I
Found:	56.75
Calc'd for $C_{10}H_{11}NO_3I_2$:	56.92

V. Preparation of 3,5-diiodo-l-tyrosine ethyl ester hydrochloride.

8.7 g of 3,5-diiodo-l-tyrosine (0.02 mole) was covered with 60cc. of absolute ethyl alcohol (1.0 mole). Dry hydrogen chloride was passed into the suspension. The solid gradually dissolved and the mixture became hot. The

x Analysis by H. W. Galbraith, Knoxville, Tenn.

hydrogen chloride was continued to be passed in until needles began to appear. The mixture was cooled well and the crystalline material collected. The filtrate was treated with 30cc. of concentrated hydrochloric acid and cooled well to yield an additional crop of crystals. The combined crops of crude ethyl ester hydrochloride weighed 8.1 g (81.0%) and decomposed at 206-207° at 10°/minute.

This crude hydrochloride could be recrystallized from 1.5N hydrochloric acid at the rate of 30cc./gram in a 73.5% yield. The purified hydrochloride decomposed at 212-214° at 10°/minute.

Analysis (78)	% Cl
Found:	7.13
Calc'd. for $C_{11}H_{14}NO_3I_2Cl$:	7.11

VI. Preparation of 3,5-diiodo-l-tyrosine ethyl ester.

6.5 g. of the crude 3,5-diiodo-l-tyrosine ethyl ester hydrochloride was dissolved in 80% methyl alcohol at the rate of 25 cc./gram. Concentrated ammonia was added dropwise to the clarified solution while swirling to precipitate the free ester. There was obtained 5.8 g. of the crude ester (96.7%), decomposing at 172-3° at 10°/minute.

The crude ester was recrystallized from methyl cellosolve. By using 20 cc. of methyl cellosolve per gram of crude ethyl ester, a temperature of only 80° was required to effect solution. The hot, clarified solution was cooled

rapidly to 50-60° and then allowed to cool undisturbed in the refrigerator. There resulted a 85% recovery of white, fine crystals. This represented an overall yield of 66.5% of pure ethyl ester from 3,5-diiodo-l-tyrosine, decomposing at 174.5-175.5° at 10°/minute.

Analysis (*)	% C	% H	% N	% I
Found:	28.60	2.71	3.02	54.92
Calc'd. for $C_{11}H_{13}NO_3I_2$:	28.65	2.84	3.04	55.01

VII. Preparation of 3,5-diiodo-l-tyrosine n-propyl ester hydrochloride.

8.7 g. of 3,5-diiodo-l-tyrosine (0.02 mole) was covered with 150 cc. of n-propyl alcohol (2.0 mole). The ester hydrochloride was prepared and isolated in the same manner as detailed for 3,5-diiodo-l-tyrosine methyl ester hydrochloride. There was obtained 8.1 g. of the crude hydrochloride (79.3%), decomposing at 210.5-212° at 10°/minute.

This crude ester hydrochloride was recrystallized from 1N hydrochloric acid at the rate of 60cc/gram in an 84% yield. The colorless needles decomposed at 211-213° at 10°/minute.

Analysis (78)	% Cl
Found:	6.89
Calc'd. for $C_{12}H_{16}NO_3I_2Cl$:	6.93

* Analysis by H. W. Galbraith, Knoxville, Tenn.

VIII. Preparation of 3,5-diiodo-l-tyrosine n-propyl ester.

7.9 g. of the crude hydrochloride was dissolved in 90% methyl alcohol at the rate of 12 cc./g. Concentrated ammonia was added dropwise with swirling, and the crystalline precipitate was collected. The filtrate was poured into 350 cc. of water to yield a second crop of crystals. The combined crude ester weighed 6.7 g. (91.7%).

The crude n-propyl ester was recrystallized from ethylene dichloride at the rate of 15 cc./g. in a yield of 90%. The overall yield of pure n-propyl ester from 3,5-diiodo-l-tyrosine was 65.5%. This decomposed at 151-152° at 10°/minute.

Analysis (*)	% I
Found:	53.69
Calc'd for $C_{12}H_{15}NO_3I_2$:	53.39

IX. Preparation of 3,5-diiodo-l-tyrosine iso-propyl ester hydrochloride.

8.7 g. of 3,5-diiodo-l-tyrosine (0.02 mole) was covered with 225 cc. of iso-propyl alcohol (3.0 mole). The suspension was saturated with dry hydrogen chloride to yield a clear solution which was refluxed for about one hour. Dry hydrogen chloride was then passed in until crystallization commenced; on cooling in an ice-bath there was collected 7.5 g. of the crude ester hydrochloride. The addition of 115 cc.

* Analysis by H. W. Galbraith, Knoxville, Tenn.

of concentrated hydrochloric acid to the filtrate caused the separation of an additional 0.8 g. The combined yield of crude n-propyl ester hydrochloride was 81.3%, decomposing at 219.5-220.5° at 10°/minute.

The crude ester hydrochloride was recrystallized from 0.25N hydrochloric acid at the rate of 50 cc./g. A yield of 78.3% was obtained, decomposing at 226-228° at 10°/minute.

Analysis (78)	% Cl
Found:	6.90
Calc'd. for $C_{12}H_{16}NO_3I_2Cl$:	6.93

X. Preparation of 3,5-diiodo-l-tyrosine iso-propyl ester.

8.0 g. of the crude iso-propyl ester hydrochloride was dissolved in 70% methyl alcohol at the rate of 15 cc./g. Concentrated ammonia was added dropwise to the swirled solution to yield 5.9 g. of the crude ester (79.8%), decomposing at 157-58° at 10°/minute.

The crude ester was recrystallized from 95% ethyl alcohol at the rate of 25 cc./g. in a yield of 76%. The overall yield of pure iso-propyl ester from 3,5-diiodo-l-tyrosine was 49.5%. It decomposed at 156-157° at 10°/minute.

Analysis (*)	% I
Found:	53.40
Calc'd. for $C_{12}H_{15}NO_3I_2$:	53.39

* Analysis by H. W. Galbraith, Knoxville, Tenn.

XI. Preparation of 3,5-diiodo-l-tyrosine n-butyl ester hydrochloride.

10.8 g. of pure 3,5-diiodo-l-tyrosine (0.025 mole) was covered with 92 cc. of dry n-butyl alcohol (1.0 mole). The suspension was saturated with dry hydrogen chloride and then refluxed gently until the diiodotyrosine was completely dissolved. The dark yellow solution was refluxed for an additional 15 minutes, resaturated with dry hydrogen chloride and allowed to cool, with final cooling in an ice-bath. There was obtained 8.8 g. of yellow colored crude ester hydrochloride (67.2%). Long colorless needles were obtained by recrystallizing from 0.25N hydrochloric acid (40 cc./g.; 73.5% yield). The purified hydrochloride decomposed at 188-190° at 10°/minute.

Analysis (78)	% Cl
Found:	6.70
Calc'd. for $C_{13}H_{18}NO_3I_2Cl$:	6.74

XII. Preparation of 3,5-diiodo-l-tyrosine n-butyl ester.

8.0 g. of the crude yellow-colored ester hydrochloride was dissolved in 80 cc. of 90% methyl alcohol, which was decolorized to yield a clear, colorless solution. While swirling, concentrated ammonia was added dropwise to precipitate 7.1 g. of the crude n-butyl ester (95.3%). This decomposed at 140-142° at 10°/minute.

The crude ester was recrystallized from 95% ethyl alcohol at the rate of 20 cc./g., giving a 95% yield of

colorless needles decomposing at 139-141° at 10°/minute. The overall yield of pure n-butyl ester from 3,5-diiodo-l-tyrosine was 63%.

Analysis (*)	% I
Found:	51.76
Calc'd. for $C_{13}H_{17}NO_3I_2$:	51.94

XIII. Preparation of 3,5-diiodo-l-tyrosine iso-butyl ester hydrochloride.

8.7 g. of colorless 3,5,-diiodo-l-tyrosine (0.02 mole) was covered with 73 cc. of dry iso-butyl alcohol (0.8 mole). The suspension was saturated with dry hydrogen chloride and then refluxed gently until the 3,5-diiodo-l-tyrosine was completely dissolved. The reddish-brown solution was refluxed for an additional 15 minutes, resaturated with dry hydrogen chloride and allowed to cool, with final cooling in an ice-bath. There was obtained 8.0 g. of reddish-brown crude ester hydrochloride (76.2%). A colorless solid was obtained by recrystallizing from 0.25N hydrochloric acid (40 cc./g.; 77.2%). The purified hydrochloride decomposed at 205-206° at 10°/minute.

Analysis (78)	% Cl
Found:	6.75
Calc'd for $C_{13}H_{18}NO_3I_2Cl$:	6.74

* Analysis by H. W. Galbraith, Knoxville, Tenn.

XIV. Preparation of 3,5-diiodo-l-tyrosine iso-butyl ester.

9.7 g. of the crude discolored ester hydrochloride was dissolved in 97 cc. of 90% methyl alcohol. Treatment with decolorizing charcoal yielded a clear, colorless solution. While swirling, concentrated ammonia was added dropwise to precipitate 7.4 g. of the crude ester (82.3%). This decomposed at 149.5-151.5° at 10°/minute.

The crude ester was recrystallized from 95% ethyl alcohol at the rate of 15 cc./g. There was obtained a 90.5% yield of colorless needles, which represented an overall yield of 56.6% of pure iso-butyl ester from 3,5-diiodo-l-tyrosine. It decomposed at 151-153° at 10°/minute.

Analysis (*)	% I
Found:	51.94
Calc'd. for $C_{13}H_{17}NO_3I_2$:	51.94

XV. Preparation of 3,5-diiodo-l-tyrosine n-amyl ester hydrochloride.

10.8 g. of 3,5-diiodo-l-tyrosine (0.025 mole) was covered with 108 cc. of dry n-amyl alcohol (1.0 mole). The suspension was saturated with dry hydrogen chloride and then refluxed gently until the 3,5-diiodo-l-tyrosine was completely dissolved. The reddish-brown solution was refluxed for an additional 15 minutes, then resaturated with dry hydrogen chloride and allowed to cool with final cooling in an ice-bath.

*Analysis by H. W. Galbraith, Knoxville, Tenn.

There was obtained 10.1 g. of reddish-brown crude ester hydrochloride (74.9%). Colorless needles of the pure hydrochloride were obtained by recrystallization from 0.25N hydrochloric acid (40 cc./g.; 78.3%). The purified hydrochloride decomposed at 190-191° at 10°/minute.

Analysis (78)	% Cl
Found:	6.53
Calc'd. for $C_{14}H_{20}NO_3I_2Cl$:	6.57

XVI. Preparation of 3,5-diiodo-l-tyrosine n-amyl ester.

7.4 g. of the crude colored hydrochloride was dissolved in 95% methyl alcohol at the rate of 20 cc./g. The yellow solution was decolorized to yield a clear, colorless solution. While swirling, concentrated ammonia was added dropwise to precipitate 5.9 g. of the crude ester (85.5%), decomposing at 123-127° at 10°/minute.

The crude ester was recrystallized from methyl cellosolve at the rate of 10 cc./g., the crude ester completely dissolving at 70°. The solution was decolorized and cooled rather rapidly to give an 82.5% yield of fine, colorless crystals, decomposing at 113-116° at 10°/minute. This represented a 52.8% overall yield of pure n-amyl ester from 3,5-diiodo-l-tyrosine.

Analysis (*)	% I
Found:	50.52
Calc'd. for $C_{14}H_{19}NO_3I_2$:	50.40

* Analysis by H. W. Galbraith, Knoxville, Tenn.

XVII. Preparation of the silver salt of l-tyrosine.

Since this silver salt is light sensitive, this preparation was performed in subdued light. 18.1 g. of l-tyrosine (0.1 mole) was placed in a Morton flask with an efficient stirrer. A solution of 5.6 g. of potassium hydroxide (0.1 mole) in 300 cc. of water was added, and the stirrer started. Not all of the l-tyrosine dissolved. While stirring the suspension vigorously, a solution of 17 g. of silver nitrate (0.1 mole) in 200 cc. of water was added over a period of 15 minutes. The white precipitate was collected by filtering through a sintered-glass funnel, washed twice with water and finally washed thoroughly with three separate portions of acetone. Complete removal of water before drying resulted in a white product, which was dried in a vacuum oven (1 mm.) at 70° for 20 hours. The dried, colorless product weighed 27.0 g. It was analyzed for silver by the Volhard method (78).

Analysis	% Ag
Found:	35.20
Calc'd. for $C_9H_{10}O_3NAg$:	37.45

The discrepancy was believed due to unreacted l-tyrosine.

XVIII. Reaction of the silver salt of l-tyrosine with β-diethylaminoethyl bromide in di-isopropyl ether.

5.2 g. of recrystallized β-diethylaminoethyl bromide hydrobromide (0.02 mole) was dissolved in water. The

cold solution was made alkaline with cold sodium hydroxide solution and immediately extracted with 100 cc. of di-isopropyl ether. The ether solution was dried overnight over anhydrous calcium sulfate.

6.4 g. of white silver l-tyrosinate (0.02 mole of 90% purity) and the dried ether solution of β-diethylaminoethyl bromide were stirred in a flask in subdued light at 40-50° for 24 hours. The resultant brown mixture was filtered. All attempts to recover any recognizable product from the red-brown filtrate were to no avail.

XIX. Reaction of the silver salt of l-tyrosine with β-diethylaminoethyl chloride in absolute ethanol.

9.6 g. of silver l-tyrosinate (0.03 mole of 90% purity), 4.1 g. of freshly vacuum distilled β-diethylamino-ethyl chloride (0.03 mole) and 125 cc. of absolute ethanol were placed in a Morton flask. The colorless suspension was vigorously stirred for 6 hours at 50° in subdued light. The resultant chocolate-brown mixture was filtered to yield a red-brown filtrate. All attempts to recover any recognizable product from the filtrate were to no avail.

XX. Attempted synthesis of ethyl-l-tyrosinate by the silver salt method.

14.4 g. of silver-l-tyrosinate (0.05 mole), 5 cc. of

colorless ethyl iodide (0.062 mole) and 75 cc. of purified methyl isobutyl ketone were stirred in a Morton flask in subdued light at 35° for 19 hours. The initially colorless suspension had turned yellow. An additional 125 cc. of methyl isobutyl ketone were added, and the mixture was stirred and heated at 55-60° for 30 minutes and filtered through a sintered glass funnel to yield a colorless filtrate. The yellow residue was stirred thoroughly with 1N nitric acid to dissolve unreacted silver-l-tyrosinate. Analysis of this nitric acid solution for silver by the Volhard method showed that only 28% of the silver-l-tyrosinate had reacted.

The original colorless filtrate was concentrated in vacuo to 25 cc. and refrigerated overnight. There was no precipitation the next day. The remainder of the solvent was removed in vacuo and the very small amount of residual oil was dissolved in a small amount of hot ethyl acetate. Cooling of the ethyl acetate solution yielded no ethyl ester, although ethyl-l-tyrosinate crystallizes well from this solvent to give colorless crystals, m.p. 108-9°.

XXI. Preparation of carbobenzoxyglycine.

The benzyl chloroformate required as well as the carbobenzoxyglycine were both prepared according to the method of Farthing (55). From one mole of glycine, there was obtained 142 g. of purified carbobenzoxyglycine (68%).

XXII. Preparation of the silver salt of carbobenzoxyglycine.

62.7 g. of carbobenzoxyglycine (0.3 mole) was dissolved in a solution of 16.8 g. of potassium hydroxide (0.3 mole) in 1800 cc. of water. With vigorous stirring, a solution of 51.0 g. of silver nitrate (0.3 mole) in 500 cc. of water was added. Vigorous agitation was required as the precipitate was quite voluminous. The colorless silver salt was collected, air dried and finally placed in a vacuum oven at 50° for 20 hours. There resulted 76.0 g. (80.2%) of colorless silver salt, decomposing at 200°.

Analysis (78)	% Ag
Found:	33.96
Calc'd. for $C_{10}H_{10}O_4NAg$:	34.13

XXIII. Reaction of the silver salt of carbobenzoxyglycine with n-butyl bromide in absolute ethanol.

31.6 g. of silver carbobenzoxyglycinate (0.1 mole) was placed in a Morton flask and covered with 400 cc. of absolute ethanol containing 16.0 cc. of purified n-butyl bromide (0.125 mole). The mixture was stirred and refluxed for 8 hours, then filtered through a sintered-glass funnel to yield a colorless filtrate (A) and a residue (B).

The residue (B) was stirred on the sintered-glass funnel with three separate 100 cc. portions of 1N nitric acid to convert unreacted silver carbobenzoxyglycinate into soluble silver nitrate and insoluble carbobenzoxyglycine.

The nitric acid washings were collected to yield a filtrate (C) and a residue (D). A Volhard analysis (78) of this filtrate (C) showed that only 0.0383 moles (38.3%) of the original silver salt had reacted.

Residue (D) was stirred with dilute sodium hydroxide solution to dissolve the carbobenzoxyglycine and yield an alkaline filtrate (E) and a residue (F). Acidification of this filtrate gave 9.4 g. of carbobenzoxyglycine (0.045 mole). This represented a 73% recovery from the 0.0617 mole of unreacted silver carbobenzoxyglycinate. The residue of darkened silver bromide (F) weighed 6.9 g. (0.0377 mole).

The solvent ethanol and excess n-butyl bromide were removed in vacuo from the original filtrate (A). The oily residue was vacuum distilled at 0.5-1.0 mm and the following cuts were obtained:

60-180°-----2.4 g. of a yellowish, two layered oil.

180-185°----1.9 g. of colorless oil (G).

The oil (G) proved to be the wanted n-butyl ester of carbobenzoxyglycine. This represented a yield of 7.2% from the total silver salt used, or 18.7% from that amount which had actually reacted.

Analysis (77)	% N
Found:	5.25
Calc'd. for $C_{14}H_{19}O_4N$:	5.28

XXIV. Preparation of O,N-dicarboallyloxy-l-tyrosine.

The procedure of Stevens and Watanabe (62) was employed. The required allyl chloroformate was prepared from redistilled allyl alcohol and phosgene by the method used by Farthing (55) for the preparation of benzyl chloroformate.

The crude O,N-dicarboallyloxy-l-tyrosine was recrystallized from 25% ethanol at the rate of 66 cc./g. The hot solution was stirred while cooling to prevent separation of the product as an oil. The colorless product melted at 105-106°.

XXV. Removal of the carboallyloxy group by means of 36% HBr/glacial acetic acid.

3.5 g. of O,N-dicarboallyloxy-l-tyrosine (0.01 mole) was treated with the saturated HBr/acetic acid reagent according to the procedure of Ben-Ishai and Berger (56) for the removal of the carbobenzoxy group. There was recovered 2.6 g. of crude l-tyrosine hydrobromide (100%), which was recrystallized from water to yield 1.2 g. of pure l-tyrosine hydrobromide, decomposing at 255-257°. A mixed melting point with an authentic sample of l-tyrosine hydrobromide showed no change.

XXVI. Preparation of the silver salt of O,N-dicarboallyloxy-l-tyrosine.

1.75 g. of O,N-dicarboallyloxy-l-tyrosine (0.005 mole) was dissolved in 50% alcohol and 1.5 cc. of 3.5N ammonia

was added (0.0052 mole). While the clear solution was vigorously stirred at room temperature, 1N silver nitrate solution was added dropwise. Each drop caused a clouding which disappeared with stirring; when one cc. had been added, the cloudiness became permanent. The stirred solution was surrounded with an ice-bath; on cooling, very fine crystals appeared. More 1N silver nitrate was added dropwise to the cold mixture until a total of 5 cc. had been added (0.005 mole). The white solid was collected, washed with cold 50% ethanol and dried in the air at 50°. There was obtained 1.92 g. (84%) of the silver salt, which decomposed at 133.5-135.5°.

Analysis (78)	% Ag
Found:	23.33
Calc'd. for $C_{17}H_{18}O_7NAg$:	23.65

XXVII. Preparation of l-tyrosine methyl ester.

The methyl ester hydrochloride was prepared by the Fischer method in 90% yield. The dried hydrochloride was covered with ethyl acetate (17 cc./g.) in a stirred three-neck flask. While stirring and refluxing the suspension, gaseous ammonia was passed in until the hydrochloride had been neutralized. The hot mixture was filtered to remove the precipitated ammonium chloride and the filtrate cooled to yield the crystalline methyl ester. Concentration of the mother liquor gave another 20% of pure methyl ester. The combined

yield was 89.6% from the methyl ester hydrochloride.
M.P. 134.5-136°. $[\alpha]_D^{35^\circ}$ = + 25.80 in methyl alcohol (c = 5).

XXVIII. Attempted alcoholysis of l-tyrosine methyl ester with β-dimethylaminoethyl alcohol.

4.9 g. of l-tyrosine methyl ester (0.025 mole) was dissolved in 30 cc. (0.3 mole) of the dry, freshly vacuum distilled amino alcohol. The faintly yellow solution was heated on a steam bath for 7 hours. The excess amino alcohol was removed in vacuo. The red-brown glass-like residue was dissolved in 200 cc. of hot 50% alcohol and cooled in the refrigerator overnight. There was deposited one gram of l-tyrosine anhydride, m.p. 275-277° (25%). No additional product could be isolated.

XXIX. Attempted alcoholysis of l-tyrosine methyl ester hydrochloride with β-diethylaminoethyl alcohol.

4.6 g. of l-tyrosine methyl ester hydrochloride (0.02 mole) was dissolved in 40 cc. of freshly vacuum distilled amino alcohol (0.30 mole) by warming to 80°. The yellow colored solution was refluxed at 80° under a vacuum of 45 mm for 16 hours. On cooling, 2.9 g. of long, silky needles were deposited, which proved to be the amino alcohol hydrochloride. This represented a 95% yield based on the l-tyrosine methyl ester hydrochloride.

Concentration of the filtrate *in vacuo* once again resulted in about a 25% yield of l-tyrosine anhydride; no other product was recovered.

XXX. Attempted preparation and use of l-tyrosine-N-carboxy anhydride.

3.6 g. of l-tyrosine (0.02 mole) was covered with 100 cc. of purified dioxane. Phosgene was passed into the suspension, which was stirred at 30-40°. After 4 hours, all but a few particles of l-tyrosine had passed into solution. These particles were filtered out and the dioxane and excess phosgene were removed by heating *in vacuo* at 30-40°. There remained a feathery solid, which was dissolved in 100 cc. of dioxane; the resultant solution was cooled to 10°. A solution of 2.5 cc. of β-diethylaminoethyl alcohol (0.02 mole) in 100 cc. of dioxane was cooled to 10°. The amino alcohol solution was added in one portion to the dioxane solution of the l-tyrosine derivative. There was an immediate clouding; on standing, there was deposited 2.5 g. of β-diethylaminoethyl alcohol hydrochloride (81%).

XXXI. Preparation of l-tyrosinamide.

This intermediate was prepared according to the procedure of Yang and Rising (79) for α-amino acid amides. A solution of 100 g. of l-tyrosine methyl ester in one liter of dry methanol was saturated with dry ammonia and held at

room temperature for 72 hours. The methanol was removed in vacuo and the residue dissolved in 1100 cc. of hot isopropyl alcohol. On cooling, there was deposited 76.5 g. of a colorless solid. Concentration of the filtrate resulted in 8.6 g. of a second crop as pure as the first. The combined yield was 91.6% of l-tyrosinamide, m.p., 154-156°.

XXXII. Preparation of l-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone.

This l-tyrosine derivative was prepared according to the procedure given by Davis and Levy (71).

XXXIII. Reaction of l-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone, with methanol containing hydrogen chloride.

1.0 g. of the thio-thiazolidone was dissolved in 5 cc. of dry methanol. 10 cc. of a saturated solution of hydrogen chloride in methanol was added and the clear solution allowed to stand at room temperature overnight. The next day the methanol was removed by evaporation. 15 cc. of ethyl acetate was added to the solid residue and heated to boiling; dry ammonia was passed in and the hot, ammoniacal mixture was filtered. On concentrating and cooling, there was obtained 0.48 g. (59%) of partly racemized tyrosine methyl ester, m.p., 111-126°. $[\alpha]_D^{35^\circ} = +20.40$ in methyl alcohol (c = 5).

XXXIV. Attempted reaction of 1-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone with β-diethylaminoethyl alcohol hydrochloride.

1 g. (0.00418 mole) of the 2-thio-5-thiazolidone and 0.55 cc. (0.00418 mole) of β-diethylaminoethyl alcohol were dissolved in 10 cc. of purified dioxane, and 4 cc. of a saturated solution of hydrogen chloride in dioxane were added. The initially precipitated amino alcohol hydrochloride dissolved in the excess HCl/dioxane to yield a clear solution. This solution was allowed to stand at room temperature overnight.

The next day, the mixture was placed under vacuum to remove the excess HCl. A second liquid phase separated and then crystallized when cooled; there was collected 0.5 g. (78%) of β-diethylaminoethyl alcohol hydrochloride. No other product could be isolated from the filtrate.

XXXV. Preparation of the triethylamine salt of methyl-l-tyrosinate dithiocarbamate.

3.0 g. of l-tyrosine methyl ester was dissolved in 66 cc. of absolute ethanol and 6 cc. of carbon bisulfide and triethylamine each were added. The resultant solution was allowed to stand at room temperature for one hour, then placed in the refrigerator for an additional hour. 4.8 g. of white crystals (84%) of the triethylamine salt were collected.

XXXVI. Reaction of the triethylamine salt of methyl-l-tyrosinate dithiocarbamate with anhydrous hydrogen chloride.

0.5 g. of the triethylamine salt of methyl-l-tyrosinate dithiocarbamate was dissolved in 10 cc. of chloroform and dry hydrogen chloride passed in until precipitation occurred. The chloroform was decanted from the precipitate and 10 cc. of ethyl acetate was added to the solid residue. The ethyl acetate was brought to a boil, dry ammonia was passed in until ammoniacal, and the hot mixture filtered. On concentrating and cooling, there was obtained 0.2 g. (76%) of l-tyrosine methyl ester, m.p., 134-135°.

XXXVII. Attempted reaction of 1-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone with methanol containing triethylamine.

0.61 g. of 1-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone was dissolved in 10 cc. of pure methanol and 1.0 cc. of triethylamine was added. The solution was allowed to stand at room temperature for six hours. A seed crystal was introduced and the mixture was refrigerated overnight. There was no precipitation the next day. The solution was concentrated to about one-third of its original volume, an additional 1.0 cc. of triethylamine added, and allowed to stand at room temperature for another 6 hours; finally seeded and refrigerated overnight. Still no precipitation the following day. This method failed to yield any of the triethylamine salt of methyl-l-tyrosinate dithiocarbamate.

XXXVIII. Attempted reaction of 1-4-(p-hydroxybenzyl)-2-thio-5-thiazolidone with β-diethylaminoethyl alcohol.

1.0 g. of the thio-thiazolidone (0.0042 mole) and 1.1 cc. (0.0084 mole) of β-diethylaminoethyl alcohol were dissolved in 10 cc. of dioxane. The clear solution was allowed to stand at room temperature overnight. The next day, the solution was deeper yellow in color. However, it was not possible to recover any recognizable product from the mixture.

XXXIX. Attempted preparation of O,N-dicarboallyloxy-l-tyrosine acid chloride.

6.0 g. of pure O,N-dicarboallyloxy-l-tyrosine (0.0172 mole) was dissolved in 35 cc. of dry ether and 3.76 g. of phosphorus pentachloride (0.0180 mole) was added in one portion. The mixture was shaken at room temperature for 15-20 minutes in the apparatus described by Karrer and Henyemann (74), wherein all but a few particles of the phosphorus pentachloride had reacted and gone into solution. The mixture was allowed to stand at room temperature for one hour and the small amount of suspended matter removed by filtering through glass wool. The clear, slightly yellow solution was heated to boiling, and 70 cc. of light petroleum ether added. On cooling, an oil separated which could be crystallized by shaking. An additional 50 cc. of petroleum

ether was added to insure complete separation of the product. The crystals were collected and recrystallized an additional two times from ether-petroleum ether. There was obtained 3.8 g. of colorless crystals (60%), m.p., 35-45°.

Based on the method of preparation, this material, though impure, was undoubtedly the wanted acid chloride. However, it proved to be quite unstable. This sample was kept for 44 hours in a vacuum desiccator in the refrigerator with no apparent change. The desiccator and contents were allowed to warm to room temperature over a period of 3 hours. At the end of this time, the crystals disappeared with gas evolution; there was left a colorless oil. This oil was not the original material. It was difficultly soluble in boiling ether; and the addition of a few cc's of petroleum ether caused the separation of an oil which could not be induced to crystallize.

XL. Preparation of l-tyrosine hydrochloride.

10 g. of l-tyrosine was dissolved in 25 cc. of 3N hydrochloric acid by warming. 150 cc. of warm, concentrated hydrochloric acid was added in one portion and the solution allowed to crystallize in an ice-bath. 9.8 g. (82%) of colorless l-tyrosine hydrochloride was collected and dried at 105-110°. M.P.--231-234°.

XLI. Preparation of β-diethylaminoethyl alcohol hydrochloride.

46.6 cc. of a standard solution of hydrogen chloride in dioxane (0.00644 mole/cc.; 0.30 mole) was added dropwise to an externally cooled and stirred solution of 39.6 cc. (0.3 mole) of freshly vacuum distilled β-diethylaminoethyl alcohol dissolved in 400 cc. of purified dioxane. The white precipitate was collected on a sintered-glass funnel, washed well with ether, and dried in a vacuum desiccator. There was obtained 45.3 g. (98.5%) of colorless β-diethylaminoethyl alcohol hydrochloride. M.P.--134.5-136°.

XLII. Attempted esterification of l-tyrosine hydrochloride and β-diethylaminoethyl alcohol hydrochloride in dimethylformamide.

10.9 g. of l-tyrosine hydrochloride (0.05 mole) and 8.1 g. of β-diethylaminoethyl alcohol hydrochloride (0.0525 mole) were covered with 50 cc. of dimethylformamide. Solution was effected by heating to 90-100° and passing in dry hydrogen chloride. The solution was partly refluxed at 100° under a vacuum of 100 mm, the distillate being passed through an anhydrous calcium sulfate column on its return to the boiling solution. Heating was continued under these conditions for 65 hours. An aliquot portion of the deep-red solution was diluted with an equal volume of water and the pH adjusted to 5; there was no precipitation of l-tyrosine

(a 24 hour heating period resulted in a 14% recovery). On cooling, 6.0 g. of dimethylamine hydrochloride (0.0735 mole) was deposited and collected by filtration. The deep red filtrate was made alkaline with dry ammonia and the precipitated salts filtered out. The dimethylformamide was removed as well as possible in vacuo, leaving a red-brown oil. This oil was dissolved in hot 50% alcohol; on cooling, there was deposited 0.3 g. of l-tyrosine anhydride. Removal of the solvent from the filtrate left a red oil which was soluble in dilute hydrochloric acid. The pH was adjusted to 5. There was no phase change over a period of two days.

XLIII. Esterification of carbobenzoxyglycine and β-diethylaminoethyl alcohol p-toluene sulfonate in ethylene dichloride.

36.5 g. of carboxybenzoxyglycine (0.175 mole), 11.6 cc. of β-diethylaminoethyl alcohol (0.0875 mole) and 17.9 g. of p-toluene sulfonic acid monohydrate (0.0943 mole) were dissolved in 175 cc. of boiling ethylene dichloride. The solution was partly refluxed for 17 hours and the distillate passed through an anhydrous calcium sulfate column on its return to the boiling solution. The cooled solution was extracted with 5% carbonate solution, and the organic layer dried. The aqueous later was acidified to yield 17.0 g. (0.081 mole) of unreacted carbobenzoxyglycine.

The ethylene dichloride was removed *in vacuo* from the dried organic layer. There remained 23.7 g. of a deep yellow oil. A small portion of this oil was vacuum distilled @ 0.5-1.0 mm. The main part of the distillate was collected at 195-203° and was water insoluble, but soluble in acid solution. Reaction of this oil with 36% HBr/acetic acid reagent evolved copious amounts of gas. This seemed to be the desired crude ester; however, it was not characterized further due to the successful preparation of this ester by the succeeding procedure.

XLIV. Preparation of the β-diethylaminoethyl ester of carbobenzoxyglycine.

20.9 g. of carbobenzoxyglycine (0.10 mole) and 14.8 cc. of β-diethylaminoethyl chloride (0.10 mole) were dissolved by warming in 80 cc. of absolute isopropyl alcohol. The clear, colorless solution was refluxed for two hours and the isopropyl alcohol removed *in vacuo*, to leave a slushy, colorless mixture. This mixture was dissolved in 80 cc. of water and extracted with ether. The water layer (A) was retained and the ether layer was extracted with cold carbonate solution to give a water layer (B) and ether layer (C). Acidification of the water layer (B) precipitated 1.0 g. of unreacted carbobenzoxyglycine (5%). Removal of the ether *in vacuo* from the ether layer (C) gave 0.6 g. of a colorless oil, which was not characterized, but was probably the isopropyl ester of carbobenzoxyglycine.

The water layer (A) was made alkaline with cold carbonate solution and the oil which had separated was taken up in ether. The water layer was discarded and the ether layer dried. Removal of the ether in vacuo left a nearly colorless oil; vacuum distillation at 0.5-1.0 mm gave 20.6 g. (70%) of the pale yellow β-diethylaminoethyl ester of carbobenzoxy-glycine, distilling at 187-189°. n_D^{35} 1.5049.

Analysis (*)	% C	% H	% N
Found:	62.53	7.64	9.08
Calc'd. for $C_{16}H_{24}O_4N_2$:	62.31	7.85	9.09

XLV. Preparation of the β-diethylaminoethyl ester of O,N-dicarboallyloxy-l-tyrosine.

A solution of 17.5 g. (0.05 mole) of white O,N-dicarboallyloxy-l-tyrosine and 7.4 cc. (0.05 mole) of freshly vacuum distilled β-diethylaminoethyl chloride in 40 cc. of absolute isopropyl alcohol was refluxed for two hours. The isopropyl alcohol was removed in vacuo and the residual colorless oil was dissolved in 75 cc. of water and extracted with ether. The water layer (A) was retained and the ether layer was extracted with cold carbonate solution to give a water layer (B) and ether layer (C). Acidification of the water layer (B) gave 0.9 g. of unreacted O,N-dicarboallyloxy-l-tyrosine (5%). Removal of the ether in vacuo from the

* Analysis by F. Schwarzkopf, Woodside, N. Y.

dried ether layer (C) gave 0.1 g. of a colorless oil, not further characterized.

The water layer (A) was made alkaline with cold carbonate solution and the liberated oil taken up in ether. The water layer was discarded and the ether layer was dried. The ether was removed *in vacuo* to leave a colorless, non-distillable oil. This oil was placed under a vacuum of 1 mm at 60° for six hours to remove all volatile materials. There remained 17.2 g. (80.5%) of the colorless, very viscous β-diethylaminoethyl ester of O,N-dicarboallyloxy-l-tyrosine. n_D^{35} 1.5017.

Analysis (*)	% C	% H	% N
Found:	61.70	7.39	6.22
Calc'd. for $C_{23}H_{32}O_6N_2$:	61.58	7.19	6.25

XLVI. Preparation of the β-diethylaminoethyl ester of l-tyrosine.

16.5 g. (0.037 mole) of the β-diethylaminoethyl ester of O,N-dicarboallyloxy-l-tyrosine was covered at room temperature with 86 g. of a 36% solution of hydrogen bromide in glacial acetic acid (0.3 mole of HBr). There was an immediate exothermic evolution of carbon dioxide, the reaction being moderated by means of an ice-bath. After the gas

* Analysis by F. Schwarzkopf, Woodside, N. Y.

evolution seemed complete, the yellow colored solution was allowed to stand at room temperature for 30 minutes. 300 cc. of dry ether was added to precipitate a gum which was triturated with three separate 50 cc. portions of ether. This gummy residue was dissolved in 50 cc. of water and extracted twice with ether. The water layer was made alkaline with a cold, saturated solution of potassium carbonate and the liberated oil extracted into ether. The ether layer was dried and the ether removed in vacuo to leave a nearly colorless, non-distillable oil. This oil was placed under a vacuum of 1-2 mm at 60° for about two hours to remove volatile impurities. There was left 5.1 g. (50%) of the nearly colorless β-diethyl-aminoethyl ester of l-tyrosine. n_D^{35} 1.5104.

Analysis (*)	% C	% H	% N
Found:	64.34	8.56	9.83
Calc'd for $C_{15}H_{24}O_3N_2$:	64.26	8.63	9.99

Numerous attempts were made to prepare a solid salt derivative of this oily ester. The best results were obtained in an attempt to prepare the mono-hydrobromide. To a swirled solution of the ester in dioxane was added dropwise a standard solution of HBr/dioxane. The amount of HBr added represented only 75% of the theoretical amount for formation of the mono-hydrobromide; the addition of the remaining 25% caused the free-flowing solid that had separated to gum. This very

(*) Analysis by F. Schwarzkopf, Woodside, N. Y.

hygroscopic solid was collected and kept in a vacuum desiccator. A bromide analysis gave 22.9% (theory for the mono-hydrobromide---22.1%). The solid showed no sharp melting point, but had a range from 50-80°.

An attempt to prepare a mono-picrate was likewise a failure.

SUMMARY

Seven simple esters of 3,5-diiodo-l-tyrosine were prepared and characterized; the hydrochlorides of these esters were likewise obtained as crystalline solids. These iodinated esters are to be evaluated for possible use as diagnostic agents in X-ray visualization.

The silver-salt method was studied as a means of obtaining amino esters of the α-amino acid, l-tyrosine. No amino ester could be obtained from the silver salt. It was also shown that the simple ethyl ester could not be obtained by this method. The use of the silver salt of a "protected" α-amino acid, carbobenzoxyglycine, was likewise shown to give very poor yields of ester.

Five additional methods were shown to be of no value as a means of obtaining amino esters of l-tyrosine: 1), alcoholysis of the methyl ester of l-tyrosine; 2), use of l-tyrosine-N-carboxy anhydride; 3), use of the 2-thio-5-thiazolidone obtained from l-tyrosine; 4), use of the acid chloride of O,N-dicarboallyloxy-l-tyrosine; and 5), the direct esterification of l-tyrosine hydrochloride and the amino alcohol hydrochloride in dimethylformamide solution.

It was shown that ethylene dichloride could be used as a solvent for direct esterification of a "protected" α-amino acid and the salt of an amino alcohol. Further study of the conditions of this method are needed to completely characterize the products which were obtained.

The method of Horenstein and Pahlicke was found to be applicable to the preparation of β-amino esters of the "protected" α-amino acids, carbobenzoxyglycine and O,N-dicarboallyloxy-l-tyrosine. The β-amino ester of the α-amino acid was obtained by removing the "protecting" group with 36% HBr/acetic acid reagent. The synthesis of additional amino esters of α-amino acids by this procedure would probably show that it is quite generally applicable.

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