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AMINO ACID DERIVATIVES OF AMINOSALICYLIC ACIDS

By

ROBERT LEE HULL

**A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of**

DOCTOR OF PHILOSOPHY

at the

UNIVERSITY OF WISCONSIN

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TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION.	1
HISTORICAL.	3
Preparation of Aminosalicyclic Acids	3
Preparation of Derivatives of the Amino- salicyclic Acids	5
DISCUSSION.	19
EXPERIMENTAL.	34
A. Synthesis of 4-Nitrosalicyclic Acid.	34
2,4-Dinitrophenylacetic Acid.	34
Methyl 2,4-Dinitrophenylacetate	34
Isocamyl Nitrite	34
3-Carbomethoxy-6-Nitrobenzisoxazole	34
4-Nitrosalicylonitrile.	34
4-Nitrosalicyclic Acid	35
B. Synthesis of 5-Nitrosalicylamides	35
5-Nitrosalicylyl-dl-alanine	35
2-Acetoxo-5-Nitrobenzoic Acid	36
Ethyl 5-Nitrosalicylate	37
2-Benzoyloxy-5-nitrobenzoic Acid	37
Ethyl Glycine Hydrochloride	38

	<u>Page</u>
Ethyl dl-Alanine Hydrochloride	38
Ethyl dl-Leucine Hydrochloride	38
Ethyl 2-Benzylloxy-5-nitrobenzoylglycine. . .	39
2-Benzylloxy-5-nitrobenzoylglycine.	39
Ethyl 2-Benzylloxy-5-nitrobenzoyl-dl-leucine.	40
C. Synthesis of 4-Nitrosalicylamides.	41
Ethyl 4-Nitrosalicylate.	41
2-Benzylloxy-4-nitrobenzoic Acid.	41
Ethyl 2-Benzylloxy-4-nitrobenzoylglycine. . .	41
Ethyl 2-Benzylloxy-4-nitrobenzoyl-dl-alanine.	42
Ethyl 2-Benzylloxy-4-nitrobenzoyl-dl-leucine.	43
D. Synthesis of 3-Nitrosalicylamides.	44
Ethyl 3-Nitrosalicylate.	44
2-Benzylloxy-3-nitrobenzoic Acid.	44
Ethyl 2-Benzylloxy-3-nitrobenzoylglycine. . .	45
E. Reduction of the Nitrosalicylamides.	45
Ethyl 2-Benzylloxy-5-aminobenzoylglycine Hydrochloride.	45
Ethyl 2-Benzylloxy-5-aminobenzoyl-dl-leucine Hydrochloride.	46
Ethyl 2-Benzylloxy-4-aminobenzoylglycine Hydrochloride.	47
Ethyl 2-Benzylloxy-4-aminobenzoyl-dl-alanine.	47
Ethyl 2-Benzylloxy-4-aminobenzoyl-dl-leucine.	48
Ethyl 2-Benzylloxy-3-aminobenzoylglycine Hydrochloride.	48
SUMMARY.	50
BIBLIOGRAPHY	52

INTRODUCTION

Since the discovery by Lehmann (1, 2) that 4-aminosalicylic acid has a pronounced bacteriostatic effect upon Mycobacterium tuberculosis, his work has been confirmed by many independent groups who observed not only the action of 4-aminosalicylic acid but also the action of many related compounds. It was concluded by Hirt and Hurni (3), after comparing the activities of over one hundred derivatives and analogs of 4-aminosalicylic acid, that some derivative of this compound was probably formed in vivo which greatly extended its anti-tubercular activity beyond that expected. If this hypothesis is true some conjugates of 4-aminosalicylic acid with such naturally-occurring compounds as amino acids, peptides, sugars, aliphatic acids or others may be expected to be the active agent.

In general, substitutions on the carboxyl group of 4-aminosalicylic acid have not lowered the activity to any extent; substitutions on the amine group have lowered the activity only slightly while those on the hydroxyl group have lowered the activity considerably. It has also been shown by a number of investigators that 3- and

5-aminosalicylic acid possess antitubercular activity (2) but in a much lesser degree than 4-aminosalicylic acid.

The purpose of this research is to prepare a series of amino acid derivatives of 3-, 4-, and 5-aminosalicylic acids for pharmacological evaluation in an effort to find more active antitubercular agents.

HISTORICAL

Preparation of Aminosalicyclic Acids

One of the most straight-forward and logical methods of preparing aromatic amine compounds is by the reduction of the corresponding nitro compounds. In the case of the aminosalicyclic acids, the 3- and the 5- isomers are available from this source since the nitration of salicylic acid has been shown to result in a mixture of 3- and 5-nitrosalicyclic acid, which can be separated by their solubility characteristics (4, 5, 6).

These nitro compounds can then be reduced to the corresponding 3- and 5-aminosalicylic acids by the usual methods. Zinc dust in acetic acid (7), ammonium sulfide (8), and stannous chloride in hydrochloric acid (9) have been used successfully for these reductions.

4-aminosalicylic acid may also be made by the reduction of the corresponding nitro compound but in this case the nitro compound is not so readily available. One source of 4-nitrosalicylic acid has been reported by Borsche (10). This method consists of the formation of 3-carbomethoxy-6-nitrobenzisoxazole from the methyl ester of 2,4-dinitrophenylacetic acid followed by hydrolysis of the isoxazole.

The methyl ester of 2,4-dinitrophenylacetic acid is made from phenylacetic acid by nitration and subsequent esterification with methanolic hydrogen chloride (11).

4-nitrosalicylic acid has also been made by the diazotization and subsequent hydrolysis of 2-amino-4-nitrobenzoic acid (12).

The reduction of 4-nitrosalicylic acid to 4-aminosalicylic acid has been accomplished by standard

procedures. Tin and hydrochloric acid (13), hydrogenation over raney nickel (14), and hydrogenation over palladium (15) have been used with success.

4-aminosalicylic acid has also been prepared by variations of the Kolbe reaction (16, 17, 18). The starting material is m-aminophenol. A recent modification by Hultquist and Baginski (19) in which the ratio of water to m-aminophenol has been decreased from 10 to 1 to 2.5-5 to 1 is said to have increased the yield of 4-aminosalicylic acid from 61 to 85 per cent.

Preparation of Derivatives of the Aminosalicyclic acids.

Several hundred derivatives of the aminosalicyclic acids have been reported in the literature. A large portion of these are azo compounds of 5-aminosalicylic acid which have been synthesized and investigated as possible dyes or dye intermediates. The discussion of these compounds is beyond the scope of this thesis. The compounds that are included in the tables that follow

represent a selection of the more important types from a pharmaceutical standpoint and do not constitute a complete literature survey.

Probably the simplest derivatives from the preparative standpoint are the esters. These compounds have been prepared by three different methods. Simple esters have been prepared by direct esterification of the aminosalicylic acid using dry hydrogen chloride or concentrated sulfuric acid as catalyst. They have also been made in good yield by the reduction of the corresponding nitro esters. For the preparation of more complex esters the acid chlorides of the nitro acids with a "blocked" phenolic hydroxyl group have been caused to react with the complex alcohol under the influence of pyridine, followed by the reduction of the nitro group and removal of the blocking group.

Amides and simple substituted amides have been made by heating the acid or its ester in a sealed tube with ammonia or the appropriate amine. Amides have also been made through the intermediate nitro acid chloride with a "blocked" hydroxyl group as in the similar ester synthesis. Hydrazides have been made in the usual manner by the reaction of the ester with hydrazine or its salts.

N-acyl derivatives of the aminosalicylic acids have been made by the usual methods with the exception that the conditions must be controlled so that O-acylation

does not take place at the same time. In general, lower temperatures favor N-acylation over O-acylation. Both acid chlorides and anhydrides have been used as acylating agents. Related to these derivatives are the N-sulfonyl compounds or sulfonamides. The sulfonamides are prepared in a similar manner using sulfonyl chlorides.

N-alkylation of the aminosalicylic acids has been attained in some cases by reaction of the free acid with a sufficiently active alkyl or aryl halides. Good yields of N-alkylated aminosalicylic acids have been obtained by the process of reductive alkylation using alkyl aldehydes under reducing conditions.

Formation of imines has been accomplished by the reaction of aldehydes with the amino group of the aminosalicylic acid, usually in the presence of sodium acetate.

Ether formation with the phenolic hydroxyl group of the aminosalicylic acids has been accomplished by employing an ester of the acid in non-aqueous basic solution with the desired alkyl halide. Hydrolysis of the ester regenerates the etherified aminosalicylic acid.

Few esters of the phenolic function have been prepared. One method has been to acylate the corresponding nitrosalicylic acid and then reduce the nitro group to the amino group. O-acylation has also been obtained, in one instance at least, by heating the aminosalicylic acid with acetic anhydride in an inert solvent such as benzene.

The following tables list representative derivatives of the three aminosalicyclic acids under consideration. The references listed refer to sources which report the syntheses of the individual compounds.

Derivatives of 3-Aminosalicyclic Acid

1. Derivatives of the Carboxyl Group (R-COOH):

<u>Compound</u>	<u>Reference</u>
R-COOCH ₃	(20)
R-COOC ₂ H ₅	(20)

2. Derivatives of the Amine Group (R-NH₂):

<u>Compound</u>	<u>Reference</u>
R-NHCOH	(21)
R-NHCOCH ₂ N(C ₂ H ₅) ₂	(22)
R-NHCONH ₂	(21)
R-NHCOOC ₂ H ₅	(21)
R-NHSC ₂	(21)
R-NHSO ₂ NH ₂	(23)

<u>Compound</u>	<u>Reference</u>
$R-NHCH_2COOH$	(21)
$R-NH$ NO_2	(24)
$R-N=CH$	(21)
$R-N=CH$	(21)

3. Mixed Derivatives:

<u>Compound</u>	<u>Reference</u>
(25)

Derivatives of 4-Aminosalicylic Acid

1. Derivatives of the Carboxyl Group ($R-COOH$):

<u>Compound</u>	<u>Reference</u>
$R-COOCH_3$	(26)
$R-COOC_2H_5$	(26, 27)
$R-COOCH_2CH_2CH_3$	(26)
$R-COOCH(CH_3)_2$	(26)
$R-COO(CH_2)_3CH_3$	(26)

$R-COOCH_2CH(CH_3)_2$	(26)
$R-COO(CH_2)_4CH_3$	(26)
$R-COO(CH_2)_2CH(CH_3)_2$	(26)
$R-COOCH_2CH_2OH$	(26)
$R-COO(CH_2)_2N(CH_3)_2$	(28)
$R-COO(CH_2)_2N(C_2H_5)_2$	(28)
$R-COO(CH_2)_2N\langle\text{5}\rangle$	(28)
$R-COO(CH_2)_2N\langle\text{5}\rangle$	(28)
$R-COO(CH_2)_3N(CH_3)_2$	(28)
$R-COO(CH_2)_3N(C_2H_5)_2$	(28)
$R-COO(CH_2)_3N\langle\text{5}\rangle$	(28)
$R-COO(CH_2)_3N\langle\text{5}\rangle$	(28)
$R-CONH_2$	(26, 29)
$R-CONHC_2H_5$	(30)
$R-CONH(CH_2)_3CH_3$	(29)
$R-CONH\langle\text{6}\rangle$	(30)
$R-CONHCH_2COOH$	(30)
$R-CONHNH_2$	(26)

2. Derivatives of the Amino Group ($R-NH_2$):

<u>Compound</u>	<u>Reference</u>
$R-NHCOCH_3$	(26, 31)
$R-NHCOCH_2Cl$	(31)
$R-NHCOCHCl_2$	(31)
$R-NHCOCCl_3$	(31)
$R-NHCOCH=CHCOOH$	(31)
$R-NHCOCH_2CH_2COOH$	(31)
$R-NHCO$	(31)
$R-NHCO$	(31)
$R-NHSO_2$ NH_2	(26)
$R-NHSO_2$ $NHCOCH_3$	(26)
$R-NHCH_3$	(30)
$R-NHCH_2CH=CH_2$	(30)
$R-NHCH_2$	(30)
$R-N(C_2H_5)_2$	(30)

3. Derivatives of the Phenol Group (R-OH):

<u>Compound</u>	<u>Reference</u>
$R-OCH_3$	(32)
$R-OCOCH_3$	(31)

4. Mixed Derivatives:

<u>Compound</u>	<u>Reference</u>
(31)
(26)
(26)
(26)
(26)

(26)

(33)

(33)

(28)

(28)

(28)

(28)

Derivatives of 5-Aminosalicylic Acid

1. Derivatives of the Carboxyl Group (R-COOH):

<u>Compound</u>	<u>Reference</u>
R-COOCH ₃	(34)
R-COOC ₂ H ₅	(35)

2. Derivatives of the Amino Group (R-NH₂):

<u>Compound</u>	<u>Reference</u>
R-NHCONH ₂	(36)
R-NHCOCH ₂ COOC ₂ H ₅	(37)

3. Derivatives of the Phenol Group (R-OH):

<u>Compound</u>	<u>Reference</u>
$R-OCH_3$	(47)

4. Mixed Derivatives:

<u>Compound</u>	<u>Reference</u>
(48)
(46)
(25)
(47)
(47)

Several derivatives of 4-aminosalicylic acid were also listed by Hirt and Hurni (3) for which preparative procedures were not found in the chemical literature. These

include the following:

This historical survey was abstracted from the chemical literature up to January 1, 1952.

DISCUSSION

For the preparation of the amine acid derivatives of 3-, 4-, and 5-aminosalicylic acid, which involves amide formation between the carboxyl group of the aminosalicylic acid and the amino group of the amine acids, it was decided to utilize as starting materials the corresponding 3-, 4-, and 5-nitrosalicylic acids. The nitro acids offered the advantage of having no amino group to interfere in the proposed sequence of reactions and yet could be converted by reduction in the final step to the desired amine compound. The 3- and 5-nitrosalicylic acids are available commercially and were obtained from this source. 4-Nitrosalicylic acid is not commercially available and so was synthesized in the laboratory.

The method chosen for the laboratory syntheses of 4-nitrosalicylic acid was that of Borsche (10). Commercial phenylacetic acid was used as starting material. (For equations representing the preparation of 4-nitrosalicylic acid, see p. 4 of this thesis). Nitration gave an 85 to 87 per cent yield of 2,4-dinitrophenylacetic acid which in turn gave on esterification with methyl alcohol an 87 per cent yield of the methyl ester of 2,4-dinitrophenylacetic acid. Conversion of this

ester into 3-carbomethoxy-6-nitrobenzisoxazole by means of isoamyl nitrite and sodium in methyl alcohol resulted in a 53 to 58 per cent yield and not an 85 per cent yield as reported by Borsche.

Hydrolysis of 3-carbomethoxy-6-nitrobenzisoxazole with boiling 5 N hydrochloric acid resulted in an 89 per cent yield of 4-nitrosalicylonitrile which Borsche had obtained by alkaline hydrolysis. He reported that hydrolysis of the isoxazole with 5 N hydrochloric acid at 130° gave an 80 per cent yield of 4-nitrosalicylic acid. However this temperature cannot be obtained with boiling 5 N hydrochloric acid at atmospheric pressure.

Conversion of the 4-nitrosalicylonitrile into 4-nitrosalicylic acid was attained in 90 per cent yield by hydrolysis with 70 per cent sulfuric acid.

5-Nitrosalicylic acid was chosen as the compound with which to begin the synthetic studies, since it is more readily available than the other two nitrosalicylic acids involved.

Numerous attempts were made to prepare the acid chloride of 5-nitrosalicylic acid by using the sodium salt of the acid. Conventional procedures for preparing acid chlorides were not applicable because of the free phenolic hydroxyl group present in the molecule. Reaction of the sodium salt of 5-nitrosalicylic acid with thionyl chloride under various experimental conditions failed to

produce any isolatable acid chloride.

It was thought that probably the reactivity of the acid chloride made its isolation difficult if not impossible. Thus it was decided to use the crude reaction mixture after evaporation of solvent and excess thionyl chloride. In a typical example, the sodium salt of 5-nitrosalicylic acid was suspended in dry benzene. Excess thionyl chloride was added dropwise with stirring. After about 30 minutes, the mixture was evaporated to dryness under reduced pressure leaving a colorless residue thought to consist of a mixture of the acid chloride and sodium chloride. This crude product was then allowed to react with the amino acid under the usual Schotten-Baumann procedure in 10 per cent alkali. In only one case did this procedure yield any of the desired product. Using dl-alanine, 13 per cent of the desired 5-nitrosalicylyl-dl-alanine was obtained.

This procedure however was not dependable and could not be repeated with other amino acids. Variation in the experimental conditions was of no benefit in obtaining the desired reaction product. In all cases the main product isolated was the starting material, 5-nitrosalicylic acid.

The reaction of acetyl chloride with the sodium salt of 5-nitrosalicylic acid was investigated as a possible source of a reactive intermediate which would lead to the desired product.

This too failed to give the desired product and only 5-nitrosalicylic acid could be isolated from the reaction mixture. No attempt was made to isolate the intermediate mixed anhydride.

A synthesis similar to that used for making phenyl salicylate (50) was attempted using 5-nitrosalicylic acid, the amino acid and phosphorous oxychloride.

This synthesis also failed to give the desired product.

It was thought that if the phenolic hydroxyl group of the 5-nitrosalicylic acid could be successfully "blocked" with some substituent which could easily be removed in subsequent operations the desired synthetic reactions might be achieved. To this end 2-acetoxy-5-nitrosalicylic acid was synthesized in 70 per cent yield using acetic anhydride and catalytic amounts of sulfuric acid.

The acid chloride of 2-acetoxy-5-nitrobenzoic acid was then prepared using thionyl chloride in dry benzene. The acid chloride was not purified but was used as a semi-crystalline mass which was recovered after evaporation of the excess thionyl chloride and benzene. The crude acid chloride was allowed to react with the amino acid under conditions of the Schotten-Baumann reaction. In all cases, none of the desired product was obtained but the unacetylated 5-nitrosalicylic acid was the only product isolated. Amino acids used included L-glutamic acid, dl-alanine and glycine. That none of the desired products was obtained indicated that competing side reactions were occurring that used up the acid chloride. At the same

time hydrolysis of the acetoxy group was taking place. Under the aqueous alkaline conditions of the Schotten-Baumann reaction hydrolysis of the acetoxy group might be expected. Side reactions involving the acid chloride which might be expected to take place include hydrolysis to the acid and elimination of sodium chloride from the sodium salt of the amino acid and the acid chloride. The latter would also lead to the formation of the free acid. It should be noted here that Doub and coworkers (30) were able to prepare amides of 4-nitrosalicylic acid by reaction of 2-acetoxy-4-nitrobenzoyl chloride with amino compounds including glycine.

Clinton and coworkers (28), however, had published an article on alkylaminoalkyl esters of 4-amino-salicylic acid. In the course of their work they studied the H6renstein-P6hlicke reaction between 4-nitrosalicylic acid and dialkylaminoalkyl chlorides (58). They found that using an acetyl group to block the phenolic hydroxyl group was unsatisfactory because a mixture of compounds was obtained. A suitable blocking agent was found in the labile benzyl group which could subsequently be removed by hydrogenation. Furthermore, it was stated that excellent yields of 2-benzylloxy-4-nitrobenzoyl chloride could be obtained from the corresponding acid.

Thus it was decided to employ the benzyl group as the blocking agent for the phenolic hydroxyl group. Furthermore, it was decided to use the amino acid esters.

in nonaqueous solution to minimize the possibility of hydrolysis and other side reactions.

The benzylation of 5 nitrosalicylic acid was carried out in a manner similar to that used by Clinton and coworkers for 4-nitrosalicylic acid. The ethyl ester of 5-nitrosalicylic was allowed to react with benzyl chloride in ethanol in the presence of sodium carbonate and sodium iodide. The ester was not isolated but hydrolyzed directly by the addition of water to the reaction mixture. The overall yield of 2-benzyloxy-5-nitrobenzoic acid from ethyl-5-nitrosalicylate was 50 per cent.

Instead of using the amino acid esters as such, they were used as the hydrochloride salts, since the salts are solids and are more easily prepared and handled and are also more stable. The ethyl ester hydrochlorides of glycine, dl-alanine and dl-leucine were prepared by established methods. Glycine ethyl ester hydrochloride was prepared by the method of Harries and Weiss (51) in 91 per cent yield. dl-Alanine ethyl ester hydrochloride was prepared in 83 per cent yield by the method of Curtius

and Koch (52). dl-Leucine ethyl ester hydrochloride was obtained in 85 per cent yield by the method of Fischer (53). In each case the amino acid was suspended in absolute alcohol and dry hydrogen chloride was bubbled in until the solid present had all gone into solution.

Amide formation between 2-benzyloxy-5-nitrobenzoic acid and glycine ethyl ester hydrochloride was attained in moderate yield (39 per cent). The acid chloride of 2-benzyloxy-5-nitrobenzoic acid was prepared in benzene solution by reaction of the acid with thionyl chloride in the presence of pyridine. The glycine ethyl ester hydrochloride was then added to the benzene solution of the acid chloride at an optimum temperature of 40-50°. It was found that above this temperature considerable decomposition took place as evidenced by darkening of the reaction mixture. Below this temperature the reaction proceeded very slowly if at all. The product, ethyl-2-benzyloxy-5-nitrobenzoylglycine, was obtained by diluting the filtered benzene solution with Skellysolve B.

It was found that this ester, ethyl-2-benzoyloxy-5-nitrobenzoylglycine could be hydrolyzed to the corresponding acid by aqueous alkali in 83 per cent yield. Ordinarily, the ester group was allowed to remain intact in the final compounds since these products are more stable, especially in alcoholic solution into which dry hydrogen chloride is introduced.

Essentially the same procedure was followed in the attempted preparation of the ethyl alanine amide of 2-benzoyloxy-5-nitrobenzoic acid. However the crude reaction product was exceedingly difficult to purify even though several recrystallizations from benzene and aqueous isopropyl alcohol were employed. Analysis indicated the final product to be impure. In a second attempt to prepare the compound, the final benzene solution was washed with 5 per cent hydrochloric acid to remove any pyridine present and then with 10 per cent sodium carbonate solution to remove any acidic substances. However after drying and dilution of the benzene solution with Skellysolve the same impure product was obtained.

The ethyl leucine amide of 5-nitrosalicylic acid was obtained in 24 per cent yield by a modification of the

previous procedures. The final benzene solution of the reaction products was evaporated to dryness under reduced pressure. Washing of the residue with 5 per cent hydrochloric acid, 10 per cent sodium carbonate and water followed by several recrystallizations from aqueous alcohol gave pure ethyl-2-benzyloxy-5-nitrobenzoyl-dl-leucine.

Again the optimum temperature for the reaction was found to be 40-50°.

The preparation of 2-benzyloxy-4-nitrobenzoic acid according to the method of Clinton and coworkers (28) as previously mentioned offered no difficulties. An 88 per cent yield of the benzylated acid was obtained from the ethyl ester of 4-nitrosalicylic acid.

Preparation of ethyl-2-benzyloxy-4-nitrobenzoyl-glycine was achieved in 27 per cent yield. It was made in a manner similar to that used in the preparation of the corresponding 5-nitro compound previously described.

The ethyl dl-alanine amide of 2-benzyloxy-4-nitrobenzoic acid as well as the ethyl dl-leucine amide of 2-benzyloxy-4-nitrobenzoic acid were prepared in an analogous manner with minor variations in the technique of isolation and purification.

The benzylation of 3-nitrosalicylic acid in a manner similar to that used for the preparation of the benzylated 4- and 5-nitro-salicylic acid resulted in a yield of only 35 per cent.

The failure of this reaction to proceed in better yield may be attributed, at least in part, to the steric hindrance presented by the two substituents present in the

ring which are both ortho to the phenolic hydroxyl group. For some reason the resulting 2-benzyloxy-3-nitrobenzoic acid is not particularly stable. On drying a sample prepared for analysis at 100° considerable decomposition was evident. At room temperature the product could be dried under vacuum with no decomposition.

Ethyl-2-benzyloxy-3-nitrobenzoylglycine was obtained in only 3 per cent yield by the condensation of the acid chloride of 2-benzyloxy-3-nitrobenzoic acid with glycine ethyl ester hydrochloride in the presence of triethyl amine. None of the desired product was obtained when pyridine was used in place of triethyl amine.

The substituted amides of 5-nitrosalicylic acid were hydrogenated at 45° and 40 p.s.i. in the presence of 5 per cent palladium on charcoal in an attempt to remove the blocking benzyl group and at the same time reduce the nitro group to the amine. A solution was obtained, after removal of the catalyst, which darkened rapidly on standing. The procedure followed was essentially that of Clinton and coworkers (28) for the conversion of esters

of 2-benzyloxy-4-nitrobenzoic acid to the corresponding esters of 4-aminosalicylic acid. Rehydrogenation of the dark-colored solution produced a clear solution which gave a positive amine test with nitrous acid and resorcinol, a positive phenol test with ferric chloride and which, as before, darkened quickly on standing in air. The most probable explanation for this behavior is that the amino-phenol produced by hydrogenation is readily oxidized by atmospheric oxygen to the corresponding quinoneimine which may be then hydrolyzed to the quinone.

In every case in which the palladium reduction of the 2-benzyloxy-5-nitrobenzamides was applied, the theoretical amount of hydrogen was taken up but only the above results were obtained and attempted isolation of any product was fruitless.

It was then decided to allow the blocking benzyl group to remain on the compounds and to reduce the nitro group to the amine. This was also found desirable for all the final products in order to obtain the same types of derivatives from all the aminosalicylic acids for pharmacological evaluation.

The hydrogenation of ethyl-2-benzyloxy-5-nitro-

benzoylglycine was carried out with Adams' catalyst at room temperature and 40 p.s.i., using only enough hydrogen for reduction of the nitro group. The product, ethyl-2-benzyloxy-5-aminobenzoylglycine, was isolated as the hydrochloride in 71 per cent yield.

In a similar manner ethyl-2-benzyloxy-5-amino-di-leucine was prepared and isolated as the hydrochloride in 50 per cent yield.

Ethyl-2-benzyloxy-4-aminobenzoylglycine was prepared in the same manner by hydrogenation of the corresponding nitro compound over Adams' catalyst at room temperature and 40 p.s.i. It was isolated as the hydrochloride in 60 per cent yield.

Similarly was prepared ethyl-2-benzyloxy-4-aminobenzoyl-dl-alanine. The product was isolated as the free base in 65 per cent yield.

Ethyl-2-benzyloxy-4-aminobenzoyl-dl-leucine was prepared in a similar manner. The hydrochloride salt did not crystallize well however and the product was isolated as the free base in 97 per cent yield.

Ethyl-2-benzyloxy-3-nitrobenzoylglycine was reduced with hydrogen over Adams' catalyst at room temperature and 40 p.s.i. The product, ethyl 2-benzyloxy-3-aminobenzoylglycine, was isolated as the hydrochloride in 29 per cent yield. The low yield was due, at least in part, to the small quantities of material which were handled.

EXPERIMENTAL

A. Synthesis of 4-Nitrosalicylic Acid2,4-Dinitrophenylacetic Acid

The acid was prepared in 87% yield by the nitration of phenylacetic acid according to the method of Borsche (11). m.p. 177-179°.

Methyl 2,4-Dinitrophenylacetate

The ester was prepared in 87% yield by the esterification of 2,4-dinitrophenylacetic acid according to the method of Borsche (11). m.p. 82-83°.

Isoamyl Nitrite

Isoamyl nitrite was prepared in 81% yield from isoamyl alcohol, sodium nitrite and sulfuric acid according to the method of Noyes (54). b.p. 94-99°.

3-Carbomethoxy-6-Nitrobenzisoxazole

The compound was prepared in 53% yield by the method of Borsche (55) by the reaction of methyl 2,4-dinitrophenylacetate with isoamyl nitrite. m.p. 130-131°.

4-Nitrosalicylonitrile

In a 100-ml. round-bottom flask equipped with a reflux condenser was placed 4.5 g. (.020 mole) of 3-carbo-

methoxy-6-nitrobenzoxazole and 25 ml. of 5 N hydrochloric acid. The mixture was refluxed for 4 hours. The flask and contents were cooled in an ice-bath and the yellow-white precipitate was collected by filtration. Recrystallization from hot water gave 3.0 g. (83%) of light yellow needles, m.p. 160-161°, which agrees with the reported value (55).

4-Nitrosalicylic Acid

In a 100-ml. round-bottom flask equipped with a reflux condenser was placed 1.0 g. (.0055 mole) of 4-nitrosalicylonitrile and 25 ml. of 70% sulfuric acid. The mixture was heated to reflux for 20 minutes and then cooled in an ice bath. After dilution with 75 ml. of cold water the mixture was filtered by suction. The precipitate was recrystallized from water giving 0.90 g. (90%) of fine, cream-colored needles, m.p. 233-235°, which agrees with the reported value (10).

B. Synthesis of 5-Nitrosalicylamides

5-Nitrosalicylyl-dl-alanine

In a 200-ml. round-bottom three-necked flask equipped with a reflux condenser, mechanical stirrer, and dropping funnel was suspended 10.0 g. (.049 mole) of dry sodium 5-nitrosalicylate in 75 ml. of dry benzene. To this was added slowly through the dropping funnel 16.5 g.

(0.139 mole) of thionyl chloride. The mixture was stirred for 30 minutes at room temperature and then evaporated to dryness at reduced pressure, leaving an almost colorless residue. To the residue was added to a solution of 4.4 g. (.049 mole) of dl-alanine and 6.0 g. (0.150 mole) of sodium hydroxide in 125 ml. of water. The flask was stoppered and shaken vigorously for 15 minutes. The reaction mixture was then heated on a steam bath for 30 minutes. The warm solution was filtered to remove foreign particles and insoluble impurities. The filtrate was cooled and acidified with 1:1 hydrochloric acid. The white precipitate was collected by filtration and recrystallized from aqueous alcohol giving 6.9 g. of 5-nitrosalicylic acid, m.p. and mixed m.p. $228-9^{\circ}$. The filtrate was evaporated to one-half its original volume and allowed to cool. A slow crystallization took place. After 24 hours the solution was cooled in an ice bath and the precipitate was collected by filtration. Two recrystallizations from aqueous alcohol gave 1.5 g. (13%) of 5-nitrosalicylyl-dl-alanine, m.p. $184-186^{\circ}$.

Anal. Calcd. for $C_{10}H_{10}N_2O_6$: C, 47.25; H, 3.97.

Found: C, 47.06; H, 3.78.

2-Acetoxy-5-Nitrobenzoic Acid

In a 250-ml. Erlenmeyer flask was placed 5.0 g. (.027 mole) of 5-nitrosalicylic acid, 10.8 g. (.106 mole) of acetic anhydride and 0.5 ml. of sulfuric acid. The

mixture was shaken vigorously, allowed to stand 10 minutes and then cooled in an ice bath. The stiff paste which formed was diluted with 100 ml. of water. The mixture was shaken well and again cooled in an ice bath. The precipitate was collected by filtration and dissolved in 100 ml. of ether. The solution was filtered to remove impurities and 100 ml. of Skellysolve A was added. On cooling in an ice bath 4.2 g. (70%) of colorless crystals were obtained, m.p. 167-168°.

Anal. Calcd. for $C_9H_7NO_6$: C, 48.01; H, 3.13.

Found: C, 49.04; H, 3.23.

Ethyl 5-Nitrosalicylate

The ester was prepared in 85% yield by the esterification of 5-nitrosalicylic acid according to the method of Hirsch (56), m.p. 95-96°.

2-Benzylloxy-5-nitrobenzoic Acid

A mixture of 40.7 g. (0.194 mole) of ethyl 5-nitrosalicylate, 27.8 g. (0.22 mole) of benzyl chloride, 14.8 g. (0.4 mole, 0.28 equiv.) of anhydrous sodium carbonate, 3 g. of sodium iodide and 200 ml. of alcohol was stirred and refluxed for 8 hours. There was then added 41.2 g. (0.29 mole) of anhydrous sodium carbonate and 200 ml. of water, and stirring and refluxing were continued for an additional 8 hours. The insoluble precipitate of sodium bicarbonate was removed by filtration,

and the filtrate was evaporated to about one-half its original volume to remove most of the alcohol. The resulting solution was poured into 1 l. of cold water and acidified with concentrated hydrochloric acid. The precipitate was removed by filtration, washed with water and pressed as dry as possible. Additional product was obtained by concentration of the filtrate and cooling. The combined precipitates were recrystallized from isopropyl alcohol giving 26.3 g. (50%) of small colorless needles, m.p. 169-170°.

Anal. Calcd. for $C_{14}H_{11}O_5N$: C, 61.54; H, 4.06.

Found: C, 61.76; H, 3.95.

Ethyl Glycine Hydrochloride

The ester was prepared in 91% yield by the esterification of glycine in the presence of dry hydrogen chloride according to the method of Harries and Weiss (51). m.p. 142-143°.

Ethyl dl-Alanine Hydrochloride

The ester was prepared in 83% yield by the esterification of dl-alanine in the presence of dry hydrogen chloride according to the method of Curtius and Koch (52). m.p. 70-74°.

Ethyl dl-Leucine Hydrochloride

The ester was prepared in 85% yield by the esteri-

fication of dl-leucine in the presence of dry hydrogen chloride according to the method of Fischer (53).

m.p. 110-112°.

Ethyl 2-Benzoyloxy-5-nitrobenzoylglycine

To a stirred mixture of 10.0 g. (.0366 mole) of 2-benzoyloxy-5-nitrobenzoic acid, 9.25 g. (.117 mole) of pure, dry pyridine and 100 ml. of dry benzene was added dropwise 4.52 g. (.0380 mole) of thionyl chloride during a period of five minutes at room temperature. The heterogeneous mixture (heavy layer of pyridine hydrochloride) was stirred and refluxed for 15 minutes, cooled to 40°, and there was added slowly with stirring 5.11 g. (.0366 mole) of finely powdered ethyl glycine hydrochloride. After stirring for thirty minutes at 40-50° the mixture was filtered while warm to remove the pyridine hydrochloride. The filtrate was diluted with an equal volume of Skellysolve B and cooled in an ice bath. The resulting orange colored precipitate was removed by filtration and crystallized from alcohol after decolorization with Norite. Two recrystallizations from alcohol gave 5.1 g. (39%) of golden yellow plates, m.p. 147-148°.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06.

Found: C, 59.95; H, 4.78.

2-Benzoyloxy-5-nitrobenzoylglycine

In a 125-ml. Erlenmeyer flask was placed 3.9 g. (.0109 mole) of ethyl-2-benzoyloxy-5-nitrobenzoylglycine

and 33 ml. (.033 mole) of 1 N sodium hydroxide. The suspension was heated gently on a hot plate and stirred continuously until practically all the solid had gone into solution. The solution was filtered to remove solid impurities, cooled in an ice bath and acidified with concentrated hydrochloric acid. The resulting precipitate was removed by filtration and crystallized twice from alcohol giving 3.0 g. (83%) of small colorless crystals, m.p. 190-191°.

Anal. Calcd. for $C_{16}H_{14}N_2O_6$: C, 58.18; H, 4.27.

Found: C, 57.96; H, 4.26.

Ethyl 2-Benzoyloxy-5-nitrobenzoyl-dl-leucine

To a stirred solution of 10.0 g. (.0366 mole) of 2-benzoyloxy-5-nitrobenzoic acid, 9.25 g. (0.117 mole) of pure, dry pyridine and 150 ml. of dry benzene was added dropwise 4.52 g. (.0380 mole) of thionyl chloride during a period of five minutes. The heterogeneous mixture was stirred and refluxed for twenty minutes, cooled to 40°, and there was added slowly with stirring 7.16 g. (.0366 mole) of finely powdered ethyl dl-leucine hydrochloride. After stirring for two hours at 40-50° the mixture was filtered while warm to remove the pyridine hydrochloride. The filtrate was evaporated to dryness at reduced pressure and the residue was washed successively with 5% hydrochloric acid, 10% sodium carbonate, and water. The residue was dissolved in aqueous alcohol and the supernatant solu-

tion was decanted from a yellow oil which remained. On cooling, the alcoholic solution deposited yellow crystals. Two recrystallizations from alcohol gave 3.7 g. (24%) of fine colorless crystals, m.p. 126-127.5°.

Anal. Calcd. for $C_{22}H_{26}N_2O_6$: C, 63.75; H, 6.32.

Found: C, 64.56; H, 6.40.

C. Synthesis of 4-Nitrosalicylamides

Ethyl 4-Nitrosalicylate

The ester was prepared in 74% yield by the esterification of 4-nitrosalicylic acid according to the method of Borsch (10). m.p. 87°.

2-Benzoyloxy-4-nitrobenzoic Acid

The compound was prepared in 88% yield by benzylation and hydrolysis of ethyl-4-nitrosalicylate according to the method of Clinton (28). m.p. 171.5-173°.

Ethyl 2-Benzoyloxy-4-nitrobenzoylglycine

To a stirred solution of 10.0 g. (.0366 mole) of 2-benzoyloxy-4-nitrobenzoic acid, 9.25 g. (0.117 mole) of pure, dry pyridine and 150 ml. of dry benzene was added dropwise 4.52 g. (.0380 mole) of thionyl chloride during a period of five minutes. The heterogeneous mixture was stirred and refluxed for twenty minutes, cooled to 40°, and there was added slowly with stirring 5.11 g. (.0366 mole) of finely powdered ethyl glycine hydrochloride. After stirring for thirty minutes at 40-50° the mixture was filtered while warm to remove pyridine hydrochloride.

The filtrate was cooled and washed successively with 5% hydrochloric acid, 5% sodium carbonate, and water. After drying over Drierite the benzene solution was diluted with an equal volume of Skellysolve B and cooled in an ice bath. The resulting precipitate was removed by filtration and crystallized twice from alcohol giving 3.5 g. (27%) of pale yellow leaflets, m.p. 133-134°.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06.

Found: C, 59.97; H, 5.03.

Ethyl 2-Benzoyloxy-4-nitrobenzoyl-dl-alanine

To a stirred mixture of 10.0 g. (.0366 mole) of 2-benzoyloxy-4-nitrobenzoic acid, 9.25 g. (0.117 mole) of pure, dry pyridine and 150 ml. of dry benzene was added dropwise 4.52 g. (.0380 mole) of thionyl chloride during a period of five minutes. The heterogeneous mixture was stirred and refluxed for twenty minutes, cooled to 40°, and there was added slowly with stirring 5.62 g. (.0366 mole) of finely powdered ethyl dl-alanine hydrochloride. After stirring for thirty minutes at 40-50° and an additional 14 hours at room temperature, the mixture was filtered to remove pyridine hydrochloride. The filtrate was cooled and washed successively with 5% hydrochloric acid, 5% sodium carbonate, and water. The benzene solution was then evaporated to dryness at room temperature. The residue was recrystallized several times from alcohol giving 2.5 g. (18%) of pale yellow crystals, m.p. 72-74°.

Anal. Calcd. for $C_{19}H_{20}N_2O_6$: C, 61.28; H, 5.41.

Found: C, 62.81; H, 5.25.

Ethyl 2-Benzoyloxy-4-nitrobenzoyl-dl-leucine

To a stirred solution of 10.0 g. (.0366 mole) of 2-benzoyloxy-4-nitrobenzoic acid, 9.25 g. (0.117 mole) of pure, dry pyridine and 150 ml. of dry benzene was added dropwise 4.25 g. (.0380 mole) of thionyl chloride during a period of five minutes. The heterogeneous mixture was stirred and refluxed for twenty minutes, cooled to 40° , and there was added slowly with stirring 7.16 g. (.0366 mole) of finely powdered ethyl dl-leucine hydrochloride. After stirring for six hours at $40-50^{\circ}$ the mixture was filtered to remove the pyridine hydrochloride. The filtrate was cooled and washed successively with 5% hydrochloric acid, 5% sodium carbonate, and water. The benzene solution was then evaporated to dryness at room temperature leaving a yellow oil. The oil was dissolved in alcohol and allowed to stand at room temperature. After four days crystal growth had stopped. The solid was removed by filtration and recrystallized twice from alcohol, three to four hours being necessary for complete crystallization. A final recrystallization from alcohol gave 3.3 g. (22%) of almost colorless finely crystalline product, m.p. $75-77^{\circ}$.

Anal. Calcd. for $C_{22}H_{26}N_2O_6$: C, 63.75; H, 6.32.

Found: C, 64.67; H, 6.51.

D. Synthesis of 3-Nitrosalicylamides

Ethyl 3-Nitrosalicylate

The ester was prepared in 89% yield by esterification of 3-nitrosalicylic acid according to the method of Zacharias (57). m.p. 43.5-44.5°.

2-Benzyloxy-3-nitrobenzoic Acid

A mixture of 61.5 g. (0.291 mole) of ethyl 3-nitrosalicylate, 44.3 g. (0.350 mole) of benzyl chloride, 21.2 g. (0.200 mole, 0.400 equiv.) of anhydrous sodium carbonate, 4 g. of sodium iodide, and 315 ml. of alcohol was stirred and refluxed for nineteen hours. There was then added 59.4 g. (0.561 mole) of anhydrous sodium carbonate and 315 ml. of water, and stirring and refluxing were continued for an additional nine hours. The insoluble precipitate of sodium bicarbonate was removed by filtration and the filtrate was evaporated to about one-half its original volume to remove most of the alcohol. The resulting solution was poured into 1400 ml. of cold water and acidified with concentrated hydrochloric acid. The precipitate was removed by filtration, washed with water and pressed as dry as possible. Two crystallizations from isopropyl alcohol gave 28.0 g. (35%) of colorless crystals, m.p. 128-129°.

Anal. Calcd. for $C_{14}H_{11}O_5N$: C, 61.54; H, 4.06.

Found: C, 62.04; H, 4.26.

Ethyl 2-Benzoyloxy-3-nitrobenzoylglycine

To a stirred mixture of 10.0 g. (.0366 mole) of 2-benzoyloxy-3-nitrobenzoic acid, 11.3 g. (0.117 mole) of pure, dry triethyl amine and 150 ml. of dry benzene was added dropwise 4.52 g. (.0380 mole) of thionyl chloride during a period of five minutes. The mixture was heated to reflux but began to darken rapidly so was cooled to about 40°. There was then added slowly with stirring 5.11 g. (.0366 mole) of finely powdered ethyl glycine hydrochloride. After stirring for thirty minutes the mixture was filtered to remove the triethyl amine hydrochloride. The filtrate was diluted with an equal volume of Skellysolve B and cooled in an ice bath. The resulting reddish gummy precipitate was removed by filtration and crystallized from aqueous alcohol after decolorization with Norite. Two recrystallizations from aqueous alcohol gave 0.35 g. (37%) of almost colorless needles, m.p. 63-64°.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06.

Found: C, 60.56; H, 5.06.

E. Reduction of the Nitrosalicylamides

Ethyl 2-Benzoyloxy-5-aminobenzoylglycine Hydrochloride

A mixture of 0.75 g. (.00209 mole) of ethyl 2-benzoyloxy-5-nitrobenzoylglycine, 0.1 g. of Adams' catalyst, and 100 ml. of absolute alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical

amount of hydrogen was taken up in about fifteen minutes. The catalyst was removed by filtration and the filtrate was saturated with dry hydrogen chloride. The cooled solution was diluted with 100 ml. of dry ether and placed in a refrigerator for one hour. The white precipitate was removed by filtration, washed with dry ether, and recrystallized twice from alcohol-ether solution giving 0.55 g. (71%) of fine white feathery needles, m.p. 206-207°.

Anal. Calcd. for $C_{18}H_{21}N_2O_4Cl$: C, 59.26; H, 5.80.
Found: C, 59.27; H, 5.93.

Ethyl 2-Benzoyloxy-5-aminobenzoyl-dl-leucine Hydrochloride

A mixture of 2.15 g. (.0051 mole) of ethyl 2-benzoyloxy-5-nitrobenzoyl-dl-leucine, 0.1 g. of Adams' catalyst, and 100 ml. of absolute alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical amount of hydrogen was taken up in about fifteen minutes. The catalyst was removed by filtration and the alcoholic solution was saturated with dry hydrogen chloride. To the cooled solution was added 100 ml. of dry ether. When crystallization did not take place on cooling, the solution was evaporated to a volume of about 50 ml., and another 100 ml. of dry ether was added. The solution was placed in a refrigerator over night. White feathery crystals were obtained by filtration. Recrystallization from alcohol-ether gave 1.1 g. (50%) of white feathery crystals which darkened slightly on standing. m.p. 163-

165° d.

Anal. Calcd. for $C_{22}H_{29}N_2O_4Cl$: C, 62.77; H, 6.94.

Found: C, 62.02; H, 6.15.

Ethyl 2-Benzylloxy-4-aminobenzoylglycine Hydrochloride

A mixture of 3.58 g. (.0100 mole) of ethyl 2-benzylloxy-4-nitrobenzoylglycine, 0.1 g. of Adams' catalyst, and 150 ml. of absolute alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical amount of hydrogen was quickly taken up. The catalyst was removed by filtration and an excess of dry hydrogen chloride was passed into the resulting solution. The solution was cooled and 400 ml. of dry ether was added. After cooling overnight in a refrigerator the white precipitate was removed by filtration. Recrystallization from alcohol-ether solution gave 2.0 g. (60%) of fine white crystals, m.p. 162-164°.

Anal. Calcd. for $C_{18}H_{21}N_2O_4Cl$: C, 59.26; H, 5.80.

Found: C, 59.15; H, 5.87.

Ethyl 2-Benzylloxy-4-aminobenzoyl-dl-alanine

A mixture of 1.0 g. (.00269 mole) of ethyl 2-benzylloxy-4-nitrobenzoyl-dl-alanine, 0.1 g. of Adams' catalyst, and 50 ml. of alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical amount of hydrogen was quickly taken up. The catalyst was removed by filtration and the resulting solution was heated to boiling. Water was added until cloudiness appeared

and the solution was allowed to cool. After standing in a refrigerator over night, the precipitate was removed by filtration and recrystallized from aqueous alcohol giving 0.6 g. (65%) of small white crystals, m.p. 124-125°.

Anal. Calcd. for $C_{19}H_{22}N_2O_4$: C, 66.65; H, 6.48.

Found:

Ethyl 2-Benzylloxy-4-aminobenzoyl-dl-leucine

A mixture of 1.0 g. (.00241 mole) of ethyl 2-benzylloxy-4-nitrobenzoyl-dl-leucine, 0.1 g. of Adams' catalyst, and 50 ml. of absolute alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical amount of hydrogen was quickly taken up. The catalyst was removed by filtration and the resulting solution was heated to boiling. Water was added until cloudiness appeared and the solution was allowed to cool. The precipitate was removed by filtration and recrystallized from aqueous alcohol giving 0.9 g. (97%) of fine cream colored needles, m.p. 119-120°.

Anal. Calcd. for $C_{22}H_{28}N_2O_4$: C, 68.72; H, 7.34.

Found: C, 67.53; H, 7.27.

Ethyl 2-Benzylloxy-3-aminobenzoylglycine Hydrochloride

A mixture of 0.35 g. (.00098 mole) of ethyl 2-benzylloxy-3-nitrobenzoylglycine, 0.1 g. of Adams' catalyst, and 50 ml. of absolute alcohol was shaken with hydrogen at room temperature and 40 p.s.i. The theoretical amount of

hydrogen was taken up in about ten minutes. The catalyst was removed by filtration and the filtrate was saturated with dry hydrogen chloride. The cooled solution was diluted with 200 ml. of dry ether and placed in an ice bath for one hour. The white precipitate was removed by filtration and recrystallized from alcohol-ether solution giving ca. 0.1 g. (29%) of fine white crystals, m.p. 102-103°.

Anal. Calcd. for $C_{18}H_{21}N_2O_4Cl$: C, 59.26; H, 5.80.

Found: C, 59.02; H, 5.96.

SUMMARY

1. A number of amino acid amides of 3-, 4-, and 5-aminosalicylic acid have been prepared for pharmacological evaluation as antitubercular agents.

2. A general method for the preparation of amino acid amides of the aminosalicylic acids has been devised which consists of acid chloride formation of the O-benzylated nitrosalicylic acid, followed by condensation with the amino acid ester hydrochloride. Catalytic reduction converts the nitro group to the amine.

3. The following new compounds have been prepared:

- a. 5-Nitrosalicylyl-dl-alanine.
- b. 2-Acetoxy-5-nitrobenzoic acid.
- c. 2-Benzyloxy-5-nitrobenzoic acid.
- d. Ethyl 2-benzyloxy-5-nitrobenzoylglycine.
- e. 2-Benzyloxy-5-nitrobenzoylglycine.
- f. Ethyl 2-benzyloxy-5-nitrobenzoyl-dl-leucine.
- g. Ethyl 2-benzyloxy-4-nitrobenzoylglycine.
- h. Ethyl 2-Benzyloxy-4-nitrobenzoyl-dl-alanine.
- i. Ethyl 2-benzyloxy-4-nitrobenzoyl-dl-leucine.
- j. 2-Benzyloxy-3-nitrobenzoic acid.
- k. Ethyl 2-benzyloxy-3-nitrobenzoylglycine.

- l. Ethyl 2-benzyloxy-5-aminobenzoylglycine hydrochloride.
- m. Ethyl 2-benzyloxy-5-aminobenzoyl-dl-leucine hydrochloride.
- n. Ethyl 2-benzyloxy-4-aminobenzoylglycine hydrochloride.
- o. Ethyl 2-benzyloxy-4-aminobenzoyl-dl-alanine.
- p. Ethyl 2-benzyloxy-4-aminobenzoyl-dl-leucine.
- q. Ethyl 2-benzyloxy-3-aminobenzoylglycine hydrochloride.

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