of $\alpha$-aminotricarballylic acid, the $\omega$-configuration of each of the diastereomeric amino acids presented in Table II has been satisfactorily established. Since it was previously noted[8] that the change in the partial rotation of the $\omega$-asymmetric center, from water to acid, was in the same direction for several amino acids of identical $\omega$-configuration, it becomes of interest here to explore briefly whether a comparable consistent behavior with regard to the partial rotation of this center is indicated in the present rotatory dispersion studies. Thus, at all of the wave lengths herein investigated, the direction of the contribution of the $\omega$-center of the L-antipodes of alloisoleucine,[13] O-methylthreonine, threonine,[14] hydroxyproline[15] and phenylserine,[16] all of which have an $\omega$-D-configuration, is negative in every instance. It is of additional interest to note that in the case of the latter three diasymmetric amino acids, all of which contain a hydroxyl group on the $\omega$-asymmetric carbon atom, there is an apparently consistent increase in the magnitude of rotation with decreasing wave length. In the absence of more abundant data, however, it would be both superfluous and indiscreet to speculate whether such consistencies can be correlated with optical configuration or are merely fortuitous.

**Reliability of Optical Rotation for Configurational Determination.**—That the use of optical or rotatory dispersion data for the configurational determination of the $\alpha$-asymmetric center of a diasymmetric amino acid requires more data, and is somewhat more complex, than the utilization of these same methods for the determination of the configuration of an amino acid with a single center of asymmetry, becomes readily apparent. In any event, since the use of such data is contingent upon different magnitudes, and sometimes varying shades of optical rotation, these rotational values must, of necessity, be derived from amino acids of the highest degree of optical and chemical purity. With the sole exception of the stereomers of aminotricarballylic acid which could not be so measured, routine purity determinations with amino acid oxidases, developed in this Laboratory,[17] demonstrated that the optical rotation values, on which the foregoing calculations were based, were determined with amino acids with a minimal optical purity of 99.9%. By a somewhat indirect method whereby the four stereomers of aminotricarballylic acid were converted to the corresponding isocitric acid lactones[18] and then subjected to the action of the highly specific isocitric dehydrogenase-triphosphopyridine nucleotide system,[19] no detectable optical contamination was indicated.

Dependent upon the magnitude and the direction of the contribution to the total molar rotation of the $\omega$-center of asymmetry, the use of optical data can be expected to give the $\alpha$-configuration of at least one and sometimes both amino acids of a diastereomeric pair. In the absence of other data, calculations based on the four theoretical *l,d*-allo, *l,l*-allo, *d,l*-allo, and *d,d*-allo combinations can be effected, in a manner shown previously,[8] in order to permit a proper choice of the L,L-allo diastereomeric pair.

(13) M. Winitz, S. M. Birnbaum and J. P. Greenstein, THIS JOURNAL, **77**, 3106 (1955).
(14) C. E. Meyer and W. C. Rose, *J. Biol. Chem.*, **115**, 721 (1936).
(15) A. Neuberger, *J. Chem. Soc.*, 429 (1945).
(16) W. S. Fones, *Arch. Biochem. Biophys.*, **36**, 486 (1952).
(17) A. Meister, L. Levintow, R. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **192**, 535 (1951).
(18) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955).
(19) A. Adler, H. von Euler, G. Günther and M. Plass, *Biochem. J.*, **33**, 1028 (1939).

BETHESDA, MARYLAND

---

[CONTRIBUTION FROM THE U. S. PLANT, SOIL AND NUTRITION LABORATORY, AGRICULTURAL RESEARCH SERVICE]

# The Identification of (+)S-Methyl-L-cysteine Sulfoxide in Plants

BY CLAYTON J. MORRIS AND JOHN F. THOMPSON

RECEIVED OCTOBER 19, 1955

An unusual amino acid has been isolated from turnip roots using ion-exchange methods. This compound has been shown to be (+)S-methyl-L-cysteine sulfoxide by comparison with synthetic material. It has been shown that this compound did not arise as an artifact during the isolation procedure. Quantitative data on the amount of S-methylcysteine sulfoxide in turnips and related plants are presented.

A brief report[1] has been made of the isolation of an amino acid from turnip roots and its identification as (+)S-methyl-L-cysteine sulfoxide. A complete account of this work is presented here along with further evidence of its occurrence in other crucifers.

**Detection, Isolation and Identification of (+)S-Methyl-L-cysteine Sulfoxide.**—In studying the amino acids of the non-protein fraction of turnip roots (*Brassica rapa*) by chromatography on paper using butyl alcohol–acetic acid and phenol,[2] a prominent ninhydrin-reactive substance was noted next to glutamine. This material was striking because of the relatively large amount present and the unusual brownish-blue color it produced with ninhydrin. This compound gave $R_F$ values of 0.30 in collidine, of 0.62 in phenol and 0.09 in butyl alcohol–acetic acid, was unstable to acid hydrolysis, and behaved like an $\alpha$-amino acid on passage

(1) C. J. Morris and J. F. Thompson, *Chemistry & Industry*, 951 (1955).
(2) R. J. Block, E. L. Durrum and G. Zweig, "A Manual of Paper Chromatography and Paper Electrophoresis," Academic Press, New York, N. Y., 1955, pp. 77–80.

through a column of copper carbonate and alumina.[3]

The alcohol-soluble amino acids of turnip roots were purified and then fractionated in the manner of Partridge and Westall.[4] The only contaminant, aspartic acid, was removed by passage through Amberlite Resin IR-4B and the unknown compound was purified by recrystallization.

The nitrogen content of the isolate by both the Kjeldahl method and by ninhydrin activity[5] was identical and hydrolysis yielded approximately half of the nitrogen as ammonia. Although the latter suggested the presence of an amide group, the fact that all the nitrogen was active toward ninhydrin made a labile amino group more reasonable. Rapid reduction of potassium permanganate by the isolate was found to be due to the presence of sulfur in the molecule. An ultimate analysis gave an empirical formula of $C_4H_9O_3NS$ and molecular weight determinations indicated that this was a four carbon compound. Reduction of the compound with hydriodic acid[6] showed the presence of a sulfoxide group and indicated that most likely the isolated material was S-methylcysteine sulfoxide. S-Methyl-L-cysteine sulfoxide was synthesized, the diasteroisomers separated, and the dextrorotatory form was found to be identical with the isolate by chromatography in three solvent systems, melting point, optical rotation and infrared spectra. The product of reduction with hydriodic acid proved to be identical with synthetic S-methyl-L-cysteine.

The possibility that the sulfoxide had arisen from S-methylcysteine by oxidation during the isolation was considered because methionine is readily oxidized to its sulfoxide, particularly in the presence of acids,[6] a condition obtained on the acid resins. To test this, a small amount was isolated in a relatively crude state. This material had a specific rotation of +114°, which is close to that (125°) of the purest isolate. Since the synthetic material had no optical rotation, this suggests that possibly some S-methylcysteine was oxidized during the isolation, but that over 95% of the isolate was present in the original tissue.

S-Methylcysteine sulfoxide has an odor characteristic of turnips and related plants, and it may account for much of this odor. Toennies and Kolb[6] have characterized the odor of methionine sulfoxide, the next higher homolog, as that of "boiled cabbage."

The products of acid hydrolysis of methylcysteine sulfoxide were investigated because of the unusual ease of breakdown of this compound. One prominent product of the hydrolysis, other than ammonia, was methylcysteine which accounted for nearly half of the ninhydrin activity.

Examination of acid hydrolysates of turnip proteins gave no evidence of methylcysteine indicating that neither it nor its sulfoxide occurred in the protein.

## Experimental

**Isolation Procedure.**—Three bushels of turnip roots were ground with an equal weight of 95% ethanol and allowed to stand three days. The extract was filtered and the extraction repeated twice with 10 gallons of 50% ethanol as before. The combined extracts were put through a column of acidic ion-exchange resin (Dowex-50-X4, 50–100 mesh) in the acid form until ninhydrin active materials were present in the effluent. All previous effluents containing sugars and anions were discarded and the resin column was washed with water until negative sugar and chloride tests were obtained. The resin was then eluted with 2 *N* $NH_4OH$. The excess ammonia was removed by passage through a column of carboxylic ion-exchange resin, IRC-50. The resultant solution contained neutral and acidic amino acids and very little else.

The amino acids were then adsorbed on a column of Dowex-50-X4 (200–400 mesh) in such a way that about one-third of the resin was saturated with amino acids. The amino acids were displaced from the resin with 0.15 *N* $NH_4OH$ as in the procedure of Partridge and Westall.[4] The effluent was collected in fractions and the unknown compound was found by paper chromatography to be one of the first to be eluted from the column.

The majority of the unknown was separated from the other amino acids, with a small portion being contaminated with aspartic acid. The aspartic acid was removed by passing the solution through a column of a weakly basic resin (Amberlite IR-4B). The effluent was concentrated, and the unknown crystallized on the addition of ethanol. After four recrystallizations from aqueous ethanol, about 5 g. of pure material was obtained, m.p. about 164° dec., $[\alpha]^{25}D$ +125 (*c* 2.5).

*Anal.* Calcd. for $C_4H_9O_3NS$: C, 31.75; H, 6.00; N, 9.27; S, 20.15; mol. wt., 151. Found: C, 32.10; H, 6.18; N, 9.50; S, 20.35; mol. wt., 126.

**Identification of Unknown Compound.**—Adsorption on a mixture of copper carbonate and alumina[3] had indicated that the unknown compound was an α-amino acid. Instability to acid hydrolysis, with the liberation of ammonia, suggested that an amide group was also present. The isolate was treated under conditions of amide hydrolysis (3 hours at 100° in 1 *N* HCl) and the ammonia was distilled off after the addition of NaOH; 48.5% of the nitrogen was recovered as ammonia. The liberation of ammonia was repeated under mild conditions[7] (*p*H 8.5 and 40°) with the same result. The nitrogen content, as measured by micro-Kjeldahl procedure, was 9.50%, which was identical with the value obtained by ninhydrin reaction.[5] Since amide groups do not usually react with ninhydrin, the presence of an amide group seemed unlikely.

The proof that there was no amide group was obtained as follows. The unknown compound was not adsorbed by passage through a mixed bed of weakly basic and weakly acidic resins (IR-4B and IRC-50) which absorbs all acidic and basic amino acids. The compound was therefore neutral, having an amino group for each carboxyl group. A formol titration of the unknown gave an equivalent weight of 150.2, which is close to the minimal molecular weight of 147.4, calculated from the nitrogen content. If there were also an amide group present, the equivalent weight would have to be 300 or more. Therefore, every nitrogen must be in an amino group.

The isolated material reduced potassium permanganate readily in the cold. Catalytic hydrogenation with palladium on charcoal was negative. Fehling and Tollen tests for aldehyde groups were negative. The presence of sulfur was confirmed by the use of lead acetate after sodium fusion. Sulfur would vitiate the hydrogenation test.

The molecular weight as determined by freezing point lowering in water was 126. The nitroprusside test showed no sulfhydryl or disulfide groups.

S-Methylcysteine was prepared by modification of the methods of Clarke and Inouye[8] and of du Vigneaud, Loring and Craft.[9] The product after several recrystallizations had $[\alpha]^{25}D$ −30.0° (*c* 2.5). This was oxidized with hydrogen peroxide to the sulfoxide.[6] In contrast to the formation of methionine sulfoxide at room temperature,[6] the oxidation of methylcysteine with hydrogen peroxide required warm-

(3) J. F. Thompson, J. K. Pollard and F. C. Steward, *Plant Physiol.*, **28**, 401 (1953).

(4) S. M. Partridge and R. G. Westall, *Biochem. J.*, **44**, 418 (1949).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(6) G. Toennies and J. J. Kolb, *ibid.*, **128**, 399 (1939).

(7) G. W. Pucher, H. B. Vickery and C. S. Leavenworth, *Ind. Eng. Chem., Anal. Ed.*, **7**, 152 (1935).

(8) H. T. Clarke and J. M. Inouye, *J. Biol. Chem.*, **94**, 541 (1931–1932).

(9) V. du Vigneaud, H. S. Loring and H. A. Craft, *ibid.*, **105**, 481 (1934).

ing at 50–60° for two hours. The product of this reaction had no optical rotation.

The diastereoisomers were separated by fractional crystallization from ethanol and water. The pure (+)S-methyl-L-cysteine sulfoxide gave a specific rotation of $[\alpha]^{25}D$ +124° (*c* 2.5), and the L(−) isomer had a specific rotation of $[\alpha]^{25}D$ −127° (*c* 2.5). The isolated material and the synthetic L(+) compound gave identical infrared spectra, melting points and mixed melting points. They were inseparable on paper in phenol–water, collidine–lutidine and butyl alcohol–acetic acid.

Eight hundred mg. of the isolate was reduced by dissolving in 20 ml. of freshly distilled 47% hydriodic acid. After three hours, at room temperature, the solution was diluted and most of the iodine was removed by extraction into ether. The remaining iodine and hydriodic acid were removed by passage through a weakly basic resin (IR-4B). The S-methylcysteine was recrystallized several times from aqueous methanol. The reduced isolate and the synthetic S-methyl-L-cysteine gave identical infrared patterns, were inseparable by paper chromatography in phenol, collidine–lutidine and butyl alcohol–acetic acid, and gave the same melting point (236°) with decomposition. The specific rotation of the reduced unknown was $[\alpha]^{25}D$ 28.0° (*c* 2.5).

**Preparation of Crude Concentrate.**—It seemed possible that the dextro and levo rotating isomers could have been separated, had they both been present, during the purification of the unknown. It was evident from the chromatographic data that the unknown came off the column in only one band. Some of the impure material from this fraction was dissolved in as little water as possible and then precipitated out by the addition of about 100 times the volume of absolute ethanol. This removed considerable of the colored material without any appreciable amount of ninhydrin active material remaining in the supernatant. The optical rotation on this relatively impure material gave a specific rotation of $[\alpha]^{25}D$ +114° (*c* 2.5) showing that over 95% was in the dextrorotatory form.

**Acid Hydrolysis of S-Methylcysteine Sulfoxide.**—S-Methylcysteine sulfoxide was hydrolyzed with 1 *N* HCl for 3 hours at 100°. The HCl was driven off and the residue chromatographed on paper. The methylcysteine sulfoxide had disappeared and the main ninhydrin activity appeared to be due to methylcysteine. The identity of methylcysteine was confirmed by chromatography in several solvents. By quantitative chromatography,[10,11] the amount of methylcysteine was found to account for 38% of the original material. It seemed likely that acid breakdown of the methylcysteine accounted for the figure being below 50%. There was also a trace of alanine present, which probably resulted from removal of sulfhydryl groups from cysteine and methyl mercapto groups from methylcysteine.

**The Occurrence of S-Methylcysteine Sulfoxide in Plants.**—S-Methylcysteine sulfoxide is a relatively prominent constituent of the amino acid fraction of turnips. Table I shows its contribution to the soluble nitrogen fraction as compared with some of the common amino acids in turnip leaves and roots. S-Methylcysteine sulfoxide also contributes most of the amino acid sulfur in turnip leaves and half of it in the roots (Table I).

TABLE I

PARTIAL AMINO ACID COMPOSITION OF NON-PROTEIN FRACTION OF TURNIP LEAVES AND ROOTS

| Compound | Total soluble nitrogen, % Leaf | Root | Total soluble amino acid sulfur, % Leaf | Root |
|---|---|---|---|---|
| Methylcysteine sulfoxide | 3.7 | 1.5 | 87.4 | 50.5 |
| Cysteine[12] | 0.30 | 1.3 | 7.1 | 42.9 |
| Methionine sulfoxide[a] | 0.23 | 0.20 | 5.4 | 6.6 |
| Aspartic acid | 4.2 | 2.9 | | |
| Glutamic acid | 5.8 | 5.1 | | |
| Serine | 5.9 | 3.5 | | |
| Threonine | 2.6 | 2.4 | | |
| Alanine | 4.4 | 3.6 | | |
| Valine | 1.1 | 1.4 | | |

[a] Methionine content was so low that satisfactory values could not be obtained by microbiological assay with *Leuconostoc mesenteroides*.[12]

Synge and Wood[13] have reported the occurrence of S-methylcysteine sulfoxide in cabbage leaves. This and several other plants related to the turnip have been examined for the presence of the sulfoxide. The results as shown in Table II indicate not only the widespread presence of the sulfoxide in other plants but that the turnip is a relatively poor source. The data on the sulfoxide content of cabbage leaves is close to the value obtained by Synge and Wood[13] of 4.2% of total soluble nitrogen. These data also demonstrate that the sulfoxide is a more prominent constituent of the non-protein fraction in young turnip tissue than in old.

The data of Table II also reveal that methylcysteine sulfoxide is in much higher concentrations than is cysteine in the non-protein fraction of crucifers.

TABLE II

THE CYSTEINE AND S-METHYLCYSTEINE SULFOXIDE CONTENT OF NON-PROTEIN FRACTION OF SEVERAL CRUCIFERS

| Plant | Tissue | Cysteine content μg./g. fresh wt. | S-Methylcysteine content μg./g. fresh wt. | S-Methylcysteine content Total soluble nitrogen, % |
|---|---|---|---|---|
| Turnip | Old leaves | 6.4 | 79 | 3.1 |
| Turnip | Middle aged leaves | 6.5 | 100 | 3.7 |
| Turnip | Young leaves | 6.1 | 167 | 4.4 |
| Turnip | Old root | 29.5 | 43 | 1.5 |
| Turnip | Young root | 15.3 | 202 | 5.1 |
| Cabbage | Leaves | 12.0 | 304 | 5.3 |
| Chinese cabbage | Leaves | 23.3 | 396 | 5.5 |
| Cauliflower | Flowers | 57.2 | 2380 | 20.2 |
| Kohlrabi | Leaves | 5.2 | 558 | 13.1 |
| Kohlrabi | Stem | 8.5 | 1069 | 9.3 |
| Radish | Root | 14.3 | 117 | 4.8 |
| Mustard | Leaves | 7.2 | 297 | 4.1 |
| Broccoli | Flowers | 35.3 | 2406 | 11.6 |
| Broccoli | Stem | 40.2 | 851 | 5.9 |
| Broccoli | Leaves | 8.3 | 1770 | 15.0 |

Examination of extracts of bean and potato leaves have not revealed the presence of any sulfoxide. Our experience with non-crucifiers indicates the sulfoxide to be a minor constituent of the non-protein fraction if it is present at all.

It appears reasonable that S-methylcysteine sulfoxide is formed in the plant by oxidation of S-methylcysteine. However, a careful examination of the plants listed in Table II revealed no methylcysteine. This fact suggests that either the sulfoxide is formed by another route or that methylcysteine is rapidly converted to the sulfoxide.

**Analysis for S-Methylcysteine Sulfoxide and S-Methylcysteine. Extraction.**—Plant materials were ground thoroughly with sufficient 95% ethanol to make the concentration 80%. The mixture was centrifuged and the supernatant removed. The residue was reground with 80% alcohol and centrifuged. The supernatant was combined with the first. This procedure was repeated twice more and then a final leaf extraction was made by refluxing 16 hours. The combined supernatant solutions were made to volume.

**Preparation of the Extract for Chromatography.**—Ninhydrin activity was[5] determined, and an aliquot containing 500 μg. of nitrogen was cleared of salts and sugars by adsorption of amino acids on a column of ion-exchange resin Dowex-50. The Dowex-50 was eluted with ammonia and the excess ammonia removed by passage through ion-exchange resin (IRC-50). Since methylcysteine sulfoxide tends to overlap glutamine on phenol, butyl alcohol–acetic acid chromatograms, glutamine was cyclized by adjusting the

(10) J. F. Thompson, R. M. Zacharius and F. C. Steward, *Plant Physiol.*, **26**, 375 (1951).

(11) J. F. Thompson and F. C. Steward, *ibid.*, **26**, 421 (1951).

(12) B. Steele, H. E. Sauberlich, M. S. Reynolds and C. A. Baumann, *J. Biol. Chem.*, **177**, 533 (1949).

(13) R. L. M. Synge and J. C. Wood, *Biochem. J.*, **60**, XV (1955).

*p*H of the solution to 7 and heating at 100° for 3 hours.[14] Methylcysteine superimposed γ-aminobutyric acid on these chromatograms, but the latter was effectively removed by using an excess of ion-exchange resin IRC-50.

Aliquots containing 50 μg. of nitrogen were then chromatographed and the amino acids determined quantitatively.[10,11] Standard curves were obtained by treating the pure compounds in the same way as the samples.

Cysteine determinations were made by microbiological assay with *L. mesenteroides*.[12]

(14) P. B. Hamilton, *J. Biol. Chem.*, **158**, 375 (1945).

ITHACA, N. Y.

---

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

# The Formation of N-(N-Acetyl-N-arylglycyl)-N-arylglycines in the Acetylation of N-Arylglycines

BY PHILIP L. SOUTHWICK, HAROLD L. DIMOND[1] AND ROGER E. STANSFIELD[2]

RECEIVED OCTOBER 21, 1955

Treatment of certain N-arylglycines with an excess of acetyl chloride at the reflux temperature resulted in the formation of N-(N-acetyl-N-arylglycyl)-N-arylglycines (II) in yields as high as 72%. N-Arylglycines which yielded products of this type included N-phenylglycine, N-*m*-chlorophenylglycine, N-*p*-tolylglycine and N-*p*-nitrophenylglycine. Experiments with N-phenylglycine and N-*p*-chlorophenylglycine showed that under certain conditions products of the same type are obtained when acetic anhydride is used. An explanation for the formation of these products has been formulated, based upon the observation that N-(N-acetyl-N-arylglycyl)-N-arylglycines (II) also can be obtained following treatment of a mixture of an N-acetyl-N-arylglycine (III) and an N-arylglycine ester (VI) with an excess of acetyl chloride.

The present investigation was prompted by the observation that treatment of N-*p*-chlorophenylglycine with a large excess of acetyl chloride at the reflux temperature led to an acidic product melting at 216–217°, and not to the expected N-acetyl-N-*p*-chlorophenylglycine, m.p. 175–176°. Similar experiments with N-phenylglycine and with N-*p*-tolylglycine subsequently revealed that under the same conditions other N-arylglycines (I) also yielded products other than those which would result from simple acetylation. It therefore became of interest to investigate the structures of the compounds obtained in this manner and to seek an explanation for the unexpected type of reaction which had occurred.

The composition and neutralization equivalents of the compounds indicated that they were N-(N-acetyl-N-arylglycyl)-N-arylglycines (II),[3] formed by the over-all reaction

$$2\underset{\text{I}}{\text{ArNHCH}_2\text{CO}_2\text{H}} + 2\text{CH}_3\text{COCl} \longrightarrow \underset{\text{II}}{\text{CH}_3\text{CONCH}_2\text{CONCH}_2\text{CO}_2\text{H}} + \text{CH}_3\text{CO}_2\text{H} + 2\text{HCl}$$

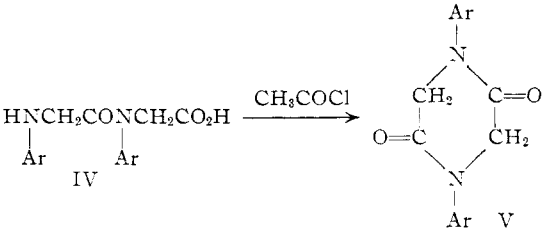
(Ar on the first N and on the second N)

The infrared absorption spectrum which was determined for the product from N-*p*-chlorophenylglycine seemed in accord with this interpretation, for the bands which were observed in the 5–7 μ region occurred at 6.22 and 6.70 μ (assigned to the substituted phenyl groups) and at 5.76 and 5.95 μ (assigned to carboxylic acid and amide carbonyl groups, respectively). In order to confirm the structure, however, and at the same time to gain some insight into the manner in which the compounds, II, were formed, a number of attempts were made to achieve the synthesis of a compound of this type from intermediates which might conceivably be involved in the reaction process.

The compounds, II, evidently did not arise as a result of an acyl replacement brought about in any manner by a reaction involving two molecules of an intermediate N-acetyl-N-arylglycine (III); N-acetyl-N-*p*-chlorophenylglycine (III, Ar = *p*-$ClC_6H_4$) was recovered unchanged after being heated with excess acetyl chloride

$$\underset{\text{III (Ar)}}{2\text{CH}_3\text{CONCH}_2\text{CO}_2\text{H}} \xrightarrow{\text{CH}_3\text{COCl}} \text{II} + \text{CH}_3\text{CO}_2\text{H}$$

Moreover, these compounds did not result from acetylation of an intermediate N-(N-arylglycyl)-N-arylglycine (IV), for treatment of N-(N-phenylglycyl)-N-phenylglycine (IV, Ar = $C_6H_5$) with acetyl chloride produced 1,4-diphenyl-2,5-diketopiperazine (V, Ar = $C_6H_5$), and the diketopiperazine was recovered unchanged after heating at the reflux temperature with acetyl chloride




It seems reasonable to conclude, therefore, that the acetylated dipeptides II were formed during the acetylation process by the reaction of an acylating agent derived from an intermediate N-acetyl-N-arylglycine (III) with a portion of the original N-arylglycine (I) which had not yet been acetylated on nitrogen.

(1) Institute Fellow in Organic Chemistry, 1951–1953.

(2) Research Corporation Fellow, 1953–1954. This paper is based mainly on a portion of a thesis submitted by Roger E. Stansfield in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Carnegie Institute of Technology, July, 1954.

(3) This result is reminiscent of the observation of T. Curtius, *J. prakt. Chem.*, [2] **24**, 239 (1881); [2] **26**, 145 (1882), that hippurylglycine is one of the products of the reaction of benzoyl chloride with the silver salt of glycine. It should be noted, however, that the acylation of α-amino acids with acid chlorides to give the expected simple N-acyl derivatives has been carried out successfully in a number of instances; see E. Ronwin, *J. Org. Chem.*, **18**, 127 (1953).