[CONTRIBUTION FROM THE FULMER CHEMICAL LABORATORY, THE STATE COLLEGE OF WASHINGTON]

Amino Acid Derivatives. I. Carboallyloxy Derivatives of α-Amino Acids[1,2]

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Recent interest in α-amino acids and amino acid derivatives has resulted in the development of improved synthetic methods for many amino acids and in their commercial availability. Practical laboratory methods for the resolution of the synthetic amino acids into the optically active forms and for the synthesis of peptides and other derivatives of amino acids are therefore increasingly valuable.

An important development both for the resolution of amino acids and for peptide synthesis was the introduction by Bergmann and Zervas[3] of the reagent benzyl chloroformate or "carbobenzoxy chloride." These investigators prepared crystalline N-carbobenzoxyamino acids and showed that the compounds could be cleaved by catalytic hydrogenolysis to regenerate the free amino acids. Thus, a method was provided for effectively "protecting" the amino group when desired and for its subsequent "liberation." The applicability to peptide synthesis was demonstrated. Since 1932, the method has been widely used and its utility has been further increased by the development of other procedures[4,5] for cleaving the carbobenzoxy group under mild conditions.

Aside from too frequent difficulty in crystallization of the products, perhaps the main disadvantage of the carbobenzoxy method is the instability of the reagent, necessitating its repeated preparation in the laboratory from phosgene. The commercial availability and stability of allyl chloroformate,[6] together with the structural analogy of "allyl" and "benzyl" compounds, suggested that it might prove to be a useful substitute. A study was therefore made of the reaction of allyl chloroformate with amino acids.

Using essentially the procedure for the preparation of the carbobenzoxy compounds,[7] a series of N-carboallyloxy amino acids has been isolated in good yield. Those which have been obtained in crystalline form are listed in Table I. The corresponding derivatives of glycine, DL-valine, DL-isoleucine, L-leucine, L-cystine and the dicarboallyloxy derivative of L-lysine have not been crystallized as yet, though in several instances they have been utilized for further synthetic work. In general, the new compounds appear to crystallize about as readily as the carbobenzoxyamino acids.

TABLE I

Compound	M. p., °C.	Solvent for recrystn.	Formula	N Analyses, % Calcd.	Found
N-Carboallyloxy-DL-alanine	60–61	Benzene–pentane	$C_7H_{11}O_4N$	8.1	8.0
N-Carboallyloxy-DL-leucine	41–42	Benzene–pentane	$C_{10}H_{17}O_4N$	6.5	6.4
N-Carboallyloxy-DL-methionine	44–45	Benzene–pentane	$C_9H_{15}O_7NS$	6.0	5.9
N-Carboallyloxy-DL-phenylalanine	83–84	Benzene–pentane	$C_{13}H_{15}O_4N$	5.6	5.5
N-Carboallyloxy-DL-tryptophan	115–116	Ethanol–benzene	$C_{15}H_{16}O_4N_2$	9.7	9.4
O,N-Dicarboallyloxy-L-tyrosine	105–106	Ethanol–water	$C_{13}H_{15}O_5N$	4.0	4.1
ε-N-Carboallyloxy-L-lysine	225–230	Water	$C_{10}H_{18}O_4N$	12.2	11.9
ε-N-Carboallyloxy-DL-lysine	237	Water	$C_{10}H_{18}O_4N$	12.2	12.1

The various procedures utilized for the cleavage of carbobenzoxy groups were tested on representative carboallyloxy derivatives. It seemed possible that catalytic hydrogenolysis of the allylic ester grouping in these compounds might be complicated by a competing reaction leading to hydrogenation of the double-bond in the allyl group.[8] The success or failure of the procedure might thus depend upon the relative rates of the two reactions. A variety of catalysts and experimental conditions were employed. The best results were obtained using Adams platinum oxide catalyst,[9] the yield of recrystallized amino acid amounting to 65–75% of the theoretical amount. Under these most favorable conditions, however, hydrogenation occurs to a significant extent. From the reaction of N-carboallyloxy-DL-phenylalanine, some 15–25% of the weight of starting material was recovered as a crystalline, ether-soluble product which was shown by comparison with an authentic sample to be N-carbopropyloxy-DL-phenylalanine. This compound was recovered unchanged when subjected to the catalytic hydrogenolysis procedure. Thus, it is clearly established that hydrogenation represents a competing side-reaction leading to reduction in yield of free amino acid.

Chemical procedures for the cleavage of the carboallyloxy group proved quite satisfactory. Treatment of the derivatives with metallic sodium in

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(2) Presented in part at the 112th Meeting of the American Chemical Society, New York, N. Y., September 16, 1947.

(3) Bergmann and Zervas, *Ber.*, **65**, 1192 (1932).

(4) Harington and Mead, *Biochem. J.*, **29**, 1602 (1935).

(5) Sifferd and du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(6) Generous samples of this compound were furnished by the Hooker Electrochemical Company, Niagara Falls, N. Y.

(7) "Organic Syntheses," **23**, 13 (1943).

(8) *Cf.* Carter, Glick, Norris and Phillips, *J. Biol. Chem.*, **170**, 285 (1947).

(9) Obtained from American Platinum Works, Jersey City, N. J.

liquid ammonia yielded 80–85% of the corresponding amino acids. It is noteworthy that this treatment failed to cleave the N-carbopropyloxy derivative to any significant extent. Experiments using phosphonium iodide in glacial acetic acid for cleavage of the carboallyloxy group proceeded smoothly with yields of 70–75% of the amino acid. Under these latter conditions, even the N-carbopropyloxy compound was converted to the amino acid in good yield.

Experimental

Preparation of Derivatives. (a) **Simple α-Amino Acids.**—The derivatives of amino acids not containing other reactive groups were prepared as follows: The amino acid (0.1 mole) was dissolved in 25 ml. of 4 *N* sodium hydroxide. The solution was cooled in an ice-bath and treated with 10.6 ml. of allyl chloroformate and 25 ml. of 4 *N* sodium hydroxide, added in eight equal portions with vigorous shaking for a few minutes after each addition, and intermittent cooling. The reaction mixture was kept alkaline to phenolphthalein throughout. After the last addition, the mixture was shaken vigorously, allowed to stand for fifteen minutes at room temperature, extracted with ether, and then acidified to congo red with concd. hydrochloric acid. After cooling for several hours, the derivatives which crystallized were collected, dried and recrystallized from the appropriate solvent. If the product was non-crystalline, the cold solution was extracted with ether, and the ether solution evaporated to a sirup which was further dried *in vacuo* over phosphorus pentoxide. The material was either used directly or recrystallized when possible. The derivatives of DL-alanine and glycine are quite soluble in water. The various derivatives were obtained in 80–95% yield. The compounds were characterized by determination of the neutralization equivalent, by elementary analysis and by reconversion to the corresponding amino acids.

(b) **Tyrosine.**—Treatment of L-tyrosine with allyl chloroformate according to the procedure just described, except that two molecular equivalents of allyl chloroformate and sodium hydroxide were added, gave the crystalline O,N-dicarboallyloxy derivative in good yield. If one molecular equivalent of allyl chloroformate was used and the alkaline reaction mixture allowed to stand for a few hours, the product isolated was non-crystalline but was characterized as N-carboallyloxy-L-tyrosine by conversion to the crystalline phenylhydrazide.[10]

(c) **Lysine.**—Treatment of L-lysine with two molecular equivalents of allyl chloroformate gave a high yield of sirupy N,N-dicarboallyloxy-L-lysine, characterized by conversion to the crystalline phenylhydrazide.[10]

The crystalline ε-N-carboallyloxy-DL-lysine was prepared from the copper complex.[11] Fifty grams of DL-lysine monohydrochloride was dissolved in 375 ml. of water and heated under reflux for thirty minutes with 25 g. of black cupric oxide. The hot suspension was filtered, and the residue washed with a small amount of water. The filtrate was cooled in an ice-bath and to it was added 10 ml. of 4 *N* sodium hydroxide, followed by 40 ml. of allyl chloroformate and 140 ml. of 4 *N* sodium hydroxide, each added in 10 portions. The mixture was shaken vigorously between additions and kept below room temperature by intermittent cooling. The *p*H was maintained at approximately 8. The blue precipitate was collected and the nearly colorless filtrate discarded. The precipitate was suspended in 1 liter of hot water and decomposed with hydrogen sulfide. The filtrate from the copper sulfide was evaporated to a small volume *in vacuo* and cooled. The yield of crystalline material was 38–39 g., (60%), m. p. 237°. N-Carboallyloxy-L-lysine was prepared by the same procedure from L-lysine.

(10) Milne and Stevens, THIS JOURNAL, in press.

(11) Kurtz, *J. Biol. Chem.*, **122**, 477 (1938).

Catalytic Hydrogenolysis. (a) **Using Adams Platinum Oxide.**—A 5-g. sample of the compound (N-carboallyloxy derivatives of DL-phenylalanine and DL-leucine were used in most experiments) was dissolved in 75 ml. of glacial acetic acid, and 2 ml. of concd. hydrochloric acid and 0.1 g. of platinum oxide[9] were added. The mixture was shaken in a Parr hydrogenation apparatus under a pressure of 2 atmospheres of hydrogen for six to ten hours. The catalyst was removed by filtration, washed with glacial acetic acid and dilute hydrochloric acid, and the filtrate and washings evaporated to a sirup *in vacuo*. The remainder of the acid was removed as completely as possible by the gradual addition of water at the same rate at which it evaporated. The residue was dissolved in water and the solution extracted with ether. The ether layer was allowed to evaporate *in vacuo* and further dried over phosphorus pentoxide until crystallization ensued. The material was recrystallized from benzene–pentane. The aqueous phase was concentrated *in vacuo* to a small volume, cooled and adjusted to *p*H 5.5–6, whereupon a large quantity of precipitate formed. Two volumes of alcohol were added, the mixture cooled for several hours, and the product collected. A second crop could be obtained from the mother liquors. The combined crops were recrystallized from alcohol–water.

In experiments using N-carboallyloxy-DL-phenylalanine, it was shown that the ether soluble material amounted to approximately 15–25% and consisted almost entirely of N-carbopropyloxy-DL-phenylalanine, m. p. 74–76° (mixed m. p. with authentic sample, 74–76°). The yield of amino acid was 70–75% and was identified as DL-phenylalanine by conversion to the N-acetyl derivative, m. p. 145–146° (mixed m. p. with authentic sample, 145–146°).

Experiments in which the conditions of hydrogenolysis were varied indicated that a decrease in the amount of hydrochloric acid decreased the yield of free amino acid, though a 50% yield was obtained when no hydrochloric acid was added.

During the hydrogenolysis, a white solid separated, presumably the amino acid hydrochloride. This could be prevented by adding a few ml. of water, but the yield of amino acid was again reduced to approximately 50%. On the other hand, the use of concentrated sulfuric acid in place of concentrated hydrochloric acid gave no increase in yield.

Experiments with N-carboallyloxy-DL-leucine gave essentially similar results wherever a comparison was made. Using N-carboallyloxy-DL-methionine, results were unsatisfactory, no appreciable quantity of DL-methionine being recovered.

(b) **Using Palladium-Charcoal.**—The catalyst was prepared according to the procedure of Mozingo,[12] except that an equal volume of water was added to the palladium chloride solution to ensure complete mixing. In the most successful experiments a total of 3–4 g. of catalyst was added in 2 or 3 portions throughout a ten-hour reaction period, under a pressure of 2 atmospheres of hydrogen. The solvent was glacial acetic acid containing hydrochloric acid as before. The reaction mixture was worked up essentially as described in the previous section with one important exception. It was found that a considerable and variable proportion of the product was adsorbed on the catalyst. By prolonged extraction with 3 *N* hydrochloric acid, the total yield of amino acid could be raised to 65–70%.

(c) **Using Palladium Black.**—The catalyst was prepared by the method of Tausz.[13] In the few experiments with this material, the yields of free amino acid were less satisfactory (*ca.* 50%) than from the previous procedures.

Other Cleavage Procedures. (a) **With Metallic Sodium in Liquid Ammonia.**—The general procedure of du Vigneaud and co-workers[5] was used. A solution of the derivative in liquid ammonia was treated with small pieces of metallic sodium until a blue color persisted for two to three minutes. Approximately 2 atomic equiva-

(12) Mozingo, "Organic Syntheses," **26**, 77 (c) (1946).

(13) Tausz and von Putnoky, *Ber.*, **52**, 1573 (1919).

lents of sodium were required. The ammonia was allowed to evaporate spontaneously and the final traces removed *in vacuo*. The residue was dissolved in water, the solution acidified to congo red and extracted with ether. No appreciable quantity of ether-soluble material was recovered from the experiments with the carboallyloxy derivatives. The aqueous phase was concentrated *in vacuo* until precipitation occurred. Then 2 volumes of alcohol was added, the *p*H adjusted to 5.5–6 and the mixture cooled. The product was recrystallized in the usual manner. The yields of DL-leucine, DL-methionine and DL-phenylalanine from the corresponding N-carboallyloxy derivatives were 65–85%.

(b) **With Phosphonium Iodide in Acetic Acid.**—The method of Harington and Mead[4] was followed. The reaction was stopped when the evolution of carbon dioxide had ceased. The solvent was removed *in vacuo* and the residue worked up as described above. Experiments with the N-carboallyloxy derivatives of DL-leucine, DL-methionine and DL-phenylalanine gave 70–75% yields of the pure amino acids.

***n*-Propyl Chloroformate.**—One mole of *n*-propyl alcohol was placed in a cylinder arranged with inlet and outlet tubes so that phosgene from a tank could be bubbled into it. The outlet tube was connected to a trap containing toluene. Both solutions were kept in an ice-bath and the final exit tube passed into a good hood. Approximately 0.9 mole (87 g.) of phosgene was allowed to bubble into the propyl alcohol over a period of ten hours. The mixture was then allowed to stand at room temperature for twenty-four hours. Without further purification, the solution was used in the following preparation.

N-Carbopropyloxy-DL-phenylalanine.—Five grams of DL-phenylalanine was dissolved in 9 ml. of 4 *N* sodium hydroxide, the solution cooled in an ice-bath, and treated with seven 1-ml. portions of *n*-propyl chloroformate (above). Simultaneously, sufficient amounts of 4 *N* alkali were added to keep the mixture alkaline to phenolphthalein. The mixture was vigorously shaken after each addition and cooled in an ice-bath. After all the reagents had been added, the mixture was shaken for an additional ten minutes and then allowed to stand for twenty minutes at room temperature. The cold solution was extracted with two 10-ml. portions of ether and the aqueous phase was aerated to remove ether. The solution was cooled and acidified to congo red with concd. hydrochloric acid. The mixture was extracted with two 25-ml. portions of ether and the ether fraction was concentrated *in vacuo*. The residue was dissolved in 45 ml. of warm benzene, an equal volume of petroleum ether was added and the solution was cooled for several hours. The crystalline product amounted to 7.5 g. (97%), m. p. 74–76°, neutralization equivalent 250 (calculated 251).

Experiments with N-carbopropyloxy-DL-phenylalanine showed that it was recovered unchanged in almost quantitative yield when subjected to the catalytic hydrogenolysis procedure using Adams catalyst, or to treatment with sodium in liquid ammonia. However, when treated with phosphonium iodide in glacial acetic acid very little of the starting material was recovered, and a reasonably good yield (55%) of pure DL-phenylalanine was obtained.

Summary

1. A series of naturally-occurring amino acids has been shown to react with allyl chloroformate to yield the corresponding carboallyloxy derivatives.

2. The ready cleavage of the carboallyloxy group has been demonstrated: (1) by catalytic hydrogenolysis using platinum or palladium catalysts, (2) by the use of metallic sodium in liquid ammonia and (3) by the use of phosphonium iodide in glacial acetic acid. The yield from catalytic hydrogenolysis has been shown to be somewhat reduced by the competing reaction leading to hydrogenation of the allyl group.

3. The probable utility of the procedures in the synthesis of peptides and related compounds has been pointed out.

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Allylic Chlorides. IX. Preparation of *cis* and *trans* Crotyl Chloride

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References to crotyl chloride have appeared frequently in the literature but in no case have the two geometrical isomers been separated and identified.[1,2,3,4] Young and Andrews[5] have noted, however, that crotyl chloride which had been obtained by the chlorination of a butene mixture showed a slightly higher refractive index than any previously reported. In addition they found that the rate of reaction with sodium ethoxide was somewhat higher for this crotyl chloride than for crotyl chlorides obtained by other methods. This was interpreted to be evidence for the presence of the *cis* isomer.

The present paper describes the preparation of both the *cis* and *trans* isomers of crotyl chloride from the corresponding crotyl alcohols by treatment of the alcohol with phosphorus trichloride in the presence of pyridine. The synthesis of *trans*-crotyl alcohol was effected by reduction of the commercially available *trans*-crotonaldehyde by the use of both lithium aluminum hydride and aluminum propoxide.

The *cis*-crotyl alcohol was synthesized by two methods each of which was designed to give an alcohol of known configuration. One procedure consisted of the hydrogenation of 2-butyn-1-ol by use of a palladium catalyst suspended on barium sulfate. This catalyst has been shown to cause the *cis* addition of hydrogen to acetylenic bonds.[6] The 2-butyn-1-ol was obtained by dehydrochlorination of 3-chloro-2-buten-1-ol, a reaction which will be discussed in detail in a forthcoming paper. The

(1) Baudrenghien, *Bull. soc. chim. Belg.*, **31**, 160 (1922).

(2) Henne, Chanan and Turk, THIS JOURNAL, **63**, 3474 (1941).

(3) Kharasch, Kritchevsky and Mayo, *J. Org. Chem.*, **2**, 489 (1937).

(4) Gredy, *Bull. soc. chim.*, [5] **2**, 1029 (1935).

(5) Young and Andrews, THIS JOURNAL, **66**, 421 (1944).

(6) Kharasch, Walling and Mayo, *ibid.*, **61**, 1559 (1939).