[CONTRIBUTION FROM DEPARTMENT OF AGRICULTURAL CHEMISTRY, NORTH DAKOTA AGRICULTURAL COLLEGE AND EXPERIMENT STATION]

New Syntheses of the Selenium Analogs of *dl*-Cystine and Cysteine Derivatives[1,1a]

BY EDGAR PAGE PAINTER

Since the discovery of selenium in plants,[1b] numerous reports have appeared on studies related to selenium in agriculture. These have been devoted primarily to the occurrence of selenium in soils,[2] in plants,[3] and the toxic effects on animals.[4] The subject has been reviewed in two articles.[5,6]

Relatively little of the work has been directed toward identifying the compounds of selenium in plants. It was shown soon after the discovery of selenium in plants that it was present in organic form and, in the cereal grains, concentrated in the protein.[7] Upon hydrolysis of seleniferous proteins a soluble selenium compound was one of the products. These results have been accepted as presumptive evidence for the occurrence of selenium in a new amino acid. Aside from the similarity of selenium to sulfur in many chemical properties, evidence has indicated that selenium may be present in plants in the same forms as sulfur. When the hydrolyzates of seleniferous proteins are fractionated, those high in sulfur are usually high in selenium. Like sulfur in proteins, selenium is cleaved when seleniferous proteins are hydrolyzed in alkaline plumbite.[8] By analogy from the cleavage of selenium from some known selenides and diselenides[9] in alkaline solution, it was concluded that part of the selenium in the cereal grains was present as a diselenide or easily cleaved selenide. The results pertinent to the likely forms of selenium in plants have been discussed in detail.[5]

The problem of separating selenium compounds of cereals, in case selenium replaces sulfur in amino acids, appears difficult since the selenium and sulfur compounds would be expected to have similar properties and the selenium content of cereal grains is usually so low that the molar sulfur/selenium ratio is nearly always above 100.[10] It appeared, therefore, that the synthetic route might be the simplest way of showing whether or not the selenium amino acids occur in plants. From the chemical properties and toxicity to animals, it seemed probable that we should be able to answer the speculations relative to the status of these compounds.

One selenium compound has been reported isolated from plant material by Horn and Jones.[11] The product isolated also contained sulfur and the structure which would account for the elementary composition was possibly a mixture of the amino acid cystathionine [S-(β-amino-β-carboxyethyl)-homocysteine][12] and its selenium analog in the ratio of one molecule of the sulfur compound to two of selenium. The source of this product was *Astragalus pectinatus*, a plant which absorbs several-fold more selenium than the cereal grains. The advantage of using as source materials plant species which absorb large amounts of selenium is obvious, but little is known of the sulfur compounds of these plants and the structure suggested for the amino acid differs from that of any of known natural occurrence. The properties of selenium in those plant species[3] which absorb large amounts of selenium (as high as 15,000 p. p. m.) seem to be quite different from that in cereals and forage plants.

Fredga[13] synthesized the selenium analog of *dl*-cystine by treating α-amino-β-chloropropionic methyl ester hydrochloride (I) with potassium diselenide in aqueous alkaline solution. He reported a yield of nearly 30% but the writer has been unable to carry out the synthesis by his method with a satisfactory yield. Diselenides are generally unstable in alkaline solution so a method avoiding prolonged solution in aqueous alkali was sought.

The syntheses are shown in Fig. 1. When the α-amino-β-chloro acid ester, I, reacted with potassium hydrogen selenide (or sodium hydrogen selenide) in alcohol, the selenium analog of *dl*-cystine (β,β′-diselenodialanine, V) was isolated, after the ester was hydrolyzed with acid, in poor

(1) Presented at the Atlantic City meeting of the American Chemical Society, April, 1946.

(1a) Published by permission of the Director, North Dakota Agricultural Experiment Station.

(1b) Robinson, *J. Assoc. Off. Agr. Chem.*, **16**, 423 (1933).

(2) (a) Byers, Miller, Williams, and Lakin, U. S. Dept. Agr. Tech. Bull. 601 (1938); Williams, Lakin and Byers, *ibid.*, No. 758 (1941); (b) Knight and Beath, *Wyoming Agr. Expt. Bull.*, 221 (1937); (c) Moxon, Olson and Searight, *S. Dakota Agr. Expt. Sta. Tech. Bull.*, No. 2 (1939).

(3) Ref. 2a; Beath, Gilbert and Eppson, *Am. J. Botany*, **26**, 257 and 296 (1939); Moxon, Olson, Searight and Sandals, *ibid.*, **25** 794 (1938); Hurd-Karrer, *ibid.*, **25**, 666 (1938); Miller and Byers, *J. Agr. Research*, **55**, 59 (1937).

(4) Franke, Rice, Johnson and Schoening, U. S. Dept. Agr. Circ. 320 (1934); Draize and Beath, *J. Am. Vet. Med. Assoc.*, **86**, 753 (1935); Franke, *J. Nutrition*, **8**, 597 (1934); Franke and Moxon, *J. Pharmacol.*, **61**, 89 (1937); Franke and Potter, *J. Nutrition*, **8**, 615 (1934); Franke and Painter, *Cereal Chem.*, **15**, 1 (1938); Smith, Stohlman and Lillie, *J. Pharmacol.*, **60**, 449 (1937).

(5) Painter, *Chem. Rev.*, **28**, 179 (1941).

(6) Moxon and Rhian, *Physiol. Rev.*, **23**, 305 (1943).

(7) Franke and Painter, *Cereal Chem.*, **13**, 67 and 172 (1936); Horn, Nelson and Jones, *ibid.*, **13**, 126 (1936); Jones, Horn and Gersdorff, *ibid.*, **14**, 130 (1937); Painter and Franke, *J. Biol. Chem.*, **111**, 643 (1935).

(8) Painter and Franke, *ibid.*, **134**, 557 (1940).

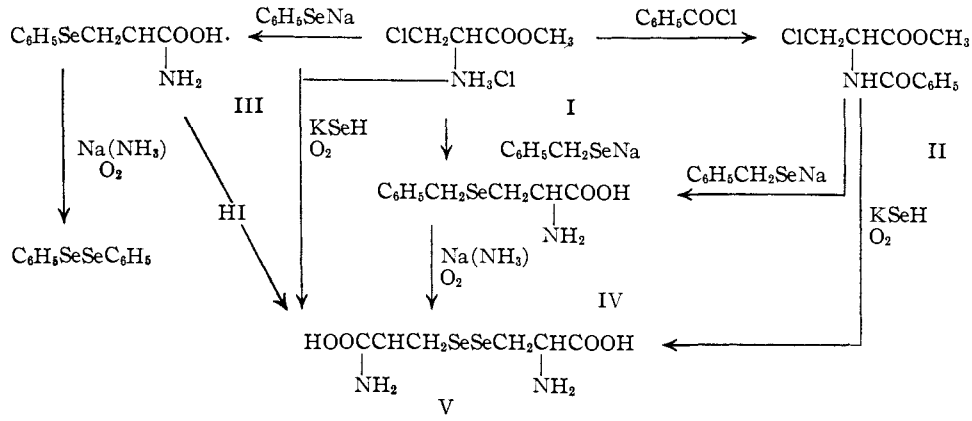
(9) Painter, Franke and Gortner, *J. Org. Chem.*, **5**, 579 (1940).

(10) Franke and Painter, *Am. J. Botany*, **27**, 336 (1940).

(11) Horn and Jones, *J. Biol. Chem.*, **139**, 649 (1941).

(12) Brown and du Vigneaud, *ibid.*, **137**, 611 (1941); du Vigneaud, Brown and Chandler, *ibid.*, **143**, 59 (1942).

(13) Fredga, *Svensk Kem. Tids.*, **48**, 160 (1936); **49**, 124 and 139 (1937).



yields. The compound *dl*-β-(phenylseleno)-alanine (III) was then prepared by treating sodium phenyl selenide with I in alcohol. III was surprisingly stable to hydrolysis with concentrated hydrobromic acid but concentrated hydriodic acid slowly cleaved the phenyl group. Yields of V by hydriodic acid hydrolysis of III were so low as to discourage use of this method. In order to avoid using additional alkaline reagent to neutralize the hydrochloride of I, the benzamido derivative, II, was prepared. This compound did not give yields of V any more promising than did similar reactions using I.

Since the reduction of S-derivatives of cysteine and homocysteine with sodium in liquid ammonia[14] has been carried out successfully, III was reduced in this way. Cleavage took place between the selenium and methylene carbon to give, after oxidation with air, diphenyl diselenide in nearly theoretical yields.

Benzyl selenomercaptan was then prepared and sodium benzyl selenide reacted wtih I to give *dl*-β-(benzylseleno)-alanine (IV). This compound was cleaved with sodium in liquid ammonia to give a good yield of the selenium analog of cystine (V).

Experimental

Serine.—Part of the serine used to prepare I was kindly supplied by Merck and Company. Additional lots were prepared by the method of Dunn, Redemann and Smith.[15]

Anal. Calcd. for $C_3H_7O_3N$: N, 13.33. Found: N, 13.26.

Serine Methyl Ester Hydrochloride.—Fifty grams of serine was suspended in 850 ml. of absolute methyl alcohol (distilled from magnesium methoxide) and dry hydrogen chloride added until saturated at room temperature. After removal of the alcohol with suction (water pump) while warmed in a water-bath, 400 ml. of methyl alcohol was added and the solution again saturated with hydrogen chloride. This was repeated four times. The serine ester hydrochloride was finally dissolved in 350 to 400 ml. of warm methyl alcohol and crystallized by slow addition of approximately one liter of dry ethyl ether. After standing overnight in a refrigerator, the compound was filtered in a sintered glass crucible, washed well with dry ether, and stored in a vacuum desiccator over sulfuric acid, m. p. 134°. Yields ranged from 70 to 72 g., 96%.

Anal. Calcd. for $C_4H_{10}O_3NCl$: N, 9.00. Found: N, 8.98.

α-Amino-β-chloropropionic Acid Methyl Ester Hydrochloride, I.—Prepared by the method of Fischer and Raske.[16] Yields ranged from 65 to 75%, m. p. 131–133°.

Anal. Calcd. for $C_4H_9O_2NCl_2$: N, 8.05. Found: N, 7.90.

Methyl α-Benzamido-β-chloropropionate, II.—Twenty grams of I was suspended in 150 ml. of dioxane, 40 g. of potassium carbonate and 6 ml. of water added, the flask cooled in ice water and 32 ml. of benzoyl chloride added over half an hour. The flask was then allowed to come to room temperature and stirring was continued four hours. The contents were then poured into 800 ml. of ice water and set in a refrigerator overnight. The product was filtered, washed with ice water and allowed to dry in air. After dissolving in boiling ligroin (70–90°) it crystallized as fine needles upon cooling, m. p. 114°. The yield was 23 g., 83%.

Anal. Calcd. for $C_{11}H_{12}O_3NCl$: N, 5.80; Cl, 14.67. Found: N, 5.76; Cl, 14.5.

β-(Phenylseleno)-alanine, III.—After the oxygen in a flask containing 150 ml. of absolute alcohol was displaced by a stream of nitrogen, 34.3 g. of phenyl selenomercaptan, (0.2 mole + 10%) prepared by addition of selenium to phenylmagnesium bromide,[17] was added. Sodium (5 g.) was then added in small pieces. A small amount of selenium cleaved as the solution became red when the last of the sodium dissolved; 17.4 g. of I (0.1 mole) was dissolved in 75 ml. of absolute alcohol and added to the sodium phenyl selenide. The solution was refluxed fifteen minutes and after cooling to room temperature, 5 ml. of concentrated hydrochloric acid added and a stream of air pulled through to oxidize unreacted phenyl selenomercaptan to diphenyl diselenide. Six hundred ml. of water and 90 ml. of concentrated hydrochloric acid were added and the solution heated to boiling. The volume was reduced almost to dryness under the water pump vacuum and then approximately 400 ml. of water plus 1 ml. of concentrated hydrochloric acid were added. The solution was warmed to melt the diphenyl diselenide and poured into a separatory funnel containing about 200 ml. of benzene. After shaking, the aqueous layer was drained off, heated to boiling and evaporated to dryness on a steam bath by use of the vacuum of the water pump. The solid was again dissolved by addition of dilute hydrochloric acid, warmed on the steam-bath and again taken to dryness at reduced pressure. The solid was dissolved in 200 ml. of water (plus a few drops of hydrochloric acid), extracted with about 100 ml. of benzene and the aqueous solution heated with a small amount of decolorizing car-

(14) Wood and du Vigneaud, *J. Biol. Chem.*, **131**, 267 (1939); Patterson and du Vigneaud, *ibid.*, **111**, 393 (1935).

(15) Dunn, Redemann and Smith, *ibid.*, **104**, 511 (1934).

(16) Fischer and Raske, *Ber.*, **40**, 3717 (1907).

(17) Foster and Brown, THIS JOURNAL, **50**, 1182 (1928).

bon and filtered. The compound was crystallized by addition of sodium hydroxide to a *p*H of 5.5. After standing overnight in the refrigerator, the white crystals were filtered out in a sintered glass crucible, washed with water, alcohol and ether. The compound was then suspended in 200 ml. of water and sodium hydroxide added until dissolved. The solution was heated almost to boiling, a small amount of carbon added and filtered. The compound crystallized as small, transparent, irregular sided plates by addition of dilute hydrochloric acid to a *p*H of 5.5. The yield was 13.7 g., 56%, m. p. 176–177°.

Anal. Calcd. for $C_9H_{11}O_2NSe$: Se, 32.34; N, 5.74. Found: Se, 32.0; N, 5.72.

Benzyl Selenomercaptan.—To 2 moles of benzylmagnesium chloride,[18] prepared in a 5-liter 3-necked flask, 2 moles of metallic selenium was added in small portions to the well-stirred solution. After each addition of selenium the stopper was immediately inserted and was not removed for the next addition until the vigorous reaction subsided. After all of the selenium was added, the solution was refluxed for one hour. To decompose the benzylselenomagnesium chloride, hydrogen chloride (not dried with concentrated sulfuric acid) was passed through the solution until the ether ceased to reflux. Dilute hydrochloric acid was then added slowly until the magnesium chloride dissolved. The contents of the flask were transferred to a large separatory funnel and the water layer drawn off. The ether solution was washed with water, then the ether layer drained off and dried with anhydrous sodium sulfate. After the ether was distilled off, the benzyl selenomercaptan was distilled *in vacuo*. The product collected at 100 to 105° at approximately 20 mm. was redistilled. The fraction distilling at 101–102° at 21 mm. was used in subsequent reactions. The best yields were about 45%. When dissolved in 95% alcohol and exposed to air oxidation, dibenzyl diselenide, m. p. 93°, crystallized in almost quantitative yields.

β-(Benzylseleno)-alanine, IV.—After the air in a flask containing 350 ml. of absolute alcohol was displaced by a stream of nitrogen, 68.4 g. (0.4 mole) of benzyl selenomercaptan was added and 9.2 g. of sodium added in small pieces until dissolved. Seventeen and four-tenths grams of I (0.1 mole) was dissolved in 75 ml. of alcohol and added to the sodium benzyl selenide. The solution was refluxed fifteen minutes. After the solution cooled down, 25 ml. of concentrated hydrochloric acid was added and the solution aerated for about two hours to oxidize unreacted benzyl selenomercaptan to dibenzyl diselenide. The solvent was then removed at the water pump vacuum. The product was dissolved in about 200 ml. of water plus a few ml. of concentrated hydrochloric acid, heated on the steam-bath, and the dibenzyl diselenide extracted in the same way as in the preparation of β-(phenylseleno)-alanine. The solution was reduced to dryness on a steam-bath under reduced pressure (water pump), dissolved in water, heated to boiling, and the solvent again removed. After dissolving in about 500 ml., the solution was extracted with ether, filtered, and β-(benzylseleno)-alanine precipitated by adding sodium hydroxide to a *p*H near 5. After standing overnight in the refrigerator the compound was filtered out, washed with ice water, then with alcohol and ether. The compound was dissolved in dilute sodium hydroxide (400 ml.), heated almost to boiling with decolorizing carbon and filtered. To crystallize, the solution was heated to about 80° and concentrated hydrochloric acid added dropwise until a copious white precipitate formed. After the solution cooled to room temperature, the *p*H was adjusted to 5.5, and the product filtered out and washed with water, alcohol and ether. The yield was 15.4 g., 60%, of a compound melting at 185°. The compound crystallized in transparent, predominantly rectangular plates or sheets.

Anal. Calcd. for $C_{10}H_{13}O_2NSe$: N, 5.41; Se, 30.52. Found: N, 5.43; Se, 30.2.

β-(Benzylseleno)-alanine was also prepared by treating sodium benzyl selenide with II (2 to 1 mole ratio). The procedure was similar to that using I except the reaction product was heated with dilute hydrochloric acid on the steam-bath to cleave the benzoyl group. The yield was 48%.

Reduction of β-(Phenylseleno)-alanine.—Five grams of β-(phenylseleno)-alanine was dissolved in about 100 ml. of liquid ammonia in a 3-neck flask equipped with a stirrer held at a temperature near −65°. Small pieces of sodium were added while stirring until the blue color persisted for ten minutes. The flask was removed from the dry-ice-bath and before the last of the ammonia evaporated 3 g. of ammonium chloride was added. The contents of the flask were dissolved in about 150 ml. of water, the *p*H adjusted to 6 and a current of air pulled through the solution. A precipitate formed slowly. This was filtered out and found to be soluble in ether. Upon recrystallization from 95% alcohol, 3 g. of diphenyl diselenide, m. p. 82°, was obtained.

β,β′-Diselenodialanine, V. A. By Reduction of β-(Benzylseleno)-alanine.—To about 150 ml. of liquid ammonia in a 3-necked flask equipped with a stirrer, 5 g. of β-(benzylseleno)-alanine was added at the boiling point of liquid ammonia. Small pieces of sodium were added until the blue color persisted for fifteen minutes. The ammonia was allowed to evaporate at room temperature and when the volume was about 50 ml. the sodium added was neutralized with ammonium iodide. Just before all of the ammonia evaporated, 75 ml. of ethyl ether was added so that when the flask was warmed to drive off the last of the ammonia, the product would not be heated above the boiling point of ether. The ether was gently boiled for about half an hour and the residue (still covered with ether) dissolved in about 100 ml. of water. Concentrated hydrobromic acid was added dropwise and the change in *p*H followed closely. No β-(benzylseleno)-alanine separated at a *p*H of 5 so cleavage of the benzyl group must be nearly quantitative. The *p*H was reduced to 4, the ether layer separated and the aqueous solution filtered free of a small amount of metallic selenium. The volume was approximately 200 ml. About 0.1 g. of hydroxylamine hydrochloride was added and a current of air drawn through the flask to oxidize to the diselenide. A small amount of selenium deposited on the side of the flask during air oxidation. Apparently a small amount of inorganic selenide is produced during the sodium reduction. β,β′-Diselenodialanine (V) precipitates as air is drawn through the solution. Completeness of oxidation can be determined by drawing off a small portion of the clear solution and a current of air drawn through to see whether any more insoluble β,β′-diselenodialanine forms. During oxidation, the *p*H of the solution increases. It was adjusted to 6, the volume reduced under reduced pressure to 75 ml. and the flask allowed to set overnight in the refrigerator.

The compound was filtered, washed with ice water, then with alcohol and ether. It was suspended in 75 ml. of water and dissolved by dropwise addition of concentrated hydrobromic acid and filtered. About 0.1 g. of hydroxylamine hydrochloride was added and the *p*H adjusted to 6 with dilute ammonium hydroxide. After standing overnight in the refrigerator, the product was filtered, washed with cold water, alcohol, and ether. The yield of slightly yellowish β,β′-diselenodialanine was 3 g., just over 90%. The compound crystallized as very small plates (some hexagonal, some rectangular) which melted with decomposition at 222°.

Anal. Calcd. for $C_6H_{12}O_4N_2Se_2$: Se, 47.27; N, 8.39. Found: Se, 46.9; N, 8.35.

A few crystals of a compound believed to be dibenzyl selenide, m. p. 44°, were obtained from the ether extract of the sodium in liquid ammonia reduction.

B. By Reactions of I and II with Sodium Hydrogen Selenide.—To 0.1 mole of sodium in 50 ml. of absolute alcohol in a 500-ml. flask a stream of hydrogen was passed through to drive out the air, hydrogen selenide[19] was ab-

(18) "Organic Syntheses," IV, 59 (1925).

(19) Green and Bradt, *Proc. Ind. Acad. Sci.*, **43**, 116 (1934).

sorbed until the insoluble sodium selenide was converted to sodium hydrogen selenide. Then 1/20 mole of I in 25 ml. of absolute alcohol was added and the flask heated to a boil. The product was hydrolyzed with hydrochloric acid in a manner similar to the preparation of III and IV. Yields of β,β'-diselenodialanine were low, never above 20%, and the products upon reprecipitation always deposited a small amount of metallic selenium.

With II the results were also discouraging. The products were never pure β,β'-diselenodialanine as the selenium content was 2 to 4% lower than the theoretical.

C. **Hydrolysis of III with Concentrated Hydriodic Acid.**—Four grams of III was refluxed seven hours with concentrated hydriodic acid. After the hydriodic acid was removed at the pump, water was added to dissolve the residue and the aqueous solution extracted with ether. Upon neutralization unchanged III, 1.7 g. was obtained. The solution was then aerated, one volume of alcohol added, and 0.6 g. of impure β,β'-diselenodialanine precipitated.

Selenium was determined by oxidation in the Parr bomb[20] using potassium chlorate, nitrogen by a micro-Kjeldahl method,[21] and halogen gravimetrically after reduction with sodium in alcohol.

Acknowledgment.—The author gratefully acknowledges the assistance of Mr. Arnold Stoutland in the preparation of the hydrochloride of methyl α-amino-β-chloropropionate, Mr. L. L. Nesbitt for the determinations of nitrogen, and Mr. Harold Klosterman for selenium determinations. A generous grant of serine from Merck and Company is gratefully acknowledged.

Summary

By treating the hydrochloride of methyl α-amino-β-chloropropionate with sodium benzyl selenide the amino acid β-(benzylseleno)-alanine was prepared. When reduced with sodium in liquid ammonia the benzyl group cleaved to give, after air oxidation, the selenium analog of cystine.

(20) Shaw and Reid, THIS JOURNAL, **49**, 2330 (1927).

(21) Ma and Zuazaga, *Ind. Eng. Chem., Anal. Ed.*, **14**, 280 (1942).

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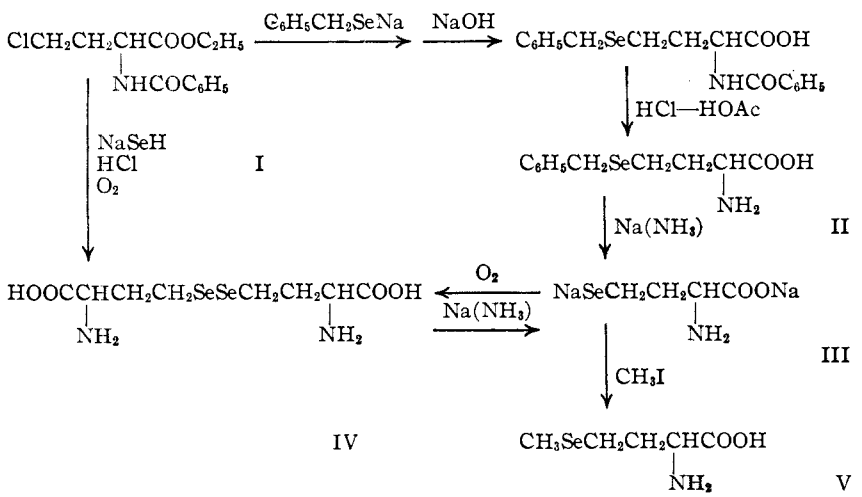
A Synthesis of Selenium Analogs of *dl*-Methionine and *dl*-Homocystine[1,1a]

BY EDGAR PAGE PAINTER

The objective of synthesizing amino acids with selenium in place of sulfur has been stated in the accompanying paper.[2] Cystine (or cysteine) and methionine carry nearly all of the sulfur in cereal proteins but the amino acid homocystine, although it has never been identified in plants, is of interest because it can take care of animals' requirement for a sulfur containing amino acid. The selenium analog of *dl*-homocystine was therefore prepared as well as the selenium analog of *dl*-methionine.

The most direct synthesis appeared to be by alkylation of β-chloro-ethyl methyl selenide with a compound like acetamido malonic ester. This was abandoned in favor of alkylation with β-chloroethyl benzyl selenide similar to the syntheses of S-benzylhomocysteine by Patterson and du Vigneaud,[3] because benzyl selenomercaptan is far easier to prepare and handle than methyl selenomercaptan. Compounds of the type $RSeCH_2CH_2X$ were prepared, where X = OH, but all attempts to prepare the same compound where X = halogen failed.



Selenium was successfully introduced into an amino acid in the γ position by use of the compound ethyl α-benzamido-γ-chlorobutyrate (I) first prepared by Hill and Robson.[4] When I reacted with sodium hydrogen selenide and the product was hydrolyzed a poor yield of the selenium analog of homocystine (IV)

(1) Presented at the Atlantic City meeting of the American Chemical Society, April, 1946.

(1a) Published by permission of the Director, North Dakota Agricultural Experiment Station.

(2) Painter, THIS JOURNAL, **69**, 229 (1947).

(3) Patterson and du Vigneaud, *J. Biol. Chem.*, **111**, 393 (1935).

(4) Hill and Robson, *Biochem. J.*, **30**, 248 (1936).