to give 12.2 g. (90%) of glycinamide sulfate. This product was dissolved in 20 ml. of water and reprecipitated with 150 ml. of ethanol to give 11.9 g. of product after drying at 100°.

Anal. Calcd. for $C_4H_{14}O_6N_4S$ (244): N, 22.8; S, 13.0. Found: N, 22.9; S, 12.6.

Ethylenediamine Sulfate.[13]—A solution of 10 g. of twice-distilled ethylenediamine in 50 ml. of water was added with stirring to a cooled solution of 17 g. of 96% sulfuric acid in 125 ml. of water. To the resultant solution was added 150 ml. of ethanol, the crystalline precipitate collected, washed with absolute ethanol and air-dried to give 24.2 g. (90%) of the desired product which was reprecipitated from an aqueous solution by the addition of an equal volume of ethanol.

Anal. Calcd. for $C_2H_{10}O_4N_2S$ (158): C, 15.2; H, 6.4; N, 17.7; S, 20.3. Found: C, 15.5; H, 6.7; N, 17.4; S, 20.4.

***p*-Sulfo-DL-phenylalanine.**[12]—The reaction of 33 g. of DL-phenylalanine and 50 ml. of 100% sulfuric acid essentially as described by Erlenmeyer and Lipp[12] gave 20.8 g. of a white solid and 15 g. of a glassy amber resin. The white solid proved to be *p*-sulfo-DL-phenylalanine monohydrate.[12]

Anal. Calcd. for $C_9H_{13}O_6NS$ (263): C, 41.1; H, 5.0; N, 5.3; S, 12.2. Found: C, 41.1; H, 4.9; N, 5.5; S, 12.1.

Reaction of Benzhydrazide with 96% Sulfuric Acid.—(A) Five grams of benzhydrazide, m.p. 113–114°, was dissolved in 15 ml. of 96% sulfuric acid with the temperature being maintained below 25°. Five minutes after solution was effected the mixture was poured into 50 ml. of ice-water, the precipitate collected, washed with water and dried. The weight of the solid, completely soluble in aqueous sodium bicarbonate, was 1.0 g. This product was benzoic acid, m.p. 121–122°. (B) In a second experiment a solution of benzhydrazide, 5 g., in 15 ml. of 96% sulfuric acid was heated on a steam-bath for 1.5 hours, the solution cooled, poured into 50 ml. of ice-water, the precipitate collected and washed with water. This product was recrystallized from 200 ml. of water to give 3.7 g. of benzoic acid, m.p. 121–122°. (C) The above experiment was repeated using benzamide instead of benzhydrazide. The product isolated was insoluble in aqueous sodium bicarbonate and proved to be benzamide, m.p. 125–126°. The yield was 3.1 g.

Reaction of Benzhydrazide with 100% Sulfuric Acid.—(A) To 15 ml. of a solution of benzhydrazide in 100% sulfuric acid obtained from a freezing point determination (containing 0.35 g. of benzhydrazide) was added an additional 5.0 g. of benzhydrazide maintaining the temperature of the solution below 25°. The solution was allowed to stand at 25° for 30 minutes and then poured into 50 ml. of ice-water. The precipitate was collected and washed with cold water. This precipitate was fractionated into bicarbonate-soluble and bicarbonate-insoluble fractions. The bicarbonate soluble fraction, 1.2 g., proved to be benzoic acid, m.p. 121–122°. The bicarbonate-insoluble fraction, 0.9 g., was recrystallized from 30 ml. of 95% ethanol to give dibenzhydrazide, m.p. 241–242°, lit.[24] 241–242°. (B) To 35 ml. of a solution of benzhydrazide in 100% sulfuric acid employed in a freezing point determination was added sufficient benzhydrazide, *i.e.*, 9.2 g., to bring the total amount to 10.0 g. The solution was heated on a steam-cone for 90 minutes, the clear yellow-orange solution cooled and poured into 150 ml. of ice-water. The copious colorless precipitate was collected, washed with water and then triturated with aqueous sodium bicarbonate. The insoluble fraction was collected and dried at 105° to give 4.8 g. of product. This product was dissolved in 50 ml. of glacial acetic acid, the solution poured into 250 ml. of water, the precipitate collected and dried to give 3.6 g. of product, m.p. 138.5–139.5°. Recrystallization of this product from 95% ethanol gave 2,5-diphenyl-1,3,4-oxadiazole, m.p. 139–140°.

Anal. Calcd. for $C_{14}H_{10}ON_2$ (222): C, 75.7; H, 4.5; N, 12.6. Found: C, 75.7; H, 4.6; N, 12.5.

Stollé[25] gives a m.p. of 138° for the above compound. Acidification of the sodium bicarbonate solution obtained above gave 1.7 g. of benzoic acid, m.p. 121.5–122.5° after recrystallization from water. It will be noted that the yield of 2,5-diphenyl-1,3,4-oxadiazole was 59% based upon the crude product.

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Some Reactions of α-Phthalimidonitriles Including Those Leading to the Synthesis of α-Aminoamidoximes and α-Aminothioamides[1]

BY PAUL E. PETERSON AND CARL NIEMANN[2]

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It has been shown that DL- and L-α-phthalimido-β-phenylpropionitrile may be prepared by the dehydration of the corresponding amides. The reaction of DL- and L-α-phthalimido-β-phenylpropionitrile with hydroxylamine has been found to give the corresponding amidoximes which in turn may be transformed, with the aid of hydroxylamine, into the corresponding α-aminoamidoximes. These latter compounds were acylated to give the corresponding O,N-diacetyl- and O,N-dibenzoylamidoximes which were then converted into the corresponding α-acetamido- and α-benzamidoamidoximes by reaction with methanolic sodium methoxide. DL-α-Phthalimido-β-phenylpropionitrile, after preliminary ammonolysis, was shown to react with hydrogen sulfide to give DL-α-phthalamamido-β-phenylthiopropionamide which, with the aid of a new cleavage procedure, was converted into DL-α-amino-β-phenylthiopropionamide. This latter compound was acetylated to give DL-α-acetamido-β-phenylthiopropionamide. DL-α-Phthalimido-β-phenylpropionitrile was observed to react with methanolic hydrogen chloride to give the corresponding imino ester and to be reduced with stannous chloride to give the corresponding aldehyde.

It is well known that esters, hydroxamides, amides and hydrazides of certain α-amino acids or acylated α-amino acids may serve as specific substrates for at least one of the proteolytic enzymes, *i.e.*, α-chymotrypsin. However, in all of the above derivatives the carbonyl group associated with the hydrolyzable bond is a common structural feature and nothing is known of the behavior of those derivatives in which the oxygen atom of this carbonyl group is replaced by another atom or group. Therefore, in order to investigate the consequences of such a structural change in a molecule otherwise capable of functioning as a specific substrate for α-chymotrypsin, we have directed our attention to the development of synthetic procedures for the preparation of α-amino acid derivatives in which the carbonyl oxygen atom associated with the potential carboxyl group of the α-amino acid is replaced by another atom or group. In this com-

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munication we shall limit our discussion to the preparation of several such α-amino acid derivatives which may be obtained *via* an α-phthalimidonitrile and in particular *via* α-phthalimido-β-phenylpropionitrile. The transformations with which we shall be concerned are summarized in Fig. 1.

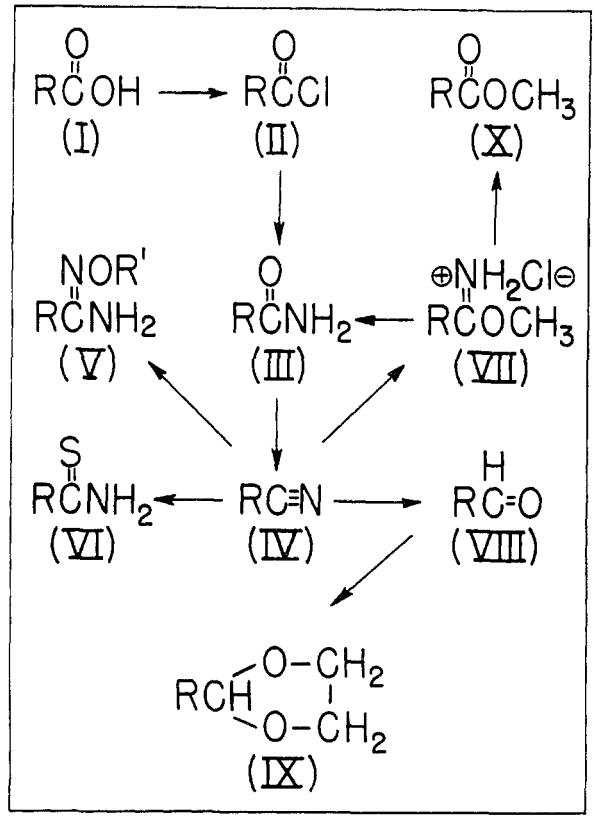

Fig. 1.—Summary of reactions investigated: a, R = $C_6H_4CO_2NCHCH_2C_6H_5$; b, R = $H_2NCHCH_2C_6H_5$; c, R = $CH_3CONHCHCH_2C_6H_5$; d, R = $C_6H_5CONHCHCH_2C_6H_5$; e, R = o-$C_6H_4(CO_2H)CONHCHCH_2C_6H_5$; f, R = o-$C_6H_4(CONH_2)CONHCHCH_2C_6H_5$; g, R = $C_6H_4CO_2NCHCH_2C_6H_5$, R′ = H; h, R = $H_2NCHCH_2C_6H_5$, R′ = H; i, R = $CH_3CONHCHCH_2C_6H_5$, R′ = CH_3CO; j, R = $CH_3CONHCHCHCH_2C_6H_5$, R′ = H; k, R = $C_6H_5CONHCHCH_2C_6H_5$, R′ = C_6H_5CO); l, R = $C_6H_5CONHCHCH_2C_6H_5$, R′ = H.

Because of an ultimate interest in optically active α-phthalimidonitriles, it was decided to restrict attention to those methods of preparation which could be based upon the relatively available optically active α-amino acids. However, in the initial stages of investigation the capabilities of the various methods of preparation were assessed on the basis of prior experience with the DL-mixtures.

It is known[3,4] that both DL- and L-α-phthalimido-β-phenylpropionic acid (DL- and L-Ia) may be converted into the corresponding acid chlorides DL- and L-IIa by the reaction of DL- or L-Ia with phosphorus pentachloride. We have found that the same transformations may be carried out with the aid of thionyl chloride and that both DL- and L-α-phthalimido-β-phenylpropionamide (DL- and L-IIIa) may be prepared from the corresponding acid chlorides DL- and L-IIa by the controlled reaction of these latter compounds with aqueous ammonia.

Preliminary attempts to dehydrate DL-α-phthalimido-β-phenylpropionamide (DL-IIIa) to the corresponding nitrile DL-IVa by the reaction of DL-IIIa at elevated temperatures with either thionyl chloride, phosphorus oxychloride, phosphorus pentachloride in phosphorus oxychloride or phosphorus pentachloride in chloroform led only to the recovery of the starting material DL-IIIa. However, it was noted that hydrocinnamamide was dehydrated rapidly to the corresponding nitrile by the reaction of the former compound with thionyl chloride under refluxing conditions.

The reaction of DL-α-phthalimido-β-phenylpropionamide (DL-IIIa) with phosphorus pentachloride in warm dioxane followed by exposure of the reaction mixture to the atmosphere gave a crystalline reaction product which may have been DL-N-(α-phthalimido-β-phenylpropionyl)-phosphoramidic dichloride,[5] since the subsequent reaction of this product with methanol gave a second crystalline product whose elementary analysis indicated that it was dimethyl DL-N-(α-phthalimido-β-phenylpropionyl)-phosphoramidate. Furthermore, heating transformed the presumed DL-N-(α-phthalimido-β-phenylpropionyl)-phosphoramidic dichloride to DL-α-phthalimido-β-phenylpropionitrile (DL-IVa). Although the above sequence of reactions represented the first transformation of DL-IIIa to DL-IVa, the method employed did not appear attractive from a preparative point of view and attention was directed to methods involving the use of phosphorus pentoxide.

The reaction of DL-α-phthalimido-β-phenylpropionamide (DL-IIIa) with phosphorus pentoxide in boiling chloroform gave, after three days, the desired nitrile DL-IVa, but when the same reaction was conducted with D-IIIa, it was noted that several recrystallizations were necessary to obtain a product of constant melting point and rotation. With this indication of partial racemization during dehydration with phosphorus pentoxide, it was decided to investigate a recently described procedure for the conversion of amides to nitriles by the reaction of the former compounds with benzenesulfonyl chloride and pyridine.[6]

The reaction of DL- and L-α-phthalimido-β-phenylpropionamide (DL- and L-IIIa) with benzenesulfonyl chloride and pyridine[6] gave the corresponding nitriles DL- and L-IVa in yields of the order of 90% with no evidence of significant racemization in the reaction involving the optically active species. From these results and the ready conversion of phthalimidoacetamide to the known phthalimidoacetonitrile[7] by the same procedure, it may be concluded that optically active α-phthalimidonitriles are readily prepared from the corresponding carboxamides by the method of Stephens,

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(4) J. C. Sheehan, D. W. Chapman and R. W. Roth, *ibid.*, **74**, 3822 (1952).
(5) W. Steinkopf, *Ber.*, **41**, 3571 (1908).
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(7) E. Radde, *Ber.*, **55**, 3174 (1922).

Bianco and Pilgrim.[6] Since this work was completed, it has been reported[8] that nitriles may be obtained in excellent yields by the reaction of carboxamides with phosphorus oxychloride in the presence of pyridine. The application of this latter procedure to amides of the type considered in this communication has not been attempted.

Hydroxylamine in the presence of hydroxylamine hydrochloride was observed to react with DL-α-phthalimido-β-phenylpropionitrile (DL-IVa) to give DL-α-phthalimido-β-phenylpropionamidoxime (DL-Vg) which was characterized by elementary analysis and by the fact that this compound did not react with ferric chloride in a manner characteristic of hydroxamic acids. The behavior of the above reaction product toward ferric chloride was similar to that of other α-acylaminoamidoximes to be described later in that a red or purple color was produced with ferric chloride in absolute ethanol but not in aqueous ethanol. Other amidoximes are known to give a color with ferric chloride which is destroyed by acids,[9] a behavior which distinguishes these compounds from the hydroxamic acids.

When DL-α-phthalimido-β-phenylpropionamidoxime (DL-Vg) was heated under refluxing conditions with one mole equivalent of hydroxylamine and one of sodium methoxide, the phthaloyl group was cleaved. The products obtained from this reaction were DL-α-amino-β-phenylpropionamidoxime (DL-Vh) and the known deep red colored sodium salt of phthaloxime[10] which was subsequently titrated with standard acid to give the colorless phthaloxime.[11]

In the cleavage described above, and in the cleavage involving a phthalimido aldehyde derivative to be described later, the desired amino compound was separated readily from the sodium salt of phthaloxime by extracting the evaporated reaction mixture with ethyl acetate in which the sodium salt of phthaloxime is relatively insoluble. With regard to future applications of the above procedure for the cleavage of the phthaloyl group, it should be noted that the possibility of a concomitant partial racemization is not excluded although the ease with which the optically active acylated α-aminoamidoximes were purified, *vide post*, suggests that there was little or no racemization in these cases. A strongly basic reagent might be expected to racemize some types of amino acid derivatives. However, it is possible that the cleavage reagent is less basic than the sodium methoxide solution from which it is prepared due to the conversion of methoxide ion to hydroxylamine anion, *i.e.*, NH_2O^-. Finally it should be noted that there is no evidence available at the present time to indicate that in general cases where both methods are applicable cleavage of a phthaloyl group by the hydroxylamine–sodium methoxide reagent is to be preferred to cleavage *via* the usual reagent, *i.e.*, hydrazine.[3]

Acylation of the crystalline DL-α-amino-β-phenylpropionamidoxime (DL-Vh) gave the corresponding O,N-diacetyl and O,N-dibenzoyl derivatives DL-Vi and DL-Vk which were then partially solvolyzed with dilute methanolic sodium methoxide to give DL-α-acetamido- and DL-α-benzamido-β-phenylpropionamidoxime (DL-Vj and DL-Vl). Repetition of the above reaction sequence starting with L-phenylalanine gave the L-isomers of all the above compounds, although in this instance the L-α-amino-β-phenylpropionamidoxime (L-Vh) was obtained only in the form of a sirup.

It has been reported[12] that phthalimidothioacetamide may be prepared by passing hydrogen sulfide into an ethanolic solution of phthalimidoacetonitrile containing 0.1 mole equivalent of triethanolamine. When DL-α-phthalimido-β-phenylpropionitrile (DL-IVa) was subjected to the same reaction conditions, only a small amount of DL-α-phthalimido-β-phenylthiopropionamide (DL-VIa) was obtained. However, when DL-IVa was first dissolved in a methanolic solution of ammonia and the resulting solution saturated with hydrogen sulfide, a good yield of product which appeared to be a methanol solvate of DL-α-phthalamamido-β-phenylthiopropionamide (DL-VIf) separated in a crystalline form. In connection with the above observations it should be noted that DL-IVa was found to react with aqueous ethanolic sodium hydroxide to give DL-α-(*o*-carboxybenzamido)-β-phenylpropionitrile (DL-IVe)[3,13] and that DL-IIIa upon prolonged contact with aqueous ammonia was transformed into DL-α-phthalamamido-β-phenylpropionamide (DL-IIIf).

The reaction of DL-α-phthalamamido-β-phenylthiopropionamide methanol solvate (DL-VIf) with one equivalent of hot aqueous sodium hydroxide gave a poor yield of the desired DL-phenylalanine thioamide (DL-VIb). However, DL-VIb was obtained in a 55% yield when DL-VIf was heated with one mole equivalent of methanolic hydrogen chloride. The other major product obtained from this reaction mixture was phthalimide.

The above two step procedure for cleaving the phthaloyl group, *i.e.*, reaction with ammonia followed by reaction with hydrogen chloride, is of interest because the intermediate phthalamamido compound is clearly more stable toward nucleophilic reagents than is the phthalimido derivative. However, it should be noted that the yield of the amino compound formed on cleavage of the phthalamamido derivative may be diminished by a competing reaction in which the phthalamamido derivative is converted to the corresponding phthalimido derivative through the loss of ammonia. This latter reaction was observed in the case at hand by the isolation of a small amount of DL-α-phthalimido-β-phenylthiopropionamide (DL-VIa) from the reaction of the corresponding phthalamamido derivative DL-VIf with methanolic hydrogen chloride as described above.

The preparation of the DL- and L-amidoxime and the DL-thioamide of phenylalanine described in this communication appear to be the first examples of the conversion of an α-amino acid to the corre-

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sponding α-aminoamidoxime or α-aminothioamide. While DL-phenylalanine thioamide is mentioned in the literature,[14] its melting point and method of preparation is not disclosed in the abstract. It may be inferred that it was prepared from an α-aminonitrile obtained in a Strecker reaction. Such α-aminonitriles have served as starting materials for the preparation of several α-acylaminothioamides[15,16] and of at least one α-acylaminoamidoxime.[17] We have not considered such procedures in the present study because it was our aim to develop synthetic methods which were capable of giving optically active compounds starting from optically active α-amino acids.

The reaction of DL-α-phthalimido-β-phenylpropionitrile (DL-IVa) with methanol and hydrogen chloride in benzene solution gave crystalline DL-α-phthalimido-β-phenylpropionimido methyl ester hydrochloride (DL-VIIa). This latter compound, like other imido ester hydrochlorides, gave DL-α-phthalimido-β-phenylpropionamide (DL-IIIa) upon heating. When the imido ester hydrochloride (DL-VIIa) was heated under refluxing conditions with methanol, no ortho ester was formed. Instead, there was obtained DL-α-phthalimido-β-phenylpropionamide (DL-IIIa) and DL-α-phthalimido-β-phenylpropionic acid methyl ester (DL-Xa). A similar behavior has been noted for other α-substituted imido esters.[18] When DL-VIIa was dissolved in water, a clear solution was first obtained and then within a few seconds a crystalline precipitate of the methyl ester DL-Xa began to form. This behavior is in striking contrast to that of DL-α-phthalimido-β-phenylpropionamidoxime (DL-Vg) which partially dissolved in dilute aqueous hydrochloric acid and then gave a precipitate of DL-α-phthalimido-β-phenylpropionamidoxime hydrochloride which could be reconverted to the starting material by treatment with base.

Phthalimidoacetonitrile was converted readily to the methyl imido ester hydrochloride by the method described immediately above. This observation is of interest in view of the previous unsuccessful attempt to carry out this reaction.[7] However, the present reaction conditions differed from those used earlier[7] in that in the present study benzene was used as a solvent. When phthalimidoacetoimido methyl ester hydrochloride was dissolved in water, a precipitate of methyl phthalimidoacetate soon formed. The product was identified by a mixed melting point with the ester prepared from phthalimidoacetyl chloride and methanol.

A Stephen reduction[19] of DL-α-phthalimido-β-phenylpropionitrile (DL-IVa) gave the corresponding aldehyde (DL-VIIIa) in a 34% yield. A recent report[20] describes the preparation of the same aldehyde by the Rosenmund reduction[21] of the acid chloride, a method which has been used with other α-phthalimido acid chlorides.[22,23] However, in this latter case[20] the reported melting point was about twenty degrees lower than that observed in this study. Consequently we repeated the preparation of the aldehyde DL-VIIIa by the Rosenmund reduction[20-23] and upon the addition of hexane to the toluene reaction mixture obtained a mixture of oil and crystals. The crystalline portion was separated and recrystallized to give a product which melted only three degrees lower than the product obtained from the Stephen reduction. A mixed melting point of these two products showed no significant depression. From a comparison of these two routes to the aldehyde DL-VIIIa it may be seen that the route *via* the nitrile involves more steps but that in the case investigated this procedure gave a product of greater purity.

Repetition of the Stephen reduction with L-α-phthalimido-β-phenylpropionitrile (L-IVa) gave the optically active aldehyde L-VIIIa in a 43% yield. An unexpected property of both DL- and L-VIIIa was their formation of alcohol adducts which appeared to be relatively stable hemiacetals. These latter products had characteristic decomposition points and could be recrystallized unchanged from hydrocarbon type solvents.

In order to provide another example of cleavage of the phthaloyl group with the hydroxylamine–sodium methoxide reagent, *vide ante*, DL-α-phthalimido-β-phenylpropionaldehyde (DL-VIIIa) was converted into the corresponding ethylene glycol acetal DL-IXa,[23] and the latter compound was subjected to treatment with the hydroxylamine–sodium methoxide reagent. Benzoylation of the ethyl acetate solution of DL-IXb gave DL-α-benzamido-β-phenylpropionaldehyde ethylene glycol acetal (DL-IXd).

Experimental[24,25]

DL-α-Phthalimido-β-phenylpropionamide (DL-IIIa).—The fusion of an equimolar mixture of DL-phenylalanine (DL-Ib) and phthalic anhydride at 185–200° as described previously[3,4] led to the isolation of crude DL-α-phthalimido-β-phenylpropionic acid (DL-Ia). The crude DL-Ia was converted into the solid DL-α-phthalimido-β-phenylpropionyl chloride (DL-IIa) with the aid of thionyl chloride, and the acid chloride obtained from 191 g. of DL-Ib was allowed to react with 1 liter of cold concd. ammonium hydroxide for a period of *ca.* 30 minutes to give 288 g. (85%) of crude DL-α-phthalimido-β-phenylpropionamide (DL-IIIa). Recrystallization of this latter product from either 95% ethanol or methyl Cellosolve gave 70–80% of DL-IIIa, m.p. 236.8–237.2°.

Anal. Calcd. for $C_{17}H_{14}O_3N$ (280): C, 69.4; H, 4.8; N, 9.5. Found: C, 69.4; H, 4.8; N, 9.5.

L-α-Phthalimido-β-phenylpropionamide (L-IIIa).—L-IIIa was prepared essentially as described for DL-IIIa except that the initial fusion was conducted at 185° for 8 minutes or at 150° for 30 minutes. From 5.05 g. of L-Ib there was obtained 7.4 g. (85%) of L-IIIa, m.p. 226–227.5° after recrystallization from 95% ethanol.

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(24) All melting points are uncorrected.

(25) Microanalyses by Dr. A. Elek.

Anal. Calcd. for $C_{17}H_{14}O_3N$ (280): C, 69.4; H, 4.8; N, 9.5. Found: C, 69.8; H, 5.0; N, 9.9.

Reaction of DL-IIIa with Phosphorus Pentachloride.—To a suspension of 12 g. of DL-IIIa in 170 ml. of dioxane was added 16.8 g. of phosphorus pentachloride, and the stirred reaction mixture was warmed to *ca.* 40° to effect solution of the DL-IIIa. The clear supernatant solution was decanted from the excess phosphorus pentachloride and diluted with 520 ml. of hexane. Exposure of the resulting solution, contained in a number of crystallizing dishes, to the atmosphere resulted in the formation of a crystalline precipitate which was collected and recrystallized from a 1:1 mixture of chloroform and benzene to give 14.6 g. of what appeared to be DL-N-(α-phthalimido-β-phenylpropionyl)-phosphoramidic dichloride. A solution of 1 g. of this latter substance in 5 ml. of methanol was heated under refluxing conditions for a few minutes, the reaction mixture cooled, the crystalline precipitate collected and recrystallized from methanol to give dimethyl DL-N-(α-phthalimido-β-phenylpropionyl)-phosphoramidate, m.p. 207–209° with resolidification and remelting at *ca.* 220°.

Anal. Calcd. for $C_{19}H_{19}O_6N_2P$ (402): C, 56.7; H, 4.8; P, 7.7. Found: C, 56.1; H, 4.7; P, 7.1.

Thermal decomposition of the presumed phosphoramidic dichloride at 180° followed by extraction of the reaction mixture with benzene and dilution of the decolorized benzene extract with hexane gave a crystalline product, m.p. 134–135° after partial melting and resolidification at 125°. When this substance was mixed with an authentic sample of DL-α-phthalimido-β-phenylpropionitrile (DL-IVa), m.p. 134–136° after partial melting and resolidification at 125°, no depression of the m.p. was observed.

DL-α-Phthalimido-β-phenylpropionitrile (DL-IVa).[6]—To a refluxing suspension of 75 g. of DL-IIIa in 2200 ml. of reagent grade chloroform was added, in the course of 72 hr., 90 g. of phosphorus pentoxide. Partial evaporation of the solvent gave 54.6 g. (78%) of DL-IVa, m.p. 134–136° after partial melting and resolidification at 125°, after recrystallization from chloroform.

Anal. Calcd. for $C_{17}H_{12}O_2N_2$ (276): C, 73.9; H, 4.4; N, 10.1. Found: C, 73.9; H, 4.5; N, 10.1.

A mixture of 2.76 g. of DL-IIIa, 10 ml. of pyridine and 6 ml. of benzenesulfonyl chloride was refluxed for 10 minutes. The addition of water to the cooled reaction mixture gave 2.56 g. (99%) of crude DL-IVa, m.p. 126–131° with partial resolidification at 127°. Recrystallization of the crude DL-IVa from methanol gave DL-IVa, m.p. 134–135° after partial melting and resolidification at 125°.

D-α-Phthalimido-β-phenylpropionitrile (D-IVa).—The reaction of 0.39 g. of D-IIIa[26] in 15 ml. of chloroform with 0.40 g. of phosphorus pentoxide under the same conditions used for the preparation of DL-IVa gave 0.38 g. of product, m.p. 138–149°. Recrystallization of this product first from a mixture of chloroform and benzene and then repeatedly from a mixture of benzene and hexane gave 0.23 g. of D-IVa, m.p. 149–152°, $[\alpha]^{27}D$ +100 ± 2° (*c* 4.6% in chloroform).

L-α-Phthalimido-β-phenylpropionitrile (L-IVa).[6]—The reaction of 5 g. of L-IIIa with 25 ml. of pyridine and 10 ml. of benzenesulfonyl chloride for 10 minutes at the boiling temperature of the reaction mixture gave 4.6 g. (97%) of crude L-IVa. This product was recrystallized from methanol to give 4.2 g. (90%) of L-IVa, m.p. 150–153.2°, $[\alpha]^{25}D$ −103 ± 1° (*c* 2% in chloroform).

Anal. Calcd. for $C_{17}H_{12}O_2N_2$ (276): C, 73.9; H, 4.4; N, 10.1. Found: C, 74.0; H, 4.5; N, 10.1.

DL-α-(*o*-Carboxybenzamido)-β-phenylpropionitrile (DL-IVe).—To a solution of 1.6 g. of DL-IVa in 40 ml. of methanol was added 20 ml. of water. To this oily suspension was added 15 ml. of 1 *N* aqueous sodium hydroxide, the reaction mixture stirred for 10 minutes and the clear solution acidified with 4 ml. of 6 *N* aqueous hydrochloric acid. The resulting precipitate was collected and recrystallized from ethanol three times to give DL-IVe, m.p. 165° dec.

Anal. Calcd. for $C_{17}H_{14}O_3N_2$ (294): C, 69.4; H, 4.8; N, 9.5. Found: C, 69.5; H, 5.0; N, 9.6.

DL-IVe was heated at 165° until bubbles no longer formed and the residue was then recrystallized from a mixture of benzene and hexane to give DL-IVa which was identified by a mixed m.p. with an authentic sample.

DL-α-Phthalamamido-β-phenylpropionamide (DL-IIIf).—Crude DL-IIIa was allowed to remain in contact with concd. aqueous ammonia for a period of 36 hours and the resulting product recrystallized from ethanol to give transparent prisms which were transformed into a colorless powder, m.p. 205–210° dec., when the prisms were collected by filtration and air-dried.

Anal. Calcd. for $C_{17}H_{17}O_3N_3$ (311): C, 65.6; H, 5.5; N, 13.5. Found: C, 65.5; H, 5.6; N, 13.4.

When DL-IIIf prepared as described above was heated to its melting point, ammonia was lost and the melt resolidified to give DL-IIIa, m.p. 236–237°.

DL-α-Phthalimido-β-phenylpropionamidoxime (DL-Vg).—To a solution prepared from 30 ml. of 1 *M* methanolic sodium methoxide and 69 ml. of 0.5 *M* methanolic hydroxylamine hydrochloride was added 8.28 g. of DL-IVa in 120 ml. of methanol, and the resulting mixture was refluxed for 3 hr. From the cooled reaction mixture there was obtained 5.74 g. of DL-Vg, m.p. 198–204° dec. Concentration of the filtrate gave an additional 0.83 g. of DL-Vg bringing the total yield to 71%.

Anal. Calcd. for $C_{17}H_{15}O_2N_3$ (309): C, 66.0; H, 4.9; N, 13.6. Found: C, 66.1; H, 5.0; N, 13.6.

L-α-Phthalimido-β-phenylpropionamidoxime (L-Vg).—The reaction of 10.2 g. of L-IVa in 290 ml. of methanol with a mixture of 37 ml. of 1 *M* methanolic sodium methoxide and 42.4 ml. of 1 *M* methanolic hydroxylamine hydrochloride was conducted under refluxing conditions for 3 hr., the volume of the reaction mixture reduced to *ca.* 100 ml. and 60 ml. of water added to the concentrate to give 8.35 g. (73%) of L-Vg, m.p. 164–171° dec., $[\alpha]^{25}D$ −108 ± 1° (*c* 2.7% in methanol).

Anal. Calcd. for $C_{17}H_{15}O_3N_3$ (309): C, 66.0; H, 4.9; N, 13.6. Found: C, 66.2; H, 4.9; N, 13.5.

DL-α-Amino-β-phenylpropionamidoxime (DL-Vh).—To a methanol solution containing 0.0107 mole equivalent of hydroxylamine and 0.0107 mole equivalent of sodium methoxide was added 3.3 g., 0.0107 mole, of DL-Vg in 150 ml. of methanol, and the mixture was refluxed for 2 hr. Evaporation of the deep red reaction mixture to dryness *in vacuo* gave a solid which was extracted with 25 ml. of hot ethyl acetate. From the ethyl acetate extract there was obtained 0.93 g. (52%) of crude DL-Vh, m.p. 115–117.5°. Recrystallization of the crude product from 10 ml. of water gave 0.65 g. of DL-Vh, m.p. 117–118°.

Anal. Calcd. for $C_9H_{13}O_3N_3$ (211): C, 60.3; H, 7.3; N, 23.5. Found: C, 60.3; H, 7.3; N, 23.5.

DL-O-Acetyl-α-acetamido-β-phenylpropionamidoxime (DL-Vi).—A solution of 0.5 g. of DL-Vh in ethyl acetate was acetylated with acetic anhydride and aqueous potassium carbonate to give 0.56 g. (76%) of DL-Vi, m.p. 160–162° dec.

Anal. Calcd. for $C_{13}H_{17}O_3N_3$ (263): C, 59.3; H, 6.5; N, 16.0. Found: C, 59.3; H, 6.6; N, 16.0.

L-O-Acetyl-α-acetamido-β-phenylpropionamidoxime (L-Vi).—An ethyl acetate solution of L-Vh prepared from 4.15 g. of L-Vg as described for the DL-compound was acetylated with acetic anhydride and aqueous potassium carbonate to give 1.44 g. (49%) of L-Vi.

DL-α-Acetamido-β-phenylpropionamidoxime (DL-Vj).—A solution of 0.56 g. of DL-Vi in 15 ml. of methanol containing 4 drops of 1 *N* methanolic sodium methoxide was heated to boiling for 9 minutes, the solution evaporated to dryness *in vacuo* and the residue recrystallized from hot water to give 0.31 g. (66%) of DL-Vj, m.p. 156–158° dec.

Anal. Calcd. for $C_{11}H_{15}O_2N_3$ (221): C, 59.7; H, 6.8. Found: C, 59.8; H, 6.6.

L-α-Acetamido-β-phenylpropionamidoxime (L-Vj).—Partial solvolysis of 1.44 g. of L-Vi as described for the DL-compound gave 0.89 g. (74%) of L-Vj, m.p. 167–169° dec., $[\alpha]^{25}D$ −11.1 ± 0.4° (*c* 2.1% in ethanol).

Anal. Calcd. for $C_{11}H_{15}O_2N_3$ (221): C, 59.7; H, 6.8; N, 18.0. Found: C, 59.8; H, 7.0; N, 17.7.

A mixture of L-Vj, m.p. 167–169°, and of DL-Vj, m.p. 156–158°, gave a m.p. of 140–150°.

DL-O-Benzoyl-α-benzamido-β-phenylpropionamidoxime (DL-Vk).—DL-Vh, 0.43 g. in 200 ml. of ethyl acetate, was allowed to react with 1 ml. of benzoyl chloride and 4.8 ml. of 1 *M* aqueous potassium carbonate. The ethyl acetate

(26) This compound was prepared from D-Ib by the same procedure used for the preparation of L-IIIa.

phase was dried over magnesium sulfate and the solvent removed to give 0.7 g. (75%) of crude DL-Vk. The crude product was recrystallized from 140 ml. of acetonitrile to give 0.4 g. of DL-Vk, m.p. 206–207° dec.

L-O-Benzoyl-α-benzamido-β-phenylpropionamidoxime (L-Vk).—The phthaloyl group present in L-Vg, 5 g., was cleaved with the methanolic hydroxylamine–sodium methoxide reagent as described for the DL-compound, the ethyl acetate extract of L-Vh[27] diluted to 200 ml. with ethyl acetate and allowed to react with 5 ml. of benzoyl chloride and 5 ml. of 1 *M* aqueous potassium carbonate. The desired dibenzoyl derivative crystallized from the ethyl acetate phase to give 2.91 g. (47%) of L-Vk, and this product was recrystallized from acetonitrile to give L-Vk, m.p. 204–211° dec., $[\alpha]^{25}D$ −38.0 ± 1° (*c* 2.4% in dimethylformamide).

Anal. Calcd. for $C_{23}H_{21}O_3N_3$ (387): C, 71.3; H, 5.5; N, 10.9. Found: C, 71.4; H, 5.5; N, 10.9.

DL-α-Benzamido-β-phenylpropionamidoxime (DL-VI).—To a solution of 0.4 g. of DL-Vk in 50 ml. of methanol was added 2–3 drops of 1 *N* methanolic sodium methoxide and the resulting mixture heated to boiling for a few minutes. The addition of water to one-half of the above solution gave 0.09 g. (62%) of DL-VI. This product was recrystallized from acetonitrile to give DL-VI, m.p. 200–202° dec.

Anal. Calcd. for $C_{16}H_{17}O_2N_3$ (283): C, 67.8; H, 6.1; N, 14.8. Found: C, 67.9; H, 6.1; N, 14.8.

The structure of DL-VI, and all of the other monoacylated α-aminoamidoximes described in this communication, was confirmed by the fact that these compounds gave a reddish-purple color with ferric chloride in anhydrous methanol and an intense yellow-green color with a very little dilute Fehling solution. The copper complexes of a number of amidoximes have been described.[28] The diacylated α-aminoamidoximes gave no color with either of the above reagents.

L-α-Benzamido-β-phenylpropionamidoxime (L-VI).—L-Vk, 1 g., was debenzoylated by the procedure described for the DL-compound to give 0.32 g. (44%) of L-VI, m.p. 200–203° dec., $[\alpha]^{25}D$ −85.2 ± 1° (*c* 2.0% in dimethylformamide).

DL-α-Phthalimido-β-phenylthiopropionamide (DL-VIa).—Hydrogen sulfide was slowly bubbled through a heated solution of 13.8 g. of DL-IVa and 0.75 g. of triethanolamine in 300 ml. of ethanol for a period of 2 days. The reaction mixture was cooled, the unreacted DL-IVa recovered by filtration and a portion of the filtrate evaporated to give but a few large prisms which were recrystallized from aqueous ethanol to give DL-VIa, m.p. 160.5–162°.

Anal. Calcd. for $C_{17}H_{14}O_2N_2S$ (310): C, 65.8; H, 4.6; N, 7.0; S, 10.3. Found: C, 66.0; H, 4.6; N, 7.0; S, 10.3.

DL-α-Phthalamamido-β-phenylthiopropionamide (DL-VIf).—A solution of 8 g. of DL-IVa in 120 ml. of ethanol previously saturated with ammonia was saturated with hydrogen sulfide, and the reaction mixture was allowed to stand at room temperature overnight. The crystalline product was collected, washed with ether and air-dried to give 9.72 g. of product. A second and similar preparation was conducted using methanol instead of ethanol as a solvent. In this latter case the air-dried product was heated at 160° *in vacuo*, and it was observed that the product partially melted and then resolidified to give a product m.p. 173–177° dec. after recrystallization from acetonitrile. When this latter substance was again recrystallized from methanol it again melted and resolidified in the range between 100–160° with a loss in weight of 7.35%. The loss of a molecule of methanol would require a loss in weight of 8.5%. It is therefore concluded that DL-VIf was obtained as a methanol solvate from the methanolic reaction system and that when this product is heated, there is formed DL-VIf, m.p. 173–177° dec.

Anal. Calcd. for $C_{17}H_{17}O_2N_3S \cdot CH_3OH$ (359): C, 60.1; H, 5.9; S, 8.9. Found: C, 59.9; H, 5.6; S, 8.3.

DL-α-Amino-β-phenylthiopropionamide (DL-VIb).—A suspension of 4.1 g. of DL-VIf in one mole equivalent of aqueous sodium hydroxide was heated to 85° and the clear yellow solution cooled to give 0.5 g. (24%) of DL-VIb, m.p. 135–136.3° dec. after recrystallization from aqueous ethanol.

Anal. Calcd. for $C_9H_{12}N_2S$ (180): C, 60.0; H, 6.7; N, 15.5; S, 17.8. Found: C, 60.1; H, 6.7; N, 15.6; S, 17.7.

DL-VIb, m.p. 135–136.3° dec., was readily soluble in aqueous hydrochloric acid.

To a solution of 25 ml. of methanol containing 0.0071 mole of hydrogen chloride was added 2.0 g., 0.0056 mole, of DL-VIf and mixture refluxed for 4 hr. Partial concentration of the reaction mixture led to the precipitation of phthalimide, identified by a mixed m.p. determination with an authentic sample, which was removed and the filtrate evaporated to dryness *in vacuo* over both sulfuric acid and solid potassium hydroxide. The residue was triturated with 15 ml. of water, the suspension filtered and the filtrate made alkaline with aqueous sodium hydroxide to cause the precipitation of DL-VIb which was collected and dried to give 0.55 g. (55%) of DL-VIb, m.p. 134–135° dec., after recrystallization from aqueous ethanol. The water-insoluble fraction was identified as DL-VIa. A second preparation starting with 10.2 g. of DL-VIf gave 2.48 g. (47%) of DL-VIb, m.p. 134–135° dec., and a mixed m.p. of this product and that obtained by the first procedure showed no depression.

DL-α-Phthalimido-β-phenylpropionimido Methyl Ester Hydrochloride (DL-VIIa).—To a solution of 7.72 g. of DL-IVa in 60 ml. of benzene was added one mole equivalent of methanol and the solution saturated with hydrogen chloride. After a lapse of 2 days the crystalline precipitate was collected to give 8.04 g. (83%) of DL-VIIa, m.p. 234–236, with conversion to DL-IIIa, m.p. 236.8–237.2°, identified by a mixed m.p. with an authentic sample.

Reaction of DL-VIIa with Methanolic Sodium Methoxide. To a solution of 8 g. (0.0344 mole) of DL-VIIa in 100 ml. of dry methanol was added one mole equivalent of methanolic sodium methoxide, the reaction mixture evaporated to dryness and the residue extracted with ether. From the residue there was obtained DL-IIIa, m.p. 236–237°, and from the ethereal extract, after the addition of hexane, oily crystals which were collected and recrystallized from aqueous methanol to give 0.43 g. (6%) of methyl α-phthalimido-β-phenylpropionate (DL-Xa), m.p. 124.8–126.8°.

Anal. Calcd. for $C_{18}H_{16}O_3N_2$ (308): C, 70.1; H, 5.2; N, 9.1. Found: C, 70.1; H, 5.3; N, 9.1.

DL-α-Phthalimido-β-phenylpropionaldehyde (DL-VIIIa).—Hydrogen chloride was passed into a suspension of 15.12 g. of anhydrous stannous chloride in 160 ml. of dry ether until the stannous chloride phase was present as a clear liquid. To this mixture was added 11.04 g. of DL-IVa in 80 ml. of benzene and 80 ml. of dry ether and the mixture again saturated with hydrogen chloride until the heavy phase became clear. The mixture was then allowed to stand for 5 hours at room temperature and for 36 hr. at 4° whereupon the ether layer was decanted, the excess hydrogen chloride blown off with nitrogen and the residue shaken for 10 minutes with 40 ml. of water and 240 ml. of benzene. The benzene layer was separated, the extraction repeated with an additional 240 ml. of benzene, the benzene extracts combined, dried over magnesium sulfate and the solvent removed to give 8.3 g. of an oil which soon solidified. The extraction of 6.56 g. of this product with 175 ml. of hot ligroin, b.p. 85–100°, gave an extract from which there was obtained on cooling 3.04 g. (34%) of crude DL-VIIIa, m.p. 94.5–96°. Recrystallization of this product from hexane gave DL-VIIIa, m.p. 96.3–96.8°. The reported[20] m.p. is 75–78°.

Anal. Calcd. for $C_{17}H_{13}O_3N$ (279): C, 73.1; H, 4.7; N, 5.0. Found: C, 73.4; H, 4.8; N, 5.1.

DL-VIIIa was converted into the corresponding semicarbazone, m.p. 238–240° dec. after recrystallization from acetonitrile. The reported[20] m.p. of this compound is 215–216°.

Anal. Calcd. for $C_{18}H_{16}O_2N_4$ (320): C, 64.3; H, 4.8; N, 16.7. Found: C, 64.2; H, 4.8; N, 16.6.

The addition of water to a solution of DL-VIIIa in ethanol led to the formation of a crystalline product which was recrystallized from a mixture of benzene and hexane to give the ethyl hemiacetal of DL-VIIIa, m.p. 122–124° dec.

Anal. Calcd. for $C_{19}H_{17}O_3N$ (307): C, 70.1; H, 5.9; N, 4.3. Found: C, 70.6; H, 5.9; N, 4.1.

The addition of hexane to a hot 1-butanol solution of DL-VIIIa gave upon cooling a crystalline product which when recrystallized from a mixture of benzene and hexane gave the *n*-butyl hemiacetal of DL-VIIIa, m.p. 102–104°.

(27) Evaporation of the solvent from this extract failed to give a crystalline product. L-Vh was obtained only as a water-soluble sirup.

(28) E. Nordmann, *Ber.*, **17**, 2746 (1884).

Anal. Calcd. for $C_{21}H_{23}O_4N$ (343): C, 71.4; H, 6.6; N, 4.0. Found: C, 71.8; H, 6.6; N, 4.0.

L-α-Phthalimido-β-phenylpropionaldehyde (L-VIIIa).—L-IVa, 1.69 g., was converted into L-VIIIa by the same procedure used above for the corresponding DL-compound. The crude L-VIIIa was recrystallized from ligroin, b.p. 85–100°, to give 0.73 g. (43%) of L-VIIIa, m.p. 115–117°, $[\alpha]^{25}D$ −157 ± 2° (*c* 2% in chloroform).

Anal. Calcd. for $C_{17}H_{13}O_3N$ (282): C, 73.1; H, 4.7. Found: C, 73.4. H, 5.1.

DL-α-Phthalimido-β-phenylpropionaldehyde Ethylene Glycol Acetal (DL-IXa).—The procedure of Balenović, *et al.*,[23] was employed to convert 4 g. of DL-VIIIa to DL-IXa, 3.74 g. (81%), m.p. 108–109.5° after recrystallization from hexane.

Anal. Calcd. for $C_{19}H_{17}O_4N$ (323): C, 70.6; H, 5.3; N, 4.3. Found: C, 70.4; H, 5.4; N, 4.3.

DL-α-Benzamido-β-phenylpropionaldehyde Ethylene Glycol Acetal (DL-IXd).—A solution of 1.08 g., 0.00333 mole, of DL-IXa in 40 ml. of methanol was heated for 3 hr. under refluxing conditions with 3.33 ml. of 1 *M* methanolic hydroxylamine and 3.33 ml. of 1 *M* methanolic sodium methoxide. The deep red solution was evaporated to dryness and the residue was extracted with hot ethyl acetate. The ethyl acetate extract was then allowed to react with 0.00333 mole of benzoyl chloride and 3.33 ml. of 1 *M* aqueous potassium carbonate, the ethyl acetate phase separated, dried over magnesium sulfate and evaporated to give 0.36 g. (36%) of DL-IXd, m.p. 118.5°.

Anal. Calcd. for $C_{18}H_{19}O_3N$ (297): C, 72.7; H, 6.4; N, 4.7. Found: C, 72.4; H, 6.7; N, 4.7.

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[CONTRIBUTION FROM THE QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES]

Chemical Effects of Ionizing Radiation on Proteins.[1,2] I. Effect of γ-Radiation on the Amino Acid Content of Insulin

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This study was undertaken to determine the radiosensitivity of the constituent amino acids of a protein in order to provide information which might indicate the source of objectionable qualities of aroma and flavors that have been found to occur in the sterilization of food proteins by ionizing radiation. One per cent. insulin in basic (*p*H 8.5) and in acidic (*p*H 3.0) solutions was subjected to 0, 10, 20 and 40 million r.e.p. γ-radiation doses. Cystine, tyrosine, phenylalanine, proline and histidine are demonstrated to be very radio-sensitive. Leucine, valine, lysine and arginine are significantly destroyed at the high irradiation dose level. The nitrogen-terminal amino acids of insulin, glycine and phenylalanine, are shown to be deaminated. Cysteic acid is identified in the hydrolysates of the irradiated insulin. An increase in molecular size of the irradiated insulin is reported.

Introduction

A potential peacetime use of ionizing radiation is in the preservation of foods. Such use is predicated upon the solution of certain problems, one of which is the prevention or masking of disagreeable irradiation-produced odors and flavors. Although proteins have been identified as primary sources of these odors, no correlation between odors produced and amino acid content has been found.[4] Irradiation of free amino acids has resulted principally in deamination,[5] aldehyde production,[6] H_2S liberation from cysteine/cystine,[7] and hydroxylation and ring-splitting of aromatic amino acids and histidine.[8] Only two previous investigations have attempted to determine whether amino acids making up the protein structure are destroyed by irradiation of the protein. In one study,[9] fish muscle irradiated by high-voltage electrons to a dose of 5.7×10^6 r.e.p. (roentgen equivalent physical) showed no significant destruction of the "essential" amino acids. In the other,[10] irradiation of aqueous 10^{-5} *M* serum albumin solutions with X-rays (44,600 and 66,900 r.e.p.) produced 30% destruction of glycine, alanine and glutamic acid, and 21, 18, 16 and 13% destruction of lysine, threonine, tyrosine and isoleucine, respectively.

The chemical compounds responsible for the disagreeable irradiation-produced aromas and flavors are not known. A quantitative amino acid analysis of irradiated proteins of known character should indicate those amino acids in a protein which are most radio-sensitive. Further research effort could then be directed toward the characterization of the irradiation-breakdown products from those amino acids and their relation to the observed quality defects in some protein foods.

Insulin was chosen as the first protein to be investigated because of its availability in a very pure form, and its characterization as to amino acid content.[11] Since insulin does not contain cysteine, methionine or tryptophan, the necessity for hydrolysis in base and the interference of acid-hydrolysis breakdown products of tryptophan is eliminated, and cystine is left as the only sulfur-containing amino acid.

(1) Paper No. 654 in series of papers approved for publication. The views or conclusions are those of the authors and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. The mention of commercial products does not imply they are endorsed or recommended by the Department of Defense over similar products not mentioned.

(2) Taken in part from a thesis submitted by M. P. Drake in partial fulfillment of the requirements for the M.Sc. degree, Dept. of Biochemistry, Northwestern University. Presented at Symposium on Radiation Sterilization. Agr. & Food Chem. Div., 130th Nat'l ACS Meeting, 1956.

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