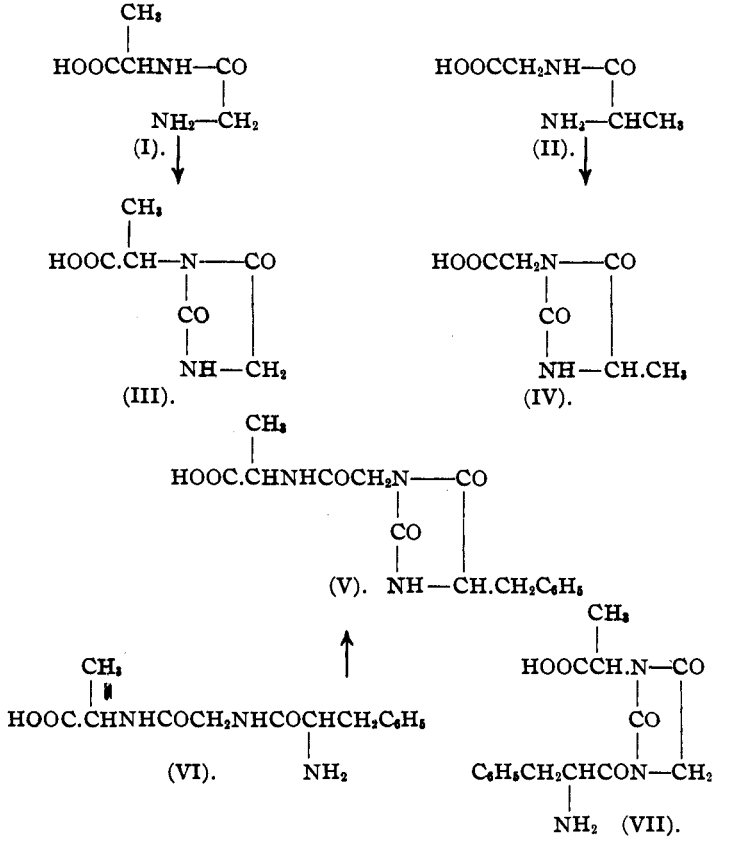
[CONTRIBUTIONS FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

RESEARCHES ON HYDANTOINS. XXXVII. SYNTHESIS OF THE POLYPEPTIDE-HYDANTOIN—PHENYLALANYL-GLYCINE-HYDANTOIN.[1]

BY TREAT B. JOHNSON AND JOSEPH S. BATES.

Received March 14, 1916.

A *polypeptide-hydantoin*, as we shall apply the term in our work, is a cyclic derivative of a polypeptide containing a hydantoin ring, which has been incorporated by joining together two nitrogen atoms of the peptide with CO in the form of a urea, and in which the characteristic grouping of the polypeptide has been preserved. For example, the hydantoins represented by Formulas III and IV are two representatives of this type of compounds and are the only hydantoins that can be constructed from their corresponding isomeric dipeptides—glycylalanine I, and alanyl-

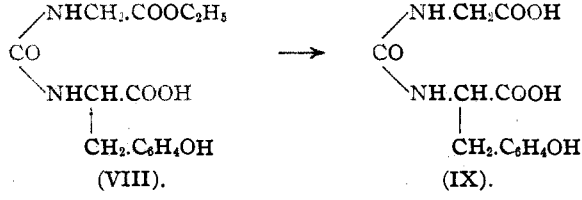


[1] Part of a dissertation presented by Mr. Joseph Sumner Bates to the Faculty of the Graduate School of Yale University, 1915, in candidacy for the Degree of Doctor of Philosophy.

glycine II. In other words, a polypeptide-hydantoin is a cyclic combination of a peptide which does not contain an unsubstituted, diacidamide grouping —CO.NH.CO— and therefore cannot break down on hydrolysis with evolution of ammonia. In the case of the higher polypeptides the possibilities of forming cyclic combinations are much greater. For example, it is possible, theoretically, to derive from the tripeptide-phenylalanylglycylalanine VI, two isomeric polypeptide-hydantoins corresponding to Formulas V and VII.

Combinations of these types have never been prepared and, as we have indicated in a preliminary paper,[1] a knowledge of their properties is very desirable from a biochemical standpoint. The writer also anticipates that an investigation of certain combinations, which may be derived from the hydantoins III and IV, will be productive of results from which more definite conclusions may be drawn regarding the tautomeric behavior of the hydantoin ring.

Symmetrically substituted ureas of α-aminoacids have been described by Morel[2] and were prepared by the action of the isocyanate—$OCN.CH_2COOC_2H_5$—on α-aminoacids in the presence of dilute alkali. He prepared in this manner from tyrosine the urea represented by Formula VIII. This was not isolated but was converted by hydrolysis with alkali into its corresponding acid urea represented by Formula IX. He also applied a



(VIII). (IX).

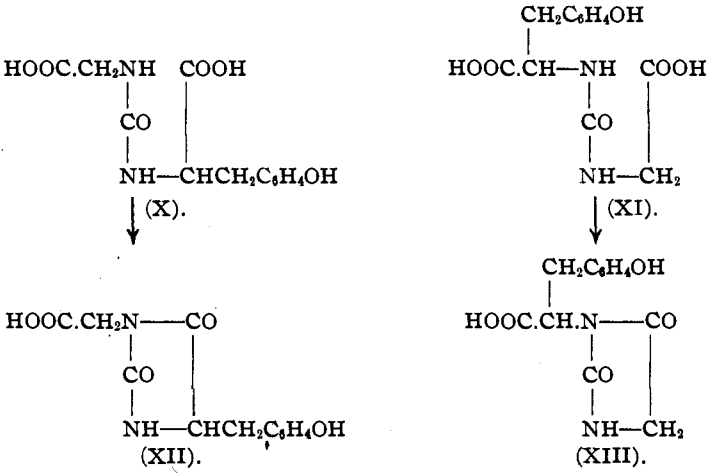
similar reaction with leucine. Combinations corresponding to Formula IX are closely related to the compounds under discussion in this paper, being derivatives of hydantoic acid, and theoretically should condense to *polypeptide-hydantoins* by the action of mineral acids. Morel,[3] however, did not consider apparently the possibility of accomplishing such condensations, perhaps because he recognized the impossibility of establishing the constitution of the resulting hydantoin from that of its precursor—the urea. For example, the urea IX, may be viewed either as a N-derivative of the hydantoic acid of tyrosine or of plain hydantoic acid as represented by Formulas X and XI, respectively. By digestion of such a compound with acids, therefore, either one of the two carboxyl groups might be involved in the condensation, leading theoretically to the

[1] Johnson, *Proc. Nat. Acad. Sci.*, **2**, 69 (1916); *Chem. News*, **113**, 127 (1916) (Paper XXXVI).

[2] *Compt. rend.*, **143**, 119 (1906).

[3] *Loc. cit.*

formation of two isomeric polypeptide-hydantoins as represented by Formulas XII and XIII, respectively. It would be a difficult matter to decide which isomer was formed here, unless some method of synthesis was available whereby the two compounds could be prepared in another manner. In this paper, we shall now describe methods of synthesizing such cyclic combinations and give a description of the synthesis and properties of the cyclic peptide—*phenylalanylglycine-hydantoin* (XXIII).



That N-substituted hydantoins may be prepared by interaction of the alkali or silver salts of hydantoins with halides has been known for a long time and the experimental data, so far obtained, leads to the conclusion that the structure of the products formed depends upon the saturation of the grouping occupying the 4-position of the hydantoin ring. In the case of plain hydantoin and related, saturated methylene derivatives, so far examined, the 1-position of the ring is the point of attack and the formation of 3- and 1,3-alkyl derivatives has never been observed.[1] In order to render both nitrogen atoms of the hydantoin susceptible to attack under the above conditions, it is apparently necessary to destroy the influence of the methylene combination in position 4 and increase the negative character of the ring by incorporation of an unsaturated grouping in this position. In such cases dialkyl-compounds can be obtained by alkylation, but it still holds here also that the 1-position of the ring is the first point of attack and that isomeric 3-alkyl hydantoins have never been isolated.[2]

[1] Franchimont and Klobbie, *Rec. trav. chim.*, **8**, 283 (1889); Harries and Weiss, *Ann.*, **327**, 355 (1902); Harries, *Ibid.*, **361**, 69 (1908); Siemonsen, *Ibid.*, **333**, 101 (1904); Neubauer, *Ibid.*, **137**, 288 (1866); Biltz, *Ber.*, **41**, 1379 (1908); Weitzner, *Ann.*, **362**, 125 (1908).

[2] Johnson and Nicolet, THIS JOURNAL, **34**, 1048 (1912); *Am. Chem. J.*, **47**, 459 (1912); Johnson and Bengis, THIS JOURNAL, **35**, 1606 (1913).

The starting point of the work to be described in this paper was 4-benzalhydantoin (XIV), which was prepared according to the method described by Wheeler and Hoffmann[1] namely, by condensing benzaldehyde with hydantoin. The *cis*-modification of the hydantoin[2] was the form used in our investigation. We now find that the sodium salt of this hydantoin interacts with ethyl chloroacetate in alcohol solution with substitution in position 1 forming chiefly the ester represented by Formula XVII. There is also formed at the same time a very small amount of an isomeric product which we have represented as a stereoisomeric modification of the hydantoin as represented by Formula XVIII. We were unable to establish definitely its constitution on account of our inability to obtain it in sufficient quantity for experimental purposes. The unsaturated hydantoin (XVII) is easily reduced at the double bond by the action of stannous chloride in hydrochloric acid and also by zinc in acetic acid giving the saturated ester—ethyl 4-benzylhydantoin-1-acetate (XXI). Hydrolysis of the ester (XVII) with hydrobromic acid leads smoothly to the formation of the unsaturated acid (XX). We succeeded in identifying only one modification of this hydantoin. Its potassium salt is easily obtained by digestion of the ester (XVII) with an alcoholic solution of potassium hydroxide. When the acid (XX) was reduced by treatment with tin and hydrochloric acid it was converted into the corresponding 4-benzylhydantoin-1-acetic acid or *phenylalanylglycine-hydantoin* represented by Formula XXIII.

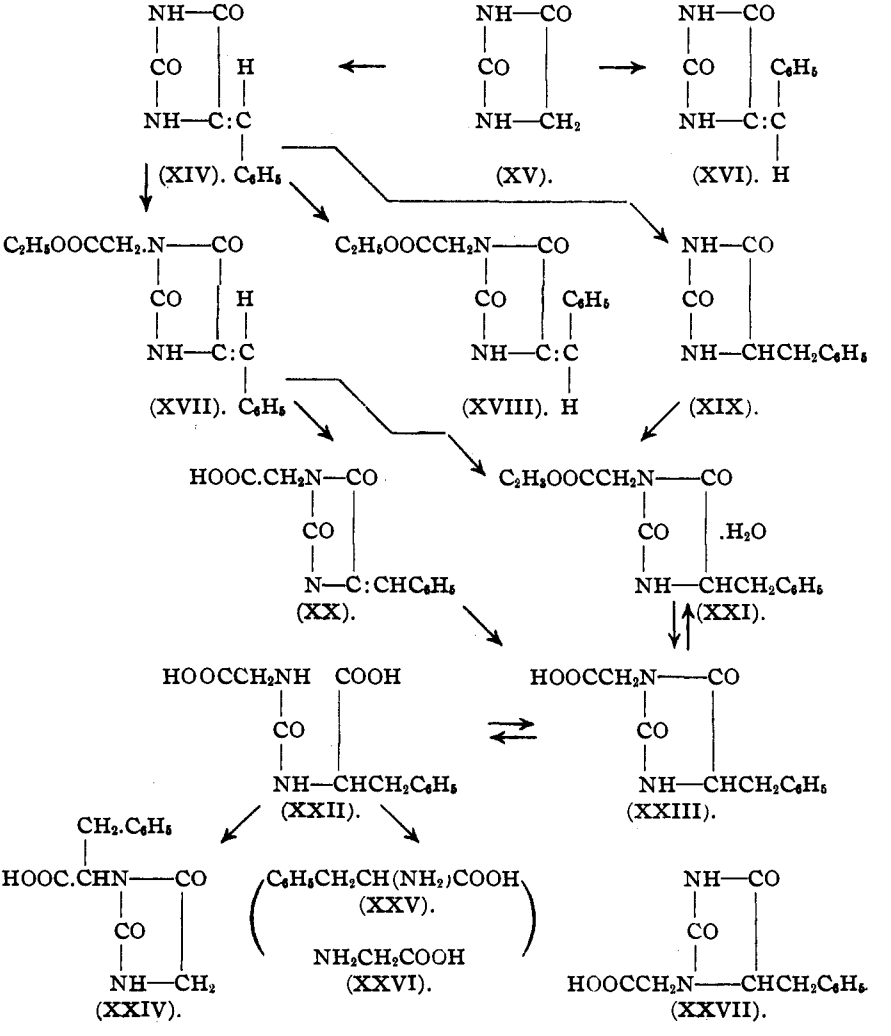
4-Benzylhydantoin-1-acetic acid (XXIII), on esterification with alcohol, is transformed smoothly into the ester (XXI), which undergoes hydrolysis by digestion with hydrochloric and hydrobromic acids, giving again the polypeptide-hydantoin (XXIII). The same saturated ester (XXI) described above is also obtained in excellent yield by alkylation of 4-benzylhydantoin (XIX) with ethyl chloroacetate. Therefore the same ester (XXI) is obtained from 4-benzalhydantoin (XIV) whether one first alkylates with ethyl chloroacetate and then reduces the double bond of the resulting hydantoin (XVIII), or first reduces to 4-benzylhydantoin and finally alkylates with the halogenated ester $ClCH_2COOC_2H_5$.

The hydantoin ring of the polypeptide-hydantoin (XXIII) is easily hydrolyzed by digestion of the hydantoin with an excess of potassium hydroxide forming the dipotassium salt of the acid (XXII). When this is heated above its melting point, water is given off and it is transformed into the original hydantoin (XXIII) melting at 184°. The same change is accomplished by digesting the acid (XXII) with hydrochloric acid. In other words, the carboxyl of the glycine group is not involved in this inner condensation leading to the formation of the isomeric polypeptide-

[1] *Am. Chem. J.*, **45**, 368 (1910).

[2] Johnson and Bates, THIS JOURNAL, **37**, 383 (1915).

hydantoin (XXIV). Complete hydrolysis of the polypeptide-hydantoin (XXIII) is easily accomplished by heating with hydrochloric acid at 140°. It breaks down completely under these conditions with formation of phenylalanine (XXV), glycocoll (XXVI) and carbon dioxide. We obtained no evidence of the formation of ammonium chloride by this treatment, proving thereby that the acetic acid group was substituted in the 1-position of the hydantoin ring. The isomeric hydantoin represented by Formula XXVII would break down on hydrolysis with formation of ammonia, carbon dioxide and the imino acid—$HOOC.CH_2NHCH(COOH)CH_2C_6H_5$. The various transformations described above are represented by the following formulas:



Experimental Part.

All the hydantoin employed in this investigation was prepared according to the directions of Siemonsen.[1]

4-Benzalhydantoin, $CO.NH.CO.NH.C:CHC_6H_5$.—This was prepared by condensation of hydantoin with benzaldehyde as described by Wheeler and Hoffmann.[2]

3-Acetyl-4-benzalhydantoin, $CO.NH.CO.N(COCH_3).C:CHC_6H_5$.—This new hydantoin is easily prepared by the action of acetic anhydride on benzalhydantoin. Two grams of the hydantoin were dissolved in the anhydride and the solution heated in an oil bath at 155–165° for 4 hours. On cooling the solution this acetyl derivative separated. It was purified by crystallization from glacial acetic acid and deposited in the form of colorless, transparent plates melting at 223° to a red oil with slight effervescence. A mixture of this compound with 4-benzalhydantoin melted at 197–198°.

Calc. for $C_{12}H_{10}O_3N_2$: N, 12.16. Found: N, 12.14, 12.01.

Ethyl *cis*-4-Benzalhydantoin-1-acetate (XVIII).—This is the chief product of the reaction when the sodium salt of 4-benzalhydantoin interacts with ethyl chloroacetate in ethyl alcohol. To prepare the hydantoin 10 g. of the benzal derivative were suspended in a solution of 1.3 g. of sodium in 100 cc. of absolute alcohol. This mixture was then heated to boiling until the hydantoin was completely transformed into its sodium salt, which separated as a colorless powder insoluble in the alcohol. Seven grams of ethyl chloroacetate and a molecular proportion of potassium iodide were then added and the digestion continued, by heating on the steam bath, until the alcohol gave a neutral reaction towards moist litmus and turmeric paper. The solution was then cooled and the insoluble material separated by filtration. Practically all the above hydantoin separated here with the inorganic salts. By trituration with cold water, it was obtained in a crystalline condition and was finally purified by crystallization from hot alcohol. It separated from this solvent in the form of rhombic prisms which melted at 174° to a clear oil without apparent decomposition. The yield of purified material was 8.5 g. or 60% of the theoretical quantity.

Calc. for $C_{14}H_{14}O_3N_2$: N, 10.21. Found: N, 10.12, 10.15.

In a second alkylation experiment the following proportions were used: 12.5 g. of the benzalhydantoin, 1.7 g. of sodium dissolved in 100 cc. of absolute alcohol, 8.3 g. of ethyl chloroacetate and 12.5 g. of potassium iodide. After digestion on the steam bath until the reaction was complete

[1] *Ann.*, **333**, 101 (1904).

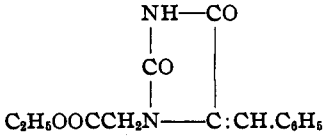
[2] *Loc. cit.*

the solution was cooled and the hydantoin-acetate filtered off with inorganic salts. This material was then washed with a small volume of cold water and finally dissolved in boiling alcohol to purify the hydantoin. The latter dissolved easily and separated, on cooling, in the form of plates melting at 174°. There was a small amount of material, however, which would not dissolve in the alcohol. This contained inorganic material and dissolved immediately in hot water. On acidifying this solution there was an immediate precipitate of a crystalline substance, which proved to be *4-benzalhydantoin-1-acetic acid* (see below). This crystallized from boiling alcohol in the form of rhombic prisms or tables which melted at 257–258° to a clear oil.

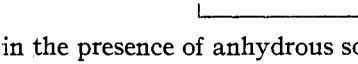
The alcohol filtrates from experiment one (above) were concentrated and cooled when more of the hydantoin-acetate (m. 174°) was obtained together with a secondary product which melted several degrees lower. The latter was very soluble in alcohol and separated as needles which melted at 158° to a clear oil. The experimental evidence so far obtained points to the conclusion that this substance is a stereoisomeric modification of *ethyl 4-benzalhydantoin-1-acetate* (*trans* form). When boiled with strong potassium hydroxide solution no ammonia was evolved. We did not obtain enough of the compound to reduce it to the corresponding 4-benzylhydantoin.

Calc. for $C_{14}H_{14}O_2N_2$: N, 10.21. Found: N, 10.10.

The corresponding structural isomer *ethyl 4-benzalhydantoin-3-acetate* has never been described. An attempt to prepare this substance by condensation of ethyl hydantoin-3-acetate[1]



with benzaldehyde, in acetic acid and in the presence of anhydrous sodium acetate, was unsuccessful. This result is in accord with previous observations on the inactivity of 3-substituted hydantoins towards aldehydes.

4-Benzalhydantoin-1-acetic Acid,  —This acid is easily obtained by saponification of the above ester with acids and alkalies. Ten grams of the ester were digested with 75 cc. of hydrobromic acid for about 5 hours. The hydantoin remained insoluble throughout the operation and ethyl bromide was evolved. After removal of the excess of hydrobromic acid by evaporation at 100°, the acetic acid derivative was then washed with cold water, in which it is

[1] This hydantoin was kindly sent to me by Professor J. R. Bailey of the University of Texas (see THIS JOURNAL, **37**, 935 (1915)).

practically insoluble, and purified by crystallization from hot alcohol. It separated from this solvent, on cooling, in the form of diamond shaped prisms which melted at 258° to a clear oil. From 10 g. of the ester were obtained 7.5 g. of the purified acid or 84% of a theoretical yield. The same acid was also obtained in the form of its potassium salt by saponifying the above ester with potassium hydroxide. Two grams of the ester and 0.4 g. of potassium hydroxide were digested with 50 cc. of 95% alcohol at 100° for one hour. The reaction was apparently complete at the end of this time and the potassium salt of the acid had separated as colorless prisms. These were separated and dissolved in water. On acidifying the aqueous solution with hydrochloric acid the hydantoin separated at once and melted at 256–258°.

Calc. for $C_{12}H_{10}O_4N_2$: N, 11.38. Found: N, 11.36, 11.30.

4-Benzylhydantoin, $CO.NH.CO.NH.CH.CH_2C_6H_5$.—Wheeler and Hoffmann[1] prepared this compound by reduction of 4-benzalhydantoin with hydriodic acid and phosphorus, and also with aluminum amalgam in alkaline solution. Wheeler, Hoffmann and Johnson[2] showed that the same change can be effected by digesting the benzal derivative with tin and hydrochloric acid but obtained a poor yield of the reduced hydantoin. By slight changes in procedure we find that the reduction can be accomplished quantitatively by means of these reagents. Ten grams of the 4-benzalhydantoin were digested, on a sand bath, with 20 g. of mossy tin and 300 cc. of 20% hydrochloric acid. When complete reduction had taken place the hydantoin had completely dissolved giving practically a colorless solution. The solution was filtered and the excess of hydrochloric acid removed by evaporation at 100°. The residue of tin chloride and reduced hydantoin was then dissolved in hot water and the tin precipitated by addition of an excess of aqueous ammonia. The precipitate of stannous hydroxide was filtered off and the filtrate, after acidifying with hydrochloric acid, evaporated to dryness. The residue left behind was then triturated with cold water to remove ammonium chloride when the benzylhydantoin remained undissolved. This was purified by crystallization from hot alcohol and separated, on cooling, in the form of colorless prisms melting at 190° to a clear oil. From 10 g. of the unsaturated hydantoin we obtained 9 g. of this purified benzalhydantoin or 90% of a theoretical yield.

This same hydantoin can also be prepared by reduction of 4-benzalhydantoin with zinc and acetic acid. Two grams of the benzal derivative were dissolved in 40 cc. of hot, glacial acetic acid and 5 g. of powdered zinc added to the solution. The mixture was then boiled for several hours

[1] *Am. Chem. J.*, **45**, 372 (1911).

[2] *J. Biol. Chem.*, **10**, 147 (1911).

and the solution evaporated to dryness after filtering off undissolved zinc. The residue obtained was then triturated with 10 cc. of water acidified with hydrochloric acid to remove zinc and the insoluble hydantoin separated. It was purified by crystallization from alcohol and melted at 190°. We obtained 1.8 g. of benzylhydantoin corresponding to a yield of 90% of the theoretical.

4-Benzylhydantoin-1-acetic Acid, $CO.N(CH_2COOH)CONH.CH.CH_2C_6H_5$.—This acid is easily prepared by reduction of the corresponding benzal derivative with tin and hydrochloric acid. Five grams of the benzal derivative and 10 g. of mossy tin were digested with 150 cc. of 20% hydrochloric acid. At the end of 4 hours the hydantoin had completely dissolved indicating complete reduction. After filtering, the acid solution was evaporated to dryness and the tin removed by precipitation with ammonia. On evaporating the ammoniacal filtrate and finally triturating the residue obtained with cold water the above acid remained undissolved. It was purified by recrystallization from boiling water and separated, on cooling, as flat, colorless prisms or rhombic plates, which melted at 184–185° to a clear oil without effervescence.

Calc. for $C_{12}H_{12}O_4N_2$: N, 11.29. Found: N, 11.02.

This same acid was also prepared from ethyl 4-benzylhydantoin-1-acetate (see below) by saponification with hydrobromic and hydrochloric acid. The simplest means of accomplishing the change was found to be the evaporation in an open dish, of a solution of the ester in a considerable excess of 25–30% hydrochloric acid. The ester dissolved almost immediately in the acid on boiling. After evaporation, the residue consisted of pure 4-benzylhydantoin-1-acetic acid melting at 184° to a clear oil. From 9.7 g. of the ester we obtained 7.8 g. of the purified acid.

Calc. for $C_{12}H_{12}O_4N_2$: N, 11.29. Found: N, 11.10, 11.18.

Ethyl 4-Benzylhydantoin-1-acetate, $C_2H_5OOCCH_2.N{-}CO{-}CH(CH_2C_6H_5).NH{-}CO.H_2O$.—This ester is easily obtained by alkylation of 4-benzylhydantoin, in alcohol solution and in the presence of sodium ethylate, with ethyl chloroacetate. After the reaction was complete, the alcohol was removed by evaporation and the residue triturated with cold water to remove sodium chloride. The undissolved hydantoin was then purified by crystallization from boiling water. It separated from this solvent, on cooling, as beautiful, colorless needles, which melted at 157° to a clear oil. The hydantoin is soluble in alcohol. From 10 g. of 4-benzylhydantoin we obtained 11.6 g. of the purified ester. This same hydantoin is also formed by esterification of the corresponding acid (see above). Two grams of the acid and 2 cc. of concentrated sulfuric acid were dissolved in 25 cc. of absolute alcohol and the mixture

boiled for 5 hours. The excess of alcohol was then removed by evaporation at 100° and the resulting ester purified by recrystallization from alcohol. It melted at 157°.

Ethyl 4-benzylhydantoin-1-acetate was also prepared by a third method, namely, by reduction of ethyl 4-benzalhydantoin-1-acetate with zinc and acetic acid. Three grams of the unsaturated ester were dissolved in 50 cc. of hot glacial acetic acid and 5 g. of powdered zinc suspended in the solution. After boiling the solution for 25 to 30 hours (using return condenser) the undissolved zinc was removed by filtration and the solution finally evaporated to dryness. The crystalline residue obtained was triturated with 10 cc. of cold dilute hydrochloric acid to remove any zinc and the hydantoin purified by crystallization from alcohol. It separated in the form of needles and melted at 155–156°.

We also prepared this ester by reducing ethyl 4-benzalhydantoin-1-acetate with stannous chloride. Eight grams of the unsaturated hydantoin and 10–12 g. of stannous chloride ($SnCl_2.2H_2O$) were dissolved in 200 cc. of 95% alcohol and the solution saturated hot with hydrochloric acid gas. After reducing in the acid solution for 8–10 hours the alcohol was then evaporated and the residue obtained triturated with cold water to remove tin chloride. The hydantoin-acetate remains behind undissolved and was purified by crystallization from boiling alcohol. It melted at 157° to a clear oil. Repeated nitrogen determinations, both by the Kjeldahl and Dumas methods, consistently indicated that this ester crystallized with a molecule of water. The compound did not lose weight when heated at 100°.

Calc. for $C_{14}H_{16}O_4N_2.H_2O$: N, 9.52. Found: N, 9.52, 9.53, 9.55, 9.60, 9.48.

Sym-**Phenylalanine-glycine Urea,** (XXII).—This acid was obtained in the form of its dipotassium salt by saponification of the ethyl 4-benzylhydantoin-1-acetate with potassium hydroxide. Three grams of the ester and 2 g. of potassium hydroxide (3 molecular proportions) were dissolved in 50 cc. of water and the solution heated on a steam bath for 5 hours. Dilute hydrochloric acid was then cautiously added until the mixture was neutral to turmeric and litmus and the solution finally evaporated nearly to dryness. This concentrated solution was then desiccated in a vacuum over concentrated sulfuric acid. The crystalline residue obtained was then triturated with cold alcohol when the potassium salt of the urea acid dissolved and could be separated easily from potassium chloride. On concentrating the alcoholic solution and cooling the potassium salt of the above acid finally deposited in the form of colorless needles. They decomposed when heated at 268–269°.

Calc. for $C_{12}H_{12}O_5N_2K_2$: K, 22.87. Found: K, 22.40.

In order to obtain the free acid the dipotassium salt was suspended in 25 cc. of dry benzene and dry hydrochloric acid gas passed into the

mixture at room temperature for 2 hours. Neither the salt nor the corresponding acid was soluble in this solvent. The benzene was finally allowed to evaporate spontaneously and the potassium chloride, formed during the reaction, dissolved by trituration with cold water. The urea-acid was not dissolved by this solvent (cold) but was soluble in hot water and crystallized on cooling in colorless, elongated prisms which melted at 176–177° with violent effervescence. When this acid was melted and the resulting oil was cooled it solidified again. On reheating this product it was not changed at 176–7°, but melted sharply at 184° to a clear oil. In other words, the acid loses water at the temperature of its melting point and is converted into 4-benzylhydantoin-1-acetic acid (m. 184°) or (phenylalanine-glycine hydantoin).

Calc. for $C_{12}H_{14}O_5N_2$: N, 10.39. Found: N, 10.36.

Hydrolysis of Ethyl 4-Benzylhydantoin-1-acetate with Hydrochloric Acid.—One gram of the hydantoin and 15 cc. of concentrated hydrochloric acid were heated in a pressure tube at 140–146° for 3.5 hours. When the tube was opened there was slight pressure, due to the presence of carbon dioxide, and a crystalline product was suspended in the acid solution. This material was extremely soluble in cold water and was apparently a mixture of the hydrochlorides of glycocoll and phenylalanine. This material was separated by filtration and examined as follows: A portion of the material was dissolved in water and the solution made strongly alkaline by addition of sodium hydroxide. On boiling this solution there was no evolution of ammonia. The original acid filtrate likewise gave no ammonia when digested with an excess of alkali. The remainder of the material was then repeatedly recrystallized from dilute hydrochloric acid when a definite crystalline product was easily obtained which deposited in the form of rosets of prisms. The substance contained chlorine and decomposed with effervescence when heated above 235°. It was dried at 90–100°. A nitrogen determination indicated that we were dealing with the hydrochloride of phenylalanine originally described by Erlenmeyer and Kunlin.[1]

Calc. for $(C_9H_{11}O_2N)_2HCl$: N, 7.69. Found: N, 7.9.

Hydrolysis with an Aqueous Solution of Potassium Hydroxide.—Five-tenths of a gram of ethyl 4-benzylhydantoin-1-acetate and 1.0 g. of potassium hydroxide were dissolved in 10 cc. of water and the solution boiled for one-half hour in order to obtain the dipotassium salt of *sym*-phenylalanine-glycine urea. There was no evolution of ammonia during this operation. The aqueous solution was then evaporated to dryness at 100° and the residue dissolved in an excess of dilute hydrochloric acid and the solution evaporated again. The crystalline residue obtained here was then triturated with cold water to remove potassium chloride

[1] *Ann.*, **307**, 160 (1899).

and the crystalline product left behind purified by recrystallization from boiling water. It separated, on cooling, in the form of prismatic crystals which melted at 183–184° to an oil. A mixture of this with *phenylalanylglycine-hydantoin* (m. 184°) melted at the same temperature.

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE CHEMICAL DEPARTMENT KANSAS AGRICULTURAL EXPERIMENT STATION.]

A STUDY OF CERTAIN CONDITIONS WHICH AFFECT THE ACTIVITY OF PROTEOLYTIC ENZYMES IN WHEAT FLOUR.

BY C. O. SWANSON AND E. L. TAGUE.

Received February 16, 1916.

Introduction.

A study on conditions which affect the activity of amylolytic enzymes in wheat flour was reported in a previous paper.[1] In that study several facts were noticed which deserve further investigation. In planning for such work it was seen that the proteins of the flour were involved, and therefore it was thought best first to make a study of certain conditions which affect the activity of proteolytic enzymes of wheat flour.

In this investigation we used a high-grade patent flour, made by the Department of Milling Industry. Such a flour is desirable because it consists mostly of the pure floury endosperm of the wheat kernel, and has a low ash content.

The proteins of wheat flour have been the object of a large amount of investigation. Osborne and Voorhees[2] separated the nitrogenous substances in wheat flour into five distinct proteins known as gliadin, glutenin, globulin, leucosin, and proteose. Because of the number and complex nature of the proteins of wheat flour it was decided to confine this study to the protein leucosin freely soluble in pure water.

It is recognized that the figures obtained would have been different had we used the whole ground wheat kernel, or flour less free from proteins of the bran coat and germ. The results would probably also have been different had the other proteins of the flour been included. It is planned to continue this experiment under conditions where the other proteins of the flour are included, and also on the whole ground wheat kernel.

Methods of Experimentation.

A large number of preliminary trials were made in order to determine the best methods of procedure. In this report are presented only the results of trials that were found workable and of value in this study. The data obtained in the different trials are not given in the chronological order of performance, but in a manner calculated to make the report clear.

[1] THIS JOURNAL, 35, 1635–1643 (1913).

[2] *Am. Chem. J.*, 15, 392–471 (1893).