

It is also obvious that the apparent contradiction pointed out in the literature is due to the fact that the two investigators did not succeed in isolating a sufficient number of products from the resulting mixture to establish all the intermediate steps in the progressive course of the reaction.

From the results given above, we then conclude that the products obtained in the oxidation of inosite with nitric acid, in the order formed, are hexahydroxybenzene, tetrahydroxyquinone, rhodizonic acid, croconic acid and triquinoyl, and finally leuconic acid. While neither triquinoyl nor leuconic acid has been isolated in this investigation, we include them among the products for reasons given in the text.

SYRACUSE, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

SYNTHESIS OF THE POLYPEPTIDE-HYDANTOIN, N-3-METHYL-5-TYROSYL-HYDANTOIN-1-PHENYLACETIC ACID

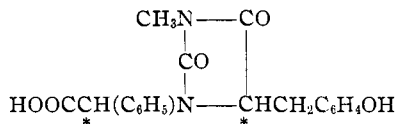
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The present investigation was undertaken with a view to extending the knowledge of polypeptide-hydantoin. The particular combination was chosen in order to confirm if possible a previous observation² that the presence of two asymmetric carbon atoms in the polypeptide-hydantoin molecule is associated with isomerism of the meso and racemic type and at the same time to continue a study of the effect of substitutions in the N-1-position upon the mobility of the molecule as shown in its tendency to isomerize and also to split off groups in the N-1-³ and in the C-5-positions.⁴

With these ends in view the following substance was selected for synthesis, the asymmetric carbon atoms being indicated by asterisks.⁵



A substance of the above configuration has further interest in that it may be expected to add two molecules of water on hydrolysis to give the N-phenyl-acetic acid derivative of tyrosine

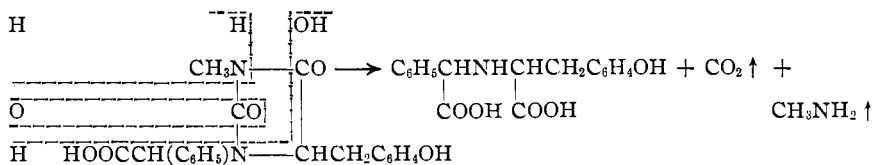
¹ This work was offered in partial fulfillment of the requirements for the degree of Master of Arts at Mount Holyoke College.

² Hahn and Gilman, *THIS JOURNAL*, **47**, 2944 (1925).

³ Hahn and Renfrew, *ibid.*, **47**, 156 (1925).

⁴ Hahn and Evans, *ibid.*, **49**, 2878 (1927) and **50**, 810 (1928).

⁵ The system of numbering the positions of the atoms in the hydantoin ring has been changed from that used in previous papers to the one now in use by T. B. Johnson, *cf. ibid.*, **51**, 255 (1929), and in the subject index of *Chemical Abstracts*.



and the fact that polypeptide-hydantoin of this type decompose in this way affords an excellent and clear method for preparing imino dibasic acids which contain aromatic residues.⁶

The synthesis of N-3-methyl-5-tyrosylhydantoin-N-1-phenylacetic acid was accomplished by means of a series of reactions which are indicated by arrows on the accompanying chart. The latter is somewhat complicated because of the fact that all of the intermediate compounds exist in two or more isomeric modifications. The primary reaction consisted in condensing the sodium derivative of N-3-methyl-5-anisalhydantoin with ethyl phenylbromo-acetate. The principal product consisted of the more stable modification of ethyl N-3-methyl-5-anisalhydantoin-N-1-phenylacetate, I, in yields averaging 74% of the theoretical, while small quantities of a labile form, II, were separated from the filtrates. Of these the former was employed in all subsequent major operations while the latter was used only where necessary in establishing the various chemical relationships. The principal transformations by means of which the polypeptide-hydantoin XXI was obtained consisted in reduction (to XI and XII), saponification (to the acids XV to XIX) and finally demethylation in the presence of hydrogen iodide.

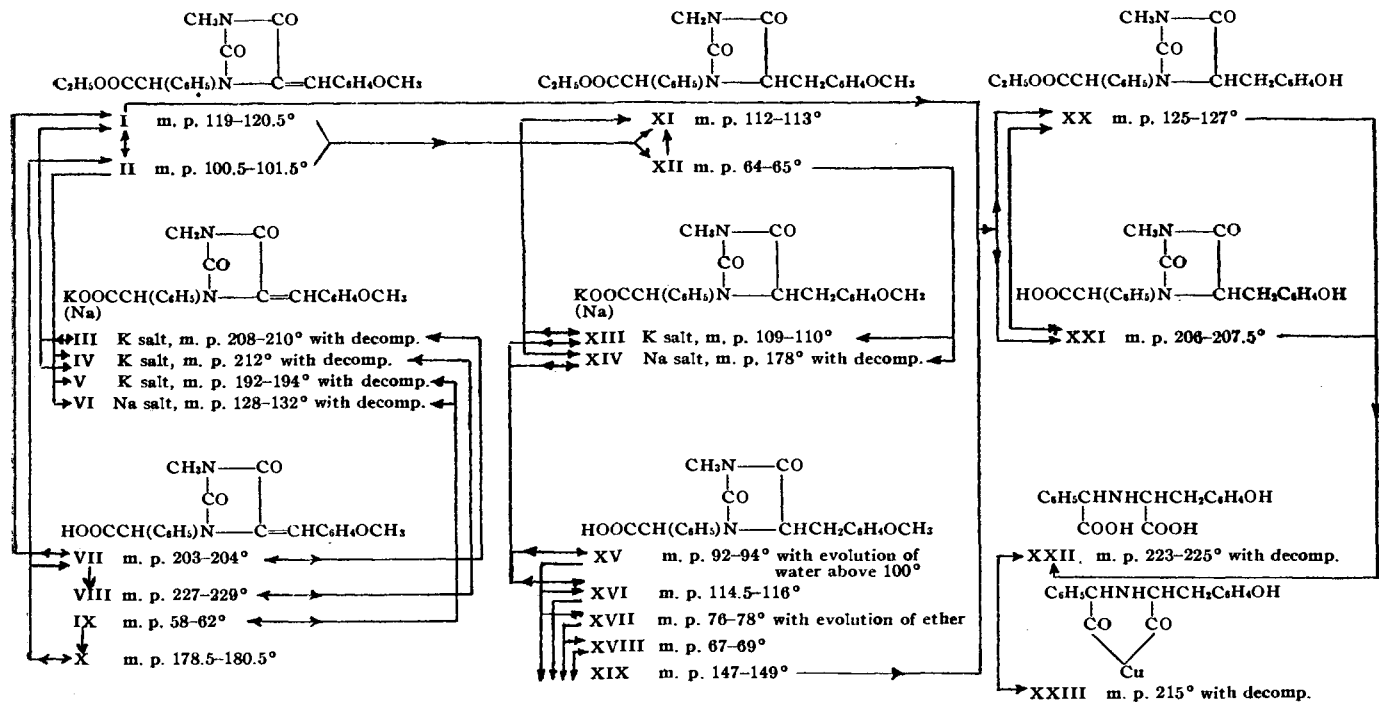
The constitutional formulas given in the chart are supported not only by evidence obtained from a study of the chemical relationships of the various substances, but by the fact that a corresponding relationship in their physical properties has been established as the result of spectrographic analyses.⁷ In this connection it is interesting to note that the absorption curves plotted for the esters I and II are almost identical with those previously obtained in the case of the geometrically isomeric modifications of N-1-methyl-5-benzalhydantoin-N-3-acetic acid,⁸ a fact which must be construed as furnishing additional evidence in support of the configuration of the former substances and of their derivatives. Still further confirmation of these formulas is shown by the fact that the polypeptide-hydantoin XXI decomposes to give methylamine and the imino dibasic acid XXII, the latter having been identified by means of analyses both of the free acid⁹ and its corresponding copper salt.

⁶ Hahn and Renfrew, *THIS JOURNAL*, **47**, 148 (1925).

⁷ A full discussion of the data has been reserved for a second paper, "The Isomerism of Certain Acids Obtained in the Synthesis of N-3-methyl-5-tyrosylhydantoin-N-1-phenylacetic Acid," *ibid.*, **52**, 2505 (1930).

⁸ Hahn and Evans, *ibid.*, **50**, 808, 809, 810 (1928).

⁹ Other derivatives of this acid have been prepared and will be reported upon later.



In conclusion emphasis must be given to the fact that the substitution of phenyl for hydrogen in the acetic acid group in the N-1-position has been observed greatly to increase the mobility of the molecule.¹⁰ This is shown by the facts (a) that a greater number of isomers has been obtained than would ordinarily be expected in the case of both the saturated and unsaturated molecules of a given configuration, (b) that certain of these compounds show a marked tendency to associate water or ether molecules (*i. e.*, the acids XV and XVII), (c) that the rate of isomerization of the labile to the stable modifications is very rapid¹¹ and finally (d) that the unsaturated esters I and II show an increased disposition under the action of alkali to split off either the acid group in the N-1-position¹² or the aldehyde group in the C-5-position.

The existence in certain cases of a number of isomeric modifications greater than was to be expected on the basis of geometrical¹³ or stereo-¹⁴ isomerism offers difficulties in the way of interpretation. Of these the unsaturated acids have been set apart for discussion in a separate paper.⁷ The saturated isomers may be explained by assuming that XVI and XIX represent stereoisomeric inactive forms and that XVIII is a desmotropic modification¹⁵ of the latter.

Experimental

Ethyl-N-3-methyl-5-anisalhydantoin-1-phenylacetate, I, m. p. 119–120.5°,¹⁶ was prepared by alkylating the sodium derivative of N-3-methyl-5-anisalhydantoin with α -phenyl- α -bromo-acetic ester. The latter was purchased from Kahlbaum while the N-3-methyl-5-anisalhydantoin was prepared in the usual way.¹⁷ The following procedure was found to give the best percentage yields.

Fifty grams of N-3-methyl-anisalhydantoin, m. p. 212–215°, was suspended in 300 cc. of 95% ethyl alcohol to which 1.2 equivalents of sodium had been added. The mixture was shaken vigorously to prevent caking of the methyl-anisalhydantoin, which did not dissolve, but was held in suspension during the process of conversion into the corresponding sodium derivative which was also insoluble. After boiling with a reflux condenser for one and one-half to two hours, 1.2 equivalents of ethyl phenyl-bromoacetate was added along with 100 cc. of alcohol used for rinsing. Within twenty minutes after the addition of this material a vigorous bubbling took place, which was accompanied

¹⁰ Compare the behavior of these substances with the corresponding derivatives of ethyl N-3-methyl-5-anisalhydantoin-N-1-acetate, Hahn and Renfrew, *THIS JOURNAL*, **47**, 147 (1925).

¹¹ XII isomerizes so rapidly that substantial losses are suffered in one recrystallization from alcohol.

¹² Compare Hahn and Renfrew and Hand and Evans, *THIS JOURNAL*, **47**, 156 (1925); **50**, 811 (1928).

¹³ Compare the unsaturated acids VII to X on the chart.

¹⁴ Compare the saturated acids XVI, XVIII and XIX on the chart.

¹⁵ The equilibrium XVIII \rightleftharpoons XIX represents a thermal relationship, one acid existing only at relatively high and the other at ordinary temperatures.

¹⁶ This substance was first prepared by Miss Marie A. Dobbrow.

¹⁷ (a) Johnson and Nicolet, *Am. Chem. J.*, **47**, 469 (1912); (b) Hahn and Renfrew, *THIS JOURNAL*, **47**, 156 (1925).

by the solution of the yellow sodium derivative and the precipitation of a fine white powder. In the preliminary experiments the latter was filtered hot and identified as a mixture of methyl-anisalhydantoin and sodium bromide. Ordinarily no separation was attempted at this point, but the boiling was continued for about two hours or until the solution became neutral to turmeric. On cooling a heavy white precipitate was formed which melted between 110 and 200°. This was filtered and when dry extracted with 100 cc. of cold chloroform in order to separate the reaction product from any unchanged methyl-anisalhydantoin and sodium bromide. The extract was evaporated to small volume in order to remove the chloroform and 200 cc. of hot alcohol was added. On cooling, 34 g. of the ester, m. p. 116–118°, crystallized out. The residue, insoluble in chloroform, yielded 23 g. of methyl-anisalhydantoin, m. p. 212–214°, along with varying quantities of sodium bromide.

This amount of reaction product (averaging about 74% as calculated on the basis of the methyl-anisalhydantoin which reacted) remained practically constant even when the period of the reaction was extended from two and one-half to fifty-two hours, but was decreased if the heating was continued for a longer time. In cases where the heating was continued for five days the product consisted of an oil from which it was impossible to separate any solid product.

The crude ester was readily purified by recrystallization from alcohol, from which it separates in the form of white silky rosettes which fill the entire solution, m. p. 119–120.5°. One gram dissolves in 3.5 cc. of boiling alcohol, and in 70 cc. of cold alcohol.

Anal. Calcd. for $C_{22}H_{22}O_4N_2$: N, 7.10. Found: N, 7.14, 7.04.

The ester was partly transformed into the lower-melting isomer II by saturating its alcohol solution with hydrogen chloride gas. On alkaline hydrolysis the ester I was changed into a mixture of the potassium salts III and IV. The latter when separated and acidified in aqueous solution gave the acids VII and VIII, respectively. Acid hydrolysis resulted in the formation of the acid III alone. Reduction of the ester I to the isomeric saturated esters XI and XII took place readily under the action of hydrogen in the presence of palladium. By treatment of the ester with hydrogen iodide the demethylated saturated acid XXI was formed.

Ethyl-N-3-methyl-5-anisalhydantoin-1-phenylacetate, II, m. p. 100.5–101.5°, was obtained by the action of dry hydrogen chloride gas on an alcohol solution of the higher-melting isomer, I.

Forty grams of the ester, m. p. 119–120.5°, was dissolved in a mixture of 300 cc. of 95% alcohol and 150 cc. of cold chloroform. During a period of twenty-five to thirty hours the solution was alternately saturated with hydrogen chloride and heated for one hour on a water-bath. The product of the reaction consisted of a hard gum which would not crystallize in the presence of even small quantities of hydrogen chloride. It was, therefore, taken up in chloroform and washed free from acid. The chloroform extract was dried, concentrated to small volume, diluted with alcohol to bring the volume to 600 cc., and cooled, when 10 g. of hard, pale yellow needles, m. p. 96–100°, separated. After two recrystallizations from boiling alcohol a pure substance of m. p. 100.5–101.5° was obtained. The filtrates from this substance (which contained the remainder of the original 40 g. of ester I) when concentrated and treated again with hydrogen chloride gave additional amounts of the lower-melting isomer in about the same ratio as before, *i. e.*, approximately 1:4. No loss of material was involved in this procedure, since after washing free from hydrogen chloride the mixture of the two esters could be separated mechanically. The higher-melting modification crystallized from alcohol in the form of white, fluffy needles and could be readily floated off with alcohol from the heavy compact crystals which formed on the bottom of the beaker and which represented the lower-melting isomer.

Anal. Calcd. for $C_{22}H_{22}O_5N_2$: N, 7.10. Found: N, 6.92, 6.93.

The lower-melting ester, II, was transformed into the higher-melting ester I by the action of sunlight on its ether solution. Five-tenths of a gram of ester, m. p. 100.5–101.5°, was dissolved in 250 cc. of dry ether and allowed to stand in the sunlight in a glass bottle for three and one-half months. After evaporation of the ether a gummy product was obtained which on treatment with alcohol yielded 0.1 g. of ester, m. p. 113–118°. The crystalline product after one recrystallization from alcohol did not lower the melting point of an analyzed specimen of the ester I, m. p. 119–120.5°.

The ester II is very soluble in boiling alcohol (1 g. in 4.4 cc.) and slightly soluble in cold alcohol (1 g. in 70 cc.). It yields on acid hydrolysis two isomeric acids VII and X. On alkaline hydrolysis the corresponding sodium and potassium salts VI and V were obtained which were transformed into the acid IX as primary product and finally into the acid X. By reduction with hydrogen in the presence of palladium the lower-melting ester is changed into the same saturated esters, XI and XII, that were obtained from the higher-melting isomer.

Ethyl N-3-methyl-5-anisylhydantoin-1-phenylacetate exists in the form of two isomeric esters: I, m. p. 112–113°, and XI, m. p. 64–65°. Both result from the reduction of either of the unsaturated esters I or II with hydrogen in the presence of palladium.¹⁸ For example, twenty grams of the ester, m. p. 112–113°, was dissolved in 250 cc. of 95% alcohol and shaken with 0.6 g. of colloidal palladium (25%) in an atmosphere of hydrogen for two days. For the first hours of this time it was necessary to heat the mixture with steam in order to keep the unsaturated ester in solution, but after a certain amount of hydrogen had been absorbed this was no longer necessary. At the end of the reaction the colloid was precipitated by boiling with 1 cc. of acetic acid and filtered. The filtrate was concentrated to 200 cc. and allowed to cool, when the reduced ester XI separated from the solution in almost pure condition, m. p. 111–112°. A further quantity of the same substance was obtained from the filtrate, making the immediate yield of crystalline product 50% of the theoretical. On recrystallization from alcohol the ester melted at 112–113°.

Anal. Calcd. for $C_{22}H_{24}O_5N_2$: N, 7.07. Found: N, 6.90, 7.04.

The filtrates from the above crystals, on evaporation, yielded an oil which, after standing for several weeks and being periodically agitated with alcohol, gave a series of crystalline precipitates which melted, respectively, at 68–71, 71–100°, etc. The sample having the lowest melting point, when recrystallized from alcohol, finally yielded a new substance, XII, m. p. 64–65°, which was identified as an ester isomeric with XI.

Anal. Calcd. for $C_{22}H_{24}O_5N_2$: N, 7.07. Found: N, 7.07, 7.07.

That the entire product resulting from the reduction of either of the unsaturated esters I or II consisted of the above two isomeric saturated esters was shown by the fact that both the crystalline ester XI and the oil obtained from its filtrates gave the same crystalline sodium salt, XIV, in amounts corresponding almost exactly with the theoretical. A slight amount of decomposition during the process of reduction was evident from the appearance of traces of methyl-anisalhydantoin in the reaction product. Both saturated esters were also obtained by reduction of the unsaturated ester II. Due to the extreme solubility of the latter in alcohol, the reaction could be carried out without heating.

The saturated ester XI is soluble in boiling alcohol (1 g. in 8 cc.) and less soluble in cold alcohol (1 g. in 55 cc.), from which it separates in the form of large transparent

¹⁸ Acknowledgment is made to Lillian Nelson and Katherine Haring for preparing these esters.

rhombic crystals. The ester may be transformed quantitatively into the potassium salt XIII or sodium salt XIV by alkaline hydrolysis.

The ester XII is extremely unstable and when recrystallized from warm alcohol with even the minimum amount of heating is partially isomerized to the ester XI. For example, 1.8 g. of ester, m. p. 64–65°, when dissolved in 20 cc. of hot alcohol, gave on cooling first 0.5 g. of crystalline precipitate, m. p. 107–109°, and then 0.6 g., m. p. 64–65°. The combined filtrates from these crystals on evaporation gave an oil which crystallized only after long standing and which was found to consist of a mixture of these two isomers. The ester XII was transformed quantitatively into the same potassium and sodium salts, XIII and XIV, that were obtained from its higher-melting isomer.

Potassium N-3-methyl-5-anisylhydantoin-1-phenylacetate, XIII, m. p. 109–110°, and **sodium N-3-methyl-5-anisylhydantoin-1-phenylacetate, XIV**, m. p. 178° with decomposition, were both prepared in quantity by saponifying the ester XI. The reactions, which were carried out by boiling in alcohol solution with an equivalent of potassium hydroxide or sodium alcoholate, were complete in ten minutes, and on cooling the salts crystallized in the form of fine white needles. When recrystallized from alcohol the potassium salt separated as large transparent plates which melted at 88–90°. On exposure to the air the crystals rapidly became opaque, lost alcohol of crystallization and the melting point changed to 94–110°. When alcohol-free (after heating in the oven at 80°) the potassium salt melted sharply at 109–110°. The sodium salt when purified by recrystallization from alcohol or water melted with decomposition at 178°.

Anal. Calcd. for $C_{20}H_{19}O_6N_2K$: N, 6.90. Found: N, 6.81, 6.61. Calcd. for $C_{20}H_{19}O_6N_2Na$: N, 7.18. Found: N, 6.85, 6.73.

Both salts when dissolved in cold absolute alcohol and treated with hydrogen chloride passed quantitatively into the ester XI. When acidified in aqueous solution both passed quantitatively into the acids XV or XVI depending on the conditions of the experiment.

N-3-methyl-5-anisylhydantoin-1-phenylacetic Acids.—Five different and well-defined modifications were obtained by the hydrolysis of the potassium or sodium salts, XIII or XIV, respectively, each salt giving one or another of these products depending upon the conditions under which the acid was precipitated and subsequently treated. The relationships between these different substances is shown in the preceding chart.¹⁹

The acid XV was the primary product usually obtained from the sodium salt XIV or potassium salt XIII. For example, when a cold aqueous solution of either salt was acidified, a gummy mass was precipitated, which on standing, for two hours in the cold solution hardened and disintegrated with the formation of a white powder. This melted at 88–90° and gave off water vapor above 100°. When purified by solution in twice its volume of warm alcohol and precipitated by the addition of an equal volume of water, it melted at 92–94° and began to give off water vapor at about 100°. When heated for one and one-half hours in an oven at 80° it was completely transformed into the anhydrous modification, XVI, melting at 114.5–116°, and suffered a loss in weight corresponding to one molecule of water. The same change took place on standing for several days at room temperature. When recrystallized from concentrated alcohol solutions or from ether, the acid XV passed quantitatively into the stable modification XIX, m. p. 147–149°.

Anal. Calcd. for $C_{20}H_{20}O_6N_2 \cdot H_2O$: N, 7.25. Found: N, 7.10, 7.04.

The acid XVI may be obtained from XV as above, or directly in crystalline form by acidifying an aqueous solution of either the potassium salt XIII or the sodium salt

¹⁹ Page 2496.

XIV. However, although it was prepared by the latter method in two experiments, the conditions which favored the precipitation of XVI rather than XV could not be determined. When an analyzed specimen of XVI was heated at 87° for twelve hours, it passed quantitatively without loss in weight into the stable isomer XIX. The same transformation was readily effected by recrystallization from ether.

Anal. Calcd. for $C_{20}H_{20}O_6N_2$: N, 7.61. Found: N, 7.51, 7.40.

The acid XVII, m. p. 76–78° with evolution of ether, was formed when the gummy acid precipitated from the salt solution was not allowed to stand under water, but was immediately taken up in ether. On evaporation of the ether the acid crystallized in the form of small transparent plates. When a sample of this acid was heated to constant weight, a mole of ether was given off and the acid XIX was formed.

Anal. Calcd. for $C_{20}H_{20}O_6N_2 \cdot (C_2H_6)_2O$: N, 6.34. Found: N, 6.34, 6.26.

The acid XVIII, m. p. 67–69°, was formed by fusing at high temperatures either the acid XVII or XIX. When the acid XVII was heated on an oil-bath and the temperature raised slowly to 140° during a period of one and one-half hours, the liquid on cooling solidified as a hard crystalline mass which now melted at 67–69°. This was ground in a mortar, heated in the oven at 50° to drive off the last traces of ether, and analyzed.

Anal. Calcd. for $C_{20}H_{20}O_6N_2$: N, 7.61. Found: N, 7.63, 7.50.

The same product was obtained when the acid XIX was held at a temperature of 160–170° for one and one-half hours, and then cooled. A sample of the analyzed specimen, m. p. 67–69°, which was kept in the oven at 50° for four weeks showed a gradual rise in melting point to 139–144°, approaching that of the acid XIX. The same change took place more gradually at ordinary temperatures on standing and was brought about quickly by one recrystallization of the substance melting at 67–69° from ether. Under these conditions it was transformed quantitatively into the acid melting at 147–149°. These experiments were repeated a number of times, so that it would seem reasonable to conclude that the acid XVIII represents a modification which is stable only at higher temperatures and which at ordinary temperatures reverts to a more stable form.

The acid XIX, m. p. 147–149°, represents a modification which is most stable at ordinary temperatures and to which all of the other acids of this series, *viz.*, XV, XVI, XVII and XVIII, revert either on recrystallization or upon long standing. It is most readily purified by crystallization from chloroform–ether mixtures (1:10), from which it separates upon cooling in the form of transparent plates.

Anal. Calcd. for $C_{20}H_{20}O_6N_2$: N, 7.61. Found: N, 7.68, 7.67.

The acid neutralizes a theoretical quantity of standard base when titrated in alcohol solution with phenolphthalein as indicator. The acid is transformed into the ester XI in 70% yield when dissolved in alcohol previously saturated with hydrogen chloride and boiled for one hour on the water-bath. The acid is rapidly demethylated when heated with hydrogen iodide and red phosphorus at a temperature of 100–110°.

N-3-methyl-5-*p*-hydroxybenzylhydantoin-N-1-phenylacetic Acid, XXI.—Acids corresponding to the above formula exist in the form of at least two and probably more isomers but because of what appears to be a tendency of these substances to isomerize in all solvents, it was never possible to isolate more than one product, namely, XXI, m. p. 206–207.5°, in pure condition. Various reactions all leading to the formation of such isomers were made the subject of intensive study over a period of more than one year and represent (a) demethylation of XIX, (b) saponification and demethylation of XI and (c) reduction, saponification and demethylation of I. In all cases the reaction was brought about by the action of hydrogen iodide in the presence of red phosphorus

and in every case the practical results were the same, the product consisting of a mixture of acids partly crystalline and partly oil.

(a) In this case the reaction could be followed quantitatively by using the Zeisel method and measuring the amount of methyl iodide evolved. For example, 8 g. of N-3-methyl-5-anisylhydantoin-N-1-phenylacetic acid, m. p. 147–149°, when suspended along with 2 g. of red phosphorus in 14 cc. of hydrogen iodide (sp. gr. 1.7) and heated for half an hour at 100–110°, gave off methyl iodide in the exact amount required by theory. The product consisted of a mixture of acids, m. p. 175–178°.

(b) and (c) In this case the reactions were carried out with hydrogen iodide in the manner described except that the period of heating was extended to one and one-half to two hours. The product after removing the excess of hydrogen iodide was partly crystalline and partly oil and the melting point of the crystalline fraction varied considerably. As a result of attempts to resolve the crystalline fraction into its components by a prolonged process of fractional recrystallizations, acids melting at 161–163 and 183–186° were obtained but these melting points could not be improved by further recrystallizations. Although obviously representing mixtures, analyses of these lower-melting and higher-melting fractions were made and both were found to correspond in composition to the polypeptide acid expected by theory.

In general, it was found convenient not to attempt to separate this mixture of acids into its components, but after removal of the excess of hydrogen iodide to proceed with it in either of two ways: (I) to esterify by treating an alcohol solution with dry hydrogen chloride, when the ester XX was obtained as the sole crystalline product in yields averaging 20%; (II) to hydrolyze the entire product (crystals and oil) under the action of barium hydroxide, in which case the sole product consisted of the imino acid XXII in yields averaging about 75%.

Since the results of a fairly large number of experiments were practically identical, it seems reasonable to conclude that the product consisted in all cases of a mixture of isomeric acids corresponding to the above formula.

The acid XXI, m. p. 206–207.5°, was prepared by dissolving this product (obtained by treating 20 g. of the ester I with hydrogen iodide) in ether and then extracting with dilute sodium hydroxide. The aqueous alkaline extract was concentrated on a water-bath, cooled and acidified, when a gum was precipitated. The latter was taken up in ether, which when washed, dried and allowed to evaporate, deposited a fine white crystalline powder. This was further purified by recrystallization from aqueous acetone from which it separated in the form of long pale yellow needles which contained water of crystallization, and which melted at 144° with evolution of water vapor. When pure and anhydrous this acid melted at 206–207.5°. The total yield was about 3 g.

Anal. Calcd. for $C_{19}H_{18}O_5N_2$: N, 7.91. Found: N, 7.96, 8.05.

Ethyl N-3-methyl-5-*p*-hydroxybenzylhydantoin-N-1-phenylacetate, XX, m. p. 125–127°.—This substance would also appear to exist in isomeric modifications although here again difficulties were encountered in the separation of a second isomer. The preparation involved: (a) the esterification under the action of hydrogen chloride of alcohol solutions of the acid XXI and also of what seemed to be mixtures of isomeric crystalline modifications having the same composition, as for example the above-mentioned crystalline fractions melting at 161–163° and 183–186°, respectively. In every case the product consisted of a crystalline precipitate XX, m. p. 125–127°, while filtrates from this on concentration gave an oil which on long standing continued to yield additional quantities of this substance. (b) The repeated additions of alcohol (and its subsequent evaporation in open beakers on a steam-bath) to the crude product obtained by the action of hydrogen iodide on the unsaturated ester I. In this way the excess of hydrogen iodide present in the mixture acted as a catalyst in the esterification of the

acids and was at the same time gradually eliminated in the form of ethyl iodide. Here again the product consisted of a crystalline ester, XX (in yields approximating 50% of the theoretical), and an oil which on standing continued to furnish additional quantities of this solid. The ester was purified by recrystallization from aqueous alcohol and also from chloroform-ether mixtures.

Anal. Calcd. for $C_{21}H_{22}O_5N_2$: N, 7.33. Found: N, 7.10, 7.08.

It may be noted that this ester appears to isomerize during the process of recrystallization since the filtrates obtained during this process on concentration yield crystalline precipitates having lower melting points than the original crystals. The ester, m. p. 125–127°, on hydrolysis under the prolonged action of barium hydroxide, is transformed into the imino acid, XXII.

The N-phenylacetic acid derivative of tyrosine,²⁰ XXII, m. p. 223–225° with decomposition, was prepared from XX and XXI by the action of barium hydroxide. It was also prepared by digesting the unsaturated ester I with hydrogen iodide in the presence of red phosphorus. The excess of hydrogen iodide was then removed by vacuum distillation and by digestion with small quantities of water and the residue without further purification was hydrolyzed with barium hydroxide.²¹ After heating on a steam-bath for twenty-four hours, the reaction was stopped and the contents of the flask distilled with steam in order to remove the methylamine. The mixture was then filtered hot to remove barium carbonate and excess of barium hydroxide. The filtrate when exactly neutralized as to barium with sulfuric acid and separated from the precipitated barium sulfate, was evaporated to small volume at ordinary temperature. Under these conditions the imino acid separated in the form of a fine white crystalline precipitate in yields averaging 75% of the theoretical. When purified by recrystallization from alcohol it melted with decomposition at 223–225°.

Anal. Calcd. for $C_{17}H_{17}O_3N$: N, 4.44. Found: N, 4.36, 4.45, 4.46.

The acid is only very slightly soluble in hot water (1 g. in 500 cc.) and hot alcohol (1 g. in 250 cc.). It is readily changed into its copper salt from which it may be regenerated quantitatively. Other derivatives of the acid have also been prepared but these will be reported in a later paper.

The copper salt of the N-phenylacetic acid derivative of tyrosine, XXIII, m. p. 215° with decomposition, was prepared by suspending 4 g. of the acid, m. p. 223–225°, and 1.2 equivalents of basic copper carbonate in 300 cc. of water and boiling the mixture for twenty minutes or until the acid had entirely disappeared and the effervescence of carbon dioxide had ceased. The hot solution was then filtered from excess copper carbonate and on cooling yielded 4.1 g. of a blue crystalline copper salt. The filtrates from this precipitate when concentrated by heating turned dark green and then black, due to decomposition, and no further quantities of salt were obtained. After recrystallizing twice from boiling water,²² from which it separated almost completely on cooling in the form of fine blue needles which contain water of crystallization, the substance was found to melt partially at 110° with the evolution of water vapor and completely with decomposition at 215°. The loss of water is accompanied by a change in color from blue to green. In order to determine the water of crystallization, the salt was heated in an air-bath at 110° to constant weight, when the following results were obtained:

²⁰ Acknowledgment is made to Dorotlea Haas and Anne L. White for assistance in preparing this substance.

²¹ The same general procedure was followed that has been already described in connection with other similar operations. (a) Hahn and Gilman, *THIS JOURNAL*, **47**, 2951 (1925); (b) Hahn and Evans, *ibid.*, **50**, 817 (1928).

²² One gram is soluble in 100 cc. of boiling and 400 cc. of cold water.

Anal. Calcd. for $3C_{17}H_{16}O_6NCu \cdot 10H_2O$: H_2O , 13.74. Found: H_2O , 13.89, 13.74, 13.37.

The green anhydrous form melts fairly sharply with decomposition at 215° and is extremely hygroscopic.

Anal. Calcd. for $C_{17}H_{16}O_6NCu$: N, 3.72. Found: N, 3.72, 3.64. Calcd. for $C_{17}H_{16}O_6NCu$: Cu, 16.89. Found:²³ Cu, 16.43, 16.53.

When the salt was dissolved in water and treated with hydrogen sulfide, it was transformed almost quantitatively into the corresponding imino acid, XXII. For example, when hydrogen sulfide was passed into a hot aqueous solution containing 2 g. of the salt, the blue color was discharged and copper sulfide was precipitated. If the solution was of sufficient volume (700 cc. for 2 g. of salt), no imino acid was precipitated at this point. The solution was filtered from sulfide, concentrated on the steam-bath to a volume of 120 cc. and cooled, when 1.5 g. of imino acid was precipitated in pure condition. Mixed melting-point determinations showed that the acid obtained in this way was identical with the original acid XXII from which the copper salt was prepared.

Summary

The introduction of the phenylacetic acid group in the N-1-position of the hydantoin ring by the synthesis of N-3-methyl-5-tyrosyl-hydantoin-1-phenylacetic acid has produced a highly labile hydantoin combination.

(a) The unsaturated derivatives exist in isomeric modifications of which two esters and three distinct acids have been isolated.⁷ (b) The saturated derivatives also exist in isomeric modifications which may be explained on the basis of stereoisomerism. Two esters and three distinct acids were isolated, the third of which may be assumed to represent a desmotropic modification. The polypeptide-hydantoin itself also would appear to consist of a mixture of unstable forms, from which only one stable modification could be isolated. (c) The hydrolysis of the polypeptide-hydantoin resulted in the formation of a dibasic imino acid.

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²³ Copper was determined by ignition to form copper oxide. Fresenius, "Quantitative Chemical Analysis," 1904, Vol. I, p. 373.