benzophenone in 450 cc. of isopropyl alcohol contained in a 500-cc. round-bottomed Pyrex flask is supported in an inverted position on a tripod and exposed to direct sunlight. After three hours crystals of benzopinacol are present. After one week 45 g. of benzopinacol is filtered off. To the filtrate is added a further 50 g. of ketone and the solution is again exposed to sunlight. This procedure was repeated until 300 g. of pinacol was obtained.

In one experiment a solution of 400 g. of benzophenone in 400 cc. of isopropyl alcohol and 400 cc. of benzene was exposed. After one month a quantitative yield of pinacol was obtained. The disadvantage of using benzene lies in the fact that the pinacol crystallizes with benzene of crystallization in the form of a compact mass which is difficult to remove from the flask.

## Summary

Benzophenone and certain substituted benzophenones are reduced nearly quantitatively to hydrols when solutions of the ketones and a small amount of sodium isopropylate in isopropyl alcohol are exposed to sunlight.

The mechanism of the reaction has been elucidated. The ketone is reduced initially to the corresponding pinacol by a photochemical reaction; the pinacol is then converted to a mixture of ketone and hydrol by the action of the sodium alcoholate and alcohol; the ketone that is regenerated in this manner goes through the series of reactions again.

ANN ARBOR, MICHIGAN RECEIVED JULY 29, 1932
PUBLISHED JANUARY 11, 1933

---

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

# Researches on Nitrogenous Glycosides. II. The Synthesis of Glycosido Ureides[1]

BY KATHERINE M. HARING AND TREAT B. JOHNSON

The present investigation[2] was undertaken in order to develop a general method for the preparation of glycosido ureides of the hydantoic acid and hydantoin types. Glycosidohydantoins and their reduced forms, the unknown glycosidoimidazoles, are of interest because of their structural relationship to the natural purine nucleosides. In addition, it was believed that a study of such nitrogenous glycosides would be of biochemical importance in relation to the problem of the structure of naturally occurring protein-carbohydrate complexes.

The interest in the latter field has recently received an added stimulus in the discovery that carbohydrate units form integral parts of the proteins of blood serum[3] and egg white,[4] which were formerly considered simple

(1) Constructed from a dissertation presented by Katherine M. Haring to the Faculty of the Graduate School of Yale University in June, 1932, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) The research described was supported in part by a grant from the Therapeutic Research Committee of the American Medical Association for 1930–1931 and 1931–1932.

(3) Rimington, *Biochem. J.*, **23**, 430 (1929); **25**, 1062 (1931).

(4) Levene and Mori, *J. Biol. Chem.*, **84**, 49 (1929).

proteins. Furthermore, since bacterial polysaccharides which in the free state show typical hapten reactions, exist in the bacteria in a state which renders them not only type specific but also antigenic, it is believed that they must be joined to another molecule—probably protein. The most striking evidence in favor of this view has been contributed by the researches of Goebel and Avery.[5]

Since carbohydrate fractions appear to be intimate parts of the structure of certain simple proteins and since there is evidence that bacterial polysaccharides may be associated with proteins in the parent cell, it becomes very important to study the kinds of linkages which might exist between them. The carbohydrates of serum proteins and of egg proteins have been found to be very firmly bound in the combined molecules, whereas several bacterial polysaccharides have easily been obtained in the free state. It is probable, therefore, that the types of linkages involved in such cases vary widely.

The method of coupling sugars with amino acids by direct union with the free amino groups gives combinations which are easily susceptible to hydrolysis. A synthetic compound resulting from such a union was obtained by Euler and Zeile,[6] who condensed glycine ester with glucose in the presence of active aluminum. Maurer and his co-workers[7] had previously studied the reaction between bromoacetoglucose and sarcosine amide. They were able to deacetylate the resulting compound to obtain sarcosine amide glycoside. An extension of this method to other amino acids and peptides led to the formation of a number of acetylated derivatives. Euler was able to apply Maurer's method further to the preparation of glycylglycine glycoside. Both Maurer and Euler found that these derivatives which contained glucose directly linked to the amino acids or peptides were unstable in aqueous alkali solution.

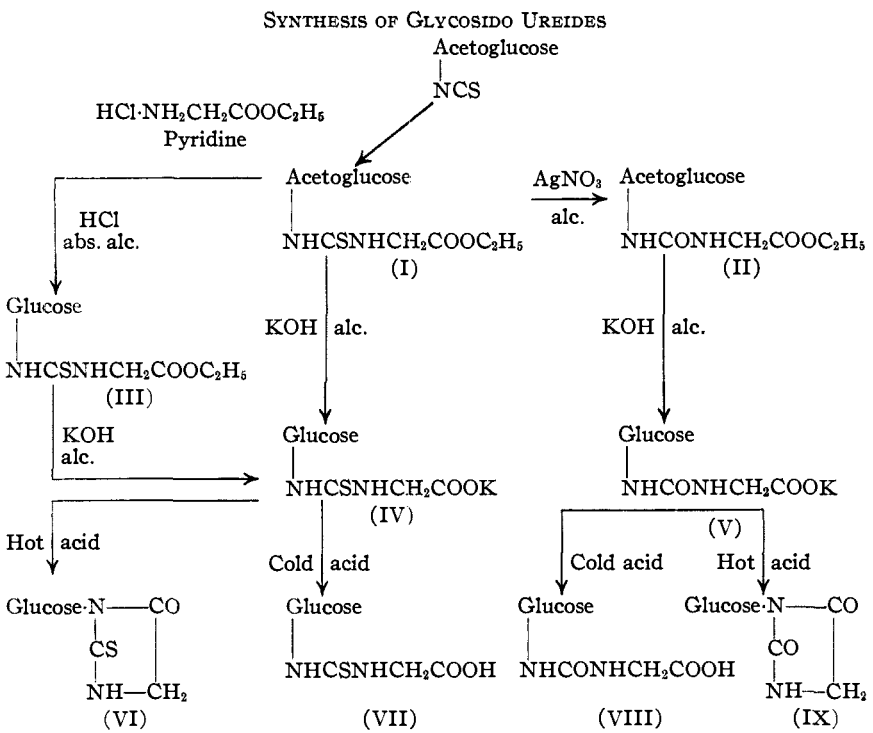
The main object of the research presented here has been to develop a general method for coupling amino acids with sugars through an urea bridge. The synthetic methods which are involved in the preparation of glycosidothiohydantoic acid (VII), N-1-glycosido-2-thiohydantoin (VI), glycosidohydantoic acid (VIII), and N-1-glycosidohydantoin (IX) are shown in the accompanying chart.

Tetracetylglucose isothiocyanate, which was described originally by Fischer,[8] condensed smoothly and almost quantitatively with glycine ethyl ester hydrochloride, in the presence of pyridine, to give ethyl tetracetyl glycosidothiohydantoate (I). The practically quantitative removal of the acetyl groups from (I) with the formation of ethyl glycosidothiohydantoate (III) was accomplished by the use of alcoholic hydrogen chloride. It was

(5) Goebel and Avery, *J. Exp. Med.*, **54**, 431, 437 (1931).
(6) Euler and Zeile, *Ann.*, **487**, 163 (1931).
(7) Maurer and Schiedt, *Z. physiol. Chem.*, **206**, 125 (1932).
(8) Fischer, *Ber.*, **47**, 1382 (1914).

more practical, however, to simultaneously deacetylate and deësterify (I) by saponification with alcoholic potassium hydroxide. The potassium salt (IV) which resulted could be subsequently hydrolyzed with cold acid to give glycosidothiohydantoic acid (VII) or with hot acid to give N-1-glycosido-2-thiohydantoin (VI).

The oxygen analogs of the compounds which have been described were prepared from ethyl tetracetylglycosidohydantoate (II). This substance resulted when ethyl tetracetylglycosidothiohydantoate (I) was treated with alcoholic silver nitrate solution according to the procedure described by Dixon[9] for the desulfurization of thioureas. Deacetylation and deesterification of (II) was accomplished by saponification with alcoholic potassium hydroxide. The resulting potassium salt (V) was then transformed with cold acid into glycosidohydantoic acid (VIII) or with hot acid into N-1-glycosidohydantoin (IX).




The glycosido ureides prepared in this investigation resemble the glycosido amino acid and peptide combinations of Euler and Zeile and Maurer and Schiedt in their behavior toward hydrolytic agents. On the other hand, they show a marked contrast to the purine and pyrimidine glycosides described in the literature. The purine nucleosides are readily hydrolyz-

(9) Dixon, *J. Chem. Soc.*, 556 (1895).

able by acids but remain intact on treatment with alkalies, whereas the pyrimidine nucleosides are much more resistant even to acid hydrolysis.[10] Glycosidohydantoic acid (VIII) and N-1-glycosidohydantoin (IX), however, represent a type of glycoside which is much more susceptible to hydrolysis than the purine nucleosides since they readily reduce Fehling's solution. In the case of glycosidothiohydantoic acid (VII) and N-1-glycosido-2-thiohydantoin (VI) colored side reactions make it impossible to observe any reduction with Fehling's solution although hydrolysis probably does occur. The ease with which these compounds are hydrolyzed by hot acid has been shown to be one of the factors which causes the small yields in the preparation of both N-1-glycosido-2-thiohydantoin and N-1-glycosidohydantoin. Research will be continued in this field in order to extend the method of synthesis, which has been described, to other sugars and to other amino acids and peptides.

## Experimental Part

**Tetracetyl-*d*-glucose Isothiocyanate.**—For the preparation of tetracetylglucose isothiocyanate a modification of Fischer's method[8] was found necessary.

A solution of 25 g. of bromoacetoglucose in 150 cc. of xylene was treated with 16 g. of solid silver thiocyanate. The mixture was refluxed over a small free flame for one to two hours until the supernatant liquid became reddish-brown. The solution was then allowed to cool, filtered from the silver salts, and diluted with about 100 cc. of petroleum ether. The red-brown gum which in most cases was thrown out on the stirring rod and sides of the beaker was discarded. Tetracetylglucose isothiocyanate was precipitated by slowly decanting the supernatant liquid from the red gum into 250 cc. of iced petroleum ether. The total yield varied from 50–87%.

The crude isothiocyanate could be recrystallized from a hot alcohol solution which was cooled quickly in an ice-bath or from a chloroform solution which was poured slowly into a large volume of petroleum ether. The latter method was more satisfactory. The products from the two methods of recrystallization did not show the different characteristics which Fischer described. In either case the purified compound melted at 112–114°.[11]

**Ethyl Tetracetyl-*d*-glycosidothiohydantoate** (I).—Eight grams of glycine ethyl ester hydrochloride was suspended in a solution of 20 g. of tetracetylglucose isothiocyanate and 6 cc. of pyridine in 150 cc. of chloroform and the mixture was refluxed on the steam-bath for at least three hours. When heating was discontinued the chloroform solution was evaporated to dryness *in vacuo*. The residual gum was stirred vigorously with 100–150 cc. of water and was alternately warmed on the steam-bath and cooled in ice until it hardened. Incomplete removal of the chloroform occasionally prevented the hardening of the gum. After standing in the ice-bath the caked product could be crushed to a powder. The yield was 96%.

Ethyl tetracetylglucosidothiohydantoate was very soluble in acetone and hot ethyl alcohol, soluble in cold ethyl alcohol (1 g. in 20 cc.), cold methyl alcohol, and cold benzene, and practically insoluble in ether, petroleum ether and water. Twenty-two grams of crude material was recrystallized from about 300 cc. of 50% alcohol. The purified substance separated in rosets of short, creamy-white needles which melted at 151–152.5°. Its specific rotation was $[\alpha]_D^{22}$ +6° in chloroform solution. A sample was dried *in vacuo* at 100° for analysis.

(10) Levene and Bass, "Nucleic Acids," Chemical Catalog Co., New York, 1931, pp. 144–145.

(11) The melting points given are corrected melting points.

*Anal.* Calcd. for $C_{19}H_{28}O_{11}N_2S$: C, 46.34; H, 5.72; N, 5.69; S, 6.50. Found: C, 46.43; H, 5.68; N, 5.68; S, 6.50.

If the time of heating the original reaction mixture was less than three hours, small amounts of tetracetylglucose thiourethan, melting at 156–157°, were isolated from the recrystallization filtrate, showing that the reaction had been incomplete. Mixed with tetracetylglycosidothiohydantoic ester, this compound melted at 129–131°.

**Ethyl *d*-Glycosidothiohydantoate (III).**—Ethyl tetracetylglycosidothiohydantoate (I) could be deacetylated either in acid or alkaline medium. When alcoholic hydrogen chloride was the reagent used, the product was ethyl glycosidothiohydantoate.

Five cc. of absolute alcohol saturated with dry hydrogen chloride was added to a warm solution of 10 g. of ethyl tetracetylglycosidothiohydantoate in 100 cc. of absolute alcohol. After this solution had stood overnight at room temperature it was concentrated to a thick sirup, diluted with absolute alcohol and concentrated again. It was then diluted once more with 100 cc. of absolute alcohol and again allowed to stand in the ice box. The total yield of ester obtained by carrying out this procedure repeatedly was 95%.

Ethyl glycosidothiohydantoate crystallized in iridescent, white plates which contained alcohol of crystallization. It was very soluble in water, soluble in hot absolute alcohol (1 g. in 50 cc.) and hot ethyl acetate (1 g. in 230 cc.), but much less soluble in cold absolute alcohol (1 g. in 400 cc.) and cold ethyl acetate (1 g. in 1100 cc.). It could be recrystallized from either of the latter solvents. The melting point of the purified product was indistinct. It softened at about 110° and melted with the evolution of gas at 119–121°. If the heating was more rapid it melted only partially with the evolution of gas at 119–121° and liquefied completely at 154–155°. Its optical rotation was $[\alpha]_D^{22}$ −31.3° in water solution. A sample, dried to constant weight *in vacuo* at room temperature, gave analyses showing the presence of one mole of alcohol of crystallization.

*Anal.* Calcd. for $C_{11}H_{20}O_7N_2S \cdot C_2H_5OH$: N, 7.57. Found: N, 7.52, 7.44.

This crystalline form of the product could be freed from alcohol only with great difficulty due to its tendency to decompose on prolonged heating. The best result was obtained when a sample was heated in an Abderhalden drier over calcium chloride at 76° for six weeks and then at 100° for two days. The total loss in weight was 12.97%. This corresponded to the theoretical loss in weight of 12.44% due to one mole of alcohol of crystallization. The alcohol-free product was a tan colored powder which melted at 152–155° and decomposed at about 162°.

*Anal.* Calcd. for $C_{11}H_{20}O_7N_2S$: N, 8.64. Found: N, 8.69.

When this alcohol-free modification was recrystallized from absolute alcohol it reverted to the original crystalline form.

When a large excess of alcoholic hydrogen chloride was used to catalyze the deacetylation of (I), the monohydrochloride of ethyl glycosidothiohydantoate was formed as a by-product. It was precipitated by pouring dropwise into a large volume of dry ether the sirupy alcoholic filtrate from which ethyl glycosidothiohydantoate itself had been separated. Purification of the hydrochloride could be accomplished by dissolving it in absolute alcohol and reprecipitating it with dry ether. The purified substance was a tan, amorphous powder which was very hygroscopic. When dry it melted with the evolution of gas at 73–75°.

*Anal.* Calcd. for $C_{11}H_{20}O_7N_2S \cdot HCl$: N, 7.76; HCl, 10.11. Found: N, 7.58, 7.50; HCl, 10.25.

**Potassium *d*-Glycosidothiohydantoate (IV).**—When alcoholic potassium hydroxide was the reagent used to deacetylate (I), the ester grouping was saponified simultaneously and potassium glycosidothiohydantoate was formed. Alcoholic potassium hydroxide

was used in preference to alcoholic ammonia because the potassium salt which resulted was more satisfactory to handle than the corresponding ammonium salt.

A solution of 1.5 g. of potassium hydroxide in 20 cc. of alcohol was added to a warm solution of 5 g. of ethyl tetracetylglycosidothiohydantoate in 75 cc. of alcohol. A flocculent pink precipitate was formed immediately when the two solutions were mixed. When the supernatant liquid had become clear the pink solid was filtered and washed with alcohol. When dry it was a hygroscopic, pink powder which decomposed at 137–141°. It could be prepared equally well by the saponification of (III) with alcoholic potash. Potassium glycosidothiohydantoate was used in the crude state for the preparation of glycosidothiohydantoic acid (VII) and N-1-glycosido-2-thiohydantoin (VI).

*d*-**Glycosidothiohydantoic Acid (VII).**—A water solution of 5 g. of potassium glycosidothiohydantoate was treated with 4 cc. of approximately 6 *N* sulfuric acid and the mixture was allowed to stand for four hours at room temperature. It was then concentrated *in vacuo* and poured into about 200 cc. of absolute alcohol. The filtrate from the precipitated potassium sulfate was concentrated again *in vacuo* until crystals of glycosidothiohydantoic acid separated. The maximum yield was 54%. If a considerable excess of sulfuric acid was used originally to neutralize the potassium salt, the mother liquor yielded finally crystals of ethyl glycosidothiohydantoate which evidently resulted from the esterification of the free acid.

The crude acid was recrystallized from alcohol. One gram dissolved very slowly in about 300 cc. of boiling alcohol. Since the solubility of the acid was not appreciably greater in hot than in cold alcohol, the solution was concentrated to half volume or less before cooling. The recrystallized compound decomposed at 180.5–181° when it was heated slowly. Rapid heating raised the decomposition point several degrees. Its specific rotation was $[\alpha]_D^{22}$ −36.1° in water solution. A sample was dried at 100° *in vacuo* for analysis.

*Anal.* Calcd. for $C_9H_{16}O_7N_2S$: C, 36.49; H, 5.45; N, 9.46. Found: C, 36.45; H, 5.50; N, 9.46, 9.39.

When a water solution of (VII) was boiled with Fehling's solution it became dark gray-green. The precipitate which settled was dark brown. Probably the sulfur caused side reactions which made it impossible to observe the reduction.

**N-1-*d*-Glycosido-2-thiohydantoin (VI).**—N-1-Glycosido-2-thiohydantoin was prepared by heating a solution of 2 g. of potassium glycosidothiohydantoate in 50 cc. of approximately 6 *N* hydrochloric acid for one-half hour on the steam-bath. The solution was then concentrated *in vacuo* to about 10 cc. and poured into 50 cc. of absolute alcohol. The precipitate of potassium chloride which formed was filtered out and the filtrate was concentrated *in vacuo* to a thin sirup which was diluted with about 50 cc. of amyl alcohol. The cloudy solution which resulted was concentrated *in vacuo* until an amorphous precipitate began to form on the sides of the flask. This product, which shriveled at about 70° and decomposed at 111–121°, was discarded. The filtrate, when concentrated further *in vacuo*, yielded fine, yellow crystals of glycosidothiohydantoin, which decomposed at about 226°. The maximum yield was 33%.

This compound was recrystallized from absolute alcohol. One gram dissolved slowly in about 85 cc. of boiling alcohol and was about one-half as soluble cold. The purified product separated in the form of pale yellow plates which darkened when heated at about 216° and melted with decomposition at 224–225°. Its specific rotation was $[\alpha]_D^{22}$ +22.8° in water solution. A sample was dried at 100° *in vacuo* for analysis.

*Anal.* Calcd. for $C_9H_{14}O_6N_2S$: C, 38.86; H, 5.08; N, 10.07. Found: C, 39.22; H, 5.17; N, 9.87.

When a water solution of N-1-glycosido-2-thiohydantoin was added to Fehling's solution it turned yellowish-brown in the cold and formed a dark yellow precipitate on

heating. Since N-1-glycosidohydantoin reduced Fehling's solution under similar conditions, it is probable that the sulfur in the former case caused the colored side reactions.

In addition to glycosidothiohydantoin, the amyl alcohol mother liquor from the reaction previously described yielded a second type of crystalline compound. When heated, it turned dark brown at about 199° and decomposed at 221–231°. It could be recrystallized from absolute alcohol but was more soluble in amyl alcohol than glycosidothiohydantoin and much less soluble in water. Nitrogen analyses did not serve to identify the substance as thiohydantoic acid or thiohydantoin but indicated clearly that the glucose part of the molecule was no longer present. The latter conclusion was supported further by the fact that this was the only crystalline product which resulted when the original reaction mixture was heated for periods longer than one-half hour. N-1-Glycosido-2-thiohydantoin was apparently not very stable toward acid hydrolysis.

**Ethyl Tetracetyl-*d*-glycosidohydantoate (II).**—Ethyl tetracetylglycosidohydantoate was prepared by desulfurizing (I).

A hot solution of 10 g. of ethyl tetracetylglycosidothiohydantoate in 150 cc. of alcohol and a solution of 7.5 g. of silver nitrate in 20 cc. of water were mixed and heated on the steam-bath. After five minutes an alcoholic solution of 2.2 g. of potassium hydroxide was added to neutralize the nitric acid set free from the silver nitrate. This promoted the coagulation of the silver sulfide and helped to prevent the nitric acid from hydrolyzing off the acetyl groups of the sugar. The solution was heated until the silver sulfide coagulated. Then it was cooled and filtered through a norit mat. The filtrate was concentrated *in vacuo* to about 50 cc. and diluted with 3–4 volumes of water. When the solution was stirred vigorously to start precipitation the desulfurized ester was obtained in 48% yield.

Ethyl tetracetylglycosidohydantoate was recrystallized from absolute alcohol. One gram was soluble in about 10 cc. of hot and about 30 cc. of cold absolute alcohol. It was practically insoluble in water but was much more soluble in 50% than in absolute alcohol. The recrystallized product separated in the form of long, white needles which melted at 149–149.5°. Its specific rotation was $[\alpha]_D^{22}$ −3° in chloroform solution. A sample was dried at 100° *in vacuo* and analyzed.

*Anal.* Calcd. for $C_{19}H_{28}O_{12}N_2$: C, 47.89; H, 5.92; N, 5.88. Found: C, 47.57; H, 5.93; N, 5.93.

**Potassium *d*-Glycosidohydantoate (V).**—A solution of 1.5 g. of potassium hydroxide was added to a warm solution of 5 g. of ethyl tetracetylglycosidohydantoate in 75 cc. of alcohol. A white precipitate of potassium glycosidohydantoate formed immediately. The crude salt, which decomposed at 134–137°, was used in the preparation of glycosidohydantoic acid (VIII) and N-1-glycosidohydantoin (IX).

***d*-Glycosidohydantoic Acid (VIII).**—Twenty cc. of approximately 1 *N* hydrochloric acid was added to a water solution of 4 g. of potassium glycosidohydantoate and the mixture was allowed to stand at room temperature. After four hours the solution was concentrated *in vacuo* to about 10 cc. and diluted with 100 cc. of absolute alcohol. When the filtrate from the precipitated potassium chloride was allowed to stand in the ice-bath crystals of glycosidohydantoic acid separated. The total yield was 55%.

The crude product was recrystallized from alcohol. One gram dissolved in 80 cc. of boiling and in 500 cc. of cold alcohol. The purified acid readily reduced Fehling's solution. It melted with decomposition at 169.5–170° when it was heated slowly and had a specific rotation of $[\alpha]_D^{27}$ −25.8° in water solution. A sample was dried at 100° *in vacuo* and analyzed.

*Anal.* Calcd. for $C_9H_{16}O_8N_2$: C, 38.57; H, 5.71; N, 10.00. Found: C, 38.40; H, 5.95; N, 9.82, 9.90.

**N-1-*d*-Glycosidohydantoin (IX).**— A solution of 2 g. of potassium glycosidohydanto-

ate in 25 cc. of approximately 6 *N* hydrochloric acid was heated on the steam-bath for one-half hour. It was then concentrated *in vacuo* to about 10 cc. and poured into 100 cc. of absolute alcohol. The filtrate from the precipitated potassium chloride was concentrated *in vacuo*, diluted with more absolute alcohol and concentrated again to about 25 cc. This thin sirup was diluted with about 75 cc. of amyl alcohol, cooled in an ice-bath and filtered from the sticky, amorphous precipitate which formed. This product, which shriveled at 60° and decomposed at 110–115°, was discarded. When the mother liquor was cooled further in the ice-bath fine, white crystals of N-1-glycosidohydantoin were deposited on the walls of the beaker. The total yield was 25%.

The crude product was very slightly soluble in hot absolute or 95% alcohol. It could best be recrystallized by dissolving it in cold 50% alcohol, adding 3–4 volumes of absolute alcohol and concentrating the resulting solution until crystals just began to separate. When the concentrated solution was allowed to stand in the ice box, N-1-glycosidohydantoin crystallized in rosets of very short, fine, white needles, which decomposed at 270–271°. Its specific rotation was $[\alpha]_D^{22}$ +5° in water solution. When it was boiled with Fehling's solution there was a decided reducing effect. A sample was dried *in vacuo* at 100° and analyzed.

*Anal.* Calcd. for $C_9H_{14}O_7N_2$: C, 41.22; H, 5.39; N, 10.69. Found: C, 40.98; H, 5.46; N, 10.63.

The amyl alcohol mother liquor yielded, in addition to N-1-glycosidohydantoin, small amounts of a second crystalline compound which melted with decomposition at about 178–194°. Further work will be necessary to identify it. Probably, however, as in the case of the second compound isolated from the preparation of N-1-glycosido-2-thiohydantoin, this compound had also lost the glucose part of the molecule.

## Summary

1. A general method has been developed for the synthesis of four new glycosido ureides, *d*-glycosidothiohydantoic acid (VII), N-1-*d*-glycosido-2-thiohydantoin (VI), *d*-glycosidohydantoic acid (VIII), and N-1-*d*-glycosidohydantoin (IX). This investigation involved the preparation of a number of intermediate acetylated and deacetylated derivatives of glycosidothiohydantoic acid and glycosidohydantoic acid.

2. A study of the final glycosido ureides has shown that they differ in chemical properties from the synthetic glycosido pyrimidines and purines, but resemble the synthetic glycosido amino acids and peptides which have been described in the literature.

NEW HAVEN, CONNECTICUT RECEIVED AUGUST 1, 1932
PUBLISHED JANUARY 11, 1933