forms. However, as shown in the present work, secondary reactions occur so that the equilibrium is only an apparent one. Undoubtedly this is the reason the reactions do not follow the first-order equation.

Experimental

By the use of pyridine and acetic anhydride at 0°, glucosylaniline was acetylated to give a mixture of two products. The mixture of glucosylaniline tetraacetates was fractionated by crystallization from methanol. After recrystallization, the most insoluble (α) isomer had a m.p. of 153° and a rotation of $[\alpha]^{30}D$ +185.0° ($CHCl_3$, c 3.2).

The reaction of pentaacetyl-β-D-glucopyranose with aniline according to the method of Frèrejacque[2] gave a mixture of glucosylaniline tetraacetates from which the β-isomer was separated by use of an addition product with carbon tetrachloride. In order to remove the carbon tetrachloride, it was found necessary to carry out the final crystallizations from ethyl ether containing petroleum ether. The pure β-isomer had a m.p. of 98–98.5°, and a rotation of $[\alpha]^{28}D$ −52.8° ($CHCl_3$, c 4). No mutarotation was noted after 24 hours. Acetylation of this product with cold acetic anhydride and pyridine did not introduce additional acetyl groups. The α-isomer recovered from the mother liquors had properties identical with that obtained by the direct acetylation of glucosylaniline, and the melting points of mixtures of the two showed no depression.

By the reaction of aniline with tetraacetyl-D-glucosyl bromide according to the method of Baker,[3] only the tetraacetyl-β-D-glucosylaniline was obtained. This was shown to be identical with the product obtained above by the Frèrejacque method.

Acetyl determinations on the α- and β-isomers gave the following results. Alkaline saponification for 8 hours at 0°: β-isomer; found, 40.1% acetyl; α-isomer; found, 40.4%. Acid deacetylation (p-toluenesulfonic acid in ethanol): β-isomer; found, 40.3% acetyl; α-isomer, 42.3%. Theory for tetraacetate, 40.7% acetyl; theory for pentaacetate, 46.2%.

BIRMINGHAM, ALABAMA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

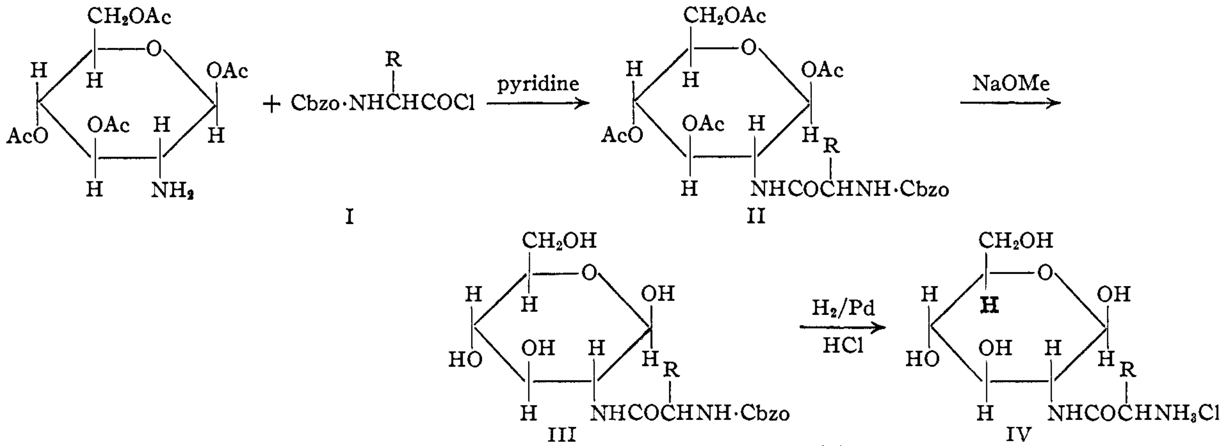
Amino Acid Derivatives of D-Glucosamine[1]

BY DAVID G. DOHERTY,[2] EDWIN A. POPENOE AND KARL PAUL LINK

RECEIVED FEBRUARY 28, 1953

A series of derivatives of D-glucosamine substituted on the nitrogen atom by acyl amino acid residues has been prepared. By coupling 1,3,4,6-tetraacetyl-β-D-glucosamine with an acylamino acid chloride in the presence of pyridine in an anhydrous solvent, the following compounds have been prepared: N-hippuryl-1,3,4,6-tetraacetyl-β-D-glucosamine, dicarbobenzoxy-L-cystyl-di-(1,3,4,6-tetraacetyl-β-D-glucosamine), N-(dicarbobenzoxy-L-lysyl)-1,3,4,6-tetraacetyl-β-D-glucosamine, N-(carbobenzoxy-L-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine and N-(carbobenzoxy-D-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine. With carbobenzoxy-L-glutamic anhydride N-(carbobenzoxy-L-α-glutamyl)-1,3,4,6-tetraacetyl-β-D-glucosamine was obtained. Of these "glucopeptide" acetates, only the first and last gave crystalline products on alkaline deacetylation.

The carbobenzoxy derivatives of amino acids were originally used by Bergmann and Zervas[3] for coupling with 1,3,4,6-tetraacetyl-β-D-glucosamine in the first definitive synthesis of so-called "glucopeptides." For example, carbobenzoxyglycyl chloride (Ia) and 1,3,4,6-tetraacetyl-β-D-glucosamine were coupled in the presence of pyridine to give N-(carbobenzoxyglycyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IIa), followed by deacetylation and hydrogenolysis of the carbobenzoxy group to give N-glycyl-D-glucosamine (IVa). In this way N-glycyl-D-glucosamine and N-alanyl-D-glucosamine (IVb) were obtained.



I, II: (a) R = H
(b) R = CH_3
(c) R = $CH_2CH_2CH_2CH_2NH$·Cbzo
(d) R = CH_2CH_2—S—CH_3
Cbzo = $COOCH_2C_6H_5$

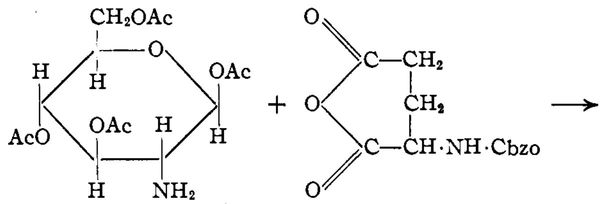
III, IV: (a) R = H
(b) R = CH_3

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. This work is from theses submitted to the faculty of the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy by David G. Doherty, June, 1948, and by Edwin A. Popenoe, June, 1950. This paper was presented in part before the Division of Sugar Chemistry and Technology at the 117th meeting of the American Chemical Society, Detroit, April, 1950.

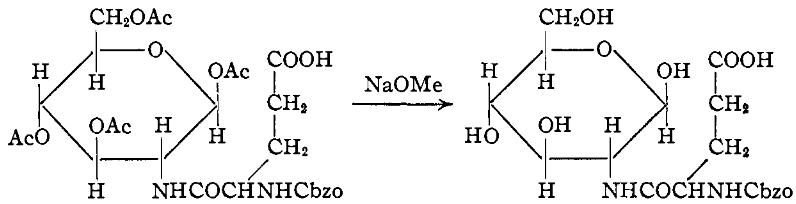
(2) Junior Fellow, National Institutes of Health, 1946–1948.

(3) M. Bergmann and L. Zervas, *Ber.*, **65**, 1201 (1932).

In conjunction with other research in this Laboratory this method was used to prepare a group of acyl "glucopeptides" containing the amino acid residues of hippuric and glutamic acid, lysine, cystine and methionine. Thus acyl "glucopeptides" with a representative aliphatic, acidic, basic and sulfur-containing amino acid are made available for further study. In all but one of these cases the method employed by Bergmann and Zervas was used—namely, reaction of an acylamino acid chloride with 1,3,4,6-tetraacetyl-β-D-glucosamine in the presence of pyridine. For the preparation of the glutamic acid derivative, carbobenzoxy-L-glutamic anhydride (V) was used as the acylating reagent. This anhydride has been shown by Bergmann, *et al.*,[4,5] to react with amines or alcohols with opening of the anhydride ring and formation of α-amides or α-esters. It was found to react smoothly with 1,3,4,6-tetraacetyl-β-D-glucosamine to produce N-(carbobenzoxy-L-α-glutamyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (VI) which could be deacetylated with sodium methoxide to give N-(carbobenzoxy-L-α-glutamyl)-β-D-glucosamine (VII).

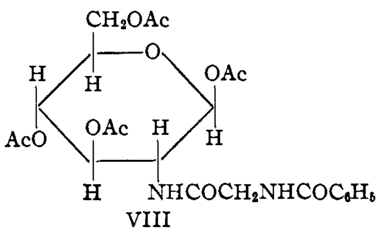


V

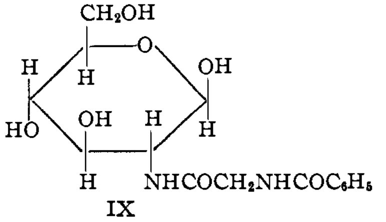


VI VII

Hippuryl chloride gave the expected tetraacetate VIII, which could be deacetylated with sodium methoxide to give N-hippuryl-β-D-glucosamine (IX).



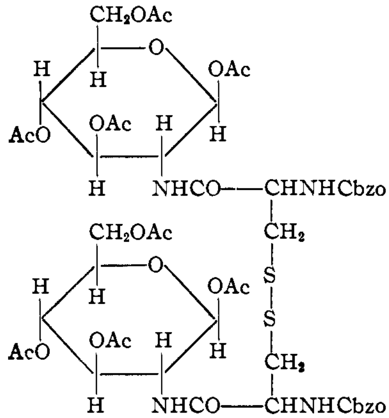
VIII



IX

Efforts to acylate 1,3,4,6-tetraacetyl-β-D-glucosamine with dicarbobenzoxy-L-lysine azide did not lead to the desired product. Therefore the sirupy dicarbobenzoxy-L-lysyl chloride was used in the synthesis of N-(dicarbobenzoxy-L-lysyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IIc). Attempts to deacetylate this product by the usual procedures resulted in gelatinous products which could not be crystallized.

In a like manner N-(carbobenzoxy-L-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine and N-(carbobenzoxy-D-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IId) were prepared from the corresponding acid chlorides. When DL-methionine was used in this preparation, a mixture of isomers resulted which could not readily be resolved by fractional crystallization, due to the similarity in solubility characteristics of the two diastereomers. When dicarbobenzoxy-L-cystyl chloride was coupled with 1,3,4,6-tetraacetyl-β-D-glucosamine in the presence of pyridine, the expected compound X, containing two glucosamine residues was obtained.



X

Deacetylation of IIc, IId and X did not give crystalline products.

Experimental

N-Hippuryl-1,3,4,6-tetraacetyl-β-D-glucosamine (VIII).—Hippuryl chloride (7.9 g.), prepared from 10 g. of hippuric acid by the procedure of Fischer,[6] was added quickly to an ice cold solution of 14 g. of 1,3,4,6-tetraacetyl-β-D-glucosamine and 13 ml. of pyridine in 150 ml. of dry chloroform. The flask was immediately stoppered and shaken vigorously. Within 15 seconds a gel had formed which made further shaking useless. After the mixture stood three hours in the ice-bath and an additional 24 hours at room temperature, the gel was mixed with three volumes of chloroform, extracted twice with *N* hydrochloric acid, twice with a 5% solution of potassium bicarbonate and twice with water. The gel disappeared with the first extraction with acid. The yellow chloroform solution was then filtered through a dry filter paper and the solvent removed *in vacuo*. The sirupy residue was dissolved in a small amount of ethanol and scratched repeatedly to promote crystallization; yield 12.9 g., 63%. The product at this stage was yellow. The color could not be removed satisfactorily by treatment with carbon or by recrystallization from methanol, ethanol or 2-propanol, but a colorless product melting at 170–171°[7] could be obtained by two recrystallizations from ethyl acetate; $[\alpha]^{23}D$ +13.75 (*c* 5, pyridine).

Anal. Calcd. for $C_{23}H_{28}O_{11}N_2$: C, 54.31; H, 5.55. Found: C, 54.58; H, 5.68.

(4) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(5) M. Bergmann, L. Zervas and L. Salzmann, *ibid.*, **66B**, 1288 (1933).

(6) E. Fischer, *ibid.*, **38**, 605 (1905).

(7) All melting points are uncorrected.

N-Hippuryl-β-D-glucosamine (IX).—For the deacetylation, hippuryl glucosamine tetraacetate was dissolved in dry methanol, cooled in an ice-salt-bath at −10° and treated with 4 moles of *N* sodium methoxide in methanol. The flask was shaken in the bath for 10 minutes. The reaction mixture was then allowed to come to room temperature over a period of 20 minutes. *N* sulfuric acid was added in an amount slightly in excess of that needed to neutralize the sodium methoxide and the mixture was evaporated to dryness *in vacuo*. The residue was extracted with several portions of hot absolute ethanol and the combined extracts were evaporated to dryness *in vacuo*. The product was usually crystalline at this stage and was transferred to a buchner funnel with the aid of a little cold absolute ethanol; yield 78%. After two recrystallizations from water and one from ethanol-water (50-50) the product melted at 210.5-212°; $[\alpha]^{22}D$ +43.4° 45 minutes after solution, changing to +73.0° after 72 hours, constant thereafter (*c* 1.3, pyridine).

Anal. Calcd. for $C_{15}H_{20}O_7N_2$: C, 52.93; H, 5.92. Found: C, 52.65; H, 6.05.

This compound has been prepared previously by another method.[8] However, a melting point of 200° and $[\alpha]^{21}D$ +43.47 was reported. The mutarotation observed by us was not mentioned.

N-(Carbobenzoxy-L-α-glutamyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (VI).—1,3,4,6-Tetraacetyl-β-D-glucosamine (13.2 g.) was dissolved in 100 ml. of pure dry chloroform and 10.2 g. of carbobenzoxy-L-glutamic anhydride[4] was added. The flask was shaken over a period of about 10 minutes during which time the mixture warmed slightly and a gel formed. After 24 hours at room temperature the gel was stirred up thoroughly with *N* hydrochloric acid, then with a 5% solution of potassium bicarbonate, and finally with water. Sufficient ethanol was then added to give a homogeneous solution and the solvent was removed *in vacuo*. The residue was taken up in 200 ml. of hot 95% ethanol. Crystallization occurred readily on cooling; yield 14.4 g., 62%. After one recrystallization from ethanol the product melted at 219° with decomposition and showed $[\alpha]^{28}D$ −7.56 (*c* 5, pyridine).

Anal. Calcd. for $C_{27}H_{34}O_{14}N_2$: C, 53.09; H, 5.69. Found: C, 53.28; H, 5.62.

N-(Carbobenzoxy-L-α-glutamyl)-β-D-glucosamine (VII).—The above product VI was deacetylated in a manner like that used for N-hippuryl-1,3,4,6-tetraacetyl-β-D-glucosamine except that five moles of sodium methoxide was used, one to neutralize the free carboxyl group. After neutralization with sulfuric acid and evaporation *in vacuo* the residue was extracted with several portions of hot absolute ethanol and the combined extracts were evaporated *in vacuo*. As the evaporation proceeded a gel formed. After as much solvent as possible had been removed the residue was taken up in a minimum amount of hot water. On cooling a gel formed which crystallized on standing; yield 88%, m.p. 182-183° with decomposition, $[\alpha]^{23}D$ +65.7 (*c* 1.5, pyridine). The product tended to decompose during recrystallization from water or methanol so that an analytically pure sample could not be obtained.

Dicarbobenzoxy-L-cystyl-di-(1,3,4,6-tetraacetyl-β-D-glucosamine) (X).—To an ice cold solution of 5 g. of 1,3,4,6-tetraacetyl-β-D-glucosamine in 50 ml. of dry chloroform was added 4.8 g. of dicarbobenzoxy-L-cystyl chloride[4] and 8 ml. of dry pyridine. The mixture was shaken in the ice-bath until solution occurred and kept in the ice-bath for 4 hours at room temperature for 36 hours. By this time the contents of the flasks were gelatinous. An equal volume of chloroform was added and the slurry was extracted first with *N* hydrochloric acid, then with a 5% solution of potassium bicarbonate and finally twice with water. During these extractions considerable difficulty with emulsions was encountered and it was necessary to resort to centrifugation to separate the two phases. The chloroform layer was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The resulting sirup was dissolved in warm dioxane and some water was added. This caused the product to separate as a gel which became partially crystalline after standing 2 days at room temperature. The solid material was separated by suction filtration and digested with a little hot ethanol which brought about complete crystallization; yield 6.1 g., 36%. The product was recrystallized from dilute dioxane; m.p. 236°, $[\alpha]^{23}D$ −3.36 (*c* 5, pyridine).

Anal. Calcd. for $C_{50}H_{62}O_{24}N_4S_2$: C, 51.41; H, 5.39. Found: C, 51.23; H, 5.39.

N-(Dicarbobenzoxy-L-lysyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IIc).—A cold solution of α,ε-dicarbobenzoxy-L-lysyl chloride (prepared according to Bergmann, *et al.*,[9] from 13 g. of α,ε-dicarbobenzoxy-L-lysine) in 60 ml. of absolute ethyl acetate was added to a cold solution of 9.9 g. of 1,3,4,6-tetraacetyl-β-D-glucosamine and 10 ml. of dry pyridine in 150 ml. of absolute ethyl acetate. A gel formed almost immediately. The flask was shaken in an ice-bath for 30 minutes and then kept at 5° for 2 days. At the end of this time an equal volume of ethyl acetate was added and the crystalline product was filtered off. After one recrystallization from dilute dioxane this product weighed 6.5 g., melted at 186-186.5°, and showed $[\alpha]^{20}D$ −3.8 (*c* 7, pyridine). By working up the filtrate in a manner like that used in the preceding preparations, an additional 5.8 g. could be obtained which, after recrystallization from 95% ethanol showed the same physical constants as the first fraction; total yield 58%.

Anal. Calcd. for $C_{36}H_{45}O_{14}N_3$: C, 58.13; H, 6.10. Found: C, 58.45; H, 6.26.

N-(Carbobenzoxy-L-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IId).—L-Methionine (2.83 g., 0.019 mole) in 19 ml. of *N* sodium hydroxide was treated with 6 g. of benzyl chlorocarbonate in the usual manner.[4] The alkaline solution was extracted twice with ethyl acetate, acidified with concentrated hydrochloric acid to congo red and the resulting oil was extracted with ethyl acetate. This solution was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. A little anhydrous ether was added and the evaporation was repeated. The sirup was then dissolved in 50 ml. of anhydrous ether, cooled in ice and 4 g. of finely powdered phosphorus pentachloride was added. After shaking the flask in the ice-bath for 30 minutes, the ether solution was filtered through a pad of glass wool and evaporated to a sirup *in vacuo* at 0° or below. A little anhydrous ethyl acetate was added and this was removed by evaporation *in vacuo* in the cold. The sirupy residue was washed with cold, anhydrous petroleum ether and then dissolved in a small amount of absolute ethyl acetate, cooled in ice, and a solution of 6.6 g. (0.019 mole) of 1,3,4,6-tetraacetyl-β-D-glucosamine and 6 ml. of pyridine in 75 ml. of ethyl acetate was added. A gel formed instantly. The reaction mixture was allowed to stand for about one hour in the ice-bath and 2 days at room temperature. The mixture was then repeatedly extracted with *N* hydrochloric acid until the gel disappeared, twice with a 5% solution of potassium bicarbonate and twice with water. The ethyl acetate solution was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. The residue, on recrystallization from 75 ml. of hot absolute ethanol, yielded 1.3 g. of needles. After a second recrystallization from absolute ethanol the product melted at 216-217° and showed $[\alpha]^{24}D$ +2.3 (*c* 3, pyridine).

Anal. Calcd. for $C_{27}H_{36}O_{12}N_2S$: C, 52.91; H, 5.93. Found: C, 52.55; H, 6.07.

N-(Carbobenzoxy-D-methionyl)-1,3,4,6-tetraacetyl-β-D-glucosamine (IId).—This was prepared from 2.83 g. of D-methionine in a manner exactly like that used for the L-methionine derivative (IId); yield 1.6 g. After three recrystallizations from absolute ethanol the product melted at 183-184° and showed $[\alpha]^{24}D$ +19.6 (*c* 6, pyridine).

Anal. Calcd. for $C_{27}H_{36}O_{12}N_2S$: C, 52.91; H, 5.93. Found: C, 53.03; H, 5.99.

MADISON, WISCONSIN

(8) A. Bertho, F. Hölder, W. Meiser and R. Rüther, *Ann.*, **485**, 127 (1931).

(9) M. Bergmann, L. Zervas, H. Rinke and H. Schleich, *Z. physiol. Chem.*, **224**, 26 (1934).