arabinosamine hydrochloride that is readily distinguished from the hydrochlorides of ribosamine, xylosamine and lyxosamine.[16]

2-Acetamido-2-deoxy-β-D-arabinose (VI) from IV.—For the preparation of crystalline N-acetyl-β-D-arabinosamine (VI) 80.2 mg. of 3-acetamido-3-deoxy-D-glucose (IV) in 10 ml. of water (equilibrium solution) was oxidized with 77.5 mg. of sodium metaperiodate as described above. However, the formyl ester produced was not isolated but was subjected, instead, to immediate hydrolysis with sodium bicarbonate as described earlier[7] for the periodate degradation of 3-acetamido-3-deoxy-β-D-mannose including the working-up of the resulting pentose. With the alkaline conditions involved being very mild no epimerization of the pentose was observed.[18] The yield of crude VI with m.p. 157–159° was 52.5 mg. (76%). After one recrystallization from 95% ethanol–ethyl acetate (1:1) the m.p. was 161–162° and a mixed m.p. with authentic 2-acetamido-2-deoxy-β-D-arabinose[7,12a] was 160–162°. The identity of VI with N-acetyl-β-D-arabinosamine was further confirmed by the infrared spectra and through chromatography.[16]

(18) *Cf.* B. Coxon and L. Hough, *Chemistry & Industry*, 374 (1960).

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On β-D-Glucosylamides of L-Amino Acids and of Nicotinic Acid

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4,6-O-Benzylidene-D-glucose (I) is converted by methanolic ammonia into 4,6-O-benzylidene-D-glucosylamine (II). N-Acylation of II with the appropriate derivatives of L-amino acids and of nicotinic acid, followed by removal of the protective groups, yields the N-β-D-glucosylamides of L-phenylalanine (IVc), L-aspartic acid (IVd), L-glutamic acid (IVe) and of nicotinic acid (IVf).

Introduction

The increased interest in glycosylamines and N-acylated glycosylamines[3] has made desirable the development of general methods for their synthesis. These acyl derivatives, especially those derived from the L-amino acids, are of particular significance since it has been shown that N-ribosyl derivatives of glycinamide are intermediates in the biosynthesis of purines.[4]

Direct condensation of amides with sugars has not been accomplished, except in the special case of urea.[5] N-Acylglycosylamines have been isolated, in low yields, as by-products of the deacylation of fully O-acylated sugars with ammonia[6,7] or of the Wohl degradation of fully O-acylated aldonitriles.[7,8] The partial hydrolysis of the fully acylated glycosylamines has been used only in the preparation of N-acetylglycosylamines.[9–12] Similarly, selective N-monoacylation of free 1-amino sugars has been restricted to the N-monoacetyl compounds[9,11,13] and only recently a more general method, involving free glycosylamines as starting material, has been used[14] for the preparation of N-monoacetyl and N-monobenzoyl derivatives.

None of the above methods appears to be applicable to the synthesis of N-α-aminoacylglycosylamines and the only reported procedure[15] is a rather tedious one, involving the conversion of O-acylated glycosyl halides to O-acylated 1-amino sugars which are coupled with N-protected amino acids.

In the present work, 4,6-O-benzylidene-D-glucosylamine (II) is proposed as a new, readily available starting material for the general synthesis of N-acyl-D-glucosylamines and especially of N-(α-aminoacyl)-D-glucosylamines (IV). Compound II is obtained from 4,6-O-benzylidene-α-D-glucopyranose (I),[16] which represents a peculiar case of a sugar with an "acidic" glycosidic hydroxyl group capable of forming quite stable crystalline salts with alkali in aqueous solution.[16] In the same way, I dissolves immediately in ammonium hydroxide to form an ammonium salt, which according to findings of this Laboratory,[17] is slowly converted to the sparingly soluble 1-amino compound II. The yield is not high in this case, but the formation of the 1-amino sugar in aqueous ammonia is interesting, in view of the known sensitivity of the free 1-amino sugars to hydrolysis. The existence of a six-membered *m*-dioxane ring in I ap-

(1) This paper is based in part on the doctoral dissertation of Charalambos Coutsogeorgopoulos, Faculty of the Natural Sciences (Chemistry Section), University of Athens, Greece, 1958. Now at Department of Pharmacology, Yale University, School of Medicine, New Haven, Conn.

(2) This investigation was partly supported by the Royal Hellenic Research Foundation to which I am greatly indebted.

(3) G. P. Ellis and J. Honeyman, *Adv. in Carbohydrate Chem.*, **10**, 95 (1955); W. Pigman, K. Nisizawa and S. Tsuiki, *Ann. Rev. Biochem.*, **28**, 15 (1959).

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(7) R. C. Hockett and L. B. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).

(8) P. Brigl, H. Mühlschlegel and R. Schinle, *Ber.*, **64**, 2921 (1931); V. Deulofeu and J. O. Deferrari, *Nature*, **167**, 42 (1951); *J. Org. Chem.*, **17**, 1087 (1952).

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(11) R. Kuhn and G. Krüger, *Chem. Ber.*, **87**, 1544 (1954).

(12) H. S. Isbell and H. L. Frush, *J. Org. Chem.*, **23**, 1309 (1958).

(13) C. Niemann and J. T. Hays, *J. Am. Chem. Soc.*, **62**, 2960 (1940).

(14) K. Onodera and S. Kitaoka, *J. Org. Chem.*, **25**, 1322 (1960).

(15) J. Baddiley, J. G. Buchanan, R. Hodges and J. F. Prescott, *Proc. Chem. Soc.*, 148 (1957); J. Baddiley, J. G. Buchanan, R. E. Handschumacher and J. F. Prestcott, *J. Chem. Soc.*, 2818 (1956).

(16) L. Zervas, *Ber.*, **64**, 2289 (1931).

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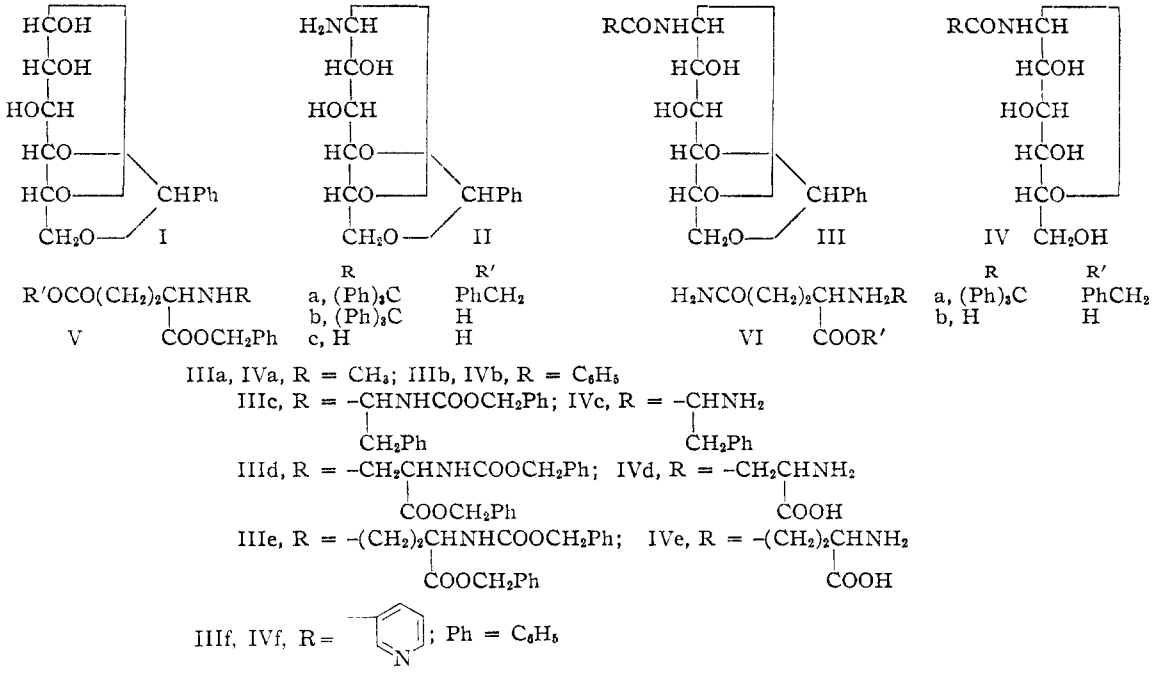

parently stabilizes the second six-membered, lactole, ring and renders the glycosidic hydroxyl "acidic." The stability of II in neutral or basic aqueous solutions probably must also be attributed to this *m*-dioxane ring.

A convenient method for the preparation of II, involving the reaction of I with methanolic ammonia at 60°, is described in the Experimental part. Compound II, dissolved in 10% acetic acid, was hydrolyzed to the starting material I after 10 min. at 25°. Similarly, reaction of II with phenylhydrazine, in the presence of acetic acid, resulted in either the phenylhydrazone or the osazone of I, depending on the quantity of phenylhydrazine used.[17] The N-acyl derivatives of II usually did not reduce Fehling solution, unless preheated with dilute acid. These facts indicate the structure of II as being that of an 1-amino sugar. The substance, probably a β-anomer, shows in pyridine solution "mutarotation" ($[\alpha]_D$ −67° → −58°) which may be attributed to an equilibrium involving partial conversion into its α-anomer. In the meantime, several N-alkyl and N-aryl derivatives of II had been prepared by the interaction of benzylideneglucose (I) and the corresponding amines.[18]

In this report, the above 4,6-O-benzylidene-D-glucosylamine(II) was coupled with compounds bearing a carboxylic function, mainly by the "mixed anhydride" method,[19] to give intermediates of the general structure III. These were subsequently freed from the benzylidene group by mild acid hydrolysis or by catalytic hydrogenolysis. In the case of the α-aminoacylglucosylamines, hydrogenolysis removed simultaneously any protective carbobenzoxy or benzyloxy groups on the amino acid moiety. By this method the β-glucosylamides[20] of several naturally occurring amino acids, *e.g.*, L-phenylalanine(IVc), L-aspartic acid (IVd) and L-glutamic acid (IVe), of nicotinic acid (IVf) and of benzoic acid (IVb) were synthesized. The amino acids used were coupled as the N-carbobenzoxy derivatives and in the case of the aminodicarboxylic acids as the N-carbobenzoxy-α-monobenzyl esters. The α-benzyl L-glutamate (Vc) used for the preparation of its carbobenzoxy derivative[21] was obtained in good yield through a series of reactions starting with dibenzyl L-glutamate. This ester was first tritylated to Va and then selectively saponified to the trityl α-monoester Vb in a manner analogous to a method developed in this Laboratory for the synthesis of N-trityl α-ethyl-L-glutamate.[22] Detritylation of Vb to α-benzyl L-glutamate (Vc) proved its structure to be that of an α-ester. Compound Vb constitutes a convenient starting material for the synthesis of α-peptides of glutamic acid. Additional evidence that Vb was an α-ester was obtained by its conversion to L-glutamine (VIb) through Nα-trityl benzyl-L-glutaminate (VIa).

In the case of the L-phenylalanine derivative IIIc, hydrogenolysis only partially removed the benzylidene group and its removal was completed by mild acid hydrolysis with 0.1 *N* hydrochloric acid. Similarly with the N-glucoside of nicotinamide IIIf, hydrolysis did not completely remove the benzylidene group; moreover the reduction of the pyridine ring was initiated as an undesirable side reaction. This difficulty was easily circumvented by removing the protective group in IIIf by mild acid hydrolysis. Under the conditions used, the acid did not hydrolyze the C–N bond. Neither type III nor IV of the synthesized N-

(18) F. Micheel and A. Frowein, *Chem. Ber.*, **90**, 1599 (1957); **92**, 304 (1959).

(19) R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951).

(20) All these N-glucosides are formulated as β-anomers, because N-acetylation of II followed by removal of the benzlidene group leads to the formation of the known N-acetyl-β-D-glucosylamine (IVa).[9,10,13,14]

(21) H. Sachs and E. Brand, *J. Am. Chem. Soc.*, **75**, 4610 (1953).

(22) L. Zervas and D. Theodoropoulos, *ibid.*, **78**, 1359 (1956).

glucosylamides reduced Fehling solution on heating; the only exception was the carbobenzoxy-L-asparagine derivative IIIc.[23] However, after removal of the protective groups the resulting glucosylasparagine IVc did not reduce Fehling solution.

Experimental

Dry solvents were used. Hydrogenations were carried out on suspensions of the compounds in 50% ethanol containing 1–2 ml. of acetic acid with palladium black as the catalyst; during the hydrogenation the compounds went into solution. Evaporations were carried out *in vacuo* at 40°. The melting points are not corrected. Prior to analysis, IVc was dried at 56°, Vb at 20° in high vacuum; other compounds were dried at 78°.

4,6-O-Benzylidene-β-D-glucosylamine (II).—4,6-O-Benzylidene-D-glucose[16] (20.0 g., 0.075 mole) was added to a chilled (ice–salt) solution of ammonia (35–40 g.) in methanol (120 ml), contained in a steel bomb. The bomb was closed, and with shaking the temperature was raised gradually and maintained at 60° for 3 hr. At the end of this period, the bomb was allowed to reach room temperature and then cooled to 0–5°. Upon careful removal of the excess of ammonia by means of a water-pump, compound II, which had already begun to crystallize, separated out in a mass. It was kept at 4° overnight, filtered off, washed with small portions of methanol and dried over calcium chloride and potassium hydroxide; yield 9.2 g. (46%),[24] m.p. 166–168° dec., unchanged on recrystallization from methanol or dioxane. The spec. rotation 8 minutes after dissolution was $[\alpha]^{18}D$ −67.2°; after 48 hours $[\alpha]D$ was −58° (*c* 2, pyridine).

Anal. Calcd. for $C_{13}H_{17}O_5N$: C, 58.4; H, 6.4; N, 5.2. Found: C, 58.3; H, 6.6; N, 5.4.

For the subsequent experiments unrecrystallized material was used.

N-Acetyl-4,6-O-benzylidene-β-D-glucosylamine (IIIa).—Acetic anhydride (0.5 ml.) was added dropwise, with shaking and cooling at 10°, to a suspension of II (1.35 g., 0.005 mole) in a mixture of dioxane (7 ml.) and pyridine (3 ml.). Shaking was continued for 45 minutes at room temperature. Upon addition of 50 ml. of water and concentration to a small volume, IIIa precipitated out; yield 1.25 g. (82%), m.p. 240° dec. After recrystallization from ethanol the m.p. was raised to 242–243° dec., $[\alpha]^{25}D$ −41.7° (*c* 2, pyridine).

Anal. Calcd. for $C_{15}H_{19}O_6N$: C, 58.2; H, 6.1; N, 4.5. Found: C, 58.3; H, 6.3; N, 4.4.

N-Acetyl-β-D-glucosylamine(IVa).—Compound IIIa (0.92 g., 0.003 mole) was catalytically hydrogenated and the filtrate was evaporated to dryness; the remainder was dissolved in water and evaporated again to dryness. The crystalline residue was dissolved in 2 ml. of hot water. Upon addition of methanol, IVa precipitated out; yield 0.5 g. (75%), m.p. 255° dec., $[\alpha]^{20}D$ −25° (*c* 2, water). The reported values are m.p. 257°,[9] 260°,[12] 255°,[13] 256°[14] and $[\alpha]D$ −22°,[9] −22.8°,[12] −22.4°,[13] −20°[14] (in water).

Anal. Calcd. for $C_8H_{15}O_6N$: C, 43.4; H, 6.8; N, 6.3. Found: C, 43.5; H, 6.8; N, 6.4.

N-Benzoyl-4,6-O-benzylidene-β-D-glucosylamine (IIIb) was prepared from II (1.35 g., 0.005 mole) and benzoyl chloride (0.62 ml.) in the same manner as IIIa. On addition of ice-water to the dioxane–pyridine solution, IIIb precipitated out. It was triturated with cold 3% sulfuric acid, filtered off and triturated again with potassium hydrogen carbonate solution; yield 0.8 g. (43%) after recrystallization from ethanol; m.p. 265–268° dec. (reported[17] m.p. 250°), $[\alpha]^{15}D$ −65.8° (*c* 2, pyridine).

Anal. Calcd. for $C_{20}H_{21}O_6N$: C, 64.7; H, 5.7; N, 3.8. Found: C, 64.6; H, 5.8; N, 3.7.

N-Benzoyl-β-D-glucosylamine (IVb).—The above substance IIIb (0.74 g., 0.002 mole) was catalytically hydrogenated. After evaporation of the filtrate the remainder was dissolved in water and evaporated again to dryness. The crystalline residue IVb was recrystallized from isopropyl alcohol; yield 0.50 g. (75%), m.p. 238–242° dec. (reported[17] m.p. 229°), $[\alpha]^{17}D$ −13.8° (*c* 2, water).

Anal. Calcd. for $C_{13}H_{17}O_6N$: C, 55.1; H, 6.1; N, 4.9. Found: C, 55.3; H, 6.1; N, 4.8.

N-(N'-Carbobenzoxy-L-phenylalanyl)-4,6-O-benzylidene-β-D-glucosylamine (IIIc).—Carbobenzoxy-L-phenylalanine[25] (3 g., 0.01 mole), triethylamine (1.4 ml.) and ethyl chloroformate (0.97 ml.) were allowed to react in dioxane (25 ml.) for 10 minutes at 10°. The mixed anhydride formed was added with shaking to a suspension of II (2.7 g., 0.01 mole) in dioxane (80 ml). Shaking was continued at room temperature for 4 hours, and the solvent was removed; the residue crystallized on the addition of water. The crude product was washed in the manner described for the preparation of IIIb and recrystallized from ethanol; yield 3.5 g. (60%), m.p. 245–247° dec., $[\alpha]^{20}D$ −25.6° (*c* 2, pyridine).

Anal. Calcd. for $C_{30}H_{32}O_8N_2$: C, 65.7; H, 5.9; N, 5.1. Found: C, 65.9; H, 6.0; N, 5.0.

N-(L-Phenylalanyl)-β-D-glucosylamine (IVc).—Compound IIIc (1.1 g., 0.002 mole) was catalytically hydrogenated until the evolution of carbon dioxide stopped. The solvent was evaporated to dryness. The evaporation was repeated after the addition of water. The white crystalline residue was dissolved in 20 ml. of 0.1 *N* hydrochloric acid and heated in a boiling water-bath for 30 minutes. The solution was then evaporated to dryness; absolute ethanol was added and the evaporation was repeated. Upon dissolving the residue in absolute ethanol (3 ml.), addition of triethylamine and cooling in the refrigerator, IVc precipitated out; it was collected by suction, washed with a minimal volume of cold absolute ethanol and dried over phosphorus pentoxide and potassium hydroxide. The substance melted at *ca.* 95°; after drying at 56° in high vacuum it sintered at 115–120° and melted not sharply at a higher temperature. Recrystallization from isopropyl alcohol did not change the m.p.; yield 0.5 g. (77%), $[\alpha]^{20}D$ −2.6° (*c* 9, 0.3 *N* hydrochloric acid).

Anal. Calcd. for $C_{15}H_{22}O_6N_2$: C, 55.2; H, 6.8; N, 8.6. Found: C, 54.9; H, 6.9; N, 8.6.

Benzyl Nα-Carbobenzoxy-Nγ-(4,6-O-benzylidene-β-D-glucosyl)-L-asparaginate (IIId).—N-Carbobenzoxy-α-benzyl-L-aspartate[26] (3.6 g., 0.01 mole), triethylamine (1.4 ml.) and ethyl chloroformate (0.97 ml.) reacted in dioxane (20 ml.) at 10° for 10 minutes. The coupling of the mixed anhydride thus formed with II (2.7 g., 0.01 mole) and the isolation of IIId was accomplished in the same manner as in the case of IIIc; yield 4.0 g. (66%), after recrystallization from ethanol, m.p. 200–203° (reported[17] m.p. 209°), $[\alpha]^{20}D$ −21.3° (*c* 2, pyridine).

Anal. Calcd. for $C_{32}H_{34}O_{10}N_2$: C, 63.3; H, 5.65; N, 4.6. Found: C, 63.5; H, 5.8; N, 4.5.

N-(L-β-Aspartyl)-β-D-glucosylamine (IVd).—After the catalytic hydrogenation of IIId (1.2 g., 0.002 mole) the filtrate was evaporated to dryness. Absolute ethanol was added and evaporated repeatedly. Crystallization of the residue was induced by dissolving in a small volume of absolute ethanol, cooling and scratching. After standing for 2–3 days in the refrigerator the crystals were filtered off and washed with a small volume of cold absolute ethanol. Recrystallization from ethanol–water (2.5:1) yielded 0.5 g. (86%), m.p. 253° dec., $[\alpha]^{18}D$ −16.5° (*c* 2, water).

Anal. Calcd. for $C_{10}H_{18}O_8N_2$: C, 40.8; H, 6.2; N, 9.5. Found: C, 40.8; H, 6.2; N, 9.6.

N-Trityl-dibenzyl-L-glutamate (Va).—Trityl chloride (5.6 g., 0.02 mole) was added with shaking and cooling (ice-water) to a solution of dibenzyl L-glutamate benzenesulfonate[27] (9.7 g., 0.02 mole) in chloroform (60 ml.) and triethylamine (6.0 ml.). The mixture was kept at room temperature overnight. Then it was washed with water, dried with sodium sulfate and evaporated to dryness. The residue, on heating with 30 ml. of methanol, went partially into solution. By cooling and scratching, crystal-

(23) This unusual behavior of IIIc may be explained assuming that under the action of alkaline solution on IIIc N-carbobenzoxy-L-aspartic imide is split off and a reducing sugar derivative is formed.

(24) Practically the same yield and m.p. of II were obtained whether the benzylidene-D-glucose used was recrystallized (m.p. 180°) or not.

(25) M. Bergmann, L. Zervas, H. Rinke and H. Schleich, *Z. physiol. Chem. Hoppe-Seyler's*, **224**, 33 (1934); W. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 462, (1958).

(26) M. Bergmann, L. Zervas and L. Salzmann, *Ber.*, **66**, 1288 (1933).

(27) B. Helferich, P. Schellenberg and J. Ullrich, *Chem. Ber.*, **90**, 700 (1957).

lization of Va was initiated. After 2 days at 4° the crystals were filtered off and washed with cold methanol; yield 10.3 g. (90%), m.p. 88–90° (reported[28] m.p. 87–89°).

N-Trityl-α-benzyl-L-glutamate (Vb).—To a solution of Va (11.4 g., 0.02 mole) in acetone (25 ml.), 4 *N* lithium hydroxide (5.5 ml.) was added and the precipitated lithium hydroxide dissolved by gentle heating. The hot solution was shaken continuously at room temperature until insoluble lithium hydroxide reappeared. Heating and shaking was repeated as described above until (*ca.* 4 hours) a homogeneous solution at room temperature resulted. The mixture was diluted tenfold with distilled water and repeatedly extracted with ether. The aqueous phase, after complete removal of the ether, was further diluted to 500 ml. with distilled water, cooled in ice and acidified with acetic acid (25 ml.). Compound Vb separated out as a white flocculent precipitate. After 24 hours at 4°, it was collected by suction, washed with distilled water and dried first in the air and then over calcium chloride; yield 8.5 g. (90%). The product which sintered at 58–60° and melted with decomposition at 105–115° was sufficiently pure for the subsequent reactions. For further purification, it was (8.5 g.) dissolved in methanol (15 ml.) and 30% sodium hydroxide (1.5 ml.). The solution was diluted with distilled water (150 ml.) and extracted twice with ether. The aqueous phase was acidified with acetic acid as previously; yield 7.8 g., m.p. the same as before the purification, $[\alpha]^{15}D$ +40.3° (*c* 2, ethanol).

Anal. Calcd. for $C_{31}H_{29}O_4N$: C, 77.6; H, 6.1; N, 2.9. Found: C, 77.3; H, 6.2; N, 2.8.

Nα-Trityl-benzyl-L-glutaminate (VIa).—Compound Vb (2.4 g., 0.005 mole), triethylamine (0.7 ml.) and ethyl chloroformate (0.48 ml.) were allowed to react in dioxane (10 ml.) at 10° for 5 minutes. To the mixed anhydride which formed, dioxane (30 ml.) saturated with ammonia at room temperature was added and after shaking for a while the dioxane was removed. Crystallization of the residue was initiated on addition of water. The supernatant aqueous layer was decanted and the semi-crystalline residue was taken up in chloroform (20–30 ml.). The chloroform solution was washed with water, dried with sodium sulfate and concentrated to dryness. The residual sirup was dissolved in hot ethanol (3 ml) and began to crystallize on cooling and scratching. After 2 days at 4° the crystals were filtered off, and washed with a minimal volume of cold ethanol; yield 2.0 g. (84%), m.p. 119–121°, unchanged on recrystallization from ethanol or isopropyl alcohol; $[\alpha]^{15}D$ + 49.7° (*c* 2, ethanol).

Anal. Calcd. for $C_{31}H_{30}O_3N_2$: C, 77.8; H, 6.3; N, 5.8. Found: C, 77.4; H, 6.3; N, 5.9.

L-Glutamine (VIb).—A solution of VIa (4.8 g., 0.01 mole) in methanol was hydrogenated (palladium black) until the uptake of hydrogen stopped. The mixture of the precipitated product with the catalyst was filtered off and triturated on the filter with hot water. The combined filtrates were evaporated to dryness. The residue was washed with acetone and dissolved in water; ethanol was added to incipient cloudiness and the glutamine was allowed to crystallize at 4° overnight. Filtration and washing with ethanol yielded 1.05 g. (70%), m.p. 184–185° dec.; mixed m.p. with an authentic sample of L-glutamine showed no depression; $[\alpha]^{15}D$ +7.6° (*c* 4, water).

α-Benzyl L-Glutamate (Vc).—A suspension of Vb (4.8 g., 0.01 mole) in acetic acid (5 ml.) and water (0.5 ml.) was heated to the boiling point. After cooling, acetone was added until the precipitated triphenylcarbinol was dissolved. On addition of ether Vc crystallized out. It was kept overnight at 4°, filtered off and washed thoroughly with ether; yield 1.95 g. (82%), m.p. 148–149° dec., unchanged on recrystallization from water; $[\alpha]^{15}D$ +12.0° (*c* 2.7, 0.1 *N* hydrochloric acid); reported[21] m.p. 147–148°, $[\alpha]^{20}D$ +12.2° (*c* 2.9, 0.1 *N* hydrochloric acid).

Benzyl N-Carbobenzoxy-N-(4,6-O-benzylidene-β-D-glucosyl)-L-glutaminate (IIIe).—N-Carbobenzoxy-α-benzyl-L-glutamate[21] (3.7 g., 0.01 mole), triethylamine (1.4 ml.) and ethyl chloroformate (0.96 ml.) reacted in dioxane (20 ml.) at 10° for 10 minutes. The mixed anhydride was coupled with II (2.7 g. in 50 ml. of dioxane) as described for IIIc. After the coupling reaction was completed, the triethylamine hydrochloride was filtered off and the filtrate was evaporated to dryness. Ethanol was added to the residue and the evaporation was repeated. The residue crystallized on addition of water; it was filtered and washed like IIIb. Recrystallization of the crude product from ethanol yielded 4.0 g. (64%) of IIIe, m.p. 192–193°, $[\alpha]^{16}D$ −11.0° (*c* 2, dioxane; dissolution occurred by gentle heating).

Anal. Calcd. for $C_{33}H_{36}O_{10}N_2$: C, 63.9; H, 5.8; N, 4.5. Found: C, 63.7; H, 5.9; N, 4.4.

N-(L-γ-Glutamyl)-β-D-glucosylamine (IVe).—Compound IIIe (1.25 g., 0.002 mole) was hydrogenated and the filtrate was evaporated to dryness. The crystalline residue was suspended in ethanol and filtered off; yield 0.65 g., m.p. 209–211°. Recrystallization from ethanol–water (3:1) yielded 0.6 g. (79% for tetrahydrate), m.p. 211°. The product contained 4 moles of water of crystallization. The anhydrous substance had the same m.p. as the tetrahydrate; $[\alpha]^{17}D$ −9.7° (*c* 2, for anhydrous, in water).

Anal. (anhydrous) Calcd. for $C_{11}H_{20}O_8N_2$:C, 42.85; H, 6.5; N, 9.1. Found: C, 42.7; H, 6.7; N, 9.1.

N-Nicotinoyl-4,6-O-benzylidene-β-D-glucosylamine (IIIf), a.—Freshly prepared nicotinoyl chloride hydrochloride (1.8 g., 0.01 mole) was added in four portions and in a period of 15 minutes to a suspension of II (2.7 g., 0.01 mole) in pyridine (25 ml.), with shaking and cooling. The mixture was shaken at room temperature for an additional 30 minutes until the chloride was completely dissolved, and then poured into ice-water (150 ml). The precipitate was filtered off, washed with water and air-dried; yield 2.4 g., m.p. 238–240° dec. After two recrystallizations from methanol, 1.2 g. (32%), m.p. 250–252° dec., was obtained, $[\alpha]^{15}D$ −60.0° (*c* 0.5, pyridine; dissolution occurred by mild heating).

b.—Triethylamine (1.4 ml.) was added to a suspension of dry nicotinic acid (1.2 g., 0.01 mole) in dioxane (20 ml.) and the resulting solution treated with ethyl chloroformate (0.95 ml.) for 10 minutes at 10°. The mixed anhydride was coupled with II (2.7 g. in 50 ml. of dioxane) as described for the preparation of compound IIIc. The product was isolated in the same manner, except that 10% acetic acid was used in place of 3% sulfuric acid for the extraction of the unreacted II; yield 1.5 g., m.p. 244–246° dec. Recrystallization from methanol yielded 1.4 g. (38%), m.p. 250–252° dec., $[\alpha]^{15}D$ −60.0° (*c* 0.5, pyridine).

Anal. Calcd. for $C_{19}H_{20}O_6N_2$: C, 61.2; H, 5.4; N, 7.5. Found: C, 61.1; H, 5.6; N, 7.4.

N-(Nicotinoyl)-β-D-glucosylamine (IVf).—A solution of IIIf (2.25 g., 0.006 mole) in 0.1 *N* hydrochloric acid (60 ml.) was heated in a boiling water-bath for 20 minutes and subsequently evaporated to dryness. Absolute ethanol was added repeatedly and evaporated to dryness. The residue was dissolved by gentle heating in a mixture of absolute ethanol (3 ml.) and triethylamine (0.8 ml). On cooling, IVf crystallized out. After filtration, the precipitate was washed with a small portion of absolute ethanol; yield 1.35 g. (82%), m.p. 234–236° dec. Recrystallization from ethanol–water (5:1) did not change the m.p.; $[\alpha]^{16}D$ −8.3° (*c* 3, water).

Anal. Calcd. for $C_{12}H_{16}O_6N_2$: C, 50.7; H, 5.7; N, 9.9. Found: C, 50.5; H, 5.8; N, 9.8.

(28) G. Amiard, J. Anatol, R. Heymes, V. Torelli and L. Velluz, *Bull. soc. chim., France*, 97 (1956).