SYNTHESIS AND STUDY OF THE PROPERTIES OF 6-O-(α-L-ASPARTYL)-D-GLUCOSE

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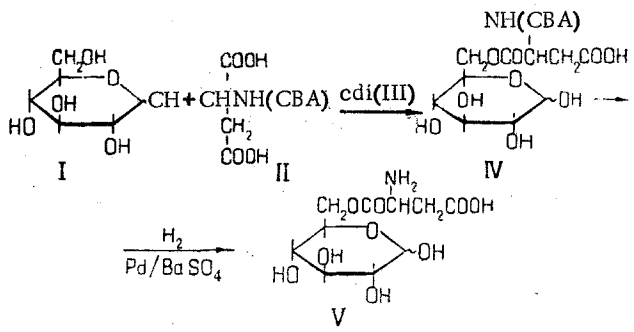
The synthesis of model glycopeptides with aspartic acid as the amino acid component and the study of their properties, particularly their stability under various conditions, are very important for the investigation of natural mixed biopolymers, since aspartic acid is the connecting link between the carbohydrate and the peptide components in many natural glycopeptides [1-4]. In continuation of our work on the synthesis of model glycopeptides, we have carried out the synthesis of 6-O-(α-L-aspartyl)-D-glucose.

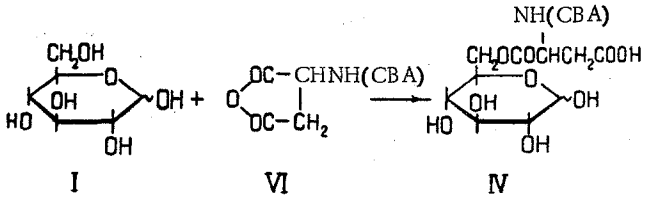
The possibility of obtaining this compound was first confirmed by the carbodiimide method [5]. The condensation of glucose (I) with N-carbobenzoxyaspartic (CBA) acid (II) was carried out in absolute pyridine in the presence of N,N'-dicyclohexylcarbodiimide (cdi) (III) by the method previously developed [5]:



When the reaction is carried out with a molar ratio (I-II-III) of (2:1:1), condensation mainly takes place between the primary hydroxyl group of the glucose and the α-carboxyl group of the aspartic acid (IV). The latter follows from the characteristic coloration of (V) with ninhydrin [6] and its mobility on electrophoresis [7]. Simultaneously, small amounts of the corresponding β-ester of aspartic acid and of the product of condensation at a secondary hydroxyl group of the glucose are formed. Attempts to free the main product (IV) from these by treating the mixture with benzaldehyde in the presence of $ZnCl_2$ [5] did not lead to the desired result. In view of this, the unpurified condensation product was subjected to hydrogenation in aqueous methanol in the presence of $Pd/BaSO_4$ by the method used previously [5].

The presence of a free carboxyl group in (IV) offered the possibility of carrying out hydrogenation without the addition of acids and of isolating the 6-O-(α-L-aspartyl)-D-glucose (V) in the free state. It was impossible to purify (V) from contamination with the β-ester and the products of condensation at a secondary hydroxyl group completely by reprecipitation with acetone. The substance was isolated in the analytically pure state only by the method of preparative paper electrophoresis. The use of this fairly laborious method considerably complicates the preparative production of (V).

To simplify the synthesis of (V) we have developed a new method of aminoacylating a primary hydroxyl group of a monosaccharide by means of N-carbobenzoxyaspartic (CBA) anhydride (VI), which permits (IV) to be obtained in high yield:



The condensation with glucose was carried out in dry pyridine for 24 hr at room temperature, a two-fold excess of glucose being used in the reaction. It was found that under these conditions a high yield (about 60%) of 6-O-(N-CBA-α-L-aspartyl)-D-glucose (IV), only very slightly contaminated with the β-ester and other condensation products, is formed. These impurities are so small in amount that even simple reprecipitation of the reaction products with acetone after removal of the CBA protection led to the pure aspartylglucose (V), completely identical chromatographically and electrophoretically with the substance obtained by the carbodiimide method.

The structure of the samples of (V) was shown by periodate oxidation [8]. The amount of HIO_4 consumed was 4 moles, which is possible only in the case of 6-O-(α-L-aspartyl)-D-glucose. The condensation of monosaccharides with CBA-aspartic anhydride (VI) in pyridine is a convenient general method for the preparation of derivatives of monosac-

charides acylated by the α-carboxyl of aspartic acid. Preliminary experiments on the condensation of (VI) with 1, 2;5, 6-diisopropylidene-D-glucofuranose in dry pyridine showed that in this case the smooth introduction of the residue of (II) at the free hydroxyl group takes place.

TABLE

Decomposition of (V) and (IV) on hydrolysis for a day, %

Substance	pH								
	0.7	1.2	2.2	3	4	5	6	7	8
6-O-(α-L-aspartyl)-D-glucose (V)	17	8.5	10.8	14	17	19	49.5	Half-decomposition period 3-5 hours	
6-O-(N-CBA-α-L-aspartyl)-D-glucose (IV)	23.5	—	10.3	—	5.5	—	13.5	—	22.5

We have also studied the stability of the aspartyl derivatives produced, both those with a protected (IV) and those with a free (V) amino group, to hydrolysis and to hydroxylaminolysis. Hydrolysis was studied at pH 0.7-8 and 37°. The percentage decomposition of the ester linkage after a day was taken as a measure of stability, this being determined by the hydroxamic reaction with ferric chloride by the method described by Derevitskaya et al [9].

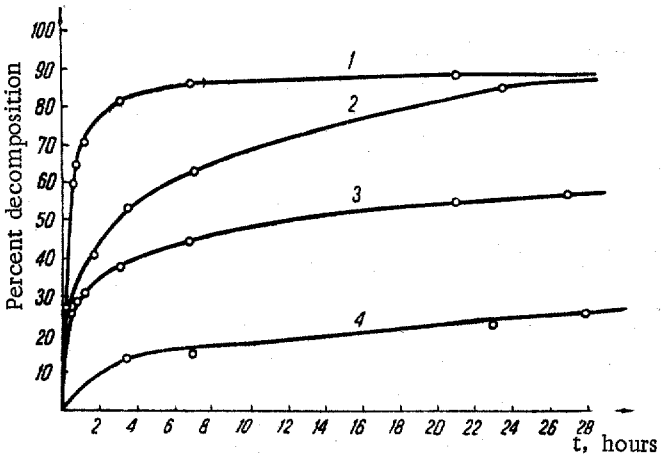


Fig. 1. Kinetic curves of the hydrolysis and hydroxylaminolysis of 6-O-(α-L-aspartyl)-D-glucose and 6-O-(N-CBA-α-L-aspartyl)-D-glucose: 1) Hydroxylaminolysis of 6-O-(α-L-aspartyl)-glucose; 2) Hydrolysis of 6-O-(α-L-aspartyl)-glucose; 3) Hydroxylaminolysis of 6-O-(N-CBA-α-L-aspartyl)-glucose; 4) Hydrolysis of 6-O-(N-CBA-α-L-aspartyl)-glucose.

As the results of hydrolysis (Table) showed, 6-O-(α-L-aspartyl)-D-glucose is a more stable compound than the derivatives of monobasic amino acids studied previously [10]. While for 6-O-glycylglucose the period of half-decomposition at pH 6 is 8 hr, for 6-O-(α-L-aspartyl)-D-glucose it is 24 hr, which is apparently due to the interaction of the free carboxyl group of the aspartic acid with the amino group and, in turn, to the weakening of the labilizing influence of the amino group on the stability of the ester bond. The optimum pH for stability is displaced to pH ~1. As in the cases reported previously [10], the presence of CBA protection markedly increases the stability of the ester bond.

We may mention that when a free carboxyl group is present the introduction of CBA protection into the amino group does not lead to such a marked increase in stability of the ester linkage as, for example, in the case of glycylglucose, for which the half-decomposition period is increased by a factor of approximately 40. The difference in the rates of hydrolysis for aspartylglucose is only 3.5, which confirms the lower labilizing action of the amino group as a consequence of the presence of a free carboxyl group. The optimum pH for stability is found, as for glycylglucose, at pH 4.

The hydroxylaminolysis of 6-O-(α-L-aspartyl)-D-glucose (V) and the CBA derivatives (IV) was carried out by the method described previously [9] with a 0.5 M solution of hydroxylamine at 21° and pH 8.0. A comparison of the results of hydrolysis and hydroxylaminolysis shows that at the same pH's and temperatures the rates of hydroxylaminolysis of V are considerably greater than of (IV) (Fig. 1). This confirms the stabilizing action of the CBA protection in this case also.

EXPERIMENTAL

The chromatography and electrophoresis were carried out on type 'M' paper of the Leningrad No. 2 mill. The chromatograms were descending. The following systems of solvents were used for chromatography:

1) CH_3COCH_3—n-C_4H_9OH—H_2O—CH_3COOH (10:2:1:0.1);

2) n-C_4H_9OH—H_2O—CH_3COOH (4:1:1);

3) CH_3COCH_3—s-C_4H_9OH—CH_3COOH—H_2O (3:3:1.5:2.5).

The electrophoresis was carried out on an EFA-1 instrument at 900 v in buffer solutions consisting of pyridine (2 ml), CH_3COOH (40 ml), H_2O (to 1 liter), pH 4.3-4.2 (No. 1); and pyridine (0.5 ml), CH_3COOH (30 ml), H_2O (to 1 liter), pH 2.8-2.9 (No. 2). The spots were revealed by means of silver nitrate and ninhydrin.

6-O-(N-carbobenzoxy-α-L-aspartyl)-D-glucose (IV). A. Carbodiimide method. To a solution of 3.6 g (0.02 M) of anhydrous glucose in 70 ml of dry pyridine were added 2.68 g (0.01 M) of N-carbobenzoxy-L-aspartic acid (II) and 25 g (0.012 M) of cdi (III). The reaction mixture was left at 5° for 48 hr. The N,N'-dicyclohexylurea was filtered off, the pyridine was distilled off in vacuum (10-15 mm), the residue was treated with a mixture of water and ether (1:1), the ether was separated off, and the aqueous layer was extracted five times with ether. A few drops of acetic acid were added to the aqueous solution and it was extracted six times with butan-1-ol. The butanolic extracts were washed six times with small portions of water, the butanol was distilled off in vacuum, and the residue (1.38 g) was dried. An additional amount of condensation products was isolated from the aqueous fraction by means of partition chromatography on cellulose in system No. 1. The total yield of (IV) was 1.98 g [44%, calculated on (II)].

B. The anhydride method. In the course of three hr, 1.25 g (0.005 mole) of N-carbobenzoxyaspartic anhydride (VI) [11] was added in portions to 1.8 g (0.01 mole) of I dissolved in 40 ml of dry pyridine, and the mixture was left for 24 hr at room temperature. The pyridine was distilled off in vacuum (10-15 mm), and the residue was dissolved in water and extracted with ether six times. A few drops of acetic acid was added to the aqueous layer, and it was extracted six times with butan-1-ol. The combined extract was washed 5-6 times with water and evaporated, and the residue was dried. Yield of (IV) 1.4 g 64% calculated on (VI)], $[\alpha]_D^{20}$ + 23.5° (c 0.68; CH_3OH).

Found %: C 51.52; 51.36; H 5.74; 5.95; N 3.43; 3.40. $C_{18}H_{23}O_{10}N \cdot O \cdot 5H_2O$. Calculated %: C 51.18; H 5.73; N 3.32.

R_f 0.56 (system 2). On electrophoresis with a potential gradient of 25.5 v/cm in buffer solution No. 1, (IV) migrates to the anode at the rate of 3.5 cm in 1.5 hr.

6-O-(α-L-aspartyl)-D-glucose (V). At room temperature with cautious stirring, 0.4 g of 6-O-(N-carbobenzoxy-α-L-aspartyl)-glucose (IV) dissolved in the minimum amount of 50% methanol was hydrogenated for 2 hr in the presence of 0.2 g of 5% $Pd/BaSO_4$. The catalyst was separated by centrifuging and the solution was evaporated in vacuum at 20°. The residue was dissolved in the minimum amount of water and the (V) was thrice reprecipitated with acetone. The substance was obtained in the form of an amorphous powder. Yield 0.16 g 60%, calculated on (IV)]. $[\alpha]_D^{20}$ + 32° (c 0.39; water). Literature data [12]: $[\alpha]_D^{20}$ + 33.5° (c 1.5; water).

Found %: C 41.10; 40.90; H 5.90; 5.86. $C_{10}H_{17}O_9N$. Calculated %: C 40.68; H 5.80.

R_f 0.37 (system 3). On electrophoresis in buffer solution No. 2 with a potential gradient of 40 v/cm, (V) migrates to the cathode at the rate of 5.2 cm in 45 minutes.

Hydrolysis of 6-O-(N-carbobenzoxy-α-L-aspartyl)-D-glucose (IV) and 6-O-(α-L-aspartyl)-D-glucose (V). One-milliliter portions of 0.01 M solutions of the substances in buffers with pH 0.7, 2.2, 4, 6, and 8 were taken. They were incubated for a day at 37°.

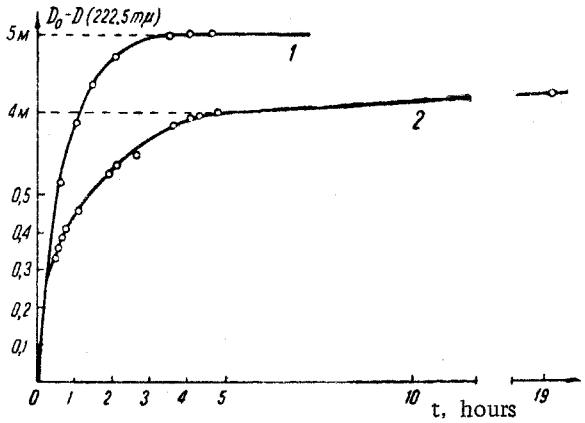


Fig. 2. Periodate oxidation of glucose (1) and 6-O-(α-L-aspartyl)-D-glucose (2).

0.2-ml samples were added to 1-ml portions of a 2 M solution of NH_2OH with pH 12.0 [for (IV)]or pH 10.6 [for (V)] and the mixtures were left for 10 min at room temperature, after which 1-ml portions of 2 HCl were added and the mixtures were shaken and added to 2-ml portions of a 15% solution of ferric chloride in 0.25 N HCl. The mixtures were subjected to colorimetry, and the percentage decomposition after a day was determined by means of a calibration curve. To study the stability for pH 8 at 20°, 2 ml of 0.01 M solution was used. The percentage decomposition was estimated after predetermined intervals of time.

Hydroxylaminolysis of 6-O-(N-carbobenzoxy-α-L-aspartyl)-D-glucose (IV) and 6-O-(α-L-aspartyl)-D-glucose (V). Two milliliters of a 0.01 M solution of the substance in 0.5 M NH_2OH with pH 8.0 was incubated at 21°. After predetermined intervals of time, 0.2-ml samples were taken, and each was treated with 0.2 ml of 0.5 N HCl, diluted with 1.8 ml of water, mixed with 2 ml of a 15% solution of ferric chloride in 0.25 N HCl, and subjected to colorimetry, and the kinetic hydroxylaminolysis curve was constructed from a calibration curve.

Periodate oxidation of 6-O-(α-L-aspartyl)-D-glucose. To 0.21 ml of an aqueous solution containing $1.176 \cdot 10^{-4}$ g of 6-O-(α-aspartyl)-D-glucose was added 0.12 ml of a 0.5% solution of sodium metaperiodate in water, and the time was reckoned from the moment of mixing. A quartz cell 0.1 mm thick was charged with 0.2 ml of the resulting solution and the optical density at 222.5 mμ was measured on a SF-4 instrument after predetermined intervals of time. An aqueous solution of 6-O-(α-aspartyl)-D-glucose of the same concentration was used as control. D-Glucose was oxidized in parallel under similar conditions, serving as a standard substance. The complete oxidation of 1 mole of 6-O-(α-aspartyl)-D-glu-

cose consumed 4.08 moles of sodium metaperiodate (Fig. 2). Under the conditions of periodate oxidation, practically no hydrolysis of the ester linkage takes place, as was shown by paper electrophoresis of the oxidation product.

SUMMARY

1. The carbodiimide method has been used for the first time for the synthesis of aminoacyl derivatives of glucose with aspartic acid.

2. A new method of synthesizing 6-O-(N-CBA-α-L-aspartyl) derivatives of sugars at a primary hydroxyl group by condensing N-carbobenzoxy-L-aspartic anhydride with the unprotected monosaccharide in pyridine has been developed.

3. The stability of 6-O-(N-carbobenzoxy-α-L-aspartyl)-D-glucose and 6-O-(α-L-aspartyl)-D-glucose to hydrolysis at various pH's and to hydroxylaminolysis at pH 8 has been studied.

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