substituted ring was not determined.[3,14] The n.m.r. spectrum of this product indicates three kinds of cyclopentadienyl ring protons by the presence of three frequencies at 4.83, 5.07, and 5.23 τ with intensities of 2.0:1.0:5.0. The lines represent a doublet, a triplet, and a singlet, respectively, with J = 2.5 c.p.s. The frequency of the doublet matches closely the value for the α-protons in benzoylruthenocene (4.82 τ), and the frequency of the triplet is close to that of the β-protons in benzoyl- (5.16 τ) and 1,1'-dibenzoylruthenocene (5.12 τ). A 1,2-dibenzoyl structure is thus indicated for the isomer with m.p. 141.8–142.4°. For 1,3-dibenzoylruthenocene, a low field triplet and a higher field doublet near 4.76–4.83 τ with an intensity ratio of 1:2 may be anticipated, in addition to a frequency for the unsubstituted ring hydrogens at about 5.2 τ.[15]

The identification of the homoannular dibenzoylruthenocene as the 1,2-isomer lends some support to the prediction, based on molecular orbital calculations,[16,17] that in electrophilic substitution reactions of ferrocene derivatives bearing electron-withdrawing groups, the 2-position is somewhat favored over the 3-position. By contrast, the descriptive resonance treatment indicates the 3-position as the preferential site of attack. The identification of homoannular diacetylferrocene as the 1,2-isomer[6,16] is thus paralleled by the isolation and identification of 1,2-dibenzoylruthenocene, and both support the molecular orbital prediction concerning site reactivities in monoacylmetallocene derivatives. The support for this interpretation is rendered even stronger when one considers the increased bulkiness of the benzoyl substituent.

The occurrence of well resolved and well separated lines in the n.m.r. spectra of various metallocene derivatives designate these compounds as suitable substrates for the study of substituent effects on the ring protons. The simplicity of the spectra allows the study of substituents and the assignment of modes in cases where the analogous operations in benzenoid systems are rendered difficult due to the multiplicity of lines resulting from long-range interactions.

Experimental

1,1'-Diacetylosmocene was isolated from the reaction of osmocene with a large molar excess of acetic anhydride and phosphoric acid. The general procedure of Hill and Richards was used,[18] except that the reaction was made on a fourfold larger scale. Chromatography of the reaction product from such a run, using benzene and benzene–ethyl ether mixtures on alumina, produced 0.050 g. of osmocene, m.p. 227–228°, 1.64 g. of acetylosmocene (91%), m.p. 130.5–131.5° (lit.[18] m.p. 129.5–130°), and 2.0 mg. of a yellow crystalline solid. The latter was recrystallized from *n*-heptane to produce 1.6 mg. (0.1%) of 1,1'-diacetylosmocene, m.p. 148–152°. The n.m.r. spectrum of this product in deuteriochloroform solution is consistent with the proposed formulation (see Table I).

Homoannular dibenzoylruthenocene was generously supplied by Prof. W. E. McEwen. All other metallocenes used in this study were analytically pure samples and were prepared by methods reported in the literature.[12,18]

N.m.r. spectra were determined on a Varian Model A-60 spectrometer as 10% (weight to volume) solutions in deuteriochloroform. In several comparative spectra in which the sample concentrations were varied from 5 to 10%, no effect due to dilution could be detected.

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The Synthesis of a Glucosamine–Asparagine Compound. Benzyl N^2-Carbobenzyloxy-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginate[1]

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In the course of the study of the link between the carbohydrate and protein parts of egg albumin, a glycosylamine type of linkage has been proposed by Johansen, Marshall, and Neuberger[3] and by Nuenke and Cunningham.[4] The carbon at position 1 of 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine) would be linked to the amide group of asparagine. Such a structure also has been suggested by other investigators[5] for egg albumin and seems also to agree with the experimental data obtained in the study of γ-globulins.[6,7] It recently has been proposed for the linkage of the carbohydrate part to the protein part of the α_1-acid glycoprotein of human plasma.[8,9]

As a part of the study carried out on the structure of the carbohydrate component of the α_1-acid glycoprotein of human plasma,[10] the synthesis of a glucosamine–asparagine compound, possessing the above-proposed structure, namely benzyl N^2-carbobenzyloxy-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-asparaginate (VIII), was undertaken.

A first approach was an attempt to condense L-aspartic acid with a benzylidene derivative of D-glucosamine: a β-D-glucosylamide compound of D-glucose and L-aspartic acid has been first obtained by

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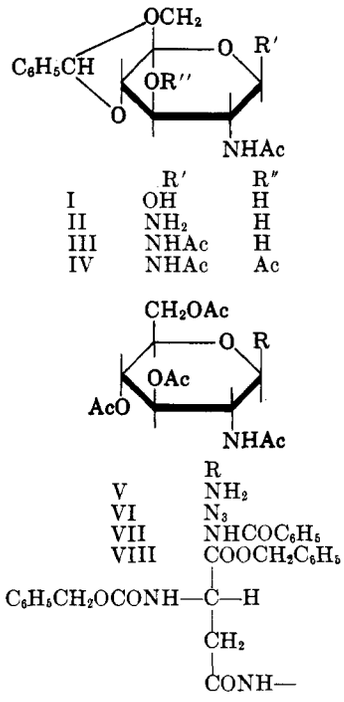

Coutsogeorgopoulos and Zervas,[11] who condensed 4,6-*O*-benzylidene-α-D-glucopyranose with ammonia to give 4,6-*O*-benzylidene-β-D-glucosylamine, which was in turn condensed with 1-benzyl-*N*-carbobenzyloxy-L-aspartate. In the present investigation, 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose (I) was condensed in the same way, but for a prolonged period of time, with ammonia in dry methanol. No crystalline glycosylamine II could, however, be isolated, but the presence of II could be ascertained, since *N*-acetylation and total acetylation afforded in low yields crystalline 1,2-diacetamido-4,6-*O*-benzylidene-1,2-dideoxy-β-D-glucopyranose (III) and 1,2-diacetamido-3-*O*-acetyl-4,6-*O*-benzylidene-1,2-dideoxy-β-D-glucopyranose (IV), respectively. No crystalline material could be isolated from the condensation of impure II with 1-benzyl-*N*-carbobenzyloxy-L-aspartate.

In order to condense aspartic acid with a pure glycosylamine, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (V) was prepared. The intermediate azide VI had been previously obtained by Micheel and Wulff[12] by acetylation of the 2-amino-2-deoxy-β-D-glucopyranosyl azide synthesized by Bertho and Révész.[13] In the present investigation, a shorter route was used in which 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide, prepared according to Inouye, *et al.*,[14] was condensed directly with silver azide according to the method of Bertho and Révész.[13] Reduction of VI gave the crystalline glycosylamine V, which was further characterized by preparation of the 1-benzamido derivative VII.

Condensation of V with 1-benzyl-*N*-carbobenzyloxy-L-aspartate in the presence of dicyclohexylcarbodiimide[15] or *N*-ethyl-5-phenylisoxazolium-3′-sulfonate[16] gave only very small amounts of a homogeneous crystalline product. Thus the use of another derivative was considered and 1-benzyl-*N*-carbobenzyloxy-L-aspartyl chloride[17] was condensed with V, giving VIII in a 28% yield. The use of an acyl chloride for the preparation of an *N*-acylglycosylamine has been previously reported by Baddiley, *et al.*[18] After this work had been completed, the condensation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine with 1-benzyl-*N*-carbobenzyloxy-L-aspartate by Marks and Neuberger[19] came to our attention, and we were informed by Dr. Neuberger of the synthesis of VIII using the carbodiimide method. Since the removal of the *O*-acetyl groups of VIII[20] may cause partial migration of the glycosylamine residue from position 4 to position 1 of aspartic acid, no attempt was made to prepare the de-*O*-acetylated derivative of compound VIII.

Experimental

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro- or micro- (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph Photoelectric Polarimeter Attachment, Model 200; the chloroform used was A.R. grade and contained approximately 0.75% of ethanol. Chromatograms were made with the flowing method on "Silica Gel Davison," from the Davison Co., Baltimore 3, Md. (grade 950; 60–200 mesh), which was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was obtained by heating to 170–200° (manufacturer's instructions). The sequence of eluants was hexane, benzene or chloroform, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. The proportion of weight of substance to be adsorbed to weight of adsorbent was 1 to 50–100. The proportion of weight of substance in grams to volume of fraction of eluent in milliliters was 1 to 100. The ratio of diameter to length of the column was 1 to 20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zürich, Switzerland.

2-Acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranosylamine (II).—A solution of 0.24 g. of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucopyranose (I), prepared according to the method of Roth and Pigman,[21] in dry methanol (30 ml.) was saturated at 0° with ammonia gas, and heated in a sealed tube at 60–70° for 4 days. After cooling, the excess of ammonia was removed from the solution with nitrogen. The yellow solution was decolorized by filtration through a pad of Darco G-60 and Celite, and concentrated to dryness, yielding 0.25 g. of sirup. This material could not be crystallized, and attempts to purify it by silica gel chromatography were unsuccessful. It was, therefore, used without further purification. When the reaction was attempted for a shorter time, most of the starting material was recovered unchanged.

1,2-Diacetamido-4,6-*O*-benzylidene-1,2-dideoxy-β-D-glucopyranose (III).—A solution of 0.2 g. of II in 2 ml. of methanol containing 0.2 ml. of acetic anhydride was allowed to stand at room temperature for 24 hr. The product crystallized from the solution yielding 0.04 g. of needles. It was recrystallized from a mixture of dimethyl sulfoxide and methanol, $[\alpha]^{26}D$ +16° (*c* 1.07, in dimethyl sulfoxide). It did not melt below 385° though some decomposition took place above 300°.

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Anal. Calcd. for $C_{17}H_{22}N_2O_6$: C, 58.28; H, 6.28; N, 8.00. Found: C, 58.17; H, 6.36; N, 8.11.

1,2-Diacetamido-3-*O*-acetyl-4,6-*O*-benzylidene-1,2-dideoxy-β-D-glucopyranose (IV).—A solution of 0.12 g. of II in a mixture of 0.6 ml. of pyridine and 0.2 ml. of acetic anhydride was allowed to stand for 24 hr. at room temperature. Water (2 drops) was added, the mixture was allowed to stand a further 5 min., and it was then poured into ice–water (70 ml.). The precipitate was filtered, washed with much water, and dissolved in chloroform. The solution, after drying, was treated with Darco G-60 and Celite and concentrated. The product (0.027 g.) crystallized into needles, and was recrystallized from a mixture of methanol and chloroform. The sample did not melt below 365°, though some decomposition took place above 300°.

Anal. Calcd. for $C_{19}H_{24}N_2O_7$: C, 58.15; H, 6.17; N, 7.14. Found: C, 58.06; H, 6.23; N, 7.40.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl Azide (VI).—A freshly prepared, dried solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl bromide, prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose according to Inouye, *et al.*,[14] (*ca.* 1 g.), in 20 ml. of chloroform was added to a suspension of silver azide in chloroform. The suspension had been prepared by mixing aqueous solutions of sodium azide (0.45 g.) and silver nitrate (1.1 g.) and washing the precipitate by decantation with water, ethanol, ether, and chloroform. The mixture was refluxed for 30 min., then it was filtered and the filtrate concentrated to dryness. The residue crystallized readily, and recrystallization from a mixture of chloroform and ether afforded 0.62 g. (68%) of needles with the same properties as those described by Micheel and Wulff.[12]

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine (V).—A solution of 2.87 g. of azide VI in 25 ml. of ethanol was hydrogenated at room temperature and atmospheric pressure for 4 hr. in the presence of 0.28 g. of Adams' platinum oxide catalyst. After removal of the catalyst by filtration through a Darco G-60 Celite pad, the filtrate was concentrated to dryness. The residue was crystallized from a mixture of ethyl acetate and pentane, yielding 0.8 g. (30%) of needles, m.p. 225–230° dec.; $[\alpha]^{28}D$ −5.2° (*c* 1.27, in chloroform).

Anal. Calcd. for $C_{14}H_{22}N_2O_8$: C, 48.55; H, 6.40; N, 8.09. Found: C, 48.52; H, 6.51; N, 7.97.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-benzamido-1,2-dideoxy-β-D-glucopyranose (VII).—A solution of 0.5 g. of V in 3 ml. of pyridine containing 0.3 ml. of benzoyl chloride was allowed to stand at room temperature for 3 days. After the addition of 1 drop of water, the mixture was left for a short time, and then it was poured into ice–water (75 ml.) and extracted with chloroform. The extract was washed with cold dilute hydrochloric acid, then with aqueous cadmium chloride, aqueous sodium bicarbonate, and water. The solution was dried over sodium sulfate and was concentrated to dryness. The residue was crystallized from a mixture of ethyl acetate and pentane yielding 0.173 g. (27%), m.p. 250.5–251.5°; $[\alpha]^{26}D$ −14° (*c* 1.10, in chloroform).

Anal. Calcd. for $C_{21}H_{26}N_2O_9$: C, 55.99; H, 5.82; N, 6.22. Found: C, 55.98; H, 5.89; N, 6.36.

Benzyl N^2-Carbobenzyloxy-*N*-(2-acetamido-3,4,6-tri-*O*-acetyl 2-deoxy-β-D-glucopyranosyl)-L-asparaginate (VIII).—1-Benzyl-*N* carbobenzyloxy-L-aspartyl chloride was prepared according to the method of Bergmann, *et al.*,[17] after purification of 1-benzyl *N*-carbobenzyloxy-L-aspartate according to LeQuesne and Young.[19,22]

The glucosylamine V (0.57 g.) was added to a solution of 0.76 g. of the acid chloride in 5 ml. of dry pyridine and the mixture was allowed to stand at room temperature for 3 days. The mixture was diluted with chloroform, and the resulting solution was washed with cold *N* sulfuric acid and water, then dried over sodium sulfate, and concentrated to dryness. The residue, dissolved in benzene, was purified by chromatography on silica gel. Elution with a mixture of ether and ethyl acetate (1:1) afforded VIII as a colorless sirup (0.32 g., 28.5%), which crystallized in fine needles from a mixture of chloroform and ether, m.p. 214–217° dec.; $[\alpha]^{25}D$ +28° (*c* 1.41, in chloroform).

Anal. Calcd. for $C_{33}H_{39}N_3O_{13}$: C, 57.80; H, 5.73; N, 6.13. Found: C, 57.89; H, 5.87; N, 6.19.

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A Convenient Preparation of 1,2-Mono-*O*-isopropylidene-α-D-glucofuranose[1]

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In connection with a series of reactions to introduce new heteroatoms into the D-glucose ring, a convenient method was developed for the preparation of monoacetone D-glucose, 1,2-mono-*O*-isopropylidene-α-D-glucofuranose.

Monoacetone D-glucose is a useful compound for the preparation of numerous D-glucose derivatives. It is usually prepared from diacetone D-glucose, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, by preferential hydrolysis of the more acid labile 5,6-isopropylidene group. Previous methods[2-8] have been rather long and require pH control, neutralization, filtration, and evaporation of large quantities of solvent before the first crop of crude crystals are obtained. The present method is shorter and avoids some of the manipulations required in other procedures.

The 5,6-isopropylidene group of diacetone D-glucose is hydrolyzed in 77% aqueous acetic acid and the solution completely evaporated to produce a quantitative yield of monoacetone D-glucose, free of D-glucose and diacetone D-glucose. It is suitable for direct use in many sugar reactions but may be purified by one crystallization from ethyl acetate. Isolation of almost pure crystalline monoacetone D-glucose from the hydrolysis mixture is attributed to its insolubility in 77% aqueous acetic acid. Scale-up of the preparation from 5 g. to 500 g. can be done without reduction in yield or loss of purity.

Experimental

Purity of monoacetone D-glucose preparations was determined by thin layer chromatography on 1 × 3 in. silica gel G-coated[9] microscope slides, irrigated with ethyl acetate and chloroform. Plates were sprayed with a dilute solution of sulfuric acid in ethanol and charred at 100° until permanent spots appeared. Further chromatographic identification of the components was performed on Whatman No. 1 filter paper at 25° with irrigants (A) ethyl acetate–pyridine–water (10:4:3 v./v.) and (B) 1-butanol–ethanol–water (40:11:19 v./v.). The spray indicator was (C) permanganate-periodate.

Preparation of 1,2-Mono-*O*-isopropylidene-α-D-glucofuranose.—Diacetone D-glucose (5 g.) was dissolved at 25° in a solution containing 10 ml. of acetic acid and 3 ml. of water. This solution was poured into a shallow evaporating dish and allowed to evaporate slowly in a hood at 25°. Within a few hours the entire mixture crystallized as a mass of crystalline monoacetone D-glucose. This was broken up with a spatula and recrystallized or allowed to air dry. Thin layer chromatography revealed no contamination from either the starting material or from D-glucose.

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