OPTICAL ACTIVITY AND CONFORMATION OF CARBOHYDRATES

PART III. PREPARATION AND OPTICAL ACTIVITY OF METHYL 2-ACETAMIDO-2-DEOXY-α- AND β-D-MANNOPYRANOSIDES AND THE CORRESPONDING FURANOSIDES

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ABSTRACT

The methyl 2-acetamido-2-deoxy-α- and β-D-mannopyranosides and their corresponding furanosides have been prepared by treatment of 2-acetamido-2-deoxy-D-mannose with methanol in the presence of Amberlite IR-120 (H^+) and separation on Dowex-1 ion-exchange resin and by paper chromatography. The optical rotatory dispersion and circular dichroism spectra of the four glycosides have been measured and compared with previous findings on the α and β anomers of methyl 2-acetamido-2-deoxy-D-glucopyranoside and -galactopyranoside. Differences were interpreted in terms of symmetry rules of optical activity and the axial orientation of the 2-acetamido-2-deoxy substituent in the D-mannose derivatives.

INTRODUCTION

In previous papers, it has been shown that o.r.d. and c.d. measurements are of significant value in elucidation of structure and sequence of oligo- and poly-saccharides[1–5]. Among substances investigated by these techniques in our laboratories have been simple *N*-acetylated amino sugars, milk oligosaccharides containing amino sugars and their derivatives, oligosaccharides derived from blood-group A, B, H, and Le^a substances, the blood-group substances themselves, *N*-acetylneuraminic acid, *N*-acetylneuraminyllactose, colominic acid, and several kinds of teichoic acid. In these studies, attention was focussed on the optical activity of the 2-acetamido group, a chromophore present in all the compounds mentioned. In particular, for oligo- and poly-saccharides containing 2-acetamido-2-deoxy-D-glucosyl residues, it

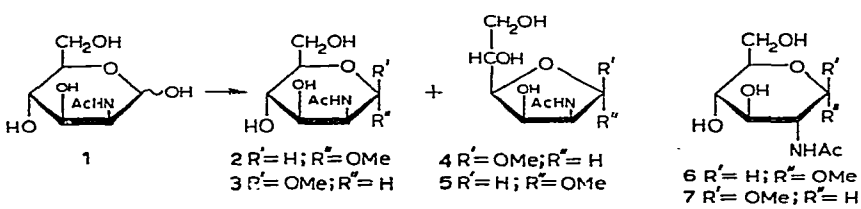
was possible to propose a set of empirical rules which allowed decisions to be made as to the nature and position of substituents on these residues[4].

The 2-acetamido (*N*-acetyl) group generates a characteristic Cotton effect centered between 210 and 215 nm (n–π^*). Shorter wavelength transitions (π–π^*) also occur and have been described by others[6]. In so far as the n–π^* transition of this chromophore is concerned, the configuration (α or β) of the glycosidic linkage, most strikingly in 2-acetamido-2-deoxy-D-glucosyl, but also in 2-acetamido-2-deoxy-D-galactosyl derivatives profoundly influences the shape, sign, and intensity of dispersion curves, and significantly affects the intensity of the c.d. bands. By comparing these Cotton effects in several compounds and applying empirical symmetry rules, Beychok and Kabat[1] were able to infer in 2-acetamido-2-deoxy-D-glucose the preferred orientation of the 2-acetamido planar group with respect to the ring. The proposed conformation was the same as that shown by Phillips to occur in crystals of lysozyme–2-acetamido-2-deoxy-D-glucose complexes[7].

It seems desirable to extend these studies to other related substances, whenever possible. Because of the importance of 2-acetamido-2-deoxy-D-mannose and its derivatives in many biological materials, and in anticipation of structural and conformational investigations of polysaccharides containing such residues, we present in this paper o.r.d. and c.d. spectra of the methyl glycosides of 2-acetamido-2-deoxy-D-mannopyranose and D-mannofuranose.

RESULTS AND DISCUSSION

Methyl 2-acetamido-2-deoxy-α- (**5**) and β-D-mannofuranoside (**4**) and methyl 2-acetamido-2-deoxy-α- (**2**) and β-D-mannopyranoside (**3**) were prepared by treatment of 2-acetamido-2-deoxy-D-mannose (**1**) with methanol in the presence of Amberlite IR-120 (H^+) and separated on an ion-exchange resin and by preparative paper chromatography. The α and β anomers of the furanosides, and of the pyranosides,



were distinguished and identified according to the specific optical rotation at the D line of sodium, on the assumption that the α anomer exhibits a more positive rotation at that wavelength in the furanoside as well as in the pyranoside series[8].

Fig. 1 shows the u.v. o.r.d. spectra of **1**, **2**, **3**, **4**, and **5**. The spectra of the corresponding methyl 2-acetamido-2-deoxy-α- (**6**) and -β-D- glucopyranoside (**7**) are also drawn in for comparison. It may be noted that the o.r.d. curve of **5** in the spectral interval shown is somewhat similar to that of **6**, and that the spectrum of **3** is similar

to that of **7**. The o.r.d. of **1**, an equilibrium mixture, resembles that of **2**. However, its c.d. spectrum (Fig. 2) is more like that of **3**.

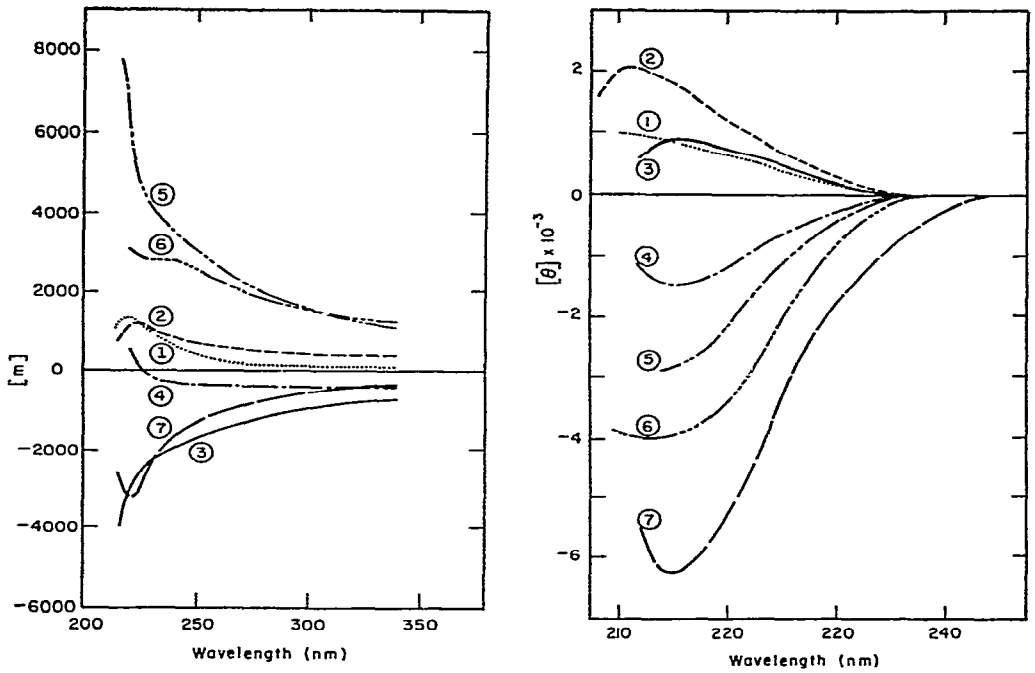


Fig. 1. O.r.d. spectra of methyl 2-acetamido-2-deoxy-α- and -β-D-mannopyranosides and -D-mannofuranosides: (1) 2-Acetamido-2-deoxy-D-mannose (**1**); (2) methyl 2-acetamido-2-deoxy-α-D-mannopyranoside (**2**); (3) methyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**3**); (4) methyl 2-acetamido-2-deoxy-β-D-mannofuranoside (**4**); (5) methyl 2-acetamido-2-deoxy-α-D-mannofuranoside (**5**); (6) methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**6**); and (7) methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**7**). Units of molecular rotation, [m], are degrees·cm²·decimole⁻¹.

Fig. 2. C.d. spectra of methyl 2-acetamido-2-deoxy-α- and -β-D-mannopyranosides and-D-mannofuranosides. (1) 2-Acetamido-2-deoxy-D-mannose (**1**); (2) methyl 2-acetamido-2-deoxy-α-D-mannopyranoside (**2**); (3) methyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**3**); (4) methyl 2-acetamido-2-deoxy-β-D-mannofuranoside (**4**); (5) methyl 2-acetamido-2-deoxy-α-D-mannofuranoside (**5**); (6) methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**6**); and (7) methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**7**). Units of molecular ellipticity, [θ], are degrees·cm²·decimole⁻¹.

In contrast to the results obtained with 2-acetamido-2-deoxy-D-glucose and its methyl α- and β-pyranosides, the Cotton effects of the 2-acetamido group in the mannosyl compounds are largely obscured by background optical rotation. In the c.d. spectra, shown in Fig. 2, these Cotton effects are well resolved and allow examination of the n–π* Cotton effect of the 2-acetamido group, unobscured by background optical activity.

With reference to the c.d. band at 212–215 nm, the main results may be summarized as follows: (a) The bands of the pyranoside derivatives **2** and **3**, which are of the same sign, are both opposite in sign to those of the pyranoside derivatives **6** and **7**. (b) The bands of the pyranoside derivatives **2** and **3** are opposite to those of

the furanoside derivatives **5** and **4**. (c) The α anomers of the D-mannosides exhibit bands more intense than those of the β anomers. The reverse is true for **6**, **7**, and the galactose analogs. (d) All four bands are less intense than either of the two bands of **6** and **7**.

Qualitatively, the first of these results is not unexpected. The chromophore, itself, is bonded to the epimeric carbon. Sterically, a favorable orientation for the *trans*-acetamido group is with the bulky carbonyl as far from the ring as possible. In this position, with the ring in a *C1* conformation, the amide plane includes the axial C–2 and C–5 bonds. The NH of the amide group points towards the ring and its CO away from it. Regardless of whether a quadrant or an octant rule is presumed to be operative, the corresponding ring substituents, as well as the ring atoms themselves, would all be in opposite sign sectors in the mannopyranoside, compared to the glucopyranoside compound. This probably accounts for the opposite sign n–π^* of the circular dichroic bands of the gluco- and manno-pyranosides. The same reasoning may explain the reversal of the relative amplitudes of the α- and β-D-anomers in the two sugars, since the anomeric substituents are also in opposite sectors, while their relative distances from the amide plane remain unchanged.

However, it would not be expected that the intensities of the c.d. bands would be equal, since the distances of ring substituents and ring atoms from the chromophore and its nodal planes are different in the two kinds of compounds. For example, the distances of the ring oxygen and of the anomeric oxygen atoms from the carbonyl group are greater in the glucopyranosides than in the mannopyranosides. Furthermore, the possibility of a *1C* ring conformation in D-mannose and its derivatives has been suggested[9], and this would alter the intensities considerably, without necessarily changing the signs.

The observation that the n–π^* c.d. bands of **5** and **4** are opposite in sign to those of the pyranoside derivatives **2** and **3** was unexpected. The result can be rationalized, since one of the main differences in the two conformations—again assuming a sterically favorable orientation of the amide group (carbonyl) pointing away from, rather than towards, the ring—is the displacement of C–4 and its substituents away from the rear, lower octant, which is positive, and towards a nodal plane. This is only a rationalization, however, since it is critical to such an argument that an octant rule, rather than a quadrant rule, is operative. These symmetry rules differ not only in respect to whether one ignores front octants; the signs of the quadrant sectors are opposite to those of rear octants[10].

Finally, in the furanoside derivatives, a hydrogen bond might form between the carbonyl of the amide group and the hydroxyl group at C–6. This would markedly alter the relationship of the chromophore to the ring from that observed in the pyranosides. The occurrence of such a conformational state, as well as the possible occurrence of an uncertain amount of a *1C* ring conformation in the mannopyranosides, may be revealed by n.m.r. measurements, which are in progress.

EXPERIMENTAL

Preparation of methyl 2-acetamido-2-deoxy-α-D-mannofuranoside (**5**), *methyl 2-acetamido-2-deoxy-β-D-mannofuranoside* (**4**), *methyl 2-acetamido-2-deoxy-α-D-mannopyranoside* (**2**), *and methyl 2-acetamido-2-deoxy-β-D-mannopyranoside* (**3**). — To a solution of 2-acetamido-2-deoxy-D-mannose (**1**, 2.20 g, Pfanstiehl, Waukegan, Illinois) dissolved in anhydrous methanol (50 ml), was added dry Amberlite IR-120 (H^+, 1.14 g), and the magnetically stirred suspension was heated at reflux for 1 h with exclusion of moisture. Methyl glycoside formation was determined by measuring the decrease in reducing power of the solution. After 30 min of reflux, 65% of the reducing sugar had reacted and, after 1 h, 81%. Since methyl glycoside formation is accompanied by deacetylation under these conditions, the reaction was stopped at this point, and the resin was removed by filtration and washed several times with 10-ml portions of methanol. The pooled filtrate and washes were concentrated *in vacuo* to a pale-yellow syrup (1.64 g). In addition, approximately 0.35 g of deacetylated hexosamine was recovered by suspension of the resin 3 times with M hydrochloric acid (2.5 ml), followed by passage of the filtrate through a column (2 × 5 cm) of Dowex-1 (AcO^-) and concentration of the eluate *in vacuo*.

Partial resolution of the resulting anomeric forms was accomplished by chromatograhy on Dowex-1(X-2, 200–400 mesh, OH^-). The syrup (1.64 g) was dissolved in carbon dioxide-free distilled water (2–3 ml) and applied to a freshly prepared Dowex-1 (OH^-) column (2 × 16.5 cm). The mixture was eluted with carbon dioxide-free distilled water (200 ml), samples (2.5 ml) being collected every 5 min. Aliquots (15 μl) were removed from alternate tubes and oxidized with sodium periodate for 2.5 h in the dark at room temperature[11]. Two well separated peaks were recovered by lyophilization with a very small intermediate peak. Fraction I (tubes 20–38), 906 mg; and Fraction II (tubes 52–80, 254 mg).

Fraction II (245 mg) was dissolved in hot, absolute ethanol (4 ml), and the solution was allowed to cool at room temperature. Needle-like crystals began to appear within 10–15 min. After storage of the suspension overnight at 3°, the crystals were removed by filtration on a sintered glass filter and dried over phosphoric anhydride to give **5** (185.7 mg) m.p. 177–178°, $[\alpha]_D^{24}$ +141° (*c* 1.3, water). Upon treatment of this material with sodium periodate, 0.96 mole formaldehyde[12] was liberated per mole of compound.

Anal. Calc. for $C_9H_{17}N_1O_6$: C, 45.95; H, 7.28; N, 5.96. Found: C, 46.12; H, 7.19; N, 6.25.

Since repeated attempts to crystallize Fraction I from a variety of solvent systems proved fruitless, further resolution was sought. To this end, the dry material (approxim. 500 mg) was dissolved in methanol (2 ml), and the solution was applied to 55 sheets of Whatman No. 1 paper measuring 17 × 57 cm. The papers were developed by descending irrigation in a solvent system consisting of the upper phase of 70:20:23 ethyl acetate–pyridine–water. After 18 h at room temperature, the papers were removed and allowed to dry. Lateral guide strips were cut and dipped in a

freshly made solution prepared by dissolving periodic acid (600 mg) in water (5 ml) and diluting to 100 ml with acetone. Following this treatment, three components were seen upon further developing the guide strips with silver nitrate, followed by sodium hydroxide[13]. The slowest moving component (I–1) formed a band of weak intensity located at 15–17 cm from the starting line. An intermediate band (I–2) was seen at 22–27 cm and a third band of greatest intensity (I–3) at 28–33 cm. After concentration, each of these fractions was applied separately to a column of Dowex-1 (OH^-) as previously described. Lyophilization of the single peak resulting in each case yielded a white amorphous solid. Paper chromatography of the isolated component in the same solvent system revealed each of the fractions to be free from contamination with the others.

Fraction I–1 (3). Yield 29.8 mg, $[\alpha]_D^{24}$ −68° (*c* 1.5, water), it gave 0.01 mole of; formaldehyde per mole of methyl glycoside on periodate oxidation[11].

Anal. Calc. for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.96. Found: C, 45.21; H, 7.18; N, 5.80.

Fraction I–2 (4). — Yield 53.8 mg, $[\alpha]_D^{24}$ −35° (*c* 2.6, water); it gave 0.95 mole of formaldehyde per mole of methyl glycoside on peridoate oxidation.

Anal. Calc. for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.96. Found: C, 45.15; H, 7.58; N, 6.18.

Fraction I–3 (2). — Yield 218 mg, $[\alpha]_D^{24}$ +50° (*c* 1.6, water); it gave 0.01 mole of formaldehyde per mole of methyl glycoside on periodate oxidation[11].

Anal. Calc. for $C_9H_{17}NO_6$: C, 45.95; H, 7.28; N, 5.96. Found: C, 46.08; H, 7.30; N, 6.18.

Optical rotatory dispersion and circular dichroism measurements were performed as described elsewhere[4].

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