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Note

Characterization of a diaminohexose (2,3-diamino-2,3-dideoxy-D-glucose) from *Rhodopseudomonas viridis* lipopolysaccharides by circular dichroism

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A 2,3-diamino-2,3-dideoxyhexose was recently isolated from the Lipid A moiety of lipopolysaccharides from various strains of the two gram-negative, photosynthetic bacteria *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*[1,2]. Based on results obtained by mass spectrometry, gas-liquid chromatography, and periodate oxidation, this amino sugar was tentatively identified as 2,3-diamino-2,3-dideoxyglucose[3].

Studies on 2-acetamido-2-deoxy sugars as well as on oligo- and polysaccharides containing such constituents have shown that circular dichroism (c.d.) measurements are a valuable method to investigate their chemical structures[4–8]. These carbohydrate compounds show Cotton effects in the 210- and 190-nm regions[4–8]. The c.d. band at 210 nm is apparently due to the n–π^* transition of the amide group, and that at 190 nm to the π–π^* transition of the same group[7]. The correlation of the characteristics of the c.d. spectra with the configuration and conformation of 2-acetamido-2-deoxy sugars and the nature and linkage of these sugar residues in oligo- and polysaccharides has been attempted. So far, however, no corresponding investigation of the relationship between optical behavior and the structural parameters of diacetamidodideoxy sugars has been performed. The c.d. method was applied, in the present study, to gain further information on the configuration of the 2,3-diamino-2,3-dideoxyhexose found in the lipopolysaccharides of *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*, to which the *gluco* configuration was tentatively assigned[3]. The c.d. of the di-*N*-acetyl derivative was investigated in aqueous as well as methanolic solution and, for comparison, an authentic sample of synthetic 2,3-diacetamido-2,3-dideoxy-D-glucose was similarly investigated. The optical properties of the just mentioned substances were also compared with those of various 2-acetamido-2-deoxyhexoses.

The 2,3-diamino-2,3-dideoxyhexose was isolated from the acid hydrolyzate of the lipopolysaccharide of *Rhodopseudomonas viridis* strain F, as described recently[3], and was selectively *N*-acetylated according to Meyer zu Reckendorf[9] to give a 2,3-diacetamido-2,3-dideoxyhexose (**1**, see Table I for structures).

TABLE I

CIRCULAR DICHROIC ABSORPTION OF 2,3-DIACETAMIDO-2,3-DIDEOXY-D-GLUCOSE AND 2-ACETAMIDO-2-DEOXYHEXOSES

Compound (source)	*Solvent*			
	Water		*Methanol*	
	λ (nm)	*Amplitude (Δε)*	*λ (nm)*	*Amplitude (Δε)*
2,3-Diacetamido-2,3-dideoxy-hexose (from *Rhodo-pseudomonas viridis* and *Rhodopseudomonas palustris*) (**1**)	198.0	−26.850	200.0	−12.350
	222.0	+0.415	225.0	+0.076
2,3-Diacetamido-2,3-dideoxy-D-glucose[9] (synthetic) (**2**)	198.5	−27.790	200.5	−12.550
	222.5	+0.448	225.0	+0.080
2-Acetamido-2-deoxy-D-glucose (**3**)	209.5	−1.275	214.5	−1.585
2-Acetamido-2-deoxy-D-galactose (**4**)	190.0	+2.495	195.0	+3.585
	211.0	−1.288	215.0	−1.030
2-Acetamido-2-deoxy-D-mannose (**5**)	205.0	+0.595	210.0	+0.267

The c.d. spectra of this compound were measured both in aqueous and in methanolic solution, in the range 185–250 nm (see Fig. 1a). Both spectra are characterized by an intense ellipticity-band near λ 198 nm and a positive one of low intensity near λ 222 nm. Although the shape of the curves is very similar, distinct differences are recognizable: on one hand, the position of the c.d. bands observed with the methanolic solution are shifted—although only slightly (2–3 nm)—to higher wavelengths; on the other hand, the dichroic absorption values are lower than the respective values with the aqueous solution, *e.g.* in water, λ_{max_1} 198 nm ($\Delta\varepsilon_1$ −26.85) and λ_{max_2} 222 nm ($\Delta\varepsilon_2$ ±0.415), and in methanol λ_{max_1} 200 nm ($\Delta\varepsilon_1$ −12.35) and λ_{max_2} 225 nm ($\Delta\varepsilon_2$ +0.076) (see Table I).

The c.d. curves of synthetic 2,3-diacetamido-2,3-dideoxy-D-glucose (**2**) in water and in methanol (see Fig. 1b) also exhibit an intense, negative and a small, positive Cotton effect in the 198- and 222-nm regions, respectively. As with the natural compound, **1**, the position of the extrema and, particularly, the magnitude of the ellipticity are influenced by the respective solvents. Comparing the spectra of both compounds **1** and **2**, one recognizes that similar c.d. curves are obtained in both solvents, *i.e.* the shape of the curves and the positions of the extrema are identical. The

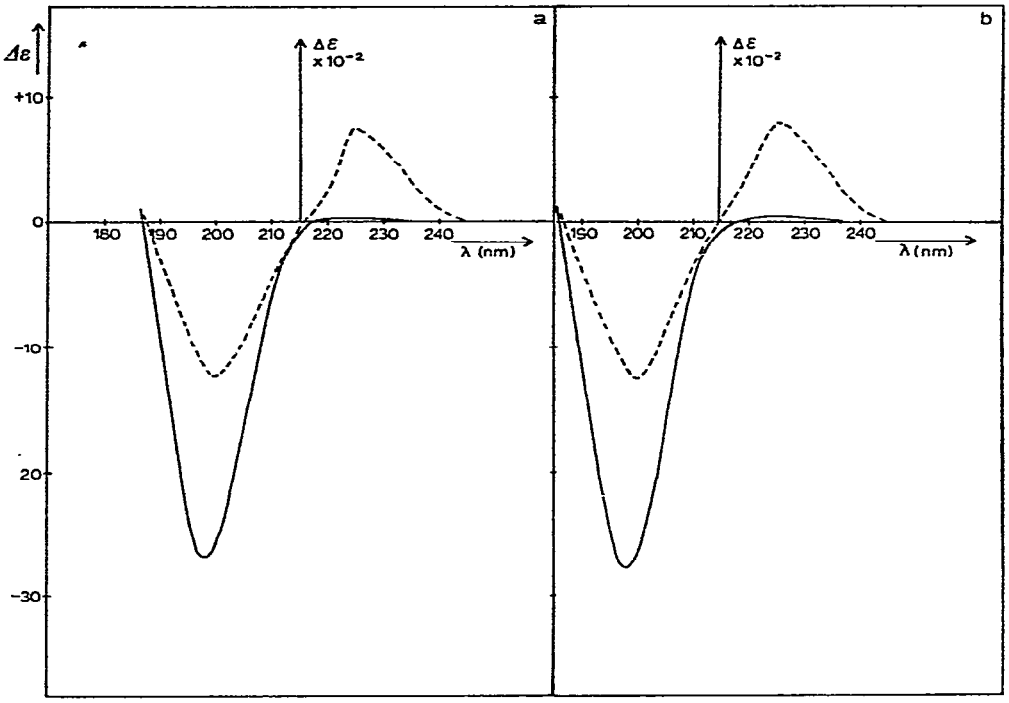

Fig. 1. (a) C.d. spectra of 2,3-diacetamido-2,3-dideoxy-D-glucose (**1**) from the lipopolysaccharides of *Rhodopseudomonas viridis* and *palustris* measured on water (———) and methanolic (— — —) solutions. (b) C.d. spectra of synthetic 2,3-diacetamido-2,3-dideoxy-D-glucose (**2**) measured on water (———) and methanolic (— — —) solutions. The scale for $\Delta\varepsilon$ was extended in the region of λ 200 nm in order to differentiate the absorption in water from that in methanol.

absolute values of the dichroic absorptions differ, however, slightly more than the allowed experimental error of $\pm 3\%$ (see Table I).

These results, which show the identical c.d. behavior of both compounds **1** and **2**, indicate that the two di-*N*-acetyl derivatives have the same steric configuration, *i.e.* the diamino sugar of the lipopolysaccharide has indeed the D-*gluco* configuration, as indicated previously by the periodate studies and the g.l.c. data[3]. The small differences in the ellipticity magnitude are not dependent on different conîgurational parameters, but could rather be caused either by small impurities or by a slightly higher water-content of the natural sample.

One characteristic effect should be emphasized, namely, both compounds **1** and **2** exhibit two Cotton effects of opposite sign, whereas 2-acetamido-2-deoxy-D-glucose (**3**) and -D-mannose (**5**) show only single c.d. bands that differ in sign and in the ellipticity magnitude (see Fig. 2 and Table I). Out of the 2-amino-2-deoxy sugars so far studied, only 2-acetamido-2-deoxy-D-galactose (**4**) shows two Cotton effects of opposite sign (Fig. 2). Comparing the c.d. curve of **4** with those of **1** or **2**, the position of the bands is shifted to shorter wavelengths (8–11 nm) (see Table I). In addition, the curves of **4** and those of **1** and **2** differ in the ellipticity magnitude of the Cotton

effects at ~195 nm: the values of the curves of **1** and **2** are about 10 times higher than that of **4**. In contrast, the compounds show reciprocal behavior near the second band at higher wavelength (see Table I).

In comparing the values of dichroic absorption of 2,3-diacetamido-2,3-dideoxy-D-glucose (**1** and **2**) with those of the 2-acetamido-2-deoxy-D-hexoses **3**, **4**, and **5**, it may be observed that, with the exception of the c.d. band at 222 nm, the former absorptions show $\Delta\varepsilon$ values higher than those of all the other compounds. Furthermore, the optical behavior of the mono- and bifunctional compounds is influenced in a similar way by use of methanol as solvent: c.d. bands shift slightly to higher wavelengths and the ellipticity magnitude decreases (see Figs. 2 and 3).

The c.d. band at 222 nm may be assigned to the n–π^* transition of the acetamido chromophore of **1** and **2**, although in the case of 2-acetamido-2-deoxy sugars this contribution was found to be localized at ~210 nm. According to Stone[7], the Cotton effects at 198 nm might be assigned to the π–π^* transition, although the position of the band of 2-acetamido-2-deoxy sugars is observed at ~190 nm. These effects seem to be characteristic for 2,3-diacetamido-2,3-dideoxyhexoses and are probably due to the direct vicinity of the chromophores. Additional c.d. measurements on these types of amino sugars are required in order to discuss the present results in a more detailed form.

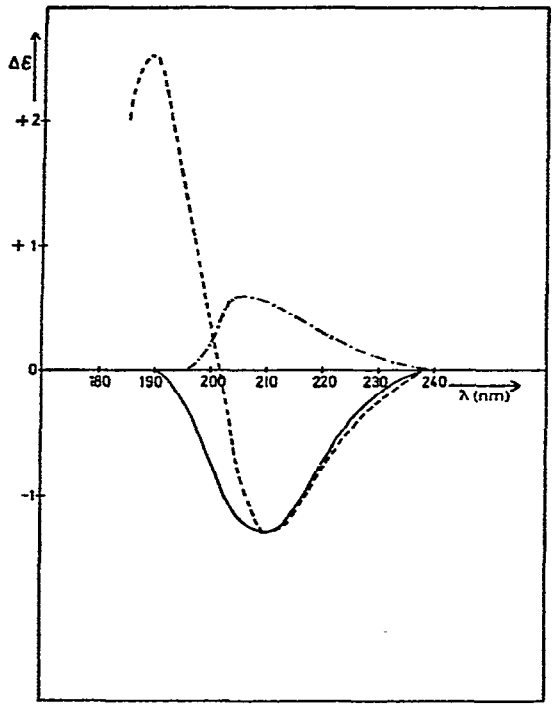

Fig. 2. C.d. spectra of 2-acetamido-2-deoxy-D-glucose (3) (———), 2-acetamido-2-deoxy-D-galactose (4) (– – –), and 2-acetamido-2-deoxy-D-mannose (5) (–• –•) on water solutions.

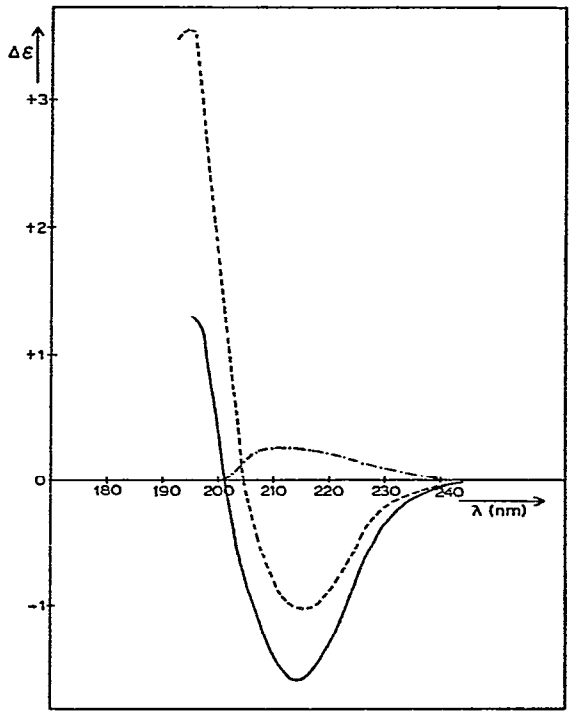

Fig. 3. C.d. spectra of 2-acetamido-2-deoxy-D-glucose (3) (———), 2-acetamido-2-deoxy-D-galactose (4) (— — —), and 2-acetamido-2-deoxy-D-mannose (5) (—· —·) on methanol solutions.

EXPERIMENTAL

Materials. — The chemical synthesis of 2,3-diamino-2,3-dideoxy-D-glucose and its di-*N*-acetyl derivative was performed by Meyer zu Reckendorf[9]. The specific optical rotation of the natural 2,3-diamino-2,3-dideoxyhexose was identical to that of the synthetic compound, $[\alpha]_D^{20}$ −46° (after 16 h, *c* 0.9, water). The natural compound showed a single spot on t.l.c. and a single peak on g.l.c. of the alditol acetate on a column of OV-17 (Ref. 3). The purity of 2-acetamido-2-deoxy-D-glucose and -D-galactose (Serva, Heidelberg, BRD) and of 2-acetamido-2-deoxy-D-mannose (Cyclo Chem. Div., Los Angeles, USA) was checked on an automatic sugar analyzer according to Keilich and Ziegler[10].

Optical measurements. — The c.d. spectra were recorded with a Roussel–Jouan Circular Dichrograph II (Roussel–Jouan et Co., Paris, France). The concentrations were 0.025–0.5%. Quartz cells having pathlengths of 0.02, 0.05, 0.1, 0.2, or 0.5 cm were used. The estimated error for c.d. measurements is ±3% for $\lambda \geqq 200$ nm and up to 10% for $\lambda \leqq 200$ nm. The coefficient of dichroic absorption $\Delta\varepsilon$ is expressed by $D/c.l.$, where D is the observed difference in the values of absorbance between left- and right-circular, polarized light; c is the molar concentration; and l is the pathlength of the cell in cm.

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