Carbohydrate Research, 254 (1994) 35–41
Elsevier Science B.V., Amsterdam

Synthesis of, and NMR and CD studies on, methyl 4-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-L-rhamnopyranoside and methyl 6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside

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(Received April 19th, 1993; accepted August 12th, 1993)

ABSTRACT

The title compounds were prepared by condensation of a suitably protected monosaccharide and (*R*)- or (*S*)-2-chloropropanoic acid. Characterization by ^{1}H and ^{13}C NMR spectroscopy revealed minor chemical shift differences between the diastereomers. CD spectra showed differences between the (*R*)- and (*S*)-1-carboxyethyl substituted monosaccharides, thereby facilitating determination of the absolute configuration of the substituent.

INTRODUCTION

Sugar residues etherified with lactic acid are found in several bacterial polysaccharides[1]. The biosynthetic pathway to the lactic acid derivatives involves the conversion of a phosphoenol pyruvate into an enol of pyruvic acid which is then reduced. Both the (*R*)- and (*S*)-forms of lactic acid substituents have been found at different positions. The position of the 1-carboxyethyl group has mainly been determined by mass spectrometry of the derived alditol acetates or the partially methylated alditol acetates after reduction of the carboxylic acid group. The configuration of the 1-carboxyethyl group has been determined by comparison of chromatographic mobilities with those of synthetic samples. A method using the nuclear Overhauser effect on a conformationally rigid lactone derivative of 1-carboxyethyl sugar ethers was recently described[2].

In our structural studies[3] of the capsular polysaccharide from *Butyrivibrio fibrisolvens* strain X6C61, we found a D-galactose residue with a 1-carboxyethyl

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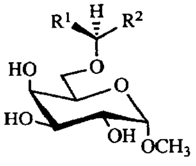
SSDI 0008-6215(93)E0302-H

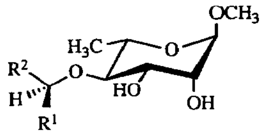
group in the 6-position. L-Rhamnose substituted in the 4-position by a 1-carboxyethyl group has also been identified in studies of polysaccharides from other strains of *B. fibrisolvens*[4]. To determine the absolute configuration of the 1-carboxyethyl group in both of these sugars, the (*R*)- and (*S*)-forms of the 1-carboxyethyl substituent were required. We report herein the synthesis of methyl 6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside (**1** and **2**) and methyl 4-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-L-rhamnopyranoside (**3** and **4**), and NMR and CD spectroscopic studies of these compounds.

RESULTS AND DISCUSSION

Compounds **1–4** were synthesized by using protected monosaccharide derivatives in which all positions but the substitution position were blocked. The derivatives chosen were methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside[5] and methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[6]. The precursor for the 1-carboxyethyl substituent was (*R*)- or (*S*)-2-chloropropanoic acid[7]. Inversion of configuration of the 2-position takes place when the alkoxy group of the sugar reacts with the (*S*)- or (*R*)-2-chloropropanoic acid forming an ether. The products were then deprotected to give **1** and **2**, respectively, and **3** and **4**, respectively.



1 R^1=COOH, R^2=CH$_3$
2 R^1=CH$_3$, R^2=COOH



3 R^1=COOH, R^2=CH$_3$
4 R^1=CH$_3$, R^2=COOH

The ^{1}H NMR chemical shifts and induced chemical shift differences on substitution are given in Table I together with the chemical shifts of the pertinent methyl glycosides. For both compounds **1** and **2**, the chemical shifts of the signals of H-1–H-4 and the *O*-methyl group are close to those of the corresponding methyl α-D-galactopyranoside, i.e., small chemical shift differences. The chemical shift differences for the H-5 and H-6 signals are up to −0.19 ppm but the differences between the diastereomers **1** and **2** are small. No difference between the signals from the 1-carboxyethyl group is observed for **1** and **2**. For the rhamnose diastereomers, **3** and **4**, the chemical shift differences for the proton at the substituted carbon are different, −0.16 and −0.05 ppm, and also for the adjacent H-3 and H-5 signals, which have a larger shift for the H-3 signal in **3** and the H-5 signal in **4**. The differences between the 1-carboxyethyl signals are smaller (0.06 and 0.03 ppm) but still significant.

The ^{13}C NMR chemical shifts and induced chemical shift differences on substitution are given in Table II in which the chemical shifts of the pertinent

TABLE I

^{1}H NMR chemical shifts [a] of compounds **1–4** and pertinent methyl glycosides; chemical shift differences [b] in parenthesis

Compound	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	OMe	H-2′	H-3′
1	4.83	3.83	3.83	4.01	4.03	3.61	3.76	3.43	3.93	1.33
	(−0.02)	(−0.01)	(0.02)	(0.02)	(0.14)	(−0.15)	(0.00)	(0.00)		
2	4.83	3.83	3.83	4.03	4.01	3.57	3.70	3.44	3.93	1.33
	(−0.02)	(−0.01)	(0.02)	(0.04)	(0.12)	(−0.19)	(−0.06)	(0.01)		
Me α-D-Gal*p*	4.85	3.84	3.81	3.99	3.89	3.76	3.76	3.43		
3	4.68	3.96	3.80	3.29	3.70	1.34		3.40	4.07	1.38
	(−0.01)	(0.03)	(0.08)	(−0.16)	(0.04)	(0.04)		(0.00)		
4	4.66	3.94	3.78	3.40	3.73	1.32		3.39	4.13	1.35
	(−0.03)	(0.01)	(0.06)	(−0.05)	(0.07)	(0.02)		(−0.01)		
Me α-L-Rha*p*	4.69	3.93	3.72	3.45	3.66	1.30		3.40		

[a] Relative to internal TSP (δ 0.00), 70°C, D_2O. [b] Compound – methyl glycoside.

methyl glycosides are also shown. The chemical shifts of the diastereomers differ, but for most signals these differences are less than 0.2 ppm. The chemical shift difference on substitution with a 1-carboxyethyl group is between 7.0 and 9.2 ppm for the signal of the linkage carbon, which is similar in magnitude to chemical shift changes induced by glycosylation. The galactose residues show a difference of 0.88 ppm for the C-5 signal between the diastereomers, and the rhamnose residues have a difference of 1.36 ppm between the signals of the substitution carbons, C-4.

The straightforward use of a structural reporter group for the determination of the relative configuration of the 1-carboxyethyl group, using NMR spectroscopy, is not possible for **1–4**. It may, however, be possible to use all the chemical shifts (differences) for determining the relative configuration of the 1-carboxyethyl

TABLE II

^{13}C NMR chemical shifts [a] of compounds **1–4** and pertinent methyl glycosides; chemical shift differences [b] in parenthesis

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OMe	C-1′	C-2′	C-3′
1	100.51	69.15 [c]	70.42 [c]	70.53	69.32	69.05	56.14	181.74	78.18	19.15
	(0.16)	(−0.02)	(−0.04)	(0.34)	(−2.22)	(6.99)	(0.18)			
2	100.45	69.14 [c]	70.20 [c]	70.25 [d]	70.20 [d]	69.22	56.16	181.82	78.71	19.19
	(0.10)	(−0.03)	(−0.26)	(0.06)	(−1.34)	(7.16)	(0.20)			
Me α-D-Gal*p*	100.35	69.17	70.46	70.19	71.54	62.06	55.96			
3	101.40	70.48	70.77	82.20	68.82	17.51	55.60	182.38	79.49	19.65
	(−0.34)	(−0.46)	(−0.53)	(9.19)	(−0.41)	(0.05)	(0.06)			
4	101.69	71.28	70.68	80.84	68.39	17.93	55.54	181.85	78.93	19.61
	(−0.05)	(0.34)	(−0.62)	(7.83)	(−0.84)	(0.47)	(0.00)			
Me α-L-Rha*p*	101.74	70.94	71.30	73.01	69.23	17.46	55.54			

[a] Relative to internal 1,4-dioxane (δ 67.40), 70°C, D_2O. [b] Compound – methyl glycoside. [c] Assignments may be reversed but are supported by the DIS-technique[9]. [d] Assignments may be reversed.

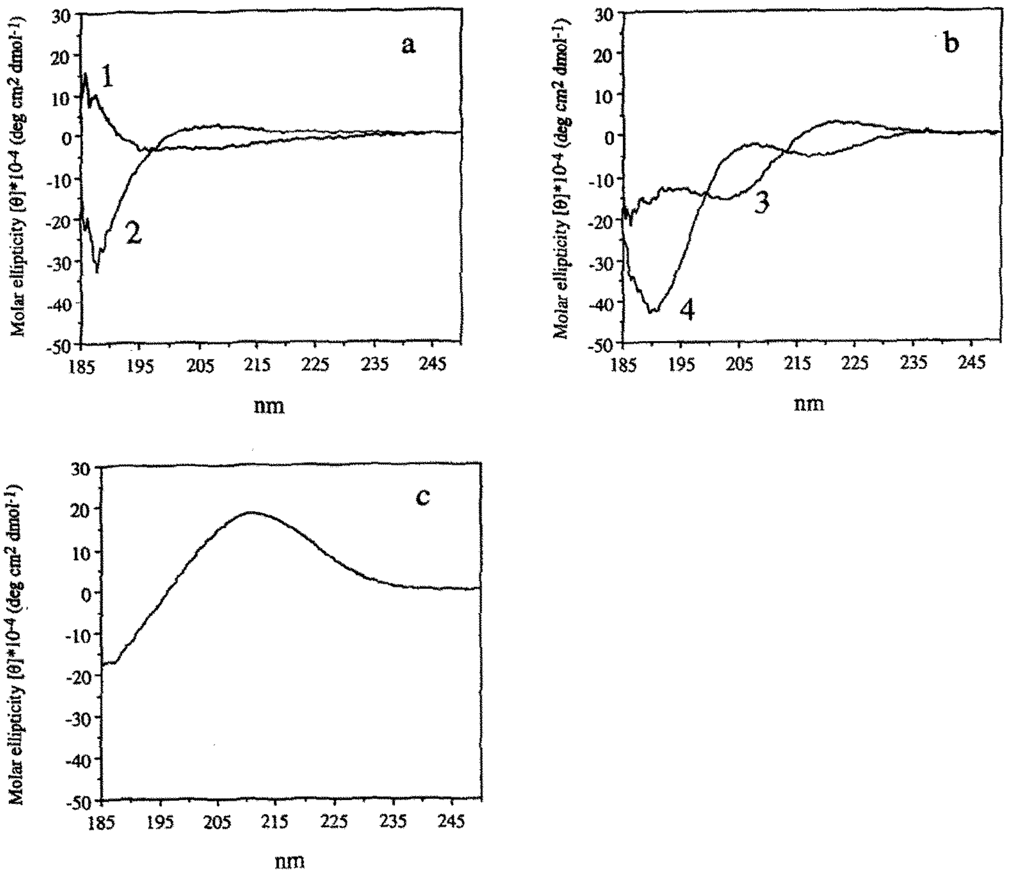


Fig. 1. Circular dichroism spectra of the sodium salts in water of compounds **1** and **2** (a), **3** and **4** (b), and (*S*)-lactic acid (c).

substituent in an approach that takes all signals into account, e.g., the computer program CASPER[8] or multivariant analysis.

Circular dichroism spectra of **1**–**4** facilitated differentiation between the diastereomers (Fig. 1). Compound **2** gives a spectrum with a positive maximum around 205 nm, whereas **1** has the opposite sign at this wavelength. In the region below 195 nm, the two diastereomers can also be differentiated but, with a mixture of saccharides or with a polysaccharide, care should be taken in interpretation below 195 nm since, e.g., both methyl α-D-galactopyranoside and methyl α-L-rhamnoside show a negative CD spectrum at these wavelengths. For **3** and **4**, the spectra were more complex, but **3** showed negative and positive maxima around 205 and 220 nm, respectively. Compound **4** had two negative maxima around 190 and 220 nm. Compounds **3** and **4** can be differentiated at 220 nm. For comparison, the CD spectrum of sodium (*S*)-lactate is also given and a positive maximum is observed around 210 nm. Thus, circular dichroism provides a rapid and sensitive technique for determining the absolute configuration of the 1-carboxyethyl substituent of sugars.

EXPERIMENTAL

General methods.—Sodium hydride (55–60%, suspension in oil) was washed with petroleum ether (bp 60–70°C) prior to use. Thin-layer chromatography (TLC) was performed on pre-coated plates (Merck Silica Gel 60 F_{254}) which were developed by charring with 8% H_2SO_4. Column chromatography of synthetic intermediates was performed on Matrex silica gel 60 Å (35–70 μm, Amicon). Gel permeation chromatography of deprotected products was carried out on polyacrylamide gel (Bio-Gel P-2, 2.6 × 90 cm) in 0.1 M pyridinium acetate buffer (pH 5.4). The purity of intermediates was first analysed by TLC, which showed only one spot, and then estimated from ^{13}C NMR spectra to be more than 95%. The purity of the final products was estimated from ^{1}H NMR spectra and shown to be $> 98\%$. Solutions were concentrated under reduced pressure at temperatures not exceeding 50°C. Measurements of **1–4** were performed on the sodium salts. Optical rotations were recorded at 22°C using a Perkin–Elmer 241 polarimeter. Circular dichroism spectra in water were recorded between 185–250 nm at ambient temperature, at a concentration of 2 mg/mL, with a Jasco J-720 spectrophotometer (cell length, 1.0 mm). NMR spectra were recorded with a Jeol GSX-270 MHz or Jeol α-400 MHz instrument. NMR spectra for solutions in $CDCl_3$ were recorded at 30°C, using Me_4Si (δ 0.00) as internal reference. NMR spectra obtained in D_2O were recorded at 70°C, using 1,4-dioxane (δ_C 67.40) or sodium 3-trimethylsilylpropanoate (δ_H 0.00) as internal reference. NMR assignments of compounds **1–4** were made using standard DEPT, H,H-COSY, and C,H-COSY experiments. ^{1}H NMR chemical shifts of overlapping signals were obtained from the center of the cross-peaks in the H,H-COSY or C,H-COSY spectra.

*Methyl 6-O-[(*R*)-1-carboxyethyl]-α-*D*-galactopyranoside* (**1**).—A solution of methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside[5] (528 mg, 1.14 mmol) and (*S*)-2-chloropropanoic acid[7] (204 mg, 1.88 mmol) in 1,4-dioxane (50 mL) was stirred with NaH (1.6 g, 36 mmol) at 50°C for 16 h, whereupon the mixture was cooled and water (25 mL) was added. Extraction with petroleum ether (bp 60–70°C, 2 × 25 mL) was followed by acidification of the water phase (pH ~ 3, AcOH). The water phase was then extracted with $CHCl_3$ (3 × 25 mL) and the combined organic phases were dried ($MgSO_4$), filtered, and concentrated to give methyl 2,3,4-tri-*O*-benzyl-6-*O*-[(*R*)-1-carboxyethyl]-α-D-galactopyranoside (**5**; 81%, 481 mg) as a syrup; $[\alpha]_{578}$ +21° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 18.1 (CH_3 carboxyethyl), 55.4 (OMe), 69.6–76.4 (C-2,3,4,5,6 and 3 $PhCH_2$), 78.9 (CH_3CH carboxyethyl), 98.7 (C-1), 127.5–138.7 (aromatic C), 177.3 (COOH carboxyethyl). Compound **5** (246 mg, 0.47 mmol) in MeOH (20 mL) was subjected to hydrogenolysis for 25 h at atmospheric pressure using Pd–C as catalyst (TLC: R_f 0.36 12:3:3:1 EtOAc–MeOH–AcOH–H_2O). Filtration of the mixture through Celite and concentration gave **1** (98%, 116 mg). Compound **1** was purified by gel permeation chromatogaphy (93% from **5**, 110 mg) and converted into its sodium salt, using an ion-exchange resin (Dowex 50, Na^+); $[\alpha]_{578}$ + 126° (*c* 1.0, sodium salt in H_2O).

Methyl-6-O[(S)-1-carboxyethyl]-α-D-galactopyranoside (**2**).—Starting from methyl 2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (504 mg, 1.09 mmol) and (*R*)-2-chloropropanoic acid (205 mg, 1.89 mmol), using the same experimental conditions as in the synthesis of **5**, methyl 2,3,4-tri-*O*-benzyl-6-*O*-[(*S*)-1-carboxyethyl]-α-D-galactopyranoside (**6**; 83%, 475 mg) was obtained; $[\alpha]_{578}$ + 15° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 18.2 (CH_3 carboxyethyl), 55.4 (OMe), 69.6–76.4 (C-2,3,4,5,6 and 3 $PhCH_2$), 78.9 (CH_3CH carboxyethyl), 98.8 (C-1), 127.5–138.7 (aromatic C), 177.1 (*C*OOH carboxyethyl). Hydrogenolysis of **6** (222 mg, 0.42 mmol), using the same conditions as above, gave, after gel permeation chromatography, **2** (92%, 98 mg). Compound **2** as its sodium form was prepared as described above; $[\alpha]_{578}$ + 63° (*c* 1.0, sodium salt in H_2O).

Methyl 4-O-[(R)-1-carboxyethyl]-α-L-rhamnopyranoside (**3**).—A solution of methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside[6] (407 mg, 1.88 mmol) and (*S*)-2-chloropropanoic acid (185 mg, 1.70 mmol) in 1,4-dioxane (25 mL) was stirred with NaH (0.6 g, 14 mmol) at 50°C for 24 h. Water (5 mL) was added and the crude mixture extracted with $CHCl_3$ (3 × 10 mL). The water phase was acidified (pH ~ 3, AcOH) and extracted with $CHCl_3$ (3 × 25 mL). The combined organic phases were dried ($MgSO_4$), decolorized with activated carbon, filtered, and concentrated to give methyl 4-*O*-[(*R*)-1-carboxyethyl]-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**7**; 52%, 258 mg) as a yellowish syrup; $[\alpha]_{578}$ −15° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 17.5 (C-6), 18.6 (CH_3 carboxyethyl), 26.1 and 27.8 (CH_3 isopropylidene), 54.9 (OMe), 64.5 (C-5), 76.0–82.8 (C-2,3,4 and CH_3CH carboxyethyl), 97.9 (C-1), 110.1 (Me_2C isopropylidene), 174.8 (*C*OOH carboxyethyl). Removal of the isopropylidene group of **7** (87 mg, 0.31 mmol) was performed with 0.1 M CF_3CO_2H (5 mL) at room temperature for 20 h. Freeze-drying and purification, first on a silica gel column (2:1 toluene–EtOAc) and then by gel permeation chromatogaphy, gave colourless **3** (71%, 47 mg) which was converted into its sodium salt using ion-exchange resin (Dowex 50, Na^+); $[\alpha]_{578}$ −17° (*c* 1.0, sodium salt in H_2O).

Methyl 4-O-[(S)-1-carboxyethyl]-α-L-rhamnpyranoside (**4**).—From methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (493 mg, 2.28 mmol) and (*R*)-2-chloropropanoic acid (197 mg, 1.82 mmol), using the same experimental conditions as for **7**, 4-*O*-[(*S*)-1-carboxyethyl]-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**8**; 35%, 182 mg) was obtained; $[\alpha]_{578}$ −54° (*c* 1.0, $CHCl_3$); ^{13}C NMR ($CDCl_3$): δ 17.6 (C-6), 19.1 (CH_3 carboxyethyl), 26.2 and 27.8 (CH_3 isopropylidene), 54.8 (OMe), 64.3 (C-5), 73.2–80.5 (C-2,3,4 and CH_3CH carboxyethyl), 97.9 (C-1), 109.4 (Me_2C-isopropylidene), 178.6 (*C*OOH carboxyethyl). Removal of the isopropylidene group of **8** (110 mg, 0.40 mmol), with purification as described for **7**, gave **4** (98%, 83 mg) which was converted into its sodium salt using ion-exchange resin (Dowex 50, Na^+); $[\alpha]_{578}$ $-111°$ (*c* 1.0, sodium salt in H_2O).

ACKNOWLEDGMENT

This work was supported by a grant from the Swedish Natural Science Research Council.

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