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Enantiomer separation of permethylated monosaccharides and 1,5-anhydroalditols and simultaneous determination of linkage positions and absolute configuration in the galactan of *Helix pomatia* [1]

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Abstract

The enantiomers of permethylated monosaccharides and 1,5-anhydroalditols were resolved using modified cyclomaltoheptaoses and cyclomaltooctaoses (β- and γ-cyclodextrins) as chiral stationary phases in capillary GLC. This method was applied to the galactan from *Helix pomatia*, which contains both D- and L-galactose. The corresponding 1,5-anhydrogalactitols which were formed by reductive cleavage of the permethylated galactan could be separated, allowing the simultaneous determination of linkage position and absolute configuration of galactose residues in snail galactan.

Keywords: Enantioselective gas chromatography; Reductive cleavage; Snail galactan; D- and L-sugars; Enantiomer separation; Cyclodextrin chiral stationary phase

1. Introduction

Primary structure elucidation of naturally occurring polysaccharides comprises the qualitative and quantitative determination of the sugar nature, the position and configuration of the glycosidic linkages, the absolute configuration of the sugar moiety, the type, number and positions of non-sugar substituents, and the sequence in the polymer chain. The interglycosidic linkages are usually determined by methylation analysis [1] or reductive cleavage [2]; both methods include permethylation of all free hydroxyl groups prior to degradation to monomer derivatives. The absolute stereochemistry of the sugar units is proved after glycosidation with (−)-2-butanol [3], acetalization with (+)-1-phenylethanethiol [4] or other deriva-

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[1] Dedicated to Professor Hans Paulsen on the occasion of his 75th birthday.

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tization process [5] by separation of the resulting diastereoisomers, or by the incubation with specific enzymes, accepting only one enantiomer. Recently, application of capillary electrophoresis to the separation of diastereomeric sugar borate complexes was reported [6]. We have already described the direct separation of sugar enantiomers as their trifluoroacetylated derivatives by enantioselective GLC [7]. Both enantiomers of at least fifteen sugars including pentoses (Ara, Xyl), hexoses (Alt, Gal, Man), deoxy sugars, aminodeoxysugars and some *O*-methyl ether derivatives have been found in nature, especially as constituents of bacterial polysaccharides [3,8,9]. D- and L-galactose occur in the galactans of some snails [10,11]. To determine the linkage position of the L-galactose residues, methylation analysis with subsequent isolation and demethylation of the galactose moiety under drastic conditions had to be performed [10]. We now report on the direct separation of per-*O*-methylated monosaccharides and anhydroalditol derivatives by enantioselective GLC, and a first application on a snail galactan in combination with the analysis of the interglycosidic linkages by reductive cleavage of the permethylated polysaccharide.

2. Results and discussion

Separation of anhydroalditol derivatives.—Initially, a number of pentoses and hexoses including arabinose, ribose, galactose, mannose, glucose, fu-

Table 1
Separation factors (α), order of elution, and GLC-conditions for the enantiomer separation of per-*O*-methylated methyl glycosides

Methyl-α,β-glycosides [a] of		Column [b]	Temp. (°C)	α	Enantiomer eluted first
Arabinose	α-Ara*f*	A	90	1.014	L
	α-Ara*p*			1.020	L
Fructose	Fru*f*	C	85	1.041	D
	Fru*f*			1.063	D
	Fru*p*			1.132	D
Fucose	Fuc*p*	A	90	1.022	D
	Fuc*f*			1.017	D
Galactose	Gal*f*	A	90	1.013	D
Glucose	β-Glc*p*	A	100	1.041	D
	α-Glc*p*			1.032	D
Glucose	β-Glc*p*	C	85	1.089	D
	α-Glc*p*			1.065	D
Mannose	α-Man*p*	A	110	1.000	–
	β-Man*p*			1.018	D
Mannose	α-Man*p*	C	100	1.032	– [c]
	β-Man*p*			1.013	D
Rhamnose	α-Rha*p*	A	90	1.022	L
	α-Rha*p*	B	100	1.046	D
Ribose	α-Rib*f*	B	100	1.036	L
	α-Rib*p*			1.059	L
	β-Rib*f*			1.013	L
	β-Rib*p*			1.043	L
Sorbose [d]		A	100	1.047	D
Sorbose		C	85	1.034	L
				1.032	L
				1.000	–
				1.031	D

[a] Up to four peak pairs were observed: α- and β-pyranosides and α- and β-furanosides.
[b] See Experimental.
[c] Not determined.
[d] Only the last of four pairs of enantiomers was separated.

cose, rhamnose, fructose, and sorbose were permethylated and directly separated on modified cyclodextrins. Chromatographic conditions, order of elution and α-values are listed in Table 1. Up to four pairs of enantiomers were formed for each glycoside: α- and β-pyranosides and -furanosides. The ring size could be differentiated by mass spectrometry [12]. By comparison with the pure enantiomers, the order of elution could unambiguously be assigned for each pair of peaks. The gas chromatograms of the α- and β-D,L-glucopyranosides and the α- and β-D,L-ribopyranosides and -furanosides are shown in Fig. 1. Reduction of the methyl glycosides, according to the reductive-cleavage method, yields only two enantiomeric pairs (1,4- and 1,5-anhydroalditols) with the exception of ketoses. The 1,5-anhydroribitol is a *meso*-form. The results of the enantiomer separation of the reduced permethylated sugars are given in Table 2. The separation of the 1,4- and 1,5-anhydro-D,L-arabinitols with an α-value of 1.295 for the latter, and the gas chromatogram of a mixture of the 1,5-anhydro-D,L-alditols obtained from glucose, galactose and mannose is shown in Fig. 2. In most cases, better resolution of the enantiomers is achieved for the per-*O*-methylated anhydroalditols compared to the corresponding methyl glycosides.

Snail galactans.—The galactan of *Helix pomatia* was permethylated and further analyzed by subsequent hydrolysis, reduction and acetylation, yielding partially methylated galactitol acetates **1–6** (Table 3). D- and L-galactose both give the same *meso*-form. Alternatively, reductive cleavage followed by acetylation was applied to the permethylated polysaccharides. From the resulting *O*-acetyl-1,5-anhydro-*O*-

Table 2
Separation factors (α), order of elution, and GLC-conditions for the enantiomer separation of per-*O*-methylated D,L-anhydroalditols

Per-*O*-methyl derivative of	Column [a]	Temp. (°C)	α	Enantiome eluted first
1,4-Anhydro-arabinitol	A	75	1.049	D
	B	90	1.066	D
1,5-Anhydro-arabinitol	A	75	1.295	D
	B	90	1.106	D
2,5-Anhydro-mannitol [b]	B	100	1.031	L
2,5-Anhydro-glucitol [b]			1.030	L
1,5-Anhydro-mannitol [c]			1.031	L
1,5-Anhydro-gulitol [c]			1.034	D [c]
1,5-Anhydro-fucitol	A	75	1.014	D
1,4-Anhydro-fucitol			1.182	L
1,4-Anhydro-galactitol	A	90	1.017	D
	C	100	1.034	D
1,5-Anhydro-galactitol	A	90	1.058	L
	C	100	1.074	L
1,5-Anhydro-glucitol	A	100	1.144	L
	B	100	1.033	L
	C	100	1.184	L
1,5-Anhydro-mannitol	A	110	1.017	L
	C	110	1.039	D
1,5-Anhydro-rhamnitol	A	85	1.017	D
	B	90	1.048	D
1,4-Anhydro-ribitol [d]	A	95	1.060	L
2,5-Anhydro-gulitol [e]/	A	100	1.155/	D
2,5-Anhydro-iditol [e]			1.131	
1,5-Anhydro-glucitol [e,f]			1.016	L [f]
1,5-Anhydro-iditol [e]			1.016	D

[a] See Experimental.
[b] From the reduction of α- and β-fructofuranosides.
[c] From the reduction of α- and β-fructopyranosides. 1,5-Anhydro-L-gulitol is the correct name for the 2,6-anhydro-D-glucitol formed from the D-fructopyranosides. Therefore, the order of elution is D (from L-Fru) before L (from D-Fru).
[d] 1,5-Anhydro-ribitol is a *meso*-form.
[e] From the reduction of α- and β-sorbofuranosides and α- and β-sorbopyranosides, respectively. Assignment of the *gulo*- and *ido*-configuration is not proved yet.
[f] 1,5-Anhydro-L-glucitol is the correct name for the 2,6-anhydro-D-gulitol formed from the D-sorbopyranosides. Therefore, the order of elution is L (from D-Sor) before D (from L-Sor).

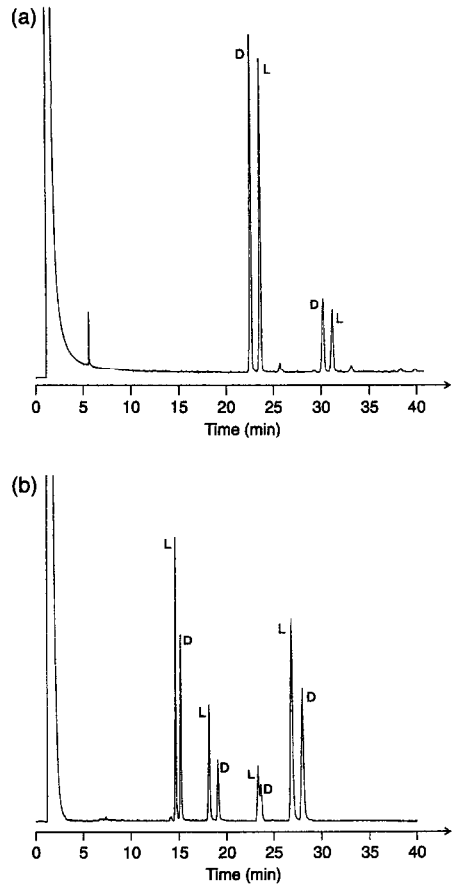

Fig. 1. Gas chromatogram of the enantiomer separation of (a) methyl β- (1. pair) and -α-D,L-glucopyranosides (2. pair) on column A at 85 °C, and (b) methyl α- and -β-D,L-ribopyranosides and -furanosides on column B at 100 °C (1. pair, α-fur; 2. pair, α-pyr; 3. pair, β-fur; 4. pair, β-pyr).

methyl-galactitols (**7–12**), the relative molar ratios of all constituents could be calculated. Results of classical methylation analysis and reductive cleavage are given in Table 3. Acetylation of the anhydrogalactitols was a critical step. Acid catalysis has to be avoided, since the terminal permethylated 1,5-anhydrogalactitol **7** is easily destroyed. Best results were obtained with acetic anhydride at room temperature after reductive cleavage with trimethylsilyl trifluoromethanesulfonate (Me_3Si-triflate) and triethylsilane. No ring isomerization can occur due to the lack of (1 → 4)-linked galactopyranosides or (1 → 5)-linked galactofuranosides [13]. 2,3,4,6-Tetra-*O*-methyl-galactitol (**7**) and 3,6-di-*O*-acetyl-1,5-anhydro-2,4-di-*O*-methyl-galactitol (**11**) were the main products.

Table 3
Relative molar ratios of the galactan constituents of *Helix pomatia*, as determined by standard methylation analysis (SMA) and the reductive cleavage method (RCM) including enantioselective GLC of the 1,5-anhydrogalactitols

Sugar moiety linked in position	Compound SMA/RCM	Molar ratio	
		SMA	RCM
terminal D-Gal*p*	**1/7**	}4.7	2.9
terminal L-Gal*p*			1.5
2-D-Gal*p*	**2/10**	1.0	1.2
3-D-Gal*p*	**3/8**	0.9	1.2
6-D-Gal*p*	**4/9**	0.2	0.4
2,6-D-Gal*p*	**5/12**	0.2	0.4
3,6-D-Gal*p*	**6/11**	4.4	4.4

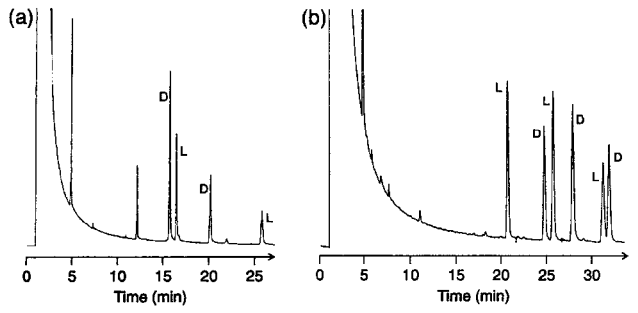

Fig. 2. Gas chromatogram of the enantiomer separation of per-*O*-methylated (a) 1,4- (1. pair) and 1,5-anhydro-D,L-arabinitols (2. pair) on column A at 75 °C, and (b) 1,5-anhydro-D,L-glucitol (1. pair), -galactitol (2. pair) and -mannitol (3. pair) on column A at 105 °C.

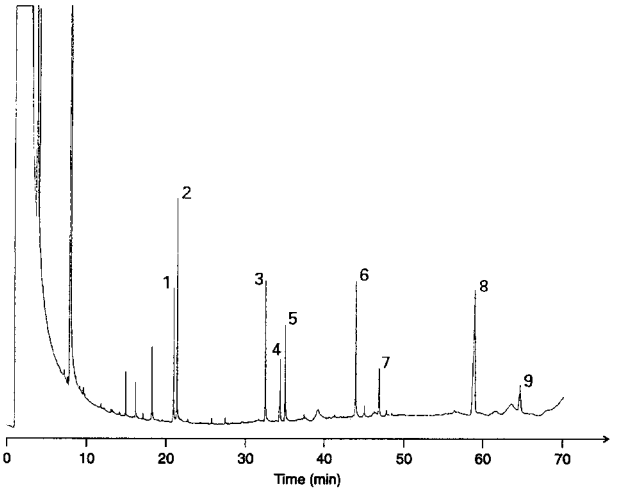

Fig. 3. Gas chromatogram of the *O*-acetyl-1,5-anhydro-*O*-methyl-galactitols obtained by reductive cleavage of the permethylated galactan from *Helix pomatia*. (Column A, 100 °C, 2 °C/min.) 1, **7** (L); 2, **7** (D); 3, **8** (D); 4, **9** (D); 5, **10** (D); 6, **11** (D); 7, **12** (D); 8 and 9, 1,3,6-tri-O-acetyl-2,4-di-O-methyl-α,β-D-galactopyranose (corresponding to **11**, from incomplete reduction due to intramolecular stabilisation of the intermediate carboxonium ion as a 1,6-anhydrogalactitol and subsequent acetylation).

On a chiral stationary phase (heptakis[2,6-di-*O*-methyl-3-*O*-pentyl]cyclomaltoheptaose, column A), only the anhydro-galactitol **7** from the terminal galactose moiety showed a separation into two peaks (Fig. 3). Since, in our experience, *O*-methyl ethers are less appropriate derivatives for enantioselective GLC compared to acetates or trifluoroacetates which may exert hydrogen bonding interactions with the chiral stationary phase, it can be assumed that all galactitols with one or more *O*-methyl groups replaced by an acetyl residue would also be separated, if the corresponding enantiomer were present. So, all the L-galactose content is represented by the terminal galactose residues. Identity of the resolved peak was proved by co-injection with the synthesized 1,5-anhydro-2,3,4,6-tetra-*O*-methyl-D- and -L-galactitols. The L-enantiomer was eluted first. The D/L-ratio of the terminal galactose was 65:35, corresponding to a total amount of 13–14% of L-galactose in the galactan, which is in good agreement with earlier investigations [10,11].

3. Conclusion

Enantiomer separation of permethylated sugar derivatives was demonstrated for a number of aldo- and keto-hexoses, 6-deoxyhexoses and aldopentoses. Therefore, the simultaneous determination of the interglycosidic linkage positions and the absolute configuration of the sugar moieties became possible by the combination of reductive-cleavage with enantioselective gas chromatography on modified cyclodextrins.

4. Experimental

General.—Arabinose, fructose, fucose, galactose, glucose, mannose, rhamnose, ribose, and sorbose (D- and L-forms) were purchased from Sigma. Triethylsilane and $Me_3SiOSO_2CF_3$ were from Fluka. Solvents were of analytical grade.

Galactan.—The galactan of *Helix pomatia* was extracted from albumen glands with 0.9% saline and purified as described earlier [14].

Methylation.—Methylation was carried out according to Ciucanu and Kerek [15]. The permethylated galactan was dialyzed and freeze dried. D- and L-sugars were extracted with CH_2Cl_2 after permethylation. In the case of D- and L-galactose, the crude product was purified on silica gel (EtOAc). MS-data were in agreement with data reported in ref. [16]. ^1H NMR ($CDCl_3$, δ in ppm): 4.12 (dd, 1 H, H-1e), 3.69 (dd, 1 H, H-4), 3.62 (ddd, 1 H, H-2), 3.58–3.44 (m, 3 H, H-6,6′, H-5), 3.09 (dd, 1 H, H-1a), 3.57, 3.53, 3.48, 3.40 (4s, 12 H, 2,3,4,6-*O*CH_3); ^{13}C NMR ($CDCl_3$, δ in ppm): 85.2 (C-3), 77.7 (C-5), 76.3 (C-2), 75.6 (C-4), 71.5 (C-6), 68.1 (C-1), 61.3, 59.2, 58.9, 57.9 (2,3,4,6-*O*CH_3)

Reductive cleavage.—Permethylated galactan (2 mg) was dissolved in CH_2Cl_2 (400 μL). Triethylsilane (5 equiv/galactosidic bond) and $Me_3SiOSO_2CF_3$ (5 equiv/galactosidic bond) were added. After 2 h at room temperature, Ac_2O was added (10 μL). After 1 h at r.t., the solution was washed twice with saturated $NaHCO_3$ soln. The dried organic phase was evaporated to dryness in a stream of nitrogen.

Standard methylation analysis was carried out as described [10].

GLC–MS.—Mass spectra were recorded on a VG Analytical VG/70-250S instrument. For CI, ammonia was used as reactant gas.

NMR spectroscopy.—^1H NMR-spectra (400 MHz) were obtained for $CDCl_3$ solutions by using a Bruker WM400 instrument. Chemical shifts were expressed as δ relative to Me_4Si as internal standard. ^{13}C NMR spectra were recorded with the same instrument at 100 MHz.

GLC.—GLC separations were carried out on a Carlo Erba GC 6000 Vega Series 2 instrument equipped with an on-column injector, a flame ionization detector, a 25 m capillary column CPSil 8CB (Chrompack) connected with a retention gap (2 m), and a Merck Hitachi D-2500 integrator. Hydrogen was used as carrier gas (80 kPa). Enantioselective GLC was carried out on heptakis[2,6-di-*O*-methyl-3-*O*-pentyl]cyclomaltoheptaose (20% dissolved in polysiloxane, column A; 50% in Fig. 2(b)), octakis[2,6-di-*O*-methyl-3-*O*-pentyl]cyclomaltooctaose (50% dissolved in polysiloxane, column B) and heptakis[2,3-di-*O*-methyl-6-*O*-TBDMS]cyclomaltoheptaose (50% dissolved in polysiloxane, column C). Hydrogen (60 kPa) was used as carrier gas. Individual temperatures are given in Tables 1 and 2, and in Fig. 3.

References

[1] H. Björndal, C.G. Hellerquist, B. Lindberg, and S. Svensson, *Angew. Chem.*, 82 (1970) 643–652; *Angew. Chem., Int. Ed. Engl.*, 9 (1970) 610–619.

[2] J.U. Bowie, P.V. Trescony, and G.R. Gray, *Carbohydr. Res.*, 125 (1984) 301–307.

[3] G.J. Gerwig, J.P. Kamerling, and J.F.G. Vliegenthart, *Carbohydr. Res.*, 62 (1978) 349–357; *ibid.* 77 (1979) 1–7.
[4] M.R. Little, *Carbohydr. Res.*, 105 (1982) 1–8.
[5] C. Bai, Y. Nishida, H. Ohrui, and H. Meguro, *J. Carbohydr. Chem.*, 15 (1996) 217–230.
[6] P. Richter, J. Freissmuth, B. Lachmann, and C.R. Noe, *Lebensmittelchem.*, 50 (1996) 82–83; C.R. Noe and J. Freissmuth, *J. Chromatogr. A*, 704 (1995) 503–512.
[7] W.A. König, P. Mischnick-Lübbecke, B. Brassat, S. Lutz, and G. Wenz, *Carbohydr. Res.*, 183 (1988) 11–17, and lit. therein.
[8] G.O. Aspinall (Ed.), *The Polysaccharides*, Vol 2, Academic Press, 1982, pp 289–292.
[9] R.J. Stack, *Abstracts of Papers*, Intern. Carbohydr. Symp. XIVth, Stockholm, August 1988, A109.
[10] H. Bretting, G. Jacobs, I. Benecke, W.A. König, and J. Thiem, *Carbohydr. Res.*, 139 (1985) 225–236, and lit. therein.
[11] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[12] K. Heyns, H.F. Grützmacher, H. Scharmann, and D. Müller, *Fortschr. Chem. Forsch.*, 5 (1966) 448–490.
[13] P. Mischnick and G.A. De Ruiter, *Carbohydr. Polym.*, 23 (1994) 5–12.
[14] H. Bretting, N.F. Whittaker, E.A. Kabat, K. Königsmann-Lange, and H.-J. Thiem, *Carbohydr. Res.*, 98 (1981) 213–236.
[15] I. Ciucanu and F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[16] L.E. Elvebak II, C. Abbott, S. Wall, and G.R. Gray, *Carbohydr. Res.*, 269 (1995) 1–15.