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THE OCCURRENCE OF L-GALACTOSE IN SNAIL GALACTANS

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ABSTRACT

The occurrence of L-galactose (L-Gal) was investigated in twelve preparations of snail galactans from nine different species. L-Gal (11–15%) was found not only in members of the family *Helicidae*, a highly developed group among the *Pulmonata*, but was detected also in a galactan of the oldest phylogenetic subclass of snails in the *Prosobranchiata*. To determine the position of the L-Gal residues, the galactans of *Arianta arbustorum*, *Cepaea nemoralis*, and *Helix pomatia* were methylated and hydrolysed, and the partially methylated Gal derivatives were isolated. Each derivative was *O*-demethylated and the ratio of D- and L-galactose was determined by g.l.c. of the trifluoroacetylated methyl glycosides using a chiral stationary phase. L-Gal (22–34%) was detected only in the tetra-*O*-methyl fractions, and hence L-Gal occupies exclusively terminal non-reducing positions.

INTRODUCTION

Galactans, composed entirely or mainly of D- or of D- and L-galactose (Gal), are the typical storage carbohydrates in the spawn of various snails. These galactans are produced in the albumen glands of the adult animals and they are added to a perivitelline fluid which accumulates around the eggs during their passage through the oviduct. The galactans are metabolised almost exclusively by the growing embryos or the freshly hatched snails[1].

Although galactans have been described from a great variety of snails of all three subclasses[2], only few have been studied in detail[1,3–9]. L-Gal has been detected only in galactans of some pulmonate snails[3,5,9,10]. It is not known if the synthesis of L-Gal only occurs in a small group within the Pulmonates, the most developed subclass among snails, or if it occurs in the phylogenetically older subclasses in the

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Prosobranchiata and the *Opisthobranchiata*. The galactan of *Ampullarius* spec., the only polysaccharide of a *Prosobranchiata* studied so far, was reported to be devoid of L-Gal[7].

L-Gal was assumed by Weinland[10] to be in terminal non-reducing positions in the galactan of *Helix pomatia* (Hp). Bretting *et al.*[9] detected, in the L-Gal-containing galactans of Hp, *Cepaea nemoralis* (Cn), and *Arianta arbustorum* (Aa), (1→3)- and (1→6)-linked β-Gal residues and ~10% of (1→2)-linked Gal residues. This percentage is close to that for the L-Gal content.

After one cycle of Smith degradation[11,12], performed with the galactans of Hp and Aa, the L-Gal content was lost and it was hypothesised that (*a*) L-Gal is in terminal positions (1→2)-linked to subterminal D-Gal, or (*b*) the Gal residues substituted at position 2 consist of L-Gal, or (*c*) the L-Gal is present in both positions.

Recently, König *et al.*[13] developed a chiral stationary phase for g.l.c. to separate the D and L enantiomers of neutral monosaccharides as the trifluoroacetylated methyl glycosides. This method has been used to investigate the occurrence of L-Gal in snail galactans from different subclasses and to determine the position of L-Gal in the galactans of Hp, Cn, and Aa.

RESULTS AND DISCUSSION

The L-galactose content of snail galactans. — L-Gal was identified first in the galactan of Hp by Bell and Baldwin[14], after Hammarsten[15] had already noticed a negative $[\alpha]_D^{20}$ value ($-15.8°$) for the native galactan caused by the α-L-Gal content. An L-Gal content of 12.5–14.3% was calculated from the optical rotation of the hydrolysed polysaccharide[9,14,16]. Comparable quantities of L-Gal were found in the galactans of Cn. Correa *et al.*[5] reported >36% of L-Gal in the galactan of *Biomphalaria glabrata* (Bg), whereas Bretting *et al.*[9] found only 12.2%, although the $[\alpha]_D^{20}$ value of the galactan was positive ($+15.5°$). More recently, Jacomini *et al.*[8] showed by enzymic studies that the galactan of Bg was devoid of L-Gal.

We have investigated, for their L-Gal content, twelve different galactan preparations from nine snails belonging to the *Pulmonata* and to the *Prosobranchiata*, two of the three subclasses of the *Gastropoda*. The results are summarised in Table I.

Among the *Stylommatophora*, the land-bound snails of the subclass *Pulmonata*, five species from three different families were studied. Hp, Aa, and Cn, which are closely related and belong to the family *Helicidae*, contained 12–14% of L-Gal in their galactans, whereas the representatives of the two other families, *Achatina fulica* (Af) (*Achatinidae*) and *Arion* spec. (*Arionidae*), were devoid of L-Gal. The galactans of Aa and Hp had lost all L-Gal residues after one cycle of Smith degradation.

No L-Gal was found in the galactans isolated from Bg and *Lymnea stagnalis* (Ls), both of which belong to the fresh-water-bound *Basommatophora*, the other order of the *Pulmonata*.

TABLE I

CONTENT OF L-Gal IN VARIOUS PREPARATIONS OF SNAIL GALACTANS

Subclass order, family and species tested	*L-Gal[a] (%)*	
	α-Galp	*β-Galp[b]*
S.C. *Prosobranchiata*		
(O. Monotocardia, F. Ampullariidae)		
Ampullarius spec.	0.1	0.1
Marisa spec.	15.2	14.3
S.C. *Pulmonata*		
(*a*) O. Basommatophora (fresh-water snails)		
F. Planorbidae		
Biomphalaria glabrata	0.1	0.0
F. Lymnaeidae		
Lymnaea stagnalis	1.1	1.0
Lymnaea stagnalis egg-shell polysaccharide	12.7	10.9
(*b*) O. Stylommatophora (land snails)		
F. *Helicidae*		
Helix pomatia	14.0	13.6
Helix pomatia after		
1. IO_4^-/BH_4^- treatment	0.4	0.1
Arianta arbustorum	14.1	14.4
Arianta arbustorum after		
1. IO_4^-/BH_4^- treatment	0.4	0.1
Cepaea nemoralis	11.5	10.9
F. *Achatinidae*		
Achatina fulica	0.1	0.1
F. *Arionidae*		
Arion spec.	0.1	0.0

[a]As trifluoroacetylated methyl glycoside. [b]Quantification of L-Gal in the β anomer is less reliable because of the small peak areas.

However, 11.8% of the Gal content was identified as the L form in a polysaccharide isolated from the egg-shells of Ls, which also contained[17] Fuc 20%, HexA 16%, and HexN 6.5%.

Only two species of the *Prosobranchiata,* the phylogenetic oldest subclass of the *Gastropoda,* were studied. The galactan of *Arion* spec. was devoid[7] of L-Gal but, in the polysaccharide obtained from *Marisa* spec., 15% of the Gal content was the L form (Fig. 1*b*). This polysaccharide also contained Fuc 10%, HexA 12%, HexN 7.5%, and ~30% of unidentified material[17], demonstrating the capability of the method for determining the D/L ratio of a monosaccharide in the presence of various other components.

Thus, it was shown for the first time that L-Gal is not restricted to a small group of evolutionary highly developed snails within the pulmonates, but is also

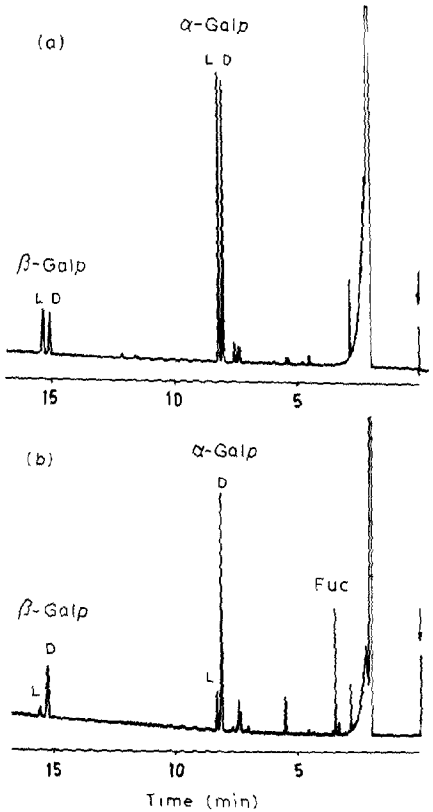


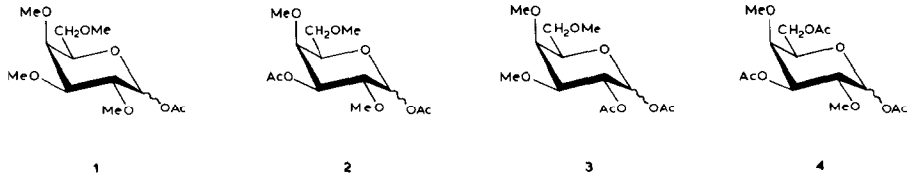
Fig. 1. G.l.c. of the methyl galactoside enantiomers after trifluoroacetylation: (*a*) standard mixture of D- and L-galactose, (*b*) hydrolysate of *Marisa* spec. galactan.

found among representatives of the subclass *Prosobranchiata,* which includes *Marisa* spec.

Isolation of the methylated derivatives by h.p.l.c. — The snail galactans are highly branched polysaccharides[9]. In the galactans of Hp, Cn, and Aa, ~80% of the Gal residues are located either in terminal, non-reducing positions or at branch points. The remaining 20% form almost equal portions of (1→2)- and (1→3)-linked sections. Each galactan contains 12–14% of L-Gal.

The →2)-Gal-(1→ structural element was absent from the galactans of Bg[9], Ls[17], and Af[18], and each was devoid of L-Gal. Nothing is known about the occurrence of this component in the galactans of *Marisa* spec and in the polysaccharide from the egg-shells of Ls. The only galactan studied so far, where galactose is substituted at C-2 but is lacking L-Gal, was from *Ampullarius* spec.

The location of the L-Gal in the galactan of Hp was suggested to be at the non-reducing end in an α-(1→6) linkage[10,19]. However, according to Bretting *et al.*[9], L-Gal could also be α-(1→2)-linked to D-Gal or it could be the structural



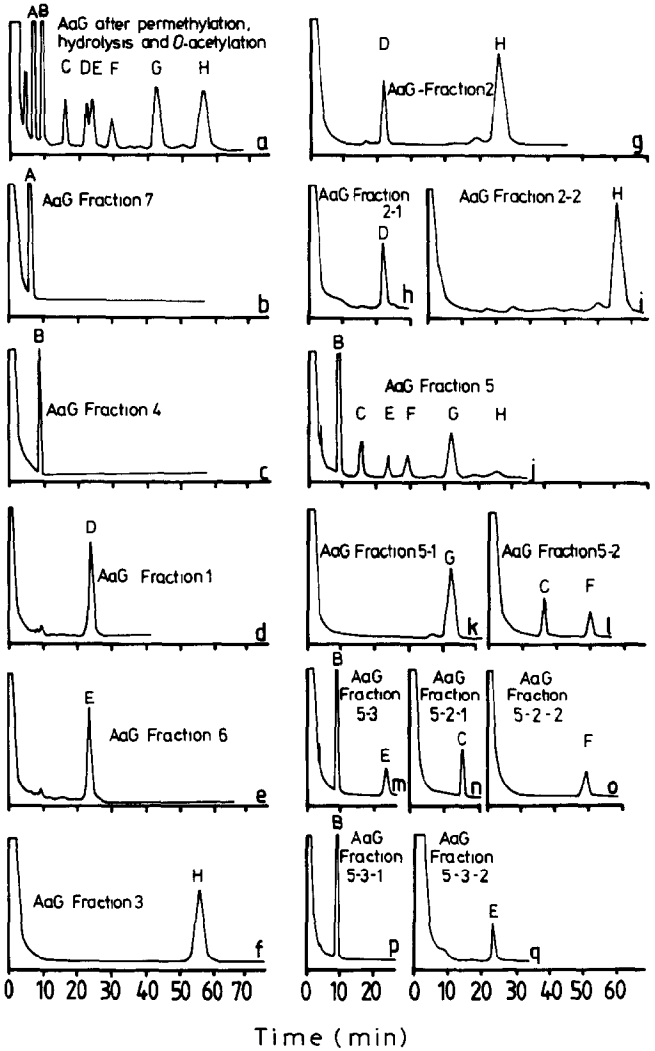


Fig. 2. G.l.c. on 3% OV-225 at 175° of acetylated galactose derivatives isolated from a hydrolysate of methylated AaG: (*a*) unfractionated hydrolysate; (*b*)–(*f*) fractions 1, 3, 4, 6, and 7 obtained by h.p.l.c. (solvent I); (*g*)–(*i*) fraction 2 and its further separation with solvent II; (*j*)–(*q*) fraction 5 and its subfractions with solvents II and III.

element substituted at position 2. To clarify whether L-Gal is either terminal or subterminal within the galactans of Hp, Aa, and Cn, the various structural elements were isolated and investigated for their L-Gal content.

The three galactans were each methylated twice by the Hakomori method and then hydrolysed, and the products were acetylated. Each product contained the α and β anomers of **1–4**. A gas chromatogram (3% OV-225) for the products from the galactan of Aa is shown. The eight components were isolated by h.p.l.c., using three solvent systems (see Experimental), and shown to be pure by g.l.c. (Fig. 2, *b–f*, *k*, *n*, and *o*).

The separation procedure for the products obtained from the galactan of Cn

TABLE II

^{1}H-N.M.R. DATA (CHEMICAL SHIFTS, δ)

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*OMe*	*OAc*
1α[a]	6.37d	3.73dd	3.51dd	3.78dd	3.96m	3.36, 3.44, 3.51, 3.56s	2.12s
1β[a]	5.45d	3.48dd	3.23dd	3.71dd		3.35, 3.51, 3.53, 3.55s	2.11s
2α[b]	6.41d	3.81dd	5.14dd	3.78dd	4.08mc	3.33, 3.39, 3.47s	2.12, 2.13s
2β[b]	5.51d	3.59dd	4.87dd	3.72dd	3.76ddd	3.30, 3.45, 3.46s	2.10, 2.13s
3α[b]	6.28d	3.52dd	3.60dd	3.81dd	3.98m	3.33, 3.43, 3.51s	1.99, 2.07s
3β[b,c]	5.59d	5.32dd	3.34dd	3.77dd	3.69ddd	3.35, 3.42, 3.55s	2.04(2)s
4α[b,d]	6.24d	3.83dd	5.15dd	3.75dd	4.09–4.19m	3.40, 3.47s	2.07, 2.13, 2.15s
4β[b,e]	5.53d	3.61dd	4.87dd	3.68dd	3.81dt	3.45(2)s	2.05, 2.13, 2.15s
8-*exo*[f,g]	5.73d	4.19dd				—	—
8-*endo*[f,h]	5.58d	4.07dd				—	—
9-*exo*[i,j]	5.66d	4.29dd	3.57dd	3.72t	4.00ddd	3.39, 3.52, 3.53s	—
9-*endo*[i,k]	5.26d	4.16dd		3.74t	4.02ddd	3.40, 3.52, 3.54s	—
10α[l,m]	5.43dd~t	5.15dd	3.70dd	3.74dd	4.15mc	3.39, 3.49, 3.56s	2.12s
10β[n]	4.53dd	5.02dd	3.32dd	3.74dd	4.15mc	3.40, 3.47, 3.57s	2.12s
11α[o]	5.12d	3.80dd		3.72dd	4.08mc	3.28, 3.44(2)s	—
11β[l]	4.43d		3.19dd	3.67dd	4.08mc	3.29, 3.45(2)s	—

[a] 270 MHz in $CDCl_3$. [b] 400 MHz in $CDCl_3$. [c] 3.61 (dd, 1 H, H-6a), 3.54 (dd, 1 H, H-6b). [d] 4.23 (dd, 1 H, H-6a), 4.09–4.19 (m, 1 H, H-6b). [e] 4.26 (dd, 1 H, H-6a), 4.19 (dd, 1 H, H-6b). [f] 270 MHz in CD_3OD, *exo*.*endo* ~2.8:1, arbitrary assignment. [g] 3.63 (q, 2 H, OCH_2CH_3), 1.16 (t, 3 H, OCH_2CH_3), 1.61 (s, 3 H, orthoester Me). [h] 3.57 (q, 2 H, OCH_2CH_3), 1.17 (t, 3 H, OCH_2CH_3), 1.51 (s, 3 H, orthoester Me). [i] 400 MHz in $CDCl_3$, *exo*:*endo* 4.1, arbitrary assignment. [j] 3.67 (q, 2 H, OCH_2CH_3), 1.18 (t, 3 H, OCH_2CH_3), 1.64 (s, 3 H, orthoester Me) [k] 3.65 (q, 2 H, OCH_2CH_3), 1.19 (t, 3 H, OCH_2CH_3), 1.53 (s, 3 H, orthoester Me). [l] 270 MHz in $CDCl_3$, **10**α·**10**β 2·1. [m] 3.87 (d, 1 H, HO-1). [n] 3.94 (d, 1 H, HO-1). [o] 270 MHz in acetone-d_6, **11**α:**11**β 3.2

TABLE III

^{1}H-N.M.R. DATA (COUPLING CONSTANTS, Hz)

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$
1α	3.6	10.2	2.0	1.0		
1β	8.0	9.6	3.0	1.0		
2α	3.8	10.4	3.0	1.0		
2β	8.0	10.2	3.0	1.0	5.8	7.4
3α	3.6	10.4	3.0	1.0		
3β^a	8.2	10.0	2.8	1.0	5.4	7.4
4α^b	3.6	10.4	3.0	1.0	6.4	
4β^c	8.0	10.0	3.0	1.0	6.6	6.2
8-*exo*^{d}	5.0	6.2				
8-*endo*^{d}	5.0	6.2				
9-*exo*^{d}	4.6	6.2	2.6	2.6	6.8	10.8
9-*endo*^{d}	4.8	6.3	2.6	2.6	6.8	10.8
10α^e	3.8	10.0	2.8	1.6		
10β^f	8.0	10.1	3.0	1.6		
11α	3.7	10.0		1.0		
11β	7.6	9.7	3.0	1.0		

$^aJ_{6a,6b}$ −8.6 Hz. $^bJ_{6a,6b}$ −10.6 Hz. $^cJ_{6a,6b}$ −11.3 Hz. $^dJ_{H,H}$ −7.1 Hz. $^eJ_{1,1\text{-OH}}$ −3.0 Hz. $^fJ_{1,1\text{-OH}}$ −9.8 Hz.

TABLE IV

RETENTION TIMES (T)^a AND RELATIVE MOLAR RATIOS OF THE VARIOUS GALACTOPYRANOSE DERIVATIVES OBTAINED FROM *Arianta arbustorum* GALACTAN AFTER METHYLATION, HYDROLYSIS, AND *O*-ACETYLATION

Peak in g.l.c.	T	*Rel. molar ratio*	*Structural assignment by n.m.r. and g.l.c.–m.s.*
A	1	17.6	α-2,3,4,6-Me_4Gal
B	1.37	19.4	β-2,3,4,6-Me_4Gal
C	2.36	6.1	α-2,4,6-Me_3Gal
D	3.24	6.0	β-2,4,6-Me_3Gal
E	3.45	8.2	α-3,4,6-Me_3Gal
F	4.31	5.8	β-3,4,6-Me_3Gal
G	6.20	16.9	α-2,4-Me_2Gal
H	8.12	20.0	β-2,4-Me_2Gal

^{a}Retention relative to that of 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-α-galactopyranose on 3% OV-225 at 175°.

was identical to that described above, but that for the products obtained from the galactan of Hp involved only the first solvent system, so that the fractions corresponding to the peaks A, B, D, E, and H were obtained for further studies.

From 60 mg of the methylated galactan of Aa, 6–7 mg of each of the four fractions corresponding to peaks A, B, G, and H were recovered, together with 2.5 mg for peaks D and E, and 1.5 mg for peaks C and F.

The galactose derivatives were identified by g.l.c.–m.s., n.m.r. spectroscopy, and comparison of the alditol acetates with those from an earlier investigation[9].

TABLE V

CONTENT OF L-Gal OF VARIOUS PARTIALLY METHYLATED AND ACETYLATED FRACTIONS OBTAINED AFTER HYDROLYSIS AND ACETYLATION OF THE METHYLATED GALACTANS FROM Aa, Cn, AND Hp

Fraction[a]	L-*Gal* (%)	
	α-Galp	*β-Galp*
AaG 1-Ac-2,3,4,6-Me_4-α-Gal	29.9	29.8
AaG 1-Ac-2,3,4,6-Me_4-β-Gal	25.0	22.5
AaG 1,2-Ac_2-3,4,6-Me_3-α-Gal	0 5	0.0
AaG 1,2-Ac_2-3,4,6-Me_3-β-Gal	1.0	0.0
CnG 1-Ac-2,3,4,6-Me_4-α-Gal	25.7	30.0
CnG 1-Ac-2,3,4,6-Me_4-β-Gal	26.7	33.9
CnG 1,2-Ac_2-3,4,6-Me_3-α-Gal	0.4	0.0
CnG 1,2-Ac_2-3,4,6-Me_3-β-Gal	0.0	1.2
HpG 1-Ac-2,3,4,6-Me_4-α-Gal	27.0	30.9
HpG 1-Ac-2,3,4,6-Me_4-β-Gal	31.1	31.2
HpG 1,2-Ac_2-3,4,6-Me_3-α-Gal	0.2	0.4

[a]The fractions were demethylated and then converted into the trifluoroacetylated methyl α- and β-galactopyranosides.

The n.m.r. data for the α and β forms of **1–4** are given in Tables II and III. The chemical shifts (Table II) and coupling constants (Table III) indicate the configurations at C-1 and the methylation/acetylation pattern.

The structures, g.l.c. retention times, and molar ratios of the eight fractions isolated from the galactans of Aa, Hp, and Cn are given in Table IV.

Identification of L-*Gal in the partially methylated galactoses.* — Trifluoroacetylated methyl glycosides of unsubstituted D- and L-monosaccharides can be separated[13] by g.l.c. on a chiral stationary phase. Since L-Gal was expected to be either in the terminal non-reducing position or (1→2)-linked, the appropriate methylated methyl α- and β-glycosides of D- and L-Gal were prepared. The methyl 2,3,4,6-tetra-*O*-methyl derivatives were prepared by the Hakomori method[20,21]. 3,4,6-Tri-*O*-methyl-α- and -β-D-galactopyranose (**11**), previously prepared[22] by a seven-step synthesis, were synthesised conveniently using the orthoester route, and were converted into their methyl glycosides and trifluoroacetylated. However, the D and L forms could not be separated by g.l.c. on a chiral stationary phase.

In an alternative approach, each isolated fraction (500 μg) was demethylated with BBr_3 in dichloromethane, and the products were converted into the trifluoroacetylated methyl glycosides, the D and L forms of which could be separated and quantified by g.l.c. on a chiral stationary phase. The results are summarised in Table V. The gas chromatograms of the fractions 7 (peak A) and 6 (peak E) derived from the galactan of Aa are shown in Fig. 3 (*a* and *b*).

In each of the three galactans studied, L-Gal (22–34%) was detected only in the products obtained after demethylation of the tetra-*O*-methylgalactose fractions. All other fractions were devoid of L-Gal. Small deviations in the D/L ratio found

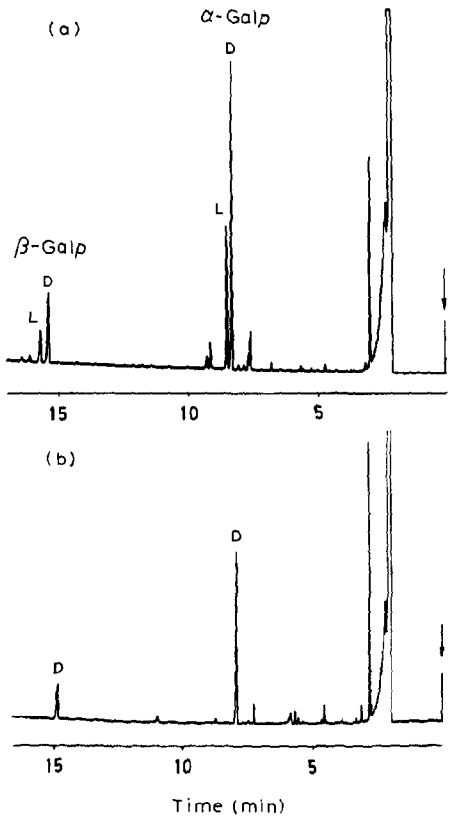


Fig. 3. Content of L-Gal in (*a*) the 2,3,4,6-tetra-*O*-methyl- and (*b*) the 3,4,6-tri-*O*-methyl-galactose fraction of AaG, corresponding to the peaks A and E, respectively, in Fig. 2a; L-Gal comprises up to 31% in (*a*), but is absent from (*b*).

between the methyl α- and β-galactosides are most likely due to impurities formed during the demethylation step. Since 2,3,4,6-tetra-*O*-methylgalactose is derived from terminal non-reducing positions in the native galactan, all the L-Gal must be present in this location. The linkage of L-Gal to the subterminal galactosyl residue remains to be determined. However, among pulmonate snails, there is a strict correlation between the appearance of L-Gal and of (1→2)-linked galactosyl residues. It is possible that α-L-Gal is added to position 2 of a terminal D-Gal residue in the growing polysaccharide and terminates elongation of this branch. Therefore, it would be more likely that L-Gal is (1→2)-linked to galactosyl residues and not (1→6)-linked as assumed by Weinland[23], who had detected traces of L-Gal in a D-Gal-(1→6)-D-Gal preparation from the galactan of Hp.

However, it is also conceivable that (1→2)-linked D-Gal is the structural requirement for the L-Gal transferase to introduce L-Gal into these polysaccharides. In this situation, L-Gal would be attached by (1→3) or (1→6) linkages, but there would still be a strict correlation between the incorporation of L-Gal and the presence of (1→2) linkages.

EXPERIMENTAL

Galactans. — The snails Aa and Cn were collected near Nürnberg, Hp snails were purchased from R. Stein (Esslingen), and *Ampullarius* spec. were a gift of Mrs. H. Moerbeck (Hamburg). The native galactans were extracted from albumen glands with 0.9% saline and purified as described earlier[9].

The snails of Bg strain Puerto Rico (a gift of the Tropeninstitut Hamburg) and *Marisa* spec. were kept in the Zoological Institute, University of Hamburg. Ls were raised in the Biological Institute of the University of Amsterdam; the galactans were extracted from their spawn and purified as for those from the albumen glands. The spawn of Ls was provided by the courtesy of Dr. W. van der Knaap. A sample of lyophilised eggs from *Arion* spec. was a gift of Professor L. Renwrantz (Hamburg). The galactan was isolated in the same way as for the other snail galactans. The galactan from Af was kindly supplied by Professor G. Uhlenbruck (Köln) as material purified from albumen glands, as described by Okotore *et al.*[24].

The polysaccharide from Ls egg-shells was prepared from the sediment of homogenised Ls spawn. This material was resuspended in 0.9% saline (50 g of sediment/1 L), stirred for 1 h, and centrifuged at 2000 r.p.m. to remove remaining galactans. The sediment was washed with distilled water (5 × 1000 mL) and a portion (100 mL) of the supernatant solution was concentrated to dryness *in vacuo*. An aqueous solution of the residue was checked for neutral sugars by the orcinol method[25]. If no carbohydrates were detected, the sediment was boiled with aqueous 25% potassium hydroxide, precipitated with ethanol at 2°, and dialysed against distilled water.

The galactans of Aa and Hp, after one cycle of Smith degradation, were those described by Bretting *et al.*[9].

Separation of the galactose enantiomers. — Samples (1 mg) of the galactans were hydrolysed in 0.3M sulfuric acid at 100° for 24 h and the products were converted[13] into the trifluoroacetylated methyl glycosides. Subsequent quantification of the D- and L-galactose was accomplished by g.l.c. on a 35-m fused-silica capillary column coated with XE-60–L-valine-(*S*)-α-phenylethylamide.

Galactans were methylated by the Hakomori method[20,21], the products were hydrolysed in 0.13M sulfuric acid, and the resulting sugars were treated with pyridine–acetic anhydride (1:1). The partially methylated sugar acetates were subjected to (*a*) g.l.c. using glass columns (2.5 m × 4 mm) containing Chromosorb Q (80–100 mesh) coated with 3% of OV-225, and a model 419 Packard Instruments gas chromatograph; and (*b*) g.l.c.–m.s. using a Finnegan MAT 311A instrument operating in the c.i. mode with isobutane as the reactant gas.

Isolation of the partially methylated and acetylated galactose derivatives by h.p.l.c. — Separation of the various derivatives was effected on a Serva steel column (25 cm × 7.6 mm) filled with silica gel Si 100 (0.003 mm); the flow rate was 1.5 mL/min and 0.5-mL samples were collected. Carbohydrate-containing fractions

were detected by t.l.c. [Silica Gel 60 (Merck)] of 5-μL aliquots of each fraction with detection by charring with sulfuric acid. Appropriate fractions were combined and checked for purity by g.l.c. The various components isolated had <2% of contaminants.

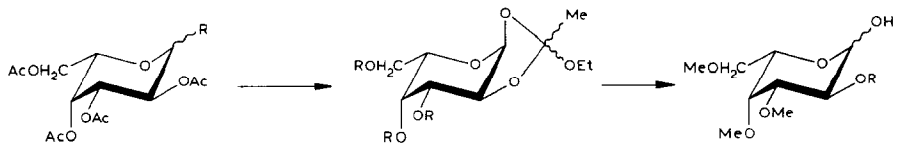
Hexane–ethyl acetate (3:2) was used first in h.p.l.c., and seven fractions were recovered, five of which were homogeneous (Fig. 2, *b–f*) and which corresponded to peaks A, B, D, E, and H in the gas chromatogram of the mixture.

Fraction 2 was subjected to h.p.l.c. with hexane–ethyl acetate (1:4). Each of the components isolated was homogeneous and was combined with the corresponding fractions 1 and 3 of the first separation step.

Fraction 5 contained five components and, using the second solvent system, three fractions (5-1, 5-2, 5-3) were obtained. Fraction 5-1 was homogeneous and was identical with peak G in g.l.c. (Fig. 2*k*). Each of the other fractions contained two components which were separated by h.p.l.c. using hexane–ethyl acetate–pyridine (5:5:1). Fraction 5-2 gave components C and F (Fig. 2, *n* and *o*) and 5-3 gave components B and E (Fig. 2, *p* and *q*). Thus, all of the constituents of the original mixture were isolated in a homogeneous state.

Demethylation was carried out with boron tribromide in dichloromethane[26] on ~0.5-mg samples. After evaporation of the solvents, each residue was extracted with dichloromethane (2 mL) to remove incompletely demethylated material.

*3,4,6-Tri-O-methyl-α- and -β-*D- *(and* -L-*)galactopyranose.* — D- or L-Gal was converted[27] into the β-penta-acetate (**5**) and thence[28] into the 2,3,4,6-tetra-*O*-acetyl-α-D(L)-galactopyranosyl bromide (**6**). Treatment with ethanol containing 2,4,6-trimethylpyridine and tetrabutylammonium bromide[29] gave a nearly quantitative yield of the acetylated orthoester derivative (**7**) with an *exo,endo*-ratio of 7:3 as previously reported[29]. Zemplén deacetylation then gave the 1,2-orthoester (**8**) (*exo,endo*-ratio 3:1), which was methylated to give **9** (*exo,endo*-ratio 4:1). On treatment (1 h, 20°) with aqueous 60% acetic acid, the 2-acetate (**10**) could be isolated. After saponification, 3,4,6-tri-*O*-methyl-α- and -β-D(L)-galactopyranose[12] (**11**) were obtained.



5 R = β-OAc
6 R = α-Br

7 R = Ac
8 R = H
9 R = Me

10 R = Ac
11 R = H

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