Identification of 4,5-Didemethyl-4-*O*-α-D-glucopyranosylsimmondsin and Pinitol α-D-Galactosides in Jojoba Seed Meal (*Simmondsia chinensis*)

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The isolation and identification of two pinitol α-D-galactosides from jojoba meal are described. The products were isolated by a combination of preparative HPLC on silica gel and TLC on amino silica gel and were identified by MS, NMR spectroscopy, and chemical derivatization as 5-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 5-α-D-galactopyranosyl-D-pinitol and 2-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 2-α-D-galactopyranosyl-D-pinitol. The same preparative HPLC method on silica gel allowed a new simmondsin derivative to be isolated and identified as 4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin mainly by NMR spectroscopy and high-resolution mass spectrometry.

Keywords: *Simmondsia chinensis; jojoba meal; water soluble carbohydrates; pinitol α-D-galactosides; simmondsins*

INTRODUCTION

The seeds of the jojoba plant (*Simmondsia chinensis Buxaceae*), an evergreen shrub native to the Sonoran desert, yield 50−60% of a liquid wax ester commonly referred to as jojoba oil. The oil is mainly used in cosmetics and lubricants. The seed flour, remaining after the oil is separated, composed of ∼30% protein along with carbohydrates and fiber, could possibly serve as an animal feed ingredient. However, when used as an animal food supplement, the meal causes food intake reduction and growth retardation. The inhibitory effect on feed intake is mainly due to the presence of simmondsin and simmondsin 2′-ferulate (*1*−*3*). The presence and composition of other simmondsin analogues and simmondsin ferulates has been described (*4*−*9*). Numerous procedures have been described to eliminate simmondsins and simmondsin ferulates from jojoba meal. More recently, extractions with water (*10*) and with organic solvents were described (*11*). The water-extracted meal is an effective animal feed supplement (*12*). The dried water extract was further separated by means of extraction with different organic solvents (*13*). Extraction with a mixture of ethanol and water (20:1, v/v) left a residue described by the authors as a "sugar residue" with a low level of simmondsins. Only pinitol was identified in the mixture by GC-MS. To optimize differential extraction procedures of jojoba meal, knowledge of the identitity of the different components of the mentioned mixture is needed. It was the aim of this work to isolate and identify the different components present in this sugar residue. The presence of carbohydrates such as galactinol in jojoba seed (*14*), pinitol in jojoba meal (*13*), and pinitol and sucrose in jojoba meal (*9*) has previously been described.

MATERIALS AND METHODS

Materials. A jojoba meal fraction (mixture A), isolated from the freeze-dried water extract from defatted jojoba meal, after extraction with a mixture of ethanol and water (20:1, v/v) was supplied by T. P. Abbott (New Crops and Process Research, USDA-ARS-NCAUR, Peoria, IL).

All solvents used were of analytical grade (Sigma-Aldrich, Bornem, Belgium).

Tri-SIL Z was obtained from Pierce (Rockford, IL).

Methods. *Thin-Layer Chromatography*. Analytical TLC was performed with 4 × 8 cm plates (Polygram SIL G/UV 254, Machery-Nagel) with a mixture of ethyl acetate, 1-propanol, and water (25:25:5, v/v/v) as eluent. The spots were vizualized with reagents specific for vicinal hydroxyls. The reagents were applied in two steps as follows. After spraying an aqueous 1% solution of sodium periodate in 3% acetic acid and heating the plates in an oven at 100 °C for 2 min, a second reagent containing 1 g of aniline, 1 g of diphenylamine, and 5 mL of 85% phosphoric acid, in 50 mL of acetone, was sprayed. Spots developed after the plates were heated in an oven at 130 °C for 5 min.

Sugars were visualized by means of the 1-naphthol spray. The spray was prepared by adding 10.5 mL of a 15% ethanolic

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solution of 1-naphthol to a mixture of 40.5 mL of ethanol, 4 mL of water, and 6.5 mL of sulfuric acid. After spraying with the reagent, the plates are heated in an oven at 100 °C for 5 min.

Preparative TLC was performed with amino silica gel HPTLC plates (SIL NH_2/UV254, Macherey-Nagel) and a mixture of ethyl acetate, pyridine, and water (2:1:2, v/v/v) as eluent.

HPLC. (1) Preparative HPLC. HPLC was performed by means of a silica gel column (Si 60 10 μm, 25 × 1 cm) with a mixture of 1-propanol, ethyl acetate, and water (25:25:5, v/v/v) as eluent at 3 mL/min and a refractometer (differential refractometer R 401, Waters) as detector. Mixture A (25 g) was taken up in 25 mL of methanol; a portion failed to dissolve and was isolated by filtration and recrystallized from water. The methanol was evaporated under vacuum, and the residue was dissolved in 250 mL of the mentioned eluent; 100 μL samples were injected. Eluents containing a single peak were evaporated under a stream of nitrogen and used for TLC, analytical HPLC, GC, GC-MS, MS, and NMR spectroscopy.

(2) Analytical HPLC. Analytical HPLC was performed by means of a silica gel column (Si 60 5 μm, 20 × 0.4 cm i.d.), with a mixture of acetonitrile and water (77:23, v/v) as eluent at 0.8 mL/min.

Mass Spectrometry. High-resolution mass spectrometry (HMRS) was performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1, v/v) mixture at 3 μL/min.

Low-resolution measurements were performed with a Kratos Concept 1H instrument by liquid surface assisted ionization mass spectrometry (LSIMS) using a 7-kV Cs beam. The spectra were obtained by dissolving the samples in a thioglycerol matrix.

Gas Chromatography. A Chrompack 9000 gas chromatograph equipped with a flame ionization detector was used for the analysis of silylated extract mixtures, silylated isolated compounds, or silylated hydrolysis products obtained from new isolated compounds. Separations were made by a 40 m × 0.2 mm i.d. glass capillary column with a chemically bonded cross-linked phenyl (50%) methyl silicone (CP Sil-24, Chrompack, Middelberg, The Netherlands) and 0.33 μm film thickness. Samples of 2 μL were injected by means of a split injector, 1:100. To protect the column, a special insert glass liner (Chrompack) was used. Injector and detector temperatures were 320 °C. Helium was used as carrier gas at 25 cm/s (set at 60 °C). Solutions were injected at 180 °C; the oven temperature was kept at 180° for 2 min and programmed to 250 °C at 10°/min and kept at 250 °C for 15 min. A Merck-Hitachi 2500 Chromatointegrator was used for measuring the chromatographic parameters.

Derivatization for Gas Chromatography. (1) Silylation. To 1 mg of carbohydrate was added 1.0 mL of Tri-SIL Z. After 30 min of reaction at 70 °C, the mixture was used for GC and GC-MS.

(2) Permethylation. The method described by Ciucanu and Kerek (*15*) with finely powdered NaOH and CH_3I as methylating agent was used.

Acid Hydrolysis. The carbohydrates (1−5 mg), isolated by HPLC, were dissolved in 1 mL of 2 N trifluoroacetic acid (TFA) in a reation tube. The capped tubes were kept in a water bath at 100 °C for 2 h. The hydrolysates were evaporated to dryness by means of a Savant Speed Vac Plus SC 110 A apparatus (Savant Instruments, Holbrook, NY), silylated, and examined by GC and GC-MS as described.

Gas Chromatography−Mass Spectrometry. Mass spectra were obtained with a Hewlett-Packard 5890 series II gas chromatograph equipped with a Hewlett-Packard 5971A mass selective detector and with an electron impact ion source (70 eV), a quadrupole mass filter, an electron multiplier detector, and an HP-1 column (cross-linked methylsilicone), 30 m × 0.25 mm i.d. and 0.25 μm film thickness. The carrier gas was He at 20 cm/s. Extracts were introduced by splitless injection at 70 °C. The oven temperature was kept at 70 °C for 2 min and programmed to 100 °C at 35 °C/min and from 100 to 270 °C at 10 °C/min. The oven temperature was then maintained at 270 °C for 30 min.

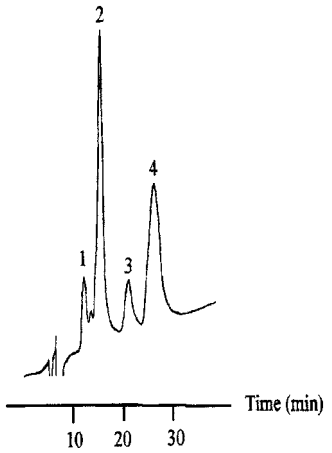

Figure 1. HPLC chromatogram of jojoba meal extract on silica gel: column, 25 cm × 1 cm i.d.; detector, refractive index; composition: pinitol (**1**), sucrose (**2**), 4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin (**3**), and a mixture of 5-α-D-galactopyranosyl-D-pinitol and 2-α-D-galactopyranosyl-D-pinitol (**4**).

Nuclear Magnetic Resonance Spectroscopy. ^1H and ^{13}C NMR spectra were recorded at 499.3 and 125.6 MHz, respectively, with a Varian Unity 500 instrument. Samples were dissolved in D_2O and measured at 27 °C. Chemical shifts are reported in parts per million (ppm) using the residual HOD signal set at 4.70 ppm as reference for the ^1H spectra and external dioxane set at 67.4 ppm for the ^{13}C NMR spectra. The coupling patterns in the ^1H NMR spectra were elucidated by homonuclear decoupling techniques and especially by 1D-TOCSY spectra (*to*tal *c*orrelation *s*pectroscop*y*). First-order analysis was used for extraction of the coupling constants. Peak assignments were based on 2D correlation experiments such as COSY (homonuclear *co*rrelation *s*pectroscop*y*) spectra or inverse detected GHSQC (*g*radient *h*eteronuclear *s*ingle *q*uantum *c*oherence) spectra. GHMBC (*g*radient *h*eteronuclear *m*ultiple *b*ond *c*oherence), NOE (*n*uclear *O*verhauser *e*ffect), and NOEDIF (*n*uclear *O*verhauser effect *dif*ference) spectra permitted unambiguous location of the point of attachment of carbohydrates to an aglycon.

RESULTS AND DISCUSSION

The fraction, from mixture A, that failed to dissolve in methanol was recrystallized from water. GC, GC-MS, LSIMS, ^{13}C NMR, optical rotation, and mp data correspond with known data for D-mannitol. Preparative HPLC, according to the described procedure, of the remaining fraction showed four peaks (Figure 1) with retention times of 12.7, 15.9, 22.4, and 27.5 min.

Isolation and Identification of D-Pinitol (1) and Sucrose (2). The first compound (t_R = 12.7 min) isolated by HPLC was identified as D-pinitol (**1**) and the second (t_R = 15.9 min) as sucrose (**2**) by comparaison of TLC, GC, GC-MS, NMR, and optical rotation values with the corresponding reference data.

Isolation and Identification of 4,5-Didemethyl-4-*O*-α-D-glucopyranosylsimmondsin (3). The same HPLC procedure allowed also 20 mg of the new compound (**3**) with t_R = 22.4 min to be isolated following 20 injections. Analytical HPLC of the isolated compound showed a single peak with t_R = 19.7 min.

*NMR Spectroscopy of Compound **3**.* The ^1H and ^{13}C NMR data of the isolated compound **3** are represented in Table 1. The ^1H and ^{13}C NMR spectra were readily

Table 1. ^{1}H and ^{13}C NMR Data for 4,5-Didemethyl-4-*O*-α-D-glucopyranosylsimmondsin and Simmondsin

	4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin (**3**)			simmondsin		
	^{13}C[a] (δ)	^{1}H	*J* (Hz)	^{13}C	^{1}H	*J* (Hz)
1′	104.34 (CH)	4.583 (d)[b]	8.0	103.96 (CH)	4.544 (d)	8.0
2′	74.02 (CH)	3.330 (dd)	8.0/9.3	73.97 (CH)	3.303 (dd)	8.0/9.2
3′	76.81 (CH)	3.509 (t)	9.2	76.95 (CH)	3.484(t)	9.2
4′	70.56 (CH)	3.436 (t)	9.2	70.37 (CH)	3.38−3.46	nd
5′	77.15 (CH)	3.422 (obs)	nd	77.07 (CH)	3.383−3.46	nd
6′	61.39 (CH_2)	3.748 (dd)	4.2/12.6	61.41 (CH_2)	3.847 (dd)	1.9/12.6
		3.857 (dd)	2.0/12.7		3.727 (dd)	4.4/12.6
1″	97.70 (CH)	5.100 (d)	3.7			
2″	72.52 (CH)	3.582 (dd)	3.7/9.6			
3″	73.95 (CH)	3.798 (t)	9.6			
4″	70.42 (CH)	3.448 (dd)	9.6/9.9			
5″	73.21 (CH)	3.932 (ddd)	2.4/4.9/10.1			
6″	61.53 (CH_2)	3.765 (dd)	4.8/12.4			
		3.831 (dd)	2.4/12.5			
1	78.15 (CH)	5.020 (t)	3.5	77.35 (CH)	4.950 (t)	3.7
2	164.63 (C)			165.09 (C)		
3	69.37 (CH)	4.832 (dd)	2.0/9.6	70.05 (CH)	4.696 (dd)	2.0/9.7
4	82.37 (CH)	3.601 (dd)	3.1/9.6	85.07 (CH)	3.284 (dd)	3.3/9.7
5	66.85 (CH)	4.378 (q)	3.3	75.52 (CH)	4.033 (q)	3.5
6	34.46 (CH_2)	1.931 (dt)	3.6/15.8	31.01 (CH_2)	2.569 (dt)	3.6/15.9
		2.448 (dt)	3.4/15.7		1.749 (dt)	3.7/15.9
7	96.87 (CH)	5.842 (d)	2.1	96.27(CH)	5.787 (d)	2.0
CN	118.01 (C)			118.02 (C)		
4-OCH_3				58.44 (CH_3)	3.451 (s)	
5-OCH_3				58.09(CH_3)	3.419 (s)	

[a] Determined by DEPT experiment. [b] Multiplicity is shown in parentheses.

assigned with the help of 2D homonuclear (COSY-45) and 2D heteronuclear GHSQC experiments together with edited DEPT (*d*istortionless *e*nhancement through *p*olarization *t*ransfer) spectra for differentiation of methine, methylene, and methyl carbons. 1D-TOCSY experiments with selective excitation at the anomeric hydrogens and using different mixing times successfully resolved overlapping signals.

From the ^{13}C NMR data it was immediately clear that the compound contains two sugar moieties: eight oxygenated methine protons together with two anomeric CH carbons at 104 and 97 ppm and two hydroxymethyl groups around 61 ppm. The presence of three separate moieties was confirmed by TOCSY experiments with selective excitation of the anomeric hydrogens at 4.583 (1′-H) and 5.100 (1″-H) ppm or at the aglycon hydrogens at 5.020 (1-H) or 4.832 ppm (3-H). Three resulting subspectra along with the normal spectrum are represented in Figure 2. At the same time these subspectra, together with the COSY and GHSQC spectra, allowed complete assignment of the ^{1}H and ^{13}C NMR spectra of the isolated compound. From the coupling constants (Table 1) it was easily concluded that the two carbohydrates were β- and α-glucopyranosyl groups both glycosidically bound to 1-OH and 4-OH, respectively, of the eight-carbon aglycon. The carbon atoms at 164.63, 118.01, and 96.87 ppm, the latter correlating with the ^{1}H signal at 5.842 ppm, are all characteristic of an *exo*-cyanomethylene group, similar to that found in simmondsin. The presence of this cyanomethylene group was confirmed by the presence of an IR absorption at 2217 cm^{-1} and a UV_{max} at 217 nm. The NMR data of the aglycon were also completely in agreement with those of simmondsin without the two methyl groups at 4-OH and 5-OH (Table 1) and with a supplementary α-glucose moiety positioned at 4-OH.

Definite confirmation of the glucose attaching points was obtained by NOEDIF and especially by heteronuclear long-range correlation GHMBC spectra.

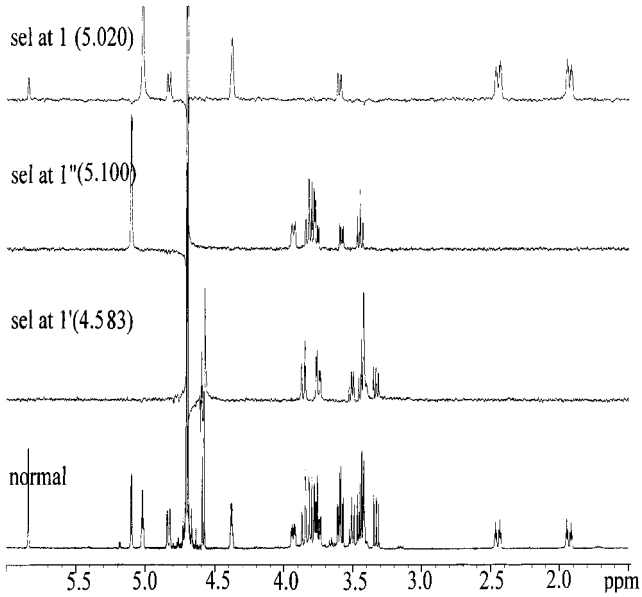

Figure 2. ^{1}H NMR spectra of 4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin (**3**) in D_2O at 500 MHz: normal and three selectively excited 1D-TOCSY subspectra for β-glucose moiety (sel of 1′-H at δ 4.583), for α-glucose moeity (sel of 1″-H at δ 5.100), and for the aglycon (sel of 1-H at δ 5.020).

Selective irradiation at 1′-H (4.583 ppm) or at 1″-H (5.100 ppm) resulted in increased peaks by NOE effect at 5.020 ppm (1-H, 12.2%), 3.509 ppm (3′-H, 8.5%), and 3.422 ppm (5′-H, 11.2%) or at about 3.59 ppm (2″-H and 4-H, 16%), respectively. In the GHMBC spectrum a cross-peak was clearly visible between 4.583 ppm (1″-H) and 78.15 ppm (C-1) for the β-glucose and between 5.100 ppm (1″-H) and 82.37 ppm (C-4) for the α-glucose.

Mass Spectrometry. The molecular formula of $[C_{20}H_{31}NO_{14} + Na]^+$ was established on the basis of high-resolution mass measurement of the $[M + Na]^+$ ion (532.1649 observed, 532.1642 calculated).

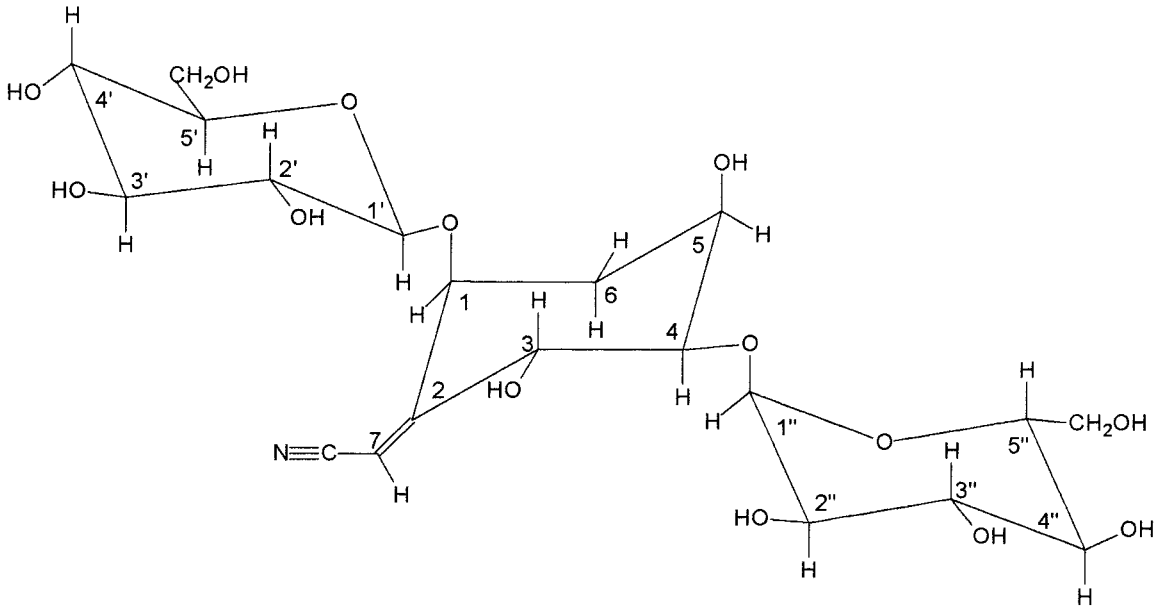

Figure 3. Structure of 4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin (**3**).

The results from NMR and HRMS in combination established the isolated compound as the 4-*O*-α-D-glucopyranosyl derivative of 4,5-didemethylsimmondsin or 4,5-didemethyl-4-*O*-α-D-glucopyranosylsimmondsin. The chemical stucture of the isolated compound is represented in Figure 3.

Isolation and Identification of Two Pinitol α-D-Galactosides (4_1) and (4_2). The chromatogram obtained by the preparative HPLC procedure also showed a peak with t_R = 27.5 (Figure 1, peak 4). GC of this silylated fraction, however, showed two peaks with t_R values of 24.75 and 25.50 min, respectively. TLC on amino silica gel of this HPLC peak also showed two spots, each corresponding with one peak in the GC. The spot with an R_f value of 0.22 (**4_1**) corresponded with the peak with t_R = 24.75 min, and the spot with an R_f value of 0.12 (**4_2**) corresponded with the peak with t_R = 25.50 min. After preparative TLC on amino silica gel, the two isolated compounds were identified by a combination of chemical (permethylation and acid degradation) and physicochemical data (NMR and MS).

GC-MS. The spectra obtained by the mentioned GC-MS method did not represent molecular ions for the two isolated products (the range of the apparatus allows only detection of fragments ions up to *m*/*z* of 650) and showed only fragmentation patterns useful for more general structural information. The most intense fragments seen for both substances are at *m*/*z* 204, a common characteristic fragment from TMS ethers of many carbohydrates.

Permethylation. Permethylation of the products **4_1** and **4_2** resulted in the same permethylated derivative, as shown by their identical retention times on two different stationary phases—a methylphenyl silicone and a methyl silicone, respectively,—and by their identical mass spectra obtained by GC-MS.

*Acid Hydrolysis of Products **4_1** and **4_2**.* GC and GC-MS analysis of the silylated hydrolysis products showed the presence of α- and β-galactose together with pinitol. Separation of the hydrolysis mixture by preparative TLC showed two dextrorotatory compounds, which were identical with reference samples of D-galactose, after equilibration by mutarotation and D-pinitol, respectively. This means that both products are positional isomers of pinitol galactosides, a structural feature to be elucidated by NMR. Because in the acid reaction medium galactose isomerizes to a mixture of α- and β-galactose, the problem of an α- or β-glycosidic bond was also solved by NMR spectroscopy.

NMR Spectroscopy. The NMR data from the two isolated compounds are shown in Table 2.

As can be seen from Table 2, the presence of galactose in both compounds **4_1** and **4_2** was clearly shown by the appearance of its typical signals in the ^{13}C as well as in the ^1H NMR spectra. The large coupling (10.4 Hz) between the axial hydrogens 2′-H and 3′-H together with the small couplings between 4′-H and its vicinal protons, typical for an equatorial proton with axial neighbors, points to a galactosyl moiety in the C1-conformation. The α-configuration at 1′ was established by the small couplings (3.7 and 3.9 Hz) of the 1′-H doublets, typical for an equatorial proton with an axial neighbor. The relatively high ^{13}C chemical shift of the axially substituted C-1′ (96.89 ppm) points to a linkage with pinitol via a glycosidic bond.

The assignments for the aglycon moiety were similarly based on 2D correlations, using as the starting point the most upfield shifted 3-H proton (due to the effect of the Me substituent at 3-OH) at 3.384 ppm and corresponding to the most deshielded carbon C-3 at ∼83.83 ppm. Starting from this signal, it was very easy with the help of a COSY spectrum to deduce the structure of the cyclitol as 3-*O*-methyl-*chiro*-inositol or pinitol. The absolute configuration of galactose and pinitol was proved for both compounds to be D by acid hydrolysis and carefully measuring the optical activity of the hydrolysis products obtained after separation and purification by preparative TLC on silica gel and using ethyl acetate/1-propanol/water (25:25:5; v/v/v) as eluent.

The attaching point between the galactosyl residue and D-pinitol was determined on the basis of 1D-NOEDIF spectra and confirmed by the observation of a long-range $^3J_{C,H}$ coupling in the GHMBC spectrum. Indeed, NOEDIF for substance **4_1** (R_f 0.22), upon selective irradiation of 1′-H (5.095 ppm) showed a 23% increased signal at ∼3.841 ppm (overlapping signals of 2′-H, 2-H, and 5-H) and also an 11% increase of the 6-H signal at 4.192 ppm. The results clearly indicate a

Table 2. ^{1}H and ^{13}C NMR Data for 5-*O*-(α-D-Galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol (4_1) and 2-*O*-(α-D-Galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol (4_2)

	5-α-D-galactopyranosyl-D-pinitol (4_1)			2-α-D-galactopyranosyl-D-pinitol (4_2)		
position	^{13}C[a] in (δ)	^{1}H (δ)	J (Hz)	^{13}C[a] (δ)	^{1}H (δ)	J (Hz)
1′	96.89 (CH)	5.095 (d)[b]	3.9	95.68 (CH)	5.108 (d)	3.7
2′	69.42 (CH)	3.841 (dd)[c]	(3.9/10.4)[c]	69.33 (CH)	3.850 (dd)	3.7/10.4
3′	70.48 (CH)	3.935 (dd)	3.2/10.4	70.77 (CH)	3.900 (dd)	3.2/10.4
4′	70.31 (CH)	4.008 (dd)	1.0/3.2	70.30 (CH)	4.089 (dd)	1.2/3.3
5′	72.00 (CH)	4.192 (dt)[c]	(1.2/6.2)	72.01 (CH)	4.100 (brt)	6.3
6′	62.04 (CH_2)	3.724 (2H,d)	6.3	61.98 (CH_2)	3.731 (2H,d)	6.3
1	72.21 (CH)	4.036 (t)	3.7	68.44 (CH)	4.216 (t)	3.4
2	70.87 (CH)	3.832 (dd)	(3.7/9.9)	75.51 (CH)	3.898 (dd)	3.0/9.5
3	83.83 (CH)	3.384 (dd)	9.1/9.9	82.48 (CH)	3.440 (t)	9.6
4	71.86 (CH)	3.778 (dd)	9.0/9.8	73.45 (CH)	3.645 (t)	9.7
5	76.88 (CH)	3.823 (dd)	6.8/9.8	71.57 (CH)	3.776 (dd)	3.2/9.8
6	68.68 (CH)	4.192 (obs)	nd	71.01 (CH)	4.025 (t)	3.4
OMe	60.83	3.581 (s)		61.40 (CH_3)	3.593	

[a] Determined by DEPT experiment. [b] Multiplicity is shown in parentheses. [c] Determined by TOCSY experiment.

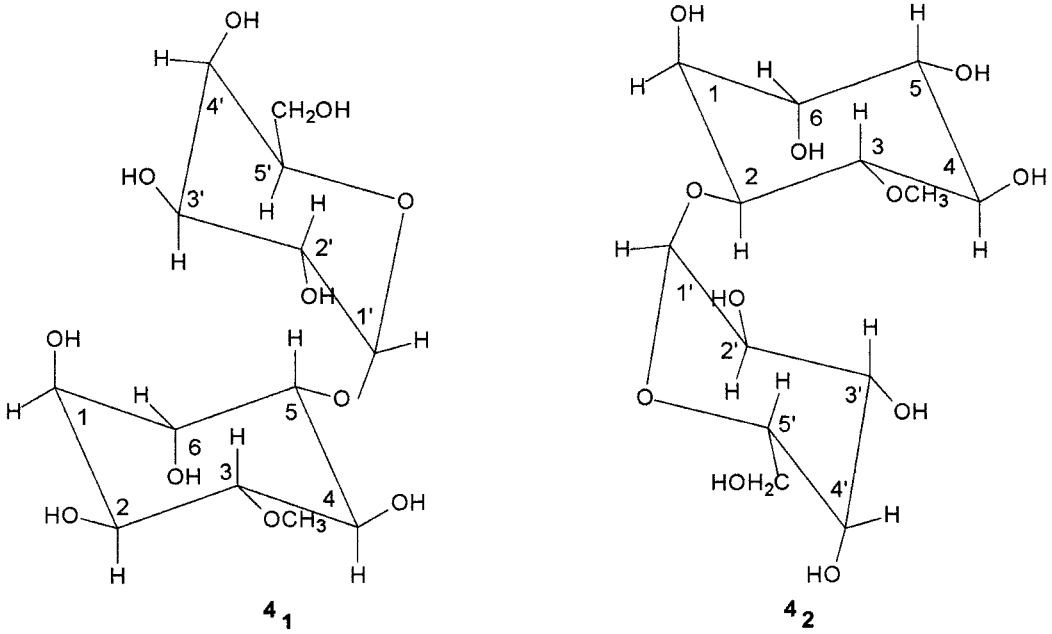

Figure 4. Structures of 5-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol (4_1) and 2-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol (4_2).

glycosidic coupling of the galactose moiety to the 5-OH or the 6-OH of pinitol. The ambiguity was resolved by the GHMBC spectrum, showing a distinct C,H-^3J coupling between 1′-H and C-5. Therefore, the galactosyl residue must be attached to the 5-OH of pinitol, and the structure of the isolated compound is 5-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 5-α-D-galactopyranosyl-D-pinitol. The spectral data of 4_1 are almost identical with the data published for galactopinitol A, a compound first described by Schweizer et al. (*16*) and subsequently synthesized by Garegg et al. (*17*).

Likewise, NOEDIF for compound 4_2 (R_f 0.12) with selective irradiation of 1′-H (5.108 ppm) showed a 17% increase of 1-H (4.216 ppm), a 12% increase of 2-H (3.898 ppm), and a 19% increase of the 2′-H signal (3.850 ppm). Those results indicate that in compound 4_2 pinitol is linked to the galactose via 1-OH or 2-OH. This ambiguity could again be resolved by a GHMBC spectrum, which clearly showed a distinct C,H ^3J coupling between 1′-H and C-2. The structure corresponds to 2-*O*-(α-D-galactopyranosyl)-3-*O*-methyl-D-*chiro*-inositol or 2-α-D-galactopyranosyl-D-pinitol. Our data compare very well with those of galactopinitol B as published by Garegg et al. (*17*).

Those results are further in accordance with the permethylation experiment, which gave the same methylated derivative when starting from either the isolated product 4_1 or 4_2, a fact that in the galactosyl pinitol isomers is only possible for two pairs of isomers, either the 2- and 5-galactosides or the 1- and 6-galactosides. The structures of both isolated compounds 4_1 and 4_2 are represented in Figure 4.

LSIMS Mass Spectrometry. LSIMS showed an $(M + 1)^+$ at *m/z* 357 for both compounds 4_1 and 4_2 as can be expected for condensation products of pinitol (194) and galactose (180).

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