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Structure of Galactosylononitol

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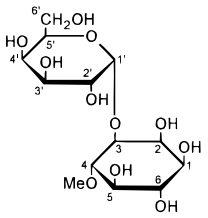
Abstract: A cyclitol galactoside was isolated from the seeds of *Vigna angularis* and shown to be identical with galactosylononitol. However, detailed 2D NMR data and methylation analysis resulted in the revision of the structure of galactosylononitol to *O*-α-D-galactopyranosyl-(1→3)-4-*O*-methyl-D-*myo*-inositol (**1**). Thus, compound **1** represents the only naturally occuring methylated derivative of galactinol.

Cyclitol galactosides accumulate during seed development in many important crop species, such as soybean, lentil, chick pea, or buckwheat.[1] Together with raffinose-series oligosaccharides, these α-galactosides are undigestible by human intestinal enzymes and therefore contribute to flatulence. However, they are thought to play an important role in the acquisition of desiccation tolerance and, hence, in storabilty and viability of seeds.[1] One of these cyclitol galactosides, a galactosylononitol, has previously been isolated from seeds of *Vigna angularis* Ohwi et Ohashi (Fabaceae). On the basis of periodate oxidation and methylation analysis, the structure of galactosylononitol was proposed to be *O*-α-D-galactopyranosyl-(1→5)-4-*O*-methyl-D-*myo*-inositol.[2] During a study on the ability of cyclitol galactosides to act as galactosyl donors in the biosynthesis of raffinose-series oligosaccharides, we observed that the ^{13}C-NMR spectral pattern of authentic (original) galactosylononitol[3] was different from the published spectrum, regardless of slight differences in detailed chemical shift values. Chemical and spectroscopic analysis led to the revision of the structure of galactosylononitol to *O*-α-D-galactopyranosyl-(1→3)-4-*O*-methyl-D-*myo*-inositol (**1**).



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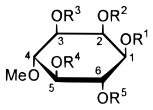

2a $R^1 = R^2 = R^3 = R^4 = R^5 = H$

2b $R^1 = R^2 = R^3 = R^5 = Me$, $R^4 = H$

2c $R^1 = R^2 = R^4 = R^5 = Me$, $R^3 = H$

Compound **1**, isolated from seeds of *V. angularis*,[4] was selectively cleaved by α-galactosidase yielding equimolar D-galactopyranose and D-ononitol[5] (1-D-4-*O*-methyl-*myo*-inositol, **2a**). The ^{1}H-NMR spectrum of **2a** showed an intense singlet at δ 3.61 corresponding to the methyl group, a triplet at δ 4.05, and five signals in the region of δ 3.36−3.66 (Table 1). The lowest field signal (δ 4.05) showed two small values of vicinal proton coupling constants ($J = 3.0$ Hz). This signal was assigned to the only equatorial proton of the ononitol ring (H-2), in agreement with the published spectrum of *myo*-inositol.[6] Large J values (9.4−10.0 Hz) of five protons (H-1, H-3 to H-6) indicated a sequential *trans*-diaxial relationship for these protons. In a one-bond ^{1}H−^{13}C correlation experiment (ge-HSQC),[7] a cross-peak was observed between the proton resonance at δ 3.40 and the lowest field carbon signal at δ 85.04. The strong deshielding of this carbon indicated methoxylation at this position, which was therefore assigned to C-4.[6] The rest of the proton and carbon resonances could then be identified and assigned by complete analysis of the DQF-COSY experiment and ^{1}H−^{13}C correlation spectra[7] (Table 1).

Analogous to the assignment of **2a**, the resonances of **1** could be identified by a combination of DQF-COSY and ^{1}H−^{13}C correlation experiments. Starting at the anomeric proton H-1′ (δ 5.16), the spin system of the

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Table 1. ^{1}H (500.1 MHz, D_2O, 25 °C) and ^{13}C-NMR (125.8 MHz, D_2O, 25 °C) Spectral Data of **1** and **2a**

	1		**2a**	
no.	δ_H[a]	δ_C	δ_H[b]	δ_C
1	3.50	73.41	3.50	73.58
2	4.29	70.32	4.05	74.97
3	3.75	77.13	3.63	73.16
4	3.52	83.80	3.40	85.04
5	3.38	76.41	3.36	76.30
6	3.70	74.89	3.66	75.05
CH_3	3.62	63.20	3.61	62.44
1′	5.16	97.45		
2′	3.89	70.73		
3′	3.94	72.19		
4′	4.07	71.72		
5′	4.11	73.55		
6′a	3.76	63.53		
6′b	3.76			

[a] Estimated J values (in Hz): $J_{1-2} = 2.7$; $J_{2-3} = 3.0$; $J_{3-4} = 9.7$; $J_{4-5} = 9.4$; $J_{5-6} = 9.4$; $J_{1-6} = 10.0$; $J_{1'-2'} = 4.0$; $J_{2'-3'} = 10.4$; $J_{3'-4'} = 3.4$; $J_{4'-5'} = <1$; $J_{5'-6'} = 6.7$. [b] $J_{1-2} = 3.0$; $J_{2-3} = 3.0$; $J_{3-4} = 9.7$; $J_{4-5} = 9.7$; $J_{5-6} = 9.4$; $J_{1-6} = 10.0$.

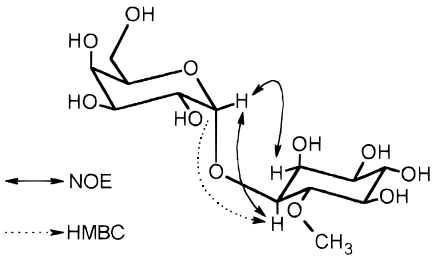

Figure 1. Selected NOE effects and an HMBC correlation for **1**.

galactosyl residue could be established (Table 1). The ^{1}H and ^{13}C NMR resonance values for this residue were very similar to those reported for galactinol (*O*-α-D-galactopyranosyl-(1→1)-L-*myo*-inositol).[8] On the basis of the fully assigned spectrum of **2**, the identification of the proton and carbon resonances of the ononitol moiety of **1** was straightforward. Compared to compound **2a**, three resonances were shifted in both the proton and the carbon spectrum (Table 1). While C-3 was strongly deshielded (+3.97 ppm), the vicinal carbons were both shifted upfield. In an alicyclic, six-membered inositol ring, the carbon atoms adjacent to a substituent are shifted upfield to various extents, depending on the configuration.[6] In the case of an adjacent carbon bearing an axial hydroxyl group, a shift of about −3.5 to −4.5 ppm can be observed, while adjacent carbons, when bearing an equatorial hydroxyl group, exhibit only small shifts (−0.2 to −1.0 ppm).[6] In the case of compound **1**, carbon C-2, bearing an axial hydroxyl group, was strongly shifted upfield (−4.5 ppm), while C-4, bearing the equatorial methoxyl group, was shifted upfield −1.2 ppm. This indicated the galactosyl residue to be attached to the ononitol ring at C-3 in compound **1**. In a NOESY experiment[7] ($t_m = 800$ ms), a cross-peak was observed between the glycoside anomeric proton H-1′ and the proton H-3, while no cross-peak could be found between H-1′ and H-5 (Figure 1).

Additionally, there was a strong cross-peak between H-1′ and H-2. It is only possible to observe an NOE interaction between the glycosidic anomeric proton and the proton on the carbon adjacent to the linkage site, if that proton is equatorial,[9−11] which apparently is only the case for H-2 in compound **1**. This provides strong evidence for the position of the linkage site to be at C-3. If the linkage site was at carbon C-5,[2] such an NOE cross-peak would not be possible. Further evidence was provided by a long-range ^{1}H−^{13}C connectivity experiment (ge-HMBC),[7] in which three-bond coupling constants across the glycosidic linkage do not depend on conformation.[11] In compound **1**, long-range couplings were observed between H-1′ and C-3 and between H-3 and C-1′, respectively (Figure 1). This provides unambigous proof of the position of the galactosyl residue to be attached to carbon C-3 of the ononitol ring. Hence, **1** has the structure *O*-α-D-galactopyranosyl-(1→3)-4-*O*-methyl-D-*myo*-inositol.

This finding is compatible with results previously obtained by periodate oxidation, which indicated a linkage site at C-3 or C-5.[2] However, permethylated, hydrolyzed galactosylononitol was reported to give a symmetrical cyclitol derivative (1,2,3,4,6-penta-*O*-methyl-*myo*-inositol, **2b**).[2] The ^{13}C NMR spectrum of **2b** was reported to have seven resonances. None of these signals was found in the region of δ 62−84,[2] implying that the signal of the carbon bearing the unsubstituted hydroxyl group must have been shifted upfield by at least 7 ppm relative to the original compound. Such large shifts are only observable in the case of a carbon bearing an axial hydroxyl group between two methoxylated carbons, as, for example, C-2 in 1,3-di-*O*-methyl-*myo*-inositol.[6,12] We therefore repeated the methylation experiment but were unable to confirm the published results.[2] The penta-*O*-methyl-*myo*-inositol (**2c**),[13] derived by permethylation and hydrolysis of **1**, revealed 11 ^{13}C-NMR resonances in accordance with the structure of **1**. A galactosylation at C-5 of the ononitol ring is not possible. Therefore, the published structure is incorrect and must be revised to **1**.

Thus, compound **1** represents a methylated derivative of galactinol, a well-known galactosyl donor in the biosynthesis of raffinose and stachyose.[1] Further investigation in the biological role of this substance in the metabolism of developing seeds and in human nutrition is urged.

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(2) Yasui, T. *Agric. Biol. Chem*. **1980**, *44*, 2253−2255.
(3) Authenthic galactosylononitol used was the original compound[2] kindly provided by T. Yasui: white powder; FABMS (glycerol) *m*/*z* [M + H]$^+$ 357; ^{13}C NMR (D_2O, 100.6 MHz) δ 96.97, 83.34, 76.67, 75.95, 74.42, 73.08, 72.95, 71.73, 71.25, 70.26, 69.85, 63.06, 62.74; published ^{13}C NMR[2] (D_2O) δ 96.22 (C-1′), 82.47 (C-4), 75.87, 75.17, 73.72, 72.24, 71.07, 70.56, 69.51, 69.19, 62.28, 61.77, 58.81 (the spectrum was not fully assigned).[2]
(4) Seeds of *V. angularis* (cvs. Wase Maruba and Takara Shodzu, kindly provided by the Asian Vegetable Research and Development Center, Tainan, Taiwan) were extracted with boiling water. The extract was deproteinized, deionized and chromatographed on charcoal−Celite,[14] followed by HPLC on an Aminex HPX-87C with distilled water at 85 °C. *O*-α-D-Galactopyranosyl-(1→3)-4-*O*-methyl-D-*myo*-inositol (**1**): white powder; FABMS (glycerol) *m*/*z* [M + H]$^+$ 357; [α]$^{20}_D$ +129.6° (*c* 0.5, H_2O); ^{1}H (D_2O, 500.1 MHz) and ^{13}C NMR (D_2O, 125.75 MHz) see Table 1.

(5) Hydrolysis of compound **1** by α-galactosidase (from green coffee beans) yielded D-galactose and D-ononitol (**2a**) in a molar ratio of 1:1.03 (as shown by comparison with authentic substances by HPLC and GC−MS of their TMSi ethers). 1D-4-*O*-Methyl-*myo*-inositol (**2a**) was isolated by HPLC (Dionex Carbopac MA1, 0.1 M NaOH, flow rate 0.4 ml min^{-1}): white powder; $[\alpha]^{20}_D$ + 6.4° (*c* 0.5, H_2O); CIMS (acetonitrile) *m/z* $[M + H]^+$ 195; EIMS of TMSi derivative (70 eV) *m/z* $[M]^+$ 554 (0.1), 318 (22.6), 305 (19.9), 260 (17.2), 247 (8.5), 217 (30.4), 191 (9.2), 147 (17.3), 89 (6.0), 73 (100); ^1H (D_2O, 500.1 MHz) and ^{13}C NMR (D_2O, 125.75 MHz) see Table 1.

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(7) 2D NMR spectra were for D_2O solutions measured on a Bruker DRX500 spectrometer operating at 500.1 MHz (^1H) or 125.75 MHz (^{13}C). ^1H and ^{13}C chemical shifts are measured relative to TSP-d_6 (sodium (trimethylsilyl)propionate) internal standard. Phase-sensitive NOESY (t_m = 800 ms) and DQF-COSY spectra[15] were typically acquired with a spectral width of 4 ppm in each dimension using a 1024 × 256 complex data matrix. The data were zero-filled to give a final data matrix of 2048 × 512 complex points, and a 90°-shifted sine-squared function was applied prior to Fourier transformation in each dimension. The gradient-enhanced HSQC[16] and HMBC[17] spectra were acquired with spectral widths of 4 and 60 ppm in the ^1H and ^{13}C dimensions, respectively, using a 1024 × 256 complex data matrix and were processed as described for the DQF−COSY spectra.

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