Plaxyloside from the Marine Sponge *Plakortis simplex*: an Improved Strategy for NMR Structural Studies of Carbohydrate Chains[‡]

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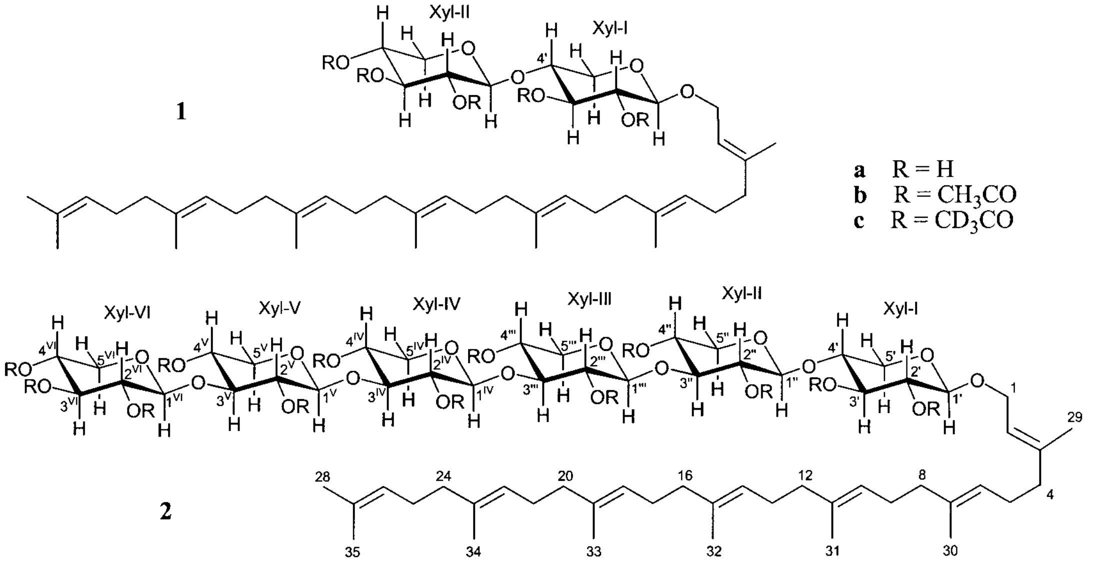
Plaxyloside, a glycolipid composed of a C_{35} linear polyisoprenoid alcohol aglycone and a linear carbohydrate chain made up of six β-xylopyranose units, has been isolated as its peracetate from the Caribbean sponge *Plakortis simplex*, and its structure determined on the basis of spectral and chemical data. Study of plaxyloside showed that: (a) carbohydrate chains with repetitive structures can be conveniently studied as their peracetates by NMR spectroscopy in C_6D_6, thanks to the peculiar conformational behavior of these derivatives, and (b) coupling constant analysis can be used to establish relative configurations in pyranose sugars even in the presence of conformational equilibria.

Introduction

Although the systematic study of glycolipids from marine sponges is quite recent, it is now well established that most marine sponges produce considerable amounts of glycolipids.[1] These are often glycosphingolipids, and sometimes glycoglycerolipids, although a number of atypical glycolipids, unclassifiable into any of the general classes into which glycolipids are divided, have also been found.

One of the richest source of unusual glycolipids is the Caribbean sponge *Plakortis simplex* (see Scheme 1 for some examples). To date, this species has been shown to contain plakosides (unique prenylated glycosphingolipids),[2] simplexides (glycosides of long-chain secondary alcohols),[3] crasserides (analogues of glycoglycerolipid first isolated from *Pseudoceratina crassa*[4] but also present in *P. simplex*), and plakopolyprenoside (**1a**, a glycolipid composed of a C_{35} linear polyprenyl alcohol and a dixylosyl carbohydrate chain).[5] We have now discovered a further novel glycolipid from *P. simplex*: plaxyloside (**2a**), composed of the same polyprenyl aglycone as in plakopolyprenoside (**1a**) and a linear chain of six β-xylopyranoses as the carbohydrate moiety. Structure elucidation of this new glycolipid was achieved by extensive spectroscopic analysis of its peracetate **2b** supplemented by microscale degradation. The peculiar conformational behavior of compound **2b** in benzene



Scheme 1

[‡] Glycolipids from Sponges, 10. − Part 9: Ref.[1]

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was the key for the structure elucidation of the repetitive carbohydrate chain of compound **2a**, and led us to an improved strategy for the structure elucidation of carbohydrate chains in the presence of conformational equilibria.

Results and Discussion

Specimens of *P. simplex* from the coast of Berry Island (Bahamas) were extracted with MeOH and then with $CHCl_3$. According to our usual procedure,[4] the extract was partitioned between water and *n*BuOH, and the organic phase was subjected to sequential column chromatography with RP-18 and normal silica gel, giving a crude glycolipid fraction that was acetylated with Ac_2O in pyridine. Repeated direct-phase HPLC separation of the peracetylated glycolipids provided 5.6 mg of pure plaxyloside peracetate (**2b**). A small portion of the crude glycolipid fraction was acetylated with trideuteroacetic anhydride instead of acetic anhydride,[5] and after the purification procedure gave the derivative **2c**, the ^{1}H NMR spectrum of which was identical to that of **2b** except for the absence of the 13 acetyl methyl singlets. This showed that none of the acetyl groups in **2b** had been present in the natural product **2a** before the acetylation reaction. The natural glycolipid **2a** could be obtained by the deacetylation of compound **2b** with Et_3N/MeOH at 40 °C for 12 h.

The negative ion FAB mass spectrum of compound **2a** showed a pseudomolecular ion peak at $m/z = 1285.6977$, indicating the molecular formula $C_{65}H_{106}O_{25}$. As usual in our work on glycolipids, all NMR experiments targeted at the structure determination of plaxyloside were performed on its peracetyl derivative **2b**.

A comparison of the ^{1}H and ^{13}C NMR spectroscopic data of compound **2b** (see Table 1) with those of plakopolyprenoside pentaacetate (**1b**)[5] showed that all the signals of the polyprenyl chain of **1b** were also present in **2b** with the same integration and multiplicity, so that the aglycone part of the two glycolipid must be the same: a geranyl-like polyprenyl chain composed of seven units.

As for the carbohydrate chain, the presence of six anomeric carbon signals between $\delta = 101.0$ and $\delta = 99.5$ in the ^{13}C NMR spectrum of **2b** pointed to the presence of six sugars. The wider range of the chemical shifts of the sugar protons in peracetylated glycolipids was particularly important in this case, since many resonances of protons belonging to different sugars, but in the same position, were almost coincident because of the repetitive structure of this glycolipid. Fortunately, in the ^{1}H NMR spectrum of **2b** recorded in C_6D_6, at least one signal for each sugar (2-H for Xyl-II and the anomeric protons for the other sugars) was not completely overlapped by other signals. Thanks to these signals, a HOHAHA 2D NMR experiment allowed all the sugar protons to be grouped into six different spin systems, each composed of six protons. An HMQC spectrum indicated that the six protons of each sugar were part of one oxymethylene and four oxymethine groups. This, along with the molecular formula, showed the carbohydrate chain of plaxyloside to be composed of six pentose units. The COSY

Table 1. NMR spectroscopic data of plaxyloside peracetate **2b** in C_6D_6 at 500 MHz

Pos.		δ_H [mult., *J* (Hz)]	δ_C (mult.)
1	a	4.37 (br. dd, 12.0, 6.0)	65.5 (CH_2)
	b	4.13 (br. dd, 12.0, 7.2)	
2		5.49 (br. t, 6.5)	120.4 (CH)
3		-	140.9 (C)
4		2.01 (br. t, 7.4)	39.8 (CH_2)
5		2.11[a]	26.7 (CH_2)
6		5.21[a]	124.2 (CH)
7		-	135.5 (C)
8, 12, 16, 20, 24		2.11[a]	40.2 (CH_2)
9, 13, 17, 21, 25		2.20[a]	27.1 (CH_2)
10, 14, 18, 22		5.30[a]	124.8 (CH)
11, 15, 19, 23		-	135.0 (C)
26		5.24[a]	124.9 (CH)
27		-	131.2 (C)
28		1.68 (br. s)	25.8 (CH_3)
29		1.52 (br. s)	16.4 (CH_3)
30		1.56 (br. s)	16.1 (CH_3)
31, 32, 33, 34		1.62−1.62 (4 singlets)	16.1 (CH_3)
35		1.56 (s)	17.7 (CH_3)
1′		4.36 (d, 7.5)	100.4 (CH)
2′		5.31[a]	71.9 (CH)
3′		5.38 (t, 9.1)	73.1 (CH)
4′		3.57 (ddd, 9.2, 9.2, 5.5)	76.1 (CH)
5′	a	3.76 (dd, 11.6, 5.4)	63.3 (CH_2)
	b	3.06 (dd, 11.6, 9.2)	
1′′		3.99 (d, 6.9)	101.0 (CH)
2′′		5.07[a]	72.8 (CH)
3′′		3.77 (t, 8.4)	77.8 (CH)
4′′		5.02 (ddd, 8.1, 8.1, 4.8)	69.8 (CH)
5′′	a	3.95 (dd, 11.8, 5.0)	62.8 (CH_2)
	b	3.06 (dd, 11.8, 8.1)	
1′′′		4.70 (d, 5.1)	100.5 (CH)
2′′′		5.14 (dd, 6.8, 5.1)	70.6 (CH)
3′′′		3.99[a]	74.9 (CH)
4′′′		5.07[a]	70.0 (CH)
5′′′	a	4.22 (dd, 12.3, 3.9)	61.4 (CH_2)
	b	3.38 (dd, 12.3, 5.7)	
1^{IV}		4.77 (d, 4.8)	99.5 (CH)
2^{IV}		5.14 (dd, 6.6, 4.7)	70.3 (CH)
3^{IV}		4.00[a]	74.4 (CH)
4^{IV}		4.97[a]	69.6 (CH)
5^{IV}	a	4.17 (dd, 12.3, 3.6)	61.1 (CH_2)
	b	3.37 (dd, 12.3, 5.6)	
1^{V}		4.69 (d, 6.0)	100.2 (CH)
2^{V}		5.21 (dd, 8.0, 6.1)	71.2 (CH)
3^{V}		3.87 (t, 7.8)	77.4 (CH)
4^{V}		4.97[a]	69.8 (CH)
5^{V}	a	3.98[a]	61.9 (CH_2)
	b	3.24 (dd, 12.1, 7.2)	
1^{VI}		4.72 (d, 6.1)	100.9 (CH)
2^{VI}		5.19 (dd, 8.0, 6.1)	70.6 (CH)
3^{VI}		5.37 (t, 7.9)	71.2 (CH)
4^{VI}		4.96[a]	69.3 (CH)
5^{VI}	a	3.98[a]	61.8 (CH_2)
	b	3.22 (12.0, 7.4)	
Ac	CH_3	2.16, 1.99, 1.97, 1.92, 1.87, 1.84, 1.81, 1.77, 1.74, 1.74, 1.70, 1.68, 1.59	20.0−20.4
	CO	-	170.6−169.7

[a] Overlapping signals.

spectrum was then used to find out the proton sequence within each sugar, thus completing the assignment of all the ^{1}H and ^{13}C signals of the carbohydrate chain (Table 1).

The subsequent steps in the structure determination of the carbohydrate chain were as follows:

(*i*) *Determination of the ring size of the sugars.* All the sugars were in the pyranose form, as shown by the HMBC correlation peak of the 5_b-H protons of each sugar with the respective C(1) carbons.

(*ii*) *Determination of the site of glycosylation in each sugar.* As we were working on the peracetylated derivative, this could easily be accomplished on the basis of the chemical shifts, the shielded oxymethine protons being the glycosylated ones. Thus, Xyl-VI is a terminal sugar (no shielded proton), Xyl-I is glycosylated at position 4 (4′-H at $\delta = 3.57$), and the remaining sugars are glycosylated at position 3 (3′′-H at $\delta = 3.77$, 3′′′-H at $\delta = 4.00$, 3^{IV}-H at $\delta = 3.99$, and 3^{V}-H at $\delta = 3.87$).

(*iii*) *Determination of the sugar sequence.* This was established by a series of HMBC correlation peaks: namely 1′-H with C(1), 1′′-H with C(4′), 1′′′-H with C(3′′), 1^{IV}-H with C(3′′′), 1^{V}-H with C(3^{IV}), and 1^{VI}-H with C(3^{V}).

(*iv*) *Determination of the nature of the sugars and of the stereochemistry of the glycosidic linkages.* As a rule, this was achieved by evaluation of the coupling constants between the sugar protons, permitting discrimination between axial and equatorial protons in a six-membered ring. Thanks to the combination of analysis of the 1D NMR spectrum and of the 2D HOHAHA experiment, we were able to measure most coupling constants, as reported in Table 2. Xyl-I and Xyl-II were readily identified as β-xylopyranoses, since the large (>7 Hz) coupling constants of 1-H, 2-H, 3-H, and 4-H showed all these protons to be axial. Xyl-V and Xyl-VI could also be shown to be β-xylopyranoses in the same way, even though the smaller coupling constant (about 6 Hz) of the anomeric protons 1^{V}-H and 1^{VI}-H raised some doubts in this case.

Unfortunately, the coupling constants of the "central" sugars Xyl-III and Xyl-IV were in the 4.8−6.6 Hz range, and prevented any easy configurational assignment. The uncertainty about the nature of the central sugars prompted us to perform a degradative analysis of plaxyloside. A small amount (200 µg) of plaxyloside was subjected to acidic methanolysis with 1 M HCl in 92% MeOH. The resulting methyl glycosides were silylated and analyzed by gas chromatography. Only two peaks were detected, with the same retention times and relative intensities as the silylated methyl glycosides obtained with the same procedure from an authentic sample of xylose. These data clearly showed that sugars III and IV were also xylopyranoses, but were not yet sufficient to elucidate their anomeric configuration, nor to explain their unusual coupling constants.

In the light of these results, the smaller coupling constants observed for Xyl-III and Xyl-IV can be explained by the assumption that these sugar residues spend a significant part of their time in the $^{1}C_{4}$ conformation instead of the most stable (for a mono-β-xylopyranoside) $^{4}C_{1}$ conformation (see Figure 1). In cases of fast chair inversion, the observed coupling constants are in fact the weighted mean of the coupling constants in the two possible chairs. Since the equatorial 2-H, 3-H, and 4-H can only show small coupling constants in a $^{1}C_{4}$ xylopyranoside, the higher the contribution from this conformation, the smaller are the observed coupling constants.

Such a conformational equilibrium is not unreasonable because, unlike the most common (gluco-, galacto-, and manno-) hexopyranosides, in which the $^{4}C_{1}$ conformation

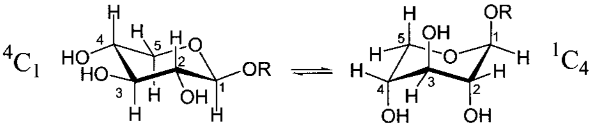


Figure 1. $^{4}C_{1}$ and $^{1}C_{4}$ conformations of a β-xylopyranoside

Table 2. Coupling constants of the carbohydrate chain of plaxyloside peracetate (**2b**) and their use to estimate the contributions from the two chair conformations in each sugar

Coupling Constant	Measured (Hz) I	II	III	IV	V	VI	Calculated (Hz)[a] $^{4}C_{1}$	$^{1}C_{4}$
1-H/2-H	7.5	6.9	5.1	4.8	6.0	6.1	7.8	1.8
2-H/3-H	9.1	8.4	6.8	6.6	7.9	8.0	9.3	3.6
3-H/4-H	9.1	8.3	N/A	N/A	7.8	7.9	9.3	3.6
4-H/5_b-H	9.2	8.1	5.7	5.6	7.2	7.4	10.0	1.3
4-H/5_a-H	5.5	5.0	3.9	3.6	N/A	N/A	5.4	0.8
% $^{4}C_{1}$ (estimated from $J_{2\text{-H}/3\text{-H}}$)[b]	96%	84%	56%	53%	75%	77%		
% $^{4}C_{1}$ (estimated from $J_{3\text{-H}/4\text{-H}}$)[b]	96%	82%	N/A	N/A	74%	75%		
% $^{4}C_{1}$ (estimated from $J_{4\text{-H}/5b\text{-H}}$)[b]	91%	78%	51%	49%	68%	70%		
% $^{4}C_{1}$ (average)	**95%**	**82%**	**53%**	**51%**	**72%**	**74%**		
Expected $J_{1\text{-H}/2\text{-H}}$ for the α anomer[c]	3.5	3.2	2.5	2.5	3.0	3.0		
Expected $J_{1\text{-H}/2\text{-H}}$ for the β anomer[c]	7.5	6.7	5.0	4.9	6.1	6.2		

[a] Coupling constants for the two chairs of xylopyranoside were estimated using the additivity rule for pyranose rings proposed by Altona et al. (ref.[7]). − [b] The contribution of the $^{4}C_{1}$ chair was estimated by using the following equation: % $^{4}C_{1} = [J(\text{exp}) - J(^{1}C_{4})]/[J(^{4}C_{1}) - J(^{1}C_{4})] \times 100$, where J(exp) is the measured coupling constant, and $J(^{4}C_{1})$ and $J(^{1}C_{4})$ are the estimated coupling constants in the $^{4}C_{1}$ and $^{1}C_{4}$ conformations, respectively. − [c] The expected coupling constants $J_{\text{H-1/H-2}}$ were calculated as weighted averages of the coupling constants of α- and β-xyclopyranoside in the $^{4}C_{1}$ conformation (1.8 and 7.8 Hz, respectively) and $^{1}C_{4}$ conformation (1.3 and 3.6 Hz, respectively) calculated using the additivity rule of Altona et al. (ref.[7]).

is strongly preferred, all the pentopyranosides except α-xylopyranosides show a significant or predominant contribution from the $^1C_4$ conformation.[6] A 19% contribution from the $^1C_4$ chair has been determined even for a β-xylopyranoside such as methyl tri-*O*-acetyl-β-xylopyranoside, the ring substituents of which are all equatorial in the $^4C_1$ chair; this is explained on the basis of the anomeric effect.

The contributions of the two chair conformations for each of the six sugars can be evaluated from the coupling constants between the sugar protons, provided that a close estimation of the coupling constants in the $^4C_1$ and in the $^1C_4$ chairs is available. We used the additivity rule of Altona et al.[7] for this estimation, and obtained the results reported in Table 2. The six sugars showed surprisingly different behaviors, the contribution of the $^4C_1$ conformation being minor for Xyl-I, significant for Xyl-II, Xyl-VI, and Xyl-V, and major for Xyl-III and Xyl-IV.

Similar, but inverse, reasoning was used to clarify the last still uncertain structural details: the anomeric configurations of Xyl-III and Xyl-IV. This time, the expected coupling constants of the anomeric protons in the cases of α and of β configurations in Xyl-III and Xyl-IV were calculated from the estimated conformational equilibrium of these sugars (see Table 2 for details). The measured 1-H/2-H coupling constants were much larger than the values expected for an α glycoside. In contrast, if the β configuration is considered, the agreement between the expected coupling constants and the experimental ones (which had not been used to estimate the conformational equilibria) is very good, with differences in the 0.1−0.2 Hz range. On one hand, this further confirmed that the atypical coupling constants of Xyl-III and Xyl-IV were dependent on equilibria between the $^4C_1$ and $^1C_4$ chairs, and on the other hand it allowed the β configuration to be assigned to Xyl-III and Xyl-IV even under the operation of such equilibria, thus completing the structure elucidation of compound **2b** and consequently of plaxyloside (**2a**).

The reason why sugars in very similar chemical environments should show such remarkably different conformational behavior is not entirely clear at the moment. However, it is interesting to note that this phenomenon does not seem to occur in the nonacetylated compound **2a**, as indicated by the following NMR evidence: firstly, those coupling constants of anomeric protons that could be measured (1′-H/2′-H, 1′′-H/2′′-H, and 1^{VI}-H/2^{VI}-H) were all 7.4 Hz, indicating a highly prevalent $^4C_1$ conformation; moreover, in the ^{1}H and ^{13}C NMR spectra of **2a** all the resonances of the same proton or carbon in Xyl-III, Xyl-IV, and Xyl-V, as well as most of those of Xyl-II, were coincident (see Exp. Sect.).

Our current hypothesis is that the flexible peracetylated carbohydrate chain has a preference for folded conformations, in which favorable electrostatic interactions between the partially charged atoms of the acetyl groups of different sugars are possible. In a sugar in the $^4C_1$ conformation, the C(3)/O and C(5)/O bonds are oriented in approximately opposite directions, so that a turn in the carbohydrate chain is only possible in connection with a sugar in the $^1C_4$ conformation. Since the most effective location for a turn is near the middle of the chain, this could explain the stronger tendencies of Xyl-III and Xyl-IV towards the $^1C_4$ conformation.

This hypothesis was supported by a preliminary molecular modeling conformation study. A conformation search was performed on model compound **3** (Scheme 2), by use of high-temperature molecular dynamics methodology in the CFF91 force field. A 1 ns dynamics simulation at 1200 K was performed, and a set of 200 structures was generated, the conformation of the molecule being saved every 5 ps; each structure was cooled to 100 K in 5.5 ps and then subjected to energy minimization. The energies of the minimized conformers were in the 49.5 kcal/mol range, but in subsequent analysis we considered only the 59 conformations within 20 kcal/mol of the lowest energy structure. As expected in view of the conformational equilibria described above, the molecule appears to be very flexible, and there is no preferential conformation; however, all the low-energy conformers have folded structures, and a sugar in the $^1C_4$ conformation generally coincides with a turn of the chain. As an example, Figure 2 shows the lowest-energy conformation obtained from this calculation: the carbohydrate chain is bent at Xyl-II and Xyl-III, and both these sugars are in the $^1C_4$ conformation. In addition, examination of the 59

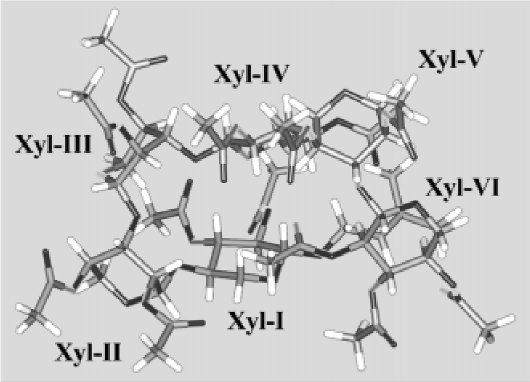
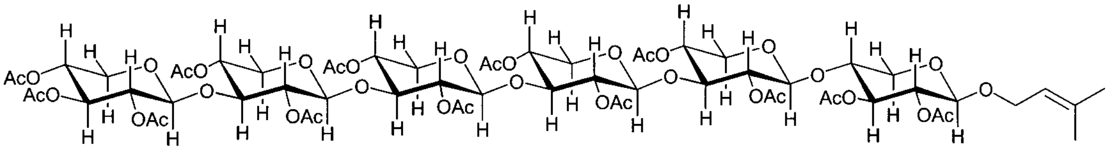


Figure 2. Lowest-energy conformation of the carbohydrate chain of plaxyloside peracetate (**2b**) as generated by the high-temperature molecular dynamics calculation



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Scheme 2

Table 3. Occurrence of the $^4C_1$, $^1C_4$, and boat conformations of each sugar of model compound **3** in the 59 low-energy conformers generated by the high-temperature molecular dynamics simulation

	Xyl-I	Xyl-II	Xyl-III	Xyl-IV	Xyl-V	Xyl-VI
$^4C_1$	64%	39%	26%	36%	39%	64%
$^1C_4$	24%	37%	59%	46%	42%	24%
boat	12%	24%	15%	18%	19%	12%

low-energy conformers showed that the occurrence of the $^1C_4$ conformation is higher for the sugars near the middle of the carbohydrate chain (Table 3), even though the calculated values are not very close to those estimated from NMR spectroscopic data. More detailed studies to clarify the origin of the atypical conformational behavior of compound **2b** are in progress.

Conclusion

Plaxyloside is a new example of the unusual class of polyisoprenoid glycolipids from *P. simplex,* first discovered with the isolation of plakopolyprenoside. In this case, however, the carbohydrate chain of the new plaxyloside is much longer, and it is the first natural example of an oligosaccharide containing more than three (1→3)-linked xylopyranosides. At first sight, the conformational equilibria of the pentapyranose sugars of plaxyloside have made the structure elucidation of the saccharide chain somewhat harder, although we have been able to explain all the spectroscopic data, and to assign the anomeric configurations of Xyl-III and Xyl-IV.

On the other hand, these equilibria affected not only the coupling constants, but also the ^{1}H and ^{13}C chemical shifts of sugars. Clearly, chemical shifts are different in the two chair conformations, and the observed chemical shifts depend on the contribution from each of them. Therefore, the different conformational behavior of the sugars in the peracetylated carbohydrate chain of compound **2b** also resulted in different ^{1}H and ^{13}C chemical shifts in different sugars. This was crucial for structure elucidation, because it avoided the complete overlap of the sugar signals to be expected for such a repetitive structure (and indeed observed for the nonacetylated compound **2a**) in both the ^{1}H and the ^{13}C NMR spectra, which would have made structure elucidation hard if not impossible. A careful choice of solvent was another important issue: signals in the ^{1}H NMR spectrum of compound **2b** showed remarkably better dispersion when the spectrum was recorded in C_6D_6 rather than in $CDCl_3$. This result can be explained by the strongly anisotropic shielding effect of the benzene molecule, which can more effectively turn differences in conformation into differences in chemical shifts. Even though more experimental work will be needed to generalize our results, we think that peracetylation and the use of C_6D_6 as the solvent should be the method of choice for NMR analysis of oligosaccharides with repetitive structures, a challenging problem even with the most recent NMR techniques.

Another important point is that the anomeric configurations of Xyl-III and Xyl-IV were determined from their 1-H/2-H coupling constants despite the almost 1:1 ratio of their conformers, and also that, more generally, the coupling constants of all six anomeric protons were predicted with a high degree of precision on the basis of the position of the conformational equilibrium. Therefore, conformational equilibria should no longer be considered a limitation to the usefulness of coupling constant analysis as a means for stereochemical determination in pyranose sugars. This further extends the scope of our strategy for the structural elucidation of carbohydrate chains, and will probably allow us to avoid chemical degradation completely in future work, especially when only low quantities of sample are available.

Experimental Section

General Remarks: FAB-MS spectra were performed in a glycerol matrix on a VG Prospec-Autospec (Fisons) mass spectrometer. Optical rotations were measured at 589 nm on a Perkin−Elmer 192 polarimeter, with a 10 cm microcell. ^{1}H and ^{13}C NMR spectra were determined on a Bruker AMX 500 spectrometer at 500.13 and 125.77 MHz, respectively; chemical shifts were referenced to the residual solvent signal (C_6D_6: δ_H = 7.15, δ_C = 128.0; CD_3OD: δ_H = 3.34, δ_C = 49.0). For accurate measurement of the coupling constants, the one-dimensional ^{1}H NMR spectra were transformed at 64 K points (digital resolution: 0.09 Hz). Homonuclear ^{1}H connectivities were determined by HOHAHA (mixing time 100 ms) and COSY experiments. Through-space ^{1}H connectivities were determined by means of a ROESY experiment with a mixing time of 500 ms. The reverse multiple-quantum heteronuclear correlation (HMQC) spectra were recorded by means of a pulse sequence with a BIRD pulse 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C; the interpulse delays were adjusted for an average $^1J_{CH}$ of 142 Hz. Gradient-enhanced multiple bond heteronuclear correlation (HMBC) was optimized for a $^3J_{CH}$ of 8.3 Hz. GLC analyses were performed on a Carlo Erba Fractovap 4160 equipped with a 25 m SPB-1 capillary column, with helium (5 mL/min) as carrier. High performance liquid chromatography (HPLC) was performed on a Varian 2510 apparatus equipped with a Varian Star 9040 refractive index detector.

Collection, Extraction, and Isolation: Specimens of *Plakortis simplex* were collected in the summer of 1998 near the coast of Berry Island (Bahamas), and identified by Prof. M. Pansini (University of Genoa, Italy). They were frozen immediately after collection and kept frozen until extraction. Reference specimens (ref. No. 98−41) were deposited at the Istituto di Zoologia, University of Genoa, Italy. The sponge (64 g dry weight after extraction) was homogenized and extracted with methanol (3 × 1 L) and then with chloroform (2 × 1 L); the combined extracts were partitioned between H_2O and *n*BuOH. The organic layer was concentrated in vacuo and afforded 20.4 g of a dark brown oil, which was chromatographed on a column packed with RP-18 silica gel. A fraction eluted with $CHCl_3$ (2.7 g) was further chromatographed on a SiO_2 column, to give a fraction [225 mg, eluent EtOAc/MeOH (9:1)] composed mainly of glycolipids. The main portion (200 mg) of this glycolipid fraction was peracetylated with Ac_2O in pyridine for 12 h. The acetylated glycolipids were subjected to HPLC separation on a SiO_2 column [eluent: *n*-hexane/EtOAc (1:9)] to afford a mixture (21 mg) containing plaxyloside together with other glycolipids. Further direct-phase HPLC purification [eluent: *n*-hexane/EtOAc (3:7)] gave 5.6 mg of pure plaxyloside peracetate (**2b**). The natural plaxyloside (**2a**) could be obtained in quantitative yield by deacetylation of the latter compound with 2 mL of $MeOH/Et_3N$ (8:2) at 40 °C for 12 h.

The remaining 25 mg of the glycolipid fractions were acetylated with $[D_6]Ac_2O$, and the same separation procedure gave 0.5 mg of plaxyloside pertrideuteroacetate (**2c**).

Plakyloside (2a): Amorphous solid, $[\alpha]_D^{25} = +21$ (MeOH, $c = 0.3$); HRFAMBS (negative ions): $m/z = 1285.6977$ ($[M - H]^-$, $C_{65}H_{105}O_{25}$ gives 1285.6945); ^1H NMR (500 MHz, CD_3OD): δ = 1.63 (br. s, 18 H, 30-H_3, 31-H_3, 32-H_3, 33-H_3, 34-H_3, 35-H_3); 1.70 (br. s, 3 H, 28-H_3), 1.71 (br. s, 3 H, 29-H_3), 2.02 (overlapped, 8-H_2, 12-H_2, 16-H_2, 20-H_2, 24-H_2), 2.09 (overlapped, 4-H_2), 2.11 (overlapped, 9-H_2, 13-H_2, 17-H_2, 21-H_2, 25-H_2), 2.16 (overlapped, 5-H_2), 3.23 (dd, J = 8.8 and 7.8 Hz, 1 H, 2′-H), 3.28 (overlapped, 5^{VI}b-H), 3.31 (overlapped, 2^{VI}-H), 3.31 (overlapped, 5′′b-H), 3.31 (overlapped, 5′′′b-H, 5^{IV}b-H, and 5^{V}b-H), 3.33 (overlapped, 5′b-H), 3.38 (t, J = 8.9 Hz, 1 H, 3^{VI}-H), 3.45 (t, J = 8.8 Hz, 1 H, 2′′-H), 3.48 (overlapped, 3′-H), 3.52 (overlapped, 2′′′-H, 2^{IV}-H, and 2^{V}-H), 3.53 (overlapped, 3′′-H), 3.55 (overlapped, 4^{VI}-H), 3.57 (overlapped, 3′′′-H, 3^{IV}-H, and 3^{V}-H), 3.63 (overlapped, 4′′′-H, 4^{IV}-H, and 4^{V}-H), 3.64 (overlapped, 4′′-H), 3.68 (m, 1 H, 4′-H), 3.93 (dd, J = 11.4 and 5.5 Hz, 1 H, 5_a^{VI}-H), 3.98 (overlapped, $5''_a$-H), 4.00 (overlapped, $5'''_a$-H, 5_a^{IV}-H, and 5_a^{V}-H), 4.04 (dd, J = 11.5 and 5.2 Hz, 1 H, $5'_a$-H), 4.21 (br. dd, J = 11.2 and 6.0, 1 H, 1b-H), 4.28 (d, J = 7.4 Hz, 1 H, 1′-H), 4.31 (br. dd, J = 11.2 and 7.2 Hz, 1 H, 1_a-H), 4.41 (d, J = 7.4 Hz, 1 H, 1′′-H), 4.56 (d, J = 7.4 Hz, 1 H, 1^{VI}-H), 4.64 (overlapped, 3 H, 1′′′-H, 1^{IV}-H, and 1^{V}-H), 5.13 (m, 6 H, 6-H, 10-H, 14-H, 18-H, 22-H, and 26-H), 5.40 (m, 1 H, 2-H); ^{13}C NMR (125 MHz, CD_3OD): δ = 16.3 (CH_3, C-30, C-31, C-32, C-33, and C-34), 16.7 (CH_3, C-29), 18.0 (CH_3, C-35), 26.1 (CH_3, C-28), 27.5 (CH_2, C-5), 27.8 (CH_2, C-9, C-13, C-17, C-21, and C-25), 40.8 (CH_2, C-4), 41.0 (CH_2, C-8, C-12, C-16, C-20, and C-24), 64.6 (CH_2, C-5′), 66.5 (CH_2, C-1), 66.6 (CH_2, C-5′′, C-5′′′, C-5^{IV}, and C-5^{V}), 67.1 (CH_2, C-5^{VI}), 69.6 (CH, C-4′′, C-4′′′, C-4^{IV}, and C-4^{V}), 71.1 (CH, C-4^{VI}), 73.7 (CH, C-2′′), 74.6 (CH, C-2′′′, C-2^{IV}, and C-2^{V}), 74.7 (CH, C-2′), 75.3 (CH, C-2^{VI}), 76.0 (CH, C-3′), 77.7 (CH, C-3^{VI}), 78.2 (CH, C-4′), 86.3 (CH, C-3′′), 86.5 (CH, C-3′′′, C-3^{IV}, and C-3^{V}), 103.4 (CH, C-1′), 103.6 (CH, C-1′′), 105.2 (CH, C-1′′′, C-1^{IV}, and C-1^{V}), 105.7 (CH, C-1^{VI}), 121.5 (CH, C-2), 125.1 (CH, C-6), 125.5 (CH, C-10, C-14, C-18, C-22, and C-26), 132.1 (C, C-27), 135.9 (C, C-7, C-11, C-15, C-19, and C-23), 142.2 (C, C-3).

Plaxyloside Peracetate (2b): Colorless oil, $[\alpha]_D^{25} = -60$ ($CHCl_3$, c = 0.5); ^1H and ^{13}C NMR: Table 1. Supporting information for compounds **2b** and **2c** is available on the WWW (see footnote on the first page of this article): ^1H NMR spectrum and COSY, HOHAHA, HMQC, and HMBC 2D NMR spectra of compound **2b**; ^1H NMR spectrum of compound **2c**.

Sugar Analysis: Plaxyloside peracetate (**2b**, 100 µg) was dissolved in 1 M HCl (1 mL) in 92% MeOH and allowed to react at 80 °C for 12 h in a sealed vial. The reaction mixture was then treated with solid Ag_2CO_3, centrifuged, and the supernatant liquid dried under a flow of nitrogen. The obtained mixture of methyl glycosides was then silylated with trimethylsilylimidazole in pyridine and analyzed by gas chromatography (oven temperature 138 °C). The chromatogram showed only two peaks, the retention times (17.5 min and 19.3 min, respectively) and relative integration (2.3:1) of which were the same as in the chromatogram of a sample of silylated methyl glycosides prepared from xylose by the same procedure.

Molecular Modeling Calculations: Molecular modeling studies were performed with the CFF91 force field in the program Discover 98.0 (Molecular Simulations Inc., San Diego, USA). Because the aglycon was expected to play a marginal role in the conformation of the carbohydrate chain, the calculations were performed on the model compound **3**. A conformer with a straight carbohydrate chain and all the sugars in the $^4C_1$ conformation was used as the starting structure. All the simulations were performed in vacuo. In order to direct the conformational search toward low-energy structures, the conformation of the carbonyl groups was restrained so that the carbonyl C=O bonds were synperiplanar to the respective C−H oxymethine bonds, since this is known to be the most stable conformation of esters.[8,9] A preliminary, unrestrained conformational study of methyl 2,3,4-tri-*O*-acetyl-β-xylopyranoside showed that all the acetyl groups adopt this conformation in the lowest energy structure. Molecular dynamics simulations were performed at 1200 K for 1 ns, and the coordinates produced by the simulation were saved every 5 ps, giving 200 structures. Each of these was cooled down 100 K in 5.5 ps and then subjected to energy minimization, using the steepest descent and the conjugated gradient methods in sequence. Finally, the restraints were removed and each structure was again minimized.

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