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Note

A conformational study of the Smith degradation product of the *Klebsiella* K40 capsular polysaccharide by 1D NOESY and molecular mechanics calculations

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A general drawback in conformational studies of oligosaccharides by NMR spectroscopy is the flexibility of glycosidic linkages and the paucity of NOE contacts [1]. Moreover, NOE measurements are often complicated by the spectral overlap typical of carbohydrates. In some cases, the latter problem can be solved by applying selective excitation techniques, such as the 1D analogue of the 2D NOESY experiment [2].

A better understanding of the spatial organisation of various biologically important polysaccharides may derive from the conformational analysis of related oligosaccharide fragments [3,4]. We describe here the approach followed to investigate the conformation of the oligosaccharide α-D-Gal*p*-(1→3)-α-L-Rha*p*-(1→2)-D-glyceraldehyde (**1**) in D_2O

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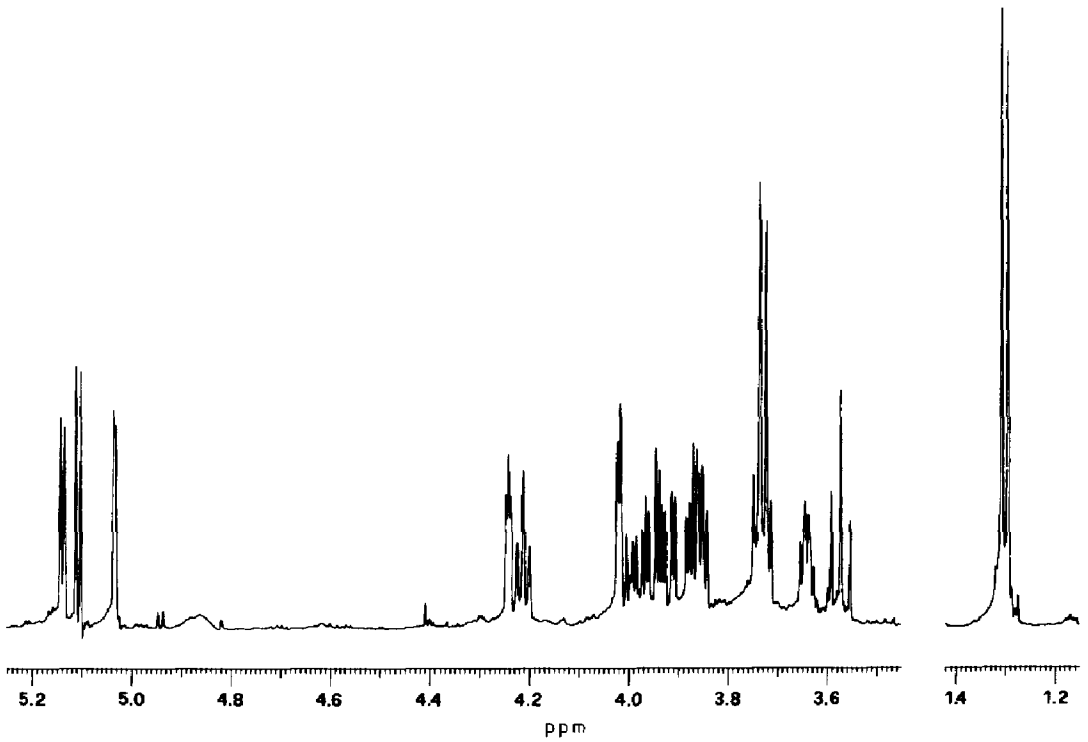

Fig. 1. The 500-MHz ^{1}H NMR spectrum of **1** in D_2O at 297 K. The small signal at 9.67 ppm attributed to H-1 of glyceraldehyde in the *aldehydo* form is not shown.

solution, using 1D NOESY measurements with half-Gaussian-shaped pulses [5] and molecular mechanics calculations with the MMP2(85) force field [6,7].

Compound **1** was obtained upon Smith degradation of the capsular polysaccharide produced by *Klebsiella pneumoniae* serotype K40, for which a revised structure has recently been published [8]. The ^{1}H NMR spectrum of **1** dissolved in D_2O (Fig. 1) was readily assigned by COSY and relayed COSY experiments (plots not shown) and by iterative simulation of the 500-MHz spectrum (Fig. 1), yielding the δ and J values given in Table 1. The signals at δ 9.67 and 5.11 were attributed to H-1 of glyceraldehyde (Glyc) in the *aldehydo* (2%) and hydrated aldehyde form (98%), respectively, and their chemical shifts and coupling constants were in agreement with published data [9]. Similarly, the ^{13}C resonance of Glyc C-1 (89.8 ppm) accorded with the reported value of glyceraldehyde hydrate [10]. Further support for the presence of glyceraldehyde hydrate was provided by the isotope effects on ^{13}C chemical shifts, when changing solvent from H_2O to D_2O. The ^{13}C NMR data of **1** in D_2O derived from heteronuclear correlation spectroscopy are given in Table 2.

The intraring $^3J_{H,H}$ coupling constants (Table 1) for **1** indicate essentially undistorted $^4C_1$ and $^1C_4$ conformations for the α-D-Gal and α-L-Rha residues, respectively. However, the observed J values for the Rha methyl and the Gal hydroxymethyl groups imply a considerable rotational freedom of these groups in solution.

The three-dimensional structure of **1** dissolved in D_2O was assessed by determination of the transient NOEs using 1D NOESY experiments. A first set of 1D NOESY experiments

Table 1
^{1}H NMR data for a solution in D_2O of the oligosaccharide **1** derived by Smith degradation of the *Klebsiella* K40 capsular polysaccharide

Residue	Atom	δ	$J_{H,H}$	(Hz)
α-D-Gal	H-1	5.14	$J_{1,2}$	3.9
	H-2	3.86	$J_{2,3}$	10.4
	H-3	3.95	$J_{3,4}$	3.2
	H-4	4.02	$J_{4,5}$	0.1
	H-5	4.21	$J_{5,6}$	6.3 [a]
	CH_2-6	3.73		
α-L-Rha	H-1	5.03	$J_{1,2}$	1.6
	H-2	4.24	$J_{2,3}$	3.2
	H-3	3.92	$J_{3,4}$	9.7
	H-4	3.57	$J_{4,5}$	9.7
	H-5	3.99	$J_{5,6}$	6.2
	CH_3-6	1.30		
D-Glyc [b]	H-1	5.11	$J_{1,2}$	4.9
	H-2	3.64	$J_{2,3a}$	5.0
	H-3a	3.73	$J_{2,3b}$	3.4
	H-3b	3.87	$J_{3a,3b}$	−12.4

[a] Second-order value, due to the same chemical shift for both CH_2-protons. [b] Hydrated form.

Table 2
^{13}C NMR data (δ in ppm) for a solution in D_2O of the oligosaccharide **1** derived by Smith degradation of the *Klebsiella* K40 capsular polysaccharide

Residue	C-1	C-2	C-3	C-4	C-5	C-6
α-D-Gal	96.2	69.1	70.2	70.0	71.6	61.7
α-L-Rha	100.0	67.7	76.0	71.2	69.8	17.5
D-Glyc [a]	89.8	80.9	60.2			

[a] Hydrated form.

Table 3
Interproton distances [a] calculated from interresidue NOEs for **1** in D_2O

NOE	Distances (Å)	
	(I)	(II)
Gal H-1–Rha H-2	2.5	2.5
Gal H-1–Rha H-3	2.5	2.5
Rha H-1–Glyc H-2	2.5	2.5
Rha H-1–Glyc H-3a	3.3	2.9
Rha H-1–Glyc H-3b	3.8	n.d. [b]

[a] (I) Refers to 1D NOESY experiments [5] with longer mixing times (τ_m 400, 600, and 800 ms), whereas (II) refers to 1D NOESY experiments with shorter mixing times (τ_m 150, 250, and 400 ms) using the procedure for the suppression of zero quantum coherences [11]. [b] Distance not determined

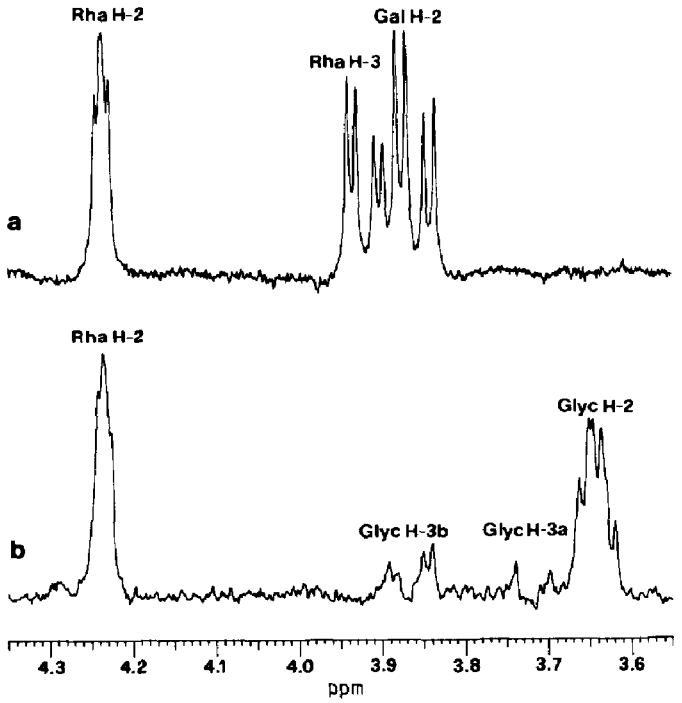

Fig. 2. Expansion of the 300-MHz 1D NOESY spectra (τ_m 800 ms) of **1** in D_2O at 305 K, obtained by selective irradiation of Gal H-1 (a), and Rha H-1 (b) using a 110-ms half-Gaussian pulse. In the case of Gal H-1, the irradiation gave rise to NOEs at two partly overlapping resonances, Gal H-2 and Rha H-3. The individual areas were then evaluated by fitting the observed signals to the required number of peaks, using the LINESIM program.

with mixing times from 400 to 800 ms was used to derive the proton–proton distances. Experiments with shorter values of mixing time (τ_m 100–200 ms) were not considered, because the presence of 1D COSY signals in the 1D NOESY spectra prevented the accurate integration of Gal H-2. Thus a second set of experiments with mixing times from 150 to 400 ms was performed using the procedure for the suppression of zero-quantum coherences [11] responsible for 1D COSY signals in the 1D NOESY spectra [12]. The 1D NOESY spectrum obtained by selective irradiation of Gal H-1 revealed close spatial proximity of this proton to Gal H-2, Rha H-3, and Rha H-2 (Fig. 2a). By irradiation of Rha H-1, two strong NOE contacts to Rha H-2 and Glyc H-2 were observed together with two weak responses of Glyc H-3a and H-3b (Fig. 2b). No NOEs suggesting spatial proximity between Rha H-2 and Glyc H-1 or H-2 were observed. Moreover, no Glyc H-2 and H-1 NOEs were detected after irradiation of Rha H-2. All interresidue distances obtained from 1D NOESY experiments are given in Table 3.

Molecular mechanics calculations, performed with the MMP2 (85) force field, gave the φ/ψ isoenergy contour maps shown in Figs. 3 and 4 for the Gal-Rha and Rha-Glyc fragments, respectively. Initially, three relaxed potential energy surfaces for the Gal-Rha fragment, which correspond to the three possible non-eclipsed conformations about ω (O-5–C-5–C-6–O-6), were computed. Comparison of these surfaces showed that the orientation about ω had no effect on the conformation of the disaccharide unit; ω was therefore allowed to assume the *gauche–trans* conformation, which is one of the two preferred non-eclipsed

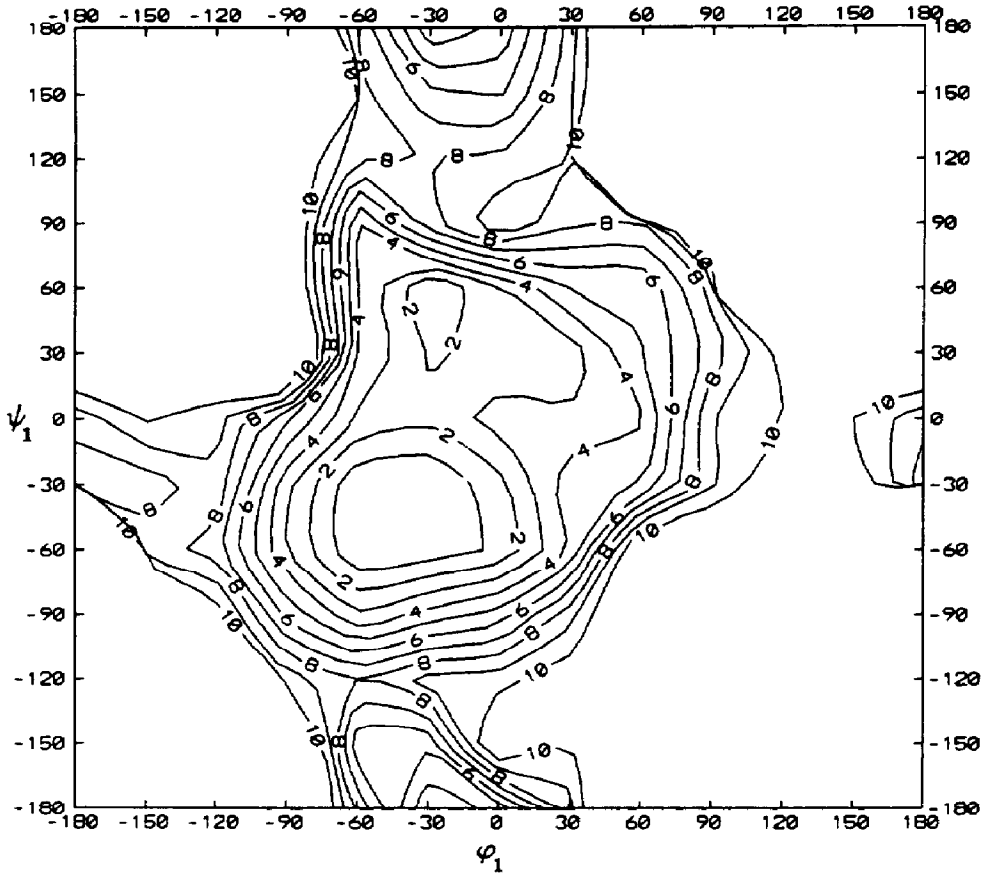

Fig. 3. Relaxed potential energy surface of α-D-Gal-(1→3)-α-L-Rha, calculated for a *gauche–trans* orientation of the primary hydroxyl group. Isoenergy contours are drawn with interpolation of 1 kcal/mol above the minimum of the map.

orientations found in the solid state for monosaccharides having the *galacto* configuration [13].

As the interatomic distances between Gal H-1 and Rha H-2, Gal H-1 and Rha H-3, and Rha H-1 and Glyc H-2 are sensitive to conformational changes about the glycosidic linkages α-D-Gal-(1→3)-α-L-Rha and α-L-Rha-(1→2)-D-Glyc, respectively, these interactions impose a serious restraint on the allowed φ_1/ψ_1 and φ_2/ψ_2 conformational space. In accordance with these restraints we considered only those conformers having interproton distances over the glycosidic linkage shorter than 2.5 Å. The geometry of the low-energy conformations was refined completely and the local minima along with selected information on geometrical parameters are summarised in Tables 4 and 5. The lowest energy conformation of **1** as obtained from MMP2(85) calculations is shown in Fig. 5.

The molecular mechanics data clearly established the presence of two preferred conformations for the α-D-Gal-(1→3)-α-L-Rha linkage (Fig. 3 and Table 4) and showed a certain similarity with those obtained before for the α-D-Glc-(1→3)-α-L-Rha linkage [14]. The examination of the low-energy molecular models revealed the presence of an intraresidue hydrogen bond involving the hydroxyl groups OH-3 (acting as donor) and OH-4 (acting as acceptor) in the galactopyranosyl residue. A hydrogen bond between one of the

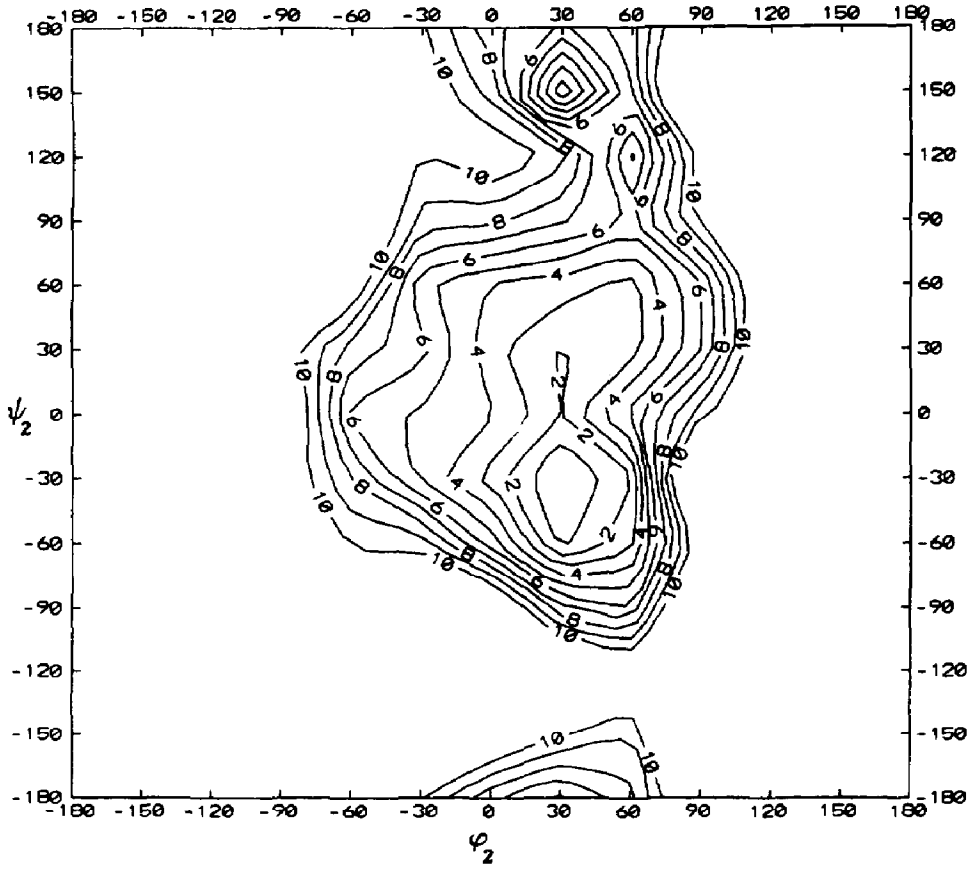

Fig. 4. Relaxed potential energy surface of α-L-Rha-(1→2)-D-Glyc. Isoenergy contours are drawn with interpolation of 1 kcal/mol above the minimum of the map.

two hydroxyl groups at C-1 of glyceraldehyde (acting as donor) and the ring oxygen O-5 in the rhamnopyranoside residue (acting as acceptor) was also observed. Thus, for a more realistic evaluation of the experimental data concerning the α-D-Gal-(1→3)-α-L-Rha linkage, weighted average distances [15] were calculated by simply considering the two minimum energy conformations [16] given in Table 4. The average distances found for Gal H-1–Rha H-3 and Gal H-1–Rha H-2 were 2.6 and 2.3 Å, respectively. Hence, the average

Table 4
Data on the minimum energy conformations of the α-D-Gal-(1→3)-α-L-Rha fragment from molecular mechanics calculations

Conformer	φ_1 [a] (°)	ψ_1 (°)	τ_1 (°)	d_1 (Å)	d_2 (Å)	E_{rel} (kcal/mol)
1a	−50	−45	114.5	2.7	2.2	0.0
1b	−30	60	116.5	2.4	3.6	1.1

[a] The torsional angles are defined as φ_1 = Gal H-1–Gal C-1–Rha O-3–Rha C-3 and ψ_1 = Gal C-1–Rha O-3–Rha C-3–Rha H-3, whereas the valence angle is defined as τ_1 = Gal C-1–Rha O-3–Rha C-3. d_1 = Gal H-1–Rha H-3. d_2 = Gal H-1–Rha H-2.

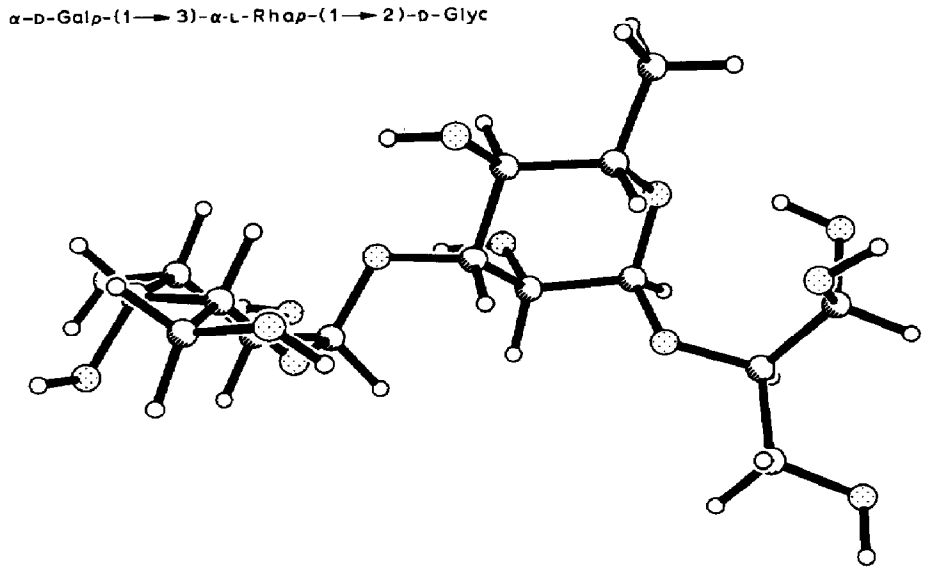

Fig. 5. The lowest energy conformation of **1** obtained from MMP2(85) calculations.

interatomic distances corresponding to the minimum energy conformations and those derived from the 1D NOESY data (both 2.5 Å) are in good agreement.

1. Experimental

The Smith degradation product **1** of the *Klebsiella* K40 polysaccharide was obtained as already described [8].

NMR spectroscopy.—For NMR experiments **1** was dissolved in D_2O, the solution freeze-dried, and **1** redissolved in 0.4 mL of D_2O at a concentration of ca. 5 mg/mL. Prior to NOE measurements, the solution was flushed with Ar and the NMR tube sealed. The ^1H and ^{13}C NMR experiments were performed on Bruker AC 200, AM 300 WB, and AM 500 spectrometers (^1H, 200.1, 300.1, and 500.1 MHz; ^{13}C, 50.3, 75.4, and 125.7 MHz), respectively. The sample temperature was 297 K, unless stated otherwise. 1D NOESY experiments [2] were performed at 305 K on a Bruker AM 300 WB equipped with a process controller and selective excitation unit (SEU), and half-Gaussian-shaped pulses [5] of 110–200 ms were used for selective irradiation. One set of 1D NOESY spectra was recorded for three values

Table 5
Data on the minimum energy conformation of the α-L-Rha-(1→2)-Glyc fragment from molecular mechanics calculations

Conformer	φ_2 [a] (°)	ψ_2 (°)	τ_2 (°)	d_1 (Å)	d_2 (Å)	d_3 (Å)
2	40	−40	115.8	2.2	3.9	4.5

[a] The torsional angles are defined as φ_2 = Rha H-1–Rha C-1–Glyc O-2–Glyc C-2 and ψ_2 = Rha C-1–Glyc O-2–Glyc C-2–Glyc H-2, whereas the valence angle is defined as τ_2 = Rha C-1–Glyc O-2–Glyc C-2. d_1 = Rha H-1–Glyc H-2. d_2 = Rha H-1–Glyc H-3a. d_3 = Rha H-1–Glyc H-3b.

of mixing time (τ_m, 400, 600, and 800 ms) using a spectral width of 4500 Hz and 32k points. The relaxation delay and acquisition time were 2.0 and 3.6 s, respectively, and up to 6400 scans were accumulated per spectrum. Another set of 1D NOESY spectra was collected for three values of mixing time (τ_m, 150, 250, and 400 ms) using the procedure for the suppression of zero-quantum coherences based on equidistant mixing time variation [11]. For both sets of experiments, an exponential multiplication giving an additional line-broadening of 0.3 or 0.5 Hz was applied prior to Fourier transformation. Chemical shifts are referenced to internal acetone (^{1}H, 2.225 ppm; ^{13}C, 31.07 ppm).

Simulation of spectra.—Simulation and fitting of the 500-MHz ^{1}H NMR spectrum were performed with the Bruker PANIC software (modified version of the LAOCN3 program [17]) running on the ASPECT 3000 computer of the NMR spectrometer. Individual areas of overlapping resonances were evaluated by fitting of the data using the software LINESIM [18].

Calculation of experimental distances.—Cross-relaxation rates were obtained by extrapolation to zero mixing time of $[I_{ij}(\tau_m)/I_{ii}(\tau_m)]/\tau_m$ vs. τ_m where I_{ij} and I_{ii} are the integrals of NOE-enhanced and selectively irradiated signals [19], respectively.

Interproton distances were calculated from the equation

$$r_{ij} = (\sigma_{cal}/\sigma_{ij})^{1/6} r_{cal}$$

where σ_{cal} and r_{cal} are the cross-relaxation rate and the distance of the proton pair used for calibration. The calibration distances Gal H-1–Gal H-2 = 2.457 Å and Rha H-1–Rha H-2 = 2.544 Å were obtained from the computed geometries.

Molecular mechanics calculations.—Molecular mechanics calculations were performed with the MMP2(85) [6,7] force field-based program PCMODEL (4.0) [20] on an IBM 386 computer. The position of the hydroxyl protons was determined following the procedure devised by Rao et al. [21] and the relaxed Ramachandran-type maps were recorded using a torsional grid increment of 30°, implying that 169 (13 × 13) different conformations were energy minimised with all internal degrees of freedom allowed to relax. The dielectric constant (ε) was set to 4.0 for all calculations.

Calculation of weighted average distances.—Weighted average distances were calculated from the equation

$$r_w = [\,1/\Sigma(1/r_i)^6 f(i)\,]^{1/6}$$

where $f(i) = \exp[-E_{rel,i}/RT]/\Sigma\exp[-E_{rel,j}/RT]$ is a Boltzmann distribution function and r_i is the interresidue distance of the optimised conformer with relative energy $E_{rel,i}$.

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