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Solution Conformation of the Two Alditols Obtained by Sodium Borohydride Reduction of N-Acetylneuraminic Acid

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Abstract. The stereochemistries and the conformations of the two alditols **1** and **2** (3 : 2) obtained upon reduction of N-acetylneuraminic acid were determined in aqueous solution using proton and carbon nuclear magnetic resonance spectroscopy. Molecular mechanics calculation was proved to be not applicable onto alditols, if the balance of the conformational equilibrium is maintained by 1,3-diaxial interactions. No measurable amount of the α-N-acetylneuraminic acid like triple bent alditol form, which is believed to be the substrate of the *Clostridium perfringens* lyase, could be detected in solution. In aqueous solution compound **2** is present in its extended form. One can expect that the necessary energy contribution of approximately 1 kcal to fold **2** into the form which is recognized by the enzyme is easily available.

Keywords. Alditols of sialic acid; Conformations; Molecular mechanics; Nuclear magnetic resonance.

Zur Konformation der beiden durch Reduktion von N-Acetyl-Neuraminsäure mit Natriumborhydrid erhaltenen Alditole in wäßriger Lösung

Bei der Reduktion von N-Acetyl-Neuraminsäure mit Natriumborhydrid in wäßriger Lösung werden die Alditole **1** und **2** im Verhältnis 3 : 2 gebildet. Die stereochemische Zuordnung und die Konformation der beiden Epimere in Lösung wurden mit Hilfe einer NMR-Analyse durchgeführt. In Ergänzung dazu wurde die Anwendbarkeit von Rechnungen des Typus Molekulare Mechanik auf flexible Moleküle dieser Art untersucht und gefunden, daß Populationen von Konformationen, welche primär durch 1,3-diaxiale Wechselwirkungen determiniert sind, durch Rechnungen dieses Typs nicht vorhersagbar sind. In wäßriger Lösung liegt Verbindung **2** in gestreckter Form vor. Die aus **2** ableitbare α-N-Acetylneuraminsäure ähnliche Konformation, welche dem Substrat der *Clostridium perfringens*-Lyase entspricht, ist um $\geqslant$ 1 kcal energiereicher als die der gestreckten Form und liegt in Lösung nicht in meßbaren Mengen vor. Man kann erwarten, daß dieser Energiebeitrag unschwer bei der Einbettung in die Enzymtasche der Lyase aufgebracht werden kann.

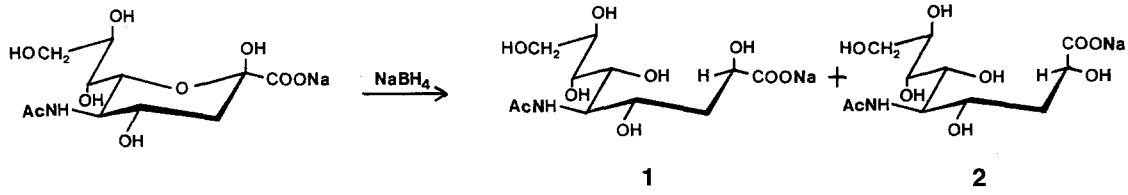
Introduction

In a series of publications we were able to find suitable structure-activity relations concerning the interaction of neuraminic acid analogues with rat liver CMP-sialate synthetase [1, 2] and *Vibrio cholerae* sialidase [3, 4]. We proposed models whose

α- as well as β-epitopes could be conveniently correlated with the mode of recognition by the aforementioned enzymes. The promising results forced us to search for a suitable model for the cleavage of neuraminic acid by *Clostridium perfringens* lyase [5]. Since the alditol obtained by reduction of neuraminic acid was reported to be an inhibitor of *Clostridium perfringens* lyase [6] we were interested in this compound.

Results and Discussion

As expected upon reduction of neuraminic acid we isolated a mixture of two alditols **1** and **2** (Scheme 1) in the ratio 3 : 2 (determined from proton n.m.r. integrals). As shown later, the major component **1** could be assigned as sodium 3,5-dideoxy-5-acetamido-*D-erythro-L-manno* nonoate and the minor component **2** as sodium 3,5-dideoxy-5-acetamido-*D-erythro-L-gluco* nonoate.



Scheme 1. Projections of the β-like (**1**) and α-like (**2**) conformation of the reduction products of N-acetyl neuraminic acid

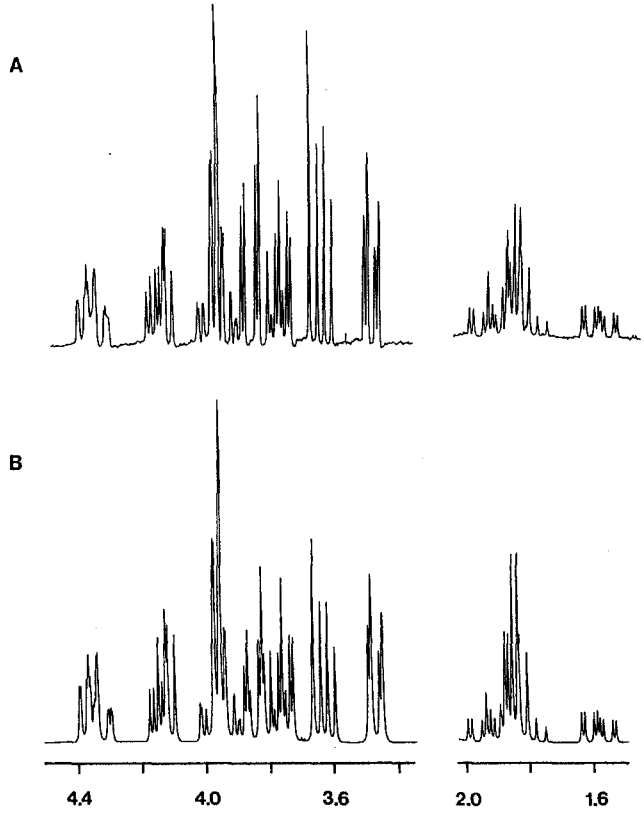


Fig. 1. Experimental (**A**) and simulated (**B**) spectrum of a 3 : 2 mixture of compounds **1** and **2**

To determine the stereochemistry of the two alditols **1** and **2** structural information was obtained from the proton and carbon n.m.r. spectra. After having assigned to proton signals starting at the H-2's whose shift values were confirmed by means of the 2-deutero derivative, the $^3J_{H,H}$-couplings were extracted. To do

Table 1. ^{1}H-Chemical shifts[a] (δ) of compounds **1–3**

Hydrogen atoms[b]	Compounds		
	1	**2**	**3**
2	4.13	4.16	
3a	1.87	1.94	
3b	1.83	1.59	
4	4.38	4.33	
(1a)			3.86
(1b)			3.78
5 (2)	4.00	3.94	4.06
6 (3)	3.96	3.98	3.89
7 (4)	3.48	3.49	3.52
8 (5)	3.77	3.77	3.76
9a (6a)	3.86	3.86	3.85
9b (6b)	3.65	3.65	3.64
CH_3	2.09	2.08	2.05

[a] Downfield from the signal of Me_4Si at 298 K
[b] Numbering of the hexitol **3** is shown in brackets

Table 2. $^3J_{H,H}$-Couplings (Hz) of compounds **1–3**

H-atom/H-atom[a]	Compounds		
	1	**2**	**3**
2/3a	5.0	3.2	
2/3b	7.9	9.9	
3a/3b	−14.2	−14.5	
3a/4	5.2	11.0	
3b/4	8.0	2.6	
4/5	1.5	1.2	
(1a/1b)			−11.0
(1a/2)			3.0
(1b/2)			6.0
5/6 (2/3)	10.5	10.5	9.8
6/7 (3/4)	0.8	0.8	1.2
7/8 (4/5)	9.0	9.0	8.8
8/9a (5/6a)	2.8	2.8	2.5
8/9b (5/6b)	6.3	6.3	6.5
9a/9b (6a/6b)	−11.6	−11.6	−11.5

[a] Numbering of the hexitol **3** is shown in brackets

Table 3. ^{13}C-Chemical shifts[a] (δ) of compounds **1–3**

Carbon atoms[b]	Compounds		
	1	**2**	**3**
1	n.d.	n.d.	
2[c]	71.29	70.46	
3	39.05	39.71	
4 (1)	67.69	66.98	61.92
5 (2)	54.22	55.22	53.09
6 (3)	68.80	68.80	69.07
7 (4)	70.46	70.46	70.42
8 (5)	71.75	71.75	71.61
9 (6)	64.23	64.23	64.10
CO	n.d.	n.d.	175.25
CH_3	22.90	22.90	22.25

[a] Downfield from the signal of Me_4Si (set at 67.40 p.p.m. upfield from the signal of 1,4-dioxane in D_2O at 298 K)

[b] Numbering of the hexitol **3** is shown in brackets

[c] Assigned using the corresponding 2-deutero derivatives

this, the proton n.m.r. spectrum was iterated in two times two parts (seven spin systems H-2 to H-7 and H-4 to H-9 b for each the major and the minor component) using the Bruker PANIC program. Fig. 1 shows the experimental spectrum and the simulated spectrum of a 3 : 2 mixture of **1** and **2**.

In analogy to the results obtained on comparison of β-Neu5*Ac* with mannitol [1] the similarity of the carbon and proton shift values (Tables 1 and 3) and proton-proton couplings (Table 2) observed for **1** and **2** with that of 2-deoxy-2-acetamido-mannitol **3** clearly indicate the carbon chain of C-5 to C-9 for both **1** and **2** to be extended. The small $^3J_{H4,\,H5}$-coupling in both **1** and **2** indicates H-4 and H-5 to be gauche. Since only one of the both gauche possibilities is free of a O//O interaction (the symbol // is used to indicate 1,3-diaxial interactions) the conformation of both **1** and **2** is extended as it concerns C-4 to C-9. Inspecting the proton-proton couplings of H-4 to H-3 a and H-3 b as well as the couplings of H-2 to H-3 a and H-3 b it turns out that only the minor alditol **2** possesses a well defined conformation (H-3 a, H-4 > 90% anti; H-2, H-3b > 90% anti − as visible from the large $^3J_{H3a,\,H4}$-coupling of 11.0 and the large $^3J_{H2,H3b}$-coupling of 9.9 Hz; 11.5 and 11.0 Hz in the related fixed geometry system 2,6-anhydro-3,5-dideoxy-5-acetamido-*D-erythro-L-gluco* nonoic acid [8]). The intermediate couplings observed for **1** ($^3J_{H3b,\,H4}$ = 8.0 Hz; H-3 b, H-4 ⩾ 60% anti; $^3J_{H2,\,H3b}$ = 7.9 Hz; H-2, H-3 b ⩾ 60% anti) indicate the major alditol to be a mixture of at least two conformers.

Writing both possible alditols using the *D-L* nomenclature (which in contrast to the *R-S* nomenclature is suited to detect immediately 1,3-interactions) one obtains *DDLLDxD* (C-8 to C-2; *x* for the CH_2 fragment) for sodium 3,5-dideoxy-5-acetamido-*D-erythro-L-manno* nonoate and *DDLLDxL* for sodium 3,5-dideoxy-5-acetamido-*D-erythro-L-gluco* nonoate. Applying the rule of Jeffrey and Kim [7]

one is now able to predict that the alditol *DDLLDxD* has to rotate between carbons 2 and 3 or carbons 3 and 4 to avoid the unfavorable O//O interaction whereas the alditol *DDLLDxL* should remain in the extended form. Considering further that in solution both mannonic [9] and galactonic acids [9] and deoxy mannitols [2] remain in the planar zig-zag forms there seems to be no doubt that the minor compound **2**, which occurs in mainly one conformation in aqueous solution and has no reason to leave the planar zig-zag form, is correctly assigned as *D-erythro-L-gluco* nonoate.

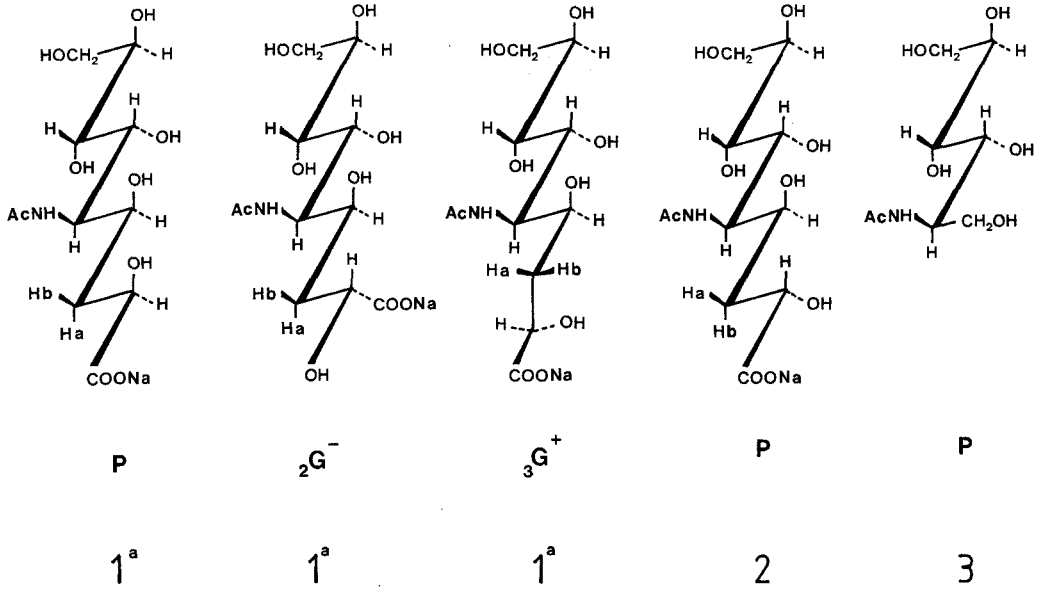
Although it is easy to explain that **2** has no reason to leave the planar zig-zag form, it is hard to explain why **1** is a conformational mixture of more than two forms. Since molecular mechanics was applied with success to predict the populations of alditols [10, 11] we calculated the conformations of **1** using MM2(87) [12]. The initial coordinates were prepared by bond modification of the X-ray structure of N-acetyl neuraminic acid [13]. All combinations of anti, g^+, and g^- concerning the bonds C 1–C 4, C 2–C 5 and C 3–C 6 were used. The C 4–C 7 angle was set to 180 degrees and the carboxylate carbon-oxygen bond was rotated to 0 and 180 degrees relative to the C 2–O 2 bond to be in accordance with the favored forms in α-hydroxy acids [14]. After the first minimization cycle hydrogen atoms and lone pairs were added to O-4, O-2, and the carboxylate oxygen in all combinations of each of the three staggered forms to all conformations with acceptable low energy above the global minimum ($32 \leqslant 3.5$ kcal above the global minimum – yielding 864 starting conformations for the next minimization cycle). Finally after having adjusted the O-8/O-9 torsion angle to ± 60 degrees, the averaged couplings of the population derived are near the observed ones. However, close inspection of the individual conformations showed beside 13% *P*, 22% ${}_2G^-$, and 36% ${}_3G^+$ a large percentage of double twisted forms (8%), and a large population which has a C-1//O-4 interaction (18% ${}_2G^+$). This forced us to calculate the set of populations of compound **2** for control purposes too. Application of the same procedure as described above onto compound **2** yielded a set of 783 conformations out of 29 starting geometries with energies $\leqslant 3.5$ kcal. In this case beside 42% of the *P* form, 16% contained an O//O; 11% an C//O interaction, and 28% were calculated to be in a doubly twisted form. For the hexitols [11] it seems to be an acceptable approach to delete manually all conformations with 1,3 C//O diaxial interactions from the generated conformation list. For **1** this procedure yields acceptable results if one deletes in addition all doubly twisted forms and for **2** if one deletes the form which contains O//O too. However, the question arises if a time consuming method is worth to be used if one has to check the results manually for plausibility.

Since MM2(87) [12] predicts both **1** and **2** to be conformeric mixtures and n.m.r. proves that one of the two occurs in mainly one conformation, MM2 in its current parametrisation is proved to be no valuable method for the alditols considered in the present study. This is in contrast to published calculations [10, 11] on hexitols (where starting geometries with diaxial interactions were omitted to save computer time). However, it cannot be accepted to neglect conformations to be calculated where it is convenient and to keep them where necessary.

An easy way to obtain approximate populations is to treat the gauche and diaxial interactions as additive energy increments. In compound **2** all conformations which deviate from the planar zig-zag form are disfavoured due to C/C gauche

and diaxial interactions (O//O, C//O, or C//N). As a result the conformation of **2** is well defined. In **1** the extended form contains one O//O interaction. If the molecule rotates at C 2 to avoid this interaction disfavouring the planar form, there are two possibilities ${}_2G^+$ and ${}_2G^-$ which both contain a gauche C 1/C 4 (/ is used to indicate a gauche relationship). In butane the magnitude of such a relation yields a disadvantage of ≈ 0.9 kcal [15] for the gauche form. The ${}_2G^+$ form in addition contains a C 1//O 4 interaction. Since the C//O interaction contributes with ≈ 0.4 kcal more [16] than that of O//O, the $2G^+$ form has not to be considered further. If the molecule rotates at C 3 to avoid the O 2//O 4 interaction, there are two possibilities ${}_3G^+$ and ${}_3G^-$ which both contain a gauche relationship at C 2/C 5. The ${}_3G^-$ form should be excluded since it contains a C 2//N 5 interaction, which in analogy to the O//N interaction should be extremely disfavoured [1]. Conformations doubly twisted in 2 and 3 can be excluded since two C/C interactions (≈ 1.8 kcal) correspond approximately to one C//O interaction. Therefore among all restraint forms both ${}_2G^-$ and ${}_3G^+$ remain, disfavoured by C/C each (≈ 0.9 kcal), and the planar form which contains a O//O interaction (≈ 1.4 kcal) but is stabilized by one more C/O interaction than the former two conformers (≈ -0.2 kcal for such an interaction [17]).

Evidence for the postulated near to 1 : 1 : 1 population of the *P*, ${}_2G^+$, and ${}_3G^-$ forms in **1** came from ^{13}C-n.m.r. If the constitutions of two molecules are the same, the major influences on carbon shifts stem from γ-effects [18]. The H//H syn-axial interaction causes a ≈ 4 p.p.m. downfield shift [18]. Thus considering that in **2** H-5 and H-3 b are always syn-axial, whereas in **1** they are only syn-axial to two third (Scheme 2; conformers 1-P and 1-${}_2G^-$), the pairwise upfield shift observed for C-3 and C-5 (≈ 1 p.p.m. for each of the carbons; Table 3) in **1** relative to the shifts in **2** can be explained. Secondly, H-4 is never syn-axial to H-2 in **2**, whereas



Scheme 2. Projections of compounds **1–3** in their preferred conformations

[a] Compound **1** is shown in all of the three forms occurring in aqueous solution (*P*, ${}_2G^-$, and ${}_3G^+$)

it is to one third in **1** (Scheme 2; conformer 1-P). This explains the pairwise upfield shift ($\approx$ 1 p.p.m. for each of the carbons; Table 3) observed for C-2 and C-4 of **1** compared to the corresponding shifts in **2**.

With respect to the observed recognition of a mixture of **1** and **2** by *Clostridium perfringens* lyase [6] (Ki = 4.1 mM) it can be assumed that since only the α-Neu5*Ac* (Km = 1.8 mM) as well as the corresponding α-methyl ketoside (Ki = 8.1 mM) is recognized by the enzyme [6] and the equatorial OH-2 is necessary for the recognition process [5], it is the minor alditol **2** (which can be folded into an α-like conformation; see Scheme 1) which binds to the enzyme. The fact that such an α-like conformation is not observed in aqueous solution is not contradictory with this result since one should not overlook that additional stabilization of the minor component might stem from the interaction of the chair-like folded form with the enzyme thus filling the void volumes [19].

Experimental Part

Sodium Boro-Hydride (-Deuteride) Reductions

Commercially available neuraminic acid (Sigma) was reduced in aqueous solution, using an excess of sodium boro-hydride (-deuteride) at 4° during 12 h. The excess boro-hydride was destroyed using acetic acid, and after neutralization with aqueous sodium hydroxide, the alditols were freed from salts by gel-permeation chromatography on Bio-Gel P-2 (Biorad). 2-Deoxy-2-acetamido-mannitol was prepared analogously with the exception that, instead of neutralizing the acetic acid, the reaction mixture was codistilled several times with methanol previous to chromatography.

N.M.R. Spectroscopy

^{1}H-n.m.r. spectra were recorded on a Bruker WM 250 instrument at 298 K in D_2O, with a deuterium lock on the water signal and an external reference of sodium (2,2,3,3-tetra ^{2}H), 4,4-dimethyl-4-silapentanoate (δ 0 in D_2O). The ^{13}C-n.m.r. spectra were recorded on the same instrument at 298 K in D_2O, using an external reference of tetramethylsilane (δ 67.40 upfield from the signal of 1,4-dioxane in D_2O).

Computations

All computations were performed on an Intel 80386-16 hosted transputer system (Microway), consisting of four INMOS T800-20 transputers equipped with 4 + 3*1 MBytes of memory. The program to simulate up to 10 spin system n.m.r. spectra on a PC is available from SSC. MM2(87) [12] (Molecular Design Ltd.) was used as obtained from QCPE. The parameters necessary for the N-acetyl group were used as published [20], a dielectricity constant of 80 was chosen, and the starting coordinates of **1** and **2** were prepared by bond modification of the X-ray structure of neuraminic acid [13].

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