Carbohydrate Research, 215 (1991) 345–349
Elsevier Science Publishers B.V., Amsterdam

Note

Configurational assignment of *N*-acetylneuraminic acid and analogues *via* the vicinal C,H coupling constants

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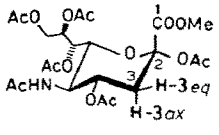
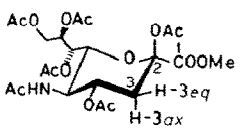
(Received September 22nd, 1990; accepted for publication, November 14th, 1990)

Sialic acids, particularly *N*-acetylneuraminic acid (Neu5Ac), frequently occur at the non-reducing ends of oligosaccharide chains of glycoproteins and glycolipids[1–3]. Although ^{1}H-n.m.r. spectroscopy has been used to elucidate the structures of these molecules[4,5], assignment of the configuration at the anomeric centre (C-2) of Neu5Ac derivatives can be difficult because C-2 is a quaternary carbon and the exocyclic C-1 is a carboxyl carbon (see **1**), and only empirical fingerprints could be given[4,6,7].



α



β

1

For several Neu5Ac derivatives, the $J_{C\text{-}2,H\text{-}3ax}$ values, determined by 2D-methods[8], were found to be ~ -8 Hz for the α anomers and ~ -4 Hz for the β anomers. However, for oligosaccharides, the identification of the sialic acid C-2 resonances may not always be accomplished easily and we have been interested in alternative methods of assignment of anomeric configuration. Haverkamp *et al.*[9] demonstrated that the β configuration of cytidine 5′-phospho-Neu5Ac can be determined on the basis of the values of $J_{C\text{-}1,H\text{-}3ax}$. We now report the results of a re-examination of this method by measuring the $J_{C\text{-}1,H\text{-}3ax}$ and $J_{C\text{-}1,H\text{-}3eq}$ values for *N*-acetyl-2,4,7,8,9-penta-*O*-acetylneuraminic acid methyl ester (**1**). The situation with **1** is complex since there is an α,β-mixture in solution[8] and the numerous carbonyl carbon resonances from the OAc groups can overlap those from the methyl ester group. 2D-N.m.r. techniques were applied in order to obtain a rigorous assignment of the resonances and a precise analysis of the coupling constants.

Vicinal C,H-coupling constants are sensitive to structure, as are vicinal H,H-coupling constants[10]. Schwarcz and Perlin[11] presented an experimental correlation diagram for vicinal C,H-couplings in mono- and di-saccharides, and recent mea-

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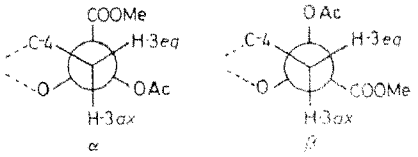
0008-6215/91/$ 03.50

TABLE I

Newman projections and C-1,H-3 dihedral angles in α-1 and β-1

Dihedral angle	α-**1**	β-**1**
φ(C-1,C-2,C-3,H-3*ax*)	180°	60°
φ(C-1,C-2,C-3,H-3*eq*)	60°	60°



surements[12] gave average $^3J_{C,H}$ values of 1.5 and 5.5 Hz for dihedral angles (φ) of 60° and 180°, respectively. These are the dihedral angles which are to be expected for α-**1**, as can be seen from the Newman projections in Table I (Neu5Ac derivatives adopt the $^2C_5$ chair conformation[4]). For β-**1**, φ is 60° for both H-3*eq* and H-3*ax*, and the couplings with C-1 are expected to be small. Only the determination of $J_{C\text{-}1,H\text{-}3ax}$ will allow configurational assignment for **1**.

The H-3*eq* and H-3*ax* resonances in the 360-MHz ^{1}H-n.m.r. spectrum of **1** have been assigned[6] by H,H-COSY. The C-1 resonances of the methyl ester groups can be assigned by a C,H-COSY experiment with polarisation transfer *via* the long-range couplings[13,14], since they correlate with the methyl protons at 3.77 p.p.m. (Fig. 1)

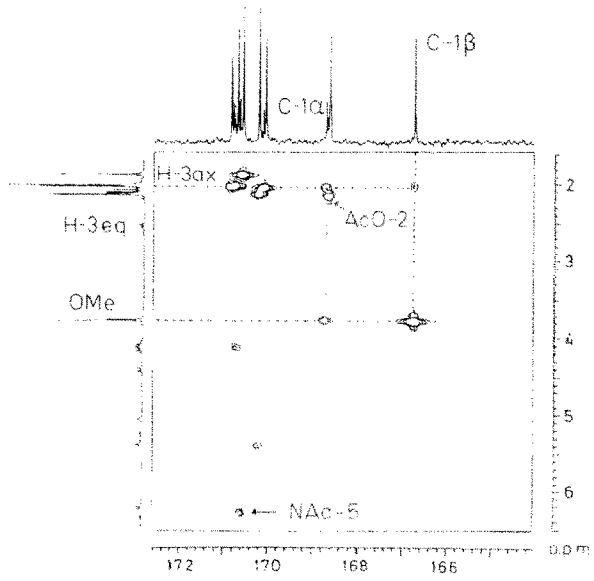


Fig. 1. 90.5-MHz C,H-COSY spectrum (CD_2Cl_2, 25°) of the region for carbonyl carbon resonances of **1**. The polarisation transfer was made *via* the long-range couplings.

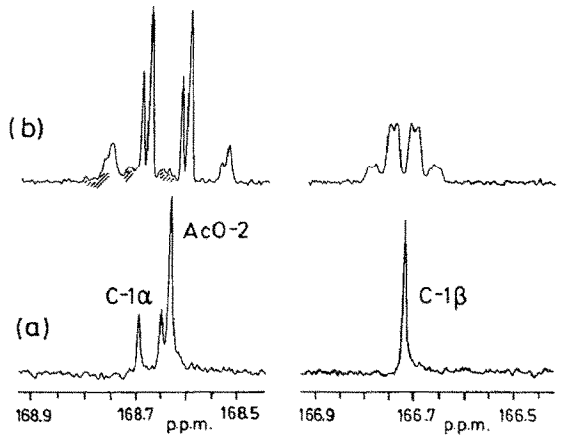


Fig. 2. Part of the region for carbonyl carbon resonances of the ^{1}H-decoupled (*a*) and ^{1}H-coupled (*b*) 90.5-MHz ^{13}C-n.m.r. spectrum of **1**. In (*b*), the multiplet of C-1α is hatched.

Resonances for C-1 were found at 166.71 and 168.74 p.p.m., which correspond to β-**1** and α-**1**, respectively (α,β-ratio ~3:1)[8]. The large difference in the chemical shifts of the C-1α and C-1β resonances may be a useful fingerprint for the assignment of anomeric configuration in sialic acids. The C-1β resonance at 166.71 p.p.m. was well separated from the other carbonyl carbon resonances. The proton-coupled ^{13}C-n.m.r. spectrum (Fig. 2b) contained a quartet of doublets, with the quartet arising from the coupling with the methyl ester protons (^3J 3.9 Hz). Additional splittings are expected from the coupling of C-1β with H-3. However, only one coupling (1.3 Hz) was resolved (Fig. 2) and the other must be less than the line width of the resonances (*i.e.*, < 1 Hz). The larger coupling should correspond to that of C-1β with H-3*ax*, since a cross-peak was observed between these resonances (Fig. 1), but not between those of C-1β and H-3*eq*.

The C-1α resonance at 168.74 p.p.m. was close to those at 168.68 and 168.66 p.p.m. that could be attributed to the AcO-2 carbonyl carbons of α-**1** and β-**1** since, in Fig. 2, they occurred as simple quartets (^3J 7.0 Hz). Consequently, the proton-coupled C-1α multiplet was markedly overlapped (Fig. 2b) and no C-1,H-3 couplings could be determined from the 1D experiment.

The heteronuclear 2D-n.m.r. techniques, gated-decoupling[15] and spin-flip[16] with selective 180° ^{1}H (DANTE) pulse[12], were applied to **1** on each H-3. Fig. 3 shows portions of the 2D gated-decoupling spectrum of **1** and the cross-sections taken at the chemical shifts of the C-1 resonance of each anomer. The C-1α multiplet was now well separated from the AcO-2 resonances. However, since the cross-sections showed multiplets where all J values were half of their actual magnitudes[14], the resolution of the couplings was not ideal. Indeed, the 2D-spin-flip experiment allowed a more precise determination of these couplings (Fig. 4). Furthermore, since the protons were selectively excited, an individual assignment of the C-1,H-3*eq* and C-1,H-3*ax* couplings was obtained. Table II collects the results for the C-1,H-3 couplings of **1**.

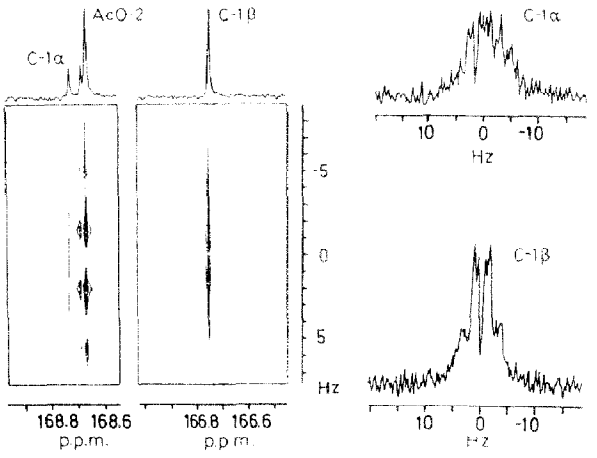


Fig. 3. The *J*-resolved 90.5-MHz ^{13}C-n.m.r. spectrum of **1** obtained by the gated decoupling method. Contour plot and cross-sections at the chemical shifts of the resonances of C-1α and C-1β.

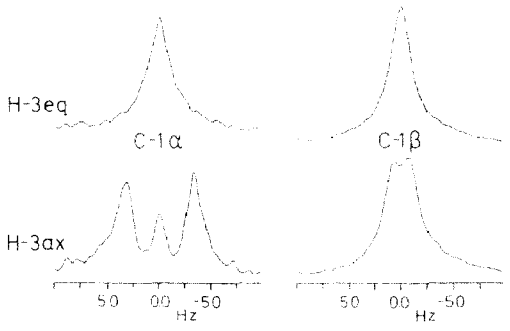


Fig. 4. The *J*-resolved 90.5-MHz ^{13}C-n.m.r. spectrum of **1** obtained by the spin-flip method; H-3*eq* and H-3*ax* of each anomer were excited selectively by a 180° DANTE pulse. Cross-sections are plotted at the chemical shifts of the C-1 resonances of C-1α and C-1β.

TABLE II

Vicinal C,H coupling constants for α-**1** and β-**1** determined by the selective 2D-spin-flip experiment

^{3}J (*Hz*)	α-**1**	β-**1**
C-1,H-3*ax*	6.6	1.3
C-1,H-3*eq*	< 1	< 1

The C-1,H-3*eq* couplings were small and similar for α-**1** and β-**1** (Table II). However, the $J_{C\text{-}1,H\text{-}3ax}$ value was definitely larger for α-**1**, *i.e.*, $(J_{C\text{-}1,H\text{-}3ax})_{\alpha} > (J_{C\text{-}1,H\text{-}3ax})_{\beta} \approx (J_{C\text{-}1,H\text{-}3eq})_{\alpha,\beta}$. Thus, $J_{C\text{-}1,H\text{-}3ax}$ values indicate unambiguously the anomeric configuration in Neu5Ac derivatives and are an alternative to $J_{C\text{-}2,H\text{-}3ax}$ values[5].

EXPERIMENTAL

Compound **1** was prepared as described[8]. N.m.r. spectra were recorded with a Bruker AM-360 instrument as reported[8].

ACKNOWLEDGMENTS

This work was supported by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.

REFERENCES

1 R. Schauer, *Angew. Chem.*, 85 (1973) 128–140.
2 R. Jäckh, *Chem. Unserer Zeit*, 10 (1975) 139–146.
3 R. Schauer, *Adv. Carbohydr. Chem. Biochem.*, 40 (1982) 131–234.
4 L. Dorland, J. Haverkamp, J. F. G. Vliegenthart, G. Strecker, J. C. Michalski, B. Fournet, G. Spitz, and J. Montreuil, *Eur. J. Biochem.*, 87 (1978) 323–329.
5 J. Haverkamp, H. van Halbeek, L. Dorland, J. F. G. Vliegenthart, R. Pfeil, and R. Schauer, *Eur. J. Biochem.*, 122 (1982) 305–311.
6 H. Ogura, in R. Schauer and T. Yamakawa (Eds.), *Sialic Acids 1988: Proceedings of the Japanese German Symposium on Sialic Acids*, Kieler Verlag Wissenschaft + Bildung, Kiel, 1988, pp. 32–33.
7 K. Okamoto, T. Kondo, and T. Goto, ref. 6, pp. 34–35.
8 S. Prytulla, J. Lambert, J. Lauterwein, M. Klessinger, and J. Thiem, *Magn. Reson. Chem.*, 28 (1990) 888–901.
9 J. Haverkamp, T. Spoormaker, L. Dorland, J. F. G. Vliegenthart, and R. Schauer, *J. Am. Chem. Soc.*, 101 (1979) 4851–4853.
10 P. E. Hansen, *Prog. Nucl. Magn. Reson. Spectrosc.*, 14 (1981) 175–295.
11 J. A. Schwarcz and A. S. Perlin, *Can. J. Chem.*, 50 (1972) 3667–3676.
12 C. Morat, F. R. Taravel, and M. R. Vignon, *Magn. Reson. Chem.*, 26 (1988) 264–270.
13 J. Wernly and J. Lauterwein, *J. Chem. Soc., Chem. Commun.*, (1985) 1221–1222.
14 G. E. Martin and A. S. Zektzer, *Magn. Reson. Chem.*, 26 (1988) 631–652.
15 R. Freeman, G. A. Morris, and D. L. Turner, *J. Magn. Reson.*, 26 (1977) 373–378.
16 A. Bax and R. Freeman, *J. Am. Chem. Soc.*, 104 (1982) 1099–1100.