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Note

Two dimensional *J*-resolved and SECSY ^{1}H-n.m.r. spectroscopy of the characteristic sequence of *O*-type carbohydrate–peptide linkage*

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During the last few years, ^{1}H-n.m.r. spectroscopy has been shown to be a rapid, sensitive and non-destructive technique for the structural investigation of complex carbohydrates[1–6]. The use of high-resolution spectrometers has allowed determination of the chemical shifts and coupling constants of the anomeric resonances and other selected resonances for these carbohydrates; nevertheless, unambiguous attribution of all the other resonances remained impossible. It was hoped that the development of two-dimensional ^{1}H-n.m.r. spectroscopy[7,8], which has already enabled complete resolution of various complex spectra such as the ^{1}H-n.m.r. spectrum of α,β-D-glucose[9], could overcome this problem. In this paper, two-dimensional, *J*-resolved and SECSY ^{1}H-n.m.r. spectroscopy was used to analyze the sequence α-D-Gal*p*NAc-(1→3)-L-Ser. This sequence of biological interest is characteristic of the *O*-type carbohydrate–peptide linkage present in mucins and numerous glycoproteins. In these glycoconjugates, the L-serine residue is included in a peptide chain, and its amino and carboxylic groups therefore have substituents. To obtain a model as close as possible to the biological structures, we used the synthetic derivative *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-*N*-acetyl-L-serine methyl ester (**1**).

The conventional spectrum observed at 500.13 MHz showed distinct resonances at δ 4.885 for H-1, 4.113 for H-2, 4.750 for H-2′, 3.750 for H_2-6, and

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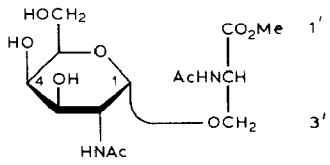

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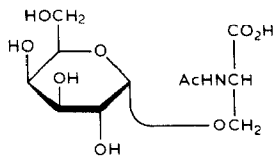

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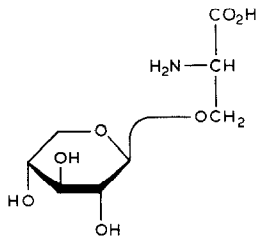

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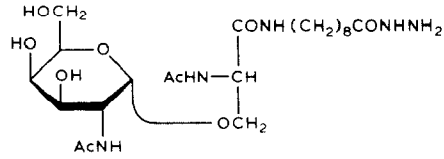

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1.776, 2.043, and 2.083 for protons of the three methyl groups. The protons of the pyranose ring gave a first-order, five-spin system. However, the resonance of three of them, H-3, -4, and -5, fell into a very small range of chemical shifts (0.1 p.p.m.) and, furthermore, extensively overlapped with the signals originating from H_2-3′ (Fig. 1).

Two-dimensional, *J*-resolved spectroscopy allowed a clear observation of the multiplets arising from H-3 and -4, and from H_2-3′, although the signal of H-5 was partially masked, the contour plot (Fig. 2a) showed a centered multiplet for this proton. The cross sections drawn in Fig. 2b allowed accurate measurement of coupling constants.

Two-dimensional, SECSY spectroscopy permitted correlation of the coupled protons. The contour plot is given in Fig. 3 (full range, except for the *N*-acetyl signal). Correlations were shown between the protons of the pyranose ring and H-2′ and H_2-3′ of the serine residue. A correlation between H-5 and H_2-6 was clearly demonstrated.

The parameters of the ^{1}H-n.m.r. spectrum of **1** (Table I) were compared with the data previously reported for the similar compounds **2**–**4**. As regards chemical shifts and coupling constants, the protons of the serine residue appeared to be strongly dependent on the substitution of the amino and carboxylic groups. H-2′ shifted from δ 3.79 in the *N*-acetylated α-D-galactopyranosyl derivative[10] **2** to 4.243 in the β-D-xylopyranosylamino acid[11] **3**, to 4.53 in the *N*-acetylated hydrazide[10] **4**, and to 4.736 in compound **1**, presently studied. Only partial data are available for H_2-3′, the chemical shifts of which seemed less sensitive to substitution. The constants for coupling between H-2′ and each H-3′, which are very different when the amino group is free[11], were very similar in all the derivatives studied by Pavia and

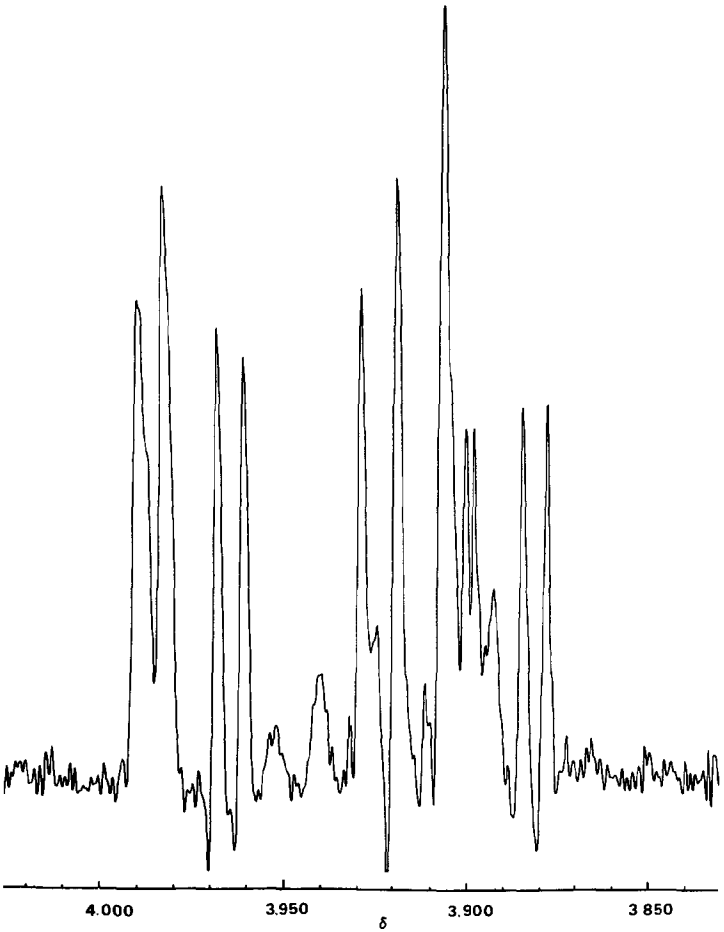

Fig. 1. Conventional 500-MHz ^{1}H-n.m.r. spectrum of compound **1**, δ 3.850–4.000 region. H-3, -5, and -3′a appeared between δ 3.850 and 3.950, and H-4 and -3′b between δ 3.950 and 4.000.

Ferrari[10], and in this work. Similarly, the absolute value of the *gem*-H-2′a,H-2′b constant changed dramatically from a low value[11] ($\approx$ 3 Hz) to a high one ($\approx$ 11 Hz) in all these derivatives. These changes might be due to a modification of the conformational equilibrium of the serine residue.

As regards to protons of the sugar residue, the chemical shifts observed for H-1, H-4, and the *N*-acetyl group protons were compared to those previously reported for the α-D-Gal*p*NAc-(1→3)-L-Ser(or -Thr) sequences present both in a glycopeptide derived from the B-chain of human plasma α_2HS-glycoprotein[12] and in the glycopeptides obtained from human mucolipidosis-1 urine[13]. In the spectrum of **1**, H-1 and the *N*-acetyl group protons appeared to be in the same range of chemical shifts as previously reported, but H-4 exhibited a large downfield shift (0.2 p.p.m.), perhaps originating from the non-substitution of the 2-acetamido-2-

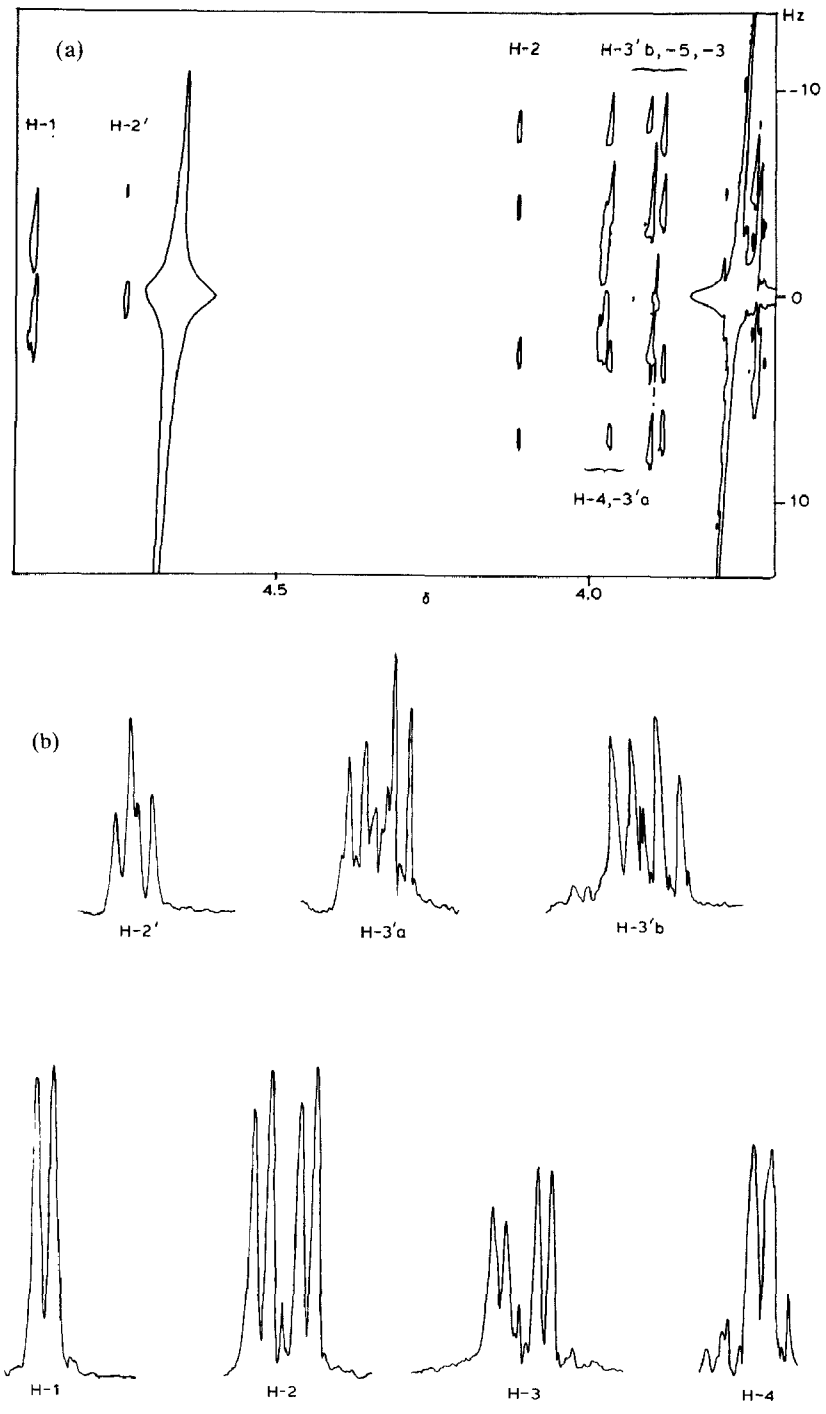

Fig. 2. Two-dimensional, *J*-resolved ^{1}H-n.m.r. spectrum of compound **1**: (a) Contour plot and (b) cross sections.

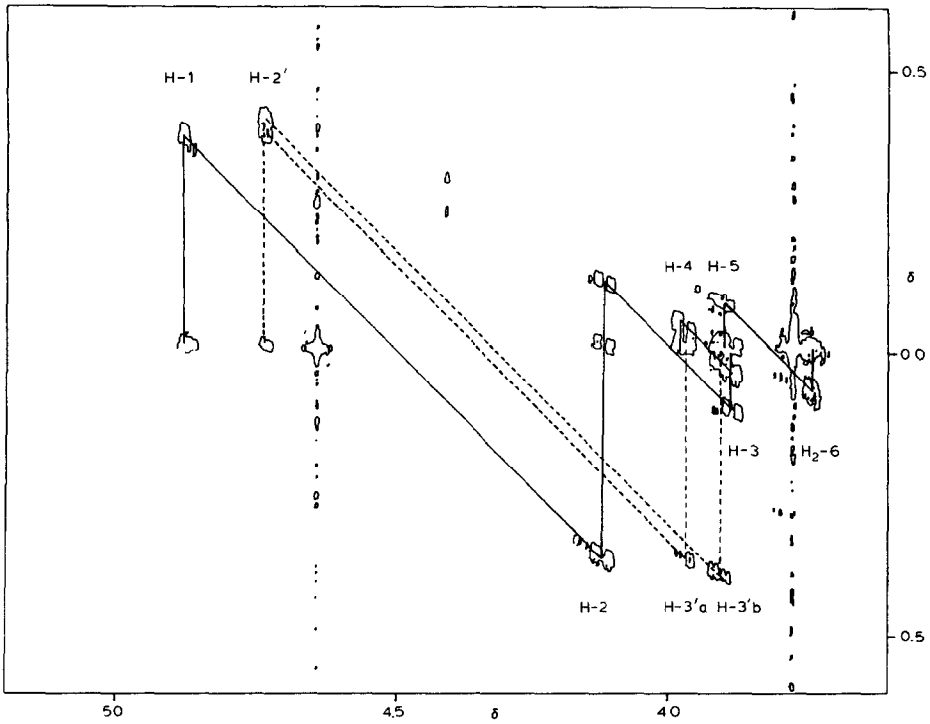

Fig. 3. Two-dimensional, ^{1}H-n.m.r. spectrum of compound **1**; SECSY contour plot full range, except for NHAc signals. (· · · · · ·) Correlation between H-2′, -3′a, and -3′b-β' serine protons; (———) correlation between sugar residue protons.

TABLE I

^{1}H-NMR DATA FOR *O*-(2-ACETAMIDO-2-DEOXY-α-D-GALACTOPYRANOSYL)-(1→3)-*N*-ACETYL-L-SERINE METHYL ESTER (**1**)

Protons	*Chemical shifts (δ)*	J	*(Hz)*
H-1	4.879	1,2	4.0
H-2	4.116	2,3	11.2
H-3	3.887	3,4	3.4
H-4	3.983	4,5	<1.0[a]
H-5	3.902	5,6	6.4
H-6a,H-6b	3.743		
H-2′[b]	4.736	2′,3′a	4.0[c]
H-3′a	3.975	2′,3′b	5.0[c]
H-3′b	3.915	3′a,3′b	11.2[c]
OCH_3	3.777		
$NHCOCH_3$	2.080		
	2.042		

[a]The signal for H-4 was broadened by the unresolved small coupling with H-5. [b]The signal is in fact the degenerate X part of an ABX system. [c]These coupling constants were obtained from a computer-simulated spectrum by use of a Bruker PANIC program.

deoxy-D-galactose residue. The H_2-6 of this residue were equivalent and gave one doublet. A similar doublet was also observed for free 2-acetamido-2-deoxy-D-galactose[14] at δ 3.74; though, a small non-equivalence ($\Delta\delta$ <0.01 p.p.m.) is usually reported for these two protons in D-galactose and 2-acetamido-2-deoxy-D-galactose[6,11]. Comparison between free 2-acetamido-2-deoxy-D-galactose[14] and the *O*-linked sugar residue of **1** also showed some differences in this respect for H-1 (δ 4.88 *vs.* 5.23) and H-5 (δ 3.91 *vs.* 4.10). The upfield shift exhibited by H-1 might have resulted from the involvement of the hydroxyl group in the *O*-glycosyl linkage, but the shift observed for H-5 is more difficult to explain. It might, for instance, be the consequence of a particular conformation of the serine molecule around the glycoside bond. Thus, the use of 2D-n.m.r. technique led to the complete attribution of chemical shifts and coupling constants of all the protons of a complex carbohydrate structure. In addition, this technique can give new insights in the conformational analysis of compounds of biological interest.

EXPERIMENTAL

Compound **1** was prepared[15] according to the procedure previously described[16]; m.p. 182–187°, $[\alpha]_D^{20}$ +101.4° (*c* 1, methanol).

^{1}H-N.m.r. spectra were recorded with a Bruker WM 500 spectrometer connected to an Aspect 2000 data system. The chemical shifts (δ) are reported relative to the shift of 4,4-dimethyl-4-silapentane-1-sulfonic acid, and acetone was used as internal secondary standard (δ 2.225). The concentration of **1** was 10 mg/mL in ^2H_2O.

The two-dimensional experiments were recorded with an FTQ n.m.r. 810–515 instrument for data acquisition and the FTQ n.m.r.-2D program for data handling. In the *J*-resolved experiments, the sizes of the time domains were 2000 Hz (8192 data points) and 31.2 Hz (256 data points) in the f2 and f1 directions, respectively. With zero filling in the f1 direction, the final digital resolutions were 0.49 Hz/pt for the f2 direction, and 0.12 Hz/pt for the f1 direction. Sixteen scans were made.

The SECSY experiments were recorded with a spectrum width of 1600 Hz and 400 Hz in the f2 and f1 directions, respectively; and the time domains in these directions corresponded to 2048 and 256 data points. Zero filling in both directions gave digital resolutions of 0.78 and 1.56 Hz/pt. Quadrature detection was used in both types of two dimensional experiments. Unshifted Sine Bell function was applied in the t1 and t2 domains before Fourier transformation.

REFERENCES

1 L. DORLAND, B. L. SCHUT, J. F. G. VLIEGENTHART, G. STRECKER, B FOURNET, G. SPIK, AND J. MONTREUIL, *Eur. J. Biochem.*, 73 (1977) 93–97.
2 J. P. CARVER AND A. A. GREY, *Biochemistry*, 20 (1981) 6607–6616.
3 J. C. BYRD, A. L. TARENTINO, F. MALEY, P. H. ATKINSON, AND R B. TRIMBLE, *J. Biol. Chem.*, 257 (1982) 14 657–14 666.

4 R. E. COHEN AND C. E. BALLOU, *Biochemistry*, 19 (1980) 4345–4358.
5 J. F. G. VLIEGENTHART, L. DORLAND, AND H. VAN HALBEEK, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 209–374.
6 R. U. LEMIEUX, K. BOCK, L. T. J. DELBAERE, S. KOTO, AND V. S. RAO, *Can. J. Chem.*, 58 (1980) 631–653.
7 L. D. HALL AND S. SUKUMAR, *J. Chem. Soc., Chem. Commun.*, (1979) 292–294.
8 J. DABROWSKI, H. EGGE, AND U. DABROWSKI, *Carbohydr. Res.*, 114 (1983) 1–9.
9 W. CURATOLO, L. J. NEURINGER, D. RUBEN, AND R. HABERHORN, *Carbohydr. Res.*, 112 (1983) 297–300.
10 A. A. PAVIA AND B. FERRARI, *Int. J. Pept. Protein Res.*, 22 (1983) 539–548.
11 H. VAN HALBEEK, L. DORLAND, G. A. VELDINK, J. F. G. VLIEGENTHART, P. J. GAREGG, T. NORBERG, AND B. LINDBERG, *Eur. J. Biochem.*, 127 (1982) 1–6.
12 F. GEJYO, J. L. CHANG, W. BURGI, K. SCHMID, G. D. OFFNER, R. F. TROXLER, H. VAN HALBEEK, L. DORLAND, G. J. GERWIG, AND J. F. G. VLIEGENTHART, *J. Biol. Chem.*, 258 (1983) 4966–4971.
13 D. LECAT, M. LEMONNIER, C. DERAPPE, M. LHERMITTE, H. VAN HALBEEK, L. DORLAND, AND J. F. G. VLIEGENTHART, *Eur. J. Biochem.*, 140 (1984) 415–420.
14 D. M. MEYER, M. LEMONNIER, C. DERAPPE, N. SELLIER, AND N. PLATZER, *FEBS Lett.*, 172 (1983) 99–102.
15 B. FERRARI AND A. A. PAVIA, unpublished results.
16 B. FERRARI AND A. A. PAVIA, *Bioorg. Chem.*, 11 (1982) 85–95.