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Note

Structure of the sugar moiety in the nephritogenic glycopeptide from rat glomerular, basement membrane

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Recently, the role of chemical substances in inducing particular types of disease has attracted increasing attention both of chemists and physicians. Shibata *et al.*[1] isolated from the glomerular basement-membrane of rats, and purified, a new glycopeptide (**1**) that has an activity consisting of induction of glomerulonephritis in homologous animals (nephritogenic activity)[2]. From g.l.c. analyses[3], it has been made clear that the components of the sugar moiety of **1** are almost all glucose, and that the percentages of galactose, mannose, and other sugar residues are almost negligibly small. It has been also indicated that the saccharide chain is, on the average, composed of three glucose residues. Concerning this point, after alkaline degradation of **1**, paper-chromatographic study showed[4] that the number of glucose residues is 3 or 4.

We now report the chemical structure of the sugar moiety of **1**, ascertained by investigating the ^{13}C-n.m.r. spectra of **1** and analogous sugar compounds.

EXPERIMENTAL

N.m.r. spectra were recorded with a Varian XL-100-15A spectrometer operating at 25.16 MHz, for a solution of **1** (39 mg) in deuterium oxide (0.4 mL) in a Pyrex tube (5 mm o.d.). The Fourier-transform mode was used, with 4096 data points. The other parameters were: spectral width, 5 kHz; acquisition time, 0.40 s; pulse width, 41 μs (corresponding to a pulse of 90°); pulse delay, 0 s; and total transients, 180,000. A little aggregation occurred. A trace of *tert*-butyl alcohol was added as the standard compound. The chemical shifts were read from the signal of the methyl carbon atoms of *tert*-butyl alcohol, and were tentatively corrected as the chemical shifts from tetra-

methylsilane [δ (from Me_4Si) = δ (from *tert*-BuOH) + 32.2 p.p.m.]. The temperature of the sample was 30°.

RESULTS

The ^{13}C-n.m.r. spectrum of **1** is shown in Fig. 1. It is well known, from a number of ^{13}C-n.m.r. investigations[5,6], that the chemical-shift range of C-1, which is bonded to two oxygen atoms in glucose and its analogs, is 110–90 p.p.m. from Me_4Si, and that that of C-2–C-6, which are bonded to one oxygen atom, is 85–60 p.p.m. The patterns obtained for these regions of the spectrum of **1** (see Fig. 1) are unexpectedly simple, suggesting that the structure of the sugar chain of glycopeptide **1** is very simple. It is evident from study of the intensities of the signals in the region of 110–60 p.p.m. that the number of carbon atoms in the sugar moiety is less than 24. This conclusion, and the results of g.l.c. and paper-chromatographic analyses[3,4], indicate that the number of sugar residues in **1** is 3 or 4, because a glucose residue has six carbon atoms, and there is a possibility that the signals of other carbon atoms, in the amino acid part of **1**, lie in this region.

The spectrum of **1** has only two peaks, A (104.2 p.p.m.) and B (100.2 p.p.m.), in the region of the chemical shifts of C-1 of the glucose residues. Interestingly, the intensities of these two signals are almost the same, eliminating the possibility of the presence of four glucose residues in **1**. Thus, it is evident that **1** has a three-glucose component.

A methine carbon atom that is bonded to one oxygen and one nitrogen atom (-O-CH-N<) would be expected to resonate at much higher field (probably in the range of 85–70 p.p.m.) than one bonded to two oxygen atoms. Taking into account

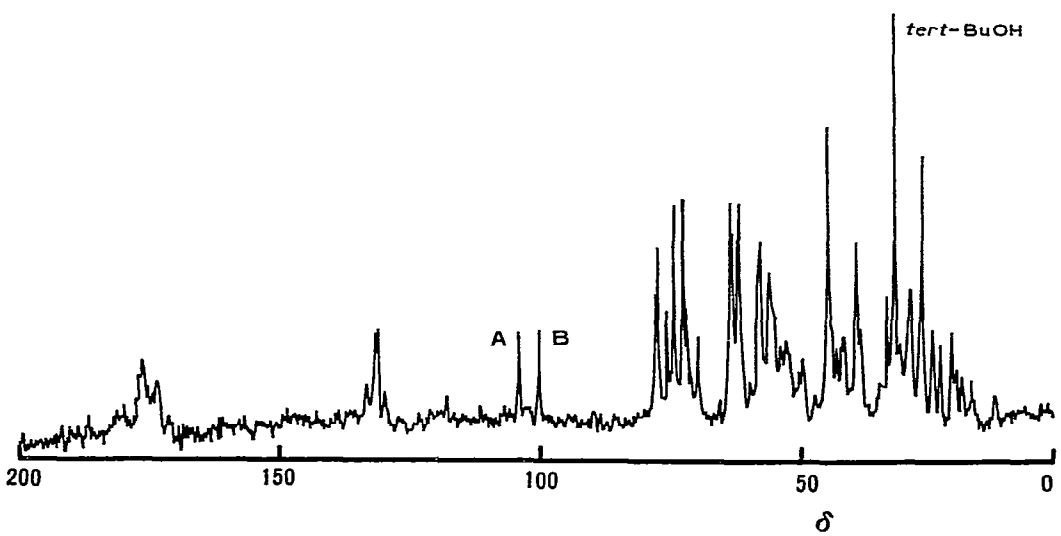
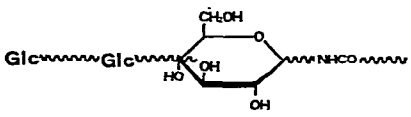


Fig. 1. Total ^{13}C-n.m.r. spectrum of **1** (δ in p.p.m. from Me_4Si.).

this probability and the observation of only two signals (having the same intensities) for C-1 in the three glucose components in **1**, it may be concluded that **1** has a glycosyl–N linkage at C-1 of a terminal glucosyl residue, as in **2** or **3**, instead of a peptide linkage[7] through the amino group of a 2-amino-2-deoxyglucose residue (**4**). Many other possibilities for the glucose–glucose chain and glycosyl linkage, for example, a glycosyl-*O* linkage[8] and a glycosyl ester linkage, are excluded. This is the first example, among natural compounds, of a direct, glycosyl–N linkage between a glycosyl and an amino acid residue. Therefore, **1** contains a new type of carbohydrate–protein linkage.



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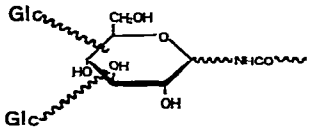

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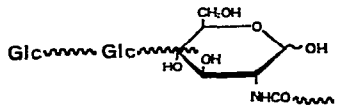

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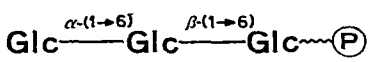
In order to assign the signals of carbon atoms in the sugar moiety of **1**, the ^{13}C-n.m.r. spectra of 27 kinds of analogous sugars and their derivatives were measured under almost the same conditions. From analysis of these spectra, it became evident that signal B, at 100.2 p.p.m. (see Fig. 1), can be assigned to C-1 of an α-(1→6) glucose–glucose linkage, because the observed chemical shift (100.5 p.p.m.) of isomaltose [having an α-(1→6)-linkage] is very close to that of B. However, signal A (104.2 p.p.m.) cannot be immediately assigned, because no peaks of analogous sugars are at values close to that of A. As the chemical shifts of carbon atoms of one glucose ring are influenced by the type of linkage of the second or the third glucose residue, or both, substituent effects by the glucose residue on the chemical shifts were obtained by detailed analysis of the chemical shifts of glucose–glucose chain-compounds having α- or β-(1→1)-, -(1→2)-, -(1→3)-, -(1→4)-, and -(1→6)-linkages. Using the values of the substituent effects and the chemical shifts of glucose thus obtained, the expected chemical-shifts of many types of combinations of three glucose residues



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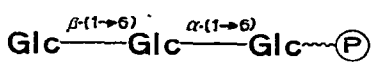

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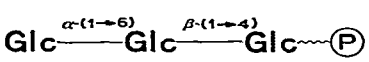

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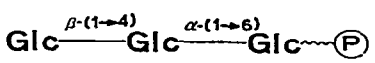

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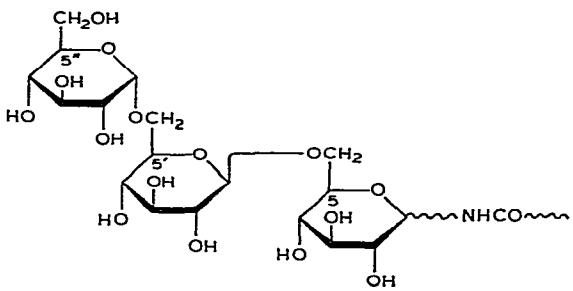
where (P) = protein residue

were calculated. Thus, five types (**5**–**9**) were chosen as appropriate combinations that fit the two peaks, A and B, of **1** (from the analysis of C-1 chemical-shifts).

In the analysis of the chemical-shift range of 85–60 p.p.m., it was to be expected that the chemical shift of the bonded, C-4 atom of the glucose engaged in a β-(1→4)-linkage must be near 81.4–81.2 p.p.m., considering the experimentally obtained value (81.2 p.p.m.) for cellobiose [β-(1→4)-linkage] and the results of the calculations. However, we could find no such signal in the spectrum of **1**. The chemical shift of its nearest peak is 77.7 p.p.m., but that is too far away. Therefore, types **7**, **8**, and **9** are excluded. From the ^{13}C-n.m.r. data alone, it is not possible to decide whether **5** or **6** is the structure, because any signals of the glucose residue linked to the peptide ("reducing" terminus) cannot at present be assigned, due to the unknown configuration (α or β) of the glycosyl–N linkage.

From the concanavalin A test[9], it has been made clear that the (nonterminal) glucosyl residue in **1** has the α configuration. This fact and n.m.r.-spectral analyses indicate that **1** has the α-(1→6)-β-(1→6) structure shown in **10**.



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REFERENCES

1 S. SHIBATA, Y. MIYAKAWA, T. NARUSE, AND T. TAKUMA, *J. Immunol.*, 102 (1969) 593–601.
2 S. SHIBATA AND T. NAGASAWA, *J. Immunol.*, 106 (1971) 1284–1294.
3 (*a*) S. SHIBATA, T. NARUSE, Y. MIYAKAWA, AND T. NAGASAWA, *J. Immunol.*, 104 (1970) 215–223; (*b*) S. SHIBATA AND H. SAITO, *J. Biol. Chem.*, to be submitted.
4 S. SHIBATA, *Jpn. J. Clin. Chem.*, 7 (1978) 110–120.
5 J. B. STOTHERS, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972, chap. 11.
6 E. BREITMAIER AND W. VOELTER, *^{13}C NMR Spectroscopy, Methods and Applications*, Verlag Chemie, Weinheim, Germany, 1974, chap. 5.
7 M. TANAKA AND I. YAMASHINA, *Carbohydr. Res.*, 27 (1975) 175–183.
8 P. HALLGREN, A. LUNDBLAD, AND S. SVENSSON, *J. Biol. Chem.*, 250 (1975) 5312–5314.
9 S. SHIBATA, T. NAGASAWA, AND K. MIURA, *Biochim. Biophys. Acta*, 499 (1977) 392–403.