Use of Carbon-13 Spin–Lattice Relaxation Times for Sugar Sequence Determination in Steroidal Oligosaccharides

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Summary Carbon-13 spin–lattice relaxation times have been measured for *k*-strophanthoside (**1**) in order to show that this technique may be useful for the sugar sequence determination in steroidal oligosaccharides.

MANY steroidal oligosaccharides are medically important compounds.[1] Determination of the sequence of their carbohydrate units may be carried out by partial hydrolysis either enzymatically or chemically.[1,2] We present here an alternative approach based on ^{13}C n.m.r. spectroscopy and which does not involve chemical degradation.

Carbon-13 spin–lattice relaxation times have been measured for a number of steroidal mono-, di-, tri-, and tetra-saccharides and as the first example† we report here results for *k*-strophanthoside (**1**), the constitution of which is known.[1] Carbon-13 signals were unambiguously assigned for (**1**) by spectral comparison with the following models: the aglycone cardenolide strophanthidin,[3] the steroidal monosaccharide cymarin,[4] and the disaccharide methyl-β-gentiobioside;[5] these data are also in conformity with the structure reported earlier.[1] The T_1 values obtained in 0·23 M [$^2H_5$]pyridine solution at 90 °C for the carbon atoms of (**1**) are indicated in the Figure.‡

The following conclusions can be drawn. (i) The differences between the average NT_1 values (N = number of hydrogen atoms attached to a particular carbon atom) of the constituent units of (**1**) are important. Relaxation time measurements allow assignment of a given carbon signal either to the steroid or to the oligosaccharide unit. The carbohydrate carbon signals can be further divided into three groups, representing the three sugars, on the basis of the T_1 values. (ii) The average NT_1 values for the

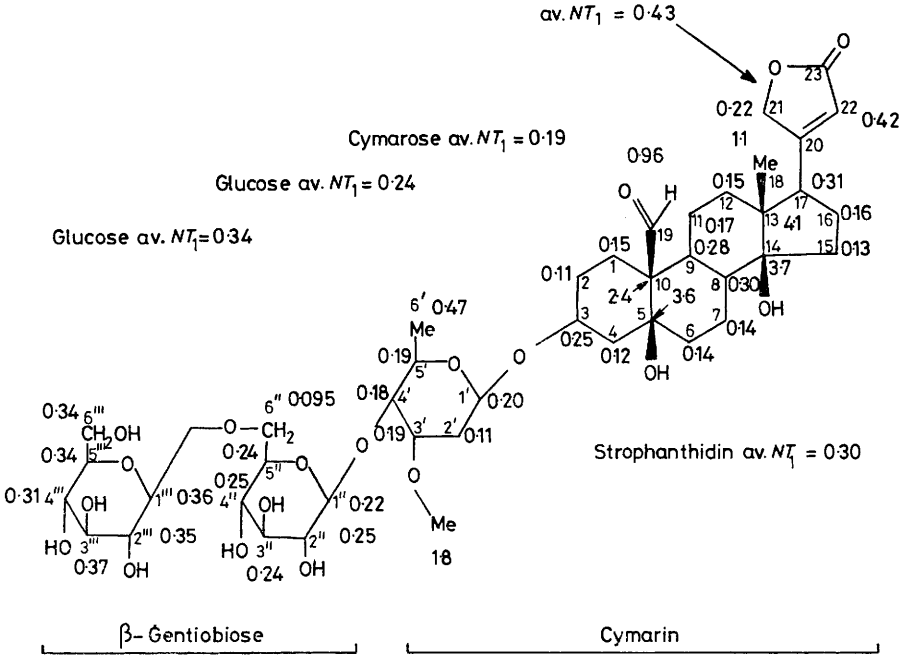

FIGURE. T_1 values measured for *k*-strophanthoside (**1**); values for C-20 and C-23 were not measured. The small numbers designate the carbon chain.

† Results related to other oligosaccharides will be presented elsewhere.

‡ The ^{13}C n.m.r. spectra were recorded on a Varian XL-100-15 FT n.m.r. spectrometer equipped with a Varian 620/1 computer. For atoms with short T_1 values (< 0·4 s) the inversion recovery technique was employed while longer relaxation times were measured by progressive saturation. Reproducibility of the measured T_1 values was ± 5—10%.

three sugars reflect their sequence with respect to the steroid [NT_1 terminal glucose $> NT_1$ central glucose $> NT_1$ inner sugar (cymarose)]. The relatively high average NT_1 value of the terminal glucose unit is due, in agreement with previous results,[6] to the nature of its $1 \rightarrow 6$ type linkage to the central sugar.

For the steroidal carbon atoms, the butenolide E-ring has a higher average NT_1 value than the tetracyclic steroidal skeleton.[7] The T_1 value of C-19 indicates an additional degree of freedom for the C-10–C-19 axis[7] [comparable T_1 values are obtained for C-18 (13-Me) and C-19 (10-CHO)]. The 13-Me group rotates freely.[7]

With the help of appropriate model compounds the carbon signals of steroidal oligosaccharides can be easily assigned.[8] However, we emphasize that it is not necessary to carry out an absolutely unambiguous assignment for all the carbon signals in order to apply the spin–lattice relaxation time technique for the sugar sequence determination. The value of one or two identified carbon signals of each carbohydrate component should shed light on the relative situation of a given sugar in the steroidal oligosaccharide.

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[1] T. Reichstein and E. Weiss, *Adv. Carbohydrate Chem.* 1962, **17**, 65.
[2] R. Tschesche and G. Wulff, *Fortsch. Chem. Org. Naturstoffe*, 1973, **30**, 462.
[3] K. Tori, H. Ishii, Z. W. Wolkowski, C. Chachaty, M. Sangaré, F. Piriou, and G. Lukacs, *Tetrahedron Letters*, 1973, 1077.
[4] Unpublished results.
[5] T. Usui, N. Yamaoka, K. Matsuda, K. Tuzimura, H. Sugiyama, and S. Seto, *J.C.S. Perkin I*, 1973, 2425.
[6] A. Neszmelyi, S. Omura, and G. Lukacs, *J.C.S. Chem. Comm.*, 1976, 97; A. Allerhand and D. Doddrell, *J. Amer. Chem. Soc.*, 1971, **93**, 2777; K. Yamasaki, M. Kaneda, and O. Tanaka, *Tetrahedron Letters*, 1976, 3965.
[7] F. W. Wehrli, *Topics Carbon-13 N.M.R. Spectroscopy*, 1976, **2**, 343.
[8] K. Tori, T. T. Thang, M. Sangaré, and G. Lukacs, *Tetrahedron Letters*, 1977, 717.