Tetrahedron Letters,Vol.26,No.33,pp 3951-3954,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

THE USE OF ONE-BOND, HETERONUCLEAR COUPLING CONSTANTS ($^1J_{C,H}$) AS STRUCTURE REPORTERS IN ^{13}C-N.M.R. OF OLIGOSACCHARIDES CONTAINING 3-DEOXY-D-MANNO-2-OCTULOSONIC ACID

RUDOLF CHRISTIAN*, GERHARD SCHULZ, AND FRANK M. UNGER
Sandoz-Forschungsinstitut, A-1235 Wien, Österreich

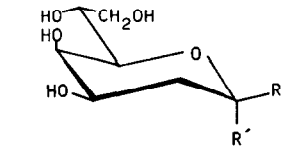
ABSTRACT. In proton-coupled, ^{13}C-n.m.r.-spectra of 3-deoxy-D-manno-2-octulopyranosylono (KDOp-)derivatives, the $^1J_{C\text{-}5,H\text{-}5}$ is significantly larger (148 Hz) than the respective coupling constants of the other carbon signals, and the chemical shift of the signal displaying this spacing indicates whether position 5 is glycosidated or unsubstituted.

The one-bond, heteronuclear n.m.r. coupling constants ($^1J_{C,H}$) corresponding to the carbon atoms of carbohydrate derivatives can be accurately determined from proton coupled, ^{13}C-n.m.r. spectra. For anomeric carbon atoms of pyranose derivatives, the values of the $^1J_{C1,H1}$ are ca. 170 Hz when the oxygen function is axial, or ca. 160 Hz when it is equatorial[1]. This empirical rule has been formulated by Bock, et al. for the determination of anomeric configurations, particularly where the three-bond, homonuclear coupling constants $^3J_{H1,H2}$ are not informative, as is the case, e. g., in the mannose or rhamnose series.

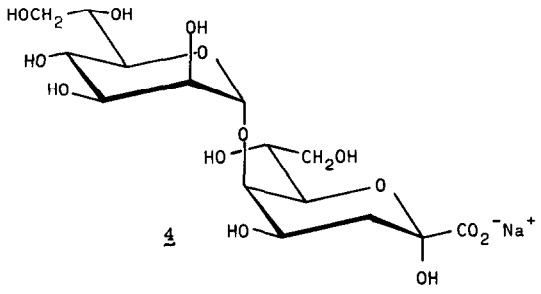
In pyranoid rings, the values of $^1J_{C,H}$ of carbon atoms other than anomeric centers range from 148 to 152 Hz for those having axial, and 140 to 146 Hz for those having equatorial oxygen functions. These values remain essentially unchanged when the respective center becomes substituted by an ether-function, as has been shown in the mannose series[2], or by a glycosyl residue[3].

Among the mean values of $^1J_{C,H}$ for carbon atoms 3 through 8 of six pyranoid derivatives (compounds 1 - 6) of 3-deoxy-D-manno-2-octulosonic acid (KDO), the one for C-5 (bearing an axial oxygen function) is significantly larger (148 Hz) than those for the other carbon atoms, as expected (Table 1). Owing to their relatively large spacings, the C-5-signals may be distinguished from others having similar chemical shifts. This is particularly useful with regard to the signal due to C-4 in the α-ketopyranose derivatives of KDO such as 1α and 2.

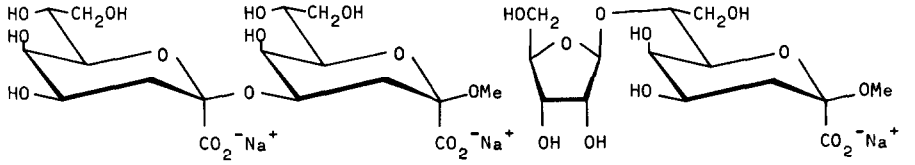
Table 2 shows the chemical shift values corresponding to the coupling constants listed in Table 1. In this manner, the empirical assignments made previously[4,5] for C-4 and C-5 of some α-ketosidic derivatives of KDO now have to be reversed (e. g., compounds 1α and 2 in Table 2).



1α, R=$CO_2^-NH_4^+$, R´=OH
1ß, R=OH , R´=$CO_2^-NH_4^+$
2 , R=$CO_2^-Na^+$, R´=OMe
3 , R=OMe , R´=$CO_2^-Na^+$



4



5 6

Table 1. $^1J_{C,H}$-couplings[a] of mono- and disaccharide derivatives of KDO (Hz)

	C-3(dd)	C-4	C-5	C-6	C-7	C-8(dd)
1-αp	131	144	149	143	144	144
1-ßp	131	145	148	140	-	144
2	132	145	149	142	144	144
3	132	142	148	144[b]	144[b]	144
4	132	143	148	145	145	145
5'	132	141	148	136[c]	139[d]	143
5	132	141	148	136[c]	-[d]	143
6	133	142	148	140	142	144

a) The couplings are of first order and are measured by hand on enlarged 62.9 MHz spectra; the estimated error is ± 1 Hz; couplings of carbons shifted through substitution are shown in boxes.

b) These couplings are determined from a 22.6 MHz spectrum.

c) These couplings are part of a strongly-coupled system ($\Delta\delta$ H6,H7 = 72 Hz; $^3J_{H6,H7}$ = 9 Hz); best simulated value for the $^1J_{C6,H6}$ is 142 Hz and for the $^2J_{C6,H7}$ = -6 Hz.

d) Strongly coupled system; the best simulated value for the $^1J_{C7',H7'}$ is 144 Hz and for the $^2J_{C7',H6'}$ = -5 Hz; determination of the $^1J_{C7,H7}$ was not possible due to signal overlap.

Compound 4 is a disaccharide containing an α-L-glycero-D-manno-heptopyranosyl residue linked to position 5 of a reducing KDO moiety[6]. The proton-coupled, ^{13}C-n.m.r. spectrum of 4 (Fig. 1) reveals the expected glycosylation shift ($\Delta\delta$ = + 8.9 ppm) of the C-5-signal (δ = 76.49 ppm; $^1J_{C5,H5}$ 148 Hz; Tables 1 and 2).

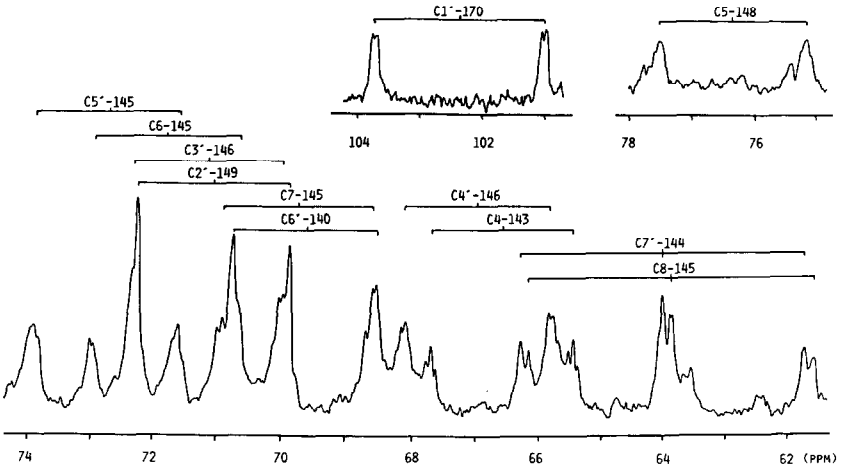


Fig. 1: 62.9 MHz proton-coupled ^{13}C-n.m.r. spectrum of compound 4 in D_2O at 297 K, referenced to dioxane = 67.40 ppm relative to TMS.

Table 2. ^{13}C-n.m.r. shifts of the compounds listed in Table 1[a,b,c,d].

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8
1-αp	177.5	97.3	34.6	67.1	67.6	72.2	70.3	64.0
1-βp	176.3	98.2	36.1	68.5	66.4	74.6	70.1	64.7
2	176.1	101.3	34.9	66.8	67.1	72.2	70.2	63.9
3	174.5	102.1	35.3	68.2	66.2	74.3	69.9	64.8
4'	102.5	71.2	71.3	67.1	72.9	69.8	64.1	
4	177.0	97.3	35.1	66.7	76.5	72.1	70.0	64.0
5'	174.4	103.0*1	35.9	68.1	66.2	73.8*2	69.8*3	65.1*4
5	174.4	102.0*1	34.1	72.8	66.2	74.3*2	69.9*3	64.8*4
6'	105.8	75.7	71.2	83.5	63.2			
6	174.4	102.1	35.3	68.2	66.0	72.9	75.4	60.8

a) Shifts are in ppm downfield from TMS, whose shift was set at 67.40 ppm relative to dioxane in deuterium oxide.

b) Assignments of compounds 1 and 6 were proven by means of proton-carbon heteronuclear correlated 2D-spectra.

c) Additional signals were 51.4 ppm (144 Hz) OMe of compound 2; 52.3 ppm (145 Hz)OMe of compound 3; 52.2 ppm (144 Hz) OMe of compound 5 and 52.4 ppm (144 Hz) OMe of compound 6.

d) Additional couplings for 4' (the heptosyl unit of 4) were from C1 to C7, 170, 149, 146, 146, 145, 140, 144 Hz (dd) and for 6' (the ribofuranosyl unit of 6) from C1 to C5, 174, 155, 148, 148 and 142 Hz (dd).

*1 - *4) assignments may be interchanged.

In this case, the occurrence of a signal having a large spacing in the shift range of linkage carbon atoms confirms C-5 as the linkage site of the disaccharide. By similar considerations, the chemical shift of C-2' of the heptopyranosyl moiety, bearing likewise an axial oxygen function, can be identified (δ = 71.19 ppm; $^{1}J_{C2',H2'}$ 149 Hz; Fig. 1).

The proton coupled ^{13}C-n.m.r. spectra of the disaccharide derivatives 5 and 6 provide examples of C-4- and C-7-signals subject to glycosidation shifts[7,8]. The spacings of both these signals are in the range expected for the $^{1}J_{C,H}$ of pyranoid ring carbons bearing equatorial oxygen functions, or of the carbon atoms of the side chain. Thus if compounds 5 and 6 were to be analyzed as unknowns, C-5 of KDO could be eliminated from consideration as site of glycosidation in both 5 and 6. This would narrow down the choice to positions 4 or 7, as the C-8-signal would appear as a doublet of doublets, and a decision could easily be made on the basis of ß-shifts.

Aside from the applications with octulopyranosylono and L-glycero-D-manno-heptopyranosyl residues, observations of $^{1}J_{C,H}$-values have contributed to the identification of C-2-positions of rhamnopyranosyl residues as linkage sites in a regular rhamnan from Bacillus stearothermophilus[3,9]. It is expected that the localisation of relatively large spacings ($^{1}J_{C,H}$) in proton-coupled ^{13}C-n.m.r. spectra can be employed to advantage in the analysis of oligosaccharides containing sugars in pyranoid form, having one axially-disposed hydroxyl function. Due to the absence of conformational equilibria, which would lead to average couplings[10], the glycopyranosyl residues of oligosaccharides or regular polysaccharides are especially suitable for study in this manner.

REFERENCES

1. K. BOCK, I. LUNDT, and C. PEDERSEN, Tetrahedron Lett., (1973), 1037-1040.
2. K. BOCK and C. PEDERSEN, J. Chem. Soc., Perkin Trans. 2 (1974), 293-297.
3. R. CHRISTIAN, G. SCHULZ, F.M. UNGER, P. MESSNER, Z. KÜPCÜ, and U.B. SLEYTR, submitted for publication.
4. R. CHRISTIAN, G. SCHULZ, P. WALDSTÄTTEN, and F.M. UNGER, Tetrahedron Lett., (1984), 3433-3436.
5. H. BRADE, U. ZÄHRINGER, E.T. RIETSCHEL, R. CHRISTIAN, G. SCHULZ, and F.M. UNGER, Carbohydrate Res., 134 (1984), 157-166.
6. W. DRÖGE, V. LEHMANN, O. LÜDERITZ, and O. WESTPHAL, Eur. J. Biochem., 14, (1970), 175-184.
7. A. NESZMÉLYI, P. KOSMA, R. CHRISTIAN, G. SCHULZ, and F.M. UNGER, Carbohydrate Res., 138 (1985), in press.
8. P. WALDSTÄTTEN, R. CHRISTIAN, G. SCHULZ, F.M. UNGER, P. KOSMA, C. KRATKY, H. PAULSEN, ACS Symposium Series 231, (1983), 121-140.
9. Z. KÜPCÜ, L. MÄRZ, P. MESSNER, and U.B. SLEYTR, FEBS Letters, 173 (1984), 185-190.
10. K. BOCK and C. PEDERSEN, Act. Chem. Scand. B29, (1975), 258-262.

(Received in Germany 17 May 1985)