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Elucidation of the structure and conformation of a methylated tetrasaccharide-alditol acetate by n.m.r. spectroscopy

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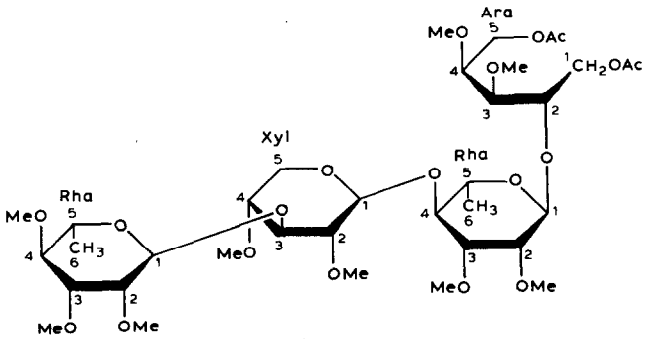
ABSTRACT

The structure of the methylated derivative (**1**) of the tetrasaccharide-alditol-*O*-β-L-rhamnopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-L-rhamnopyranosyl-(1→2)-1,5-di-*O*-acetyl-L-arabinitol has been determined solely on the basis of n.m.r. data.

INTRODUCTION

The methylated alditol acetate **1**, derived from the tetrasaccharide β-L-Rha*p*-(1→3)-β-D-Xyl*p*-(1→4)-β-L-Rha*p*-(1→2)-L-Ara, was obtained[1] in studies of triterpenoid glycosides from *Crossopterix febrifuga* after methylation followed by reductive cleavage with lithium aluminium hydride and acetylation. These saponins show analgesic, mucolytic, and antiedemic activities.



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* This paper is dedicated to the memory of Dr. Antonio De Marco who died on 16 February 1990.
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The structure and conformation of **1**, now reported, were determined solely on the basis of n.m.r. data. Use has been made of 2D ^1H–^1H homonuclear correlated (DQF-COSY, HOHAHA, NOESY, and ROESY) and ^{13}C–^1H heteronuclear correlated experiments with proton observation that has higher sensitivity than the conventional carbon observation[2], and has been applied to oligosaccharides[3,4].

Semiselective excitation with Gaussian pulses[5] has also been used to obtain high-resolution spectra, where severe overlap prevented the extraction of multiplet patterns and coupling constants.

1D COSY, COSY-RELAY, and HOHAHA were performed as recently described by Kessler *et al.*[6].

EXPERIMENTAL

Compound **1** was obtained as described[1] and the n.m.r. spectra were obtained on a 73mM solution in C_6D_6 (internal Me_4Si) at 25° with a Bruker AM-500 spectrometer, using a 5-mm reverse probe and a selective pulse generator.

All 2D experiments were performed in the phase-sensitive mode. For the homonuclear 2D experiments, 2K data were accumulated in the F2 dimension, with 512 t_1 increments, zero-filled to 1024, before Fourier transformation. The experimental data were weighted in both the t_2 and t_1 dimensions with a cosine-bell function. For the HOHAHA and for the ROESY experiments, a sweep width of 3550 Hz was employed in each dimension. A 14- and 53-ms MLEV-17 spin-lock train, preceded and followed by trim pulses of 2.5 ms, was applied for the HOHAHA experiments. The mixing time for the ROESY was 0.216 s.

When using the selective excitation unit, a 90° soft pulse of 80 ms was applied with an external attenuation of 40 db and transmitter waveform power (WP1) of 40. The selective HOHAHA experiment was performed in the reverse mode with the soft and hard signals mixed within a directional coupler. A sweep width of 4000 Hz was employed and 64K data were acquired, which gave a digital resolution of 0.12 Hz/pt.

2D Heterocorrelated spectra *via* direct connectivities (HMQC) were obtained by using the standard sequence with the addition of a bilinear pulse in the preparation period, in order to obtain[7] a better suppresion of resonances due to protons bound to ^{12}C. The delay T, calibrated to correspond to the zero crossing of the inverted magnetisation, was 1.2 s.

The spectra based on ^{13}C–^1H coupling across more than one bond (HMBC) were performed using the scheme described by Bax and Summers[8]; the correlation was created *via* heteronuclear zero and double-quantum coherences using the inverse mode, optimised on long-range couplings ($D1 = 1/2\,{}^1J_{C,H}$) with a low-pass J-filter to suppress one-bond correlations ($D2$ = 60 ms). Sweep widths of 3550 and 22000 Hz were used in the F2 and F1 dimensions, respectively.

RESULTS AND DISCUSSION

The ^{13}C-n.m.r. spectrum of **1** (Fig. 1) contains 33 resonances in five regions: (*1*) 2 C = O near 170 p.p.m. (off-scale), (*2*) 3 C–1 near 100 p.p.m., (*3*) 76–86 p.p.m. (12 C), (*4*) 57–69 p.p.m. (14 C), and (*5*) near 20 p.p.m. (4 C).

The ^{1}H-n.m.r. spectrum is even less informative. There are signals for H-1 (3 d, 4.8–5.6 p.p.m.) and OMe (9 s, 3.0–3.6 p.p.m.) together with four sharp resonances (2 s and 2 d) in the range 1.4–1.8 p.p.m., which parallel those observed in the ^{13}C-n.m.r. spectrum. The remaining ^{1}H resonances are in the range between 2.7–4.6 p.p.m. Integration of the spectrum indicated 23 protons plus the OMe groups.

2D ^{1}H–^{1}H Correlated spectra. — The ^{1}H–^{1}H connectivities were determined by DQF-COSY[9] with homonuclear HOHAHA optimised[10] for *J* 7.5 and 2.0 Hz, to select for vicinal and long-range interactions, respectively.

The three 2D contour plots are shown in Fig. 2 for the region 2.7–4.6 p.p.m., *i.e.*, omitting resonances for H-1 and OMe which are assigned readily and give clear cross-peaks to the coupled protons.

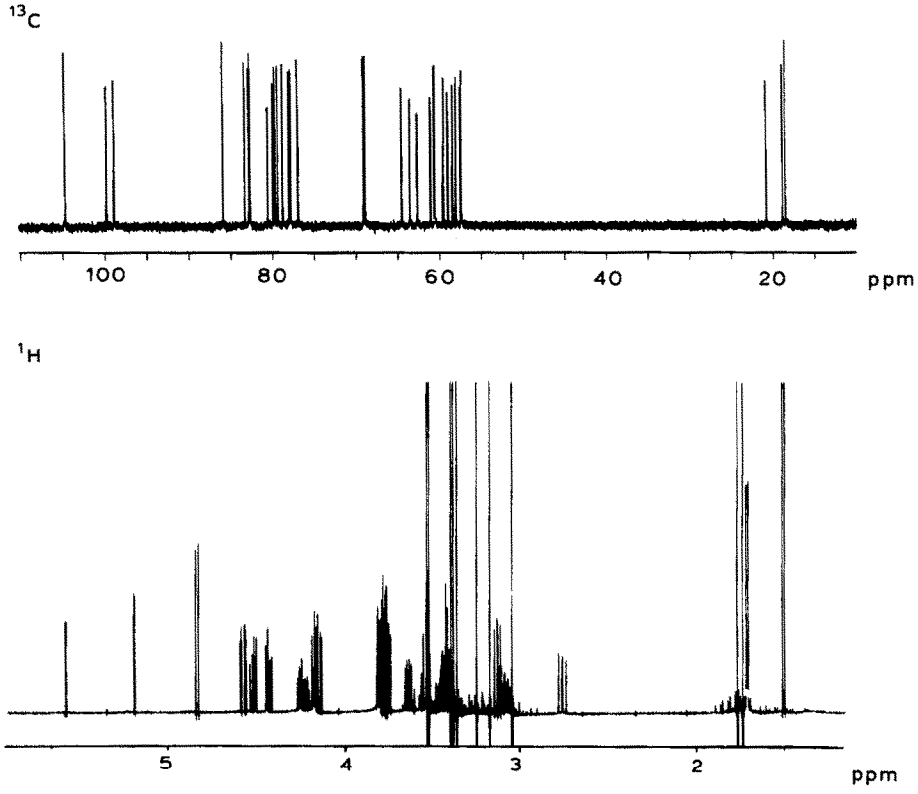

Fig. 1. 1D ^{1}H- and ^{13}C-n.m.r. spectra of $R^{II}XR^{1}A$ (**1**).

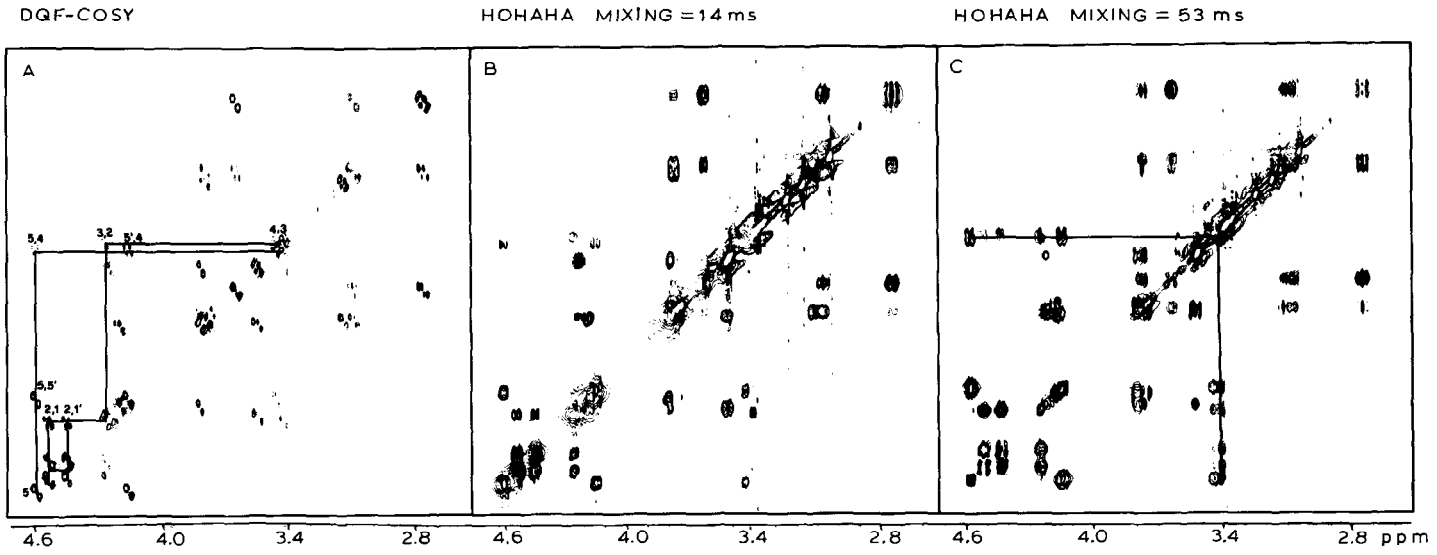

Fig. 2. 2D N.m.r. spectra of $R^{II}XR^{I}A$ (**1**): 2.5–4.7 p.p.m. region (in F1 and F2) of *A*, DQF-COSY; *B*, HOHAHA experiment optimised for $J_{H,H}$ 7.5 Hz; *C*, HOHAHA experiment optimised for $J_{H,H}$ 2.0 Hz. In *A*, the connectivities for the arabinose protons are indicated. In *C*, the same connectivities appear on the same row (or column), because of the long-range interactions: starting from H-3 on the main diagonal, H-5, H-1, H-1′, H-2′, H–5′,and H-4 are found from low to high field.

The DQF-COSY identified one-bond interactions, but the phase properties of the signals prevented analysis of the multiplets in terms of coupling constants. However, the HOHAHA cross-sections give well-resolved multiplets amenable to interpretation. On going from *A* to *B* (Fig. 2), a few new peaks appear, and in *C* the information is of the RELAY type. The combined analysis of the three spectra *A*–*C* identifies the four independent spin systems:

(1,1) 2 3 4 (5,5′)	1 2 3 4 (5,5′)	1 2 3 4 5 6
$-CH_2-CH-CH-CH-CH_2$	$-CH-CH-CH-CH-CH_2-$	$-CH-CH-CH-CH-CH-CH_3$
A	X	R^I and R^{II}

The assigments at this stage are arbitrary. As an example, the connectivities are shown for the spin system *A* identified from the DQF-COSY spectrum (Fig. 2A); in the HOHAHA spectrum optimised for small *J* values (Fig. 2C), the resonances of H-1 and H-1′ also show up in the row of the H-3 resonance. The resonances of H-3 and H-4 are in close proximity, and their connectivity is identified only in the DQF-COSY spectrum, in which the diagonal is not very intense.

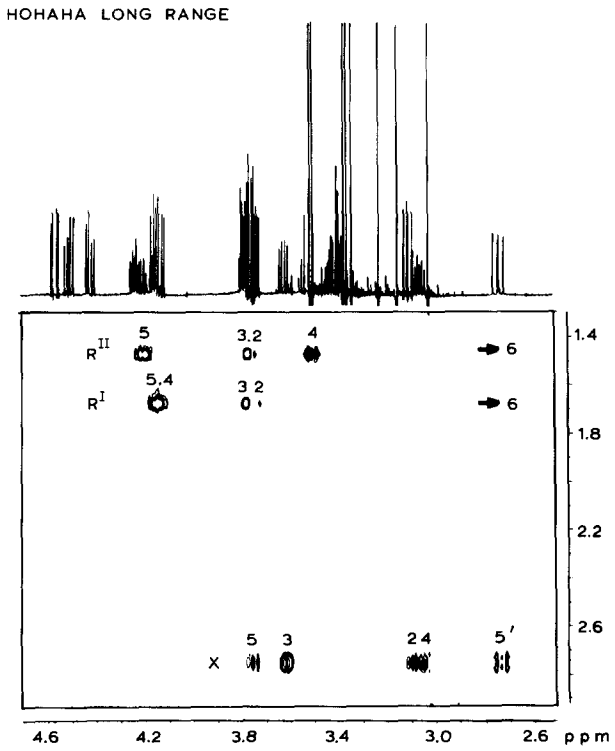

Fig. 3. 2D N.m.r. spectra of $R^{II}XR^IA$ (**1**): the 2.5–4.7 p.p.m. (F2) and 1.2–2.9 p.p.m. (F1) regions of the HOHAHA experiment optimised for $J_{H,H}$ = 2.0 Hz. Starting from the methyl resonances 6 of R^I and R^{II}, and from H-5′ of X, the ^1H resonances of the three rings are identified.

Fig. 3 shows an expansion of the HOHAHA long-range spectrum, including the methyl resonances in the F1 dimension. Starting from the two CH_3 doublets 6, the spin system for R^I and R^{II} were identified. Similarly, from the diagonal peak at 2.75 p.p.m., the spin system of X was determined. By transferring the assignments from COSY and HOHAHA to the 1D spectrum, analysis in terms of couplings is possible. For H-4 and H-5 of the spin system R^I and for H-3 of X, severe overlap made it difficult to resolve individual resonances of a multiplet in the 1D spectrum. The multiplet structure of H-3 was determined by 1D COSY, COSY-RELAY, and HOHAHA experiments, using soft Gaussian pulses for the excitation[5,6].

Fig. 4C shows how the selective excitation of H-1(X) at 4.85 p.p.m. reveals the signal of the coupled proton H-2(X) at 3.12 p.p.m. and the RELAY experiment (Fig. 4B) identifies that of H-3(X). The multiplet for H-3(X), obtained from 1D HOHAHA and COSY-RELAY experiments, when compared with the same multiplet taken from a

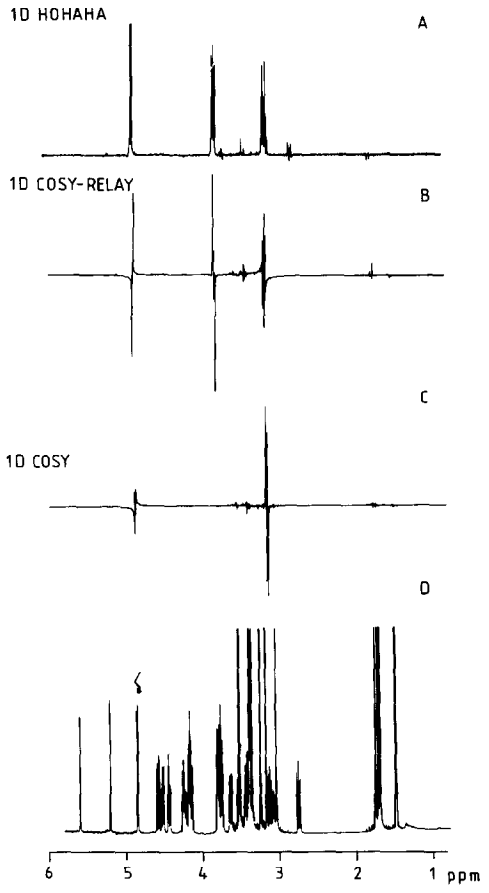

Fig. 4. Selective excitation experiments involving the anomeric proton at 4.85 p.p.m.: *A*, 1D HOHAHA; *B*, 1D COSY-RELAY; *C* 1D COSY; *D*, reference spectrum.

cross-section of the corresponding 2D HOHAHA spectrum, was shown to have a triplet structure owing to coupling to H-2,4(X). The low digital resolution of the 2D experiment does not allow the detection of possible differences between the two J values, and the triplet for H-3(X) obtained with the high-resolution (0.1 Hz/point) 1D HOHAHA experiment, suggests that this multiplet could be a deceptive triplet. The multiplet obtained from the COSY-RELAY experiment (Fig. 4B) shows four peaks in phase alternation, with partial cancellation of the central lines. It is not possible, however, to extract correct $J_{2,3}$ and $J_{3,4}$ values due to the inherent inaccuracy of the peak-to-peak measurements.

The resonances of H-4 and H-5 of R^I are in close proximity and are coupled. Although their chemical shifts were determined from the 2D spectra, the coupling constants could not be determined. The problem was solved by heteronuclear ^{13}C–^1H correlation (see below). The ^1H-n.m.r. data are listed in Tables I and II (the OMe singlets are reported in Table IV).

At this point, it is reasonable to close the X, R^I, and R^{II} systems with oxygens to form pyranoid rings. The A system lacks a resonance characteristic of H-1 and is therefore acyclic as suggested by (*a*) the J values, which are consistent with a high degree of free rotation, and (*b*) the fact that the two CH_2 groups of A are the only positions where the acetyl groups can be located. Inspection of the J values (Table II) gives information on the configurations and conformations of the units R′, R″, and X. The values are consistent with two β-L-Rha*p* units (R′ and R″) in $^1C_4$ conformations and with a β-D-Xyl*p* unit (X) in the $^4C_1$ conformation. Since acid hydrolysis of the original

TABLE I

^1H Chemical shift data[a] (δ in p.p.m.) for $R^{II}XR^IA$ (1)

Residue	*H-1*	*H-1′*	*H-2*	*H-3*	*H-4*	*H-5*	*H-5′*	*H-6*
A	4.52	4.43	4.25	3.41	3.44	4.58	4.14	
R^I	5.21		3.74	3.80	4.18	4.16		1.68
X	4.85		3.12	3.80	3.07	3.63	2.75	
R^{II}	5.59		3.76	3.79	3.53	4.23		1.47

[a] See Table IV for data on OMe and OAc groups.

TABLE II

$^3J_{H,H}$ Values (Hz) for $R^{II}XR^IA$ (1)

Residue	*1,1′*	*1,2*	*1′,2*	*2,3*	*3,4*	*4,5*	*4,5′*	*5,5′*	*5,6*
A	11.5	6.7	5.1	4.3	6.3	3.0	4.7	12.1	
R^I		1.9		3.1	9.3	10.0			5.8
X		7.8		9.0	9.0	5.3	10.2	11.6	
R^{II}		1.8		3.3	9.3	9.5			6.2

tetrasaccharide afforded[1] D-xylose, L-rhamnose, and L-arabinose in the ratios 1:2:1, the formation of A must have occurred during the preparation of **1**.

A straightforward assignment of sequence was obtained from the ROESY experiment (data not shown) which showed the following dipolar connectivities, H-1(X)–H-4(R′), H-1(R′)–H-2(A), and H-3(X)–H-1(R″).

Thus, **1** is the methylated derivative of *O*-β-L-rhamnopyranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-L-rhamnopyranosyl-(1→2)-1,5-di-*O*-acetyl-L-arabinitol ($R^{II}XR^{I}A$).

2D ^{13}C–^{1}H Correlated spectra — The ^{1}H-n.m.r. data were transferred to the ^{13}C resonances *via* heteronuclear ^{13}C–^{1}H correlation in the reverse mode (*i.e.*, with proton

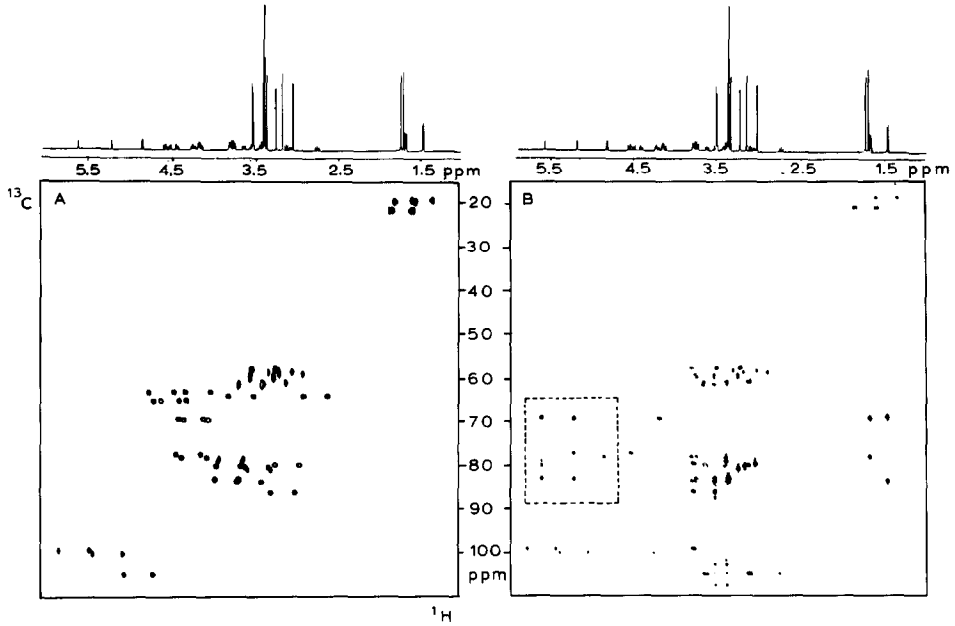

Fig. 5. 2D ^{1}H-^{13}C COSY spectrum of $R^{II}XR^{I}A$ (**1**) in the reverse mode: *A*, correlation optimised for ^{1}J coupling; *B* for $> {}^{1}J$ coupling (see Experimental).

TABLE III

^{13}C Chemical shift data[a] (δ in p.p.m.) for C-1/6 of $R^{II}XR^{I}A$ (**1**)

Residue	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
A	64.20	76.61	80.17	79.58	62.28	
R^{I}	99.38	77.64	82.45	77.43	68.73	18.49
X	104.32	85.99	79.33	79.10	63.17	
R^{II}	98.49	78.42	82.29	82.93	68.52	18.14

[a] See Table IV for data on the OMe and OAc groups

observation[2,11]), which has the advantages of increased sensitivity by a factor of 32 and there are no limits in the digital resolution of the ^{1}H-n.m.r. spectrum.

Fig. 5A shows the HMQC spectrum of $R^{II}XR^{I}A$ (1). Since no heteronuclear decoupling was applied, the spectra exhibit heteronuclear coupling in the F_2 dimension. In the lower-left corner are the resonances from the three C-1/H-1 and in the opposite corner are the signals for CMe of R^I and R^{II} and the OAc groups. The bulk of the ^{13}C resonances are in the ranges 56–70 and 76–86 p.p.m., and the chemical shifts are listed in Table III.

Heteronuclear ^1J ^{13}C–^{1}H correlation gave the J values for H-4 and H-5 of R^I, which could not be extracted from the homonuclear ^{1}H experiments as mentioned above. Approximate chemical shifts of the ^{1}H resonances were obtained easily from any of the 2D spectra shown, but the strong second-order effects gave a complex pattern that is not readily interpreted. Fig. 6C shows a small region of the ^{1}H-n.m.r. spectrum, which contains the resonances for H-4,5 of R^I, H-2,5a of A, and H-5 of R^{II}. Only the last three signals have the multiplets that were identified easily.

The cross-sections taken at the positions of the ^{13}C resonances in F1 and parallel to F2 can be regarded as 1D ^{1}H-n.m.r. spectra of molecules containing one ^{13}C with the remainder being ^{12}C. Figs. 6A and 6B show the cross-sections for H-4,5 of R^I and indicate the three molecular species which generate the spectra *A*–*C*. The $^1J_{C,H}$ splitting of 150 Hz allows precise measurement of the parameters for H-4,5. Thus, H-4 is a triplet

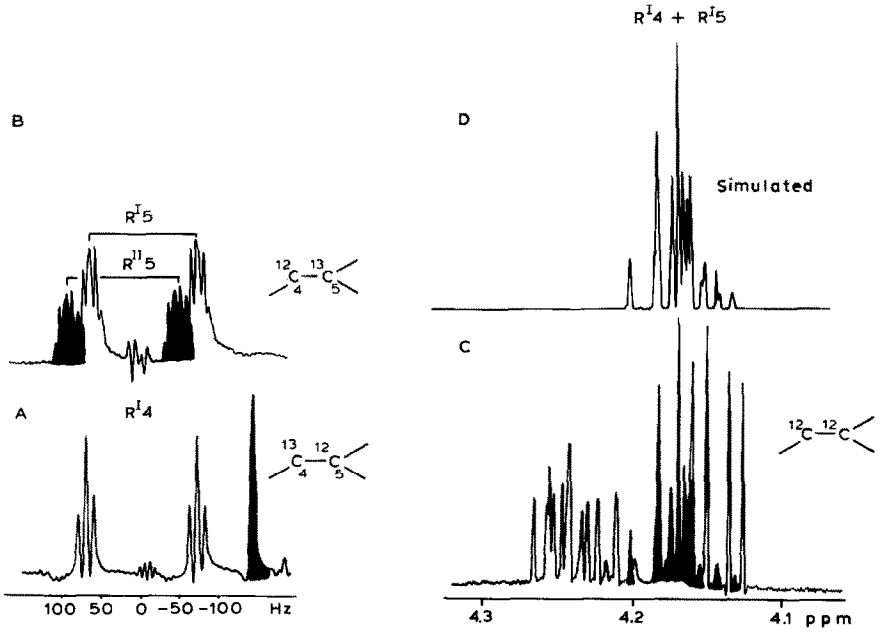

Fig. 6. Identification of the resonances for H-4,5 of R^I: *A* and *B*, cross-sections extracted from the spectrum in Fig. 7; *C* 1D ^{1}H-n.m.r. spectrum of the same region; *D*, simulation of the H-4,5 resonances based on the parameters (δ, J) in *A* and *B*, neglecting the ^{1}H,^{13}C coupling.

with $J_{3,4}$ and $J_{4,5}$ values of ~ 10 Hz. The signal for H-5 is more complex: the dotted resonances come from H-5 of R^{II}, the resonance of the attached carbon of which is close to that of R^{I} and below the separation allowed by the digital resolution in F1. The multiplets for H-5 of R^{I} can be interpreted as a 5.8-Hz quartet, further split by a 10-Hz interaction. The latter effect reflects the coupling to H-4, discussed above, and the former is due to interaction with H-6,6,6. The data obtained from the spectra in Figs. 6A and 6B were used to simulate 6C. The result, shown in Fig. 6D, is satisfactory and can be obtained only in the reverse mode.

Fig. 5B shows the HMBC ^{13}C–^{1}H reverse experiment based on interactions across more than one bond (see Experimental). All of the cross-peaks are assigned easily to interactions across 2 and 3 bonds. For example, those for ^{1}H at 1.68 p.p.m. and for ^{13}C at 77.43 and 68.73 p.p.m. represent the interaction of H-6,6,6 of R^{I} and C-5 (2 bonds) and C-4 (3 bonds). The same correlations hold for the R^{II} methyl group at 1.47 p.p.m. Most correlations are in the same sugar moiety and confirm the assignments made, but with three notable exceptions.

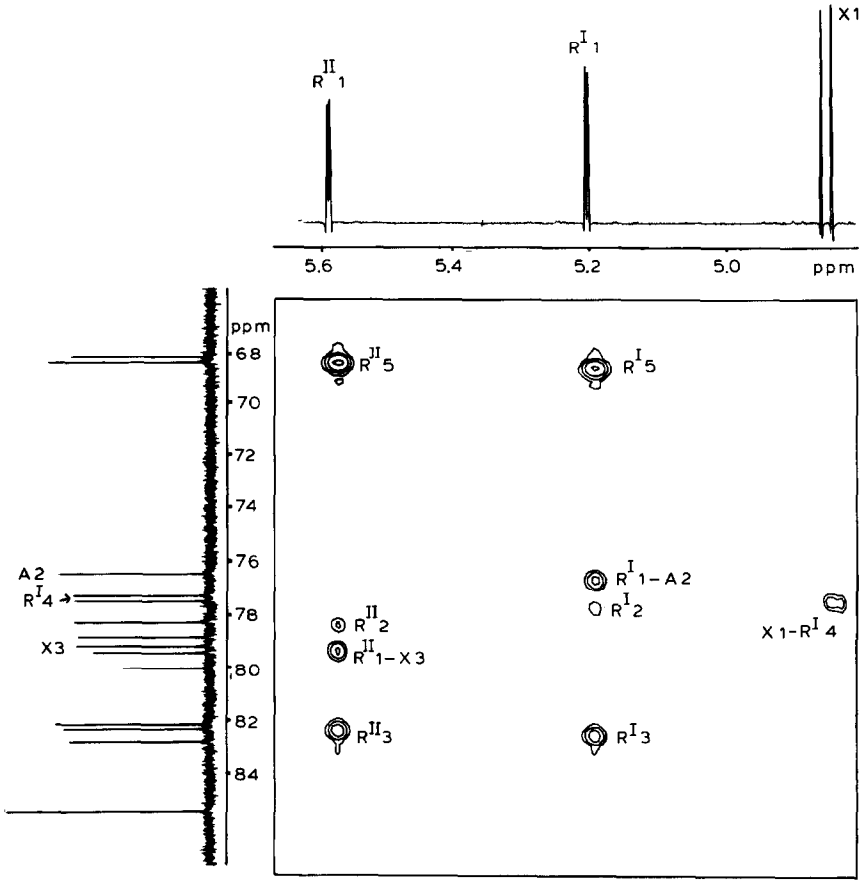

Fig. 7. 2D ^{1}H–^{13}C COSY spectrum of $R^{II}XR^{I}A$ (1) in the reverse mode (expansion of the dotted box in Fig. 6B), which shows the long-range correlations between the anomeric protons and neighbouring carbons.

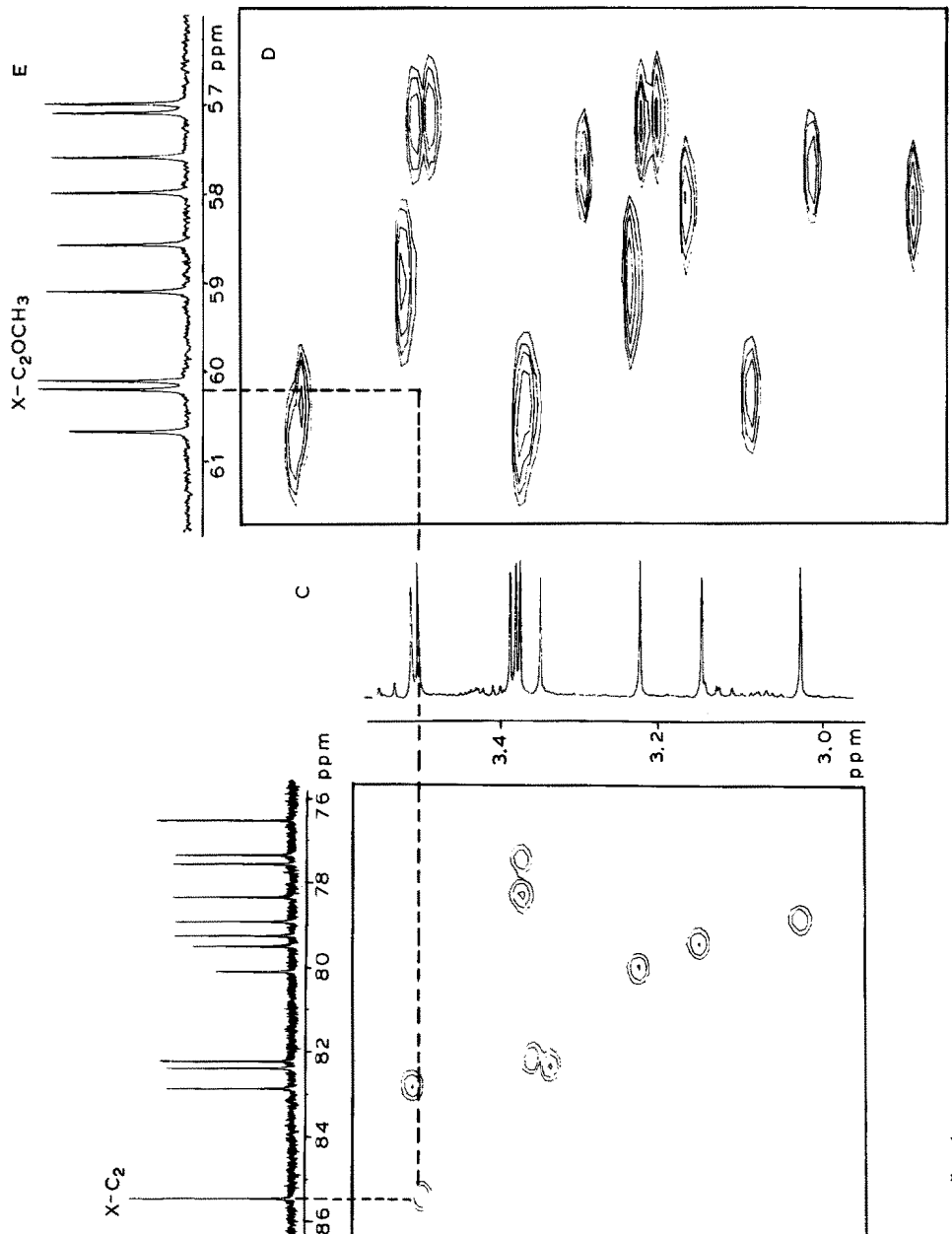

Fig. 8. 2D ^{1}H–^{13}C COSY of $R^{II}XR^{I}A$ (1) in the reverse mode for assignment of the ^{1}H and ^{13}C resonances of the OMe groups: *A*, region of the 1D ^{13}C-n.m.r. spectrum in which 9 carbon atoms show long-range interactions to the protons of the OMe groups (*B*); *C*, OMe protons correlated to the carbons; *D*, correlation of the ^{1}H resonances in *C* to the attached carbons *via* ^{1}H $J_{C,H}$ values; *E*, assignment of the OMe protons.

Fig. 7 is an expansion of the dotted box in Fig. 5B, which contains the cross-peaks between the anomeric protons and the aliphatic carbons in the range 68–86 p.p.m. R^{I} and R^{II} show interactions to C-2, C-3 and C-5 in the same ring. This effect is not observed for X and may depend on the different geometry of the ring. However, for each of the three rings, there was an interaction of H-1 with the carbon atom across the glycosidic linkage. The result is identical to that obtained by ROESY and both methods can be used to determine the sequence of monosaccharides in oligosaccharides.

The HMBC experiment also allows assignment of the MeO resonances. The ^{13}C resonances in the range 76–86 p.p.m. show ^{3}J correlations to the protons of the OMe groups. The ^{1}H resonances are correlated, through ^{1}J interactions, with the corresponding OMe carbons (Fig. 8E). The data for the OMe groups are reported in Table IV, which also contains data for the OAc groups that are not assigned specifically.

TABLE IV

^{1}H and ^{13}C Chemical shift data [C(H), δ in p.p.m.] for the OMe and OAc groups of $R^{II}XR^{I}A$ (**1**)

Residue	*OMe Groups*		
	2	*3*	*4*
A		60.17 (3.52)	57.65 (3.15)
R^{I}	59.15 (3.38)[a]	57.05 (3.35)	
X	60.26 (3.24)		58.05 (3.03)
R^{II}	58.63 (3.39)[a]	57.15 (3.38)	60.74 (3.53)

[a] Reverse assignment is possible.

OAc Groups		
CO	170.04	169.86
$^{13}C\ CH_3$	20.46	20.43
$^{1}H\ CH_3$	1.74	1.71

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