Tetrahedron, 1964, Vol. 20, pp. 883 to 889. Pergamon Press Ltd. Printed in Northern Ireland

A PROTON MAGNETIC RESONANCE STUDY OF D-GLUCAL TRIACETATE

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(*Received* 24 *November* 1963)

Abstract—The PMR spectrum of D-glucal triacetate has been determined at both 60 and 100 Mc/s and fully assigned. The spectral assignments have been confirmed by "field-sweep" double-resonance experiments and the effect of weak field "tickling" experiments is illustrated and discussed. From the first-order coupling constants it is shown that this molecule adopts the so-called half-chair conformation, having C_4 "above" and C_6 "below" the plane defined by C_1, C_2, C_3 and O_5.

EXPERIMENTAL

This compound was a commercial sample from the Aldrich Chemical Co. and was used without further purification. The 60 Mc/s spectra were measured on a Varian V-4302 Spectrometer with a Varian V-3521 Integrator for baseline stabilization. Calibration was by the usual side-band technique with tetramethylsilane as internal reference. Field-sweep double-resonance experiments at 60 Mc/s were performed with the Varian Integrator modified by the method of Johnson.[1] The 100 Mc/s spectra were measured with a Varian HR-100 Spectrometer and the double-resonance experiments at this frequency were performed in the same manner as those at 60 Mc/s.

DISCUSSION

PROTON Magnetic Resonance (PMR) spectroscopy has proved to be one of the most generally useful physical techniques available to organic chemists. For carbohydrate chemists in particular PMR represents an ideal method for determining the configurations[2–4] of carbohydrates and for measuring their precise conformation[5–7] in solution. Sometimes such information cannot be deduced from a direct study of the PMR spectrum because it is not sufficiently resolved, and in these cases recourse must be made to more recently developed instrumentation such as double-resonance and higher radio frequency spectrometers. Although these techniques are well known to PMR spectroscopists, relatively few carbohydrate investigations have embodied them and the following discussion is presented, in part, as a demonstration of both the potential and the limitations of these methods.

It has recently been shown[8] that the conformation of shikimic acid 3,4,5-trihydroxy-1-cyclohexene-1-carboxylic acid (1) closely approximates to the half-chair

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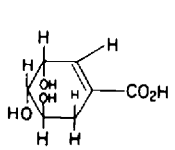
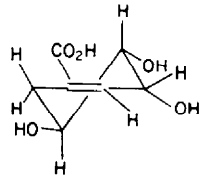
[1] L. F. Johnson, *Varian Tech. Bul.* Vol. 3, No. 3 (1963).
[2] R. U. Lemieux, R. K. Kullnig, H. J. Bernstein and W. G. Schneider, *J. Amer. Chem. Soc.* **79**, 1005 (1957); **80**, 6098 (1958).
[3] P. K. W. Woo, H. W. Dion and L. F. Johnson, *J. Amer. Chem. Soc.* **84**, 1066 (1962).
[4] W. Hofzein, H. Grisebach and H. Friebolin, *Tetrahedron* **18**, 1265 (1962).
[5] C. D. Jardetsky, *J. Amer. Chem. Soc.* **83**, 2919 (1961) and preceding papers.
[6] R. J. Abraham, L. D. Hall, L. Hough and K. A. McLauchlan, *J. Chem. Soc.* 3699 (1962).
[7] L. D. Hall, L. Hough and K. A. McLauchlan and K. G. R. Pachler, *Chem. & Ind.* 1465 (1962).
[8] L. D. Hall, unpublished results.

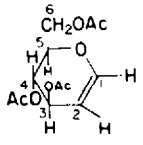


I

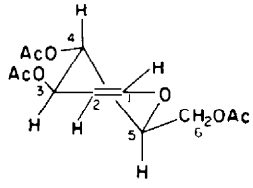


Ia

conformation (Ia). D-Glucal triacetate (II) should similarly adopt a half-chair conformation (IIa) and accordingly its PMR spectrum was determined to investigate if this were so.



II



IIa

The spectrum of D-glucal triacetate at 60 Mc/s (Fig. 1A) was insufficiently resolved to permit any conformational deductions. The low field quartet at δ6·53 (6·53 ppm downfield from the resonance of dissolved $SiMe_4$) was assigned to H_1, and the quartet δ4·81 to H_2: these chemical shifts are characteristic of cyclic α,β-unsaturated ethers since they are similar to the values observed[9] for 2,3-dihydrofuran (δ6·23, 4·86) and 2,3-dihydropyran (δ6·22, 4·54). The unresolved pattern (intensity two) at δ5·3 was assigned to H_3 and H_4 (both these protons are shifted to low field by the adjacent acetoxy substituents) and the remaining group at 4·2 (intensity three) to the H_5 and H_6 protons. Two peaks were observed in the acetate region near 2·1. From this spectrum the only coupling constant which could be measured was $J_{1,2} = 6{\cdot}4$ cs.

TABLE 1. FIRST-ORDER SPECTRAL PARAMETERS FOR D-GLUCAL TRIACETATE IN $CDCl_3$ SOLUTION

Chemical shifts (δ-values)									
H_1	H_2	H_3	H_4	H_5	H_{6a}	H_{6e}		OAc.	
6·53	4·81	5·34	5·20	4·19	4·09	4·29	2·11	2·07	1·99
Coupling constants (cs)									
	$J_{1,2}$	$J_{1,3}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6e}$	$J_{6a,6e}$	
	6·4	1·3	3·2	6·4	6·8	6·3	2·4	14·0	

Since the 60 Mc/s spectrum was insufficiently resolved the spectrum was measured at 100 Mc/s and the effect of this can be clearly seen in Fig. 1(B). The multiplets at δ5·3 and δ4·2 are now clearly resolved and complete assignment of the spectrum could be made by matching the multiplet splittings in the usual way. No attempt was made

[9] L. M. Jackman, *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry* Pergamon Press (1959). p. 62.

to analyse the spectrum beyond the first-order values shown in Table 1. An explicit analysis would have involved the solution of a seven-spin problem which is rather complex, and in any case unnecessary. Inspection of Fig. 1B shows that the ring hydrogens may be considered for analysis purposes as being an $A(H_3)$, $B(H_4)$, $M(H_2)$, $X(H_1)$ system in which the J/δ ratios are sufficiently large to justify a first-order analysis. The C_6-methylene protons with H_5 form an ABC-systems so that the first-order values for these protons are likely to be somewhat inaccurate: however, the coupling constants between these protons were not required for the subsequent conformational discussion and hence a second-order analysis was not attempted. Owing to the large chemical shift between H_5 and H_4 no correction was necessary to allow for the fact that H_4 was coupled with the ABC-system. The spectrum was also measured in a variety of solvents in an attempt to effect some solvent shift which might simplify the spectrum. The most significant effect was observed with benzene (Fig. 1C) as solvent which exposed one of the C_6-hydrogens as a quartet.

Although the spectral assignments were fairly certain it was of interest to comfirm them by double-resonance experiments. This technique has only been applied to two previous carbohydrate investigations[10,11] although many applications have been made in other fields. Essentially a "field-sweep" double-resonance experiment may be considered as the passage of two radiofrequency fields across the spectrum, the usual low intensity "observing" frequency (ω_1) and an additional higher intensity "decoupling" frequency (ω_2). Actually ω_1 and ω_2 are audio frequency sideband components of the radio frequency field which is held constant while the D.C. field is swept. However, it is more convenient to think of ω_1 and ω_2 as individual r.f. fields which are scanned while the D.C. field is constant. The separation between these two frequencies ($\omega_1 - \omega_2$) is set equal to the measured chemical shift between the two hydrogen to be decoupled and the two fields are then scanned *as a pair* with constant separation across the spectrum. This illustrated diagrammatically in Fig. 2 for an AX-system. Initially ω_1 excites the resonance of H_A which is then observed as a doublet in the usual manner. However, by the time ω_1 has reached the resonance position of H_X and is ready to excite its resonance, ω_2 has reached H_A. It causes the resonance transitions of this proton to occur so rapidly that H_X "sees" only a single averaged energy for H_A and hence H_X is observed as a singlet instead of the usual doublet. If the two frequencies were passed across the spectrum in the reverse order, then H_X would be observed as a doublet and H_A as a singlet. Had one of the protons been further coupled then this experiment would have eliminated the AX-coupling and left the residual couplings unaltered.

The effect of double-resonance experiments on the 100 Mc/s spectrum of D-glucal triacetate is shown in Fig. 3. Initially $\omega_2 - \omega_1$ was set approximately equal to the chemical shift between H_1 and H_3 (119 cs) which effected the removal of their small coupling constant leaving the larger H_1, H_2 coupling unaltered (Fig. 3A). It was indeed fortunate that the frequency separation actually used (105 cs) was also approximately to the chemical shift between H_4 and H_5 (101 cs) so that the same experiment also decoupled H_4 from H_5. The experiment was repeated with the order of ω_1 and ω_2 reversed, but with the same frequency separation: this gave the spectrum shown in

[10] R. J. Abraham, R. Freeman, L. D. Hall and K. A. McLauchlan, *J. Chem. Soc.* 2080 (1962).
[11] D. W. Turner, *J. Chem. Soc.* 847 (1962).

Fig. 3B in which H_3 was decoupled from H_1 and H_5 was decoupled from H_4. Experiments were also performed at 60 Mc/s in which H_1 was decoupled from H_2 and *vice-versa*.

The behaviour outlined above in which the coupling between interacting protons is *fully* removed only occurs when the strength of the decoupling field ω_2 (measured in cs) is greater than the coupling to be removed also (measured in cs). If this is not the case, or if $\omega_2 - \omega_1$ is significantly different from the chemical shift between the two protons, then the spectrum can become more complex rather than simplified. This is because the decoupling field only slightly perturbs the populations of the energy levels of the proton instead of effectively averaging them completely: these so-called "tickling" experiments have been described elsewhere.[12,13] An illustration of the effect of deliberately offsetting a weak decoupling field is shown in Fig. 4, which is the effect on H_1 of a decoupling field in the vicinity of H_2. The strength of ω_2 was kept constant and its frequency was systematically offset from the optimum* decoupling by $\pm$ ncs until decoupling was no longer observed. The symmetry of the effect should be noticed. It follows from this that all routine decoupling experiments should be conducted with as strong a decoupling field as possible to avoid such complications.

The determination of carbohydrate comformations using a Karplus[14] equation to relate the coupling constant of adjacent ring hydrogens with their dihedral angles has been described previously.[5–8] It is now apparent that considerable care should be exercised to the application of this method for determining carbohydrate conformations. For example, Williamson[15] has unequivocally demonstrated that allowance must be made for the electronegativity of other substituents attached to the carbon atoms bearing the hydrogens under consideration; hence the precise values of the Karplus parameters are likely to vary from system to system. However, the method probably gives semi-quantitative results which are still of value. Hence converting the ring coupling constants H_3, H_4, H_5 to angles using the Karplus parameters described previously[6] gave angle (H_3, H_4) $\simeq$ 140° and angle (H_4, H_5) $\simeq$ 150°. Even assuming a relatively large error in these values, due to errors in the coupling constants and Karplus parameters, we can still make some comment on the conformation. Firstly it approximates to the expected half-chair conformation shown in IIa. However, some flattening of the ring is evidenced from the fact that angle (H_4, H_5) is less than the expected value of 180°. The difference between the two values cannot be accounted for on the basis of "skewed-boat" conformations which are excluded by the conformational rigidity of the double bond. Moreover, the existence of boat conformations in conformational equilibrium with IIa is unlikely in view of their unfavourable energy and of the results found for shikimic acid.[8] An epoxide substituent should also cause a pyranose carbohydrate ring to adopt a half-chair conformation. When carbon-carbon double bonds or epoxide groups form part of a five membered ring, the latter is forced to adopt the so-called "envelope" conformation.[16]

* It should be emphasized that the optimum decoupling frequency is not exactly equal to the observed chemical shift. The difference between the two is usually small but increases as the strength of ω_2 increases. Cf. reference 11.

[12] R. Freeman and W. A. Anderson, *J. Chem. Soc.* **37**, 2053 (1962).

[13] F. A. L. Anet, *J. Amer. Chem. Soc.* **84**, 3767 (1962).

[14] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959).

[15] K. L. Williamson, *J. Amer. Chem. Soc.* **85**, 516 (1963).

[16] L. D. Hall, *Chem. & Ind.* 950 (1963).

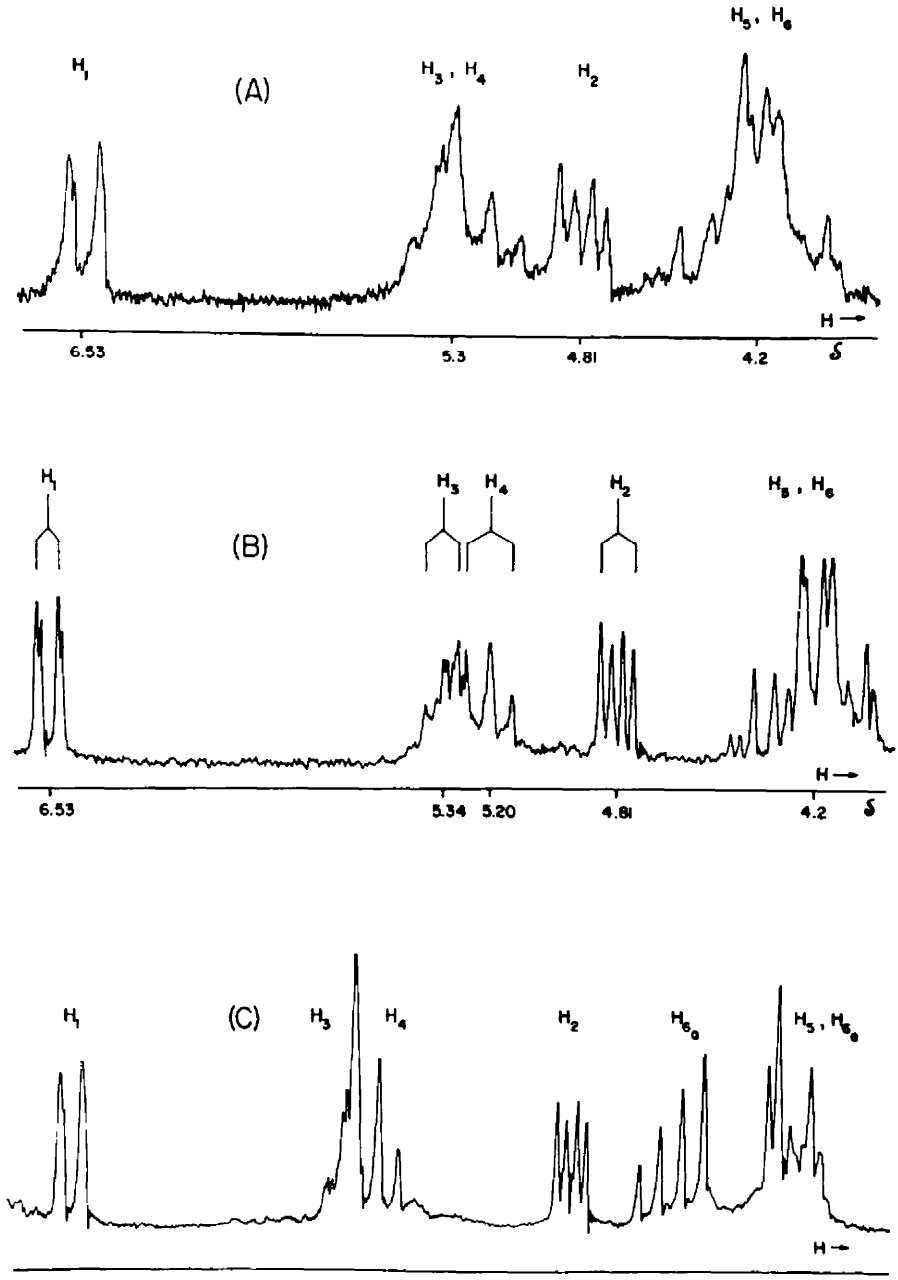


FIG. 1. PMR spectra of D-glucal triacetate: (A) in $CHCl_3$ at 60 Mc/s, (B) in $CDCl_3$ at 100 Mc/s and (C) in benzene at 100 Mc/s.

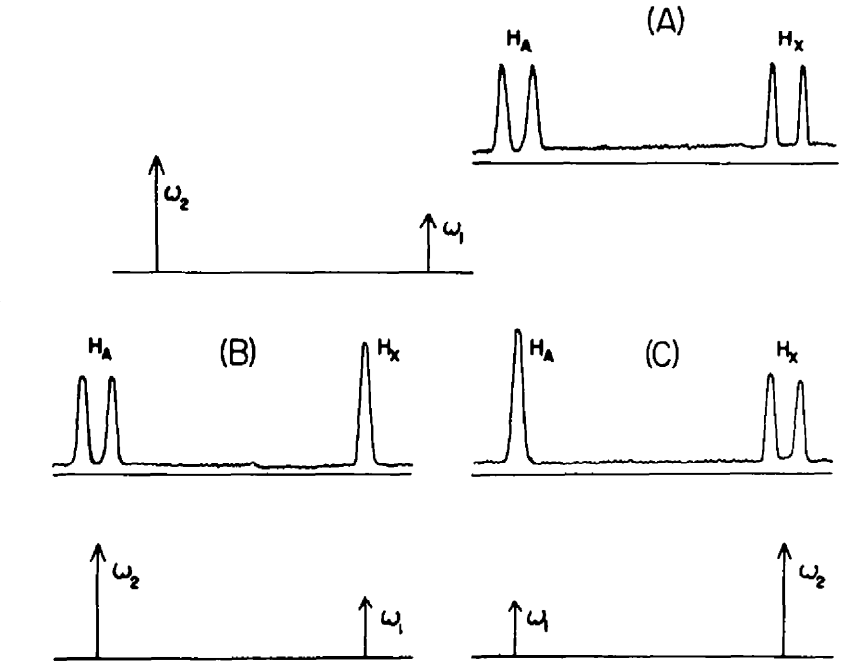


FIG. 2. Diagrammatic representation of field-sweep double-resonance experiment.

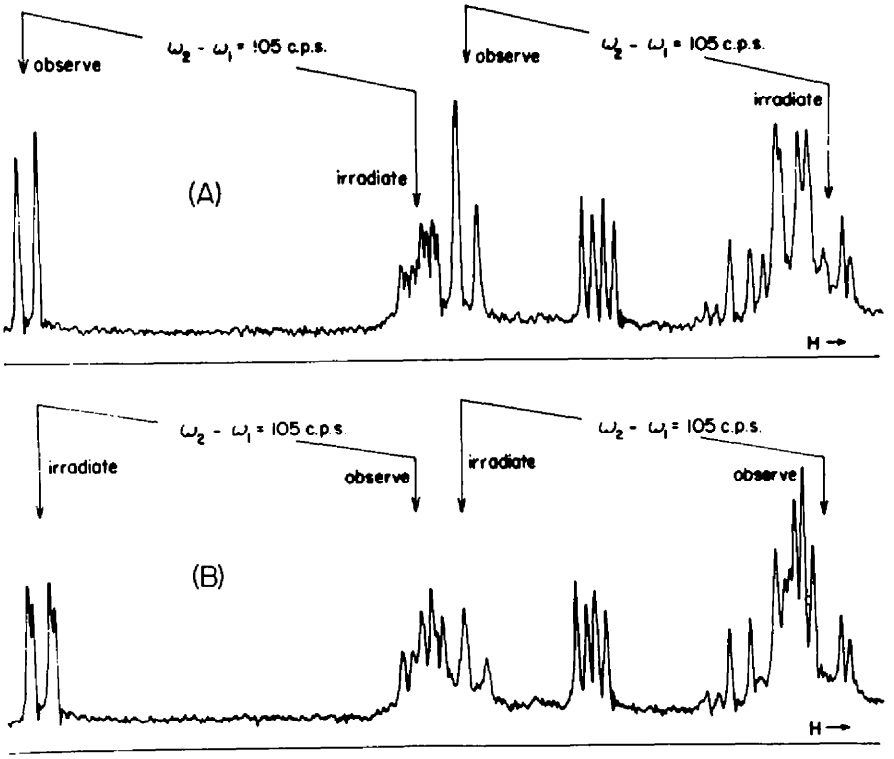


FIG. 3. Double-resonance at 100 Mc/s: (A) decoupling H_1 from H_3 and H_4 from H_5, and (B) decoupling H_3 from H_1, and H_5 from H_4.

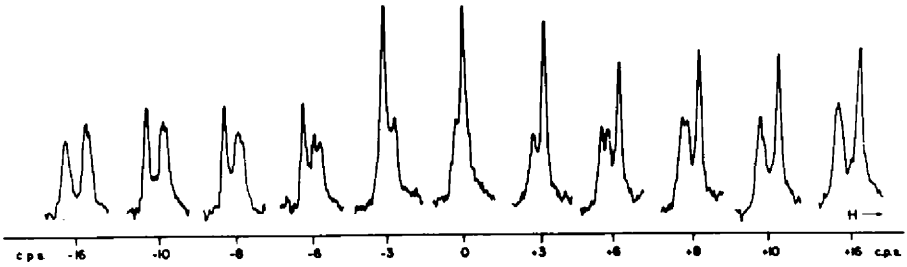


FIG. 4. Double-resonance "tickling" experiment at 60 Mc/s: the effect on H_1 of a weak decoupling field set in the vicinity of the H_2 resonance.

This study illustrated quite clearly the superior resolution of PMR spectra measured at 100 Mc/s and the potential of double-resonance as an aid to carbohydrate PMR studies. It is necessary to emphasise that whilst a Karplus approach to the determination of conformations can give *semi*-quantitative results, these may be subject to considerable errors and should not be accepted *quantitatively* unless some more independent check is applied, such as calculating the "theoretical" conformation.

Acknowledgements—One of us (L. D. H.) thanks Dr. F. A. L. Anet for facilities used in this investigation and the National Research Council for financial assistance.

Note added in proof—Since the submission of this paper, Karplus has commented {*J. Amer. Chem. Soc.*, **85**, 2870 (1963)} on the need for caution when applying the results of his calculations to the determination of conformations.