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THE PROTON RESONANCE SPECTRUM OF METHYL 4,6-O-BENZYLIDENE-3-DEOXY-3-PHENYLAZO-α-D-GLUCOSIDE 2-ACETATE

PARTIAL VIRTUAL COUPLING IN A FOUR-SPIN SYSTEM

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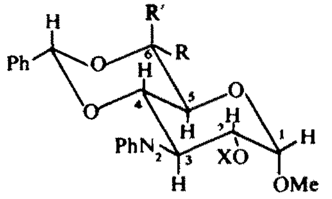
Abstract—A detailed interpretation of the PMR spectrum of the title compound is presented. Parameters include coupling constants across four bonds. Partial virtual coupling in the system ABMX is discussed in relation to this compound. With spin decoupling, this permits unambiguous determination of the relative signs of *geminal* and (*trans*) vicinal coupling.

1. INTRODUCTION

In the course of investigation of the ORD of 3-phenylazo-glucosides,[1] we have made a detailed analysis of the PMR spectrum of methyl 4,6-O-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside 2-acetate (I) at 100 Mc/s. This appears to be the first complete non-first-order analysis of the ring PMR spectrum of a sugar derivative, and is moreover a good example of long range and "virtual" coupling.



I: R = R' = H, X = Ac
II: R = R' = H, X = H
III: R = R' = D, X = H

2. INTERPRETATION OF THE SPECTRUM

The spectral lines for compound I are grouped in three regions: (i) the two Ph substituents produce three multiplets at 775, 740 and 700 c/s corresponding to 2, 2 and 6 protons per molecule respectively, which have been discussed elsewhere;[2] (ii) the methoxy and acetoxy substituents each produce a single, sharp peak at 375 and 145 c/s respectively; (iii) between the above regions, protons directly bound to the carbon framework produce a series of well-resolved multiplets in the region 590 to 340 c/s, with a single peak also at 4·73 ppm (527 c/s from TMS) due to the benzylidene proton. Region (iii) is isolated in a spin-coupling sense from the rest of the spectrum (see later), and alone is of interest in this paper. The solvent was selected to maximize the chemical shift separations and display the region of the spectrum to best effect as shown in Figs 1a and b. Integration shows that each of the distinct multiplets (quartet, singlet, doublet, triplet and the modified triplet at highest field) corresponds to one proton, and the remaining three skeletal protons give rise to the more complicated group of lines at 440 to 390 c/s from TMS. The four multiplets to low field are sufficiently close to the first order condition to allow unambiguous assignment to the C_2, benzylidene, C_1 and C_3 protons respectively. This is evident from the *trans* and *gauche* vicinal coupling constants established in many related compounds,[3] and was confirmed by double resonance experiments under spin-decoupling conditions. Irradiation of the C_3H triplet affected the remainder of the spectrum at two places only; first it caused a collapse of the C_2H quartet to a sharp doublet, and second, it caused a partial collapse of lines from 440 to 420 c/s. The latter locates approximately the chemical shift of C_4H. Assignment of the modified triplet centred upon 360 c/s was more difficult. It was initially assigned to C_5H, but extensive trial calculations failed to reproduce the experimental spectrum adequately. Tentative re-assignment as one of the protons at C_6 received strong support from the spectra of the corresponding 2-hydroxy compound II and its 6,6′-d_2 analogue III. The PMR spectrum of II resembles that of I in C_6D_6 solution, particularly in giving an isolated simple triplet in almost the same position (350 c/s) as the modified triplet of I. This signal is absent from the spectrum of III, showing conclusively that it was due to one of the C_6 protons. Further trial calculations showed that the general appearance of the spectrum could be reproduced by assigning it as 6′-H,[*] the remaining protons 6-H and 5-H to chemical shifts close to the values given below, and setting geminal and vicinal coupling constants to the expected order of magnitude.

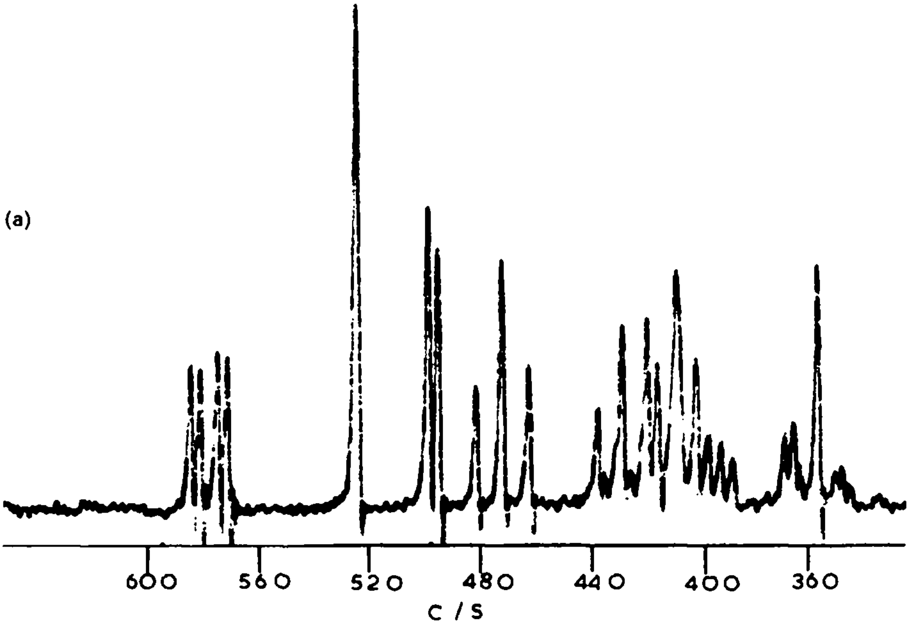
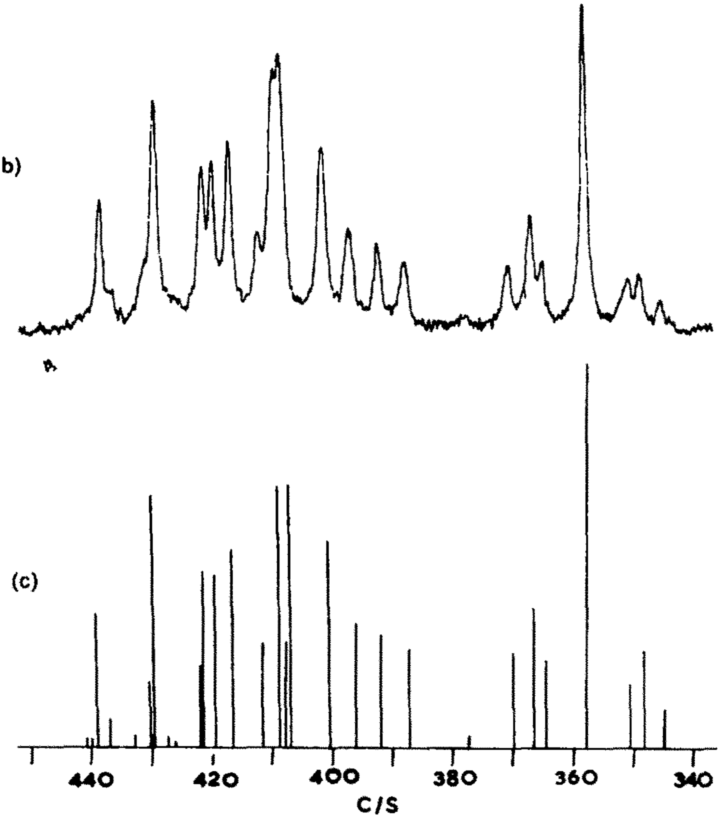


Fig. 1 PMR spectrum of compound I at 100 Mc/s. (a) Complete experimental spectrum of the pyranose ring protons, and (b) high-field region expanded. (c) The calculated part-spectrum corresponding to (b), with close transitions coalesced. The remainder of the calculated spectrum is not shown, being close to first order with closely grouped transitions. Experimental line positions used in the iterative solution are as follows: 588·95, 585·25, 578·75, 575·05, 501·6, 497·6, 481·7, 472·2, 262·0, 438·2, 429·1, 416·7, 409·4, 401·3, 396·8, 392·3, 387·8, 377·7, 370·7, 366·8, 365·0, 357·8, 350·6, 348·7, 345·2 c/s from TMS.

[*] The axial proton on C_6 is referred to as 6′-H; the equatorial one as 6-H.



The trial calculations employed Part 1 of the computer programme LAOCOON II. The parameters were modified "by hand" until sufficient theoretical lines could be matched with their experimental counterparts. Forty transitions, assigned to 25 experimental well-resolved lines, were then employed as a basis for iterative convergence of the theoretical to the experimental part-spectrum, using Part 2 of LAOCOON II. All possible parameters for the seven protons were allowed to vary independently in this stage of the calculation, and the final values are given in Table 1. The root mean square deviation between the selected experimental line positions and the final calculated values was only 0·063 c/s, and the calculated spectrum (Fig. 1c) agrees well with the experimental one.

TABLE 1. PARAMETERS FOR COMPOUND I

ν_2	582·3 c/s	J_{12}	3·77 c/s	J_{45}	9·41 c/s
ν_1	499·9 c/s	J_{13}	−0·16 c/s	J_{46}	−0·30 c/s
ν_3	471·8 c/s	J_{23}	10·41 c/s	$J_{46'}$	−0·53 c/s
ν_4	428·9 c/s	J_{24}	−0·12 c/s	J_{56}	5·06 c/s
ν_6	413·7 c/s	J_{34}	9·66 c/s	$J_{56'}$	10·16 c/s
ν_5	400·2 c/s	J_{35}	−0·28 c/s		
$\nu_{6'}$	359·2 c/s			$J_{66'}$	−10·29 c/s

All other J's < 0·05 c/s. Probable errors ±0·2 c/s (ν's);
±0·1 c/s (J's).

3. GENERAL DISCUSSION

A feature of immediate interest in the above results is the calculation of small negative coupling constants between all pairs of skeletal protons separated by four bonds, H—C—C—C—H. This could not have been deduced from a first order interpretation since the couplings are too small to cause resolvable first order line splittings. Although the precise values are open to question (see probable error), the effect is almost certainly real since all longer range couplings remain zero during the iteration, even between nuclei which are relatively close in chemical shift (e.g. $J_{35} = -0{\cdot}24$ c/s but $J_{36\,\mathrm{eq}} = 0{\cdot}00$ c/s). The values are not unexpected from data on related compounds, and preserve a regular sign alternation (−, +, −) of J_{HH} across two, three and four bonds respectively. It may be noted that the *geminal* 6-H, 6′-H coupling is very similar in *magnitude* to axial–axial (*trans*) vicinal coupling, although opposite in sign.

The above analysis was conducted upon the skeletal protons as a general 7-spin system (ABCDEFG). Possible couplings to protons in the rest of the molecule were neglected due to limitation of the programme and computer capacity. By way of justification, (a) such couplings are known to be generally very small; (b) agreement with experiment is satisfactory without them; (c) additional interactions would be very close to first order and their omission unlikely to affect the parameters found in Table 1, and (d) there is no direct evidence for them in the spectrum. Slight broadening of the benzylidene peak is removed on irradiation of the ortho-protons in the adjacent Ph group, and is therefore due to coupling to these rather than to the pyranose ring protons. Of the latter, coupling to protons at C_5 and C_6 might just be detectable, being across four bonds (H—C—O—C—H). Similarly, coupling between the 1-MeO group and 1-H might be envisaged. However, it may be noted that one possible coupling across H—C—O—C—H (J_{15}) was included in the analysis, and remained very close to zero (0·02 c/s) on iteration.

Long range couplings in sugar molecules have been reported by several workers (see 4, 5 and 6 and refs therein), although on a first-order basis only. It is hoped to compare these with further results obtained in this Laboratory, in a future paper.

4. THE C_6H (AXIAL) MULTIPLET

This is the most unusual feature of the spectrum. Although there is some resemblance to a 1:2:1 triplet, the further splitting of the outer components is in marked contrast to the simple triplet observed for 3-H. Further, the *absence* of splitting of the strong central peak gives a pattern which is unlike that associated with "second order" effects. In each case, there are two large coupling constants to the nucleus in question, and these are almost equal in magnitude. In the absence of any other coupling effects, these would cause a splitting pattern resembling a 1:2:1 triplet, being in this case a group of four lines with the inner components too close to be resolved. This is shown clearly in Fig. 2a, which is the pattern calculated by including $J_{56'}$, $J_{66'}$ only, setting all other coupling constants to zero. There is some distortion from the 1:2:1 intensity distribution that would have corresponded to a first order spectrum, due to the relative proximity of the chemical shifts concerned. It should be noted that the relative signs of the two coupling constants have little effect on the calculated pattern. The effect of introducing the coupling J_{56} is shown in Fig. 2b. At this stage, 6′-H is still being considered as part of a three-spin system (ABC tending to ABX), comprising the protons at C_5 and C_6. The calculated pattern now has six lines, which is the maximum number that could be produced by interaction of 6'-H with two other protons only. The outermost two lines would be regarded as "combination" lines in an ABX system, and are rather weak. Clearly this cannot account for the observed pattern, which must involve at least a fourth proton. In Fig. 2c, a pattern very close to the experimental one is achieved by introducing J_{45}. This shows that 4-H is essential to the unusual 6'-H pattern, but it is *not* due to the small direct coupling $J_{46'}$ given in Table 1, since this is set equal to zero in Fig. 2c. Furthermore, the direct coupling $J_{46'}$ would be expected to have a rather similar effect on each line of the 6'-H multiplet, and experimentally it is seen to be too small to split the strong central peak. The marked influence of 4-H upon the outer lines of this multiplet is therefore of the type described as "virtual coupling", and depends on finite coupling of 4-H to 5-H and/or 6-H, whilst the latter are in turn coupled to 6'-H. Since the central line is not split, the effect is further qualified as "partial virtual coupling", which is discussed below. In favourable cases it can be eliminated, just as direct coupling, by double resonance—and this is used to demonstrate the involvement of the C_4 proton. Fig. 2d gives the appearance of the 6'-H multiplet in the presence of irradiation at the 4-H chemical shift. The C_4 proton is *approximately* decoupled from all other nuclei, and the 6'-H multiplet collapses to a simpler pattern which is close to that of Fig. 2b.

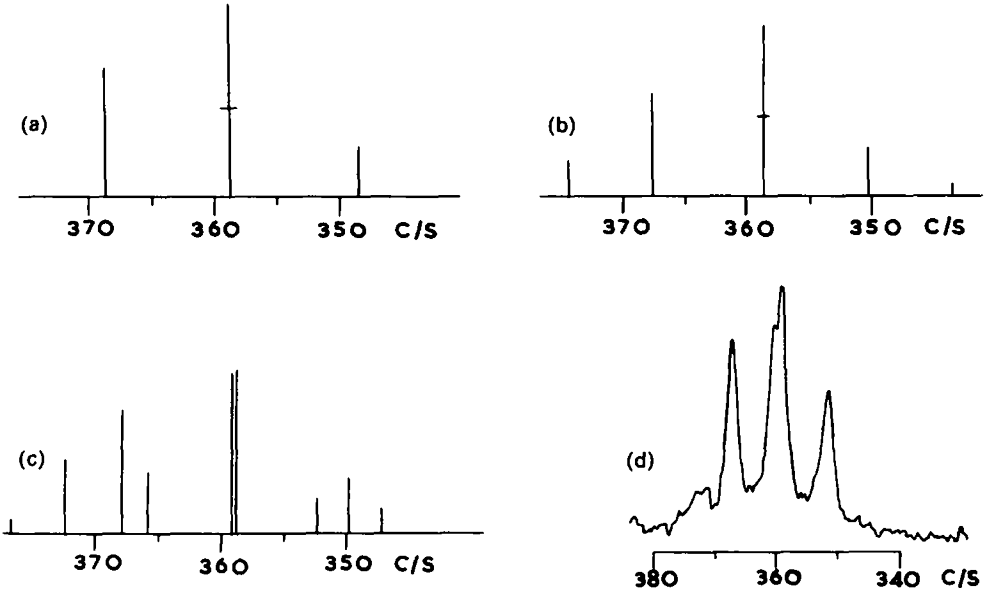


FIG. 2 Calculated appearance of the 6'-H (Axial) multiplet under various spin coupling conditions; (a) with $J_{56'}$ and $J_{66'}$ only; (b) with J_{56}, $J_{56'}$ and $J_{66'}$ only; (c) with J_{45}, J_{56}, $J_{56'}$ and $J_{66'}$ only. (d) Experimental 6'-H multiplet with irradiation at 4-H chemical shift; approximate decoupling from 4-H.

5. PARTIAL VIRTUAL COUPLING IN FOUR SPIN SYSTEMS

The protons at C_1, C_2 and C_3 have almost no effect upon the 6'-H multiplet, so the latter may be discussed in terms of a systems of four spins only (ABCD tending to ABMX), comprising the protons at C_4, C_5 and C_6.

Diagonalization of the nuclear spin Hamiltonian matrix can be achieved only by numerical methods in the general ABCD case. The high field modified triplet is therefore discussed in terms of the ABMX case (protons 5,6, 4, and 6′ respectively), and the results illustrate the conclusions of Hoffman *et al.*[7] concerning partial virtual coupling in this system. They also provide a unique example of the combined use of this phenomenon with spin decoupling to yield the relative signs of coupling constants. The requirement that J_{MX} must be approximately zero for partial virtual coupling to be observed, is stressed in the following short algebraic rationale.

The ABMX system has 16 spin basis functions compared with 8 for ABX. With $J_{MX} = 0$ in ABMX, the unmixed X transitions are pairwise degenerate, and occur at the same frequencies as the unmixed X transitions for the ABX case having the same parameters. J_{AX} and J_{BX} occur in the diagonal elements only of the Hamiltonian matrix. Hence, their participation in the unmixed eigenvalues is of a linear form, and they disappear upon taking differences to find the corresponding transition frequencies. This correspondence to the ABX case no longer holds for mixed transitions. There are eight non-degenerate, mixed, transitions in the X region of ABMX, and these occur at the following positions, where ν_X is the chemical shift of nucleus X:

$$\nu_X \underset{\text{indep}}{\pm} \tfrac{1}{2}[\{\nu_A - \nu_B + \tfrac{1}{2}(J_{AX} - J_{BX}) \pm \tfrac{1}{2}(J_{AM} - J_{BM})\}^2 + J_{AB^2}]^{\frac{1}{2}}$$
$$\underset{\text{indep}}{\pm} \tfrac{1}{2}[\{\nu_A - \nu_B - \tfrac{1}{2}(J_{AX} - J_{BX}) \pm \tfrac{1}{2}(J_{AM} - J_{BM})\}^2 + J_{AB^2}]^{\frac{1}{2}}$$

Hence, the mixed transitions depend on J_{AM}, J_{BM} whereas the unmixed ones do not. This demonstrates the algebraic significance of partial virtual coupling in the ABMX system. For values of J_{MX} which are neither zero nor small, *all* transitions are affected by spin decoupling and the distinction *partial* virtual coupling is no longer relevant. The very small magnitude of the direct coupling $J_{46'}$ $(=J_{MX})$ validates the use of partial virtual coupling in the description of the present system.

Reducing J_{AM} and J_{BM} approximately to zero by irradiating at the chemical shift of proton M (the proton on C_4, i.e. making J_{46}, $J_{46'}$, $J_{46} = 0$) allows immediate identification of the unmixed transitions as those which are unaffected by the presence of the decoupling field. The central strong peak of the "modified triplet" is essentially unperturbed by the "removal" of M (Fig. 2d) allowing the unambiguous assignment of this peak as being due to the unmixed transitions. A separation of $|J_{AB} + J_{BM}| = |J_{66'} + J_{56'}|$ characterizes the pure transitions. As the width at half height of the centre peak is only slightly greater than line width in other multiplets, it can be concluded that

$$J_{66'} + J_{56'} = 0$$

Hence J (*geminal*) $= -J$ (*vicinal, trans*) for the 5, 6 and 6′ protons.

It may be noted that Figure 2a of Ref. 7 shows a calculated "X" multiplet with coupling constants rather similar in magnitude to the above, but all having the same sign. The unmixed transitions then give rise to a strong line at each side of the multiplet, giving an appearance totally different from the present case.

In conclusion, it must be emphasized that long range and virtual coupling may be encountered together in the spectra of sugar derivatives but that complete analysis is necessary for their effects to be separately recognized and understood.

6. EXPERIMENTAL

NMR spectra were recorded on a Varian HA-100 spectrometer for saturated solns in benzene-d_6 containing 1% TMS as an internal standard. Line positions and chemical shifts (ν) are recorded in c/s from TMS. They should not be converted to the τ-scale; the latter refers strictly to infinite dilution in CCl_4, in which solvent the spectrum is appreciably different.

Methyl α-D-glucopyranoside-6,6′-d_2. D-Glucuronolactone (3·0 g, in excess), dry MeOH (12 ml), and methanolic HCl (24% W/W; 1·2 ml) was boiled under reflux for 16 hr. The yellow soln was cooled and neutralized (pH 6) with Amberlite IR4B (OH^-) resin. The resin was removed and washed well with dry MeOH (4 × 20 ml). Washings and the bulk soln were concentrated (20 ml). TLC [silica gel $CHCl_3$–MeOH (3:1)] showed one spot with a greater R_f value than starting material. The substance was not isolated but was assumed to be the methyl ester of methyl α-D-glucuronic acid.

Sodium borodeuteride (1·00 g; 98% isotopically pure) was added to the soln over a period of 2 hr. The soln was acidified (pH 4) with Amberlite IR120 (H^+) resin. The resin was removed and washed well with dry MeOH (6 × 20 ml). The washings and bulk soln were evaporated to give a colourless syrup. TLC in $CHCl_3$–MeOH (3:1) showed two spots; the faster moving one had the same R_f as the ester starting material and the other the same R_f as methyl α-D-glucopyranoside. The syrup was dissolved in hot, abs EtOH and the soln filtered, cooled, and then seeded with methyl α-D-glucopyranoside to give white crystals of methyl α-D-glucopyranoside-6,6′-d_2 (0·585 g) m.p. 162–165° (cf methyl α-D-glucopyranoside prepared by this method here m.p. 164–166°). The IR spectrum showed weak peaks at 2115 and 2245 cm^{-1} (ν_{C-D}).

Methyl 4,6-O-benzylidene-α-D-glucoside-6,6′-d_2 was prepared as described for the non-deuterium substituted compound[8] from methyl α-D-glucopyranoside-6,6′-d_2 in 68% yield and had m.p. 163–165°, $[\alpha]_D^{23}$ +113° (c, 0·5) Lit.[8] m.p. 163–164°, $[\alpha]_D^{20}$ +110 (c, 2) for methyl 4,6-O-benzylidene-α-D-glucoside. The IR spectrum showed weak peaks at 2103 and 2240 cm^{-1}.

Periodate-oxidized methyl 4,6-O-benzylidene-α-D-glucoside-6,6′-d_2 was prepared from methyl 4,6-O-benzylidene-α-D-glucoside-6,6′-d_2 as described for the non-deuterium substituted compound,[9] in 81% yield and had m.p. 139–141°, Lit.[9] m.p. 142°, for periodate-oxidized methyl 4,6-O-benzylidene-α-D-glucoside.

Methyl 4,6-O-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside-6,6′-d_2 (III) was prepared from periodate-oxidized methyl 4,6-O-benzylidene-α-D-glucoside-6,6′-d_2 as described for the non-deuterium substituted compound.[10] The crude product (65%) was purified by several recrystallizations from $CHCl_3$–light petroleum (b.p. 40–60°) to give yellow crystals m.p. 182–183° (23%). Lit.[10] m.p. 182–183° for methyl 4,6-O-benzylidene-3-deoxy-3-phenylazo-α-D-glucoside.

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