Pyridine-induced Shifts in the ^1H Nuclear Magnetic Resonance Spectra of 20-Hydroxypregnane Derivatives

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Solutions of compounds of the pregnane-3,20-diol, -3,16α,20-triol, and -3,17α,20-triol series in [^2H]chloroform and in [$^2H_5$]pyridine show ^1H n.m.r. spectral differences which can be used to assign configurations at C-20. The C-18 protons exhibit a chemical shift difference [$\Delta = \delta(C_5D_5N) - \delta(CDCl_3)$] which is greater for the pregnan-20β-ols than for the -20α-ols; the reverse is generally true of the C-21 proton signal, except when a 16α-hydroxy-group is present. The main features of the pyridine-induced shifts are rationalised in terms of the preferred conformations of 20α- and 20β-hydroxypregnanes, and of the OH · · · py hydrogen-bonded complexes.

THE ^1H n.m.r. spectra of steroids with hydroxy-groups in skeletal positions in [^2H]chloroform and in [$^2H_5$]pyridine show distinctive solvent-induced shifts of the C-18 and C-19 proton signals.[1-3] The shifts [$\Delta = \delta(C_5D_5N) - \delta(CDCl_3)$] are generally to low field. Their magnitude (0.05—0.32 p.p.m.) appeared[1] to be almost linearly related to the OH · · · Me (O · · · C) distance over the range 3.8—2.45 Å (respectively), although variations arise for distances exceeding 3.8 Å, where Δ is small. More recently, a logarithmic relationship has been proposed.[2] Solvent-induced shifts were approximately additive for a series of steroidal diols and triols,[1] suggesting that hydroxy-groups are independently hydrogen-bonded to pyridine molecules, even when pairs of hydroxy-groups occupy vicinal positions which might be considered to permit OH · · · OH bonding, or mutual interference of OH · · · py complexes.

The magnetic anisotropy of pyridine, associated with the induction of an aromatic ring current,[4] is such that

[1] G. S. Ricca, B. Rindone, and C. Scolastico, *Gazzetta*, 1969, **99**, 1284.
[2] R. A. W. Johnstone and C. C. Howard, *J.C.S. Perkin II*, 1974, 1583.
[3] P. V. Demarco, E. Farkas, D. Doddrell, N. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, 1968, **90**, 5480; T. Nambara, H. Hosoda, and M. Usui, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1687; M. Fétizon, J.-C. Gramain, and P. Mourgues, *Bull. Soc. chim. France*, 1969, 1673.
[4] L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, Oxford, 1969, p. 94.

steroid–[$^2H_5$]pyridine complexes of the types (1) and (2) are consistent with the induced shifts observed.[1] The possibility that observed shifts are of dipole origin has been excluded by studies with triethylamine as an alternative base.[2] The $C_5D_5N \cdots H{-}O$ system is believed to be linear. Equatorial alcohols are thought to form complexes which favour a conformation of type (1), with O–H · · · py *anti* to the methine C–H bond. Axial alcohols, and particularly those which are sterically hindered, appear to favour a *syn* conformation of type (2). Since an eclipsed conformation about the C–O bond of an alcohol represents a potential energy maximum, we assume that the preferred conformations of axial alcohol–pyridine complexes will be of synclinal[5] (skew or gauche) type. *Two* such conformations may have to be considered in some cases.

Most of the published data [1-3] refer to hydroxy-groups in conformationally defined locations, where OH · · · Me distances and the probable conformations of the OH · · · py complexes are readily estimated from Dreiding models.

TABLE 1

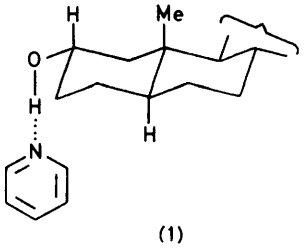
^1H N.m.r. data; solvent-induced shifts [$\Delta = \delta(C_5D_5N) - \delta(CDCl_3)$] for methyl signals of pregnane-3,20-diols, -3,16α,20-triols, and -3,17α,20-triols (Me_4Si internal standard)

Compound	18-H_3 (s)			19-H_2 (s)			21-H_3 (d, *J ca.* 6.5 Hz)			Differences	
	δ($CDCl_3$)	δ(C_5D_5N)	Δ	δ($CDCl_3$)	δ(C_5D_5N)	Δ	δ($CDCl_3$)	δ(C_5D_5N)	Δ	[Δ(20β) − Δ(20α)] 18-H_3	21-H_3
3,20-Diols											
5β-Pregnane-3α,20β-	0.76	0.895	0.135	0.945	0.935	−0.01	1.145	1.315	0.17	+0.055	−0.06
5β-Pregnane-3α,20α-	0.65	0.73	0.08	0.915	0.925	0.01	1.20	1.43	0.23		
5α-Pregnane-3β,20β-	0.74	0.89	0.15	0.81	0.825	0.015	1.12	1.29	0.17	+0.08	−0.03
5α-Pregnane-3β,20α-	0.68	0.75	0.07	0.83	0.84	0.01	1.225	1.43	0.205		
Pregn-5-ene-3β,20β-	0.76	0.91	0.15	1.01	1.05	0.04	1.14	1.29	0.15	+0.06	−0.06
Pregn-5-ene-3β,20α-	0.66	0.75	0.09	0.99	1.05	0.06	1.21	1.42	0.21		
5β-Pregn-6-ene-3α,20β-	0.77	0.92	0.15	0.83	0.92	0.09	1.14	1.30	0.16	+0.10	−0.03
5β-Pregn-6-ene-3α,20α-	0.70	0.75	0.05	0.86	0.90	0.04	1.23	1.42	0.19		
3,16α,20-Triols											
5α-Pregnane-3β,16α,20β-	0.77 *	1.00	0.23	0.82 *	0.82	0.0	1.35 *	1.73	0.38	+0.17	+0.19
5α-Pregnane-3β,16α,20α-	0.64	0.70	0.06	0.78	0.80	0.02	1.24	1.43	0.19		
5α-Pregnane-3α,16α,20β-	0.78	1.04	0.26	0.78	0.84	0.06	1.33	1.74	0.41	+0.19	+0.23
5α-Pregnane-3α,16α,20α-	0.66	0.73	0.07	0.77	0.82	0.05	1.26	1.44	0.18		
Pregn-5-ene-3β,16α,20β-	0.85	0.99	0.14	1.05	1.01	−0.04	1.43	1.75	0.32	+0.12	+0.13
Pregn-5-ene-3β,16α,20α-	0.68	0.70	0.02	1.00	1.00	0.00	1.28	1.47	0.19		
3,17α,20-Triols											
5β-Pregnane-3α,17α,20β-	0.79	0.94	0.15	0.93	0.94	0.01	1.17	1.41	0.24	+0.07	−0.05
5β-Pregnane-3α,17α,20α-	0.71	0.79	0.08	0.92	0.95	0.03	1.18	1.47	0.29		
Pregn-5-ene-3β,17α,20β-	0.80	0.95	0.15	0.99	1.05	0.06	1.15	1.40	0.25	+0.08	−0.04
Pregn-5-ene-3β,17α,20α-	0.74	0.81	0.07	1.02	1.08	0.06	1.21	1.50	0.29		
5β-Pregn-6-ene-3α,17α,20β-	0.81	0.995	0.185	0.84	0.96	0.12	1.16	1.45	0.29	+0.15	0.00
5β-Pregn-6-ene-3α,17α,20α-	0.79	0.825	0.035	0.89	0.96	0.07	1.22	1.51	0.29		
3-Oxo-17α,20-diols											
5β-Pregn-6-ene-17α,20β-	0.85	1.01	0.16	0.96	0.91	−0.05	1.19	1.45	0.26	+0.11	−0.05
5β-Pregn-6-ene-17α,20α-	0.80	0.85	0.05	0.96	0.92	−0.04	1.21	1.52	0.31		

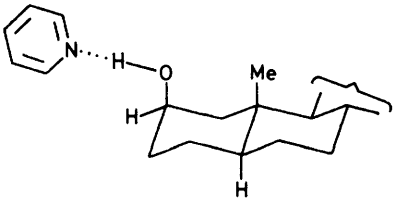
* Values for solution in 100% $CDCl_3$ by extrapolation (see text).

We now report a similar study of 20-hydroxypregnane derivatives, including 3,20-diols, 3,16α,20-triols, and 3,17α,20-triols, where the 17β-side chain is formally free to rotate, although it is generally thought to prefer a particular conformation depending upon the C-20 configuration.[6,7] Table 1 lists n.m.r. data for twenty-two compounds of the 20-hydroxypregnane type, in [^2H]-chloroform and in [$^2H_5$]pyridine. Some of these compounds were synthesised recently in our laboratories.[8,9]

Chemical shifts, and solvent-induced shifts, are listed



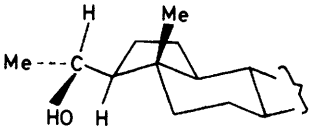
(1)



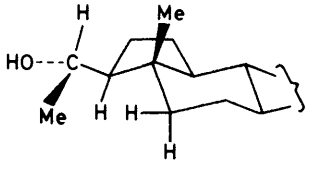
(2)

[5] W. Klyne and V. Prelog, *Experientia*, 1960, **16**, 521.
[6] H. Lee, N. S. Bhacca, and M. E. Wolff, *J. Org. Chem.*, 1966, **31**, 2692; H. Lee and M. E. Wolff, *ibid.*, 1967, **32**, 192.
[7] C. Altona and M. Sundaralingam, *Tetrahedron*, 1970, **26**, 925.
[8] D. N. Kirk and D. R. A. Leonard, *J.C.S. Perkin I*, 1973, 1836.
[9] G. Cooley and A. E. Kellie, *J.C.S. Perkin I*, 1976, 452.

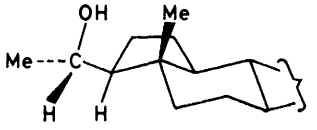
for the protons at C-18, C-19, and C-21, the most easily identifiable signals in the spectra. Assignments of methyl proton signals were generally straightforward. The C-21 protons give a doublet (J *ca.* 6.5 Hz). The exceptional cases the relative positions of these signals were interchanged in [$^2H_5$]pyridine, giving the possibility of erroneous assignments. Where a crossover was suspected, spectra of solutions in $CDCl_3$–C_5D_5N were



(3) 20β-OH

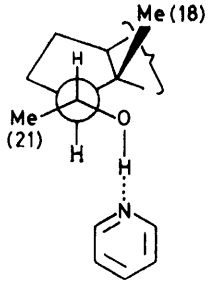


(a)

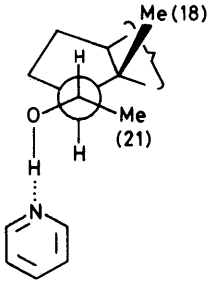


(b)

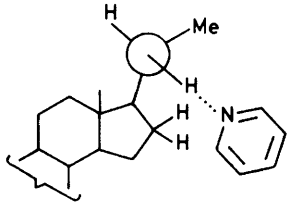
(4) 20α-OH



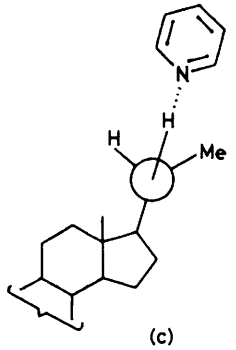
(5) 20β-OH
Newman projection about
C(20)—C(17) bond



(a)
Newman projection about
C(20)—C(17) bond



(b)



(c)

Newman projections about O—C(20) bond

(6)
(20α-OH)

18-H_3 peak is usually at higher field than that for 19-H_3, especially in [^{2}H]chloroform. Moreover the solvent shift is normally greater for protons at C-18 than for those at C-19, which is relatively far from hydroxy-groups in the present series of compounds. In a few examined: these showed that in a few cases the 18-H_3 signal does move past that for 19-H_3 as the concentration of C_5D_5N is increased. Experiments with 5α-pregnane-3β,20β-diol and -3β,16α,20β-triol, and with pregn-5-ene-3β,16α,20α-triol, showed that the chemical shifts of all

the methyl protons in $CDCl_3$–C_5D_5N mixtures are linearly related to solvent composition, to within the limits of experimental accuracy. The last-named of these three compounds, alone of all those examined, was essentially insoluble in 100% $CDCl_3$, but could be dissolved to sufficient concentration in the presence of 25% C_5D_5N; the chemical shifts tabulated for $CDCl_3$ were obtained in this case by extrapolation to 100% $CDCl_3$.

We note the following main features of the data in Table 1.

(a) C-18 *Protons*. The solvent-induced shift in pyridine (downfield) is consistently larger for the 20β- than for the 20α-isomer. The commonest values are *ca.* 0.14—0.15 and *ca.* 0.05—0.08 p.p.m., respectively, although a few fall outside these ranges.

(b) C-19 *Protons*. Shifts are generally rather small, and show no correlation with configurations at C-20.

(c) C-21 *Protons*. Shifts are pronounced for both C-20 isomers; the 20α-isomer shows the larger value except in compounds which also have a 16α-hydroxy-substituent, where the effect is larger for the 20β-isomer.

Characteristics associated specifically with the presence of 16α- or 17α-hydroxy-groups are listed in Table 2. Differences in solvent-induced shifts associated with configurations at C-3 and C-5, or with 5,6-unsaturation, are minor, and are ignored in the present study.

TABLE 2

Solvent effects due to 16α- and 17α-hydroxy-groups in the n.m.r. spectra of some pregnane-3,20-diols

Compound type	18-CH_3			19-H_3			21-H_3		
	$D(CDCl_3)$	$D(C_5D_5N)$	$D(C_5D_5N) - D(CDCl_3)$	$D(CDCl_3)$	$D(C_5D_5N)$	$D(C_5D_5N) - D(CDCl_3)$	$D(CDCl_3)$	$D(C_5D_5N)$	$D(C_5D_5N) - D(CDCl_3)$
Shift increments due to 16α-OH (p.p.m.)									
5α-Pregnane-3β,16α,20-triols									
20β-OH	+0.03	+0.11	+0.08	+0.01	−0.005	−0.015	+0.23	+0.044	+0.21
20α-OH	−0.04	−0.05	−0.01	−0.05	−0.04	+0.01	+0.015	0.00	−0.015
5α-Pregnane-3α,16α,20-triols									
20β-OH	+0.04	+0.15	+0.11	−0.03	+0.015	+0.45	+0.21	+0.45	+0.24
20α-OH	−0.02	−0.02	0.00	−0.06	−0.02	+0.04	+0.035	+0.01	−0.025
Pregn-5-ene-3β,16α,20-triols									
20β-OH	+0.09	+0.08	−0.01	+0.04	−0.04	−0.08	+0.29	+0.46	+0.17
20α-OH	+0.02	−0.05	−0.07	+0.01	−0.05	−0.06	+0.07	+0.05	−0.02
Shift increments due to 17α-OH (p.p.m.)									
5β-Pregnane-3α,17α,20-triols									
20β-OH	+0.03	+0.045	+0.015	−0.015	+0.005	+0.02	+0.025	+0.095	+0.07
20α-OH	+0.06	+0.06	0.0	+0.005	+0.025	+0.02	−0.02	+0.04	+0.06
Pregn-5-ene-3β,17α,20-triols									
20β-OH	+0.04	+0.04	0.0	−0.02	0.0	+0.02	+0.01	+0.11	+0.10
20α-OH	+0.08	+0.06	−0.02	+0.03	+0.03	0.0	0.0	+0.08	+0.08
5β-Pregn-6-ene-3α,17α,20-triols									
20β-OH	+0.04	+0.075	+0.035	+0.01	+0.04	+0.03	+0.02	+0.15	+0.13
20α-OH	+0.09	+0.075	−0.015	+0.03	+0.06	+0.03	−0.01	+0.09	+0.10

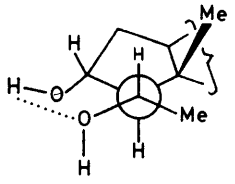
DISCUSSION

Data from n.m.r.[6] and 'molecular mechanics'[7] imply that the pregnan-20β-ol side chain has a normal preference for the staggered conformation (3), in which 20α-H occupies the most hindered position, lying over ring D. The hydroxy-group lies near C-12, and the relatively bulky C-21 methyl group is exposed in a synclinal position with respect to C-16. Reversal of the locations of the hydroxy- and methyl groups in the 20α-isomer raises the strain energy of the corresponding conformation (4a), and apparently permits the existence of a significant proportion of the rotamer (4b) in which the 20-OH group rather than 20-H projects above ring D.

A hydrogen-bonded 20β-OH · · · C_5D_5N complex, with pyridine *anti* to 20α-H (5), would cause quite strong deshielding of C-18 by the pyridine, as observed [(a) above]. An analogous complex (6a) of the 20α-isomer places the pyridine ring further from C-18, and in an orientation where its deshielding effect at C-18 would be less pronounced. Pyridine complexes (6b and c) of the alternative rotamer (4b) seem unlikely to be as important, for the following reasons. The complex (6b), with the *anti*-conformation about the C(20)–O bond, although strongly deshielding C-18, is judged from models to be destabilised by pyridine–16β-H compression, which should shift the conformational equilibrium in favour of form (6a). The alternative (6c), with a synclinal relationship of hydrogen atoms about the C(20)–O bond, would not suffer steric compression, but like (6b) would very strongly deshield C-18 (*cf.* 1,3-diaxial OH–Me effects [1,2]). We therefore discount the synclinal conformation (6c) on the basis of the experimental evidence. In summary, the larger 18-H solvent-induced shift for the 20β-alcohol is consistent with the preferences for conformations (5) and (6a), respectively. It is not surprising, since other conformations are likely also to be significantly populated, that the magnitudes of the solvent-induced shifts of the C-18 protons in particular were found not to fit well with either of the shift–distance relationships established for conformationally rigid molecules.[1,2]

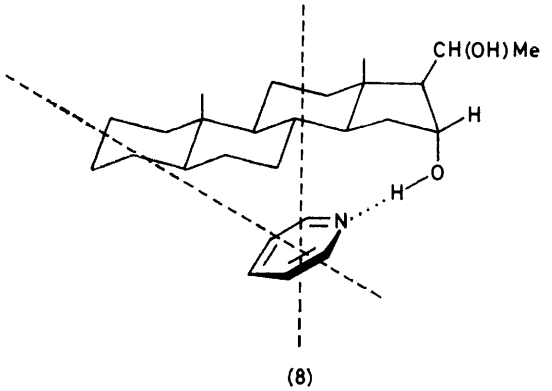
The reason for the sensitivity of 21-H solvent shifts to

the configuration at C-20 is less obvious, for the hydrogen-bonded complexes (5) and (6a) appear to be quasienantiomeric with respect to the py · · · H–O–CH–CH_3 component. To a first approximation the isomers should show identical solvent-induced shifts at C-21, but data show that the shift is on average *ca.* 0.04 p.p.m. larger for the 20α-isomer of each pair. Several factors may



(7)

(16α, 20α—diol)



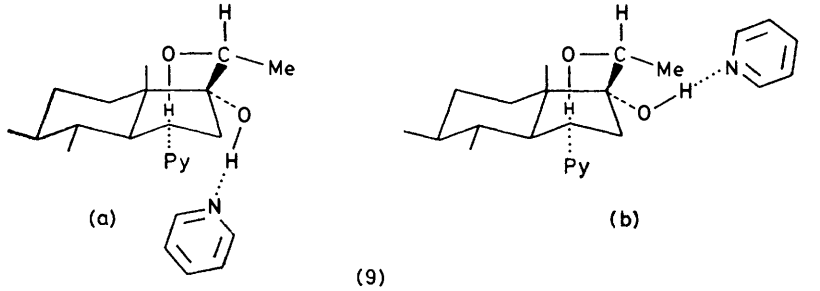
(8)

contribute to this rather small difference. One is the disturbance of the conformational equilibrium for the 20α-alcohol by pyridine, suggested above. If complexing with pyridine forces the side chain more into the conformation (6a) in which C-21 is compressed by proximity to C-12 and C-18, a small van der Waals contribution [10,11] would be added to the deshielding experienced by the C-21 protons. A second possibility is a slightly less favourable equilibrium constant for 20β-OH · · · py complexing, as a result of the proximity of the 20β-OH group to the bulk of the steroid framework, with consequent steric hindrance to association with solvent as compared with the 20α-alcohols. Any difference in the stabilities of complexes would have to be rather small, however, for its effect is not apparent in shifts of the 18-H_3 signals. Altered orientations of an associated pyridine molecule at the hindered 20β-OH as compared with the unhindered 20α-OH may also contribute, but the direction of such an effect cannot be assessed.

The additional solvent shift contributions imposed upon the three sets of methyl protons by 16α-hydroxy- or 17α-hydroxy-groups were obtained by pair-wise comparisons of the data in Table 1, for compounds with and without these substituents. The findings are collected in Table 2. To interpret these effects, an additional pyridine molecule is assumed to be hydrogen-bonded to the 16α- or 17α-hydroxy-group.

16α-*Hydroxy*. A 16α-hydroxy-group causes only small additional shifts of 18-H and 19-H signals in $CDCl_3$. The 21-H signal is also little affected by a 16α-hydroxy-group in the 20α-hydroxy-isomers, although i.r. spectra of 16α,20α-diols indicate substantial intramolecular hydrogen bonding,[12] which must hold the side chain in a conformation of type (7). The only proton signal which experiences a major shift due to 16α-OH is that of 21-H in the 20β-hydroxy-derivatives. A substantial deshielding of 21-H_3 by the 16α-OH group is consistent with their proximity, implied by the preferred conformation (3) of the pregnan-20β-ol side chain.

The effect of a 16α-hydroxy-group on the spectra in [$^2H_5$]pyridine is generally evident as a small upfield displacement of methyl signals for the 20α-isomer, but of only the 19-H signals for the 20β-isomer; 18-H signals show small but erratic shifts. The signal due to 21-H is moved strongly downfield in the 20β-isomer, but very slightly upfield in the 20α-isomer. Upfield 19-H shifts for *both* C-20 isomers in [$^2H_5$]pyridine suggest that a pyridine molecule attached to 16α-OH mainly adopts the conformation (8) in which it lies below the steroid framework, and *anti* to 16β-H. The C-19 methyl group would then experience slight shielding by this pyridine ring. The C-18 methyl group, and probably also C-21 in the 20α-isomer, would lie near the boundary surface between deshielded and shielded regions of space for pyridine bonded to 16α-OH. In the 20β-alcohol, however, C-21 lies close to the plane defined by the pyridine ring, accounting for the observed additional deshielding at C-21.



(9)

(17α, 20β—diol)

[10] Ref. 4, p. 71.

[11] N. S. Bhacca and D. H. Williams, 'Applications of N.M.R. Spectroscopy in Organic Chemistry,' Holden–Day, San Francisco, 1964, p. 189.

[12] J. C. Danilewicz and W. Klyne, *J. Chem. Soc.*, 1965, 1306.

17α-*Hydroxy*. Spectra in $CDCl_3$ show rather small shifts, mainly downfield, although there are a few exceptions involving very small upfield shifts. The effect of pyridine is almost negligible except for a definite downfield shift of the 21-H signals, very slightly larger for the 20β- than for the 20α-isomers. Dreiding models show that the 17α-OH · · · py complex is unlikely to be formed *syn* to the 20-OH · · · py system because of steric congestion. A pyridine molecule attached to 17α-OH therefore has a choice between two other conformations, illustrated here for the 20β-isomer: (i) the conformation (9a) in which pyridine lies below ring D, and would *deshield* the C-21 methyl group, or (ii) the conformation (9b) in which pyridine lies below the C-21 methyl group, which should then experience *shielding*. It seems likely that conformation (9a), with pyridine below ring D, is preferred. Analogous arguments show that the same should be true of a 17α,20α-diol.

A pyridine molecule so located, as a consequence of bonding to 17α-OH, would probably exert a very weak deshielding effect at C-19. Its effect at C-18 is not clearly defined, since C-18 lies close to the boundary surface between shielding and deshielding regions of the pyridine molecule. Data in Table 2 are entirely consistent with this interpretation.

In general, the present results support the conclusion [1,2] that OH · · · py complexes are generally formed in the conformation *anti* to the methine proton at the carbon atom carrying the hydroxy-group [*cf.* (1)]. The exceptions noted for hindered axial alcohols (2) in previous work [1] presumably depend upon the conformational rigidity of a steroid-like framework; they have no counterpart in the present series of compounds with a side chain which is able to rotate to relieve steric strains.

EXPERIMENTAL

Spectra were recorded at 100 MHz for solutions in $CDCl_3$ or C_5D_5N (dried over molecular sieves) (Me_4Si as internal standard).

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