^{14}N Quadrupole Cross-Relaxation Spectroscopy of a Compound of Pharmacological Interest*

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Cross-relaxation spectroscopy has been used to record the ^{14}N quadrupole resonance spectrum of two of the polymorphic forms of the pharmacologically-important compound: 2-4-(5-Bromo-3-methylpyrid-2-yl)butylamino-5-(6-methylpyrid-3-yl)methyl-pyrimidin-4-one.

Three ^{14}N frequencies for four of the non-equivalent nitrogen sites within the molecule have been identified by combined irradiation and cross-relaxation techniques and quadrupole coupling constants and asymmetry parameters deduced therefrom; the absence of ^{14}N (and ^{79}Br) signals from the bromine-substituted pyridine ring suggests that this part of the molecule is disordered in the crystal. The spectral intensities have been used to derive approximate values for the ^{14}N and ^{1}H relaxation times, which show clear differences between the two polymorphs.

Introduction

Level-crossing in radio-frequency spectroscopy can be applied in several distinct ways to the measurement of nuclear quadrupole resonance frequencies and relaxation times [1]. In cross-relaxation spectroscopy [2], the quadrupolar (Q) and proton (P) levels are matched in a finite magnetic field, the P levels being initially cooled by adiabatic demagnetisation [1]; after a short period, T_{CR}, in which the two spin systems come to a common spin temperature, the whole spin system, P + Q, thereafter relaxes with a time constant

$$(T_1)_{\text{eff}} = \left\{ \left(\frac{\varepsilon}{1+\varepsilon} \right) \frac{1}{T_{1Q}} + \left(\frac{1}{1+\varepsilon} \right) \frac{1}{T_1} \right\}^{-1} \quad (1)$$

in which T_{1Q} is the Q spin-lattice relaxation time, T_1 the ^{1}H spin-lattice relaxation time in the applied magnetic field, and ε the ratio of the heat capacities of the Q to P spins [3]. Stepping the matching field applied to the spin system, and monitoring the P spin magnetisation after each step, usually by transferring the sample to a much higher magnetic field, gives the Q quadrupole cross-relaxation spectrum.

For ^{14}N, the technique has a number of advantages over more common double resonance studies by level crossing [4]. No r.f. radiation need be applied to the spin system: the method has high sensitivity, particularly if $T_{1Q}/\varepsilon \ll T_1$, when a large proton magnetisation can be relaxed by a much smaller number of Q nuclei: all three transitions of a spin-1 nucleus, ν_x, ν_y, ν_z, can be detected, often with almost equal sensitivity, making reliable assignments an easier matter; and the line intensities depend on the relaxation times, which can in principle be deduced from the spectra. The method has the disadvantage that the Q spins are "observed" in a finite magnetic field so their resonance lines are broadened and distorted in shape [5]. For ^{14}N, with a low gyromagnetic ratio ($\gamma_N = 19.34 \times 10^6$ rad s^{-1} T^{-1}) and spin $I=1$, the matching fields are not large, being usually less than 0.1 T, and the line broadening is small unless the asymmetry parameter is close to zero [5]. However, distortions of the line shape may be significant [3]. All the ^{14}N frequencies quoted have been determined by irradiation combined with cross-relaxation (see Results and Discussion section), which overcomes this problem by recording all the frequencies with the proton frequency matched with ν_z; under these conditions the Zeeman broadening is insignificant.

The method has already been used successfully to detect ^{14}N quadrupole resonance in pyrazole, 1,2,4-triazole and tetrazole [6]; in this article, we discuss

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its application to a compound of pharmacological interest, which exists in several polymorphic forms.

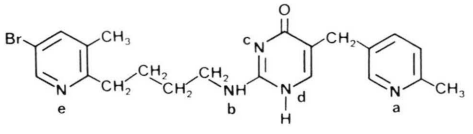
Experimental

The cross-relaxation experiments were conducted on a double resonance spectrometer, details of which have already been published [7]. The Helmholtz coils round the Q coil were replaced by an air-cooled 1100-turn, seven-layer, solenoid (of 20 s.w.g. varnished copper wire) supplied by a Kingshill 60 × 15 power supply with a maximum current of 15 amps. It was usually necessary to apply a magnetic field to the sample whilst in transit (the so-called "field pipe" [8] or "transfer field" [9]) in order to reduce losses in the proton magnetisation; the glass transfer tube was therefore wound with a 900-turn solenoid through which a current of 2 amps supplied a "transfer-field" of 4 mT. Both the matching and transfer fields were switched off during the monitoring of the residual ^{1}H magnetisation and the polarization phase in high field (0.9438 T). All experiments were conducted at room temperature (291 K).

The Q quadrupole spectrum was recorded by slowly changing the matching field by means of a potentiometer drive, and continuously monitoring the current; the current was converted to magnetic field strength by measuring the ^{1}H magnetic resonance frequency at several values. The deviation from linearity was usually less than 1% and the magnetic field could be measured to an accuracy of ± 0.2 mT.

Results and Discussion

The compound to be discussed in this paper (A) was prepared in the laboratories of Smith Kline and French Research (SK & F 93944). In direct contact with liquid water between 20 and 40 °C, it forms a hydrate which is the stable form at ambient temperatures. On heating, all the water is easily lost (verified by thermo-gravimetric analysis), to give an anhydrous form which is the subject of this investigation.



A

The anhydrous form of (A) exists in several polymorphic forms [10]; at least two of these, (II) and (III), can be considered to be well characterised in the sense that mixtures give rise to resolved melting endotherms in differential scanning calorimetry (DSC). Two other polymorphs, (I) and (IV), are believed to exist but are difficult to obtain reproducibly. The investigation therefore concentrated on polymorphs (II) and (III). Polymorph II is obtained by heating the hydrate to 100 – 105 °C; the loss of water is followed by an exotherm near 90 °C in a DSC experiment above which the sample is all phase (II). Polymorph III is prepared from solution in methanol/ethyl acetate by azeotropic distillation of the methanol and cooling to crystallization. The purity of both forms was checked by C, H, N, Br analyses and HPLC.

Apart from the melting endotherms in DSC experiments, the two polymorphs are exceedingly difficult to characterize spectroscopically. Their infra-red spectra are virtually identical, as are their X-ray powder patterns. Both polymorphs give good ^{14}N quadrupole cross-relaxation spectra; Figure 1 shows an example for polymorph III, with proton magnetisation increasing from bottom to top of the diagram. Twelve peaks are seen, each representing a loss in proton magnetisation, as predicted by equation (1) when $T_{1Q}/\varepsilon < T_1$. Two other feature of this typical cross-relaxation spectrum should be noted. Firstly, the sloping baseline, which moves towards a smaller recovered proton magnetisation as the applied field diminishes. This behaviour may be attributed to variation in the ^{1}H T_1 with magnetic field [11]; in this sample, it lengthens as the field increases. The base-line is usually seen with a value proportional to

$$M = M_0\, e^{-\tau_Q/T_1}, \qquad (2)$$

where M_0 is the initial proton magnetisation at the beginning of each cycle and τ_Q the time the sample resides in the low-field solenoid. A second feature is the sharp peak at 335 kHz, the lowest frequency signal to be observed. It occurs at half the frequency of the broad shoulder at 670 kHz and is therefore assigned to a two-proton relaxation jump. Multi-proton jumps are frequently found in cross-relaxa-

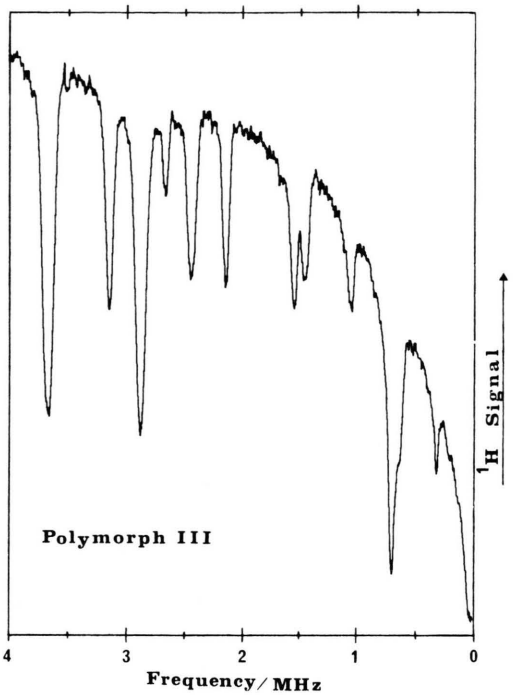


Fig. 1. ^{14}N Quadrupole cross-relaxation spectrum of polymorph (III) at room temperature; $\tau_P = 15$ s, $\tau_Q = 0.5$ s, ca. 1000 field steps.

tion spectroscopy [12], particularly at low frequencies; further examples of this behaviour will be discussed elsewhere [13]. This leaves eleven signals to assign, out of an expected total of fifteen for molecule (A).

These frequencies were grouped into consistent sets by combining irradiation with cross-relaxation. The magnetic field in the solenoid was adjusted such that the proton splitting matched a suspected $\nu_z(\nu_0)$ frequency; r.f. radiation was then sent to the Q coil and swept through the frequency range in which the $\nu_y(\nu_-)$ and $\nu_x(\nu_+)$ signals were expected to lie. At exactly one or other of these frequencies, there occurs a perturbation in the equilibrium population of the $\nu_z(\nu_0)$ levels, which as the ^{1}H nuclei are in thermal contact with them, can be monitored as a change in the recovered ^{1}H magnetisation in high field. Thus sweeping the irradiation frequency under these conditions gives just two signals, corresponding to ν_y and ν_x, ν_y being seen as a positive enhancement and ν_x as a negative enhancement (Figure 2) [13]. In Fig. 3, spectrum (a) was obtained with the proton frequency matched with the peak in the cross-relaxation spectrum at 708 kHz and the two peaks are seen at 2966 kHz and 3674 kHz; spectrum (b) shows the same frequency range recorded with the proton matching frequency reduced to 670 kHz. The two peaks seen in this case are at 2490 kHz and 3160 kHz. The transition frequencies grouped in this way are shown in Table 1, along with the most plausible assignment based on ^{14}N quadrupole coupling constants and asymmetry parameters in structurally similar compounds, such as 1-methylpyridine [14] and cytosine [15]. It will be noted that one complete set of nitrogen frequencies is missing, tentatively assigned to the pyridine nitrogen nucleus labelled (e). The main reason for this choice is that, despite extensive searches, it has proved impossible to detect the ^{79}Br quadrupole resonance signal from the bromine substituent in this ring, suggesting that there is static or dynamic disorder in this part of the molecule within the crystal.

Table 1. ^{14}N peak frequencies in (A), together with quadrupole coupling constants ($e^2 q Q/h$) and asymmetry parameters (η).

Nuclei	^{14}N frequencies ν_z, ν_y, ν_x (MHz)	$e^2 q Q/h$ (MHz)	η
N (*a*)	0.708, 2.966, 3.674	4.426 ± 0.005	0.320 ± 0.005
N (*b*)	0.670, 2.490, 3.160	3.676 ± 0.005	0.356 ± 0.005
N (*c*)	1.100, 1.600, 2.700	2.867 ± 0.010	0.767 ± 0.010
N (*d*)	0.650, 1.520, 2.170	2.460 ± 0.010	0.453 ± 0.010

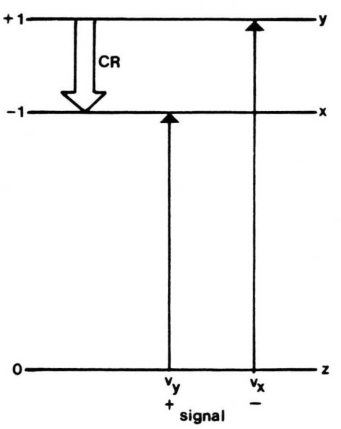


Fig. 2. Irradiating at $\nu_y(\nu_-)$ whilst the cross-relaxation magnetic field is matched to $\nu_z(\nu_0)$ causes positive enhancement of the recovered ^{1}H magnetisation, negative at ν_x.

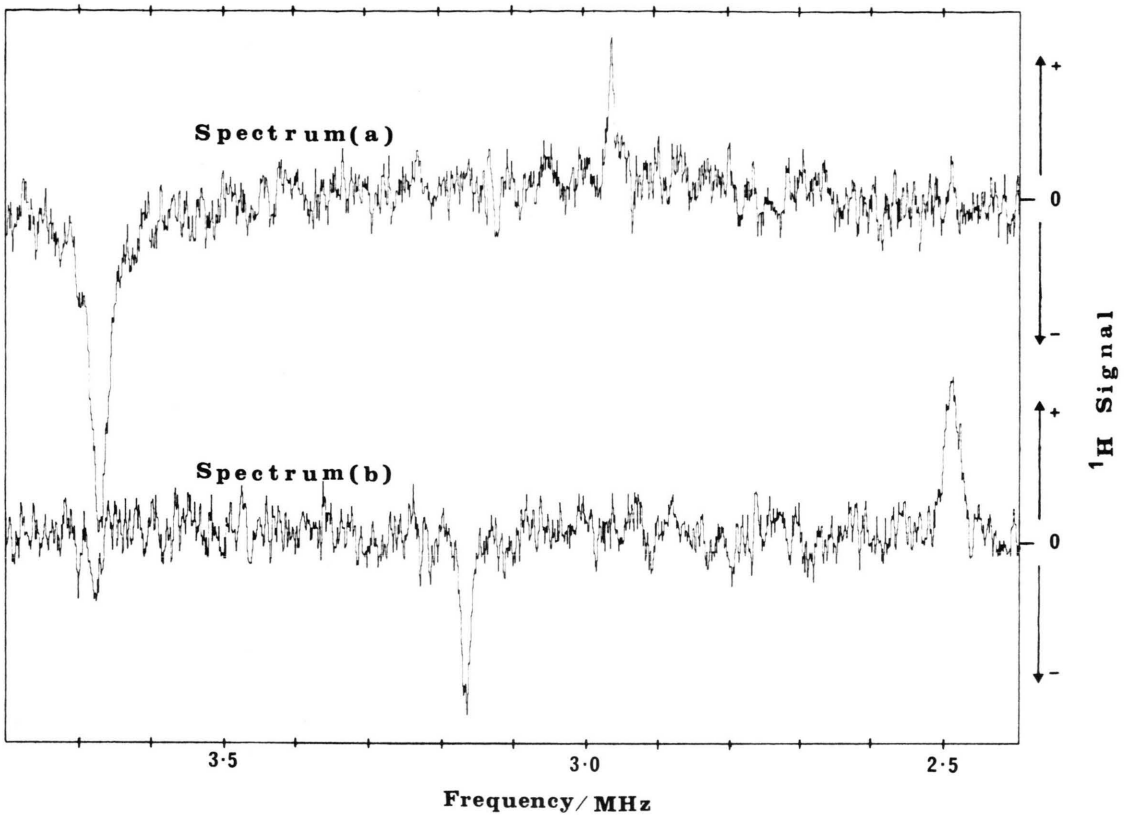


Fig. 3. Combined irradiation and cross-relaxation spectra of (A), polymorph III; spectrum (a) for nucleus N(*a*) with the proton frequency matched to ν_z at 708 kHz; spectrum (b) for nucleus N(*b*) with the proton frequency matched to ν_z at 670 kHz.

Polymorph II gave ^{14}N frequencies which were identical within experimental error to those observed in polymorph III. However, the signal-to-noise ratio of the spectrum was appreciably lower, and there were significant differences in the relative intensities of corresponding peaks in the two spectra, suggesting differences in both the ^{1}H and ^{14}N spin-lattice relaxation times. It is possible to derive rough estimates of the magnitudes of these quantities from (1) and (2) by recording the spectrum with different residence times τ_Q in the low field solenoid. From the position of the baseline in these spectra, T_1 for ^{1}H can be determined as a function of magnetic field by means of (2). With this information and a knowledge of the rate at which the ^{14}N peak intensity increases with the time spent under cross-relaxation conditions, relaxation times T_{1Q} for the three ^{14}N frequencies can be evaluated from (1). It must be emphasized that these experiments assume exponential decays in both cases. This is certainly not the case for ^{1}H relaxation in high field, which is bi-exponential, and is unlikely to be true for ^{14}N relaxation, which is generally bi-exponential, with two relaxation times T_{1s}, T_{1l} related to the three relaxation probabilities of a three-level system [16, 17]. Unfortunately too few experimental points were obtained in these experiments to check bi-exponential behaviour; all data were fitted to a single exponential decay, and the time constants so measured are recorded in Table 2 for polymorphs (II) and (III). It is not possible to resolve separately the relaxation times of the two peaks at 650 and 670 kHz because of overlap, so values for these two transitions are excluded. In view of errors and approximations in both theory and experiment, it cannot be argued that there are significant differences between N (*b*) and N (*c*) and that for N (*d*) is uncertain because of

Table 2. Comparison of the ^{14}N decay time constants for the observed transitions in polymorphs (II) and (III) at 291 K.

Nuclei	Polymorph	Decay time constants (ms)		
		ν_z	ν_y	ν_x
N (*a*)	II	8	51	37
N (*a*)	III	17	32	26
N (*b*)	II	–	44	35
N (*b*)	III	–	53	42
N (*c*)	II	59	42	92
N (*c*)	III	71	48	90
N (*d*)	II	–	43	25
N (*d*)	III	–	55	47

the absence of value for ν_z. However, the two sets for N (*a*) do appear to differ significantly, there being a much greater "anisotropy" in polymorph (II), suggesting differences in relaxation mechanism at this site.

The ^{1}H T_1 dispersion also differs between the two polymorphs (Fig. 4); the single-exponential decay times for polymorph (II) are shorter, and show a linear dependence on frequency, compared to the longer values for polymorph (III), in which the decay times are proportional to the square of the frequency. Behaviour of the latter kind has been observed in other compounds in low magnetic fields [9] and attributed to molecular reorientation.

To summarize, a combination of ^{14}N cross-relaxation and ^{1}H relaxation time measurements has provided a clear distinction between the two polymorphs (II) and (III) of (A), giving a simple experimental method of distinguishing between them. The

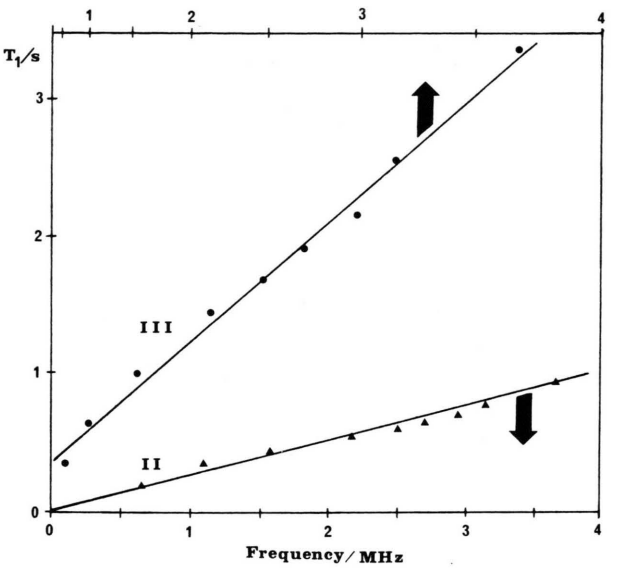


Fig. 4. Frequency variation of the ^{1}H decay time for the two polymorphs at room temperature.

interpretation of the results suggests that the 3-bromo-5-methylpyridine ring, and possibly its 2-$(CH_2)_4$ sidechain, are disorded in both crystalline forms, and that there are slight differences between the two polymorphs in the environment of the nitrogen atom in the other pyridine ring which lead to significant changes in relaxation mechanism.

Acknowledgements

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