Endor Studies of Cation Radicals from Pharmacologically Active Phenothiazines

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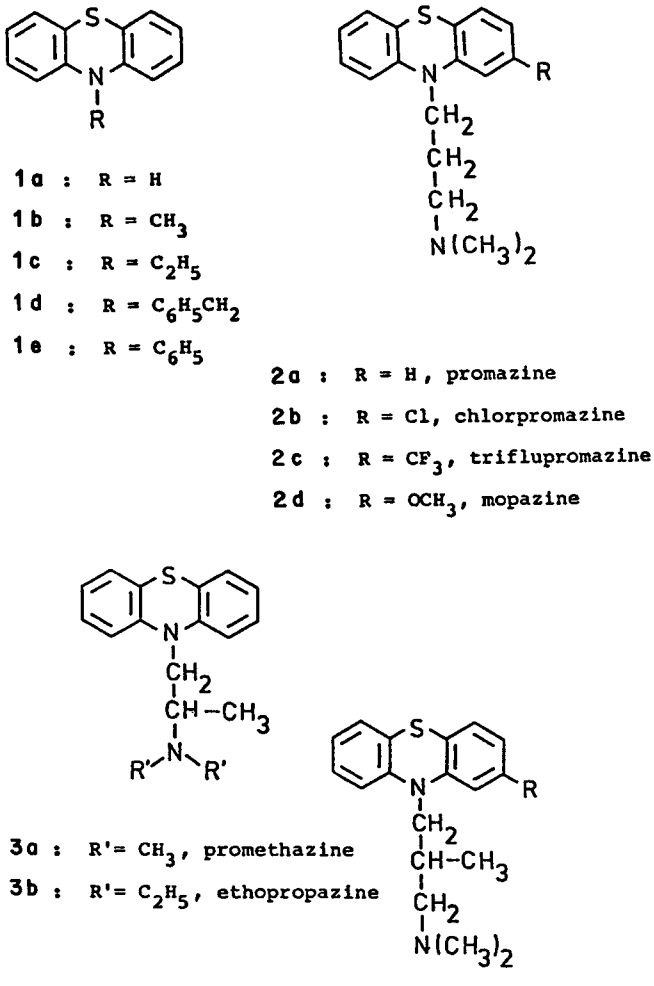
A variety of substituted phenothiazine cation radicals, including those from pharmacologically active derivatives, e.g. chlorpromazine, alimemazine and laevomepromazine, have been studied by means of ENDOR and TRIPLE resonance spectroscopy. These techniques allowed accurate determinations of hyperfine coupling constants, including their signs. Conclusions concerning molecular structure (e.g. twist angles) could be drawn, supporting previous investigations of the interrelationship of molecular conformations with the pharmacological potential, i.e. neuroleptic, antihistaminic or anti-Parkinsonian.

INTRODUCTION

Phenothiazines have attracted great interest because of their various pharmacological properties, which are strongly dependent on the size and conformation of the straight or branched side-chains connected to the N-10 position of the parent heterocyclic system.[1] For example, whereas chlorpromazine (**2b**) is a neuroleptic agent, alimemazine (**4a**) is a potent antihistamine and ethopropazine (**3b**) has been used for treatment of Parkinson's disease. Previously, a theory was proposed linking the biochemical potential of phenothiazines to their ability to act as electron donors.[2] The involvement of radical states has also been discussed in the context of the studies of the mode of action of these drugs.[3] A variety of papers have been devoted to the generation and EPR spectroscopy of neutral and cationic phenothiazine radicals. The limited resolving power of EPR, however, prevented an unambiguous interpretation of the EPR data or the identification of the radicals.

In studies of low-symmetry organic radicals, electron nuclear double resonance (ENDOR) spectroscopy has proved to be a powerful tool in unravelling complex hyperfine interactions.[4] Kennedy *et al.*[5] were the first to apply the ENDOR technique to the neutral radical of the parent and of the chloro-substituted phenothiazine. In this paper we report a thorough ENDOR investigation on the cation radicals of a variety of N-10 substituted phenothiazine model compounds and on some biologically relevant derivatives (Scheme 1). The relative signs of the hyperfine coupling (HFC) constants were determined by applying the electron nuclear triple resonance technique (TRIPLE). In order to achieve a correct assignment of the HFC constants to molecular



Scheme 1

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positions, some bromo-substituted and selectively deuteriated compounds are included.

RESULTS AND DISCUSSION

N-10 unsubstituted model compounds

According to symmetry, from the ENDOR spectrum of the parent phenothiazine cation radical five different ^1H HFC constants, including that of the N-10 proton and the N HFC constant, could be extracted. Whereas the signal-to-noise ratio of the ^1H ENDOR spectrum taken at 260 K is excellent, the ^{14}N ENDOR signals only show up at elevated temperatures and higher microwave and RF power levels (Fig. 1, bottom right). As can be seen by inspection of Fig. 1, the EPR spectrum can be well simulated using the ENDOR HFC constants. These data are in good agreement with the spin density distributions previously obtained from quantum mechanical calculations[6] (Table 1). Moreover, the assignments of the HFC constants to specific molecular positions given in the cited paper[6] are supported by investigation of the 1,3,7,9-tetradeuterioderivative, showing only two remaining small HFC constants with opposite signs for the ring protons (Fig. 1, bottom right). The HFC constant of the proton at the N-10 position could be readily assigned by an H/D exchange experiment. In spite of the fact that the EPR spectra of the 3,7-dibromo- and the 1,3,7,9-tetrabromo derivative are badly resolved, and that no ENDOR signals could be observed for these radicals (large g factor anisotropy caused by the large

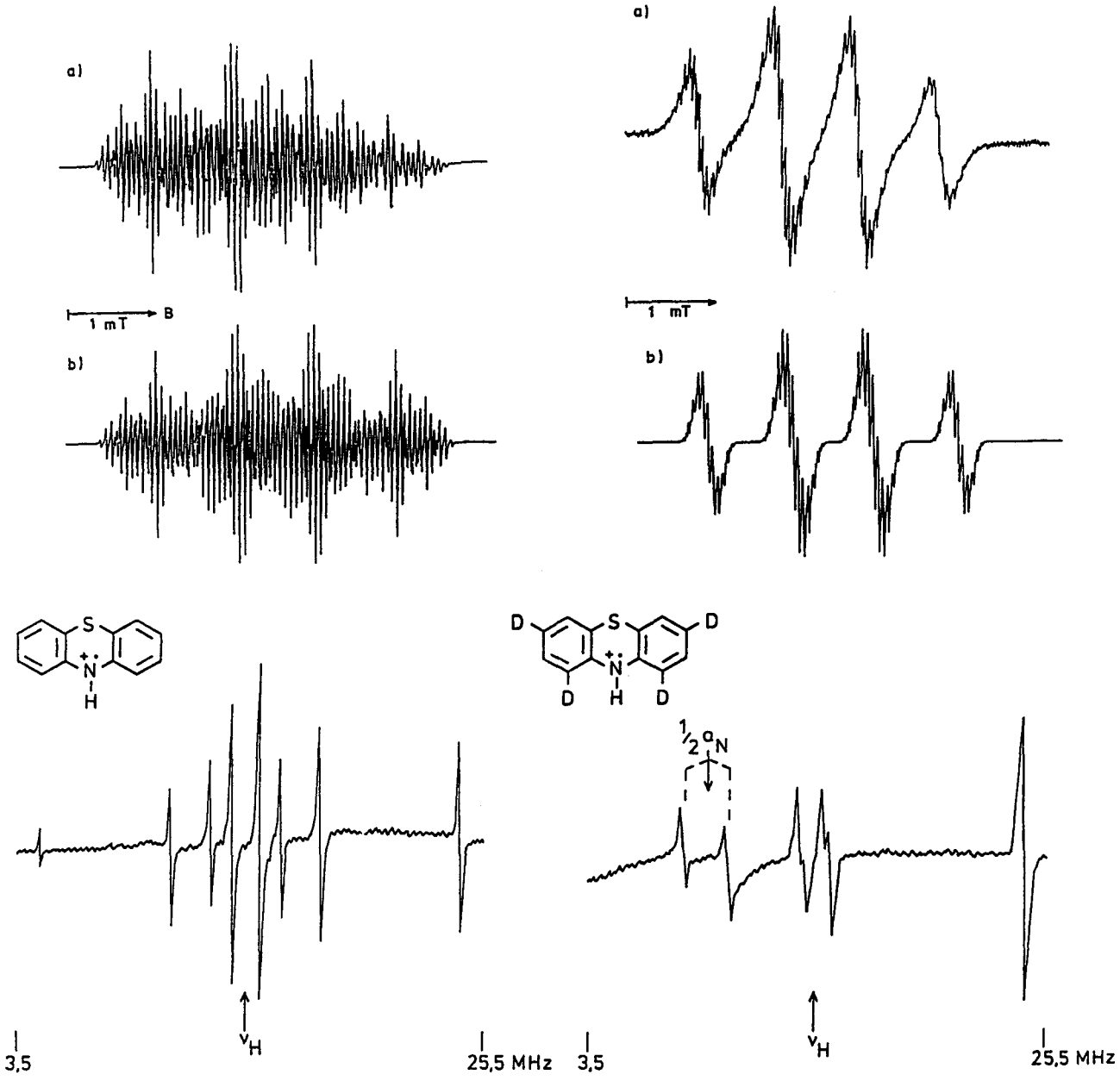

Figure 1. Top: (a) experimental and (b) simulated EPR spectra of the radical cations of phenothiazine (**1a**$^{+\cdot}$) and 1,3,7,9-tetradeuteriophenothiazine. Bottom: ENDOR spectra recorded at 250 and 290 K, respectively.

Table 1. ^{1}H and ^{14}N hyperfine coupling constants (MHz) of 10-substituted phenothiazine cation radicals

Compound	a_N	$a_{\beta\text{-H}}$	$a_{H_{1,9}}$	$a_{H_{2,8}}$	$a_{H_{3,7}}$	$a_{H_{4,6}}$
1a$^{+\cdot}$	18.68	20.85(N – H)	−3.40	−0.93	−7.20	+1.49
1b$^{+\cdot}$	20.88	+18.82	−2.66	−2.02	−5.94	+0.53
1c$^{+\cdot}$	20.80	+10.43	−2.72	−2.13	−6.00	+0.45
1d$^{+\cdot}$	19.09	+10.82	−2.61	−2.16	−5.49	+0.34
1e$^{+\cdot}$[a]	19.20		−2.38	−2.38	−5.89	+0.56

[a] Hyperfine coupling constants of the phenyl substituent: $a_{ortho} = -0.92$, $a_{meta} = +0.56$, $a_{para} = -0.25$.

spin–orbit coupling of the heavy bromo atoms preventing any detectable ENDOR response[7]), additional information could be achieved from these species by measuring the g values. In fact, with an increasing number of substituents considerable g-value shifts were observed, namely phenothiazine$^{+\cdot}$ ($g = 2.00491$, relative value), 3,7-dibromo- (2.00585) and 1,3,7,9-tetrabromo- (2.00635). This increase in the g values indicates positive spin densities at the substituted positions (see, e.g. Ref. 8), being again in agreement with the calculations.

N-10 substituted model compounds

The EPR spectra of the N-10 substituted phenothiazine radical cations are, of course, more complex and the ENDOR technique has to be applied for an unambiguous interpretation. Figure 2 shows the EPR and ENDOR spectra of the ethyl (**1c**$^{+\cdot}$) and benzyl (**1d**$^{+\cdot}$) substituted derivatives serving as typical examples. The HFC constants of several N-10 substituted compounds are collected in Table 1, including the relative signs obtained from the general TRIPLE resonance spectroscopy. Again, good agreement between experimental and simulated EPR spectra could be achieved using the HFC constants deduced from the ENDOR spectra. Considerable spin density redistribution is observed within the heterocyclic system on comparison with the unsubstituted parent compound. Regarding the HFC constants obtained for the phenyl ring of compound **1e**$^{+\cdot}$ (Fig. 3), it is interesting that the sequence $|a_{Ho}| \approx |a_{Hp}| > |a_{Hm}|$ normally found for phenyl substituents must be replaced by the sequence $|a_{Ho}| \cong |a_{Hm}| > |a_{Hp}|$, in contrast to the results from HMO McLachlan calculations.[9] Apparently, the phenyl ring is highly twisted (*ca* 75°, HMO[9]), and the so-called phenyl hyperconjugation mechanism can no longer be neglected in order to account for this sequence. Since this effect has already been exhaustively described in the literature,[10] it will no longer be discussed in the context of this investigation. Figure 3 (bottom) depicts the superimposed general TRIPLE spectra of **1e**$^{+\cdot}$ observed at two different settings of the RF pump frequency.

Phenothiazine drugs

Whereas the EPR spectra of the pharmaceuticals with a straight side-chain are fairly well resolved, those with a branched chain only exhibit a typical 1:2:2:1 four-line pattern. The ENDOR spectra, however, yield valuable information on the spin density distributions (see Table 2 and Figs 4 and 5). The sets of HFC constants extracted for the series of cations promazine (**2a**$^{+\cdot}$), chlorpromazine (**2b**$^{+\cdot}$), and trifluprom azine (**2c**$^{+\cdot}$) clearly reveal that substitution at position 2 significantly changes the spin density distributions within the thiazine moiety, and an increased number of non-equivalent protons is observed. The non-symmetrical spin density distribution of the trifluprom azine (**2c**$^{+\cdot}$) and of the laevomepromazine (**4b**$^{+\cdot}$) cation radical, for example, is reflected in the non-equivalence of the ^{1}H HFC constants of positions 1 and 9, and 3 and 7, respectively (Table 2). It should be noted that the ^{19}F ENDOR signals of the CF_3 group can be easily detected for **2c**$^{+\cdot}$ (Fig. 5). The salient feature of the data of the cation radicals is apparently the significant increase of one of the β-proton HFC constants when the straight side-chain is replaced with a branched side-chain, e.g. chlorpromazine (**2b**$^{+\cdot}$) and trifluprom azine (**2c**$^{+\cdot}$) with alimemazine (**4a**$^{+\cdot}$) or promethazine (**3a**$^{+\cdot}$). In other words, the equivalence of the two β-protons of the species bearing straight chains is removed if a branched chain is present, resulting in two different, i.e. a considerably larger and a much smaller, HFC constants. A closer inspection of Table 2 reveals two exceptions, as follows.

(a) For trifluprom azine$^{+\cdot}$ (**2c**$^{+\cdot}$), two slightly different HFC constants are found. These values, however, are in the same range as those in the straight side-chain molecules. Obviously this has to be attributed to the pronounced non-symmetrical spin density distribution of the parent phenothiazine (see above).

(b) A striking exception is exhibited by laevomepromazine$^{+\cdot}$ (**4b**$^{+\cdot}$), showing a *ca.* 9 MHz HFC constant for two equivalent β-protons in spite of the fact that the side-chain is branched (see below).

As already pointed out in the Introduction, it is tempting to look for a correlation between the pharmacological activities and the geometrical and conformational properties of the phenothiazines. For any conclusions to be drawn from the hyperfine data extracted from the ENDOR spectra of the cation radicals, two structural influences have to be discussed, viz. the conformation of the side-chain and the folding of the phenothiazine ring system and, in

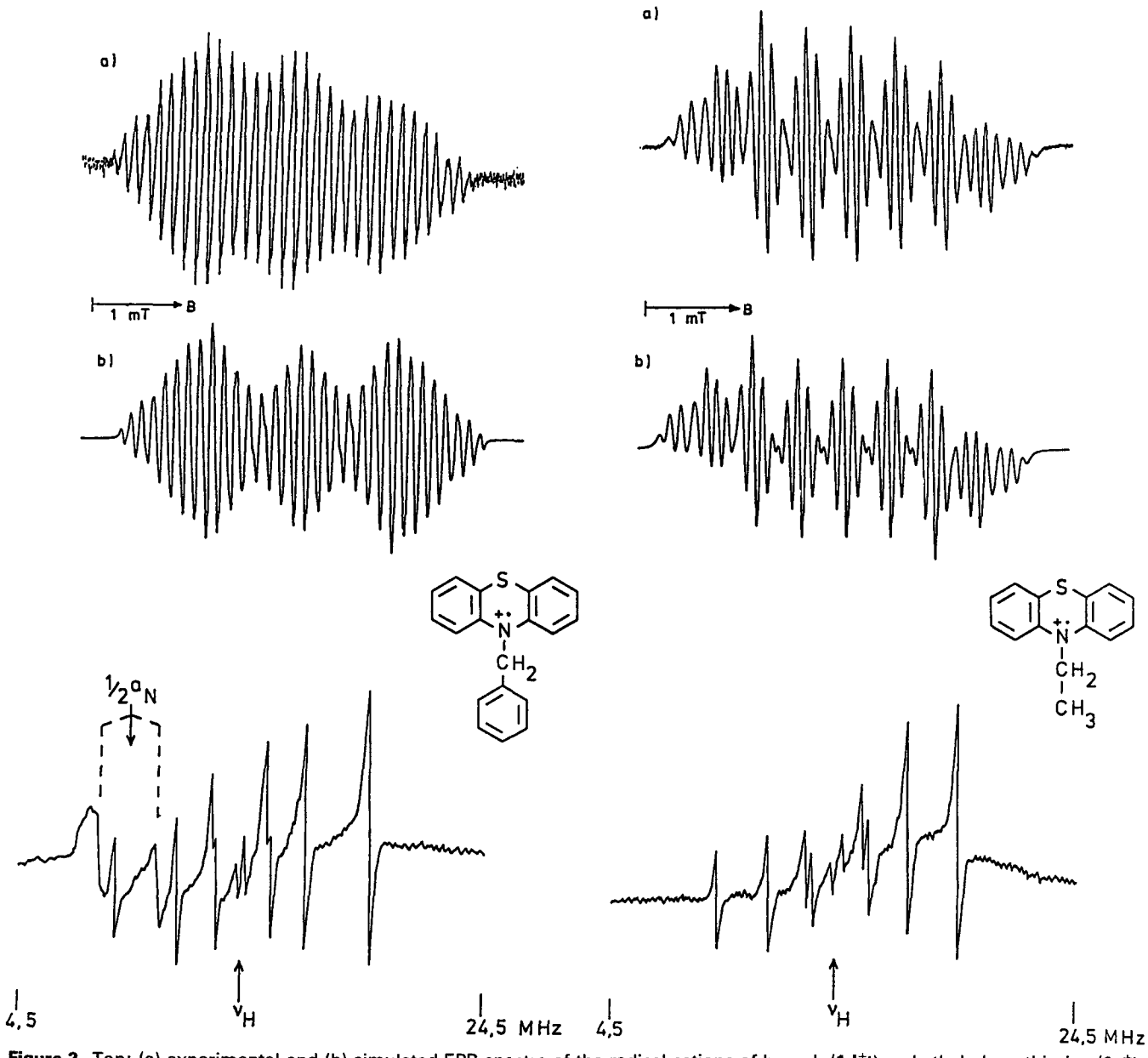

Figure 2. Top: (a) experimental and (b) simulated EPR spectra of the radical cations of benzyl- (**1d**$^{+\cdot}$) and ethyl-phenothiazine (**1c**$^{+\cdot}$ Bottom: ENDOR spectra (250 K).

addition, the mutual dependence of these geometrical arrangements.

Provided hyperconjugation is the predominant mechanism, the following equation applies for calculating the β-proton HFC constant[9,11]:

$$a(\beta - \mathrm{H}) = \rho_{\mathrm{N}} B \cos^2 \theta + B_0 \qquad (1)$$

where ρ_{N} is the π-spin density at the nitrogen, θ is the dihedral angle between the nitrogen $2p(\pi)$ orbital containing the unpaired electron and the β-C—H bond, B is a constant and B_0 is a correction term, which can be neglected to a first approximation.[9] Provided that the methyl group is free to rotate (which is known to be true even below 70 K[12]), the $\rho_{\mathrm{N}}B$ term can be replaced by twice the HFC constant of the methyl protons. Hence,

$$\cos^2 \theta = \frac{a(\beta - \mathrm{H})}{2a(\mathrm{N} - \mathrm{CH_3})} \qquad (2)$$

The twist angles thus obtained are collected in Table 2. In the case of the straight side-chain derivatives, the twist angle is approximately 60°, whereas significantly decreased angles are obtained for the phenothiazines bearing branched side-chains, but a prerequisite for a sound comparison of these angles is a constant flattening of the heterocyclic ring system. In fact, from an HMO and an x-ray study of the diamagnetic neutral compounds previously published,[13] it is obvious that in all systems similar to those studied in this paper the folding angle is *ca* 140°. These results allow the conclusion that the drugs having neuroleptic properties exhibit a twist angle of *ca* 60°, whereas those exhibiting other pharmacological activities display different angles, e.g. alimemazine$^{+\cdot}$ (**4a**$^{+\cdot}$) 53°, promethazine$^{+\cdot}$ (**3a**$^{+\cdot}$) 46° (Table 2).

As already mentioned, at first glance laevomepromazine$^{+\cdot}$ (**4b**$^{+\cdot}$) offers a striking exception, inasmuch as the side-chain is branched but

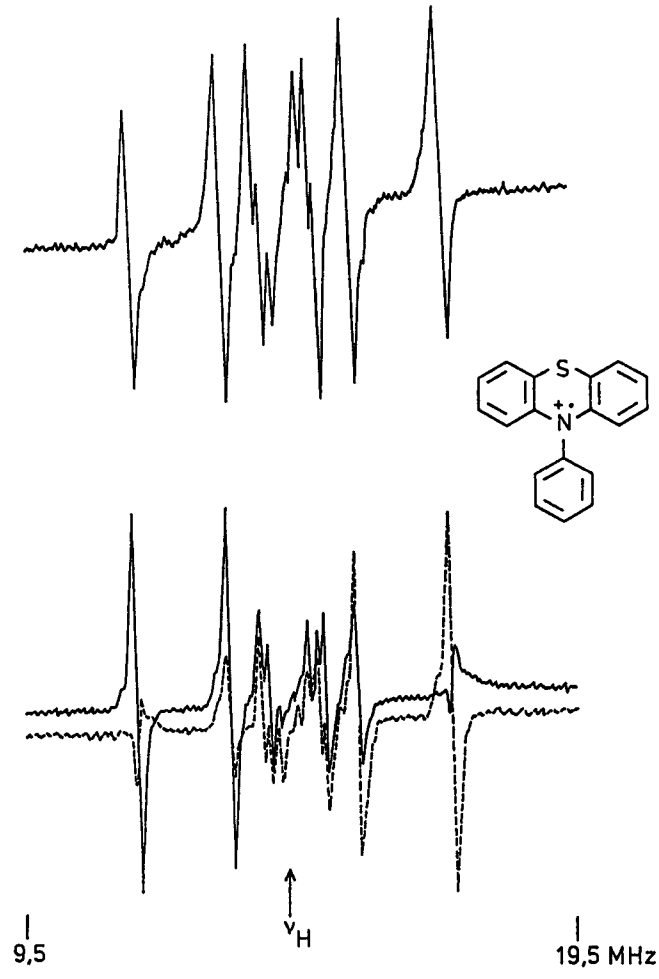

Figure 3. ENDOR and general TRIPLE spectra of the cation radical of phenylphenothiazine (**1a**$^{+\cdot}$), recorded at 260 K.

nevertheless the twist angle amounts to *ca* 60°; the HMO and x-ray study previously cited, however, clearly demonstrated that methoxy substitution in the 2-position drastically flattens the phenothiazine ring [the authors used mopazine (**2d**) rather than laevomepromazine (**4b**), also bearing a 2-methoxy substituent]. Hence, different geometries of the ring system require different conformational energy minima, and these systems therefore belong to different

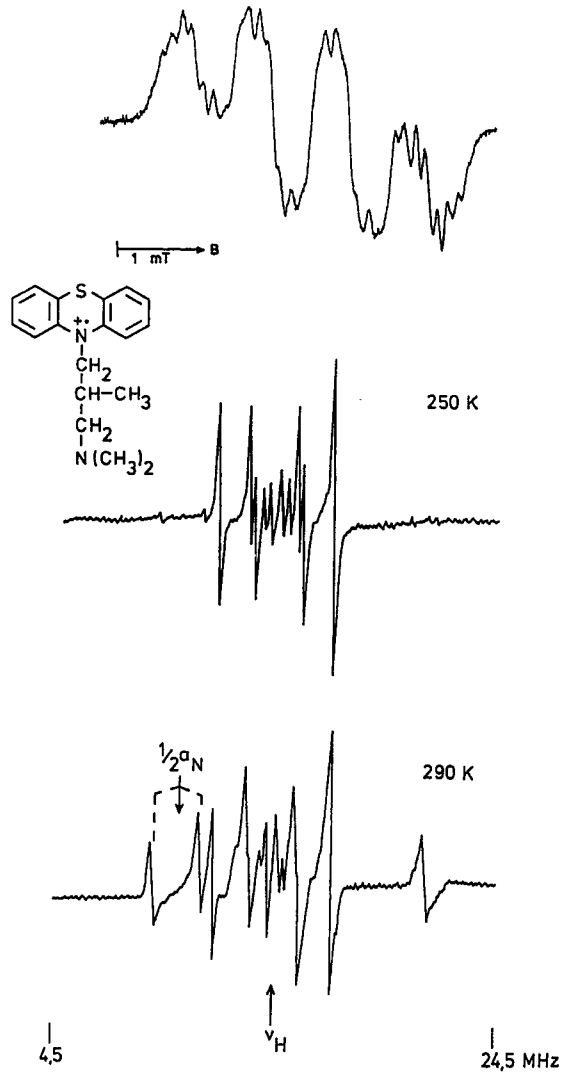

Figure 4. EPR (270 K) and ENDOR spectra (centre, 250 K; bottom, 290 K) of the cation radical of alimemazine (**4a**$^{+\cdot}$).

structural families. Changes in the N—C bond angles change the N-10 hybridization and also the steric interactions of the side-chain with the two peri-protons at positions 1 and 9.

Finally, a short comment is in order regarding the

Table 2. ^{1}H and ^{14}N hyperfine coupling constants (MHz) of cation radicals from phenothiazine drugs, and calculated twist angles between the β-C—H Bond and the 2p$_z$ orbital of Nitrogen

Compound	a_N	$a_{\beta-H}$	$a_{H_{1,9}}$	$a_{H_{2,8}}$	$a_{H_{3,7}}$	$a_{H_{4,6}}$	$\theta_{\beta-H}$(°)
2a$^{+\cdot}$	19.90	+9.98	−2.61	−2.21	−5.72	+1.15	60
2b$^{+\cdot}$	19.09	+9.58	−3.00	−1.71	−5.61	+1.15	60
2c$^{+\cdot}$	19.90	+10.59	−2.77	−1.21	−6.28	+0.65	59.4
		+9.86	−2.30	+3.28(F)	−4.46		
4a$^{+\cdot}$	20.04	+14.43	−2.52	−1.93	−5.44	+0.50	52.9
		+5.44					
3a$^{+\cdot}$	20.04	+18.92	−2.94	−1.65	−5.07	+0.56	46.3
		+1.12					
3b$^{+\cdot}$	20.15	+19.98	−2.89	−1.71	−5.07	+0.56	44.8
		+1.12					
4b$^{+\cdot}$	19.90	+9.67	−2.91	−1.88	−7.71	+1.15	60
			−2.41		−3.67		

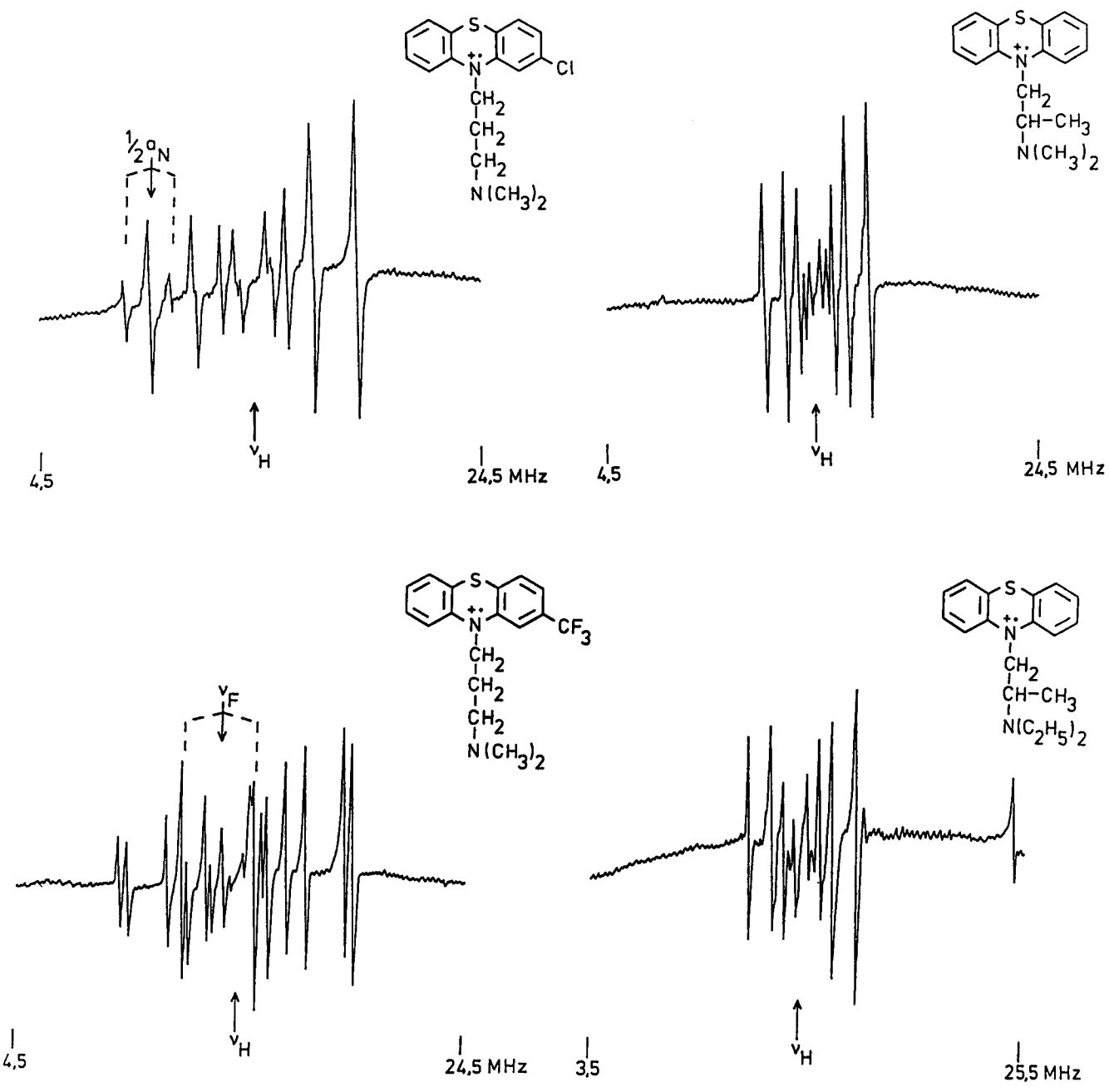

Figure 5. ENDOR spectra of the cation radicals of chlorpromazine (**2b**$^{+\cdot}$), promethazine (**3a**$^{+\cdot}$), triflupromazine (**2c**$^{+\cdot}$) and ethopropazine (**3b**$^{+\cdot}$) recorded at 250 K.

correlation between pharmacological activities and molecular structure. The pronounced alterations to the biochemical properties caused by small geometrical changes are not surprising, and in the case of 'specific' neuroleptic action stereoselectivity has long been recognised.[1] This behaviour is explained in terms of an apparent competition between, e.g., dopamine receptors and neuroleptics for specific binding.

CONCLUSIONS

It has been demonstrated that the ENDOR technique can be applied without difficulty in investigations of the paramagnetic derivatives of phenothiazine-type drugs, yielding valuable information on spin density distributions and, thereby, the geometrical arrangements of these systems. We feel that, in future work, this approach may contribute to a better insight into the very delicate interplay between structure and pharmacological activities.

EXPERIMENTAL

Compounds

The N-10 substituted phenothiazines were synthesized following published procedures: **1b**,[14] **1c**,[15] **1d**[16] and

1e.[17] 1,3,7,9,Tetradeuteriophenothiazine was obtained by repetitive H/D exchange using a D_2O–tetrahydrofuran mixture as the solvent. The deuterium contents and distributions were determined by mass spectrometry and NMR analysis. The degree of deuteriation was at least 95%. The phenothiazine pharmaceuticals **2a**, **2b**, **3a** and **3b** are available from commercial sources. Alimemazine (**4a**), laevomepromazine (**4b**) and triflupromazine (**2c**) were gifts from Rhone-Poulenc Pharma GmbH, Norderstedt, Tropon-Werke GmbH and Co. KG, Köln, and Von Heyden GmbH, München, respectively.

Preparation of samples

Samples were prepared by reacting 10^{-4} M trifluoroacetic acid–toluene solutions of the parent phenothiazine with dibenzoyl peroxide. The solutions were carefully deoxygenated prior to EPR/ENDOR measurements by flushing with purified nitrogen, or by means of the freeze–pump–thaw technique on a high vacuum line. The latter technique proved to be superior since (a) in the sealed tubes the radicals were stable for several weeks, (b) deoxygenation was more effective and (c) the sample tube allowed distillation of the solvent under vacuum conditions for concentration or dilution (the ENDOR enhancement critically depends on, among other conditions, the radical concentration).

Instrumentation

The ENDOR and TRIPLE instrumentation consists of a Bruker ER 220D EPR spectrometer equipped with a Bruker ENDOR cavity (ER 200ENB) and home-built NMR facilities described elsewhere.[4] ENDOR spectra were accumulated by using a Nicolet 1170 signal averager employing 1K data points; 32 or 64 scans were taken, 30 s per scan. The microwave power level was 40–80 mW, the RF power *ca* 170–220 W, corresponding to a field strength of 0.6–0.7 mT in the rotating frame (not constant over the frequency range) and the modulation amplitudes ±15 to ±30 kHz. The temperature was varied with a Bruker B-VT 1000 temperature control unit, constant to ±1 K and checked by means of a thermocouple. The *g* values were determined using a Bruker NMR gauss meter (B-NM 12) and an HP 5340A frequency counter.

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