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Selective Paramagnetic Relaxation Reagents (SPRR). Study of Line Broadening Effect of Cu^{2+} Ions on the ^{13}C-NMR Spectral Lines of N-Containing Molecules

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Summary. The selective line broadening effect of the paramagnetic Cu^{2+} on the ^{13}C-NMR linewidths of nitrogen compounds has been studied. It operates through electron-nuclear hyperfine coupling, however, chemical exchange processes also contribute significantly. The observed selective broadenings make possible the assignments of carbon atoms one or two bonds away from a basic nitrogen (earlier assignment of quinidine has been modified), the determination of likely metal binding sites of a complex and the relative basicity of different nitrogen atoms of the same molecule. The broadenings depend also on the spatial vicinity of the Cu^{2+} ion and the interacting nuclei. While its main advantage is simplicity, the main drawback is that it is limited to molecules capable to form complexes with Cu^{2+} ion.

Keywords. Selective paramagnetic relaxation agent; Cu^{2+}, application in ^{13}C-NMR analysis.

Selektive paramagnetische Relaxationsreagenzien (SPRR). Untersuchung des Linienverbreiterungseffekts von Cu^{2+}-Ionen auf die ^{13}C-NMR-Signale in N-Verbindungen

Zusammenfassung. Es wurde der selektive Verbreiterungseffekt von paramagnetischen Cu^{2+}-Ionen auf ^{13}C-NMR-Linienbreiten von Stickstoff-Verbindungen untersucht. Diese Verbreiterung erfolgt durch Elektron-Kern-Hyperfeinkopplung, allerdings liefern auch chemische Austauschprozesse signifikante Beiträge. Die beobachteten Verbreiterungen erlauben die Zuordnung von Kohlenstoffatomen, die eine oder zwei Bindungen vom basischen Stickstoffatom entfernt sind (die frühere Zuordnung für Chinidin mußte modifiziert werden). Außerdem können die Koordinationsstellen festgestellt und eine relative Basizitätszuordnung von verschiedenen Stickstoffatomen innerhalb eines Moleküls getroffen werden. Die Verbreiterungen hängen auch von der räumlichen Nachbarschaft der Cu^{2+}-Ionen und dem in Wechselwirkung befindlichen Kern ab. Der Hauptvorteil ist die Einfachheit der Methode, der Hauptnachteil liegt in der Limitierung der Methode auf Moleküle, die mit Cu^{2+} Komplexe bilden können.

Introduction

In the presence of paramagnetic species both the chemical shift and linewidth of magnetic nuclei can change significantly due to the interaction of the unpaired electron and the nuclei. In the collision complexes formed the chemical environment is different and through the electron–nucleus relaxation the nuclei can lose their energy much faster.

Reagents which affect primarily the chemical shifts are called shift reagents [1–3] while those which contribute mainly to the relaxation rates are the relaxation reagents. The latters can be divided in two groups depending on the general or selective nature of the effect they cause. While members of the first group [e.g., Cr(*acac*)$_3$] are in practical use for long time in ^{13}C-NMR spectroscopy, only a few reports on the selective line broading effect of Cu^{2+} and Ni^{2+} have been published so far [4–7]. However, in these reports the problem of selective line broadening has been dealt with only marginally. The first paper, to our knowledge, devoted to the selective line broadening effect of Cu(*acac*)$_2$ was that of Doddrell and coworkers [8]. Studying the Cu(II) dropped ^{13}C-NMR spectra of simple aliphatic amines these authors emphasized the analogy of the spin-spin scalar couplings and the hyperfine coupling and pointed out the usefulness of the effect in assigning ^{13}C-NMR spectra of aliphatic amines.

This paper is an extended study of these "shiftless" paramagnetic relaxation agents. The origin and nature of the selective effects of a potential SPRR agent [Cu(2,4-dichlorobenzoate)$_2$] has been studied in detail and possible applications in the ^{13}C-NMR spectral analysis have been looked for.

Theory

The theory of electron spin-nuclear spin interaction has been laid down by the works of Kurland-McGarvey [9], Solomon [10], Bloembergen [11] and Doddrell [12]. For stable complexes the paramagnetic linewidth of spin-1/2 nuclei is governed by dipolar and/or hyperfine exchange (contact) relaxation processes.

The dipolar interaction is often dominant for ^{1}H nuclei except in delocalized systems in which a considerable unpaired electron density exists on nuclei far removed from the metal ion. For pure dipolar relaxation the spin-spin relaxation, T_2 can be expressed by the Solomon-Bloebergen equations [10, 11], provided the $w_I^2\tau_r^2 \ll 1$ condition is fulfilled (τ_r^{-1} = rate constant for rotation of the species containing the coupled pair and w_I = nuclear resonance frequency),

$$1/T_2 = S(S+1)\gamma_I^2 g^2 \mu_B^2/15\, r^6 [7\,\tau_{c1} + 13\,\tau_{c2}/(1 + w_S^2\tau_{c2}^2)],$$

where S is the total electron spin of the paramagnetic ion, r is the electron-nuclear distance, w_s is the electron resonance frequency, and τ_{c1} and τ_{c2} are the rate constants for the reorientation of the coupled magnetic moment vectors.

If the dipolar mechanism is dominant the observed linewidths, $W_{1/2} = (\pi\, T_2^{-1})$ must reflect the r^{-6} dependence on the electron-nuclear distance.

Early publications on the subject used these equations for the determination of metal-nucleus distances. Later on, it was pointed out that (i) an additional term responsible for the relaxation induced by the delocalized part of the unpaired spin must also be included [12] (ii) the often assumed dominance of dipolar contribution is rarely if ever justified for ^{13}C nuclei [13], instead (iii) the relaxation is merely dominated by the contact contribution. For pure contact (scalar) contribution T_2 can be expressed as follows [10, 11],

$$1/T_2 = S(S+1)A^2/3\,h^2[\tau_{e1} + \tau_{e2}/1 + (w_I - w_S)^2\tau_{e2}^2],$$

where τ_{e1}, τ_{e2} are electron correlation times modulating the scalar interaction.

Since the equation involves the square of the hyperfine coupling constant, A, in cases where dominant contact shifts are observed the square of the shifts must

correlate with the linewidths. For equilibrium complexes beside the contact and dipolar terms the *chemical exchange* can also contribute to the observed line widths. Under such conditions the more general form of the Solomon-Bloembergen equation should be used [13],

$$W_{1/2} = 1/T_{2P} = (M/L)\,q\,(7\,a\,\tau_c\,r^{-6} + bA^2\tau_e + \tau_M\,\Delta\,w_M^2),$$

where q is the average number of ligand bound in an identical way, a and b are constants, τ_c and τ_e are the correlation times for the dipolar and contact interactions, respectively.

The contribution of the chemical exchange depends on the magnitude of the $\tau_M\,\Delta\,w_M^2$ term, where τ_M is the lifetime of the metal-ligand (ML) complex and $\Delta\,w_M$ is the chemical shift difference of the nucleus in question in the bound and free ligand.

When $1/T_{2M} \gg \tau_M\,\Delta\,w_M^2$ it does not affect the actual linewidth, however, when the reverse inequality is true ($1/T_{2M} \ll \tau_M\,\Delta\,w_M^2$), the linewidths will be frequency and temperature-dependent. T_{2M} is the transverse relaxation time of the bound ligand.

Results and Discussions

Since the line broadening effect of different paramagnetic metal ions can be different, we made a comparative study which included Zn^{2+}, Ni^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+} and Cu^{2+} using pyridine as a common ligand.

Arbitrary terms, selectivity and strength, were used to characterize the observed effects. The former can be expressed as the relative line broadening of signals compared with the least broadened one. The strength of the effect is expressed by the metal/ligand ratio, M/L at which a given selectivity could be achieved. M/L changed between 10^{-3} and 10^{-5} throughout this study. Of all the ions studied, Ni^{2+}, Cu^{2+} and Mn^{2+} showed remarkable selectivity and strength (Table 1).

Table 1. Isotropic chemical shift, $\Delta\,\delta$ and line broadening $\Delta\,W_{1/2}$[a] values of the ^{13}C lines of pyridine doped with Ni^{2+}, Cu^{2+} and Mn^{2+} in ppm and Hz, respectively ($CDCl_3$, $\delta_{TMS} = 0$ ppm)

Ion	$M/L \cdot 10^{-5}$	C-2,6		C-3,5		C-4	
		$\Delta\,\delta$	$\Delta\,W_{1/2}$	$\Delta\,\delta$	$\Delta\,W_{1/2}$	$\Delta\,\delta$	$\Delta\,W_{1/2}$
Ni^{2+}	8.5	−0.05	0.7	0.05	1.4	0.0	0.0
	12.5	−0.1	0.95	0.1	3.4	0.0	0.0
	21.2	−0.2	1.4	0.2	4.1	0.0	0.0
Cu^{2+}	6.8	0.0	0.0	0.0	6.1	0.0	0.0
	13.6	0.0	0.0	0.0	11.1	0.0	0.0
Mn^{2+}	15.0	0.05	0.9	0.1	0.9	0.15	0.0
	40.0	0.25	3.8	0.3	3.3	0.3	0.15
	60.0	0.40	5.6	0.5	4.6	0.4	0.8

[a] $\Delta\,W_{1/2} = W_{1/2}$(bound ligand) − $W_{1/2}$(free ligand)

Table 2. ^{13}C line broadenings $\Delta W_{1/2}$[a] (in Hz) observed for 2-amino-, 3-amino- and 4-amino-pyridines on addition of Cu^{2+} and Mn^{2+} ($CDCl_3$)

Ion	$M/L \cdot 10^{-4}$	C-2	C-3	C-4	C-5	C-6
			2-Amino-pyridine			
Cu^{2+}	1.3	0.25	4.0	0.0	3.85	1.8
Mn^{2+}	1.2	4.9	1.1	0.0	0.7	2.4
			3-Amino-pyridine			
Cu^{2+}	0.64	0.5	6.6	0.15	6.1	0.2
Mn^{2+}	0.85	8.8	5.2	0.0	4.1	6.3
			4-Amino-pyridine			
Cu^{2+}	0.97	0.8	21.3	0.25	21.3	0.8
Mn^{2+}	6.32	4.7	4.7	0.3	4.7	4.7

[a] $\Delta W_{1/2} = W_{1/2}$(bound ligand) $- W_{1/2}$(free ligand)

From these Cu^{2+} shows by far the greatest selectivity; furthermore it is practically "shiftless", unlike to Ni^{2+} and Mn^{2+} which induce remarkable isotropic shifts.

It is interesting to note that the relative order of line broadenings were identical for Ni^{2+} and Cu^{2+} [$\Delta W_{1/2}$(C-3,5) > $\Delta W_{1/2}$(C-2,6) $\gg$ $\Delta W_{1/2}$(C-4)] but different for Mn^{2+} [$\Delta W_{1/2}$(C-2,6) > $\Delta W_{1/2}$(C-3,5) $\gg$ $\Delta W_{1/2}$(C-4)] (see Table 2 as well).

An organic anion (2,4-dichloro-benzoate) was used to improve the solubility in organic solvent.

To understand the reasons of the observed selectivity we had to reveal the nature of possible interactions. Amino-pyridines (Table 2) and methyl-amino-pyridines (Table 3) were used as model compounds for this purpose. As expected, C-3 and C-5 carbons show the strongest broadening in each compounds. For amino-pyridines the position of the amino group has almost no effect on the observed line broadenings. In the case of methyl-amino derivatives the position of the methyl group has some effect on the relative order. For example, while for the 4-methyl and 5-methyl derivatives the broadening of C-6 is generally larger than that of C-2, for the 6-methyl derivative the opposite is true. Since direct involvement of the methyl group in the coordination is unlikely its effect must be, at least in part, of steric origin.

For 2-amino-4-methyl-pyridine the induced isotropic chemical shifts and line broadenings were measured at different M/L ratios (Table 3).

Since squares of the observed contact shifts were negligible relative to the line broadenings the interaction can not be of purely contact origin.

Concerning the possibility of remarkable dipolar contribution it is by no means significant. The metal-nucleus distances calculated by assuming the r^{-6} dependence of the line broadenings, could not be interpreted.

Finally the possibility of slow chemical exchange also have been looked for. For this purpose we carried out variable-temperature NMR studies on 2-amino-4-methyl-pyridine, 3-amino-phenol, cyclohexylamine and penicillin-G.

Table 3. Cu^{2+} induced ^{13}C chemical shift $\Delta\delta$ and line broadening $\Delta W_{1/2}$[a] data of 2-amino-4-methyl-, 2-amino-5-methyl- and 2-amino-6-methyl-pyridines relative to the free ligands in ppm and Hz, respectively ($CDCl_3$, $\delta_{TMS} = 0$ ppm)

M/L		C-2	C-3	C-4	C-5	C-6	*Me*
		2-Amino-4-methyl-pyridine					
$2.8 \cdot 10^{-5}$	$\Delta W_{1/2}$	0.0	2.8	0.0	3.8	1.5	0.3
	$\Delta\delta$	0.01	0.02	0.03	0.08	0.03	0.03
$5.6 \cdot 10^{-5}$	$\Delta W_{1/2}$	0.0	5.9	0.0	5.5	2.4	0.4
	$\Delta\delta$	−0.06	0.01	0.02	0.05	−0.01	−0.01
$3.0 \cdot 10^{-4}$	$\Delta W_{1/2}$	0.3	9.5	0.3	9.8	2.9	0.3
	$\Delta\delta$	0.0	0.1	0.0	0.0	0.0	0.0
		2-Amino-5-methyl-pyridine					
$2.1 \cdot 10^{-4}$	$\Delta W_{1/2}$	0.0	10.3	0.3	6.7	5.0	0.3
	$\Delta\delta$	0.0	0.0	0.0	0.0	0.0	0.0
		2-Amino-5-methyl-pyridine					
$2.1 \cdot 10^{-4}$	$\Delta W_{1/2}$	1.3	4.6	0.3	6.9	0.1	0.6
	$\Delta\delta$	0.0	0.0	0.0	0.0	0.0	0.0

[a] $\Delta W_{1/2} = W_{1/2}$(bound ligand) $- W_{1/2}$(free ligand)

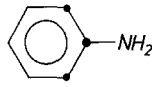
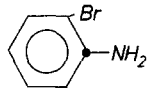
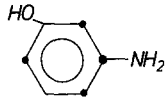
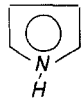
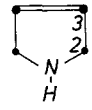
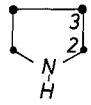
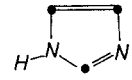
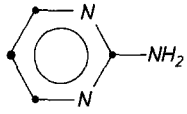
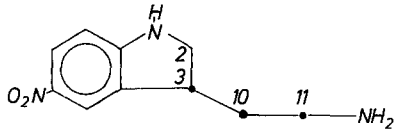
For moderately weak complexes such as cyclohexylamine Doddrell and coworkers assumed fast equilibrium between the bound and unbound states in excess of ligands [8]. However, our investigations proved the existence of slow exchange processes for all compounds studied with the exception of penicillin-G. At higher temperature line broadenings decreased or completely disappeared but after recooling the samples they were restored indicating temperature dependence of terms involved. This is reasonable since, beside τ_M, the induced contact shift, Δw_M is also reversely proportional with the temperature [9].

In addition for long electron relaxation times (as in the case of the Cu^{2+}) τ_e approaches τ_M [14] which reduces the contact contribution drastically.

Since hyperfine coupling and contact shift are generally different for the various nuclei of the ligand only extensive studies on the effect of temperature and frequency variations would allow the evaluation of their relative contributions to the observed broadenings.

The selective broadenings will be determined, after all, by the actual values of A and the lifetime of the complex, therefore parameters which determine their magnitudes have been looked for. The lifetime of the complex depends on the strength of the bond between the Cu^{2+} ion and the donor atom. For nitrogen compounds it must be related with the basicity of the nitrogen atom. The magnitude of A depends on the probability density of the unpaired electron at the given nucleus. This involves dependence on the geometrical arrangements of the interacting centres as well. From the point of ligand and/or complex stereochemistry the latter is of particular interest. For aliphatic amines a $A_2 < A_3$ relationship and spatial dependence on nitrogen lone pair orientation has been reported [8].

Table 4. ^{13}C-NMR line broadening, $\Delta W_{1/2}$[a] data of aniline derivatives and some other nitrogen heterocycles doped with Cu(2,4-dichloro-benzoate)$_2$ ($CDCl_3$)

Compound	Relative line broadenings $\Delta W_{1/2}$	M/L
Aniline 	$\Delta W_1 > \Delta W_{2,6} > \Delta W_4 > \Delta W_{3,5}$ 5.0 : 1.9 : 0.6 : 0.0 $A_2 > A_3 > A_{5,4}$	$1.0 \cdot 10^{-3}$
2-Br-aniline 	$\Delta W_1 > \Delta W_n$ 0.6 : 0.0 $A_2 > A_n$	$0.7 \cdot 10^{-3}$
3-OH-aniline 	$\Delta W_1 > \Delta W_6 > \Delta W_2 > \Delta W_4 \gg \Delta W_{3,5}$ $A_2 > A_3 > A_5 > A_4$	$1.0 \cdot 10^{-3}$
Pyrrole  No effect!	3-Pyrroline $A_3 > A_2$ 	$1.3 \cdot 10^{-3}$
Pyrrolidine  $A_3 > A_2$	Imidazole  $A_3 = A_2$	$1 \cdot 10^{-3}$
2-Aminopyrimidin 	$\Delta W_5 > \Delta W_{4,6} > \Delta W_2$ $A_3 > A_2$	$1 \cdot 10^{-3}$
3-Ethylaminoindoline 	$\Delta W_{10} > \Delta W_{11} > \Delta W_3 > \Delta W_n$ 5.0 : 1.5 : 0.3 : 0.0 $A_3 > A_2 > A_4$	$5.8 \cdot 10^{-3}$

[a] $\Delta W_{1/2} = W_{1/2}$(bound ligand) $- W_{1/2}$(free ligand)

Anilines and Other Planar Nitrogen Heterocycles

The line broading data of compounds studied are given in Table 4. Due to the delocalization of the nitrogen lone electron pair [15] aniline is weaker basic than pyridine. In agreement the observed line broadenings are rather small even at metal/ligand molar ratio, (M/L) 15–20 times higher than usual.

It is noteworthy that unlike to the pyridine derivatives, for the anilines studied two-bond hyperfine couplings were larger that three-bond ones ($A_2 > A_3$). The heteroaromatic pyrrol in line with its slightly acidic character does not form complex with Cu^{2+} ion. The more basic nitrogens in 3-pyrroline and pyrrolidine show stronger complexing strength. The $A_3 > A_2$ relative order is restored for both.

For compounds with two or more nitrogens their relative basicity can be estimated. In imidazole only the basic nitrogen atom is expected to coordinate. However, since it exists in tautomeric equilibrium, C-4 and C-5 show identical broadening.

Likewise, 1,2,4-triazole, being also in fast tautomeric equilibrium at room temperature, does not show any selective broadening. At the same time, its substituted derivatives (where such equilibrium could be excluded) do.

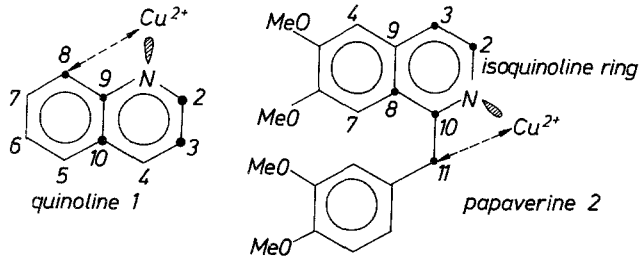
In 2-aminopyrimidin, where Cu^{2+} ions can coordinate to both ring nitrogens in a similar way, C-5 shows the strongest and C-2 the weakest line broadenings [$A_3(5) > A_2(4,6) > A_2(2)$]. The latter fact again emphasizes the much weaker complexing ability of the conjugated amine nitrogen compared with the sp^2 ring nitrogens.

The "pyrrol-like" indoline derivatives do not show selective effects, the 3-ethylamino derivative acts as a simple aliphatic amine (see Table 4).

Rigid Planar Nitrogen Heterocyclic Compounds: Quinoline (**1**) *and Isoquinoline (in Papaverine* **2***) (Table 5 and Scheme 1)*

Quinoline shows weaker complexing strength compared with pyridine. The magnitude of three-bond hyperfine couplings are generally 2–3 times larger than the two-bonds ones, furthermore $A_2(2)$ is about three times as large as $A_2(9)$. It is also noteworthy that the line broadeninig of C-8 *cis* to the nitrogen lone pair is much smaller than that of C-10 *trans* to the same electron pair.

Isoquinoline shows a somewhat different broadening pattern. While the basic trend is the same, differences between $A_2(2)$ and $A_2(10)$ or $A_3(3)$ and $A_3(8)$ are more remarkable (Table 5).



Scheme 1

Table 5. ^{13}C line broadening data, $\Delta W_{1/2}$[a] of quinoline[b] and isoquinoline[b] (in papaverine) rings on addition of Cu^{2+} ($CDCl_3$)

$M/L \cdot 10^{-3}$		C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
					Quinoline					
3.5	$\Delta W_{1/2}$	5.3	11.0	0.8	0.3	0.0	0.5	1.4	1.6	10.0
7.0	$\Delta W_{1/2}$	9.4	23.5	1.4	1.1	0.0	0.9	4.1	3.4	20.0
	$A_3(3) \geqslant A_3(10) > A_2(2) > A_3(8) \geqslant A_2(9) > A_4(4) = A_4(5) > A_4(7) > A_5(6) = 0$									
					Isoquinoline[c] (in papaverine)					
1.1	$\Delta W_{1/2}$	19.5	20.0	0.0	0.0	0.0	0.0	30.0	0.0	6.2
	$A_3(8) > A_2(3) \geqslant A_2(2) > A_3(11) > A_2(10) \gg A_n(m)$									

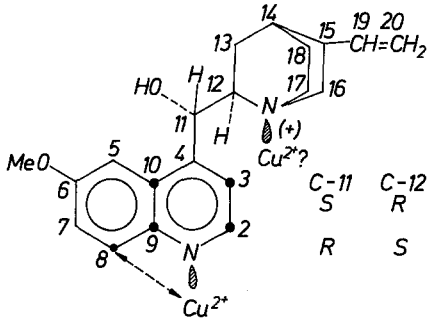
[a] $\Delta W_{1/2} = W_{1/2}$(bound ligand) $- W_{1/2}$(free ligand)

[b] The assignments were taken from Ref. [22]

[c] For C-11 the broadening was about 15 Hz!, the numbering used in quinoline is retained for ease of comparison

Quinine and Its HCl Salt (**3 a, b**), *Quinidine and Its* H_2SO_4 *Salt* (**4 a, b**) (*Table 6*)

Quinine and quinidine are diastereoisomers having opposite configuration at C-11 and C-12 but identical at C-15 and C-14 [16] (Scheme 2).



Scheme 2

In the free base **3 a** the Cu^{2+} ions are expected to coordinate primarily to the tertiary nitrogen (because of its higher basicity). However, carbons in one- and two-bond environment of the quinoline nitrogen show much stronger broadening than those of the quinuclidine ring. Furthermore, compared with the HCl salt the general strength of the effect is 7–8 times weaker (Table 6).

To interpret these results we assume fast equilibrium (shifted toward the quinoline nitrogen) between the possible coordination points. The weak performance of the amine nitrogen can also be related with the equatorial position of its lone electron pair. Similarly weak coordination ability was observed for the 1-methyl-1,2,3,6-tetrahydropyridine in which the lone pair is thought to be equatorial as well (see below).

In the hydrochloride salt **3 b** the quinuclidine ring carbons do not show any broadening which indicates that the salt formation takes place on the amine nitrogen

Table 6. ^{13}C-NMR line broadenings, $\Delta W_{1/2}$ (Hz) observed for quinine (**3a**), quinine · HCl (**3b**), quinidine (**4a**) and quinidine · H_2SO_4 (**4b**) on addition of Cu^{2+} ions ($CDCl_3$)

$M/L \cdot 10^{-3}$	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
Quinine (**3a**)																			
5.35	2.5	2.5	1.6	0.5	0.1	0.5	1.1	2.2	2.8	0.8	0.5	1.6	0.5	0.3	0.8	1.6	0.3	0.1	0.0
$A_3(10) > A_3(3) = A_2(2) > A_2(9) > A_2(4) = A_3(13) > A_2(17) > A_3(8) > A_2(11) \gg A_n(m)$																			
Quininehydrochloride salt (**3b**)																			
0.71	10	15	0.1	0.2	0.0	0.3	6.5	15	15	0.1	0.1	1.5	0.0	0.0	0.1	0.3	0.0	0.5	0.3
$A_3(10) \geqslant A_3(3) \geqslant A_2(9) \geqslant A_2(2) > A_3(8) \gg A_n(m)$																			
Quinidine (**4a**)																			
6.7	4.6	2.2	1.7	0.3	0.4	−0.2	0.5	4.7	5.0	1.7	2.2	1.6	0.8	1.2	1.3	1.4	1.4	0.6	0.4
$A_3(10) \geqslant A_2(9) \geqslant A_3(2) > A_3(3) = A_2(12) > A_3(4) = A_2(11) \geqslant A_3(13) > A_3(17) > A_3(18) \gg A_n(m)$																			
Quinidine H_2SO_4 salt[a] (**4b**)																			
2.7	10	15	0.9	0.2	0.3	0.3	0.6	15	15	3.3	3.1	4.2	0.2	0.4	2.9	2.9	3.0	0.2	0.3
$A_3(10) \geqslant A_2(9) \geqslant A_2(2) \geqslant A_3(3) > A_2(11) \geqslant A_3(12) \geqslant A_2(16, 17) \geqslant A_3(13) > A_3(18) > A_4(4) \gg A_n(m)$																			

[a] Recorded in *DMSO-d_6*, assignments were taken from Ref. [16]

exclusively. Only the quinoline nitrogen and the OH group are available for the Cu^{2+} ions. Since carbons one or two bonds apart from the latter did not broaden, coordination to the oxigen cannot be remarkable in **3b**. The increased general strength also confirms the lack of any other competitive coordination point.

Concerning the *cis-trans* effect of the nitrogen lone pair the observed A_3 values are consistently smaller for C-8 *cis* to it in both compounds.

Interestingly, in quinidine, **4a**, unlike to **3a**, C-11 and the quinuclidine ring carbons show significant broadening (see Table 6). A likely explantation is that since in this molecule the OH group and the tertiary nitrogen's lone pair are *trans* [16] the Cu^{2+} ions can coordinate freely to the nitrogen. In quinine where they are *cis* an intramolecular hydrogen bridge can block the nitrogen's lone electron pair preventing thus the coordination of Cu^{2+} ions. The fact that quinidine could afford with copper sulphate addition compounds but quinine not [17] supports this view.

It also turned out that the original assignment of C-3 and C-7 [18] must be reserved, otherwise the collapse of the 119.8 ppm signal (C-3) and the non-broadening nature of that of at 120.9 ppm (C-7) cannot be explained.

For the H_2SO_4 salt **4b** (recorded in *DMSO-d_6*) the strength of the effect was larger compared with **4a** and, unlike to **3a**, C-11 and the quinuclidine ring carbons show increased broadening in line with the expectations.

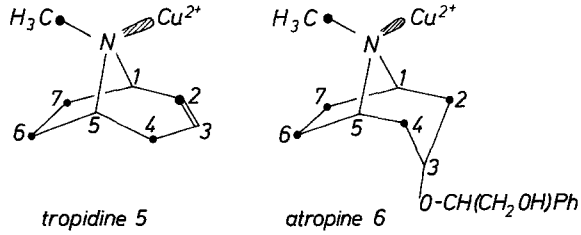
Rigid Non-Planar Molecules

To confirm the existence of a Karplus-type relationship between the actual value of A_3 and the $Cu^{2+}-N-C-C$ dihedral angle we needed molecules of rigid skeleton. In tropidine **5** and atropine **6** according to earlier reports [16] the N-methyl group is *cis* to C-6,7 carbons, i.e. it is orientated equatorially in the piperidine ring. The relative order of line broadening obtained for **5** and **6** are given below (compare Scheme 3),

$$\mathbf{5}\quad A_2(Me) > A_3(2) > A_3(6,7) > A_3(4) > A_2(1,5) \gg A_4(3),$$

$$\mathbf{6}\quad A_2(Me) > A_3(6,7) > A_3(2,4) \gg A_2(1,5) \gg A_n(m).$$

As it can be seen C-6,7 carbons *trans* to the lone pair exhibit stronger broadenings compared with C-2,4 in **6** but the broadening of the sp^2 carbon C-2 in **5** surpasses those of C-6, C-7 or C-4. Interestingly, among the two-bond interactions the $N-CH_3$ carbon shows the strongest broadening in both compounds, at the same time C-1,5, also two bonds away from the Cu^{2+}, do not broaden at all.



tropidine 5 atropine 6 **Scheme 3**

Table 7. ^{13}C line broadenings, $\Delta W_{1/2}$ of carbons 2, 3 and 4 bonds away from Cu^{2+} in Hz obtained for strychnine and brucine[a] at $M/L = 1.26 \cdot 10^{-3}$ and the relevant Cu−N−C−C dihedral angles

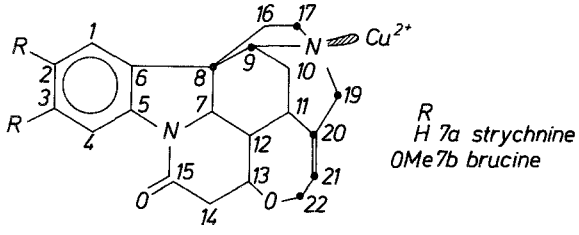
Carbon	Brucine $R = OMe$	Strychnine $R = H$	Cu−N−C−C angle[b] (°)
C-9	50.0	50.0	–
C-17	50.0	50.0	–
C-19	50.0	50.0	–
C-8	50.0	50.0	+125
C-10	2.2	1.7	−120
C-16	0.5	0.6	−120
C-20	50.0	50.0	+160
C-11	0.6	0.0	–
C-21	3.9	2.7	–
C-22	1.2	0.8	–

[a] The assignments were taken from Ref. [10]

[b] Approximate values obtained from Dreiding models (clockwise rotation was taken as positive)

In strychnine **7a** and brucine [19] **7b** (see Scheme 4 and Table 7) the relative order of hyperfine couplings is the following,

$$A_2(17) = A_2(19) = A_3(9) = A_2(8) = A_3(20) > A_4(21) > A_4(10) > A_n(m).$$



Scheme 4

While signals of carbon atoms in one-bond environment of the tertiary nitrogen practically disappear from the spectrum, the broadening of carbons two bonds away from the nitrogen show an interesting dependence on the $Cu^{2+}-N-C-C$ dihedral angle. Signals of carbons with large positive theta values (C-8 and C-20) broaden heavily ($\Delta W_{1/2} > 50$ Hz), whereas C-10 and C-16 (both have a dihedral angle of about −120°) show much weaker broadenings. Although these data confirm the dihedral angle dependance of the three-bond hyperfine coupling constants, further refinement on a larger data base is definitely required.

The unexpected broadening of C-21 and C-22 indicates that other factors such as *W*-mechanism or long range effects can also be operative over three or more bonds.

Flexible Molecules

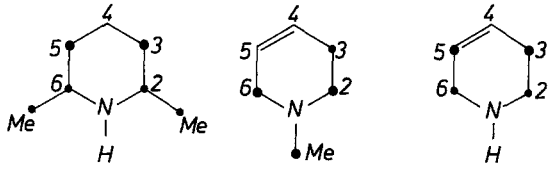
For conformationally mobile molecules the hyperfine coupling constants, metal-nucleus distances and exchange rates can be different for each conformer. We

Table 8. ^{13}C-NMR line broadening values, $\Delta W_{1/2}$ (in Hz) of piperidine and derivatives **8–10** obtained on addition of Cu^{2+} ions ($CDCl_3$)

M/L	C-2	C-3	C-4	C-5	C-6	*Me*
			Piperidine			
$5.8 \cdot 10^{-5}$	2.6	5.4	0.0	5.4	2.6	
$11.7 \cdot 10^{-5}$	4.3	8.3	0.3	8.3	4.3	
			2,6-Dimethyl-piperidine (**8**)			
$3.9 \cdot 10^{-4}$	1.2	1.2	0.3	1.2	1.2	0.0
$13.6 \cdot 10^{-4}$	2.7	4.0	0.6	4.0	2.7	0.9
			1-Methyl-1,2,3,6-tetrahydro-pyridine (**9**)			
$8.9 \cdot 10^{-5}$	0.4	0.3	0.0	0.0	0.0	0.9
$53.0 \cdot 10^{-5}$	2.0	0.6	0.0	0.0	1.4	2.8
			1,2,3,6-Tetrahydro-pyridine (**10**)			
$7.3 \cdot 10^{-5}$	1.4	3.5	0.0	4.1	1.8	
$11.7 \cdot 10^{-5}$	3.8	5.4	0.0	9.7	4.5	

studied these conformational effect on piperidine derivatives **8–10** (see Scheme 5 and Table 8).

In this respect it is interesting to compare data of the aromatic planar pyridine and the saturated flexible piperidine. While for pyridine the broadening of C-3,5



Scheme 5

is by far the greatest (see Table 1), for piperidine it is only two times as large as that of C-2,6. C-4 does not show any increase in neither case. In piperidine which undergoes fast ring and nitrogen inversions [20] in some conformers the lone pair is *cis* to C-3 and C-5. This time-averaged *cis* effect could explain the decrease of A_3 in agreement with our earlier observations. If so, we observe rather the relative decrease of A_3(3,5) that the absolute increase of A_2(2,6)!

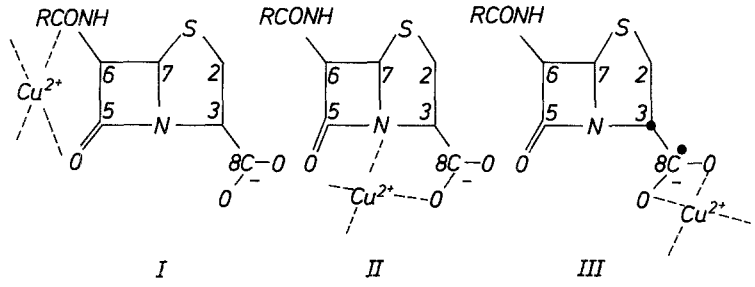
For the conformationally more stable 2,6-dimethyl derivative **8**, compared with piperidine, the general strength of the effect is about ten-times larger but its relative order is the same. A likely explanation can be that in the favoured chair conformation the nitrogen lone pair occupies an equatorial position. The much smaller broadening of the equatorial methyl groups relative to that of C-3,5 indicates a *cis* relation between the methyls and the nitrogen lone pair.

In the asymmetric tetrahydro derivative **10** the broadening pattern is similar to that of piperidine, however, the opposite trend is observed for the methyl derivative **9** [$A_2(2) > A_2(6) > A_3(3) > A_3(5)$] with a simultaneous drop of general strength by a factor of five. While the latter can be explained with the decreased basicity of the tertiary nitrogen [15], the problem of different broadening patterns is also thought to be related with the nitrogen's lone pair orientation.

In **10** a fast nitrogen inversion could explain the observed similarity to **8**, in **9** the spatial vicinity of the presumably axial lone pair to C-3 and C-5 can be credited for the decreased broadening of C-3,5.

The obtained correlations can be used in spectral assignments or in the determination of metal binding sites. An illustrative example is given below.

Originally Fazakerly and Jackson reported on the ^{1}H NMR spectra of the Cu^{2+}-benzylpenicillin complex [21]. Of the proposed structures (see Scheme 6) **II** was confirmed on the basis of the observed excessive broadening of H-3 and the non-broadening of the side chain (*R*) methylene protons.



I *II* *III*

R = phenyl

Scheme 6

Our ^{13}C NMR results do not support this view. On addition of some Cu^{2+} to the benzylpenicillin sample two signals (at 73.8 and 171.3 ppm) completely disappeared from the spectrum. While the former could easily be assigned to C-3, the assignment of the carbonyl signals is ambiguous (these were also not assigned in the original report [22]). Apart from these two lines, only C-2 showed some broadening while the rest of the lines remained unchanged. On the basis of the proton-coupled spectrum the collapsed carbonyl signal could be assigned to C-8. This suggests structure **III** rather than **II**. In the latter both C-5 and C-7 signals should have collapsed since, like C-8, they are also two bonds away from the Cu^{2+} ion.

It is noteworthy that by increasing the sample temperature (in *DMSO*) the lines of the Cu^{2+} doped benzylpenicillin broadened further. This is just the opposite of what has been observed e.g. for cyclohexylamine. A likely explanation can be the formation of *DMSO* – Cu^{2+} collision complexes that dissociate at higher temperature. Thus, an increasing number of Cu^{2+}-benzylpenicillin complexes can form. In the case of cyclohexylamine the *DMSO* molecule, being a weaker donor than the amine group, cannot compete effectively. The fact that the overall strength of the effect is 5–10 times weaker for benzylpenicillin than for cyclohexylamine supports this view.

Experimental

Cu(2,4-dichloro-benzoate)$_2$ was obtained from the reaction of $CuSO_4$ and Na-benzoate. An 5 mg/ml stock solution was prepared in $CDCl_3$. Compounds used as ligands were commercially available Merck, Fluka and Reanal products.

The estimated error of line broadening determination was about 0.5 Hz. The recording conditions were as follows: ^{13}C frequency, 20.1 MHz; instrument, Varian CFT-20; mode, FT; lock, ^2H (internal); temperature range, 303–400 K; tube size, 10 mm o.d.; solvent and concentration, 200–250 mg for 1 ml of $CDCl_3$ or *DMSO-d_6*; standard, internal *TMS*; pulse conditions, BB-decoupling (DF = 3 200 Hz, PW = 8 μs, flip angle = 42°); acquisition time, 1.024 s; spectral width 4 000 Hz, scans, ca. 3–4 K; accuracy, J = 1 Hz, 0.05 ppm.

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