Comparative conformational and dynamical study of some *N*-quaternarized UV filters: structure–activity relationships

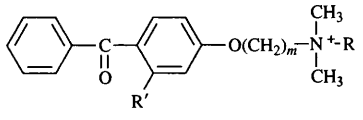
Cecilia Anselmi, Marisanna Centini, Marco Francioli and Alessandro Sega *

Istituto di Chimica Organica, Università di Siena, Piano dei Mantellini 44, 53100 Siena, Italy

A comparative conformational and dynamical study of a series of structurally related molecules with UV-filtering properties, *i.e.* *N*,*N*-dimethyl-*N*-[2-(4-benzoyl-2-hydroxyphenoxy)ethyl]-*N*-dodecyl ammonium bromide 2, *N*,*N*-dimethyl-*N*-[6-(4-benzoylphenoxy)hexyl]-*N*-dodecyl ammonium bromide 3 and *N*,*N*-dimethyl-*N*-[3-(salicyloylamino)propyl]-*N*-dodecyl ammonium bromide 5, has been performed in $CDCl_3$ and [$^2H_6$]DMSO solutions. The main conformers of 2 and 3 (extensively folded conformations) and 5 ('linear' conformations) were determined by means of selective spin-lattice proton relaxation rates, carbon spin-lattice relaxation rates and 1D nuclear Overhauser effects. On the basis of these results a possible correlation between conformation and antibacterial activity is discussed.

The synthesis of molecules with sunscreen properties has become an important field of research.[1,2] Particular interest has been shown towards substantivity,[3] the capacity of these molecules to adhere to and be retained by the keratin of the skin. A product with good substantivity resists removal by bathing and perspiration. However, substantivity must be accompained by high protective efficacy and very low toxicity.

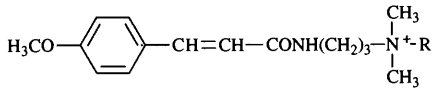
For some years, we have been concerned with the synthesis of new molecules with sunscreen properties.[4–8] We have synthesized a series of *N*-quaternarized benzophenone derivatives in which the n-C_{12} derivatives **1**–**3** have the highest



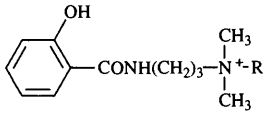
R′ = H, OH
R = n-C_{12}.n-C_{14}, n-C_{16}, n-C_{18};
m = 2,3,6

1 : R′ = H; R = n-C_{12}; *m* = 3
2 : R′ = OH; R = n-C_{12}; *m* = 2
3 : R′ = H, R = n-C_{12}; *m* = 6

substantivity, but this has always been associated with the highest antibacterial activity.[9] The same associations were found in the *N*-quaternarized derivatives of cinnamic and salicylic acids: the n-C_{12} derivatives **4** and **5** showed the highest antibacterial activity and substantivity.[10]



R = n-C_2, n-C_3, n-C_{12}, n-C_{16} **4** : n-C_{12}



R = n-C_2, n-C_8, n-C_{12}, n-C_{16} **5** : n-C_{12}

A possible key to this finding is offered by the rationale introduced by Garcia Dominguez *et al.*[11] to explain the 'odd' behaviour of cationic and anionic n-C_{12} detergents in their interaction with keratinic proteins.[11] It is well known that these n-C_{12} detergents are the most efficient agents for detergent–adsorption–denaturation–permeability–irritation interactions. The mechanism proposed by these authors is based on the fact that the n-C_{12} chain may adopt a coiled conformation: in this way the surface-to-volume ratio of the molecule is reduced to a minimum. This is presumably of great importance when the absorbed molecules migrate inside the protein structure. Significant homologies appear to exist between this mechanism and that of the antibacterial activity of quaternary cationic detergents proposed by other authors.[12] It therefore seems that the substantivity of an *N*-quaternarized sunscreen for a keratinic substrate cannot be unrelated to its antibacterial action and consequent damage to the cell membrane and cytoplasm and skin irritation phenomena.

However the n-C_{12} benzophenone derivatives (compounds **1**, **2** and **3**) show lower antibacterial activity than the corresponding n-C_{12} derivatives of cinnamic and salicylic acids (compounds **4** and **5**). Moreover within the n-C_{12} benzophenone derivatives, compounds **3** and especially **2** are somewhat less active than **1**. These experimental results suggest that appropriate modification of molecular structure may minimize antibacterial activity while retaining the high degree of substantivity.

In previous studies we demonstrated that the conformation and dynamics of compounds **1**[13] and **4**[14] differ significantly; the conformation of compound **1** is also strongly affected by the solvent ($CDCl_3$ or [$^2H_6$]DMSO). This study investigated compounds **2**, **3** and **5** in $CDCl_3$ and [$^2H_6$]DMSO solutions with the aim of enabling complete comparison of the main conformers of these molecules. We have chosen two solvents that differ widely in their properties since the environment in which the molecule–keratinic protein interaction takes place plays a fundamental role in the conformation of the molecule.

Results and discussion

^1H and ^{13}C NMR assignments were made on the basis of decoupling and 2D (COSY and HETCOR) NMR experiments. The numbering system of compounds **2**, **3** and **5** is shown in Fig. 1. ^1H NMR chemical shifts of compounds **2**, **3** and **5** are shown in Table 1; ^{13}C NMR chemical shifts and spin-lattice relaxation rates (R_1) are shown in Tables 2, 3 and 4, respectively.

It is known that ^{13}C NMR relaxation rates ($R_1 = 1/T$) are almost exclusively determined by dipolar interactions with directly bonded or nearby protons, thus allowing suitable determination of the molecular dynamics.[15,16]

The motional features of aromatic carbons were considered first since they have fewer degrees of freedom than side-chain carbons.

Tables 2, 3 and 4 clearly show that the C-1 carbons have the

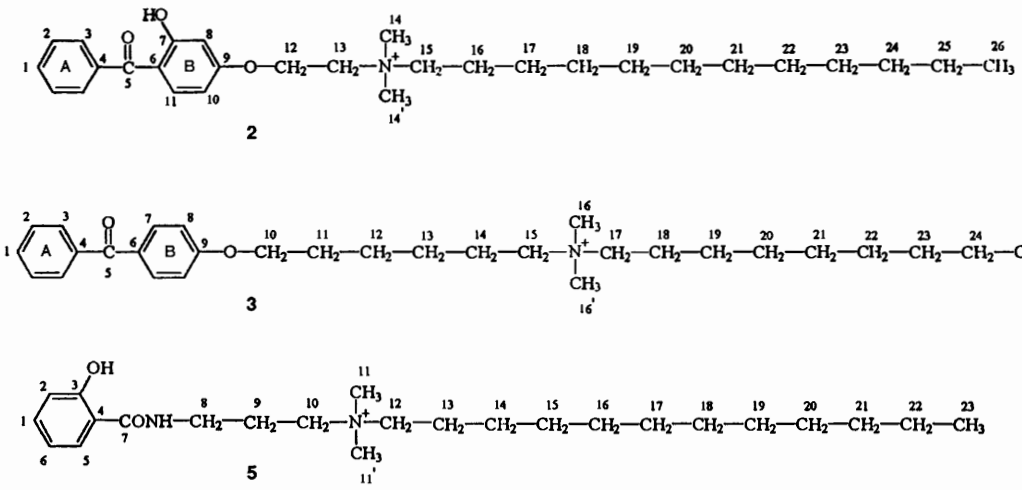


Fig. 1 Numbering system of compounds **2**, **3** and **5** used throughout the text

Table 1 ^{1}H NMR chemical shifts[a] for compounds **2**, **3** and **5** (0.1 mol dm^{-3} in $CDCl_3$ and in [$^2H_6$]DMSO, T = 298 K)

	δ							
	2			**3**			**5**	
	$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO
OH	12.53	11.89	H-7	7.80	7.73	OH	12.47	12.35
H-1	7.43–7.65	7.54–7.76	H-3	7.74	7.66	NH	8.85	8.98
H-2			H-1	7.53	7.62	H-5	8.13	7.89
H-3			H-2	7.46	7.51	H-1	7.30	7.38
H-11		7.45	H-8	6.95	7.07	H-2	6.93	6.90
H-8	6.57	6.64	H-10	4.04	4.08	H-6	6.90	6.87
H-10	6.46	6.57	H-15	3.62	3.30	H-10	3.73	3.2–3.4
H-12	4.65	4.55	H-17	3.52	3.25	H-8	3.60	
H-13	4.32	3.81	H-16,16′	3.41	3.01	H-12	3.28	
H-15	3.66	3.38	H-11	1.84	1.84	H-11,11′	3.21	3.01
H-14,14′	3.54	3.14	H-14,18	1.75	1.60–1.90	H-9	2.21	1.95
H-16	1.83	1.68	H-12,13	1.53	1.30–1.60	H-13	1.65	1.60
H-17–H-25	1.24	1.22	H-10–H-27	1.25	1.22	H-14–H-22	1.26	1.21
H-26	0.87	0.83	H-28	0.87	0.83	H-23	0.86	0.83

[a] Measured downfield from internal TMS.

fastest relaxation rates of the carbons of the aromatic ring (ring A for compounds **2** and **3**) suggesting that the C-1–C-4 axis is the main rotation axis in these moieties. On the other hand, C-2 and C-3 (C-2, C-5 and C-6 for compound **5**) have very similar relaxation rates, which makes it possible to use an anisotropic model based on rotational reorientation around the main rotation axis with some degree of internal motion[17] [eqn. (1)

$$\frac{1}{nT_1} = \frac{\hbar^2\gamma_H^2\gamma_C^2}{r_{CH}^6}\tau_c A \times \left\{A + B\frac{6\tau_g A}{6\tau_g A + \tau_c A} + C\frac{3\tau_g A}{3\tau_g A + 2\tau_c A}\right\} \quad (1)$$

with $A = 0.25(3\cos^2\alpha - 1)^2$, $B = 3(\sin^2\alpha\cos^2\alpha)$ and $C = 0.75(\sin^4\alpha)$] where $\tau_c A$ is the main rotation correlation time, $\tau_g A$ the correlation time for vibrational motions of the aromatic ring A, r_{CH} the length of the C–H bond, n the number of protons attached to the carbon under consideration and α the angle between the main rotation axis and the C–H vector. The main correlation time, $\tau_c A$, can be calculated by considering the relaxation rate R_1 of C-1: since the C-1–H-1 vector lies on the main axis eqn. (2) can be applied. This holds for a pure dipole–dipole relaxation mechanism in the extreme narrowing region.[18] As all protonated carbons exhibit maximum ^{13}C–{^1H} nuclear Overhauser effects (NOEs), eqn. (2) was used to

$$\frac{1}{nT_1} = \frac{\hbar^2\gamma_H^2\gamma_C^2}{r_{CH}^6}\tau_c \quad (2)$$

obtain $\tau_c A$, and $\tau_g A$ was then obtained by applying eqn. (1) (see Table 5).

The same analysis holds for ring B of compounds **2** and **3**: for **2** the relaxation rates of C-8, C-10 and C-11 are very similar and the main rotation axis is C-6–C-9 as would be expected for *para*-disubstituted benzene (the hydroxy group bonded to C-7 has little or no role in determining the main axis of rotation); for compound **3** the relaxation rates of C-7 and C-8 confirm that C-6–C-9 is the main rotational axis. Thus the same anisotropic model can be applied.

However, it is impossible to calculate $\tau_c B$, the main correlation time of ring B, from the relaxation rates of carbon atoms. This problem can be solved by using proton relaxation rates; indeed the H-7–H-8 (H-10–H-11 for compound **2**) vector is parallel to the main axis C-6–C-9 and by using double selective and selective relaxation rates we can write eqns. (3) and (4) (see below for a discussion of proton relaxation

$$R_{10,11}^{10} - R_s^{10} = \sigma_{10,11} = \frac{1}{2}\frac{\hbar^2\gamma_H^4}{r_{10,11}^6}\tau_c B \quad (3)$$

$$R_{7,8}^{7} - R_s^{7} = \sigma_{7,8} = \frac{1}{2}\frac{\hbar^2\gamma_H^4}{r_{7,8}^6}\tau_c B \quad (4)$$

Table 2 ^{13}C NMR chemical shifts[a] and spin-lattice relaxation rates[b] for compound **2** (0.1 mol dm^{-3} in $CDCl_3$ and [$^2H_6$]DMSO, T = 298 K)

	$CDCl_3$			[$^2H_6$]DMSO	
	δ	R_1/s^{-1}		δ	R_1/s^{-1}
C-5	200.05	0.10 ± 0.01	C-5	198.45	0.15 ± 0.01
C-9	165.82	0.26 ± 0.01	C-9	163.24	0.27 ± 0.02
C-7	163.12	0.24 ± 0.01	C-7	163.07	0.23 ± 0.02
C-4	137.80	0.11 ± 0.01	C-4	137.67	0.12 ± 0.01
C-11	135.68	2.47 ± 0.06	C-11	134.30	3.34 ± 0.09
C-1	131.72	2.32 ± 0.06	C-1	131.93	2.79 ± 0.06
C-3	128.81	0.93 ± 0.04	C-3	128.68	1.55 ± 0.07
C-2	128.31	0.90 ± 0.04	C-2	128.36	1.61 ± 0.05
C-6	114.19	0.14 ± 0.01	C-6	114.75	0.16 ± 0.01
C-10	106.59	2.49 ± 0.08	C-10	107.21	3.41 ± 0.09
C-8	102.49	2.56 ± 0.06	C-8	102.15	3.22 ± 0.08
C-15	66.08	4.57 ± 0.18	C-15	63.93	5.18 ± 0.18
C-12	62.65	4.17 ± 0.20	C-12	61.86	5.49 ± 0.20
C-13	62.34	6.53 ± 0.24	C-13	61.56	6.62 ± 0.22
C-14,14′	52.07	4.50 ± 0.21	C-14,14′	50.75	5.29 ± 0.20
C-24	31.79	0.33 ± 0.02	C-24	31.20	0.57 ± 0.02
C-21,22	29.48	0.74 ± 0.03	C-21,22	28.93	1.26 ± 0.04
C-20	29.38	0.99 ± 0.03	C-20	28.87	1.62 ± 0.05
C-19	29.32	1.24 ± 0.07	C-19	28.74	2.03 ± 0.07
C-23	29.21	0.57 ± 0.02	C-23	28.61	0.91 ± 0.03
C-18	29.17	1.51 ± 0.06	C-18	28.46	2.34 ± 0.07
C-17	26.22	2.85 ± 0.10	C-17	25.72	3.30 ± 0.08
C-16	22.91	4.33 ± 0.19	C-16	22.00	5.46 ± 0.21
C-25	22.57	0.27 ± 0.01	C-25	21.80	0.45 ± 0.02
C-26	14.01	0.16 ± 0.01	C-26	13.84	0.26 ± 0.01

[a] Measured downfield from internal TMS. [b] (±) Figures denote approximate 95% confidence limits of the exponential regression analysis.

Table 3 ^{13}C NMR chemical shifts[a] and spin-lattice relaxation rates[b] for compound **3** (0.1 mol dm^{-3} in $CDCl_3$ and [$^2H_6$]DMSO, T = 298 K)

	$CDCl_3$			[$^2H_6$]DMSO	
	δ	R_1/s^{-1}		δ	R_1/s^{-1}
C-5	195.43	0.12 ± 0.01	C-5	194.28	0.16 ± 0.01
C-9	162.55	0.22 ± 0.01	C-9	162.32	0.25 ± 0.01
C-4	138.13	0.13 ± 0.01	C-4	137.71	0.14 ± 0.01
C-7	132.40	1.54 ± 0.04	C-7	132.10	2.60 ± 0.08
C-1	131.80	1.38 ± 0.03	C-1	131.98	2.25 ± 0.06
C-6	129.90	0.15 ± 0.01	C-6	129.16	0.17 ± 0.01
C-3	129.55	0.84 ± 0.02	C-3	129.11	1.18 ± 0.03
C-2	128.09	0.80 ± 0.02	C-2	128.35	1.23 ± 0.03
C-8	113.95	1.69 ± 0.05	C-8	114.23	2.58 ± 0.07
C-10	67.68	2.91 ± 0.13	C-10	67.71	3.90 ± 0.08
C-15	63.91	5.65 ± 0.21	C-15,17	62.80	4.20 ± 0.09
C-17	63.70	6.33 ± 0.25	C-16,16′	49.90	4.78 ± 0.11
C-16,16′	51.06	4.72 ± 0.20	C-26	31.20	0.69 ± 0.02
C-26	31.75	0.38 ± 0.01	C-23,24	28.93	1.38 ± 0.04
C-23,24	29.44	0.83 ± 0.02	C-22	28.85	1.81 ± 0.04
C-22	29.32	1.23 ± 0.04	C-21	28.73	2.05 ± 0.06
C-21	29.26	1.44 ± 0.04	C-25	28.62	1.00 ± 0.03
C-25	29.17	0.64 ± 0.02	C-19,20	28.40	2.47 ± 0.08
C-20	29.10	1.89 ± 0.06	C-12	28.17	3.69 ± 0.11
C-12	28.71	2.90 ± 0.08	C-13	25.71	3.57 ± 0.12
C-19	26.17	3.02 ± 0.08	C-14	25.41	4.33 ± 0.14
C-13	25.87	3.79 ± 0.13	C-11	24.90	3.42 ± 0.10
C-11	25.57	2.75 ± 0.09	C-27	22.00	0.45 ± 0.02
C-18	22.71	4.72 ± 0.18	C-18	21.62	3.98 ± 0.08
C-14	22.64	4.85 ± 0.16	C-28	13.85	0.33 ± 0.01
C-27	22.53	0.31 ± 0.01			
C-28	13.98	0.23 ± 0.01			

[a] Measured downfield from internal TMS. [b] (±) Figures denote approximate 95% confidence limits of the expotential regression analysis.

rates for compounds **2** and **3**, respectively, where $r_{10,11}$ and $r_{7,8}$ are the H-10–H-11 and H-7–H-8 interproton distances in compounds **2** and **3**, respectively. This distance was determined from neutron scattering data:[19] r = 2.45 Å. Hence we have

Table 4 ^{13}C NMR chemical shifts[a] and spin lattice relaxation rates[b] for compound **5** (0.1 mol dm^{-3} in $CDCl_3$ and [$^2H_6$]DMSO, T = 298 K)

	$CDCl_3$			[$^2H_6$]DMSO	
	δ	R_1/s^{-1}		δ	R_1/s^{-1}
C-7	170.93	0.31 ± 0.01	C-7	169.24	0.14 ± 0.01
C-3	161.23	0.30 ± 0.01	C-3	160.01	0.20 ± 0.01
C-1	134.11	3.72 ± 0.09	C-1	133.67	3.45 ± 0.10
C-5	127.89	2.70 ± 0.07	C-5	127.77	2.26 ± 0.07
C-2	118.96	2.94 ± 0.07	C-6	118.43	2.06 ± 0.05
C-6	117.82	2.70 ± 0.06	C-2	117.27	2.24 ± 0.06
C-4	114.19	0.19 ± 0.01	C-4	115.01	0.14 ± 0.01
C-12	64.66	4.95 ± 0.11	C-12	62.81	5.99 ± 0.14
C-10	62.55	6.85 ± 0.15	C-10	60.75	5.40 ± 0.14
C-11,11′	51.17	4.74 ± 0.12	C-11,11′	50.07	4.67 ± 0.13
C-8	36.27	7.41 ± 0.21	C-8	35.92	5.71 ± 0.16
C-21	31.85	0.44 ± 0.02	C-21	31.19	0.61 ± 0.02
C-17,18	29.53	0.82 ± 0.03	C-17,18,19	28.90	1.34 ± 0.04
C-16	29.39	1.33 ± 0.04	C-16	28.71	1.83 ± 0.06
C-15	29.32	1.65 ± 0.05	C-20	28.64	0.94 ± 0.03
C-19,20	29.26	0.66 ± 0.03	C-15	28.39	2.39 ± 0.08
C-14	29.08	1.89 ± 0.06	C-14	25.68	3.31 ± 0.09
C-13	26.20	3.26 ± 0.10	C-13	22.21	5.13 ± 0.14
C-9	22.72	4.69 ± 0.13	C-22	21.99	0.41 ± 0.02
C-22	22.64	0.42 ± 0.02	C-9	21.59	5.10 ± 0.13
C-23	14.08	0.31 ± 0.01	C-23	13.84	0.30 ± 0.02

[a] Measured downfield from internal TMS. [b] (±) Figures denote approximate 95% confidence limits of the exponential regression analysis.

$\tau_cB = 1.96 \times 10^{-10}$ and $\tau_cB = 1.28 \times 10^{-10}$ s for compounds **2** and **3**, respectively, and $\tau_gB(\mathbf{2}) = 0.76 \times 10^{-10}$ and $\tau_gB(\mathbf{3}) = 0.47 \times 10^{-10}$ s from eqn. (1). The correlation times of the carbon atoms of the alkyl chains were obtained from eqn. (2) and the complete results are reported in Table 5.

Analysis of the correlation times of compound **2** presents similar dynamics in both solvents; the aromatic rings A and B have very different main correlation times (relative conformational freedom within the benzophenone moiety), the correlation times of the methylene carbons from C-12 to C-16 are very close to τ_cA [with the exception of τ_c(13)] and the motions of the carbons from C-17 to C-26 are clearly dominated by a segmental motion (correlation times steadily decrease from C-17 onward). The main difference in the dynamical behaviour of **2** in the two solutions is the slower overall motion in [$^2H_6$]DMSO and the very restricted internal motions within ring B in [$^2H_6$]DMSO [τ_c(B) and τ_g(B) are 2.00×10^{-10} and 1.82×10^{-10} s, respectively].

The τ_c data of compound **3** in Table 5 shows that the same situation found for compound **2** also applies to the benzophenone moiety: τ_cA and τ_cB are different and the motions of the two rings are disengaged (and in [$^2H_6$]DMSO, τ_cB and τ_gB are very similar). However the dynamics of the alkyl chain in $CDCl_3$ and [$^2H_6$]DMSO is more complex. In $CDCl_3$ the methylene carbon atoms C-10, C-11 and C-12 have correlation times aligned with τ_cA, but from C-13 to C-17 the corresponding τ_c steadily increase (indeed C-15 and C-17, the methylene carbon atoms bonded to the nitrogen atom, have the highest τ_c values in the molecule). From C-18 to C-28 segmental motion takes place, but C-18 and C-19 still have correlation times between τ_cA and τ_cB. In [$^2H_6$]DMSO the carbon atoms from C-10 to C-18 have correlation times aligned with τ_cA while segmental motion rules the dynamics of carbon atoms from C-19 onward.

Thus the most striking dynamical feature of compounds **2** and **3** is the tendency for a segment of the alkyl chain to move accordingly with the corresponding main correlation times of ring A, τ_cA, a trend that is most accentuated in compound **3** in [$^2H_6$]DMSO. As a consequence, in a large part of these molecules, internal motion is restricted and only the last segments of the n-alkyl chains have segmental motion.

Table 5 Correlation times ($\times 10^{-10}$ s) of carbon atoms of compounds **2**, **3** and **5** (0.1 mol dm^{-3} in $CDCl_3$ and [$^2H_6$]DMSO, T = 298 K)

	2			**3**			**5**	
	$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO
τ_cA	1.04	1.23	τ_cA	0.61	0.99	τ_cA	1.64	1.52
τ_gA	0.31	0.48	τ_gA	0.27	0.15	τ_gA	1.59	0.81
τ_cB	1.96	2.00	τ_cB	1.28	1.54	τ_c8	1.72	1.33
τ_gB	0.76	1.82	τ_gB	0.47	1.45	τ_c9	1.09	1.19
τ_c12	0.97	1.28	τ_c10	0.68	0.91	τ_c10	1.59	1.26
τ_c13	1.52	1.54	τ_c11	0.64	0.80	$\tau_c11,11'$	0.73	0.72
$\tau_c14,14'$	0.70	0.82	τ_c12	0.67	0.86	τ_c12	1.15	1.39
τ_c15	1.06	1.21	τ_c13	0.88	0.83	τ_c13	0.76	1.19
τ_c16	1.01	1.27	τ_c14	1.13	1.01	τ_c14	0.51	0.77
τ_c17	0.66	0.77	τ_c15	1.31	0.98	τ_c15	0.38	0.56
τ_c18	0.35	0.54	$\tau_c16,16'$	0.73	0.74	τ_c16	0.31	0.42
τ_c19	0.29	0.47	τ_c17	1.47	0.98	τ_c17	0.19	0.31
τ_c20	0.23	0.38	τ_c18	1.10	0.93	τ_c18	0.19	0.31
τ_c21	0.17	0.29	τ_c19	0.70	0.57	τ_c19	0.15	0.31
τ_c22	0.17	0.29	τ_c20	0.44	0.57	τ_c20	0.15	0.22
τ_c23	0.13	0.21	τ_c21	0.33	0.48	τ_c21	0.10	0.14
τ_c24	0.077	0.13	τ_c22	0.29	0.42	τ_c22	0.098	0.096
τ_c25	0.063	0.10	τ_c23	0.19	0.32	τ_c23	0.049	0.047
τ_c26	0.025	0.040	τ_c24	0.19	0.32			
			τ_c25	0.15	0.23			
			τ_c26	0.088	0.16			
			τ_c27	0.071	0.10			
			τ_c28	0.036	0.051			

[a] See the text for discussion.

In compound **5** in $CDCl_3$, internal motion in the aromatic ring is very restricted (as for ring B in **2** and **3** in [$^2H_6$]DMSO); the correlation times of methylene carbons alternate in value: $\tau_c(8)$ = 1.72 and $\tau_c(10)$ = 1.59 are close to τ_cA, but the correlation times of C-9 and C-12 drop to 1.09 and 1.15, respectively; from C-13 onward segmental motion takes place. In [$^2H_6$]DMSO this compound has less restricted internal motions in the aromatic ring, less pronounced alternation in τ_c values for the first methylene carbons and segmental motion from C-13 onward.

The dynamics of compound **5** clearly show a greater motional freedom than **2** and **3**. Information on the conformations of the aromatic moieties of **2**, **3** and **5** were obtained from proton relaxation rates. Non-selective (R^i_{ns}), mono-selective (R^i_s) and double-selective (R^{in}_{ds}) proton spin-lattice relaxation rates of selected protons of these compounds are shown in Table 6.

The measurment of ^1H non-selective, mono-selective and double-selective spin-lattice relaxation rates is a useful aid for conformational studies in solution.[20-24] The ratio (R^i_{ns}/R^i_s) for any proton i reflects the extent to which pairwise dipole–dipole interactions account for the relaxation mechanism (the ratio is 1.5 for a purely dipolar mechanism). For a 100% contribution from ^1H–^1H dipole–dipole relaxation mechanism, eqns. (5)–(7) can be derived.

$$R^i_{ns} = \sum_{i \neq j} \rho_{ij} + \sum_{i \neq j} \sigma_{ij} \quad (5)$$

$$R^i_s = \sum_{i \neq j} \rho_{ij} \quad (6)$$

$$R^{in}_{ds} = \sum_{i \neq j} \rho_{ij} + \sigma_{in} \quad (7)$$

According to theory[23,24] and within the limits of the extreme narrowing region, eqn. (8) can be derived where σ_{in} is the cross-relaxation rate for any proton pair,[25] r_{in} is the interproton distance and τ_c is the motional correlation time.

$$\sigma_{in} = \frac{1}{2} \frac{\hbar^2 \gamma_H^4}{r_{in}^6} \tau_c \quad (8)$$

Thus proton–proton distances can be obtained from double-selective and mono-selective proton spin-lattice relaxation rates if τ_c is known from other sources [or *vice versa* as with eqns. (3) and (4)].

Proton relaxation data were fundamental for defining dynamics in the benzophenone moieties of compounds **2** and **3**, whereas for compound **5**, it enabled conformation within the salicylamide moiety to be determined (see Fig. 2).

$$R^5_{5,NH} - R^5_s = \sigma_{5,NH} \quad (9)$$

Substituting $\tau_cA = 1.52 \times 10^{-10}$ or $\tau_cA = 1.64 \times 10^{-10}$ s for [$^2H_6$]DMSO and $CDCl_3$ solutions, respectively, eqn. (9) gives an estimate of the distance $r_{5,NH}$ = 2.2 ± 0.1 Å in both solvents. The angle θ between the aromatic ring and the plane of the amide unit was *ca.* 40°.

We searched the literature for molecules containing the salicylamide moiety to compare our results with published data. These molecules can be divided into two groups. In one group the phenyl ring and the amide plane are almost coplanar with θ varing from[26] 0.1 to 8.2° or θ varing from[27] 174.0 to 178.3° (in this case the hydrogen bond is between the hydroxylic oxygen and the NH proton). For the second group the angle between the two planes is sensibly higher:[28] θ = 31.9° or θ varies from[29] 137.3 to 154.3°.

For one structure[30] we found θ at an intermediate value of 15.3°. From this data it is evident that (*i*) coplanar structures outnumber (14 to 6) the other structures and (*ii*) only one structure[28] presents a conformation close to that found by us.

The geometry of the salicylamide moiety is thus consistent with hydrogen bonding between the hydroxy proton and the carbonyl oxygen; this is confirmed by OH chemical shifts of 12.47 and 12.48 ppm in 0.1 mol dm^{-3} $CDCl_3$ and [$^2H_6$]DMSO solutions, respectively, without appreciable variations on dilution to 0.01 mol dm^{-3} solutions in both solvents ($\Delta\delta \approx 0.05$). It is interesting that we can also calculate the main correlation time of the aromatic ring of compound **5** from proton relaxation rates [eqns. (10) and (11)]. From these

Table 6 Non-selective (R_{ns}/s^{-1}), mono-selective (R_s/s^{-1}) and double-selective (R_{ds}/s^{-1}) spin-lattice relaxation rates[a] for selected protons of compounds **2**, **3** and **5** (0.1 mol dm^{-3} $CDCl_3$ and [$^2H_6$]DMSO solutions)

	2			**3**			**5**	
	$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO		$CDCl_3$	[$^2H_6$]DMSO
R_{ns}^{8}	0.70	1.67	R_{ns}^{7}	0.71	0.95	R_{ns}^{NH}	5.55	3.74
R_{ns}^{10}	0.60	1.67	R_{ns}^{8}	1.16	1.38	R_{ns}^{5}	2.67	2.13
R_{ns}^{11}	–	1.23	R_{s}^{7}	0.54	0.71	R_{s}^{NH}	4.00	2.78
R_{s}^{8}	0.92	1.25	R_{s}^{8}	0.84	1.03	R_{s}^{5}	2.04	1.49
R_{s}^{10}	0.79	1.25	$R_{7,8}^{7}$	0.70	0.91	$R_{NH,5}^{5}$	2.44	1.92
R_{s}^{11}	–	0.92	$R_{7,8}^{8}$	1.00	1.23	R_{ns}^{NH}/R_{s}^{NH}	1.39	1.35
$R_{10,11}^{10}$	–	1.51	R_{ns}^{7}/R_{s}^{7}	1.32	1.33	R_{ns}^{5}/R_{s}^{5}	1.31	1.42
$R_{10,11}^{11}$	–	1.19	R_{ns}^{8}/R_{s}^{8}	1.39	1.35			
R_{ns}^{8}/R_{s}^{8}	1.31	1.34						
R_{ns}^{10}/R_{s}^{10}	1.32	1.34						
R_{ns}^{11}/R_{s}^{11}	–	1.34						

[a] Errors were evaluated at ±2% or less (all experiments were performed three times).

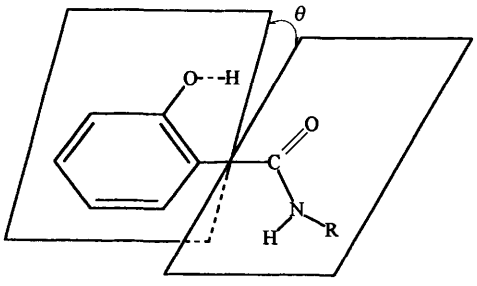


Fig. 2 Conformation of the salicylic amide moiety

$$R_{ns}^{5} - R_{s}^{5} = \sigma_{5,6} + \sigma_{5,NH} \quad (10)$$

$$\sigma_{5,6} = (R_{ns}^{5} - R_{s}^{5}) - (R_{5,NH}^{5} - R_{s}^{5}) \quad (11)$$

equations and eqn. (9) we obtain $\tau_c A = 1.73 \times 10^{-10}$ and 1.56×10^{-10} s for $CDCl_3$ and [$^2H_6$]DMSO solutions, respectively: as can be seen in Table 5 there is very good agreement with the corresponding $\tau_c A$ derived from ^{13}C relaxation rates analysis. Information on the conformation of the benzophenone moieties cannot be obtained because of the significant differences in correlation times $\tau_c A$ and $\tau_c B$ of the aromatic rings.

To investigate the possible main conformation(s) of the alkyl chains we made use of the homonuclear 1D Overhauser effect. The NOE analysis was performed in 0.1 and 0.01 mol dm^{-3} solutions of both $CDCl_3$ and [$^2H_6$]DMSO (see Table 7). For compound **5** the only significant NOE effect was between NH and H-5 protons (see Figs. 3 and 4) in 0.1 and 0.01 mol dm^{-3} solutions [$f_{H\text{-}5}$(NH) = 19.5 and 13.5% in 0.01 and 0.1 mol dm^{-3} $CDCl_3$ solutions, respectively, and $f_{H\text{-}5}$(NH) = 16.5 and 18.0% in 0.01 and 0.1 mol dm^{-3} [$^2H_6$]DMSO solutions, respectively]. These results are in line with the conformation of the salicylamide moiety obtained from proton relaxation analysis. Moreover on irradiation of protons H-2 and H-6 (selective presaturation was not possible) we observed an NOE on proton H-5 [$f_{H\text{-}5}$(H-6) = 7.3 and 4.8% in 0.01 and 0.1 mol dm^{-3} $CDCl_3$ solutions, respectively, and $f_{H\text{-}5}$(H-6) = 5.7 and 6.5% in 0.01 and 0.1 mol dm^{-3} [$^2H_6$]DMSO solutions, respectively]. Assuming that this NOE effect was due only to H-6 and that cross-saturation terms are negligible, we can write eqn. (12).

$$\frac{f_{H\text{-}5}(H\text{-}6)}{f_{H\text{-}5}(NH)} = \frac{r_{H\text{-}5,NH}^{6}}{r_{H\text{-}5,H\text{-}6}^{6}} \quad (12)$$

This gave a value of $r_{H\text{-}5,NH} = 2.1 \pm 0.1$ Å ($r_{H\text{-}5,H\text{-}6} = 2.45$ Å) and $\theta \approx 35°$ in very good agreement with the same distance and angle evaluated from proton spin-lattice relaxation rates.

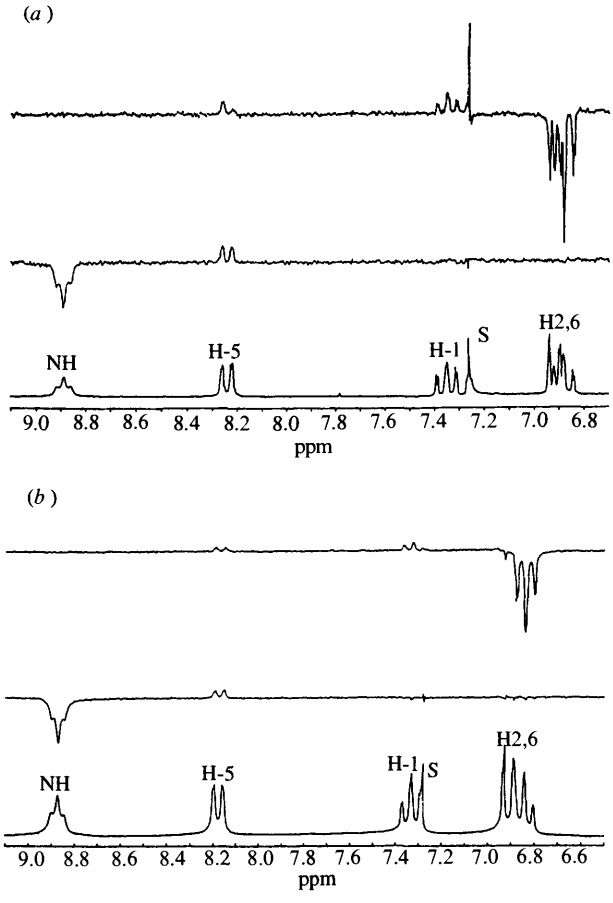


Fig. 3 NOE difference experiments on compound **5** in (*a*) 0.01 mol dm^{-3} and (*b*) 0.1 mol dm^{-3} $CDCl_3$ solutions (S = solvent)

The absence of NOEs between aromatic protons and protons of the alkyl chains coupled with great conformational freedom, deduced from ^{13}C relaxation rates, suggests that the main conformations of **5** in $CDCl_3$ and [$^2H_6$]DMSO are 'linear'. This does not exclude the possibility of folding, but stable main conformation(s) with extensive folding of the alkyl chain towards the aromatic ring can be ruled out.

For compounds **2** and **3** the situation is completely different: NOE experiments showed NOE effects between protons of the n-alkyl chains and aromatic protons of rings A and B. This situation found in 0.1 mol dm^{-3} solutions is confirmed in 0.01 mol dm^{-3} solutions in both solvents (see Table 7).

The main conformations of **2** and **3** in both solvents showed an extensive folding back of the n-alkyl chains towards and along the aromatic moieties.

Table 7 NOEs[a] for selected proton resonances of compounds **2**, **3** and **5** (0.1 and 0.01 mol dm^{-3} in $CDCl_3$ and in $[^2H_6]$DMSO, T = 298 K)

			Enhancements (%)			
			$CDCl_3$		$[^2H_6]$DMSO	
Compound	Proton(s) irradiated	Proton(s) observed	0.1 mol dm^{-3}	0.01 mol dm^{-3}	0.1 mol dm^{-3}	0.01 mol dm^{-3}
2	H-17–25	H-8	4.3	4.6	5.4	5.0
	H-17–25	H-10	2.6	2.0	3.1	3.5
	H-17–25	H-11	4.0	4.4	5.3	4.9
	H-17–25	H-1–3	4.6	4.2	5.8	6.0
	H-26	H-1–3	–	–	3.0	3.5
3	H-19–27	H-1,2	6.0	6.3	5.4	5.0
	H-19–27	H-7	4.0	4.4	3.2	3.6
	H-19–27	H-8	6.0	5.4	–	–
	H-28	H-1,2	3.0	2.8	3.2	3.3
5	NH	H-5	13.5	19.5	18.0	16.5
	H-6	H-5	4.8	7.3	6.5	5.7

[a] Errors in the NOEs were evaluated at ± 6% (all experiments were performed three times).

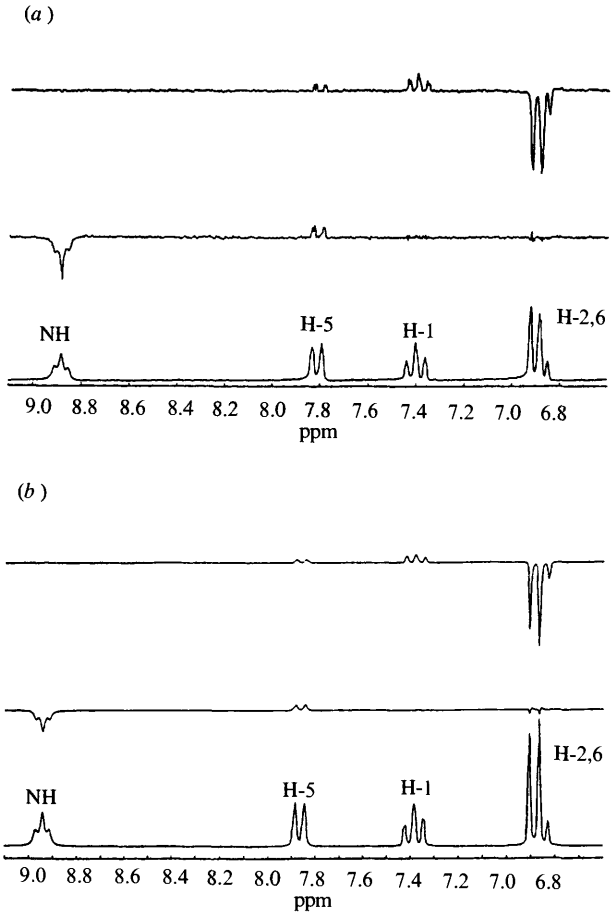


Fig. 4 NOE difference experiments on compound **5** in (*a*) 0.01 mol dm^{-3} and (*b*) 0.1 mol dm^{-3} $[^2H_6]$DMSO solutions

The dynamics of compounds **2** and **3** can now be reconsidered in the light of NOE results: (*i*) a large part of these molecules, involving benzophenone moieties up to C-16 (for **2**) and C-18 (for **3**), is subject to restricted internal motion, a fact that is presumably related to the preferred conformation(s) of **2** and **3**; (*ii*) the methylene carbon atoms of the first part of the alkyl chains have correlation times close to τ_cA and in our opinion it must be stressed that the alignment is with motion around the C-1–C-4 axis and not with τ_cB (C-6–C-9 axis) as could have been expected; (*iii*) one or both of the carbon atoms connected to the quaternary nitrogen atom have the longest τ_c among the methylene carbons and this suggests that torsional angles around the nitrogen are responsible for the folding of the n-alkyl chain. This interpretation is supported by NOE analysis: there are NOE effects only between aromatic protons and protons bonded to carbon atoms undergoing segmental motion. The mean main conformations of compounds **2**, **3** and **5** are drawn in Fig. 5 (together with those of **1** and **4**).

As stated in the introduction, the n-C_{12} derivatives of cinnamamide **4** and salicylamide **5** have the highest antibacterial activity. This activity greatly decreases in the n-C_{12} benzophenone derivative **1** and shows a further decrease in n-C_{12} benzophenone derivatives **2** and **3**. The main conformations of **4** and **5** are 'linear', **1** has a folded conformation in $[^2H_6]$DMSO and a 'linear' conformation in $CDCl_3$, and **2** and **3** have extensively folded conformations in both solvents. The presence of main extensively folded conformations in both environments ($CDCl_3$ and $[^2H_6]$DMSO) is important since **2** and **3** are less active than **1**. These results suggest that there must be a relation between conformation and antibacterial activity: main folded or extensively folded conformers strongly reduce or even inhibit antibacterial activity.

Since all these compounds have the same n-C_{12} alkyl chain the cause of the different conformational behaviour must lie in the nature of the aromatic moiety and/or the alkyl chain linking the ethereal oxygen (or the amide nitrogen for **4** and **5**) to the quaternary nitrogen atom. For compounds **1**, **4** and **5** the main difference lies in the aromatic moieties. The influence exerted by these units must be related to their capacity of acting as anchor groups for the alkyl chain; this capacity presumably depends on their relative masses and possibly their shape (salicylamide and cinnamamides are 'linear' while benzophenone has a bent structure).

In compounds **2** and **3** there are changes with respect to **1** in the benzophenone unit (an OH group bound to C-7 in **2**) and in the length of the alkyl chain linking the ethereal oxygen to the quaternary nitrogen atom (two and six methylene carbons in **2** and **3**, respectively). While the results determined by the changes introduced in **2** can be explained in terms of greater overall rigidity (hydrogen bonding between the hydroxy group and the carbonyl unit and shortening of the alkyl chain), the preference of **3** for extensively folded conformers as a result of lengthening of the alkyl chain cannot be easily rationalized.

We did not find any evidence of coiled conformations, *i.e.* the type of conformation associated with antimicrobial activity, toxicity and irritation of the skin. However, if the mechanism proposed by Garcia Dominguez *et al.*[11] is correct, this conformation is probably adopted on interaction with keratin. Hence for these molecules it is presumably easier (lower energy barrier) to force a 'linear conformation' than a folded or extensively folded one (higher energy barrier) into a coiled conformation.

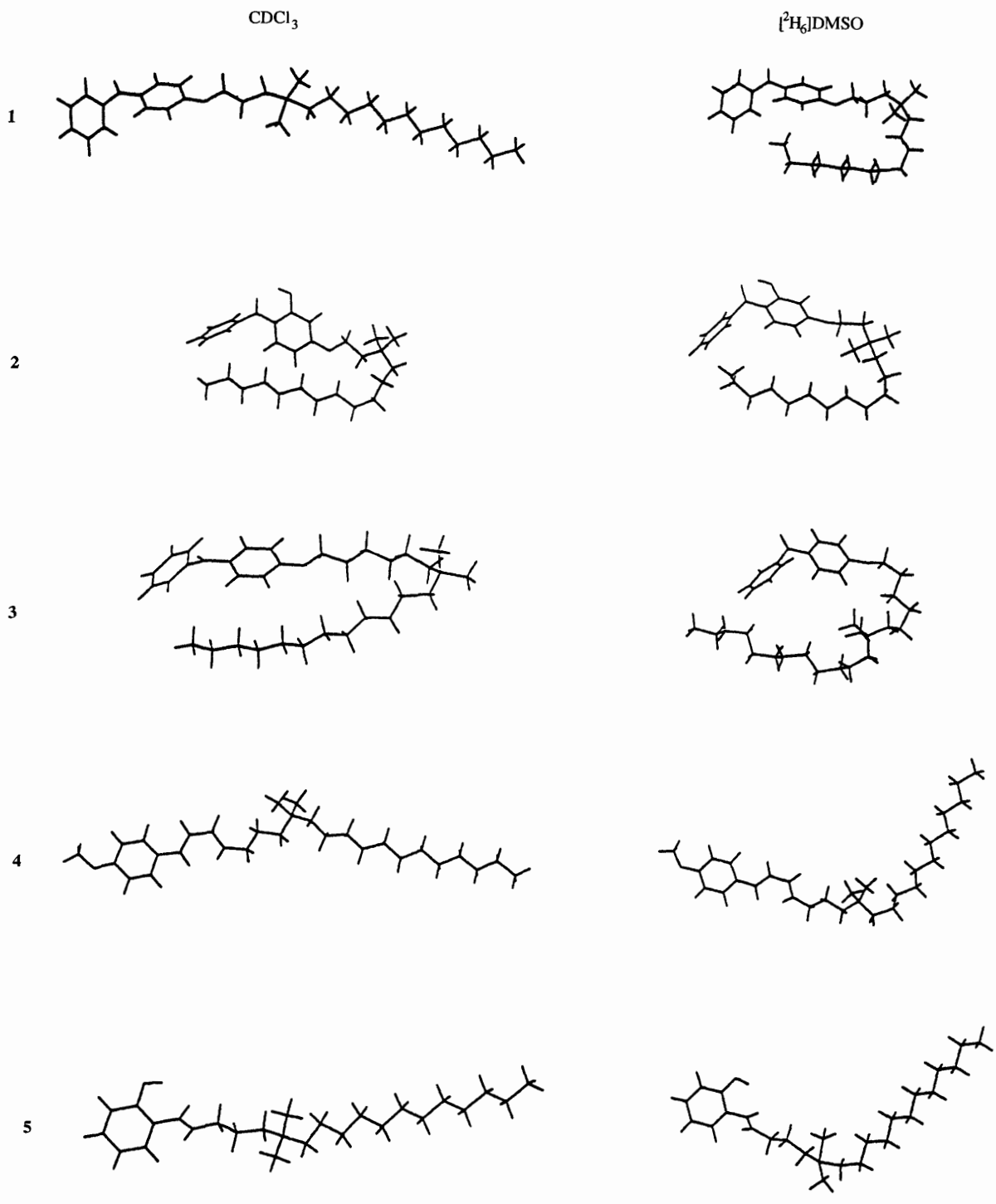


Fig. 5 'Most probable' conformations of compounds **1–5** in $CDCl_3$ and $[^2H_6]$DMSO solutions as inferred from NMR experiments

Experimental

The compounds under investigation were synthesized as previously described.[9] Solutions were made in 99.8% $[^2H_6]$DMSO (Merck) and $CDCl_3$ (Merck) and were carefully deoxygenated. NMR measurements were carried out with a Bruker AC-200 Fourier transform spectrometer. Chemical shifts were referred to internal TMS. Spin-lattice relaxation rates were measured using the inversion-recovery pulse sequence; 32 and 196 FIDs were collected for ^1H and ^{13}C T_1 measurements, respectively. Selective and double-selective relaxation rates were measured using inversion-recovery pulse sequences where the π pulse was given by the proton decoupler at selected frequencies at low power for relatively long times.[25] The selective rate was measured[31] in the initial slope approximation by considering only the first part of the recovery curve; $^{13}C-\{^1H\}$ and $^1H-\{^1H\}$ NOEs were measured with gated decoupling techniques using NOE difference pulse sequences. ^1H Homonuclear (COSY) and $^{13}C-^1H$ heteronuclear (HETCOR) shift-correlated 2D NMR experiments were performed according to standard sequences.[32,33]

Acknowledgements

This research was supported by the National Research Council (CNR Progetto Strategico).

References

1 N. A. Shaath, *Cosmet. Toilet.*, 1987, **102**, 21.
2 C. Fox, *Cosmet. Toilet.*, 1987, **102**, 41.

3 E. D. Goddard, *Cosmet. Toilet.*, 1987, **102**, 71.
4 C. Anselmi, A. Camparini and M. Scotton, *J. Heterocycl. Chem.*, 1983, **20**, 687.
5 M. Checchi, M. F. Saettone, B. Giannaccini, B. Pach and C. Anselmi, *Cosmet. Toilet. Ed. It.*, 1986, 35.
6 G. Adembri, C. Anselmi, A. Camparini, M. F. Saettone and M. Scotton, CNR IP 47509/A 86, 1986.
7 G. Adembri, C. Alderigi, C. Anselmi, B. Giannaccini, M. G. Rossetti, M. F. Saettone and M. Scotton, CNR IP 48245/A 88, 1988.
8 G. Adembri, C. Anselmi, A. Camparini, M. G. Rossetti and M. Scotton, *J. Heterocycl. Chem.*, 1988, **25**, 1019.
9 M. F. Saettone, C. Alderigi, B. Giannaccini, C. Anselmi, M. G. Rossetti, M. Scotton and R. Cerini, *Int. J. Cosmet. Sci.*, 1988, **10**, 99.
10 M. F. Saettone, B. Giannaccini, C. Morganti, A. Persi and C. Cipriani, *Int. J. Cosmet. Sci.*, 1986, **8**, 9.
11 J. Garcia Dominguez, J. L. Parra, M^{A} R. Infante, C. M. Pelejero, F. Balaguer and T. Sastre, *J. Soc. Cosmet. Chem.*, 1977, **28**, 165.
12 D. W. Blois and J. Swarbrick, *J. Pharm. Sci.*, 1972, **61**, 393.
13 C. Anselmi, M. Centini, M. Scotton and A. Sega, *Can. J. Chem.*, 1991, **69**, 913.
14 C. Anselmi, M. Centini, M. Scotton and A. Sega, *Magn. Reson. Chem.*, 1992, **30**, 944.
15 F. W. Wehrli, in *Topics in carbon-13 NMR spectroscopy*, ed. G. C. Levy, Wiley, New York, 1976, vol. 2, p. 343.
16 J. R. Lyerla, Jr. and G. C. Levy in *Topics in carbon-13 NMR spectroscopy*, ed. G. C. Levy, Wiley, New York, 1974, vol. 1, p. 74.
17 A. Allerhand, D. Doddrell and R. Komoroski, *J. Chem. Phys.*, 1971, **55**, 189.
18 F. W. Wehrli and T. Wirthlin, in *Interpretation of carbon-13 NMR spectra*, Heyden, London, 1980.
19 M. N. Frey, T. F. Koetzle, M. S. Lehmann and W. C. Hamilton, *J. Chem. Phys.*, 1973, **58**, 2547.
20 C. W. M. Grant, L. D. Hall and C. M. Preston, *J. Am. Chem. Soc.*, 1973, **95**, 7742.
21 L. D. Hall, *Chem. Soc. Rev.*, 1975, **4**, 401.
22 C. C. Constain and J. M. Dowling, *J. Chem. Phys.*, 1960, **32**, 158; J. C. Brand, D. R. Williams and T. J. Cook, *J. Mol. Spectrosc.*, 1966, **20**, 193; F. A. L. Anet and J. M. Osyany, *J. Am. Chem. Soc.*, 1967, **89**, 352; J. Karle and I. L. Karle, *J. Am. Chem. Soc.*, 1967, **89**, 804.
23 E. Gaggelli and G. Valensin, *Concepts Magn. Reson.*, 1992, **4**, 339.
24 E. Gaggelli and G. Valensin, *Concepts Magn. Reson.*, 1993, **5**, 19.
25 G. Valensin, G. Sabatini and E. Tiezzi, in *Advanced magnetic resonance techniques in systems of high molecular complexity*, eds. N. Niccolai and G. Valensin, Birkauser, Boston, 1986, p. 69.
26 (*a*) A. C. Sindt and M. F. MacKay, *Cryst. Struct. Commun.*, 1980, **9**, 1063; (*b*) A. C. Sindt and M. F. MacKay, *J. Cryst. Mol. Struct.*, 1979, **9**, 265; (*c*) T. M. Garrett, M. E. Cass and K. N. Raymond, *J. Coord. Chem.*, 1992, **25**, 241; (*d*) T. B. Karpishin, T. D. P. Stack and K. N. Raymond, *J. Am. Chem. Soc.*, 1993, **115**, 182; (*e*) H. R. Luss and J. Texter, *Acta Crystallogr., Sect. C*, 1992, **48**, 1269; (*f*) I. A. Krol', V. M. Agre and V. S. Pangani, *Zh. Strukt. Khim.*, 1988, **29**, 194.
27 (*a*) K. Vyas, V. M. Rao and H. Manohar, *Acta Crystallogr., Sect. C*, 1987, **43**, 1201; (*b*) W. J. J. Smeets, J. A. Kanters and K. Venkatasubramanian, *Acta Crystallogr., Sect. C*, 1985, **41**, 272; (*c*) D. Kobelt, E. F. Paulus and W. Kunstmann, *Z. Kristallogr.*, 1974, **139**, 15; (*d*) F. Magirius, A. Linden and H. Heimgartner, *Helv. Chim. Acta*, 1993, **76**, 1980; (*e*) M. W. Bredenkamp, J. L. M. Dillen, P. H. Van Rooyen and P. S. Steyn, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1835; (*f*) P. Domiano, A. Musatti, C. Pelizzi and G. Predieri, *Cryst. Struct. Commun.*, 1974, **3**, 531; (*g*) I. K. Larsen, *Acta Crystallogr., Sect. B*, 1978, **34**, 962.
28 A. C. Sindt and M. F. MacKay, *J. Cryst. Mol. Struct.*, 1978, **8**, 17.
29 (*a*) S. Kashino, T. Matsushita, T. Iwamoto, K. Yamaguchi and M. Haisa, *Acta Crystallogr., Sect. C*, 1986, **42**, 457; (*b*) P. R. Andrews, J. M. Gulbis, M. N. Iskander, M. F. MacKay, C. Di Paola and M. Sadek, *Aust. J. Chem.*, 1988, **41**, 493; (*c*) F. Magirius, A. Linden and H. Heimgartner, *Helv. Chim. Acta*, 1993, **76**, 1980; (*d*) M. W. Bredenkamp, J. L. M. Dillen, P. H. Van Rooyen and P. S. Steyn, *J. Chem. Soc., Perkin Trans. 2*, 1989, 1835; (*e*) M. C. Etter, Z. Urbanczyk-Lipkowska, P. M. Ameli and P. W. Panunto, *J. Cryst. Spectrosc.*, 1988, **18**, 491.
30 A. C. Sindt and M. F. MacKay, *Aust. J. Chem.*, 1980, **33**, 203.
31 R. Freeman, H. D. Hill, B. L. Tomluison and L. D. Hall, *J. Chem. Phys.*, 1974, **61**, 4466.
32 W. P. Ane, E. Barthosdi and R. R. Ernst, *J. Chem. Phys.*, 1976, **64**, 2229.
33 J. A. Wilde and P. H. Bolton, *J. Magn. Reson.*, 1984, **59**, 343.

Paper 5/05277G
Received 7th August 1995
Accepted 7th December 1995