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Stereochemical Study of a Bradicardisant Benzazepine-Type Drug. X-Ray Structure of the Chloride Salt and High-Field NMR Study of the Stereochemistry in Solution

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A chiral bradicardisant benzazepine-type drug with an asymmetric carbon and a tertiary amino nitrogen was studied as the chloride salt. X-ray analysis in the solid state of the salt formed upon protonation of the base shows the occurrence in the unit cell of two diastereomeric species with horseshoe-shaped structures. NMR parameters were obtained for the base and for the protonated species in solution. A conformational study by 2D NOESY and ROESY NMR techniques led to the conclusion that conformations similar to the solid-state structures were retained in solution. Over a wide range of pH, in particular in alkaline solution or under biological conditions, a conformation similar to that of the 1′(*R*) N(*R*) diastereomer is observed. The diastereomeric species 1′(*R*) N(*R*) and 1′(*R*) N(*S*) were clearly identified at low pH values with conformations similar to their solid-state structures.

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INTRODUCTION

The monochloride of 7,8-dimethoxy 3-(3-{[4,5-dimethoxybenzocyclobutan-1-yl)methyl]methylamino} propyl)-1,3,4,5-tetrahydro-2*H*-benzazepin-2-one is a bradicardisant and has possible therapeutic applications in angina pectoris, particularly effort-induced stable angina. A bradicardisant, by lowering the cardiac frequency, diminishes the amount of oxygen needed. This compound has the advantage of being very selective. The molecule possesses an asymmetric centre and pharmacological studies concluded that the salt of the (+)-enantiomer, S 16257-2, is the more biologically active. The structure of the base is shown in Scheme 1 with the numbering adopted for the atoms.

The molecule consists of two bicyclic moieties, each containing an aromatic ring which bears two methoxy groups. The first (part A) is further composed of a seven-membered ring which includes a lactam function. The second (part B) is further composed of a four-membered ring. These two moieties are connected by aliphatic acyclic segments linked to a tertiary amino nitrogen.

This compound is used as a quaternary ammonium chloride salt, and protonation of the amino nitrogen results in a new asymmetric centre. If the deprotonation–inversion–reprotonation process is slow enough, two diastereomers occur.

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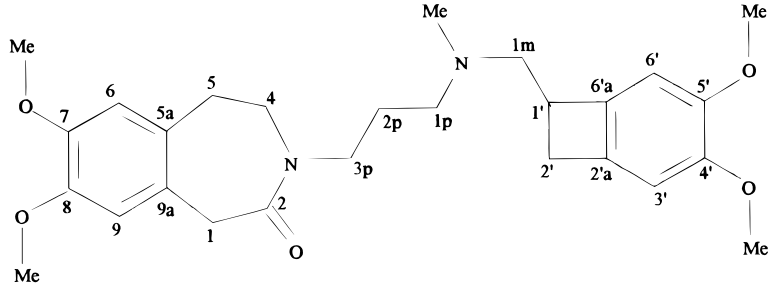

part A part B

Scheme 1

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Crystals of the chloride salt of the (−)-enantiomer, S 16260-2, were submitted to x-ray analysis High-field NMR was used to investigate the solution behaviour.

RESULTS AND DISCUSSION

X-ray analysis

Crystals suitable for x-ray analysis were obtained from the salt of the (−)-enantiomer, S 16260-2. The crystal system is orthorhombic. The asymmetric unit contains two protonated molecules with opposite configurations of the charged nitrogen. The absolute configurations of the chiral centres in these two diastereomers, 1′(*R*) N(*R*) and 1′(*R*) N(*S*), were determined using anomalous dispersion of the chlorine ions.

A representation of the crystalline structure is shown in Fig. 1(a); the bond distances and angles are available in supplementary material. Figure 1(b) shows the two diastereomers drawn with the same disposition of part B. The most significant torsion angles are given in Table 1.

Whatever the configuration at the nitrogen, the molecule has a horseshoe-shaped conformation. The rigid part B is almost identical in the two isomers. In the first part of the aliphatic linkage, the torsion angle 1′—1m—N—1 retains the same sign with slightly different values in the two isomers. Nevertheless, a configurational

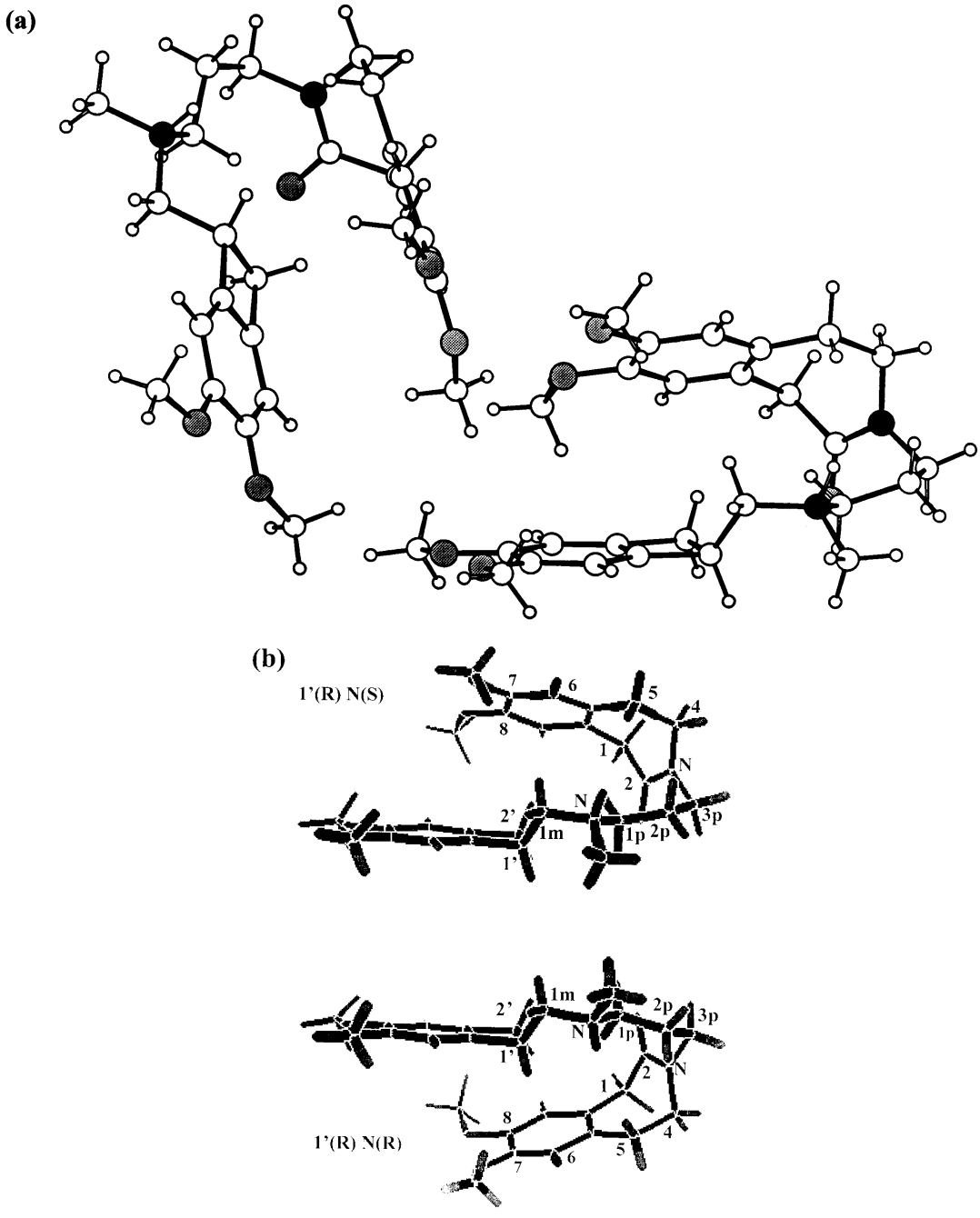

Figure 1. (a) View of the crystalline structure of S 16260 showing the two diastereomer species. (b) Comparison of the two diastereomers of S 16260 drawn with the same orientation of part B.

Table 1. Selected torsion angles (°) in the 1′(*R*) N(*R*) and 1′(*R*) N(*S*) diastereomers

Torsion angle	1′(*R*) N(*R*)	1′(*R*) N(*S*)
2′—1′—1m—N	−90.3	−92.8
6′a—1′—1m—N	170.3	170.1
1′—1m—N—1p	62.6	56.0
1′—1m—N[a]—Me	−170.5	−73.5
1′—1m—N[a]—H	−55.2	175.3
1m—N[a]—1p—2p	−171.9	158.1
N[a]—1p—2p—3p	168.8	−168.4
1p—2p—3p—N	−63.0	54.2
2p—3p—N—4	−72.0	73.9
2p—3p—N—2	109.1	−103.6
3p—N—4—5	107.2	−103.8
N—4—5—5a	57.5	−53.3
2—N—4—5	−73.9	73.7
2—1—9a—5a	−54.2	55.6
1—9a—5a—5	−2.6	4.9
4—5—5a—9a	1.1	−7.0

[a] Amino nitrogen.

change at the nitrogen atom induces significant modifications. While the bond between the methyl carbon and the nitrogen and the C1m—C1′ bond are almost antiperiplanar in the 1′(*R*) and N(*R*) isomer, they are *gauche* in the 1′(*R*) N(*S*) isomer. The methyl group on the protonated nitrogen is then always positioned outside the core of the molecule. It is worth noting that, within the second part of the aliphatic linkage, all the torsion angles have opposite signs in the two isomers, while differences between the absolute values never exceed 14°. As a result, starting from the amino nitrogen, bending of the aliphatic link occurs in opposite directions. The torsion angles have also opposite signs in the lactam ring. Thus, if each diastereomer is bent in such a way that the two bicyclic moieties are in close proximity, a major difference results for the orientation of the four-membered ring with respect to part A. Whereas the *syn* bonds C1′—H1′ and C2′—H2′* (where the asterisk denotes the proton H2′ *syn* with respect to H1′) point in the direction of the second bicyclic moiety in the 1′(*R*) N(*R*) isomer, they are situated on the external side of the molecule in the 1′(*R*) N(*S*) isomer. The *cis* protons H1′ and H2′* (in part B) are closer to the aromatic proton H6 (in part A) in the first case than in the second. In contrast, the C1m methylene group is more remote from the second bicyclic moiety in the 1′(*R*) N(*R*) isomer than in the 1′(*R*) N(*S*) isomer. Finally, in both diastereomers, the methoxy groups attached to the aromatic ring in part A, 7-OMe and 8-OMe, are in close proximity to the aromatic ring in part B. The oxygens are thus associated with an electron-enriched aromatic region. The 4′-OMe and 5′-OMe groups attached to the second aromatic ring (part B) are more remote from the aromatic ring in part A.

^{1}H and ^{13}C NMR study

The two isomers S 16257-2 and S 16260-2 gave the same spectra in achiral solvents. For ease of comparison with the crystalline structure, the NMR study will be discussed for S 16260-2 with the *R* configuration at C1′. The ^{1}H NMR spectrum and, to a lesser extent, the ^{13}C spectrum are strongly dependent on the pH for aqueous solutions, on temperature, on concentration and on the solvent characteristics.

Assignment of resonances. Spectral assignment was first made for a solution of the chloride salt in D_2O (the measured pH was 6.5, uncorrected value) and for an alkaline solution (the pH was increased to 9 by addition of NaOD). In both cases, a single set of resonances was observed. For the chloride salt, exchange-averaged resonances result from a rapid protonation–deprotonation equilibrium.

The method used for assignments is described for the spectrum recorded at pH 6.5. The high-field signal at 2.1 ppm might be ascribed to H2p, 2p′, devoid of direct deshielding effects. An AB-type, two-spin system clearly observed in the region 3.7–3.9 ppm is assigned to the protons of the isolated methylene group at C1 in the seven-membered ring. Difficulties arise from the lack of significant coupling interactions in the aromatic moieties and from severe overlap in the aliphatic region. As a result, the COSY 2D technique[1] gives only limited information. The most shielded signal assigned to H2p, 2p′ is correlated to two equivalent protons at 2.97 ppm and to a pair of more deshielded, diastereotopic protons. On the basis of chemical shifts, it seems reasonable to assign the first signal to H1p, 1p′ and the two others at 3.54 and 3.67 ppm to H3p and H3p′, respectively.

The heteronuclear ^{13}C,^{1}H correlations detected in a 2D HMQC experiment[2] allowed the assignment of the carbons linked to already identified protons and identification of new pairs of diastereotopic protons, e.g. those which give the signals at 2.63 and 2.96 ppm (H2′, H2′*) and the signals at 3.22 and 3.39 ppm (H1m). The correlation observed for the sole tertiary, aliphatic carbon (previously detected in a *J*-modulated echo ^{13}C spectrum) gives the position of H1′.

The most decisive information was obtained from an experiment combining inverse carbon–proton correlation with a HOHAHA transfer to remote protons.[3] Figure 2 shows the correlations which allow assignments of the five- and four-spin systems 1m—1′—2′ and 4—5. For example, at the frequency of C1′, correlations appear at the frequencies of H1′ and at the frequencies of the geminal protons at C1m and C2′. The former are more deshielded owing to the inductive effect of the nitrogen. The protons H2′, H2′*, *anti* and *syn* with respect to H1′, respectively were distinguished on the basis of the ^{3}J coupling constants to H1′, 2.3 and 5.5 Hz, respectively. These values do not deviate markedly from the *trans* (1.7 Hz) and *cis* (4.4 Hz) vicinal coupling constants in cyclobutene.[4]

Regarding the aromatic region and the attached methoxy groups, $^{1}J_{13C,1H}$ coupling detected in an HMQC experiment[2] and long-range $^{n}J_{13C,1H}$ coupling detected in an HMBC experiment[5] allowed, simultaneously, complete assignment of the ^{1}H and ^{13}C resonances as shown in Fig. 3. For example, H6 is correlated to C5, C8 and C9a, and the protons of the 8-methoxy group are correlated to C8 through $^{3}J_{13C,1H}$ interactions. These assignments were further confirmed

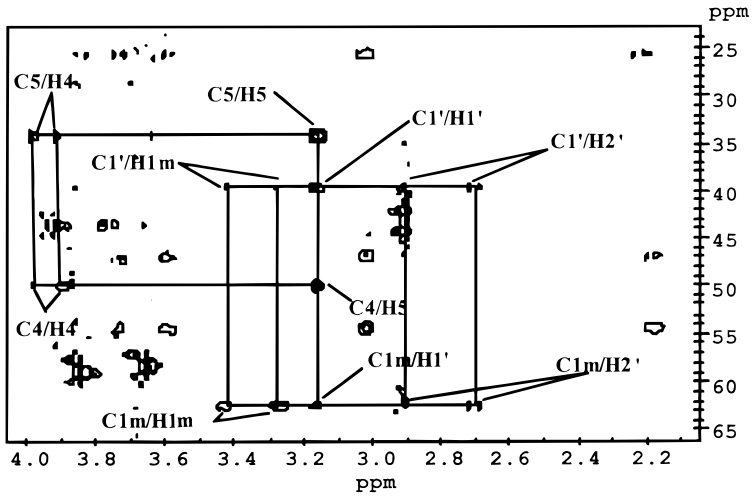

Figure 2. Inverse $\delta^{13}C$, $\delta^{1}H$ correlations through HMQC followed by $\delta^{1}H$, $\delta^{1}H$ correlations through HOHAHA transfer. Assignments of spin systems 1m—1′—2′ and 4—5.

by the observation of dipolar interactions in a NOESY experiment.[6] Each aromatic proton is correlated with the proton(s) of the aliphatic group linked in an *ortho* position, e.g. H9 with the protons at C1. Each aromatic proton is correlated with the methoxy group attached at the adjacent position, e.g. H9 with 8-OMe.

In the spectrum recorded at pH 6.5, one signal, H-6′ in the aromatic region, was severely broadened and some signals in the region 2–3.5 ppm were slightly broadened. Upon addition of NaOD the spectrum simplified, and at pH 9 well resolved singlets or multiplets were distinguished between 1.9 and 3.5 ppm.

The ^{1}H and ^{13}C chemical shift data corresponding to pH 6.5 and 9 are given in Table 2.

Following the addition of increasing amounts of DC1, severe broadening occurred for nearly all the signals. Then, when very acidic solutions were reached, narrow signals were obtained, and the spectrum appeared as the superposition of sub-spectra of the two diastereomeric species. The rate of the deprotonation–inversion–reprotonation process corresponds to the slow-exchange limit. Eight signals are easily observed in the aromatic region with equal intensities and seven for the methoxy groups (one superposition). The major difficulty associated with the identification of signals corresponding to the same diastereomer arise from considerable overlap in the region 2–4 ppm. The two species are temporarily named I and II. The distinction was first made for the group of protons H1m—H1′—H2′ on the basis of *J*-coupling interactions in a 2D COSY experiment. For the other protons it was made on the basis of Overhauser effects by means of NOESY or ROESY[7] experiments, assuming a lack of intermolecular effects in solution. Finally, most of the signals were unequivocally assigned. The ^{1}H chemical shift data corresponding to pH 2 are collected in Table 3. For each site, the interaction used for the assignment to stereoisomer I or II is indicated.

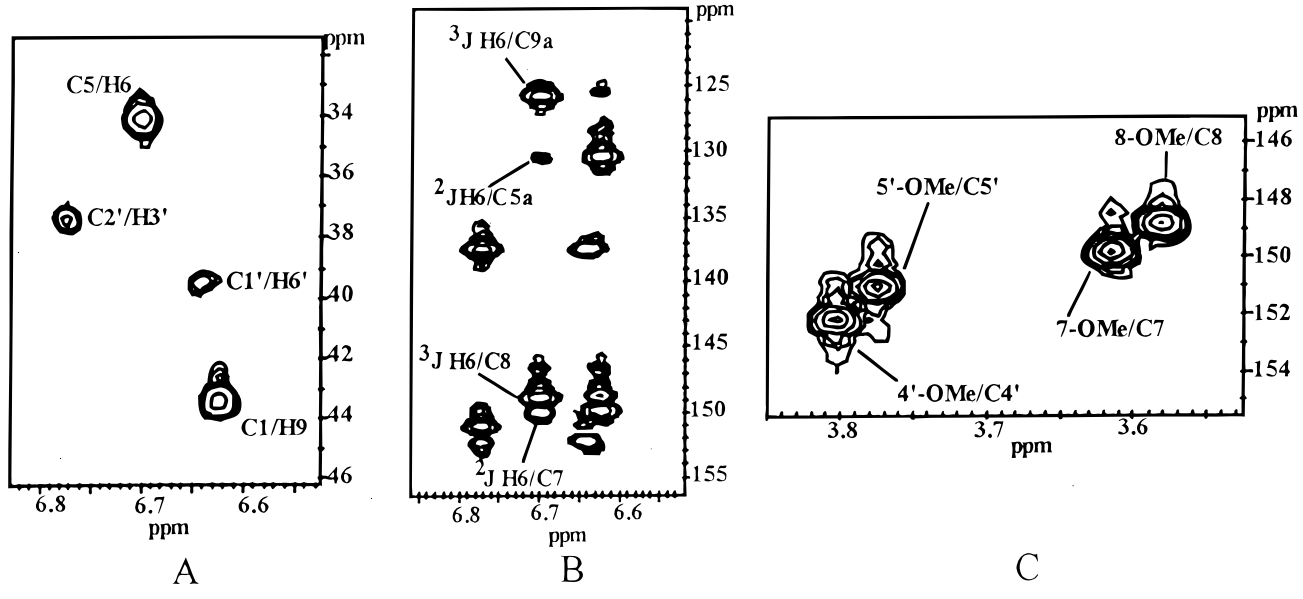

Figure 3. Long-range $\delta^{13}C$, $\delta^{1}H$ correlations through HMBC experiment. Partial contour plots allowing assignments of (A) the aromatic protons, (B) the quaternary aromatic carbons and (C) the methoxy protons.

Table 2. ^1H and ^{13}C chemical shift data in D_2O solution at pH 6.5 and 9.0, δ ppm, and diastereotopic proton chemical shift differences [ppm]

	pH 6.5		pH 9.0	
Site	δ^1H	$\delta^{13}C$	δ^1H	$\delta^{13}C$
1	3.69, 3.84 [0.15]	43.4	3.67, 3.88 [0.11]	43.6
2	—	178.4	—	178.8
4	3.84, 3.90 [0.06]	49.3	3.82, 3.93 [0.11]	49.5
5	3.08	33.9	3.09	34.2
5a	—	130.3	—	130.5
6	6.67	115.9	6.68	116.2
7	—	149.8	—	149.9
7-OMe	3.61	57.9	3.56	57.0
8	—	148.9	—	148.9
8-OMe	3.57	57.9	3.63	57.0
9	6.60	116.2	6.62	116.2
9a	—	125.3	—	125.7
1p	2.97	54.9	2.35, 2.42 [0.07]	54.4
2p	2.09, 2.11 [0.02]	25.6	1.88, 1.89 [0.01]	26.3
3p	3.54, 3.67 [0.13]	46.3	3.46, 3.65 [0.19]	46.9
N-Me	2.86	42.9	2.32	43.4
1m	3.22, 3.39 [0.17]	61.8	2.62, 2.73 [0.11]	62.4
1′	3.12	39.6	3.00	40.3
2′,2′*	2.63, 2.96* [0.33]	37.5	2.49, 2.83* [0.34]	37.6
2′a	—	137.2	—	138.3
3′	6.76	110.1	6.73	109.5
4′	—	152.3	—	152.1
4′-OMe	3.79	58.4	3.79	57.8
5′	—	151.2	—	151.1
5′-OMe	3.77	58.4	3.78	58.0
6′	6.64	109.5	6.56	109.5
6′a	—	137.2	—	137.7

Influence of pH. The spectra registered for solutions adjusted to pH 2.0, 6.5 and 9.0 are compared in Fig. 4(a) (aromatic protons) and 4(b) (aliphatic protons). Regarding the influence of pH on the ^1H chemical shifts, expected strong deshielding effects were observed upon protonation for the signals of protons at sites 1p, N-Me and 1m, in the α-position with respect to nitrogen, as well as smaller deshielding effects for the protons 2p

Table 3. ^1H chemical shift data in D_2O solution at pH 2, δ in ppm, diastereotopic proton chemical shift differences [ppm] for each stereoisomer and difference, Δδ, between the two diastereomers (the mean values are given for diastereotopic protons)

Site	Assignment *via J* or NOE	Stereoisomer I: 1′(*R*) N(*S*)	Stereoisomer II: 1′(*R*) N(*R*)	Δδ
1	NOE 9	3.73, 3.85 [0.12]	3.69, 3.86 [0.17]	0.02
4		3.80 ↔ 3.93[a]		
5		3.09	3.09	0
6	NOE 1′–2′*	6.69	6.68	0.01
7-OMe	NOE 6	3.62	3.64	0.02
8-OMe	NOE 3′	3.59	3.61	0.02
9	NOE 8-OMe	6.62	6.59	0.03
1p	NOE 6′	2.90, 2.95 [0.05]	3.03	0.04
2p	NOE 1p	2.09, 2.16 [0.07]	2.08, 2.20 [0.12]	0.02
3p	NOE 2p	3.54, 3.62 [0.08]	3.48, 3.67 [0.19]	~0
N-Me		2.89–2.89$_5$[a]		
1m	J	3.47, 3.49 [0.02]	3.28. 3.35 [0.07]	0.16
1′	J	3.17	3.15	0.02
2′,2′*	J	2.62, 2.71* [0.09]	2.69, 3.11* [0.42]	0.24
3′	NOE 2′	6.75	6.78	0.03
4′-OME		3.81	3.81	0
5′-OMe	NOE 6′	3.79	3.78	0.01
6′	NOE 1m	6.74	6.60	0.14

[a] Not assigned unequivocally.

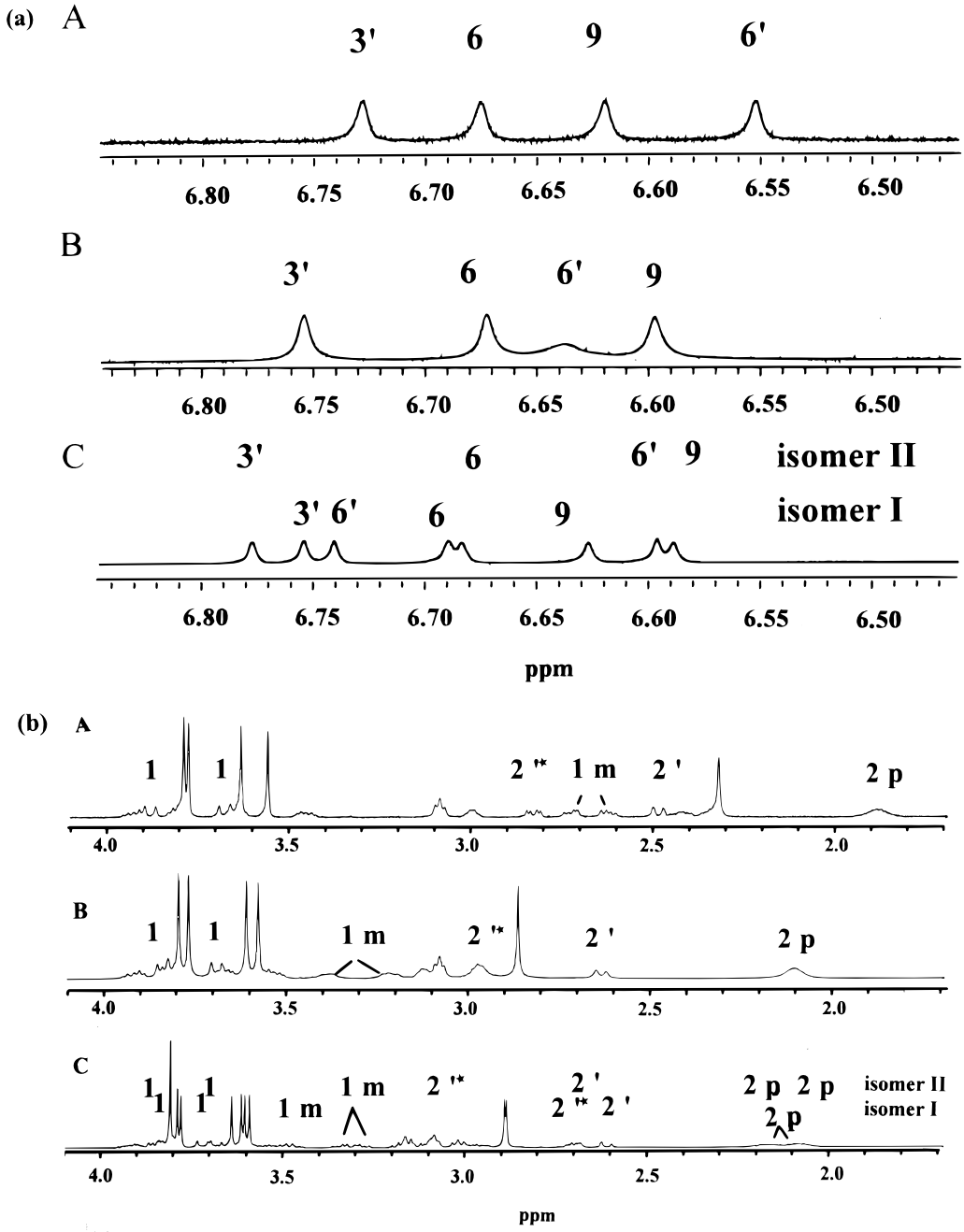

Figure 4. ^{1}H NMR spectra registered for (a) the aromatic and (b) the aliphatic protons as a function of pH: (A) pH 9; (B) pH 6.5; (C) pH 2.

and 1′ in the β position. The pK_a value was evaluated to be 8.3 from the curves $\delta = f(\text{pH})$ drawn for the *N*-methyl group or for the neighbouring protons.

Strikingly, some protons at remote sites also undergo significant shifts, particularly H2′* *syn* and H2′ *anti*, H6′ (aromatic) and the methoxy groups 7-OMe and 8-OMe.

Chemical shift differences between the diastereomers are noticeable for H2′, H1m and H6′. In contrast, signals of the *N*-methyl groups are hardly distinguished.

It is also noteworthy that the chemical shift difference between diastereotopic protons is greater in diastereomer II than in I for all the methylene groups except for methylene-1p, where the effect is more pronounced in I.

Analogous behaviour was observed for the ^{13}C spectra. Whereas at intermediate pH and in an alkaline medium one signal was observed for each carbon, most signals were split in very acidic solution. For ^{13}C resonances the observed splitting ($\Delta\delta$ ppm) decreases for the following sites: 2′(0.6), 6′a(0.4) then 3p, 1p and 1′(0.3) and to less than 0.1 ppm for the other carbons.

Concentration effects. The influence of concentration was observed for solutions in D_2O at constant pH (0.8), allowing the observation of two diastereomers. The more significant differences are noted for the chemical shifts of aromatic protons and for those of the methoxy groups. Overall, aromatic protons are shifted to low field by *ca.* 0.3 ppm when the concentration decreases. A similar trend is apparent for the methoxy groups. Stacking of the molecules should be reduced by lower-

ing the concentration, which is consistent with these observations.

Temperature effect. Signals originating from the different diastereomers in the spectra of a solution in D_2O (pH 1.5 at 300 K) progressively coalesce as the temperature is increased to 380 K. As expected, the protonated–deprotonated forms equilibrate more rapidly at higher temperatures.

Solvent effects. In the aprotic solvent $CDCl_3$, where stable ion pairs probably exist, the two diastereomeric species give distinct signals for almost all the protons. It is noteworthy that the protons of the *N*-methyl group show distinct signals which exhibit coupling to the ammonium proton.

In dimethyl-d_6 sulphoxide, which is known to solvate cationic species easily, differentiation between the stereoisomers is less significant. This is clearly seen for the aromatic protons, the *N*-methyl group and partially for the methoxy groups. The ammonium protons gave two distinct signals separated by *ca.* 0.5 ppm near 10.7 ppm.

The use of the protic solvent CD_3OD does not allow one to distinguish the diastereomeric species. In this solvent, decoalescence occurs only for two aromatic protons, H3′ and H6′, at the lowest attainable temperature (220 K).

Conformational study

Dipolar interactions detected via nuclear Overhauser effects and, to a lesser extent, chemical shift variations were used to gain information on the mean geometry of molecules in solution.

In alkaline solution where the amino group is not protonated, rapid inversion at the nitrogen atom is expected. Through 2D NOESY experiments, dipolar interactions were observed between protons, or groups of protons, belonging to the different bicyclic moieties, namely H3′–7-OMe and H3′–8-OMe, H6–H2′* and H6–H1′ and 7-OMe–H1′ and 7-OMe–H2′*. A correlation was also observed for H5, 5′ and the protons 2p and 2p′ several bonds away in the aliphatic linkage. All these results are in accordance with the hypothesis of a preferred conformation where the molecule is bent in a manner similar to that observed in the solid state for the 1′(*R*) N(*R*) isomer of the salt where the *syn* protons 1′ and 2′* of the four-membered ring are in close proximity to H6. The shielding of the 7- and 8-methoxy groups with respect to the 4′- and 5′-methoxy groups is in agreement with this hypothesis.

In slightly acidic solutions, e.g. pH 6.5, where the protonated species are largely predominant, interconversion between the diastereomers remains rapid on the NMR time-scale, and averaged spectra corresponding to the contribution of two protonated diastereomers and of the undistinguishable non-protonated forms are expected. Some dipolar interactions are still observed between protons belonging to the two parts of the molecule, e.g. H3′–7-OMe and H3′–8-OMe, H6–H1m and H6–H2′* or H1p, 1p′ (the last three signals overlap), H6–H2′ *anti* (a weak interaction) and finally H2p, 2p′ and H5. Regarding the chemical shifts, observations similar to those corresponding to the non-protonated amine are still valid. Bent conformations are then expected to contribute to the dipolar and shifts interactions.

In very acidic medium, distinct resonances are observed for most signals of the stereoisomers since the rate of interconversion lies in the slow-exchange limit. Nevertheless, in a phase-sensitive NOESY experiment a correlation due to exchange (opposite in phase to the NOE-type correlations) was observed for the protons of the 1m-methylene group which give well differentiated and isolated signals in both stereoisomers

Dipolar interactions were first used to examine each diastereomer.

The following correlations were observed between individual interacting groups, which exhibited differences in intensity for the two isomers: H5 in the lactam ring and H2p in the aliphatic linkage; H3′ and the protons of the 8-methoxy group (much more intense for isomer I); H6′ and H1m (stronger in stereoisomer I where both H1m protons are involved than in the isomer II, where the interaction is observed only for the more deshielded H1m); and between H2′ and H1m (the more shielded proton), much stronger in II than in I. A bent, horseshoe-shaped conformation, similar to that of either diastereomer in the solid state, would satisfy the above requirements.

The most significant results occur for H6. Strong NOEs are detected between H6 and H′, H2′* in stereoisomer II, whereas no such correlations appear for isomer I.

By comparison with the structures found in the solid state, it becomes reasonably certain that the 1′(*R*) N(*R*) configuration corresponds to species II, where the protons H1′ and H2′* must be oriented toward H6 and the 1′(*R*) N(*S*) configuration to species I, where the atoms H1′ and H2′* are devoid of interaction with part A of the molecule.

These results are supported by comparing the two ^1H spectra. In the aromatic region the signals are less spread out for I than for II, and the relative order of the signals is modified with H6′ being noticeably deshielded. In the aliphatic region, chemical shift differences for diastereotopic protons (for II divided by I) are as follows: 4.5 for the methylene–2′, 3.5 for the methylene–1m and *ca.* 2 for methylenes 1, 3p and 2p. In contrast, it is noteworthy that signals of sites situated at the periphery, e.g. the *N*-methyl group, methylene-5 and 4′- and 5′-methoxy, are scarcely distinct.

In the solid state, the mean distance between the two bicyclic halves is greater for the 1′(*R*) N(*S*) isomer. The spectral features outlined above for the isomers in solution, in particular the smaller chemical shift differences for diastereotopic protons at many sites for the 1′(*R*) N(*S*) isomer (I), might result from smaller steric and/or anisotropy effects.

Finally, except for the typical deshielding associated with full protonation, comparison of the spectrum of the 1′(*R*) N(*R*) isomer (II) with spectra recorded in an alkaline or moderately acidic medium reveals great similarity. For the aromatic region similar patterns are observed at pH 2 and 6.5. In the aliphatic region, chemical shift differences for diastereotopic protons of

the methylene groups at C2′, C1, C3p and C1m are similar in magnitude.

CONCLUSION

All of the results led to the conclusion that the molecule shown in Scheme 1 adopts a bent, horseshoe-shaped conformation in solution at all pH values, and in particular under biological conditions. In strongly acidic solutions, the conformation of the 1′(*R*) N(*R*) isomer is similar to the solid-state structure of the 1′(*R*) N(*R*) isomer with the *syn*-protons, linked to the four-membered ring, directed inside. Likewise, the conformation of the 1′(*R*) N(*S*) isomer is similar to the solid-state structure of the 1′(*R*) N(*S*) isomer with the *syn*-protons of the four-membered ring outside. For a given absolute configuration of carbon 1′, the absolute configuration of the nitrogen atom, upon protonation, determines the overall shape of the molecule. In weakly acidic solution, a conformational equilibrium might be expected to occur. Nevertheless, the need for the molecule to undergo a complete rearrangement on going from the structure of one diastereomer to the other probably severely limits the ability of a rapid exchange on the NMR time-scale. The results seem to reflect a mean preferred conformation similar to that of the 1′(*R*) N(*R*) isomer. Regarding basic pH at which inversion at the nitrogen atom is expected to be very fast, it seems that the interconversion between the molecular geometries of the two isomers does not occur and that a conformation similar to that of the 1′(*R*) N(*R*) isomer prevails.

EXPERIMENTAL

Crystallographic data

The crystal system is orthorhombic, space group $P2_12_12_1$. The cell dimensions are $a = 8.662(2)$, $b = 18.201(12)$ and $c = 38.65(5)$ Å. $Z = 8$. Data collection: CAD4 diffractometer. Radiation: Cu Kα. Reflections collected, 11 146; reflections used $[I > 3\sigma(I)]$, 4221. The structure was resolved with a direct method:[8] and refined by least squares.[9] $R = 3.9\%$, $R_w = 4.9\%$, $w = [\sigma^2(F) + 0.001\ F^2]^{-1}$. All non-hydrogen atoms were anisotropically refined. H atoms were located on difference Fourier maps and refined with an isotropic thermal parameter. Absolute configurations were determined using the anomalous scattering of Cl^- anions, which was incorporated in structure factors computations. The program BIJVOET[10] compares observed and calculated structure factors. Over 71 higher differences (>2 electrons), 69 have the same sign, which is in favour of the absolute configurations used.

Spectra

The 1D and 2D spectra were recorded on a Bruker AMX500 spectrometer with a 5 mm inverse probe, operating at 500.13 MHz for ^{1}H. The sample was dissolved in 500 µl of D_2O at a concentration of 20 mg ml^{-1}. DSS was used as an external reference (a calibration graph was obtained for the different pH values).

COSY experiment

The COSY experiment was performed in the phase-sensitive mode with 32 scans for each of the 256 t_1 values. The final matrix was 1K × 1K. $\pi/2$ shifted-sine bell exponential weighting was used. Baseline corrections in F_1 and F_2 were performed with a polynomial function.

NOESY experiments

2D NOESY experiments were performed in the phase-sensitive mode (TPPI) with mixing times ranging from 200 to 800 ms. A spectral width of 4000 Hz was used in both dimensions; 96 scans were acquired for each of the 400 t_1 increments. Zero-filling was applied, which led to a final matrix of 1K × 1K. $\pi/2$ shifted-sine bell exponential weighting was used in both dimensions. Baseline corrections in F_1 and F_2 were performed with a polynomial function.

ROESY experiments

ROESY experiments were performed in the phase-sensitive mode (TPPI) with mixing times of 600 and 800 ms. A spectral width of 4000 Hz was used in both dimensions; 96 scans were acquired for each of the 400 t_1 increments. Zero-filling was applied, which led to a final matrix of 1K × 1K. $\pi/2$ shift-sine bell exponential weighting was used in both dimensions. Baseline corrections in F_1 and F_2 were performed with a polynomial function.

MLEV-HMQC experiment

This 2D experiment was performed in the phase-sensitive mode (TPPI) with 96 scans for each of the 256 t_1 increments; 80 ms was used as the spin-locking time. In the F_1 dimension, a 20 000 Hz spectral width was used. After zero-filling, the final matrix was 1K × 1K. Sine bell exponential weighting was used in both dimensions.

^{1}H, ^{13}C HMQC

An HMQC experiment was performed in the phase-sensitive (TPPI) mode with 32 or 76 scans for each of the 300 t_1 increments. The delay was adjusted for $^1J_{13C,\,1H} = 145$ Hz. After zero-filling, the final matrix was 1K × 1K. Data processing used non-shifted-sine bell weighting and baseline correction in the F_1 and F_2 dimensions.

^{1}H, ^{13}C HMBC

This experiment was performed with 128 scans for each of the 400 t_1 increments. A value of 7 Hz was used for

the average long-range. C–H coupling constant. The final matrix was 1K × 1K after zero-filling. $\pi/2$ shifted-sine bell exponential weighting was used. No baseline correction was made.

Concentration effects

The pH was fixed at 0.8 and 1D spectra were acquired with concentrations ranging from 1.2 to 26.7 mg ml^{-1}.

Solvent effects

Spectra were recorded at a concentration of 20 mg ml^{-1}. DMSO-d_6, CD_3OD, $CDCl_3$ D_2O were used as solvents.

Temperature studies

Spectra were recorded at a concentration of 20 mg ml^{-1} in D_2O between 300 and 380 K. Spectra were recorded in CD_3OD between 300 and 220 K.

Supplementary material

X-ray characterization data including tables of functional atomic coordinates, distances and angles, thermal parameters (Tables s1–s7) and calculated and observed structure factors (6 pages) have been deposited at the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

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