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Differential protonation and dynamic structure of doxylamine succinate in solution using ^{1}H and ^{13}C NMR

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A protonation and dynamic structural study of doxylamine succinate, a 1:1 salt of succinic acid with dimethyl-[2-(1-phenyl-1-pyridin-2-yl-ethoxy)ethyl]amine, in solution using one- and two-dimensional ^{1}H and ^{13}C NMR experiments at variable temperature and concentration is presented. The two acidic protons of the salt doxylamine succinate are in 'intermediate' exchange at room temperature, as evidenced by the appearance of a broad signal. This signal evolves into two distinct signals below about −30 °C. A two-dimensional ^{1}H–^{1}H double quantum filtered correlation experiment carried out at −55 °C shows protonation of one of the acidic protons to the dimethylamine nitrogen. A two-dimensional rotating frame ^{1}H–^{1}H NOE experiment at the same temperature reveals that the other proton remains with the succinate moiety. Comparison of the ^{1}H and ^{13}C chemical shifts and the ^{13}C T_1 relaxation times of the salt with those of the free base further substantiate the findings. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; antihistaminic drug; doxylamine succinate; protonation; solution structure

INTRODUCTION

Doxylamine succinate[1] (DOS) has been used as an antihistaminic drug for several decades.[2] It is also used as an efficient hypnotic drug[3] and for the relief of runny nose and sneezing associated with upper respiratory tract infection.[4] It is reported that DOS crystals exhibit differences in stability and reactivity and the tablets stored at 75% relative humidity change color. These characteristics have been attributed to its polymorphic behavior. Two polymorphic forms have been crystallized.[5] The x-ray structure of one of the forms has been reported and the results show two different conformations for doxylamine cations in the crystal.[5] Another recent and independent x-ray crystal structure study on DOS also shows that the cation exists in two different conformations.[6] With a view to elucidating the protonation and structural behavior of DOS in the solution state, we made a detailed investigation using NMR in two solvents, chloroform and dichloromethane. The results obtained for the protonation and dynamic structure based on ^{1}H and ^{13}C one- and two-dimensional experiments at variable temperature in the two solvents and $^{13}C T_1$ relaxation measurements in chloroform are reported.

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EXPERIMENTAL

Synthesis of doxylamine succinate

Doxylamine base was prepared from 2-acetylpyridine and bromobenzene as described[1] and purified by flash chromatography using silica gel and dichloromethane–methanol (98 : 2) as eluent to obtain pure doxylamine base (yield 55%). To a solution of purified doxylamine base (27 g, 100 mM) in acetone (180 ml), succinic acid (11.8 g, 100 mM) was added and heated until it had dissolved completely (50–55 °C). On cooling under nitrogen to 5–6 °C, crystals of doxylamine succinate separated out. These were filtered and washed with dry acetone (50 ml) and dried at 55–60 °C for 6 h to give pure doxylamine succinate salt ($C_{21}H_{28}N_2O_5$) as white crystals (32 g, 82%).

NMR experiments

NMR experiments were performed in deuterated chloroform (99.8% D) (Aldrich, Milwaukee, WI, USA). However, for studying the broad acidic proton signals at temperatures lower than the freezing-point of chloroform, deuterated dichloromethane (99.8% D) (Aldrich) was used as the solvent. Solutions of the DOS were prepared in chloroform and dichloromethane with the same concentrations (6.6 mg per 0.5 ml). NMR experiments were performed on a Bruker Biospin Avance 400 spectrometer using a 5 mm broadband inverse probehead. Normal one-dimensional ^{1}H NMR experiments were performed at room temperature on all the samples. In order to monitor the effect of concentration on the chemical shifts of labile protons, one-dimensional

spectra of the salt were obtained in relatively dry deuterated chloroform as a function of concentration of DOS varying from 1.0 to 157.2 mg per 0.5 ml. The spectra for the DOS at lower concentrations were also obtained by varying the temperature in steps of 10 °C between room temperature and −55 °C in chloroform solution and between room temperature and −75 °C in dichloromethane solution. All one-dimensional ^{1}H spectra were obtained using one pulse sequence using the following parameters: spectral width, 8000 Hz; time domain points, 32K; relaxation delay, 5 s; pulse angle, 45°; number of scans, 16 or 64; spectrum size, 32K; and line broadening, 0.3 Hz. ^{13}C spectra were obtained at room temperature using one pulse sequence with proton decoupling using the WALTZ-16 sequence. The parameters used were as follows: spectral width, 23 100 Hz; time domain points, 32K; relaxation delay, 3 s; pulse angle, 45°; number of scans, 64; spectrum size, 32K; and line broadening, 3 Hz.

For the complete assignments of the ^{1}H and ^{13}C signals, homonuclear and heteronuclear two-dimensional experiments such as ^{1}H–^{1}H double quantum filtered correlation (DQF-COSY),[7,8] ^{1}H–^{13}C gradient enhanced heteronuclear single quantum coherence with improved sensitivity by preservation of equivalent pathways[9–11] (PFG-PEP-HSQC) and gradient enhanced heteronuclear multiple bond correlation[12] (PFG-HMBC) experiments were performed. For DOS in chloroform, two-dimensional DQF-COSY and rotating frame Overhauser effect spectroscopy (ROESY)[13,14] were also performed at −55 °C for the assignments of the acidic protons. For DQF-COSY and ROESY experiments, a spectral width of 8561 Hz was used in both dimensions. About 512 FIDs with t_1 incrementation, each of 2048 complex data points, were collected with a relaxation delay of 2.5 s; 16 transients and 1.5 s; 40 transients for DQF-COSY and ROESY data, respectively. Phase-sensitive data were obtained by the TPPI method.[15] A spin-lock mixing time of 200 ms was used for the ROESY experiment. The resulting data were zero filled to 1024 points in the t_1 dimension, multiplied by a squared sine-bell window function shifted by $\pi/2$ along both dimensions and double Fourier transformed. For the HSQC and HMBC experiments, spectral widths of 7200 and 23 100 Hz in the ^{1}H and ^{13}C dimensions, respectively, were used. For the HSQC and HMBC experiments, 256 and 400 FIDs, respectively, were collected with t_1 incrementation, each of 2048 points, 16 transients and a 2 s recycle delay. Phase-sensitive data for HSQC experiments were obtained using the echo, anti-echo method[16] and HMBC experiments were in magnitude mode. The resulting data were zero filled to 1024 points in the t_1 dimension and double Fourier transformed after multiplying by a squared sine-bell window function shifted by $\pi/2$ along both dimensions.

$^{13}CT_1$ relaxation measurements for DOS at room temperature were made using the inversion–recovery method incorporating inverse gated proton decoupling. Parameters used were as follows: spectral width, 24 000 Hz; time domain points, 32K; relaxation delay, 45 s; number of scans, 16; inversion–recovery delay, varied between 10 ms and 45 s in 26 steps; spectrum size, 64K; and line broadening, 3.0 Hz. The relaxation times of individual carbons were calculated using Bruker Xwinnmr software version 3.1.

NMR experiments for the free base, doxylamine, were performed under similar conditions in order to compare the structural and dynamic parameters of DOS with those of the free base.

RESULTS

DOS shows a single set of signals in both the ^{1}H and ^{13}C spectra in both the solvents. The ratio between doxylamine and succinate signals indicates that the base to acid ratio in the salt is 1 : 1. Analysis of all the ^{1}H and ^{13}C resonances, except the acidic protons, were made using a combination of two-dimensional DQF-COSY, PFG-PEP-HSQC and PFG-HMBC experiments. A typical PFG-HMBC spectrum of DOS along with the traces of ^{1}H and ^{13}C one-dimensional spectra and the structure of DOS with numbering of the nuclei is shown in Fig. 1. ^{1}H and ^{13}C chemical shifts of DOS assigned with reference to the internal standard, TMS, are given in Table 1. The corresponding values determined for the free base are given in parentheses.

Both the acidic protons H-*a* and H-*f* give rise to a broad signal at lower concentrations shifted to low positive values depending on the concentration of DOS and water impurity. As the concentration of DOS is increased, the signal shifts to more positive values. Figure 2 shows the spectra of DOS in

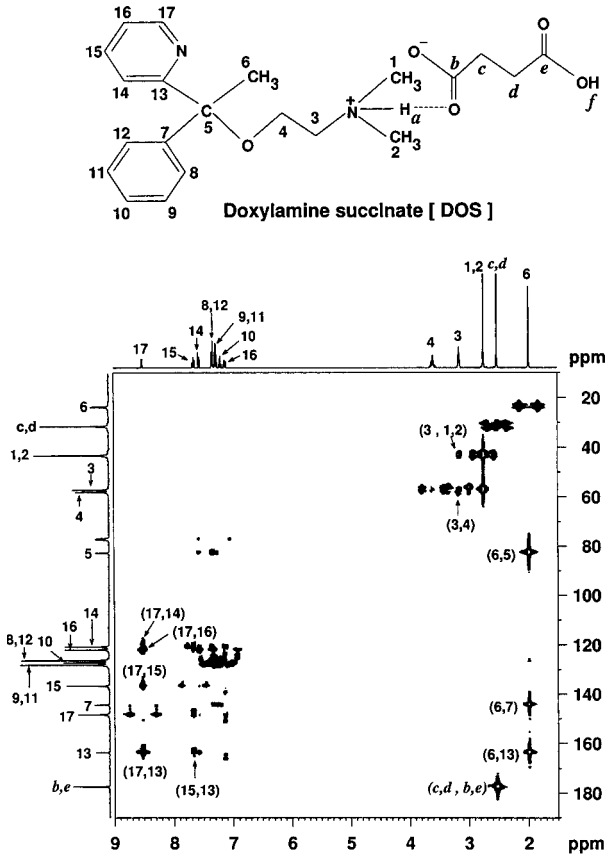

Figure 1. PFG-HMBC spectrum of doxylamine succinate in deuterated chloroform (6.6 mg per 0.5 ml) along with the traces of the ^{1}H and ^{13}C one-dimensional spectra showing the assignments of various ^{1}H and ^{13}C signals. The structure of doxylamine succinate with numbering of the nuclei is shown at the top.

Table 1. ^{1}H and ^{13}C chemical shifts and ^{13}C relaxation times of doxylamine succinate obtained in deuterated chloroform at 25 °C, with values obtained under similar conditions for the free base, doxylamine, in parentheses for comparison

$^{1}H/^{13}C$	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)	^{13}C T_1 (s)
1, 2	2.80 (2.27)	43.30 (46.04)	0.599 (1.789)
3	3.18 (2.59)	57.13 (59.46)	0.276 (1.731)
4	3.63 (3.41)	57.94 (61.22)	0.297 (1.645)
5	—	82.62 (81.90)	6.530 (23.965)
6	1.99 (1.98)	23.75 (23.90)	0.276 (0.653)
7	—	144.37 (145.60)	2.774 (11.213)
8, 12	7.32 (7.40)	126.37 (126.35)	0.619 (2.281)
9, 11	7.30 (7.27)	128.16 (128.01)	0.627 (2.172)
10	7.23 (7.18)	127.27 (126.79)	0.516 (1.597)
13	—	163.78 (164.99)	2.849 (11.586)
14	7.51 (7.60)	121.00 (120.91)	0.536 (1.934)
15	7.69 (7.61)	136.76 (136.39)	0.559 (1.979)
16	7.16 (7.09)	122.12 (121.70)	0.479 (1.598)
17	8.54 (8.51)	148.34 (148.34)	0.562 (2.059)
a	7.80 (13.5[a])	—	—
b, *e*	—	177.62	2.988
c, *d*	2.54	31.56	0.330
f	7.80 (17.1[a])	—	

[a] Chemical shift values observed at −55 °C.

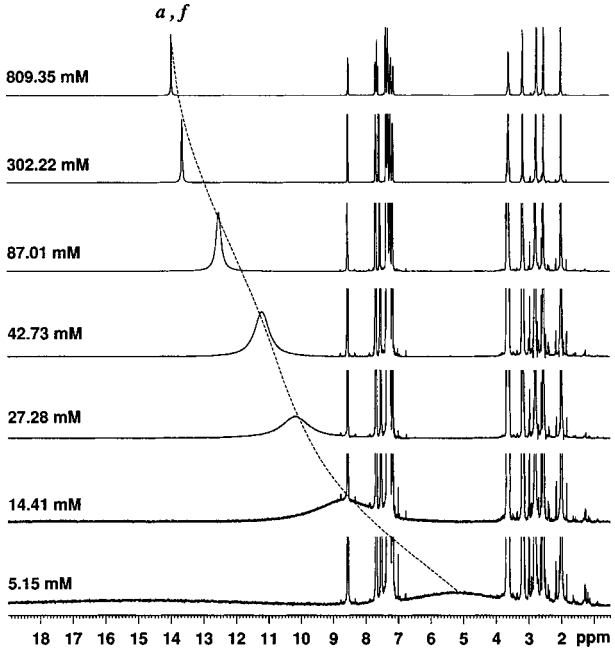

Figure 2. ^{1}H NMR spectra as a function of concentrations of DOS in deuterated chloroform. The variation of the chemical shift of protons H-*a* and H-*f* with concentration is highlighted by the dotted line. At lower concentration, the signal is shifted to less positive values owing to the exchange among protons H-*a* and H-*f* and protons from water impurity in the solvent. As the concentration of DOS is increased, the signal shifts to more positive values owing to the increase in the fraction of protons H-*a* and H-*f* relative to water protons.

deuterated chloroform as a function of the concentration of the salt.

When the solutions of DOS in chloroform and dichloromethane, at lower concentration, were cooled below room temperature, the broad signal due to the protons H-*a* and H-*f* shifts to more positive values. Below about −30 °C, two distinct broad signals appear. At about −55 °C, these signals are relatively sharp in both solvents. One of the acidic protons at this temperature is at 13.5 ppm and the other is at 17.1 ppm in chloroform solvent. Further narrowing of the signals of the acidic protons was not considerable upon cooling below −55 °C as observed in dichloromethane down to −75 °C. Figure 3 shows parts of the one-dimensional spectra showing the acidic proton signals in DOS in chloroform as a function of temperature. There was no significant difference in ^{1}H chemical shifts of DOS between chloroform and dichloromethane solvents.

The DQF-COSY spectrum recorded at −55 °C shows a cross peak of the acidic proton at 13.5 ppm with amino dimethyls H-1 and H-2 and the methylene protons H-3 whereas the signal at 17.1 ppm does not show any cross peak. However, the ROESY spectrum at −55 °C shows an NOE cross peak between the acidic proton signal at 17.1 ppm and succinate protons. Thus, from these spectra, the signal at 13.5 ppm is assigned to the proton H-*a*, protonated at the amino nitrogen of the salt DOS, and the signal at 17.1 ppm is assigned to proton H-*f*, attached to the succinate moiety. Part of the DQF-COSY spectrum showing cross peaks of the proton signal at 13.5 ppm with the protons H-1, H-2 and H-3 is shown in Fig. 4(a) and part of the ROESY spectrum showing the NOE cross peak between the acidic proton at 17.1 ppm with the succinic protons H-*c* and H-*d* is shown in Fig. 4(b).

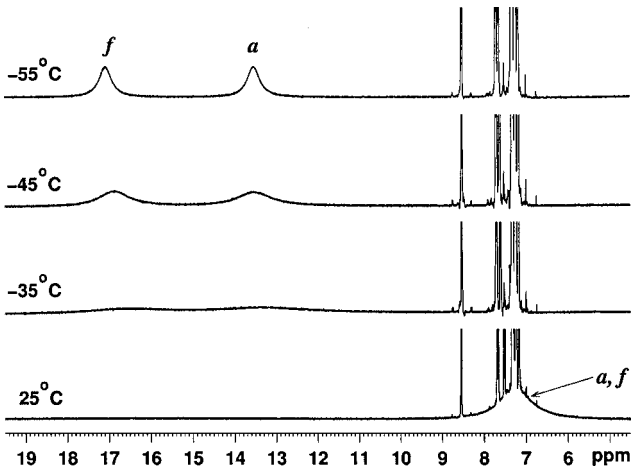

Figure 3. Parts of the one-dimensional ^{1}H spectra of doxylamine succinate in deuterated chloroform showing acidic proton signals at 25, −35, −45 and −55 °C. Both the acidic protons H-*a* and H-*f* (marked *a* and *f*, respectively) show, together, a broad signal at about 7.8 ppm at 25 °C. It gradually becomes unobservably broad and subsequently shows distinct signals upon cooling with a relatively sharp linewidth at 13.5 and 17.1 ppm, respectively, at −55 °C.

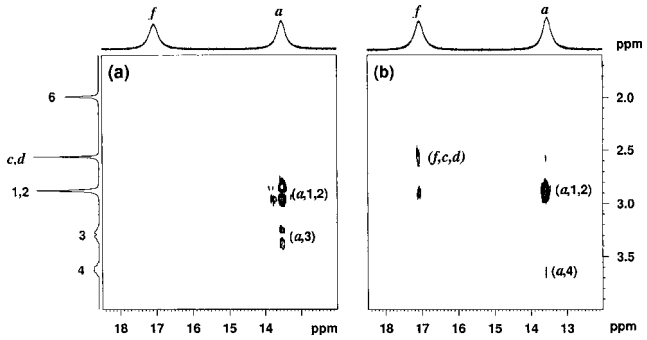

Figure 4. Parts of the two-dimensional spectra of doxylamine succinate at −55 °C in deuterated chloroform: (a) DQF-COSY spectrum showing the cross peaks between proton H-*a* and H-1, H-2 [marked (*a*, 1, 2)] and between H-*a* and H-3 [marked (*a*, 3)], establishing protonation of H-*a* with the amino nitrogen, and (b) ROESY spectrum highlighting the NOE cross peak between H-*f* and H-*c* and -*d*, establishing that the proton H-*f* is associated with the succinate moiety.

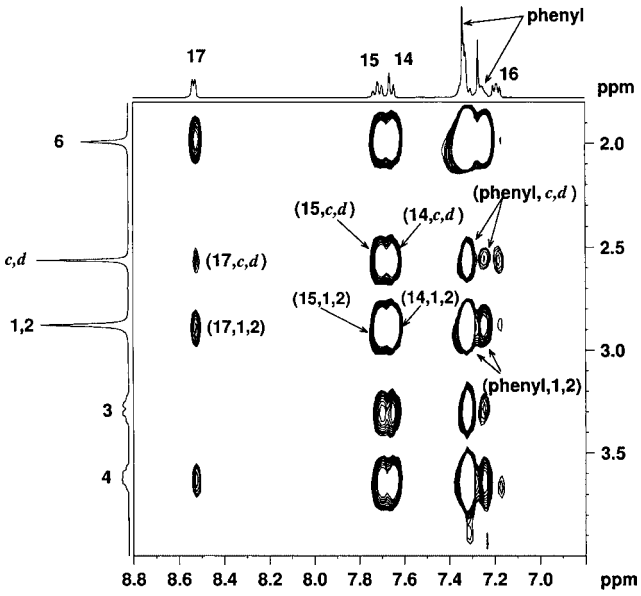

Figure 6. Part of two-dimensional ROESY spectrum of doxylamine succinate at −55 °C in chloroform solvent highlighting the NOE cross peaks of amino methyls and succinate with the aromatic ring protons. Amino methyl cross peaks with aromatic ring protons indicate the proximity of the amino nitrogen to the aromatic ring similar to that observed in the solid state[5,6].

$^{13}CT_1$ relaxation times of DOS are given in Table 1 along with those of the free base. Variations of ^{13}C relaxation times of DOS with reference to those of the free base along with the variations of ^{1}H and ^{13}C chemical shifts are depicted in Fig. 5.

The amino methyl H-1 and H-2 show strong NOE cross peaks to aromatic ring protons and pyridyl ring protons H-14 and H-15. Succinate protons H-*c* and -*d* also show strong NOE cross peaks to aromatic ring protons and pyridyl ring protons H-14 and H-15. Part of the ROESY spectrum highlighting these cross peaks is shown in Fig. 6.

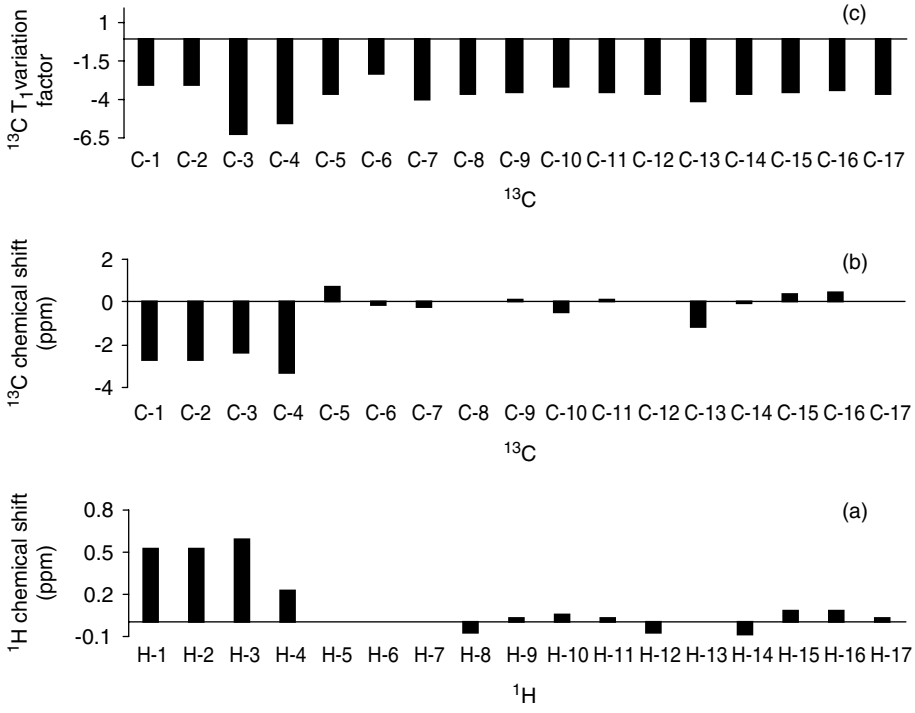

Figure 5. Depiction of variation of ^{1}H and ^{13}C chemical shifts and ^{13}C T_1 relaxation times in deuterated chloroform of doxylamine succinate relative to those of the free base, doxylamine, obtained under identical conditions: (a) difference in ^{1}H chemical shifts between the protons in the salt and the corresponding protons in the free base; (b) difference in ^{13}C chemical shifts between the carbons in the salt and the corresponding carbons in the free base. In (a) and (b), bars on the positive side indicate a more positive chemical shift of $^{1}H/^{13}C$ of doxylamine succinate compared with those in its free base and vice versa. (c) Factor by which the ^{13}C T_1 relaxation times have decreased in doxylamine succinate compared with those of the free base.

MRC

DISCUSSION

The single set of signals for DOS in both the ^{1}H and ^{13}C spectra shows that the DOS salt exists in a single conformation in solution. Since the assignments were, hitherto, not available in the literature to the best of our knowledge, we carried out complete ^{1}H and ^{13}C assignments of DOS and its free base using homonuclear and heteronuclear experiments at room temperature (Fig. 1).

The single broad signal for the two acidic protons H-*a* and H-*f* indicates that these protons exchange with one another and the shift to less positive values at lower concentrations of DOS indicates that the protons also exchange with protons of water impurity.[17] As the concentration of DOS is increased, the signal gradually shifts to more positive values owing to the gradual increase of total fraction of H-*a* and H-*f* protons relative to water protons[17] (Fig. 2). Hence the chemical shift of the protons H-*a* and H-*f* at room temperature depends on the concentration of DOS and also the water impurity in the organic solvent.

Since the protons H-*a* and H-*f* give a single resonance at room temperature, it was not possible either to assign these protons distinctly or to obtain any correlation of this broad signal with the other proton signals in the two-dimensional spectra. Since the individual assignment of the acidic protons was necessary to study the protonation of doxylamine, we carried out low-temperature experiments. As the temperature is lowered below room temperature (25 °C), the signal due to H-*a* and H-*f* (strictly also due to water impurity) pass from 'intermediate' exchange to relatively slow exchange. The signals become unobservably broad, as seen in Fig. 3, at −35 °C. On further reducing the temperature, the protons show distinct signals and they become relatively sharp at −55 °C. Under these conditions, we carried out two-dimensional NMR experiments for specific assignments.

The cross peaks of the acidic proton at 13.5 ppm, in the DQF-COSY spectrum, with amino dimethyl H-1, H-2 and the methylene protons H-3 clearly indicate that the signal at 13.5 ppm arises from proton H-*a* [Fig. 4(a)]. The absence of any cross peaks with the signal at 17.1 ppm in the DQF-COSY spectrum indicates that this proton is not protonated to doxylamine. However, the NOE cross peak between this signal and protons H-*c* and -*d* in the ROESY spectrum at −55 °C establishes that the signal at 17.1 ppm arises from the proton H-*f* [Fig. 4(b)]. Hence these data show the monoprotonation of doxylamine with succinic acid at the dimethyl amine nitrogen in solution similar to that observed in the solid.[5,6] This is further substantiated by the significant changes in the ^{1}H and ^{13}C chemical shifts and $^{13}CT_1$ relaxation times of DOS relative to those of free base, only in the vicinity of protonation. For example, as seen in Fig. 5(a) and (b), there is a significant change in the chemical shifts of H-1, H-2, H-3 and H-4 and carbons C-1, C-2, C-3 and C-4 arising from the protonation at the amine nitrogen. Similarly, the reduction in the relaxation times of carbons C-3 and C-4 is more significant compared with others [Fig. 5(c)], indicating an enhanced relaxation rate arising from the protonation at the amino nitrogen site.

The observation of strong NOE cross peaks of the amino methyl protons H-1 and H-2 with aromatic ring protons indicates the proximity of the amino nitrogen with the centroid of the aromatic ring (Fig. 6), which is stated to be important for its antihistaminic activity,[18] similar to that observed in the solid state. Further, the succinate protons H-*c* and -*d* also show strong NOE to phenyl ring protons as in addition to the protons H-14 and H-15 of the pyridine ring, indicating that the succinate moiety is also in close proximity to the aromatic and pyridine rings (Fig. 6). Correlation of the common NOE of H-1, H-2 and H-*c*, H-*d* with the ring protons with a significantly more positive shift of acidic protons H-*a* and H-*f* suggests the possibility of strong intra-ionic hydrogen bonding of acidic protons. X-ray studies also show that the structure in the solid state is stabilized by the hydrogen bonding involving the cations and anions (proton H-*a* with carbonyl group of C-*b*) as shown in Fig. 1.[5,6]

In conclusion, although NMR shows a single averaged conformation in solution for doxylamine succinate, protonation and NOE data indicate overall similarity with the solid-state conformations. The detailed NMR study of DOS presented here further demonstrates how the protonation could be established using NMR even from broad and exchangeable signals.

Acknowledgment

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