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Note

Protonation of trimipramine salts of maleate, mesylate and hydrochloride observed by ^{1}H, ^{13}C and ^{15}N NMR spectroscopy

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Protonation of the tricyclic antidepressant drug trimipramine with maleic acid, methanesulfonic acid and hydrochloric acid was studied using ^{1}H, ^{13}C and ^{15}N NMR spectroscopy at natural abundance. The effect of counter ions on the protonation was compared under identical conditions of solvent, concentration and temperature using homonuclear and heteronuclear one- and two-dimensional experiments. Differential protonation of the terminal tertiary amine nitrogen is determined from the indirect spin–spin couplings, chemical shifts, ^{13}C relaxation data and variable-temperature experiments. In the maleate salt, only one of the acidic protons is involved in protonation, the other being associated with the anion moiety. ^{15}N chemical shifts of the protonated nitrogens are nearly linearly related to the pK_a of the constituent acid.

KEYWORDS: ^{1}H NMR; ^{13}C NMR; ^{15}N NMR; antidepressant drug; trimipramine; protonation; pK_a

INTRODUCTION

Tricyclic antidepressant drugs (TCAs) have been used for a wide range of depressive disorders since their introduction into the pharmaceutical market in the 1950s. These drugs exhibit their pharmacological effects through neurotransmitter binding sites.[1] The structure–activity relations in these TCA series lack specificity, suggesting a difficulty in defining specific pharmacological activity and mechanism of therapeutic efficacy.[1] Therefore, even though TCAs have been in use for a long time, the exact mechanism of binding of these drugs to the receptor site and their location in the biological membrane is still unclear. It has been suggested that all these cyclic drugs bind to α_1-acid glycoprotein, lipids and cholesterol.[1] A number of studies have been carried out on these TCAs using NMR spectroscopy to establish the drug–receptor interaction, which includes solution dynamics, molecular flexibility and the interaction of drugs with model membranes and micellar aggregates.[2–6]

In contrast to many tricyclic antidepressants that are hydrochloride salts, trimipramine has been used as the maleate salt. In the present study, we explored the protonation of trimipramine with maleic acid using ^{1}H, ^{13}C and ^{15}N NMR spectroscopy, with a view to assessing the role of the anion on the protonation characteristics of the drug. The protonation of trimipramine by maleic acid is compared, under identical conditions, with that by methanesulfonic acid and hydrochloric acid.

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MATERIALS

Synthesis of trimipramine (free base)

2-Methyl-3-dimethylaminopropyl chloride (27 g, 0.2 mol, as a 30% solution in toluene) was added to a slurry of iminodibenzyl (19.5 g, 0.1 mol) and sodamide (7.8 g, 0.2 mol) in toluene (200 ml) under a nitrogen atmosphere and refluxed for 16 h. The reaction mass was cooled and the excess sodamide was quenched with methanol (50 ml). The reaction mass thus obtained was washed with water (400 ml) and the toluene was removed under reduced pressure to leave trimipramine as an oily viscous liquid (20.9 g, 71%). This was purified by column chromatography over silica using light petroleum–ethyl acetate (90 : 10) to afford trimipramine base as a pale yellow liquid, which solidified on cooling (17.5 g).

Synthesis of trimipramine maleate (maleate salt)

Trimipramine (6 g, 0.02 mol) was dissolved in 40 ml of dry acetone. Maleic acid (2.32 g, 0.02 mol) was added and the reaction mixture was heated for 30 min at 328–333 K. It was then cooled to 268–270 K under nitrogen to give white crystals of trimipramine maleate. The crystals thus obtained

were filtered and washed with cold acetone and dried under vacuum to leave white crystals of trimipramine maleate (7.34 g, 88.2%).

Synthesis of trimipramine mesylate (mesylate salt)

Trimipramine (6 g, 0.02 mol) was dissolved in 40 ml of dry acetone, cooled to 268–270 K under nitrogen and dry methanesulfonic acid (1.92 g, 0.02 mol) was added. The mixture was stirred for 2 h under nitrogen and then cooled to 258 K to give white crystals of trimipramine mesylate. The crystals thus obtained were filtered and washed with cold acetone and dried under vacuum to give white crystals of trimipramine mesylate (4.5 g, 57.6%). This salt is highly hygroscopic and, therefore, it was dried under nitrogen and kept under a nitrogen atmosphere.

Synthesis of trimipramine hydrochloride (hydrochloride salt)

Trimipramine (6 g, 0.02 mol) was dissolved in 40 ml of dry acetone, cooled to 268–270 K under nitrogen and dry HCl gas was purged into the reaction mass until the pH of the reaction medium was 2 as monitored with litmus paper. The crystals thus obtained were filtered and washed with cold acetone. The white crystals were dried under vacuum at 343–253 K for 4 h to give trimipramine hydrochloride (5.5 g, 83%).

NMR experiments

The NMR experiments were performed in deuterated chloroform (99.8% D, Aldrich, Milwaukee, WI, USA). For studying the broad acidic proton signals at temperatures lower than the freezing-point of chloroform, CD_2Cl_2 (99.8% D, Aldrich) was used and for 1D ^{15}N experiments, a 1 : 5 mixture of deuterated (DMSO-d_6; 99.8% D, Aldrich) and non-deuterated dimethyl sulfoxide were used as solvents. The experiments were performed on a Bruker Biospin Avance 400 spectrometer using 5 mm BBI and BBO probe heads. Normal 1D ^{1}H and ^{13}C experiments were performed at room temperature. 1D ^{1}H NMR spectra at variable temperature were obtained for all the three salts at 25 mM concentration, varying the temperature in steps of 10 K from 298 to 218 K in chloroform solution. For the maleate and the hydrochloride salts, ^{1}H spectra varying the temperature from 298 to 198 K in steps of 10 K were also obtained in dichloromethane solution. 1D ^{1}H spectra were measured with a spectral width, of 10 700 Hz, 32 K data points, 10 s relaxation delay, 45° pulse angle, 16 scans, and a spectral size of 32 K with 0.3 Hz line broadening. 1D ^{13}C spectra were obtained at room temperature using WALTZ-16 1H decoupling with spectral width 24 000 Hz, relaxation delay 3 s, time domain points 32 K, pulse angle 45°, number of scans 32 and a line broadening of 3 Hz. 1D ^{15}N experiments in DMSO-d_6 were performed at 323 K using a spectral width of 8090 Hz, 20 s relaxation delay, 32 K time domain points, 45° pulse angle, 4000 scans and a line broadening of 3 Hz.

For complete, unambiguous assignments of the ^{1}H, ^{13}C and ^{15}N signals, homonuclear and heteronuclear 2D experiments such as DQF-COSY,[7,8] ^{1}H–^{13}C gradient-enhanced heteronuclear single quantum correlation[9–11] (PFG-PEP-HSQC) and ^{1}H–^{13}C, ^{1}H–^{15}N gradient-enhanced heteronuclear multiple bond correlation[12] (PFG-HMBC) experiments were performed at room temperature. For assigning the broad signals arising from acidic protons, ^{1}H–^{13}C HMBC, DQF-COSY and ROESY[13,14] experiments were performed for the maleate salt at 223 K and for the mesylate salt at 263 K, both in $CDCl_3$.

For DQF-COSY and ROESY experiments, a spectral width of 10 700 Hz was used in both the dimensions. About 512 FIDs were obtained with t_1 incrementation, each of 2048 complex data points. Phase-sensitive data were obtained by the TPPI method.[15] The relaxation delay and the number of repetitions were 2.5 s and 16 for DQF-COSY and 1.5 s and 40 for ROESY experiments. A spin lock time of 150 ms was used for the ROESY experiment. For ^{1}H–^{13}C, HSQC and HMBC experiments, spectral widths of 10 700 Hz and 24 000 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 data points, 32 transients and a 2 s recycle delay. For ^{1}H–^{15}N HMBC experiments, a spectral width of 4800 Hz and 3650 Hz in the ^{1}H and ^{15}N dimensions, respectively, were used. About 400 FIDs were collected with t_1 incrementation, each of 2048 points, 64 transients and a 1.5 s recycle delay. Phase-sensitive data for HSQC experiments were obtained using the echo–antiecho method,[16] and for HMBC experiments, magnitude mode data were obtained. The resulting data from all 2D experiments were zero-filled to 1024 points in t_1 and double Fourier transformed after multiplying by a squared sine-bell window function shifted by $\pi/2$ along both dimensions.

^{13}C T_1 relaxation measurements were made at 298 K using the inversion–recovery method incorporating inverse gated proton decoupling. Parameters used were spectral width 24 000 Hz, time domain points 32 K, relaxation delay 50 s, number of scans 24, inversion–recovery delay varied between 25 ms and 50 s in 20 steps, spectrum size 64K and a line broadening of 3.0 Hz. From the resulting data, the relaxation times of individual carbons were calculated using Bruker Xwinnmr software version 3.1.

NMR experiments on the free trimipramine base were also performed, under identical conditions, for comparison of the results.

RESULTS AND DISCUSSION

All three trimipramine salts show a single set of signals with an anionic and cationic ratio of 1 : 1 as seen from the intensity of the NMR signals. Analyses of the ^{1}H, ^{13}C resonances were made using the combination of two-dimensional DQF-COSY, ROESY, PFG-PEP-HSQC, PFG-HMBC and variable-temperature experiments (Table 1). The structures of the free base and its three salts and the labeling are shown in Scheme 1.

For the maleate salt, a broad signal around 12 ppm (75 Hz linewidth) was observable at 298 K arising from one of the acidic protons, H-a or H-f, in both chloroform and dichloromethane. The other acidic proton signal appeared around 18 ppm, which was too broad to be clearly seen at

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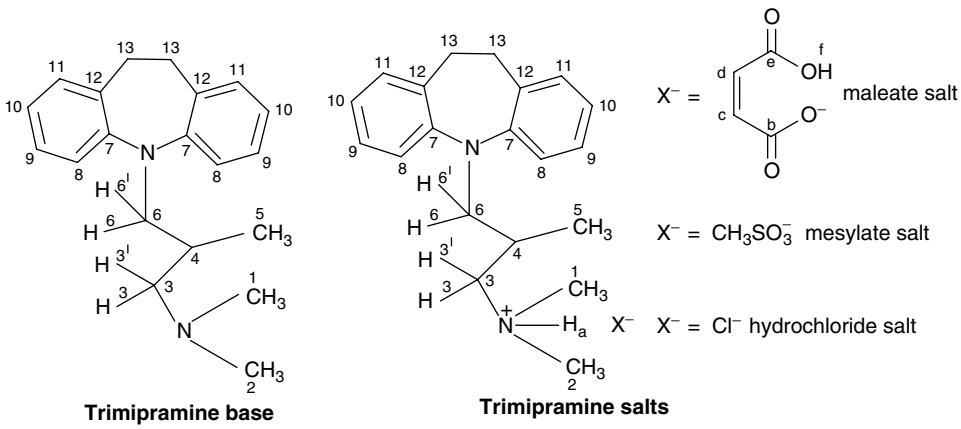
Table 1. ^{1}H and ^{13}C chemical shifts (in ppm relative to TMS) and ^{13}C T_1 relaxation times (s) of trimipramine base and its maleate, mesylate and hydrochloride salts

	Trimipramine			Maleate salt			Mesylate salt			Hydrochloride salt		
$^{1}H/^{13}C$ No.	^{1}H	^{13}C	T_1	^{1}H	^{13}C	T_1	^{1}H	^{13}C	T_1	^{1}H	^{13}C	T_1
1	2.14	46.18	0.98	2.50	41.98	0.48	2.49	42.25	0.71	2.41	42.18	0.76
2	2.14	46.18	0.98	2.71	44.83	0.48	2.84	45.55	0.65	2.73	45.18	0.76
3	2.04	65.54	0.98	2.85	62.68	0.32	2.84	62.95	0.34	2.80	62.78	0.29
3′	2.24	—	—	3.11	—	—	3.09	—	—	3.13	—	—
4	1.94	29.24	1.58	2.23	27.71	0.75	2.22	27.71	0.74	2.23	27.76	0.61
5	0.91	17.56	1.45	1.17	16.86	0.79	1.23	16.86	0.78	1.33	17.77	0.75
6	3.24	55.99	0.88	3.47	54.49	0.34	3.48	54.39	0.38	3.54	54.51	0.32
6′	3.95	—	—	3.81	—	—	3.82	—	—	3.82	—	—
7	—	148.57	10.39	—	147.35	4.36	—	147.34	4.48	—	147.36	3.81
8	7.08	119.97	1.49	7.05	119.52	0.63	7.08	119.59	0.68	7.09	119.71	0.57
9	7.11	126.26	1.46	7.16	126.87	0.62	7.16	126.90	0.63	7.16	126.91	0.56
10	6.90	122.27	1.34	6.96	123.40	0.61	6.96	123.40	0.61	6.96	123.35	0.54
11	7.10	129.79	1.50	7.12	130.21	0.66	7.12	130.18	0.65	7.11	130.11	0.60
12	—	134.08	8.23	—	133.81	3.46	—	133.77	3.55	—	133.71	2.91
13	3.18	32.25	1.16	3.17	32.10	0.66	3.16	32.10	0.65	3.17	32.09	0.60
a	—	—	—	12.39	—	—	10.72	—	—	12.08	—	—
f	—	—	—	18.46[a]	—	—	—	—	—	—	—	—

[a] At 273 K.



Scheme 1. Structures of the free base and its maleate, mesylate and hydrochloride salts.

298 K (linewidth ~800 Hz). As the solutions were cooled below room temperature, the linewidth of both the signals reduced significantly. Figure 1 shows parts of the one-dimensional spectra showing the acidic proton signals in dichloromethane as a function of temperature. For the assignment of the acidic proton signals, two-dimensional DQF-COSY, ROESY and HMBC spectra obtained at 223 K were used. The DQF-COSY spectrum recorded at 223 K shows a cross peak of the acidic proton at 12.3 ppm with amino dimethyls H-1, H-2 and the methylene protons H-3 whereas the signal at 18.98 ppm does not show any cross peak. The $^{1}H-^{13}C$ HMBC spectrum at 223 K shows correlation between carboxylic carbon of maleate and proton signal at 18.98 ppm. Further, the ROESY spectrum at 223 K shows an NOE cross peak between acidic proton signals at 18.98 ppm and maleate protons H-c and H-d. It is clear from these data that the signal at 12.3 ppm arises from H-a protonated at the terminal nitrogen and the signal at 18.98 ppm arises from H-f which is associated with the maleate moiety.

In the case of the hydrochloride and mesylate salts in chloroform, at room temperature, the signal due to acidic proton H-a appears at 12.08 and 10.72 ppm, respectively. In the DQF-COSY spectrum, these protons show correlation with protons H-1, H-2 and H-3, indicating protonation at the terminal nitrogen site.

Protonation of trimipramine in the three salts was compared using ^{1}H, ^{13}C chemical shifts and ^{13}C T_1 relaxation parameters. Figure 2 depicts the effect of protonation on the ^{1}H and ^{13}C chemical shifts and ^{13}C T_1 relaxation parameters. It was observed that for all three salts there is a significant change in the ^{1}H and ^{13}C chemical shifts only in the vicinity of protonation, the terminal nitrogen site [Fig. 2(a) and (b)]. Further, the magnitude of the reduction of the ^{13}C relaxation time of C-3 is significantly greater than for other carbons in all three salts [Fig. 2(c)]. The enhanced relaxation rate is also

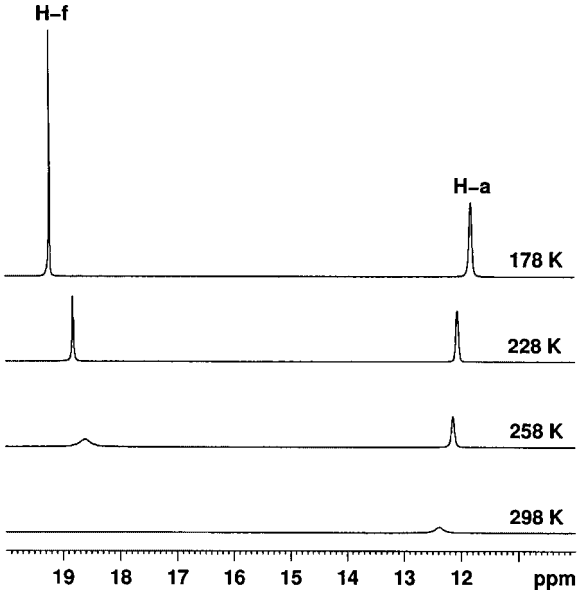


Figure 1. Parts of ^{1}H NMR spectra of trimipramine maleate in deuterated dichloromethane showing acidic proton signals at variable temperature. At 298 K the acidic proton signal at ~18 ppm (marked H-f at 178 K) is invisibly broad and the other acidic proton signal at ~12 ppm showed a linewidth of 75 Hz. As the solution is cooled below 298 K down to 178 K, the linewidths of both the signals decrease significantly.

indicative of protonation at the amino nitrogen site which is in the vicinity of C-3.

The acidic proton attached to nitrogen shifts towards less positive values with decrease in temperature for all three salts (Fig. 3). On the other hand, the acidic proton H-f of the maleate salt which is not attached to nitrogen shifts towards more positive values with decrease in temperature. The more negative shift of the protons attached to nitrogen upon cooling is typical for hydrogen-bonded protons in polar solvents where the dielectric constant increases.[17,18] As the hydrogen moves closer to the nitrogen, the dipole moment increases and the distance between the nitrogen and the anion increases because of the increase in solvent polarity. Consequently, the hydrogen bond with the anion becomes weaker. At the same time, the intramolecular hydrogen bond in maleate becomes more symmetric, leading to a low-field shift of the hydrogen bonded proton (H-f).[19]

The 1D ^{15}N spectra of trimipramine base and its three salts show two distinct resonances each, one corresponding to ring nitrogen and the other to terminal nitrogen. The $^{1}H–^{15}N$ PFG-HMBC spectra in all cases showed that the signal at more positive values correlates with aromatic proton H-8 and protons H-6, H-6′ and H-4, whereas the signal at less positive values shows correlation peaks to protons H-4, H-1 and H-2. Therefore, the signal at more positive values is assigned to the ring nitrogen and that at less positive values is assigned to terminal nitrogen. The ^{15}N chemical shifts for the three salts including the base were measured relative to an external reference, nitromethane, and are given in Table 2. The positive shift induced by the protonation from hydrochloride is maximum (18.73 ppm) followed by mesylate (15.29 ppm), while maleate induces

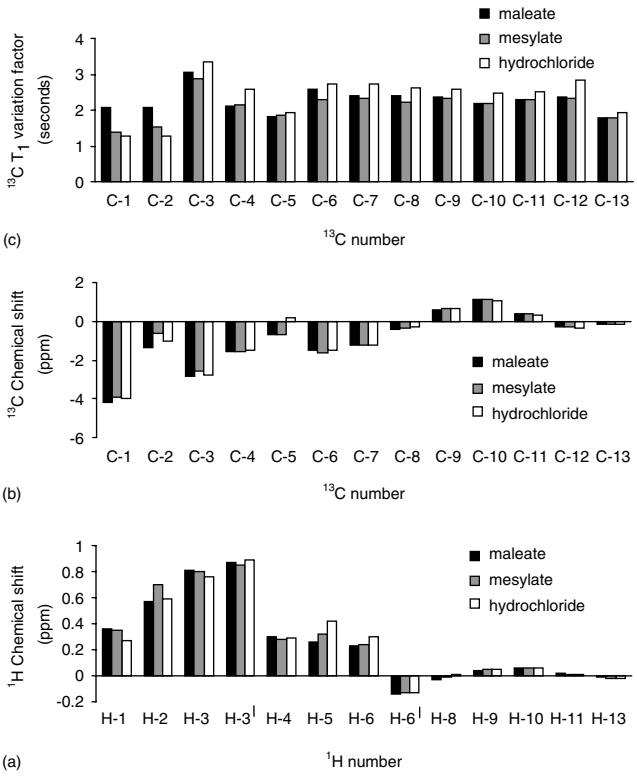


Figure 2. Depiction of variation of ^{1}H and ^{13}C chemical shifts and ^{13}C T_1 relaxation times in deuterated chloroform of trimipramine salts of maleate, mesylate and hydrochloride relative to that of free base, trimipramine, obtained under identical conditions. (a) Difference in ^{1}H chemical 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 trimipramine salts compared with those in its free base and vice versa. (c) Factor by which the ^{13}C T_1 relaxation times have decreased in trimipramine salts compared with those of the free base.

Table 2. ^{15}N chemical shifts (in ppm relative to external reference nitromethane) of trimipramine base and its maleate, mesylate and hydrochloride salts

Compound	Ring nitrogen	Terminal (protonated) nitrogen
Free base	−312.98	−358.30
Maleate salt	−314.17	−343.83
Mesylate salt	−314.16	−343.01
Hydrochloride salt	−313.66	−339.57

the least positive shift (14.47 ppm). On the other hand, the chemical shift of the ring nitrogen of the salts shifts towards less positive values compared with that of free base. However, the magnitude of the shift in this case is much less than that of the protonated nitrogen. ^{15}N chemicals shift data further unambiguously establish that only the terminal nitrogen is protonated in all the salts. The magnitude of the positive shift of the protonated nitrogen signal depends on

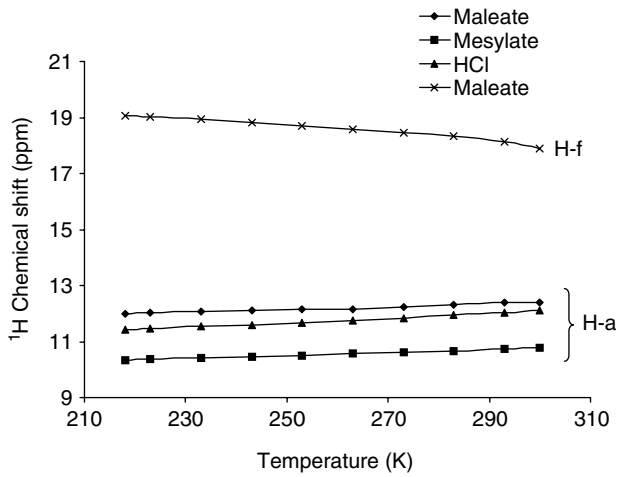


Figure 3. Plot showing the variation of chemical shift of the proton H-a for the three salts in chloroform as a function of temperature. As the temperature is decreased, the signal due to proton H-a shifts towards less positive values in all three salts. The proton H-f of the maleate salt, which is not protonated, shifts to more positive values, unlike protonated signals.

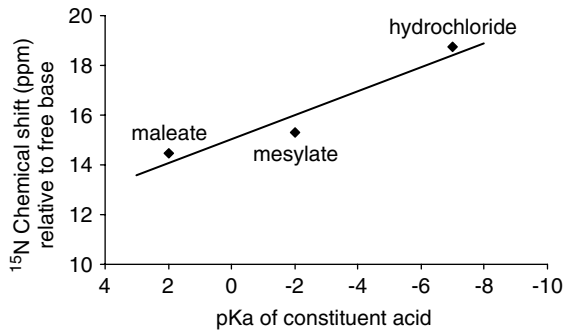


Figure 4. Plot of ^{15}N chemical shift of protonated nitrogen in trimipramine salts of maleate, mesylate and hydrochloride relative to that of the free base as a function of the pK_a of the constituent acid.

the pK_a of the constituent acid and it varies nearly linearly as shown in Fig. 4. From these data, it may be inferred that the protonation by the hydrochloride (p$K_a = -7$) is stronger compared with mesylate (p$K_a = -2$) and it is the weakest for maleate (p$K_a = +2$).

In conclusion, these studies on trimipramine salts show protonation of only the terminal nitrogen. Protonation of trimipramine appears to be stronger with hydrochloride, which has a more negative pK_a, and it is relatively weak with maleic acid, which has a more positive pK_a value. These characteristics of protonation of trimipramine may have implications for its functional activity as an antidepressant drug.

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