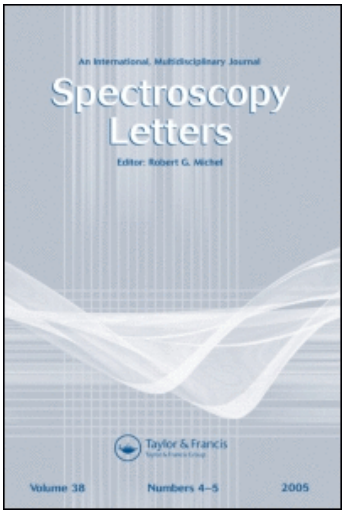

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NMR Studies of Drugs. ^{1}H and ^{13}C NMR Chemical Shift Assignments in Etidocaine and Etidocaine Hydrochloride Determined by Two-Dimensional NMR Spectroscopy

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NMR STUDIES OF DRUGS. ^{1}H and ^{13}C NMR CHEMICAL SHIFT ASSIGNMENTS IN ETIDOCAINE AND ETIDOCAINE HYDROCHLORIDE DETERMINED BY TWO-DIMENSIONAL NMR SPECTROSCOPY

Key words: 2D Heteronuclear shift correlation, Etidocaine, Etidocaine hydrochloride, Chiral nitrogen, Proton exchange.

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ABSTRACT

^{1}H and ^{13}C NMR chemical shift assignments were obtained for the local anesthetics etidocaine (1) and etidocaine hydrochloride (2) in $CDCl_3$ solution, as well as for 2 in D_2O solution. The COSY experiment was employed for proton-proton correlation, while one-bond and long-range 2D heteronuclear techniques allowed the assignments of all ^{13}C chemical shifts in each molecule. Etidocaine has a chiral carbon; etidocaine hydrochloride has, in addition to the natural chiral center, an acid-induced chirality at the protonated amine nitrogen, resulting in solvent-dependent

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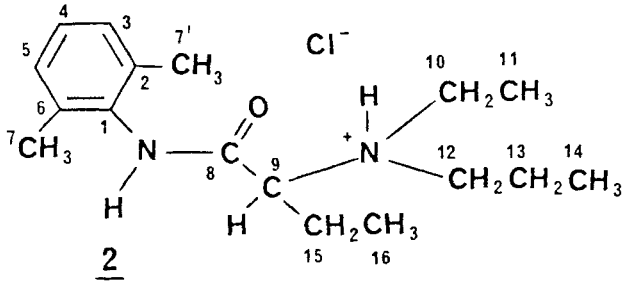
diastereomers. Ten of the fourteen magnetically nonequivalent ^{13}C nuclei of 2 exhibit doubled ^{13}C resonance peaks (50.3 MHz, 20°C, $CDCl_3$ solution) due to the presence of the two diastereomers.

INTRODUCTION

Local anesthetics, such as etidocaine, 1, are of pharmacological interest because they have the ability to block nerve conduction. The majority of the common local anesthetics consists of an aromatic ring joined to an aliphatic tertiary amine grouping by way of an amide or ester linkage. Etidocaine is of this type, with an additional interesting property: the carbon atom bonded to the aliphatic tertiary amine group is chiral (Fig. 1).

The pK_a of etidocaine (7.7 at 25°C) is close to that of the more widely used local anesthetic, lidocaine (7.9 at 25°C(1)). Therefore, at physiological pH values, the nitrogen atom of the tertiary amine group is predominantly protonated. Fig. 1 shows the structure of 2 with the numbering system used for the chemical shift assignments.

In our earlier work with lidocaine (2), the conformations of lidocaine free base were determined in CCl_4 solution via a combination of lanthanide-induced shift measurements and empirical conformational energy calculations. In these studies, we found that steric interactions between the aromatic ring methyl protons and the N-H and O atoms of the amide group force the plane of the phenyl ring to be nearly perpendicular to the plane of amide group. Since this portion of the etidocaine molecule is identical



2

Fig. 1 Structure of etidocaine hydrochloride.

to that of the lidocaine molecule, we expect the same type of orientation of ring plane to amide plane in etidocaine. No evidence of *cis/trans* isomerization about the amide bond is found. This agrees with NMR studies of pyridiniumacetanilide salts (acetanilide ring substituted in both *ortho* positions) in which only the *trans* conformation about the amide bond is found for secondary anilides (3), as well as with earlier NMR studies of similarly substituted amides (4).

In compounds of this type (acetanilide with di-ortho substituted ring) a second dynamic process is possible, namely, slow rotation about the N-C_{aryl} bond. This is usually observed only in the more sterically hindered tertiary anilides (4); we did not see any evidence of hindered rotation about the N-C_{aryl} bond for 1 or 2 at room temperature. The complexity of NMR resonances which we report for 2, as contrasted to 1, therefore, is not due to hindered rotation about either the amide bond or the N-C_{aryl} bond.

EXPERIMENTAL

Racemic etidocaine hydrochloride was a gift from Astra Pharmaceutical Products Inc., and was used without further purification. The free base 1 was prepared by neutralizing 2 with NaOH, extracting 1 into $CHCl_3$, and removing the $CHCl_3$ under vacuum. The melting points of 1 and 2 were 86.8°C and 203°C, respectively. Deuterated chloroform (99.8 atom %D), purchased from Aldrich, contained 0.03% v/v TMS as internal reference and was stored over molecular sieves. D_2O (99.8 atom %D), C_5D_5N (99.5 atom %D) and CD_3OD (99.9 atom %D) were purchased from MSD Isotopes. D_2O solutions contained a small amount of DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) which served as internal reference for ^{13}C. The reference for ^{13}C in $CDCl_3$ solutions was the central chloroform ^{13}C peak at 77.0 ppm, while the reference for ^{13}C in the C_5D_5N/CD_3OD solution was the central methanol ^{13}C peak at 49.0 ppm. Internal residual HOD served as reference for ^1H in D_2O solutions, while $CHCl_3$ at 7.26 ppm was the internal reference for $CDCl_3$ solutions. Solutions prepared for 1D studies had concentrations of approximately 0.179M (0.700 mL $CDCl_3$ added to 0.0345g 1, 0.199M (0.700 mL $CDCl_3$ added to 0.0434 g 2, or 0.243M (0.500 mL D_2O added to 0.0379 g 2) while solutions for 2D heteronuclear correlation experiments were more concentrated, approximately 0.5M for 1 in $CDCl_3$ and 0.4M for 2 in $CDCl_3$. Most solutions were measured in thin-walled 5-mm tubes; 10-mm tubes were however used to increase sensitivity for some of the COLOC experiments.

An IBM Instruments Inc. WP 200SY FTNMR spectrometer equipped with an Aspect 2000A data system, and a 10mm broadband probe was used to record ^{1}H spectra at 200.1 MHz and ^{13}C spectra at 50.3 MHz. Sample temperature was 20°C unless noted otherwise.

^{1}H spectra were accumulated with a sweep width of 2000 Hz over 16K points, giving a resolution of 0.244 Hz per point. Broadband decoupled ^{13}C spectra were accumulated with a sweep width of 10,000 Hz over 16K points, giving a resolution of 1.22 Hz per point. The Bruker microprogram GATEDEC.AU was used to acquire fully coupled ^{13}C spectra, with a delay of 5 s between scans. Exponential multiplication of the free induction decay (FID) with a line broadening of 0.1 Hz for ^{1}H or 4.0 Hz for ^{13}C was typically used before Fourier transformation.

The Bruker microprogram XHCORR.AU was used for the one-bond ^{13}C-^{1}H chemical shift correlation experiment (5). The ^{13}C spectral width was 9615 Hz for $\underline{1}$ and $\underline{2}$ over 2K points, giving a resolution of 9.39 Hz per point; 256 spectra with evolution time t_1 incremented by 0.00023 s gave a ^{1}H spectral width of 1074 Hz, which after zero filling gave a resolution of 4.98 Hz per point. The delays for transferring magnetization (Δ_1) and refocusing (Δ_2) are given in the figure legends. A 1 s delay was used between pulses. For each value of t_1, 256 transients were accumulated preceded by 4 dummy scans. The data in Fig. 5 was processed using exponential multiplication (LB = 2) in the ^{13}C dimension and a Gaussian window function (LB = -3, GB = 0.3) in the ^{1}H dimension, while that in Fig. 7 was processed with a shifted (θ = 45°) sine bell squared window function in each dimension.

The Bruker microprogram COLOC.AU was used for the long-range ^{13}C-^{1}H chemical shift correlation experiment (6). Parameters were the same as employed for the XHCORR.AU experiment with the exception of the delay times for transferring magnetization and refocusing. These were set equal to 50.0 ms and 33.0 ms, respectively, for the long-range heteronuclear shift correlation experiment.

RESULTS AND DISCUSSION

^{1}H Chemical Shift Assignments in Etidocaine

Fig. 2(a) shows the ^{1}H spectrum and Fig. 3 shows the upfield region of the COSY contour plot for 1. Cross peaks revealing ^{1}H-^{1}H couplings over two to four bonds show up in this homonuclear correlation experiment. The cross peak between the ring methyl protons and the ring protons H_3 and H_5 appears in the lower field region of the contour plot from which Fig. 3 was taken.

Assigning the highest field methyl resonance to position 14 allows the remaining protons in 1 to be assigned. Cross peaks can be seen from H_{14} at 0.87 ppm to H_{13} (complex multiplet at 1.50 ppm) and H_{12} (a triplet at 2.55 ppm). The methyl peaks at 1.07 ppm must then result from an overlapping of resonance peaks H_{11} and H_{16}. Cross peaks are observed between H_{16} and the two diastereotopic methylene protons H_{15a} and H_{15b} at 1.69 and 1.91 ppm. A small cross peak is also observed at lower contour levels (not shown) between H_{16} at 1.07 ppm, and H_9 at 3.23 ppm from coupling over four bonds to the proton on the chiral carbon (H_9). The other methyl peak resonating at 1.07 ppm, H_{11}, has a cross

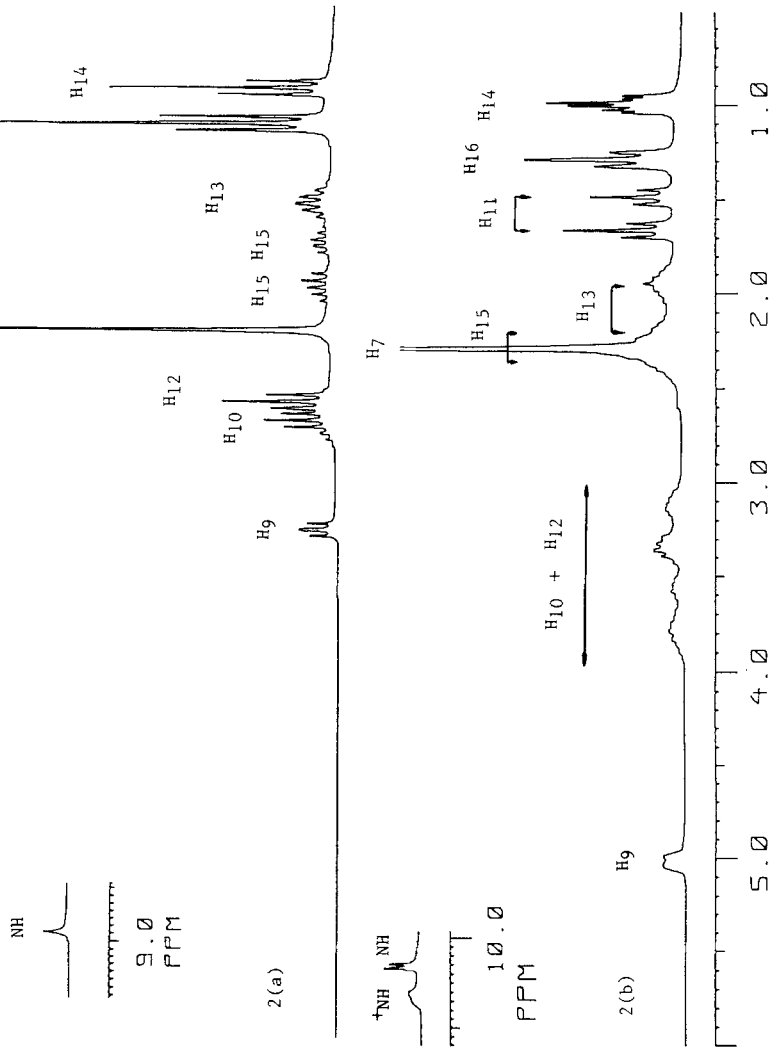


Fig. 2 200.1 MHz ^1H NMR spectra of: (a) 0.179 M etidocaine in $CDCl_3$; (b) 0.199 M etidocaine hydrochloride in $CDCl_3$.

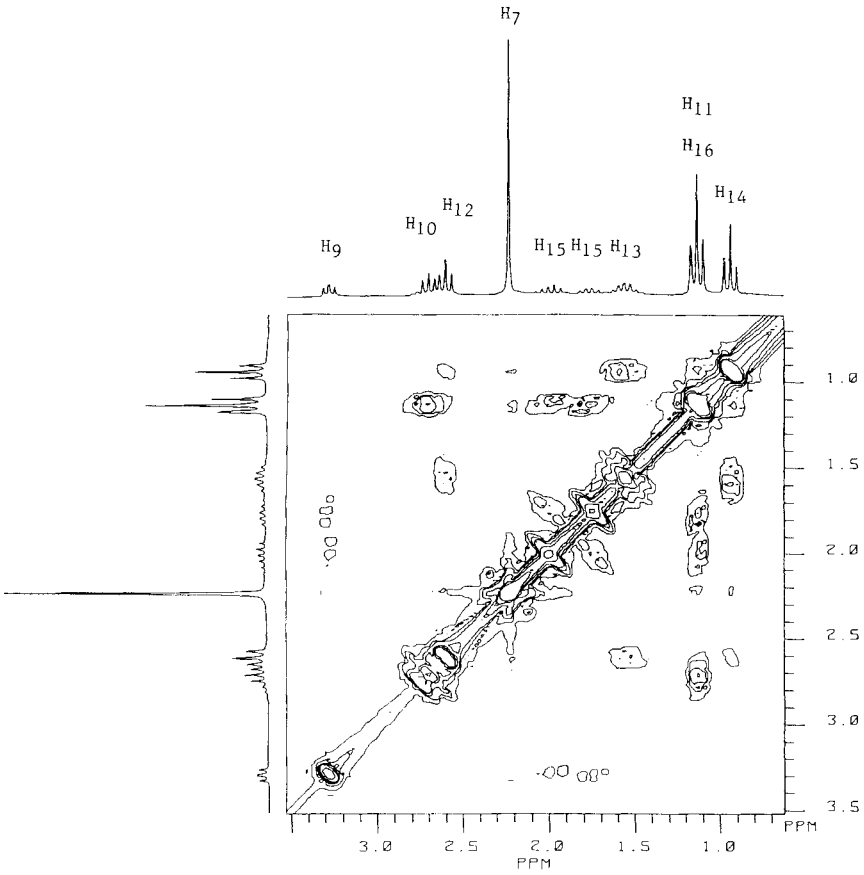


Fig. 3 Contour plot of the upfield region of the two-dimensional ^1H-^1H correlated (COSY) NMR spectrum of 0.179 M etidocaine in $CDCl_3$.

peak with the methylene protons (H_{10}) at 2.64 ppm. In the 1D spectrum, the H_{10} resonance peak is a quartet. The remaining cross peaks, confirming these assignments, occur between H_9 at 3.23 ppm and the diastereotopic methylene protons H_{15a} and H_{15b} at 1.69 and 1.91 ppm. A cross peak is also evident between H_{15a} and H_{15b}. The ^1H chemical shift assignments are summarized in Table 1.

TABLE 1

^{1}H Chemical Shifts (δ) of 1 and 2

^{1}H No.[a]	1[b]	2[c]	2[d]
3,5	7.02	7.00	7.24
4	7.02	7.00	7.24
7	2.17	2.26	2.26
9	3.23	5.10-4.95[e]	4.29
10	2.64	(3.8,3.2)[f](3.5,3.2)[g]	3.41
11	1.07	1.64,1.45[h]	1.41
12	2.55	(3.3,3.0)[i](3.6,3.0)[j]	3.27
13	1.50	2.20,1.9[k]	1.85
14	0.87	0.98,0.96[k]	1.04
15	1.91,1.69[l]	~2.2[m]	2.19
16	1.07	1.27	1.19
NH(amide)	8.90	10.34,10.29[k]	----
$^+$NH(ammonium)	----	10.6[n]	----

[a] See Fig. 1 for proton numbering.
[b] 0.179M in $CDCl_3$; see Fig. 2(a)
[c] 0.199M in $CDCl_3$; see Fig. 2(b)
[d] 0.242M in D_2O
[e] Broad X resonance of ABX spin system. Doubled due to RR and RS diastereomers.
[f] Methylene protons (δ_A and δ_B) from minor diastereomer correlated to C_{10} resonance at 46.10 ppm. (Fig. 8).
[g] Methylene protons (δ_A and δ_B) from major diastereomer correlated to C_{10} resonance at 49.94 ppm. (Fig. 8).
[h] The resonance at 1.64 ppm is from the major diastereomer.
[i] Methylene protons (δ_A and δ_B) from minor diastereomer correlated to C_{12} resonance at 55.44 ppm. (Fig. 8).
[j] Methylene protons (δ_A and δ_B) from major diastereomer correlated to C_{12} resonance at 52.28 ppm. (Fig. 8).
[k] Doubled due to RR and RS diastereomers.
[l] Doubled due to diastereotopic methylene protons.
[m] Broadened complex multiplet overlapping H_7 peak.
[n] Broad unsymmetrical peak.

^{13}C Chemical Shift Assignments in Etidocaine

Fig. 4(a) shows the fully decoupled ^{13}C spectrum of 1 in $CDCl_3$ while the 2D one-bond heteronuclear correlation (XHCORR) contour diagram is given in Fig. 5. The non-protonated carbons, C_1, C_2, C_6, and C_8 do not have cross peaks in the correlation diagram, but they are easily assigned since the carbonyl carbon, C_8, will be at lowest magnetic field, while C_2 and C_6 resonate together (135.00 ppm) at approximately twice the intensity of C_1 (134.36 ppm). The diastereotopic methylene protons H_{15a} and H_{15b} at 1.91 and 1.69 ppm have cross peaks with C_{15} at 19.80 ppm. Other ^{13}C resonances may similarly be assigned from the correlation diagram, Fig. 5, and are collected in Table 2. The only difficulty in assignment of the ^{13}C resonances came from C_{11} and C_{16}, since H_{11} and H_{16} are overlapped in the ^{1}H spectrum. To resolve this question, a long-range heteronuclear correlation experiment (COLOC) was carried out for 1 in $CDCl_3$. The lower field ^{13}C signal at 13.74 ppm is C_{11}, as it shows a correlation peak with H_{10} at 2.64 ppm. The resonance at 13.35 ppm is therefore C_{16}. Other COLOC cross peaks confirm the ^{13}C assignments already discussed.

^{1}H Chemical Shift Assignments in Etidocaine Hydrochloride

The ^{1}H NMR spectrum of 2 in $CDCl_3$ is shown in Fig. 2(b). Chemical shift assignments in 2 cannot be made directly from 1 due to the large differences in the spectrum as a result of protonation at the amine nitrogen. Resonance peaks from protons in the vicinity of the amine nitrogen are broadened and show

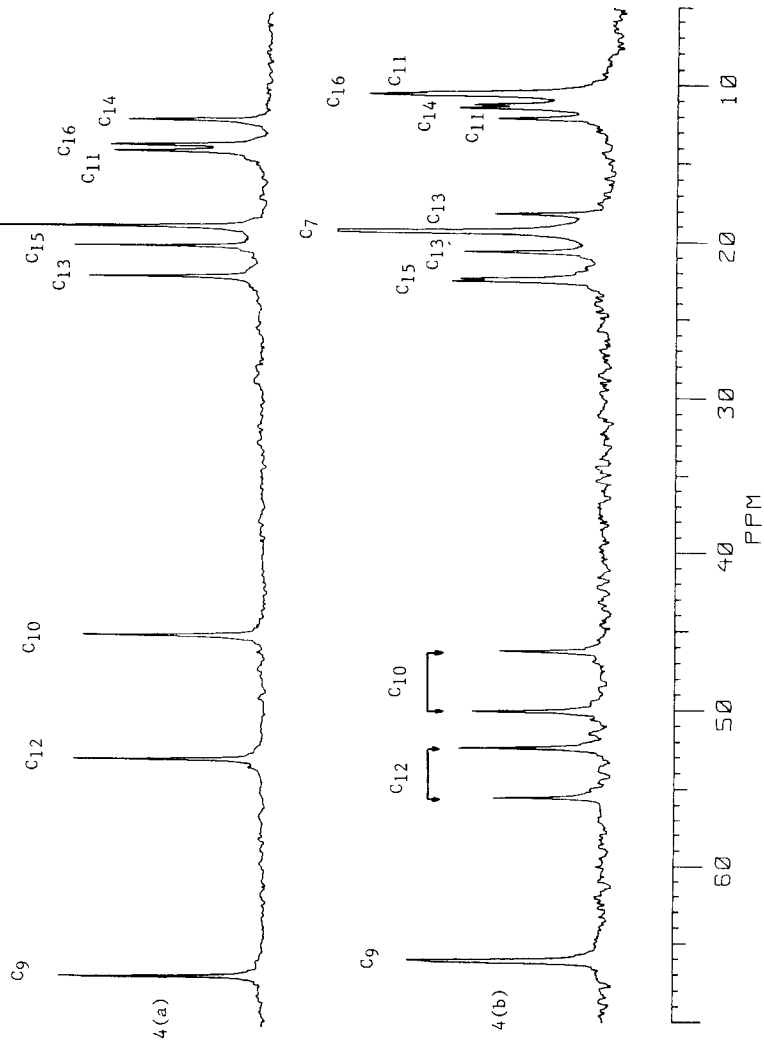


Fig. 4 50.3 MHz ^{13}C NMR spectra of: (a) 0.179 M etidocaine in $CDCl_3$; (b) 0.199 M etidocaine hydrochloride in $CDCl_3$.

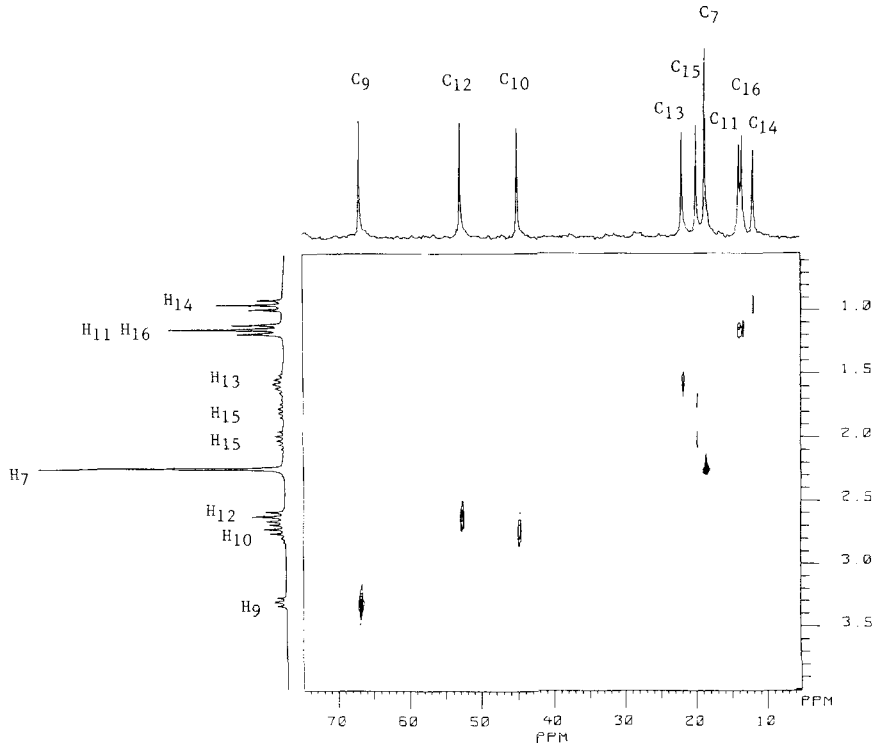


Fig. 5 Contour plot of the upfield region of the two-dimensional ^{13}C-^{1}H correlated (XHCORR) NMR spectrum of 0.5 M etidocaine in $CDCl_3$. Optimized for one-bond couplings ($\Delta_1 = \Delta_2 = 2.85$ ms).

complex multiplet structure. The complexity is due to the creation of a second chiral center at the quaternary nitrogen atom. Proton exchange at the ammonium nitrogen (in $CDCl_3$) is slow on the NMR chemical exchange timescale so that direct evidence for two pairs of diastereomers can be seen. These shall be referred to as RR (which is magnetically equivalent to SS) and RS (which is magnetically equivalent to SR). The first letter refers to the C_9

TABLE 2

^{13}C Chemical Shifts (δ) of 1 and 2

^{13}C No.[a]	1[b]	2[c]	2[d]	2[e]
1	134.36	133.34	134.60	134.99
2,6	135.00	134.79	137.99	136.32
3,5	128.09	128.18	130.90	129.25
4	126.69	127.27	130.90	128.39
7	18.53	19.24,19.18[f]	20.26	19.41
8	172.53	165.30,165.24[f]	169.76	167.85
9	66.67	66.08,65.97[f]	68.42	67.06
10	44.79	49.94,46.10[f,g]	50.16,47.33[f]	46.96
11	13.74	11.98,10.30[f,h]	11.31,9.79[f]	10.70
12	52.64	55.44,52.28[f,i]	56.01,53.08[f]	53.35
13	21.83	20.51,18.08[f]	21.54,18.62[f]	19.19
14	11.75	11.75,11.32[f]	12.58	11.50
15	19.80	22.46,22.30[f]	24.13	22.98
16	13.35	10.50,10.45[f]	11.31	10.36

[a] See Fig. 1 for carbon numbering.
[b] 0.179M in $CDCl_3$. Referenced to $^{13}CDCl_3$ at 77.0 ppm.
[c] 0.199M in $CDCl_3$. Referenced to $^{13}CDCl_3$ at 77.0 ppm.
[d] 0.242M in D_2O. Referenced to DSS at 0.0 ppm.
[e] 0.319M in C_5D_5N/CD_3OD (1:1 v/v). Referenced to $^{13}CD_3OD$ at 49.0 ppm.
[f] Doubled due to RR and RS diastereomers.
[g] The resonance at 49.94 ppm is due to the major diastereomer.
[h] The resonance at 10.30 ppm is due to the major diastereomer.
[i] The resonance at 52.28 ppm is due to the major diastereomer.

chiral center while the second letter refers to the chiral center at the protonated amine nitrogen. When the chemical shift from a given proton in the RR isomer is different from that of the RS isomer, the difference shall be referred to as $\Delta\delta_{RS}$. (These experiments distinguish between RR and RS when $\Delta\delta_{RS} \neq 0$, however they cannot be used to assign the two peaks to the RR *vs.* the RS

diastereomer). With the exception of the aromatic ring protons, all ^1H peaks move downfield upon protonation, as expected (7), Table 1. The methyl protons at H_{14}, being part of a propyl group, will resonate to highest magnetic field, at 0.96 and 0.98 ppm. The methyl triplets between 1.64 and 1.27 ppm were assigned to H_{11} and H_{16} from the magnitude of the protonation shifts in $CDCl_3$ ($\delta(\underline{2})$-$\delta(\underline{1})$, Table 1). The sum of the integrals of the triplets at 1.64 and 1.45 ppm corresponds to three protons, as does the resonance at 1.27 ppm. The H_{16} protons, being four bonds removed from the protonated nitrogen, shift only 0.20 ppm downfield upon protonation. The H_{11} protons, being three bonds removed from the protonated nitrogen, shift downfield by 0.57 and 0.38 ppm (Table 1). A chemical shift difference of $\Delta\delta_{RS} = 0.19$ ppm exists between the H_{11} resonances of the RR *vs.* the RS diastereomers of $\underline{2}$. The ratio of the integrated peak areas of the two H_{11} resonances is 53:47. Once the methyl protons have been assigned, the broad N-methylene peaks in the region 4.0-3.0 ppm and the broad C-methylene peaks in the region 2.4-1.8 ppm may be identified from the COSY contour plot (Fig. 6). The chiral center at the tertiary ammonium nitrogen atom in $\underline{2}$ greatly increases the spectral complexity of $\underline{2}$ as compared with $\underline{1}$. Separate NMR signals may be observed not only for each diasterotopic proton CH_aH_b in a given methylene group, but also, a second set of signals may result from the diasteromeric salts $\underline{2}$-(RR) versus 2-(RS). For example, the two broad COSY cross peaks with H_{14} (0.98,0.96 ppm) at about 1.9 and 2.2 ppm are combinations of $(H_{13a,b})_{RR}$ and $(H_{13a,b})_{RS}$. The

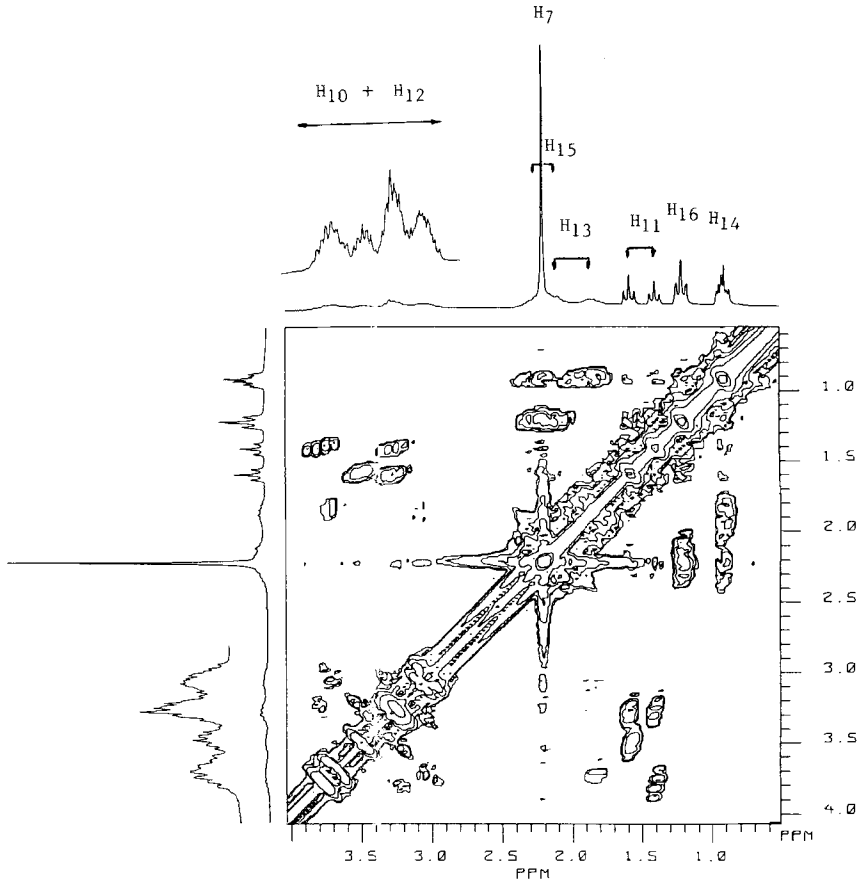


Fig. 6 Contour plot of the upfield region of the two-dimensional ^1H-^1H correlated (COSY) NMR spectrum of 0.199 M etidocaine hydrochloride in $CDCl_3$.

methyl protons H_{16} (1.27 ppm) have a broad cross peak with the $H_{15a,b}$ methylene protons between 2.1 and 2.4 ppm. The H_{11} resonance (1.45 ppm) from the minor diastereomer, $(H_{11})_{minor}$ has two COSY cross peaks in the H_{10} and H_{12} methylene region. These must be H_{10a} and H_{10b} (at about 3.2 and 3.8 ppm). $(H_{11})_{major}$ also

has two COSY cross peaks which are due to H_{10a} and H_{10b} from the major diastereomer (at about 3.2 and 3.5 ppm). Due to the width of the H_{13} resonances, and the interference from H_7, the COSY H_{12a} and H_{12b} cross peaks with H_{13} are much smaller than those between H_{11} and H_{10}. These proton assignments are discussed below in connection with the heteronuclear shift correlation spectrum.

The methine peak from H_9 (also close to the tertiary ammonium nitrogen) is also broadened (Fig. 2(b)). The proton on the ammonium nitrogen appears broad and asymmetrical at 10.6 ppm, while the amide NH proton resonance doubles, appearing at 10.34 and 10.29 ppm, with $\Delta\delta_{RS}$ therefore equal to 0.05 ppm for the latter resonance. The ^1H chemical shift assignments are collected in Table 1.

^{13}C Chemical Shift Assignments in Etidocaine Hydrochloride

The fully ^1H decoupled 1D ^{13}C NMR spectrum of 2 in $CDCl_3$ is shown in Fig. 4(b). Immediately apparent in this figure or in the expanded spectrum (not shown, but see Table 2) is the doubling of many of the resonance lines. This doubling of the ^{13}C peaks is due to the presence of the RR and RS diastereomers. All of the ^{13}C resonances may be assigned by using first the one-bond and then the long-range heteronuclear correlation experiments. The upfield region of the contour diagram for the XHCORR experiment for 2 in $CDCl_3$ is shown in Fig. 7. Not shown in this Figure therefore, is the cross peak between the broad H_9 resonance at 5.0 ppm and the two C_9 resonances, at 66.08 and 65.97 ppm. The sharp singlet resonance for the H_7 methyl triplets at 2.26 ppm has a

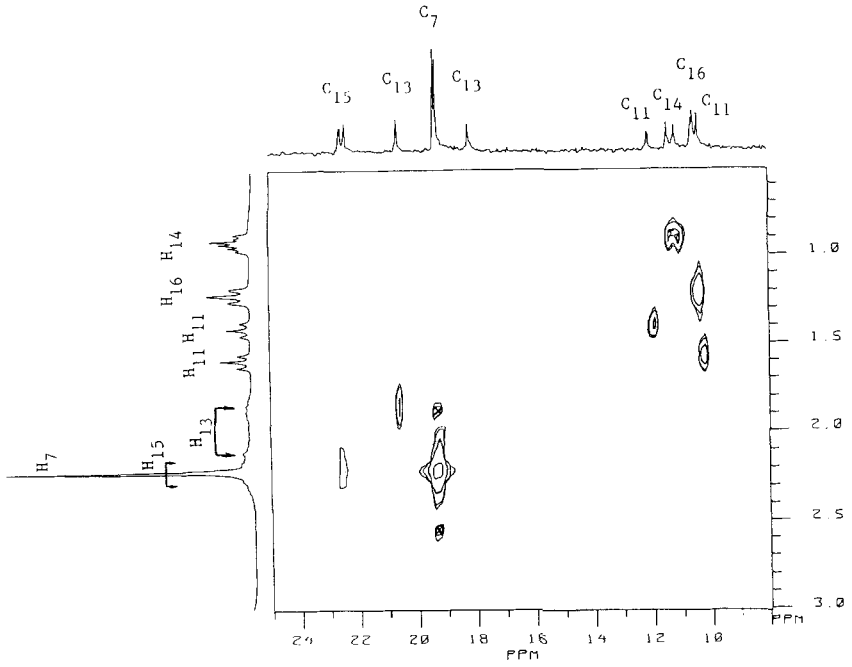


Fig. 7 Contour plot of the upfield region of the two-dimensional ^{13}C-^{1}H correlated (XHCORR) NMR spectrum of 0.4 M etidocaine hydrochloride in $CDCl_3$. Optimized for one-bond couplings (Δ_1 = 3.3 ms, Δ_2 = 2.2 ms).

cross peak with two C_7 resonances at 19.24 and 19.18 ppm. The broadened ^{1}H peaks which resonate near H_7 at 2.2 ppm, and were assigned to H_{15} from the COSY diagram (Fig. 6), have a small cross peak with the two C_{15} resonances at 22.46 and 22.30 ppm. The $(H_{11})_{major}$ peak at 1.64 ppm correlates with the $(C_{11})_{major}$ peak at 10.30 ppm, while the $(H_{11})_{minor}$ peak at 1.45 ppm correlates with the $(C_{11})_{minor}$ peak at 11.98 ppm. Due to the small value of $\Delta\delta_{RS}$ for H_{14} (0.02 ppm = 4 Hz) and to the fact that the resolution in

the ^1H dimension of the XHCORR experiment was only 4.98 Hz per point, the two H_{14} resonances at 0.98 and 0.96 ppm have a single cross peak with the two C_{14} resonances at 11.75 and 11.32 ppm. It is indeed surprising that the C_{14} nuclei, being three bonds removed from the chiral nitrogen atom, have such a large value for $\Delta\delta_{RS}$ (0.43 ppm in $CDCl_3$). The H_{16} resonance at 1.27 ppm correlates with the two closely spaced C_{16} resonances at 10.50 and 10.45 ppm. One of the broadened H_{13} protons (at 1.9 ppm) has a cross peak with C_{13} at 20.51 ppm. The other C_{13} peak at 18.08 ppm does not show a cross peak, and must be assigned from temperature studies and/or solvent effects, which will be discussed below. The shortening of the spin-spin relaxation times (T_2) of broadened protons (H_9, H_{10}, H_{12}, H_{13}, H_{15}) reduces the amount of transverse magnetization available for transfer to ^{13}C; thus some of the expected cross peaks are absent in the contour diagram.

The most difficult part of the assignment of the ^{13}C spectrum of 2 in $CDCl_3$ was the assignment of the C_{10} and C_{12} resonances. The expanded XHCORR contour plot for this region (Fig. 8) was quite interesting, as it showed that each C_{10} peak and each C_{12} peak is correlated with at least two H_{10} and H_{12} peaks (for example, H_{10a} and H_{10b} in each diastereomer). The chemical shifts which are listed for H_{10} and H_{12} in Table 1 were obtained from the intersection of the cross peaks in Fig. 8 with the ^1H spectrum on the vertical axis. The long-range heteronuclear correlation experiment (COLOC) was used to assign the C_{10} and C_{12} resonances. An expanded region of the contour diagram for the COLOC experiment

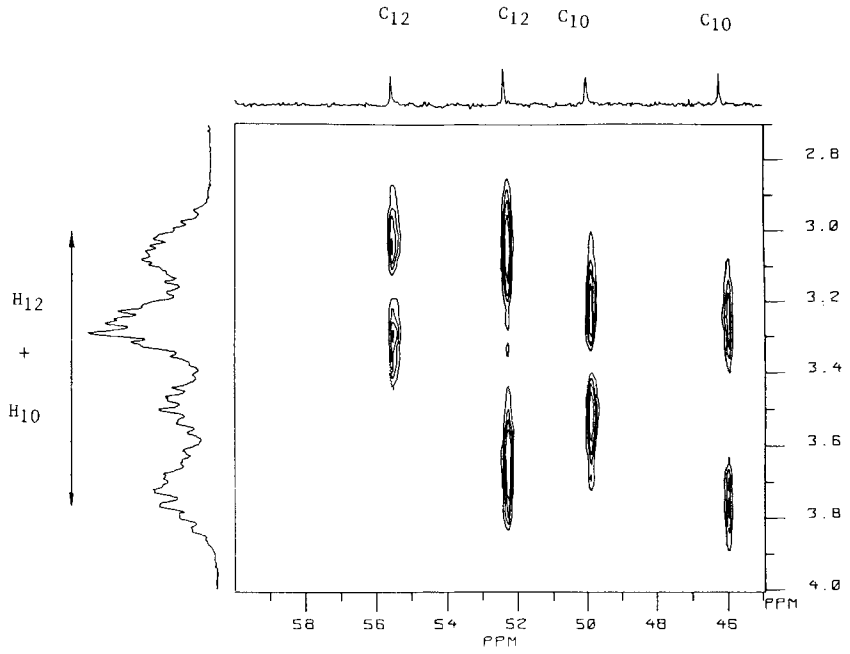


Fig. 8 See legend to Fig. 7. This is a different region of the contour plot.

on 2 in $CDCl_3$ is shown in Fig. 9. The methyl protons $(H_{11})_{major}$ at 1.64 ppm are correlated to $(C_{10})_{major}$ at 49.94 ppm, while the methyl protons $(H_{11})_{minor}$ at 1.45 ppm are correlated to $(C_{10})_{minor}$ at 46.10 ppm. This confirms the assignment of the two methylene carbons to higher magnetic field (in the 50-45 ppm region) to C_{10}, since a two-bond correlation (H_{11}/C_{10}) is expected to have a more intense cross peak than a four-bond correlation (H_{11}/C_{12}) through a nitrogen atom. There is also a three-bond correlation peak between the methyl protons H_{14} and the C_{12} resonance at 52.28 ppm.

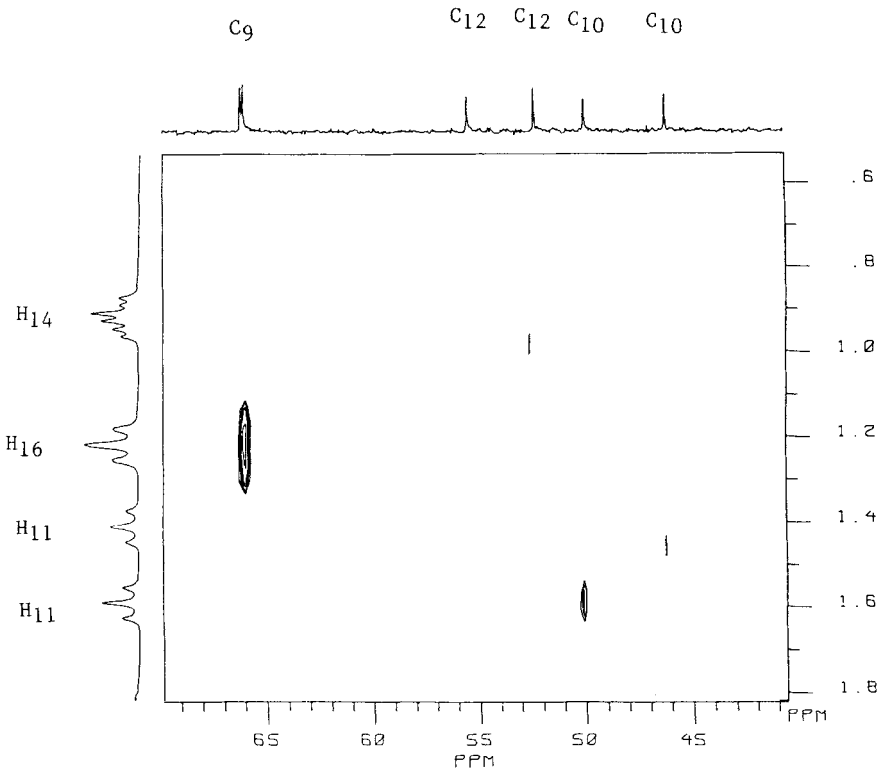


Fig. 9 Contour plot of an upfield region of the two-dimensional ^{13}C-^{1}H correlated (COLOC) NMR spectrum of 0.4 M etidocaine hydrochloride in $CDCl_3$. Optimized for long-range couplings (Δ_1 = 50.0 ms, Δ_2 = 33.0 ms).

The assignment of the fourth ^{13}C resonance in this region to C_{12} at 55.44 ppm comes from the temperature and solvent studies discussed below. Fig. 9 also shows an unusually strong COLOC cross peak over three bonds between the chiral carbon, C_9 (66.08 and 65.97 ppm) and the methyl protons, H_{16} (1.27 ppm).

The ^{13}C resonances from the phenyl ring were assigned from the XHCORR diagram as they were for 1, and the carbonyl resonance, C_8, showed splitting due again to the presence of the RR and RS diastereomers. The ^{13}C chemical shift assignments are summarized in Table 2. Note that the protonation shift in $CDCl_3$ ($\delta(2)-\delta(1)$) for carbons α to the amine nitrogen are downfield for C_{10} and C_{12}, (with however a small upfield shift for C_9). Downfield α-^{13}C protonation shifts have been observed for tertiary amines, such as for the methylene carbons of triethylamine (8). Carbons substituted β or γ to the amine nitrogen, however, usually show upfield shifts on protonation (8), as is observed for C_8, C_{11}, C_{13} and C_{16} (Table 2). C_{15} however, is an exception to the general trend for tertiary amines as it shifts approximately 2.5 ppm downfield upon protonation.

Temperature and Solvent Studies of Etidocaine Hydrochloride

The ^1H and ^{13}C chemical shifts of 1 and 2 in D_2O are given in Tables 1 and 2. Using D_2O as a solvent instead of $CDCl_3$ facilitates proton exchange, and many of the peaks which were doubled for 2 in $CDCl_3$, for example, C_7, C_8 and C_9, can be observed only as singlets at 50.3 MHz in D_2O. However, the RR and RS diastereomers must persist for 2 in D_2O, since many of the ^{13}C peaks which were singlets for 1 in $CDCl_3$ remain doubled for 2 in D_2O (for example, C_{10}, C_{11} and C_{12}). However, when D_2O is replaced with a more strongly basic solvent such as a 1:1 mixture of C_5D_5N and CD_3OD, all doubled ^{13}C peaks become singlets. In this solvent mixture, proton exchange at the ammonium nitrogen site in 2 is so

rapid that the nitrogen loses its configurational stability on the NMR timescale and there exists once again a simple mixture of enantiomers. The ^1H spectrum of 2 in the pyridine/methanol solvent mixture shows all sharp multiplets, as opposed to the broadened multiplets for 2 in $CDCl_3$ solution (Fig. 2(b)).

Similarly, temperature studies confirm that 2 in D_2O exists as a mixture of RR and RS diastereomers. As the temperature is raised, proton exchange at the ammonium nitrogen increases and the nitrogen begins to lose its configurational stability. When the temperature of a solution of 2 in D_2O is raised to 90°C, the two ^{13}C resonances assigned to C_{12} merge and are still broad at 55.5 ppm. Similarly, the two ^{13}C resonances assigned to C_{10} merge into one broad peak at 49.4 ppm. At 90°C in D_2O, all of the other ^{13}C peaks are sharp singlets. $\Delta\delta_{RS}$ is largest at 20°C for C_{10} and C_{12}; therefore a higher temperature is required to observe coalescence of their ^{13}C resonance peaks. These temperature studies confirm that the ^{13}C resonance peaks at 50.16 and 47.33 ppm for 2 in D_2O arise from the same ^{13}C (C_{10}) in two different diastereomers, while the ^{13}C peaks at 56.01 and 53.08 ppm arise from C_{12}.

Conclusion

1D NMR studies and the 2D NMR experiments COSY, XHCORR and COLOC were used to assign ^1H and ^{13}C resonances in the local anesthetics etidocaine and etidocaine hydrochloride. Near ambient temperatures, racemic etidocaine, 1, effectively exists in $CDCl_3$ as a simple mixture of enantiomers, while for etidocaine hydrochloride, 2, the proton on the tertiary ammonium nitrogen

exchanges so slowly in $CDCl_3$ that a second chiral center is created (on the NMR time scale). At 50.3 MHz, and ambient temperatures, a doubling of ^{13}C resonance peaks due to RR and RS diastereomers may be observed for 10 of the 14 magnetically nonequivalent ^{13}C nuclei of 2 in $CDCl_3$ (the aromatic carbons are presumably too far away from the chiral centers). The environment of 2 changes greatly when the relatively nonpolar solvent $CDCl_3$ is replaced by D_2O. This is reflected by the fact that many of the previously doubled ^{13}C peaks become singlets in D_2O (Table 2). Resonances for C_{10}-C_{13} are still doubled in D_2O, therefore, proton exchange at the ammonium nitrogen atom, while expected, is still slow enough to maintain the chirality at the nitrogen (on the NMR timescale). Upon changing the environment of 2 to pyridine/methanol solution, however, all ^{13}C peaks become singlets. Proton exchange with pyridine is fast enough to destroy the configurational stability of nitrogen. Heating 2 in D_2O to 90°C also renders the nitrogen achiral, i.e., the interconversion RR ⇌ RS occurs rapidly on the NMR timescale.

The ^1H and ^{13}C NMR spectra of 2 are very sensitive to environment and may be used to study solvent effects upon protonation as well as to determine the kinetic parameters for proton exchange. We have carried out ^1H temperature studies of 2 in D_2O at 500 MHz and extended these measurements to other solvents (9).

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