intensity) 244 (100, M^+). Anal. ($C_6H_{15}I_2NO$) H, N, I; C: calcd, 19.41; found, 19.83.

(Chloromethyl)(2-acetoxyethyl)dimethylammonium iodide (8), mp 66 °C, was similarly prepared in 70% yield: ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Cl), 4.57 (t, 2 H, CH_2OAc), 3.87 (t, 2 H, $-NCH_2CH_2-$), 3.30 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOH_3$); ^{13}C NMR (D_2O) δ 175.2 ($OCOCH_3$), 72.3 (NCH_2Cl), 63.9 (CH_2O-Ac), 53.0 ($N(CH_3)_2$), 23.0 ($OCOCH_3$); FAB MS *m/z* (relative intensity) 182 (33, M^+), 180 (100, M^+). Anal. ($C_7H_{15}ClINO_2$) C, H, N, Cl.

(Bromomethyl)(2-acetoxyethyl)dimethylammonium bromide (9), mp 58 °C, was similarly prepared in 66% yield: ^{1}H NMR (D_2O) δ 5.25 (s, 2 H, NCH_2Br), 4.55 (t, 2 H, CH_2OAc), 3.90 (t, 2 H, $-NCH_2CH_2-$), 3.35 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOCH_3$); ^{13}C NMR (D_2O) δ 175.8 ($OCOCH_3$), 65.0 (CH_2OAc), 61.0 (NCH_2Br), 60.7 ($-NCH_2CH_2-$), 54.1 ($N(CH_3)_2$), 23.0 ($OCOCH_3$); FAB MS *m/z* (relative intensity) 226 (37, M^+), 224 (37, M^+), 146 (100, M^+ - Br). Anal. ($C_7H_{15}Br_2NO_2$) C, H, N, Br.

(Iodomethyl)(2-acetoxyethyl)dimethylammonium iodide (10), mp 100 °C, was similarly prepared in 76% yield: ^{1}H NMR (D_2O) δ 5.30 (s, 2 H, NCH_2I), 4.55 (t, 2 H, CH_2OAc), 3.90 (t, 2 H, NCH_2CH_2), 3.35 (s, 6 H, $N(CH_3)_2$), 2.15 (s, 3 H, $OCOCH_3$); ^{13}C NMR (D_2O) δ 175.5 ($OCOCH_3$), 65.9 (CH_2OAc), 60.7 ($N(CH_2CH_2)$, 55.4 ($N(CH_3)_2$), 35.3 (NCH_2I), 23.0 ($OCOCH_3$); FAB MS *m/z* (relative intensity) 272 (100, M^+). Anal. ($C_7H_{15}I_2NO_2$) C, H, N, I.

(Iodomethyl)(2-acetoxypropyl)dimethylammonium iodide (11), mp 143-144 °C, was similarly prepared in 17% yield: ^{1}H NMR (D_2O) δ 5.60-5.05 (m, 3 H, $CH(CH_3)O$, NCH_2I), 4.20-3.45 (m, 2 H, NCH_2CH), 3.30 (s, 6 H, $N(CH_3)_2$), 2.10 (s, 3 H, $OCOCH_3$), 1.32 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 178.5 ($OCOCH_3$), 73.1 ($CH(CH_3)OAc$), 71.8 ($-NCH_2CH-$), 58.5 ($N(CH_3)_2$), 38.0 (NCH_2I), 26.5 ($OCOCH_3$), 23.4 ($CHCH_3$); FAB MS *m/z* (relative intensity) 286 (100, M^+). Anal. ($C_8H_{17}I_2NO_2$) C, H, N; I: calcd, 61.50; found, 61.07.

Preparation of 3,3-Dimethyloxazolidinium Bromide (14). To a magnetically stirred and ice-cooled solution of dimethylfluoromethylamine (12) (1.37 g, 17.8×10^{-3} mol) in dry THF (5 mL) was added bromoethanol (2.22 g, 17.8×10^{-3} mol). The solution was stirred at 0-4 °C for 15 min. The white solid was filtered and washed several times with dry THF. Removal of THF on a rotary evaporator from the solid gave the product **14**: mp 198-200 °C; yield 2.77 g (86%); ^{1}H NMR (D_2O) δ 4.85 (s, 2 H, $-NCH_2O-$), 4.40 (t, 2 H, $-NCH_2CH_2O-$), 3.75 (t, 2 H, $-NCH_2CH_2O-$), 3.25 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR (D_2O) δ 96.8 ($-NCH_2O-$), 68.5 ($-NCH_2CH_2O$), 64.0 ($-NCH_2CH_2O-$), 52.7 ($N(CH_3)_2$); FAB MS *m/z* (relative intensity) 102 (100 M^+). Anal. ($C_5H_{12}BrNO$) C, H, N.

3,3,5-Trimethyloxazolidinium bromide (15), mp 158 °C, was prepared by the same procedure in 63% yield: ^{1}H NMR (D_2O) δ 5.15-4.40 (m, 3 H, $-OCH(CH_3)CH_2-$, $-NCH_2O-$), 4.15-3.75 (d of d, 2 H, $-NCH_2CH-$), 3.33 (s, 6 H, $N(CH_3)_2$), 1.43 (d, 3 H, $CHCH_3$); ^{13}C NMR (D_2O) δ 95.6 (NCH_2O), 77.9 ($-OCH(CH_3)CH_2-$), 70.2 ($-NCH_2CH-$), 54.3 (NCH_3), 52.5 (NCH_3), 20.0 ($CHCH_3$); ^{13}C NMR-OFR (D_2O) δ 95.0 (t, NCH_2O), 77.7 (d, $-OCH(CH_3)CH_2-$), 70.2 (t, $-NCH_2CH-$), 54.34-50.33 (2 quartets, $N(CH_3)_2$), 19.7 (q, $CHCH_3$); FAB MS *m/z* (relative intensity) 116 (100, M^+). Anal. ($C_6H_{14}BrNO$) C, H, N.

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Registry No. 2, 133933-03-6; 3, 28508-20-5; 4, 28508-22-7; 5, 133933-04-7; 6, 133933-05-8; 7, 133933-06-9; 8, 133933-07-0; 9, 133933-08-1; 10, 38473-69-7; 11, 133933-09-2; 12, 25393-80-0; 14, 133933-10-5; 15, 133933-11-6; QNB, 6581-06-2; ChAT, 9012-78-6; AChE, 9000-81-1; $Me_2NCH_2CH_2OH$, 108-01-0; $Me_2NCH_2CH_2OAc$, 1421-89-2; $Me_2NCH_2CH(CH_3)OH$, 108-16-7; $Me_2NCH_2CH(CH_3)OAc$, 32188-28-6; $ClCH_2I$, 593-71-5; $BrCH_2CH_2OH$, 540-51-2; $BrCH_2CH(OH)CH_3$, 19686-73-8; ICH_2CH_2OH, 624-76-0; methylene bromide, 74-95-3; methylene iodide, 75-11-6.

An NMR and Theoretical Study of the Conformation and Internal Flexibility of Butaclamol Hydrochloride

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A theoretical (MM2) and experimental (^{1}H and ^{13}C NMR) study of butaclamol hydrochloride in $CDCl_3$ has been done in order to determine preferred conformations and internal molecular flexibility of this molecule. The theoretical calculations suggest the presence of four low-energy conformations, two of which involve a trans junction of the D and E rings, with the other two involving a cis I ring junction. An alternative cis junction (cis II) was excluded on energetic grounds. The ^{1}H NMR data strongly suggest the presence of a trans D-E ring junction and are consistent with a chair conformation of the E ring. ^{13}C spin-lattice relaxation time measurements show that most of the molecule is rigid, although there is some degree of mobility in the seven-membered B ring, associated with rapid flipping of the bridging C8 and C9 carbons between two skewed conformations, which have previously been referred to as conformer A and conformer B (Laus et al. *Heterocycles* 1984, *22*, 311).

Introduction

Since its synthesis and testing in the mid 1970s,[1] butaclamol has been used in several attempts to design antipsychotic drugs[2-6] as well as for dopamine receptor mapping.[1] This interest derives from its high affinity in dopamine receptor binding assays, in which it is enantiospecific,[7] and because it is generally assumed to have

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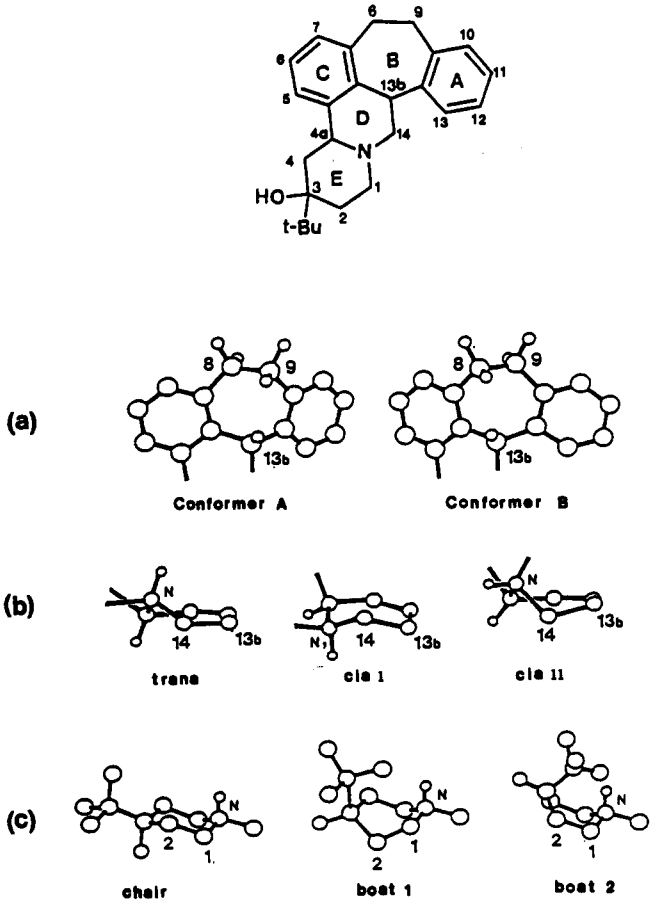

Figure 1. Major regions of conformational variation in (+)-butaclamol: (a) ring B (conformer A/conformer B), (b) the D–E ring junction (trans/cis forms), and (c) ring E (chair/boat forms).

a relatively rigid polycyclic system.[1] Because of these properties, early efforts were made to precisely define butaclamol's most probable conformers on the premise that one or more of them might represent a biologically active conformation.

The first conformational distinctions were made by Humber's group,[1] who deduced that the active conformation was trans, conformer B, despite (+)-butaclamol having an A conformation in the solid state. (A nomenclature scheme for butaclamol conformations is provided in Figure 1.) However, from a consideration of the energies of alternative conformers,[8] Froimowitz and Matthysse concluded that the biologically active conformation of butaclamol was most likely to be trans, conformer A. Both these studies recognized that butaclamol was not entirely rigid by noting the possibility of cis–trans interconversion at the D–E ring junction, and that flexing of the cycloheptane tricyclic system led to A and B conformers. However, no recognition was given in those studies to the flexibility of the E ring, which could exist

Table I. Relative Energies (kcal/mol) of Conformers of (+)-Butaclamol[a]

D–E ring junction	ring E conformation	ring B conformation A	ring B conformation B
trans	chair	19.5 (1.1)	22.4 (4.0)[a]
	boat 1	28.2 (9.8)[b]	32.6 (14.2)
	boat 2	28.2 (9.8)[b]	37.3 (18.9)
cis I	chair	18.5 (0.1)	18.4 (0.0)
	boat 1	25.0 (6.6)	23.5 (5.1)
	boat 2	37.2 (18.8)	18.5 (0.1)[c]
cis II	chair	27.5 (9.1)	30.6 (12.2)
	boat 1	23.9 (5.5)	28.2 (9.8)[b]
	boat 2	28.1 (9.7)[b]	29.6 (11.2)

[a] Values in brackets are those above the global minimum (cis I, chair, conformer B). Conformer designations match the examples given in Figure 1. [b] In these cases, ring inversion to a common twist-boat form occurred. [c] Convergence was not obtained while the system was forced to occupy a boat 2 form and the energy value cited corresponds to a conformation in which ring inversion to the near global minimum has occurred.

in either chair or boat forms, nor to the existence of a cis form, *alternative* to that observed in the solid state for (+)-isobutaclamol. Furthermore, the anomalous pK_a (5.7) available at the time of these studies, and upon which the Humber model was based,[9] was subsequently shown to be too low by a later, more accurate determination of 7.2.[10] This fact, and the recognition of two possible cis forms (I and II, Figure 1), means that the solution conformation at the D–E ring junction is not clear-cut, with the potential of both D and E rings to be quite mobile. From these considerations we decided to carry out more extensive MM2 calculations on all possible conformations and to investigate the solution conformations and dynamics of butaclamol hydrochloride using NMR spectroscopy. These results are complementary to a very recent report on the X-ray crystal structure of butaclamol hydrochloride which incorporates some updated theoretical calculations.[11]

To date, only limited ^1H NMR studies of butaclamol have been reported.[12,13] In this study we utilize ^1H NMR measurements to determine the preferred conformation of butaclamol hydrochloride in solution and ^{13}C spin–lattice relaxation times to determine internal flexibility in this system. These results are discussed in the light of the MM2 calculations.

Maryanoff et al.[13] recently reported an extensive stereochemical study of protonated bridgehead amines containing ring-fused structures found in a variety of alkaloid structures and biologically active compounds. As part of that investigation, the conformation of butaclamol was examined and the presence of trans and cis structural forms established. Some of the ^1H NMR results reported in the current paper for studies in $CDCl_3$ differ from those reported for DMSO solutions by Maryanoff et al.[13] In the accompanying paper[14] it is shown that the solvent has a significant influence on the stabilization of different low-energy conformations. This is consistent with the general

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Table II. Predicted Geometries for Energetically Feasible Conformers of Butaclamol

torsion angle[a]				conformation[b] trans A	trans B	cis I A	cis I B	NMR[c]
$H9_{eq}$	C9	C8	$H8_{ax}$	68	-73	54	-57	55
$H9_{eq}$	C9	C8	$H8_{eq}$	-46	172	-60	-171	55
$H9_{ax}$	C9	C8	$H8_{ax}$	-175	42	168	57	172
$H9_{ax}$	C9	C8	$H8_{eq}$	71	-73	54	-57	55
NH	N1	C4a	H4a	-173	177	53	49	152
NH	N1	C14	$H14_{ax}$	-172	-176	-69	-75	159
NH	N1	C14	$H14_{eq}$	70	68	-173	170	54
NH	N1	C1	$C1_{eq}$	-64	-60	64	64	38
NH	N1	C1	$C1_{ax}$	178	-178	-51	-51	160
H_{4a}	C4a	C4	H_{4eq}	-61	-52	-57	-55	47
H_{4a}	C4a	C4	H_{4ax}	-178	-170	-172	-169	158
C_{2ax}	C2	C1	C_{1eq}	55	59	57	59	65
C_{2ax}	C2	C1	C_{1ax}	172	176	172	174	145
C_{2eq}	C2	C1	C_{1eq}	-61	-57	-58	-57	65
C_{2eq}	C2	C1	C_{1ax}	56	59	57	58	57
H_{13b}	C13b	C14	H_{14ax}	-178	-159	-83	-67	158
H_{13b}	C13b	C14	H_{14eq}	-58	-40	31	47	47

[a] Defined according to the Klyne–Prelog convention.[28] [b] Conformation of the E ring is restricted to a chair conformer. [c] Magnitudes of torsion angles calculated from the Karplus equations $^3J = A\cos^2\theta + B\cos\theta + C$:

(1) ^3J(CH–NH)[18] $A = 9.8, B = -1.8, C = 0$

(2) ^3J(CH–CH)[19] $\theta < 90°$ $A = 12.5, B = 0, C = -0.3$

$90° < \theta < 180°$ $A = 14.5, B = 0, C = -0.3$

results of this and other recent work,[11] suggesting that energy differences between low-energy forms calculated in the gas phase are small.

Results

The results of the MM2 calculations are summarized in Table I and show that only four of the 18 possibilities that we considered are feasible (i.e., within 4 kcal mol^{-1} of the lowest energy conformer): the E ring favors a chair conformation, but there could be a cis I or trans arrangement at the D–E ring junction, and either conformer A or B in the cycloheptane ring, in agreement with the work of Froimowitz, Matthysse, and Cody.[8,11] Even allowing for small differences between protonated and deprotonated forms, it is unlikely that any of the other 14 isomers (which are at least 5.1 kcal mol^{-1} higher in energy) would be significantly populated. Predicted geometries (as quantified by key torsion angles) for each of the four energetically feasible structures are shown in Table II.

It was of interest to confirm that these theoretical predictions could be verified experimentally, so a series of NMR experiments was carried out. ^{1}H NMR measurements were done to obtain three-bond vicinal coupling constants for use in conformational analysis, while ^{13}C measurements of spin–lattice relaxation times were utilized to probe possible dynamic flexibility in butaclamol.

The 400-MHz ^{1}H NMR spectrum of butaclamol hydrochloride in $CDCl_3$ is shown in Figure 2. The spectrum differs significantly from the partial spectrum of the free base reported recently by Laus et al.,[12] as might be expected because protonation of the nitrogen produces significant chemical-shift changes at adjacent methylene and methine protons, as well as introducing additional spin–spin coupling into the spectrum. It is interesting to note that Maryanoff et al.[13] recently reported that in DMSO, two resonances are observed for the NH protons, an observation consistent with slow exchange between two conformers. The present study reveals differences when butaclamol hydrochloride is dissolved in $CDCl_3$. We have examined the role of solvent in differential stabilization of the various conformations of butaclamol and conclude that only one conformer is present in $CDCl_3$, in contrast

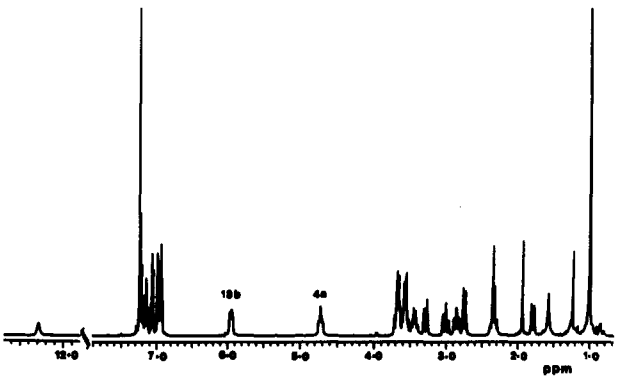


Figure 2. 400-MHz ^{1}H spectrum of butaclamol hydrochloride in $CDCl_3$.

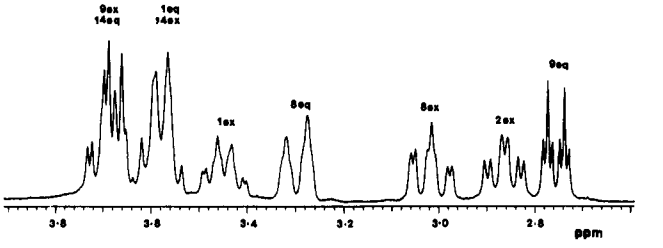


Figure 3. Expansion of 400 MHz spectrum of butaclamol hydrochloride in $CDCl_3$ from 2.7 to 3.8 ppm.

to the DMSO case. The results of that study are reported in the accompanying paper.[14] In this paper we deal exclusively with data obtained in $CDCl_3$.

Spectral assignments were made using a combination of 1D (^{1}H decoupling, NOE difference) and 2D (COSY, J-resolved, and ^{13}C, ^{1}H-correlated spectroscopy) methods and are summarized in Table III.

Table III and Figure 2 show that two aliphatic protons, i.e., H13b and H4a, are significantly shifted relative to the others, with H13b having a particularly downfield shift of 5.96 ppm. This can be attributed to a combination of effects due to the positive charge on the nearby nitrogen and the fact that the 13b proton is close to the plane of both the A and C aromatic rings and, hence, experiences

Table III. ^{1}H NMR Data for Butaclamol Hydrochloride in $CDCl_3$

proton	chemical shift (ppm)	multiplicity	coupling[a] (Hz)
1_{ax}	3.43	m	12.0, 10.2, 9.5, 3.4
1_{eq}	3.59	m	12.0, 4.7, ?, ?
2_{ax}	2.86	ddd	14.3, 9.5, 4.7
2_{eq}	1.81	br	14.3
$C(CH_3)_3$	1.03	s	
4_{ax}	2.34	m	14.5, 9.2
4_{eq}	2.41	m	14.5, 3.2
4a	4.75	m	9.2, 9.5, 3.2
5	7.00	d	7.7 (7.7, 1.6)
6	7.08	m	7.7, 7.7 (7.7, 7.9)
7	6.95	m	7.7 (7.9, 1.6)
8_{ax}	3.02	ddd	17.5, 13.9, 3.9 (16.8, 12.5, 5.0)
8_{eq}	3.30	ddd	17.5, 3.8, 3.8 (16.8, 5.0, 4.9)
9_{ax}	3.69	m[b]	14.0, 13.9, 3.8 (14.5, 12.5, 4.9)
9_{eq}	2.76	ddd	14.0, 4.0, 4.0 (14.5, 5.0, 5.0)
10	7.22	m	7.2
11	7.24	m	7.2, 7.2
12	7.16	ddd	7.2, 7.2, 2.8
13	6.97	m	7.2
13b	5.96	dd	12.1 5.6
14_{ax}	3.57	m[b]	14.0, 12.1, 10.0
14_{eq}	3.69	m[b]	14.0, 5.6, 2.0
NH	12.43	br	
OH	1.93	br	

[a] Couplings in parentheses determined by Laus et al.[12] for free-base butaclamol. ? indicates additional coupling present could not be measured. Coupling constants are accurate to ±0.25 Hz. [b] Overlapped multiplets.

a significant downfield shift due to ring current effects.

Figure 2 also shows that a large number of remaining aliphatic resonances fall between 2.7 and 3.8 ppm. An expansion of this region is shown in Figure 3, where it can be seen that overlap of the pairs of protons $H9_{ax}$, $H14_{eq}$, $H14_{ax}$, and $H1_{eq}$ occurs. A 2D, J-resolved spectrum provided a means of resolving the chemical shifts of the latter pair of protons, but $H9_{ax}$ and $H14_{eq}$ are coincident in chemical shift. The only other protons that presented some difficulty in analysis were $H4_{ax}$ and $H4_{eq}$, which form a highly coupled second-order spin system. An iterative spectral simulation which included these two protons together with H4a and NH allowed chemical shifts and coupling constants to be determined accurately. All experimentally determined coupling constants are summarized in Table III. These were used in the Karplus equation (1) to determine the NMR predicted torsion angles shown in Table II.

$$^3J = A\cos^2\theta + B\cos\theta + C \quad (1)$$

Values for the empirical parameters A, B, and C are given in the footnote to Table II.

^{13}C chemical shifts of butaclamol hydrochloride are reported in Table IV. Assignments were based on expected shifts from model compounds and were confirmed from a ^{13}C–^{1}H 2D correlated spectrum. ^{13}C spin–lattice relaxation times are also given in Table IV, presented in NT_1 values, N being the number of protons attached to the carbon of interest. These provide a measure of the relative mobility of various sites in the molecule via eq 2 where γ_H

$$\frac{1}{NT_1} = \frac{\gamma_H^2\gamma_C^2\hbar^2}{r_{CH}^6}\tau_{eff} \quad (2)$$

and γ_C are the ^{1}H and ^{13}C gyromagnetic ratios, respectively, $\hbar$ is Planck's constant divided by 2π, r_{CH} is the distance between the carbon and its attached proton, and τ_{eff} is the effective correlation time which provides a measure of overall and internal molecular flexibility. In interpreting the NT_1 values in Table IV, the aromatic carbons were used as a reference point for the measurement of internal mobility since they are least subject to internal flexing. The C8, C9, and methyl group carbons all show larger NT_1 values than do the aromatics, indicating additional motion at these sites.

Table IV. ^{13}C Chemical Shifts[a] and NT_1 Values[b] for Butaclamol Hydrochloride in $CDCl_3$

carbon	chemical shift (ppm)	NT_1 (s)
C1	52.25	0.78
C2	28.60	0.86
C3	73.76	
C4	36.49	0.82
C4a	61.13	0.81
C5	122.25	0.74
C6	126.54	0.75
C7	131.45	0.86
C8	35.30	0.96
C9	30.91	0.94
C10	127.70	0.74
C11	128.00	0.85
C12	126.23	0.86
C13	122.75	0.80
C13b	35.46	0.78
C14	55.04	0.90
tert-butyl	37.63	
$C(CH_3)_3$	24.87	2.94

[a] Aromatic quaternary carbons not reported. [b] NT_1 values reported only for protonated carbons.

Discussion

A primary question that the NMR studies were designed to address was the nature of the D–E ring junction. A consideration of couplings to protons H13b and H4a allows ready distinction between the cis and trans possibilities. H13b appears as a quartet due to couplings of 12.1 and 5.6 Hz to the C14 methylene protons. Based on the generalized Karplus equation applicable for this type of system (eq 1 and Table II), couplings of approximately 3–7 and 12–14 Hz would be expected to the two C14 protons in the trans configuration, while in the cis I form, values of approximately 6–9 and 0–2 Hz would be expected. The cis I form can thus be excluded on the basis that no small coupling on the order of 2 Hz is present and that one of the observed couplings exceeds 9 Hz. Agreement between observed and predicted couplings in the trans form is reasonable given the limitations of the Karplus equation in systems where strained bonds or electronegative substituents are present.

Confirmation that the D–E ring junction is trans can be obtained from the couplings to H4a. When resolution enhancement (Gauss–Lorentz transformation) is applied, this resonance appears as a six-line multiplet made up of couplings of 9.2, 9.2, and 3.2 Hz (Figure 4). Two of these couplings are due to the C4 methylene proton pair (whose relative orientation does not depend directly on the nature of the ring junction), and the third represents an interaction with the NH proton of the hydrochloride salt. This latter coupling is expected to depend strongly on the nature of the ring junction, since the H–N–C4a–H4a dihedral angle is approximately 180° (predicted $J \sim 10$–12 Hz) in the trans case, and 50° (predicted $J \sim 3$ Hz) in either cis case. Since again no small coupling is observed, the cis cases can be excluded. Irradiation of the NH resonance at 12.43 ppm results in loss of a 9.2-Hz coupling from the multiplet (Figure 4), confirming that $J^{NH,H4a}$ has a mag-

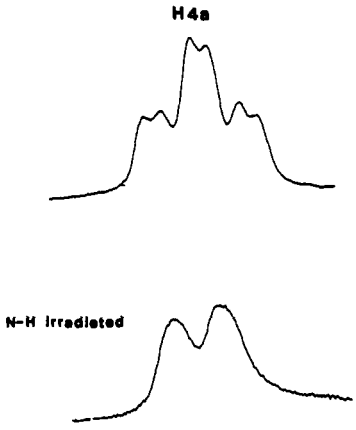


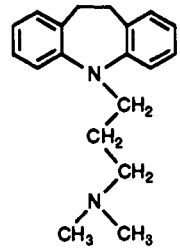
Figure 4. Expansion of a resolution-enhanced 400-MHz ^1H spectrum showing the 4a proton of butaclamol hydrochloride in $CDCl_3$ and proton 4a following irradiation of the NH proton.

nitude consistent with the trans orientation. Confirmatory evidence for the trans ring junction is also obtained from an observed NOE between the H5 aromatic proton and the overlapped multiplet due to $H4_{eq}$ and $H4_{ax}$.

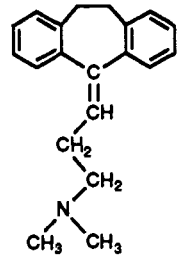
The Karplus analysis presented here is based on the assumption that a single conformation is predominant. The possibility of there being several different conformations in fast exchange can be dismissed because several observed couplings have magnitudes approaching the extreme value expected for a single trans arrangement of the geminal protons involved. This cross-corroboration of the couplings between H_{14} and H_4 with other protons, together with the NOE results, supports interpretation in terms of a single translike species.

The second major conformational feature to be addressed by the NMR study was a determination of the conformer A/conformer B equilibrium. Laus and coworkers[12] have previously suggested a preference for conformer A in butaclamol free base on the basis of an observed NOE between $H9_{ax}$ and H13b, and the absence of a measurable NOE between $H8_{ax}$ and H13b. It was of interest to determine whether this also applies in the hydrochloride salt. Figure 5 shows a control and NOE difference spectrum resulting from irradiation of H13b. As was seen for the free base, there is clearly an NOE to $H9_{ax}$, but any enhancement to $H8_{ax}$ is below the noise level. This suggests that the relative population of conformer B is at least a factor of 10 less than that of conformer A. The ^{13}C spin–lattice relaxation time data in Table IV were examined in order to obtain confirmatory information on relative lifetimes of the two forms. While NT_1's for all protonated aromatic carbons are similar and in the range 0.75–0.85 s, the values for the C8 and C9 carbons are significantly larger (0.95 s), suggesting a degree of internal flexibility. From eq 2, the effective correlation time for C8 and C9 is 4.9×10^{-11} s compared with 5.9×10^{-11} s for the aromatic carbons. Such an effect has previously been observed[15] in the structurally related tricyclic antidepressants imipramine (1) and amitriptyline (2), but in this case the ratio of T_1's for the C10, C11 carbons to the aromatic carbons was significantly greater. This suggests that the additional cyclic framework associated with butaclamol restricts the flexing motion of this portion of the molecule.

The increased mobility of C8, C9 relative to the rest of the molecule suggests that there is dynamic interconversion



imipramine (1)



amitriptyline (2)

between conformers A and B. While the "effective" correlation time calculated above provides some measure of the rate of interconversion, τ_{eff} also reflects contributions from overall motion. To obtain a more precise picture of the internal motional rates, it is necessary to quantitatively specify the nature of the internal motion. As nuclear spin relaxation for a ^{13}C site is usually dominated by local dipolar fields generated from motion of nearby protons, the specification of internal motion must be done in terms of the rate and amplitude of fluctuations of C–H vectors of interest. In the current case a transition from conformer A to conformer B results in rotation of the C–H vectors attached to C8 or C9 through approximately 90° around the axis defined by the C8–Ar or C9–Ar bonds. Eclipsing of protons in geometries intermediate to the two extreme conformers suggests that such intermediate geometries will be of high energy, and so the interconversion process can be conveniently viewed as a two-state jump between stable forms having lifetimes τ_A and τ_B. The appropriate spectral density function relating observable relaxation parameters (such as T_1) and motional parameters for such a model has been formulated,[16] and is incorporated in the program MOLDYN[17] which allows the calculation of motional parameters from observed T_1 data. Input parameters in the present case included $\tau_0 = 5.9 \times 10^{-11}$ s, as calculated above from the ^{13}C T_1 data for the aromatic carbon, $\theta = 45°$ (the half-angle of the jump) and $\beta = 109.5°$ (the angle between the C–H vectors and the jump axis). MOLDYN was then used to generate τ_A and τ_B values consistent with the observed T_1 for C8 and C9.

Based on a possible 10% error in the NT_1 values, the data could be fitted for τ_A and τ_B values, satisfying the inequality $0.02 < \tau_B/\tau_A < 50$. Taking into account the additional constraint derived earlier from the NOE data, it may be calculated that there is a 10–50-fold population preference for conformer A. This corresponds to an energy difference of 1.4–2.2 kcal/mol, which is not inconsistent with the MM2 predictions in Table I, given the limitations of gas-phase calculations.

The ^{13}C T_1 data have proved extremely useful, in conjunction with ^1H NOE measurements, in verifying conformational preferences about the C8/C9 bridge. Note that both the NMR results and the theoretical torsion angles in Table II suggest that the conformational A/B interconversion occurs with minimum disruption to the rest of the structure.

Finally, the third region of conformational interest involves the E ring, which may potentially exist in a chair or either of two boat forms. The MM2 calculations showed that interconversion may occur via an intermediate

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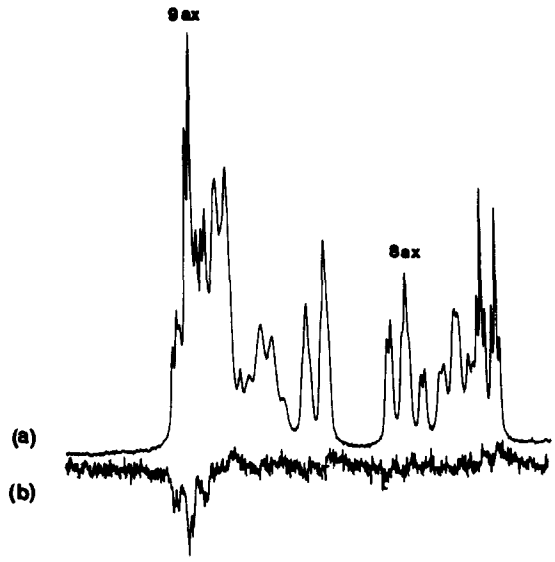

Figure 5. (a) Control spectrum and (b) NOE difference spectrum showing region containing $H9_{ax}$ and $H8_{ax}$ protons following irradiation of H13b.

twist-boat form. The boat 2 conformer can be dismissed due to a steric clash involving the tertiary butyl group and the D ring and also based on disagreement between observed and predicted coupling constant measurements for $H1_{ax}$.

The remaining E-ring conformers (chair and boat 1) are more difficult to distinguish. However, by combining the unequivocal experimental evidence for a trans D–E ring junction with the theoretical calculations, which strongly favour a chair E ring over a boat form in this case, it is highly likely that the chair form is, indeed, the prevalent E-ring conformer. While there are no NOE's or coupling constants that unequivocally distinguish boat 1 from chair for this ring, the coupling of 10.2 Hz between NH and $H1_{ax}$ is more consistent with a chair form (where the torsion angle between the protons is 180°) than the boat form (where the torsion angle is close to 0°). This is based on analogy with the H4a–NH coupling of 9.5 Hz, which applies for a torsion angle of 180°. As couplings for 0° are usually smaller than for 180°, a smaller coupling between NH and $H1_{ax}$ would be expected if the boat form were present. It should also be pointed out that it is not possible to dismiss a twist-boat form with the current NMR data available.

As was noted for the B ring, ^{13}C T_1's were examined for their potential to yield information about possible dynamics in the E ring; however, the results in Table IV indicate that there is very little, if any, additional internal (e.g., chair–boat) interconversion present in the E ring of butaclamol hydrochloride.

Having examined the three major areas of conformational interest separately, it is now useful to examine the overall agreement between the calculations and the NMR experiments. This is conveniently done by calculating mean square deviations for the magnitude of MM2-derived and NMR-derived torsion angles in Table II. This trans form (with either conformer A or B) has an RMS deviation of approximately 5° between the theoretical and experimental data, whereas for the cis I form, the RMS deviation approaches 60°.

The relationship of the new experimental (NMR spectroscopy in solution) and theoretical work (molecular mechanics calculations) to the biological activity of butaclamol is now discussed and compared with previous conformational studies. After initial synthesis and testing as an antipsychotic in 1975,[1] butaclamol was proposed as a model compound for studying dopamine D_2 receptors. This was because its tight binding as an antagonist at these receptors resides exclusively in the (+)-3*S*,4a*S*,13b*S* isomer and because it has a *relatively* inflexible pentacyclic structure compared with other neuroleptic drugs.[1] In addition, structure–activity data on butaclamol analogues showed clearly that an aromatic group, a tertiary nitrogen, and a hydrophobic group (*tert*-butyl) constituted the pharmacophore necessary for binding to D_2 receptors.[1] Butaclamol's conformations were therefore intensively studied using a variety of methods in order to define the spatial limits of these pharmacophore groups and, hence, the probable biologically active conformation of butaclamol at dopamine receptors. By comparing the single-crystal X-ray conformations of (+)-butaclamol, (+)-isobutaclamol, and the dopamine agonist (–)-apomorphine, Humber's group[1] postulated that the biologically active form of butaclamol was trans conformer B. Subsequently, Froimowitz and Matthysse[8] used molecular mechanics to compare the energies of four possible conformers of butaclamol (trans A and B, cis I A and B) with the analogous ones of isobutaclamol. From their calculations, they concluded that, of the two trans isomers of butaclamol, trans conformer A was energetically favored by a factor of 100. The two cis I isomers, although lowest in energy of those studied, were rejected after comparing nitrogen–phenyl distances with other active neuroleptics. Their conclusions were reinforced by a further comparison with the X-ray structures of the semiflexible neuroleptics loxapine and octoclothiepin.[8] These studies led to the deduction that the biologically active conformation of butaclamol was most probably trans conformer A: the one observed in the solid state for the hydrobromide by X-ray crystallography. A subsequent study by Cody and Froimowitz[11] compared these results with the X-ray derived structures for butaclamol hydrochloride and with revised MM2 calculations on this structure for the four conformers cis and trans conformers A and B, using better parameters for the ammonium group in butaclamol hydrochloride. The calculations showed that the lowest energy form was trans conformer A, which was also the conformation in the solid state, thereby supporting a previous conclusion[8] about the biologically active conformation.

An attempt has also been made to decide between conformers A and B through use of rigid analogues of butaclamol. Thus Laus et al.[12] synthesized and tested via receptor binding assays the molecules having a two- and one-methylene bridge between rings C and D (to mimic respectively the A and B conformations), but with the ethano bridge of ring B omitted, presumably to assist adoption of the desired C ring orientation relative to ring E. The resultant analogues showed significantly decreased activity compared with that of butaclamol. However, these results may be regarded as inconclusive on the grounds that the modifications may have affected binding by adversely distorting the rest of the molecule, despite having achieved each of conformers A and B. (This aspect has been retrospectively supported by use of computer graphics.[20])

In the conformational analysis presented in this paper, we have taken the molecular mechanics energy calculations further than before by considering 18 conformations that are a combination of the alternative arrangements possible in rings B, D, and E (see Figure 1). Fourteen of these could

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be eliminated because they were considerably higher in energy than the remainder, viz. trans and cis conformers A and B. Although these four conformers correspond exactly to those found by previous workers, the value of our calculations is that a wider search of butaclamol's conformational space has revealed no new viable conformations that would be feasible candidates for the biologically active form.

Turning to the NMR analysis of butaclamol, earlier work by Laus et al.[12] provided evidence from NOE experiments to support the fact that conformer A predominates in solution. Further work by Maryanoff et al. on the ^1H NMR of butaclamol hydrochloride in DMSO showed two species in the ratio 81:19 that were assigned as a trans and a cis form, respectively. We have extended those preliminary results by using ^1H NMR to measure the preferred conformation of butaclamol hydrochloride in $CHCl_3$ and DMSO (see next paper) and ^{13}C spin–lattice relaxation times to determine internal flexibility.

The NMR experiments for butaclamol hydrochloride in $CDCl_3$ show that the preferred conformer of this drug has a trans D–E ring system, a chair E ring and possible interconversion between conformer A and conformer B of the B ring, with conformer A being favored by at least 10:1. This result compares favorably with the MM2 theoretical calculations in that the solution conformation was one of the four low-energy conformers predicted, although the global minimum was shown to be in the cis I, chair, conformer B orientation. Taken in conjunction with the conclusions of previous workers based on analyses of the solid- and isolated-state conformations of butaclamol, our results, by including detailed considerations of the possible solution conformations, firmly establish that there is indeed an extremely limited range of flexibility for this molecule.

Experimental Section

Energy Calculations. From a consideration of Dreiding models, three regions of conformational flexibility can be discerned for butaclamol (Figure 1), namely: (1) There is flexing in the seven-membered B ring leading to conformer A (eclipsed hydrogens at positions 9 and 13b) and conformer B (eclipsed hydrogens at positions 8 and 13b). (2) The D–E ring junction may be either cis or trans. This results from the possibility of proton transfer at the tertiary nitrogen atom with intervening nitrogen inversion. Although the trans juncture exists in only one form, with the nitrogen proton cis to the 13b hydrogen, two cis forms (I and II) are possible: that in which the nitrogen proton is trans to the 13b hydrogen, and that in which these protons adopt a pseudocis geometry. (3) Ring E may adopt one chair or two boat (including a twist-boat intermediate) forms through ring inversion. Although on intuitive grounds the boat forms are unlikely, as a result of the bulky *tert*-butyl moiety causing unfavorable nonbonded interactions, they were nevertheless included. Thus there are at least 18 possible biologically relevant conformations, of which the relative energies of only four have been previously reported on in detail.[8,11]

The 18 conformations detailed above were constructed using the computer-graphics modeling program CRYS-X.[21] The structure of the tricyclic (A–B–C) cycloheptane system was based on the coordinates obtained from X-ray crystallography of (+)-butaclamol[22] and (+)-isobutaclamol,[23] while the D and E rings were built on in the desired conformation using standard geometries.[24] For the purpose of initially filtering out the highest energy conformers from this set of 18, only the protonated forms were considered since it has been shown[11] that the differences in energy from the unprotonated forms is at most 1.0 kcal mol^{-1}. A preliminary energy minimization was then performed within the CRYS-X program to ensure a reasonable starting conformation for the subsequent MM2 calculation. The CRYS-X program performs classical conformational energy calculations by pairwise summation of the van der Waals interactions between nonbonded atoms without full geometry optimization, using parameterization based on that of Giglio.[25] This initial minimization was particularly important for boat forms of the E ring, in which an axial conformation of the *tert*-butyl group tended to produce prohibitive nonbonded interactions. A full geometry optimization was then carried out using the 1983 version of the MM2 program of Allinger and Yuh,[26] supplemented for aromatic compounds with additional parameters.[27] This program has previously been shown to give correct quantitative results for hydrocarbons and to be in good agreement with results from X-ray crystallography.[8] To check the validity of our calculations, the results for the four (+)-butaclamol conformers, cis and trans, A and B, were compared with results previously obtained by Froimowitz and co-workers,[8,11] with general agreement being obtained.[8] Repetition of MM2 calculations from different starting points on the energy surface produced identical minima. When specifying the geometry of butaclamol we adopted, for ease of comparison, the same atom numbering and conformer designation as previous authors[8] (Figure 1). The dihedral angles are specified according to the convention of Klyne and Prelog.[28]

NMR Measurement. (+)-Butaclamol hydrochloride was purchased from Research Biochemicals Inc. and used without further purification. ^1H NMR spectra were recorded at 300 or 400 MHz on a Bruker AM300 or a Varian VXR400 spectrometer, respectively. Measurements were carried out for 10 mM solutions of butaclamol hydrochloride in $CDCl_3$ (99.8%, Cambridge Isotope Laboratories) in 5-mm sample tubes. Spectra were recorded immediately after sample preparation and no change in spectral appearance was noted over the course of several days. Typical conditions for 1D ^1H NMR spectra included a repetition delay of 2 s, a pulse flip angle of 45°, spectral width of 3000 Hz (or 4000 Hz at 400 MHz) and a 16 K data acquisition table. Zero filling to 32 K was generally applied, leading to a digital resolution of at least 0.25 Hz/pt in the transformed spectra. ^1H NOE experiments were carried out by acquiring one free induction decay (FID) where low power, selective irradiation on the peak of interest was applied for 4 s prior to data acquisition and a second FID where the decoupler was set in a control region of the spectrum containing no peaks. FID's for these on- and off-resonance irradiations were interleaved in blocks of eight scans until a total of 64 transients was acquired for each. 2D spectra were obtained using a repetition time of 2 s, with minimum spectral widths for the region of interest and, generally, a data matrix of 1 K points in the F2 dimension and 512 points in the F1 dimension.

^{13}C NMR spectra were recorded at 75 MHz on a Bruker AM300 spectrometer for samples in 10-mm sample tubes at ambient temperature in $CDCl_3$. Typical conditions included a spectral width of 16,000 Hz, and a data acquisition table of 16 K. Prior to Fourier transformation, an exponential line-broadening factor of 2 Hz was applied. Broad-band proton irradiation was achieved using the standard composite pulse-decoupling mode supplied with the spectrometer. ^{13}C T_1's were measured using the fast inversion recovery technique,[29] with a recovery delay of 3 s between

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180-τ-90 pulse sequences. Twelve τ values in the range 0.01-8 s were used. Raw data were analyzed using a nonlinear, three-parameter, exponential fitting program on the Aspect 3000 computer on the spectrometer. Reported T_1's represent an average of two determinations and have an estimated error of 5-10%.

Acknowledgment. This work was supported in part by a grant from the Australian Research Council.

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NMR Studies of the Conformational Interconversion of Butaclamol in Solution

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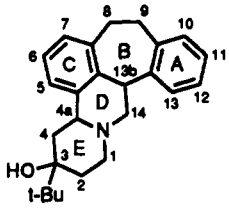
^{1}H NMR experiments at 300 MHz have been carried out to determine the identity and study the interconversion of two conformations of butaclamol in solution. The hydrochloride salt in DMSO exists as an equilibrium mixture of two conformations, which differ in their stereochemistry about the ring junction that contains the single nitrogen atom in butaclamol. The trans form has a relative population of 80% and the cis I form 20%. In $CDCl_3$ only the trans form is observed, while in $CDCl_3$-DMSO mixtures, both forms are detected in a ratio (trans:cis I) that decreases as the percentage of $CDCl_3$ decreases. For the free base in either CD_2Cl_2 or DMSO, only a single set of resonances is observed at room temperature, but as temperature is lowered, peaks from methine protons H4a and H13b near the ring junction broaden and (for samples in CD_2Cl_2) eventually split into two resonances corresponding to the cis and trans forms. It is suggested that nitrogen inversion is the dynamic process responsible for the interconversion of the two forms. Line shape analysis as a function of temperature yielded an energy barrier of 9.6 ± 0.5 kcal/mol for the interconversion, in good agreement with values obtained from saturation transfer experiments. In the hydrochloride salt, the barrier in DMSO was somewhat higher, i.e., 17.3 ± 0.9 kcal/mol, as determined by saturation transfer and variable-temperature measurements.

Introduction

In the preceding paper,[1] a theoretical and NMR analysis of the conformations of butaclamol (1) was reported. This



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compound has been extensively used as a dopamine receptor mapping agent,[2] so a knowledge of its conformational behavior is important for a full understanding of its receptor binding. The theoretical results reported in the previous paper are in general agreement with related studies[3-5] in that four low-energy conformers were identified. NMR data recorded in $CDCl_3$ support the existence of one of these low-energy conformers in solution, but there is apparent conflict with a recent NMR study in DMSO reported by Maryanoff et al.[4] That study (carried out in DMSO because of a reported difficulty in dissolving butaclamol hydrochloride in $CDCl_3$) suggested the presence of two low-energy conformations, one of which did not correspond to any of the previously identified low-energy conformers in three theoretical studies.[1,3,5]

In the present paper, the apparent anomaly regarding the conformation of butaclamol in solution is investigated. It is established that two conformers are indeed present for butaclamol hydrochloride in d_6-DMSO, both of which correspond to low-energy forms previously identified in theoretical studies. The conformational interconversion of butaclamol is analyzed and the role of solvent in stabilizing individual conformers is discussed.

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Results

(a) Comparison of Spectra in DMSO and $CDCl_3$. Spectra of butaclamol hydrochloride and its free base in $CDCl_3$ and d_6-DMSO at 297 K are shown in Figure 1. Major differences in the spectra of the free base relative to those of the hydrochloride include the absence of downfield NH resonances and chemical-shift changes of the methylene and methine protons adjacent to the nitrogen atom. There are a number of other, smaller chemical-shift differences and changes to spin-spin coupling systems in the free base which were previously complicated by the presence of the NH proton in the hydrochloride.

Changing the solvent has little influence on the spectrum of the free base, apart from effects on the OH resonance. However, there are significant differences in the spectrum of the hydrochloride in d_6-DMSO relative to that in $CDCl_3$. The emergence of a second NH resonance for butaclamol hydrochloride in d_6-DMSO, and a doubling or broadening of other resonances, are consistent with the presence of two conformers undergoing chemical exchange in this solvent. The two sets of peaks occur in a ratio of approximately 4:1. Maryanoff et al.[4] previously reported the observation of two NH peaks in an approximate ratio of 4:1. The existence of two conformations can be readily seen in the COSY spectrum in Figure 2, where two sets of peaks are observed for a number of protons. The con-

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