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CONFORMATIONS OF THE SECURININE ALKALOIDS AS STUDIED BY HIGH-FIELD ^{13}C-, ^{1}H- AND 2-D NMR, AND MOLECULAR MECHANICS CALCULATION

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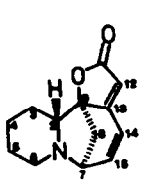
ABSTRACT

On the basis of C-H correlation experiments, COSY, and other 2-dimensional nmr evidence, the ^{1}H and ^{13}C-nmr assignments for securinine are revised from those previously proposed. Assignments are also made for the biologically relevant hydrochloride salt of securinine in D_2O. The spectra of allosecurinine are completely assigned and assignments for securinol A are presented for the first time. Implications of the nmr results for the conformational preferences of these alkaloids are discussed in light of molecular mechanics calculations.

Securinine (1) has been shown to be a stereospecific $GABA_A$ receptor antagonist.[1] Since it would appear to be a fairly inflexible molecule with a well-defined geometry, securinine offers the hope that it might aid in understanding the shape of the GABA receptor site. It was of interest, therefore, to examine the conformational preferences of securinine in solution. This was done by us recently using low-field NMR and relying on lanthanide shift reagent information.[2] We have now re-examined securinine itself as well as several alkaloids of the securinine family (2, 3) using high-field NMR and various 2-dimensional techniques. The conformational implications of these more reliable data are discussed herein.



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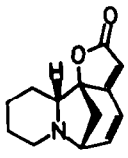

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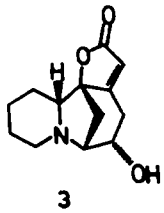

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Experimental

Securinine (**1**) and allosecurinine (**2**) were isolated as reported previously.[3] Hydrochloride salts were prepared by precipitation from ethereal solution by means of HCl gas. Securinol A was prepared by flash chromatography from Securinega suffruticosa as described, and its ir, UV and mass spectra agreed with those published [4] The ^{1}H-NMR spectra of **1** and **2** at low resolution have been reported.[5]

NMR spectra were obtained on a Bruker AM-400 spectrometer operating at a proton frequency of 400.13 MHz and a carbon frequency of 100.64 MHz. A 90 degree carbon pulse was 6.3 μsec. COSY spectra typically were obtained using 1K data points in the F2 dimension (width ca. 3200 Hz), and 256 experiments to generate the F1 dimension followed by zero-filling to 512W. Sine-bell weighting was generally used in both dimensions prior to transformation. The resulting COSY array was symmetrized to suppress F1 noise. Carbon-proton correlation spectra typically employed 2K data points in the F2 dimension (width ca. 18,500 Hz) and 256 experiments to generate the F1 dimension (width ca. 1900 Hz) followed by zero-filling to 512W. Normal proton spectra used 64K points (4000 Hz width). Resolution enhancement was done by Gaussian multiplication of the FID prior to Fourier transformation.

MMP2 calculations were done utilizing QCPE program 395 (1982 force field). One bending parameter and six torsional potentials were supplied by us. They were 2-3-6 bend; K(b) = 0.650, θ(0) = 109.100; 2-3-6-1 torsion V_1 = 3.53, V_2 = 2.30, V_3 = -3.53; 2-2-3-6 torsion V_1 = 0.00, V_2 = 15.00, V_3 = -1.06; 2-3-6-20 torsion $V_1 = V_2 = V_3$ = 0.00; 2-1-6-20 torsion $V_1 = V_2 = V_3$ = 0.00; 5-2-3-6 torsion $V_1 = V_3$ = 0.00, V_2 = 16.25; 2-1-6-3 torsion $V_1 = V_2$ = 0.00, V_3 = 0.403.

Results and Discussion

Assignment of Spectra. Tables 1 and 2 report the 400 MHz ^{1}H-NMR spectrum of securinine in $CDCl_3$, including proton-proton correlations derived from a COSY experiment. The COSY leaves little room for doubt in the assignments, especially the difficult assignments of H3, H4 and H5. For example, H2 (at 2.10 ppm) correlates only with the multiplet at 1.48 - 1 67 ppm. thus this multiplet must harbor the signals due to H3ax and H3eq. Also, H6eq (at 2 97 ppm), in addition to correlating with H6ax (at 2.42 ppm) as expected, correlates only with the multiplet at 1.48 - 1.67 ppm, and not with the multiplet at 1.24 ppm nor the one at 1.88 ppm. Thus the 1.48 - 1.67 ppm multiplet must harbor signals due to H5ax and H5eq. The protons on C4 thus are assigned to the multiplets at 1.88 and 1.24 ppm. The remaining signals in the spectrum are assigned in a straightforward way on the basis of chemical shift, multiplicity, and COSY correlations In assigning the protons on C8 we suggest that the more upfield of the two be assigned to the H8 situated over the C14-C15 double bond, viz. H8b.

Having established the identity of each signal in the ^{1}H-NMR spectrum of securinine, a C-H heteronuclear correlation experiment was run to aid in the assignment of the ^{13}C-NMR of securinine. The ^{13}C-NMR spectrum, C-H correlations and assignments are listed in Table 4. Figure 1 shows that portion of the C-H correlation spectrum used to assign C3, C4, and C5. Although the 1.48 - 1.67 ppm multiplet (proton spectrum) is complex, the COSY experiment is consistent with H3a and H3b occupying the middle of the multiplet and H5a and H5b extending to both edges of the multiplet. This allowed the assignments of C3 and C5 to be made from the C-H correlation spectrum (Figure 1). Assignments for C11 and C13 were the result of obtaining a fully proton-coupled ^{13}C-NMR spectrum. The C11 signal appears as a doublet (J = 8.9 Hz) while C13 appears as a broad peak with some unresolved fine structure. The 8.9 Hz C11-H12 splitting is reasonable when compared to the analogous coupling

Table 1. 400 MHz ^{1}H-NMR Spectrum of Securinine ($CDCl_3$). Chemical shifts (ppm), multiplicities, proton-proton correlations, and assignments.

Chemical shift	Multiplicity	COSY[a]	Assignment
6.61	d	a	H14
6.43	d of d	ab	H15
5.56	s		H12
3.83	d of d	bc	H7
2.97	d of t	e	H6eq
2.50	d of d	cd	H8a
2.42	d of d of d	e	H6ax
2.10	d of d	f	H2
1.88	m	g	H4a
1.78	d	d	H8b
1.67 - 1.48	m	efg	H3a,H3b, H5a,H5b
1.24	m	g	H4b

[a] Peaks with the same letter are correlated by a cross peak in the COSY spectrum

Table 2. Coupling constants, J (Hz), absolute values.

Protons	Securinine ($CDCl_3$)	Securinine·HCl (D_2O)	Allosecurinine ($CDCl_3$)
14-15	9.2	9.0	9.1
15-7	5.3	6.4	5.3
7-8a	4.1	4.8	4.4
8a-8b	9.2	12.2	9.8
6ax-6eq	10.5	-	-
6ax-5ax	7.5	-	-
6ax-5eq	7.0	-	-
6eq-5ax	3.7	-	-
6eq-5eq	3.7	-	-
2-3ax	11.3	12.7	13.2
2-3eq	2.5	4.8	3.5

constant reported for 2-cyclopentenone, viz. 5.62 Hz.[6] This sort of coupling constant is sensitive to ring geometry (the analogous coupling for 2-cyclohexenone is 0.5 Hz).[6] This new data shows that the previous assignments for C-14 and C-15 must be reversed. The peril of relying on lanthanide shift data to make assignments for unsaturated systems is clear in this case.

The ^{13}C-nmr and ^{1}H-nmr spectra of the hydrochloride salt of securinine in D_2O are substantially different from those of the free base. Since the protonated securinine spectra were not just a simple extension of the free base spectra, assignments were made without recourse to the previous assignments for the free base. The ^{1}H-NMR spectrum of securinine·HCl in D_2O is summarized in Tables 5 and 6. Protons alpha to nitrogen move downfield; H7 by 0.64 ppm, H2 by 1.62 ppm; and the average

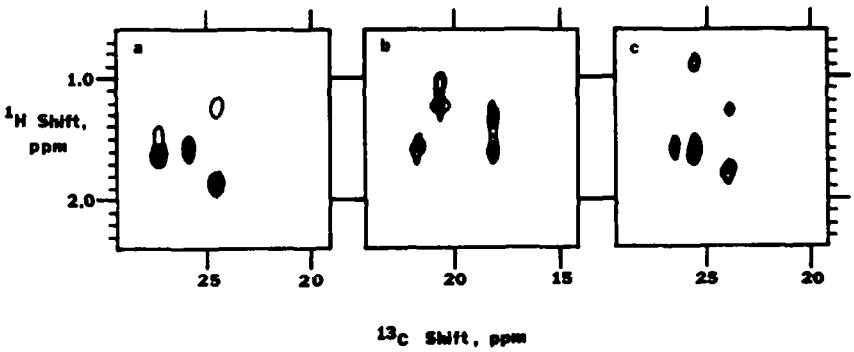


Figure 1. Proton-carbon correlation spectra of the regions corresponding to C3, C4, and C5 of Securinine (a), Allosecurinine (b), and Securinol A (c).

Table 3. 100 MHz ^{13}C-NMR Spectrum of Securinine ($CDCl_3$). Chemical shifts (ppm), proton correlations (ppm), and assignments.

Chemical Shift	Proton Correlation	Assignment
173.5	-	C11
170.0	-	C13
140.2	6.43	C15
121.3	6.61	C14
104.9	5.56	C12
89.4	-	C9
62.9	2.10	C2
58.7	3.83	C7
48.7	2.97, 2.42	C6
42.2	2.50, 1.78	C8
27.2	1.48 - 1.67	C5
25.8	1.48 - 1.67	C3
24.5	1.88, 1.24	C4

Table 4. 400 MHz ^{1}H-NMR Spectrum of Securinine·HCl (D_2O) Chemical shifts (ppm), multiplicities, proton-proton correlations, and assignments.

Chemical shift	Multiplicity	COSY[a]	Assignment
6.96	d	a	H14
6.74	d of d	ab	H15
6.02	s		H12
4.47	d of d	bc	H7
3.72	d of d	e	H2
3.39	m	f	H6ax, H6eq
2.99	d of d	cd	H8a
2.22	d	d	H8b
1.99 - 1.73	m	efg	H3ax, H3eq, H5ax, H5eq, H4a
1.40-1.31	m	g	H4b

[a] Peaks with the same letter are correlated by a cross peak in the COSY spectrum.

Table 5. 100 MHz ^{13}C-NMR Spectrum of Securinine·HCl (D_2O). Chemical shifts (ppm), proton correlations (ppm), and assignments

Chemical Shift	Proton Correlation	Assignment
175.1	-	C11
167.5	-	C13
138.2	6.74	C15
127.2	6.96	C14
112.0	6.02	C12
89.9	-	C9
63.9	4.47	C7
62.6	3.72	C2
47.8	3.39	C6
37.2	2.99, 2.22	C8
20.9	1.87 - 1.99	C3
18.2	1.87, 1.74	C5
15.2	1.87, 1.35	C4

Table 6. 400 MHz ^{1}H-NMR Spectrum of Allosecurinine ($CDCl_3$). Chemical shifts (ppm), multiplicities, proton-proton correlations, and assignments.

Chemical shift	Multiplicity	COSY[a]	Assignment
6.83	d of d	ab	H15
6.66	d	a	H14
5.73	s		H12
3.91	d of d	bc	H7
3.65	d of d	e	H2
2.75	m	f	H6ax, H6eq
2.68	d of d	cd	H8a
1.93	d	d	H8b
1.70	m	fg	H5ax, H5eq, H4a
1.42	m	gh	H4b
1.34	m	eh	H3eq
1.15	m	e	H3ax

[a] Peaks with the same letter are correlated by a cross peak in the COSY spectrum.

position of H6ax and H6eq by 0.70 ppm. Further, the chemical shifts of H6ax and H6eq, separated by 0.55 ppm in the free base, are merged into one non-first-order multiplet (separation ca. 0.06 ppm) in the hydrochloride. The protons on C3, C4 and C5 appear differently too. The protons on C4 are still widely separated in chemical shift as they were in securinine free base, but in the hydrochloride the downfield H4 multiplet is now part of the main multiplet at 1 73 - 1.99 ppm. The ^{1}H nmr spectrum of securinine·HCl in 50/50 (v/v) trifluoroacetic acid (TFA)/$CDCl_3$ was examined with the hope of observing H-C-N^+-H coupling as reported by Infarnet, Duplan and Huet for N-methylhexahydroisoindoline, 4.[7] While we did not observe this type of coupling, we did note the nmr appearance of protonated securinine in this solvent system was quite different than its appearance in D_2O Notably $H6_{ax}$ and $H6_{eq}$ were separated by 0.42 ppm

The ^{13}C-nmr spectrum of protonated securinine in D_2O was obtained and was assigned completely and unambiguously with reference to the results of a C-H correlation experiment (Table 5). In order to probe whether the major upfield shift of C3, C4, and C5 from 25.8, 24.5 and 27.2 ppm respectively in securinine free base ($CDCl_3$) to 20.9, 15.2, and 18.2 ppm respectively in securinine·HCl (D_2O) was primarily a solvent effect or not, the ^{13}C spectrum of securinine·HCl in $CDCl_3$ solvent was obtained (T = 55 C for adequate solubility). Under these conditions, C3, C4, and C5 appeared at 20.8, 14 4, and 17.2 ppm.

Allosecurinine is an epimer of securinine whose configuration at C2 is inverted. Its ^{1}H and ^{13}C spectra are readily assignable on the basis of COSY and C-H correlation experiments, and are reported in Tables 6 and 7. It is interesting to note the relative chemical shifts of H14 and H15 are reversed relative to their chemical shifts in securinine free base. The C3, C4, and C5 signals in the C-H correlation spectrum are shown in Figure 1.

Table 7. 100 MHz ^{13}C-NMR Spectrum of Allosecurinine ($CDCl_3$). Chemical shifts (ppm), proton correlations (ppm), and assignments.

Chemical Shift	Proton Correlation	Assignment
172.6	-	C11
167.5	-	C13
148.6	6.83	C15
122.6	6.66	C14
108.9	5.73	C12
91.7	-	C9
60.8	3.65	C2
58.8	3.91	C7
43.7	2.75	C6
42.7	2.68, 1.93	C8
22.2	1.70	C5
21.1	1.34, 1.15	C3
18.5	1.70, 1.42	C4

Table 8. 400 MHz ^{1}H-NMR Spectrum of Securinol A ($CDCl_3$). Chemical shifts (ppm), multiplicities, proton-proton correlations, and assignments.

Chemical shift	Multiplicity	COSY[a]	Assignment
5.70	t	a	H12
4.36	m	b	H15
2.99	d of "t"	abc	H14a
2.89	m	d	H7, H6a
2.80	m	ac	H14b
2.69 - 2.76	m	be	H8a, H6b, H2
1.80	m	f	H4a
1.58	m	df	H3a, H5a
1 48	d of d, m	bce	H8b, H5b
1.28	m	bfg	H4b
0.88	m	efg	H3b

[a] Peaks with the same letter are correlated by a cross peak in the COSY spectrum.

Our investigation of securinol A was made difficult by the availability of only a very small sample which contained modest amounts of impurities. Fortunately, our examination of the resulting complex spectra could be guided by our previous unraveling of the other securinine alkaloid spectra. The results are presented in Tables 8 and 9. The only point of worry was the assignment of H2 and H7. However a COSY cross peak coupling 0.88 ppm with 2.69 - 2.76 ppm laid this worry to rest since H7 would not be expected to couple to any proton at as high a field as 0.88 ppm. A signal at that high a field must have arisen from a proton of the piperidine ring. Thus 2.69 - 2.76 ppm must harbor H2 and 0.88 ppm must be one of the H3's.

Conformation of Securinine Alkaloids in Solution. Charts 1 and 2 depict several compounds containing a piperidine ring for which nmr data exist and for which a conformational assignment has been made. Table 10 summarizes pertinent nmr data for these compounds and for securinine(1), allosecurinine (2), and securinol A (3). The difference in chemical shift between the equatorial and axial protons of the CH_2 alpha to nitrogen in the piperidine ring ($\Delta = \delta_{eq} - \delta_{ax}$) is a parameter which is sensitive to the conformation of the ring. Compounds **5** - **14** all are purported to be locked in the conformations drawn for them in Chart 1, viz. all have the nitrogen lone pair anti to the proton on the adjacent carbon of the ring fusion. For these compounds, Δ is around +0.9 ppm with a scatter ranging from +0.41 ppm for **8** to +1.16 ppm for **6**. When the nitrogen lone pair is gauche to the proton on the adjacent carbon of the ring fusion, as in **15** - **20**, Δ is about -0.1 ppm (negative meaning H_{ax} downfield of H_{eq}) with a scatter from -0.49 ppm for **16** to +0.28 ppm for **18**. Clearly, securinine (**1**) with Δ = +0.55 ppm falls into the former group (lone pair anti to H2), and allosecurinine and securinol A fall into the latter group (lone pair gauche to H2). The fact that securinine's Δ is notably smaller than the typical value of 0.9 ppm suggests another interpretation: securinine is involved in a conformational equilibrium such as the ones depicted in Chart 2. For the compounds in Chart 2 Δ lies between the two extremes of +0.9 ppm and -0.1 ppm, depending on the position of equilibrium.

Table 9. 100 MHz ^{13}C-NMR Spectrum of Securinol A ($CDCl_3$). Chemical shifts (ppm), proton correlations (ppm), and assignments.

Chemical Shift	Proton Correlation	Assignment
173.94	-	C11 or C13
173.89	-	C11 or C13
111.5	5.70	C12
84.4	-	C9
65.2	2.7	C2
65.0	4.36	C15
58.9	2.89	C7
52.7	2.89, 2.7	C6
40.9	2.7, 1.48	C8
29.4	2.99, 2.80	C14
26.6	1.58	C5
25.6	1.58, 0.88	C3
23.9	1.80, 1.28	C4

Table 10. ^{1}H-NMR Data for Piperidine Derivatives

Compound	Δ (ppm)[a]	Reference
5	+1.00	b
6	+1.16	b
7	+0.47	c
8	+0.41	c
9	+0.96	b
10	+1.13	d
11	+0.90	e
12	+0.93	e
13	+0.76	e
14	+0.92	e
15	-0.25	b
16	-0.49	d
17	-0.35	c
18	0.0	e
19	+0.28	e
20	+0.25	e
21	+0.61	b
22	+0.65	b
23	+0.73	b
Securinine (**1**)	+0.55	f
Allosecurinine (**2**)	0.0	f
Securinol A (**3**)	+0.19	f

[a] This parameter is defined as $\delta H_{eq} - \delta H_{ax}$ for the CH_2 alpha to nitrogen in the piperidine ring. [b] Ref. 8 [c] Ref. 9 [d] Ref. 10 [e] Ref 11 [f] this work

Chart 1. Conformationally Rigid Piperidine Derivatives.

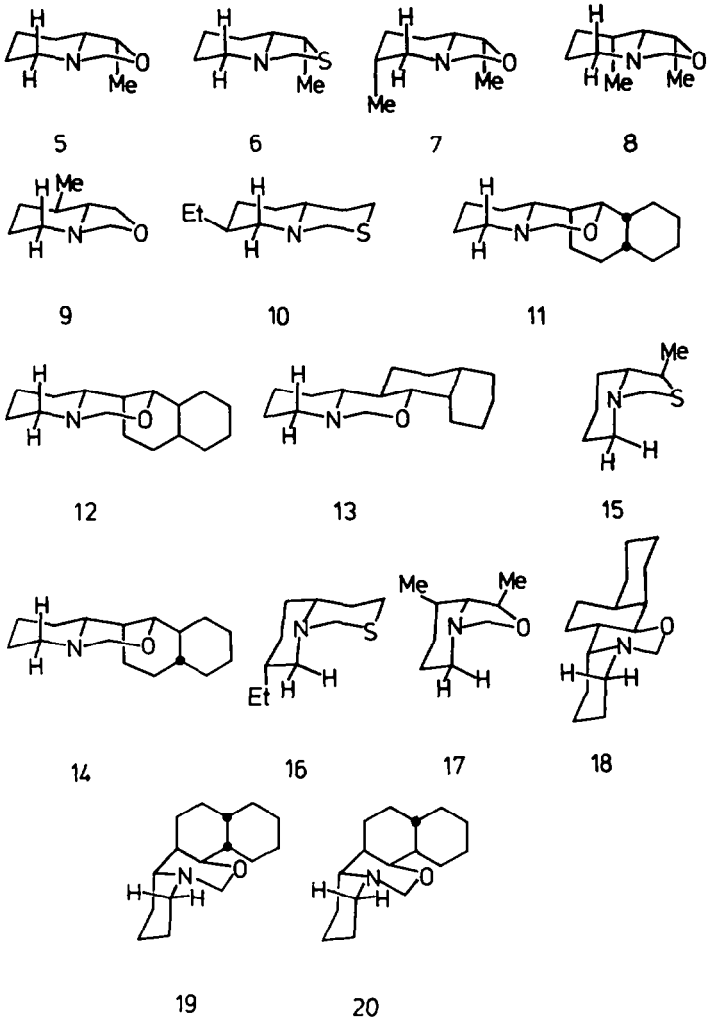
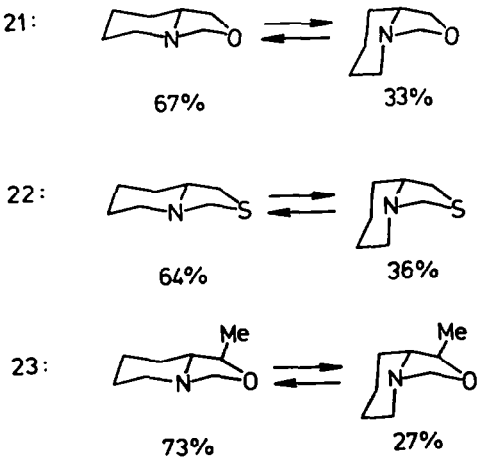
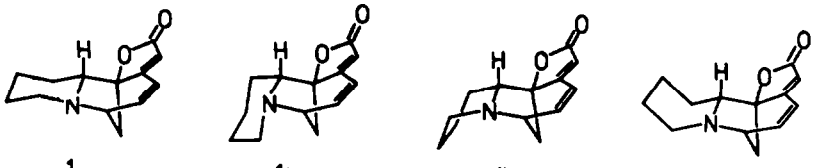


Chart 2. Piperidine Derivatives Involved in Conformational Equilibria.

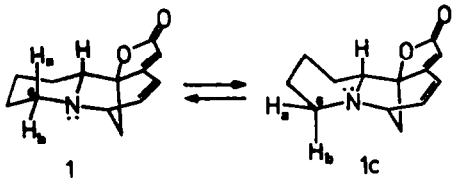


The question of a conformational equilibrium may be addressed by a molecular mechanics calculation This was done using Allinger's MMP2 force field, including SCF calculations of the dienone system of securinine. The steric energy of securinine itself was set to zero and three conformations corresponding to nitrogen inversion were considered. The results are shown below. Obviously **1a** and **1b** are too high in energy relative to **1** to be involved in a conformational equilibrium in any significant way.



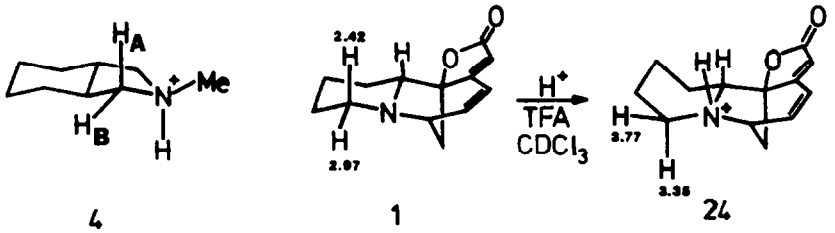
	1	1a	1b	1c
E_{rel} (kcal/mol)	0.00	+4 24	+4.34	+0.82

However, **1c** is a very reasonable partner for **1** in an equilibrium. At room temperature, K_{eq} for **1** $\rightleftharpoons$ **1c** is predicted to be 0.25. As can be seen below, H6a is anti to the nitrogen lone pair in **1** but becomes gauche to it in **1c**. Also H6b is gauche to the lone



pair in **1**, but becomes anti to it in **1c**. If we assume Δ = +0 9 ppm for **1** (i.e the average value for **5** - **14** from Chart 1), then **1c** would have Δ = -0.9 ppm, and a 4:1 equilibrium mixture of **1** and **1c** respectively would exhibit Δ = +0 54 ppm. This is remarkably (and most likely fortuitously) close to the observed value of +0.55 ppm. To test the notion of a conformational equilibrium, the proton spectra of securinine at 0 °C, -25 °C, -45 °C, and -60 °C were obtained. As expected, Δ increased to 0 62 at -60 °C as a result of a greater preponderance of **1** in the equilibrium mixture at the lower temperature. The H6ax-H5ax and H6ax-H5eq coupling constants, 7.5 Hz and 7.0 Hz respectively at room temperature, were 10.7 Hz and 3.1 Hz respectively at -60 °C. This too is consistent with an increase in the proportion of chair **1** at the expense of boat **1c** as the temperature is lowered. At -60 °C, K_{eq} is predicted to be 0.14, from which a Δ of 0.67 ppm may be calculated, as above. This compares to the observed Δ of 0.62 ppm. The concept of an equilibrium involving **1** and **1c** is supported by molecular mechanics calculations, and is consistent with securinine's spectrum at room temperature and its response to the lowering of temperature.

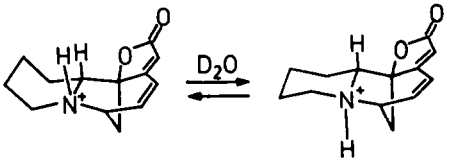
In TFA/$CDCl_3$ solvent, protonated securinine exhibits Δ = -0.42 ppm A plausible explanation follows from the work of Huet, et al.[7]. Using the same solvent system, they observed a protonation shift for proton A of +0.2 ppm and for proton B of



4 **1** **2a**

+1.12 ppm in N-methylhexahydroisoindoline, **4**. If protonated securinine exists predominantly in the boat form corresponding to **1c**, viz. **2a**, the protonation shifts are then +0.38 and +1.35 ppm, which are similar to the +0.2 and +1 12 ppm reported by Huet, et al.

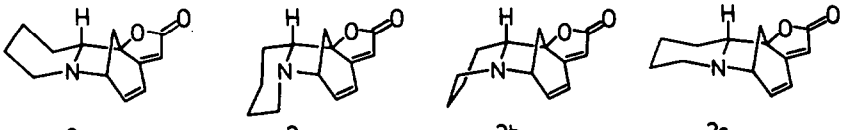
In D_2O, protonated securinine (viz. the hydrochloride) gives a ^{1}H-NMR spectrum quite different than that obtained in TFA/$CDCl_3$. The H6 protons give one multiplet with $\Delta < 0.06$ ppm. This suggests that in D_2O both **24** and **25** exist. The postulation of **24** as the preferred conformation in TFA/$CDCl_3$, and an equilibrium between **24** and **25** in D_2O is consistent with



24 **25**

the proton chemical shift of H-2. In **24** H-2 eclipses the N-H proton and appears at 3.99 ppm. When **25** contributes to the overall picture in D_2O, a conformation in which H-2 is anti to the N-H proton is being mixed in. The chemical shift of H-2 in **25** is expected[7] to be smaller (more upfield) than H-2 in **24**, thus H-2 in the **24** ⇌ **25** equilibrium should be upfield of H-2 in **24**, and indeed appears at 3.72 ppm. One may estimate, from the work of Huet et al., the chemical shift of H-2 in **25** as (2.10 + 0.2) = 2.30 ppm. This rough estimate would predict an equilibrium in D_2O involving 84% **24** and 16% **25**. We emphasize that this is a very crude estimate and it neglects solvent effects. Preliminary MMP2 calculations on the protonated forms of **1**, **1a**, **1b**, and **1c** indicate no significant changes in the energetic ordering, so the postulation of an equilibrium between **24** and **25** is not contradicted by calculation.

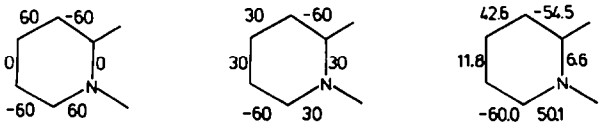
Allosecurinine's solution conformation may be addressed by MMP2 calculation. Results are shown below, with energies given relative to **1**. Here the most plausible equilibrium, **2** ⇌ **2c**, favors **2** by 1.87 kcal/mole. At room temperature this corresponds to $K_{eq} = 0.042$ or roughly 96% **2** and 4% **2c**. Clearly the nmr results will not be explained by recourse to the notion of a conformational equilibrium. Allosecurinine, it may be said, has the conformation shown for **2**. The chemical shift of H2, 3.65 ppm, indicates unambiguously that H2 is not anti to the nitrogen lone pair. The anti relationship produces a characteristic



2 **2a** **2b** **2c**

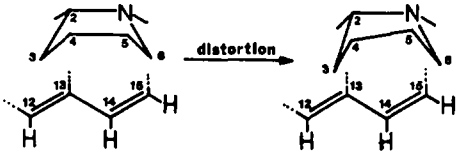
E_{rel} (kcal/mol)	-0.35	+4.96	+4.17	+1.52

upfield shift as is observed for H2 in securinine free base (2.10 ppm). As can be seen by the calculated torsion angles the piperidine ring of allosecurinine is perhaps better described as a boat distorted toward a twist-boat. The distortion is such as to



Boat Twist-Boat Calculated for **2**

move C3 more squarely into the shielding region of the C12-C13-C14-C15 pi system and to move C6 away from it. Thus one observes H3ax and H3eq at 1.15 and 1.34 ppm respectively, dramatically upfield of their position in the chair piperidine of



securinine (1.48 - 1.67 ppm).Another consequence of this distortion is to destroy the anti relationship between H6ax and the nitrogen lone pair, attenuating the upfield shift of this proton, and causing both H6ax and H6eq to absorb at 2.75 ppm. The calculated dihedral angles between the nitrogen lone pair and H6ax and H6eq are 170.7° and 52 4° respectively. (By comparison, the corresponding calculated dihedral angles in **1** are 174.0° and 67.0°).

Securinol A also exhibits the tell-tale upfield shift of one of the H3 protons, indicative of the twist-boat seen for allosecurinine (viz. H3 for allosecurinine 1 34, 1.15 ppm; H3 for securinol A 1.58, 0.88 ppm; but H3 for securinine 1.48 - 1.67 ppm). However, the downfield shift of H2 of allosecurinine (3.65 ppm) versus securinine (2.10 ppm) is not as pronounced in the case of securinol A (2.69 - 2.76 ppm) versus securinine

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