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CMR ASSIGNMENTS OF THE SECURININE ALKALOIDS

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ABSTRACT.—The cmr spectrum of securinine (**1**), allosecurinine (**2**), and their borohydride reduction products (**3** and **4**) have been assigned on the basis of lanthanide shift experiments and specific deuteration.

The securinine alkaloids comprise a group of about a dozen alkaloids sharing a unique ring structure and biosynthesis (1,2). It has recently been demonstrated that securinine is a stereospecific $GABA_A$ receptor antagonist of restricted conformation (3), making it a useful tool for understanding GABA receptor topography. It was therefore of interest to assign the cmr spectra in this series in order to facilitate identification of new alkaloids and to understand better the limited conformational mobility of the alkaloids.

Single-frequency off-resonance decoupling (SFORD) experiments were of limited utility in assigning resonances because the respective methylene, methine, and quaternary carbon resonances occurred in closely spaced groups that could not be completely assigned without further experimental techniques. The lanthanide shift reagent $Eu(fod)_3$ provided invaluable information, and the trends in chemical shifts throughout the series were correlated with conformation predicted by MM2 molecular mechanics calculations. Specific deuteration was helpful in assigning the resonances of dihydrosecurinine (**3**). Finally, the previously assigned spectrum of indolizidine (4) provided confirmation. Assignments are tabulated in Table 1 for securinine (**1**), allosecurinine (**2**), 14,15-dihydrosecurinine (**3**), and 14,15-dihydroallosecurinine (**4**). Detailed comparisons of predicted and observed shifts are not presented for all cases, because a simple graphical comparison of the induced shifts of each set of two or three ambiguous resonances gave identical asignments. The distance term (r^3) in the McConnell-Robertson equation (5,7) is dominant over the angle term (cos 2 θ) for the securinine alkaloid structures.

DISCUSSION

SECURININE (**1**).—Pmr lanthanide shift experiments were performed to confirm that the shift reagent coordinated preferentially to the carbonyl oxygen of securinine. Calculations of the predicted relative magnitudes of the induced shifts were made using the McConnell-Robertson equation (5). It was assumed that the europium atom was located 2.8 Å from the carbonyl oxygen colinear with the C-O bond (5). Interatomic angles and distances used were those predicted by the molecular mechanics program, using X-ray data (6) as a starting point for the calculations. In the case of securinine, the preferred conformation was predicted to be a chair in the piperidine ring, with the nitrogen lone pair transoid to the C-2 proton (Figure 1). This conformation was favored by 4.37 kcal/mole over a piperidine ring boat with the nitrogen lone pair cisoid to the C-2 proton. Thus, the less-favored conformation was not expected to contribute to the nmr spectrum.

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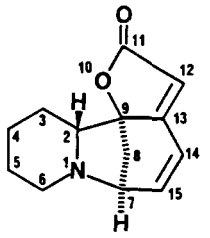

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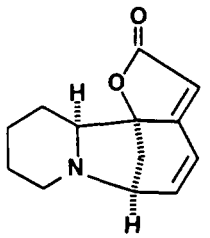

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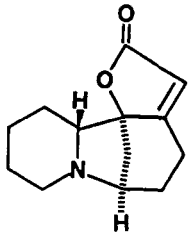

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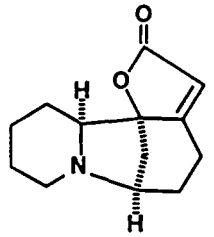

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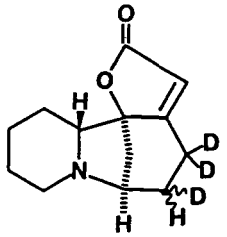

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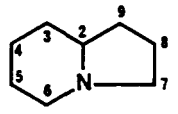

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TABLE 1. Shift Assignments for Securinine Alkaloids.
(Reference is middle $CDCl_3$ signal=76.89 ppm)

Carbon No.	1	2	3	4	5
C-2	62.7	60.5	61.3	67.2	64.1
C-3	27.4	20.8	25.5	23.8	30.7
C-4	24.6[a]	21.9	21.5	23.8	24.2
C-5	26.0[a]	18.3	23.4	26.6	25.1
C-6	48.8	43.4	48.0	50.2	52.7
C-7	58.9	58.5	59.3	56.3	53.9
C-8	42.4	42.4	38.3	40.8	20.3
C-9	89.5	91.0	91.0	89.7	30.1
C-11	173.4	172.4	175.4	172.7	—
C-12	105.0	110.6	109.1	111.6	—
C-13	170.2	167.3	172.9	172.7	—
C-14	140.3	148.4	32.2	32.5	—
C-15	121.4	122.4	22.9	23.8	—

[a]Assignment tentative. Entries may be reversed.

Three singlets are seen in the SFORD spectrum of securinine. The resonance at 89.5 ppm can be unambiguously assigned to C-9. The two others (170.2 ppm and 173.4 ppm) belong to C-11 and C-13. The resonance at 173.4 ppm shows a higher lanthanide-induced shift and therefore can be assigned to the carbonyl carbon, C-11.

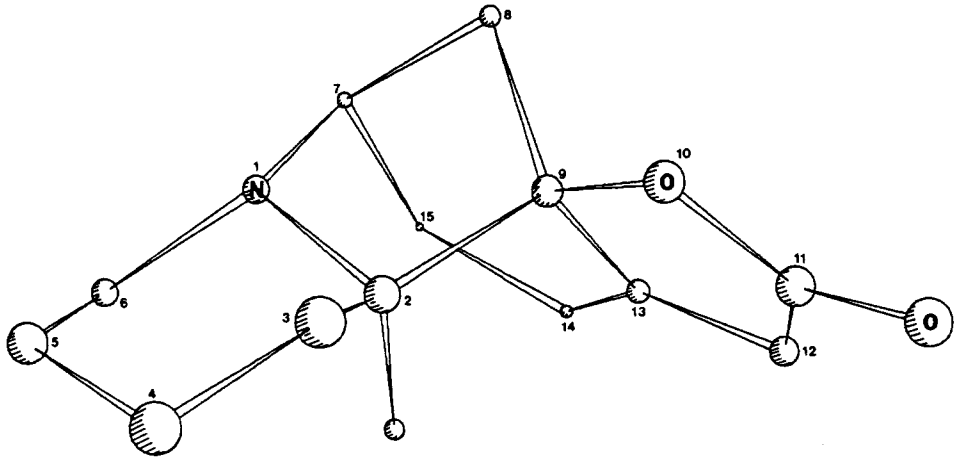

FIGURE 1. Structure of securinine predicted by molecular mechanics calculation.

Five doublets appear in the SFORD spectrum: three unsaturated carbons at greater than 100 ppm (105.0, 121.4, 140.3 ppm). Of these three, the peak at 105.0 ppm shows the largest lanthanide-induced shift and thus is assigned to C-12, which is closest to the europium atom. The peak at 121.4 ppm shows an intermediate induced shift and is assigned to C-14. The peak at 140.3 ppm shifts the least and thus belongs to C-15. Unsaturated system carbons are expected to deviate from the McConnell-Robertson predictions and, indeed, do not show close agreement; however, the overall trend of smaller induced shift with increased distance is maintained. The remaining two doublet resonances (62.7 and 58.9 ppm) belong to C-2 and C-7. The signal at 62.7 ppm shows a larger induced shift and is assigned to C-2.

The SFORD spectrum of securinine shows five triplets; the two at lower field (42.4 ppm, 48.8 ppm) are attributed to methylene attached to nitrogen, (C-6), and to C-8. The peak at 42.4 ppm shows a larger induced shift and is thus due to C-8. The three triplet resonances in the range of 20 to 30 ppm can also be distinguished. The peak at 27.4 ppm has the largest induced shift and is assigned to C-3. The peaks at 24.6 ppm and 26.0 ppm have similar induced shifts and cannot be differentiated in the same way. Consideration of the assigned spectrum of indolizidine (**5**) whose piperidine ring should probably assume a chair conformation similar to that of securinine and therefore should present a similar magnetic environment, suggests that the peak at 24.6 ppm should be assigned to C-4 and that at 26.0 to C-5 because the corresponding carbons of indolizidine have similar shifts (4).

ALLOSECURININE (**2**).—The ^{13}C-spectrum of allosecurinine shows several differences from that of securinine. The lower three methylene resonances as a group shift from 24-28 ppm to 18-22 ppm. The unsaturated resonances also change, and these differences can best be explained by a through-space shielding interaction of the *pi*-system upon the piperidine ring carbons. Alternatively, a γ-gauche interaction of C-3 and C-6 can be postulated; however, hydrogenation of the 14, 15 double bond eliminates the effect (lowest methylene of **4** is at 23.8 ppm). The shielding effect is as great as 8 ppm for C-5 comparing securinine and allosecurinine. Molecular mechanics calculations for allosecurinine show the most favored piperidine ring conformation to be a boat with C-4 and C-5 pointing away from the *pi*-system at a distance of 4 Å. Predicted induced shifts for this conformation of allosecurinine were found to differentiate between ambiguous resonances in the same manner as with securinine, and detailed arguments will not be presented. Differences from securinine include a predicted and observed difference in

the induced shifts between C-4 and C-5, making differential assignment possible; also, a very slight difference in observed induced shift for C-11 and C-13 conflicted with the predicted wide difference. This has been reported to occur with carbons nearest to the europium atom (7). Assignments for allosecurinine are given in Table 1.

DIHYDROSECURININE (**3**) AND DIHYDROALLOSECURININE (**4**).—Assignment of resonances for the reduction products **3** and **4** presents further difficulties because the number of aliphatic triplets is increased by two. The quaternary and methine carbons were assigned on the basis of induced shift, as for the parent compound. A detailed correlation of the predicted and observed shifts for the dihydrosecurinine (DHS) triplets is made in Table 2. C-14 is the only allylic position and was expected to be deshielded. Confirmation of this assignment was obtained by deuteration of securinine with $NaBD_4$ in EtOD. This d_3-DHS (**3a**) showed a conversion of the resonance at 32.3 ppm to a weak multiplet (pentet predicted) centered at 31.7 ppm, giving an isotope shift of −0.47 ppm. Thus, the 32.3 ppm resonance is due to C-14 since the allylic protons at this position should both exchange with the solvent. The signal at 22.9 ppm in d_3-DHS showed reduced intensity and an isotope shift of −0.34 ppm. These isotope shifts are in rough agreement with predicted values of −0.25 per deuteron for directly bonded carbons and −0.10 for adjacent carbons (8). A small shift (−0.04 ppm) was also detected to C-7. The remaining methylene resonances can be assigned using shift reagent data as before.

TABLE 2. Comparison of Predicted and Observed $Eu(fod)_3$ Induced Shift, for Dihydrosecurinine (**3**)

Carbon No.	Chemical Shift	Observed[a]	Predicted[b]
C-8	38.3	6.5	6.7
C-14	32.2	4.1	5.8
C-3	25.5	4.2	5.1
C-15	22.9	4.3	4.3
C-6	48.0	4.0	3.0
C-5	23.4	2.9	2.8
C-4	21.5	2.8	2.8

[a]Induced shift extrapolated to 1.0 Mole ratio of shift reagent: alkaloid.

[b]Shift predicted by McConnell-Robertson equation, values normalized to induced shift of C-4 resonance.

The spectrum of **4**, assigned on the basis of $Eu(fod)_3$ data, is unusual in that the resonances for C-3, C-4, and C-15 all occur at 23.8 ppm. Addition of shift reagent separates the three signals. As mentioned above, the shielding effect seen in allosecurinine is eliminated on reduction, while molecular mechanics calculations show a similar favored conformation for both **2** and **4**.

Examination of Table 1 shows a coherence to the independently made assignments. C-9 is virtually the same for all four compounds, occurring from 89.5-91.0 ppm. C-8 is identical in securinine and allosecurinine and is slightly shielded by reduction of the adjacent *pi* system. The piperidine ring carbons occur at similar resonances with the exception of allosecurinine.

EXPERIMENTAL

Spectra were run in $CDCl_3$ solution on a Varian CFT-20 spectrometer at 20 MHz. Chemical shift reference was made to TMS but precise measurements were based on the middle signal of $CDCl_3$ equivalent to 76.89 ppm.

Securinine and allosecurinine were isolated by silica gel flash chromatography of the crude base fraction extracted from *Securinega suffructicosa* (Pellas) Rehder. Both alkaloids gave spectra (ms, ir, uv) consistent with literature values (1). Elemental analyses of the hydrochloride salts were within 0.4 per cent for C,

H, and N. **3** and **4** were prepared by reduction of the parent compound with $NaBH_4$ in EtOH at room temperature, and purified by flash chromatography. $Eu(fod)_3$ experiments were run using at least three concentrations of shift reagent between 0.2 and 0.6 mole ratio to the alkaloid (7). Molecular mechanics calculations were made using the MM2 program (9).

ACKNOWLEDGMENTS

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