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STUDIES ON THE NMR SPECTROSCOPIC PROPERTIES OF GELSEMINE—REVISIONS AND REFINEMENTS

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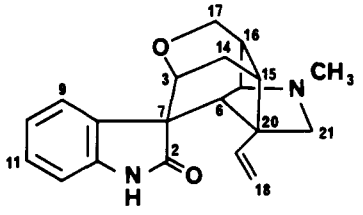
Gelsemium sempervirens (L.) Jaume St.-Hilaire has been used in medicine for its antispasmodic and analgesic properties. It was at one time recognized in several pharmacopoeias and found use in the treatment of influenza (1,2). For toxicity reasons, it is no longer included in the current pharmacopoeias.

The principal alkaloid, gelsemine, was first isolated by Wormley in 1870, and its physiological properties in vivo have been studied for many years (3-8). It is not the most potent alkaloid in this series thus far known; gelsemicine has that distinction. The structure of gelsemine (**1**) was established through



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X-ray crystallographic analysis (9) more than 90 years after its initial discovery. Mass spectrometry proved to be a useful tool for the identification of gelsemine and its analogs (10) and 220 MHz ^{1}H-nmr (11) and ^{13}C-nmr spectra (11,12) of gelsemine (**1**) have been described.

As part of our program on the isolation of plant anticancer agents, we found that extracts of *G. sempervirens* displayed cytotoxic activity in the KB or P-388 test system (13). In order to assist in future studies of alkaloids in this series, we decided to reestablish the ^{1}H- and ^{13}C-nmr assignments obtained previously.[1] Although most of the assignments in the ^{1}H- and ^{13}C-nmr spectra (Table 1) compare favorably with those derived earlier (11,12), a number of revisions were found to be necessary.

Figure 1 shows the homonuclear 2-D correlation spectrum of gelsemine in the range 1.9-4.1 ppm. It is clear that the original assignments for H-14a, H-14e and H-15 and H-16 should be revised. Thus, the signal at 2.43 ppm can now be attributed to H-16, rather than H-14a, since it is the only proton with coupling to the H-17 methylene protons. Consequently, the signal at 37.93 ppm could now be ascribed to C-16. The axial proton at C-14, coupling to both H-3 (J=2.8 Hz) and H-14e (J=14.2 Hz) but not to H-15, was assigned to the signal at 2.83 ppm. This is also clearly shown in the heteronuclear correlation spectrum of gelsemine, in which this signal correlates with that for C-14, which is the most upfield carbon at 22.67 ppm. The equatorial proton at C-14 appears as a doublet of doublets of doublets (J=14.2, 6.1, 3.2 Hz), as expected from Dreiding models where only the equatorial proton is predicted to couple with H-15. Revision in the assignment of H-15 is also necessary, though its precise chemical shift and multiplicity remain obscured below one of the C-21 methylene protons. The chemical shift is perhaps more clearly seen in Figure 1 where the coupling to H-14e is apparent. The corresponding carbon chemical shift is 35.54 ppm.

The only remaining protons to be discussed are therefore those on C-21. Assignment of these protons was made possible through the observation of long-range coupling between one of these

[1]Gelsemine was actually the first alkaloid whose ^{13}C-nmr spectrum was assigned (12).

TABLE 1. Original and Revised Proton and ^{13}C-nmr Assignments of Gelsemine (**1**)[a]

Proton	Original[b] Chemical Shift	Multiplicity	Revised[d] Chemical Shift	Multiplicity	Carbon	Original[e]	Revised[f]
					C-2	179.0	179.25
H-3	3.79	d, 2.8	3.81	s	C-3	69.2	69.24
H-5	3.47	s	3.46	s	C-5	71.7	71.81
H-6	1.97	s	1.98	s	C-6	40.2	50.47
H-9	7.43	dd, 7.5, 2.0	7.40	d, 7.6	C-7	53.7	53.96
H-10	6.97	ddd, 7.5, 7.5, 2.0	6.99	ddd, 7.2, 7.2, 1.0	C-8	131.8	131.74
H-11	7.15	ddd, 7.5, 7.5, 2.0	7.17	ddd, 7.2, 7.2, 1.0	C-9	127.7[c]	127.97
H-12	6.65	dd, 7.5, 2.0	6.74	d, 7.6	C-10	121.4	121.41
H-14a . . .	2.37	d, 8.0	2.83	dd, 14.2, 2.8	C-11	128.0[c]	127.70
H-14e . . .	ca. 2.0	d, 8.0	2.01	ddd, 14.2, 6.1, 3.2	C-12	108.7	108.80
H-15	2.83	dd, 14.5, 2.8	ca. 2.30		C-13	140.3	140.52
H-16	ca. 2.30	ddd, 14.5, 2.0, 2.0	2.43	bd d, 8.3	C-14	22.6	22.67
H-17a . . .	3.91	dd, 11.0, 2.0	3.92	dd, 11.0, 2.0	C-15	37.8	35.54
H-17e . . .	4.10	dd, 11.0, 2.0	4.11	dd, 11.0, 2.0	C-16	35.6	37.93
H-18c . . .	4.95	dd, 18.0, 1.8	4.95	dd, 17.8, 1.1	C-17	61.1	61.33
H-18t . . .	5.09	dd, 11.0, 1.8	5.09	dd, 11.0, 1.1	C-18	111.9	111.89
H-19	6.28	dd, 18.0, 11.0	6.26	dd, 17.8, 11.0	C-19	138.5	138.50
H-21 exo . .	2.78[c]	d, 10.0	2.78	d, 10.4	C-20	53.7	53.82
H-21 endo .	2.32[c]	d, 10.0	2.32	d, 10.4	C-21	65.9	65.88
N-CH_3 . . .	2.24	s	2.25	s	N-CH_3	50.4	40.40
NH	—		8.71	s			

[a]$CDCl_3$ was used as a solvent; chemical shifts in ppm; coupling constants in Hz.
[b]Obtained at 220 MHz, data from Wenkert *et. al.* (12).
[c]Assignments may be reversed.
[d]Obtained at 360 MHz.
[e]Obtained at 55.51 MHz, data from Wenkert *et. al.* (12).
[f]Obtained at 90.84 MHz.

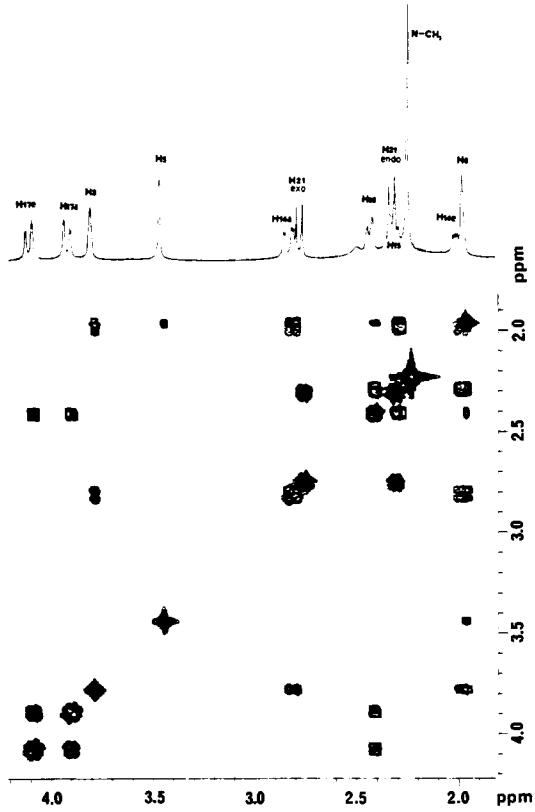

FIGURE 1. Two-dimensional correlation spectrum of gelsemine (**1**) in the range 1.9-4.1 ppm.

(2.78 ppm) and H-5 at 3.46 ppm. Molecular models indicate that this should be the *exo* (α) proton at C-21, leaving the doublet at 2.32 ppm to be assigned to the endo proton. Unfortunately, the close chemical shifts of H-15 and H-21 *endo* preclude observation of any nOe between these protons. However, a NOESY experiment did reveal an interaction between H-6 and H-21 *exo*, which substantiated the proton assignments. The N-CH_3 displayed nOe effects with both H-6 and H-16, as well as with both 21-protons, suggesting that inversion of the lone pair occurs with ease.

In the ^{13}C-nmr spectrum, besides the revisions indicated previously, the most significant correction is for C-6 and the N-CH_3 group, whose assignments must now be reversed; C-6 at 50.47 ppm and N-CH_3 at 40.4 ppm. The heteronuclear COSY spectrum also allowed the assignment of C-9 (127.97 ppm) and C-11 (127.70 ppm) to be made unambiguously based on their proton multiplicities.

Two quaternary carbons, C-7 and C-20, also have very close chemical shifts. They were distinguished through a SINEPT experiment (14) irradiating H-9 and H-18. The signal at 53.96 ppm was selectively enhanced following irradiation of H-9, and the resonance at 53.82 ppm was enhanced on irradiating H-18. These signals are therefore C-7 and C-20, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H-nmr spectra were recorded on a Nicolet Model NMC 360 (360 MHz) instrument with a Nicolet Fourier-Transform attachment using $CDCl_3$ as the solvent and TMS as an internal standard. The ^{13}C-nmr spectrum was obtained in $CDCl_3$ at 90.84 MHz on the same instrument.

ISOLATION OF GELSEMINE (**1**).—Gelsemine (**1**) was isolated from the stem material of *G. sempervirens* through MeOH extraction, acid-base workup and column chromatography as described in a subsequent publication (15).

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