Table III—Effect of Time of Ball Milling of *P. bracteatum* Capsule Tissues on Distribution of Particle Size and Thebaine Content [a]

Milling Time, min	Mesh Size	Sieve Weight g	Sieve Weight %	Thebaine Weight, %	$\bar{x}$	Δ%[b]
1.0	>40	4.77	27	1.12		
	40–70	4.62	26	1.20		
	70–140	6.41	36	1.31	1.264	−1.7
	140–260	1.19	6	1.60		
	<260	0.95	5	1.64		
2.0	>40	0.92	4	0.99		
	40–70	4.14	17	1.06		
	70–140	11.63	47	1.21	1.286	—
	140–260	4.59	19	1.51		
	<260	3.26	13	1.62		
3.0	>40	0.22	2	0.95		
	40–70	1.18	8	0.96		
	70–140	7.30	48	1.07	1.229	−4.4
	140–260	1.90	13	1.35		
	<260	4.30	29	1.53		
4.5	>40	0.09	1	0.83		
	40–70	0.33	2	0.89		
	70–140	7.12	47	1.06	1.227	−4.6
	140–260	3.10	20	1.31		
	<260	4.57	30	1.47		

[a] Capsules were deseeded, crushed, freeze dried for 24 hr, and ball milled. [b] Percent difference from 2 min of milling time thebaine yields.

sponse were 0.1–1.0 μg of thebaine/sample. Detector and/or integrator saturation occurred when the thebaine concentration per GLC injection exceeded ~2 μg. Reproducibility between triplicate analyses at each concentration of alkaloid was ±1%. Integrator responses to samples yielding $<10^5$ μv/sec were reanalyzed to give counts $>10^5$ either by adjustment of the injection volume or by increased electrometer sensitivity.

Linear detector responses for isothebaine, papaverine, alpinigenine, laudanosine, salutaridine, reticuline, and several additional alkaloids from *Papaver* except morphine were similar. The detector response was not linear with morphine because it severely tailed under GLC conditions, possibly because of adsorption onto the column. Morphine was calibrated as its *N,O*-bis(trimethylsilyl)acetamide derivative for samples suspected of containing this alkaloid but was not usually included in standard calibration mixtures.

GLC flame-ionization responses to varying concentrations of thebaine *versus* a constant level of cholesterol acetate were linear throughout the range analyzed. Linear ratio detector responses also were obtained when tetrahydrothebaine was used as an internal standard. The disadvantage of tetrahydrothebaine in routine analyses, alone or in combination with cholesterol acetate, was its relatively short retention time, especially at high column temperatures. Tetrahydrothebaine usually eluted from the GLC column as a rider to the solvent peak, which may have complicated manual peak area determinations. Laudanosine, antipyrine, and tetrahydropalmatine were not suitable for use as internal standards in GLC analyses, although they were recommended by the Third Working Group on *P. bracteatum* (9).

A small quantity of thebaine (<1%) always adsorbed to new OV-17–Gas Chrom Q columns. Saturating the columns with an alkaloid standard containing thebaine (~0.2–0.4 μg) by injection of standard mixtures obviated this problem. Calibration solutions consisted of 1 μg of cholesterol acetate plus 0.2–1 μg each of thebaine, isothebaine, and codeine in 1 ml of absolute ethanol.

By carefully standardizing and checking each step of the procedure for loss of thebaine, it was determined that this procedure can be used for comparison of intra- and interlaboratory analyses.

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^{13}C-NMR Spectra of Strychnos Alkaloids: Brucine and Strychnine

SHIVA P. SINGH *, VIRGIL I. STENBERG ‡, SURENDRA S. PARMAR *ˣ, and SYLVIA A. FARNUM ‡

Received October 14, 1977, from the **Department of Physiology, School of Medicine, and the* ‡*Department of Chemistry, University of North Dakota, Grand Forks, ND 58202.* Accepted for publication June 27, 1978.

Abstract □ The natural abundance ^{13}C-NMR spectra of brucine and strychnine were obtained using the pulse Fourier transform technique. The chemical shifts of various carbon resonances were assigned on the basis of substituent effects on benzene shifts, intensities of signals, multiplicities generated in single-frequency off-resonance-decoupled spectra, and comparisons with the chemical shifts of structurally related compounds.

Keyphrases □ Brucine—^{13}C-NMR spectrometry, chemical shifts assigned □ Strychnine—^{13}C-NMR spectrometry, chemical shifts assigned □ ^{13}C-NMR spectrometry—brucine and strychnine, chemical shifts assigned

Several literature reports show the correlation of the structure of the various classes of alkaloids with their ^{13}C-NMR spectra (1–6). The structure and stereochemistry of strychnos alkaloids, brucine (I) and strychnine (II), were characterized (7, 8), but no reports are available concerning the assignments of their carbon resonances.

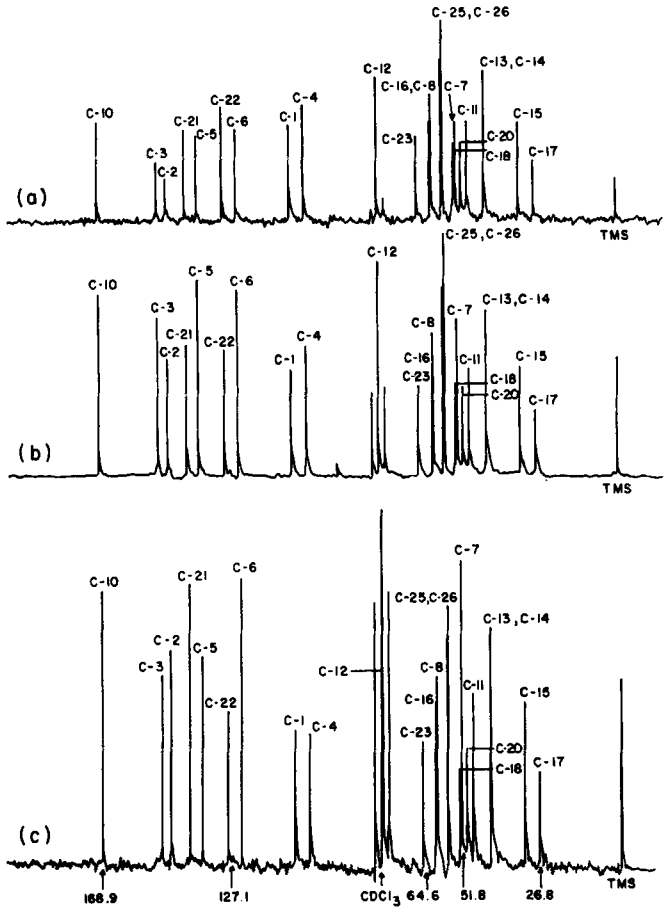
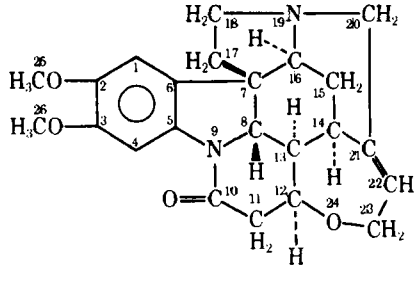


Figure 1—*Proton noise-decoupled ^{13}C-NMR spectrum of brucine. Key: (a), pulse repetition of 2.5 sec; (b), pulse repetition of 7 sec; and (c), pulse repetition of 25 sec.*

Previous efforts in assigning the ^{13}C-NMR chemical shifts of synthetic and natural therapeutic agents (9–12) initiated the ^{13}C-NMR analysis of brucine and strychnine. Strychnine is a powerful convulsant and is not used therapeutically because of its high toxicity.



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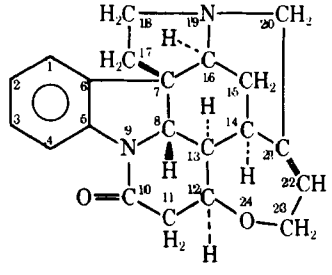


II

Table I—^{13}C-Chemical Shifts of Brucine

Assignment	Multiplicity	Chemical Shift
C-10	s	168.9
C-3	s	149.2
C-2	s	146.2
C-21	s	140.6
C-5	s	136.0
C-22	d	127.1
C-6	s	123.6
C-1	d	105.6
C-4	d	101.1
C-12	d	77.3
C-23	t	64.6
C-16[a]	d	60.3
C-8[a]	d	59.9
C-26[b]	q	56.4
C-25[b]	q	56.1
C-18	—[c]	52.6
C-7	—[c]	51.8
C-20	—[c]	50.2
C-11	—[c]	48.2
C-13	d	42.4
C-14	d	42.4
C-15	t	31.6
C-17	t	26.8

[a] These values may be interchanged. [b] These values may be interchanged. [c] The signal multiplicities are not clear.

The natural abundance ^{13}C-NMR spectra of brucine and strychnine were recorded in 30% (w/v) deuterochloroform as an internal lock and solvent with tetramethylsilane as a reference. In each case, a proton noise-decoupled and single-frequency off-resonance-decoupled (SFORD) spectra were run. The SFORD spectra differentiated the various types of carbons. The assignments of the carbon resonances were made on the basis of chemical shift theory, multiplicities generated in SFORD spectra, signal intensities, and comparisons with other structurally related compounds.

EXPERIMENTAL

The ^{13}C-NMR spectra[1] of brucine[2] and strychnine[3] (recrystallized from chloroform) were run in 10-mm tubes with spectrometer settings as follows: spectra width, 4 kHz; pulse width, 12 μsec (90°); repetition rate, 2.5, 7, 25, and 35 sec; and data points, 4 K.

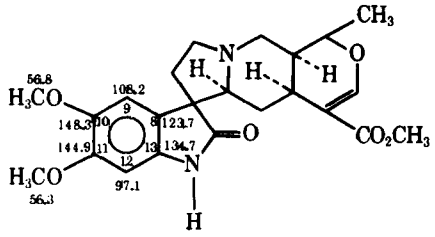
RESULTS AND DISCUSSION

Brucine—The ^{13}C-NMR chemical shifts of brucine are recorded in Table I. The various carbon resonances are illustrated in Fig. 1. Twenty-two separate signals account for all 23 carbon resonances of I. The nine signals in the lower field region of the 102–169-ppm chemical shift are due to aromatic carbons, amidic carbon, and ethylenic carbons. The 13 signals in the higher field region of the 26–78-ppm chemical shift are due to the remaining 14 carbons.

The six singlets in the lower field region represent the carbon resonances of C-2, C-3, C-5, C-6, C-10, and C-21. The singlet at the farthest downfield region (168.9 ppm) is assigned to C-10 on the basis of the chemical shift theory of the carbonyl carbons of amides (13). Earlier studies indicated that a directly bonded methoxy group to the benzene nucleus produces a 31.4-ppm downfield shift at the same carbon and a 14.4-ppm upfield shift at the *ortho*-carbon (13). Since C-2 and C-3 in I are attached to a methoxy group and are *ortho* to the methoxy group, the singlets observed at 149.2 and 146.2 ppm are thus assigned to C-3 and C-2, respectively. The resonance of C-3 observed at the lower field in comparison to C-2 is due to the *meta* and *para* effect of nitrogen (13) on C-3 and C-2, respectively.

The assignments of various carbon resonances of I compare well with the chemical shifts of carbons in III and IV (1), with the exception of the

[1] Jeol FX60 spectrometer operating at 15.00 kHz.
[2] Fisher Scientific Co., New York, N.Y.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.



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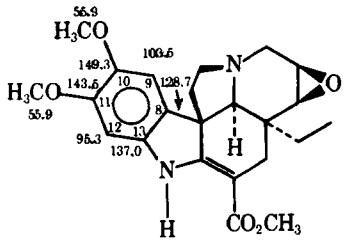

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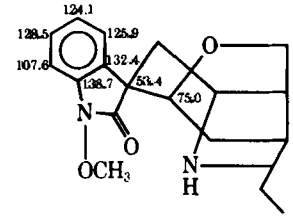

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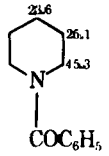

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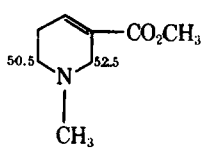

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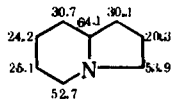

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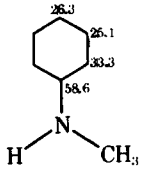

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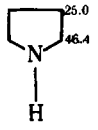
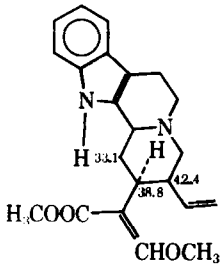


XII



XIII

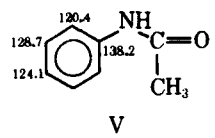
chemical shifts of C-10 and C-11. Contrary to the earlier studies (1), the assignments of C-10 (148.3 ppm) and C-11 (144.9 ppm) in III and C-10 (149.3 ppm) and C-11 (143.5 ppm) in IV should be reversed since in both cases C-11 is *meta* and C-10 is *para* to the nitrogen atom (13) and the chemical shifts assigned for C-3 (128.7 ppm) and C-4 (124.1 ppm) of acetanilide (V) (14). The carbon resonances of C-5 and C-6 are represented by chemical shifts at 136.0 and 123.6 ppm, respectively, by comparison with the chemical shift of the corresponding carbons of the analogous compounds, III and IV (1).

The C-22 has one α-carbon while C-21 has two α-carbons and two β-carbons, and these carbons account for the downfield chemical shift (13). Since oxygen produces an upfield chemical shift to its β-carbon and nitrogen exhibits a downfield chemical shift to its β-carbon, the remaining singlet at 140.6 ppm and a doublet centered at 127.0 ppm are thus attributed to C-21 and C-22 resonances, respectively. The assignments of these signals are in agreement with the chemical shifts reported for pimaradiene (VI) (15). Similar signals are also present in the ^{13}C-NMR spectra of II where the electronic environment of all aromatic carbons is completely different than that of I.

By comparing the assignment of chemical shift of III and IV, it can be assumed that the two doublets centered at 105.1 and 101.1 ppm are best assigned to C-1 and C-4, respectively.

The assignment of the chemical shift of carbon resonances in the lower field region is somewhat difficult. The C-12 and C-23 are directly attached to an oxygen atom, which produces more downfield shift at the same carbon than the nitrogen (13). Since the C-12 has the greater number of α- and β-carbons as compared to C-23, the chemical shift observed at the lower field is due to C-12. These observations provide support for the assignments of the doublet centered at 77.3-ppm and the triplet centered at 64.6-ppm chemical shifts to C-12 and C-23, respectively. The assignment of the chemical shifts of the analogous compound, gelsedine (VII) (16), provides further support for the assignments in I.

The quartets centered at 56.4 and 56.1 ppm are attributed to C-25 and C-26, respectively, as in III and VI (1), which, however, changed. This assignment is also supported by the absence of these signals in II. The C-8, C-16, C-18, and C-20 are directly bonded to nitrogen atoms, and thus the resonances due to these carbons are observed at a lower field in comparison to the remaining carbons (13). By comparison with the assignments of the chemical shift in VIII, IX, X (3), and XI (13), the doublets centered at 60.3 and 59.9 ppm and the signals at 52.6 and 50.2 ppm are assigned to C-16, C-8, C-18, and C-20, respectively, and the resonances for C-8 and C-16 are interchangeable. The methylene carbon C-11 is represented by the signal at 48.2 ppm.



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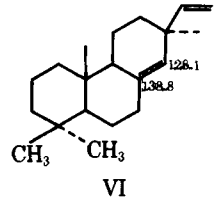


VI

The farthest upfield triplet at 26.5 ppm is assigned to C-17 due to the splitting pattern and comparison of the assignments of X and XII (3). This assignment is at a lower field in comparison to the corresponding carbon resonance of X, because it possesses a greater number of β-carbons. The chemical shift of C-15 is observed at 31.6 ppm while the carbon resonances of both C-13 and C-14 are represented at 42.4 ppm by comparing the assignment of chemical shifts of XIII (3) and XI (13).

In the present study, the proton noise-decoupled spectrum of I was also recorded at three different pulsing sequences of 2.5, 7.0, and 25.0 sec. These results, exhibiting the relaxing time (T_1) of different carbon atoms, are represented in Fig. 1. The presence of a longer T_1 for quaternary carbons as compared to other carbons indicated that the increase in time between pulsing sequences provides these carbons more time to relax, which consequently results in their larger peaks with respect to the peaks observed with carbons with a shorter T_1.

These results provide a clear distinction between quaternary carbons and other carbon atoms. The heights of the signal due to the carbon resonances of C-3, C-2, C-21, C-5, C-6, and C-7 are longer with an increase in the pulse time as compared to other carbons. The signal at 51.8 ppm is thus assigned to the quaternary carbon C-7, which is also supported by the chemical shifts of VII and XIV (1). The chemical shifts observed with II exhibited a similar signal at 52.0 ppm for C-7 in the proton noise-decoupled spectra of II (Fig. 2).

Strychnine—The ^{13}C-NMR chemical shifts of strychnine relative to tetramethylsilane are recorded in Table II. The various carbon resonances of II are shown in Fig. 2. The nine separate signals downfield in the 116–170-ppm region are assigned to aromatic carbons, amidic carbon, and ethylenic carbons. The 11 separate signals upfield in the 26–77-ppm

Table II—^{13}C-Chemical Shifts of Strychnine

Assignment	Multiplicity	Chemical Shift
C-10	s	169.3
C-5	s	142.3
C-21	s	140.5
C-6	s	132.7
C-3	d	128.6
C-22	d	127.5
C-1	d	124.2
C-2	d	122.3
C-4	d	116.3
C-12	d	77.2
C-23	t	64.6
C-8 and C-16	d	60.2
C-18	—[a]	52.8
C-7	—[a]	52.0
C-20	—[a]	50.4
C-11	—[a]	48.2
C-13[b]	d	42.9
C-14[b]	d	42.5
C-15	t	31.7
C-17	t	26.9

[a] The signal multiplicities are not clear. [b] These values may be interchanged.

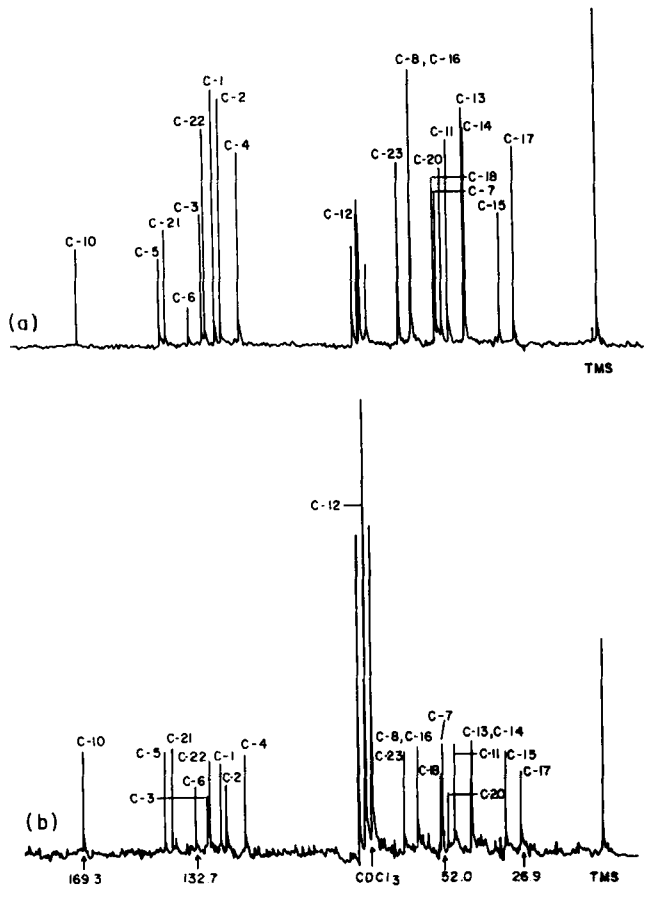
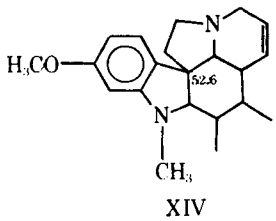


Figure 2 *--Proton noise-decoupled ^{13}C-NMR spectrum of strychnine. Key: (a), pulse repetition of 3 sec; and (b), pulse repetition of 35 sec.*

chemical shift region represent the carbon resonances of the remaining 12 carbons of II.

The farthest downfield singlet in the ^{13}C-NMR spectrum of II is attributed to C-10 on the basis of chemical shift theory of the carbonyl carbons of amides (13). By comparison with the chemical shifts of I, the singlet at 140.4 ppm and the doublet centered at 127.4 ppm are assigned to C-21 and C-22, respectively. The remaining two singlets at 142.3 and 132.6 ppm are due to the carbon resonances of C-5 and C-6, respectively. These assignments are comparable with the chemical shifts of VII (16) and XV (3). On the basis of the chemical shift theory for aromatic substitution and comparison with the chemical shifts of VII and XV, the four doublets centered at 124.2, 122.2, 128.6, and 116.3 ppm are represented by the carbon resonances of C-1, C-2, C-3, and C-4, respectively.

The remaining carbons, which exhibit their resonances in the upfield region, are in the same electronic environment as the corresponding carbons of I. Hence, the chemical shifts of these carbons observed in the ^{13}C-NMR spectrum of II are assigned by comparing the chemical shift observed due to the corresponding carbons of I (Table II).



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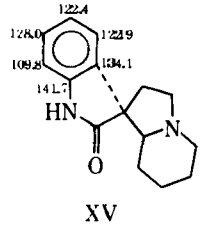

XV

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