Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Nicotine, Quinine, and Some *Amaryllidaceae* Alkaloids[1]

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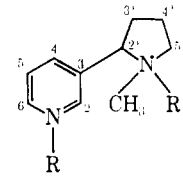
Abstract: The natural-abundance ^{13}C magnetic resonance spectra of nicotine, quinine, several *Amaryllidaceae* alkaloids, and selected derivatives of these have been determined at 15.8 MHz. With the aid of proton decoupling techniques and the shifts which occur on formation of specific derivatives, it has been possible to make self-consistent and unambiguous assignments of nearly all the resonances for these alkaloids.

The alkaloids constitute one of the largest classes of naturally occurring compounds. Because of the great structural variety within this class, prodigious amounts of work have gone into alkaloid structure elucidation. Modern techniques such as infrared spectroscopy, high-resolution mass spectroscopy, X-ray crystallography, and proton magnetic resonance (pmr) spectroscopy have all greatly facilitated structural determination of the alkaloids. Pmr spectroscopy has proven to be especially useful in determining and confirming structural assignments. However, the pmr spectra of an alkaloid often are too complex to be useful. The complexity results from extensive spin–spin coupling among the protons, overlap of the numerous resonance patterns, line broadening arising from intermolecular association as well as ^{14}N quadrupolar relaxation effects, or a combination of these. Carbon-13 magnetic resonance (cmr) spectroscopy has proven to be especially helpful in just such cases as this.[4] We have applied the recently developed natural-abundance cmr techniques to the problem of alkaloid structure identification. The instrumentation[5,6] and standard procedures such as noise-modulated total proton decoupling[6,7] and specific and off-resonance, single-frequency proton decoupling[4b,8] have been described in detail elsewhere.

In addition to nicotine and quinine, a series of *Amaryllidaceae* alkaloids were chosen for this study because there are a number of different ring systems exhibited within this family and examples of the various ring systems were available in the form of compounds whose structures had been unambiguously established by other means. Also, these compounds were known to have reasonably satisfactory solubility characteristics in common solvents. The literature concerning the chemistry of the *Amaryllidaceae* alkaloids has been extensively reviewed by one of us.[9]

Nicotine and Quinine. The general procedure for the assignment of the carbon resonances for each of the compounds reported here will be demonstrated with nicotine (**1a**). Both the noise and single-frequency



1a, no R group
b, R = CH_3

off-resonance (SFOR) proton-decoupled spectra of nicotine are shown in Figure 1. In the noise-decoupled spectrum (Figure 1A), a single peak is observed for each carbon. From the large and growing body of data concerning carbon chemical shifts, it is usually possible to make tentative assignments on the basis of the chemical shifts exhibited by these single peaks. The SFOR proton-decoupled spectrum (Figure 1B) has in place of the single peak for each carbon a multiplet pattern with one more line than the number of hydrogens directly attached to that carbon. By means of these multiplicities it is possible to check the assignments made on the basis of chemical shifts. When a further check is necessary, it is often possible to determine exact proton-decoupling frequencies by means of specific single-frequency proton decoupling, and to make assignments on the basis of absorption frequencies of the hydrogen(s) directly bonded to particular carbons. However, this technique is of limited usefulness in the case of the alkaloids, for it requires concentrated solutions and readily interpretable pmr spectra, neither of which are generally available for the alkaloids. Finally, it may be possible to form simple derivatives of the compound under investigation and correlate the changes in shifts with special structural features[4b,6] (*vide infra*).

The peaks in the nicotine spectra appear as two groups, those corresponding to unsaturated carbons at lower field and those corresponding to saturated carbons at higher field. This is generally the case for cmr spectra and greatly simplifies the task of assigning chem-

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(4) (a) D. E. Dorman and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **65**, 19 (1970); (b) H. J. Reich, M. Jautelat, M. T. Meese, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, **91**, 7445 (1969).
(5) F. J. Weigert and J. D. Roberts, *ibid.*, **89**, 2967 (1967).
(6) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, 60, 1152 (1968).
(7) (a) L. F. Johnson and M. E. Tate, *Can. J. Chem.*, 47, 63 (1969); (b) R. R. Ernst, *J. Chem. Phys.*, **45**, 3845 (1966).
(8) M. Jautelat, J. B. Grutzner, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **65**, 288 (1970).
(9) W. C. Wildman, *Alkaloids*, **6**, 290 (1960); **11**, 307 (1968).

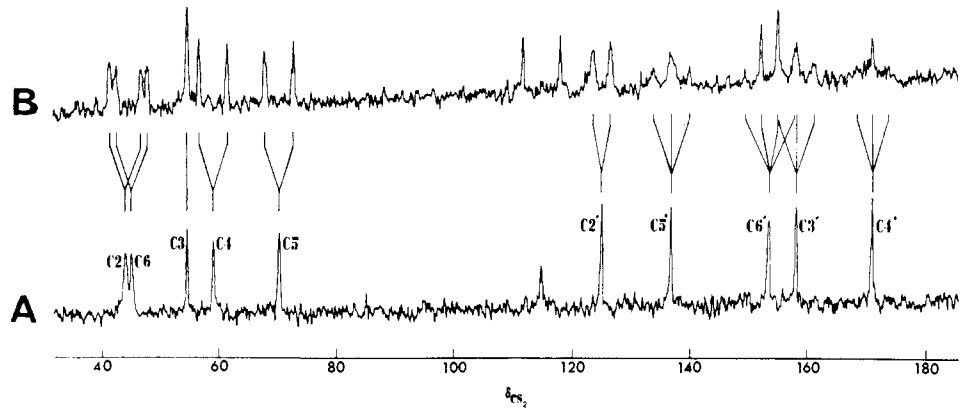
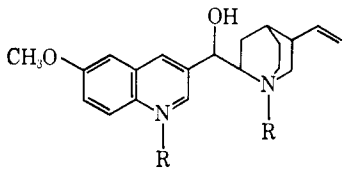


Figure 1. ^{13}C spectrum at 15.08 MHz of nicotine (**1a**) in chloroform: A, 20 scans, noise decoupled; B, 65 scans, off-resonance decoupled.

ical shifts in that the two types of carbons may be considered separately.

In the low-field portion of the nicotine spectra, carbons C-2, C-6, and C-5 may be assigned, by comparison with the spectrum of 3-picoline,[10] from their chemical shifts. It is to be expected that C-3 should be shifted downfield relative to C-5 because of α substitution,[10,11] but it is difficult to decide between the remaining two peaks on the basis of chemical shifts alone. The problem is easily resolved, however, by consideration of the multiplicities in the SFOR-decoupled spectrum (Figure 1B). One of the two remaining carbons appears as a singlet and is thus assigned as C-3, while the other appears as a doublet and is assigned as C-4. The relative positions of C-3 and C-4 are the reverse of that in 3-picoline.[10] This may be explained by a downfield shift of C-3 in nicotine as a result of a higher degree of β substitution.[11]

the saturated portion of quinine (**2a**) which were determined in a similar manner.



2a, no R group
b, R = CH_3

Amaryllidaceae **Alkaloids. Unsaturated Part.** Most of the *Amaryllidaceae* alkaloid ring systems isolated and characterized thus far contain a single benzenoid ring with methoxy or methylenedioxy substitution.[9] In addition, one alkenic linkage is observed. The low-field portion of the cmr spectra of these compounds (see Figure 2) shows peaks corresponding to these unsaturated carbons as well as any saturated carbons which have more than one directly bonded oxygen atom, *e.g.*, a methylenedioxy carbon.

Table I. Carbon-13 Chemical Shifts of 3-Picoline, Nicotine, and Quinine[a]

Carbon	3-Picoline[b]	Nicotine	Nicotine·2MeI	Carbon	Quinine	Quinine·2MeI
2	43.2	43.7*	45.4‡	2	135.9	128.6
3	59.9	54.2	62.7	3	153.0	155.5
4	56.4	58.5	45.6‡	4	165.0	166.9
5	69.6	69.9	63.8	5	171.2	173.2
6	45.9	44.7*	45.8‡	6	149.8	143.5
2′		124.8	118.2	7	165.0	167.3
3′		158.1	166.1	8	132.7	128.7
4′		170.8	173.2	9	121.5	126.0
5′		136.7	125.5	11	78.9	76.1
$-NCH_3$		153.3	140.8†	$-OCH_3$	136.8	135.8
			142.8†	$-NCH_3$		138.3
			145.7†			

[a] All shifts are in parts per million upfield from CS_2. [b] Reference 10. *, ‡, and † represent groups of almost identical chemical shifts where the assignment may possibly be reversed.

In the upfield portion of the nicotine spectra, the chemical shifts allow assignment of carbons C-2′, C-4′, and C-5′. The remaining carbons are again assigned without ambiguity on the basis of the multiplicities exhibited in the SFOR-decoupled spectrum (Figure 1B). C-6′, a methyl carbon, appears as a quartet, while C-3′, a methylene carbon, appears as a triplet. Table I shows the nicotine chemical shifts, along with those for

The low-field portions of representative noise-decoupled spectra for each of the ring systems reported here are given in Figure 3 in bar graph form. Each of the compounds represented, with the exception of buphanamine (**6**), has a pair of ortho oxygen substituents. The ring carbons bearing these substituents are assigned as the pair of peaks approximately 45 ppm up-

(10) P. C. Lauterbur, *J. Chem. Phys.*, **43**, 360 (1965).
(11) (a) G. B. Savitsky and K. Namikawa, *J. Phys. Chem.*, **68**, 1956 (1964); (b) D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, **86**, 2984 (1964).

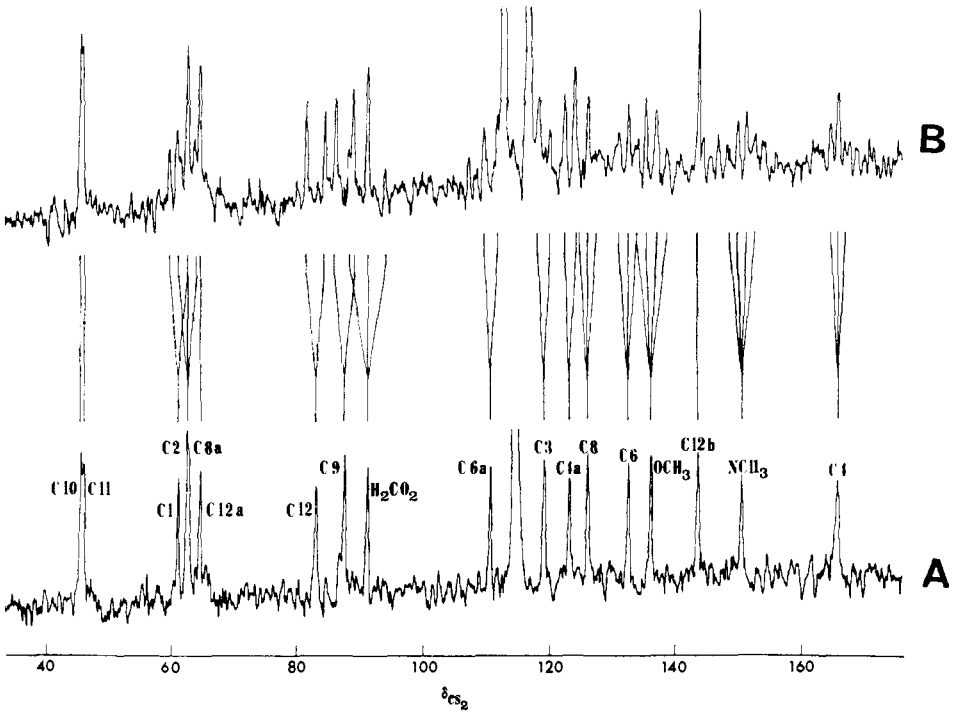
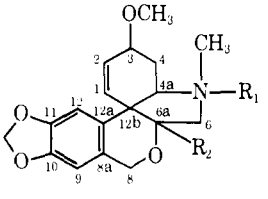
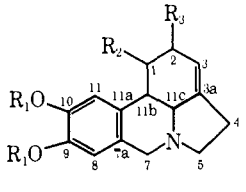


Figure 2. ^{13}C spectrum at 15.08 MHz of deoxytazettine (**3b**) in chloroform: A, 135 scans, noise decoupled; B, 184 scans, off-resonance decoupled.

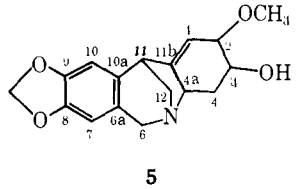
field from CS_2, the lowest field peaks in each case but **5**. The two carbons have virtually the same chemical shifts and it is impossible to choose between them. There is a slight upfield shift upon going from the dimethoxy to





3a, no R_1 group; R_2 = OH
b, no R_1 group; R_2 = H
c, R_1 = H; R_2 = OH
d, R_1 = CH_3; R_2 = OH

4a, R_1 = CH_3; R_2 = OH; R_3 = OCH_3
b, R_1 = CH_2; R_2 = OAc; R_3 = H
c, R_1 = CH_2; R_2 = R_3 = OAc



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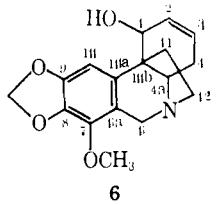

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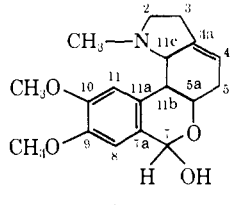

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methylenedioxy substitution. The other two aromatic carbons without directly bonded hydrogens, C-d and C-e, are assigned by chemical shifts and SFOR-decoupled multiplicities. However, there is no clear-cut

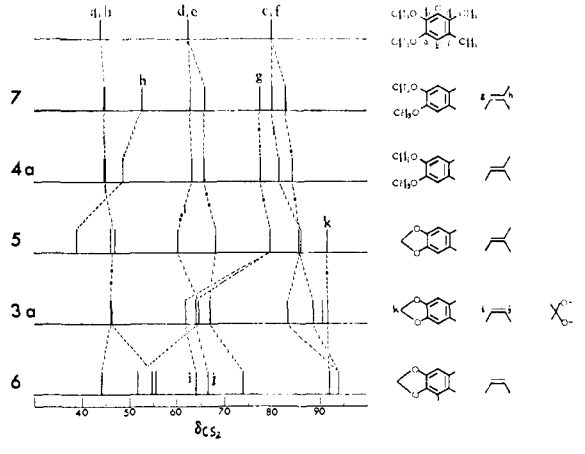

Figure 3. Correlation of low-field ^{13}C chemical shifts for 1,2-dimethyl-4,5-dimethoxybenzene (calculated), lycorenine (**7**), galanthine (**4a**), tazettine (**3a**), montanine (**5**), and buphanamine (**6**).

way to distinguish between them, and carbon D-d has been assigned as the lower field peak because the degree of β substitution is greater at this position than at C-e. The last two aromatic carbons C-c and C-f may be assigned from their chemical shifts and exact proton-decoupling frequencies. Partial assignments of the pmr spectra of lycorenine[12] (**7**) and tazettine[13] (**3a**) are found in the literature. For these alkaloids, C-c and C-f (as well as C-g in the case of lycorenine) were distinguished from one another by this means.

Two types of alkenic bonds are exhibited by these alkaloids, di- and trisubstituted. The fully substituted C-h is easily recognized by its rather low-field chemical shift as well as its degree of substitution in the SFOR-

(12) W. A. Hawksworth, P. W. Jeffs, B. R. Tidd, and T. P. Toube, *J. Chem. Soc.*, 1991 (1965).
(13) R. D. Haugwitz, P. W. Jeffs, and E. Wenkert, *ibid.*, 2001 (1965).

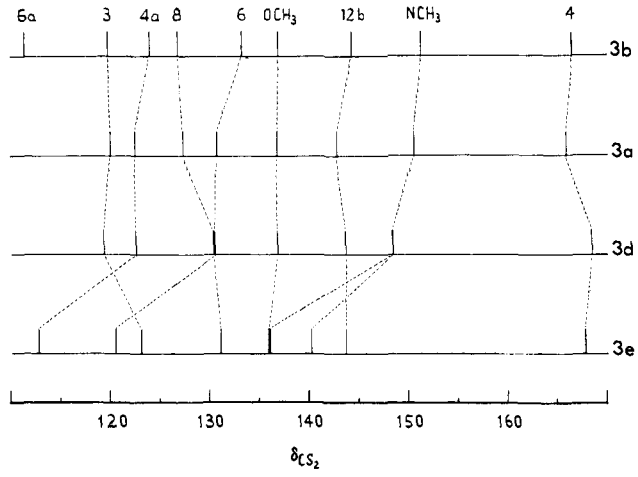

Figure 4. Correlation of high-field ^{13}C chemical shifts for tazettine (**3a**), deoxytazettine (**3b**), tazettine hydrochloride (**3d**), and tazettine methiodide (**3e**).

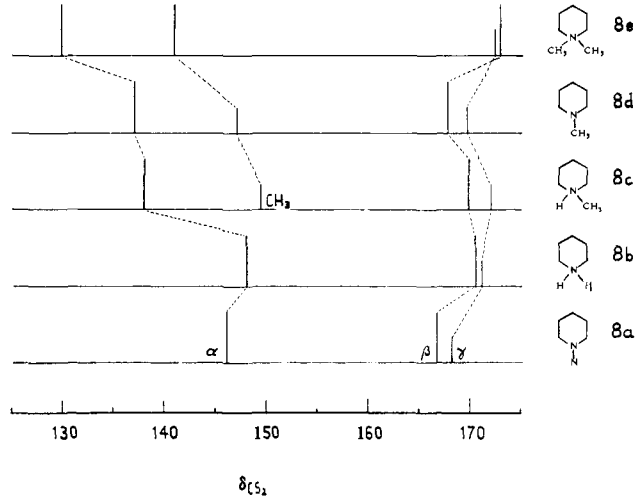

Figure 5. Correlation of ^{13}C chemical shifts for piperidine (**8a**), piperidinium hydrochloride (**8b**), *N*-methylpiperidinium hydrochloride (**8c**), methylpiperidine (**8d**), and *N,N*-dimethylpiperidinium iodide (**8e**).

decoupled spectrum. The carbons forming a disubstituted alkenic bond, C-i and C-j, absorb in the same region as aromatic C-d and C-e, but are easily distinguished from these by their degree of substitution.

The addition of a third oxygen substituent, as in buphanamine (**6**), drastically alters the absorption pattern of the aromatic carbons. Because each of the aromatic carbons, except C-c, is now fully substituted, the assignments become quite difficult. The assignments made here are based upon comparison with chemical shifts calculated by adding average benzene substituent effects.[14] Account was also taken of the observation by Lauterbur[15] that ortho oxygen substitution causes a downfield shift over and above that expected. These assignments are, of course, subject to the errors inherent in treatments of this sort.

The methylenedioxy carbon is easily identified as the only carbon in this region which gives rise to a triplet in the SFOR-decoupled spectrum. Lycorenine (**7**) and tazettine (**3a**) contain a hemiketal carbon and it may be assigned by elimination.

Saturated Part. One feature common to each of these alkaloids is methoxyl substitution, either on the aromatic ring or on the saturated rings, or both. These methoxy carbons are easily assigned from their chemical shift (δ 137.0 ± 0.5 ppm) and the quartet pattern observed in the SFOR-decoupled spectra. Aside from these, the carbons corresponding to the lowest field peaks in this portion of the spectra are those bonded to oxygen. Those occurring at highest field positions are the unsubstituted methylene carbons, as there are no methyl carbons in these compounds other than those bonded to oxygen or nitrogen. The remainder of the saturated carbon atoms in these alkaloids have resonance absorptions intermediate between these extremes.

For the alkaloid tazettine (**3a**), the lowest field peaks which correspond to saturated carbons are those of the methylenedioxy and hemiketal carbons. Both of these appear with the unsaturated carbons. When the hydroxyl group of the hemiketal is replaced by a hydrogen atom in deoxytazettine (**3b**), the corresponding resonance peak C-6a is shifted back upfield into the saturated region (Figure 4). The lone unsubstituted methylene carbon in tazettine shows a peak substantially upfield from any other carbon and is therefore assigned by its chemical shift. The single quaternary carbon in this region, C-12b, is readily determined from the SFOR-decoupled spectrum. The nitrogen methyl is also assigned in this manner, the partially decoupled quartet appearing at much higher field than the one corresponding to the methoxy carbon. This is also a distinctive chemical shift ($\delta \sim 150$ ppm) for a methyl bonded to nitrogen.

The remaining methylene carbons, C-6 and C-8, may be distinguished from the two methine carbons, C-3 and C-4a, by the multiplicities exhibited in the SFOR-decoupled spectrum. The final assignments are made by considering the chemical shifts. The lower field peaks are assigned to the carbons bonded to oxygen. Generally, a carbon bonded to nitrogen will absorb about 10 ppm higher field than a similarly substituted

Table II. Carbon-13 Chemical Shifts of Tazettine and Its Derivatives[a]

Carbon	3a	3b	3c[b]	3d	3e
1	61.9*	61.7*	60.2*	61.4*	63.0*
2	64.0*	63.0*	65.1*	65.4*	64.4*
3	120.0	119.8	120.3	119.4	123.1
4	165.8	166.4	165.4	168.4	167.8
4a	122.5	123.9	125.6	122.6	112.8
6	130.7	133.3	129.0	130.6	120.5
6a	90.7	111.4	116.2	92.7	93.3
8	127.4	126.8	128.7	130.6	131.1
8a	64.6‡	63.4‡	59.9‡	67.7‡	66.4‡
9	88.7	88.2	84.2	88.4	87.7
10	46.1†	46.1†	45.3†	45.4†	46.0†
11	46.1†	46.5†	46.8†	45.7†	46.0†
12	83.3	83.7	80.5	84.0	84.3
12a	67.0‡	65.2‡	59.7‡	68.4‡	66.4‡
12b	142.3	144.3	137.2	143.7	143.7
O–CH_2–O	91.8	91.8	91.5	91.3	91.0
O–CH_3	136.8	136.9	137.0	136.8	136.0
N–CH_3	150.5	151.3	152.1	148.4	136.0
					140.2

[a] All shifts are in parts per million upfield from CS_2. [b] Tazettadiol (*cf.* ref 9). *, ‡, and † represent groups of almost identical chemical shifts where the assignments may possibly be reversed.

(14) H. Spieseke and W. G. Schneider, *J. Chem. Phys.*, 35, 731 (1961).
(15) P. C. Lauterbur, *J. Amer. Chem. Soc.*, 83, 1846 (1961).

Table III. Carbon-13 Chemical Shifts of Piperidines and Piperidinium Salts[a]

Carbon	8a	8b	8c	8d	8e
2,6	146.6	148.1	138.0	137.0	129.9
3,5	166.7	170.5	169.8	167.7	172.9
4	168.2	171.1	172.0	169.6	172.4
N-CH_3			149.4	147.1	140.9

[a] All shifts are in parts per million upfield from CS_2.

correlated. Unfortunately, different solvents are required for the free alkaloid and the salts, so that solvent effects are superimposed upon the derivative effects. The methiodide double salts of nicotine and quinine were also studied and their chemical shifts are given in Table I.

Assignments for the other *Amaryllidaceae* alkaloids were made in the manner described above. The chemical shifts for these are given in Table IV. In the instances where it was not possible to decide between two peaks, the assignments were made in a manner to provide consistency with those of the other alkaloids.

Table IV. Carbon-13 Chemical Shifts of Other *Amaryllidaceae* Alkaloids[a]

Carbon	4a	4b	4c	Carbon	5	6	Carbon	7
1	124.3	123.4	126.3					
2	111.6	122.0	159.5	1	79.7	128.2	2	135.7
3	77.7	79.1	78.8	2	112.9	64.1	3	164.5
3a	48.8	46.6*	53.2	3	124.2	66.6	3a	52.8
4	164.2	164.2	164.3	4	159.7	164.2	4	77.5
5	139.0	139.4	139.2	4a	131.9	133.4	4	160.7
7	135.6*	136.1	135.9	6	134.1	135.3	5a	125.1
7a	63.3‡	63.3‡	63.2*	6a	60.3*	74.1	7	101.7
8	81.6	85.6	85.6	7	85.6‡	52.0*	7a	62.9*
9	44.9†	46.6*	46.5†	8	4.61*	55.0*	8	80.1
10	44.9†	46.6*	46.5†	9	46.9†	44.4	9	44.8‡
11	84.3	87.7	87.8	10	86.1‡	94.1	10	44.8‡
11a	65.8‡	66.3‡	65.0*	10a	68.2*	55.8*	11	83.0
11b	151.2	152.4	149.4	10b		144.3	11a	65.9*
11c	131.8	131.9	131.6	11	146.9	153.4	11b	148.6†
OCH_2O		91.9	92.0	11a	38.8		11c	126.0
OCH_3	136.2*			12	135.5	140.9	OCH_3	136.3
	136.6*			OCH_2O	91.7	92.2		136.5
	136.8*			OCH_3	137.4	133.4	NCH_3	148.6†
O=C		23.2						
		23.5						
O=CCH_3		172.0	171.8					
		172.4						

[a] All shifts are in parts per million upfield from CS_2. *, ‡, and † represent groups of almost identical chemical shifts where the assignments may possibly be reversed.

carbon bonded to oxygen.[16] The greater degree of β substitution for C-4a and C-6 apparently accounts for the similarity of their chemical shifts to those of C-3 and C-8, respectively.[11]

In order to substantiate the assignments described above, several derivatives of tazettine were studied (see Table II). Deoxytazettine is easily prepared from tazettine,[9] and is useful for comparison. However, in search of a more generally useful derivative, the HX and RX salts of the alkaloids were investigated. Data for a series of such salts of piperidine and *N*-methylpiperidine are given in Figure 5 and Table III. Upon formation of the hydrochloride salts, α, β, and γ carbons are shifted upfield by from 1 to 4 ppm. In the methiodide salts, however, the α carbons are shifted downfield approximately 10 ppm. The methiodide should, therefore, be the more diagnostic derivative. This is shown to be the case in Figure 4, in which the chemical shifts of the various derivatives of tazettine are

(16) Unpublished results, this laboratory.

Experimental Section

The ^{13}C spectra were collected, using the DFS spectrometer which has been previously described.[5,6] The samples were examined as 0.5–1.5 *M* solutions in chloroform (free alkaloids), dimethyl sulfoxide–H_2O (alkaloid salts), or 50% dioxane–H_2O (piperidines and salts) solutions. The carbon of the solvent chloroform served as internal reference in the first instance, and the chemical shifts were referenced to external carbon disulfide by the relation $\delta_c^{CS_2} = \delta_c^{CHCl_3} + 115.3$ ppm. In the latter two cases, 1,4-dioxane served as internal reference and the chemical shifts were referenced to external carbon disulfide by the relation $\delta_c^{CS_2} = \delta_c^{diox} + 126.1$ ppm.

The methiodide salts were prepared by stirring the tertiary amine and methyl iodide in methanol at room temperature. The hydrochloride salts were prepared by mixing an ethanol solution of the amine with a saturated solution of hydrogen chloride in ethanol. In both cases, the salts were isolated by filtration and used without further purification.