Carbon-13 and Nitrogen-15 Nuclear Magnetic Resonance of Physostigmine (Eserine)

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The ^{15}N and ^{13}C nmr spectra of physostigmine are discussed along with complete assignment of the signals. This alkaloid ^{15}N nmr spectrum is notable because it contains nitrogens in three different environments.

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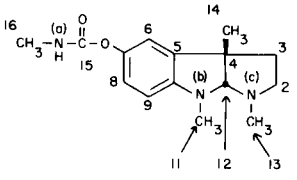
During the course of our continuing interest in the photochemistry of physostigmine (1) and other alkaloids (2,3), we have studied the ^{13}C and ^{15}N nmr spectra of physostigmine **1**. Physostigmine is the active constituent of the calabar bean, and its fatal dose for man is approximately 10 mg. (4). It is isolated from *Physostigma venenosum* and has been used by natives of West Africa for the administration of "divine justice" for criminal offences. The simultaneous occurrance of an emetic in the bean saved the lives of several prisoners and declared them innocent.



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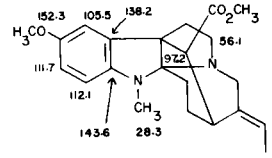
Spectroscopically, physostigmine's ^{15}N nmr is notable because it contains nitrogens in three different environments. One is directly coupled to a proton. Though this is one of the few reports of ^{15}N nmr for alkaloids, ^{13}C nmr of these compounds currently is providing an interesting set of data for structural analysis (5).

Assignments of the signals in the carbon nuclear magnetic resonance spectrum of physostigmine were made on the basis of ^{13}C nmr chemical shift theory, verification by proton - induced splitting in the off resonance decoupled and proton coupled spectra of physostigmine and comparision with structurally related compounds. Recently Robinson and his coworkers reported the ^{13}C nmr spectra of physostigmine (6). Our experimental data and assignments are in agreement with the reported assignments except for four shift groupings: C-2 and C-4; C-6, C-8 and C-9 (order of the aromatic carbons): C-11, C-13, C-14 and C-16 (the methyl carbons, and C-7 and C-15 (cf. Table 1 and Figure 1).

A complicating feature of the spectrum is that the signal for C-4 appears in the vicinity of C-2. However, the two are readily distinguished by the splitting pattern in the undecoupled spectrum (7) and the relative intensities (7) (Figure 1).

The assignments and relative order of the six signals between 105.6-148.3 ppm, which are in the range of aromatic carbons, are derived by direct comparison with the alkaloids vincorine **2** (8), vincarodine **3** (9) and N_b-methyltetrahydroharmine **4** (5d) and several of the cinchona alkaloids (5b).



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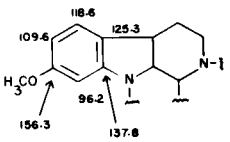

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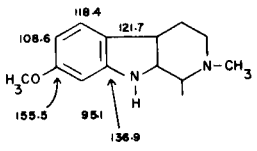


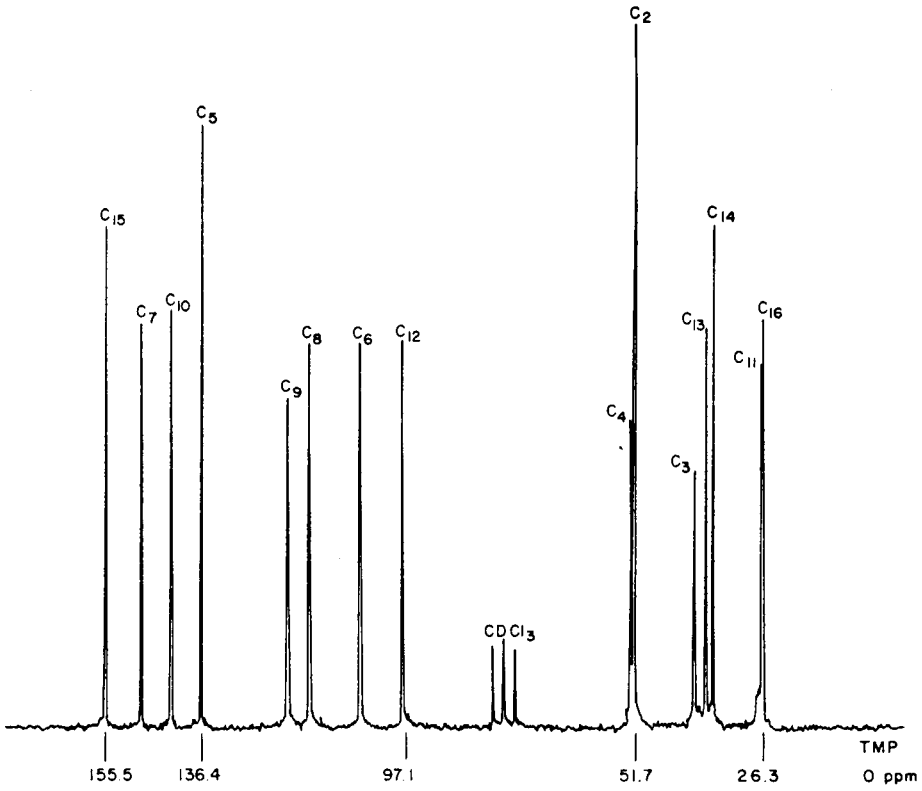
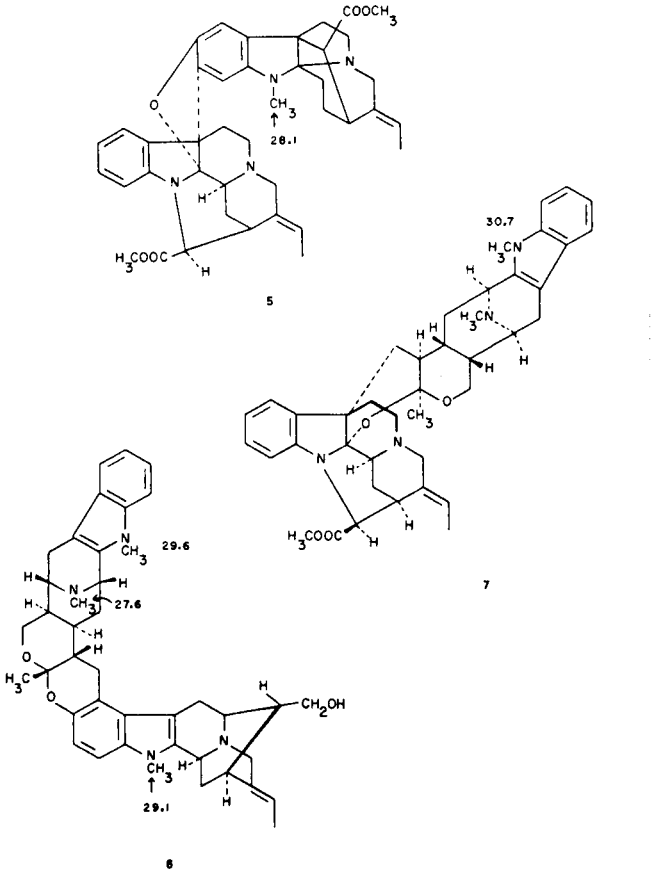


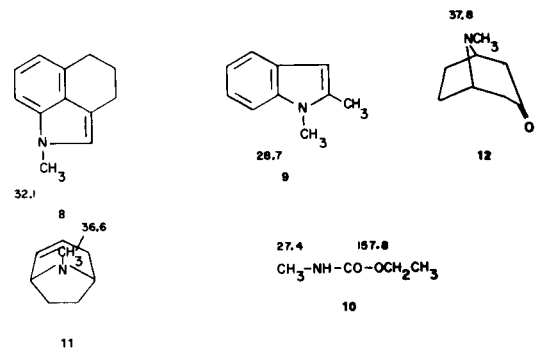
Figure 1. The proton noise decoupled ^{13}C nmr spectrum of physostigmine.

The signals representing the four methyl carbons, C-11, C-13, C-14 and C-16, were assigned by analogy but with some difficulty. The quartets centered at 26.6 and 26.3 ppm were assigned to C-11 (7-10) **2**, **5-9** and C-16 (11) **10**, respectively, due to conjugation of the nitrogen lone pairs, one with an aryl and the other with a carbonyl group: however, the assignments could be reversed. One of the remaining methyl signals at 37.4 ppm was attributed to C-13 on the basis of the analogous structures **11** and **12** (5d), while that of 36.0 ppm was assigned to C-14 by difference.

The reversal of the C-7 and C-15 assignments to that of Crooks, *et al.*, is due to secondary coupling in the gated I (NOE) decoupled spectrum of physostigmine. The signal at 155.5 ppm in the completely decoupled spectrum becomes a doublet separated by 3.9 Hz in the gated mode. This is attributed to a secondary coupling to the meta proton at C-9 and, consequently, should be assigned to C-7. The signal at 148.3 (Table 1) remains unsplit however broad in the gated mode and is assigned to C-15.

Recently, high resolution nmr spectroscopy of nitrogen became feasible. Improvements in the experimental techniques have created a remarkable surge of interest in nitrogen nmr since 1964 for both ^{14}N and ^{15}N nuclei (12,13).





The proton decoupled ^{15}N spectrum of physostigmine (Figure 2) has three resonances at 44.8, 44.1 and 30.1 ppm adjusted to $^{15}NH_4Cl$ as an external reference set at 0.0 ppm. Positive values indicate downfield shifts. The gated decoupled spectrum (NOE only, Figure 3) exhibited

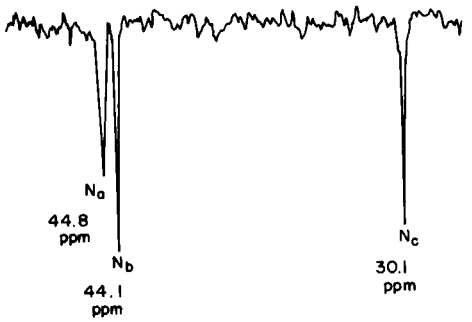


Figure 2. The proton decoupled ^{15}N spectrum of physostigmine in deuteriochloroform.

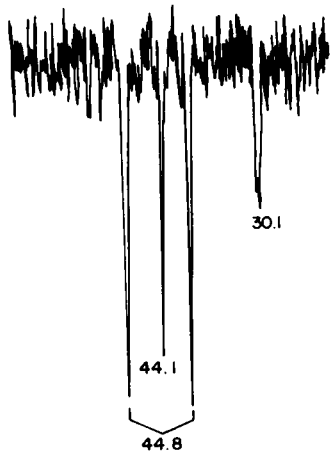


Figure 3. The gated decoupled ^{15}N spectrum of physostigmine.

a doublet centered at 44.8, a singlet at 44.1 and a poorly resolved multiplet at 30.1 ppm.

The singlet at 44.8 ppm in the completely decoupled ^{15}N spectrum, which appeared as a doublet in the gated decoupled spectrum, can be attributed to N_a on the basis of its coupling to the attached proton. Though in the initial analysis of the physostigmine molecular structure one is tempted to conclude N_b and N_c would have signals in the ^{15}N spectrum close to one another, the two actually occur at quite some distance apart. This is due to the fact that one is conjugated with the aromatic ring. Hence the singlet at 44.1 ppm, which remained unsplit in the gated decoupled spectrum, was assigned to N_b. The remaining singlet at 30.1 ppm, a poorly resolved multiplet (Figure 3) because of the long-range coupling with the C-2 and C-12 protons, was safely attributed to N_c. As with the corresponding ^{13}C *N*-methyl signals, N_a and N_b chemicals shifts are close together for the same reason.

Table 1

^{13}C Nmr Spectral Data of Physostigmine (a)

Chemical Shift (ppm) (b)	Multiplicity	Assignments	Chemical Shift (ppm) (c)
155.5	s	C-7	156.3
148.3	s	C-15	149.3
142.5	s	C-10	143.3
136.4	s	C-5	137.4
119.6	d	C-9	106.5
115.3	d	C-8 (f)	120.4 (e)
105.6	d	C-6 (f)	116.1 (e)
97.1	d	C-12	98.1
52.2	s	C-4	52.6
51.7	t	C-2	53.2
39.7	t	C-3	40.7
37.4	q	C-13	36.9
36.0	q	C-14	27.2
26.6	q	C-11 (d)	38.4
26.3	q	C-16 (d)	27.5

(a) The shifts are reported in ppm with respect to tetramethylsilane. (b) This experiment. (c) Data from reference 6. (d, e, f) May be interchanged.

EXPERIMENTAL

The ^{15}N spectra were obtained on a JEOL FX 100 multinuclear spectrometer operating at 10.09 MHz and the ^{13}C spectra were obtained on a JEOL FX 60 spectrometer operating at 15.03 MHz. The samples were run in 10 mm tubes using deuteriochloroform (*ca.* 1 g. in 1 ml.) as an internal lock and tetramethylsilane as reference. The spectra were obtained using the following typical conditions: spectra width 4000 Hz, pulse width 4 μ seconds (45°), repetition rate 3 seconds, data points 8K, number of pulses 223 (complete decoupling), 956 [gated I (NOE)]. Nitrogen shifts were measured relative to external $^{15}NH_4Cl$ (set at 0.0 ppm) at 30°. It was obtained under the following conditions: spectra width 2000 Hz, pulse width 5 μ seconds (13°), repetition rate 2.2 seconds, data point 8K, window Ex 20, number of pulses 4268; for NOE only spectral width 200 Hz, pulse width 10 μ seconds (26°), repetition rate 4 seconds, data points 8K, window Ex 20 and number of pulses 4268. Physo-

stigmine was obtained from Sigma Chemicals, St. Louis, MO.

Acknowledgment

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