Interpretation of the ^{13}C NMR Spectrum of Physostigmine

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The 25.1 MHz ^{13}C nmr spectrum of physostigmine in deuteriochloroform has been reanalysed with the aid of high resolution proton coupled spectrum and selective proton irradiation experiments. In addition to unambiguous assignment the analysis yielded values for carbon-proton coupling constants.

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As an extension of our studies on irreversibly acting anticholinesterases of the organophosphorus type (1) we have investigated the naturally occurring alkaloid and reversibly acting enzyme inhibitor physostigmine. The principal alkaloid of *Physostigma venenosum*, physostigmine has been familiar to pharmacologists since the latter part of the nineteenth century.

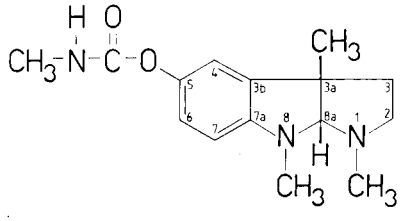

Table 1

^{13}C Chemical Shifts (in ppm, relative to internal tetramethylsilane) and $^{n}J_{CH}$ Coupling Constants (in Hz) of Physostigmine in deuteriochloroform

Carbon	δ_C	Assignment of Ref 2	Ref 3	$^{1}J_{CH}$	$^{n}J_{CH}$ (n = 2,3 and 4)
2	53.1	2	3a	136.5	
3	40.6	3	3	133.4	
3a	52.6	3a	2	—	
3b	137.1	3b	3b	—	
4	115.9	4	6	158.7	$^{3}J_{CH6}$ = 5.5, $^{4}J_{CH7}$ = 1.2
5	142.9	7a	7a	—	$^{2}J_{CH4/H6}$ = 4.3 $^{3}J_{CH7}$ = 9.5
6	120.2	6	7	160.8	$^{3}J_{CH4}$ = 4.6
7	106.3	7	4	160.5	
7a	149.0	5	CO	—	
8a	97.8	8a	8a	153.7	
1-Me	38.2	8-Me	1-Me	133.7	
3a-Me	27.1	3a-Me	NH-Me	126.9	$^{3}J_{CH8a}$ = 5.2, $^{3}J_{CH3}$ = 3.4
NH-Me	27.6	NH-Me	8-Me	138.3	$^{2}J_{CNH}$ = 5.2
8-Me	36.9	1-Me	3a-Me	135.2	$^{3}J_{CH8a}$ = 3.4
CO	155.9	CO	5	—	$^{3}J_{CNCH}$ = 3.6

Two papers (2,3) on the ^{13}C nmr spectrum of physostigmine are available in the literature, but the assignments in both are based more on a comparison with structurally related compounds than on independent nmr data. We now report the ^{13}C nmr spectrum of physostigmine in deuteriochloroform conclusively analysed with the aid of high resolution proton coupled spectrum and selective proton irradiation experiments.

The resulting assignments along with the ^{13}C chemical shifts and carbon-proton coupling constants are presented in Table 1. Excluding the assignments of carbons 5 and 7a and of methyl carbons 1-Me and 8-Me our results substantiate the work carried out by Crooks *et al.* (2). Since there are eleven discrepancies between our assignment and that made by Stenberg *et al.* (3), the interpretation made here will be discussed in detail (see Table 1).

The 100 MHz ^1H nmr spectrum of the sample was identical within ±0.05 ppm with the very early 60 MHz spectrum recorded by Bhacca *et al.* (4). It has been taken as a basis in the selective proton irradiation experiments used to interpret the ^{13}C spectrum.

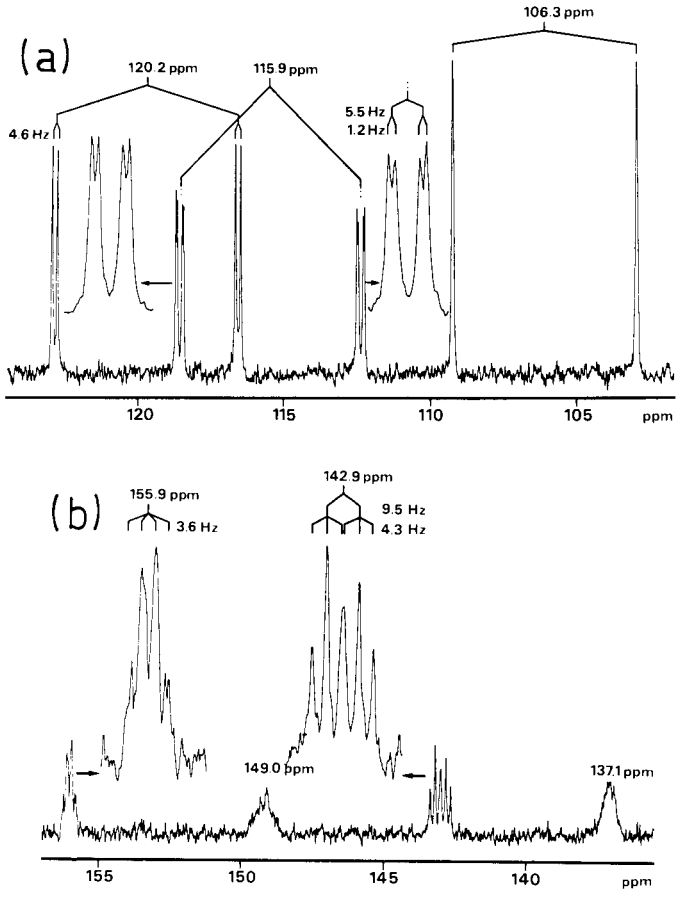

Figure 1. The two portions of the high resolution proton coupled ^{13}C nmr spectrum of physostigmine. The resonances (a) of the protonated aromatic carbons and (b) of the low-field quaternary carbons showing the splittings due to carbon-proton couplings.

The triplets at 40.6 ppm ($^1J_{CH}$ = 133.4 Hz) and 53.1 ppm ($^1J_{CH}$ = 137.4 Hz) were assigned to C−3 and C−2, respectively. Selective irradiation of 3a-methyl protons at 1.42 ppm eliminated most of the fine structure of the resonance at 40.6 ppm confirming the assignment based on the chemical shifts. This experiment also confirmed the assignment of C−3a (52.6 ppm), the only quaternary carbon nucleus resonating at high field. C−8a was distinguished from aromatic methine carbons by its complicated fine structure in the proton coupled spectrum. High-power irradiation centered at the 8-methyl protons at 2.91 ppm collapsed almost all the fine structure, corroborating this assignment.

The resonances of the four methyl carbons were assigned without ambiguity, too. When the decoupler frequency was set to the resonance of the 3a-methyl protons at 1.42 ppm, the highest field quartet converted to a singlet at 27.1 ppm which was assigned to the 3a-methyl carbon. Medium-power irradiation at 2.91 ppm collapsed the 8a-methyl carbon quartet at 36.9 ppm to a singlet. Another quartet at 27.6 ppm reduced to a broad singlet under the same irradiation, confirming its assignment to the carbamate methyl carbon, since the corresponding methyl protons resonate at 2.82 ppm, near the irradiation frequency. Finally, the quartet at 38.2 ppm with indicative very complex fine structure was safely assigned to 1-methyl carbon.

The resonances of the quaternary carbons 3b, 5, 7a and the carbonyl carbon were assigned with aid of selective irradiation experiments and the proton coupled spectrum (Figure 1b). High-power irradiation of the 3a-methyl protons at 1.42 ppm reduced the broad multiplet structure of the signal at 137.1 ppm to a doublet revealing the three-bond coupling between C−3b and H−7. The resonance at 149.0 ppm could be unambiguously assigned to C−7a by irradiating the protons of 8-methyl at 2.91 ppm. The complex multiplet converted to a nice triplet due to the couplings to the two *meta*-protons. The irradiation decoupled also the carbamate methyl protons and collapsed the quartet at the lowest field to a singlet. Hence the resonance at 155.9 ppm was assigned to the carbonyl carbon. The doublet of triplets of C−5 at 142.9 ppm (Figure 1b) could be analysed in terms of two *ortho*-couplings of 4.3 Hz and one *meta*-coupling of 9.5 Hz. The assignment was confirmed by high-power irradiation of the aromatic protons, which reduced the multiplet to a singlet.

The doublet at 106.3 ppm ($^1J_{CH}$ = 160.5 Hz) with no three bond couplings was easily assigned to C−7. However, an individual assignment of the resonances at 115.9 ppm and 120.2 ppm was not possible on the basis of

the proton coupled spectrum (Figure 1a) and their assignment to the aromatic carbons C−4 and C−6 was made with the aid of the residual splittings. The protons H−4 and H−6 resonate at 6.75 and 6.80 ppm, respectively. Low-power irradiation at 6.7 ppm decreased more drastically the one-bond coupling of the signal at 115.9 ppm than that of the signal at 120.2 ppm. Moreover, the three-bond coupling on the signal at lower field was collapsed. Irradiation at 7.3 ppm reversed the magnitudes of the one-bond residual couplings asserting that the signal at 115.9 ppm belonged to C−4 and the signal at 120.2 ppm to C−6.

EXPERIMENTAL

Free physostigmine was obtained through neutralisation of the sulphate (Burroughs Wellcome & Co) with dilute ammonium hydroxide.

A Jeol FX-100 spectrometer equipped with a $^{1}H/^{13}C$ dual 5 mm probe was used to obtain the 99.6 MHz proton and 25.1 MHz carbon-13 spectra at room temperature. Sample concentration was approximately 0.25 M in deuteriochlorform. The proton coupled ^{13}C nmr spectrum (with NOE) was a result of 20 000 pulses of 23° pulse angle repeated every 3.3 seconds. The FID over 5000 Hz was stored into 32 k data points producing a digital resolution of 0.31 Hz.

Acknowledgement.

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