2D ^{1}H- ^{13}C HETERONUCLEAR NMR STUDY OF 9-METHOXYELLIPTICINE AND OF THE RELATED INDOLE AND CARBAZOLE DERIVATIVES

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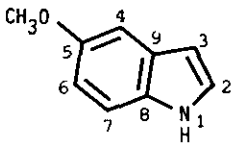
Abstract - Unambiguous assignment based on 2D ^{1}H-^{1}H homonuclear and ^{1}H-^{13}C heteronuclear correlations is given for the ^{13}C-NMR spectrum of 9-methoxyellipticine (3) in DMSO-d6, settling the discrepancy existing between the earlier data. The ^{13}C-NMR spectra of 5-methoxyindole (1), and 1,4-dimethylcarbazole (2), which have been used as model compounds for the earlier ^{13}C assignments of (3) have also been reinvestigated.

INTRODUCTION

Since the antitumour activity of naturally occurring ellipticines has first been recognized[1], there have been intensive searches for more active and less toxic analogs.

^{1}H-NMR spectroscopy has been routinely used for identifying the intermediates as well as the final products obtained in the course of many syntheses of ellipticine and ellipticine derivatives that have thus been carried out[2,6,7]. The more recent interest in the ^{13}C-NMR spectra of these derivatives is concerned with the possibility of using ^{13}C together with ^{1}H-NMR spectroscopy, either for studying their intercalation between nucleic acid bases[3], or for recognizing the tetracyclic system characteristic of ellipticines, when a ring closure is involved in the synthetic process[4]. Complete ^{13}C assignments of a number of ellipticine derivatives, including (3), have been published[3-5,8], most of these assignments[3-5] being deduced from careful chemical shift comparisons with closely related compounds such as the relevant indole, carbazole, isoquinoline, or pyridine derivatives. The assignments of these model compounds were usually based on data obtained from classical one-dimensional (1D) NMR experiments while the most recent study[8] used the INEPT

technique together with substituent increments to reassign the ^{13}C spectrum of ellipticine.



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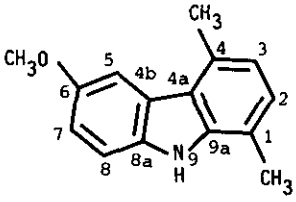

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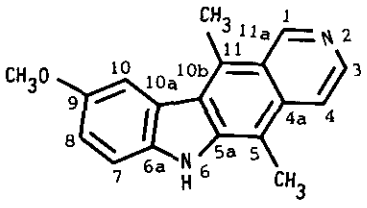

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However, even when every precaution regarding the experimental conditions to ensure meaningful comparisons is taken, conclusions drawn from empirical chemical shift comparison are not always unambiguous. This seems indeed to be the case for (3) as there is no full agreement between the data already published[3-5]. We decided therefore to engage on an NMR study using the results of homonuclear ^{1}H-^{1}H dipolar correlated as well as heteronuclear ^{1}H-^{13}C chemical shift correlated two-dimensional (2D) NMR experiments to reinvestigate the ^{13}C assignments of (3), and of the two model compounds (1) and (2).

The ^{1}H spectrum was assigned first, followed by the assignment of the ^{13}C spectrum using ^{1}H-^{13}C shift correlations either via $^{1}J_{CH}$ to provide the ^{13}C assignments for protonated carbons or via long-range ^{13}C-^{1}H couplings to provide assignments of quaternary carbons. The ^{13}C assignments thus obtained are clearly unambiguous.

RESULTS AND DISCUSSION

Earlier[9] we had completely assigned the ^{1}H spectrum of (1), (2) and (3) using selective homonuclear decoupling and 1D-NOE experiments. In the case of intercalating drugs such as ellipticine derivatives[10], the ^{1}H chemical shifts may be expected to be concentration-dependent due to the existence of intermolecular interactions leading to vertical stacking of the aromatic heterocyclic system characteristic of this series of molecules. As the solutions used for the previous 1D-experiments were at least five times less concentrated than the solutions used in the present work, we reassigned the ^{1}H spectra by performing ^{1}H-^{1}H dipolar correlated 2D-NMR experiments (NOESY) on the more concentrated solutions used for obtaining the ^{1}H-^{13}C connectivities. Though the ^{1}H chemical shifts were indeed found to be sensitive to concentration, and particularly for (3), the independent assignments obtained here confirmed the earlier ones (Table 1).

With the ^{1}H assignments complete and certain, unequivocal assignments of protonated and quaternary carbon signals were obtained for (1), (2) and (3) by using 2D ^{1}H-^{13}C shift correlation methods (Table 2).

Among the earlier ^{13}C assignments of (1)[4,12-15] only one[15] has been obtained for the compound in solution in DMSO-d6 and our results fully confirm the data given by Ernst and Kang[15]. In the case of (2), one[4] of the two previous studies[3,4] used also DMSO-d6 as the solvent. Comparison with our results shows that the assignments of the carbon pairs C-2 and C-3, C-4a and C-4b, C-5 and C-7 proposed by Sainsbury et al.[4] must be reversed. The two earlier[3,4] ^{13}C assignments of (3) were both given for (3) in solution in DMSO-d6. From the comparison of the older data[3] with ours, it follows that the assignment proposed by Ahond et al. is correct, though it results from the comparison of the chemical shifts of (2) in solution in $CDCl_3$ with those of (3) in solution in DMSO-d6, whereas the more recent assignment[4] proposed for (3) disagrees with ours for C-4, C-5, C-7, C-8, C-10, C-10a, C-10b, C-11 and C-11a.

Table 1. ^1H spectrum assignments of (1), (2), and (3)

	Concentration	NH	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-10	Me-1	Me-4	Me-5	Me-11	OMe
(1)	0.2×10^{-1}M	10.97	-	7.39	6.46	-	-	6.85	7.40	-	-	-	-	-	-	3.87
	10^{-1}M	11.09	-	7.45	6.52	7.21	-	6.93	7.48	-	-	-	-	-	-	3.89
(2)	0.2×10^{-1}M	10.89	-	7.05	6.80	-	7.59	-	7.05	7.43	-	2.49	2.76	-	-	3.85
	5×10^{-1}M	11.00	-	7.16	6.92	-	7.71	-	7.16	7.55	-	2.61	2.88	-	-	3.97
(3)	0.2×10^{-1}M	11.20	9.69	-	8.41	7.91	-	-	7.49	7.20	7.89	-	-	2.77	3.27	3.91
	2.4×10^{-1}M	11.25	9.80	-	8.52	8.01	-	-	7.61	7.31	8.00	-	-	2.89	3.39	4.03

EXPERIMENTAL

The NMR experiments were performed on a Bruker WM 250 spectrometer equipped with an Aspect 3000 computer. The probe temperature was (313 ± 1K) for all the experiments and quadrature detection was used throughout. Chemical shifts are measured relative to the central peak of the dimethylsulphoxide multiplet and converted to the tetramethylsilane scale using δ_{DMSO} = 2.62 ppm for ^1H and δ_{DMSO} = 39.60 ppm for ^{13}C.

The pulse sequence used for the NOESY experiments was D_1-($\pi/2$,^1H)-τ-($\pi/2$,^1H)-D_2-($\pi/2$,^1H) acquire. For the ^1H-^{13}C chemical shift correlations, the applied pulse sequence was that of Bax and Morris[11]. The polarization transfer delays were optimized either for direct ^1H-^{13}C couplings to

obtain direct ^{1}H-^{13}C connections or for 5 Hz long-range couplings which permitted unequivocal assignment of the quaternary carbons.

Table 2. ^{13}C spectrum assignments of (1), (2), and (3)

C	(1)	(2)	(3)
1	-	117.4	149.6
2	125.8	125.6	-
3	101.1	119.5	140.3
4	102.0	129.6	115.9
4a	-	120.5	132.3
4b	-	123.8	-
5	153.5	105.5	107.8
5a	-	-	141.2
6	111.3	152.9	-
6a	-	-	137.3
7	112.1	113.4	111.0
8	131.3	111.3	115.1
8a	-	134.8	-
9	128.3	-	153.1
9a	-	139.9	-
10	-	-	107.8
10a	-	-	123.6
10b	-	-	121.6
11	-	-	123.3
11a	-	-	128.0
OMe	55.4	55.6	55.7
Me-1	-	16.6	-
Me-4	-	20.1	-
Me-5	-	-	11.7
Me-11	-	-	14.2

The chemical shifts were found to be insensitive to concentration in the range 5×10^{-2} M to 5×10^{-1} M.

CONCLUSION

The assignment of some of the ^{13}C-NMR chemical shifts of (3) was uncertain and even contradictory. The use of 2D ^{1}H-^{1}H homonuclear and ^{1}H-^{13}C heteronuclear correlations has enabled us to assign unambiguously the ^{13}C NMR spectrum of (1), (2), and (3) in solution in DMSO-d6 ; some of the earlier assignments of (2), and (3) are thus corrected, whereas those of (1) are fully confirmed. Definitive ^{13}C data are thus provided for these compounds containing the important indole chromophoric group.

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REFERENCES

1. L.K. Dalton, S. Demerac, B.C. Elmes, J.W. Loder, J.M. Swan, and T. Teitei, Aust. J. Chem., 1967, 20, 2715.
2. M. Sainsbury and R.F. Schinazi, J. Chem. Soc. Perkin Trans. 1, 1976, 1155 and references therein.
3. A. Ahond, C. Poupat, and P. Potier, Tetrahedron, 1978, 34, 2385.
4. M. Sainsbury, D. Watkins, and D.K. Weerasinghe, Org. Magn. Reson., 1982, 18, 117.
5. V.N. Rheinhold and R.J. Bruni, Biomed. Mass Spectrom., 1976, 3, 335.
6. M. Sainsbury, D. Weerasinghe, and D. Dolman, J. Chem. Soc. Perkin Trans. 1, 1982, 587.
7. S. Michel, F. Tillequin, and M. Koch, Tetrahedron Letters, 1980, 21, 4027.
8. T.S. Mansour, T.C. Wong, and E.M. Kaiser, Org. Magn. Reson., 1983, 21, 71.
9. R.C. Rao, unpublished results.
10. A. Delbarre, B.P. Roques, J.B. Lepecq, J.Y. Lallemand, and N. Guyen-Dat-Xuong, Biophys. Chem., 1976, 4, 275.
11. A. Bax and G. Morris, J. Magn. Reson., 1981, 42, 501.
12. W. Bremser, L. Ernst, B. Franke, R. Gerhards, and A. Hardt, "Carbon-13 NMR Spectral Data" Verlag Chemie, Weinheim, New-York, 1979.
13. E. Rosenberg, K.L. Williamson, and J.D. Roberts, Org. Magn. Reson., 1976, 8, 117.
14. F. Ladhar, N.G. Horbel, and M. Damak, J. Soc. Chim. Tunisia, 1981, 5, 43.
15. L. Ernst and S. Kang, J. Chem. Res. (M), 1981, 3019.

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