*J. Chem. Research (S)*,
1998, 448–449†

# A Simple Aid to the Assignment of $^{13}$C NMR Spectra, based on the Visual Differences caused by Deuteriation†

**John E. O'Brien, T. Brian H. McMurry and Conor N. O'Callaghan***

*University Chemical Laboratory, Trinity College, Dublin 2, Ireland*

Selective deuteriation of complex polyheterocyclic compounds effects changes in the intensities of the $^{13}$C NMR signals which can be used to solve assignment problems.

Deuteriation of exchangeable C–H to C–D brings about a substantial diminution in the height of the carbon signal, and also effects a decrease in the height of the signals of nearby carbons.[1] Similarly, deuteriation of exchangeable functional groups (OH, NH) diminishes the height of the signal of the attached carbon atom. These signal changes may be attributable to various causes – loss of heteronuclear nuclear Overhauser effect (NOE) effects, the fact that C–D coupling constants are smaller than C–H coupling constants, and signal 'splitting' owing to the presence of partially and fully deuteriated species; in a molecule where deuteriation takes place at different sites, different factors are obviously involved. In each case, however, the effect of deuteriation is made evident by an obvious decrease in the height of the signals of the relevant carbons.

The potential of this deuteriation effect for determining assignment has been referred to before now.[2] We find that it provides a particularly useful, wide-ranging aid to the assignment of the $^{13}$C NMR spectra of complex molecules where several exchangeable protons are present.

Selective deuteriation is achieved by the normal laboratory technique of adding deuterium oxide to solutions of the compounds in dimethyl sulfoxide ([$^2$H$_6$]DMSO). This procedure causes a small solvent effect; other small positional changes also occur, owing to the 'isotope shift' which is manifested by small changes in the resonance positions of substituted carbons. This has been described for various types of molecules;[3,4] shielding effects of 0.2 ppm per deuterium atom for the substituted $\alpha$-carbon and $\sim$0.01 ppm for the $\beta$-carbon have been reported.[5] This is particularly evident in the case of compounds containing a C—NH$_2$ group, where partial deuteriation may afford a split signal or 'isotopic multiplet'.[6] Apart from the latter, however, which is diagnostically useful, the small changes in resonance positions are not regarded as significant for the present work, which depends on signal intensity as measured by height rather than position.

The assignment of a group of complex polyheterocyclic compounds, which are not readily amenable to normal assignment methods, serves to illustrate the usefulness of the technique. The compounds are closely related amino-, hydroxy- and cyano-derivatives of benzopyrans, dibenzo[*b*,*d*]pyrans, benzopyrano[2,3-*b*]pyridines and benzopyrano[4,3,2-*de*]naphthyridines,[7] the formulae of which are shown in Fig. 1, together with their $^{13}$C NMR chemical shifts.

In the case of the relatively simple compounds **1** and **2**, deuteriation helps to resolve the main assignment problems. Thus, the signal at $\delta$ 163.4 is immediately established by deuteriation as the C–NH$_2$ signal; the effect is manifested as a split signal or isotopic multiplet, owing to the separate resonances of fully and partially deuteriated species. The intensity of each part of the split signal is quoted in Fig. 2, together with its chemical shift. Direct deuteriation of carbon occurs at the exocyclic CH, where the acid proton is readily replaced; this effects considerable reduction of the C signal (and thus allows the latter to be distinguished from the endocyclic CH signal, which is also reduced but to a lesser extent). The intensities of the neighbouring carbon signals are also reduced, further facilitating assignment. In compounds **1** and **2**, the aromatic CH signals are not affected by deuteriation, but can be assigned on the basis of combined HH, COSY experiments. The configuration of other assignments is provided by long-range CH coupling constants.

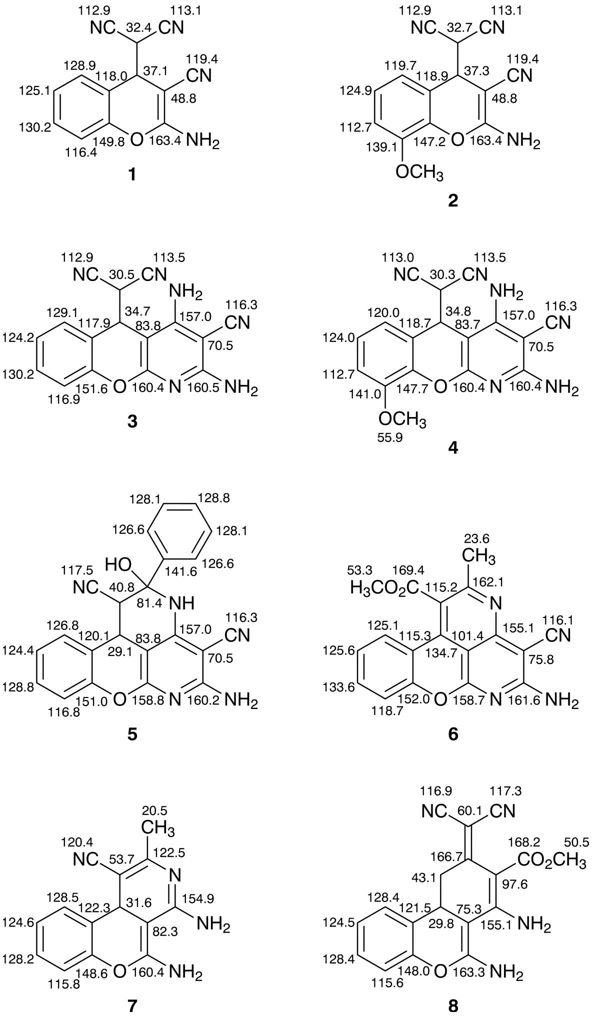

**Fig. 1** $^{13}$C NMR chemical shifts of compounds **1**–**8**

Similar considerations apply in the case of the more complex compounds **3**–**8**; the effects of selective deuteriation on intensity are summarised in Fig. 2. The results obtained

---

*To receive any correspondence.

†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

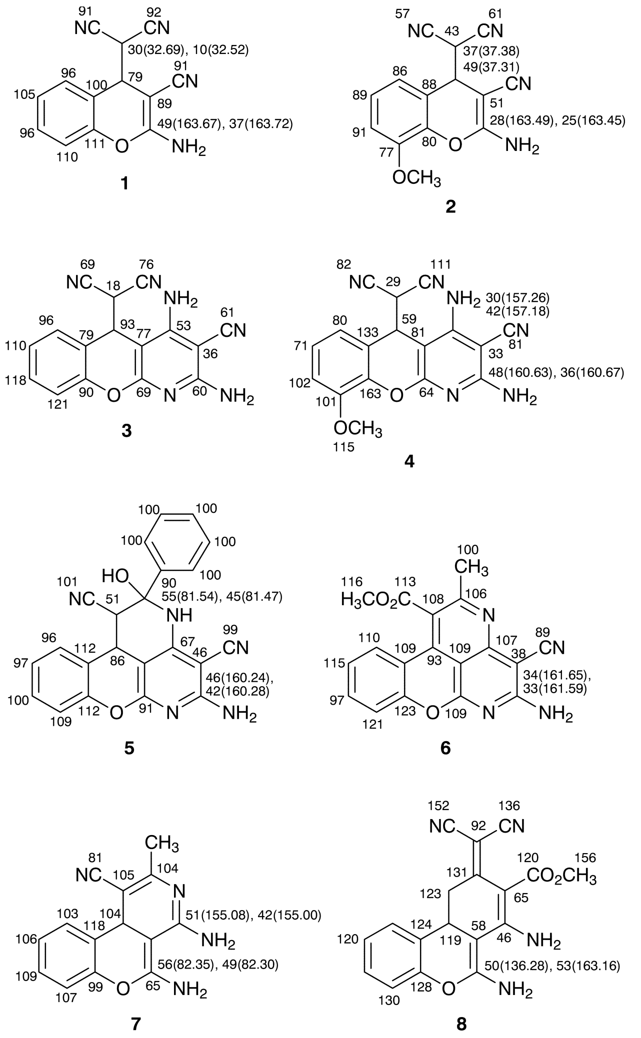

**Fig. 2** Effect of selective deuteriation on signal intensities. The numerical data represent the intensity of each carbon signal in the deuteriated compound, expressed as a percentage of the intensity of the corresponding signal in the non-deuteriated compound. (Data in brackets are the chemical shifts for 'split' signals.)

show that the highly characteristic effects of deuteration on $^{13}C$ NMR spectra of polyheterocyclic compounds, where several exchangeable protons are present, provide a valuable aid to formulating and assigning these compounds. It is considered that this technique may prove to be applicable to a wide range of organic compounds.

## Experimental

The preparation of compounds **1**–**8** has been described elsewhere.[7] All $^{1}H$ and $^{13}C$ NMR spectra were recorded at 297 K in [$^{2}H_6$]DMSO on a Bruker ML300 spectrometer. The $^{1}H$ spectra were recorded at 300.12 MHz with 16 k points zero filled to 32 K, with a resolution of 0.2 Hz pt$^{-1}$ and TMS as an internal reference. The $^{13}C$ spectra were at 75.468 MHz with a digital resolution of 1.1 Hz pt$^{-1}$. Four types of $^{13}C$ NMR experiments were used: a standard proton-decoupled $^{13}C$ (Waltz-16), DEPT 135°, gated $^{1}H$ coupled $^{13}C$ and selective $^{1}H$ decoupled $^{13}C$ gated. The 2D HH COSY had $1024 \times 512$ points with 128 increments; resolution varied from 1–2 Hz pt$^{-1}$. The CH COSY had $1024 \times 1024$ points with 128 increments; resolution was 10–15 Hz pt$^{-1}$ in $F_2$ and 1–3 Hz in $F_1$. Selective deuteriation was achieved by the addition of $D_2O$ (24 mg) to a solution containing 20 mg of compound. The spectra of both solutions were recorded for all compounds under investigation. The concentration of all the compounds (%, w/w) was 3%, except for compound **6** (1%). Signal intensities (using peak heights, not integrals) were measured by setting aromatic carbons (C-1 to C-4) to 100% and comparing the relative intensities of the remaining peaks in the [$^{2}H_6$]DMSO and [$^{2}H_6$]DMSO/$D_2O$ solutions.

*Received, 3rd February 1998; Accepted, 14th April 1998*
*Paper E/8/00939B*

## References

1 R. E. Echols and G. C. Levy, *J. Org. Chem.*, 1974, **39**, 1321.
2 M. J. Shapiro, M. X. Kolpak and T. Lemka, *J. Org. Chem.*, 1984, **49**, 187.
3 For a review, see C. F. Jameson, in *Isotopes in Physical and Biochemical Sciences*, ed. E. Buncel and J. R. James, Elsevier, Amsterdam, vol. 2, pp. 1–55.
4 P. E. Hansen, S. N. Ibsen, T. Kristensen and S. Bolvig, *Magn. Reson. Chem.*, 1994, **32**, 399 and refs. therein.
5 R. A. Bell, C. L. Chen and B. G. Sayer, *J. Chem. Soc., Chem. Commun.*, 1972, 67.
6 J. Reuben, *J. Am. Chem. Soc.*, 1987, **109**, 316.
7 C. N. O'Callaghan, T. B. H. McMurry and J. E. O'Brien, *J. Chem. Soc., Perkin Trans. 1*, 1995, 417.