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Two-Dimensional Nuclear Magnetic Resonance Spectra of Selected Tricyclic Antidepressants

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Two-dimensional nuclear magnetic resonance (2D NMR) spectroscopy has been used to assign ^{13}C spectra of the tricyclic antidepressants imipramine and chlorimipramine. The 2D-INADEQUATE method was used to unambiguously assign the aromatic spectral region for the former compound. Errors in previous literature assignments based on 1D methods were corrected. For chlorimipramine the pitfalls of classical substituent chemical shift arguments for ^{13}C assignments and the difficulties of 1D selective ^1H irradiation in overlapped systems are contrasted with the power and relative simplicity of the 2D- ^{13}C , ^1H -correlated and ^{13}C , ^1H RELAY methods.

Keywords—NMR; 2D NMR; imipramine; chlorimipramine; antidepressants; spectral assignment; ^{13}C -NMR

Introduction

Tricyclic antidepressants (TCA's) are widely used in the treatment of depression. This action is thought to be related, at least in part, to their ability to inhibit re-uptake of amine neurotransmitters into presynaptic nerve endings, although the detailed mechanism by which TCA's exert their effects remains unknown. Nuclear magnetic resonance (NMR) spectroscopy has the potential to increase our knowledge of the molecular events involved in antidepressant action, since it provides a means of examining interactions between drugs and membranes or receptor proteins.¹⁾ However, before such studies can be contemplated a thorough understanding of the solution properties and spectral assignments of the antidepressants is required. Unfortunately, some of the early studies in this area^{2,3)} have been marred by incorrect assignments due largely to the inadequacies of conventional assignment techniques. In this paper these errors are corrected and the power of modern two-dimensional (2D) methods in the assignment of ^1H - and ^{13}C -NMR spectra of representative antidepressants is discussed.

The 2D NMR methods of interest are 2D-INADEQUATE,⁴⁾ carbon-hydrogen correlated spectroscopy⁵⁾ and carbon-hydrogen relayed coherence transfer (RELAY).⁶⁾

The 2D-INADEQUATE⁴⁾ experiment is potentially the most powerful technique available for deducing connectivities in molecular frameworks as it provides direct detection of one-bond carbon-carbon couplings. Such couplings are observable only in molecules containing two adjacent ^{13}C nuclei (*i.e.*, 1 in every 10000 molecules at natural abundance) and thus in a conventional ^{13}C spectrum would appear as satellite signals centred about the hundred-fold stronger resonance arising from molecules containing a single ^{13}C nucleus. Limitations of dynamic range and resolution make observation of these satellite signals difficult in practical cases, except with a technique such as INADEQUATE,⁷⁾ which uses phase cycling of the applied radio frequency pulses to suppress the strong central band. For carbons attached to more than one other carbon, significant overlap of the separate satellites

could be expected in a 1D experiment, but in the 2D-INADEQUATE method an appropriate pulse sequence separates the satellites according to the double quantum frequency of the coupled nuclei. The resultant 2D spectrum has the carbon chemical shift as one axis (F_2) and the double quantum frequency, which is the sum of the chemical shifts of the coupled nuclei (relative to the carrier frequency) as the second axis (F_1). While this technique provides very direct connectivity information, it has the disadvantage of being time-consuming in terms of data acquisition because only molecules containing two adjacent ^{13}C nuclei are detected.

The heteronuclear shift-correlated 2D pulse experiment provides a rapid method for correlating the chemical shift of proton-bearing carbons with the chemical shift of their respective protons. Heteronuclear shift-correlated 2D spectra are normally represented in contour form, with the ^{13}C chemical shift (δC) along the horizontal (F_2) axis and the proton chemical shift (δH) along the vertical (F_1) axis. Correlations appear at (δC , δH). A major advantage of this method is that it uses the carbon chemical shift dispersion to resolve the proton spectrum and in so doing, it has the potential to reveal overlapping proton multiplicities and coupling constants.

Detection of remote carbon-proton connectivities may be achieved by the 2D technique of relayed coherence transfer heteronuclear correlated spectroscopy (RELAY). The resultant 2D spectrum correlates the chemical shifts of ^{13}C nuclei (along the F_2 axis) with the ^1H chemical shifts of both directly coupled neighbouring protons and the remote protons which are coupled to the neighbouring protons. The 2D spectrum therefore displays not only neighbouring cross-peaks of two protonated carbons, A and B, at (δC_A , δH_A) and (δC_B , δH_B), similar to the CH correlated 2D spectrum, but in addition relayed cross-peaks at (δC_A , δH_B) and (δC_B , δH_A). These four signals appear at the corners of a rectangle in the 2D spectrum and provide evidence that the two carbon signals at δC_A and δC_B are from sites in the immediate vicinity of each other within the molecular framework. The method thus directly provides connectivity information, as does 2D-INADEQUATE, but is more sensitive since it does not rely on natural abundance double- ^{13}C labelling.

Experimental

Materials—Imipramine hydrochloride was a generous gift from Ciba-Geigy. Chlorimipramine hydrochloride was kindly donated by Dr. D. Taylor, Victorian College of Pharmacy Ltd. Deuteriochloroform (99.8% D) was supplied by Aldrich Chemical Co., Michigan, U.S.A.

NMR Spectroscopy— ^1H - and ^{13}C -NMR spectra were recorded on a Bruker AM 300WB spectrometer operating at 300.13 and 75.48 MHz respectively. Fourier transform (FT) ^1H -NMR spectra were normally recorded with a 60° pulse (3 μs), repetition time 3 s, spectral width 3 kHz, and 16 k data points. 16 transients were accumulated. Chemical shifts are referenced to the residual chloroform resonance at 7.24 parts per million (ppm).

1D ^{13}C -NMR spectra were obtained with a 45° pulse (6 μs), repetition time 2 s, spectral width 16 kHz and 8 K data points. Proton decoupling was achieved using low-power Waltz decoupling.⁸⁾ Free-induction decays (FID's) were zero-filled to 16 K data points, and 2 Hz exponential line-broadening was applied before Fourier transformation. Chemical shifts were referenced to the centre peak of deuteriochloroform at 77.0 ppm.

2D-INADEQUATE spectra⁴⁾ were obtained with the pulse sequence $90^\circ\text{-}\tau\text{-}180^\circ\text{-}\tau\text{-}90^\circ\text{-}t_1\text{-}135^\circ\text{ FID } (t_2)$ with spectral widths $F_1 = 5000$, $F_2 = 2500$ Hz, a 90° pulse of 13.5 μs , quadrature detection in both dimensions, Waltz proton decoupling, $\tau = 1/4J(\text{C-C}) = 4.2$ ms, a repetition time of 1.2 s, a 128×2048 word data matrix, and a total acquisition time of 12 h. The data was processed as a 256×2048 word data matrix with a sine-bell window function in both dimensions.

The 2D- ^1H , ^{13}C CORRELATED spectra⁵⁾ were acquired with the pulse sequence

^1H : $90\text{-}t_1/2\text{-}\tau\text{-}t_1/2\text{-}\tau\text{-}90\text{-}\tau\text{-}2\text{-Waltz decoupling}$

^{13}C : $180 \quad 90 \quad \text{-FID } (t_2)$

with spectral widths $F_1 \pm 100$ Hz, $F_2 = 2000$ Hz, quadrature detection in both dimensions, $\tau_1 = \tau_2 = 1/2 J(\text{CH}) = 3$ ms, a 128×2048 word data matrix, and a repetition time of 4 s. The data were processed as a 512×2048 word data matrix with a sine-bell window in F_1 and 2 Hz exponential line-broadening in F_2 .

The 2D- ^1H , ^1H , ^{13}C RELAYED spectra⁶⁾ were acquired with the pulse sequence

^1H : $90-t_1/2- -t_1/2-90-t_m/2-180-t_m/2-\tau_1-90-\tau_2$ -Waltz decoupling

^{13}C : 180 90 FID (t_2)

with the same spectral and acquisition parameters used in the ^1H , ^{13}C correlated spectra. The mixing time, t_m , was 16 ms and total acquisition time 15 h. The data were processed as a 256×2048 word data matrix with a sine-bell window in F_1 and a 2 Hz exponential line-broadening in F_2 .

Results and Discussion

Imipramine (**1**) was the first of the TCA's to be developed and it is still in widespread clinical use.

Its ^{13}C -NMR spectrum in the aromatic region is shown in Fig. 1 with proposed assignments of Abraham *et al.*²⁾ and Saito and coworkers.¹⁾ The original assignments of Abraham, which were presumably based on model compounds, appear to be plausible; however, very recently Saito and coworkers used selective ^1H decoupling to reassign the ^{13}C shifts for protonated carbons. In this technique each proton is separately irradiated during acquisition of the ^{13}C spectrum and hence only the carbon attached to the irradiated proton appears as a sharp singlet. Other carbons are only partially decoupled and appear as multiplets or broadened peaks. The results of these experiments suggested that the original C_2 and C_4 assignments should be reversed.

This ^{13}C assignment technique of course requires a prior assignment of the aromatic region of the ^1H -NMR spectrum, which in the case of imipramine consists of two doublets and two triplets due to *ortho* coupling (*meta* coupling was not resolved in the spectra shown by Saito and coworkers). The doublets were assigned to H_1 and H_4 and the connectivity of the peaks was determined by selective ^1H homonuclear decoupling. To complete the assignment a determination of which of the doublets was H_1 and which H_4 was required, and this was made from chemical shift arguments relating to the central nitrogen's effect on H_1 . Such arguments must, however, be used with caution since the substituent effect of a nitrogen varies markedly with its state of protonation (*e.g.*, NH_2 induces an upfield shift of -0.8 ppm at the *ortho* proton in aniline relative to benzene, while for NH_3^+ the shift is opposite in sign, $+0.4$ ppm.⁹⁾ To some extent this ambiguity was resolved by noting that on treatment with DCl, the ^1H -NMR signals from H_2 and H_4 disappeared, as expected for sites *ortho* and *para* to the nitrogen, but again this relies on assumptions about the chemical-directing effect of this group, which may well change with protonation.

Another difficulty with Saito's assignment is that C_{1a} is shown to be the most downfield peak, in contrast to Abraham's assignment and to expectations based on the inductive effect of the nitrogen having its major effect on C_{4a} . In Saito and coworkers' discussion of this assignment, it was suggested that the peak assigned to C_{1a} is broadened by unresolved long-range coupling to the benzylic bridge protons. While this is reasonable, it would be equally valid to argue that C_{4a} should be broadened due to rapid quadrupolar relaxation of the adjacent nitrogen, and hence peak broadening cannot be used as an assignment criterion.

To overcome the difficulties noted above, and to resolve the anomalies in the two literature assignments of imipramine, we have applied a technique which requires no assumptions and does not require specific isotope labelling. This is the 2D-INADEQUATE method. A contour plot of the 2D-INADEQUATE spectrum for the aromatic region of imipramine is shown in Fig. 2. In this figure the F_2 dimension shows pairs of AX doublets arising from molecules containing two adjacent ^{13}C nuclei, while the F_1 dimension resolves these doublet pairs according to their double quantum frequency (equal to the sum of the shifts of A and X with respect to the radio frequency carrier).

The assignment proceeds by noting that the only doublet (marked X on Fig. 2) which has a coupling partner outside the spectral range must be C_{1a} , since it is the only aromatic carbon coupled to an aliphatic carbon. In addition, this peak shows couplings to two aromatic carbons, one of which is a quaternary carbon and thus must be C_{4a} , and the other is thus C_1 . The coupling network can be further traced around the ring, as shown in the figure, to provide a completely unambiguous assignment. Our results show that the assignments of Saito *et al.* are correct for the protonated aromatic carbons but that the non-protonated aromatic carbon assignments should be reversed to correspond with the original assignment of Abraham *et al.* The correct assignments are summarized in Fig. 1.

Imipramine represents a favourable case for the 2D-INADEQUATE experiment in that it has a low molecular weight and is relatively soluble, so that sensitivity problems can be

Fig. 1. The Aromatic Region of the ^{13}C -NMR Spectrum of Imipramine with Proposed Assignments of (a) Saito and Coworkers,¹⁾ (b) Abraham *et al.*,²⁾ and (c) This Work Based on the 2D-INADEQUATE Technique

Fig. 2. 2D-INADEQUATE ^{13}C Spectrum of the Aromatic Region of Imipramine

TABLE I. Predicted ^{13}C Shifts for Chlorimipramine

Carbon number	Imipramine shifts (ppm)	Cl-SCS (ppm)	Predicted chlorimipramine shifts (ppm)	Observed chlorimipramine shifts (ppm)
1	129.9	1.3	131.2	131.4
2	123.1	0.4	123.5	122.7
3	126.5	6.2	132.7	131.4
4	119.5	0.4	119.9	119.4
6	119.5	—	119.5	120.2
7	126.5	—	126.5	126.8
8	123.1	—	123.1	124.0
9	129.9	—	129.9	129.7
1a	133.9	-1.9	132.0	131.7
4a	147.1	1.3	148.4	148.2
6a	147.1	—	147.1	146.5
9a	133.9	—	133.9	134.9

Fig. 3. ^{13}C -NMR Spectrum of the Aromatic Region of Chlorimipramine

alleviated by using relatively concentrated solutions. (In the case above the concentration was 150 mg/ml.) The symmetry of the molecule on the NMR time-scale also doubles the effective concentration of each aromatic carbon, thus reducing the accumulation time by a factor of four relative to a similarly sized non-symmetrical TCA such as chlorimipramine.

In this case, the relative insensitivity of the 2D-INADEQUATE technique is compounded by chlorimipramine's lower solubility relative to imipramine and thus alternative assignment methods are of interest. The usual technique for assignment of a substituted derivative when that of the parent is available, as in this case, is the use of substituent chemical shifts¹⁰⁾ (SCS) to predict ^{13}C shifts in the substituted derivative. Using standard SCS values (derived from chlorobenzene) for a Cl substituent, the predicted shifts for chlorimipramine are shown in Table I. It can be seen that predicted shifts produced in this way are sufficiently close to observed shifts (Figure 3) to assign the non-protonated carbons as well as C_1 , C_3 , C_7 and C_9 , but the pairs C_2 , C_8 and C_4 , C_6 remain ambiguous. Indeed, when the shifts are compared with those established to be correct by 2D NMR (see below), it is seen (Table I) that predictions that C_2 should be downfield of C_8 , and C_4 downfield of C_6 are incorrect. This occurs because SCS values of a given substituent may be significantly different in a polysubstituted benzene from those in mono-substituted benzenes.¹¹⁾

It is interesting to note, for example, that better predictions for C_2 , C_8 and C_4 , C_6 can

be obtained by using chlorine SCS increments derived from aniline and *m*-chloroaniline.¹²⁾ These SCS values are shown in structure (2).

Using these values, the predicted shifts (C_2 , 123.0; C_8 , 123.1; C_4 , 119.3; C_6 , 119.5) now show the correct order. Even with these improvements, however, SCS values are not generally a totally conclusive assignment aid and other methods to distinguish the pairs C_2 , C_8 and C_4 , C_6 are required. The 2D- ^{13}C , ^1H correlated spectrum in Fig. 4 provides this distinction.

In the F_1 (^1H) dimension, H_4 should be the only peak to show just *meta* coupling and hence this unique coupling pattern in the contour plot identifies the most upfield peak (119.4 ppm) as being due to the carbon correlated with H_4 , *i.e.*, C_4 . The peak at 120.2 ppm must therefore be due to C_6 . Similarly, of the remaining ambiguous peaks for C_2/H_2 , C_8/H_8 at 122.7 and 124.0 ppm, the lower field carbon signal appears as an *ortho* doublet with *meta* coupling, *i.e.*, it is connected to H_2 and is hence C_2 . The peak at 124.0 ppm is thus C_8 .

The projection of the 2D- ^{13}C , ^1H correlated spectrum in the proton dimension provides the conventional proton spectrum (Fig. 4), which is heavily overlapped in the H_8 , H_1 , H_4 , H_6 region. This would make the traditional technique of selective ^1H irradiation to assign the carbon spectrum difficult and, indeed, is the reason why Saito *et al.* were unable to unambiguously assign the spectra of the structurally related compound chlorpromazine. The 2D- ^{13}C , ^1H -correlated technique overcomes the problem of overlap in the ^1H spectrum by spreading it into a second dimension based on ^{13}C chemical shifts.

It should be noted that although the 2D- ^{13}C , ^1H -correlated technique was extremely valuable in distinguishing between C_2 and C_8 , and between C_4 and C_6 , the assignment is not without assumption, since chemical shift arguments were used to roughly predict shifts for C_1 , C_9 , C_7 , C_8/C_2 and C_6/C_4 . Such chemical shift arguments can break down and, for example, the 2D-correlated spectrum alone could not be used to distinguish between C_7 and C_8 , since both have similar ^1H multiplicity. In principle 1D-selective homonuclear decoupling experiments could be used to further assign the 1D proton spectrum, which would then lead to an assignment of the ^{13}C spectrum *via* the 2D- ^{13}C , ^1H -correlated spectrum. However, in the

Fig. 4. 2D-Carbon-Hydrogen-Correlated Spectrum of the Aromatic Region of Chlorimipramine

Fig. 5. 2D-RELAY Spectrum of the Aromatic Region of Chlorimipramine

current case the 1D ^1H spectrum is significantly overlapped, making the selective ^1H irradiation time-consuming and subject to substantial interpretation.

The RELAY technique can be used to overcome these difficulties and provides an assignment essentially free of assumption. The RELAY spectrum of the aromatic region of chlorimipramine shown in Fig. 5 displays all of the peaks noted in Fig. 4, but in addition contains cross-peaks indicative of two carbons sharing a common ^1H coupling partner. The way in which this provides connectivity information can be seen by noting that once, say, C_6 is assigned, then connection to, and hence assignment of, C_7 , then C_8 , and then C_9 is immediately established. Similarly, connection between the protonated carbons C_1 and C_2 is established.

In summary, it can be seen that even for relatively simple compounds of pharmaceutical interest, 1D methods are often inadequate for NMR spectral assignment. On the other hand, 2D methods are now easy to implement and have proven useful in correcting previous ambiguous assignments.

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