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NMR analysis of a series of imidazobenzoxazines

Mirko Rivara,[a] Marco Fantini,[a]* Domenico Acquotti[b] and Valentina Zuliani[a]

The complete ^{1}H and ^{13}C NMR assignment of a series of imidazobenzoxazines by a combination of one- and two-dimensional experiments (COSY, HSQC and HMBC) is studied. Moreover, 2D NOESY and 1D selective NOESY are reported. This procedure allows the identification of the regioisomers obtained.

Keywords: NMR; 2D NMR; HSQC; HMBC; COSY; NOESY; imidazobenzoxazines

Introduction

As part of our research on the design and synthesis of new sodium channel blockers potentially useful as anticonvulsants,[1,2] we have been involved in the preparation of a series of imidazole-based privileged structures (Fig. 1), obtained both under conventional as well as microwave heating conditions in high yields.[3] The synthetic route employed to obtain the imidazobenzoxazines **1–6** allowed to the formation of two regioisomers, separated and isolated. These new polycyclic heterocycles have been described by Mahesh only in 1985[4] and detailed information about ^{1}H and ^{13}C NMR signals of this class of compounds is still lacking. Thus, the present study deals with the complete ^{1}H and ^{13}C assignments of these structures, also focusing on the identification of the regioisomers obtained.

Results and Discussion

The complete proton and carbon chemical shifts' assignments were achieved by a combination of one- and two-dimensional NMR experiments. Considering the structure of **1** (Fig. 2), the 1D ^{1}H NMR spectrum shows two singlets at $\delta = 5.74$ and 7.12 ppm, integrating for 1 and 2 protons, respectively: these data suggest that the signal at $\delta = 5.74$ belongs to H2 and that at $\delta = 7.12$ ppm to H7. The heteronuclear single quantum coherence (HSQC) shows that H2 correlates with the carbon at $\delta = 75.0$ ppm and the proton H7 with the carbon at $\delta = 111.1$ ppm (Table 1). The long-range heteronuclear multiple bond coherence (HMBC), showing ^{2}J and ^{3}J CH coupling constants, allows to assign a number of quaternary carbons. In particular, the proton H2 correlates with the previously assigned C7 and with two quaternary carbons at $\delta = 141.1$ and 152.1 ppm; the proton H7 shows a long-distance correlation with C2 and with two quaternary carbons at $\delta = 141.1$ and 143.2 ppm. Their only possible common correlation must be with C4, assigned to the carbon signal at $\delta = 141.1$ ppm. Consequently, the remaining two quaternary carbons considered can be identified as C6 ($\delta = 143.2$ ppm) and C8 ($\delta = 152.1$ ppm) correlated to H7 and H2, respectively.

The HMBC analysis allows to discriminate H10 and H13: considering the structure **1**, only H10 can correlate (^{3}J) with the already assigned C4. It is interesting to observe that for this rigid scaffold, a number of ^{4}J correlation peaks, such as H11-C8 and H13-C4, are present in the spectrum. Moreover, a similar ^{1}H and ^{13}C chemical shift behavior was observed in a series of benzoxazine derivatives previously published.[5] Once H10 is assigned and considering the correlations obtained by 2D COSY, H11, H12 and H13 can be endowed easily; the respective carbons were allotted from HSQC. In the HMBC spectrum, H10 with $\delta = 8.01$ ppm showed a ^{2}J coupling constant with the quaternary carbon signal at $\delta = 117.02$ ppm, identified as C9. The protons of the second aromatic ring (H15–H17) are easily assigned on the basis of multiplicity and 2D COSY relations; the only doublet not assigned in 1D ^{1}H NMR spectrum ($\delta = 7.79$ ppm) belongs to H15 and, consequently, H16 ($\delta = 7.36$ ppm) and H17 ($\delta = 7.23$ ppm) were identified. The corresponding carbons are directly assigned through a HSQC experiment. The same methodology described was applied to the imidazobenzoxazine **2**. The correct conformation of each regioisomer was obtained with 2D NOESY and 1D selective NOESY. The 1D selective NOESY spectrum of structure **1** shows two spatial contacts between H7 and both H2 and H15, whereas for the structure **2** only one cross-peak, between H2 and H15, is detected. Figure 2 shows the differences between the spatial interactions of the two isomers, clearly identified both in the 2D NOESY as well as in the 1D selective NOESY spectra.

All ^{1}H and ^{13}C data regarding the two isomers 2-phenyl-5*H*-imidazo[1,2-c][1,3] benzoxazine (**1**) and 3-phenyl-5*H*-imidazo[1,2-c][1,3]benzoxazine (**2**) are given in Table 1.

For compounds **3**, **4**, **5** and **6**, the protons and carbons were assigned using the same strategy and the data obtained are reported in Tables 2 and 3.

* *Correspondence to: Marco Fantini, Dipartimento Farmaceutico, Università degli Studi di Parma, Viale G.P. Usberti 27/A, I-43124 Parma, Italy. E-mail: marco-fantini@libero.it*

a *Dipartimento Farmaceutico, Università degli Studi di Parma, V.le G.P. Usberti, 27/A, I-43124 Parma, Italy*

b *Centro Interdipartimentale Misure 'Giuseppe Casnati', V.le G.P. Usberti, 23/A, I-43124 Parma, Italy*

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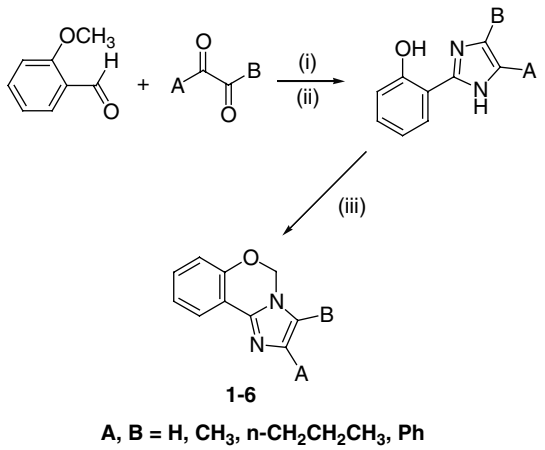


1-6

A, B = H, CH_3, n-$CH_2CH_2CH_3$, Ph

Figure 1. Synthesis of the imidazobenzoxazines **1–6**.

Reagents and conditions: (i) CH_3COONH_4, CH_3OH, rt, 2 h. (ii) 48% HBr, MW, 150 °C, 200 W, 10 min. (iii) CH_2I_2, Cs_2CO_3, CH_3CN, MW, 80 °C, 150 W, 10 min.

The NOESY spectra allow to identify the two isomers of each couple; they show two spatial contacts H2–H18 and H18–H15 for **3** while for **4** H2 presents a NOE cross-peak with H15 and H18, indicating that the phenyl ring is linked in position 6 in **3** and in position 7 in **4**. For **5** only the H2–H14 contact is detected while the spectrum of **6** shows cross peaks of H2 with the chain protons H15, H16 and H17.

Experimental

Synthesis

The imidazobenzoxazines reported in this study were prepared in our laboratories as previously described.[4]

NMR measurements

The ^{1}H NMR experiments were performed in $CDCl_3$ on a Varian Inova 600 spectrometer operating at 599.74 MHz using a Standard Bore 5 mm inverse triple resonance Z gradient probe. ^{13}C NMR experiments were performed on the same instrument operating at 150.82 MHz.

^{1}H and ^{13}C NMR, gCOSY, NOESY, gHSQC, gHMBC spectra were acquired from samples in $CDCl_3$ (0.6 ml) solutions (50 mg ml^{-1}). ^{1}H and ^{13}C NMR spectra were measured at 25 °C in standard 5-mm o.d. tubes (Norell, S600). All experiments were performed at 298°K without spinning. Chemical shifts are reported as δ (ppm); coupling constants (J) are expressed in Hz. ^{1}H NMR spectra were referenced to residual $CDCl_3$ at $\delta = 7.24$ ppm; ^{13}C assignments were referenced to $CDCl_3$ at $\delta = 77.00$ ppm. In 1D ^{1}H NMR data, 16 transients were collected with a 30° pulse flip angle and 1.5-s relaxation delay using 32 K data points. ^{13}C NMR spectra were run operating typically with a 45° pulse flip angle, a relaxation delay time of 3–5 s and a spectral width of 25 000 Hz with 64 K data points and zero-filled to a digital resolution of 0.4 Hz.

The data for the gCOSY, gHSQC and gHMBC spectra were collected with 1 K data points for F_2 and 128–256 increments for F_1 with pulse sequences that allowed gradient selection; spectral windows were 5500–6000 and 27 000–37 000 Hz in the ^{1}H and ^{13}C dimensions, respectively. In the homonuclear ^{1}H, 2D COSY and NOESY experiments, the relaxation delay was set to 1.5–2 s. The resulting FIDs were zero-filled to a 2 K × 1 K data matrix, apodized with a sine function for COSY, and a shifted sine function for NOESY in both the ω 1 and ω 2 dimensions prior to Fourier transformation. In NOESY spectra mixing time was 0.7 s.

The 1D NOESY was achieved using the standard Varian DPFGSE NOE pulse sequence using the pulse sequence using a q3 shape pulse (Gaussian cascade 180° inversion); the selective 180° pulse was arrayed between 25.5 and 49.4 ms. The 1D NOESY spectra were acquired with a mixing time of 700 ms, a relaxation delay of 1 s and acquisition time of 1.7 s.

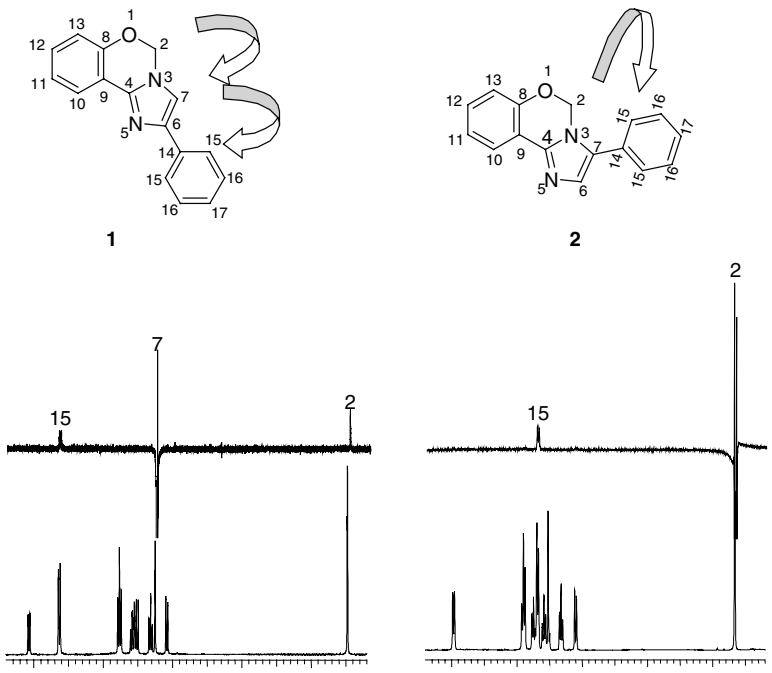
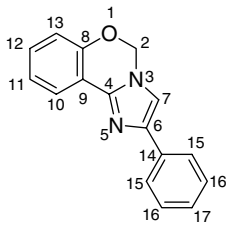
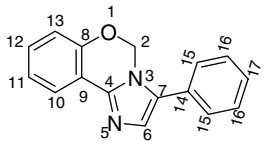


Figure 2. 1D selective NOESY spectra of compounds **1** and **2**.

Table 1. ^{1}H and ^{13}C chemical shifts (in ppm) and HMBC correlations of **1** and **2**[a]



1

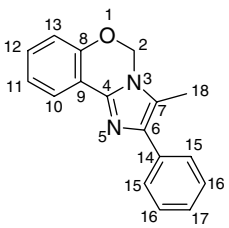


2

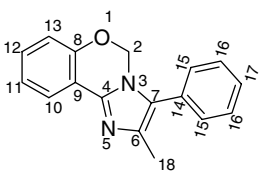
Position	^{1}H	^{13}C	HMBC	Position	^{1}H	^{13}C	HMBC
2	5.74 (s)	75.0	4, 7, 8	**2**	5.82 (s)	73.9	4, 7, 8
4	–	141.1	–	**4**	–	141.6	–
6	–	143.2	–	**6**	7.24 (s)	128.8	2, 4, 7
7	7.12 (s)	111.1	2, 4, 6	**7**	–	131.0	–
8	–	152.1	–	**8**	–	152.2	–
9	–	117.1	–	**9**	–	117.5	–
10	8.01 (d)	123.7	4, 8, 9, 11, 12	**10**	7.96 (d)	123.6	4, 8, 11, 12, 13
11	7.13 (t)	123.7	8, 9, 10, 12, 13	**11**	7.14 (t)	123.8	8, 9, 10, 12, 13
12	7.27 (t)	130.1	8, 10, 11, 13	**12**	7.28 (t)	130.1	8, 9, 10, 11, 13
13	7.02 (d)	116.9	8, 9, 11, 12	**13**	7.04 (d)	116.9	8, 9, 11, 12
14	–	133.7	–	**14**	–	128.7	–
15	7.79 (d)	125.1	6, 16, 17	**15**	7.33 (d)	127.8	7, 16, 17
16	7.36 (t)	128.6	14, 15, 17	**16**	7.43 (t)	129.1	14, 15, 17
17	7.23 (t)	127.1	15, 16	**17**	7.35 (t)	128.1	15, 16

Multiplicities: s, singlet; d, doublet, t, triplet.
[a] Recorded in $CDCl_3$.

Table 2. ^{1}H and ^{13}C chemical shifts (in ppm) and HMBC correlations of **3** and **4**[a]



3

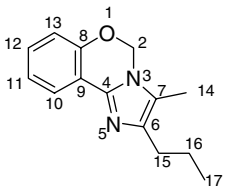


4

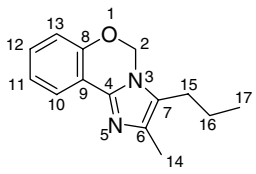
Position	^{1}H	^{13}C	HMBC	Position	^{1}H	^{13}C	HMBC
2	5.69 (s)	73.3	4, 7, 8	**2**	5.66 (s)	74.0	4, 7, 8
4	–	139.5	–	**4**	–	139.8	–
6	–	139.2	–	**6**	–	136.8	–
7	–	120.7	–	**7**	–	126.0	–
8	–	151.8	–	**8**	–	152.3	–
9	–	117.2	–	**9**	–	117.3	–
10	7.99 (d)	123.5	4, 8, 9, 11, 12	**10**	7.95 (d)	123.4	4, 8, 9, 11, 12
11	7.11 (t)	123.7	8, 9, 10, 12, 13	**11**	7.12 (t)	123.6	8, 9, 10, 12, 13
12	7.23 (t)	129.8	8, 10, 11, 13	**12**	7.25 (t)	129.8	8, 10, 11, 13
13	7.01 (d)	116.8	8, 9, 11, 12	**13**	7.01 (d)	116.8	8, 9, 10, 11, 12
14	–	134.6	–	**14**	–	129.0	–
15	7.65 (d)	127.3	6, 14, 16, 17	**15**	7.27 (d)	129.1	7, 14, 16, 17
16	7.39 (t)	128.4	14, 15, 17	**16**	7.45 (t)	128.9	14, 15, 17
17	7.25 (t)	126.6	15, 16	**17**	7.36 (t)	127.9	15, 16
18	2.38 (s)	9.4	6, 7	**18**	2.33 (s)	13.2	6, 7

Multiplicities: s, singlet; d, doublet, t, triplet.
[a] Recorded in $CDCl_3$.

Table 3. ^{1}H and ^{13}C chemical shifts (in ppm) and HMBC correlations of **5** and **6**[a]



5



6

Position	^{1}H	^{13}C	HMBC	Position	^{1}H	^{13}C	HMBC
2	5.57 (s)	73.3	4, 7, 8, 9	**2**	5.61 (s)	73.4	4, 7, 8, 9
4	–	138.7	–	**4**	–	138.7	–
6	–	139.7	–	**6**	–	135.4	–
7	–	119.6	–	**7**	–	124.4	–
8	–	151.6	–	**8**	–	151.6	–
9	–	117.4	–	**9**	–	117.4	–
10	7.89 (d)	123.1	4, 8, 9, 11, 12, 13	**10**	7.87 (d)	123.0	4, 8, 9, 11, 12, 13
11	7.08 (t)	123.5	4, 8, 9. 10, 12, 13	**11**	7.08 (t)	123.5	4, 8, 9, 10, 12, 13 1311313
12	7.21 (t)	129.3	8, 10, 11, 13	**12**	7.21 (t)	129.4	4, 8, 10, 11, 13
13	6.99 (d)	116.7	8, 9, 11, 12	**13**	6.98 (d)	116.7	8, 9, 11, 12
14	2.17 (s)	8.0	6, 7, 15	**14**	2.22 (s)	12.6	6, 7, 15
15	2.51 (t)	29.1	6, 7, 16, 17	**15**	2.51 (t)	24.7	6, 7, 16, 17
16	1.66 (m)	23.4	6, 15, 17	**16**	1.50 (m)	23.4	7, 15, 17
17	0.93 (t)	13.9	15, 16	**17**	0.92 (t)	13.4	15, 16

Multiplicities: s, singlet; d, doublet, t, triplet.
[a] Recorded in $CDCl_3$.

Heteronuclear spectra were recorded with 1 K × 256 data points, zero-filled only in F_1 to a 1 K × 512 data matrix, and apodized in both dimensions with a shifted sine function. HSQC experiments were acquired using adiabatic pulses for inversion of ^{13}C, optimized for $^{1}J(CH) = 145$ Hz. The 90° ^{13}C pulse length was 13.8 µs. The gHMQC was acquired with ^{13}C sweep width of 32 000 Hz and 256 t_1 increments. Each increment was acquired with 32 transients. Sinebell weighting was applied to the F_2 dimension before zero-filled to 2 K points, and a sinebell was applied to the F_1 and zero-filled to 2 K points before Fourier transformation. A one-bond coupling constant delay was set using 140 Hz and WURST decoupling was applied during acquisition. The gHMBC was acquired using 64 transients per increment with 256 F_1 increments. The one-bond coupling constant of 140 Hz and long-range coupling constant varying between 5 and 12 Hz were used to set the delays in the pulse sequence. A sinebell and Gaussian weighting functions were applied to ^{13}C and ^{1}H dimensions and zero-filled to 1 and 4 K, respectively.

Acknowledgements

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