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Long-Range Correlations ($^{n}J_{C,H}$ n > 3) in the HMBC Spectra of 3-(4-Oxo-4*H*-chromen-3-YL)-acrylic Acid Ethyl Esters

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Um estudo sistemático das correlações de longo alcance em espectros de HMBC foi feito para uma série de ésteres etílicos do ácido cis- e trans-3-(4-oxo-4H-chromen-3-il) acrílico e os derivados 5-hydroxilados. Os resultados foram comparados com os já observados para espectros IMPEACH-MBC e CIGAR-HMBC. Um acoplamento incomum do tipo $^{5}J_{C,H}$ entre prótons metilicos e o C_{α} do grupo carbonilico foi observado nesses sistemas, assim como no crotonato etílico. A atribuição completa dos espectros ^{1}H e ^{13}C RMN foi realizada.

A systematic study about the long-range correlations observed in the HMBC spectra of a series of cis and trans 3-(4-oxo-4H-chromen-3-yl)-acrylic acid ethyl esters, and 5-hydroxy derivatives, was carried out. The results were compared with those observed in IMPEACH-MBC and CIGARHMBC spectra. An uncommon $^{5}J_{C,H}$ between the methyl protons and the C_{α} to the carbonyl group was observed in these systems as well as in ethyl chrotonate. The complete assignments of the ^{1}H and ^{13}C NMR spectra are provided.

Keywords: ^{1}H and ^{13}C NMR , HMBC, CIGAR-HMBC, IMPEACH-MBC, $^{5}J_{C,H,}$ acrylic acid ethyl esters, chromone

Introduction

The Heteronuclear Multiple Bond Correlation (HMBC)[1,2] NMR experiment has probably been the most widely used 2D heteronuclear NMR method in the field of structural elucidation of complex organic molecular structures. Nowadays, it has become one of the most popular techniques for multiple-bond (or long-range) heteronuclear chemical shift correlation. It provides a wealth of structural information, and eventually the full assignment, through long-range correlation signals for $^{13}C,^{1}H$ spin pairs. It can span quaternary carbons or heteronuclei, providing a way to link different molecular fragments into a complete structure.[3-5] The use of HMBC together with Heteronuclear Multiple Quantum Coherence, HMQC,[6] or Heteronuclear Single Quantum Correlation, HSQC,[7] has proved to be extremely useful in the total structure elucidation and NMR spectral assignments of numerous complex organic molecules.

The HMBC experiment was highly improved since the application of pulsed field gradients (PFG) in the early 1990's. High quality spectra, without artefacts, are obtained in standard conditions, allowing the analysis of very small but informative cross peaks. Today the advantages offered by the incorporation of pulsed field gradients into high-resolution NMR pulse sequences, combined with advanced software tools available to acquire and process multidimensional NMR experiments, has allowed to record and process the HMBC sequence with PFG just by clicking a button or executing a predefined macro, making it available to the non-experienced NMR user.[8]

Despite the widely recognized structural elucidation capabilities of HMBC, it is usually described as a technique that allows the assignment of structural fragments through the correlation between protons and carbons separated by two or three bonds.[3-5] However, the early observation of a crucial four bond C,H correlation in the HMBC spectrum

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of antibiotic distamicyn A[9], along with another examples of this kind of correlations (${}^{n}J_{C,H}$ n > 3) subsequently reported, validate HMBC as a technique that allow to obtain more valuable structural information.[10] Moreover, a frequent source of contradictions in computer-assisted structure elucidation programs, has been the observation of correlations in COSY and in long-range heteronuclear shift-correlation experiments, which corresponds to four or more bonds.[11]

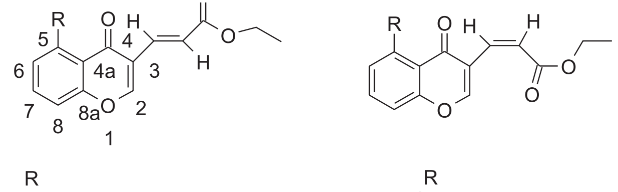
With regard to the observation of ${}^{n}J_{C,H}$ n>3, two extreme opinions have been argued: the first position emphasizes the disadvantages associated with the increase in the number of H,C correlation peaks for which the number of bonds is uncertain, even worst if the cross-peaks intensities are low, as usually occurs. Because of this, the valuable information that might be obtained through heteronuclear long-range correlations, across four or more bonds, has been generally discarded. It is important to mention, even though it is obvious, that if the number of intervening bonds assigned to a cross-peak is different from the actual value, wrong connectivities will be made. Moreover, these correlations can be crucial in structural elucidation.[9,12,13] The other position focuses on the large amount of structural information that can be obtained with a larger number of long-range correlation peaks, which can be translated to bonding network, despite the lack of discrimination. The pulse_sequences: D-HMBC,[14] 3D-HMBC,[15] CT-HMBC,[16] ACCORD-HMBC,[17] IMPEACH-MBC[18] and CIGAR-HMBC,[19] which sample a great number of long-range coupling constants, were developed based on the last point of view.

The tuning of the HMBC experiment is achieved by setting the Δ_2 preparation period, the so called long-range delay, to a sufficiently long time to allow the small long-range proton-carbon couplings to evolve and produce the antiphase displacement of vectors required for the subsequent generation of heteronuclear multiple quantum coherence, and is calculated from $\Delta_2 = 1 / (2\ {}^{n}J_{C,H})$.[5] Since molecules have a range of ${}^{n}J_{CH}$ values, typically from 2 to 15 Hz,[4,5] Δ_2 should be at least 100ms. In practice a delay shorter than the theoretical value is employed in order to avoid the ^{1}H magnetization to decay during this delay, particularly for large molecules. For routine applications Δ_2 is usually set from 60 to 80 ms.[3-5] This delay significantly attenuate cross-peaks intensities arising from values of ${}^{n}J_{CH}$ which are far away from the average value.

Since small molecules tend to have slower relaxation rates, longer delays can be successfully used in the search for connectivities through smaller couplings. A maximum of 200 ms has been recommended to detect cross-peaks arising from four bonds correlations, which are described as most likely to occur when the coupling pathway contains unsaturations or when it has the planar zig-zag (W coupling) configuration,[4] as commonly observed in long-range proton-proton coupling. However these generalizations might induce to confusion. For example values of Δ_2 between 50 to 400 ms to record HMBC spectra of phenolic compounds, has been reported,[20] moreover the observation of five bonds correlations by using standard value of Δ_2 (65 or 80 ms) in HMBC spectra of quinones has also been reported.[12,13,21] In a similar way, several examples of ${}^{4}J_{CH}$ has been observed in a great variety of molecules and coupling pathways.[10]

Generally the choice of Δ_2 is made on arbitrary basis rather than from knowledge of the actual value of the couplings. On this basis, a first approximation to observe a greater number of long-range correlations is to perform several HMBC experiments with different long-range delays and using a deeper threshold in the contour plot. Hereby new correlations could be observed and some of the already observed ones might disappear. Alternatively, some of the new pulse sequences focused on long-range delay optimisation, of which CIGAR-HMBC has been described as the best,[5] can be used.

In order to explore this subject in a systematic way and hence to obtain further information that can be used for a more effective choice of Δ_2 values, we selected compounds **1-4** (Figure 1) to study the long range correlations that can be observed at different Δ_2 values. The selection of these structures was made considering that the oxygen atom of the heterocyclic moiety and the quaternary carbons impose an assignment relying on long-range correlations. Additionally, it is interesting because the chromone system has received attention due to the possibility of aromatic character of the heterocyclic moiety.[22] On the other hand, from a synthetic point of view, the π system of the acrylic ester conjugated with heterocyclic double bond conform an attractive push-pull diene with *E* or *Z* configuration that has been used in cycloadditions.[23] In this paper we present a study to obtain a criterion that allows a more appropriate choice of Δ_2 in this type of molecular systems. For this purpose, several HMBC experiments increasing



R
1: OH
2: H

R
3: OH
4: H

Figure 1. 3-(4-Oxo-4*H*-chromen-3-yl)-acrylic acid ethyl esters derivatives studied.

Δ_2 long-range delays were carried out to observe the necessary $^{n}J_{CH}$ correlations for the assignments. In this manner we have assigned the ^{13}C NMR spectra of these compounds by the concerted use of ^{1}H-detected one bond heteronuclear multiple quantum coherence HMQC,[6] or HSQC,[7] and long-range heteronuclear multiple bond connectivity, HMBC.[1,2]

Experimental

*General procedure for preparation of chromones **1-4***

The chromones **1-4** were prepared starting from the corresponding 3-formylchromone,[24] through a Wittig reaction according to the following experimental conditions: one equivalent of carboethoxymethylene-triphenylphosphorane was added to a solution of 0.5 g of the corresponding 3-formylchromone in 10 mL of toluene and the mixture heated to reflux for 2h. The triphenylphosphine oxide formed in the reaction was removed through vacuum filtration and then rotatory evaporation of the solvent from the filtrate affords a mixture of *cis* and *trans* acrylic acid ethyl esters derivatives. Purification by column chromatography through silica gel using ethyl acetate/hexane 1: 2 as eluent and further crystallization from a mixture of ethyl acetate/hexane yields the corresponding pure compounds **1-4** as white needles. *trans*-5-hydroxy-3-(4-oxo-4*H*-chromen-3-yl)-acrylic acid ethyl ester **1** was obtained with 55% yield (m.p. 158.5-159.5 ºC), Anal. Calc. For $C_{14}H_{12}O_5$: C, 64.61; H, 4.65 Found: C, 64.40; H, 4.42. IR (KBr) ν_{max}/cm^{-1}: 3442, 1706, 1652, 1296, 1267, 1170. *cis*-5-hydroxy-3-(4-oxo-4*H*-chromen-3-yl)-acrylic acid ethyl ester **3** was obtained with 40% yield (m.p. 69.5-70 ºC). Anal. Calc. For $C_{14}H_{12}O_5$: C, 64.61; H, 4.65 Found: C, 64.10; H, 4.47. IR (KBr) ν_{max}/cm^{-1}: 3440, 1703, 1655, 1265, 1196, 1158. *trans*-3-(4-oxo-4*H*-chromen-3-yl)-acrylic acid ethyl ester **2** was obtained with a 50% yield (m.p. 111.5-112.5 ºC lit. 96-98 ºC[23]). *cis*-3-(4-oxo-4*H*-chromen-3-yl)-acrylic acid ethyl ester **4** was obtained with a 49 % yield (m.p. 66,5 - 67.5 ºC).

Table 1. ^{1}H NMR Chemical shift assignments (ppm TMS) for chromones **1 -4**

Atom	**1**	**2**	**3**	**4**
H-2	8.10	8.12	9.10	9.13
H-5	-	8.28	-	8.26
H-6	6.85	7.45	6.82	7.43
H-7	7.56	7.70	7.55	7.69
H-8	6.93	7.49	6.93	7.49
H-9	7.38	7.42	7.05	7.16
H-10	7.21	7.28	6.12	6.10
CH_2	4.30	4.26	4.20	4.19
CH_3	1.34	1.33	1.30	1.29
OH	12.49	–	12.41	–

Table 2. ^{13}C NMR Chemical shift assignments (ppm TMS) for chromones **1-4**

Atom	**1**	**2**	**3**	**4**
C-2	157.88	157.40	159.18	158.28
C-3	118.10	119.40	117.41	118.73
C-4	181.48	176.00	181.24	176.08
C-4a	110.90	124.30	110.71	123.86
C-5	161.25	126.30	161.98	126.15
C-6	112.24	125.90	111.70	125.44
C-7	135.78	133.90	135.65	133.79
C-8	107.12	118.20	107.27	118.19
C-8a	155.67	155.60	156.13	155.99
C-9	134.11	135.20	132.14	133.88
C-10	122.42	122.30	121.21	120.60
CH_2	60.57	57.70	60.53	60.39
CH_3	14.21	10.10	14.08	14.11
CO (ester)	167.06	167.40	166.01	166.17

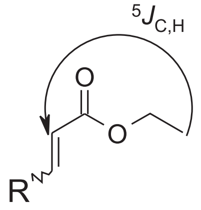


Figure 2. Five-bonds correlation observed in the HMBC spectrum of chromones (Δ_2 = 130 ms) and ethyl chrotonate (65 ms).

NMR Spectra

^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVANCE 300 and a Bruker AVANCE 400 spectrometers operating at 300.13 and 400 MHz (^{1}H) or 75.47 and 100 MHz (^{13}C) respectively. All measurements were carried out at a probe temperature of 300 K, using solutions of **1-4** (20-30 mg mL^{-1} in $CDCl_3$ containing tetramethylsilane as internal standard). All two dimensional spectra were acquired, in both spectrometers, with a Bruker inverse 5 mm Z gradient probe. HMQC spectra were recorded using the standard Bruker software. These spectra were collected with 512 x 512 data points, with eight scans acquired for every 256 increments in t_1, and the Δ_1 delay was set to 3.45 ms. Spectral widths of 3000 Hz and 18000 Hz were used in the F_2 (^{1}H) and F_1 (^{13}C) domains, respectively. HMBC, CIGAR-HMBC and IMPEACH-MBC spectra were obtained using the standard pulse sequence from the Bruker software. The spectral widths were 3900 Hz (F2) and 18000Hz (F1). The Δ_2 delay in the HMBC pulse sequence was varied from 65 to 390 ms. Data was processed using an exponential decay window in F_2, with 1b = 5Hz, and a qsine window function in F_1.

Results and Discussion

The ^{1}H-NMR spectra of compounds **1** and **3** display two doublets corresponding to an AB system with *J* 15.9 Hz, characteristics of *trans* double bond (**1**), and *J* 12.8 Hz for the *cis* compound (**3**). The H-10 proton doublet, δ 7.21 for compound **1,** is shifted to lower field respect to compound **3** (δ 6.12), probably due to the anisotropic effect of C-4 carbonyl group in the *s-trans* conformation of the diene moiety. Both compounds exhibit three signals for the aromatic protons: two doublets and an apparent triplet, besides the two characteristic triplet and quartet of the ethyl chain. The singlet corresponding to the heterocyclic proton appears at very low field, as reported before for this class of heterocyclic systems,[23,25] due to oxygen substitution and the conjugation effect of the carbonyl group (C-4), although the postulated aromatic character of heterocyclic ring of the chromone[22] may also contribute. The absolutes magnetic shielding at the rings centers and at the carbon nuclei has been previously reported.[22] The H-2 resonance in compound **3** (δ 9.10) is shifted to low field with respect to compound **1** (δ 8.10), probably due to the anisotropic effect of the carbonyl ester group in the *s-cis* conformation.

The protonated carbons can be easily assigned through an HMQC or HSQC experiments, except in the case of C-6 and C-8 because H-6 and H-8 exhibit similar chemical shifts and multiplicities. Therefore, these carbons together with the quaternary centers must be assigned through long-range correlations.

The HMBC spectra of both compounds obtained with a 300 MHz spectrometer and using a standard long-range delay of 65 ms, displayed a three bond correlation between the hydroxyl proton and C-6, and did not correlate with C-8, allowing the discrimination between them. A three bond correlation between the same proton and C-4a is also observed allowing discrimination between C-4a and C-3. The $^3J_{C,H}$ between H-10 with C-3 and the $^2J_{C,H}$ between this carbon with H-2 assures these assignments.

The HMBC spectrum of compound *cis* (3), with Δ_2 = 65 ms, shows seven $^4J_{C,H}$ correlations, of which only four of them take place through a W coupling path: H-10 with C_2, H-6 with C-4, H-8 with C-4 and OH with C-7. The other three correlations occur between a proton and the carbon located at the *para* position: H-6 with C-8a, H-7 with C-4a and H-8 with C-5. With the purpose of confirming that these cross-peaks are real and not artifacts, we performed duplicate experiments in a 400 MHz spectrometer. The HMBC spectrum for the cis compound **3** displays the same cross-peaks in both spectrometers. The 300 MHz HMBC spectrum with Δ_2 = 65 ms of compound *trans* **1** shows only four $^4J_{C,H}$ correlations: the hydroxyl proton with C-7, H-7 with C-4a, OH with C-4 and H-8 with C-4. However in the spectrum recorded at 400 MHz the correlation between OH and C-4 is not observed. However other four correlations were observed: H-6 with C-8a, H-8 with C-5, H-10 with C-2 and H-9 with C-4a. At present we have no explanation for these differences.

The 300 MHz spectra of both compounds, with a little increment in Δ_2 (130 ms), lead to observation of several additional $^4J_{C,H}$ correlations, (12 cross-peaks). Interestingly, a long-range correlation across five bonds between the methyl protons with C-10 is also observed. This is a very long-range correlation through a coupling path with a high degree of conformational freedom. As far as we know[10] this kind of long-range correlation has not been reported before.

In the 400 MHz HMBC spectrum of compound **1**, with Δ_2 = 130 ms, only the same correlations obtained using Δ_2 = 65 ms were observed. However the HMBC spectrum of compound **3** showed a $^4J_{C,H}$ correlation between the OH and C-4, but the cross-peaks due to H-7 with C-4a and H-8 with C-5 disappeared. In the spectra of both compounds, **1** and **3**, the $^5J_{C,H}$ correlations were not observed, but in the 400 MHz spectra of both compounds with Δ_2 = 195 ms the $^5J_{C,H}$ correlations between CH_3 and C-10 can be observed, beside eleven $^4J_{C,H}$ other correlations. In the 300 MHz HMBC spectrum (Δ_2 = 195 ms) of compound **1** thirteen $^4J_{C,H}$ correlations are shown, in addition to a $^5J_{C,H}$ correlation. For compound *cis* twelve $^4J_{C,H}$ correlations are observed, beside two $^5J_{C,H}$: CH_3 with C-10 and H-10 with C-4a.

The IMPEACH-MBC experiment (Δ_2 = 50-200 ms, *J* 2.5-10 Hz) of compound *trans* shows several although not all correlations observed in the series of HMBC experiments. This experiment shows the $^4J_{C,H}$ correlations between the aromatic protons with carbon atoms in the *para* positions: H-8 with C-4, H-10 with C-2 and H-9 with C-4, but is unable to show correlations of the ethyl chain with C_α. A CIGAR-HMBC (Δ_2 = 50-200 ms, *J* 2.5-10 Hz) of the same compound shows two additional $^5J_{C,H}$: CH_3 with C-10 and OH with C-8 , but several artifacts are obtained, mainly for H-2.

The ^{1}H spectra of compounds **2** and **4** display the known AB system for olefinic protons with coupling constants of 12.8 and 15.9 Hz for compounds *cis* (**4**) and *tran*s (**2**) respectively. For compound **2**, H-5 appears as broad doublet with coupling constant of 8.3 Hz, at δ 8.28, H-8 appears as broad doublet with *J* 8.3 Hz at δ 7.45. For compound **4**, H-5 appears at δ 8.26 as doublet of doublet, with coupling constants of 9.4 and 1.6 Hz, and H-8 appears at δ 7.49 as a broad doublet with J 8.5 Hz.

The conjugation of the carbonyl group shifts the aromatic protons H-5 and H-7 to low field and the conjugation of the heterocyclic oxygen atom shifts the H-6 and H-8 to high field. In this way was possible to discriminate between H-5 and H-8 or H-6 and H-7, together with the reported chemical shifts of chromone derivatives.

H-2 appears at 8.12 ppm and 9.13 ppm for molecules **2** and **4** respectively, in a similar way as compounds **1** and **3**. The standard HMBC spectrum (Δ_2 = 65 ms) of compound **2** displays only one $^4J_{C,H}$ correlation (H-8 with C-4), however the HMBC spectrum of compound **4** shows four $^4J_{C,H}$ correlations: H-8 with C-4, H-6 with C-8a, H-2 with C-10, and H-10 with C-2. Increments of Δ_2 allows to observe a bigger number of these $^4J_{C,H}$ as discussed previously for compounds **1** and **3**. Using Δ_2 = 130 ms or larger, the $^5J_{C,H}$ correlation between the methyl protons with C-10 is observed for both compounds, in a similar way as compounds **1** and **3**.

The HMBC spectrum of ethyl chrotonate shows the correlation between the methyl protons (δ 1.89) and C_α to carbonyl with Δ_2 = 65 ms, indicating that this very long-range coupling is a general characteristic for the unsaturated ester moiety.

Conclusions

From this study, an additional HMBC spectrum using a long Δ_2 delay (200 – 300 ms) is recommended in order to obtain valuable structural information through long range correlations ($^nJ_{C,H}$ n > 3).

An uncommon $^5J_{C,H}$ between the methyl protons and C_α to carbonyl, as well as several $^4J_{C,H}$ through no W coupling paths, were observed in these systems.

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References

1. Bax, A.; Summers, M.F.; *J. Am. Chem. Soc*. **1986**, *108*, 2093.
2. Bax, A.; Marion, D.; *J. Magn. Reson.* **1988**, *78*, 186.
3. Braun, S.; Kalinowski, H.-O.; Berger, S.; *150 and More Basic NMR Experiments*, Wiley-CCH: Weinheim, 1998.
4. Claridge, T.D.W.; *High-Resolution NMR Techniques in Organic Chemistry, Tetrahedron Organic Chemistry Series*, Pergamon: Oxford, 1999.
5. Reynolds, W.F.; Enríquez, R.G.; *J. Nat. Prod*. **2002**, *65*, 221.
6. Bax, A.; Subramanian, S.; *J. Magn. Reson*. **1986**, *67*, 565.
7. Bodenhausen,G.; Ruben, D.J.; *Chem. Phys. Lett*. **1980**, *69*, 185.
8. Parella, T.; *Magn. Reson. Chem.* **1998**, *36*, 467.
9. Williamson, D.S.; Smith, R.A.; Nagel, D.L.; Cohen, S.M.; *J. Magn. Reson.* **1989**, *82*, 605.
10. Araya-Maturana, R.; Delgado-Castro, T.; Cardona, W.; Weiss-López, B.E.; *Curr. Org. Chem*., **2001**, *5*, 89.
11. Martin, GE.; Hadden, CE.; Russell, D.J.; Kaluzny, B.D.; Guido. J.E.; Duholke, W.K.; Stiemsma, B.A.; Thamann, T.J.; Crouch. R.C.; Blinov, K.; Elyashberg. M.; Martirosian, E.R.; Molodtsov, S.G.; Williams. A.J.; Schiff, P.L.; *J. Het. Chem*. **2002**, *39*, 1241.
12. Araya-Maturana, R.; Delgado-Castro, T.; Cardona, W.; Jullian, C.; *Magn. Reson. Chem.* **2000**, *38*, 135.
13. Araya-Maturana, R.; Cassels, B.K.; Delgado-Castro, T.; Hurtado-Guzman, C.; Jullian, C.; *Magn. Reson. Chem.* **1999**, *37*, 312.
14. Furihata, K.; Seto, H.; *Tetrahedron Lett*. **1995**, *36*, 2817.
15. Furihata, K.; Seto, H.; *Tetrahedron Lett*. **1996**, *37*, 8901.
16. Furihata, K.; Seto, H.; *Tetrahedron Lett*. **1998**, *39*, 7337.
17. Wagner, R.; Berger, S.; *Magn. Reson. Chem.* **1998**, *36*, S44.
18. Hadden, C.E.; Martin, G.E.; Krishnamurthy, K.K.; *J. Magn. Reson.* **1999**, *140*, 274.
19. Hadden, C.E.; Martin, G.E.; Krishnamurthy, K.K.; *Magn. Reson Chem.* **2000**, *38*, 143.
20. Li, X.-C.; Elsohly, H.N.; Hufford, C.D.; Clark, A.M.; *Magn. Reson. Chem.* **1999**, *37*, 856.
21. Tapia, R.A.; Prieto, Y.; Pautet, F.; Fenet, B.; Fillion, H.; *Magn. Reson. Chem.* **2002**, *40*, 165.
22. Polly, R.; Taylor, P.R.; *J. Phys. Chem. A*. **1999**, *103*, 10343.
23. Bodwell, G.J.; Hawco, K.M.; daSilva, R.P.; *Synlett* **2003**, 179.
24. Nohara, A.; Umetani, T.; Sanno, Y.; *Tetrahedron* **1974**, *30*, 3553.
25. Araya-Maturana, R.; Heredia-Moya, J.; Pessoa-Mahana, H.; Weiss-López, B.; *Synthetic Commun*. **2003**, *33*, 3225.

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