THE USE OF LONG RANGE C-H COUPLING IN THE STRUCTURE DETERMINATION AND ^{13}C ASSIGNMENT OF PYRONES

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■ ABSTRACT: Fully coupled ^{13}C NMR spectra and selective long range heteronuclear C-H decoupling experiments were used in the structure elucidation and ^{13}C NMR assignment of some pyrones. It is show that the products of the reaction of malonyl dichloride with benzoyl acetone are the 5-acetyl-4-hydroxy-6-phenyl-2-pyrone (7), the isomeric 5-benzoyl-4-hydroxy-6-methyl-2-pyrone (8) and the new fused dipyrone 8-benzoyl-4-hydroxy-7-methylpyrano[3.4-e]pyran-2.5-dione (9). The technique was also used to determine the structure of the natural antibiotic lachnellulone (3) and to assign the ^{13}C NMR spectrum of the model compound 4.5-dihydro-3-carboethoxy-2-ethoxyfuran-4-one (4).

■ KEYWORDS: Pyrones; dipyrones; lachnellulone; ^{13}C NMR; long range coupling; selective decoupling.

Introduction

The structure elucidation and ^{13}C NMR assignment of organic compounds containing molecular cores with low hydrogen content is a difficult task that is often accomplished using long range two-dimensional heteronuclear correlation NMR techniques (2D-HETCOR).[1] When using this technique it is necessary to define an average long range coupling constant for the system in study in order to optimize polarization transfer for all the long range coupled carbons.[1] As it is often the case, for a given compound the long range coupling constants (J) vary from 1 to 15 Hz, being necessary to carry out two 2D-HETCOR experiments to detect all the long range correlations efficiently. Sometimes it is possible to obtain good correlations for such cases using a single J value of 9 Hz.[2] The 2D-HETCOR method does not provide the chemist with the values of J,[3] which are very useful to determine molecular conformations by calculating the C-H dihedral angles.[4] The selective long range

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heteronuclear technique first used by Takeuchi[5] provides not only the long range C-H correlations, but also the values of J. In this work, this very useful technique was used to determine the structure and to assign the ^{13}C NMR spectra of the pyrones 3, 4, 6, 7 and 8.

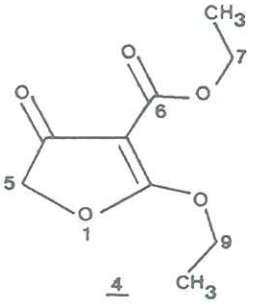
Results and discussion

Pyrones and polypyrones are an important class of compounds of natural[6,9] and synthetic[10,13] source. This kind of compound usually has molecular cores that contain few hydrogen atoms, thus making difficult their structure elucidation and their complete unambiguous ^{13}C NMR assignment. We have applied the gated decoupling-long range heteronuclear selective decoupling method to study this kind of compounds. To obtain clear multiplets for the coupled ^{13}C signals, the gated decoupled spectra were recorded and the data zero filled, followed by resolution enhancement by gaussian multiplication of the free induction decays before Fourier transformation. When the simple inspection of the ^{13}C coupled spectrum was not enough to solve the problem, a well tuned selective decoupled pulse was used to eliminate the desired long range coupling constant only. The correlation was established by comparison with the simple gated decoupled spectrum. The power of the decoupling pulse could be determined by trial an error, or more conveniently from the Paul-Grant equation.[14]

$$H_2 = \frac{J(\gamma_H^2 - \gamma_C^2)^{1/2}}{2\,\gamma_H\,\gamma_C} = (0.452 \text{ m Gauss/Hz})\ J$$

The first problem solved using this methodology was the structure of the natural antibiotic lachnellulone.[9] The one-dimensional homonuclear correlation of this natural product showed the existence of the spin systems corresponding to the fragments A and D, shown in the Scheme 1.[9] The spin system corresponding to fragment A contains a *geminal* pair of protons at δ 3.59 (1H,m,H3'a) and δ 3.26 (1H,m,H3'b), which are coupled to a second *geminal* pair at δ 2.37 (1H,m,H4'a) and δ 1.87 (1H,m,H4'b). This last pair is coupled to a low field one hydrogen multiplet at δ 4.94 (H5'), which in turn is coupled to two methylenic hydrogens at δ 1.95 (H6'a) and δ 1.75 (H6'b) that are part of an aliphatic chain. The spin system corresponding to the partial structure D contains just one proton doublet of doublets at δ 4.45 (H5) that is coupled to a hydroxylic hydrogen at δ 3.92 (1H,d, exchanges with D_2O) and which chemical shift is little affected by changes in the sample solvent or concentration, indicating that it makes part of an intramolecular hydrogen bridge. The proton at δ 4.45 is also coupled to another hydrogen geminal to oxygen at δ 4.57 (1H,ddd,H6), which is coupled to a methylene that makes part of an aliphatic chain. The noise decoupled ^{13}C NMR spectra showed the existence of a highly polarized carbon-carbon double bond in an unsaturated fragment corresponding to fragment B in agreement with the structure of lachnelluloic acid,[8] a compound isolated from the same microorganism than lachnellulone and that contains a 5,6-dihydro-4-hydroxy-2-pyrone ring. In consequence, the joining of the fragments A, B and D could lead preferentially to structure 2 (obtained from joining a2 with b4, a1 with b1, d1 with b2 and d2 with b3) or 3 (obtained from the joining of a1 with b2, a2 with b4, d1 with b1 and d2 with b3). The ^{13}C NMR data could also correspond to the uncommon fragment C, which would lead only to structure 1, a structure that contains an extremely polarized carbon-carbon double bond, with one of the carbons linked to two oxygens and the other to two carbonyl carbons. There are no reports of the ^{13}C NMR chemical shifts for such carbons in the literature. In order to have comparative data the model compound 4[15] was prepared and its ^{13}C NMR spectra assigned. In this last compound it was necessary to distinguish without ambiguities between the carbons C2, C4 and C6. The coupled ^{13}C NMR spectrum was easily distinguished between the C4 and the other two carbons. The C2 appeared as a clear quintet (J = 3.2 Hz) at 183.8 ppm, C4 and C6 appearing as clear triplets were distinguished by selective low power (7 Db) decoupling of the methylene hydrogens, being assigned at 190.0 and 160.3 ppm, respectively. The chemical shift of the high electronic density carbon in the polarized double bond (C3) was easily assigned to the low intensity singlet at 88.9 ppm. The complete and unambiguous ^{13}C NMR assignments for 4 are shown in Table 1. The chemical shifts of the C2 and C3 carbons on the model compound 4 suggest that 2 and 3 are the more likely structures for lachnellulone. The distinction between the three possibilities was carried out using gated decoupled and selective long range decoupled spectra of lachnellulone. The results are summarized in Table 2. It can be observed that the lactone carbonyl carbon in lachnellulone gives origin to a doublet at 165.3 ppm (J = 8.0 Hz), which correlates to H6 at δ 4.57. If we consider lachnellulone to be formed from fragments A, B and D, the latter result indicates that the connection points d2 and b3 are linked together. Decoupling of the H5' signal (δ 4.45) leads to the modification of the multiplicity of the carbon signal at 198.0 ppm (ketone carbonyl carbon) from a multiplet (m') to a simpler multiplet (m'), and therefore a1 and b1 are linked together. Since lachnellulone contains only five oxygen atoms and no enolic hydroxyl group ($FeCl_3$ test negative) b4 and a2 are also linked. At the same time, it is important to notice that both, H5'(δ 4.98) and H3' (δ3.32) correlate to the same carbon, the enol carbon at δ191.1. Of the two possible structures obtained from the junction of fragments A, B and D, only 3 explain all the obtained results and the existence of an intramolecular hydrogen bond in lachnellulone. If we consider lachnellulone to be formed from the junction of the fragments A, C and D, the only possible structure is 1, in which case both hydrogens H6 (δ4.57) and H5' (δ 4.98) would correlate to the same sp^2 carbon (the

doubly oxygenated carbon), which would show its signal as a triplet or doublet of doublets. Since this is not observed, this possibility is also ruled out, and the structure of the natural product must be 3. This conclusion have been confirmed by the X-rays diffraction pattern analysis of a derivate of the natural product.[9]



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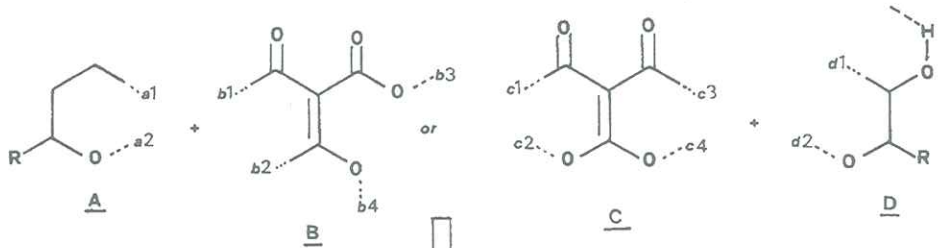
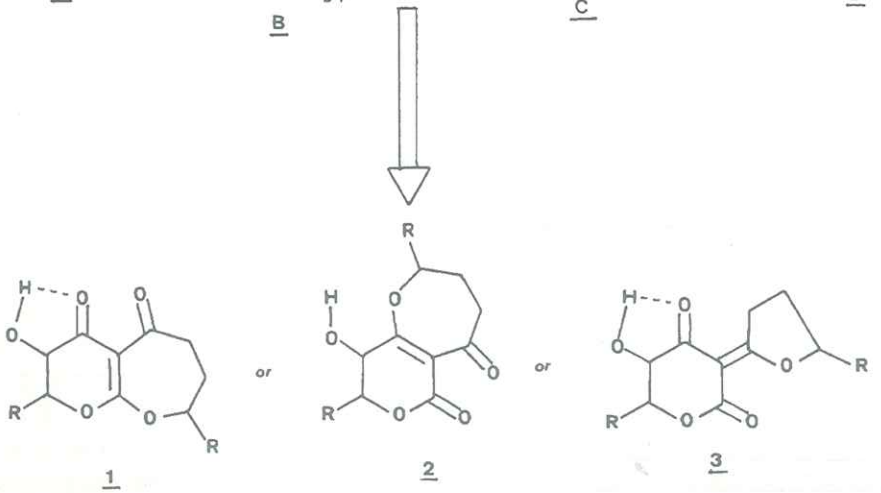
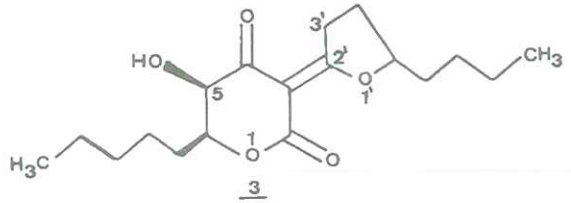


A + B or C + D



1 or 2 or 3



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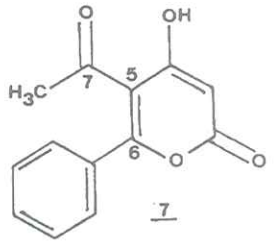

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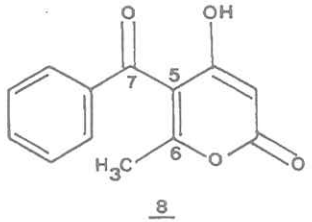

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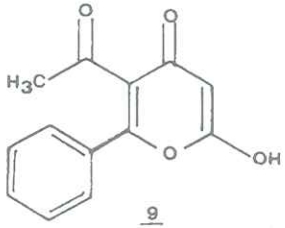

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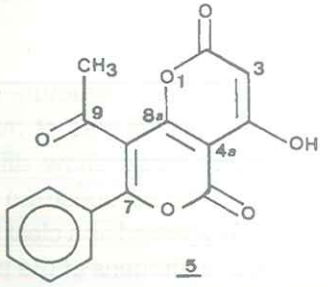

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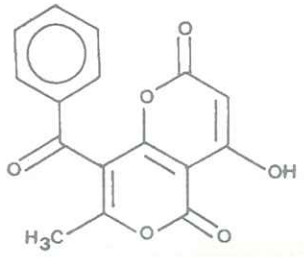

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Table 1 – ^{13}C NMR assignment and selective long range heteronuclear decouplings of 4

Carbon No.	Multiplicity and δ (ppm)	Multiplicity on decoupling at		
		δ 4.70 (H5)	δ 4.60 (H9)	δ 4.20 (H7)
2	183.8 (m, ~3.2 Hz)	t	t	quintet
3	88.9 (broad s)	s	broad s	s
4	190.7 (t, 3.5 Hz)	s	t	t
5	75.4 (t, 152 Hz)	t	t	t
6	161.4 (t, 3.5 Hz)	t	t	s
7	58.9 (qt, 4.5 Hz)*	qt	qt	qt
8	13.1 (tq, 2.5 Hz)*	tq	tq	q
9	67.9 (qt, 4.5 Hz)	qt	qt	qt
10	13.0 (tq, 2.7 Hz)	tq	q	tq

* Only the range coupling constant is given.

Table 2 – ^{13}C NMR assignment and selective long range heteronuclear decoupling of the sp_2 of lachnellulone*

Carbon No.	Multiplicity and δ (ppm)	Multiplicity on decoupling at δ			
		4.98(H5')	4.57(H6)	4.45(H5)	3.32(H3)
2	165.3 (d, 8.0 Hz)	d	s	d	d
3	100.2 (s)	s	s	s	s
4	191.1 (dt, 10, 2.5 Hz)	dd	dt	dt	dd
2'	198.0 (m)	m	m'	m	m

* For a complete ^{13}C and ^{1}H NMR assignment of 3, see reference 9.

In a similar problem, this technique was used to confirm the structure of the product of the condensation of benzoyl acetone with two equivalents of malonyl dichloride.[16] The two possible isomeric products, 5 and 6, would show different coupling patterns for C7 and C9. In fact, when the coupled ^{13}C NMR spectrum of the only reaction product was recorded, it was observed that C9 appeared as a clear triplet at δ 189.6 due to coupling of this carbon with the two *ortho* hydrogens of the phenyl group, thus indicating that the structure of the compound was 6 and not 5 (see Table 3). This result was confirmed by the observed multiplicity of C7, which appeared as a clear quartet (δ 164.7), and by selective decoupling of the methyl hydrogens leading to a singlet. The same procedure was applied to the pyrones 7 and 8, obtained by condensation of benzoyl acetone with one equivalent of malonyl dichloride under controlled conditions.[16,18] As shown in Table 4, the multiplicities of C7 and C6 in the two compounds were inverted. In 7 C6 appeared as a triplet at δ 167.7 and C7 as a quartet at δ 198.83. For 8 C6 is a quartet at δ 168.0 and C7 a triplet at δ 191.9. This result confirms the proposition of Ziegler & Kappe[17] that the two products of the reaction of benzoyl acetone with malonyl dichloride are the pyrones 7 and 8, and not the pyrones 7 and 9, as it was first believed by Butt & Elvidge.[18]

Table 3 – ^{13}C NMR assignment and selective long range heteronuclear decoupling of 6

Carbon No.	Multiplicity and δ (ppm)	Multiplicity on decoupling at δ	
		8.10 – 7.50 (arom. H)	2.20 (–Me)
2	156.4 (d, 1.6 Hz)	d	d
3	89.0 (d)	d	d
4	167.8 (m)	m	m
4a	96.8 (broad s)	broad s	broad s
5	159.1 (d, 3.4 Hz)	d	d
7	164.7 (q, 6.6 Hz)	q	s
8	111.5 (broad s)	broad s	s
8a	164.6 (s)	s	s
9	189,6 (t, 4.3 Hz)	s	t
–Me	18.63 (q)	q	q
1'	(m)	m	m
2', 6'	(m)	m	m
3', 5'	(m)	m	m
4'	(m)	m	m

* Only the long range coupling constants are given.

Table 4 – Chemical shifts and multiplicities of carbons C5, C6 and C7 in compounds 7 and 8

Carbon No.	Compound 7 δ (ppm) and multiplicity	Compound 8 δ (ppm) and multiplicity
5	116.5 (s)	113.5 (s)
6	167.6 (t)	168.0 (q)
7	198.8 (q)	191.9 (t)

Conclusion

These practical examples demonstrate the simplicity, importance and practical value of the application of coupled ^{13}C NMR spectra to determine the structure and the ^{13}C NMR assignment of low hydrogen content molecules, with the characteristic that this kind of experiences can be easily carried out in NMR spectrometers in which 2D spectra are not practical.

Materials and methods

The spectra of compounds 5 to 8 were recorded in deuterated dimenthyl sulfoxide (DMSO-d_6) in a Varian VXR-300 spectrometer (CENPES/PETROBRAS), and the spectra of compounds 3 and 4 were recorded in deuterated chloroform ($CDCl_3$) in a Bruker WH-200 spectrometer (Chemistry Department, University of Alberta). All the spectra were recorded in dual ^{13}C ^{1}H 5 mm probes, using samples with a 0.5 M concentration and tetramethyl silane (TMS) as internal reference. In all cases, the time delays for the gated decoupled spectra were 0.5 and 5 milliseconds, with a 5 second relaxation delay between pulses. The decoupler power for the long range decoupling experiments varied between 7 and 5 Db. The zero filling used was obtained by increasing the data points from 16K to 32K (50 MHz) or from 32K to 64K (75 MHz). Resolution enhancement was accomplished using a negative line broadening (LB) between -11.5 and -13.0, and a Gaussian Multiplier controlled by a Gaussian Broadening function (GB) varying from 0 (no enhancement) to 1 (maximum enhancement). For each case, the degree of enhancement was optimized as a function of the desired resolution and the signal to noise ratio.

Compound 4 was prepared according to the literature procedure.[15] M.p. 90-91 0C. Ir.($CHCl_3$ film)ν_{max} 1700, 1590, 1485 cm^{-1} Uv.(MeOH, 0.05 g/L) λ_{max} (ε) 222 (12500) and 248 (17800) nm ^{1}H NMR (80 MHz, $CDCl_3$) δ 4.70 (s,2H,H5), 4.60 (q,2H,H9), 4.20 (q,2h,H7), 1.48 (t,3H,H10) and 1.20 (t,3H,H9) ppm.

The dipyrone 6 was prepared by the reaction of two equivalents of malonyl dichloride with benzoyl acetone in refluxing trifluoroacetic acid (TFA) in 40% yield. M.p. 210-212 0C. Iv. (KBr) ν_{max} 3416, 1747, 1708, 1668, 1639 and 1595 cm^{-1} Uv.(MeOH, 0.05 g/L) λ_{max} (ε) 258 (20500) and 330 (4700) nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.10-7.50 (m,5H, arom.H), 5.45 (s,1H,H3) and 2.20 (s,3H,-Me) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ 189.6, 167.8, 164.7, 164.6, 159.1, 156.4, 136.1, 134.8, 129.6, 129.2, 111.5, 96.8, 89.0 and 18.6 ppm. Mass spectrum e/z (%) 298 (M^+, 100, $C_{16}H_{10}O_6$).

Pyrones 8 and 9 were prepared accordind to the procedure of Elvidge and col.,[18] and separated using flash chromatography on a silica gel column eluted with chloroform hexane mixtures. Data for 7: m.p. 160-161 0C. Ir.(KBr) ν_{max} 3422, 1709, 1682, 1619, 1600 and 1579 cm^{-1} Uv (Dioxane 0.05g/L) λ_{max} (ε) 260 (5400) and 310 (2800) nm. ^{1}H NMR (300 MHz. DMSO-d_6) δ 7.78 (broad s, 5H,arom.H), 5.70 (s,1H,H3) and 2.44 (s,3H,-Me)ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ 198.9, 167.6, 161.6, 159.3, 131.5, 130.9, 128.7, 128.0, 116.5, 89.6 and 32.12 ppm. Mass spectrum e/z (%) 230 (M^+, 20, $C_{13}H_{10}O_4$). Data for 8: M.p. 215-217 0C. Ir.(KBr)ν_{max} 3850, 1768, 1728, 1671, 1643, 1618 and 1580 cm^{-1} Uv (Dioxane, 0.05 g/L) λ_{max} (ε) 252 (18000) and 282 (8500) nm. ^{1}H NMR (75 MHz, DMSO-d_6) δ 8.28-7.60 (m,5H,arom,H), 5.62 (s,1H,H3) and 2.17 (s,3H,-Me) ppm ^{13}C NMR (75 MHz, DMSO-d_6) δ 191.9, 168.0, 162.4, 162.0, 136.8, 134.0, 131.1, 128.9, 125.4, 113.5, 98.3, 89.6 and 17.95 ppm. Mass spectrum e/z (%) 230 (M^+, 44, $C_{13}H_{10}O_4$).

For the espectra of 3, an original sample of lachnellulone was used.[9] (For complete spectral data of 3 see reference 9.)

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FIGUEROA VILLAR, J. D., OLIVEIRA, A. M. de. O uso de acoplamento C-H a longa distância na determinação estrutural e assinalamento de RMN de ^{13}C. *Ecl. Quím.*, São Paulo, v. 17, p. 7-16, 1992.

- *RESUMO: Espectros totalmente acoplados de RMN de ^{13}C e desacoplamentos seletivos heteronucleares C-H foram usados na elucidação estrutural e assinalamento de RMN de ^{13}C de algumas pironas. Mostram-se que os produtos de reação entre o cloreto de malonila e a benzoil acetona são a pirona 5-acetil-4-hidroxi-6-fenil-2-pirona (7), a pirona isomérica 5-benzoil-4-hidroxi-6-metil-2-pirona (8) e a nova dipirona 8-benzoil-4-hidroxi-7-metilpirano[3,4-e]piran-4-ona (9). Esta técnica também foi usada na determinação estrutural do antibiótico natural lachnelulona (3) e no assinalamento do espectro de ^{13}C do composto modelo 4,5-diidro-3-carboetoxi-2-etoxifuran-4-ona (4).*

- *UNITERMOS: Pironas; dipironas; lachnelulona; RMN de ^{13}C; acoplamento heteronuclear; desacoplamento seletivo.*

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