

CARBON-13 MAGNETIC RESONANCE CHEMICAL SHIFT ADDITIVITY RELATIONSHIPS OF CLINICALLY USED FUROCOUMARINS AND FUROCHROMONES

MAHMOUD M.A. HASSAN, M. UPPAL ZUBAIR AND M.A. LOUTFY*

Department of Pharmaceutical Chemistry, King Saud University, Riyadh, Saudi Arabia

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The natural abundance carbon-13 nuclear magnetic resonance spectra of various clinically used furocoumarins and furochromones have been studied. The assignment of carbon chemical shift values were based on the theory of chemical shift, additivity rules, SFORD spectra and model compounds.

Key words: C-13, magnetic resonance, Furocoumarins, Furochromones

Introduction

The furocoumarins, methoxsalen (III), trioxsalen (IV) and bergapten (V) are widely used in the treatment of leukoderma. The furochromones, khellin (VIII) and visnagin (VII) are well known as potent vasodilators. Although their physico-chemical and physiological properties have been thoroughly investigated [1-8], yet only few reports on their ¹³C-NMR studies have been published [9-14]. The present work deals with further studies on the ¹³C-NMR characteristics of these compounds and some related derivatives. These are psoralen (II) and dimethoxypsoralen (isopimpinellin) (VI). Model compounds are also designed in an attempt to derive chemical shift additivity values so that carbon chemical shifts of related structures can be assigned unambiguously. Furthermore, to compare carbon chemical shifts of the active sites of furocoumarins and their relative photosensitizing activity*. This might be helpful in studying the structure-activity relationship in this series as well as in designing more active derivatives.

Experimental

The proton noise decoupled and single-frequency off-resonance decoupled (SFORD) ¹³C-NMR spectra were obtained at 50.30 MHz on a Varian XL-200-200 MHz Fourier transform NMR spectrometer using a broad band 10 mm probe. The samples were run at concentrations ca 1-2 M in deuterated chloroform with tetramethylsilane as an internal reference standard. Spectra were recorded with 8K data points at a probe temperature of 23°. The chemical shifts were measured at 5 KHz spectral width. Typical pulse widths were 10 μs, and the delay time between pulses was fixed at 2.0 sec.

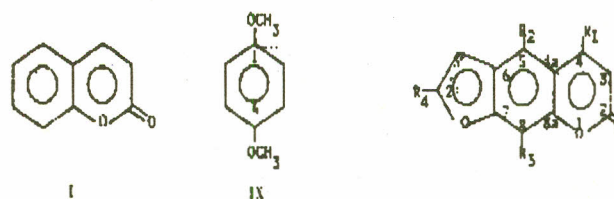
Chemicals. Methoxsalen, bergapten and visnagin were obtained from Memphis Company, Cairo, Egypt, trioxsalen

and khellin are commercially available and were used without further purification.

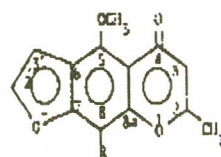
Results and Discussion

Previously, the carbon-proton coupling constants and the effect of the substituents were used for the carbon chemical shift assignments in furocoumarins and furochromones [11]. In the present study, however, carbon chemical shift assignments are mainly based on chemical shift values derived from model compounds, the single-frequency off-resonance decoupled spectra (SFORD) as well as the effect of substituents compared to some model compounds. The multiplicities generated in the SFORD spectra enabled distinction between methyl, methylene, methine and quaternary carbon resonances.

Table 1 shows the carbon chemical shift of furocoumarin



	R ₁	R ₂	R ₃	R ₄
(II) Psoralen	H	H	H	H
(III) Methoxsalen	H	H	OCH ₃	H
(IV) Trioxsalen	CH ₃	H	CH ₃	CH ₃
(V) Bergapten	H	OCH ₃	H	H
(VI) Dimethoxy-psoralen	H	OCH ₃	OCH ₃	H



(VII) Visnagin	H	H		
(VIII) Khellin	OCH ₃			

* Central Laboratory and blood Bank, Riyadh, Saudi Arabia.

† This work will be published later.

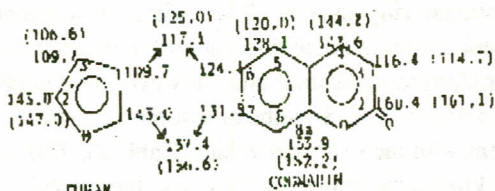
TABLE 1. ¹³C-NMR CHEMICAL SHIFT VALUES OF FUROCUMARINS AND FUROCHROMONES.*

Compd. No.	Chemical Shift ppm											Others					
	C2	C3	C4	C4a	C5	C6	C7	C8	C8a	C2'	C3'	8-OCH ₃	4-CH ₃	8-CH ₃	2'-CH ₃	5-OCH ₃	2-CH ₃
I	160.4	116	4143.6	118.8	128.1	124.4	131.8	116.4	153.9								
II	161.1	114.7	144.2	115.6	120.0	125.0	156.6	99.9	152.2	147.0	106.5						
III	160.4	114.5	144.5	116.4	113.0	126.2	147.6	132.6	142.9	146.7	106.8	61.2	—	—	—	—	—
IV	161.5	112.7	155.4	109.2	112.1	125.4	153.2	116.0	148.9	157.3	102.6	—	14.2	8.5	19.2	—	—
V	160.3	112.8	139.4	106.7	149.6	113.0	158.5	94.0	152.7	145.0	105.3	—	—	—	—	60.3	—
VI	160.5	112.8	139.4	107.7	144.4	114.9	149.9	128.3	143.7	145.3	105.3	61.7	—	—	—	60.9	—
VII	163.9	110.8	178.2	112.5	157.8	117.1	153.6	95.2	156.0	145.2	105.3	—	—	—	—	61.8	20.0
VIII	164.0	110.6	178.2	113.8	147.4	119.5	148.9	130.0	147.2	145.5	105.3	62.4	—	—	—	61.5	20.1

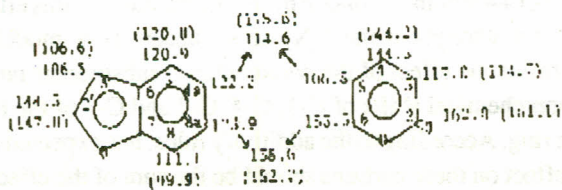
*Chemical shift values in ppm relative to TMS, solvent CDCl₃.

compounds (II-VI), furochromones (VII and VIII) and coumarin (I).

Furocoumarins. In complex molecules of natural products, it is generally advisable to use model compounds of single structures for assigning carbon chemical shifts with great accuracy. In our work on furocoumarins, two main models have been designed to obtain the parent compound psoralen. These are models A and B. Model B psoralen can be obtained by fusing 2-pyrone [11] and benzofuran [15], while fusion of coumarin and furan [9-16] afforded model A psoralen. The carbon chemical shift values derived for model A and B, calculated and found values for psoralen and other furocoumarins are listed in Table 2. It is evident from Table 2 that the overall carbon chemical shift differences between



PSORALEN (MODEL A)



PSORALEN (MODEL B)

the found for psoralen and that calculated from model A and B are -1.9 and +1.7, respectively. Therefore, the two models can be equally applied for carbon chemical shifts prediction in furocoumarins.

However, it should be pointed out that in fusing the C-5 (106.8 ppm) of the 2-pyrone ring system (Model B) with the C-5 (121.2 ppm) of the benzofuran ring system, a carbon hybrid is produced C-4a (115.6 ppm). The net result of this fusion is an increase of electron density of the C-5 of the benzofuran and a decrease of the electron density of the C-5 of the 2-pyrone. This amounts to a downfield shift of the C-5 of the 2-pyrone ring system by +8.8 ppm. Also, fusion of the C-6 (153.3 ppm) of the 2-pyrone ring system with C-6' (123.9 ppm) of the benzofuran ring system, produced a carbon hybrid C-8a (152.2 ppm) with a downfield shift of +28.3 ppm. The effect on C-5 of the 2-pyrone ring might be due to the extended conjugation caused by the fusion of the benzene nucleus of the benzofuran ring. These two values could be utilized in elucidation of similar ring structures via their carbon chemical shifts.

The predicted chemical shift values for methoxypsoralen (except for C-7 and C-8a) are in agreement with the found values. The two carbons C-7 and C-8a have suffered a downfield shift of 5.6 and 5.4 ppm, respectively than the predicted values. So, the effect of a methoxy substituent in a furocoumarin ring system will have a shielding effect on ortho carbons of -9.2 ppm, (CF. benzene -14.6 ppm). However, in case of 5-methoxypsoralen (bergapten) the predicted chemical shift values due to the effect of 5-methoxy substituent are in agreement with the calculated values except for the C-8,

TABLE 2. CALCULATED, FOUND AND DIFFERENCE IN CARBON CHEMICAL SHIFT VALUES OF PSORALENS.

Compound		Chemical shift in δ ppm													Accumulative difference
		C2	C3	C4	C4a	C5	C6	C7	C8	C8a	C2'	C3'	5-OCH ₃	8-OCH ₃	
II Psoralen	Found	161.1	114.7	144.2	115.6	120.0	125.0	156.6	99.9	152.2	147.0	106.6			
	Model A	160.4	116.4	143.6	118.8	128.1	117.1	137.4	116.4	153.9	143.0	109.7			
	Diff.*	+0.7	-1.7	+0.6	-3.2	-8.1	+7.9	+19.2	-16.5	-1.7	+4.0	-3.1			
	Model B	162.0	117.0	144.3	114.6	120.9	127.2	154.7	111.1	138.6	144.5	106.5			
	Diff.	-0.9	-2.0	-0.1	+1.0	-0.9	-2.2	+1.9	-11.2	+14.1	+2.5	+0.1			
III Methoxy- salen	Found	160.4	114.5	144.5	116.4	113.0	126.2	147.6	132.6	142.9	146.7	106.8	—	61.2	
	Calc.**	—	—	—	116.4	112.0	125.8	142.0	131.4	137.6	—	—	—	—	
	Diff.	—	—	—	—	+1	+0.4	+5.6	+1.2	+5.4	—	—	—	—	
IV Trioxsalen	Found	161.5	112.7	155.4	109.2	112.1	125.4	153.2	116.0	148.9	157.3	102.6	14.2	8.5	19.2
	Calc.	—	115.5	153.5	116.4	117.1	128.0	157.4	109.2	153.0	156.3	107.4	21.2	21.2	21.2
	Diff.	—	-2.8	+1.9	-7.2	-5.0	-2.6	-4.2	+6.8	-4.1	+1.0	-4.8	-7.0	12.7	-2.0
V Bergapten	Found	160.3	112.8	139.4	106.7	149.6	113.0	158.5	94.0	152.7	145.0	105.3	60.3		
	Calc.	—	—	—	106.5	150.2	113.9	157.4	91.9	153.0	—	—	—		
	Diff.	—	—	—	+0.2	-0.6	-0.9	+1.1	+2.1	-0.3	—	—	—		
VI Isopim- pinellin	Found	160.5	112.8	139.4	107.7	144.4	114.9	149.9	128.3	143.7	145.3	105.3	60.9	61.7	
	Diff.	—	—	—	+5.9	+0.9	+3.7	+7.1	+4.9	+5.3	—	—	—	—	

*Diff. = Difference **Calc. = Calculated

which suffered the downfield shift of 2.1 ppm. Thus, the overall effect of a methoxyl substituent in the 5- position is about -6.0 ppm, instead of -8.0 ppm observed in case of benzene. However, this effect has not been observed on C-5 of methoxsalen. This shows that electron density at position 5 and 8 in psoralen molecule is of great importance in mediating its photo-sensitizing activity. The methyl substituent of the methoxyl group in both, bergapten and methoxsalen, has a chemical shift value of 61.2 ppm which is more deshielded by 6.5 ppm than the methoxyd group of anisole [17].

In case of 2',4,8-trimethylpsoralen, the predicted chemical shift values showed significant differences from the observed values, which range from -7.2 to +1.9 ppm. Derivation of any general additivity rule was thus not possible. The chemical shift values of the methyl substituents were also very different. The 2'-methyl was similar to that of toluene while the 4- and 8- methyls suffered a shielding effect of 7 and 12.7 ppm, respectively. This difference in electron density may be attributed to the mesomeric effect of the furocoumarin ring system.

Furochromones. The predicted chemical shift values were calculated on the basis of values obtained in case of

chromone and psoralens, considering the effect of the methyl substituent at C-5 in visnagin and C-5, C-8 positions in case of khellin. The data derived for visnagin were in good agreement with the observed values while those of khellin were also in agreement with the observed values, except for C-8 of the furochromone ring system. The effect of the methoxyl substituent on the chemical shift value of this carbon is less by 11.6 ppm than the predicated value. However, the data obtained for 5- methoxy and 8-methoxy psoralens were in good agreement with the expected values (Table 2). This unusual effect in khellin molecule may be due to the presence of two methoxyl groups in the system (Table- 3).

This effect has also been noticed for the 5,8- dimethoxypsoralen (isopimpinellin) but it is a deshielding effect of +4.9 ppm. To obtain more information for this effect, 1,4- dimethoxybenzene (IX) was chosen as a model to determine the effect of the two methoxyl substituents on the carbon chemical shifts of C-1, C-4, C-2 and C-3 of the benzene ring. According to the additivity rules, it is expected that the effect on these carbons should be the sum of the effect of the two methoxyl substituents. It turns out however that the found carbon chemical shifts for these carbons are more than

TABLE 3. CALCULATED, FOUND AND DIFFERENCE IN CARBON CHEMICAL SHIFT VALUES OF VISNAGIN AND KHELLIN.

Compound	Chemical Shift δ ppm											
	C2	C3	C4	C4a	C5	C6	C7	C8	C8a	C-2'	C-3'	
Visnagin	Found	163.9	110.8	178.8	112.5	157.8	117.1	153.6	95.2	156.0	145.2	105.3
	Calc.**	164.3	113.5	177.1	111.5	156.3	111.1	157.6	110.2	157.2	147.0	106.6
	Diff.*	-0.4	-2.7	+1.1	+1.0	+1.5	+6.0	-4.0	-15.0	-1.2	-1.8	-1.3
Khellin	Found	164.0	110.6	178.2	113.8	147.4	119.5	148.9	130.0	147.2	145.5	105.3
	Calc.	164.3	113.5	177.1	111.5	148.6	112.1	143.2	141.6	142.8	147.0	106.6
	Diff	-0.3	-2.9	+1.1	+2.3	-0.8	+7.4	+5.7	-11.6	+4.4	-1.5	-1.3

Diff. = Difference **Calc. = Calculated.

that predicted by +4.28 ppm for C-1, C-4 and +1.8 ppm for C-2, C-3, i.e., the four carbons have been deshielded (Table 4).

TABLE 4. CARBON CHEMICAL SHIFT VALUES FOR 1,4-DIMETHOXY BENZENE

	Chemical Shift δ ppm	
	C-1, C-4	C-2, C-3
Calculated	150.00	113.2
Found	154.28	115.0
Differences	+4.28	+1.8

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