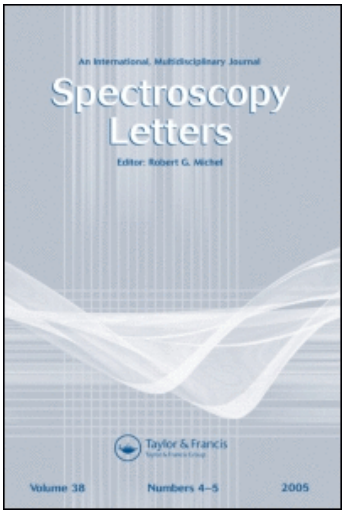

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Two-Dimensional NMR Spectroscopy in Complete Analysis of the ^{1}H and ^{13}C NMR Spectra of the Ionophore A 23187 and its Calcium Salt

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TWO-DIMENSIONAL NMR SPECTROSCOPY II

COMPLETE ANALYSIS OF THE ^{1}H and ^{13}C NMR SPECTRA OF THE IONOPHORE A 23187 AND ITS CALCIUM SALT.

KEY WORDS : A 23187. Calcium Ionophore

2D ^{1}H-^{1}H homonuclear correlation.

2D ^{1}H homonuclear J-resolved.

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ABSTRACT

The complete assignment of the proton carbon spectra of the A 23187 Ionophore and its calcium salt is presented.

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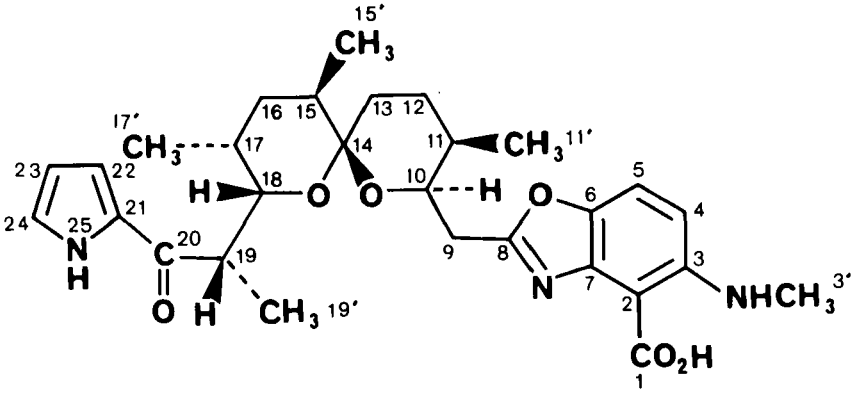

A

FIG. 1 : Ionophore A 23187 free acid (A).

The interpretation of the spectra was achieved from concerted application of two-dimensional homonuclear correlation and J-resolved and selective decoupling experiments for ^{1}H and ^{13}C measurements respectively.

INTRODUCTION

A 23187 (A) is a carboxylic ionophorous antibiotic which provides a powerful experiment tool for investigating the role of calcium transport in cell physiological processes (1). Therefore, complete unambiguous ^{1}H and ^{13}C chemical shift assignment of this compound is essential for the NMR studies of its complexing ability with various

cations or drugs, such as calcium antagonists widely used in cardiovascular diseases (2).

^1H and ^{13}C chemical shifts for A 23187 and its calcium complex (B) are partially available in the literature (3-7). Unfortunately, since marked overlap of resonances occurs in the ^1H NMR spectra, a definite and complete assignment was not possible even at high-field (5). Moreover no analysis of the aliphatic region of ^{13}C spectra had been performed. Recent advances in NMR spectroscopy (8) have opened up new possibilities for total assignment of complex ^1H and ^{13}C spectra. In this paper, we report complete analysis of the ^1H NMR spectra of the ionophore A 23187 and its calcium salt by the means of two-dimensional ^1H-^1H correlation (9) and J-resolved (10) spectroscopy. The ^{13}C chemical shifts assignment follows then from selective decoupling experiments of various previously reported protons.

EXPERIMENTAL

A 23187 and its calcium salt were purchased from Sigma. All spectra were recorded on Bruker AM-200 Spectrometer (Centre Interuniversitaire de RMN de Marseille) in $CDCl_3$ solutions. Tetramethylsilane was used as internal standard for both the ^1H and ^{13}C measurements. Typical experimental

conditions for the recording of ^1H selective decoupled ^{13}C spectra were as follows : spectral width, 12 KHz ; pulse width, 8 µs ; acquisition time, 0.688 s ; number of transients, 5000-10000 ; number of data points, 16 K ; decoupling, CW mode ; decoupling power, 20-30 H.

^{13}C resonance multiplicities were established via the acquisition of DEPT (11) spectra obtained for proton pulses P_θ = 90° (CH only) and 135° (CH and CH_3 differentiated from CH_2). For the DEPT sequence, the width of a ^{13}C 90° pulse was 13 µs, the width of a ^1H 90° pulse was 29 µs and the $(2J)^{-1}$ delay was set equal to 3.7 ms.

The homonuclear ^1H-^1H chemical shift correlated two-dimensional diagrams were obtained using COSY-45 pulse sequence. The spectral widths were F_2 = 2118 Hz and F_1 = ± 1059 Hz, allowing a digital resolution of 2.06hz point. The spectra were collected as 2048 x 1024 blocks of data and were processed using sinusoïdal multiplication in each dimension followed by symmetrization of the final data matrix. Other parameters were as follows : number of increments in t_1 : 512 ; scans : 32 ; phase cycling : 16 ; relaxation delay : 2 s ; pulse width 4,3 µs.

The basic pulse sequence (10) was used for the two-dimensional homonuclear proton J-resolved diagram. The F_2

spectral width was 1750 Hz and F_1 was ± 62 Hz. A 16-phase cycling with 16 scans and 64 increments, followed by zero filling and weighting with sine bell functions in both dimensions gave a matrix of 2 K x 128 data points, providing a digital resolution of 1.71 Hz in F_2 and 0.97 Hz in F_1 ; the recycle delay was 2 s.

RESULTS AND DISCUSSION

^{1}H NMR Spectral analysis

^{1}H spectra assignment of compounds A and B is for the most part straightforward with the exception of the upfield region which contains the four methyl signals and the most methylene and methine protons of spiroketal backbone. The analysis of aromatic and central region protons can be done directly from the one-dimensional ^{1}H spectra ; our results (Table 1) are in good agreement with those previously reported (3-7).

Since the remaining resonances are highly crowded, their assignments can be made through concerted use of two-dimensional NMR spectroscopy. On the one hand chemical shift information is provided by 2D-J technique, and on the other hand, the establishement of the proton-proton connectivities is easily available from homonuclear correlation since this

experiment allows correlation of the coupled protons even if their signals overlap with others.

For the free acid form, our results agree well with those determined by homo-INDOR and double resonance experiments (5), with the exception of the three methyl resonances of the spiroketal backbone. Since the detection of weak long-range couplings in the COSY spectrum indicated a trans diaxial disposition between the C-CH_3 and C-H bonds (12), 2D results reversed the previously reported (5) assignment and configurations of C-11', and C-15' and C-17 (Table 1).

In the case of compound B, 2D measurements also led to a fully assigned ^1H NMR spectrum. Unfortunately, due to the marked overlapping of cross correlation peaks, no direct conformational information could be obtained from the contour plot of the COSY spectrum.

^{13}C NMR spectral analysis

Our results allow inambiguous ^{13}C spectra assignment. The relatively small quantity of material available precluded the use of the heteronuclear ^{13}C-^1H shift correlation technique. Hence, information about the proton-carbon connectivities had to be obtained from selective decoupling experiments. These results are reported in

Table 1 - ^1H Chemical shifts of A 23187[a]

Atoms	Free acid (A)	Calcium salt (B)
4	6.65	6.71
5	7.59	7.46
9a	3.08	2.65
9b	2.93	2.50
10	4.28	3.80
11	1.47	1.50
12a	1.39 ax.	0.88[b]
12b	1.09 eq.	0.78[b]
13a	1.04 ax.	0.94[c]
13b	1.74 eq.	1.59[c]
15	1.63	1.45
16a	1.26 ax.	1.07[d]
16b	1.66 eq.	1.42[d]
17	1.69	1.23
18	3.70	2.61
19	3.21	3.06
22	6.93	7.02
23	6.25	6.27
24	7.06	7.56
3'	2.98	2.97
11'	0.86 ax.	0.70
15'	0.87 ax.	0.80
17'	0.97 eq.	0.79
19'	0.92	0.73
NH-3	8.09	9.19
NH-25	9.70	13.98

[a] In ppm from TMS.

[b,c,d] Assignments may be reversed.

Table 2 - ^{13}C Chemical shifts of A23187[a]

Atoms	Group[b]	Free acid (A)	Calcium salt (B)	Δ[c]
1	C	166.36	171.87	5.51
2	C	98.50	106.57	8.07
3	C	151.16	151.40	0.24
4	CH	108.54	108.71	0.17
5	CH	116.82	130.28	13.46
6	C	141.95[d]	141.92[d]	-0.03
7	C	141.12[d]	140.96[d]	-0.16
8	C	168.27	168.62	0.35
9	CH_2	32.52	32.27	-0.25
10	CH	68.62	71.23	2.61
11	CH	28.56	29.01	0.44
12	CH_2	25.70	25.59	-0.11
13	CH_2	35.49	35.40	-0.09
14	C	98.80	98.30	-0.50
15	CH	32.66	32.27	-0.39
16	CH_2	25.91	25.15	-0.76
17	CH	29.06	26.25	-0.81
18	CH	73.11	74.86	1.75
19	CH	42.78	42.96	0.18
20	C	193.91	197.90	-3.99
21	C	133.51	134.45	0.94
22	CH	116.32	121.23	4.91
23	CH	110.27	110.67	0.40
24	CH	124.30	113.18	-11.12
3'	CH_3	30.08	30.37	0.29
11'	CH_3	13.19	11.19	-2.00
15'	CH_3	16.23	16.00	-0.23
17'	CH_3	11.49	11.66	0.17
19'	CH_3	10.85	10.73	-0.12

[a] In ppm from TMS

[b] Determined from DEPT measurements

[c] $\Delta = \delta\ (2) - \delta\ (1)$

[d] These assignments may be reversed

Table 2 with the chemical shift changes upon complex formation. The most striking differences concern, first, the carbons directly involved in the calcium binding (C-1, C-2, C-20, C-22 and C-24), and, second, the C-5, C-11' pair. As suggested by the X-ray crystallographic results, the changes in chemical shifts observed for these latter atoms could be explained in terms of strong steric interaction between these two carbons.

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