Tetrahedron Vol. 37, pp. 1943 to 1949, 1981
Printed in Great Britain.

0040-4020/81/101943-07$02.00/0

SELECTIVE ^1H–^{13}C CORRELATION BY LOW POWER SINGLE FREQUENCY OFF-RESONANCE DECOUPLING ASSISTED BY EXHAUSTIVE SIMULATION TECHNIQUES

J. C. BELOEIL
Laboratoire de Synthèse Organique, Ecole Polytechnique, 91128 Palaiseau Cedex France

and

C. LE COCQ, V. MICHON and J. Y. LALLEMAND*
Laboratoire de Chimie de l'Ecole Normale Supérieure, 24, rue Lhomond, 75231 Paris Cedex 05, France

(*Received in France* 21 *October* 1980)

Abstract—Selectivity in the ^1H–^{13}C correlation for assignments of ^{13}C spectra is enhanced by recording single frequency off-resonance ^{13}C NMR spectra with low decoupling field strength ($\leq 2J_0$), distortion of signal patterns is avoided by submitting the spin system to a previous noise broad band decoupling. Correlation is obtained by comparison of observed and simulated 2-D spectra (δ ^{13}C vs decoupler frequency) joined to fitting of the curve $J_R = f$ (decoupler frequency) with the equation of Freeman and Anderson.[1]

Single Frequency Off-Resonance Decoupling (SFORD) is one of the most widely used techniques for the assignment of ^{13}C signals. Usually, its application is restricted to the separation of quaternary carbons, CH, CH_2 and CH_3 groups according to their signal multiplicity. Actually the off-resonance spectrum contains more precise information, through the measure of the "residual splitting" J_R which is given by the equation of Anderson and Freeman:[1]

$$J_R = \sqrt{\left(\left(\Delta f - \frac{1}{2}J_0\right)^2 + D^2\right)} - \sqrt{\left(\left(\Delta f + \frac{1}{2}J_0\right)^2 + D^2\right)} \quad (1)$$

where Δf is the difference between the proton chemical shift f_1 and the decoupler frequency f_2, J_0 is the heteronuclear one bond coupling constant (^{13}C–^1H) and D is the decoupling field strength ($D = \gamma H_2$).

Provided that J_0 and D are known and J_R is measured, Δf and f_1 may be calculated. Then the comparison of f_1 with ^1H chemical shifts furnishes a ^{13}C–^1H correlation. Generaly the ^1H spectrum is much easily assigned, and ^{13}C assignments are deduced from it, but the reverse may also be useful.

According to this scheme, several methods have been described[2–6] which often use a simplified relationship between J_R and Δf:[7,8]

$$J_R = \frac{J_0}{D}\Delta f \quad (2)$$

$$\Delta f = \frac{DJ_R}{\sqrt{(J_0^2 - J_R^2)}}. \quad (3)$$

Recently we reported briefly in a preliminary communication,[9] the assignment of the ^{13}C spectrum of the ionophorous antibiotic lonomycin (Fig. 5). We noticed that the methods already described were not well suited to the study of large molecules because of the poor selectivity in the determination of ^1H chemical shifts.

We report in this paper, a technique of correlation $^{13}C \rightleftarrows ^1H$, specially effective in the field of natural products. The assignment of the ^{13}C spectrum of the antibiotic lonomycin is described and used to illustrate the method.

RESULTS

Low Power Single Frequency Off Resonance Decoupling (LPSFORD)

From Fig 1 which displays the dependance of J_R versus decoupler offset at different decoupling powers, it can be seen that increased sensitivity of J_R to the variation of Δf, and therefore selectivity enhancement in $^1H \rightleftarrows ^{13}C$ correlation is obtained for low Δf and decoupling strength (D) values. For example, when $\Delta f \simeq J_0$, if $D = 2\ J_0$ is used instead of $4\ J_0$, $\Delta J_r/\Delta f$ is multiplied by 2.5. This advantage is rapidly limited by the apparition of anomalous lines' intensities, higher multiplicities (up to 16 lines for a CH_3 group), and even transitions in emission[10–12] (Fig. 2a). These perturbations could make the determination of the residual splitting J_R impossible at low decoupling strengths.

According to Bain *et al.*,[10] part of this phenomena may be explained by population effects coming from two factors, the tilting of the effective field in the rotating frame and the relaxation effect.

As suggested by Freeman *et al.*,[13] we submitted the spin system to an incoherent proton irradiation which completely modifies the spin's populations just before excitation pulse, acquisition being recorded under low power single frequency off-resonance decoupling (LPSFORD). Comparison of spectra recorded in LPSFORD conditions, with and without preliminary noise broad band decoupling (e.g. Figs. 2a and 2b) shows that the "preparation" of the spin system restores the usual off-resonance pattern. The reduced splitting J_R may be easily measured, giving a better precision of the determination of the related proton chemical shifts. According to this technique, we have recorded several LPSFORD spectra ($J_0 \leq D \leq 2J_0$), no significant distortions of the signal patterns have been observed (e.g. Fig. 3a).

Determination of the connected ^1H chemical shifts f_1

From equation[1], it is theoretically possible to obtain Δf and f_1 by collecting J_R from only one or two

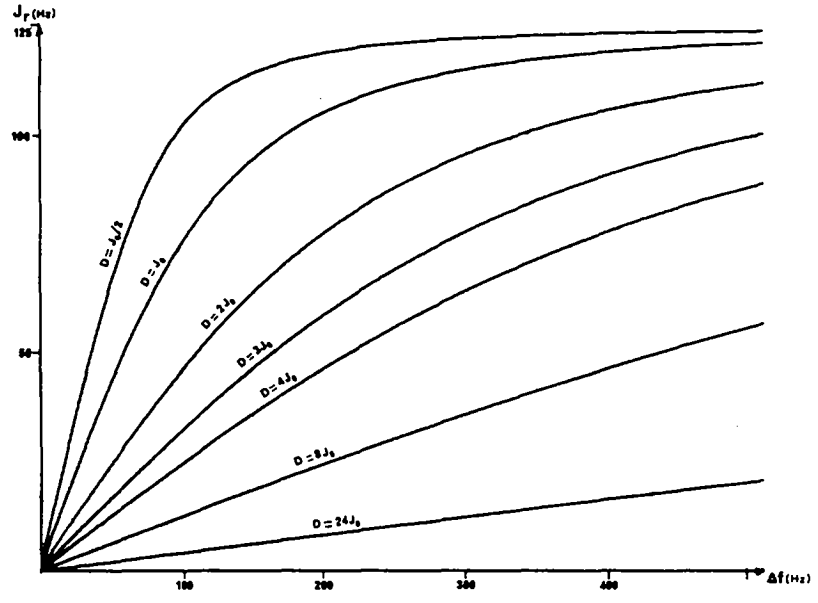

Fig. 1. Plot of $J_R = \sqrt{((\Delta f - (1/2)J_0)^2 + D^2)} - \sqrt{((\Delta f + (1/2)J_0)^2 + D^2)}$ $J_0 = 125$ Hz; $D = \gamma B_2$.

LPSFORD spectra (with different decoupling frequencies), provided that D and/or J_0 are known.[6] In fact, this scheme is limited to the study of small molecules because identification of doublet, triplet or quadruplet patterns in ^{13}C spectra of large molecules remains difficult.

Practically, all J_R values cannot be extracted from only one or two spectra. Moreover some regions of the ^1H spectra are often very crowded, thus it is necessary to obtain a high selectivity (≤ 10 Hz at 250 MHz) in the computation of the ^1H chemical shifts.

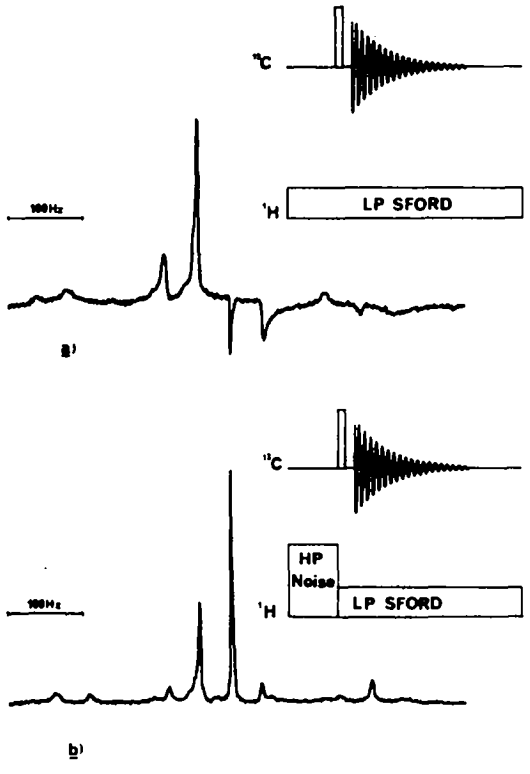

Fig. 2. LPSFORD ($D = 1J_0$, $\Delta f = 50$ Hz) ^{13}C NMR spectra of $I^{13}CH_3$. a, without preparation of the spin system; b, with previous noise broad band decoupling.

We describe below a six stages method which overcomes these difficulties:

(1) A set of LPSFORD spectra ($D \leq 2J_0$), with incremental variation of the decoupling frequency f_2 is recorded. The observation of the resulting 2-D spectrum (Figs. 7 and 8) allows to follow a signal pattern from one spectrum to another.

(2) The decoupler field strength $D = \gamma H_2$ is computed by fitting the curve $J_R = f(f_2)$ with equation[1]. This operation is made on the TMS signal for which J_0 and f_1 are known.

(3) A very rough assignment of the ^{13}C spectrum is done, partly by comparison with known parent compounds, to initialize the process.

(4) For a given value of f_2, assuming in a first approximation that all ^{13}C–^1H coupling constants are equal to 125 Hz for sp3 carbons, the LPSFORD spectrum is simulated (first order only, see below) and compared with the experimental one (Fig. 3). Wrong assignments can be detected and corrected (Fig. 3). The process is repeated until good agreement between observed and calculated spectra is obtained. Then the decoupler frequency f_2 is changed, a new simulation is made, attributions are checked and modified if necessary.

To keep this stage quick and useful we had to develop a simplified conversational simulation program, all previously existing sophisticated programs which need time consuming matrice's inversion had to be discarded.

This program generates singlets, doublets and quadruplets with adjustable line width and only takes into account the one bond heteronuclear coupling constants (the simulation program is written in FOCAL and was developed on a NICOLET 1180 computer; a copy can be sent upon request). Second order interactions and secondary effects like NOE are neglected. In the case of CH_2 groups the two protons are considered to be non-

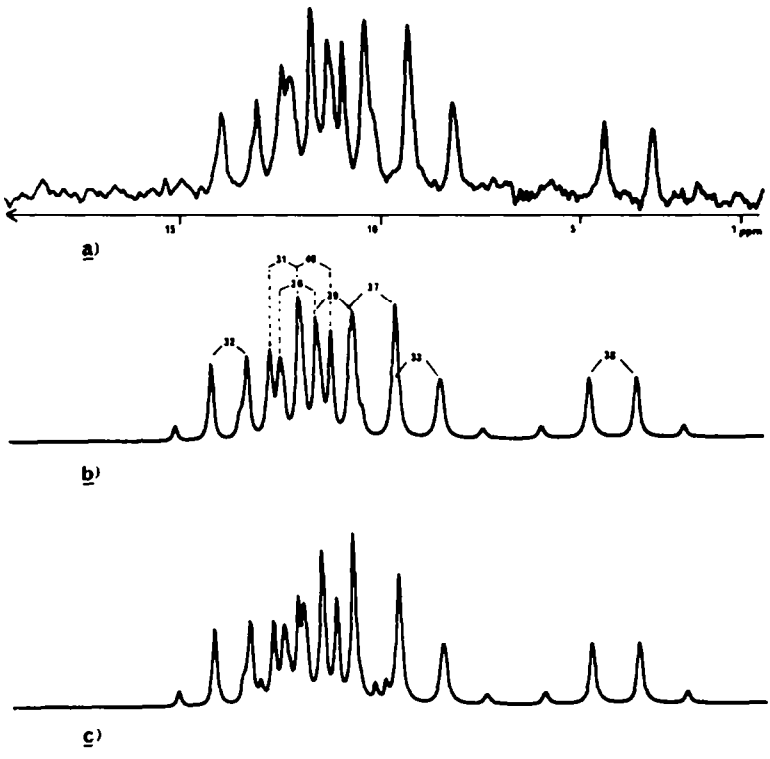

Fig. 3. (a). 8 ≃ 14 ppm region of the observed LPSFORD ^{13}C NMR spectrum of lonomycin (D = 243 Hz, f_2 ≃ 342 Hz); (b) Simulated spectrum using Table 1 values; (c). Simulated spectrum using Table 1 values, except that assignments of C_{39} and C_{40} are exchanged.

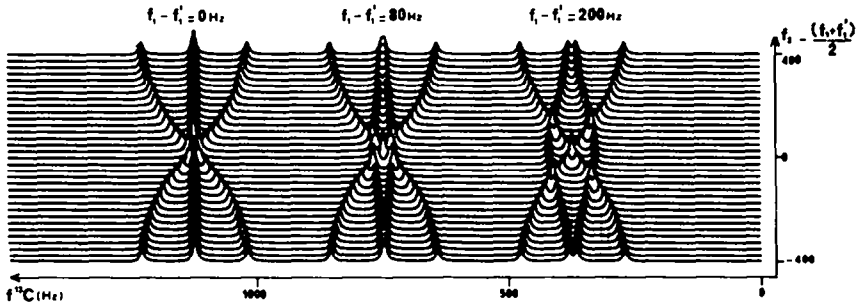

Fig. 4. 2-D representation (δ ^{13}C vs f_2) of CH_2 signal patterns with various protons chemical shift differences ($f_1 - f_1'$) (D = 250 Hz, J_0 = 125 Hz).

equivalent and the corresponding independant residual coupling constants are computed (Fig. 4).

On the practical example reported on Fig. 3 it can be seen that the simplified simulated spectrum is highly sufficient to identify the signal patterns, for example the inversion of the assignments of the carbon 39 and 40 of lonomycin is easily detected.

We must underline that the computational time needed for the simulation of a twenty carbons SFORD spectrum is in the order of a few minutes. At this stage the connection scheme of even signals (CH, CH_3 groups) is well established. On the other hand the identification of the CH_2 groups, by comparison of observed and simulated spectra is difficult and time consuming because a CH_2 which bears non-equivalent protons give much complicated signal patterns (Fig. 4). Moreover the assignments of CH_2 signals in the ^1H spectrum are sometimes less accurate than those of CH and CH_3, or even impossible.

(5) Most signal patterns are now assigned. Thus it is possible to recognize the unidentified signals and to extract the curve $J_R = f(f_2)$ for each of it. This curve is fitted with the equation[1] by a classical least square method,[14] the values of the proton chemical shifts f_1 and ^{13}C–^1H coupling constants J_0 are obtained. It must be noticed that the precise J_0's values of previously identified signals (Stage 4) can be obtained in the same manner.

(6) A very effective verification of the total assignment can be made if the observed 2-D spectrum (δ ^{13}C vs f_2) is compared with the simulated one which was computed from final f_1's values and from J_0's values calculated at stage 5 (see Figs. 7 and 8).

Application: assignment of the ^{13}C NMR spectrum of the sodium salt of the antibiotic lonomycin

Lonomycin $C_{44}H_{76}O_{14}$ (Fig. 5) is an ionophorous antibiotic produced from *Streptomyces hygroscopicus*;[15] its ^1H NMR spectrum (Fig. 6a) has been well studied.[16] From the classical ^{13}C NMR "SFORD" spectrum, 6 singlets, 18 doublets, 5 triplets and 15 quadruplets were identified. The carbonyl carbon C-1 was immediately attributed to the signal at 180.1 ppm. Obviously decoupling methods are ineffective for the assignment of non-substituted carbons and quaternary methyls. Therefore C-3, C-29, C-13, C-16, C-20, C-30, C-34 and C-35 were attributed by comparison with spectra of similar products.[17] The four quadruplets in the 54 ≈ 66 ppm region correspond to the methoxy groups, they couldn't be individually assigned but selective decoupling could give the ^1H-^{13}C correlation.

The 3 ≈ 5 ppm region of the ^1H NMR spectrum was well resolved. It was easy to assign C-27, C-11, C-5, C-17, C-7, C-25, C-24, C-23, C-21, C-9, C-2, C-28, C-22 and C-6 by heteronuclear selective decoupling. The region A (8 ≈ 14 ppm) and B (20 ≈ 40 ppm) of the ^{13}C NMR spectra (Fig. 6b) remained unassigned. The related regions of the ^1H NMR spectrum were very badly resolved, therefore the selective decoupling techniques were useless.

We recorded a set of "LPSFORD" spectra ($D \approx 2J_0$, decoupler frequency step = 25 Hz), with "preparation" of the spin system by broad band decoupling (Figs. 7a and 8a). A rough assignment was done, partly by comparison with known ionophorous antibiotics.[17] Then the method described above was entirely applied (Figs. 3, 7 and 8). The results are reported in Table 1. The selectivity is better than 10 Hz.

Final observed and simulated 2-D spectra of the regions 8 ≈ 14 ppm and 20 ≈ 40 ppm are represented in Figs. 7 and 8.

It can be seen in Figs 8a and 8b that the simulation of CH_2 signal patterns is very effective (C-12 for example). Protons signals of C-14, C-15, and C-19 (CH_2 groups) are unidentified in the ^1H spectrum, C-15 and C-19 which have very similar environment are attributed to two very closed signals in the ^{13}C spectrum at 33.7 and 33.4 ppm. C-14 is then assigned by default; the corresponding ^1H chemical shifts could be computed (Table 1) and thus predicted. This operation is impossible for C-15 and C-19, their ^{13}C signal patterns are too overlapped, it is impossible to collect J_R values.

The high selectivity obtained by this technique forced us to modify three of our previous assignments[9] (C-12, C-14 and C-18), and to reverse the C-39 and C-40

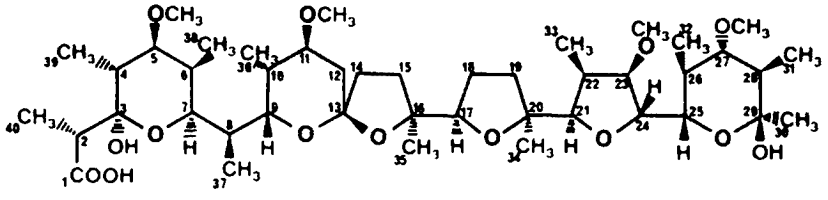

Fig. 5. Lonomycin.

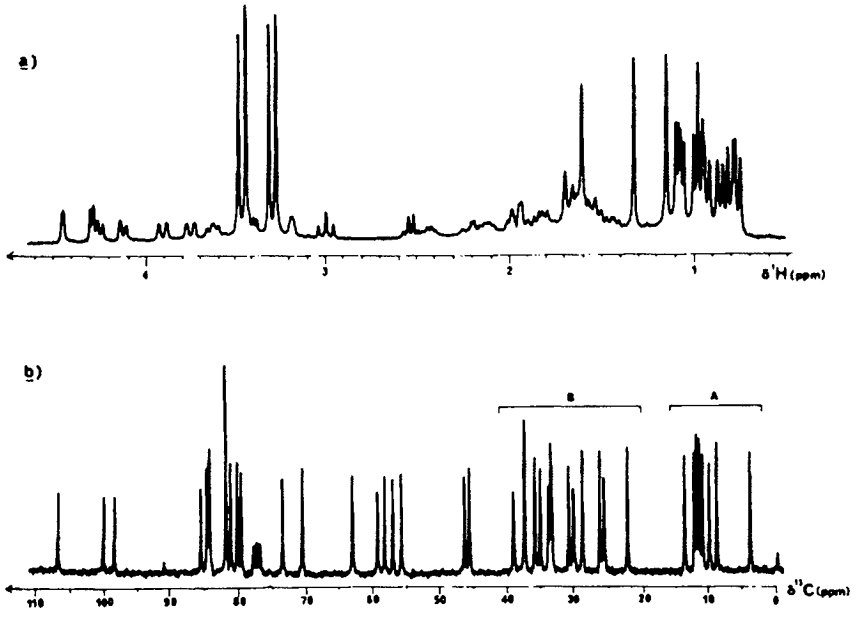

Fig. 6. (a). ^1H NMR spectrum of lonomycin ($CDCl_3$, 250 MHz); (b). Noise decoupled ^{13}C NMR spectrum of lonomycin ($CDCl_3$, 62.9 MHz).

Table 1. Comparison of the ^1H chemical shifts (250 MHz) obtained from experimental spectrum and from computation-simulation

carbon N°	^1H chemical shifts in Hz from TMS (at 250 MHz)		
	experimental	simulation	diff.
4	457	460	3
6	530	533	3
8	385	390	5
10	417	423	6
18	395	389	6
	455	458	3
12	427	426	1
	497	499	2
14	not identified	397	
	not identified	562	
22	607	614	7
26	417	409	8
30	332	340	8
31	274	276	2
32	247	254	7
33	212	221	9
34	287	299	12
35	405	406	1
36	232	239	7
37	200	207	7
38	190	197	7
39	240	245	5
40	265	272	7

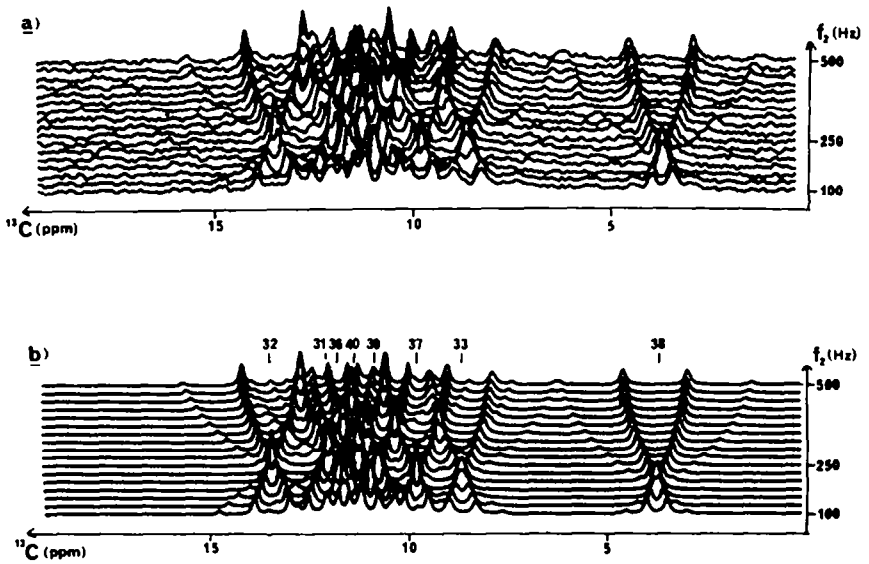

Fig. 7. (a). 8 ≃ 14 ppm region of the 2-D "LPSFORD." ^{13}C NMR spectrum of Ionomycin (^{13}C chemical shift vs decoupling frequency), (b). Simulated spectrum.

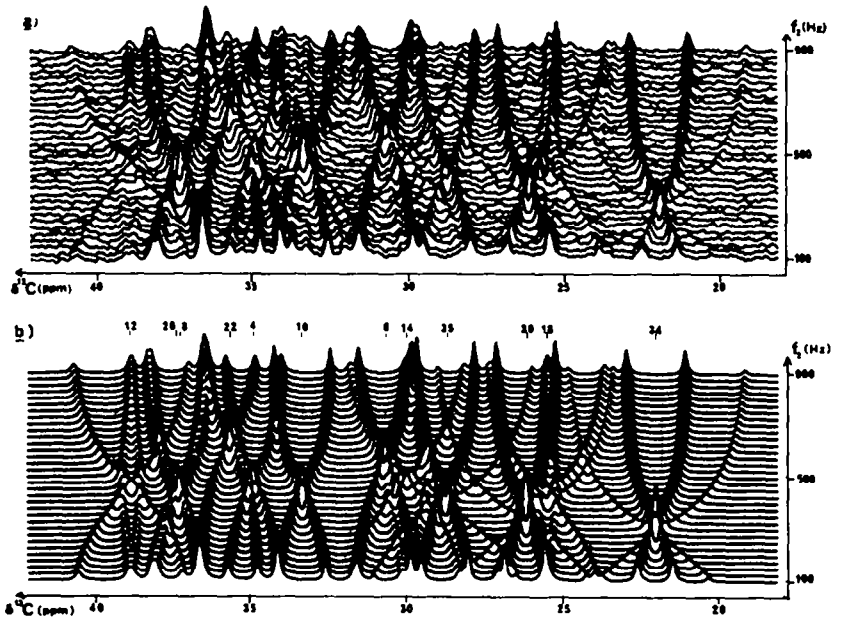

Fig. 8 (a). 20 ≃ 40 ppm region of the 2-D "LPSFORD" ^{13}C NMR spectrum of lonomycin. (b). Simulated spectrum (except for C_{15} and C_{19} signals which are not represented).

Table 2. Chemical shifts of the ^{13}C and related ^{1}H of the lonomycin sodium salt (in ppm from TMS). Attributions with * and + can be respectively reversed

Carbon N°	^{13}C chemical shifts in ppm from TMS	^{1}H chemical shifts in ppm from TMS	
1	180.1		
2	45.4	2.54	
3	99.6		
4	34.9	1.83	
5	81.6	3.38	
6	30.7	2.12	
7	70.3	3.75	
8	37.3	1.54	
9	62.8	4.43	
10	33.4	1.67	
11	81.6	3.20	
12	38.9	1.99	1.71
13	106.3		
14	30.0		
15	33.7 *		
16	84.1 +		
17	80.9	3.61	
18	25.5	1.58	1.82
19	33.4 *		
20	85.4 +		
21	84.4	4.28	
22	35.7	2.43	
23	79.9	4.24	
24	79.3	4.12	
25	73.2	3.90	
26	37.4	1.67	
27	84.1	3.01	
28	46.2	1.44	
29	98.0		
30	26.2	1.33	
31	12.0	1.10	
32	13.5	0.99	
33	8.7	0.85	
34	21.9	1.15	
35	28.8	1.62	
36	11.7	0.93	
37	9.8	0.80	
38	3.7	0.76	
39	10.8	0.96	
40	11.3	1.06	
MeO	59.3	3.49	
MeO	58.1	3.46	
MeO	56.9	3.33	
MeO	55.6	3.29	

attributions of Seto *et al.*[18] The complete assignment of the ^{13}C NMR spectrum of lonomycin is reported in Table 2.

CONCLUSION

The preparation of the spin system, by high power noise decoupling, before the LPSFORD experiment allows the use of low decoupling power which give its high sensitivity to the described method. The comparison of the observed and simulated 2-D spectra (δ ^{13}C versus f_2); joined to the fit of the curve $J_R = f(f_2)$ with equation[1] give it the self-consistency. However it remains a routine technique because of the very short computational times needed by its conversational program.

This method should be a highly attractive counterpart to the well known but dangerous empirical chemical shift correlation between model compounds.

EXPERIMENTAL

The spectra at 18° in $CDCl_3$ were obtained on a 250 MHz CAMECA apparatus, equipped with a NICOLET 1180 computer. Modification of the proton decoupler was provided in the following way: decoupler power supply voltage was switched between various levels adjusted by potentiometers. Pulses originally used for field gradient spoiling were used to select the various voltage levels. The obtained switching time was $\simeq 50\,\mu s$ and dynamic $\simeq$ 40 dB.

REFERENCES

[1] W. A. Anderson and R. Freeman, *J. Chem. Phys.* **37**, 85 (1962).
[2] R. Freeman and H. D. W. Hill, *Ibid.* **54**, 3367 (1971).
[3] B. Birdsall, N. J. M. Birdsall and J. Feeney, *J. Chem. Soc. Chem. Comm.* 316 (1972).
[4] M. Tanabe, T. Hamasaki, D. Thomas and Le Roy Johnson, *J. Am. Chem. Soc.* **93**, 273 (1971).
[5] P. H. McCabe and C. R. Nelson, *J. Magn. Res.* **22**, 183 (1976).
[6] J. C. McDonald and M. Mazurek, *Ibid.* **28**, 181 (1977).
[7] R. R. Ernst, *J. Chem. Phys.* **45**, 3845 (1966).
[8] K. G. R. Pachler, *J. Magn. Res.* **7**, 442 (1972).
[9] J. Y. Lallemand, C. Le Cocq, V. Michon, C. Derouet and R. C. Rao, *Comptes Rendus Acad. Sci. C.* **288**, 383 (1979).
[10] A. D. Bain and R. M. Lynden-Bell, *J. Magn. Res.* **25**, 315 (1977).
[11] J. C. McDonald and M. Mazurek, *Ibid.* **38**, 107 (1980).
[12] J. C. McDonald and M. Mazurek, *Ibid.* **19**, 51 (1975).
[13] R. Freeman, J. B. Grutzner, G. Morris and D. L. Turner, *J. Am. Chem. Soc.*, **100**, 5637 (1978).
[14] W. E. Wentworth, *J. Chem. Ed.* **42**, 96 (1965).
[15] S. Omura, M. Shibata, S. Machida, J. Sawada and N. Otake, *J. Antibiotics* **29**, 15 (1976).
[16] M. J. O. Anteunis and N. A. Rodios, *Bull. Soc. Chim. Belg.* **87**, 447 (1978).
[17] H. Seto, K. Mizoue, H. Nakayama, K. Furihata, N. Otake, and H. Yonehara, *J. Antibiotics* **32**, 239 (1979).
[18] H. Seto, K. Mizoue, N. Otake, M. Yamagishi, T. Mizutani, H. Hara and S. Omura, *Ibid.* **31**, 929 (1978).