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MICROBIAL CONVERSION OF GRISORIXIN : CONFORMATIONAL PROPERTIES OF A BIOCONVERSION PRODUCT

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Abstract - On the basis of ^{1}H and ^{13}C NMR spectra, the conformation of a bioconversion product of grisorixin is elucidated.

Grisorixin[1] belongs to the carboxylic ionophore family[2] which possesses the ability to transport cations through biological membranes by forming complexes[3].

This group of compounds, especially monensin[4,5], is used as anticoccidials in poultry and as growth factors in cattle[6]. They show some toxicity[7] and their metabolism, until now, has not been extensively studied. To investigate the structural modifications involved in the metabolism of ionophorous antibiotics, we used the method of microbial models described by Rosazza[8], on grisorixin ; this gave us a major bioconversion product $\underline{G}_1$ (Fig.1), obtained by adding grisorixin to a culture of Streptomyces rimosus NRRL 2234. This work was described in a previous paper[9].

The bioconversion reaction affects rings E and F, by oxidation of Me32 to COOH and Me33 to CH_2OH.

The properties of this bioconversion product are completely different from those of grisorixin :

- it has lost all antibiotic activity,
- it shows a slight solubility in water, which helps detoxication as it implies a partial solubility in biological liquids,
- it no longer transports cations through a bulk liquid membrane, nor through mitochondrial membranes.

Spectral data (IR, mass, ^{13}C NMR) enabled us to elucidate the structure of $\underline{G}_1$[9].

Since its biological properties were markedly altered, it was interesting to determine whether its conformational properties were also modified, and to what extent.

This paper describes the conformation of the potassium salt of the bioconversion product ($\underline{G}_{1b}$) of grisorixin and compares it with that of grisorixin potassium salt ($\underline{GRI}_b$). Insolubility of $\underline{G}_{1b}$ in $CDCl_3$ led us to use CD_3OD as solvent. These conformations were obtained by a high-field NMR proton and carbon-13 study.

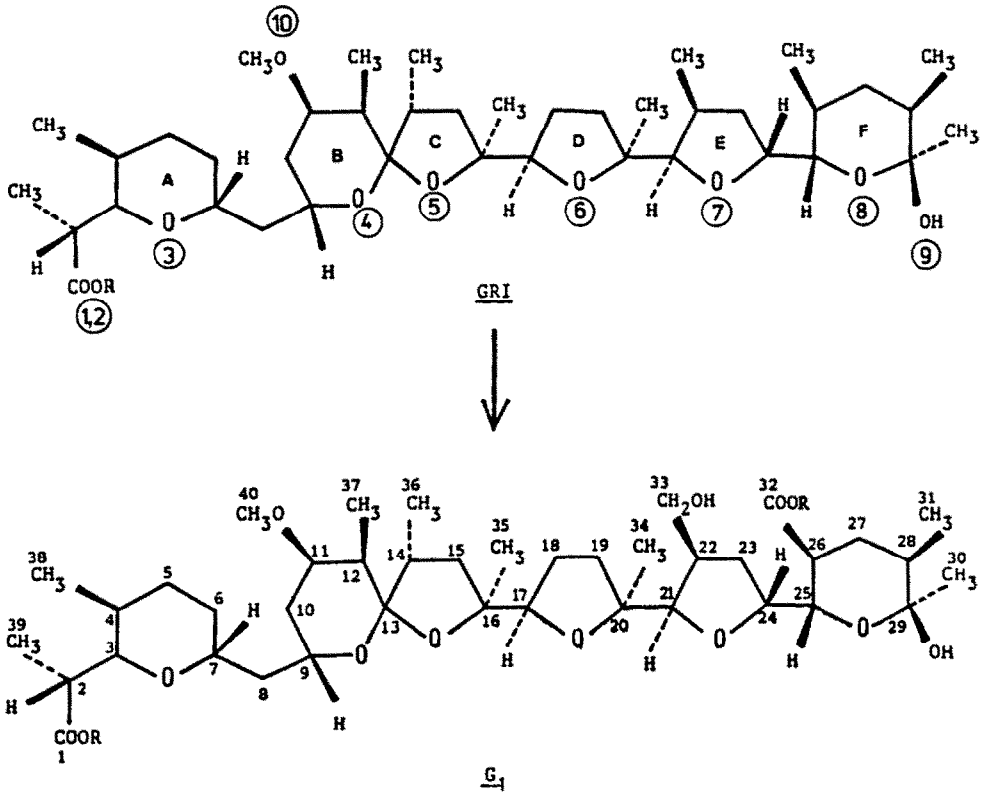


Figure 1 : Grisorixin (GRI) and its bionconversion product (G_1).
R = H, a
R = K, b

RESULTS AND DISCUSSION

To obtain ^{1}H and ^{13}C chemical shifts and ^{3}J ^{1}H-^{1}H scalar coupling constants, we used NMR in two dimensions, as even with a high-field spectrometer (400 MHz) and double resonance methods, numerous regions of the 1D ^{1}H spectrum remain unassignable.

The proton assignment of GRI_b was achieved using a ^{1}H-^{1}H SECSY[10] chemical shift correlation spectrum. All protons were assigned except those of methyl groups carried by quaternary carbons. From this assignment and the ^{1}H -^{13}C [10-13] chemical shift correlation spectrum, we deduced ^{13}C assignments except for C16, C20 and C29 quaternary carbons, and the 3 methyl groups borne by them. We identified with certainty these last 6 carbons by the use of "long-range" ^{1}H-^{13}C chemical shift correlation, which relates ^{1}H and ^{13}C through ^{2}J or ^{3}J scalar couplings (Fig.2). The uncertainties in the ^{13}C data of the preceding paper[9] are thus removed ; in addition, inversions of assignment are rectified (C24 and C25, Me31 and Me32). 2D NMR (δ-δ ^{1}H-^{1}H, δ-δ normal and "long-range" ^{13}C-^{1}H) gave complete assignment, without having to compare with similar molecules[14], an insecure method[15] which can lead to errors of assignment. In the ionophore series, the only other reported ^{13}C assignments by 2D NMR are those of monensin by δ-δ ^{13}C-^{13}C correlation INADEQUATE[16] and that of X14547A[17].

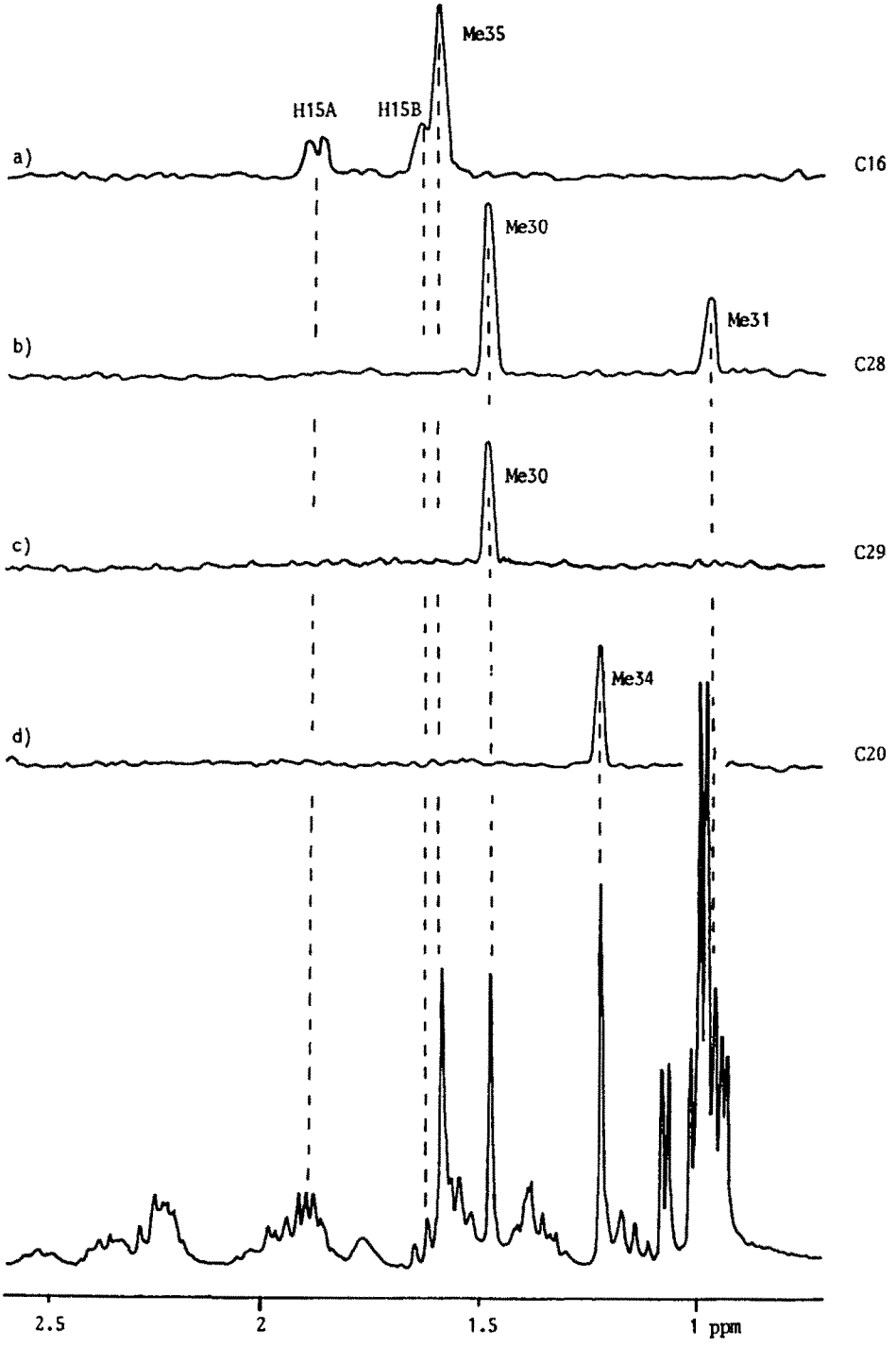


Figure 2 : ^{1}H-^{13}C 2D long-range chemical shift correlation lower trace ^{1}H NMR (400 MHz, CD_3OD ; upfield region) spectrum of GRI_b presented as cross-section plots .
a) C16 b) C28 c) C29 d) C20

We used a similar method for the bioconversion product $\underline{G}_{1b}$: a partial assignment was obtained using a 2D NMR δ-δ ^{1}H-^{1}H COSY [10,18] spectrum (Fig.3) ; then, using a 2D NMR ^{13}C-^{1}H spectrum (Fig.4), we identified the corresponding carbons ; using a return process between the two spectra[17], we completely assigned all carbons and protons of the molecule.

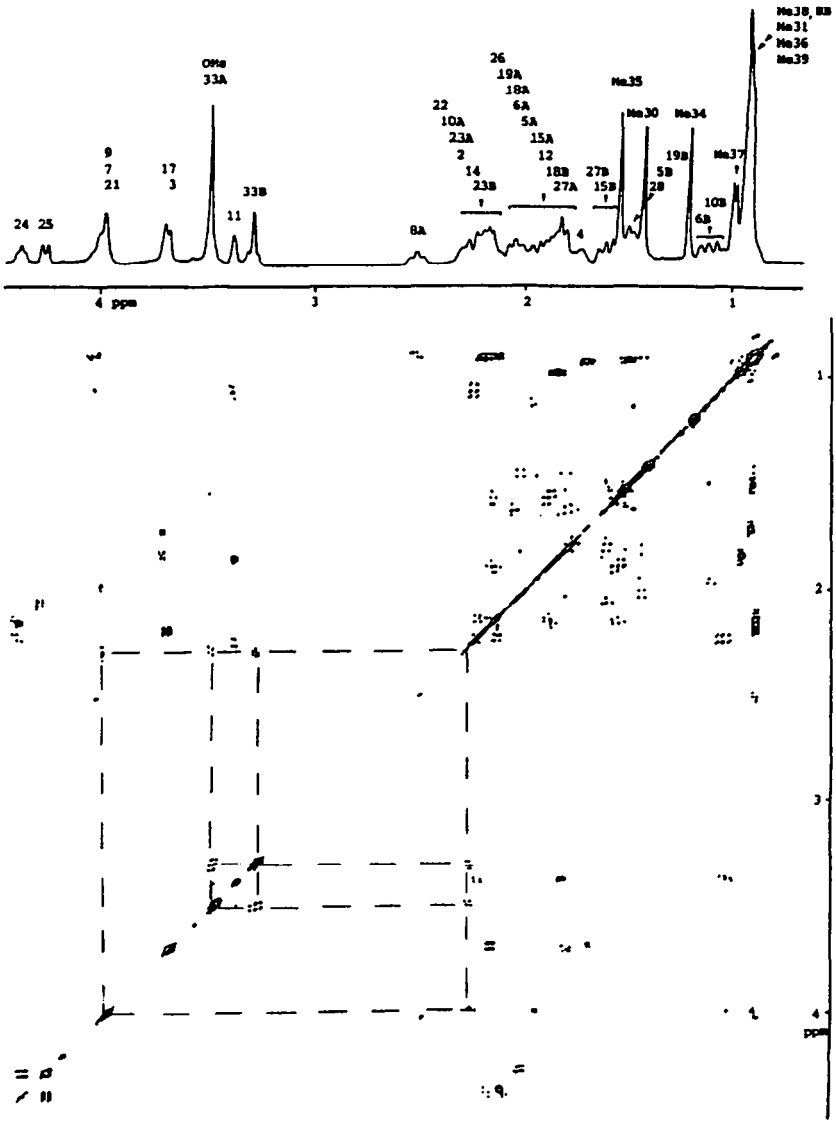


Figure 3 : COSY (400 MHz, CD_3OD) spectrum of $\underline{G}_{1b}$ presented as a contour plot (δ ^{1}H in two dimensions) {– – – – – for example, H_{21}-H_{22}-H_{33A},H_{33B} filiation }

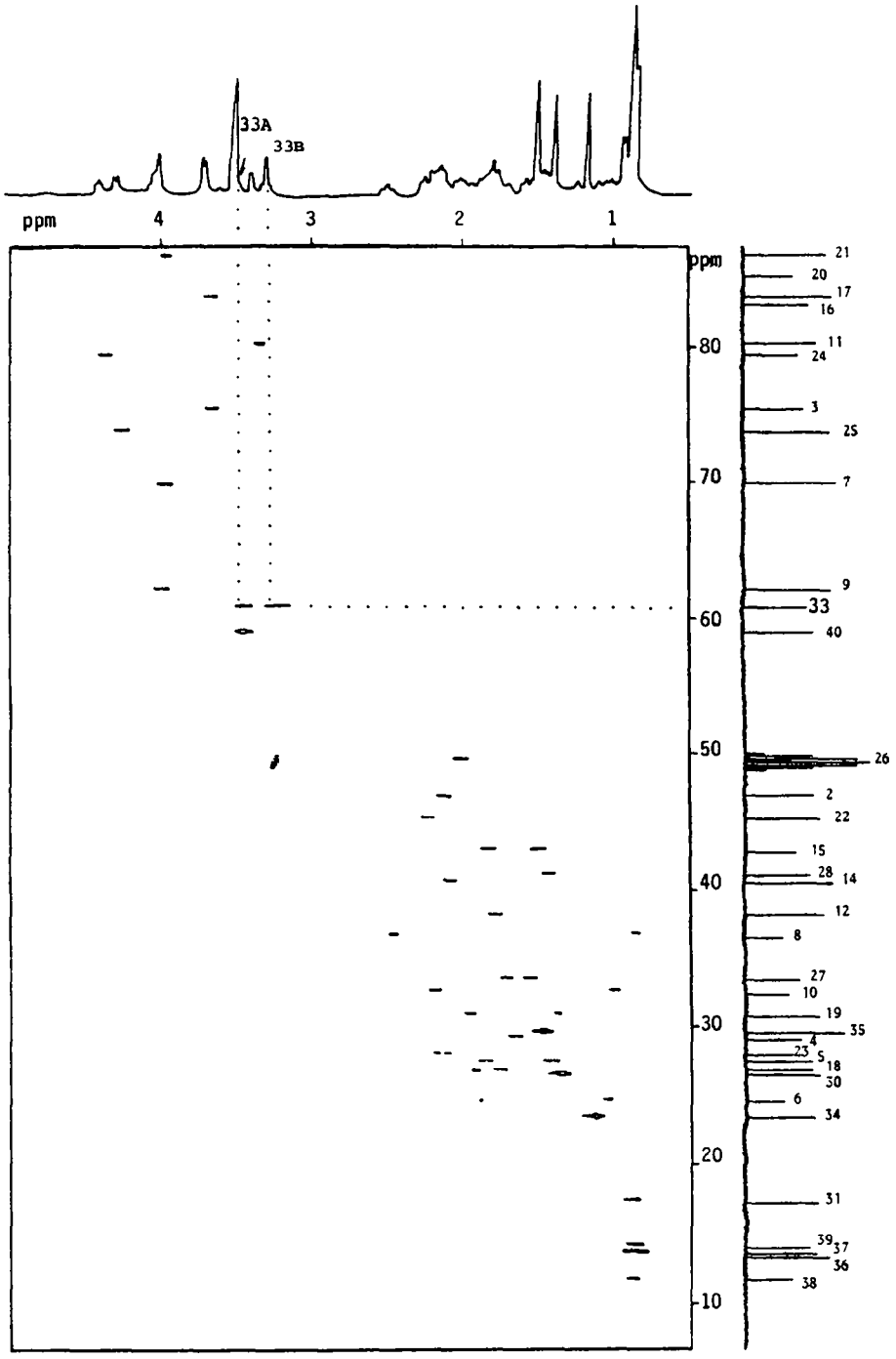


Figure 4 : ^{1}H-^{13}C 2D chemical shift correlation NMR (100 MHz, CD_3OD) spectrum of G_{1b} presented as a contour plot.

^{13}C and ^{1}H chemical shifts are shown in table 1.

Table 1 : ^{13}C and ^{1}H chemical shifts of $\underline{GRI}_b$ and $\underline{G}_{1b}$ in CD_3OD* .

		$\underline{GRI}_b$		$\underline{G}_{1b}$	
C-N°.	FUNCTIONAL GROUP	$\delta\ ^{13}C$ IN PPM	$\delta\ ^{1}H$ IN PPM	$\delta\ ^{13}C$ IN PPM	$\delta\ ^{1}H$ IN PPM
1	$-COO^-$	183.8	--	183.8	--
2	$-CH(CH_3)$	46.7	2.19	46.7	2.19
3	-CH(O)	75.3	3.68	75.3	3.71
4	$-CH(CH_3)$	29.3	1.74	29.2	1.73
5	$-CH_2$	27.4	1.97 - 1.51	27.3	1.94 - 1.48
6	$-CH_2$	24.6	1.99 - 1.18	24.6	1.96 - 1.12
7	-CH(O)	69.6	3.96	69.6	3.99
8	$-CH_2$	36.9	2.56 - 0.99	36.7	2.49 - 0.92
9	-CH(O)	62.0	3.97	61.9	4.01
10	$-CH_2$	32.6	2.22 - 1.14	32.5	2.26 - 1.06
11	$-CH\ (OCH_3)$	80.2	3.41	80.1	3.40
12	$-CH(CH_3)$	38.0	1.88	38.0	1.86
13	-O-C-O	109.3	--	109.4	--
14	$-CH(CH_3)$	40.7	2.18	40.6	2.16
15	$-CH_2$	42.9	1.93 - 1.62	42.9	1.90 - 1.57
16	$-C-O(CH_3)$	83.2	--	83.0	--
17	-CH(O)	83.2	3.69	83.6	3.72
18	$-CH_2$	26.6	1.84 - 1.84	26.7	2.02 - 1.82
19	$-CH_2$	30.8	2.21 - 1.08	30.8	2.05 - 1.46
20	$-C-O(CH_3)$	85.6	--	85.0	--
21	-CH(O)	87.4	3.93	86.7	3.98
22	-CH(R)°	36.6	2.34	45.2	2.27
23	$-CH_2$	33.2	2.44 - 1.55	27.9	2.22 - 2.15
24	-CH(O)	78.7	4.43	79.3	4.37
25	-CH(O)	77.9	3.66	73.7	4.28
26	-CH(R')°	33.6	1.37	49.5	2.07
27	$-CH_2$	37.9	1.36 - 1.36	33.5	1.81 - 1.63
28	$-CH(CH_3)$	41.5	1.51	41.1	1.51
29	-O-C-OH	98.4	--	98.4	--
30	$-CH_3$	26.3	1.46	26.4	1.43
31	$-CH_3$	17.3	0.96	17.3	0.91
32	$-CH_3$	17.8	0.94	--	--
32	$-COO^-$	--	--	181.6	--
33	$-CH_3$	15.8	0.98	--	--
33	$-CH_2OH$	--	--	60.7	3.46 - 3.29
34	$-CH_3$	23.0	1.21	23.3	1.20
35	$-CH_3$	29.4	1.57	29.4	1.54
36	$-CH_3$	13.5	0.98	13.5	0.90
37	$-CH_3$	13.5	1.07	13.6	0.97
38	$-CH_3$	11.7	1.00	11.6	0.92
39	$-CH_3$	14.1	0.98	14.0	0.90
40	$-O-CH_3$	58.6	3.48	58.9	3.49

° R = R' = CH_3 in $\underline{GRI}_b$
R = CH_2OH and R' = COO^- in $\underline{G}_{1b}$

* Spectra were recorded on a Brüker WM-400 spectrometer. Chemical shifts (δ) are in ppm downfield from internal TMS.

By means of a 2D NMR J-δ ^{1}H spectrum giving scalar coupling constants *versus* chemical shifts[10], selective irradiations and 1D NMR spectra, we determined the scalar coupling constants (^{1}H-^{1}H) of $\underline{GRI}_b$ and $\underline{G}_{1b}$ (table 2).

Table 2 : Apparent coupling constants of $\underline{GRI}_b$ and $\underline{G}_{1b}$ in CD_3OD *.

$\underline{GRI}_b$		$\underline{G}_{1b}$	
H	^{3}J(Hz)	H	^{3}J(Hz)
2 - 3a	10.3	2 - 3a	10.0
2 - Me39	7.0	2 - Me39	7.0
3a - 4e	2.8	3a - 4e	2.5
4e - Me38	7.0	4e - Me38	7.3
4e - 5Aa	5.0	4e - 5Aa	5.0
4e - 5Be	1.1	4e - 5Be	1.0
5A - 5B	12.0	5A - 5B	
5Aa - 6Aa	11.8	5Aa - 6Aa	
5Aa - 6Be		5Aa - 6Be	
5Be - 6Aa	3.0	5Be - 6Aa	
5Be - 6Be		5Be - 6Be	
6A - 6B	13.5	6A - 6B	13.4
6Aa - 7e	5.5	6Aa - 7e	5.3
6Be - 7e	~1	6Be - 7e	~1
7e - 8A	13.1	7 - 8A	13.1
7e - 8B	3.7	7 - 8B	3.7
8A - 8B	13.2	8A - 8B	13.1
8A - 9a	4.6	8A - 9a	4.6
8B - 9a	11.5	8B - 9a	12.0
9a - 10Ae	2.0	9a - 10Ae	2.0
9a - 10Ba	10.9	9a - 10Ba	12.0
10A - 10B	14.3	10A - 10B	14.0
10Ae - 11e	2.0	10Ae - 11e	2.0
10Ba - 11e	3.0	10Ba - 11e	3.0
11e - 12a	4.0	11e - 12a	4.0
12a - Me37	7.0	12a - Me37	7.0
14 - 15A	8.6	14 - 15A	8.7
14 - 15B	11.5	14 - 15B	12.0
14 - Me36	7.0	14 - Me36	7.0
15A - 15B	11.5	15A - 15B	12.0
17 - 18A 17 - 18B	Σ 17.0	17 - 18A 17 - 18B	Σ 16.5
21 - 22	4.0	21 - 22	4.0
22 - 23A	7.0	22 - 23A	8.0
22 - 23B	~1	22 - 23B	~1
22 - Me33	7.0	22 - 33A	3.9
23A - 23B	12.3	22 - 33B	8.6
23A - 24	9.0	23A - 23B	12.0
23B - 24	7.1	33A - 33B	13.7
24 - 25a	2.9	23A - 24	8.9
25a - 26a	10.3	23B - 24	7.1
26 - Me32	6.5	24 - 25a	2.9
26 - 27A		25a - 26a	10.6
26 - 27B		26a - 27Aa	12.0
27A - 27B		26a - 27Be	3.7
27A - 28a		27A - 27B	12.5
27B - 28a		27Aa - 28a	12.0
28 - Me31	6.9	27Be - 28a	3.5
		28 - Me31	6.5

* The codings A and B refer respectively to the proton at lowest and highest field side.

X-ray studies of grisorixin silver[19] and thallium[20] salts revealed their cyclic conformation. The molecule forms a cavity, buttoning shut by hydrogen bonding between the hemiacetalic OH and one of the oxygens of the head carboxylate, which is blocked in this conformation by the interactions between the trapped cation and five oxygens of the molecule (01, 05, 06, 07 and 010).

Grisorixin is selective for monovalent cations. Studies carried out on association constants with alkali cations in methanol solution showed that it preferentially complexes potassium[21] ; this liposoluble complex induces K^+ efflux and penetration of H^+ into mitochondria[22].

The ^{13}C and ^{1}H high resolution NMR study of $\underline{GRI}_b$ in $CDCl_3$ solution[23] shows it to be in a globular form similar to that found in its solid state thallium salt[20] ; it exhibits head-to-tail hydrogen bonding (characterized by the proton chemical shift of the OH group at 9.92 ppm). The results obtained in this work in methanol are the same as above.

- Rings A, B and C

In methanol solution, rings A, B and C of $\underline{GRI}_b$ and $\underline{G}_{1b}$ present the same NMR characteristics : ^{1}H and ^{13}C chemical shifts, and (^{1}H,^{1}H) scalar coupling constants are identical. We particularly note the similarity of the $J_{2,3}$ coupling constant in the two molecules (10.0 Hz for $\underline{GRI}_b$ and 10.3 Hz for $\underline{G}_{1b}$), this agrees with H_2 and H_3 in antiperiplanar positions. The 7-8-9 hinge is the same for the two molecules. Interestingly, the C_7-C_8 bond is axial, an unusual conformation in the ionophore family.

- Ring D

Conformation of ring D of $\underline{G}_{1b}$ is given in figure 5.

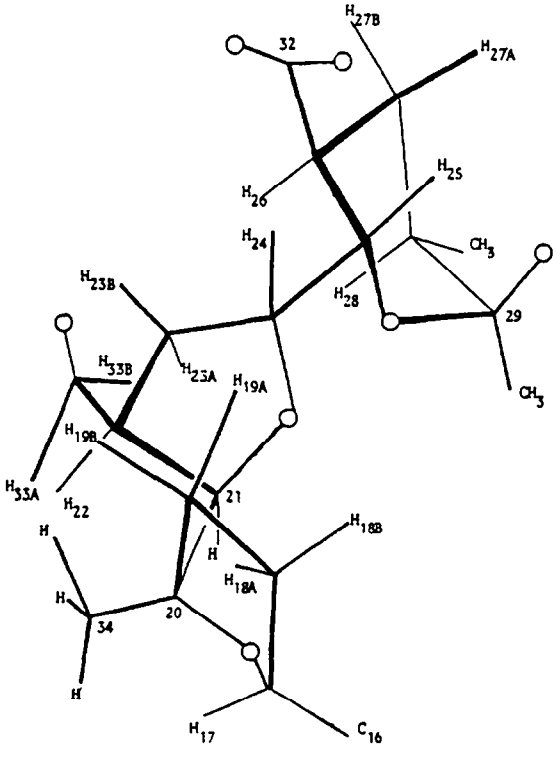


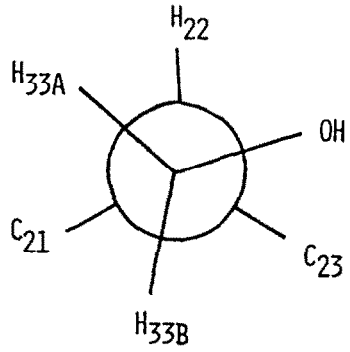
Figure 5 : Stereodrawing of conformation and relative positions of rings D, E and F of $\underline{G}_{1b}$ in CD_3OD solution.

H_{19B} suffers steric hindrance from the protons H_{33A} and H_{33B} which explains its low field chemical shift (1.46 instead of 1.08 ppm in the case of $\underline{GRI}_b$). H_{18A} is deshielded and must therefore be situated on the same side as H_{19B}. The remaining proton chemical shifts in the two molecules are identical. Therefore, we can deduce a similar conformation as well as a close spatial position.

- Rings E and F

E and F, the last two rings, are those affected by the bioconversion ; they give numerous NMR perturbations due to the effects of the substitution of Me_{32} and Me_{33} respectively by COOH and CH_2OH.

In ring E, $J_{21,22}$, $J_{22,23A}$ and $J_{22,23B}$ coupling constants are the same in both $\underline{GRI}_b$ and $\underline{G}_{1b}$. Therefore these protons are in the same position and the conformation of ring E is identical in the two molecules (Fig. 5). In the course of bioconversion, Me33 is converted into CH_2OH. The proton NMR spectrum of $\underline{G}_{1b}$ shows that the two methylene protons H_{33A} and H_{33B} have different chemical shifts (3.46 and 3.29 ppm respectively) ; this gives a blocked structure for the CH_2OH group. The two carboxylic functions of $\underline{G}_{1a}$ have been converted into COO^-K^+ in $\underline{G}_{1b}$; the first K^+ is trapped within the structure, the second stays close to the COO^- of ring F ; an interaction can then occur between the free doublets of oxygen belonging to close CH_2OH and K^+. The CH_2OH position with regard to ring E is given by examination of scalar coupling constants $J_{22,33A}$ and $J_{22,33B}$ which are respectively 3.9 and 8.6 Hz. Only one position is possible ; this is shown in the following scheme :



Proton H_{33B} stays practically antiperiplanar to H_{22}, H_{33A} forming an angle of approximately 50° with H_{22}.

Proton H_{23B} is strongly deshielded in $\underline{G}_{1b}$ with regard to $\underline{GRI}_b$ ($\Delta\delta$ = 0.60 ppm) ; it is subjected to "long-range shielding" effects from neighbouring OH and COO^- groups. Proton H_{23A} situated under the ring E plane undergoes practically no influence (0.22 ppm shielding).

Coupling value $J_{24,25}$ = 2.9 Hz is the same in both potassium salts ; the hinge 24-25 is in the same position.

In ring F of $\underline{G}_{1b}$, all scalar coupling constants were defined. Coupling value $J_{25,26a}$ is practically identical in the two molecules (10.6 Hz for $\underline{G}_{1b}$ and 10.3 Hz for $\underline{GRI}_b$). Therefore they possess the same chair conformation for ring F (Fig. 5).

Protons H_{25}, H_{27A} and H_{27B} are deshielded by the β effect of the carboxylate group. Protons H_{27A} and H_{27B} which have the same chemical shift in $\underline{GRI}_b$, are distinct in $\underline{G}_{1b}$ (1.81 and 1.63 ppm respectively) ; by means of the 2D NMR J-δ ^1H spectrum, we can define their respective positions from

scalar coupling constants. We observe a large value ($J_{26a,27A}$ = 12.0 Hz) and a small one ($J_{26a,27B}$ = 3.7 Hz) which agree with H_{27A} axial and H_{27B} equatorial ; this stereochemistry is confirmed by the scalar coupling constant values of these two protons with H_{28a} ($J_{28a,27A}$ = 12 Hz and $J_{28a,27B}$ = 3.5 Hz).

Therefore H_{27A} is axial and on the same side as the carboxylate ; its deshielding effect can be explained by the proximity of this group.

To conclude, the differences between the NMR characteristics of $\underline{GRI}_b$ and $\underline{G}_{1b}$ (δ ^1H, δ ^{13}C and J ^1H) can be explained by the local effects of substitution. The conformation of the six rings, the relative positions of the atoms and the hinge angles are very similar.

The potassium salt of the bioconversion product of grisorixin has an entirely blocked globular structure, wrapped around the trapped cation, very similar to that of $\underline{GRI}_b$ (Fig. 6). The substitution of the two methyl groups of grisorixin by the polar CH_2OH group and the dissociable COOH func-

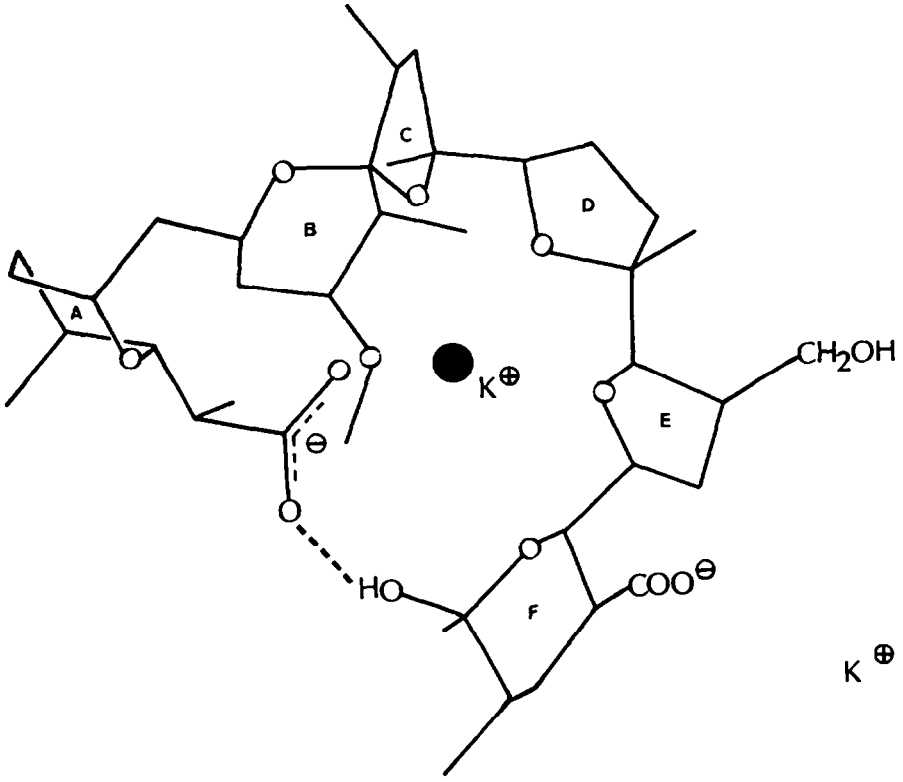


Figure 6 : Schematic representation of $\underline{G}_{1b}$.

tion on the lipophilic region of the molecule markedly modifies the amphiphilic balance of the molecule. Accordingly, even though the bioconversion product has in solution the same conformation as grisorixin, and wraps the cations in the same way, it does not carry them as efficiently[9]. Loss of ability to transport cations through the biological membranes entails loss of antibiotic properties.

In conclusion, the antibiotic character of the carboxylic polyether ionophores is undoubtedly linked to their cation transport aptitudes. Structural modifications, even if they have little effect on the backbone, can have important consequences for the biological properties by reducing these transport abilities.

EXPERIMENTAL

Isolation of the bioconversion product G_1.
The experimental process was described in a preceding paper[9].

Preparation of the potassium salt G_{1b}.
To a solution of G_{1a} in ethanol:water(1:1)was added 0.1N KOH to pH 10 (pH meter). The mixture was evaporated, washed with Et_2O and filtered. m.p. 235-238°C ; FAB-MS : m/z $(M+H)^+$; $(\alpha)_D^{20}$ = + 14.0° (C 0.025, Me_2CO) ; IR ν (KBr) 1575 cm-1. Anal. calc. for $C_{40}H_{64}O_{13}K_2$: C 57.80, H 7.76, K 9.41, O 25.03 ; found : C 57.68, H 7.79, K 9.44.

NMR spectra.
All the 1D and 2D spectra were recorded on a Brüker WM-400 in CD_3OD solution (0.6 ml).

COSY. The two-dimensional correlated ^1H NMR experiment was performed on 80 mg of G_{1b}. The applied pulse sequence was $(\pi/2)-(t_1)-(\pi/4)-(FID,t_2)$. The spectral width in F_1 and F_2 was 1818 Hz ; the number of data points in t_2 was 2048, and 512 increments were recorded. Before Fourier transformation, the data were multiplied with unshifted sine bell. Total acquisition time was 4h. The π/2 pulse was 13 µs.

SECSY. The two-dimensional correlated ^1H NMR experiment was performed on 70 mg of GRI_b. The applied pulse sequence was $(\pi/2)-(t_1/2)-(\pi/2)-(t_1/2)-(FID,t_2)$. The spectral width in F_1 was 2000 Hz and in F_2 4000 Hz ; the number of data points in t_2 was 1024, and 512 increments were recorded. Before Fourier transformation, the data were multiplied with Exponential in F_2 and Lorentz-Gauss in F_1. Zero filling was applied in each dimension. Total acquisition time was 5 h. The π/2 pulse was 13 µs.

^1H-^{13}C shift correlation of G_{1b}. The experiment was performed on 80 mg of G_{1b}. The applied pulse sequence was $(\pi/2, {}^1H)-(t_1/2)-(\pi, {}^{13}C)-(t_1/2)-(\tau_1)-(\pi/2, {}^1H ; \pi/2, {}^{13}C)-(\tau_2)-(BB, {}^1H ; FID, t_2)$ with τ_1 = 0.00357 s and τ_2 = 0.001785 s. The spectral width in F_1 was 1779 Hz and in F_2 10700 Hz ; the number of data points in t_2 was 4096, and 256 increments were recorded. Before Fourier transformation, the data were multiplied with Lorentz - Gauss. Zero filling was applied in each dimension. Total acquisition time was 24 h. The π/2 pulse was 11 µs for ^{13}C, and the decoupler π/2 pulse for ^1H was 47 µs.

^1H-^{13}C shift correlation of GRI_b. The experiment was performed on 70 mg of GRI_b. Id to ^1H-^{13}C shift correlation of G_{1b} except : the spectral width in F_1 was 1798 Hz and in F_2 8772 Hz.

^1H-^{13}C "long-range" shift correlation of GRI_b. The experiment was performed on 70 mg of GRI_b. Id to ^1H-^{13}C shift correlation of GRI_b except : the spectral width in F_2 was 10870 Hz, $\tau_1 = \tau_2$ = 0.0417s.

J-δ ^1H correlation of GRI_b and G_{1b}. The experiments were performed on 80 mg of G_{1b}, and 70 mg of GRI_b. The applied pulse sequence was $(\pi/2)-(t_1/2)-(\pi)-(t_1/2)-(FID, t_2)$. The spectral width in F_2 was 4000 Hz and in F_1 62.5 Hz. The number of data points in t_2 was 4096, and 64 increments were recorded. Before Fourier transformation, the data were multiplied with Lorentz-Gauss. Zero filling was applied in each dimension. Total acquisition was 4 h. The π/2 pulse was 13 µs.

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REFERENCES

1- P. GACHON, A. KERGOMARD, T. STARON and C. ESTEVE : J. Antibiotics, 28, 345 (1975).

2- J.W. WESTLEY : "Polyether Antibiotics - NaturallyOccurring Acid Ionophores", Vol.1 : Biology, Vol.2 : Chemistry, M. Dekker Inc., New-York (1982).

3- R.W. TAYLOR, R.F. KAUFFMAN and D.R. PFEIFFER : in reference 2, Vol.1, Chap.4, 103.

4- R.F. SHUMARD and M.E. CALLENDER : Antimicrob. Agents Chemother., 1967, 369 (1968).

5- A.P. RAUN, C.O. COOLEY, E.L. POTTIER, R.P. RATHMACHER and L.F. RICHARDSON : J. Anim. Sci., 43 670 (1976).

6- M.D. RUFF : in reference 2, Vol. 1, Chap. 6, 303.

7- B.C. PRESSMAN and M. FAHIM : Annual Rev. Pharmac. Toxicol., 22, 465 (1982).

8- J.P. ROSAZZA and R.V. SMITH : Adv. Appl. Microbiol., 25, 169 (1979).

9- A. CUER, G. DAUPHIN and J.C. BELOEIL : J. Antibiotics, 36, 20 (1983).

10- A. BAX : "Two-Dimensional Nuclear Magnetic Resonance in Liquids", Delft University Press, London (1982).

11- G. BODENHAUSEN and R. FREEMAN : ^{13}C-^{1}H shift correlation by polarization transfer. J. Magn. Res., 28, 471 (1977).

12- A.A. MAUDSLEY and R.R. ERNST : Chem. Phys. Letters, 50, 368 (1977).

13- R. FREEMAN and A. MORRIS : J.C.S. Chem. Comm., 648 (1978).

14- H. SETO and N. OTAKE : Heterocycles, 17, 555 (1982).

15- F.N. WEHRLI and T. WIRTHLIN : "Interpretation of Carbon-13 NMR Spectra", P.37, Heyden, New-York, (1976).

16- J.A. ROBINSON and D.A. TURNER : J.C.S. Chem. Comm., 148 (1982).

17- J.C. BELOEIL, M.A. DELSUC, J.Y. LALLEMAND, G. DAUPHIN and G. JEMINET : J. Org. Chem., 49, 1797 (1984).

18- A. BAX and R. FREEMAN : J. Magn. Res., 44, 542 (1981).

19- M. ALLEAUME and D. HICKEL : J.C.S. Chem. Comm., 1422 (1970).

20- M. ALLEAUME and D. HICKEL : J.C.S. Chem. Comm., 175 (1972).

21- P. GACHON, G. CHAPUT, G. JEMINET, J. JUILLARD and J.P. MOREL : J.C.S. Perkin Transactions II, 907 (1975).

22- M. CHAPEL, G. JEMINET, P. GACHON, R. DEBISE and R. DURAND : J. Antibiotics, 32, 740 (1979).

23- A. CUER, G. DAUPHIN, G. JEMINET, J.C. BELOEIL and J.Y. LALLEMAND : Nouv. J. Chim. In press.