Structure and Conformation of Bioconversion Products of a Carboxylic Ionophorous Antibiotic, Grisorixin, by Means of Two-dimensional Nuclear Magnetic Resonance

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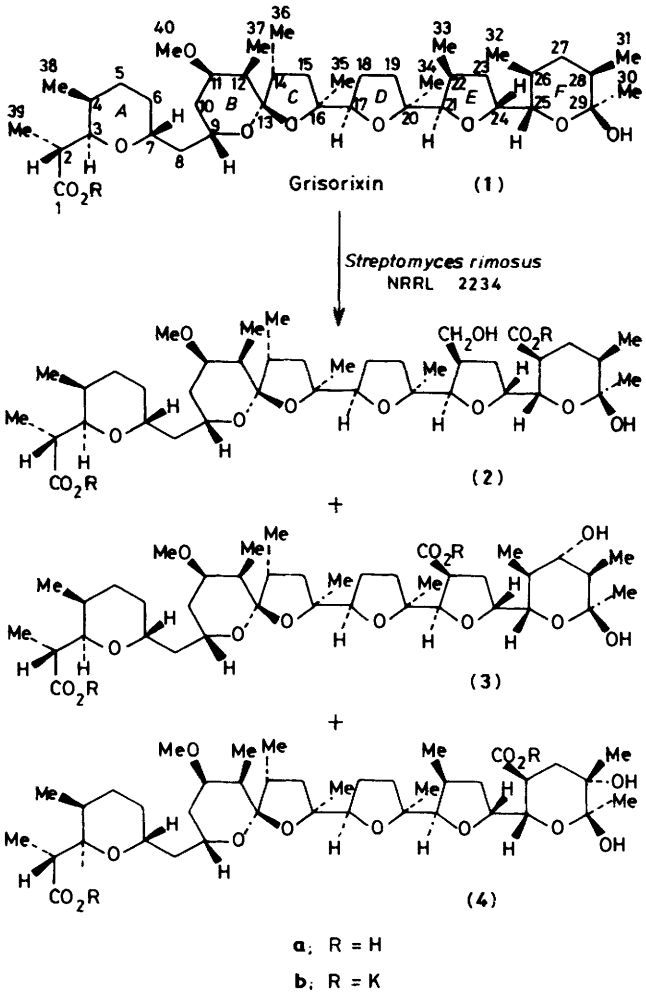
The structures of two bioconversion products of a carboxylic ionophorous antibiotic, grisorixin, have been determined by means of two-dimensional n.m.r. The potassium salt conformations of grisorixin and its bioconversion products are very similar. Grisorixin metabolites still complex the cations, but no longer transport them, and have no antibiotic activity.

Grisorixin[1] (**1a**), produced by a strain of *Streptomyces griseus* 2142N6, belongs to the polyether carboxylic ionophore family,[2] which are known for their ability to complex and transport cations through biological membranes.[2a] This ability results in a variety of biological properties which include antimicrobial activity against Gram-positive bacteria and fungi,[2b] anticoccidial activity against *Eimeria*,[2b] growth promotion in ruminants,[2c] and cardiovascular effects.[2d]

We have carried out a study of grisorixin metabolism using the microbial model method described by Rosazza,[3] with the aim of investigating detoxication mechanisms and the ensuing modifications of antibiotic properties, cation transport, and complexation of grisorixin.

Grisorixin is bioconverted by *Streptomyces rimosus* NRRL 2234 into three products: (**2a**), (**3a**), and (**4a**). One of these, (**2a**), has already been identified.[4] As large-scale separation of (**3a**) and (**4a**) was not feasible, two-dimensional n.m.r. correlation spectroscopy[5] (COSY and SECSY) was employed, as it allows structure determination with quantities of 5—10 mg.



Results and Discussion

The COSY ^1H–^1H chemical shift correlation[6] of (**3b**) (Figures 1 and 2) provides a ^1H scalar coupling relationship. Four groups of coupled protons, separated by quaternary carbons or heteroatoms, can be detected. The COSY procedure gives unequivocally the connections Me(39)–H(2)–H(3)–H(4)–Me(38) H(5A),H(5B)–H(6A),H(6B)–H(7)–H(8A),H(8B)–H(9)–H(10A),H(10B)–H(11)–H(12)–Me(37) (Figure 1), Me(36)–H(14)–H(15A),H(15B) (Figure 2), and H(17)–H(18A),H(18B)–H(19A),H(19B) (Figure 2). These three groups of coupled protons are identical in (**3b**) and (**1b**). In addition, the COSY procedure gives the last group of coupled protons of (**3b**) corresponding to ring *E* and *F*: H(21)–H(22)–H(23A),H(23B)– H(24)–H(25)–H(26)–Me(32) H(27)–H(28)–Me(31) (Figure 1). This group differs from that of grisorixin because H(22) lacks a methyl connection, and in the existence of a single H(27) signal at δ 3.10 (CHOH region), deshielded by 1.74 p.p.m. in relation to H(27) of (**1b**). Me(33) and one H(27) of grisorixin (**1a**) are substituted respectively by CO_2H and OH in (**3a**); substitutions are in agreement with the molecular formula found. The stereochemistry of H(27) is determined by the values of $J_{27,26}$ and $J_{27,28}$ (10.2 Hz), H(27) being antiperiplanar to both axial H(26) and H(28).

A similar procedure determines the structure (**4**). The SECSY ^1H–^1H chemical shift correlation[7] of (**4b**) (Figure 3) also gives four groups of coupled protons. The first three are identical with those of (**1b**) and (**3b**), and correspond to rings *A* and *B* for the first, *C* for the second, and *D* for the third. Chemical shifts for (**1b**) and (**4b**) are almost identical. The fourth group corresponds to rings *E* and *F*, and differs from the equivalent group in (**1b**) in the lack of Me(32) and H(28) (Figure 3): both are substituted, respectively by CO_2H and OH, in (**4a**), in agreement with the molecular formula found.

The ^{13}C n.m.r. spectrum (100 MHz) confirms the structures (**3b**) and (**4b**). Only carbon atoms 22, 23, 26, 27, 28, 31, 32, and 33 for (**3b**), and 25, 26, 27, 28, 30, 31, and 32 for (**4b**), give differences in chemical shifts (Table 1), and the differences are consistent with the substitution effects.[8] Straightforward application of the substitution effects method in ^{13}C n.m.r. to structure determination is often an insecure method, whereas the use of

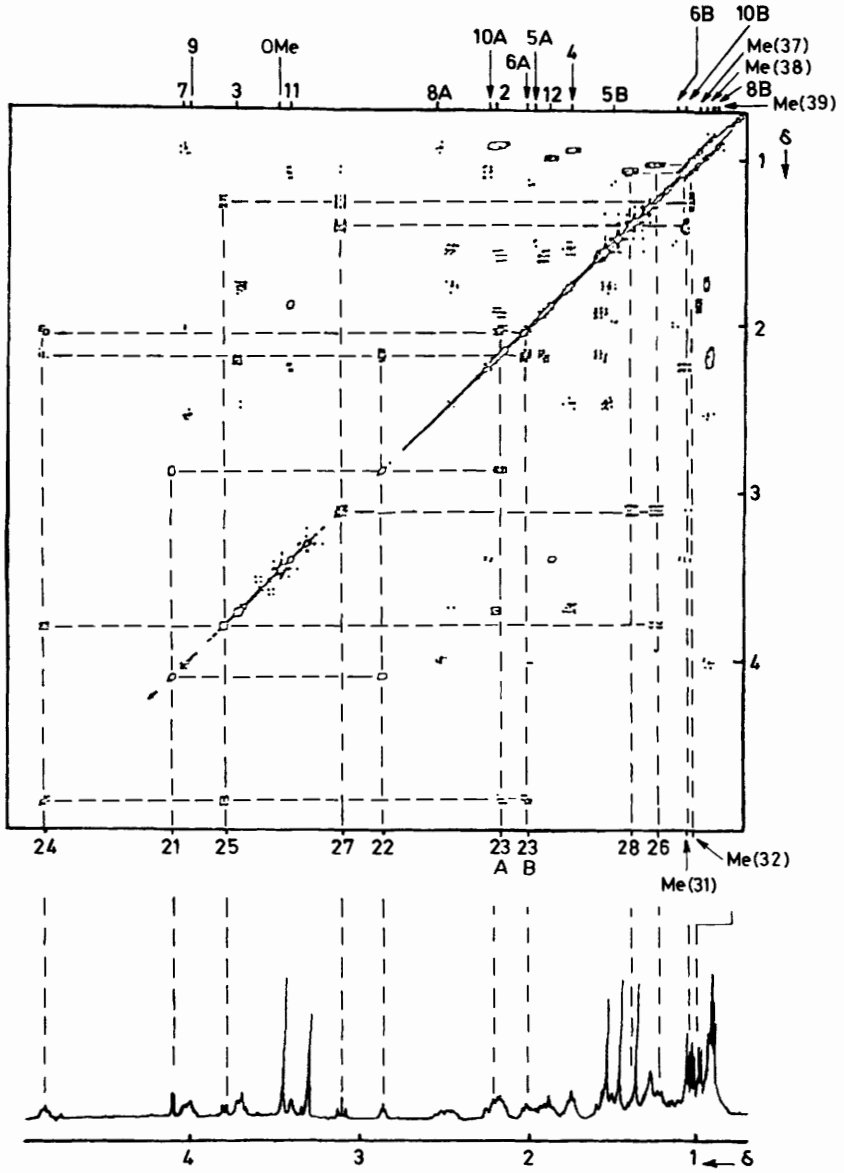


Figure 1. 400 MHz COSY spectrum of (**3b**) in CD_3OD at 298 K presented as a contour plot

^1H–^1H correlation spectroscopy gives accurately the structures (**3**) and (**4**).

Determination of coupling constants obtained from the one-dimensional ^1H spectrum through double-resonance experiments, or from a two-dimensional J–$\delta(^1H)$ correlation [5] (Table 2), allows us to elucidate the conformations (**3b**) and (**4b**) in CD_3OD solution. N.m.r. data of rings *A*, *B*, and *C* are the same as for grisorixin potassium salt (**1b**) (Tables 1 and 2).

In ring *D* of (**3b**), H(18A) and H(19B) are deshielded by 0.60 and 0.44 p.p.m., respectively, because of steric hindrance by the carboxylate group; $\delta(^{13}C)$ values are the same. In (**4b**), ^1H chemical shifts of ring *D* are distinct (Table 1); coupling constants have been determined (Table 2). Ring *D* has the same conformation in (**3b**) and (**4b**) as in (**1b**).

Rings *E* and *F* are affected by bioconversion in (**3b**), but only ring *F* in (**4b**). The coupling constants observed for these two rings are the same as in (**1b**); except for local substitution effects, $\delta(^{13}C)$ and $\delta(^1H)$ are not changed.

The positions of the acyclic moieties of these molecules are of great importance because they determine their conformations. The C(2)–C(3) system is equatorial to ring *A*; $J_{2,3}$ of (**3b**) and (**4b**) is the same as in (**1b**) (Table 2): H(2) is antiperiplanar to H(3). The 'hinge' C(7)–C(8)–C(9) in (**3b**) and (**4b**) is characterized by large coupling constants $J_{7,8A}$ and $J_{8B,9}$, which indicate antiperiplanar positions for these protons, as in (**1b**). The C(7)–C(8) bond is axial to ring *A* and C(8)–C(9) equatorial to ring *B*. The value of $J_{24,25}$ is the same in (**3b**) and (**4b**) as in (**1b**), and corresponds to a torsion angle smaller than 60°.

Without taking into account local effects of substitution, similiar n.m.r. data lead to the conformation shown, nearly the same in the three molecules (**1b**), (**3b**), and (**4b**).

Data obtained for grisorixin potassium salt in $CDCl_3$ [9] (head-to tail hydrogen bonding) and for the bioconversion product (**2b**) [10] lead to the same conformation.

Conductimetric measurements were carried out on the 1:1 neutral complexes of grisorixin with alkali cations in methanol [11] and show a selectivity maximum for K^+: this is related to the existence of an equilibrium complexing cavity

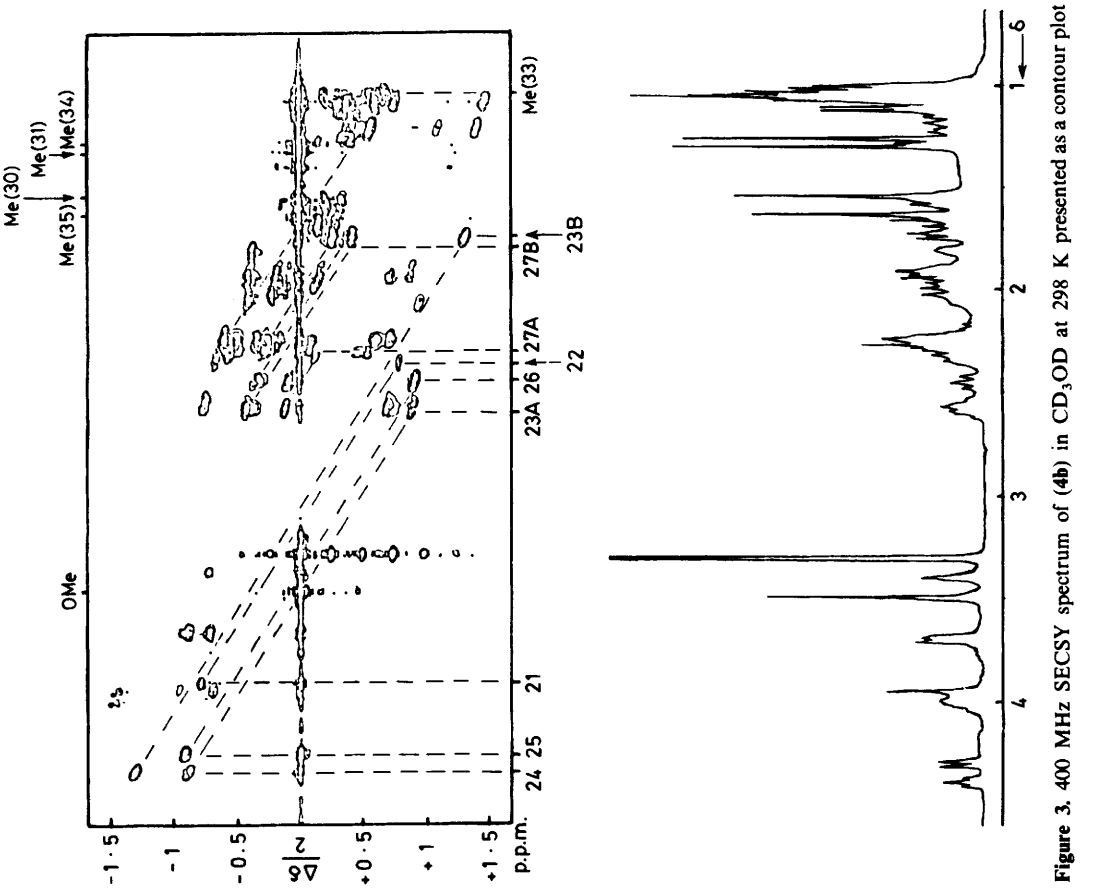


Figure 3. 400 MHz SECSY spectrum of (**4b**) in CD_3OD at 298 K presented as a contour plot

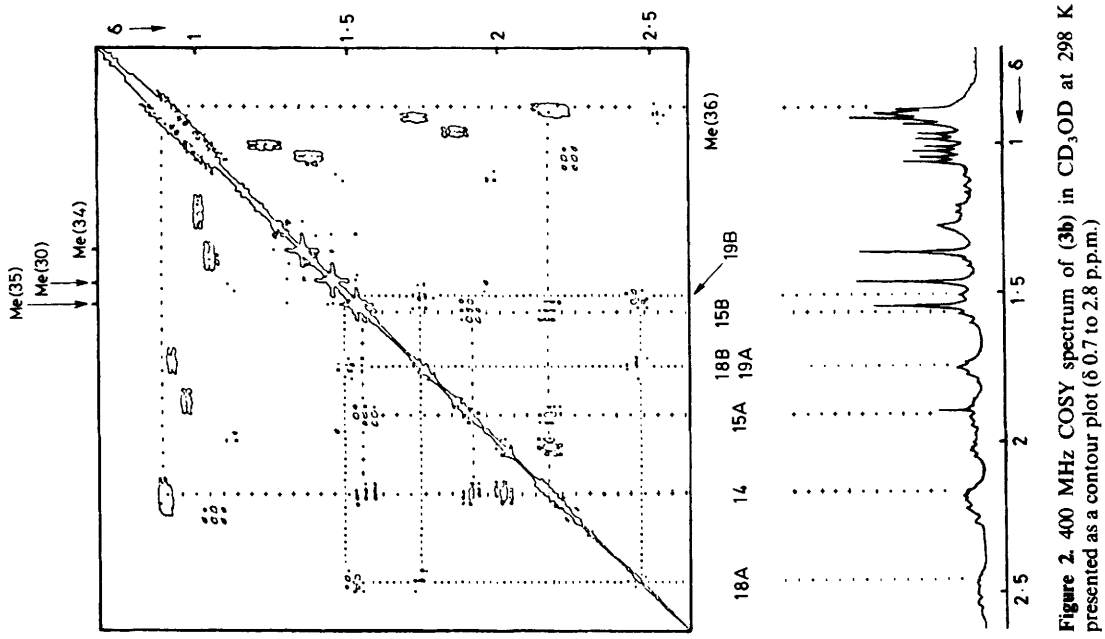


Figure 2. 400 MHz COSY spectrum of (**3b**) in CD_3OD at 298 K presented as a contour plot (δ 0.7 to 2.8 p.p.m.)

Table 1. Chemical shifts (^{13}C and ^{1}H) of (**1b**), (**3b**), and (**4b**) in CD_3OD

	(1b)		(3b)		(4b)	
Carbon	δ(^{13}C)[a]	δ(^{1}H)	δ(^{13}C)[a]	δ(^{1}H)	δ(^{13}C)[a]	δ(^{1}H)
1	183.8		184.1		183.9	
2	46.7	2.19	46.9	2.09	46.8	2.22
3	75.3	3.68	75.3	3.70	75.3	3.70
4	29.3	1.74	29.2	1.73	29.2	1.79
5	27.4	1.97—1.51	27.4	1.97—1.49	27.4	2.01—1.56
6	24.6	1.99—1.18	24.6	2.00—1.12	24.5	2.05—1.22
7	69.6	3.96	69.7	4.03	69.7	3.97
8	36.9	2.56—0.99	36.7	2.51—0.93	38.7	2.55—1.03
9	62.0	3.97	62.0	4.00	62.0	4.01
10	32.6	2.22—1.14	32.5	2.24—1.07	32.5	2.29—1.16
11	80.2	3.41	80.2	3.38	80.2	3.39
12	38.0	1.88	38.0	1.86	38.1	1.92
13	109.3		109.4		109.4	
14	40.7	2.18	40.6	2.18	40.6	2.23
15	42.9	1.93—1.62	42.9	1.91—1.58	42.9	1.95—1.64
16	83.2		83.2		83.2	
17	83.3	3.69	83.8	3.67	83.2	3.69
18	26.6	1.84—1.84	26.9	2.44—1.76	26.4	1.89—1.54
19	30.8	2.21—1.08	30.7	1.76—1.52	30.7	2.25—1.10
20	85.6		85.6		85.6	
21	87.4	3.93	88.7	4.09	87.3	3.94
22	36.6	2.34	50.9	2.85	36.7	2.33
23	33.2	2.44—1.55	29.1	2.16—2.12	33.3	2.58—1.72
24	78.7	4.43	76.2	4.82	79.4	4.38
25	77.9	3.66	75.3	3.78	73.7	4.29
26	33.6	1.37	41.3	1.24	44.3	2.45
27	37.9	1.36—1.36			30.6	2.26—1.73
27			80.5	3.10		
28	41.5	1.51	46.9	1.39		
28					72.5	
29	98.4		99.8		99.7	
30	26.3	1.46	26.8	1.46	22.4	1.53
31	17.3	0.96	13.4	1.06	25.6	1.28
32	17.8	0.94	13.4	1.02		
32					181.6	
33	15.8	0.98			15.6	0.98
33			180.9			
34	23.0	1.21	23.7[b]	1.36	22.9[b]	1.24
35	29.0	1.57	29.5[b]	1.55	29.5[b]	1.62
36	13.5	0.98	13.4	0.90	13.6	1.02
37	13.5	1.07	12.9	0.98	13.4	1.09
38	11.7	1.00	11.6	0.94	11.6	1.04
39	14.1	0.98	14.1	0.91	13.9	1.02
40	58.6	3.48	58.5	3.45	58.9	3.49

[a] Multiplicity given by *J*-modulated spin-echo spectrum. [b] Attributed by comparison with grisorixin potassium salt in CD_3OD solution.[10]

Table 2. Apparent coupling constants of (**1b**), (**3b**), and (**4b**) in CD_3OD; the codings A and B refer respectivly to the proton at lower and higher field site

(1b)		(3b)		(4b)	
Proton pair	^{3}J/Hz	Proton pair	^{3}J/Hz	Proton pair	^{3}J/Hz
2,3a	10.3	2,3a	10.1	2,3a	10.0
2,Me(39)	7.0	2,Me(39)	7.1	2,Me(39)	7.0
3a,4e	2.8	3a,4e	2.6	3a,4e	
4e,Me(38)	7.0	4e,Me(38)	7.0	4e,Me(38)	7.0
4e,5Aa	5.0	4e,5Aa		4e,5Aa	
4e,5Be	1.1	4e,5Be		4e,5Be	1.0
5A,5B	12.0	5A,5B		5A,5B	12.2
5Aa,6Aa	11.8	5Aa,6Aa		5Aa,6Aa	
5Aa,6Be		5Aa,6Be		5Aa,6Be	
5Be,6Aa	3.0	5Be,6Aa		5Be,6Aa	3.6
5Be,6Be		5Be,6Be		5Be,6Be	1.0
6A,6B	13.5	6A,5B		6A,6B	
6Aa,7e	5.5	6Aa,7e		6Aa,7e	
6Be,7e	1.0	6Be,7e		6Be,7e	
7e,8A	13.1	7e,8A	13.2	7e,8A	14.0
7e,8B	3.7	7e,8B	3.0	7e,8B	3.0
8A,8B	13.2	8A,8B	13.2	8A,8B	14.0
8A,9a	4.6	8A,9a	4.8	8A,9a	4.8
8B,9a	11.5	8B,9a	11.0	8B,9a	10.5
9a,10Ae	2.0	9a,10Ae	2.2	9a,10Ae	3.0
9a,10Ba	10.9	9a,10Ba	11.0	9a,10Ba	10.5
10A,10B	14.3	10A,10B	14.0	10A,10B	14.1
10Ae,11e	2.0	10Ae,11e	3.0	10Ae,11e	2.0
10Ba,11e	3.0	10Ba,11e	3.0	10Ba,11e	2.8
11e,12a	4.0	11e,12a	4.1	11e,12a	3.9
12a,Me(37)	7.0	12a,Me(37)	7.2	12,Me(37)	7.2
14,15A	8.6	14,15A	8.9	14,15A	8.8
14,15B	11.5	14,15B	11.5	14,15B	11.5
14,Me(36)	7.0	14,Me(36)	7.0	14,Me(36)	7.0
15A,15B	11.5	15A,15B	12.6	15A,15B	12.6
17,18A	17.0	17,18A	16.0	17,18A	8.7
17,18B		17,18B		17,18B	8.7
21,22	4.0	21,22	4.8	18A,18B	12.0
22,23A	7.0	22,23A	7.5	18A,19A	10.2
22,23B	1.0	22,23B	1.0	18A,19B	1.0
22,Me(33)	7.0	22,Me(33)		18B,19A	5.1
23A,23B	12.3	23A,23B	12.3	18B,19B	1.0
23A,24	9.0	23A,24	9.4	19A,19B	12.1
23B,24	7.1	23B,24	7.3	21,22	4.0
24,25a	2.9	24,25A	2.9	22,23A	6.8
25a,26a	10.3	25a,26a	10.8	22,23B	1.0
26,Me(32)	6.5	26,Me(32)	6.7	22,Me(33)	7.0
26,27A		26a,27a	10.2	23A,23B	12.8
26,27B				23A,24	10.0
27A,27B				23B,24	6.9
27A,28a		27a,28a	10.2	24,25a	2.8
27B,28a				25a,26a	10.7
28a,Me(31)	6.9	28,Me(31)	6.7	26a,27Aa	13.2
				26a,27Be	3.7
				27A,27B	13.2

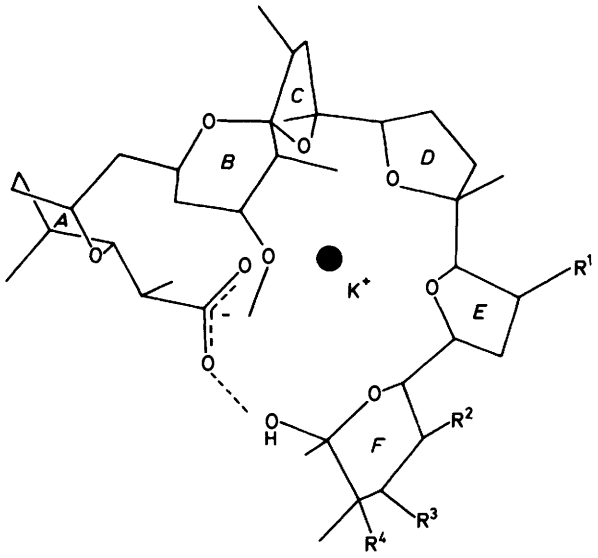
assumed to be similar to that described in the crystallographic studies on Ag^+ or Tl^+.[12] Thus (**3**) and (**4**) should complex cations in the same manner as grisorixin.

The principal difference between grisorixin and its bioconversion products is the alteration of the amphiphilic balance: this is due to the introduction of polar groups in the external lipophilic region of the molecule, and corresponds to a detoxification procedure. Grisorixin metabolites still complex the cations, but no longer transport them, and have lost all antibiotic activity: this result demonstrates the relationship between transport and antibiotic character.

Experimental

Isolation of the Bioconversion Products (**2a**), (**3a**), *and* (**4a**).—Grisorixin was added to a *Streptomyces rimosus* NRRL 2234 culture as previously described.[4] After reaction, the culture was filtered; the filtrate was saturated with ammonium sulphate and extracted with ethyl acetate. The extract was separated by medium-pressure column chromatography [silica gel; $CHCl_3$–MeOH (9:1)] and by preparative thin-layer chromatography (silica gel LS 254 plates). Pure bioconversion products were obtained in 20% yield for (**2a**), 12% for (**3a**), and 8% for (**4a**).

Preparation of the Potassium Salts (**3b**) *and* (**4b**).—To a solution of (**3a**) [or (**4a**)] in ethanol–water (1:1) was added 0.1N-KOH to pH 10 (pH meter). The mixture was evaporated and the residue washed with Et_2O and filtered off; (**3b**) had m.p. 169—170 °C; $[\alpha]_D^{20}$ +2.0° (*c* 0.025 in Me_2CO); fast-atom bombardment (FAB) positive ion, *m/z* 831, $(M + H)^+$; (**4b**) had m.p. 211—212 °C; $[\alpha]_D^{20}$ +3.6° (*c* 0.025 in Me_2CO); FAB positive ion, *m/z* 831, $(M + H)^+$.



	R^1	R^2	R^3	R^4
(1b)	CH_3	CH_3	H	H
(3b)	$CO_2^-K^+$	CH_3	OH	H
(4b)	CH_3	$CO_2^-K^+$	H	OH

N.m.r. Data.—The n.m.r. spectra were recorded with a Brüker WM-400 spectrometer.

COSY. The applied pulse sequence was [90°–t_1–45°–FID(t_2)]. The spectral width in F_1 and F_2 was 4 000 Hz; the number of data points in t_2 was 2 048, and 512 increments were recorded. Before Fourier transformation and symmetrisation, the data were multiplied with unshifted sine bell. Total acquisition time was 10 h.

SECSY. The applied pulse sequence was [90°–t_1/2–90°–t_1/2–FID(t_2)]. The spectral width in F_1 was 2 000 Hz and in F_2 4 000 Hz; the number of data points in t_2 was 1 024, 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.

J–δ correlation. The applied pulse sequence was [90°–t_1/2–180°–t_1/2–FID(t_2)]. The spectral width in F_2 was 4 000 Hz and in F_1 62.5 Hz; the number of data points in t_2 was 4 096, and 512 increments were recorded. Before Fourier transformation, the data were multiplied with Lorentz-Gauss. Zero filling was applied in each dimension. Total acquisition time was 4 h.

Acknowledgements

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