EPIGRISORIXIN, A NEW POLYETHER CARBOXYLIC ANTIBIOTIC

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Grisorixin[1] (**1**) belongs to the group of carboxylic polyether antibiotics. Some of them are used as feed additives acting as anticoccidial agents and growth promoters in ruminants[2].

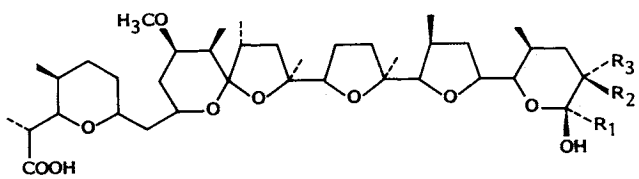
The biosynthesis of this antibiotic family has attracted a great deal of interest. In the course of our study on the biosynthesis of the carboxylic polyether antibiotic, nigericin (**2**), produced by *Streptomyces hygroscopicus* NRRL B-1865, the addition of methyl oleate (1%) to the original fermentation culture increased the antibiotic titer[3]. Under these conditions, three nigericin-type antibiotics were isolated, grisorixin (**1**) (unpublished data), abierixin[3] and epinigericin[4] (**3**).

The comparative biological activity of the two epimeric antibiotics, nigericin and epinigericin on a protist *Tetrahymena pyriformis* GL[5] and *Bacillus cereus*[4] showed that epinigericin was less toxic than nigericin.

The present paper describes the ability of the microorganism *S. hygroscopicus* NRRL B-1865 to biotransform grisorixin to epigrisorixin (**4**) which may be also less toxic and the isolation and identification of the bioconversion product.

After incubation in the antibiotic production medium[3] contained in a 100 ml-Erlenmeyer, at 27°C on a rotatory shaker for 24 hours before the antibiotic biosynthesis began, the *S. hygroscopicus* cells were collected by centrifugation and filtration and washed with a 0.3 M sucrose solution. Then the cells were suspended in 100 ml of bioconversion medium (0.4 g/liter sucrose, 0.05 g/liter $MgCl_2$, 0.05 g/liter $CaCl_2$ and 0.05 g/liter TES buffer) at pH 7.0. Grisorixin was added as a DMSO solution (20 mg/ml). The reaction was allowed to proceed for 48 to 72 hours. Under these conditions, grisorixin was weakly transformed to a novel product G1. The Rf of this metabolite is 0.35 (TLC, Merck 60F-254, cyclohexane - EtOAc - formic acid 60 : 40 : 0.5), whereas the Rf of grisorixin is 0.5. No product of bioconversion could be detected in test experiments where grisorixin or cells were omitted. The biotransformation yield was greatly increased with induced cells. In the *S. hygroscopicus* antibiotic production medium, a small amount of grisorixin (1 to 5 mg/100 ml) was added 2 hours after incubation. Cells were collected 24 hours after incubation. Grisorixin was then added into the bioconversion medium under the same conditions as above.

The new metabolite G1 was isolated by ethanol extraction of the mycelium and purification was achieved by a flash-chromatography column[6] (Merck silicagel 0.025 ~ 0.040 mm, using cyclohexane with increasing amounts of EtOAc). The antibiotic was obtained as a colorless powder soluble in organic solvents but not in water. Mass spectrometry combined with FAB desorption[7] using *meta*-nitrobenzylalcohol as matrix resulted in the molecular mass determination of biotransformed product. Actually, under these experimental



Grisorixin (**1**)	$R_1 = CH_3$	$R_2 = CH_3$	$R_3 = H$
Epigrisorixin (**4**)	$R_1 = CH_3$	$R_2 = H$	$R_3 = CH_3$
Nigericin (**2**)	$R_1 = CH_2OH$	$R_2 = CH_3$	$R_3 = H$
Epinigericin (**3**)	$R_1 = CH_2OH$	$R_2 = H$	$R_3 = CH_3$

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conditions†, cationized molecules are formed such as: *m/z* 731 $(M+Na)^+$ and *m/z* 747 $(M+K)^+$. This ion assignment was confirmed by using a mixture of KCl solution to matrix which enhanced the desorption of cationized molecules with potassium: $(M+K)^+$ at *m/z* 747 and $(M+2K-H)^+$ at *m/z* 785. With addition of INa, only $(M+Na)^+$ at *m/z* 731 was observed. A similar behavior characterizes grisorixin desorption in the same experimental conditions.

By using MS/MS methods[8,9], the assumed cationized molecule, *m/z* 731, submitted to low energy collisions†† in collision cell of tandem mass spectrometer led to formation of the *m/z* 23 ion $(Na)^+$ as the main daughter ion. Furthermore, in the CAD spectrum of *m/z* 731 under lower collision energy conditions ($E_{lab}=50$ eV), fragment ions at *m/z* 713, 699 and 688 appeared, due to low mass neutral eliminations such as H_2O, CH_3OH and CO_2, respectively[10]. These fragmentations suggest the presence of OH, CH_3O– and CO_2– groups. Cleavage of ionophore skeleton also takes place and led to formation of *m/z* 485 and *m/z* 237. The same daughter ions are displayed in CAD spectrum of grisorixin (recorded in the same collision conditions). However, a significative abundance change characterized the abundance of the produced daughter ions. This similarity observed for both ion species (*m/z* 731) generated from biotransformed compound and grisorixin indicates that these polyethers contain a common skeleton with one (or more) stereochemical changes at certain sites (molecular weight 708).

The 13-carbon NMR spectra (recorded on Brücker MSL 300 spectrophotometer) of K-salt of grisorixin and epigrisorixin (in C_6D_6) gave further structural information. Both antibiotics contained 40 carbons. The *J*-modulated spin-echo ^{13}C NMR spectra gave multiplicities leading to preliminary ^{13}C assignments. All assignments were then made by first comparison with grisorixin (Table 1). The ^{13}C NMR spectra revealed chemical changes for eight carbons, principally for the F-ring carbons. The shift changes, including a marked upfield shift of the C-26 signal, are compatible with a structure in which the methyl-31 of the new metabolite is epimeric to that of grisorixin (γ-gauche effect). We observed the same results with epinigericin whose structure was investigated by 1D and 2D ^1H and ^{13}C NMR spectrometry[4]. The epimerization at C-28 of grisorixin was explained by the opening of the ring F to the ketone intermediate.

Table 1. Assignments of the ^{13}C NMR spectra of K-salt of grisorixin and biotransformed product G1 in C_6D_6, relative to TMS.

C	Functional group	K-Grisorixin	K-product G1
1	–COOH	181.5	181.4
2	$-CH(CH_3)$	46.0	46.1
3	–CH(O)	74.5	74.6
4	$-CH(CH_3)$	28.2	28.2
5	$-CH_2$	27.0	27.0
6	$-CH_3$	23.9	23.9
7	–CH(O)	68.4	68.4
8	$-CH_2$	36.1	36.1
9	–CH(O)	61.3	61.4
10	$-CH_2$	31.3	31.3
11	$-CH(OCH_3)$	78.5	78.6
12	$-CH(CH_3)$	37.3	37.1
13	–O–C–O	108.0	108.0
14	$-CH(CH_3)$	39.7	39.7
15	$-CH_2$	42.3	42.3
16	$-C-O-(CH_3)$	81.9	81.9
17	–CH(O)	82.2	82.2
18	$-CH_2$	25.9	25.7
19	$-CH_2$	29.8	29.9
20	$-C-O(CH_3)$	84.2	84.3
21	–CH(O)	85.9	85.9
22	$-CH(CH_3)$	35.4	35.4
23	–CH	32.1	32.3
24	–CH(O)	77.5	77.5
25	–CH(O)	76.3	76.8
26	$-CH(CH_3)$	32.9	25.3
27	$-CH_2$	37.1	35.7
28	$-CH(CH_3)$	41.4	38.6
29	–O–C–OH	98.0	98.0
30	$-CH_3$	25.9	25.3
31	$-CH_3$	17.4	16.5
32	$-CH_3$	14.4	17.7
33	$-CH_3$	15.4	15.4
34	$-CH_3$	22.6	22.7
35	$-CH_3$	29.1	29.1
36	$-CH_3$	13.4	13.4
37	$-CH_3$	13.4	13.4
38	$-CH_3$	11.4	11.4
39	$-CH_3$	14.9	14.7
40	$-OCH_3$	58.3	58.3

The minimal inhibitory concentration (MIC) of grisorixin and epigrisorixin against *Bacillus cereus* are 2×10^{-4} and 5×10^{-4} μg/ml, respectively (MIC for nigericin and epinigericin are 1×10^{-4} and

† FAB mass spectra were performed on a triple quadripole instrument (R30-10 Nermag) equipped with a fast-atom-bombardment gun.

†† $E_{lab}=50$ eV as parent ion kinetic energy, within single collision conditions.

2×10^{-4} μg/ml in the same conditions). Epigrisorixin and epinigericin are less toxic than grisorixin and nigericin respectively. The epimerization by *S. hygroscopicus* NRRL B-1865 corresponds to a detoxification process. The increased transformation yield of grisorixin with induced cells showed that epimerization was produced by enzymatic pathway.

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