MICROBIAL CONVERSION OF NIGERICIN IN THREE SUCCESSIVE STEPS, BY *SEBEKIA BENIHANA*

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A strain of *Sebekia benihana* NRRL 11111 was found to transform nigericin in three successive steps, giving three compounds which were isolated. Their structure were determined by IR, ^{1}H and ^{13}C NMR, and fast atom bombardment mass spectra.

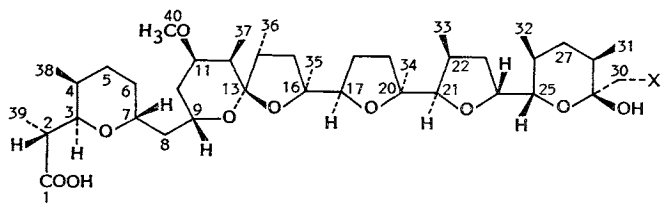
The first compound resulted from the reduction of the terminal hemiketal ring it was then transformed into the two other compounds as a result of the oxidation of methyl (C-33) into a CH_2OH and COOH group respectively. All these products had lost the ionophoric and antibiotic properties of nigericin and thus were products of a detoxification process.

Carboxylic polyether antibiotics belong to a well-documented ionophore family[1], among which several components are currently used as anticoccidial compounds for poultry[2] or to improve the efficiency of feed utilization in ruminant animals[3].

Apart from specific work carried out on monensin[4], calcimycin[5] and more recently on salinomycin[6], little information is to our knowledge available on the enzymatic transformation of these compounds by soil-decontaminating microorganisms.

We started a few years ago with the study of grisorixin (**1**) bioconversion by a strain of *Streptomyces rimosus* NRRL 22343[7~9]. This approach to detoxification was based on a microbial method described by ROSAZZA and SMITH[10]. The main difference between grisorixin (**1**) and its biotransformed products was the alteration of the amphiphilic balance of the skeleton, polar groups being introduced in the external lipophilic region of the molecule. Under the same conditions nigericin (**2**), closely related to grisorixin (**1**), was not significantly transformed. However, it must be noted that the high instability of the *S. rimosus* strain used prevented a thorough investigation.

Thus, we searched for another strain capable of bioconverting nigericin (**2**). *Sebekia benihana* NRRL 11111, described recently for its ability to hydroxylate novobiocin[11], proved to be of interest for this purpose. This paper deals with the structure determination of the three major products obtained by this process. Furthermore, the detoxification mechanism will be discussed in connection with chemical work previously done in our group[12].



Grisorixin (**1**) X=H
Nigericin (**2**) X=OH

Experimental

Nigericin was obtained from cultures of the strain *Streptomyces hygroscopicus* NRRL 1860 in our laboratory.

Culture and Reaction Conditions

(a) Bioconversion with Whole Cells: The procedure for biotransformation of nigericin by *S. benihana* NRRL 11111 was as follows. The strain was maintained as frozen mycelium. 7 ml of which were incubated into 100 ml of the seed medium (glucose 3%, cornsteep solids 0.5%, soyabean meal 0.5%, yeast extracts 0.3% and $CaCO_3$ 0.5% in tap water, pH adjusted to 7.0 by NaOH). The flask was cultivated on a rotary shaker at 27°C for 72 hours. A 5-ml aliquot was inoculated in 500-ml Erlenmeyer flasks containing 100 ml of TYG medium (Tryptone 0.5%, yeast extract 0.3%, glucose 1%, pH adjusted to 7.2 by NaOH). After agitation for 48 hours, nigericin in the acid form was added as an ethanolic solution (20 mg in 2 ml). The reaction was allowed to proceed for 24, 48 or 72 hours, respectively.

(b) Bioconversion with Cell Free Extracts: The strain of *S. benihana* NRRL 11111 was cultivated as previously described. After shaking for 48 hours, the cells were collected by filtration and washed three times with 0.05 M Tris-HCl buffer, at pH 7.0. The washed cells were resuspended to 1/10 of the original culture volume in 0.05 M Tris-HCl buffer and put in the French press maintained at −25°C. After freezing for 5 hours, cells were passed four times through the French pressure cell at 2,000 kg/cm^2. After thawing, the cell free extract was stored frozen in 5-ml fractions at −25°C.

For the bioconversion process, 25 ml of cell free extracts were diluted with 25 ml of Tris-HCl buffer in 100-ml flasks, and 50 mg of nigericin were added 6 each flask as a solid. Reaction mixtures were incubated at 27°C for 1, 2 or 5 hours, respectively.

(c) Detection and Isolation of the Bioconversion Products: The kinetics of bioconversion were followed by taking a 20-ml analytical sample, filtering, saturating the filtrate with ammonium sulfate and extracting with ethyl acetate. The extract was chromatographed on Silica gel TLC plates (Merck 60F-254) with $CHCl_3$ - MeOH (9 : 1). The conversion products were detected by using a spray reagent (4 g of vanillin, 4 ml concentrated sulfuric acid, 100 ml MeOH) followed by heating at 100°C. Rf values of conversion products were nigericin: 0.69, N1: 0.20, N2: 0.069, N3: 0.023. Ethyl acetate extracts containing mainly N1 (after 24 hours), N2 (after 48 hours) or N3 (after 72 hours) were chromatographied. The bioconversion of nigericin, from TLC estimations, was complete, and after extraction and purification the final yield was about 50%.

Chemistry

Nigericin was reduced by sodium borohydride[13], the two diastereoisomers obtained (70% DN1, 30% N1) were separated by column chromatography using a MeOH - $CHCl_3$ gradient (2 to 5% MeOH in $CHCl_3$), N1 was eluted first (Rf N1: 0.20, DN1: 0.12, TLC, $CHCl_3$ - MeOH (9 : 1)).

Spectroscopy

IR spectra in KBr were recorded on a Perkin-Elmer 377 spectrometer, fast atom bombardment mass spectra (FAB-MS) on a VG 70F spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 25°C, with the wavelength λ 578 nm which is the most frequently used by authors.

All the 1D and 2D spectra of nigericin, N1, N2, N3 and DN1 were recorded on a Bruker MSL 300 spectrometer in $CDCl_3$ solution (from 30 to 70 mg of products in 500 μl).

COSY: The two-dimensional correlated ^{1}H NMR experiments were performed at 300.13 MHz. The applied pulse sequence was $(\pi/2)-t_1-(\pi/4)-$FID, t_2. The spectral width in F1 and F2 was 1,119 Hz; the number of data points in t_2 was 2,048, and 128 increments were recorded (NS=96). Before Fourier transformation, the data were multiplied with an unshifted sine bell. The $\pi/2$ pulse was 8.5 μs.

^{1}H-^{13}C Shift Correlation: The applied pulse sequence was $(\pi/2, {}^1H)-(t1/2)-(\pi, {}^{13}C)-(t1/2)-(\tau_1)-(\pi/2, {}^1H; \pi/2, {}^{13}C)-(\tau_2)-$(BB, ^{1}H; FID, t_2) with τ_1: 0.003570 second and τ_2: 0.001785 second. The spectral width in F1 was 1,119 Hz and in F2 6,667 Hz; the number of data points in t_2 was 2,048,

and 128 increments were performed (NS=768). Before Fourier transformation, the data were multiplied with an unshifted sine bell in F2 and with an exponential in F1. The $\pi/2$ pulse was 3.5 μs for ^{13}C and the decoupler $\pi/2$ pulse was 10 μs.

For ^{1}H-^{13}C "long range" shift correlations, the parameters were identical to those used for ^{1}H-^{13}C shift correlation except for τ_1=0.05 second and τ_2=0.025 second.

Results and Discussion

S. benihana, a new soil bacterium[11], was shown to be quite resistant against nigericin, for which the MIC, measured by the conventional dilution method in TYG medium at pH 7, is above 300 μg/ml. In our experiment we used nigericin at a concentration of 200 μg/ml. Under these conditions nigericin was quantitatively converted to N1 after 24 hours. Twenty-four hours later N1 was converted to N2 which finally transformed into N3 after 24 more hours (Fig. 1).

The order of polarity for the bioconverted products was as follows: N3>N2>N1>nigericin. None of these products could be detected in a test experiment where nigericin was omitted.

Each bioconversion product was isolated and analyzed at each step according to the methods described in Experimental section (c).

An alternative method was the bioconversion of nigericin using cell free extracts. After 2 hours nigericin was converted to N1. The advantage of this method is its speed, provided a stock of frozen cell free extracts is available.

Identification of N1, N2 and N3 Structures

Physico-chemical properties of nigericin, N1, N2 and N3 (free acid products) are listed in Table 1. IR spectra of free acids in KBr reveal the same general features for these compounds. The main

Fig. 1. Bioconversion of nigericin by *Sebekia benihana*.

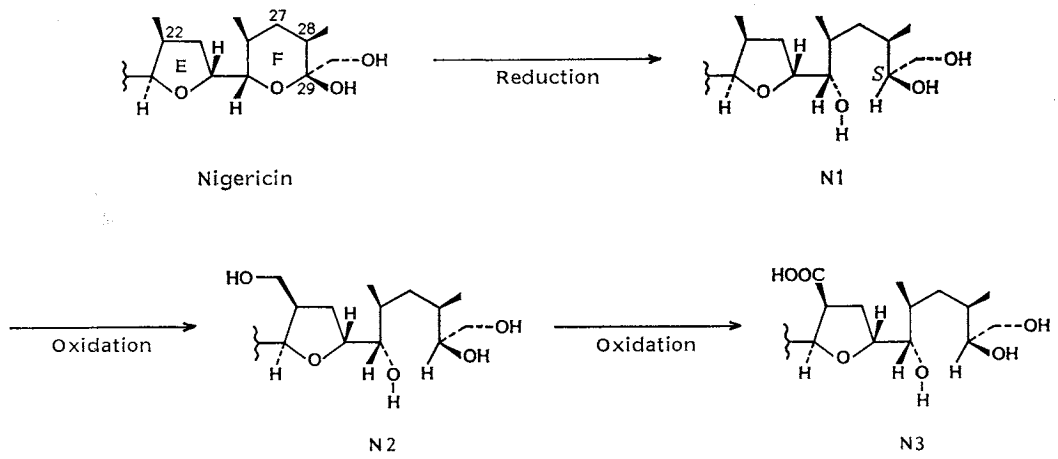
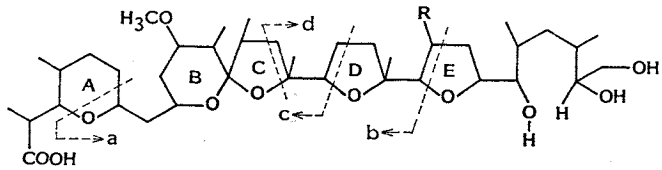


Table 1. Physico-chemical properties of nigericin and derivatives (free-acids).

	Nigericin	N1	N2	N3	DN1
MW	724	726	742	756	726
MP (°C)	108~110	99~101	92~95	96~101	78~82
$[\alpha]_D^{20}$ (Me_2CO)	+26.2 (c 19.5×10^{-3})	+26.13 (c 6.2×10^{-3})	+21.69 (c 11.8×10^{-3})	+17.31 (c 5.2×10^{-3})	+17.5 (c 14×10^{-3})

Fig. 2. Main fragmentations of bioconversion products obtained in FAB experiments.



N1 R=CH_3
N2 R=CH_2OH
N3 R=COOH

Table 2. FAB-MS data of nigericin and derivatives.

Proposed ion assignments	N1 (acid)[a]	N2 (acid)[a]	N3 (acid)[a]	N3 (Na)[b]	DN1 (acid)[a]
FAB(+)					
MNa^+	749	765	779	801	749
MK^+	765	781	795		765
a	607*	623*	637*		607*
b	545	561	575		545
d	393	408.9*	423		
e	308.9				
MH^+				779	
$M(-H^+)+Na^+$				823	
FAB (−)					
$M(-H^+)$	725	741	755		725
$M(-2H^+)+Na^+$			777		
$M(-H^+)$				777	
$M(-Na^+)$				755	

[a] Acid form M(N1)=726, M(N2)=742, M(N3)=756, M(DN1)=726.
[b] Na^+ form M=756 (acid form)−1 (H^+)+23 (Na^+)=778.
* Weak intensity.

absorption frequencies present in nigericin[14] can be found; a broad band between 3400 and 3100 cm^{-1} corresponding to ν OH, an intense band at 1725 cm^{-1} due to ν C=O (COOH) and the usual sharp intense band at 950 cm^{-1} caracteristic of ν_{as} C-O-C in a five membered ring. In the ν_{as} C-O-C complex region between 1120 and 1020 cm^{-1}, the relative intensities of the different bands are clearly modified if one compares the nigericin spectrum to N1, N2 and N3 spectra, which indicate some important modification in the heterocycle structure.

The analysis of FAB-MS (positive and negative ionization) of N1, N2, N3 (free acids) and N3 (Na) can be patterned according to the fragmentation scheme proposed by SIEGEL *et al.*[15] in the case of nigericin (Fig. 2).

FAB-MS are reported in Table 2.

The same fragmentations as for nigericin (**2**) can be observed in the case of N1, N2 and N3, except for the c fragment that could not be detected. The molecular weights deduced from these data were in good agreement with the structure proposed for N1, N2 and N3.

Complete assignments of ^1H and ^{13}C NMR spectra of N1, N2, N3 in $CDCl_3$ were performed by ^1H-^1H chemical shift correlations, ^1H-^{13}C chemical shift correlations[16~19], long range ^1H-^{13}C correlation experiments and *J*-modulated spectra. The general strategy for these assignments wa sdescribed previously in the case of the grisorixin potassium salt[20] and the bioconverted products of gri-

Table 3. ^{13}C Chemical shifts (δ) of nigericin, N1, N2, N3 and DN1 free acids in $CDCl_3$.

Carbon No.	Parity[a]	Nigericin	N1	N2	N3	DN1
1	q	177.5	176.0	176.6	176.7	176.4
2	t	44.3	44.1	44.1	44.4	44.2
3	t	73.1	73.6	73.3	73.2	73.7
4	t	28.0	27.8	27.9	27.7	27.9
5	s	26.3	25.5	26.1	26.1	26.3
6	s	23.6	23.7	23.6	23.5	23.8
7	t	69.1	68.8	69.0	69.1	68.9
8	s	35.6	35.9	35.6	35.4	35.8
9	t	60.4	60.4	60.5	60.5	60.6
10	s	32.1	31.8	31.7	31.8	31.9
11	t	78.4	78.9	77.8	79.4	79.0
12	t	36.1	37.0	37.1	37.4	37.1
13	q	108.3	107.7	107.9	108.1	107.8
14	t	39.2	39.2	39.3	39.2	39.3
15	s	42.7	42.1	41.7	42.4	42.2
16	q	81.8	80.8	80.8	81.9[b]	80.9
17	t	82.6	83.3	77.2	84.7	83.4
18	s	26.0	27.4	27.8	26.1	27.6
19	s	31.0	31.8	31.7	31.8	31.9
20	q	83.7	84.9	85.1	82.7[b]	84.8
21	t	86.0	85.9	84.7	87.4	88.1
22	t	35.4	35.5	44.7	45.4	35.6
23	s	31.0	32.0	26.1	29.2	32.0
24	t	74.7	78.7	79.2	80.3	78.8
25	t	77.4	77.3	77.8	77.6	77.1
26	t	32.8	34.1	34.1	34.4	34.3
27	s	37.7	39.9	38.8	38.8	39.0
28	t	37.4	35.5	34.9	35.2	32.9
29	t	97.2	77.2	77.0	77.1	73.4
30	s	68.5	64.7	64.5	64.5	65.1
31	p	16.4	17.7	17.6	17.6	15.7
32	p	17.4	18.1	17.8	17.9	17.7
33	p/s/q	15.7	15.5	61.0	176.3	15.6
34	p	22.8	24.4	24.2	24.0	24.5
35	p	27.7	28.2	28.2	28.3	28.2
36	p	13.2	13.0	13.0	13.1	13.0
37	p	13.1	13.0	13.3	13.1	13.2
38	p	10.9	11.1	11.0	11.0	11.2
39	p	13.3	12.9	13.0	13.1	12.9
40	p	57.6	57.4	57.2	57.1	57.5

[a] Multiplicity given by *J*-modulated spin echo spectrum. [b] May be inverted.
Abbreviations: p, primary; s, secondary; t, tertiary; q, quaternary.

sorixin[7~9]. Quaternary carbons such as C-20 and C-16 and methyl groups directly attached to these carbons (C-34 and C-35) were attributed to long range ^{13}C-^{1}H chemical shift correlations. ^{13}C and ^{1}H chemical shifts of N1, N2, N3 and nigericin (acid forms) are listed in Tables 3 and 4, respectively.

A detailed analysis of these data shows clearly that the A, B, C and D rings of these molecules remain similar to nigericin rings, in agreement with IR and MS. By contrast, E and F rings undergo great modifications (Fig. 1).

Table 4. ^1H Chemical shifts (δ) of nigericin, N1, N2, N3 and DN1 free acids in $CDCl_3$.

Carbon No.	Parity[a]	Nigericin	N1	N2	N3	DN1
1	q	—	—	—	—	—
2	t	2.57	2.28	2.31	2.36	2.33
3	t	3.97	3.72	3.74	3.72	3.75
4	t	1.48	1.81	1.78	1.84	1.81
5	s	1.80~1.35	1.86~1.47	1.92~1.46	2.00~1.55	1.99~1.44
6	s	1.98~0.98	1.93~1.10	2.00~1.13	2.03~1.19	2.01~1.14
7	t	4.07	4.04	4.07	4.06	4.08
8	s	2.74~1.02	2.45~0.94	2.46~0.97	2.53~1.02	2.49~0.97
9	t	4.52	4.06	4.09	4.06	4.09
10	s	2.26~0.85	2.19~1.03	2.21~0.99	2.27~1.13	2.23~1.05
11	t	3.13	3.36	3.34	3.45	3.40
12	t	1.67	1.72	1.73	1.84	1.79
13	q	—	—	—	—	—
14	t	2.12	2.04	2.05	2.10	2.10
15	s	1.86~1.77	1.73~1.55	1.74~1.54	1.79~1.61	1.79~1.81
16	q	—	—	—	—	—
17	t	3.54	3.46	3.59	3.62	3.49
18	s	2.00~1.59	1.83~1.61	1.82~1.63	1.68~1.55	1.83~1.64
19	s	2.41~1.31	2.26~1.35	2.11~1.48	2.22~2.22	2.29~1.38
20	q	—	—	—	—	—
21	t	4.22	3.91	3.94	3.72	3.94
22	t	2.02	2.20	2.53	3.06	2.24
23	s	2.38~1.28	2.28~1.36	2.08~1.89	2.13~2.13	2.30~1.36
24	t	4.40	4.23	4.19	4.56	4.24
25	t	4.36	3.58	3.48	3.63	3.57
26	t	1.28	1.48	1.61	1.59	1.39
27	s	1.72~1.00	1.97~0.83	1.93~0.86	1.97~0.95	1.94~0.83
28	t	1.84	1.71	1.74	1.77	1.64
29	t	—	3.41	3.45	3.48	3.68
30	s	4.04~3.98	3.68~3.44	3.67~3.45	3.68~3.46	3.65~3.50
31	p	1.37	0.85	0.86	0.93	0.86
32	p	0.70	0.84	0.86	0.94	0.85
33	p/s/q	0.81	0.88	3.80	—	0.89
34	p	1.07	1.15	1.26	1.38	1.18
35	p	1.86	1.38	1.35	1.45	1.43
36	p	1.08	0.87	0.87	0.94	0.89
37	p	1.10	1.03	1.00	1.07	1.05
38	p	0.83	0.91	0.91	0.99	0.93
39	p	1.04	1.01	1.02	1.09	1.04
40	p	3.56	3.38	3.35	3.40	3.40

[a] Multiplicity given by *J*-modulated spin echo spectrum.
Abbreviations: See footnote in Table 3.

F ring: From ^1H data, a new proton appears on C-29 in N1, N2 and N3 molecules suggesting the opening of this ring. Consequently the ^{13}C chemical shift of C-29 moves upfield. Modification of this ring induces chemical shift variations for neighboring carbons and protons (see C-26, C-27, C-28, C-30, C-31; 25-H, 26-H, 27-H_A, 27-H_B, 28-H, 30-H_A, 30-H_B).

E ring: The main event in this ring is the disappearance of the methyl group (C-33) for N2 and N3 (from ^1H and ^{13}C data). At the same time a new signal corresponding to a secondary carbon (N2) or quaternary carbon (N3) appears in *J*-modulated spectra, confirmed by the presence of two protons

corresponding to a CH_2O (N2) or the signal absence in ^1H spectra (N3). Clearly, the methyl group (C-33) in nigericin and N1 is replaced by CH_2OH in N2 and COOH in N3. Consequently 22-H and C-22 are shifted markedly downfield (especially for N3) in agreement with rules on substituent effects. In the same way C-23 and 23-H_B are shifted downfield. In addition, the COSY procedure (cross peaks) unequivocally shows linkings of rings A-B, C-D and E-F. All these connections are visible for N1, N2 and N3.

In conclusion, NMR, IR and FAB data are consistent in supporting the structures of N1, N2 and N3 proposed in Fig. 1.

The N1 structure could be explained by an enzymatic reduction of the δ-hydroxy ketone which is the tautomeric form of the hemiketal in the equilibrium.

The reduction could be realized by a glycerol dehydrogenase (GDH) that recognizes the sequence: $COCH_2OH$[21]. This could also explain why grisorixin whose terminal structure is $COCH_3$ was not reduced under the same conditions.

This prompted us to carry out the same reaction chemically. Nigericin (**2**) was reduced by $NaBH_4$, two diastereoisomers were isolated and analyzed in the same way as N1, N2 and N3. One of them had the same structure as N1 and its diastereoisomer was named DN1 (Tables 1~4).

The problem was then to attribute the right configuration to C-29. ^{13}C chemical shifts of N1 and DN1 (Table 3) are very similar except for C-31 (2 ppm) and C-29 (3.8 ppm). The most probable energetically favored conformations of the rotor C-28, C-29 are presented in Fig. 3.

The *S*-configuration of C-29 can be attributed to N1 because the sum of the "OH γ gauche" and CH_2OH *trans* effects on the C-31 are of -7 ($0-7$) for this configuration, although the sum of the CH_2OH– "gauche" and OH *trans* effects are -9.4 ($-6.4-3$) for the *R*-configuration[22~24]. For C-29, the difference of 4 ppm can be explained by a decrease of the β effect of C-31 due to a sterical compression in the case of DN1 (*R*-configuration).

Biological Properties of the Bioconversion Products

The antibiotic properties of the bioconversion products N1, N2, N3 and of N1, DN1, obtained from chemical reduction were compared with the activity of nigericin, using the conventional dilution method with *Bacillus cereus* ATCC 14579 in Mueller-Hinton broth at pH 7 (Table 5).

N1 is still weakly antibiotic but its MIC value is much higher than that of nigericin (1/500). It is worth noting that N1 obtained by bioconversion has the same MIC value as DN1 and N1 prepared chemically. This suggests that the configuration of C-29 does not play any role in biological properties.

The significant increase of MIC values for N1 and DN1 can be directly linked to a decrease in the ionophoric properties. Earlier experiments[12] had shown by two complementary methods that these compounds indeed could not transport K^+ efficiently; first by using a liquid

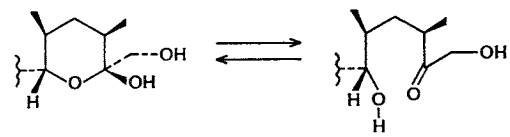


Fig. 3. Energeticaly favored conformations of N1 and DN1.

C-28~C-29 rotors.

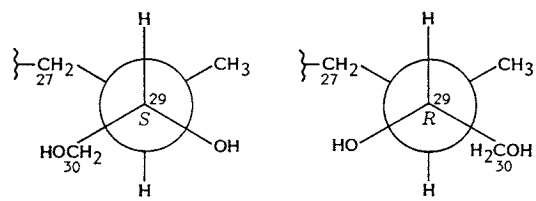


Table 5. MIC values (μg/ml) measured on *Bacillus cereus*.

Nigericin	N1[a]	N2[a]	N3[a]	N1[b]	DN1[b]
0.05	25	na	na	25	25

[a] Bioconversion product.
[b] Resulting from $NaBH_4$ reduction of nigericin.
na: No activity.

membrane electrode system, secondly by testing their transporting abilities in rat liver mitochondria.

All these results focussed on the fact that the opening of the terminal hemiketal ring drastically reduces the ionophoric and antibiotic properties of nigericin. The additional presence of polar functions such as CH_2OH (N2) or COOH (N3) modifies the lipophilic external envelope to such an extent that N2 and N3 completely lack the original antibiotic properties of nigericin.

Conclusion

As in the case of grisorixin, only the E and F rings are affected by enzymatic reactions. The same type of modifications, *i.e.* oxidation of CH_3 to CH_2OH or COOH occur for nigericin and grisorixin. The process corresponds to a classical detoxification pathway; molecules are becoming more polar to be eliminated in the culture medium.

For nigericin, a new type of reaction was found in a first additional step; the opening of the terminal ring, due to reduction. This modification directly affects the complexation site of the molecule.

In these two examples of detoxification of carboxylic polyether antibiotics, the resulting products have lost their linked cationic carrier and antibiotic properties.

Previous studies[8,9] had shown that the bioconversion products of grisorixin keep the same globular conformation as grisorixin in solution, since the six heterocycles are still present in defined orientations relative to each of their neighbors. This could not be deduced for the bioconversion products N1, N2 and N3 of nigericin and a thorough conformational study by NMR is required to answer this question. Work on this is currently in progress.

Acknowledgments

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References

1) WESTLEY, J. W.: Chapter 1. Notation and classification. *In* Polyether Antibiotics. Naturally Occurring Acid Ionophores. Vol. 1. Biology. *Ed.*, J. W. WESTLEY, pp. 1~20, Marcel Dekker, Inc., New York, 1982
2) OSBORNE, M. W.; J. WENGER, F. KOVZELOVE, R. BOYD & M. ZANKO: Chapter 7. Effects of lasalocid and monensin on chickens. *In* Polyether Antibiotics. Naturally Occurring Acid Ionophores. Vol. 1. Biology. *Ed.*, J. W. WESTLEY, pp. 333~340, Marcel Dekker, Inc., New York, 1982
3) LIU, C. M.: Chapter 3. Microbial aspects of polyether antibiotics: Activity, production, and biosynthesis. *In* Polyether Antibiotics. Naturally Occurring Acid Ionophores. Vol. 1. Biology. *Ed.*, J. W. WESTLEY, pp. 43~102, Marcel Dekker, Inc., New York, 1982
4) BRANNON, D. R. & D. R. HORTON (Eli Lilly): Metabolite A-27106 and processes for its preparation and use. U.S. Pat. Appl. 3,932,619, Jan. 13, 1976
5) ABBOTT, B. J.; D. S. FUKUDA, D. E. DORMAN, J. L. OCCOLOWITZ, M. DEBONO & L. FARHNER: Microbial transformation of A23187, a divalent cation ionophore antibiotic. Antimicrob. Agents Chemother. 16: 808~812, 1979
6) VÉRTESY, L.; K. HEIL, H.-W. FEHLHABER & W. ZIEGLER: Microbial decomposition of salinomycin. J. Antibiotics 40: 388~390, 1987

7) CUER, A.; G. DAUPHIN & J. C. BELOEIL: Microbial conversion of grisorixin, a monovalent cation ionophorous antibiotic. J. Antibiotics 36: 20~24, 1983
8) CUER, A. & G. DAUPHIN: Microbial conversion of grisorixin: Conformational properties of a bioconversion product. Tetrahedron 41: 3725~3736, 1985
9) CUER, A. & G. DAUPHIN: Structure and conformation of bioconversion products of a carboxylic ionophorous antibiotic, grisorixin, by means of two-dimensional nuclear magnetic resonance. J. Chem. Soc. Perkin Trans. II 1986: 295~299, 1986
10) ROSAZZA, J. P. & R. V. SMITH: Microbial models for drug metabolism. Adv. Appl. Microbiol. 25: 169~208, 1979
11) SEBEK, O. K. & L. A. DOLAK: Microbial hydroxylation of novobiocin and related compounds. J. Antibiotics 37: 136~142, 1984
12) DAVID, L.; M. CHAPEL, J. GANDREUIL, G. JEMINET & R. DURAND: The importance of the hemi-acetal group for the ionophoric properties of nigericin. Experientia 35: 1562~1563, 1979
13) KUBOTA, T. & S. MATUTANI: Studies on the antibiotic nigericin (polyetherin A). J. Chem. Soc. (C) 1970: 695~703, 1970
14) MELLIER, A. & O. AUGE: Spectre infra-rouge d'antibiotiques polyethers carboxyliques: grisorixine et nigéricine. C.R. Acad. Sci. Paris. 305: 17~21, 1987
15) SIEGEL, M. H.; J. M. WILLIAM, K. B. TOMER & T. T. CHANG: Applications of fast atom bombardment mass spectroscopy and fast atom bombardment mass spectrometry-mass spectrometry to the maduramicins and other polyether antibiotics. Biomed. Environ. Mass Spectrom. 14: 29~38, 1987
16) BAX, A. (*Ed.*): Two Dimensional Cosy Nuclear Magnetic Resonance in Liquids. Deft University Press, D. Reidel Publishing Company, London, 1982
17) BAX, A.; R. FREEMAN & G. MORRIS: Correlation of proton chemical shifts by two-dimensional Fourier transform NMR. J. Magn. Reson. 42: 164~168, 1981
18) BODENHAUSEN, G. & R. FREEMAN: Correlation of proton and carbon-13 NMR spectra by heteronuclear two-dimensional spectroscopy. J. Magn. Reson. 28: 471~476, 1977
19) FREEMAN, R. & G. MORRIS: Experimental chemical shift correlation maps in nuclear magnetic resonance spectroscopy. J. Chem. Soc. Chem. Commun. 1978: 684~686, 1978
20) CUER, A.; G. DAUPHIN, G. JEMINET, J. C. BELOEIL & J. Y. LALLEMAND: Complete assignment of the ^{1}H and ^{13}C spectra of the grisorixin potassium salt by means of two dimensional NMR method. Application to the solution conformation of the molecule. Nouv. J. Chim. 9: 437~441, 1985
21) LEE, L. G. & G. M. WHITESIDES: Preparation of optically active 1,2-diols and α-hydroxy ketones using glycerol deshydrogenase as catalyst: Limits to enzyme-catalysed synthesis due to non-competitive and mixed inhibition by-product. J. Org. Chem. 51: 25~36, 1986
22) WHERHLI, F. N. & T. WIRTHLIM: Interpretation of C-13 NMR spectra. *Ed.*, Heyden & Son Ltd., p. 45, Spectrum House, London, 1976
23) DALLING, D. K. & D. M. GRANT: Carbon-13 magnetic resonance. XXI. Steric interactions in the methylcyclohexanes. J. Am. Chem. Soc. 94: 5318~5324, 1972
24) SCHNEIDER, H.-J. & V. HOPPEN: Equilibria and ^{13}C NMR shifts of cyclohexane conformers. Tetrahedron Lett. 1974: 579~582, 1974