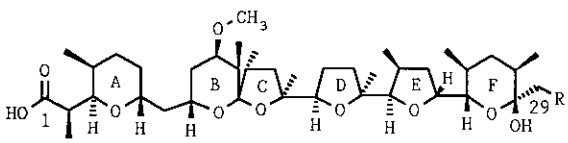
SECONDARY METABOLITES BY CHEMICAL SCREENING. 11[1] INFLUENCE OF THE C-1 AND C-29 MOIETIES IN NIGERICIN ON COMPLEXATION BEHAVIOUR AND BIOLOGICAL ACTIVITY

Susanne Grabley, Peter Hammann*, Robert Klein,
and Michael Magerstädt
Hoechst AG, Postfach 80 03 20, D-6230 Frankfurt a. Main, FRG

Abstract-A reversible blocking of the C-29 hemiacetal in nigericin (1) was performed with LiBr in methanol to the acetal (6). Conversion of the carboxylic acid to the C-1 alcohol (7) and the ketone (9) and the subsequent deprotection yielded 8 and 10, respectively leaving F-rings intact. Complexation- and molecular modelling-studies with the nigericin alcohols (7) and (8) demonstrated the importance of noncyclic conformations.

Nigericin (1), a polyether isolated from Streptomyces hygroscopicus,[2] is a very potent antibiotic against Gram positive bacteria[3] and viruses.[4] The mode of action can be explained by complexation of cations, especially potassium, and transportation through biological membranes. It can be assumed that the carboxyl group in 1 which participates directly in cation liganding, is playing an important role for this biological effect, thus the present study was designed to investigate this possibility.



Nigericin (1) R = OH
Grisorixin (2) R = H

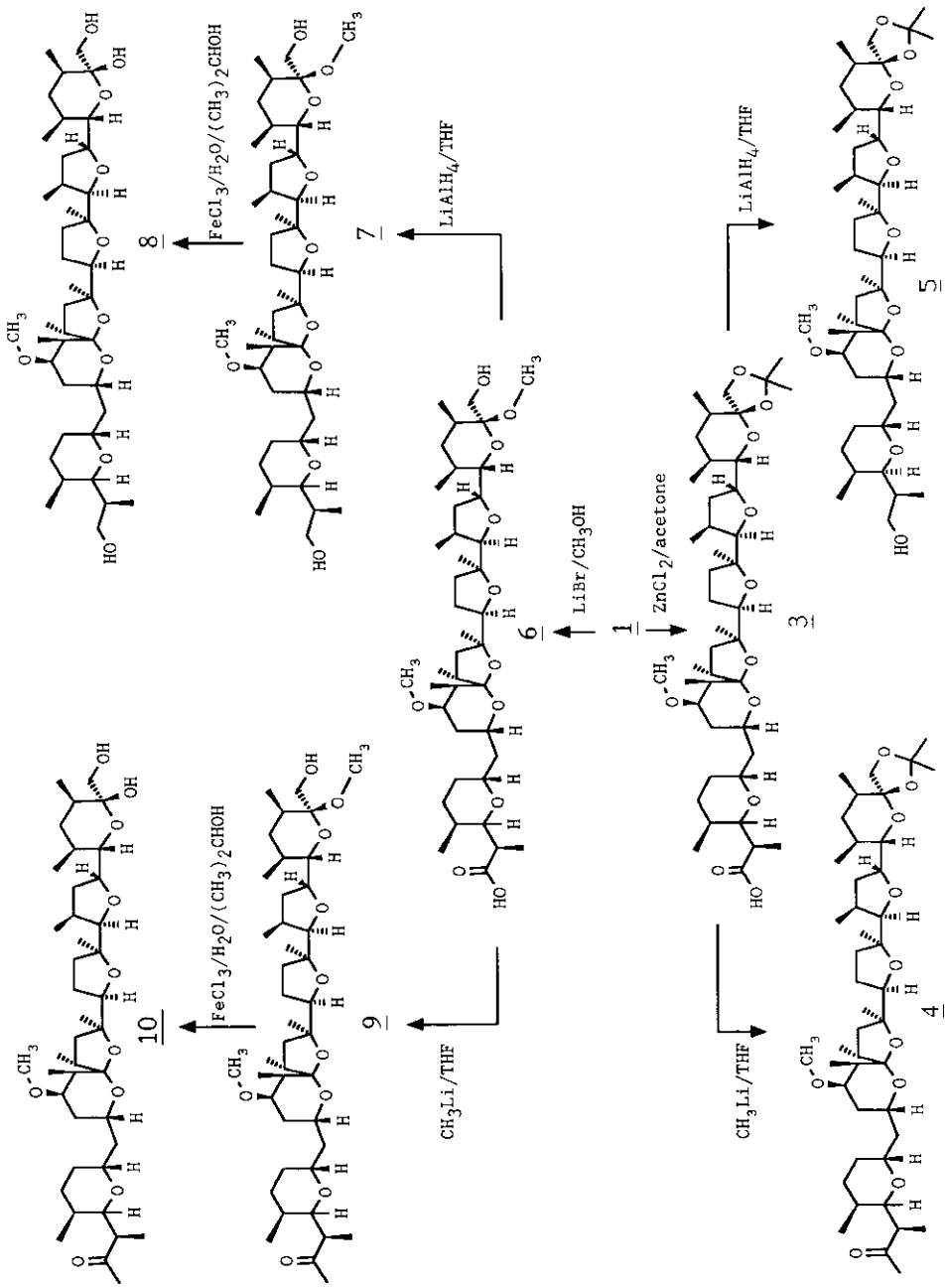
The transformation of the C-1 carboxyl group into the keto or alcohol group could not be performed without any competing side reaction at the C-29 acetal group. A suitable reversible protection of C-29 is possible by

acetalisation.[5] While grisorixin (2) undergoes acetalisation at C-29 with methanol at room temperature, nigericin (1) showed no reaction with alcohols even under reflux. Strong acid catalysts, e.g. H_2SO_4, $AlCl_3$, or p-toluenesulfonic acid, led to decomposition of 1 .

Reaction of 1 with acetone in the presence of $ZnCl_2$[6] for 4 h under reflux gave the spiroacetal (3), $[\alpha]_D^{20}$ +12.9° (c = 1, CH_2Cl_2) in quantitative yield. Reduction of 3 with $LiAlH_4$ in THF[5] for 5 h at 60°C yielded the C-1 alcohol 5 in 93%. Reaction of 3 with methyllithium in THF at 0°C for 1 h yielded 4 in 42%. A cleavage of the C-29 spiro-ketal group in 3, 4 and 5 is obviously not possible without any side reaction at the C-13 spiro-ketal group in nigericin (1). No biological activity of 4 and 5 was observed up to now.

Another route for protecting the hemiacetal group is the acetalisation with methanol. Suprisingly, we found that only LiBr is a suitable catalyst for acetalisation of 1. We assumed that the interaction of the polyether with the inorganic salt is responsible for the observed reaction. When 1 was refluxed in methanol with LiBr for 6 h, 6 was formed in 82% yield, $[\alpha]_D^{20}$ +25° (c = 1, CH_2Cl_2). The characteristic ^{13}C-nmr signals are located at C-1 δ = 176.4, C-13 δ = 107.6, C-29 δ = 99.1 ppm. The reduction of 6 with $LiAlH_4$ in THF for 3 h under reflux yielded the C-1 alcohol (7) in 91%, $[\alpha]_D^{20}$ +31° (c = 1, CH_2Cl_2). The cleavage of the protecting group is performed by heating 7 in isopropanol/ water (1 : 1) with $FeCl_3$ for 4 h at 60°C to yield 8 in 78%, $[\alpha]_D^{20}$ +35.5° (c = 1, CH_2Cl_2). The characteristic ^{13}C-nmr signals are located at C-1 δ = 67.7, C-13 δ = 108.0 and C-29 δ = 97.2 ppm.

Reaction of 6 with methyllithium in THF at 0°C gave the keto derivative (9) in 40% yield, $[\alpha]_D^{20}$ +24.4° (c = 1, CH_2Cl_2). The protecting group was removed by heating 9 in isopropanol/water (1 : 1) with $FeCl_3$ for 4 h at 60°C to yield 10 in 78%, $[\alpha]_D^{20}$ +21.1° (c = 1, CH_2Cl_2). The structure was confirmed by the characteristic ^{13}C-nmr signals at C-1 δ = 212.6, C-13 δ = 107.4 and C-29 δ = 97.4 ppm. For the first time it became possible to investigate the biological activity and complexation behaviour of nigericin alcohols or corresponding keto derivatives by the present conversion. Because 7 and 9 as well as 8 and 10 showed similiar activities against bacteria we selected 7


1
2
3
4
5
6
7
8
9
10
ZnCl2/acetone
LiBr/CH3OH
LiAlH4/THF
CH3Li/THF
FeCl3/H2O/(CH3)2CHOH

and 8 for the determination of the K_{mL} values and compared them with those of nigericin (1).

The thermodynamic complex stability constants K_{mL} represents the ratio of complex formation to complex dissociation at equilibrium. They were determined by acid-base titration of the free ligands and the ligand-metal mixtures in water/methanol solutions and subsequent iterative optimization using the fortran program BEST.[7]

Table: Complex stability constants (log K_{mL})[8] and MICs[9] (minimal inhibitory concentration) in μg/ml

Compound	Na^+ (log K_{mL})	K^+ (log K_{mL})	Herpes Virus I	S. aureus S 285	S. pyogenes S 308A	S. pyogenes S 77A
1	4.1	5.1	<0.02	0.098	0.026	0.013
6	nd	nd	<0.02	1.56	0.39	0.39
7	2.9	4.6	4.94	>100	>100	>100
8	2.5	4.1	<0.18	6.25	1.56	1.56
9	nd	nd	40.0	>100	>100	>100
10	nd	nd	4.44	3.13	1.56	1.56

nd = not determined

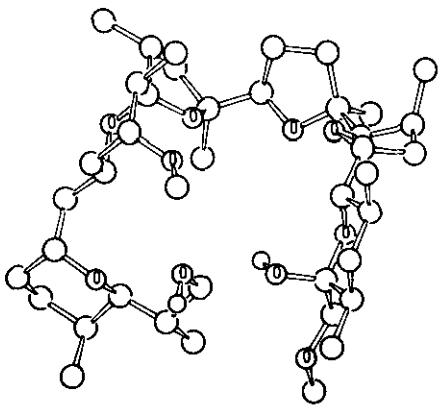
In the case of potassium, the K_{mL}-values for 7 and 8 were in the same range as for 1, so that the carboxylic acid moiety is not so important first assumed. Furthermore, the data in the table demonstrated that no correlation excists between biological activities against bacteria and viruses and complex stability with sodium and potassium.[10] Obviously in the alcohols (5) and (7) the protecting group blocks any biological activity.

The molecules (1, 6, 7 and 8) as well as the corresonding sodium and potassium complexes have been subjected to a conformation search using the valence force field method for calculating conformational energies.[11] In order to take solvent effects into account 40 water molecules have been added to each molecule. Up to 400 conformations of each molecular system have been generated randomly and subsequently energy minimized using the molecular

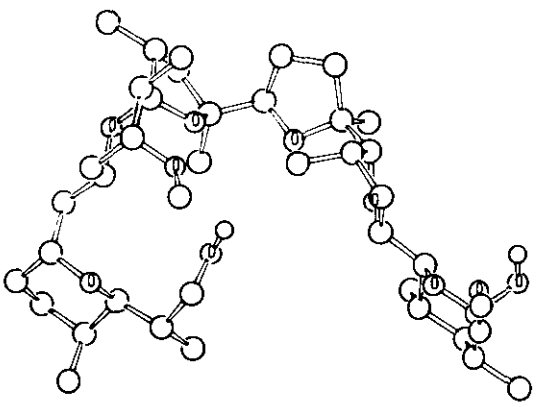
mechanics program MOLMEC.[12] The force field parameters have been taken from the program system AMBER.[13]

Nigericin (1) exhibits a cyclic conformation, caused by head-to-tail hydrogen bonding, and is further stabilized by the twists in the asymmetric centers and rings of the backbone. The hydrogen bonding is weakened by acetalisation and reduction in 6, 7 and 8 when compared to 1. According to the conformational energies an open conformation gains in probability in the order of 8, 6 and 7. A noncyclic conformation of 1 in protic solvents determined by ^{1}H-nmr spectroscopy was already discussed by Anteunis.[14]

By complexation with cations the cyclic conformation is prefered. Thus, the stability of the cyclic structure in the complex increases from 7 to 8 and due to salt formation from 8 to 6. The stabilities of the cation complexes depend on both salt formation and conformational effects. Because of the larger ionic radius the conformational contribution is more important in potassium complexes than in sodium complexes. This explains the differently pronounced trends in the corresponding K_{mL}-values 1, 7 and 8.



cyclic conformation of 8



noncyclic conformation of 8

The difference in the activity of 7 and 8 may be explained by assuming that only the cyclic structure can produce the biological effect.

Further studies on nigericin derivatives and their biological properties, based on the results reported, are in progress.

ACKNOWLEDGEMENTS

We like to thank the Bundesministerium für Forschung und Technologie for financial support. Furthermore, we like to thank G. Seibert and I. Winkler (Hoechst AG) for biological-testing and A. E. Martell and R. J. Motekaitis, Texas A and M university for a copy of the BEST-program and valuable hints.

REFERENCES AND NOTES

1. Part 10 of this series: M. Schönewolf, R. Machinek, A. Zeeck, K. Hütter, J. Wink, S. Grabley, and J. Rohr, Liebigs Ann. Chem., in press 1990.
2. J. Shoji, S. Kozuki, S. Matsutani, T. Kubota, H. Nishimura, M. Mayama, K. Motokawa, Y. Tanaka, N. Shimaoka, and H. Otsuka, J. Antibiotics, 1986, 21, 402, R. L. Harned, P. H. Hidy, C. J. Corum, and K. L. Jones, Antibiotics and Chemotherapy, 1951,1, 594 , S. Grabley, P. Hammann, W. Raether, J. Wink, and A. Zeeck, J. Antibiotics, 1990, 43, 657.
3. T. Kubota, S. Matsutani, and M. Mayama, JP 44-75411, 22. 9. 1969 (Chem. Abstr., 1972, 76, 478).
4. S. Dundarov, P. Andonov, T. Todorov, S. Tishkov, E. Minkov, N. Boyadzhieva, A. Marinov, N. Vulova, and M.Alkusheva, EP 294,538, 14. 12. 1988 (Chem. Abstr., 1989, 111, 66).
5. For acetalisation of monensin see: P. W. Smith and W. Clark Still, J. Am. Chem. Soc., 1988, 110, 7917, C. Gaboyard, G. Dauphin, F. Vaufrey, and G. Jeminet, Agric. Biol. Chem., 1990, 54, 1149.
6. T. Kubota and S. Matsutani, J. Chem Soc. (C), 1970, 696.
7. R. J. Matekaitis and A. E. Martell, Can. J. Chem., 1982, 60, 2403.
8. The constants for 1 have been published previously and could be confirmed by our titrations; see: R. W. Taylor, R. E. Kaufmann, and D. R. Pfeiffer, "Polyether antibiotics," Vol. I, Biology, ed. by J. Westley, Marcel Dekker, New York, 1982, p. 103.
9. The MIC values were estimated as described in reference 2.

10. G. Dauphin, L. David, A. Kergomard, and G. Sainz, Agric. Biol. Chem., 1989, 53, 2947.

11. H. Frühbeis, R. Klein, and H. Wallmeier, Angew. Chem. Int. Ed. Engl., 1987, 26, 403.

12. R. Klein and B. Hientzsch, unpublished results.

13. P. K. Weiner and P. A. Kollman, J. Comp. Chem., 1981, 2, 287.

14. N. A. Rodios and N. J. Anteunis, Bull. Soc. Chim. Belg., 1980, 89, 537.

Received, 27th August, 1990