Secondary Metabolites by Chemical Screening. 17.[1] Nigericinol Derivatives:[†] Synthesis, Biological Activities, and Modeling Studies

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The synthesis and the biological activity of C-1-reduced nigericin derivatives (nigericinols) are described and discussed. The dichloronigericinol **7** impressively demonstrated that the C-1 carboxylic acid moiety was not required for a distinct activity against bacteria and viruses. Based on the correlation between K^+/H^+ antiport activities and antibacterial activities it was deduced that the mode of action of the described nigericinols are related to their ionophoric properties. Molecular modeling studies showed that the efficiency of the nigericinols as ionophores correlates, qualitatively, with the probability of forming a cyclic structure, with the exception of **7**.

Nigericin (1) is a polyether which originally was isolated from *Streptomyces hygroscopicus*[2,3] and is now available in large quantities by fermentation of other *Streptomyces* sp.[4] Two different groups independently elucidated the structure.[5,6] It has antibiotic activity against Gram-positive bacteria[7] and viruses.[8] The mode of action against bacteria was explained by cation complexation and its action as an ionophore in biological membranes.[9] X-ray crystallographic studies showed that nigericin (1) exhibited a cyclic conformation caused by head-to-tail hydrogen bonding[10] and is further stabilized by the rings of the backbone. It is noteworthy that the oxygen at C-1 is involved in a hydrogen bond with OH-29, exhibiting a bond length of 2.63 Å, and with OH-30, exhibiting a bond length of 2.73 Å, as determined from the X-ray crystal structure of the potassium salt. Hereby, the carboxylic acid moiety participated directly as a ligand to the cation. Furthermore, the three oxygen atoms of the tetrahydrofuran moieties and the ether oxygen at C-11 coordinated the cation.

There are only a few derivatives of 1 reported to date, which were originally prepared for the structure elucidation of 1.[11] With regard to the biological activity, 30-acyl,[12] 29-dihydro, and 30-de(hydroxymethyl)[13,14] derivatives of 1 have been synthesized. Furthermore, a microbial conversion of 1[15] and a rearrangement induced by sodium hydroxide[16] was described. We already reported the C-1-reduced derivatives of 1[17] and were able to demonstrate that nigericinol **5** also exhibited antibacterial and antiviral properties. In contrast to **5**, the C-29-protected derivative **4** was inactive against bacteria and viruses, which was explained by the higher probability of forming a noncyclic conformation.

The disadvantage of nigericin 1 and derivatives were their high cytotoxicities, expressed at the maximal tolerated dose (MTD) value, and their lack of biological selectivity. This was one of the reasons why polyether ionophores were not considered for human application. Up to now it was not possible to synthesize a derivative which acts selectively against bacteria or viruses. This study was therefore designed to investigate the potential of nigericinol derivatives and to study their structure–activity relationships.

Results and Discussion

Chemical Derivatization. Nigericinol derivatives with an open F-ring could be easily prepared by $NaIO_4$ cleavage of 1 to the lactone **2** and following reduction with $LiAlH_4$ to **3** (Scheme I).

[†]We named the C-1 reduced nigericin derivative **6** nigericinol to present a convenient nomenclature for the discussed compounds.

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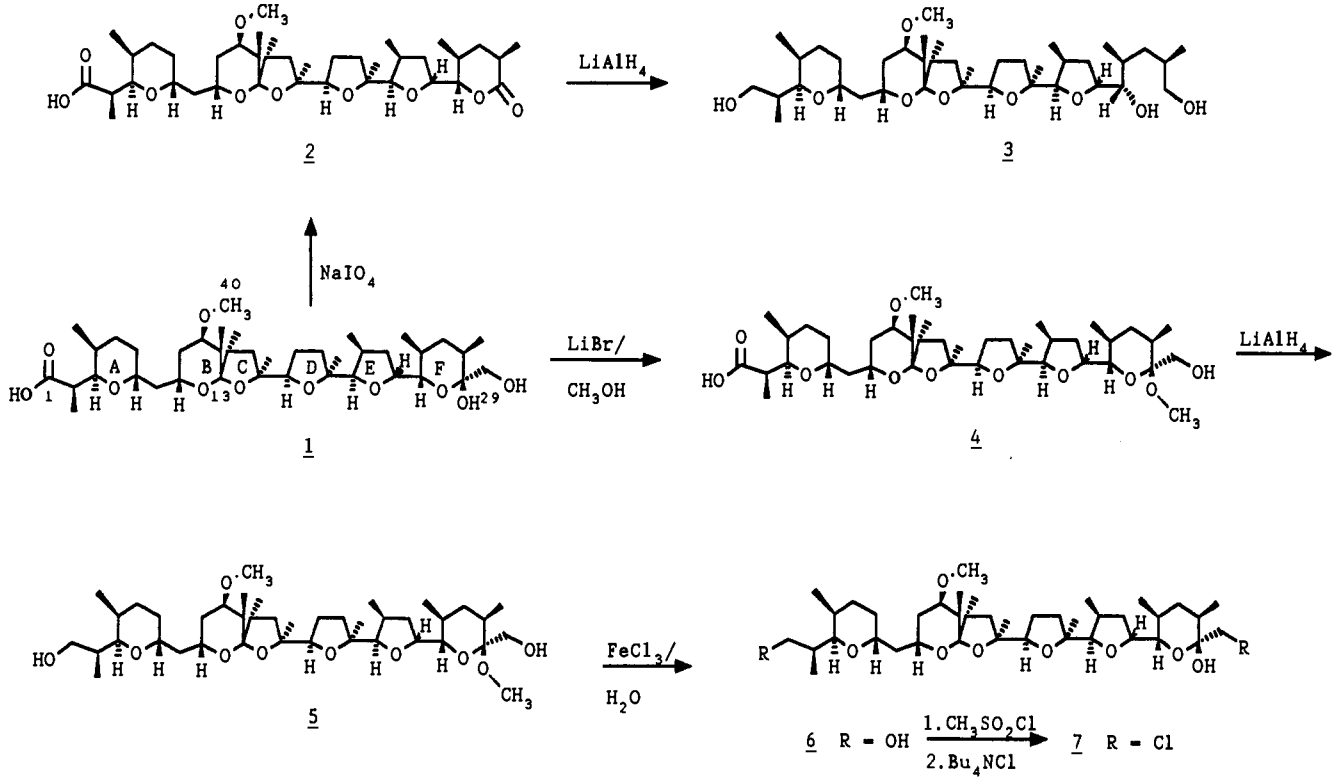
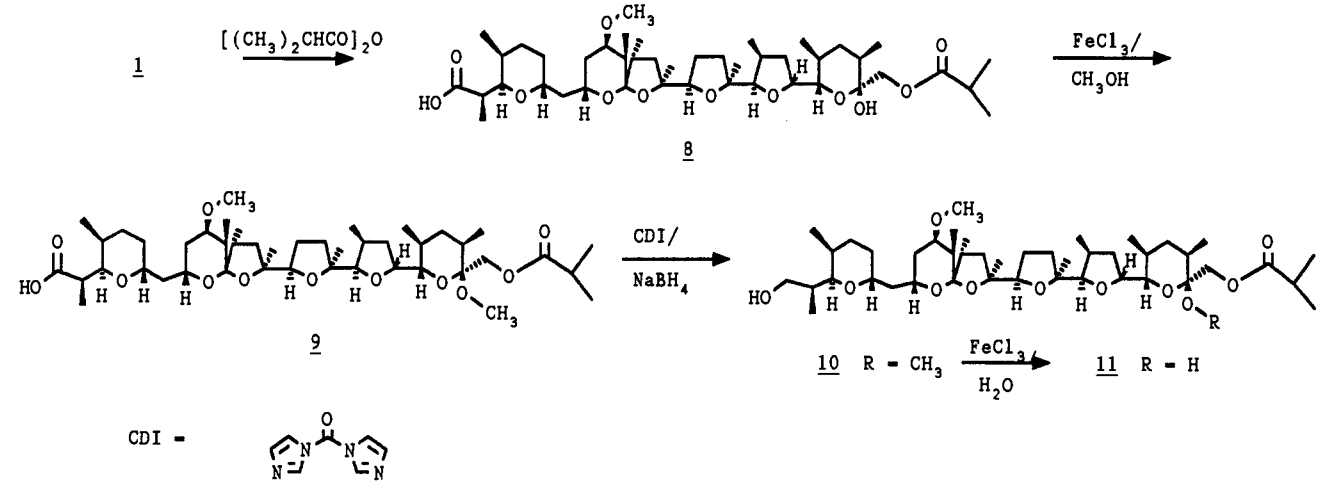
Scheme I



Scheme II



The selective reduction of the C-1 carboxylic acid group could not be performed without any competing side reaction at the C-29 acetal group. For monensin, a related polyether which also incorporates the hemiacetal moiety of **1**, an acetalization with $ZnCl_2$/benzaldehyde[18] or camphorsulfonic acid (CSA)/methanol[19] was described. We showed that LiBr was a favorable catalyst for the reaction of **1** with methanol to **4**.[17] Reduction of **4** at C-1 with $LiAlH_4$ yielded the protected nigericinol **5**. The protecting group could be removed with 2-propanol/water in the presence of $FeCl_3$ to afford **6**. Mesylation of **6** at OH-1 and OH-30, followed by nucleophilic displacement with chloride ion, gave the dichloronigericin **7**.

For the investigation of structure–activity relationships it was interesting to study acyl derivatives of **6**, either acylated at C-1 or C-30. An approach to C-30 acyl derivatives was performed by acylation of **1** with isobutyric acid anhydride to form **8** (Scheme II). Acetalization with LiBr/methanol yielded **9**, which could be reduced with CDI/$NaBH_4$ selectively at C-1 to give **10**. Cleavage of the protecting group yielded the 30-acylnigericinol **11**.

A convenient preparation of the 1-acylnigericinol started with the reaction of **1** with an excess of SEM-Cl to afford the tri-SEM derivative **12** (Scheme III). By using only one reagent, three different functional groups were generated in **12**: at C-1 an ester group, at C-29 an acetal moiety, and at C-30 an ether group. This molecule is an excellent intermediate for regioselective reactions at the

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Scheme III

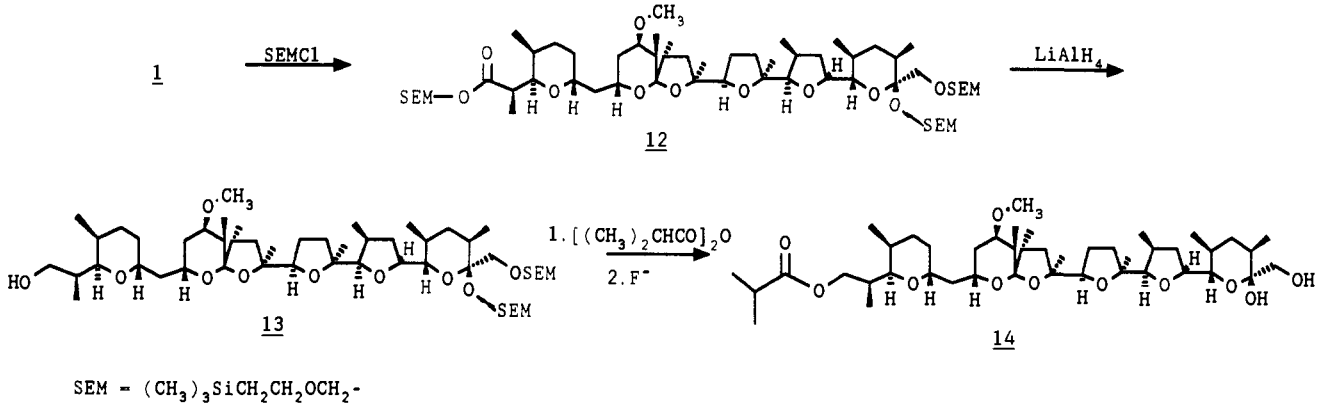

Table I. Antimicrobial Activities of Nigericin Derivatives (MIC in μg/mL)

	bacteria					viruses		
	S. aureus			*S. pyogenes*		HSV		
compd	SG511	285	503	308 A	77 A	I	II	MTD[a]
1	0.098	0.098	0.098	0.025	0.013	<0.02	0.05	1.4
2	50.0	50.0	50.0	25.0	25.0	4.44	13.3	40
3	6.25	3.13	1.56	1.56	50.0	40.0	133	40
4	1.56	1.56	1.56	0.39	0.39	<0.02	0.5	4.4
5	>100	>100	>100	>100	>100	4.94	0.5	14.5
6	>100	6.25	3.13	1.56	1.56	<0.18	<0.18	14.8
7	0.39	0.39	0.098	0.098	0.78	<0.02	<0.02	14.8
8	0.39	0.39	0.19	0.049	0.049	<0.02	<0.02	1.4
9	>100	100	25.0	25.0	>100	>400	>400	>400
10	>100	>100	>100	>100	>100	>400	>400	>400
11	12.5	6.25	3.13	3.13	12.5	0.49	0.49	4.4
12	>100	>100	>100	>100	>100	4.44	13.3	>40
13	>100	>100	>100	50.0	50.0	>400	>400	>400
14	>100	>100	>100	>100	>100	>400	>400	>400

[a] Maximum tolerated dose.

mentioned positions. Reduction of the ester group with $LiAlH_4$ yielded the 29,30-di-SEM-nigericinol **13**. Acylation at C-1 and deprotection with Bu_4NF resulted in the required 1-acylnigericinol **14**.

Biological Activities. All the described derivatives were tested against bacteria and Herpes viruses type I and II (Table I). As already mentioned, the activity of **1** against bacteria and viruses was described.[8,17] Derivatives with an intact C-1 carboxylic acid moiety, e.g., C-30 isobutyryl derivatives, were all potent antibiotics against Gram-positive bacteria and viruses. The alcohol **3** with the open F ring showed activity against bacteria but was inactive against viruses. As it was already described, acetalization and reduction of **1** to **5** gave rise to an antibacterial inactive compound which became active again by deacetalization to **6**.[17] Alcohol **6** was also active against Herpes viruses, whereas **5** exhibited a distinctly lesser activity. Through substitution of the C-1 and C-30 hydroxyl group by chlorine in **7**, the activity against bacteria and viruses was increased when compared to **6**. Remarkable is the fact that **7**, in relation to nigericin **1** exhibited one-fourth of the activity against bacteria and viruses, yet only one-tenth of the cytotoxicity.

The 29-*O*-methyl-30-isobutyryl derivative **10** was inactive against bacteria and viruses. In contrast to this observation, the 30-isobutyryl derivative **11** showed biological activity in our test systems. However, **11** was less active than **6** against viruses.

The C-1 isobutyryl derivative **14**, as well as the intermediates in the synthesis of this compound, was inactive.

Discussion

The presented methodology allowed for the first time the preparation of nigericinol derivatives, either C-1 or C-30 acylated, which could be used in further derivatizations. It was shown that the carboxylic acid at C-1 was not required for the antiviral and antibacterial activity, although the alcohol **6** was less active than nigericin (**1**) itself. Remarkable was the biological effect of the dichloronigericin derivative **7**. In our opinion this impressively demonstrates that the carboxylic acid moiety at C-1 is not required for the expression of the antiviral and antibacterial activities. Although the OH-29/Cl-30 moiety gives rise to the assumption that **7** possesses alkylating properties which would lead to a different mode of action. The C-30 acylated polyether **11** exhibited a decreased biological activity compared to that of nigericinol **6**. In the C-1 acylated compound **14** the activity was totally lost.

The compounds **1**, **3**, **4**, **5**, **6**, **7**, **11**, and **14** as well as their corresponding potassium complexes have been subjected to a conformation search using the valence force field method for calculating conformational energies.[17] Furthermore, the complex stabilities have been estimated by comparing the energies of the polyether potassium complexes and the corresponding neutral compounds.

Nigericin (**1**) exhibits a cyclic conformation stabilized by a head-to-tail hydrogen bond. This bond is weakened by acetalization and reduction. According to the conformational energies, which have been calculated in the presence of 40 water molecules to account for solvation effects, a noncyclic conformation gains in probability in the order of **1**, **4**, **6**, **5** = **11**, **7**. In order to outline the effects of different substituents in the 1- and 29-positions on this probability, compounds **6** and **7** are compared as follows. In **6** a distance of 2.86 Å between O-1 and O-29 is found which is typical of a hydrogen bond. The calculated in-

Table II. Nigericinols as K^+/H^+ Antiporters in Liposomal Membranes Compared to Nigericin 1

compd	N, nmol/g phospholipid	turnover number W, s^{-1}
1	0.5–50	1170
3	158–50000	2.5
4	0.5–158	430
5	15000–50000	0.13
6	158–50000	1
7	1.58–500	120
11	1580–50000	0.13

teraction energy between the hydrogen pairs H-1 and O-29 is about −10 kcal/mol. In compound 7 only an electrostatic interaction between Cl-1 and the hydrogen of the 29-hydroxyl group is possible. The calculated distance between Cl-1 and O-29 is 3.37 Å, and the Cl-1/H-29 interaction energy is about −4 kcal/mol, resulting in a less pronounced preference for the cyclic conformation.

In 14 the cyclic structure is disfavored in the neutral compound by disruption of the OH-1/OH-29 hydrogen bond through acylation of OH-1. It was already demonstrated that nigericin (1), 5, and 6 had comparable complex stability constants for potassium, for 1, lg K_{ml} = 5.1, for 5, lg K_{ml} = 4.6, and for 6, lg K_{ml} = 4.1.[17] Based on these values the calculated complex stabilities can be classified. The calculation shows that in the presence of potassium all molecules prefer the cyclic structure. The complexation ability for potassium was estimated to be in the order of 14 = 11 > 6 = 7. The activity of the derivatives could not be correlated with complex properties for potassium, which is in contrast to data obtained for monensin.[19]

To assess the efficiency of the nigericinols as ionophores the H^+/K^+-antiport activities of selected nigericinol derivatives in the membranes of liposomes were measured.[20] After addition of the compounds 1, 3, 4, 5, 6, 7, or 11, the external pH was shifted to 7.5. The velocity of internal pH increase (dpH/dt), which was caused by the external pH shift, was recorded fluorometrically. The proton flux (J) was calculated according to equation a using the internal buffering capacity (B) of liposomes.

$$\text{(a)}\quad J/N = (\mathrm{dpH}/\mathrm{d}t)B/N$$

The turnover numbers in Table II represent the ratios (J/N) of the initial proton flux (pH_{int} 6.5; pH_{ext} 7.5) and the concentration (N) of the tested compounds within the membrane.

Nigerin (1) exhibits the highest turnover number (1170 s^{-1}), with N ranging between 0.5 and 50 nmol/g phospholipid (Table II), which is decreased by acetalization of OH-29 in 4. The nigericinols 3 and 6 were 3 orders of magnitude less effective than 1.

A plot of the logarithms of the MIC values for *S. aureus* 308A and *S. pyogenes* 77 versus the logarithm of the turnover numbers of nigericinols results in an approximate linear correlation (Figures 1 and 2). From these data it was deduced that the antibacterial activity of the nigericinols should be based on an ionophoric mechanism. On the basis these plots, the activity of the dichloronigericinol 7 against bacteria can also be explained by its ionophoric activity.

The transport of potassium and protons by polyethers is related to a cyclic structure. The K^+/H^+ antiport ac-

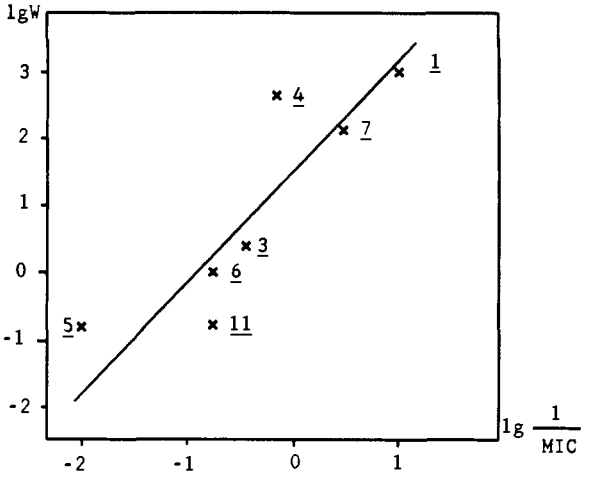

Figure 1. Plot of the log (turnover number W) vs log 1/MIC for *S. aureus* 285.

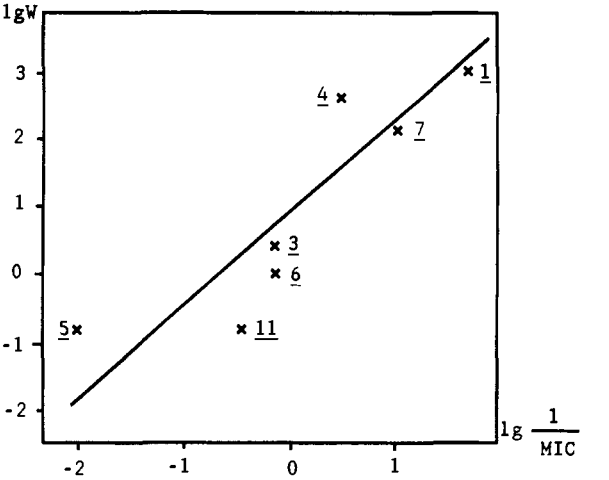

Figure 2. Plot of the log (turnover number W) vs log 1/MIC for *S. pyogenes* 308A.

tivities are decreased in the same sequence as the open conformation gains in probability, with the exception of 7. Therefore, the antibacterial activity correlates, qualitatively, with the amount of the cyclic form in the equilibrium.

The biological selectivity for the prepared derivatives was observed in principle for 3 and 5. By opening of the F ring in 3 a selectivity for bacteria were observed. In contrast, the alcohol 5 exhibited only an antiviral effect. Based on these results, it was not possible to deduce if the ionophoric character of the discussed compounds was involved in the antiviral effect.

Further experimental studies concerning the mode of action of nigericin derivatives are presently under investigation.

Experimental Section

General. The fermentation and isolation of 1 was carried out as already described.[4] NMR spectra were recorded on a Bruker AM 300 in $CDCl_3$. Chemical shifts are expressed in ppm with TMS as internal standard. The assignments of the chemical shifts were performed by 2D NMR methods and furthermore compared to the empirical rules described.[21] FAB-MS were recorded on a MS 50 Kratos Analytical with 3-nitrobenzyl alcohol as matrix. The physicochemical properties of the derivatives were summa-

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Table III. Physical Constants and Spectral Data of Compounds 2–14

no.	formula (analysis)	MW	observed mass (MK$^+$)	$[\alpha]^{20}_D$ (c = 1, $CHCl_3$)	characteristic ^{13}C NMR data ($CDCl_3$) δ in ppm, carbon position in parentheses
2	$C_{39}H_{64}O_{10}$ (C, H)	692.94	715[a]	−9.3	180.7 (29), 17.9 (1), 108.8 (13), 68.8 (7), 58.2 (40)
3	$C_{39}H_{70}O_9$ (C, H)	682.99	721	+8.7	107.6 (13), 69.1 (7), 67.0 (1), 60.6 (9), 57.7 (40)
4	$C_{41}H_{70}O_{11}$ (C, H)	739.01	762[a]	+31.7	176.1 (1), 107.6 (13), 99.1 (29), 69.3 (7), 64.0 (30), 60.3 (9), 58.3 (40), 48.5 (CH_3)
5	$C_{41}H_{72}O_{10}$ (C, H)	725.02	763	+31	107.6 (1), 99.0 (29), 69.1 (7), 64.8 (1), 64.1 (30), 60.4 (9), 58.6 (40), 48.5 (CH_3)
6	$C_{40}H_{70}O_{10}$ (C, H)	711.0	749	+35.5	108.0 (13), 97.2 (29), 69.0 (7), 67.7 (30), 64.7 (1), 60.6 (9), 58.0 (40)
7	$C_{40}H_{68}O_8Cl_2$ (C, H, Cl)	911.15	949	+15.2	107.9 (13), 96.3 (29), 69.4 (7), 60.7 (9), 57.9 (40), 50.0 and 49.5 (1 and 30)
8	$C_{44}H_{74}O_{12}$ (C, H)	795.07	834	+34.2	176.3, 176.4 (1, est), 108.2 (13), 96.3 (29), 69.0 (7), 67.9 (30), 60.4 (9), 58.5 (40)
9	$C_{45}H_{76}O_{12}$ (C, H)	809.1	848	+36.0	176.6, 176.4 (1, est), 107.6 (13), 98.4 (29), 69.4 (7), 64.3 (30), 60.4 (9), 58.3 (40), 48.3
10	$C_{45}H_{78}O_{11}$ (C, H)	795.1	833	+40.9	176.3 (est), 107.7 (13), 98.4 (29), 69.2 (7), 64.9 (1), 64.3 (30), 60.5 (9), 58.8 (40) 48.3
11	$C_{44}H_{76}O_{11}$ (C, H)	781.09	819	+24.2	177.2 (est), 108.0 (13), 96.2 (29), 69.0 (7), 67.9 (30), 64.7 (1), 60.7 (9), 57.9 (40)
12	$C_{58}H_{110}O_{14}Si_3$ (C, H)	1115.77	1137[a]	+28.3	175.3 (1), 107.4 (13), 100.9 (29)
13	$C_{52}H_{98}O_{12}Si_2$ (C, H)	971.5	1009	+38	107.5 (13), 100.8 (29), 64.5 (1)
14	$C_{44}H_{76}O_{11}$ (C, H)	781.09	819	+21.4	177.2 (est), 107.5 (13), 94.3 (29), 73.4 (1), 69.4 (7), 66.9 (30), 60.6 (9), 58.3 (40)

[a] (MNa^+).

rized in Table II. Investigation of the biological activities against Gram-positive bacteria[4] and viruses[22] were performed as described. The valence force field energy calculations were carried using the molecular mechanic program MOLMEC.[23] The force field parameters have been taken from the program system AMBER.[24]

H^+/K^+ Antiport Activity in Liposomes. Liposomes (average diameter 110 nm) were prepared from soybean phospholipids (Sigma no. 5638) as described[20] and suspended (0.6 g phospholipid/L) in a medium (pH 6.5 and 22 °C) containing 4-morpholinoethanesulfonate, glycylglycine, and KCl (0.1 M each). The internal space of the liposomes contained the same medium supplemented with the fluorescent pH-indicator 8-hydroxy-1,3,6-pyrenetrisulfonate (0.1 mM). The nigericinol derivatives tetsted were applied in dimethyl sulfoxide solution. After shifting the external pH to 7.5 the change of the internal liposomal pH (dpH/dt in equation a) was recorded, from which the proton flux (J) across the membrane was calculated using the internal buffering capacity of the liposomes B = 160 μmol H^+/g phospholipid.

29-De(hydroxymethyl)-29-oxonigericin (2). Nigericin (1) (725 mg, 1 mmol) was dissolved in 20 mL of methanol and 20 mL of water and was allowed to stir with $NaIO_4$ (1.2 g, 5.6 mmol) for 10 h. The reaction mixture was extracted 3× with EtOAc, and the organic phase was washed 4× with 40 mL of water. The organic solvent was dried over Na_2SO_4 and was evaporated in vacuo. Chromatography of the residue on silica gel with $CHCl_3/CH_3OH$ (20:1 to 9:1) resulted in **2** in a yield of 430 mg (62%).

29-De(hydroxymethyl)-29-dihydronigericinol (3). Compound **2** (400 mg, 0.57 mmol) was dissolved in 40 mL of THF and was allowed to reflux with $LiAlH_4$ (150 mg, 4 mmol) under nitrogen for 8 h. The reaction was quenched carefully with water followed by addition of 1 N HCl to pH 2. The reaction mixture was extracted 3× with 30 mL of EtOAc, and the organic phase was washed twice with 20 mL of water and dried over Na_2SO_4. The solvent was evaporated in vacuo, and the residue was chromatographed on silica gel with $CHCl_3/CH_3OH$ (20:1 to 9:1). The compound **3** was obtained in a yield of 320 mg (82%).

29-*O*-Methylnigericin (4). Nigericin (1) (10 g, 13.8 mmol) was refluxed with anhydrous LiBr (5 g, 57 mmol) for 6 h. After filtration the reaction mixture was concentrated, and the residue was chromatographed on silica gel with $CHCl_3/CH_3OH$ (15:1). The compound **4** was obtained in a yield of 8.4 g (82%).

29-*O*-Methylnigericinol (5). Compound **4** (8 g, 10.8 mmol) was refluxed with $LiAlH_4$ (1.5 g, 40 mmol) in 100 mL of THF for 3 h under nitrogen. The workup procedure was performed as described for compound **3**. 29-*O*-Methylnigericinol (**5**) was obtained in a yield of 7.1 g (91%).

Nigericinol (6). Compound **5** (7.1 g, 9.8 mmol) was heated at 60 °C with $FeCl_3$ (1 g, 6 mmol) in 40 mL of water and 40 mL of 2-propanol for 4 h. After filtration over silica gel the organic residue was evaporated. Nigericinol (**6**) was obtained in a yield of 5.4 g (78%).

1,30-Didehydroxy-1,30-dichloronigericinol (7). Nigericin (1) (710 mg, 1 mmol) was allowed to stir with methanesulfonyl chloride (340 mg, 3 mmol) in 10 mL of pyridine for 0.5 h at room temperature. After addition of water the mixture was allowed to stir for 20 min. The aqueous phase was extracted 3× with 20 mL of EtOAc, and the organic phase was washed 2× with 10 mL of 0.1 M HCl and 2× with 10 mL of water. Drying over Na_2SO_4 and concentration gave a syrup which was dissolved in 30 mL of toluene. After tetra-*n*-butylammonium chloride (1.4 g, 5 mmol) was added, the reaction mixture was heated at 60 °C for 15 h. The organic phase was washed 2× with 10 mL of water and subsequently concentrated in vacuo. Chromatography on silica gel with $CHCl_3/CH_3OH$ gradient from 40:1 to 9:1 resulted in the compound **7** in a yield of 565 mg (62%).

30-*O*-Isobutyrylnigericin (8). Nigericin (1) (724 mg, 1 mmol) was dissolved in 10 mL of pyridine, and isobutyric acid anhydride (240 mg, 1.5 mmol) was added. The reaction mixture was allowed to stir at room temperature for 24 h. After addition of 40 mL of water the mixture was stirred for 20 min. The aqueous phase was extracted 3× with 30 mL of EtOAc, and the organic phase was washed 2× times with 0.1 N HCl and 2× with 20 mL of water. Drying over Na_2SO_4 was followed by evaporation. Chromatography on silica gel with $CHCl_3/CH_3OH$ from 30:1 to 9:1 furnished the compound **8** in a yield of 680 mg (86%).

30-*O*-Isobutyryl-29-*O*-methylnigericin (9). Compound **8** (500 mg, 0.63 mmol) dissolved in 30 mL of CH_3OH was allowed to reflux with LiBr (600 mg, 2.8 mmol) for 8 h. The workup procedure was performed as described for compound **2**. The yield of **9** was 440 mg (86%).

30-*O*-Isobutyryl-29-*O*-methylnigericinol (10). Compound **9** (300 mg, 0.37 mmol) was dissolved in 30 mL of THF and was allowed to stir with *N,N'*-carbonyldiimidazole (81 mg, 0.5 mmol) for 1 h at room temperature under nitrogen. After the addition of $NaBH_4$, 40 mg (1.1 mmol) of the reaction mixture was allowed to stir for 6 h. The reaction was quenched by the addition of 4 mL of acetic acid. After neutralization with a concentrated $NaHCO_3$ solution, the organic phase was extracted 3× with 30 mL of EtOAc. The organic phase was washed 2× with 30 mL of water. Drying over Na_2SO_4, evaporation of the solvent, and chromatography on silica gel with $CHCl_3/CH_3OH$ from 30:1 to 9:1 furnished compound **10** in a yield of 210 mg (72%).

30-*O*-Isobutyrylnigericinol (11). Compound **10** (210 mg, 0.26 mmol) was heated at 60 °C with $FeCl_3$ (50 mg, 0.3 mmol) in 8 mL of water and 8 mL of 2-propanol for 4 h. Workup as it was described for compound **6** resulted in compound **11** in a yield of 145 mg (72%).

1,29,30-Tris-*O*-{[2-(trimethylsilyl)ethoxy]methyl}nigericin (12). Nigericin (1) (5 g, 6.9 mmol) was dissolved in 40 mL of CH_2Cl_2. Under nitrogen, ethyldiisopropylamine (12 mL, 72 mmol) and [2-(trimethylsilyl)ethoxy]methyl chloride (6 mL, 35.4 mmol) were added, and the reaction mixture was allowed to stir at room

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temperature for 24 h. After CH_3OH was added, the mixture was concentrated in vacuo. Chromatography on silica gel with EtOAc/hexane from 40:1 to 2:1 furnished 12 in a yield of 6 g (78%).

29,30-Bis-*O*-[[2-(trimethylsilyl)ethoxy]methyl]nigericinol (13). Compound 12 (6 g, 5.4 mmol) was allowed to reflux with $LiAlH_4$ (1.5 g, 40 mmol) in 100 mL of THF for 3 h under nitrogen. The workup was performed as described for compound 3; the yield of 13 was 4.4 g (86%).

1-*O*-Isobutyrylnigericinol (14). Compound 13 (4 g, 4.1 mmol) was heated at 40 °C in 100 mL of THF in the presence of tetra-*n*-butylammonium fluoride (800 mg) for 6 h. After filtration and evaporation of the solvent, the residue was chromatographed on silica gel with EtOAc/hexane from 40:1 to 2:1. Compound 14 was isolated in a yield of 2.3 g (72%).

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Perfluorocarbon-Based Antidiabetic Agents[⊥]

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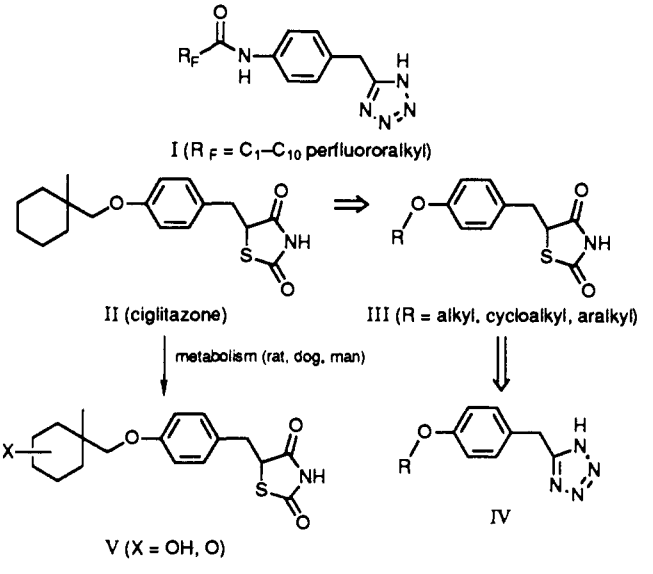
In a preliminary communication (*J. Med. Chem.* **1989**, *32*, 11-13) a series of perfluoro-*N*-[4-(1*H*-tetrazol-5ylmethyl)phenyl]alkanamides (perfluoro anilides I), designed as novel analogues of ciglitazone, were reported to possess oral antidiabetic activity in two genetic animal models of non-insulin-dependent diabetes mellitus (NIDDM): obese (ob/ob) and diabetic (db/db) mice. In this report, the results from a structure-activity relationship (SAR) study of the series I are described. Comprehensive statistical analysis among the 86 analogues screened for blood glucose lowering in ob/ob mice was achieved by a new application of a general statistical procedure which made it possible to make meaningful comparisons between more than 140 separate experiments (N = 2966). Perfluoro anilides I lowered plasma glucose in the hyperglycemic ob/ob and db/db mice but not in euglycemic normal rats. In the hyperinsulinemic ob/ob mouse, decreases in plasma insulin levels paralleled the decline in plasma glucose. Potency and efficacy in the series was shown to be dependent on the length of the perfluorocarbon chain (R_F) of I. Optimal activity occurred with the C_7 and C_8 R_F chains. The more extensive SAR studies reported here, indicated that the lipophilic R_F chain is the most important structural element of I since neither the phenyl nor tetrazole rings present in anilides I were necessary for antihyperglycemic activity while medium length (C_7-C_8) R_F chains, especially the C_7F_{15} chain, were shown to confer antihyperglycemic activity in ob/ob mice to a wide variety of structures.

Recently we reported the synthesis and antidiabetic activity of a series of perfluoro-*N*-[4-(1*H*-tetrazol-5ylmethyl)phenyl]alkanamides[1] (perfluoro anilides, I, Scheme I). These compounds, based on in vivo characterization,[2] appear to have a pharmacologic profile similar to that of ciglitazone[3] (II), the prototype for a new generation of oral antihyperglycemic agents.[4]

In several types of insulin-resistant animals (models of non-insulin-dependent diabetes mellitus (NIDDM)), ciglitazone lowers plasma glucose and improves glucose tolerance by, at least in part, improving insulin responsiveness in peripheral tissues.[5] Ciglitazone does not lower plasma glucose in nondiabetic (normal) or in insulin-deficient animals (models of IDDM). Very large doses of the drug (e.g. > 10 times the amount necessary to lower plasma glucose in insulin resistant animals) do not lower plasma glucose below euglycemic levels, unlike the widely used oral hypoglycemic sulfonylureas.[6]

We began a search for structurally novel antihyperglycemic agents which would possess the pharmacologic profile of ciglitazone but would not contain the antihyperglycemic pharmacophore III[7] (Scheme I). Substitution of a tetrazole nucleus for the acidic thiazolidinedione

Scheme I. Ciglitazone and Analogues



heterocycle generated a lead in this search, but hybrid compounds which combined the benzyltetrazole IV with

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