Ganomycins A and B, New Antimicrobial Farnesyl Hydroquinones from the Basidiomycete *Ganoderma pfeifferi*

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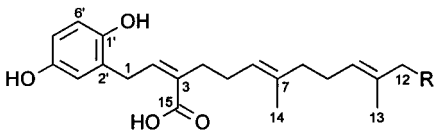
Two new farnesyl hydroquinones named ganomycin A (**1**) and ganomycin B (**2**) were isolated from *Ganoderma pfeifferi*, and their structures were elucidated by spectroscopic methods. Both carboxylic acids exhibit antimicrobial activity against several Gram-positive and Gram-negative bacteria.

Ganoderma pfeifferi Bres. (syn. *Ganoderma cupreolaccatum* Kalchbr., *Ganoderma soniese* Steyaert),[1] is a weak parasitic basidiomycete, later a saprophyte, which is only found in Europe,[2] preferring to live on *Fagus* and a variety of other deciduous trees such as *Aesculus*, *Acer*, *Fraxinus*, *Prunus*, and *Quercus*. The species is easily recognized by its cracked and wrinkled resinous layer on the pileus and by its sweet scent in winter. It is immediately distinguished from old specimens of *Ganoderma lucidum* and *Ganoderma resinaceum* by its dark brown context.[3] *G. pfeifferi* is one of the lesser known phytochemically investigated species of the family Ganodermataceae. However, a number of biologically and pharmacologically interesting triterpenes, polysaccharides, and steroids have been isolated from *G. lucidum* and from *Ganoderma applanatum*.[4−6] Sesquiterpenes have not been reported from species of the family Ganodermataceae. The present paper deals with the isolation and structure elucidation of two new farnesyl hydroquinones, 2-[2-(2,5-dihydroxyphenyl)-ethyliden]-11-hydroxy-6,10-dimethyl-undeca-5,9-diene acid (**1**) and 2-[2-(2,5-dihydroxyphenyl)-ethyliden]-6,10-dimethyl-undeca-5,9-diene acid (**2**), which have been named ganomycins A (**1**) and B (**2**). Aside from the widespread ubiquinones, related compounds are not common among fungi. However, they have been reported in a broad spectrum of marine organisms, including brown algae and sponges,[7,8] and some previous reports have noted the cytotoxic and antimicrobial activities of such compounds.[9,10] Other related farnesyl hydroquinones have been isolated from the plant *Seseli farreyni*[11] and from the exudate of the glandular trichomes of *Turricola parryi*.[12] Nothing is known about their antimicrobial activity.



1: R = OH
2: R = H

In the course of a random screening program for antibacterial metabolites in fungal extracts, the dichloromethane extract of *G. pfeifferi* showed activity against several Gram-positive and -negative bacteria. Air dried and powdered fruiting bodies of *G. pfeifferi* were extracted with dichloromethane in a Soxhlet apparatus for 24 h. The extract was fractionated on Sephadex LH 20 with *n*-hexane−CH_2Cl_2 (2:7) and finally with methanol to afford eight fractions (A−H). Further separation of the only antibacterially active fraction, H, led to 12 subfractions. Subfractions H-8 and H-11 were further purified on Sephadex LH-20 with CH_2Cl_2−MeOH (4:1) and CH_2Cl_2−acetone (1:2) to give ganomycins A and B (**1** and **2**).

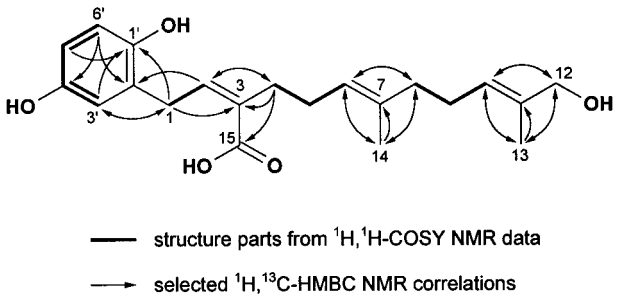


Figure 1. Selected NMR correlations in the structure elucidation of ganomycin A (**1**).

Ganomycin A (**1**) was obtained as a yellow oil. Because EIMS of **1** failed to produce a molecular ion, the molecular mass *m*/*z* 360 was derived from (−)- and (+)-DCIMS with ammonia, which showed highly abundant molecular-ion pairs at *m*/*z* 358/360 for $[M - 2]^-$ and $[M]^-$ and at *m*/*z* 376/378 for $[M - 2 + NH_4]^+$ and $[M + NH_4]^+$. The appearance of such pairs is characteristic of hydroquinones.[13] From the ^{1}H and ^{13}C NMR spectral data, together with the HMQC data, the 21 signals in the ^{13}C NMR spectrum were assigned to two methyl, one oxymethylene, and six aliphatic methylene groups; five sp^2 methine groups, two oxygen-bearing sp^2 carbons; and one carboxyl group. These accounted for the even-numbered molecular ion, except for four exchangeable protons. Together with the latterone acid and three hydroxy protons the elemental composition of $C_{21}H_{28}O_5$ was proposed for **1**.

The corresponding structure **1** was derived via four short structure parts recognized in the ^{1}H−^{1}H COSY NMR spectrum, which could be interconnected according to long-range correlations in the ^{1}H−^{1}H COSY and ^{1}H−^{13}C HMBC NMR spectra as shown in Figure 1. The *E* configuration of the double bonds was assigned according to the correlations in the ^{1}H−^{1}H NOESY NMR spectrum. The MS- and NMR-derived structure of ganomycin A (**1**) further accounts for

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Table 1. NMR Data for Ganomycin A (**1**)[a]

position	^{1}H δ[b]	m	J [Hz]	^{13}C δ[c]	m
1′				149.30	s
2′				128.04	s
3′	6.61	d	2.8	117.76	d
4′				151.17	s
5′	6.52	dd	8.5, 2.8	114.78	d
6′	6.64	d	8.5	116.91	d
1	3.69	d	7.7	31.75	t
2	5.98	t	7.8	140.52	d
3				133.32	s
4	2.33	t	7.2 (br)	35.88	t
5	2.19	dt	7.2, 7.3 (br)	28.49	t
6	5.14	tq	7.3, 0.9	124.61	d
7				136.73	s
8	2.00	t	7.8 (br)	40.38	t
9	2.10	dd	7.1, 7.8 (br)	27.27	t
10	5.39	tq	7.1, 1.0	126.76	d
11				135.75	s
12	3.94	s	br	69.03	t
13	1.66	s	(br)	13.73	q
14	1.61	d	1.0 (br)	16.15	q
15				172.32	s

[a] All NMR data were recorded using CD_3OD solution. [b] Recorded at 400 MHz. [c] Recorded at 100 MHz.

Table 2. Antimicrobial Activity of Ganomycins A and B[a]

strain	zones of inhibition (mm)	
	ganomycin A	ganomycin B
S. aureus ATCC 6538	19	20
S. aureus ATCC 25923	15	17
S. aureus ATCC 29213	16	18
S. aureus SG 511	24	24
B. subtilis SBUG 14	16	15
M. flavus SBUG 16	25	26
P. aeruginosa ATCC 15442	b	b
E. coli SBUG 13	4	5
P. mirabilis SBUG 47	15	15
S. marcescens SBUG 9	15	16

[a] 100 μg/paper disk. [b] No activity.

the UV absorptions at 298 nm (log ϵ 3.65, hydroquinone) and 210 nm (log ϵ 4.42, unsaturated carboxyl) as well as for the main IR absorptions of hydroxy groups (about 3400 cm^{-1}) and an unsaturated carboxy group (1626 cm^{-1}).

Ganomycin B (**2**) was also obtained as a yellow oil. Because IR and UV spectra of **2** nearly matched those of **1**, the close relationship of the ganomycins was evident. The molecular formula of **2** could be established as $C_{21}H_{28}O_4$ by HREIMS of the molecular ion m/z 344. The DCIMS spectra again showed pairs of molecular ions. The difference of one oxygen in the structure of **2** is reflected in the NMR spectra by the replacement of the signals of the primary alcohol by an additional methyl group.

Ganomycins A and B showed moderate growth inhibition of several bacterial strains, particularly Gram-positive strains such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Micrococcus flavus* (zones of inhibition, 15−25 mm) (Table 2). The minimum inhibitory concentrations (MIC) for both ganomycins A and B against *M. flavus* were determined as 2.5 μg/mL (MIC ampicillin 0.25 μg/mL). Against *S. aureus* a MIC of 25 μg/mL for each of the ganomycins could be measured (MIC ampicillin 0.05 μg/mL). None of these compounds were active in assays against *Candida albicans* and *Candida maltosa* at 100 μg/paper disk. The structurally related coenzyme Q 10, which plays a role in photosynthesis and electron transport, did not show antimicrobial activity in our investigations. Further biological evaluation of ganomycins A and B is in progress.

Experimental Section

General Experimental Procedures. TLC was carried out on Si gel 60 F_{254} plates (Merck) with the solvent toluene−EtOAc−HCO_2H (5:5:0,1). Chromatograms were inspected under UV light at wavelengths 254 and 366 nm, and by spraying with anisaldehyde−H_2SO_4 reagent. Column chromatography was carried out on Sephadex LH 20 (Pharmacia Biotech). IR spectra (in KBr) were recorded on a Perkin-Elmer FTIR 1650 spectrometer and UV spectra in MeOH with Uvikon 930 spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker spectrometer AM 400 with the solvent signal as internal reference.

Fungal Material. Mature fruiting bodies of *G. pfeifferi* were collected in the vicinity of Greifswald (Germany) in September 1995. They were kindly identified by Prof. Dr. H. Kreisel, Department of Biology, Ernst-Moritz-Arndt-Universität, D-17487 Greifswald, Germany. A voucher specimen is deposited at the Department of Pharmaceutical Biology, Ernst-Moritz-Arndt-Universität, D-17487 Greifswald, Germany.

Extraction and Isolation. Air-dried and powdered fruiting bodies of *G. pfeifferi* (100 g) were extracted with CH_2Cl_2 (4 × 700 mL) in a Soxhlet apparatus for 24 h. The extract (4 g) was separated repeatedly on Sephadex LH 20 column [column 3.5 × 35 cm, flow 0.4 mL/min] by elution with *n*-hexane−CH_2Cl_2 (2:7) and finally with MeOH to yield eight fractions (A−H). Fraction H (1.1 g) was separated further on Sephadex LH 20 [column 2.5 × 35 cm, flow 0.3 mL/min] with MeOH−H_2O (2:1) and provided 12 fractions (H-1 to H-12). Fraction H-8 (110 mg) was purified repeatedly on Sephadex LH-20 (column 1 × 65 cm, flow 0.2 mL/min) with CH_2Cl_2−MeOH (4:1) to yield 22 mg of compound **1**. Further purification of H-11 (100 mg) on Sephadex LH-20 column (column 1 × 65 cm, flow 0.2 mL/min) with acetone−CH_2Cl_2 (2:1) afforded 20 mg of compound **2**.

Ganomycin A (**1**): 2-[2-(2,5-dihydroxyphenyl)-ethyliden]-11-hydroxy-6,10-dimethyl-undeca-5,9-diene acid; yellow oil; TLC toluene−EtOAc−HCO_2H (5:5:0.1) R_f = 0.46 (green spot after spraying with anisaldehyde−H_2SO_4 reagent); UV (MeOH) λ_{max} (log ϵ) 210 (4.42) and 298 (3.65) nm; IR (KBr) ν_{max} 3327, 2925, 2852, 2360, 2341, 1626, 1573, 1449, 1244, and 668 cm^{-1}; NMR, see Table 1; MS (−)-DCI (NH_3) m/z (%) 360 (100) $[M]^-$, 358 (21) $[M - 2]^-$; (+)-DCI (NH_3) m/z (%) 378 (88) $[M + NH_4]^+$, 376 (100) $[M + NH_4 - 2]^+$.

Ganomycin B (**2**): 2-[2-(2,5-dihydroxyphenyl)-ethyliden]-6,10-dimethyl-undeca-5,9-diene acid; a yellow oil; TLC toluene−EtOAc−HCO_2H (5:5:0.1) R_f = 0.58 (black spot after spraying with anisaldehyde−H_2SO_4 reagent); UV (MeOH) λ_{max} (log ϵ) 214 (4.30) and 297 (3.53) nm; IR (KBr) ν_{max} 3272, 2968, 2855, 2355, 2341, 1683, 1630, 1459, 1380, 1248, 1200, 961, and 813 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) similar to **1** but δ = 5.13 [1H, m (with 6-H at 5.10 ppm)], 2.05 (2H, dt, 7.1/7.3 Hz, 9-H), 1.68 (3H, d, 1 Hz, 13-H_3), 1.62 (3H, s, 12-H_3); ^{13}C NMR (CD_3OD, 100 MHz) similar to **1** but δ = 132.0 (C-11), 125.5 (C-10, assignment interchangeable with C-6 at δ 124.4), 27.7 (C-9), 25.9 (C-13), 17.8 (C-12); EIMS (200 °C) m/z (%) 344 (40) $[M]^+$, 326 (23), 189 (73), 69 (100); (−)-DCI (butane) m/z (%) 344 (100) $[M]^-$, 343 (35) $[M - 2H]^-$; (+)-DCI (butane): m/z (%) 345 (100) $[M + H]^+$, 343 (41) $[M + H - 2]^+$; HREIMS found 344.1927, calcd 344.1987 for $C_{21}H_{28}O_4$.

Antibiotic Test System. The antimicrobial tests were performed with Gram-positive and Gram-negative bacteria (Table 2) by the agar diffusion method. Ampicillin (SIGMA) and coenzyme Q 10 (SIGMA) were used as controls. Substance-containing paper disks (6 mm diam) were deposited on the surface of agar plates (nutrition agar, SIFIN, Berlin) seeded with overnight cultures of the test microorganisms. The plates with *S. aureus*, *B. subtilis*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* were incubated for 18 h at 37 °C. The plates with *Serratia marcescens* were incubated, at 30 °C and those with *M. flavus* for 48 h at 25 °C. After incubation the inhibition zones were measured and recorded as the diameter of the zone. The minimum inhibitory concentration (MIC) values were determined by standard serial broth microdilution assay in nutrition medium II (SIFIN, Berlin), starting from a 250-μg/mL solution. The end point in this assay

was indicated by the absence of detectable growth after 18 h of incubation at 37 °C.

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