GRISEOCHELIN, A NOVEL CARBOXYLIC ACID ANTIBIOTIC FROM *STREPTOMYCES GRISEUS*

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Griseochelin, $C_{33}H_{60}O_7$, isolated from an asporogenous strain of *Streptomyces griseus* represents a novel carboxylic acid antibiotic. The metabolite, which is active against Gram-positive bacteria, forms water-insoluble salts with mono- and divalent cations and binds alkaline-earth metal ions specifically in 2: 1 (X_2M) stoichiometry. Detailed spectral (IR, MS and NMR) studies provide full characterization of its constitution featuring a carboxylic acid function, a substituted tetrahydropyran ring, an allylic OH group which are accomodated within a tetrahydroxylated-octamethyl-C_{25} diene backbone.

Griseochelin was isolated in the course of our investigations into the biochemistry of streptomycin-non-producing Amy$^-$ mutants derived from high-yielding streptomycin-producing strains of *Streptomyces griseus*[1~3]. The metabolite which is active against Gram-positive bacteria exhibits physicochemical properties reminiscent of polyether antibiotics: It forms water insoluble complex salts with various metal ions by virtue of its carboxylic acid and hydroxyl groups; its salts with alkaline-earth metal ions show particularly high stability and are formed in 2: 1 (ligand/metal) stoichiometry. Still, the metabolite is unusual: Its oxygen content is lower than that required by the general formula for polyethers, $(C_{3\sim4}H_{5\sim7}O)_n$[4], its molecular backbone contains one single tetrahydropyran ring and the molecule shows a number of features uncommon for polyethers. The present communication describes the production, isolation, chemical and spectral characterization and biological activity of the new metabolite.

Strain

The griseochelin-producing strain, *S. griseus* ZIMET 43681, was taken from the strain collection of the Central Institute of Microbiology and Experimental Therapy, Jena. It represents a stable prototropic Amy$^-$ Str$^-$ mutant of the high-yielding, streptomycin-producing (Amy$^+$ Str$^+$) *S. griseus* HP strain[2]. Derived from the parent strain *via* glycerol-limited chemostat cultivation, this mutant exhibits a number of modified biochemical properties. In particular, it excels in its capability to produce griseochelin under submerged cultivation in enhanced yields that may exceed those of the parent strain by a factor of 10 to 30[3].

Fermentation

Lyophilized stock mycelia[1] were first propagated in agar slant cultures at 27°C for 8 days. The medium consisted of baker's yeast 1.5%, maize starch 1%, NaCl 0.5%, peptone (Difco 0.1%) and agar 2% and had a pH 7.0. Samples of the slant cultures were then used to inoculate the growth medium

consisting of glucose 2%, soybean flour 2%, NaCl 0.5%, KH_2PO_4 0.1% and $CaCO_3$ 0.1% at pH 6.7. Incubation was at 28°C for 2 days by stirring at 240 rpm. Further stage of the propagation was carried out in 2.5-liter flasks using the same growth medium and conditions. The culture was then inoculated to 500 liters of the fermentation medium consisting of glucose 3%, soybean flour 3%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% with pH 6.2. The medium was sterilized at 120°C for 20 minutes. Incubation was at 28°C for 4 days, stirring at 280 rpm and aerating at 1 liter air/minute/liter medium. Griseochelin was found to accumulate predominantly in the mycelium and attain its highest concentration (20 to 40 μg/ml) at the end of the cultivation period. The production of the metabolite was practically unaffected by the addition of either inorganic phosphates or initiator molecules (sodium acetate, sodium propionate) to the culture at the start of the fermentation.

Isolation of Griseochelin

The fermentation broth was separated by filtration yielding 14 kg (wet) mycelial residue. This was extracted twice with acetone and the collected extracts evaporated. The oily residue (160 g) was chromatographed in portions of 5 g on a column (1 m × 6 cm) filled with alumina (Chemiewerk, Greiz, G.D.R.) using benzene as the eluent. The metabolite-containing fractions were pooled and evaporated to yield 45 g of the crude product. Five gram of the crude product was then rechromatographed in 1-g portions on a silica gel column (Kieselgel 60, Merck, 1.2 m × 3 cm) using $CHCl_3$ - MeOH (95: 5) as the eluent. The antibiotic-containing fractions were again collected, evaporated and the residue (2.0 g) was dissolved in 100 ml 1 M aqueous triethylamine. To the filtered solution 50 ml of 1 M aqueous $CaCl_2$ was added that caused the calcium salt of griseochelin to precipitate. The calcium salt (1.5 g) was dried, dissolved in small amounts of $CHCl_3$ and subjected to column chromatography on Sephadex LH-20 using MeOH as the eluent. From the metabolite-containing fractions the calcium salt of griseochelin crystallized in large colorless cubes. Repeated chromatography on Sephadex LH-20 afforded the calcium salt in high purity. To obtain the free acid in high purity, a solution of the calcium salt in $CHCl_3$ - C_6H_{12} - AcOH (2: 7: 1) was chromatographed on a silica gel column (Kieselgel 60, Merck). Evaporation of the solvent gave griseochelin as a colorless glassy solid which crystallized after 10 to 20 days upon standing at ambient temperatures.

Chromatographic fractions containing griseochelin were identified by spraying the TLC sheets with 1% solution of vanillin in conc sulfuric acid. This approach has also been adopted for quantitative assays of the metabolite where use was made of the linear correlation existing between the area of the stained spots and the logarithm of the concentration of the metabolite. The correlation was established by means of standard solutions of griseochelin. Chromatograms were made on precoated TLC sheets (Silufol, Kavalier, CSSR) using benzene - ether (1: 1) as the eluent.

Characterization of Griseochelin

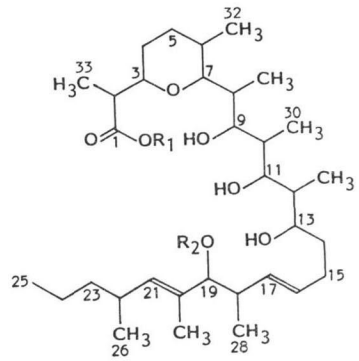
Physico-chemical properties of the free acid (**1**) and its magnesium salt (**2**) are summarized in Table 1.

The elemental composition, $C_{33}H_{60}O_7$, and constitution of the new metabolite portrayed in **1** were initially inferred from a separate high-field two-dimensional (2D) NMR study[5]. It has been shown that the metabolite has a carboxylic acid function attached at one end of a tetrahydroxylated-octamethyl-C_{25} alkyl chain that accomodates one ether linkage resulting in a substituted tetrahydropyran ring, and two unsaturated bonds. Another characteristic structural feature that greatly influences the chemical and physico-chemical behavior of the metabolite is the presence of an allylic OH function in the molecular backbone. Subsequent chemical and spectroscopic studies have confirmed the NMR-based

Table 1. Physico-chemical properties of griseochelin (**1**) and its Mg-salt (**2**).

	1	**2**
Nature	Colorless crystals	Colorless crystals
Mp (°C)	72~74	229~231
$[\alpha]_D^{25}$ (c 1.0, $CHCl_3$)	0°	+13.0°
MW (MS)	568.4309 (M^+, Calcd 568.4339)	1,158.8492 (M^+, Calcd 1,158.8367)
MW (Osmometry)	—	1,140±25
Formula	$C_{33}H_{60}O_7$	$C_{66}H_{118}O_{14}Mg$
Anal Found:	C 69.7, H 10.6, O 19.7	C 68.4, H 10.2, O 19.3, Mg 2.1
Calcd:	C 69.8, H 10.5, O 19.7	C 68.5, H 10.1, O 19.4, Mg 2.0
IR ($CHCl_3$) (ν cm^{-1})	3547 (ν_{OH}), 3400 (ν_{OH}), 3284 (ν_{OH}), 3187 (ν_{OH}), 2650 (ν_{COOH}), 1735 (ν_{CO})	3560 (ν_{OH}), 3370 (ν_{OH}), 3240 (ν_{OH}), 2700 (ν_{OH}), 1565 ($\nu_{CO_2^-}$)
Characteristic MS peaks *m/z* (%)	568 (0.02) $C_{33}H_{60}O_7$, 428 (100) $C_{24}H_{44}O_6$, 287 (7) $C_{15}H_{27}O_5$, 229 (15) $C_{12}H_{21}O_4$, 171 (92) $C_9H_{15}O_3$, 153 (11) $C_9H_{13}O_2$, 141 (5) $C_9H_{17}O$	1,158 (1) $C_{66}H_{118}O_{14}Mg$, 1,157 (0.5) $C_{66}H_{117}O_{14}Mg$, 1,114 (21) $C_{65}H_{118}O_{12}Mg$, 1,017 (5) $C_{57}H_{101}O_{13}Mg$, 591 (100) $C_{33}H_{59}O_7Mg$, 573 (10) $C_{33}H_{57}O_6Mg$, 451 (10) $C_{24}H_{43}O_6Mg$, 171 (10) $C_9H_{15}O_3$, 153 (10) $C_9H_{13}O_2$, 141 (5) $C_9H_{17}O$

elemental composition and provided corroborative evidence for the proposed constitution (**1**). Details of these complementary works are outlined in the following.



1 $R_1=R_2=H$
2 $R_1=Mg/2$, $R_2=H$
3 $R_1=CH_3$, $R_2=H$
4 $R_1=R_2=CH_3$

Griseochelin is readily converted into its methyl ester derivative (**3**) through the addition of equimolar amounts of diazomethane dissolved in dry ether at ambient temperature. Acid-catalized methylation with MeOH - HCl (5%) in dry ether at room temperature affords the griseochelin-methyl ester-19-*O*-methylate (**4**) as a mixture of two separable C19 epimers, in full accord with the proposed allylic nature of the hydroxyl group at C19. Acetylation of **1** with Ac_2O - pyridine at 0°C for 24 hours yields a mixture of mono- to tetraacetates with a net predominance of the lower (C13 and C19 mono- and di-) acetates of griseochelin. Attempts to separate the product mixture into its components proved as yet unsuccessful.

The free acid (**1**) forms labile salts with alkali metal ions when treated with corresponding bases or large excesses of the metal ions. Preliminary data suggest the stability constant to decrease in the order $Li^+ > Na^+ > K^+$. By contrast, the salts with alkaline earth metal ions and some other divalent inorganic cations (*e.g.* Cd^{2+}, Zn^{2+}, Mn^{2+}, *etc.*) show high stability and, according to all evidence, contain two molecules of the antibiotic for one metal ion.

Griseochelin and its salts dissolve readily in benzene, $CHCl_3$ and other non-polar organic solvents; they are less soluble in lower alcohols, ethers and ketones. The free acid exhibits low (approximately 0.15 g/liter at 25°C) solubility while its alkaline-earth metal salts are practically insoluble in water.

IR Spectrometry

The IR spectrum of the free acid (Fig. 1) shows the presence of a carboxylic acid function (ν_{CO} 1735, ν_{COOH} 2650 cm^{-1}) and at least three, differently H-bonded, OH groups (ν_{OH} 3547, 3400 and 3258 cm^{-1}). Computer-aided iterative bandshape analysis revealed that the band at 3258 cm^{-1} arises from the superposition of two separate OH bands at ν_{OH} 3284 and 3187 cm^{-1}. Comparison of IR spectra recorded in KBr and dilute $CHCl_3$ solutions disclosed that the carboxylic acid OH and the three lower frequency alcoholic OH groups are H-bonded intramolecularly. Methylation of the allylic OH groups as in **4** results in the disappearance of one of the H-bonded OH band and in a net displacement of the ν_{CO} band to higher wave-numbers. The latter finding provides support to other spectroscopic observations[5] according to which the free acid in solutions assumes such a form that allows for H-bonded interaction to occur between COOH and C19-OH groups. On going to the magnesium salt of the metabolite (**2**), one observes the characteristic shift in the carbonyl frequency accompanying the transformation of the acid function into carboxylate anion ($\nu_{CO_2^-}$ 1565 cm^{-1}) and the displacement toward lower frequencies and simultaneous broadening of two OH bands (ν_{OH} 3240 and 2700 cm^{-1}) which suggests that two of the OH functions become involved in the metal ion-ligand interactions. The IR spectra

Fig. 1. FT IR spectrum of griseochelin free acid in 0.001 M $CHCl_3$ solution. Solvent bands are subtracted.

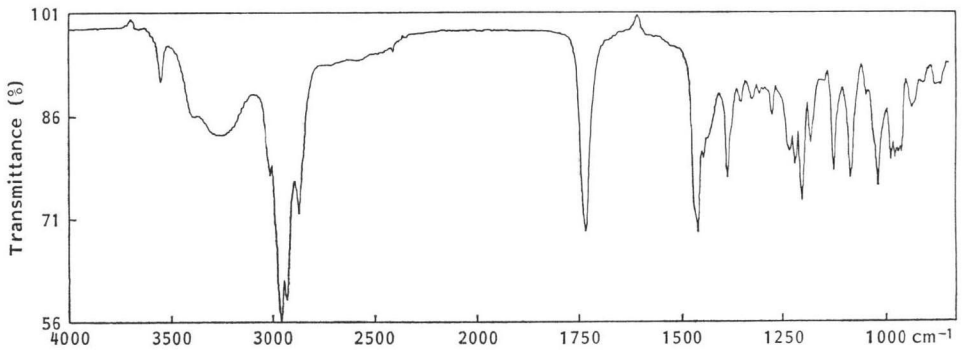


Fig. 2. 400 MHz ^1H NMR spectrum of griseochelin Mg-salt in $CDCl_3$.

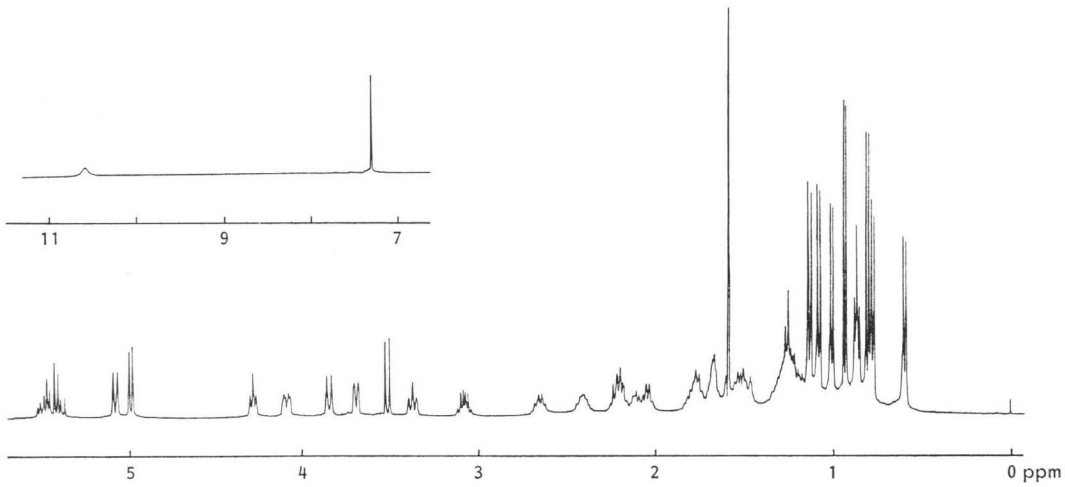
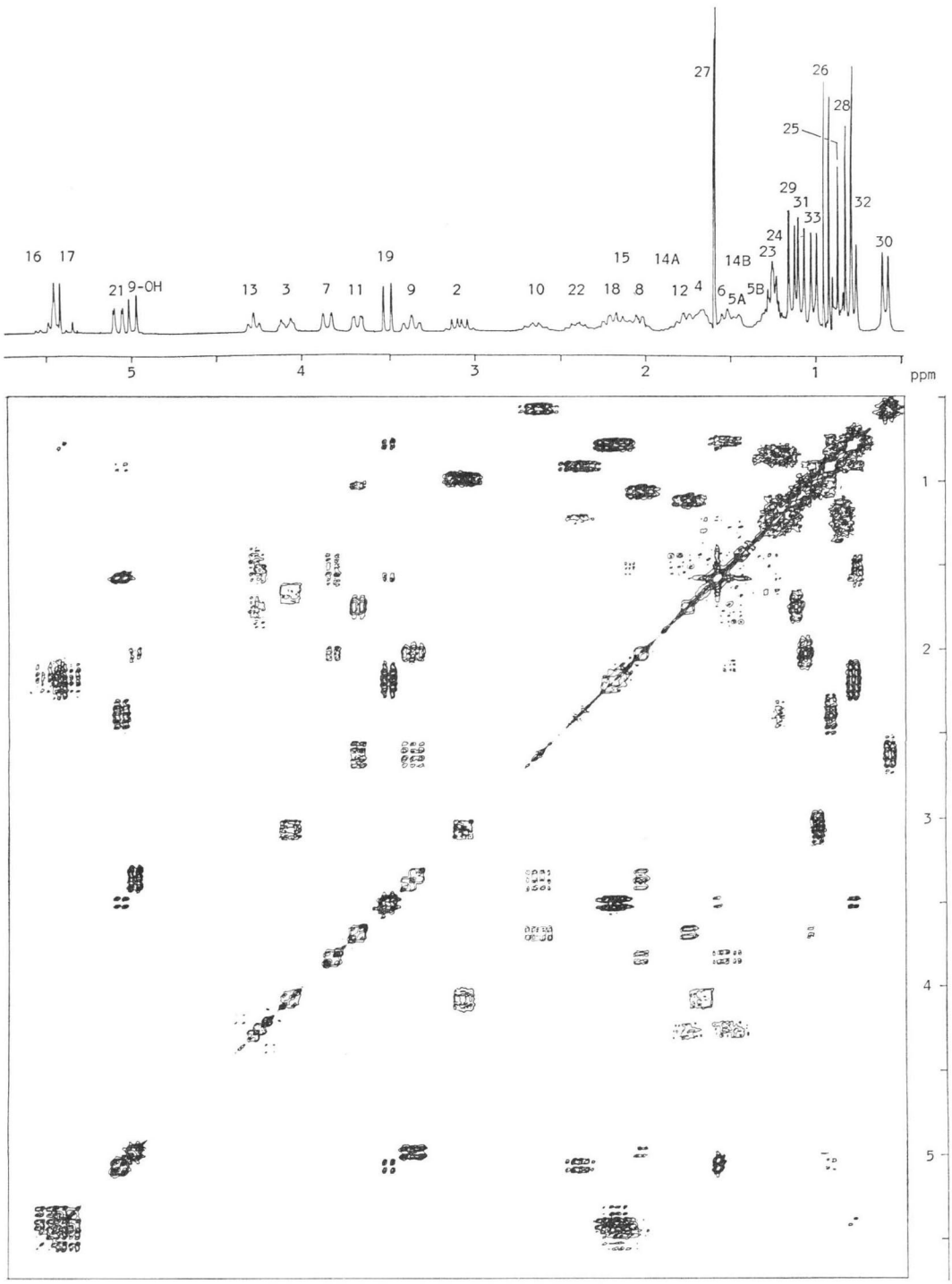


Fig. 3. 200 MHz conventional and chemical shift correlated 2D NMR spectra of griseochelin Mg-salt in $CDCl_3$.



of the salts with alkali metal ions show great similarity to those of the Mg^{2+}-salt. However, as attested by IR spectra, salts obtained in the presence of excess concentrations of alkali metal ions contain various amounts of the free acid, the salt/acid ratio being related to the nature of the cation.

Mass Spectrometry

The accurate mass data for the free acid and its Mg^{2+}-salt are given in Table 1. The mass fragmentation of **1** is dominated by a rearrangement process that proceeds *via* the cleavage of C18/C19 bond and concomitant proton transfer from C19-OH to the carboxyl group facilitated presumably by the spatial proximity of the two functions[6]. This gives the base peak at *m/z* 428 (M−140) and the lower intensity ion at *m/z* 141 (M−427, 5%). The proposed fragmentation route is fully supported by the mass spectra of derivatives **3** and **4**.

In spite of the apparent complexity, most of the ions in the mass spectrum of the magnesium salt (**2**) can be rationalized by considering the loss of CO_2 and the cleavage of various fragments encountered in the spectrum of the free acid (**1**). A more detailed account of the mass spectrometry of griseochelin and its derivatives will be the subject of a subsequent paper.

NMR Spectrometry

To derive the elemental composition and constitution of the new metabolite, **1**, two-dimensional (2D) proton chemical shift correlated (COSY)[7] spectra of griseochelin were recorded at 400 MHz[5]. The same experimental approach has been adopted here to interpret the ^1H NMR spectrum of the magnesium salt (Fig. 2) in terms of chemical shifts and connectivities of protons that form a virtually uninterrupted sequence along the molecular backbone. Displayed as the contour map of the absolute value 2D data matrix, the COSY spectrum of **2** is shown in Fig. 3. Chemical shift correlations mediated

Table 2. ^1H NMR chemical shifts in griseochelin (**1**) and its Mg-salt (**2**).[a]

Proton	1	2	Proton	1	2
H2	3.29	3.07	H18	2.24	2.19
H3	4.03	4.08	H19	3.58	3.16
H4A	1.75	1.65	H21	5.11	5.08
H4B	1.64	1.46	H22	2.42	2.41
H5A	1.50	1.45	H23A	1.28	1.28
H5B	1.32	1.28	H23B	1.20	1.20
H6	1.62	1.50	H24	1.24	1.26
H7	3.75	3.85	H25	0.89	0.86
H8	2.02	2.01	H26	0.93	0.94
H9	3.46	3.37	H27	1.59	1.59
H10	2.00	2.64	H28	0.85	0.81
H11	3.69	3.69	H29	1.12	1.14
H12	1.74	1.75	H30	0.67	0.59
H13	4.03	4.27	H31	1.08	1.08
H14A	1.75	1.78	H32	0.78	0.78
H14B	1.35	1.46	H33	1.16	1.01
H15A	2.18	2.18	C9-OH	4.43[b]	4.99
H15B	2.12	2.08	C11-OH	5.93[b]	7.23
H16	5.49	5.49	C13-OH	4.12[b]	10.7
H17	5.37	5.37	C19-OH	2.24[b]	2.3

[a] In ppm relative to internal TMS, $CDCl_3$, 27°C.

[b] Because of fast proton-exchange, these resonances do not appear in the spectrum of **1**; the shift values were therefore measured in the methyl ester derivative **3**.

by ^2J, ^3J and longer range interproton couplings are manifested by the occurrence of off-diagonal ('cross') peaks. Evaluation of the chemical shift coordinates (δ_1, δ_3) of the cross peaks affords a consistent labelling of the sequentially coupled protons which, in turn, leads to the assignment of the resonances to the individual proton sites of the molecule. The fully assigned chemical shifts of the free acid (**1**) and its magnesium salt (**2**) are compared in Table 2.

Because of the COOH-induced intramolecular exchange processes characteristic of many carboxylic acid antibiotics[8], detection of the OH resonances in the ^{1}H NMR spectrum of **1** proved unsuccessful even in highly aprotic solvents and at low temperatures[5]. By contrast, these resonances do appear in the spectrum of **2** (see Fig. 2) and some of the OH protons retain their (residual) coupling to the respective carbinyl methine proton which allows for ready assignment. Inspection of the pertinent chemical shift data in Table 2 shows that resonances due to C11-OH and C13-OH groups become substantially deshielded in **2** with respect to their position in the spectrum of the methyl ester derivative (**3**). This finding suggests that binding of the Mg^{2+} ions by the metabolite occurs with the participation of these hydroxyl groups.

Fig. 4. 50 MHz ^{13}C NMR spectrum of griseochelin free acid in $CDCl_3$.

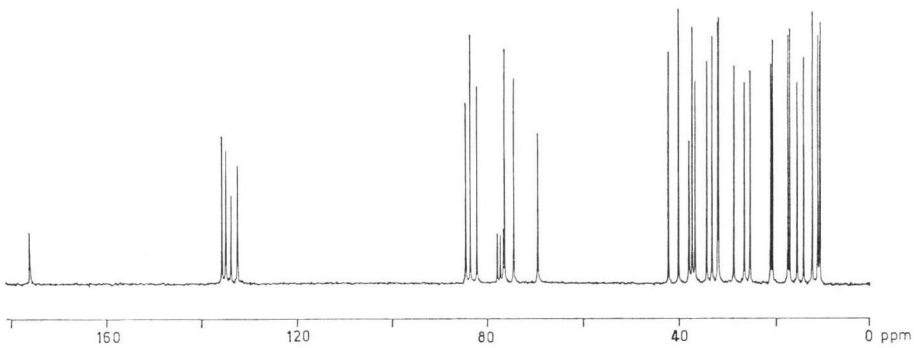


Table 3. ^{13}C NMR chemical shift data for griseochelin (**1**) and its Mg-salt (**2**).[a]

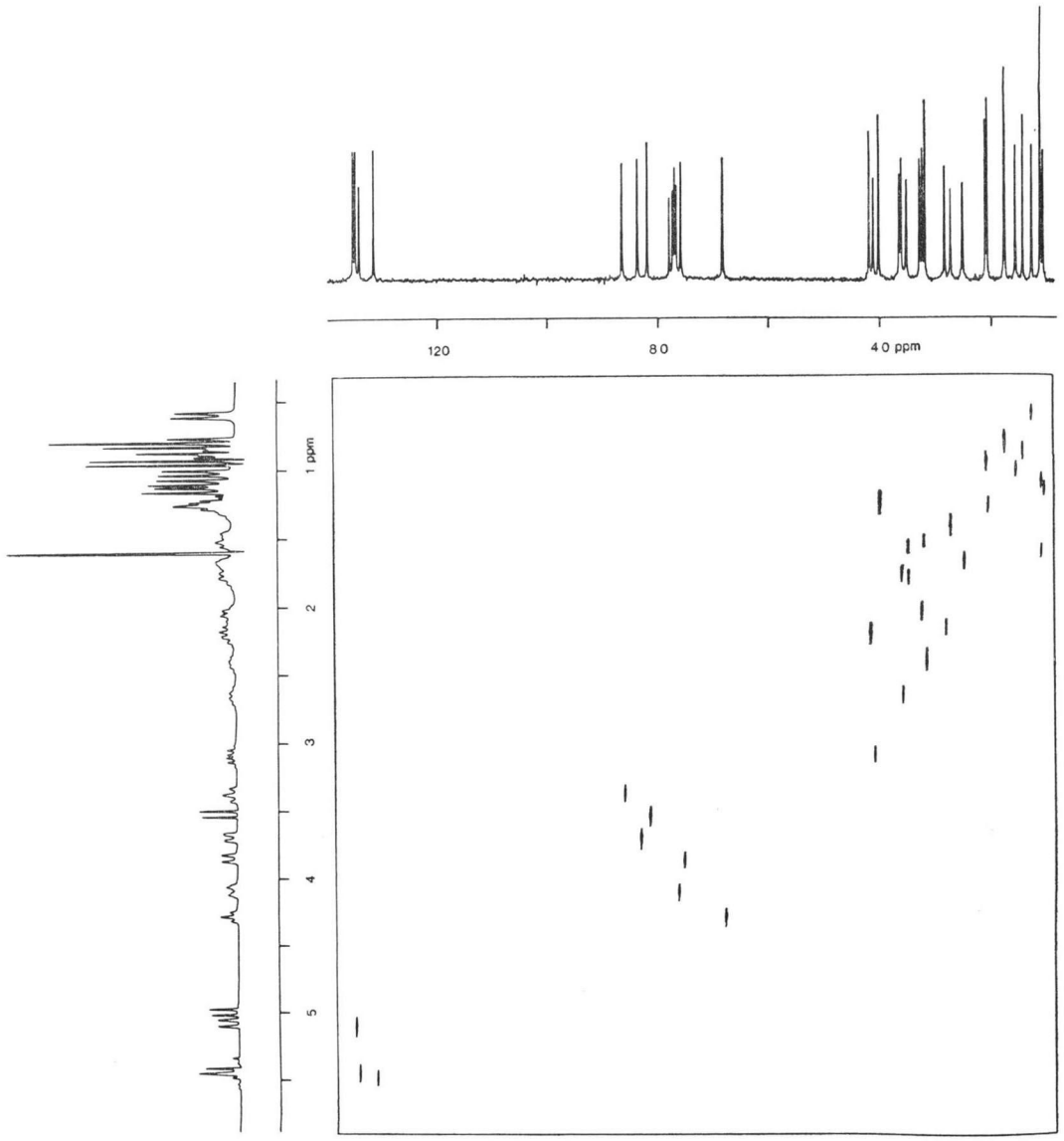
Carbon	1	2	Carbon	1	2
C1	176.09 (s)	182.85 (s)	C18	42.21 (d)	41.72 (d)
C2	37.94 (d)	41.04 (d)	C19	82.14 (d)	81.80 (d)
C3	74.36 (d)	76.47 (d)	C20	133.57 (s)	133.88 (s)
C4	25.19 (t)	25.01 (t)	C21	135.63 (d)	135.09 (d)
C5	26.39 (t)	27.23 (t)	C22	31.86 (d)	31.82 (d)
C6	32.03 (d)	32.28 (d)	C23	40.04 (t)	40.03 (t)
C7	76.32 (d)	75.69 (d)	C24	20.67 (t)	20.59 (t)
C8	33.24 (d)	32.74 (d)	C25	14.18 (q)	14.18 (q)
C9	84.46 (d)	86.33 (d)	C26	20.99 (q)	20.89 (q)
C10	37.28 (d)	36.02 (d)	C27	10.87 (q)	10.99 (q)
C11	83.45 (d)	83.52 (d)	C28	17.48 (q)	17.49 (q)
C12	36.72 (d)	36.29 (d)	C29	10.77 (q)	10.50 (q)
C13	69.33 (d)	68.23 (d)	C30	12.38 (q)	12.57 (q)
C14	34.37 (t)	35.13 (t)	C31	11.27 (q)	10.99 (q)
C15	28.64 (t)	28.34 (t)	C32	17.11 (q)	17.44 (q)
C16	132.33 (d)	131.33 (d)	C33	15.58 (q)	15.50 (q)
C17	134.70 (d)	134.58 (d)			

[a] In ppm relative to internal TMS, $CDCl_3$, 28°C; s singlet, d doublet, t triplet, q quadruplet.

The ^{13}C NMR spectrum of the free acid is displayed in Fig. 4. The chemical shifts and multiplicities of the assigned individual resonances are collected in Table 3 where they can be compared with the respective data for the magnesium salt (**2**). In both cases the order of carbon atoms was inferred from multiplicity-selected DEPT spectra[9] while the assignment of the carbon resonances was conveniently performed by means of 2D heteronuclear chemical shift correlation experiment[10]. Displayed as the contour map of the absolute value 2D spectrum of the magnesium salt (**2**), the result of the ^{1}H-^{13}C correlation experiment mediated by one-bond carbon-proton couplings is shown in Fig. 5.

Fig. 5. 50/200 MHz conventional and carbon-proton chemical shift correlated 2D NMR spectra of griseochelin Mg-salt in $CDCl_3$ with broad band decoupling in the proton dimension.

The resonance at 35.13 ppm (C14) shows two correlation peaks because of large non-equivalence of pertinent methylene protons.



Evaluation of the chemical shift coordinates (δ_{C_i}, δ_{H_i}) of the correlation peaks gives immediately and unambiguously the assignment of the carbon chemical shift to the respective proton sites of the molecule.

Interpretation of the ^1H and ^{13}C chemical shift differences between the free acid and its magnesium salt in terms of the ion binding properties of the new metabolite requires a detailed knowledge of the stereochemistry of both species. Studies directed toward this end are now being carried out in our laboratories.

The C_{25} backbone of griseochelin suggest propionic acid as initiator and a number of propionic acid subunits involved in the biosynthesis[11]. The relatively low oxygen content of the metabolite may by accounted for by assuming either excessive reduction or dehydratation following the assembly of the polyketide chain. Studies on the biosynthesis of griseochelin are now in progress in our laboratories.

Biological Properties

Griseochelin and its calcium salt exhibit broad antibiotic activity against Gram-positive bacteria. The minimum inhibitory concentration (MIC) values for representative strains obtained by agar plate assay are given in Table 4. No biological activity has been observed against Gram-negative bacteria, yeasts and fungi. (In view of the low water solubility, prior to these tests the free acid was solubilized by the addition of Tris and Tween 80 detergent to the water suspension of the metabolite in approximately 2: 1: 1 (w/w/w) ratio while the calcium salt was dissolved in MeOH.) LD_{50} was found to range from 500 to 750 mg/kg when tested orally in male hybrid mice ($ABD2F_1$) and to vary from 1 to 2 mg/kg when administered intravenously to JeLei WIST rats. The ammonium and sodium salts of griseochelin exhibited significant anticoccidal activity against *Eimeria tenella* W/CAM in quantities above 100 μg/embryo when using the mortality if chicken embryos as a measure.

Table 4. MIC (μg/ml) of griseochelin (**1**) and its Ca-salt against Gram-positive bacteria.

Organism	**1**[a]	Ca-salt[b]
Bacillus subtilis ATCC 6633	1.0	1.8
B. subtilis SG 119	1.0	1.5
Micrococcus luteus 125a	1.0	1.5
M. pyogenes var. *aureus* 511	50	75

[a] In H_2O (see text). [b] In MeOH solution.

Experimental

The IR spectra were recorded on a Nicolet 7000 FT IR instrument in KBr pellets and in dilute $CHCl_3$ solutions using 0.086 mm cells. A Jeol JMS D-100 (EI MS, direct inlet, 150°C) and an AEI MS 902 (EI MS, direct inlet, 120~140°C) mass spectrometers were used to obtain the mass spectra. Detection of the low intensity molecular ions was achieved by evaporating the sample from the surface of a quarz road deeply inserted into the ion source.

The NMR spectra were recorded on a Bruker WM-400-WB and a Bruker WP-200/SY instruments. ^1H Homonuclear chemical shift correlated 2D spectra were obtained by incrementing in a regular manner the length of the period t_1 of the 90-t_1-90-t_2 COSY sequence[7] and accumulating the NMR response during t_2. The ^1H-^{13}C heteronuclear chemical shift correlated 2D spectra were measured with a modified 90(H)-$t_{1/2}$-180(C)-$t_{1/2}$-τ_1-90(H)90(C)-τ_2-t_2 (acquire with broad band proton decoupling) sequence[10]. The modification consisted in the replacement of the non-selective 180(C) pulse by a selective proton inversion ('BIRD') sequence[12] resulting in broad band decoupling of the proton dimension.

Griseochelin Mg^{2+}-salt (**2**)

Method I: To a solution of 56 mg (0.1 mM) griseochelin free acid (**1**) in $CHCl_3$ (5 ml) a solution

of 5 mg (0.05 mM) $MgCl_2$ in H_2O (2 ml) was added and the heterogeneous liquid was stirred for 2 hours at ambient temperature. The organic phase was separated, washed twice with H_2O, dried over molecular sieve (Merck) and the solvent was evaporated to yield **2** as a colorless glassy solid (49 mg).

Method II: To a solution of 56 mg griseochelin free acid in 10 ml H_2O containing 100 mg Tris base 5 mg $MgCl_2$ was added. The precipitated Mg^{2+}-salt was filtered, washed twice with H_2O and dried in air to yield **2** as a white amorphous solid (41 mg). Crystallization from MeOH gave the magnesium salt as colorless elongated cubes. Physico-chemical properties are in Table 1.

Griseochelin Methyl Ester (3)

To a solution of 56 mg griseochelin in $CHCl_3$ equimolar amount (0.1 mM) of diazomethane in dry ether was added at ambient temperature. After evaporation of the solvents the product was purified column chromatography (Kieselgel 60, Merck) using hexane - EtOAc (1: 1) as the eluent (Rf 0.64). Colorless glassy solid (40 mg); IR ($CHCl_3$) 3547, 3438, 3359 (ν_{OH}), 1730 (ν_{CO}) cm^{-1}; MW (MS) 582.4495 (Calcd 582.4487 for $C_{34}H_{62}O_7$).

Griseochelin Methyl Ester 19-*O*-Methylate (4)

A solution of griseochelin sodium salt (0.4 g) in dry MeOH with 5% HCl was stirred at ambient temperature for 48 hours. After addition of 20 ml H_2O, the product was extracted twice with 40 ml $CHCl_3$. The organic layer was dried ($MgSO_4$) and evaporated. The crude product (0.35 g) was subjected to column chromatography (Kieselgel 60, Merck) using benzene - ether (2: 1) as the eluent. Chromatography yielded **4** as two separable colorless oily fractions (Rf 0.51, 160 mg **4a** and Rf 0.40, 48 mg **4b**) exhibiting identical IR and MS data. IR ($CHCl_3$) 3495, 3440, 3363 (ν_{OH}), 1738 (ν_{CO}) cm^{-1}. MW (MS) 596.4637 (Calcd 596.4652 for $C_{35}H_{64}O_7$). The site of *O*-methylation followed from the ^{13}C NMR spectra exhibiting for both isomeric products an approx 10 ppm downfield shift of the C19 carbon resonance relative to its position in the spectrum of the free acid (**1**).

Acknowledgments

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