The Constitution of Griseochelin

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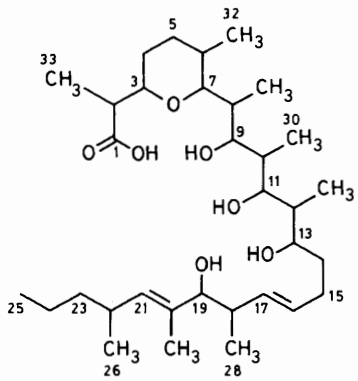
High field 2 dimensional n.m.r. spectra disclose the unusual constitution of a novel carboxylic acid ionophore antibiotic, griseochelin.

Most of the known carboxylic acid ionophore antibiotics produced by strains of *Streptomycetes* are polyethers.[1] Formally, these have the general empirical formula $(C_{3-4}H_{5-7}O)_n$ (usually: $9 < n < 15$) and contain a considerable number of tetrahydro-furans and -pyrans in their backbone.[1] Structural studies reported in this communication show that griseochelin, a novel carboxylic acid antibiotic isolated recently by Gräfe *et al.* from cultures of a modified strain of *S. griseus*,[2] represents an interesting exception to this formal definition. Its empirical formula, $C_{33}H_{60}O_7$, and constitution (**1**) are suggested by the following evidence.

The only pieces of structural information available at the outset of the present work were that the metabolite contained a carboxylic acid group, at least two hydroxy functions [i.r., $\nu_{max}(CHCl_3)$ 1735, 3400, 3545 cm^{-1}], and had a molecular mass of around 550 a.m.u.‡ A reasonable estimate of the elemental composition was readily accessible through multiplicity-selected ^{13}C n.m.r. spectra.[3] The inventory, chemical shift values, and multiplicities of the carbon-13 resonances [9 CH_3 groups resonating between δ 10 and 20 p.p.m., 6 CH_2 and 7 CH carbon lines between δ 20 and 40 p.p.m., 6 CH(OR) groups resonating between δ 70 and 84 p.p.m., 3 proton-bearing and 1 quaternary olefinic carbon signals at δ *ca.* 130 p.p.m., and 1 CO_2H line at δ 176 p.p.m.] indicated that, depending on the number of OH (R = H) and ether functions in the molecule, the empirical formula of the new metabolite should vary from $C_{33}H_{58}O_6$ to $C_{33}H_{62}O_8$. Observation of just two quaternary carbon atoms in the product suggested the use of ^1H n.m.r. spectroscopy to elucidate its constitution and stereochemistry.



(**1**)

From the 400 MHz ^1H n.m.r. spectrum (Figure 1) it became evident that, owing to extensive overlap of tightly coupled multi-spin resonances (δ 1.2—2.3), full identification of inter-proton connectivities by conventional n.m.r. methods would

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‡ Owing to facile intramolecular rearrangements and associated fragmentation processes, detection of M^+ ions in early mass spectra proved unsuccessful.

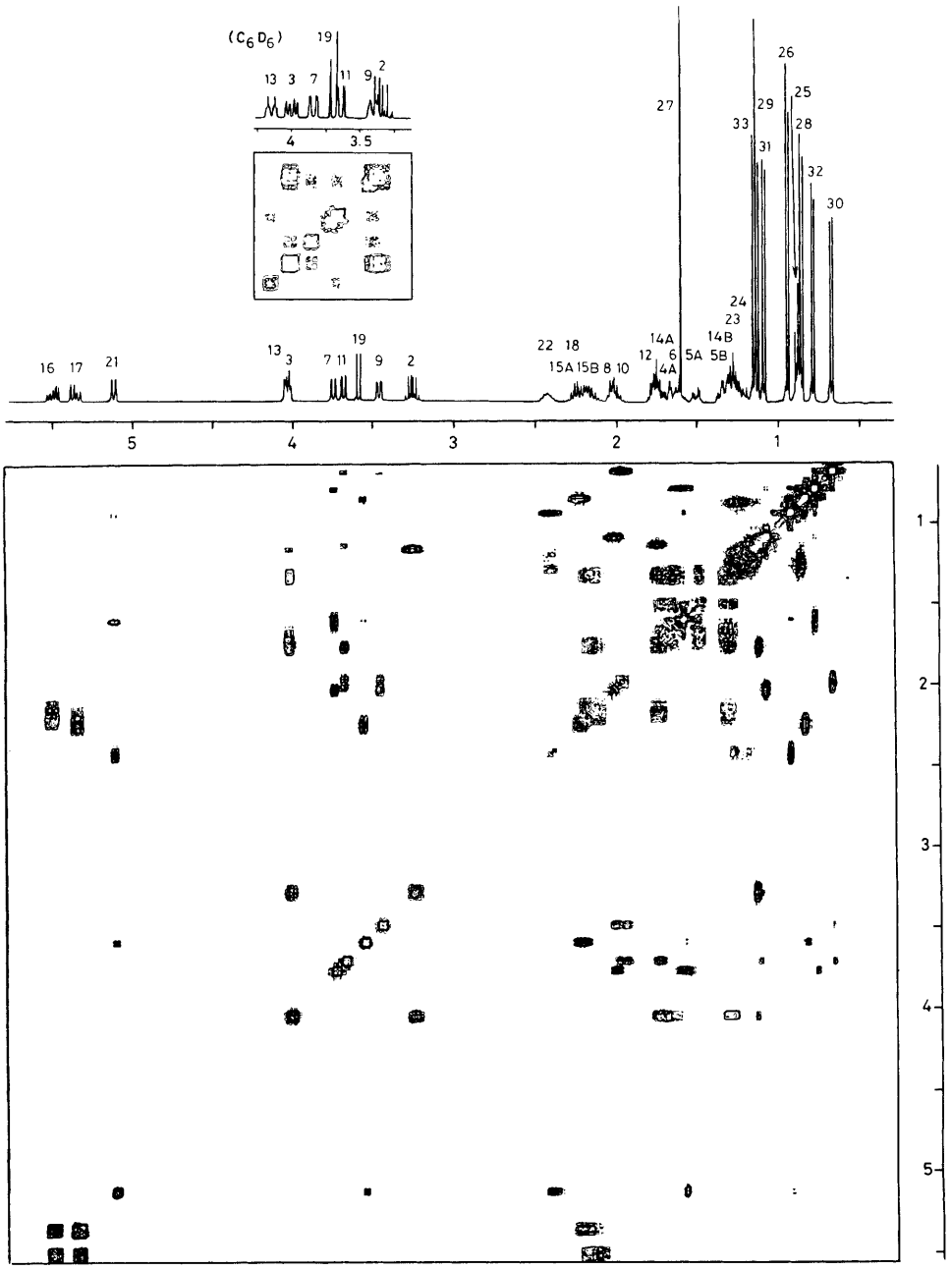

Figure 1. Conventional and chemical shift correlated 2D ^{1}H n.m.r. spectrum of 0.1 M griseochelin in $CDCl_3$ at 313 K and 400 MHz (Bruker WM-400-WB). COSY Parameters: spectral width (in both dimensions): 2200 Hz; acquisition time: 0.465 s; recycle time: 2.5 s; number of t_1 increments: 512; quadrature detection. Transform with sine-bell multiplication in both dimensions and zero-filling to 1 K in the F_1 dimension. Absolute value presentation of the symmetrized data matrix. Inset: partial contour map of the COSY spectrum run in C_6D_6 solution at 200 MHz (Bruker WP-200/SY) with a fixed delay of 0.5 s inserted before and after the mixing pulse (ref. 6). Transform with squared sine-bell window function in both dimensions.

be impractical (if not impossible) even at the high magnetic fields used. A two-dimensional (2D) time domain data matrix was therefore set up by incrementing in a regular manner the length of the t_1 period of the familiar 90-t_1–90-t_2 COSY sequence[4] and accumulating the n.m.r. response during t_2. Complex Fourier transformation afforded the 2D frequency domain data matrix displayed as a contour map of the absolute value spectrum in Figure 1 where chemical shift correlations mediated by scalar ^{1}H–^{1}H couplings are manifested by the occurrence of off-diagonal ('cross') peaks. Evaluation of the chemical shift co-ordinates (δ_i, δ_j) of cross peaks afforded a consistent labelling of the sequentially coupled protons (see Table 1) which, in turn, led directly to the constitution (**1**). Although the sequence of protons was inferred primarily from correlations *via* two- and three-bond couplings, of considerable assistance were the numerous cross peaks owing to long range ^{1}H–^{1}H interactions. Thus, interruption of the sequence of vicinally coupled protons by quaternary C-20 is seen to be more than compensated for by the appearance of cross peaks mediated by $^4J_{19,21}$, $^4J_{19,27}$, $^4J_{21,27}$, and $^6J_{26,27}$.

CO_2H-Induced intramolecular exchange processes[5] made detection of the OH resonances (as expected from the i.r.

Table 1. ^{1}H Chemical shift correlations (δ_i,δ_j) in (**1**) ($CDCl_3$).

$\delta(y)_i$				$\delta(x)_i$	$^nJ_{ij}$[a]							
5.49	5.37	$^3J_{16,17}$	(15.3)	2.18	$^3J_{16,15A}$		2.12	$^3J_{16,15B}$				
5.37	2.24	$^3J_{17,18}$	(8.7)	2.12	$^4J_{17,15B}$							
5.11	3.58	$^4J_{21,19}$		2.42	$^3J_{21,22}$	(9.5)	1.59	$^4J_{21,27}$		0.93	$^4J_{21,26}$	
4.03	3.69	$^4J_{13,11}$[b]		1.75	$^3J_{13,14A}$		1.74	$^3J_{13,12}$	(2.5)	1.35	$^3J_{13,14B}$	1.12 $^4J_{13,29}$
4.03	3.75	$^4J_{3,7}$[b]		3.29	$^3J_{3,2}$	(11.2)	1.73	$^3J_{3,4A}$	(1.3)	1.64	$^3J_{3,4B}$	(5.0)
3.75	3.46	$^4J_{7,9}$[b]		2.02	$^3J_{7,8}$	(2.1)	1.62	$^3J_{7,6}$	(10.2)	0.78	$^4J_{7,32}$	
3.69	3.46	$^4J_{11,9}$[b]		2.00	$^3J_{11,10}$	(9.6)	1.74	$^3J_{11,12}$	(2.5)	1.12	$^4J_{11,29}$	0.67 $^4J_{11,30}$
3.58	2.24	$^3J_{19,18}$	(9.2)	1.59	$^4J_{19,27}$		0.85	$^4J_{19,28}$				
3.46	2.02	$^3J_{9,8}$	(2.7)	2.00	$^3J_{9,10}$	(10.1)	0.67	$^3J_{9,30}$				
3.29	1.16	$^3J_{2,33}$										
2.42	1.28	$^3J_{22,23A}$		1.20	$^3J_{22,23B}$		0.93	$^3J_{22,26}$				
2.24	0.85	$^3J_{18,28}$										
2.18	2.12	$^2J_{15A,15B}$		1.75	$^3J_{15A,14A}$		1.35	$^3J_{15A,14B}$				
2.12	1.75	$^3J_{15B,14A}$		1.35	$^3J_{15B,14B}$							
2.02	1.08	$^3J_{8,31}$										
2.00	0.67	$^3J_{10,30}$										
1.75	1.35	$^2J_{14A,14B}$										
1.74	1.12	$^3J_{12,29}$										
1.73	1.64	$^2J_{4A,4B}$		1.50	$^3J_{4A,5A}$		1.32	$^3J_{4A,5B}$				
1.62	1.50	$^3J_{6,5A}$		1.32	$^3J_{6,5B}$		0.78	$^3J_{6,32}$				
1.59	0.85	$^6J_{27,28}$										
1.28	1.24	$^3J_{23A,24}$		1.20	$^2J_{23A,23B}$							
1.24	0.89	$^3J_{24,25}$										

[a] Cross peak co-ordinates [$\delta(y)_i$,$\delta(x)_j$] below the diagonal (in p.p.m. relative to internal $SiMe_4$) for coupling path $^nJ_{ij}$. Coupling constants (Hz), in parentheses, were assigned by double resonance experiments. [b] Cross peaks shown in the inset of Figure 1. See also text.

spectra) unsuccessful even at reduced temperatures and in highly aprotic solvents. Discrimination between the methine protons in –CHOCH– and –CH(OH)– was possible by noting cross peaks between resonances of these protons: magnetization transfer between resonances of structurally distant 3-H and 7-H indicated four-bond H–COC–H coupling across the ether linkage, whereas the cross peaks for the other methine protons indicated long range interaction between neighbouring CH(OH) groups *via* $^4J_{HCCCH}$: $^4J_{7,9}$, $^4J_{9,11}$, and $^4J_{11,13}$. Owing to unfavourable conditions for magnetization transfer (small ^4J and short T_2 values of interacting protons) the latter cross peaks were of low intensity and do not appear in the full correlation map of Figure 1. They could, however, be observed by reprocessing the time domain data matrix using appropriate window-functions resulting in enhanced signal-to-noise ratios for these low intensity cross peaks or by inserting a fixed delay time before and after the mixing pulse of the COSY sequence[6] as illustrated in the partial contour map in Figure 1 [obtained in C_6D_6 wherein the CH(OR) methine resonances appear without substantial overlap]. The location and nature of CH(OR) groups thus inferred has received full support by converting the free acid (**1**) into its methyl ester derivative (*via* the addition of a few drops of diazomethane in dry diethyl ether to the $CDCl_3$ solution) and then rerunning the ^{1}H n.m.r. spectrum which revealed the presence of four OH protons vicinally coupled to their respective methine CH: δ_H ($CDCl_3$) 5.93 (d, *J* 2.2 Hz, 11-OH), 4.43 (d, *J* 8.6 Hz, 9-OH), 4.12 (d, *J* 1.8 Hz, 13-OH), and 2.24 (d, *J* 2.0 Hz, 19-OH).

Also collected in Table 1 are some relevant couplings related to the steric arrangements of substituent groups along the aliphatic chain.§ Model studies, carbon-13 T_1 data, and the low H/D exchange rates of OH protons in the methyl ester derivative (inaccessibility to solvent molecules) suggest that, as in polyether ionophores,[1,5] griseochelin assumes a bent, hairpin-like, solution conformation. This conformation is maintained by intramolecular H-bonds between polar groups whose restricted polar region is responsible for the ionophore activity of the metabolite. Griseochelin forms stable complexes with alkaline-earth metal ions in a 2:1 (X_2M) stoicheiometry.[2] The substitution pattern of the C_{25} backbone suggests propionic acid as the initiator and that a number of propionic acid subunits are involved in the biosynthesis of the polyketide chain.[7]

The constitution of griseochelin is the first of such a complex ionophore antibiotic to be determined by direct n.m.r. spectroscopic methods.

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§ The stereochemistry at all 13 chiral centres in (**1**) remains undefined.

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