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THE STRUCTURES OF GRISEUSINS A AND B, NEW ISOCHROMANQUINONE ANTIBIOTICS

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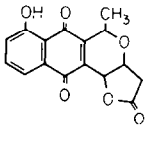
Abstract—The structures of griseusins A and B have been elucidated from chemical and spectroscopic data, and their absolute configurations have also been determined by CD spectroscopy as **4a** and **5**, respectively. The griseusin is a new type of isochromanquinone antibiotic including a spiro-ring system fused with a juglone moiety. The conversion reaction of griseusin B to griseusin A suggests the biogenetic pathway of the γ-lactone ring formation in some isochromanquinone antibiotics.

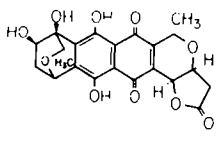
Griseusins A and B are antibacterial antibiotics produced by a strain of *Streptomyces griseus*, and it was suggested from preliminary characterization that both antibiotics contain a 5 - hydroxy - 1,4 - naphthoquinone (juglone) chromophore, and have molecular formulae, $C_{22}H_{20}O_{10}$ and $C_{22}H_{22}O_{10}$, respectively.[1] This paper is concerned with the structure determinations of the antibiotics.

The chromophore of griseusin A was confirmed by its ^{1}H NMR spectrum (Fig. 1) which shows a peri-OH signal at δ_H 11.80 (1H, s) and an ABC-type signal due to three aromatic protons (vicinally arranged) at 7.1–7.8, and by the natural-abundance ^{13}C FT NMR spectrum which exhibits two quinone CO signals at δ_C 187.4 and 181.5 and eight ^{13}C signals (three doublets and five singlets in ^{1}H single-frequency off-resonance decoupled spectra) in the region of 100–165. Since no ^{1}H signals attributable to the hydrogens on the quinone ring are found, the naphtho-quinone should be substituted at the C-2 and C-3 positions (see partial structure **1**).

Regarding other oxygen-containing groups, the presence of an aliphatic OAc group (1740 cm^{-1}; δ_H 2.08, 3H, ; δ_C 170.5, s and 21.2, q) is evident. An IR band at 3580 cm^{-1} and a broad ^{1}H signal at 2.9 (1H) which disappeared by addition of D_2O (see Figs. 1a and b) suggest the presence of an additional OH group. The addition of D_2O also converts a multiplet signal at δ_H 4.87 (1H) to a double ; therefore, the OH group should be secondary alcohol. In fact, treatment of griseusin A with acetic anhydride in the presence of *p*-toluenesulfonic acid and of pyridine gave a monoacetate and a diacetate, respectively. From their ^{1}H NMR spectra (Fig. 2), the monoacetate was found to be an acetylation product of the alcoholic OH group and the diacetate to be a product which had been fully acetylated. The large downfield shift of the signal at δ_H 4.87 by the acetylation (+1.03 ppm) clearly proves the presence of the secondary OH group (see Table 1). Further, a CO IR band at 1795 cm^{-1} and ^{1}H signals (centred at δ_H 2.90, 2H) assignable as the AB part of an ABX spin system (J_{AB} = 18.0, J_{AX} = 4.0, and J_{BX} < 1.0 Hz) resemble those of kalafungin[2] and granaticin;[3] thus, it is likely that griseusin A has a γ-lactone system similar to those found in the



Kalafungin

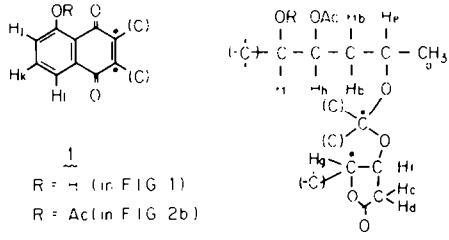


Granaticin

both compounds. ^{13}C NMR signals at δ_C 174.0 (s) and 36.5 (t) are compatible with this assumption. The remaining two oxygen atoms should be ethereal and be in a form of

as judged from the ^{13}C signal at δ_C 96.6 (s).

The sequence of the carbon atoms bearing hydrogen atom(s) were determined by ^{1}H NMR spin-decoupling experiments at 100 MHz on the diacetate, and the assignment of ^{1}H signals was given in Fig. 2(b). Thus, the partial structure of griseusin A can be formulated as structure **1**. This partial structure includes all the



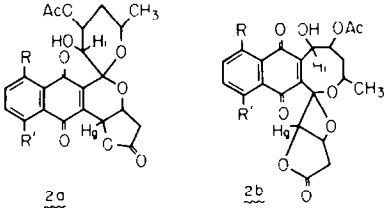
1

R = H (in FIG 1)
R = Ac (in FIG 2b)

constituent atoms and is in accord with the ^{13}C NMR results. Therefore, the completion of the linkages between the five asterisked carbon atoms is established.

Since the linkages, CH_i–CH_g and

, can easily be excluded, only two structures **2a** and **2b** are possible for griseusin A without regard to the position of the peri-OH group. However, the latter structure **2b**



2a **2b**

R = OH, R' = H or R = H, R' = OH

seems unlikely because it has a highly strained γ-lactone system different from those of kalafungin and granaticin. Furthermore, the structure is definitely inconsistent with the requirement that the CH_g carbon should be allylic, as mentioned below.

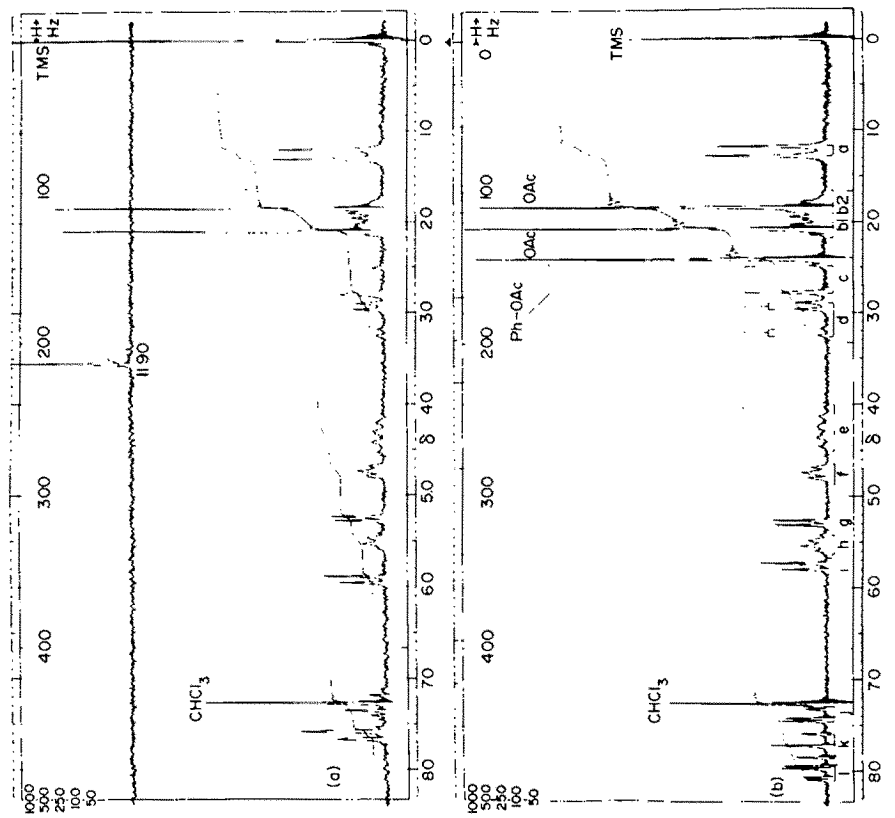


Fig. 2. The 60-MHz ^{1}H NMR spectra of (a) griseusin A monoacetate and (b) griseusin A diacetate in $CDCl_3$.

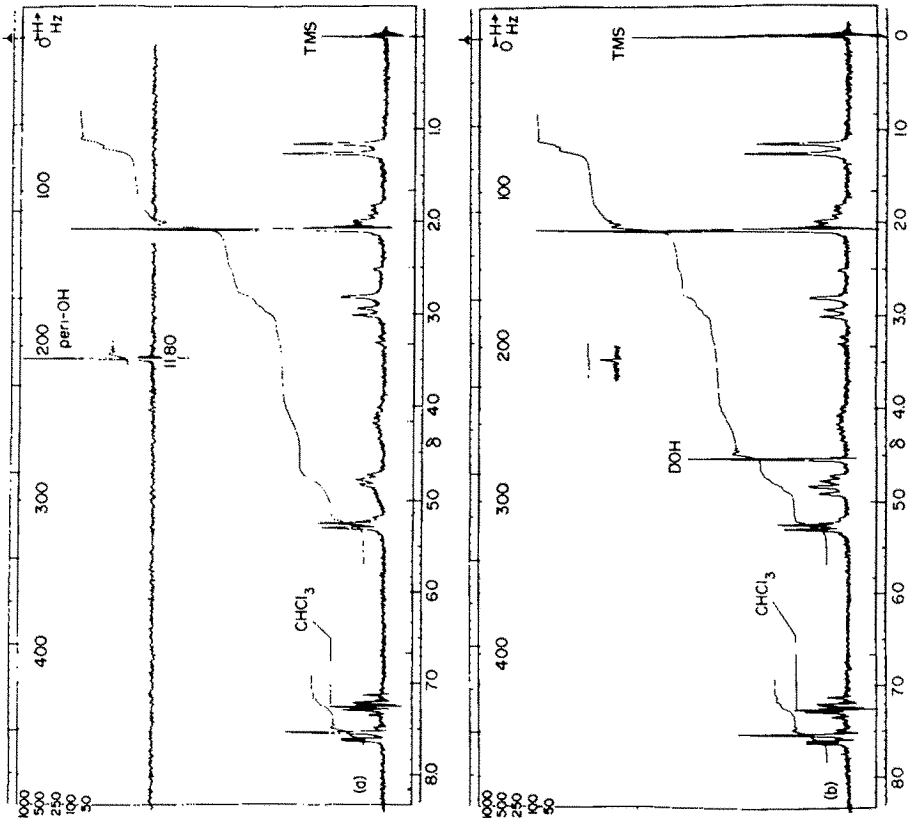
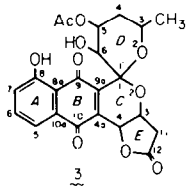


Fig. 1. The 60-MHz ^{1}H NMR spectra of griseusin A. (a) in $CDCl_3$; (b) in $CDCl_3$ with an addition of one drop of D_2O.

Table 1. Changes in ^1H chemical shifts δ_H by acetylation in $CDCl_3$

	peri-OH	CH_f	CH_g
Griseusin A	11.80	4.87	5.28
Griseusin A monoacetate	11.90	5.90	5.26
Griseusin A diacetate	---	5.76	5.28

The position of the peri-OH group was determined by the ^1H NMR spectra of griseusin A and its acetates. As shown in Table 1, acetylation of the alcoholic OH group results in a downfield shift of the peri-OH signal by +0.10 ppm, and acetylation of the peri-OH function caused and upfield shift of the CH, signal by −0.14 ppm, but no effect on the CH_g signal. These facts clearly reveal that the two OH groups are closely located through the quinone carbonyl group. Thus, griseusin A should be shown as structure **3**.



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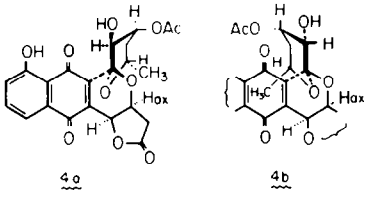


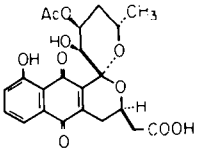
4a 4b

The stereochemistry on the aliphatic rings of griseusin A was revealed by the ^1H NMR spectrum. Since the J value between C(3)–H_f and C(11)–H_c is less than 1 Hz, the dihedral angle between the hydrogens should be near 90°. Inspection of Dreiding models suggests that the only conformation in which the C(3)–H_f is *axial* and *cis* to C(4)–H_g satisfies this situation. As mentioned above, the C(6')–OH group is located closely to the quinone carbonyl at C(9), the C(1)–C(6') bond should be quasi-equatorial. In the ^1H NMR spin decoupling experiments, it was observed that the value of $[J_{5',4'(ax)} + J_{5',4'(eq)}]$ is *ca.* 7 Hz, and that the value of $[J_{3',4'(ax)} + J_{3',4'(eq)}]$ is *ca.* 13.5 Hz. Accordingly, the conformations of C(5')–H_h and C(3')–H_e are reasonably assigned as equatorial and axial, respectively. The $J_{5',6'}$ value of 4.0 Hz corresponds to a vicinal $J_{eq,ax}$ value. Thus, the conformation of the C(6')–H_i is concluded to be axial.

Model examinations suggest that two structures having a chair conformation for the D-ring, **4a** and **4b**, are in harmony with the above stereochemistry. However, structure **4b** in which the C(6')–C(5') and O–C(3') bonds turn to the quinone ring, is unlikely because of a severe steric hindrance between the quinone carbonyl at C(9) and the C(5')–OAc group. Thus, griseusin A should be shown as structure **4a** or its antipode.

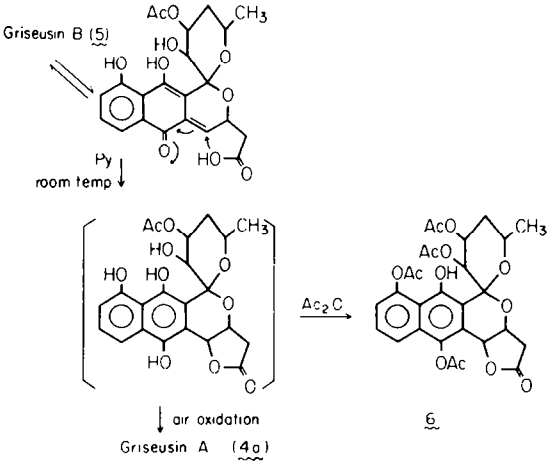
Griseusin B is more polar than griseusin A. Its IR spectrum shows no γ-lactone band but a new carbonyl band at 1722 cm^{-1}. Combination of these facts with its molecular formula suggests that griseusin B is a compound having a carboxymethyl group instead of the γ-lactone.

On treating with acetic anhydride in the presence of *p*-toluenesulfonic acid, griseusin B gave a monoacetate which had been acetylated at the alcoholic OH group as griseusin A. The comparison of its ^1H NMR spectrum with that of griseusin A monoacetate clearly shows that the ^1H signal corresponding to C(4)–H_g is absent. Further, the signal comparable to C(3)–H_f changed to a broad multiplet and two methylene signals, ABX and A_2X, appeared in the region of δ_H 2.1–3.2. These observations support the structure **5** for griseusin B.



5

Treatment of griseusin B with acetic anhydride in pyridine gave an unexpected colourless acetate. From its spectroscopic results, the acetate has a γ-lactone, four OAc groups and no quinone chromophore. Therefore, the structure of this acetate is easily assumed as structure **6**, and its formation is speculated by a path shown in Chart 1.



In fact, griseusin B was quantitatively converted into griseusin A in a pyridine solution via a colourless intermediate. This reaction not only proves the stereochemical correlation between griseusins A and B but also refuses to give griseusin A the structure **2b**. Since the conversion proceeds under such a mild condition as may be expected in the fermentation medium, the reaction should take part in the biosynthesis of the γ-lactone ring generally found in isochromanquinone antibiotics[4] such as granaticin, kalafungin, naphthocyclinones,[4,5] etc.

^{13}C NMR signals of griseusin A and its derivatives were assigned as shown in Table 2 by means of ^1H-noise and ^1H-single-frequency off-resonance decoupling techniques and esterification effects, where methoxycarbonylation of OH groups was especially useful. Esterification effects on δ_C of some fundamental alcohols and of perihydroxynaphthoquinones are reported separately.[6,7]

Griseusins are optically active, and the CD curves, shown in Fig. 3, suggest that griseusins have dihydropyran rings of which chirality is opposite to actionorhodin–indazolquinone.[4] Since the absolute configuration of the actinorhodin derivative was established,[8] the absolute stereochemistry of griseusin A should be indicated as structure **4a**.

Table 2. ^{13}C chemical shifts δ_C data on griseusin A and its diacetate and dimethoxycarbonate in $CDCl_3$[a]

Carbon No.	Griseusin A	8,6'-Diacetate	8,6'-Di-methoxycarbonate
1	96.6 s	95.2 s (-1.4)	95.2 s (-1.4)
3	66.8 d	66.2 d (-0.6)	65.9 d (-0.9)
4	65.8 d	65.8 d (0.0)	65.9 d (+0.1)
4a	143.1 s	143.8 s (+0.7)	143.3 s (+0.2)
5	119.2 d	124.6 d (+5.4)	125.1 d (+5.9)
6	136.8 d	134.6 d (-2.2)	135.1 d (-1.7)
7	125.2 d	130.5 d (+5.3)	129.8 d (+4.6)
8	161.7 s	149.7 s (-12.0)	149.9 s (-11.8)
8a	115.2 s	124.3 s (+9.1)	124.3 s (+9.1)
9	187.4 s	180.3 s (-7.1)	181.3 s (-6.1)
9a	138.5 s	135.6 s (-2.9)	136.7 s (-1.8)
10	181.5 s	181.9 s (+0.4)	182.0 s (+0.5)
10a	130.9 s	132.6 s (+1.7)	132.9 s (+2.0)
11	36.5 t	36.6 t (+0.1)	36.4 t (-0.1)
12	174.0 s	173.7 s (-0.3)	173.7 s (-0.3)
3'	62.9 d	63.2 d (+0.3)	63.6 d (+0.7)
4'	36.2 t	36.2 t (0.0)	36.1 t (-0.1)
5'	69.5 d	68.0 d (-1.5)	68.0 d (-1.5)
6'	68.5 d	68.0 d (-0.5)	71.6 d (+3.1)
3'-Me	20.5 q	20.4 q	20.4 q
5'-OCOMe	170.5 s	170.1 s	170.4 s
5'-OCOMe	21.2 q	21.0 q	21.1 q
8-OCOMe		169.1 s	
8-OCOMe		21.0 q	
6'-OCOMe		170.1 s	
6'-OCOMe		20.4 q	
8-OCOOMe			154.6 s
8-OCOOMe			56.1 q
6'-OCOOMe			153.2 s
6'-OCOOMe			55.0 q

[a] Abbreviations s, d, t, and q are multiplicities in ^1H single-frequency off-resonance spectra. Values in parentheses are esterification shifts in ppm.[6,7]

EXPERIMENTAL

^1H NMR spectra were taken with a Varian A-60A (60 MHz) and an HA-100 (100 MHz) spectrometer in $CDCl_3$ containing TMS as an internal reference. Errors of chemical shifts (δ_H) and coupling constants (J) are ±0.02 and ±0.5 Hz, respectively. ^{13}C NMR spectra were recorded on a Varian NV-14 FT NMR spectrometer using $CDCl_3$ solutions in 8-mm spinning tubes. Errors of chemical shifts relative to internal TMS (δ_C) are ±0.1. FT measurement conditions are as follows: spectral width, 3923 Hz; pulse flipping angle, 15°; acquisition time, 0.6 sec; number of data points, 4820. CD spectra were recorded on a JASCO Model ORD/UV-6 optical rotatory dispersion spectrometer.

Griseusin A monoacetate. A mixture of 43 mg griseusin A and 5 mg *p*-toluenesulfonic acid in 2 ml of Ac_2O was stirred at room temp. for 15 min. The mixture was poured into H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC on acidic silica gel plate using a solvent system of $CHCl_3$–MeOH (96:4). The main orange zone gave 45 mg of monoacetate as an amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 212.5 (4.56), 253 (4.01), 431 (3.59). (Found: C, 58.27; H, 4.98. $C_{24}H_{22}O_{11}\cdot\frac{1}{2}H_2O$ requires: C, 58.18; H, 4.68%).

Griseusin A diacetate. To a soln of 40 mg griseusin A in pyridine (2 ml) was added Ac_2O (1 ml) and the solution was allowed to stand at room temp. overnight. The soln was treated as for monoacetate. The main pale yellow zone gave 40 mg of diacetate as an amorphous powder. UV λ_{max}^{MeOH} nm (log ε): 247 (4.14), 347 (3.42). (Found: C, 58.31; H, 4.64. $C_{26}H_{24}O_{12}\cdot\frac{1}{2}H_2O$ requires: C, 58.21; H, 4.51%).

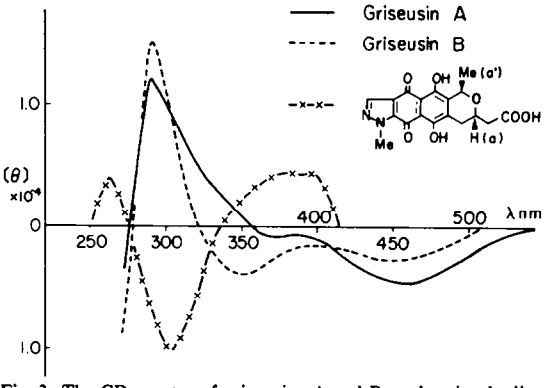


Fig. 3. The CD spectra of griseusins A and B, and actinorhodin-indazolquinone.

Griseusin A methoxycarbonates. A soln of 150 mg griseusin A in pyridine (4 ml) was cooled to 0° and to the soln excessive methyl chloroformate was added dropwise. The mixture was kept at 0° overnight and poured on ice. The mixture was extracted with $CHCl_3$ and the product was separated preparative TLC (continuous development method using $CHCl_3$:MeOH = 98.5:1.5). The upper orange zone gave 45 mg of 6'-O-methoxycarbonate and the bottom yellow zone gave 115 mg of 8,6'-dimethoxycarbonate.

Griseusin B monoacetate. A soln of 40 mg griseusin B and 20 mg *p*-toluenesulfonic acid in 15 ml of Ac_2O was allowed to stand at room temp. overnight. Working up as griseusin A monoacetate gave 18 mg of griseusin B monoacetate as an amorphous powder.

Compound 6 from griseusin B. To a soln of 30 mg griseusin B in pyridine (1 ml) was added Ac_2O (1 ml) and the soln was allowed to stand overnight. After decomposition of Ac_2O with H_2O, the mixture was extracted with $CHCl_3$. Preparative TLC on acidic silica gel with $CHCl_3$–MeOH (95:5) gave 33 mg of compound **6** (visualized with UV light) as a colourless amorphous powder. IR: $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1790 (γ-lactone, Ar–OAc), no absorption bands at 1630–1700 cm^{-1}.

Griseusin A from griseusin B. A soln of 5 mg griseusin B in pyridine (0.5 ml) was allowed to stand at room temp. overnight. The soln was evaporated *in vacuo* and the residue was purified by preparative TLC on acidic silica gel plate with $CHCl_3$–MeOH (96:4). The main orange zone gave 4 mg pure product, which was identified with griseusin A by TLC and IR spectrum.

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