Synthesis and Biological Activities of Griseofulvin Analogs

Byoung-Seob Ko, Takayuki Oritani and Kyohei Yamashita

Department of Agricultural Chemistry, Faculty of Agriculture,
Tohoku University, Aoba-ku, Sendai 981, Japan

Received December 18, 1989

In order to elucidate the structure-activity relationship of griseofulvin (1), (+)-6′-demethyl analog (3), 2′-demethoxy-6′-demethyldihydro analog (4), (+)-dechloro-6′-ethyl analog (5), (+)-dechloro-6′-epi-ethyl analog (6), (+)-6′-ethyl analog (7) and (+)-6′-epi-ethyl analog (8) were synthesized by a Diels-Alder cycloaddition of alkylidene ketones (16, 17, 18, 19 and 20) with modified 1,3-butadienes (21 or 22). Their biological activities were examined against fungi.

Griseofulvin (1) was isolated from the mycelium of Penicillium griseofulvum Dierckx by Oxford et al.\textsuperscript{1} in 1939. Orally active antifungal antibiotic 1 had been used itself as a medicine for several years, but is now out of use owing to its harmful after effects. Total syntheses of 1\textsuperscript{2} and a number of its analogs have been studied by several workers.\textsuperscript{3}

For the further development of griseofulvin analogs as antibiotic agents, the authors synthesized several analogs of 1 and evaluated their fungicidal activity. Previous works\textsuperscript{3} on the correlation of the structure and antifungal activity in griseofulvin analogs have been limited mainly to the grisane derivative containing an alkoxy or hydroxyl group on the aromatic ring.

From this point of view, we were interested in determining the influence of a 6′-methyl group in ring C of 1 on the fungicidal activity. For this purpose, we undertook the syntheses of griseofulvin analogs 3, 4, 5, 6, 7 and 8.

Danishefsky and co-workers have described a synthesis of 1 by a cycloaddition reaction of the 1,1-disubstituted diene (21) with electrophilic dienophiles.\textsuperscript{4} It was of interest to examine the extension of this reaction to dienophiles 16, 17, 18, 19 and 20 by applying Danishefsky’s method. This seemed a possible synthetic route to the griseofulvin analogs.

Lithium enolates of coumaranones 11 and 12 were reacted with aldehyde 13 in tetrahydrofuran (THF) at −78°C to afford a diastereomeric mixture of β-ketols 14 in good yields. Treatment of 11 with gaseous formaldehyde in the presence of lithium diisopropylamide (LDA) gave two products, a dihydroxymethyl derivative (23, 14%) and a monohydroxymethyl derivative (14a, 51%). The structures of 14a and 23 were assigned by their \textsuperscript{1}H-NMR and mass spectra. The \textsuperscript{1}H-NMR spectrum of 14a in CD\textsubscript{3}COCD\textsubscript{3}
showed signals at δ 4.01 (2H, m, -CH₂) and 4.26 (1H, dd, J = 4.88 and 1.95 Hz, -OH). The ¹H-NMR spectrum of 23 (CD₃COCD₃ + D₂O) showed a signal at δ 3.85 (4H, s). The mass spectrum of 23 showed a peak at m/z (rel. int.) 289/287 (3.9/11.2, M⁺ + 2/M⁺, C₁₂H₁₃O₆CO).

The enolates of 11 and 12 were reacted with propionaldehyde to afford diastereomeric mixtures of β-hydroxy ketones 14b and 14c in high yields, respectively. Without chromatographic separation, we attempted to dehydrate diastereomers 14b and 14c. Mesylation of β-hydroxy ketones 14a, 14b and 14c with methanesulfonyl chloride in pyridine containing 4-(dimethylamino)pyridine (DMAP) gave corresponding methanesulfonates 15a, 15b and 15c, respectively. Treatment of 15a, 15b and 15c with 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) in benzene resulted in β-elimination to afford methylene ketone 16, dechloropropylidene ketones 17 and 18 as a mixture of geometrical isomers, and propylidene ketones 19 and 20 as a mixture, respectively. Dechloropropylidene ketones 17 and 18 were separated by thin-layer chromatography (TLC) and recrystallization in a 1:4 ratio. By the same method, propylidene ketones 19 and 20 were separated in a 1:3 ratio. The structures of dechloropropylidene ketones 17 and 18 and propylidene ketones 19 and 20 were elucidated by ¹H-NMR (CDCl₃).

It is possible, in the case of the geometrical isomers of certain α,β-unsaturated carbonyl compounds, to make configurational assignments on the basis of the chemical shifts of the β-protons. In the (Z)-isomer, due to the deshielding influence of the carbonyl group, the β-proton gave a signal in a lower field than the signal of the corresponding proton in the (E)-isomer. Thus, it was deduced that isomers 18 and 20 (δ 5.96 and 6.10 ppm for each β-proton) were of (Z)-form, and that isomers of 17 and 19 (δ 5.88 and 6.04 ppm for each β-proton) were of (E)-form.

Cyloaddition of methylene ketone 16 with the known modified-1,3-butadienes (21 and 22) was carried out in dry toluene in a sealed tube at 110–140°C overnight. The adducts were treated with aqueous 0.1 n-HCl in THF. The crude products were chromatographed on TLC to afford (±)-6'-demethylgriseofulvin (3) and 2'-demethoxy-6'-demethyldihydrogriseofulvin (4), respectively, in high yields.

By the same method, a mixture of dechloropropylidene ketones 17 and 18 was reacted with 21 to give a 1:5 mixture of (±)-dechloro-6'-ethyl analog 5 and (±)-dechloro-6'-epi-ethyl analog 6, in a 72% yield. A 1:4.5 mixture
Synthesis and Activities of Griseofulvin Analogs

(±)-6'-ethyl analog 7 and (±)-epi-ethyl analog 8 was obtained in a 74% yield from a mixture of propylidene ketones 19 and 20 by a series of reactions applied to 16. The structures of (±)-5, (±)-6, (±)-7 and (±)-8 were determined by comparing their 1H-NMR spectra with those reported for griseofulvin (1) and epigriseofulvin (2). Their 1H-NMR data are summarized in Table I. The NMR spectra of 1 and 2 differ in the coupling pattern of the 6'-methine and 5'-protons (in the δ 2.3-3.1 region). The 6'-methyl group in 1 gave signals in a considerably lower field than the signals of the corresponding proton in 2. By a comparison of their 1H-NMR, ethyl analogs (±)-5 and (±)-7 were clearly distinguishable from epi ethyl analogs (±)-6 and (±)-8. The CH2 signals due to the 6'-ethyl group appeared in a lower field in (±)-5 and (±)-7 than in (±)-6 and (±)-8; moreover, the signals due to the 5'-proton and 6'-methine of (±)-5 and (±)-6 were analogous to those of 1 and different from those of 2. Thus, it was deduced that (±)-5 and (±)-7 had a griseofulvin configuration, and that (±)-6 and (±)-7 had an epigriseofulvin configuration.

The biological activities of the synthesized analogs were tested against fungi (Botrytis allii and B. cinerea). Griseofulvin as a standard was employed for comparison purposes in this test. Compounds 9 and 10 prepared from 1 by the known method9) were also tested to compare with the activity of (±)-3-8. The antimicrobial activity was determined by a paper disc method, the activities of the test compounds being listed in Table II. (±)-6'-Demethyl analog 3 showed quite weak activity, and 4'-deoxo analog 10 did not show any activity. The activities of (±)-5 and (±)-7 were considerably inferior to that of 1. However, the activity of (±)-7 was stronger than that of (±)-5. Also, it became clear that the 7-chloro group on the A ring was necessary to accelerate the biological activity.3) From the bioassay of ethyl analogs 7, the 6'-substituted analogs of 1 might be expected to show considerable activity. From these results, it was deduced that

Table I. 1H-NMR Data for the Griseofulvin Analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift (ppm)</th>
<th>5'-Proton and 6'-methine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.96</td>
<td>3.07-2.28</td>
</tr>
<tr>
<td>2</td>
<td>0.88</td>
<td>2.26-2.38</td>
</tr>
<tr>
<td>(±)-5</td>
<td>—</td>
<td>1.34 2.23-2.21</td>
</tr>
<tr>
<td>(±)-6</td>
<td>—</td>
<td>1.28 2.78-2.36</td>
</tr>
<tr>
<td>(±)-7</td>
<td>—</td>
<td>1.27 3.05-2.16</td>
</tr>
<tr>
<td>(±)-8</td>
<td>—</td>
<td>1.21 2.71-2.41</td>
</tr>
</tbody>
</table>

* The chemical shifts of compounds 1-6 were measured on a 100 MHz spectrometer, and those of (±)-7 and (±)-8 on a 270 MHz spectrometer.

Table II. Biological Activities of the Griseofulvin Analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Botrytis allii</th>
<th>Botrytis cinerea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 50 100</td>
<td>25 50 100</td>
</tr>
<tr>
<td>1</td>
<td>++ + ++ + + +</td>
<td>++ + + + + +</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>++ + ++ +++</td>
<td>++ + ++ +++</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* The inhibitory activity is represented in the order of ++ + + +, ++ + +, ++ , +, −, ++ + + + is strongly active, ++ + is more active, ++ is active, + is less active, and − is inactive.
the presence of the 6'-methyl group and 4'-oxo group on the C ring were very important factors in the biological activities of griseofulvin (1).

Experimental

Melting points (mp) were determined on micro-melting point apparatus (Yanagimoto no. 1593). All melting and boiling points are uncorrected. IR spectra were measured on a JASCO IR-810 infrared spectrometer, and $^1$H-NMR and $^{13}$C-NMR spectra were recorded on a JEOL JNM FX (100 MHz/270 MHz) spectrometer. All $^1$H-NMR and $^{13}$C-NMR data are reported as parts per million downfield from Me$_4$Si ($\delta$=0). The mass spectrum was recorded on Hitachi M-52 and JEOL JMX HX-105 spectrometers. Microanalyses were performed by the Analytical Laboratory of the Faculty of Science at Tohoku University. Preparative TLC was carried out on Merck Kieselgel 60 P$.254 of 0.7 mm thickness.

7-Chloro-4,6-dimethoxy-2-methylenecoumaran-3-one (16). To a solution of diisopropylamine (146 mg, 4.8 mmol) in 10 ml of dry THF was added n-BuLi (3.12 ml of a 1.56 M solution in hexane) at -78°C under nitrogen. This was stirred for 15 min at the same temperature, and to this solution was then added dropwise ketone 11 (900 mg, 3.96 mmol) in 180 ml of dry THF over c. 30 min. To this resulting solution was introduced formaldehyde gas, which was generated from 500 mg of paraformaldehyde by heating at c. 150°C for about 5 min. This solution was stirred for 1 min and then quenched by adding 10 ml of saturated aqueous ammonium chloride. The reaction mixture was poured into 30 ml of ether. The organic layer was generated from 500 mg of paraformaldehyde by heating at c. 150°C for about 5 min. The mixture was separated into 17 and 18 by preparative TLC and recrystallization in a 1 : 4 ratio. By the same method, 19 and 20 were separated in a 1 : 3 ratio.

A mixture of 14b (380 mg, 1.5 mmol) and 14c (720 mg, 2.5 mmol) was mesylated and eliminated with DBU by the same method as that already described to give geometric isomers of dechloropropylidene ketones (17 and 18) and (±)-7-chloro-4,6-dimethoxy-2-propylidene coumaran-3-ones (19 and 20). The lithium enolate of ketone 12 (300 mg, 1.5 mmol) and 11 (600 mg, 2.6 mmol), which had been prepared by the method already described, were reacted with propionaldehyde (2.1 eq.) and stirred for 10 min at -78°C. The usual work-up and subsequent column chromatography of a diastereomeric mixture afforded 14b (392 mg, quant.) and 14c (721 mg, 96%) as a viscous liquid.

14b. IR $\nu_{max}$ (film) cm$^{-1}$: 3450, 1692, 1618, 1598, 1500.
$^1$H-NMR (CDCl$_3$) $\delta$: 1.03 (3H, t, $\delta$=7.32 Hz), 1.66 (2H, m), 3.86 (3H, s), 3.88 (3H, s), 4.43 (1H, m), 4.49-4.52 [2H, m, +D$_2$O $\delta$ 4.50 (1H, d, $J=2.93$ Hz)], 5.99 (1H, d, $J=1.95$ Hz), 6.16 (1H, d, $J=1.95$ Hz).

dihydroxymethyl ketone 14b was recrystallized from EtOAc-hexane to give 519 mg of pure 14a as white needles, mp 194-197°C (51% yield), was recrystallized from EtOAc-hexane to give 564 mg of crude monohydroxymethyl ketone 14a, which was treated with 10 ml of CH$_2$Cl$_2$ and 10 ml of saturated ammonium chloride, and then poured into 50 ml of ether. The organic layer was washed with 0.1 N HCl and dried over anhydrous MgSO$_4$. After evaporating the solvent, 138 mg of crude 15a was obtained as a yellow solid. A solution of this crude 15a in 5.0 ml of dry benzene containing 5 mg of DBU was stirred at room temperature for 10 min and then poured into 100 ml of ether layered on 10 ml of water. Evaporation of the ethereal layer afforded 62.8 mg (75%) of 16 as a yellow solid, which was not purified further. IR $\nu_{max}$ (KBr) cm$^{-1}$: 1702, 1659, 1618, 1587, 1505. $^1$H-NMR (CDCl$_3$) $\delta$: 4.00 (3H, s), 4.02 (3H, s), 4.03 (3H, s), 4.07 (3H, s), 4.08 (3H, s), 0.90 (1H, m), 0.49 (1H, m), $+D_2$O $\delta$ 4.58 (1H, d, $J=2.68$ Hz), 6.09 (1H, s), MS $m/z$: 288 (M$^+$ + 2), 288 (M$^+$).

A mixture of 14b (380 mg, 1.5 mmol) and 14c (720 mg, 2.5 mmol) was mesylated and eliminated with DBU by the same method as that already described to give geometric isomers of dechloropropylidene ketones (17 and 18) and propylidene ketones (19 and 20) in 78% and 76% yields, respectively. The mixture was separated into 17 and 18 by TLC and recrystallization in a 1 : 4 ratio. By the same method, 19 and 20 were separated in a 1 : 3 ratio.

17. IR $\nu_{max}$ (AcOEt:Et$_2$O=1:10) cm$^{-1}$: 1705, 1698, 1628, 1600, 1502. $^1$H-NMR (CDCl$_3$) $\delta$: 1.11 (3H, t, $J=7.33$ Hz), 2.81 (2H, dq, $J=7.57$ and 7.33 Hz), 3.86 (3H, s), 3.91 (3H, s), 5.88 (1H, d, $J=7.57$ Hz), 6.06 (1H, d, $J=1.71$ Hz), 6.17 (1H, d, $J=1.71$ Hz). Anal. Found: C, 66.54; H, 5.92. Calcd. for C$_{13}$H$_{14}$O$_2$: C, 66.66; H, 6.03%.

18. IR $\nu_{max}$ (AcOEt:Et$_2$O=1:10) cm$^{-1}$: 1700, 1682, 1634, 1598, 1500. $^1$H-NMR (CDCl$_3$, 270 MHz) $\delta$: 1.13 (3H, t, $J=7.33$ Hz), 2.41 (2H, dq, 2H, $J=7.81$ and 7.33 Hz), 3.87 (3H, s), 3.91 (3H, s), 4.01 (2H, m, +D$_2$O $\delta$ 4.50 (1H, d, $J=2.93$ Hz)]. 5.99 (1H, d, $J=1.95$ Hz), 6.16 (1H, d, $J=1.95$ Hz).

i) (±)-6'-Demethylgriseofulvin (3). A solution of methylene ketone 16 (56 mg, 0.23 mmol) and diene 21 (510 mg, 3.59 mmol) in 10 ml dry toluene was heated under reflux for 30 min and then poured into 50 ml of ether layered on 50 ml of water. The aqueous layer was extracted with an additional 50 ml of ether, and the combined organic phases were washed with saturated sodium bicarbonate and brine, and dried over MgSO₄. Evaporation of the solvent gave 87 mg of a crude yellow solid. The crude product was purified on TLC to afford 53 mg of 3 (41%) as colourless needles (from benzene), mp 205–208°C (from benzene), Rf 0.47.

ii) 2'-Demethoxy-6'-demethyldihydrogriseofulvin (4). A solution of methylene ketone 16 (84 mg, 0.35 mmol) and diene 22 in 5 ml of dry toluene was heated overnight at 110–130°C. After evaporating the solvent in vacuo, the residue was taken up in 20 ml of THF, 10 ml of water, and 1 ml of 1 n HCl. This reaction mixture was stirred at room temperature for 30 min and then poured into 50 ml of ether. The aqueous layer was extracted with an additional 50 ml of ether, and the combined organic phases were washed with saturated sodium bicarbonate and brine, and dried over MgSO₄. Evaporation of the solvent gave 87 mg of a crude yellow solid. The crude product was purified on TLC to afford 53 mg of 4, mp 205–208°C (from benzene), Rf 0.32 (AcOEt:Et₂O = 1:10), Rf 0.28 (AcOEt:Et₂O = 1:10), Rf 0.16 (Et₂O:AcOEt = 10:1), mp 204–207°C.


i) (±)-6'-Demethyl-2',4,6-trimethoxybis[benzofuran-2(3H), 1'-(2-cyclohexene)]-3',4'-dione (5) and (±)-6'-epiethyl analog (8) in a 74% yield.

Reduction of griseofulvin (1). Dihydrogriseofulvin (9) and 4'-deoxogriseofulvin (10) were prepared by the method of Mulholland. 20

i) Dihydrogriseofulvin (9), mp 201–204°C (lit. 198°C). IR νmax (KBr) cm⁻¹: 1717, 1665, 1620, 1592, 1502.

ii) 2(3H)-1'-(2-cyclohexene)-3',4'-dione (6). A mixture of dechloropropylidene ketones 17 and 18 (184 mg, 0.78 mmol) and diene 21 (862 mg, 4.25 mmol) in 5 ml of dry toluene was heated in sealed tube at 145 to 160°C overnight. The usual work-up provided a crude product, purification of which by TLC and recrystallization gave a 1:5 ratio of (±)-dechloro-6'-ethyl analog (5) and (±)-dechloro-6'-epi-ethyl analog (8) in a 72% yield.

iii) (±)-(2S*,6'S*)-7-Chloro-6'-ethyl-2',4,6-trimethoxybis[benzofuran-2(3H), 1'- (2-cyclohexene)]-3',4'-dione (7) and (±)-(2S*,6'S*)-7-Chloro-6'-ethyl-2',4,6-trimethoxybis[benzofuran-2(3H), 1'- (2-cyclohexene)]-3',4'-dione (8). A mixture of propylidene ketones 19 and 20 (115 mg, 0.43 mmol) and diene 21 (953 mg, 6.71 mmol) in 8 ml of dry toluene was heated in sealed tube at 145 to 160°C overnight. The usual work-up followed by TLC and recrystallization gave a 1:4.5 ratio of (±)-6'-ethyl analog (7) and (±)-6'-epi-ethyl analog (8) in a 74% yield.

Reduction of griseofulvin (1). Dihydrogriseofulvin (9) and 4'-deoxogriseofulvin (10) were prepared by the method of Mulholland. 20
ii) 4'-Deoxogriseofulvin (10). mp 193–195°C (lit. 194–195°C). IR $\nu_{\text{max}}$ (KBr) cm$^{-1}$: 1702, 1654, 1606, 1584, 1500. $^1$H-NMR (CDCl$_3$) $\delta$: 0.86 (3H, d, $J$ = 6.35 Hz), 1.81–2.51 (4H, m), 3.43 (3H, s), 3.72 (1H, m, 4'-$\beta$-H), 3.96 (3H, s), 4.01 (3H, s), 5.11 (1H, d, $J$ = 3.17 Hz), 6.08 (1H, s).

These data were confirmed by comparing the IR spectra with those reported.

Antimicrobial assay of the griseofulvin analogs (3–8) against Botrytis allii (IFO 9430) and Botrytis cinerea (AHU 9573). The antimicrobial activity of the synthesized analogs was determined by the paper disc method in potato sucrose medium. A solution containing the test compound at a defined concentration (25, 50 and 100 $\mu$g/disc) was poured on to paper layered in a Petri dish. The treated culture was incubated at 26-28°C for 5 days, and the growth-inhibited zone around the disc was measured.

References