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Synthesis and biological activity of 6'-phenylgriseofulvin as analogs of antibiotic griseofulvin

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Abstrat : In order to study the influence of a 6'-methyl group in ring C of griseofulvin (1) on the fungicidal activity, 6'-methyl group was replaced with a larger phenyl group as (±)-6'-phenylgriseofulvin (3), (±)-6'-epiphenylgriseofulvin (4), synthesized by a Diels-Alder cycloaddition. Their biological activities were examined against *Botrytis allii* (IFO 9430) and *B. cinerea* (AHU 9573). (±)-6'-Phenylgriseofulvin (3) showed high activity in 25 μg/disc(Received August 24, 1992, accepted September 27, 1992).

Griseofulvin (1) is a classic antifungal agent still used in the treatment of dermatomycoses in animals, humans and in plant protection.[1)]

Our recent studies have concluded that the prsence of 6'-methyl group and 4'-oxo group was very important factor in the biological activities of 1.[2)] 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, when a 6'-methyl group was replaced with a larger group (Fig. 1). For this purpose, we synthesized 6'-phenyl analogs 3, 4 by Diels-Alder cycloaddition of benzylidene ketone (8, 9) with modified 1,3-butadiene (10).

The biolgical activities of synthesized analogs were tested against fungi (*Botrytis allii* and *B. cinerea*) by a paper disc method.

Materials and Method

Chemicals

Coumaran-3-one (5) was prepared according to the known method by Stork *et al.*[3)] Melting points (m.p.) were determined on micro-melting point apparatus (Yanagimoto No. 1593). All melting points are uncorrected. IR spectra were measured on a JASCO IR-810 infrared spectrometer and ^{1}H-NMR was recorded on a JEOL JNM FX (100 MHz/270 MHz) spectrometer. The mass spectrum was recorded on JEOL HX-105. Microanalyses were performed by the Analytical Laboratory of the Faculty of Science at Tohoku University. Preparative TLC (thin-layer chromatography) was carried out on Merck Keselgel 60 PF_{254} of 0.7 mm thickness.

Synthesis

(±)-7-*Chloro*-4,6-*dimethoxy*-2-*benzylidenecoumaran*-3-*one* (8 and 9) A solution of lithium diisopropylamide (LDA, 2.0 mmol) in dry tetrahydrofuran (THF, 5 ml) was added dropwise to a solution of ketone 5 (300 mg, 1.3 mmol) in dry THF (50 ml) at −78 ℃ in a Ar atmosphere. The mixture was stirred for 30 min at −78 ℃, then benzylaldehyde (2.1 eq) was added at −78 ℃ in an Ar atomsphere. The mixture was added at −78 ℃. After stirring for 10 min, the mixture was quenched by a sat. aqueous solution of NH_4Cl and extracted with Et_2O. The Et_2O layer was dried over $MgSO_4$ and concentrated. Chromatography of the residue on silica gel

Key words : Griseofulvin, epigriseofulvin, 6'-phenylgriseofulvin, dermatomycoses, paper disc method, geometrical isomer, Diels-Alder cycloaddition, acidic hydrolysis, configuration
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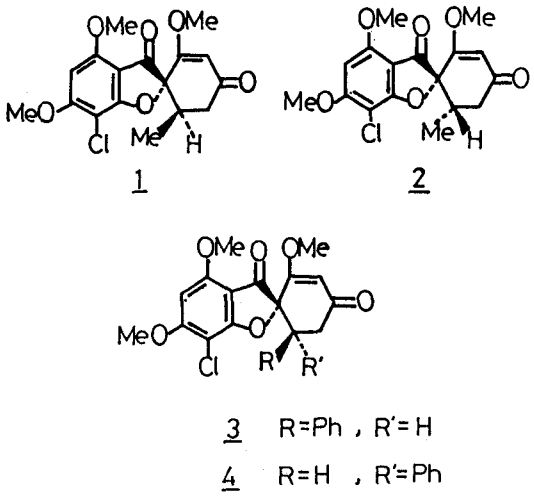


1　　2

3　R=Ph , R'=H

4　R=H , R'=Ph

Fig. 1. Griseofulvin and its analogs.

with AcOEt-Hexane (5 : 1) gave diastereomeric mixture of β-hydroxy ketone 6 (342 mg, 78%).

6; IR ν_{max} (KBr) cm^{-1} : 3420, 1698, 1618, 1592, 1500. ^{1}H-NMR ($CDCl_3$) δ : 3.53(1H, m), 3.94(3H, s), 3.97(3H, s), 4.73(1H, m), 5.02(1H, m), 6.04(1H, s), 7.42(5H, m).

To a solution of mixture 6 (130 mg, 0.4 mmol) and 4-(dimethyl-amino) pyridine (21 mg) in 4 m*l* of dry pyridine was added methane-sulfonyl chloride (120 mg, 1.04 mmol) at room temperature. The reaction mixture was stirred overnight in an Ar atomsphere. After evaporating the solvent *in vacuo*, the residual solid was treated with 10 m*l* of CH_2Cl_2 and 5 m*l* of sat. aqueous NH_4Cl and extracted with Et_2O. The Et_2O layer was washed 0.1 N HCl and dried over anhydrous $MgSO_4$. After evaporating the solvent, crude 7 was obtained as a yellow solid, which was not purified further. A solution of crude 7 in dry benzene (10 m containing 1,5-diazabicyclo[5.4.0.] undec-5-ene (DBU, 14 mg) was stirred at room temperature for 10 min, then extracted with Et_2O. Evaporating of the ethereal layer afforded geometric isomer of benzylidene ketone (8 and 9) in 72% (89 mg) yield. The mixture was seperated into 8 and 9 by TLC (Et_2O : AcOEt = 10 : 1) and 1 : 3 ratio by recrystallization.

8; IR ν_{max} (KBr) cm^{-1} : 1702, 1665, 1618, 1598, 1510. ^{1}H-NMR ($CDCl_3$)δ : 4.01(6H, s), 6.17(1H, s), 6.83(1H, s), 7.26~7.98(5H, m).

9; IR ν_{max} (KBr) cm^{-1} : 1700, 1660, 1615, 1590, 1505. ^{1}H-NMR ($CDCl_3$) δ : 4.03(6H, s), 6.21(1H, s), 6.97(1H, s), 7.23~8.13(5H, m).

(±)-(2'*S*, 6'*R*)-*7-chloro-6'-phenyl-2',4'6-trimethoxyspiro*[*benzofuran*-2-(3H), 1'-(2-*cyclohexene*)]-3,4'-*dione* (3) and (±)-(2'*S*,6'*S*)-*7-chloro-6'-phenyl-2',4', 6-trimethoxyspiro*[*benzofuran*-2(3H),1'-(2-*cyclohexene*)]-3,4'-*dione*(4). A mixture of benzylidene ketone 8 and 9(84 mg, 0.27 mmol) and diene[4] (226 mg, 1.16 mmol) in 5 m*l* of dry toluene was heated overnight under Ar at 180 ℃. The residue was taken up in 20 m*l* of THF, 10 m*l* of water and 5 m*l* of 1N HCl. The reaction mixture was stirred at room temperature for 30 min and then poured into a mixture of 30 m*l* of Et_2O, and 20 m*l* of water. The aqueous layer was extracted with an additional 50 m*l* of Et_2O, and the combined organic phase was washed with sat. sodium bicarbonate solution and brine, and dried over $MgSO_4$. After evaporation of solvent, the crude product was purified by TLC (Et_2O : AcOEt = 10 : 1) and recrystallization (from AcOEt) gave a 1 : 3 ratio of (±)-6'-phenyl analog (3) and (±)-6'-epiphenyl analog(4) in a 68% yield.

3; m.p. 234~236 ℃. IR ν_{max}(KBr)cm^{-1} : 1712, 1670, 1620, 1598, 1510. ^{1}H-NMR($CDCl_3$)δ : 2.66(1H, dd, J = 16.6 and 4.2 Hz), 3.28(1H, dd, J = 16.6 and 13.7 Hz), 3.67(3H, s), 3.85(3H, s), 3.88(1H, m), 3.91(3H, s), 5.68(1H, s), 5.89(1H, s), 7.15(5H, m). MS(m/z) 416(M^++2), 414(M^+). Anal. Found : C, 63.60; H, 4.65; Cl, 8.67. Calcd. for $C_{22}H_{19}O_6Cl$: C, 63.68; H, 4.62; Cl, 8.55%.

4; m.p. 226~228 ℃. IR ν_{max} (KBr)cm^{-1} : 1709, 1668, 1618, 1590, 1504. : ^{1}H-NMR($CDCl_3$)δ : 2.64(1H, dd, J = 16.4 and 12.5 Hz), 3.32(2H, m), 3.85(3H, s), 3.91(3H, s), 4.01(3H, s), 5.56(1H, s), 5.38(1H, s), 7.16 (5H, m). Anal. Found : C, 63.94; H, 4.76; Cl, 8.74. Calcd. for $C_{22}H_{19}O_6Cl$: C, 63.68; H, 4.62; Cl, 8.55%.

Antimicrobial assays

The antimicrobial activity was determined by the paper disc method angainst *Botrytis allii*(IFO 9340) and *Botrytis cinerea*(AHU 9573) in potato sucrose medium. A solution containing the test compound at a defined concentration (10, 25, 50 and 100 μg /disc) was pured on to paper layered in petri di-

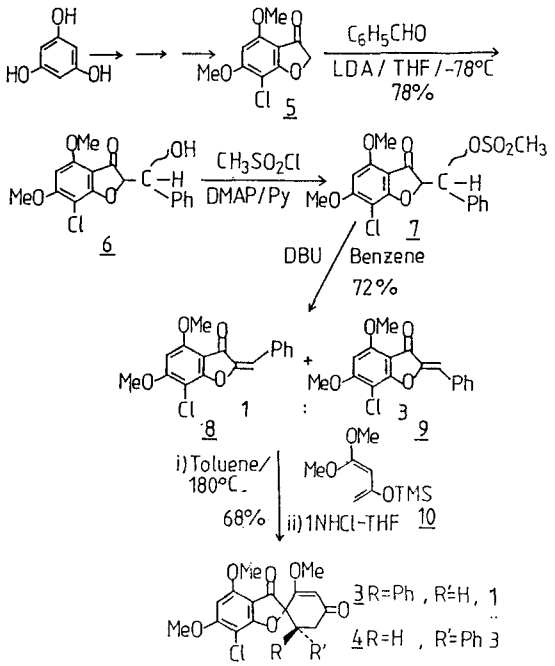


Fig. 2. Synthetic scheme of 6′-phenyl analogs of griseofulvin.

shes. The treate was incubated at 26~28℃ for 4~5 days, and the growth-inhibited zone around the disc was measured.

Result and Discussion

We defined as our first intermediate the preparation of the dienophiles 8 and 9 (Fig. 2). Phenylidene ketone 8 and 9 were seperated by TLC and recrystallization in 1 : 3 ratio. The structures of phenylidene ketones 8 and 9 were elucidated by ^{1}H-NMR ($CDCl_3$). In case of the geometrical isomers of certain α, β-unsaturated carbonyl compounds, it is possible to make configuration assignments on the β-proton.[1,5] Thus, it was deduced that isomer 8 (δ 6.21) was of (Z)-form, and that isomer 9(δ 6.17) was of (E)-form.

By cycloaddition *via* acidic hydrolysis of the Diels-Alder adduct, a mixture 8 and 9 was treated with diene 10 to given a 1 : 3 ratio of (±)-6′-phenyl analog 3 and (±)-6′-epiphenyl analog 4, in 68% yield. The structures of 3 and 4 were determined by comparing their ^{1}H-NMR spectra with those reported for griseofulvin (1) and epigriseofulvin (2).[1,6] Their ^{1}H-NMR are showed in Fig. 3. On ^{1}H-NMR, (±)-3 was clearly distinguishable from (±)-4. The 5′-proton and 6′-methine of (±)-3 gave asignal in a lower field than the singal of the corresponding proton in the (±)-4. Thus, it was concluded that (±)-3 had a griseofulvin configuration, and that (±)-4 had an epigriseofulvin configuration.

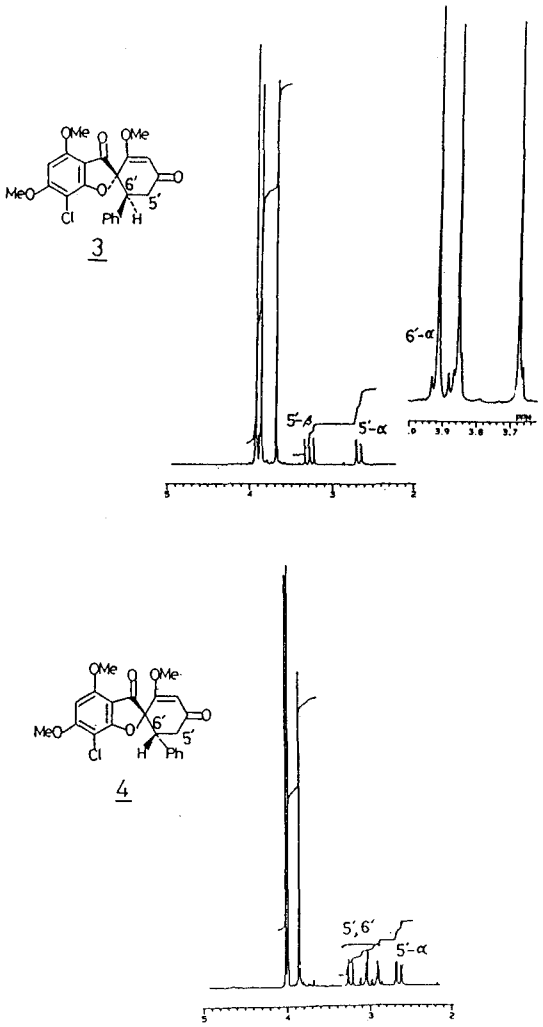
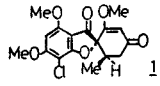
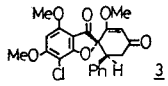
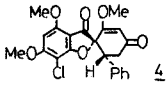
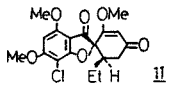
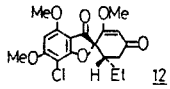


Fig. 3. ^{1}H-NMR of 3 and 4, 6′-phenyl analogs of griseofulvin.

The antimicrobial activity of 6′-phenylgriseofulvin analogs are listed Table 1. Griseofulvin was used as a standard in this test. (±)-Epiphenyl analog (4) did not showed any activity. However, (±)-6′-phenyl anlog (3) showed high biological activity in

Table 1. Biological activity of griseofulvin analogs (μg/disc)

Compund	*Botrytis allii*				*Botrytis cinerea*				Inhibited zone (mm)
	10	25	50	100	10	25	50	100	
 1	+++	+++	++++	++++	+++	+++	++++	++++	++++ : 45~50 +++ : 28~34 ++ : 18~22 + : ≧12 − : Inactive
 3	−	+++	++++	++++	+	+++	++++	++++	
 4	−	−	−	+	−	−	−	−	
 11	−	++	++++	++++	−	++	++++	++++	Ko, B. S. *et al.*, Agric. Biol. Chem. (1990)
 12	−	−	−	+	−	−	−	−	

25 μg/disc. The activity of (±)-3 was considerably inferior to that of 1, although the activity of (±)-3 was higher than that of (±)-6′-ethyl analog (11).

From these result, it was deduced that configuration at 6′-position is very critical in biological activity of griseofulvin (1), and this could be used for designing new candidate compounds of griseofulvin analogs.

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항진균성항생물질 griseofulvin 유도체인 6′-phenylgriseofulvin의 합성과 항균활성

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초록 : Griseofulvin의 구조활성상관연구에서 6′-methyl기와 4′-oxo기가 생물활성에 있어서 중요한 인자라는 것이 예견되어, 6′-methyl基를 phenyl基로 치환한 6′-phenylgriseofulvin을 diels-alder부가환화로 합성하고 *Botrytis allii* 및 *B. cinerea*에 대한 항균활성을 paper disc 법으로 연구하였다. 6′-Phenylgriseofulvin은 25 μg/disc에서 높은 항균활성을 나타냈다.