Phytochemistry, Vol. 22, No. 4, pp. 1011–1015, 1983.
Printed in Great Britain.

0031-9422/83/041011-05$03.00/0

ANTIMICROBIAL AGENTS FROM *BLETILLA STRIATA*

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(*Received* 26 *July* 1982)

Key Word Index—*Bletilla striata*; Orchidaceae; bibenzyl; dihydrophenanthrene; antimicrobial agents.

Abstract—Three new bibenzyls and two new dihydrophenanthrenes, which exhibit antimicrobial activity, were isolated from tubers of *Bletilla striata*. They were identified by various spectroscopic methods.

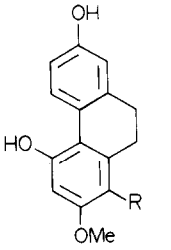
INTRODUCTION

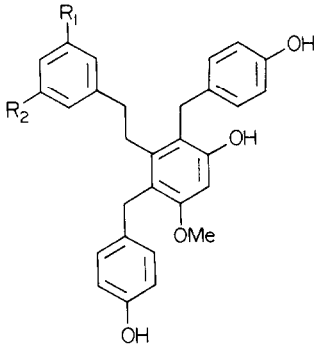
The tubers of *Bletilla striata* Reichb. fil., which are called "bái jí" in China, have been used in traditional medicine to treat pneumonorrhagia and pneumonophthisis, but no detailed chemical study on them has been reported. This paper describes the results of our chemical and antimicrobial studies on the constituents of this crude drug.

RESULTS AND DISCUSSION

The methanol-extractable material from sliced tubers of *Bletilla striata* showed antimicrobial activity *in vitro* against *Staphylococcus aureus*. Subsequent fractionation of this material showed that the bioactivity was restricted to the phenolic part of the ethyl acetate-soluble material. The active portion was repeatedly subjected to column and centrifugal liquid chromatography over Si gel and yielded five active principles, the bibenzyls **2**–**4** and the dihydrophenanthrenes **1** and **5**.

Compound **2** was isolated as colourless needles and its mass spectrum exhibited a molecular ion peak at *m/z* 456, suggesting the elemental composition of $C_{29}H_{28}O_5$. It showed a typical benzenoid absorption in the UV spectrum and absorption bands at 3500–3000 (OH), 1590 and 1510 (aromatic) cm^{-1} in the IR spectrum. Methylation of **2** gave a tetramethyl ether, $C_{33}H_{36}O_5$ (M^+ 512) and acetylation of **2** afforded the tetra-acetate, $C_{37}H_{36}O_9$ (M^+ 624), indicating the presence of four hydroxyl groups in **2**. The presence of the *p*-hydroxybenzyl group was confirmed by peaks at *m/z* 350 $[M-106]^+$, 244 $[M-106\times2]^+$ and 106 [hydroxytropylium]$^+$ in the mass spectrum and by the presence of two singlets at δ3.91 and 3.94 (each 2H) due to two benzylic methylenes and four doublets at δ6.62 and 6.86, δ6.64 and 6.94 (each 2H, J = 8.5 Hz) due to two sets of the A_2B_2 system characteristic of the *para*-substituted aromatic ring in the ^{1}H NMR spectrum. The ^{1}H NMR spectrum also contained a singlet at δ3.76 (3H) due to a methoxyl group and a pair of multiplets at δ2.29 and 2.69 (each 2H) assignable to ϕ–CH_2–CH_2–ϕ, which suggested that the two *p*-hydroxybenzyl groups were linked to C-2 and C-6. Additionally, the ^{1}H NMR spectrum of **2** showed signals corresponding to five aromatic protons, a singlet at δ6.49 (1H) due to H-4, a triplet at δ7.02 (1H, J = 8.5 Hz) due to an *o*-coupled proton (H-5′), a pair of double double





1 R = H_2C–(C$_6$H$_4$)–OH
5 R = H

	R_1	R_2
2	OH	H
3	OMe	H
4	OH	H_2C–(C$_6$H$_4$)–OH

doublets at δ6.48 and 6.56 (each 1H, J = 8.5, 2.0, 2.0 Hz) due to two *o*-coupled protons (H-4′ and H-6′), and a double doublet at δ6.50 (1H, J = 2.0, 2.0 Hz) due to H-2′. These assignments were confirmed by double resonance experiments. NOE experiments on **2** indicated a 21.8% increase in the intensity of the signal of H-4 at δ6.49, after irradiation of the singlet at δ3.76 attributable to the methoxyl group. On the basis of the above features, the structure of **2** was assumed to be the bibenzyl having two *p*-hydroxybenzyl groups, a methoxyl group and a hydroxyl group on the same benzene ring. Comparison of the ^{13}C NMR spectra of **2** and **2** acetate confirmed the substitution patterns (Table 1). These data established the structure of **2** to be 3,3′-dihydroxy-2′,6′-bis(*p*-hydroxybenzyl)-5-methoxybibenzyl.

The less polar component **3** was obtained as colourless needles and exhibited the presence of a hydroxy group (3600–3100 cm^{-1}) in the IR spectrum and a molecular ion peak at *m/z* 470 ($C_{30}H_{30}O_5$) in the mass spectrum. The UV, IR and ^{1}H NMR spectra of **3** were strikingly similar to those of **2**, suggesting that **3** possessed the same skeleton with one hydroxyl group being replaced by one methoxyl group. This was supported by the following evidence. Methylation of **3** gave a pentamethyl ether displaying spectral and chromatographic behaviour identical to those of permethylated **3**. The location of the methoxyl group was deduced by considering the acetylation shift in the ^{13}C NMR spectrum. The signals of C-2′ and C-4′ in the spectrum of the **2** acetate appeared downfield by δ5.5 and 5.3, respectively, compared with those of **2**, but the corresponding signals of the **3** acetate remained almost unaffected, compared with those of **3**. Consideration of this acetylation shift indicated that the methoxyl group is linked to C-3′ in **3**. Accordingly, the structure of **3** was established to be 2,6-bis(*p*-hydroxybenzyl)-3′,5-dimethoxy-3-hydroxybibenzyl.

The polar component **4** was obtained as a colourless powder and exhibited the presence of a hydroxy group (3500–3100 cm^{-1}) in the IR spectrum. Acetylation of **4** gave a penta-acetate, $C_{46}H_{44}O_{11}$ (M^+ 772), indicating the presence of five hydroxy groups in **4**. Its mass spectrum showed the molecular ion at *m/z* 562 and its fragmentation pattern was strikingly similar to that of **2**. Intense peaks at *m/z* 456, 350 and 244 were assigned to the fragments, $[M-C_7H_6O]^+$, $[M-C_7H_6O\times 2]^+$, $[M-C_7H_6O\times 3]^+$, respectively. This chemical and spectral evidence in-

Table 1. ^{13}C NMR data of **2**–**4** and their acetates (200 MHz, CD_3OD, $CDCl_3$, TMS as int. standard)

C No.	2	2 acetate	3	3 acetate	4	4 acetate
1	142.6	141.5	142.7	141.8	142.7	141.1
2	120.3	125.6	120.3	125.4	120.3	125.2
3	158.2	150.8	156.0	149.1	156.7	149.5
4	98.1	104.0	98.1	103.9	98.4	104.1
5	158.3	157.1	161.0	159.7	158.4	157.1
6	119.5	122.4	119.5	122.4	119.5	122.4
1′	145.2	143.0	145.2	143.1	142.8	141.5
2′	113.7	119.2	112.6	111.7	113.8	119.4
3′	155.8	149.1	158.4	157.1	156.6	149.2
4′	115.9	121.2	114.5	113.8	116.6	121.2
5′	130.2	129.3	130.2	129.4	131.5	135.6
6′	120.5	125.3	121.5	120.5	132.0	131.1
OMe	55.9	55.7	56.0	55.7	56.0	55.7
	—	—	55.9	55.2	—	—
$\underline{C}H_2$–ϕ	37.6	36.2	37.7	36.6	37.9	39.3
	33.3	31.8	33.4	32.1	34.7	33.3
	31.2	31.7	31.2	31.7	31.8	31.6
	31.1	31.0	31.0	31.0	31.2	31.0
	—	—	—	—	31.1	30.5
CH_2–$\underline{C}_6H_4$–OH	155.8 × 2	148.9	156.0 × 2	149.0	156.3	149.1
	—	148.8	—	148.8	155.9 × 2	148.9
	—	—	—	—	—	148.8
	134.4	138.4	134.4 × 2	138.5	134.2	138.2
	134.3	138.1		138.1	134.1	137.9
	—	—	—	—	133.7	137.6
	130.0 × 2	129.0 × 2	130.1 × 2	129.0 × 2	130.6 × 2	129.6 × 2
	129.9 × 2	128.8 × 2	129.9 × 2	128.8 × 2	130.1 × 2	128.9 × 2
	—	—	—	—	129.9 × 2	128.7 × 2
	115.9 × 4	121.5 × 2	115.9 × 4	121.5 × 2	116.1 × 2	121.6
	—	121.2 × 2	—	121.2 × 2	115.9 × 4	121.5 × 4
	—	—	—	—	—	121.4
OCOMe	—	169.4 × 2	—	169.4 × 2		169.4 × 3
	—	169.3 × 2	—	169.2	—	169.2 × 2
	—	21.2 × 2	—	21.1 × 2	—	21.1 × 4
	—	20.9 × 2	—	20.9	—	20.9

dicated that **4** possessed the same skeleton as that of **2** and one *p*-hydroxybenzyl group was located on the benzene ring instead of one aromatic proton. The ^{1}H NMR spectrum of **4** showed three singlets at δ3.57, 3.81 and 3.85 (each 2H) due to three benzyl methylenes, a singlet at δ3.73 (3H) due to the methoxyl group and three doublets at δ6.49, 6.53 and 6.58 (each 1H, J = 2.7 Hz) due to three *meta*-coupled protons (H-2′, H-4′ and H-6′), which showed the additional *p*-hydroxybenzyl group attached to C-5′. These considerations led to the conclusion that the structure of **4** was 3,3′-dihydroxy-5-methoxy-2,5′,6-tris(*p*-hydroxybenzyl)bibenzyl.

Compound **5** was obtained as white needles, mp 72°, $C_{15}H_{14}O_3$ (M^+ 242), and the presence of a hydroxy group (3500–3100 cm^{-1}) and an aromatic group (1610, 1450 cm^{-1}, λ_{max} 281 nm) was indicated by the IR and UV spectra. Acetylation of **5** gave the diacetate, $C_{19}H_{18}O_5$ (M^+ 326), indicating the presence of two hydroxyl groups in **5**. The ^{1}H NMR spectrum of **5** showed the presence of one methoxyl group (3H, *s*, δ3.81), and two methylene groups (4H, *s*, δ2.63) located between two aromatic rings, and five aromatic protons. The signal of the aromatic protons appeared as a pair of doublets at δ6.30, 6.39 (each 1H, J = 2.4 Hz) due to *meta*-coupled H-1 and H-3, a double doublet at δ6.60 (1H, J = 9.5, 2.7 Hz) due to *ortho*- and *meta*-coupled H-6, a doublet at δ6.61 (1H, J = 2.7 Hz) due to *meta*-coupled H-8 and a doublet at δ7.99 (1H, J = 9.5 Hz) due to *ortho*-coupled H-5, which was deshielded by the hydroxyl group at C-4. These data, coupled with the molecular formula, suggested a 2,4,7-trisubstituted dihydrophenanthrene structure for **5**. The ^{13}C NMR spectra of **5** and its acetate supported these deductions (Table 2). Hence, the structure of **5** was established to be 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene.

Compound **1** was obtained as colourless needles, mp 231 233°, $C_{22}H_{20}O_4$ (M^+ 348), and its IR and UV spectra indicated the presence of a hydroxy group (3500–3100 cm^{-1}) and an aromatic group (1590, 1510 cm^{-1}; λ_{max} 282 nm). Acetylation of **1** gave the triacetate, $C_{28}H_{26}O_7$ (M^+ 474), indicating the presence of three hydroxy groups in **1**. The presence of the *p*-hydroxybenzyl group was confirmed by the peak at m/z 242 $[M-C_7H_6O]^+$ in the mass spectrum and by the presence of a singlet at δ3.93 (2H) due to a benzylic methylene and a pair of doublets at δ6.63 and 6.93 (each 2H, J = 8.5 Hz) due to the A_2B_2 system characteristic of the *para*-substituted aromatic ring in the ^{1}H NMR spectrum. The position of the *p*-hydroxybenzyl group was confirmed by comparison of the ^{1}H NMR spectrum of **1** with that of **5**. The ^{1}H NMR spectrum of **1**, unlike that of **5**, showed no signals due to *meta*-coupled protons (H-1, H-3) at δ6.30 and 6.39 and methylene groups (H-9, H-10) at δ2.63, but did exhibit a singlet at δ6.51 (1H), assignable to the isolated aromatic proton and a multiplet at δ2.45–2.57 (4H) due to an unequivalent methylene. This evidence suggests that the *p*-hydroxybenzyl group is attached to the C-1 position. Accordingly, the structure of **1** was established as 4,7-dihydroxy-1-*p*-hydroxybenzyl-2-methoxy-9,10-dihydrophenanthrene.

Four known compounds were also isolated in their pure forms from the acidic fraction. Their chemical and spectral properties were identical to those of the authentic compounds, *p*-hydroxybenzoic acid, protocatechuic acid, *p*-hydroxybenzaldehyde and cinnamic acid.

The *in vitro* antimicrobial activity of five compounds

Table 2. ^{13}C NMR data of **1** and **5** and their acetates (conditions as in Table 1)

C No.	1	1 acetate	5	5 acetate
1	118.6	121.3	108.4	113.5
2	157.2	156.1	159.1	157.6
3	99.3	104.8	99.5	104.1
4	155.6	149.1	156.1	149.0
5	130.3	129.7	130.0	129.5
6	114.6	119.8	115.0	120.3
7	156.0	148.9	157.4	150.0
8	113.5	118.8	113.6	118.9
9	30.9	29.3	31.8	30.2
10	27.4	26.2	31.2	29.5
1a	140.9	140.7	141.8	141.1
4a	117.5	121.3	116.9	120.7
5a	126.5	130.1	126.2	129.9
8a	140.4	139.5	140.5	139.8
OMe	56.0	55.7	55.9	55.7
$\underline{C}H_2$–ϕ	31.1	31.6	—	—
CH_2–$\underline{C}_6H_4$–OH	156.0	148.9	—	—
	133.8	137.5	—	—
	130.0 × 2	128.8 × 2	—	—
	115.9 × 2	121.4 × 2	—	—
OCOMe	169.4 × 3	—	—	169.5
	—	—	—	169.4
	21.1 × 2	—	—	21.2 × 2
	20.9	—	—	—

Table 3. Antibacterial activity *in vitro* (MIC; μg/ml)

Test compound	Test organism*							
	1	2	3	4	5	6	7	8
1	200	100	100	50	—	—	—	—
2	12.5	6.25	25	6.25	—	—	—	—
3	400	400	400	400	—	—	—	—
4	50	25	25	3.12	—	—	—	—
5	200	50	200	25	—	—	—	—
p-Hydroxybenzoic acid	—	—	—	—	—	—	—	—
Protocatechuic acid	—	—	—	—	—	—	—	—
Cinnamic acid	—	—	—	—	—	—	—	—
p-Hydroxybenzaldehyde	—	100	—	—	—	—	—	—
p-Hydroxybenzyl alcohol	200	25	100	50	—	—	—	—
4,4′-Dihydroxydiphenyl methane	200	200	100	200	200	200	200	200
Berberine chloride	400	50	100	100	200	50	400	400

*1, *Bacillus subtilis*; 2, *Bacillus cereus* var. *mycoides*; 3, *Nocardia gardneri*; 4, *Staphylococcus aureus*; 5, *Shigella sonnei* I; 6, *Escherichia coli*; 7, *Klebsiella pneumoniae*; 8, *Pseudomonas aeruginosa* P_3.

Table 4. Antimycotic activity *in vitro* (MIC; μg/ml)

Test compound	*Candida albicans* ATCC 10257	*Trichophyton mentagrophytes* QM 248
1	> 100	> 100
2	> 100	100
3	> 100	> 100
4	> 100	50
5	> 100	100

and related substances are given in Tables 3 and 4. These data show that they are mainly active against Gram-positive bacteria and very weakly active against certain fungi. The data also suggest that introduction of the methoxyl group causes a decrease in the activity whereas the introduction of the *p*-hydroxybenzyl group enhances it. However, the relative narrowness of the spectrum is discouraging. In addition, these compounds have no activity against leukemic P-388.

Bibenzyls and dihydrophenanthrenes occur naturally in a few higher plant families, like Combretaceae [1, 2], Dioscoreaceae [3], Leguminaceae [4] and Pinaceae [5]. Some exhibit antibacterial or growth inhibitory properties.

EXPERIMENTAL

Mps were uncorr. Centrifugal liquid chromatography was carried out on a Hitachi CLC-5 using KT gel 2061 (Fuji gel) as adsorbent and CC using Si gel 60 (70–230 mesh, Merck). TLC was done on precoated Si gel F_{254} (Merck) and spots were detected by viewing the TLC sprayed with 1% $CeSO_4$ reagent under UV light (254 nm).

Plant material. Tubers of *Bletilla striata* were obtained as a crude drug and identified by Dr. K. Yoneda, Faculty of Pharmaceutical Sciences, Osaka University. A voucher specimen is kept in our laboratories.

Extraction and isolation. The crushed drug (10 kg) was extracted with 3 × 10 l. of MeOH at room temp. After concn of the biologically active extracts *in vacuo*, H_2O was added then extracted successively with *n*-hexane, EtOAc and *n*-BuOH. The bioactive EtOAc layer was concd *in vacuo* to obtain a residue (100 g), which was dissolved in Et_2O and extracted with 5% HCl, 5% Na_2CO_3 and 2% NaOH. The Na_2CO_3 and NaOH layers were acidified with 1% HCl and extracted with Et_2O, giving acidic and phenolic fractions, respectively. The bioactive fraction (15 g) was subjected to CC on Si gel with $CHCl_3$–MeOH (30:1) as eluent and four fractions were obtained. The first was submitted to rechromatography on a Si gel column eluted with *n*-hexane–EtOAc (10:1) and gave **5** (200 mg) and 4,4′-dihydroxydiphenylmethane (75 mg). From the second fraction, **1** (370 mg) and **3** (250 mg) were obtained by CLC using $CHCl_3$–MeOH (50:1) as eluent. The third fraction was rechromatographed on a Si gel column eluted with $CHCl_3$–MeOH (30:1) and gave **2** (920 mg). The fourth fraction was rechromatographed on a Si gel column developed with $CHCl_3$–EtOAc (5:1) and gave **4** (120 mg). The acidic fraction was chromatographed on Si gel using $CHCl_3$–MeOH (30:1) and *n*-hexane–EtOAc (20:1) and gave *p*-hydroxybenzoic acid, protocatechuic acid, cinnamic acid and *p*-hydroxybenzaldehyde, which were identified by mmp with the authentic sample and IR and ^{1}H NMR spectra.

The properties of the bioactive compounds isolated were as follows.

Compound **1** colourless needles, mp 231–233° ($CHCl_3$–Me_2CO). MS *m/z*: 348 $[M]^+$ ($C_{22}H_{20}O_4$), 242 $[M-C_7H_6O]^+$, UV λ_{max}^{MeOH} nm (log ε): 273sh (4.31), 282 (4.39), 300sh (4.20); IR ν_{max}^{KBr} cm^{-1}: 3500–3100 (*br*, OH), 1590, 1510, ^{1}H NMR (200 MHz, CD_3OD, TMS as int. standard): δ 2.45–2.57 (4H, *m*, $-CH_2-CH_2-$), 3.82 (3H, *s*, $-OCH_3$), 3.93 (2H, *s*, $CH_2-\phi$), 6.51 (1H, *s*, H-3), 6.58 (1H, *d*, *J* = 2.5 Hz, H-8), 6.60 (1H, *dd*, *J* = 8.5, 2.5 Hz, H-6), 6.63 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.93 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 7.96 (1H, *d*, *J* = 8.5 Hz, H-5). Triacetate: colourless amorphous powder. MS *m/z*: 474 $[M]^+$ ($C_{28}H_{26}O_7$), 432 $[M-Ac]^+$, 390 $[M-Ac\times2]^+$, 348 $[M-Ac\times3]^+$; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ 2.24, 2.26, 2.29 (each 3H, *s*, $-OCOCH_3\times3$), 2.62 (4H, *s*, $-CH_2-CH_2-$), 3.87 (3H, *s*, $-OCH_3$), 3.94 (2H, *s*, $CH_2-\phi$), 6.68 (1H, *s*, H-3), 6.93–6.98 (4H, H-6, H-8, H-3′, H-5′), 7.07 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 8.23 (1H, *d*, *J* = 8.5 Hz, H-5); IR ν_{max}^{KBr} cm^{-1}: 2950, 1760, 1600, 1500.

Compound **2**, colourless needles, mp 185–186° ($CHCl_3$–Me_2CO). MS *m/z*: 456 $[M]^+$ ($C_{29}H_{28}O_5$), 350 $[M-C_7H_6O]^+$, 244 $[M-C_7H_6O\times2]^+$, 106 $[C_7H_6O]^+$; UV λ_{max}^{MeOH} nm (log ε): 273sh (3.89), 281 (3.99), 289sh (3.88); IR ν_{max}^{KBr} cm^{-1}: 3500–3000

(*br*, OH), 1590, 1510; ^{1}H NMR (200 MHz, CD_3OD, TMS as int. standard): δ2.26–2.31, 2.66–2.71 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.76 (3H, *s*, $-OC\underline{H}_3$), 3.91, 3.94 (each 2H, *s*, $-C\underline{H}_2-\phi\times 2$), 6.48 (1H, *ddd*, J = 8.5, 2.0, 2.0 Hz, H-4'), 6.49 (1H, *s*, H-4), 6.50 (1H, *dd*, J = 2.0, 2.0 Hz, H-2'), 6.56 (1H, *ddd*, J = 8.5, 2.0, 2.0 Hz, H-6'), 6.62, 6.64 (each 2H, *d*, J = 8.5 Hz, H_2-3'', H_2-5''), 6.86, 6.94, (each 2H, *d*, J = 8.5 Hz, H_2-2'', H_2-6''), 7.02 (1H, *t*, J = 8, 5 Hz, H-5'). Tetra-acetate: colourless amorphous powder. MS *m/z*: 624 $[M]^+$ ($C_{37}H_{36}O_9$), 582 $[M-Ac]^+$, 540 $[M-Ac\times 2]^+$; IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1590, 1500; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ2.16, 2.29 (each 3H, *s*, $-OCOC\underline{H}_3\times 2$), 2.26 (6H, *s*, $-OCOC\underline{H}_3\times 2$), 2.41–2.50, 2.78–2.87 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.77 (3H, *s*, $-OC\underline{H}_3$), 3.90, 4.07 (each 2H, *s*, $-C\underline{H}_2-\phi\times 2$), 6.62 (1H, *s*, H-6), 6.69 (1H, *dd*, J = 2.0, 2.0 Hz, H-2'), 6.82 (1H, *ddd*, J = 8.5, 2.0, 2.0 Hz, H-4'), 6.88 (1H, *ddd*, J = 8.5, 2.0, 2.0 Hz, H-6'), 6.94, 6.95 (each 2H, *d*, J = 8.5 Hz, H_2-3'', H_2-5''), 7.06, 7.08 (each 2H, *d*, J = 8.5 Hz, H_2-2'', H_2-6''), 7.23 (1H, *t*, J = 8.5 Hz, H-5'). Pentamethylate: MS *m/z*: 512 $[M]^+$ ($C_{33}H_{36}O_5$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2850, 1600; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ2.35–2.46, 2.76–2.85 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.74, 3.82 (6H, *s*, $-OC\underline{H}_3\times 2$), 3.76 (3H, *s*, $-OC\underline{H}_3$), 4.02 (4H, *s*, $-C\underline{H}_2-\phi\times 2$), 6.51 (1H, *s*, H-6), 6.54–6.69 (3H, *m*, H-2', H-4', H-6'), 6.76 (2H, *d*, J = 8.7 Hz, H-3'', H-5''), 7.01 (2H, *d*, J = 8.7 Hz, H-2'', H-6''), 7.15 (1H, *t*, J = 7.8 Hz, H-5').

Compound **3**, colourless needles, mp 175–176° ($CHCl_3$–Me_2CO). MS *m/z*: 470 $[M]^+$ ($C_{30}H_{30}O_5$), 440 $[M-OCH_3]^+$, 364 $[M-C_7H_6O]^+$; UV λ_{max}^{MeOH} nm (log ε): 277sh (3.86), 283 (3.96), 290sh (3.89); IR ν_{max}^{KBr} cm^{-1}: 3600–3100 (*br*, OH), 1600, 1510; ^{1}H NMR (200 MHz, CD_3OD, TMS as int. standard): δ2.23–2.37, 2.64–2.73 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.73, 3.77 (each 3H, *s*, $-OC\underline{H}_3\times 2$), 3.91, 3.94 (each 2H, *s*, $-C\underline{H}_2-\phi\times 2$), 6.49–7.15 (13, aromatic H). Triacetate: colourless amorphous powder. MS *m/z*: 596 $[M]^+$ ($C_{36}H_{36}O_8$), 554 $[M-Ac]^+$, 512 $[M-Ac\times 2]^+$, 470 $[M-Ac\times 3]^+$; IR ν_{max}^{KBr} cm^{-1}: 2900, 1750, 1600, 1500; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ2.16 (3H, *s*, $-OCOC\underline{H}_3$), 2.26 (6H, *s*, $-OCOC\underline{H}_3\times 2$), 2.45–2.50, 2.78–2.83 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.76, 3.77 (each 3H, *s*, $-OC\underline{H}_3\times 2$), 3.93, 4.09 (each 2H, *s*, $-C\underline{H}_2-\phi\times 2$), 6.52–7.24 (13H, *s*, aromatic H). Pentamethylate: the spectral data and chromatographic behaviour were identical to those of **2** pentamethylate.

Compound **4**, colourless powder, mp 233–235° ($CHCl_3$–EtOAc). MS *m/z*: 562 $[M]^+$ ($C_{36}H_{34}O_6$), 456 $[M-C_7H_6O]^+$, 350 $[M-C_7H_6O\times 2]^+$, 244 $[M-C_7H_6O\times 3]^+$; UV λ_{max}^{MeOH} nm (log ε): 283 (4.09), 289sh (4.06); IR ν_{max}^{KBr} cm^{-1}: 3500–3100 (*br*, OH), 1590, 1510; ^{1}H NMR (200 MHz, CD_3OD, TMS as int. standard): δ2.42–2.47, 2.64–2.68 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.73 (3H, *s*, $-OC\underline{H}_3$), 3.57, 3.81, 3.85 (each 2H, *s*, $-C\underline{H}_2-\phi\times 3$), 6.46 (1H, *s*, H-4), 6.49 (1H, *d*, J = 2.7 Hz, H-2'), 6.53 (1H, *d*, J = 2.7 Hz, H-4'), 6.58 (1H, *d*, J = 2.7 Hz, H-6'), 6.58 (2H, *d*, J = 8.8 Hz, H-3'', H-5''), 6.60 (2H, *d*, J = 8.5 Hz, H-3'', H-5''), 6.64 (2H, *d*, J = 8.8 Hz, H-3'', H-5''), 6.77 (2H, *d*, J = 8.8 Hz, H-2'', H-6''), 6.79 (2H, *d*, J = 8.5 Hz, H-2'', H-6''), 6.86 (2H, *d*, J = 8.8 Hz, H-2'', H-6''). Penta-acetate: colourless powder, mp 138–140° (EtOH). MS *m/z*: 772 $[M]^+$ ($C_{46}H_{44}O_{11}$), 730 $[M-Ac]^+$; IR ν_{max}^{KBr} cm^{-1}: 2950, 1760, 1600, 1500; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ2.13, 2.28, 2.29, (each 3H, *s*, $-OCOC\underline{H}_3\times 3$), 2.26 (6H, *s*, $-OCOC\underline{H}_3\times 2$), 2.51–2.59, 2.73–2.80 (each 2H, *m*, $-C\underline{H}_2-\times 2$), 3.72, 3.80, 3.99 (each 2H, *s*, $-C\underline{H}_2-\phi\times 3$), 3.75 (3H, *s*, $-OC\underline{H}_3$), 6.61 (1H, *s*, H-4), 6.71 (1H, *d*, J = 2.5 Hz, H-2'), 6.82 (1H, *d*, J = 2.5 Hz, H-4'), 6.86 (1H, *d*, J = 2.5 Hz, H-6'), 6.91 (2H, *d*, J = 8.8 Hz, H-3'', H-5''), 6.92 (2H, *d*, J = 9.5 Hz, H-3'', H-5''), 6.94 (2H, *d*, J = 9.3 Hz, H-3'', H-5''), 6.96 (2H, *d*, J = 8.8 Hz, H-2'', H-6''), 7.02 (2H, *d*, J = 9.5 Hz, H-2'', H-5''), 7.05 (2H, *d*, J = 9.3 Hz, H-2'', H-6'').

Compound **5** colourless needles, mp 72° (Me_2CO). MS *m/z*: 242 $[M]^+$ ($C_{15}H_{14}O_3$); UV λ_{max}^{MeOH} nm (log ε): 274sh (4.21), 281 (4.28), 295sh (4.14); IR ν_{max}^{KBr} cm^{-1}: 3350, 1610, 1450; ^{1}H NMR (200 MHz, CD_3OD, TMS as int. standard): δ2.63 (4H, *s*, $-C\underline{H}_2-C\underline{H}_2-$), 3.81 (3H, *s*, $-OC\underline{H}_3$), 6.30 (1H, *d*, J = 2.4 Hz, H-1), 6.39 (1H, *d*, J = 2.4 Hz, H-3), 6.60 (1H, *dd*, J = 9.5, 2.7 Hz, H-6), 6.61 (1H, *d*, J = 2.7 Hz, H-8), 7.99 (1H, *d*, J = 9.5 Hz, H-5). Diacetate: colourless powder, mp 132–134° (EtOH). MS *m/z*: 326 $[M]^+$ ($C_{19}H_{18}O_5$), 284 $[M-Ac]^+$, 242 $[M-Ac\times 2]^+$; IR ν_{max}^{KBr} cm^{-1}: 2950, 2850, 1760, 1600; ^{1}H NMR (200 MHz, $CDCl_3$, TMS as int. standard): δ2.30, 2.31 (each 3H, *s*, $-OCOC\underline{H}_3\times 2$), 2.77 (4H, *s*, $-C\underline{H}_2-C\underline{H}_2-$), 3.87 (3H, *s*, $-OC\underline{H}_3$), 6.64 (2H, *s*, H-1, H-3), 6.96 (1H, *d*, J = 2.5 Hz, H-8), 6.97 (1H, *dd*, J = 9.3, 2.5 Hz, H-6), 8.26 (1H, *d*, J = 9.3 Hz, H-5).

Antibacterial testing. The agar medium used for antibacterial tests was heart infusion agar for bacteria. Assays were done by the agar dilution streak method, using the medium (9.0 ml) to which a sample soln (1.0 ml) was added. The concn of samples tested ranged from 0.78 to 400 μg/ml. The antibacterial activities were measured after 18 hr incubation at 37°. The minimal inhibitory concn (MIC) was the lowest level of sample that completely prevented growth. The results are shown in Table 3.

Acknowledgements—We are deeply grateful to Dr. K. Yoneda, Faculty of Pharmaceutical Sciences, Osaka University for identification of the plant material and to Professor T. Araki of this university for the useful suggestion related to antibacterial testing. The bioassays of antimycotic activity were performed by Dainippon Pharmaceutical Co., Ltd.

REFERENCES

1. Letcher, R. M., Nhamo, L. R. and Gumiro, I. T. (1972) *J. Chem. Soc. Perkin Trans.* 1, 206.
2. Letcher, R. M. and Nhamo, L. R. (1972) *J. Chem. Soc. Perkin Trans.* 1, 2941.
3. Hashimoto, T., Hasegawa, K., Yamaguchi, H., Saito, M. and Ishimoto, S. (1974) *Phytochemistry* **13**, 2849.
4. Mitscher, L. A., Park, Y. H., Al-Shamma, A., Hudson, P. B. and Haas, T. (1981) *Phytochemistry* **20**, 781.
5. Lindstedt, G. and Misiorny, A. (1951) *Acta Chem. Scand.* **4**, 121.