Curtisians M—Q: Five Novel *p*-Terphenyl Derivatives from the Mushroom *Paxillus curtisii*

Dang Ngoc QUANG,[a] Toshihiro HASHIMOTO,[a] Makiko NUKADA,[b] Isao YAMAMOTO,[b] Masami TANAKA,[a] and Yoshinori ASAKAWA*,[a]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan: and*
[b] *Faculty of Food Culture, Kurashiki Sakuyo University; Kurashiki 710–0290, Japan.*
Received May 19, 2003; accepted June 23, 2003

Five novel *p*-terphenyl derivatives called curtisians M—Q (1—5) were isolated from the methanolic extract of fruit bodies of the Basidiomycete *Paxillus curtisii*. Their structures were elucidated by two dimensional (2D) NMR, MS, IR, and UV spectroscopy, and chemical reaction. Their antioxidative activities were also investigated.

Key words *Paxillus curtisii*; curtisian; *p*-terphenyl

We have been studying the chemical constituents of some Japanese inedible mushrooms and isolated interesting biologically active compounds such as cryptoporic acids A—G from the fungus *Cryptoporus volvatus*, spiromentins from *Paxillus atrotomentosus*, *etc.*[1,2] Recently, we have investigated the chemical constituents of some Basidiomycete fungi and isolated *p*-terphenyl derivative series such as thelephantins A—C[3] and D—H[4] from *Thelephora aurantiotincta*, curtisians E—H[5] and I—L[6] from *Paxillus curtisii* and characterized their structures. Further fractionation of the methanolic extract of the fruit bodies of *P. curtisii* resulted in the isolation of five novel curtisians M—Q (**1**—**5**). Previously, curtisians A—D with antioxidative activity were also reported from Korean *P. curtisii* without determination of the stereochemistry of the side chains.[7] This paper describes the isolation and structural elucidation of the newly isolated terphenyl derivatives (**1**—**5**) and their radical-scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Results and Discussion

The methanolic extract of Japanese *P. curtisii* was subjected to Sephadex LH-20 and reverse-phase (C-18) column chromatography, followed by reverse-phase preparative HPLC to afford the five curtisians M—Q (**1**—**5**) as described in the Experimental section.

Curtisian M (**1**) showed a molecular ion peak at *m/z* 605 corresponding to $C_{30}H_{30}O_{12}Na$ by high resolution (HR)-FAB-MS. Its IR and UV spectra showed the absorption of a hydroxyl (3386 cm^{-1}), an ester carbonyl (1740 cm^{-1}), and an aromatic (1612, 1525 cm^{-1}; 263 nm) group. The ^{1}H-NMR of **1** (Table 1) showed the presence of *ortho*-coupling aromatic proton signals at δ 6.83 and 7.17 (d, J=8.8 Hz), one methyl signal at δ 1.08 (d, J=6.3 Hz), one methylene, and one methine group. The ^{13}C-NMR of **1** (Table 2) exhibited 13 carbon resonances including one acetyl (δ 172.0, 21.1), ester carbonyl (δ 169.9), and phenolic carbons (158.2, 142.7). The signals observed in the ^{13}C-NMR spectrum of **1** (Table 2) showed exact overlapping on the basis of free rotation of two phenyl groups, suggesting that this compound has a symmetrical structure.[7] The correlations between H-3a,b/H-2a,b and H-4a,b in ^{1}H–^{1}H correlation spectroscopy (COSY) and long-range correlations between 1) C-1a,b/H-2a,b and H-3a,b; and 2) CH_3CO-/H-3a,b in the heteronuclear multiple bond connectivity (HMBC) spectrum suggest the presence of two 3-acetoxybutyryl groups in **1**. Comparison of the ^{1}H–^{1}H COSY, nuclear Overhauser effect spectroscopy (NOESY), and HMBC spectra of **1** with those of thelephantins A—H[3,4] indicates the presence of a *p*-terphenyl derivative with two *para*-substitution groups located at the central aromatic ring. To determine the positions of two 3-acetoxybutyryl groups, **1** was oxidized by di-ammonium cerium(IV) nitrate in acetonitrile to give *para*-benzoquinone **6**. The ^{1}H- and ^{13}C-NMR data (Tables 1, 2) of **6** were similar to those of **1** except for the presence of a signal at 179.1 ppm instead of the phenolic carbon at 142.7 ppm and the low-field shift position of H-2a,b (Table 1). The IR and UV spectra of **6** showed absorption at 1671 cm^{-1} and 492 nm, respectively indicating the presence of *para*-benzoquinone in **6**.[6] Therefore the locations of two 3-acetoxybutyryl units at the central aromatic ring were determined at C-2′ and C-5′. Consequently, the structure of curtisian M (**1**) was established to be di(3-acetoxybutyric acid), 3′,4,4″,6′-tetrahydroxy-[1,1′:4′,1″-terphenyl]-2′,5′-diyl ester, as shown in Chart 1.

Curtisian N (**2**) displayed a molecular ion peak at *m/z* 519, corresponding to $C_{26}H_{24}O_{10}Na$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR spectra data (Tables 1, 2) of **2** resembled those of curtisian M (**1**), indicating that **2** is also a *p*-terphenyl except for the presence of an acetoxy group (δ_H 1.93; δ_C 20.3, 170.6) in the place of the 3-acetoxybutyryl group at C-5′ of central aromatic ring. Thus the structure of curtisian N (**2**) was determined by two dimensional (2D) NMR (HMBC, NOESY, *etc.*) to be acetic acid, 3′,4,4″,6′-tetrahydroxy-2′-(3-acetoxy-1-oxobutoxy)-[1,1′:4′,1″-terphenyl]-5′-yl ester, as shown in Chart 1.

The absolute configuration of the side chains at C-3a,b of both curtisians M (**1**) and N (**2**) was established to be *S* by chemical reactions (hydrolysis, methylation, and acetylation of the crude extract) and comparison of the retention times of the reaction product on gas chromatography-mass spectroscopy (GC-MS) equipped with chiral column with authentic (*R*)- and (*S*)-3-acetoxybutyric acid methyl ester.[6]

The molecular formula of curtisian O (**3**) was determined to be $C_{24}H_{22}O_8$ by HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data of **3** also resembled those of **2** and thelephantin A,[3] revealing that **2** was a *p*-terphenyl structure, except for the presence of an *n*-butyryl group instead of the 3-acetoxybutyryl group. NOESY investigation of **3** (Chart 1) showed NOE correlations between 1) H-2, H-6/H-2a and H-4a; 2) H-3, H-5/H-3a;

* To whom correspondence should be addressed. e-mail: asakawa@ph.bunri-u.ac.jp © 2003 Pharmaceutical Society of Japan

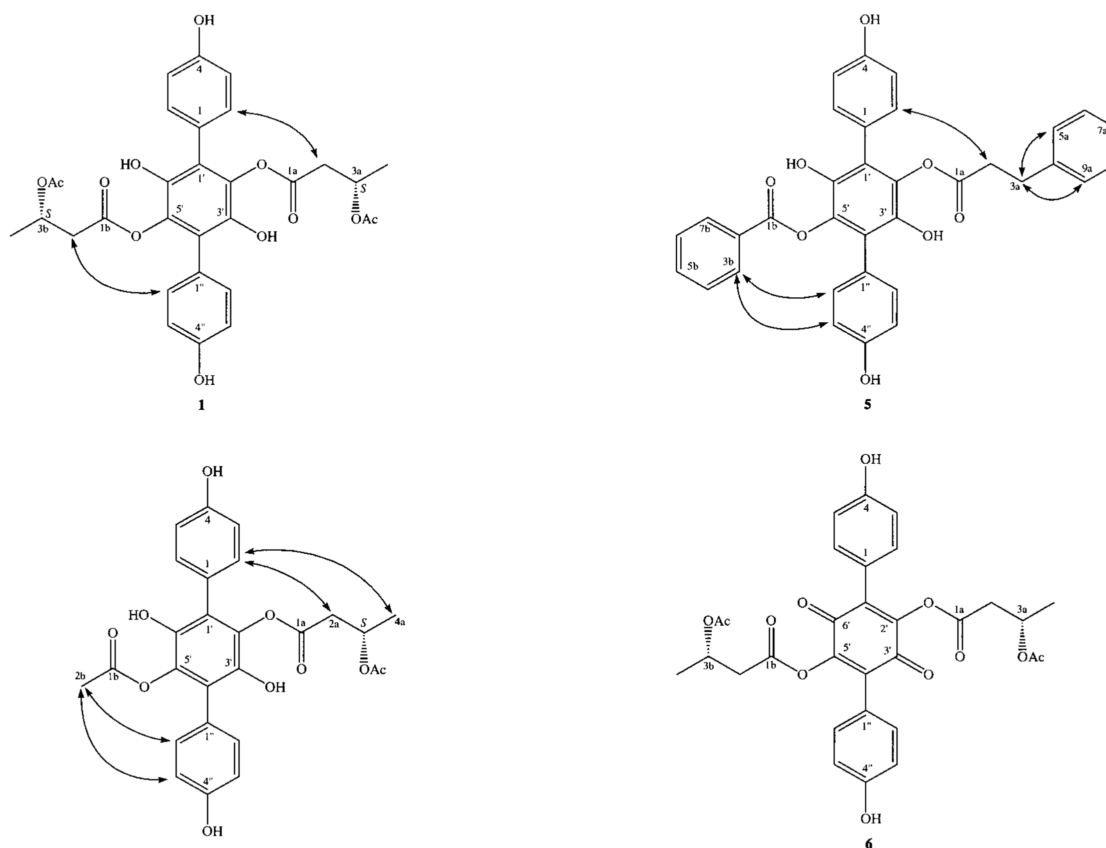
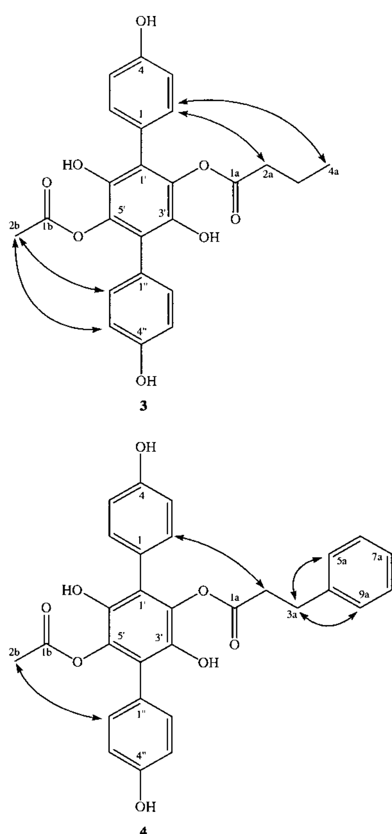


Chart 1. Important NOE Correlations of Compounds **1**—**6**



and 3) H-2b/ H-2″, H-3″, H-5″, and H-6″, indicating that the substitution pattern of *n*-butyryl and acetoxy group was attached to C-2′ and C-5′ at the central aromatic ring, respectively. Consequently, curtisian O (**3**) was found to be acetic acid, 3′,4,4″,6′-tetrahydroxy-2′-(1-oxobutoxy)-[1,1′:4′,1″-terphenyl]-5′-yl ester, as shown in Chart 1.

Curtisian P (**4**) was related to curtisian O (**3**) in its spectral data, suggesting that it was also a *p*-terphenyl with the molecular formula $C_{29}H_{24}O_8$. The ^{1}H-NMR spectrum of **4** (Table 2) showed signals due to two *ortho*-coupling aromatic protons, two methylenes, one acetyl, and one phenyl group. Its ^{1}H-NMR data were similar to those of **3**, with a notable difference being the presence of a phenylpropionyl group in the place of the butyryl group as evidenced by ^{1}H–^{1}H COSY and HMBC spectra. Thus curtisian P (**4**) was deduced to be phenylpropionic acid, 3′,4,4″,6′-tetrahydroxy-5′-(acetoxy)[1,1′:4′,1″-terphenyl]-2′-yl ester, as shown in Chart 1.

Curtisian Q (**5**) was assigned to have the molecular formula $C_{34}H_{26}O_8$ based on HR-FAB-MS. Examination of ^{1}H–^{1}H COSY and HMBC spectra of **5** revealed the presence of a phenylpropionyl group and a benzoyl group in **5**. The positions of these functional groups were determined to be C-2′ and C-5′ at the central aromatic ring, respectively, based on the NOE correlations between 1) H-2a/H-2 and H-6; 2) H-3b, 7b/H-2″, H-6″, H-3″ and H-5″ in the NOESY spectrum (Chart 1). Therefore curtisian Q (**5**) was determined to be phenylpropionic acid, 3′,4,4″,6′-tetrahydroxy-5′-(benzoyloxy)[1,1′:4′,1″-terphenyl]-2′-yl ester as, shown in Chart 1.

The free radical-scavenging activity of curtisians M—Q (**1**—**5**) was evaluated against the stable free radical DPPH.

Table 1. ^{1}H-NMR Spectral Data for Compounds **1**—**6** (CD_3OD, 600 MHz)

Position	1	2	3	4	5	6
2,6	7.17 d (8.8)	7.16 d (8.5)	7.17 d (8.8)	7.15 d (8.8)	7.18 d (8.8)	7.15 d (8.8)
3,5	6.83 d (8.8)	6.83 d (8.5)	6.83 d (8.8)	6.82 d (8.8)	6.83 d (8.8)	6.82 d (8.8)
2″, 6″	7.17 d (8.8)	7.16 d (8.5)	7.15 d (8.8)	7.15 d (8.8)	7.23 d (8.8)	7.15 d (8.8)
3″, 5″	6.83 d (8.8)	6.83 d (8.5)	6.82 d (8.8)	6.82 d (8.8)	6.73 d (8.8)	6.82 d (8.8)
2a	2.62 dd (7.1, 16.5) 2.45 dd (5.8, 16.5)	2.59 dd (7.1, 16.5) 2.44 dd (6.0, 16.5)	2.19 t (7.1)	2.52 t (7.4)	2.31 t (7.7)	2.82 dd (8.0, 17.3) 2.70 dd (5.5, 17.3)
3a	5.03 m	5.03 m	1.46 m	2.71 t (7.4)	2.54 t (7.7)	5.09 m
4a	1.08 d (6.3)	1.10 d (6.3)	0.78 t (7.4)			1.15 d (6.3)
5a, 9a				7.08 d (7.1)	6.86 d (8.2)	
6a, 8a				7.23 t (7.4)	7.10 t (7.4)	
7a				7.15 overlap	7.07 t (7.4)	
2b	2.62 dd (7.1, 16.5) 2.45 dd (5.8, 16.5)	1.93 s	1.90 s	1.76 s		2.82 dd (8.0, 17.3) 2.70 dd (5.5, 17.3)
3b	5.03 m				7.86 d (8.5)	5.09 m
4b	1.08 d (6.3)				7.44 t (7.4)	1.15 d (6.3)
5b					7.60 t (7.4)	
6b					7.44 t (7.4)	
7b					7.86 d (8.5)	
3a-Ac	1.93 s	1.94 s				1.91 s
3b-Ac	1.93 s					1.91 s

Table 2. ^{13}C-NMR Spectral Data for Compounds **1**—**6** (CD_3OD, 150 MHz)

Position	1	2	3	4	5	6
1, 1″	124.9	124.9	125.0	125.0	124.9	121.7
2, 6	132.7	132.6	132.6	132.7	132.7	132.5
3, 5	116.1	116.1	116.0	116.0	116.1	116.1
4, 4″	158.2	158.2	158.2	158.2	158.1	159.6
1′, 4′	123.9	123.9	123.8	123.8	124.0	131.7
2′, 5′	134.5	134.6	134.8	134.9	134.9	151.3
3′, 6′	142.7	142.6	142.6	142.5	142.6	179.1
2″, 6″	132.7	132.6	132.6	132.7	132.6	132.5
3″, 5″	116.1	116.1	116.0	116.0	116.0	116.1
1a	169.9	169.9	173.1	172.5	172.6	168.2
2a	40.5	40.5	36.4	36.0	36.0	40.4
3a	68.2	68.3	19.2	31.3	31.3	67.9
4a	19.7	19.6	13.8	141.7	141.4	19.6
5a, 9a				129.3	129.0	
6a, 8a				129.5	129.4	
7a				127.3	127.1	
1b	169.9	170.6	170.6	170.6	166.2	168.2
2b	40.5	20.3	20.2	20.1	130.2	40.4
3b	68.2				130.9	67.9
4b	19.7				129.8	19.6
5b					134.9	
6b					129.8	
7b					130.9	
3a-Ac	21.1, 172.0	21.1, 172.1				21.0, 171.9
3b-Ac	21.1, 172.0					21.0, 171.9

Their antioxidant activities were defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50% [IC_{50} (μM)] and compared with those of the known antioxidants ascorbic acid, *d*,*l*-α-tocopherol, and BHA. The antioxidative activities of curtisians M—Q (**1**—**5**) were almost similar to each other and about two times weaker than that of *d*,*l*-α-tocopherol, as shown in Table 3.

Table 3. Free DPPH Radical-scavenging Activity of Curtisians M—Q (**1**—**5**)

Sample	IC_{50} (μM)	Authentic sample	IC_{50} (μM)
Curtisian M (**1**)	45.9	Ascorbic acid	16.5
Curtisian N (**2**)	48.8	α-Tocopherol	22.8
Curtisian O (**3**)	58.7	BHA	31.6
Curtisian P (**4**)	44.0		
Curtisian Q (**5**)	43.4		

Experimental

General Column chromatography was carried out on silica gel 60 (0.2—0.5 mm, 0.04—0.063 mm, Merck) and Sephadex LH-20 (Amersham Pharmacia Biotech, $CHCl_3$–MeOH, 1 : 1). Preparative medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd., Japan) and a Lobar column (Merck). Preparative HPLC was performed on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II column. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. Optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH as solvent. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using CD_3OD as solvent. Chemical shifts are given relative to TMS δ 0.00 as an internal standard (^{1}H) and δ 49.0 ppm from CD_3OD as standards (^{13}C). Mass spectra including HR-MS were recorded on a JEOL JMS AX-500 spectrometer.

Fungal Material Fruit bodies of *P. curtisii* were collected in November 1995 in Kyoto, Japan, and then identified by M. N. A voucher specimen (KSU95111) has been deposited in the Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki, Japan.

Extraction and Isolation The MeOH extract (1.83 g) of fruit bodies of *P. curtisii* was divided into 5 fractions (fr. 1—5) by Sephadex LH-20 column chromatography. Fraction 2 (550.6 mg) was chromatographed on SiO_2 column using $CHCl_3$–MeOH–H_2O (25 : 2.5 : 0.1) to give four subfractions. Subfraction 2-2 (158.3 mg) was purified by reverse-phase MPLC with the solvent system $CH_3CN : H_2O$ (1 : 1) and then reverse-phase preparative HPLC with the same solvent system to give curtisian M (**1**) (4.3 mg), curtisian N (**2**) (3.7 mg), curtisian O (**3**) (3.5 mg), curtisian P (**4**) (8.8 mg), and curtisian Q (**5**) (5.5 mg).

Curtisian M (**1**): Grayish solid, $[\alpha]_D^{20}$ $-8.7°$ (c=0.75, CH_3OH). UV λ_{max} (MeOH) (log ε): 212 (4.3), 225 (4.3), 263 (4.2). IR (KBr) cm^{-1}: 3386, 1740, 1612, 1525, 1457, 1376, 1267, 1102, 984. ^{1}H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. HR-MS (FAB) *m/z*: 605.1622 ($[M+Na]^+$, Calcd for $C_{30}H_{30}O_{12}Na$: 605.1635).

Curtisian N (**2**): Grayish solid, $[\alpha]_D^{20}$ $-16.2°$ (c=0.51, CH_3OH). UV λ_{max} (MeOH) (log ε): 210 (4.5), 225 (4.4), 263 (3.3). IR (KBr) cm^{-1}: 3397, 1757, 1612, 1526, 1457, 1373, 1220, 1102, 980. ^{1}H- and ^{13}C-NMR (CD_3OD): see

Tables 1 and 2. HR-MS (FAB) *m*/*z*: 519.1242 ($[M+Na]^+$, Calcd for $C_{26}H_{24}O_{10}Na$: 519.1267).

Curtisian O (**3**): Grayish solid, UV λ_{max} (MeOH) (log ε): 208 (4.3), 224 (4.1), 263 (4.0). IR (KBr) cm^{-1}: 3394, 1749, 1613, 1525, 1467, 1369, 1216, 1175, 1105, 980. ^1H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. HR-MS (FAB) *m*/*z*: 461.1187 ($[M+Na]^+$, Calcd for $C_{24}H_{22}O_8Na$: 461.1212).

Curtisian P (**4**): Light red-brown solid, UV λ_{max} (MeOH) (log ε): 212 (4.5), 262 (4.3). IR (KBr) cm^{-1}: 3412, 1753, 1612, 1526, 1497, 1456, 1369, 1216, 1110, 980. ^1H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. HR-MS (FAB) *m*/*z*: 500.1445 (M^+, Calcd for $C_{29}H_{24}O_8$: 500.1471).

Curtisian Q (**5**): Light red-brown solid, UV λ_{max} (MeOH) (log ε): 213 (4.5), 229 (4.5), 262 (4.3). IR (KBr) cm^{-1}: 3430, 1743, 1611, 1525, 1496, 1264, 1174, 1108, 1061, 974. ^1H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. HR-MS (FAB) *m*/*z*: 562.1664 (M^+, Calcd for $C_{34}H_{26}O_8$: 562.1628).

Oxidation of curtisian M (**6**). To a solution of curtisian M (**1**) (3.1 mg) in acatonitrile (2 ml) was added di-ammonium cerium(IV) nitrate (3.6 mg). The reaction mixture was stirred at 5 °C for 20 min and then partitioned between EtOAc and water. The EtOAc layer was evaporated and then subjected to SiO_2 column chromatography using EtOAc as eluent to give compound **6** (2.8 mg). UV λ_{max} (MeOH) (log ε): 210 (4.1), 256 (4.1), 262 (4.1), 492 (3.2). IR (KBr) cm^{-1}: 3340, 1772, 1735, 1671, 1608, 1514, 1441, 1269, 1175, 978. ^1H- and ^{13}C-NMR (CD_3OD): see Tables 1 and 2. HR-MS (FAB) *m*/*z*: 621.1392 ($[M+2+K]^+$, Calcd for $C_{30}H_{30}O_{12}K$: 621.1374).

DPPH free radical-scavenging activity. The free radical-scavenging activity of curtisians M—Q (**1**—**5**) was measured using the DPPH method.[8] Curtisians, BHA, α-tocopherol, and ascorbic acid were dissolved in ethanol at different concentrations (100, 50, 10, 5, 1 μg/ml) and mixed with DPPH (180 μl). After incubation at room temperature for 20 min, absorbance at 517 nm was measured with a Spectra Max 340 PC and the IC_{50} values were calculated.

Acknowledgements The authors thank Miss Y. Okamoto (TBU, Japan) for measurement of mass spectra.

References

1) Steven M. C., Russell J. M., "Bioactive Natural Products," CRC Press, Boca Raton, FL, 1993, pp. 333—347.
2) Hashimoto T., Asakawa Y., *Heterocycles*, **47**, 1067—1110 (1998).
3) Quang D. N., Hashimoto T., Nukada M., Yamamoto Y., Hitaka Y., Tanaka M., Asakawa Y., *Phytochemistry*, **62**, 109—113 (2003).
4) Quang D. N., Hashimoto T., Nukada M., Yamamoto Y., Hitaka Y., Tanaka M., Asakawa Y., *Phytochemistry*, **63**, 919—924 (2003).
5) Quang D. N., Hashimoto T., Nukada M., Yamamoto Y., Tanaka M., Asakawa Y., *Phytochemistry*, (2003) in press.
6) Quang D. N., Hashimoto T., Nukada M., Yamamoto Y., Tanaka M., Asakawa Y., *Planta Med.*, (2003) in press.
7) Yun B. S., Lee I. K., Kim J. P., Yoo I. D., *J. Antibiot.*, **53**, 114—122 (2000).
8) Blois M. S., *Nature* (London), **181**, 1191—1200 (1958).