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Curtisians A~D, New Free Radical Scavengers from the Mushroom *Paxillus curtisii*

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In our continuous investigation for free radical scavengers from extracts of fruit body of basidiomycetes, we have isolated four new *p*-terphenyl compounds, designated as curtisians A~D, from the methanolic extract of the fruit body of *Paxillus curtisii*. These compounds were isolated by silica gel and Sephadex LH-20 column chromatographies, preparative-TLC and HPLC, consecutively. The structures of curtisians were assigned as *p*-terphenyls with substituents of acetyl, benzoyl, phenylbutyryl, 3-hydroxybutyryl and 3-acetoxybutyryl. Curtisians A, B, C and D exhibited inhibitory activity against lipid peroxidation with IC_{50} values of 0.15, 0.17, 0.24 and 0.14 μg/ml, respectively.

It is well known that free radicals such as active oxygen species are involved in the pathogenesis of various diseases such as myocardial and cerebral ischemia, atherosclerosis, diabetes, rheumatoid arthritis, cancer-initiation and the aging process[1~3]. These diseases have been reported to be ameliorated by free radical scavengers and thus many antioxidants of microbial origin have been searched[4~6]. For free radical scavengers having the potential as protective agents against these diseases, we have investigated the metabolites of basidiomycetes and ascomycetes[7~9]. As a part of our results, we have isolated four *p*-terphenyl compounds, curtisians A~D, from the methanolic extract of the fruit body of *Paxillus curtisii* (Fig. 1). In this paper, we describe the isolation, structural elucidation and free radical scavenging activity of these compounds.

Materials and Methods

General Experiment

Specific rotations were determined by using a Polartronic polarimeter. High resolution mass spectra were measured by using a JEOL JMS-HX 110/100A spectrometer in the FAB mode using glycerol matrix with polyethylene glycol as an internal standard. UV and IR spectra were recorded on a Shimadzu UV-260 and a FT-IR Equinox 55 spectrophotometer, respectively. NMR spectra were obtained on a Varian UNITY 500 NMR spectrometer with ^{1}H NMR at 500 MHz and with ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as an internal standard. All NMR experiments were performed with 1~15 mg each sample dissolved in 0.8 ml of CD_3OD. Analytical silica gel TLC (Merck, Kiesel gel $60F_{254}$, 0.25 mm) and preparative silica gel TLC (Merck, Kiesel gel $60F_{254}$, 0.5 mm) plates were used without activation. HPLC was performed on a Senshu pak ODS column (20×250 mm) with flow rate of 6 ml/minute using 50% aqueous acetonitrile as a mobile phase and by monitoring with a photodiode-array detector (190~650 nm).

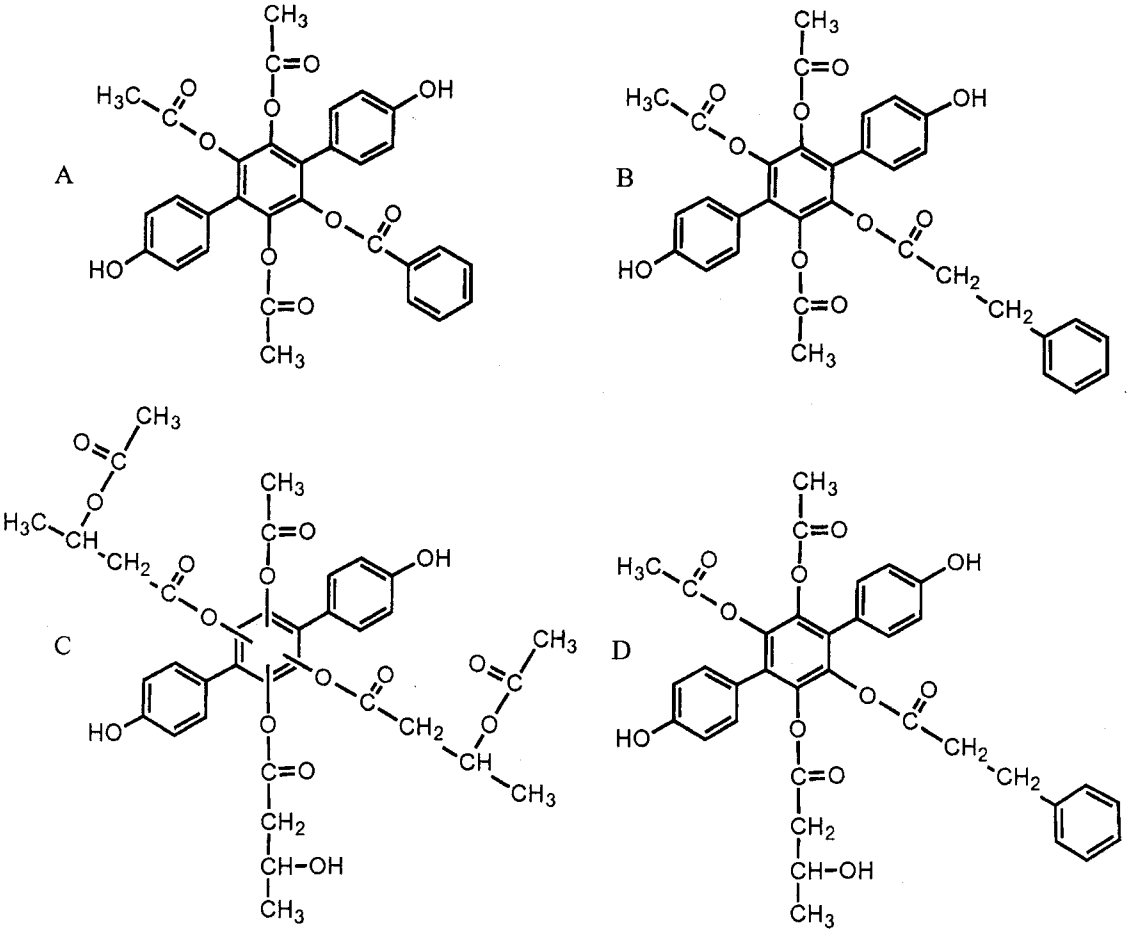
Microorganism

Paxillus curtisii was collected at Keryoung mountain in Chungnam Province, Korea and identified by the Korea Research Institute of Bioscience and Biotechnology, Korea, according to the Hongo method[10]. After dried in a dark and well-ventilated place, the fruit body of *P. curtisii* was extracted with methanol for isolation of active compounds.

Inhibitory Activity against Lipid Peroxidation in Rat Liver Microsomes

Lipid peroxidation inhibitory activity in rat liver microsomes was evaluated by the thiobarbituric acid

Fig. 1. Structures of curtisians A, B, C and D.

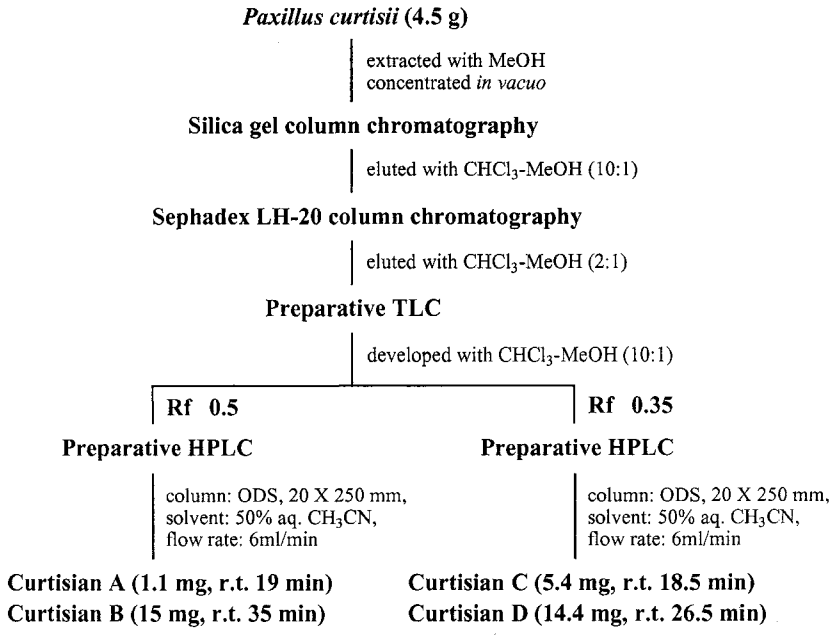


method[11] with minor modifications. Rat liver microsomes were prepared according to the method of HOGEBOOM[12] with some modifications and finally suspended in 100 mM Tris-HCl buffer (pH 7.4). Reaction was initiated by the addition of 100 μM $FeSO_4 \cdot H_2O$ (0.1 ml) into a mixture of Tris-HCl buffer (0.7 ml), 0.2 mM ascorbic acid (50 μl), 0.5 μg protein/ml microsomal suspension (40 μl) and 10 μl of sample solution. The reaction mixture was incubated at 37°C for 30 minutes. After incubation, the reaction was stopped by the addition of 0.25 ml of TCA (3 M) - HCl (2 N) 1 : 1 mixture and then centrifuged at 3500g for 10 minutes. The reaction supernatant (1 ml) was mixed with 0.67% (w/v) thiobarbituric acid (0.25 ml) and then heated in boiling water for 10 minutes. The lipid peroxidation was assessed by measuring the thiobarbituric acid reactive products at 532 nm. Lipid peroxidation inhibitory activity was calculated as follows: $[1-(T-B)/(C-B)]\times 100$ (%), in which T, C and B are absorbance values at 532 nm of the sample treatment, the control (without sample) and the zero time control, respectively. The value of IC_{50} means the concentration (μg/ml) of compounds required for 50% inhibition of microsomal lipid peroxidation.

Superoxide Dismutase (SOD) Radical Scavenging Activity

The method chosen for the assay of SOD activity was a modification of an indirect inhibition assay developed by BEAUCHAMP and FRIDOVICH[13]. Xanthine/xanthine oxidase system was utilized to generate a superoxide flux. Each well of a 96-well plate contained the final concentration of the following reagents: 50 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, 5.6×10^{-5} M NBT (nitroblue tetrazolium), 0.1 mM xanthine, enough xanthine oxidase to achieve the required reference rate (0.020 absorbance/minute) and each concentration of sample. NBT reduction to blue formazan by superoxide was followed at 560 nm in a microplate reader at room temperature. The inhibitory

Fig. 2. Purification procedure of curtisians A, B, C and D.



ratio of each compound to the formation of diformazan from NBT was calculated. The amount of inhibition was defined as a percentage of the reference rate of NBT reduction when sample was not present. The data were plotted as percentage inhibition *vs* sample concentration.

Measurement of DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Activity[14]

Each concentration of a test sample solution in DMSO (20 μl) was added to 980 μl of 150 μM DPPH ethanol solution. After vortex mixing, the mixture was incubated for 20 minutes at room temperature and the absorbance at 517 nm was measured. The differences in absorbance between a test sample and a control (DMSO) were taken and the ED_{50} values were determined as the concentration of the compound that gave a 50% decrease in the absorbance from a blank test.

Results

Isolation and Purification

The fruit body of *P. curtisii* (4.5 g, dry weight) was extracted three times with MeOH. The extracts were combined and concentrated *in vacuo*. The concentrate was chromatographed on a column of silica gel eluting with $CHCl_3$ only followed by a mixture of $CHCl_3$ - MeOH (10 : 1). The active fraction ($CHCl_3$ - MeOH mixture) in lipid peroxidation inhibition test was concentrated *in vacuo* and then subjected to a column of Sephadex LH-20 eluted with $CHCl_3$ - MeOH (2 : 1). The active eluate from the column was concentrated *in vacuo*, applied on preparative silica gel TLC plate and developed with a mixture of $CHCl_3$ - MeOH (10 : 1). Two bands of crude curtisians with Rf values of about 0.5 and 0.35 were scraped out from a TLC plate. The compound from the band of Rf 0.5 was finally purified by reverse-phase HPLC (ODS column, 50% aq. CH_3CN) to give curtisians A (1.1 mg) and B (15 mg) with retention time of 19 and 35 minutes, respectively. Curtisians C (5.4 mg) and D (14.4 mg) showing the retention time of 18.5 and 26.5 minutes, respectively, were obtained from another band of Rf value of 0.35 in the TLC using reverse-phase HPLC under the same conditions as the above (Fig. 2).

Physico-chemical Properties of Curtisians

The physico-chemical properties of curtisians A, B, C and D are summarized in Table 1. Curtisian A was obtained as a yellow solid while curtisians B, C and D were obtained as a dark brown solid. Curtisians were readily soluble in dimethyl sulfoxide (DMSO) and methanol, slightly soluble in $CHCl_3$ and insoluble in *n*-hexane and water. The

Table 1. Physico-chemical properties of curtisians A, B, C and D.

	A	B	C	D
Appearance	Yellow solid	Dark brown solid	Dark brown solid	Dark brown solid
Molecular weight	556	584	710	628
Molecular formula	$C_{31}H_{24}O_{10}$	$C_{33}H_{28}O_{10}$	$C_{36}H_{38}O_{15}$	$C_{35}H_{32}O_{11}$
$[\alpha]_D$			+36 (*c* 0.22, MeOH)	+6 (*c* 1.08, MeOH)
HRFAB-MS (*m/z*)				
found	579.1254 $(M+Na)^+$	584.1669 $(M+H)^+$	733.2112 $(M+Na)^+$	651.1851 $(M+Na)^+$
calcd	579.1267	584.1682	733.2108	651.1842
UV λ_{max}^{MeOH} nm (ε)	208 (31900) 267 (12200)	207 (22100) 268 (8300)	207 (34000) 268 (15900)	208 (33700) 267 (15100)
IR ν_{max} (KBr) cm^{-1}	3450, 1777, 1745, 1613, 1525, 1452, 1372, 1262, 1188, 1115, 1057, 837	3445, 1778, 1745, 1613, 1526, 1445, 1373, 1265, 1175, 1115, 1022, 833	3432, 1773, 1735 (br), 1613, 1524, 1447, 1374, 1263, 1112, 1016, 836	3430, 1773 (br), 1745 (sh) 1612, 1524, 1450, 1371, 1263, 1202, 1112, 1019, 836
Solubility				
soluble in	MeOH, DMSO	MeOH, DMSO	MeOH, DMSO	MeOH, DMSO
slightly soluble in	$CHCl_3$	$CHCl_3$	$CHCl_3$	$CHCl_3$
insoluble in	H_2O, *n*-hexane	H_2O, *n*-hexane	H_2O, *n*-hexane	H_2O, *n*-hexane

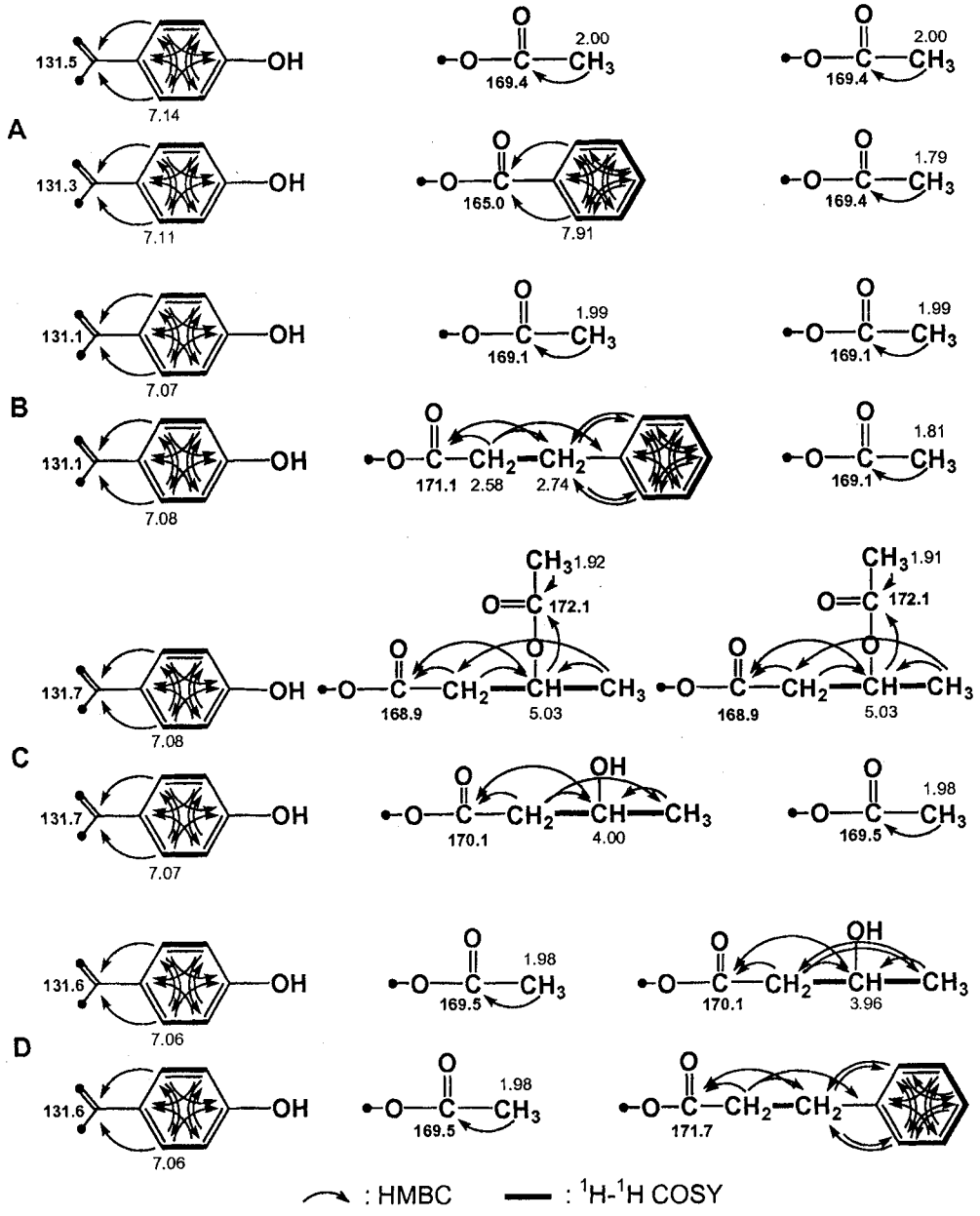
molecular formulae of curtisians A, B, C and D were determined to be $C_{31}H_{24}O_{10}$ (*m/z* 579.1254 $(M+Na)^+$ −1.3 mmu), $C_{33}H_{28}O_{10}$ (*m/z* 584.1669 $(M+H)^+$ −1.3 mmu), $C_{36}H_{38}O_{15}$ (*m/z* 733.2112 $(M+Na)^+$ +0.4 mmu) and $C_{35}H_{32}O_{11}$ (*m/z* 651.1851 $(M+Na)^+$ +0.9 mmu), respectively, by high-resolution FAB mass spectroscopy in combination with ^1H and ^{13}C NMR spectra. Their common IR absorption bands at 3450~3430, near 1775 and near 1740 cm^{-1} suggested the presence of hydroxyl group, carbonyl groups derived from phenyl alkanoate moiety and other carbonyl groups, respectively, in curtisians. The UV absorptions of curtisians near 267 nm suggested the presence of aromatic functions in their structures.

Structure Elucidation

The ^1H NMR spectrum of curtisian A revealed the signals attributable to twenty two protons including thirteen aromatic protons originated from two 1,4-disubstituted benzenes and one phenyl, and three methyl protons of the acetyl groups. The peaks observed in the ^{13}C NMR spectrum of curtisian A were seriously overlapped, suggesting that this compound has symmetrical structure. Direct ^1H-^{13}C connectivity was established by HMQC spectrum[15] and further structural information was obtained by an HMBC experiment[16]. Three methyls at 2.00, 2.00 and 1.79 ppm showed the long-range correlations to three ester carbonyl carbons at 169.4 ppm and two aromatic methine protons (7.91 ppm, H-2,6) of a phenyl group were long-range coupled with an ester carbonyl carbon at 165.0 ppm, revealing the presence of benzoyl group. Also the long-range correlations from H-2,6 of two *p*-hydroxyphenyls to quaternary carbons at 131.5 and 131.3 ppm, respectively, were observed. The six partial structures shown in Fig. 3 were unambiguously assigned by the above HMBC data. By eliminating the partial structures from the molecular formula, four remaining unassignable quaternary carbons at 140.4 (strong intensity, ×3) and 134.1 ppm suggested that the structure of curtisian A should be *ortho*- or *para*-terphenyl. The *p*-terphenyl structure for curtisian A was suggested by the NOE experiment[17], which showed no NOE correlation between the protons of two *p*-hydroxyphenyl groups. It also should be noted that *p*-terphenyl moieties are ubiquitous among mushroom metabolites[18~22]. Therefore, curtisian A was assigned as a *p*-terphenyl compound with the substituents of three acetyls and one benzoyl, as shown in Fig. 1.

Curtisian B was closely related to curtisian A in its physico-chemical properties and NMR spectra, suggesting that it was also a terphenyl with a molecular formula of $C_{33}H_{28}O_{10}$. The ^1H NMR spectrum revealed the presence of two *p*-hydroxyphenyls, one phenyl, two methylenes coupled to each other and three methyls originating from acetyl groups. The comparison of mass and NMR spectra of curtisians A and B indicated that the structure of curtisian B had additional two methylenes in that of curtisian A. HMBC data were analysed in order to clarify the location of two additional methylenes and to assign all the NMR

Fig. 3. Partial structures of curtisians elucidated by ^{1}H-^{1}H COSY and HMBC (^{13}C chemical shifts were bolded).



signals. The long-range couplings from protons of two methylenes at 2.58 and 2.74 ppm to an ester carbonyl carbon at 171.1 ppm and to the carbons of a phenyl group at 140.8 and 128.9 ppm revealed the presence of phenylbutyryl. In result, we found that the difference between curtisians A and B in chemical structure was that the benzoyl group in curtisian A was replaced by a phenylbutyryl group in curtisian B. Also the one remaining quaternary carbon of a strong intensity at 140.1 ppm extracted from the process of elimination established the structure of curtisian B as a *p*-terphenyl compound. It would require two or more remaining quaternary carbons for an *o*- or *m*-terphenyl. Therefore, the structure of curtisian B was determined as a *p*-terphenyl compound as shown in Fig. 1.

The NMR spectra and physico-chemical properties of curtisian C also suggested that this compound was a *p*-terphenyl with a molecular formula of $C_{36}H_{38}O_{15}$. The ^{1}H

NMR spectrum of curtisian C exhibited the presence of two *p*-hydroxyphenyls in the terphenyl moiety, three oxygenated methines, three methylenes, three singlet methyls of the acetyl groups and three doublet methyls (Table 2). The COSY revealed five partial structures including two *p*-hydroxyphenyls and three CH_2–CH(−O)–CH_3, and the HMBC spectrum settled the six partial structures including one acetyl, two 3-acetoxybutyryls and one 3-hydroxybutyryl groups, as shown in Fig. 3. Two acetoxybutyryls were assigned by HMBC correlations from the oxygenated methine proton at 5.03 ppm and acetyl protons at 1.92 ppm to an ester carbonyl carbon at 172.1 ppm and from the oxygenated methine proton at 5.03 ppm and acetyl protons at 1.91 ppm to ester carbonyl carbon at 172.1 ppm. In order to clarify the location of these substituents, we measured the NOESY spectrum but could not obtain any clues for structure assignment from the spectrum. We also tried to make a crystal for structure determination but could not obtain suitable crystals for X-ray analysis. Therefore, the attached sites of four partial structures on *p*-terphenyl moiety remain to be determined, as shown in Fig. 1.

Curtisian D was also suggested to be in the same class of compounds as those of curtisians A and B from its physicochemical properties and NMR spectra. The molecular

Table 2. ^{1}H NMR spectral data of curtisians A, B, C and D in CD_3OD.

Positions	A	B	C	D
p-hydroxyphenyl (1)				
H-2,6	7.14 (d, *J*=8.7 Hz)[a]	7.07 (d, *J*=8.7 Hz)	7.08 (d, *J*=8.7 Hz)	7.06 (d, *J*=8.7 Hz)
H-3,5	6.83 (d, *J*=8.7 Hz)	6.82 (d, *J*=8.7 Hz)	6.83 (d, *J*=8.7 Hz)	6.81 (d, *J*=8.7 Hz)
p-hydroxyphenyl (2)				
H-2,6	7.11 (d, *J*=8.7 Hz)	7.08 (d, *J*=8.7 Hz)	7.07 (d, *J*=8.7 Hz)	7.06 (d, *J*=8.7 Hz)
H-3,5	6.72 (d, *J*=8.7 Hz)	6.83 (d, *J*=8.7 Hz)	6.83 (d, *J*=8.7 Hz)	6.82 (d, *J*=8.7 Hz)
Acetyls				
CH_3 (1)	1.79 (s)	1.81 (s)	1.98 (s)	1.98 (s)
CH_3 (2)	2.00 (s)	1.99 (s)		1.98 (s)
CH_3 (3)	2.00 (s)	1.99 (s)		
Benzoyl				
H-2,6	7.91 (d, *J*=7.5 Hz)			
H-3,5	7.46 (br t, *J*=8.0)			
H-4	7.63 (br t, *J*=7.5)			
Phenylbutyryl				
α-CH_2		2.58 (t, *J*=7.2 Hz)		2.59 (t, *J*=6.6 Hz)
β-CH_2		2.74 (t, *J*=7.2 Hz)		2.69 (t, *J*=6.6 Hz)
H-2,6		7.08[b]		7.06[b]
H-3,5		7.24 (tt, *J*=7.2, 1.5 Hz)		7.23 (tt, *J*=7.2, 1.2 Hz)
H-4		7.16 (br t, *J*=6.9 Hz)		7.15 (tt, *J*=6.9, 1.2 Hz)
3-hydroxybutyryl				
H-2			2.46 (dd, *J*=15.5, 6.9 Hz)	2.35 (dd, *J*=15.5, 6.9 Hz)
			2.31 (dd, *J*=15.5, 5.7 Hz)	2.19 (dd, *J*=15.5, 5.7 Hz)
H-3			4.00 (m)	3.96 (m)
H-4			1.03 (d, *J*=6.0 Hz)	1.01 (d, *J*=6.0 Hz)
3-acetoxybutyryl (1)				
H-2			2.66 (dd, *J*=16.0, 6.9 Hz)	
			2.55 (dd, *J*=16.0, 5.7 Hz)	
H-3			5.03 (m)	
H-4			1.09 (d, *J*=6.3 Hz)	
$COCH_3$			1.92 (s)	
3-acetoxybutyryl (2)				
H-2			2.64 (dd, *J*=16.0, 6.9 Hz)	
			2.52 (dd, *J*=16.0, 5.7 Hz)	
H-3			5.03 (m)	
H-4			1.07 (d, *J*=6.6 Hz)	
$COCH_3$			1.91 (s)	

[a] Proton resonance multiplicity and coupling constant in parentheses.
[b] Overlapped with proton peaks of H-2,6 for hydroxyphenyl groups.

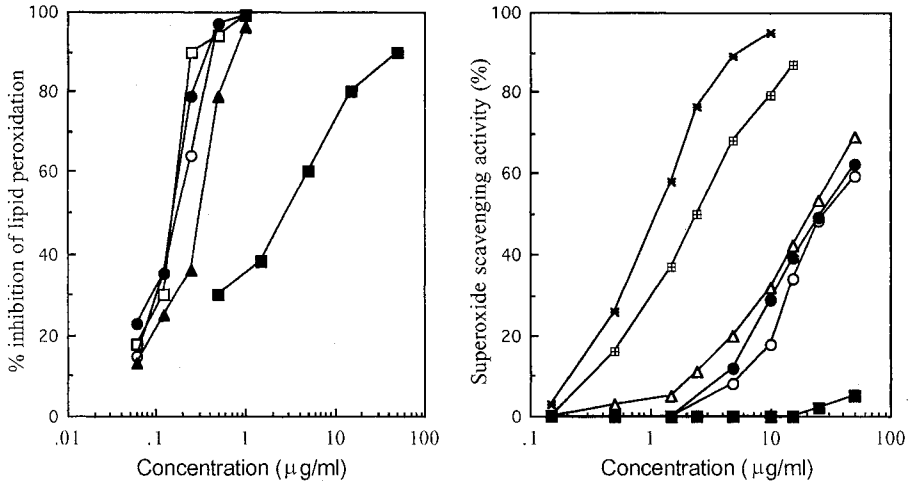
formula of curtisian D was determined as $C_{35}H_{32}O_{11}$ with combination of ^1H NMR, ^{13}C NMR and high resolution FAB-mass spectral data. The ^1H NMR spectrum of curtisian D showed the peaks from thirteen aromatic methines derived from two *p*-hydroxyphenyls of a terphenyl moiety and one phenyl, an oxygenated methine, three methylenes, two siglet methyls of the acetyl groups and one doublet methyl (Table 2). The COSY and HMBC spectrum of curtisian D unambiguously settled the six partial structures including two acetyl, one phenylbutyryl and one 3-hydroxybutyryl groups, as shown in Fig. 3. Four remaining quaternary carbons at 140.6 (strong intensity) and 140.5 ppm suggested that the structure of curtisian D is a *p*-terphenyl compound as curtisians A, B and C. The attached positions of these substituents on the *p*-terphenyl were assigned by the comparison of the ^1H NMR spectra of curtisians A, B, C and D. We found in the case of curtisians A and B that the acetyl protons situated on the *ortho*-

Table 3. ^{13}C NMR spectral data of curtisians A, B, C and D in CD_3OD.

Positions	A	B	C	D
p-hydroxyphenyl (1)				
C-1	123.1	122.9	123.2	123.3
C-2,6	131.7	131.5	132.1	132.0
C-3,5	116.0	115.8	116.2	116.1
C-4	158.5	158.1	159.0	158.4
p-hydroxyphenyl (2)				
C-1	123.0	122.9	123.2	123.3
C-2,6	131.7	131.5	132.1	132.0
C-3,5	116.0	115.9	116.2	116.1
C-4	158.4	158.1	159.0	158.4
Acetyls				
CO, CH_3 (1)	169.4, 20.1	169.1, 20.1	169.5, 20.1	169.5, 20.0
CO, CH_3 (2)	169.4, 20.1	169.1, 20.2		169.5, 20.0
CO, CH_3 (3)	169.4, 20.1	169.1, 20.2		
Benzoyl				
C-1	129.2			
C-2,6	130.8			
C-3,5	129.6			
C-4	135.0			
CO	165.0			
Phenylbutyryl				
α-CH_2		35.7		36.0
β-CH_2		30.9		31.2
C-1		140.8		141.5
C-2,6		128.9		129.2
C-3,5		129.2		129.5
C-4		127.0		127.3
CO		171.1		171.7
3-hydroxybutyryl				
C-1			170.1	170.1
C-2			44.2	44.0
C-3			65.0	65.0
C-4			23.0	23.1
3-acetoxybutyryl (1)				
C-1			168.9	
C-2			40.3	
C-3			68.1	
C-4			19.5	
CO, CH_3			172.1, 21.0	
3-acetoxybutyryl (2)				
C-1			168.9	
C-2			40.3	
C-3			68.1	
C-4			19.5	
CO, CH_3			172.1, 21.0	
Hexasubstituted phenyl				
	131.3	131.1 (x2)	131.7 (x2)	131.6 (x2)
	131.5	140.1 (x4)	140.0~	140.5
	134.1		140.5 (x4)	140.6 (x3)
	140.4 (x3)			

Fig. 4. Lipid peroxidation inhibitory activity in rat liver microsome and superoxide scavenging activity for curtisians.

Curtisians A (□), B (○), C (▲), d (●), vitamin E (■), caffeic acid (*), catechin (⊞), BHA (△).



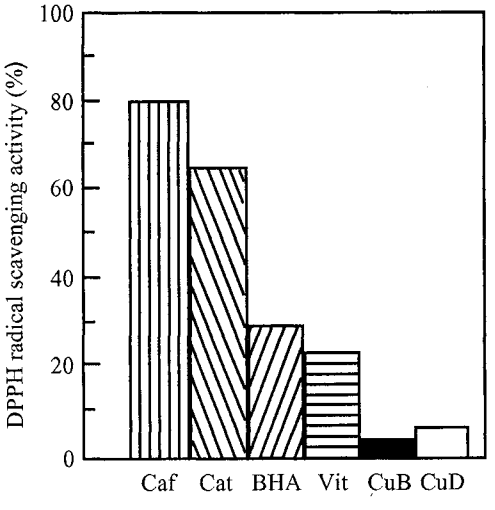
position of benzoyl or phenylbutyryl groups were shifted up-field (0.2 ppm) than those situated on *ortho*-position of acetyl (in curtisians A and B) or other non-aromatic acyl group (in curtisian C), probably due to anisotropic effect. Both of the two acetyl groups in curtisian D were not shifted up-field, appearing at 1.98 ppm. This suggested that the 3-hydroxybutyryl is located on *ortho*-position of phenylbutyryl group and the two acetyls are *ortho*-coupled to each other. Therefore, the structure of curtisian D was established as shown in Fig. 1. The ^{1}H and ^{13}C NMR spectral data of curtisians A, B, C and D are summarized in Tables 2 and 3.

Biological Activity

For the purpose of evaluating the antioxidative activity of curtisians A~D, lipid peroxidation inhibitory activity, superoxide scavenging activity and DPPH radical scavenging activity were investigated. Curtisians A, B, C and D inhibited the lipid peroxidation induced by non-enzymic Fe(II)-ascorbic acid system in rat liver microsomes with IC_{50} values of 0.15, 0.17, 0.24 and 0.14 μg/ml, respectively, in a dose-dependent fashion. These activities were about ten to twenty times higher than that of vitamin E (IC_{50}, 2.5 μg/ml) which was used as a control. Superoxide radical scavenging activities of curtisians B and D, the major components of curtisians, were also investigated and compared with those of well-known free radical scavengers

Fig. 5. DPPH radical scavenging activity of curtisians B and D.

Each sample was added at a final concentration of 6.0 μg/ml. Caf: caffeic acid, Cat: catechin, BHA: butylated hydroxyanisole, Vit: vitamin E, CuB: curtisian B, CuD: curtisian D.



such as caffeic acid, catechin, butylated hydroxyanisole (BHA) and vitamin E. Curtisians B and D scavenged superoxide radicals generated by the xanthine/xanthine oxidase system with IC_{50} values of 36.2 and 28.3 μg/ml,

respectively, which were similar to BHA (IC_{50}, 21.5 μg/ml). Although curtisians B and D were inferior to caffeic acid (1.0 μg/ml) and catechin (2.3 μg/ml), they showed comparable activity with that of vitamin E, as shown in Fig. 4. However, the curtisians did not exhibit DPPH radical scavenging activity (<10%) as shown in Fig. 5. The antioxidative test with DPPH used in this experiment was based on proton radical scavenging action which is one of the various mechanisms of antioxidation. This means that curtisians B and D are not proton-donating antioxidative compounds.

References

1) HAMMOND, B.; H. A. KONTOS & M. L. HESS: Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfusion injury, and in cerebral vascular damage. Can. J. Physiol. Pharmacol. 63: 173~187, 1985
2) HALLIWELL, B. & J. M. C. GUTTERIDGE: Role of free radicals and catalytic metal ions in human disease: An overview. Method in Enzymology 186: 1~85, 1990
3) COYLE, J. T. & P. PUTTFARCKEN: Oxidative stress, glutamate, and neurodegenerative disorders. Science 262: 689~695, 1993
4) SHIN-YA, K.; Y. HAYAKAWA & H. SETO: Structure of benthophoenin, a new free radical scavenger produced by *Streptomyces prunicolor*. J. Nat. Prod. 56: 1255~1258, 1993
5) TANAKA, M.; K. SHIN-YA, K. FURIHATA & H. SETO: Isolation and structural elucidation of antioxidative substances, carbazoquinocins A to F. J. Antibiotics 48: 326~328, 1995
6) SHIN-YA, K.; K. FURIHATA, Y. Kato, Y. HAYAKAWA, J. CLARDY & H. SETO: The structure of benthocyanin A, a new free radical scavenger of microbial origin. Tetrahedron Lett. 32: 943~946, 1991
7) LEE, I.-K.; B.-S. YUN, S.-M. CHO, W.-G. KIM, J.-P. KIM, I.-J. RYOO, H. KOSHINO & I.-D. YOO: Betulinans A and B, two benzoquinone compounds from *Lenzites betulina*. J. Nat. Prod. 59: 1090~1092, 1996
8) KIM, W.-G.; I.-K. LEE, J.-P. KIM, I.-J. RYOO, H. KOSHINO & I.-D. YOO: New indole derivatives with free radical scavenging activity from *Agrocybe cylindracea*. J. Nat. Prod. 60: 721-723, 1997
9) KIM, J.-P.; B.-S. YUN, Y.-K. SHIM & I.-D. YOO: Inoscavin A, a new free radical scavenger from the mushroom *Inonotus xeranticus*. Tetrahedron Lett. 40: 6643~6644, 1999
10) IMAZEKI, R. & T. HONGO: Colored illustrations of mushrooms of Japan. Vol. 2, Hoikusha, Osaka, 1989
11) OHKAWA, H.; N. OHISHI & K. YAGI: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95: 351~358, 1979
12) HOGEBOOM, G. H.: General methods for the isolation of liver cell components: Fraction of cell components of animal tissues. Meth. Enzymol. 1: 16~19, 1965
13) BEAUCHAMP, C. & I. FRIDOVICH: Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44: 276~287, 1971
14) BLOIS, M. S.: Antioxidant determinations by the use of a stable free radical. Nature 181: 1199~1200, 1958
15) HURD, R. E. & B. K. JOHN: Gradient-enhanced proton-detected heteronuclear multiple-quantum coherence spectroscopy. J. Magn. Reson. 91: 648~653, 1991
16) BAX, A. & M. F. SUMMERS: ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. J. Am. Chem. Soc. 108: 2093~2094, 1986
17) JEENER, J.; B. H. MEIER, P. BACHMANN & R. R. ERNST: Investigation of exchange processes by two-dimensional NMR spectroscopy. J. Chem. Phys. 71: 4546~4553, 1979
18) MOSBACH, K.; H. GUILFORD & M. LINDBERG: The terphenyl quinone polyporic acid: production, isolation and characterization. Tetrahedron Lett. No. 17: 1645~1648, 1974
19) DIVEKAR, P. V.; G. READ, L. C. VINING & R. H. HASKINS: Volucrisporin; isolation, structure, and synthesis of the methyl ether. Can. J. Chem. 37: 1970~1976, 1959
20) MCMORRIS, T. C. & M. ANCHEL: Phlebiarubrone, a basidiomycete pigment related to polyporic acid. Tetrahedron Lett. No. 5: 335~337, 1963
21) QUACK, W.; H. SCHOLL & H. BUDZIKIEWICZ: Ascocorynin, a terphenylquinone from Ascocoryne sarcoides. Phytochemistry 21: 2921~2923, 1982
22) GRIPENBERG, J.: Fungus pigments IV. aurantiacin, the pigment of *Hydnum aurantiacum* Batsch. Acta. Chem. Scand. 10: 1111~1115, 1956