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Curtisians E–H: four *p*-terphenyl derivatives from the inedible mushroom *Paxillus curtisii*

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Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

Four *p*-terphenyl derivatives named curtisians E–H (**1–4**) were isolated from the methanolic extract of fruit bodies of the Basidiomycete *Paxillus curtisii* by a combination of Sephadex LH-20, silica gel column chromatography and preparative reversed phase HPLC. The relative stereochemistries of the curtisians were elucidated by 2D NMR, MS, IR and UV spectroscopy with the absolute configurations at $C_{3a–3d}$ of the side chains being established as *S* by GC–MS on a chiral column previously used for separation of authentic standards.

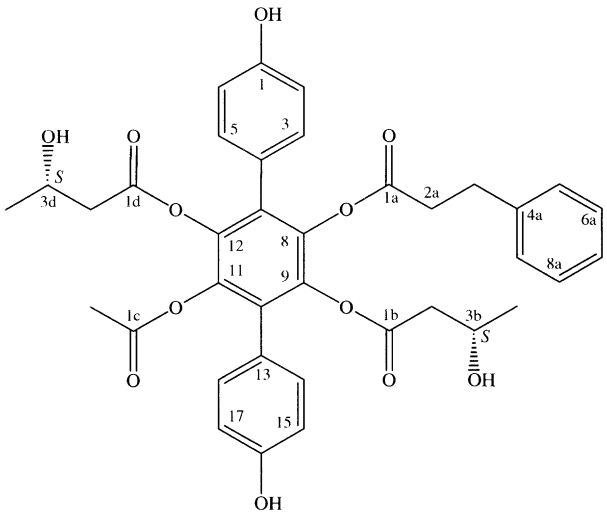
Keywords: *Paxillus curtisii*; Paxillaceace; Curtisian; Fungus; Basidiomycete; *p*-Terphenyl

1. Introduction

The fungi of genus *Paxillus* belonging to Paxillaceace grow widely in East Asia and North America on decayed pine trees (Imazeki et al., 1988). Previously, leucometin-2,-4,-5 and-6 were reported from *Paxillus panuoides* (Yun et al., 2000a,b), and the same authors later isolated curtisians A–D from *Paxillus curtisii* (Yun et al., 2000c); all of these compounds were reported as new free radical scavengers (Yun et al., 2000a–c). As part of our systematic investigation of biologically active substances from inedible mushrooms (Hashimoto et al., 1998; Quang et al., 2003), we studied the chemical constituents of *Paxillus atrotomentosus* and isolated (+)-osmudalactone, three γ-lactone and eight spiromentins (Buchanan et al., 1995) and a novel dimeric lactone *bis*-osmundalactone (Hashimoto et al., 2002). We now report the isolation and structural elucidation of four *p*-terphenyl compounds named curtisians E–H (**1–4**) from the methanolic extract of the fruit bodies of *Paxillus curtisii* collected in Kyoto, Japan.

2. Results and discussion

The methanolic extract of fruit bodies of *Paxillus curtisii* was subjected repeatedly to Sephadex LH-20 and silica gel column chromatography, followed by prep. HPLC with a reversed phase C-18 column as described in the Experimental section to give four new *p*-terphenyl derivatives (**1–4**) named curtisians E–H.



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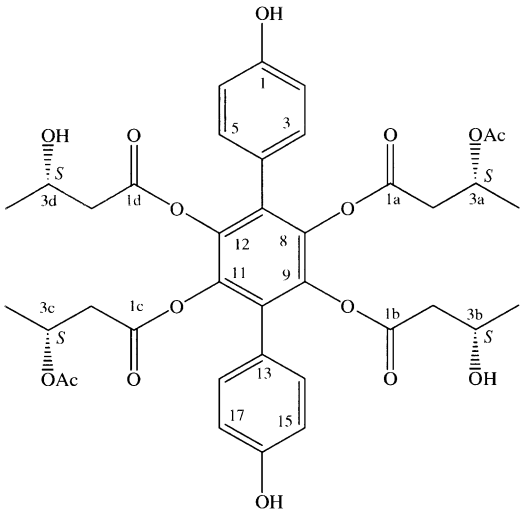

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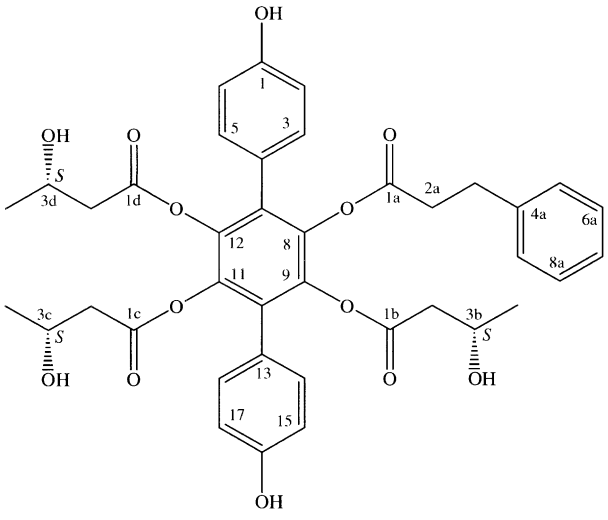

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Curtisian E (**1**) was obtained as a grayish solid, the molecular formula of which was found to be $C_{37}H_{36}O_{12}$ by HR-FABMS. The IR spectrum indicated absorption bands of hydroxyl (3362 cm^{-1}), ester (1770 cm^{-1}) and benzene (1612, 1524 cm^{-1}) groups. The ^1H NMR spectral data of **1** (Table 1) revealed signals of four aromatic protons (δ 6.80, 6.82, 7.06 and 7.08), one phenyl ring (δ 7.06, 7.15 and 7.22) and two methine protons (δ 3.96 and 4.01). The ^{13}C NMR spectral data of **1** (Table 2) showed resonances for 37 carbons including four ester carbonyls (δ 169.7, 170.1, 170,1 and 171.7) and two methyls (δ 23.1), four methylenes (δ 31.2, 36.0, 44.0 and 44.0), and one acetyl (δ 20.2 and 169.7). The NMR spectrum of **1** suggested that it was a *p*-terphenyl derivative (Hu et al., 2001; Quang et al., 2003). The COSY spectrum showed ^1H–^1H correlations between (1) H-2a and H-3a, (2) H-3b and H-2b, H-4b, (3) H-3c and H-2c, H-4c while long range correlations between (1) H-2a, H-3a/C-la, (2) H-3a/C-4a, C-5a, C-9a, (3) H-2b, H-3b/C-lb, (4) H-2c/C-1c, as well as (5) H-2d, H-3d/C-1d were observed in the HMBC spectrum, indicating the presence of one acetyl, two 3-hydroxybutyryl groups and one phenylbutyryl group in **1**. The location of the substituents on the central aromatic ring was determined by a ROESY experiment (Fig. 1), which showed the correlations between (1) H-3a and H-2, H-2b, H-4b; (2) H-15 and H-2b, H-4b; (3) H-2c and H-17,H-l8; (4) H-2d, H-4d and H-6. These correlations indicated that two 3-hydroxybutyryl groups were attached to the central aromatic ring at C-9 and C-12, while the phenylbutyryl and acetyl groups were linked at C-8 and C-l1, respectively. The NMR spectral data of **1** was very similar to those of curtisian D (Yun et al., 2000c), except for the presence of a 3-hydroxybutyryl group in the place of an acetyl group at C-12. Therefore, the structure of curtisian E (**1**) was determined as shown in Scheme 1.

Curtisian F (**2**) was obtained as a grayish solid, whose HR-FABMS showed a molecular ion peak at *m*/*z* 777.2371 ($[M+Na]^+$), suggesting the molecular formula of $C_{38}H_{42}O_{16}$. The IR spectrum of **2** showed absorptions at 3229, 1772 and 1612 cm^{-1} assignable to hydroxyl and ester groups, and an aromatic ring, respectively. The UV spectrum of **2** showed absorption maxima at 212 and 268 nm assignable to an aromatic ring. The ^1H NMR spectral of **2** (Table 1) showed the presence of four aromatic protons, four methyls and two acetyls. The signals observed in the ^{13}C NMR spectrum of **2** (Table 2) showed exact overlapping along the terphenyl bond axis, suggesting that this compound has a symmetrical structure (Yun et al., 2000c). The spectral data of **2** were similar to those of curtisian C (Yun et al., 2000c) except for the presence of a 3-hydroxybutyryl moiety in place of the acetyl group attached to C-12 of the central aromatic ring. Its ^1H–^1H COSY, NOESY and HMBC spectra indicated the presence of two 3-hydroxybutyryl substituents located at C-9 and C-12, and two 3-acetoxybutyryl units attached to C-8 and C-11. Thus, the structure of **2** was determined as shown and named curtisian F (Scheme 1).

Curtisian G (**3**) was obtained as a grayish solid, whose molecular formula was determined to be $C_{39}H_{40}O_{13}$ by HRFAB-MS. The IR and UV spectra indicated absorption bands of hydroxyl (3292 cm^{-1}), ester (1769 cm^{-1}) and benzene (1612, 1524 cm^{-1}) groups and the absorption maxima at 215 and 267 nm, respectively. The ^1H NMR spectrum of **3** (Table 1) revealed the presence of two *para*-hydroxy phenyl rings (δ 6.80, 6.82, 7.07 and 7.07), one phenyl (δ 7.06, 7.15 and 7.22) and three methyl groups (δ 1.02). The ^{13}C NMR of curtisian G (**3**) (Table 2) exhibited 39 carbon signals including two phenolic carbons (δ 159.0), four esters (δ 171.7, 170.2, 170.2 and 170.2) and three methine carbons (δ 65.1). The HMBC spectrum of **3** indicated the presence of one phenylbutyryl and three 3-hydroxylbutyryl groups. Its NMR spectral data were very similar to

Table 1
^{1}H NMR spectral data for **1–4**(600 MHz, CD_3OD)

H	1	2	3	4
2, 6	6.80 (*d*, 8.8)	6.83 (*d*, 8.8)	6.80 (*d*, 8.8)	6.80 (*d*, 8.8)
3, 5	7.06 (*d*, 8.8)	7.08 (*d*, 8.8)	7.07 (*d*, 8.8)	7.06 (*d*, 8.8)
14, 18	7.08 (*d*, 8.8)	7.08 (*d*, 8.8)	7.07 (*d*, 8.8)	7.08 (*d*, 8.8)
15, 17	6.82 (*d*, 8.8)	6.83 (*d*, 8.8)	6.82 (*d*, 8.8)	6.82 (*d*, 8.8)
2a	2.59 (*t*, 7.4)	2.67 (*dd*, 7.4, 16.8)	2.58 (*t*, 7.7)	2.59 (*t*, 7.4)
		2.55 (*dd*, 5.5, 16.8)		
3a	2.68 (*t*, 7.4)	5.01 (*m*)	2.68 (*t*, 7.7)	2.68 (*t*, 7.4)
4a		1.07 (*d*, 6.6)		
5a, 9a	7.06 (overlap)		7.06 (*t*, 7.4)	7.06 (overlap)
6a, 8a	7.22 (*t*, 7.4)		7.22 (*t*, 7.4)	7.22 (*t*, 7.7)
7a	7.15 (*t*, 7 4)		7.15 (*t*, 7.4)	7.15 (*t*, 7.4)
2b	2.30 (*dd*, 6.0, 15.4)	2.45 (*dd*, 7.1,15.7)	2.34 (*dd*, 7.1, 15.4)	2.34 (*dd*, 7.1, 15.4)
	2.20 (*dd*, 6.0, 15.4)	2.31 (*dd*, 5.8, 15.7)	2.19 (*dd*, 5.8, 15.4)	2.19 (*dd*, 5.8, 15.4)
3b	3.96 (*m*)	4.00 (*m*)	3.97 (*m*)	3.95 (*m*)
4b	1.01 (*d*, 6.3)	1.03 (*d*, 6.6)	1.02 (*d*, 6.3)	1.03 (*d*, 6.3)
2c	1.97 (*s*)	2.67 (*dd*, 7.4, 16.8)	2.31 (*dd*, 5.8, 15.7)	2.46 (*dd*, 7.1, 15.4)
		2.55 (*dd*, 5.5, 16.8)	2.47 (*dd*, 7.1, 15.7)	2.31 (*dd*, 5.8, 15.4)
3c		5.01 (*m*)	3.97 (*m*)	3.99 (*m*)
4c		1.07 (*d*, 6.6)	1.02 (*d*, 6.3)	1.01 (*d*, 6.3)
2d	2.44 (*dd*, 7.1, 15.4)	2.45 (*dd*, 7.1, 15.7)	2.47 (*dd*, 7.1, 15.7)	2.67 (*dd*, 7.1, 15.4)
	2.34 (*dd*, 7 1,15.4)	2.31 (*dd*, 5.8, 15.7)	2.31 (*dd*, 5 8, 15.7)	2.58 (*m*)
3d	4.01 (*m*)	4.00 (*m*)	3.97 (*m*)	5.02 (*m*)
4d	1.05 (*d*, 6.1)	1.03 (*d*, 6.6)	1.02 (*d*, 6.3)	1.07 (*d*, 6,3)
3a-Ac		1.92 (*s*)		
3c-Ac		1.92 (*s*)		
3d-Ac				1.92 (*s*)

those of curtisian E (**1**) except for the presence of one 3-hydroxylbutyryl moiety in the place of an acetyl group at C-l1. Thus, the structure of curtisian G (**3**) was elucidated as shown in Scheme 1.

Curtisian H (**4**) was obtained as a grayish solid whose molecular formula was $C_{41}H_{42}O_{14}$ by HR-FABMS. The IR spectrum showed absorption bands for hydroxyl (3258 cm^{-1}), ester (1771 cm^{-1}) and benzene (1612, 1525 cm^{-1}) groups. The ^{1}H NMR spectrum of **4** (Table 1) showed four aromatic proton signals belonging to two *p*-hydroxy phenyl groups, one unsubstituted phenyl, three methyls and one acetyl moiety. The ^{13}C NMR spectrum (Table 2) exhibited 41 carbon signals including four ketone, two phenolic, and five methylene carbon signals. Its ^{1}H–^{1}H COSY, NOESY and HMBC spectra also revealed that it contained one phenylbutyryl, two 3-hydroxybutyryl and one 3-acetylbutyryl groups. The spectral data of **4** resembled those of curtisian G (**3**) indicating that it was a *p*-terphenyl derivative except for the presence of one 3-acetoxybutyryl in the place of a 3-hydroxybutyryl. The substitution pattern of the central aromatic ring followed from ^{1}H–^{1}H interactions observed in the ROESY spectrum (Fig. 2), in which the ROESY correlations between (1) H-2 and H-2a, H-3a; (2) H-4b and H-15; (3) H-2c and H-17; (4) H-2d and H-6 were observed, indicating that two 2-hydroxybutyryl groups were determined to be at C-9 and C-11. In addition the phenylbutyryl and the 3-acetoxybutyryl groups were found at C-8 and C-12, respectively at the central aromatic ring. Therefore, the structure of **4** was determined as shown in Scheme 1 and named curtisian H.

To determine the absolute configuration of curtisians E–H (**1–4**), a mixture of curtisians E-H was saponified with potassium hydroxide in methanol, followed by methylation and acetylation to afford 3-acetoxy-n-butyric acid methyl ester (**5**). Compound **5** was analyzed by GC–MS on a chiral column with authentic samples (each 3R- and 3S-acetoxy-*n*-butyric acid methyl ester) derived from *3R*- and 3*S*-hydroxy-*n*-butyric acid to give the chromatograms shown in Fig. 3. Consequently, the absolute configuration at $C_{3a–3d}$ of the side chain of curtisians E–H (**1–4**) were established to be *S*.

3. Experimental

3.1. General

NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR), using CD_3OD as solvent. Chemical shifts are given with TMS used as internal standard (^{1}H NMR), and δ 49.00 (ppm) from CD_3OD as a standard. Mass spectra including high-resolution FAB mass spectra were recorded on a Jeol JMS AX-500 spectrometer. IR spectra were measured on JASCO FT/IR-5300 spectrophotometer. The UV spectra were obtained on a Shimadzu UV-16SOPC in MeOH solution. The specific

Table 2
^{13}C NMR spectral data for **1–4** (150 MHz, CD_3OD)

C	1	2	3	4
1, 16	159.0	159.1	159.0	159.0
2.6	116.2	116.3	116.2	116.2
3.5	132.1	132.1	132.1	132.1
4.13	123.3	123.2	123.3	123.3
7.10	131.6	131.8	131.7	131.7
8,9,11,12	140.5	140.5	140.6	140.5
14, 18	132.1	132.1	132.1	132.1
15,17	116.2	116.3	116.2	116.2
1a	171.1	169.1	171.7	171.7
2a	36.0	40.4	36.0	36.0
3a	31.2	68.1	31.2	31.2
4a	141.5	19.6	141.5	141.5
5a, 9a	129.2		129.2	129.2
6a,8a	129.5		129.5	129.5
7a	127.3		127.3	127.3
1b	170.1	170.1	170.2	170.1
2b	44.0	44.1	44.0	44.1
3b	65.1	65.5	65.1	65.1
4b	23.1	23.1	23.1	23.1
1c	169.7	169.1	170.2	170.1
2c	20.2	40.4	44.0	44.1
3c		68.1	65.1	65.1
4c		19.6	23.1	23.1
1d	170.1	170.1	170.2	169.1
2d	44.0	44.1	44.0	40.4
3d	65.1	65.5	65.1	68.1
4d	23.1	23.1	23.1	19.6
3a-Ac		21.1, 172.1		
3c-Ac		21.1, 172.1		
3d-Ac				21.1, 172.0

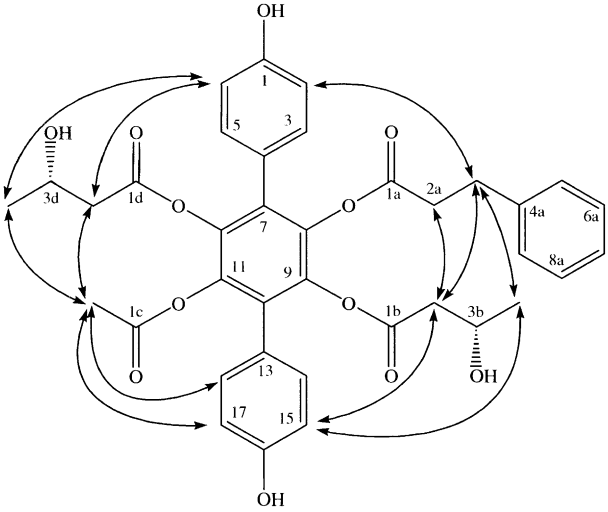
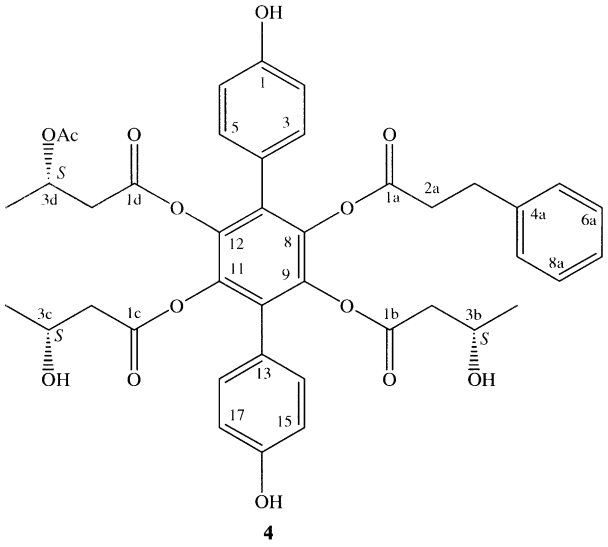


Fig. 1. Important ROESY correlations of compound **1**.

optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH as solvent. HPLC was performed on Shimadzu Liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II column. The spots on TLC were detected under UV 254 nm and by spraying with 10% H_2S0_4 or Godin reagent (Godin, 1954), followed by heating at



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Scheme 1.

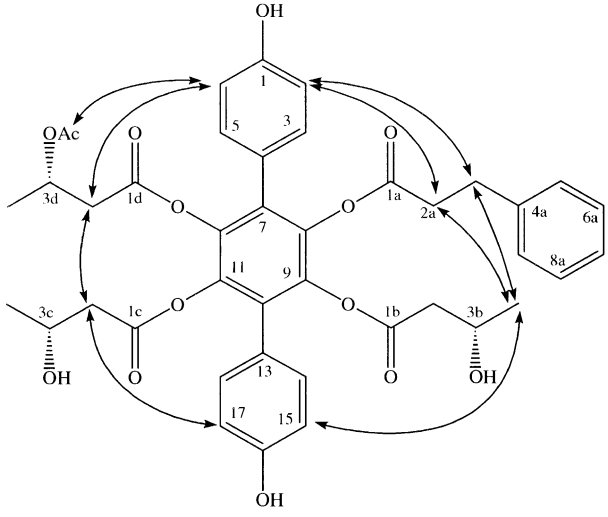

Fig. 2. Important ROESY correlations of compound **4**.

120 °C. GC–MS was carried out by a Hewlett Packard mass selective detector 5971 A and a gas chromatograph 5890 Series II with chiral column β-DEX 120 (30 m×0.25 mm, film thickness 0.25 μm). The temperature programming of GC–MS analysis performed from 50/bk7C isothermal for 3 min, then 50–230 °C at 3 °C min^{-1}, and finally isothermal at 230 °C for 20 min. Injection temperature was 250 °C.

3.2. Materials

Fruit bodies *of Paxillus curtisii* were collected in November 1995 in Kyoto city, Japan and then identified by Mrs. Makiko Nukada at Kurashiki Sakuyo University, Japan. The voucher specimen (KSU95111) has been deposited in Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki 710-0290, Japan.

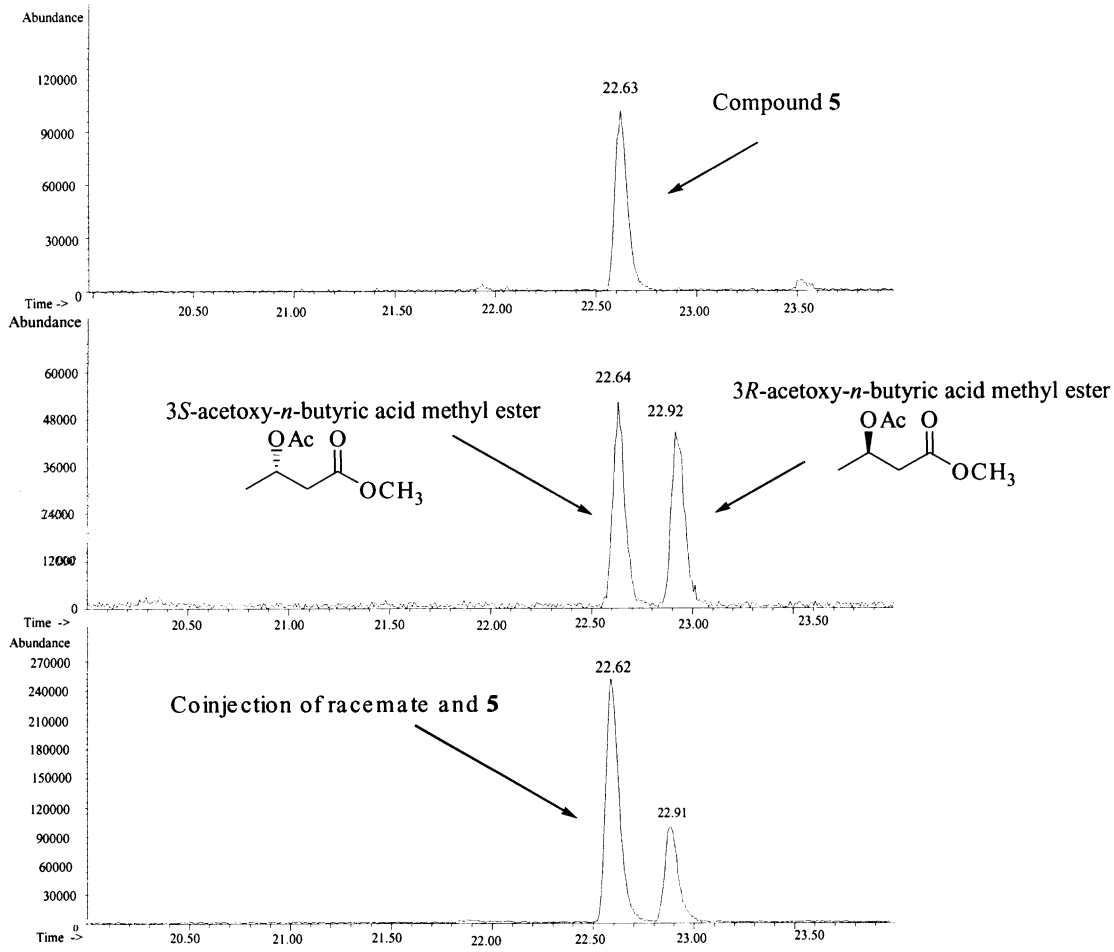

Fig. 3. Determination of the absolute configuration of 3-acetoxy-*n*-butyric acid methyl ester by GC–MS.

3.3. Extraction and isolation

Dried fruit bodies (5 g) of *P. curtisii* was extracted with MeOH at room temperature, the methanolic extract concentrated in vacuo to give a residue (1.83 g), which was fractionated by Sephadex LH-20 CC using MeOH:$CHCl_3$ (1:1) as eluent to give five fractions (Fractions 1–5). Fraction 1 (447.8 mg) was purified by silica gel CC with solvent system $CHCl_3$:MeOH:H_2O (50:5:1) to give six sub-fractions. Fraction 1-4 (38.3 mg), fraction 1-5 (27.2) and fraction 1-6 (6.3 mg) were purified by prep. HPLC with a reversed phase C-18 column, solvent system CH_3CN–H_2O (1:1) to give curtisian H (**4**) (6.1 mg) from fraction 1-4; curtisian F (**2**) (4.2 mg) and curtisian E (**1**) (11.1 mg) from fraction 1-5; curtisian G (**3**) (3.8 mg) from fraction 1-6.

3.3.1. Curtisian E (*1*)

Grayish solid, $[\alpha]_D^{20}$ −5.4° (c 1.1, CH_3OH). HR-FABMS: *m/z* 695.2129 $[M+Na]^+$, $C_{37}H_{36}O_{12}Na$, requires 695.2104. UV λ_{max} (CH_3OH) nm (log ε): 213 (4.40), 268 (4.18). IR (KBr) cm^{-1}': 3362, 2974, 1770, 1666, 1612, 1525, 1375, 1274, 1016, 988, 837. For ^1H and ^{13}C NMR (CD_3OD), see Tables 1 and 2.

3.3.2. Curtisian F (*2*)

Grayish solid, $[\alpha]_D^{20}$ −6.4° (c 0.8, CH_3OH). HR-FABMS: *m/z* 777.2355 $[M+Na]^+$, $C_{38}H_{42}O_{16}Na$, requires 777.2371. UV λ_{max} (CH_3OH) nm (log ε): 212 (4.45), 268 (4.23). IR (KBr) cm^{-1}: 3229, 2977, 1772, 1662, 1612, 1592, 1524, 1447, 1376, 1267, 990, 837. For ^1H and ^{13}C NMR (CD_3OD), see Tables 1 and 2.

3.3.3. Curtisian G (*3*)

Grayish solid, $[\alpha]_D^{20}$ +3.1° (c 0.5, CH_3OH). HR-FABMS: *m/z* 739.2378 $[M+Na]^+$,$C_{39}H_{40}O_{13}Na$, requires 739.2367. UV λ_{max} (CH_3OH) nm (log ε): 215 (4.4), 267 (4.4). IR (KBr) cm^{-1}: 3292, 1769, 1612, 1525, 1453, 1227, 1122, 980, 836. For ^1H and ^{13}C NMR(CD_3OD), see Tables 1 and 2.

3.3.4. Curtisian H(4)

Grayish solid, $[\alpha]_D^{20}$ −3.9° (c 1.0, CH_3OH). HR-FABMS: *m/z* 781.2483 $[M+Na]^+$, $C_{41}H_{42}O_{14}Na$, requires 781.2472. UV λ_{max} (CH_3OH) nm (log ε): 217 (4.42), 267 (4.29). IR (KBr) cm^{-1}: 3258, 2976, 1771, 1667, 1612, 1592, 1525, 1446, 1375, 1269, 1021, 837. For ^1H and ^{13}C NMR (CD_3OD), see Tables 1 and 2.

3.3.5. Preparation of 3-acetyl-n-butyric acid methyl ester (5)

To a mixture (4 mg) of curtisians E–H [each curtisian (1 mg)] in MeOH (1 ml) was added KOH (6 mg) and the mixture was stirred at room temperature for 3 h. The reaction mixture was neutralized by 1 N HCl until pH = 7 and then subjected repeatedly on column packing proton exchange resin (Amberlite IR-120B/H^+) using distilled water as eluent. The eluent was evaporated and methylated with $(CH_3)_3SiCHN_2$ (1 ml) in MeOH (1 ml) at 5 °C for 3 h, then acetylated with acetic anhydride (1 ml) in pyridine (1 ml). Work-up gave 3-acetyl-butyric acid methyl ester (**5**; 1.5 mg) which was subjected to chiral GCMS analysis (see Section 3.1).

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