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Terrestrins A–G: *p*-Terphenyl derivatives from the inedible mushroom *Thelephora terrestris*

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Abstract

Seven *p*-terphenyl derivatives named terrestrins A–G together with three known ganbajunin B, thelephantins F and H, were isolated from the methanol extract of fruiting bodies of the Japanese inedible mushroom *Thelephora terrestris* (Thelephoraceae). Their structures were elucidated by means of high-resolution MS, 2D NMR, IR and UV spectroscopy, and X-ray crystallographic analysis.

Keywords: *Thelephora terrestris*; Thelephoraceae; Basidimycetes; Fungi, *p*-Terphenyls; Terrestrins

1. Introduction

Previous phytochemical investigations conducted on the genus *Thelephora* (Thelephoraceae) revealed that this genus is an abundant source of *p*-terphenyl derivatives. Ganbajunins A–G have been isolated from *Thelephora ganbajun* (Hu et al., 2001a; Hu and Liu, 2001b), thelephorin A from *Thelephora vialis* (Tsukamoto et al., 2002), and thelephantins A–H (Quang et al., 2003a,b) from *Thelephora aurantiotincta*. It has been shown that *p*-terphenyl compounds from mushrooms possess a range of diverse biological activities the most strongly pronounced being that of radical scavengers, and Takahashi et al. (1992) reported that three *p*-terphenyls from *Boletopsis leucomelas* showed inhibitory effects on 5-lipoxygenase. Yun et al. (2000a,b) isolated the new radical scavengers curtisians A–D from *Paxillus curtisii* and leucometin-5 and -6 from *Paxillus panuoides*, whereas Lee et al. (2003) found that curtisians A–D protect cultured neuronal cells against glutamate neurotoxicity via iron chelation. We also reported on the antioxidant activity of curisians I–L from *Paxillus curtisii* (Quang et al., 2003c). In our search for naturally occurring, biologically active compounds from the fungus family Thelephoraceae, an investigation was undertaken on the chemical constituents of the MeOH extract of *Thelephora terrestris*. In this paper, the isolation and structure elucidation of seven new *p*-terphenyl derivatives, named terrestrins A–G (**1**–**4**, **6**–**8**), together with three known related compounds ganbajunin B (**5**), thelephantin F (**9**) and thelephantin H (**10**) are described.

2. Results and discussion

The MeOH extract of the fruiting bodies of the Japanese inedible mushroom *Thelephora terrestris* was

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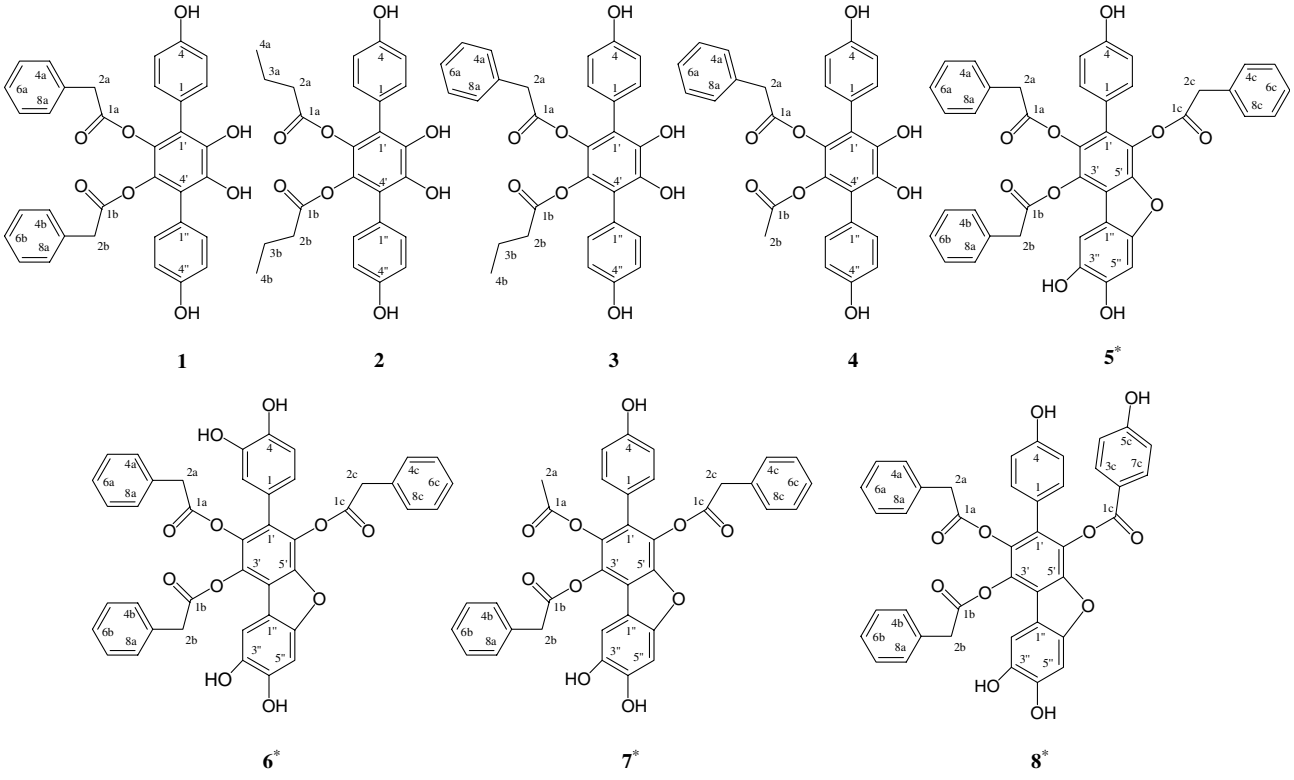

Structures of compounds **1**-**8**

*For convenience, compounds **5**-**8** are numbered like **1**-**4**, for the discussion of NMR data; see names for systematic numbering.

subjected to silica-gel and reverse phase (C-18) column chromatography, followed by MPLC on DIOL and preparative reverse phase HPLC to afford 10 compounds (**1**–**10**) as described in Section 3. Methyl *p*-hydroxybenzoate was also present in the MeOH extract but it seems to be an artefact of the isolation procedure.

Terrestrin A (**1**) was obtained as a violet material from the polar fractions of the methanol extract. Its molecular formula was deduced to be $C_{34}H_{26}O_8$ by HRFABMS ($[M + H]^+$ *m*/*z* 563.1733). The IR spectrum of **1** showed absorptions at 3384, 1766, 1612 cm^{-1} assignable to hydroxyl, ester carbonyl and aromatic double bond functionalities, respectively. The UV spectrum indicated the presence of aromatic ring(s) (λ_{max} 210 and 261 nm). The ^1H NMR spectrum (Table 1) of **1** in CD_3OD revealed an *AA′BB′* system arising from the protons of a 1,4-disubstituted benzene ring at δ 6.77, 7.09 (J = 8.8 Hz), a monosubstituted phenyl group (δ 6.96, J = 8.2 Hz, δ 7.20–7.23, two signals overlapping), and one singlet at δ 3.25. The ^{13}C NMR spectrum (Table 2) of **1** showed resonances for 13 carbons including one ester carbonyl (δ 171.2) and one phenolic carbon (δ 158.1), indicating that terrestrin A has a C_2-symmetrical structure with various aromatic ring symmetries within the structure. The presence of two phenylacetoxy groups was suggested by its FABMS (loss of two PhCH═CO moieties from $[M]^+$ *m*/*z* 444, 326). The ^1H and ^{13}C NMR spectroscopic data of **1** was very similar to those of ganbajunin E (Hu et al., 2001a) leading to a conclusion that terrestrin A is a *p*-terphenyl derivative. In addition, the ^1H–^1H COSY, DEPT, HMQC, HMBC and NOESY spectra of **1** corroborated the partial structures: phenylacetoxy and a 1,1′;4′,1″-terphenyl core. Further, the bathochromic shift (4 nm) of the absorption maximum at 261 nm in its UV spectrum observed when one and then 10 drops of H_3BO_3 solution (1% in methanol) were added, led us to believe that compound **1** is a 1,2-diol at the central ring. Definite proof of the structure of **1** was subsequently obtained from X-ray crystallographic analysis of suitable colorless crystals obtained from a water–methanol solution. The crystals of **1** are triclinic belonging to the space group *P*1. As shown in the ORTEP drawing in Fig. 1, compound **1** possesses a symmetrically substituted *p*-terphenyl skeleton and the location of two phenylacetoxy groups is at the central ring *ortho* one to each other. Consequently, terrestrin A (**1**) was determined to be 4,5′,6′,4″-tetrahydroxy-2′,3′-diphenylacetoxy-[1,1′;4′,1″-terphenyl]. As far as we are aware, this is the first example of a X-ray crystallographic analysis of a natural *p*-terphenyl possessing four substitution groups at the central aromatic ring, although the crystals of synthetic

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**8** (600 MHz, CD_3OD)

H	1	2	3	4	5	6	7	8
2	7.09 *d* (8.8)	7.15 *d* (8.8)	7.14 *d* (8.5)	7.13 *d* (8.5)	6.89 *d* (8.8)	6.58 *d* (1.9)	6.94 *d* (8.7)	7.00 *d* (8.8)
3	6.77 *d* (8.8)	6.82 *d* (8.8)	6.80 *d* (8.5)	6.81 *d* (8.5)	6.66 *d* (8.8)		6.72 *d* (8.7)	6.73 *d* (8.8)
5	6.77 *d* (8.8)	6.82 *d* (8.8)	6.80 *d* (8.5)	6.81 *d* (8.5)	6.66 *d* (8.8)	6.66 *d* (8.0)	6.72 *d* (8.7)	6.73 *d* (8.8)
6	7.09 *d* (8.8)	7.15 *d* (8.8)	7.14 *d* (8.5)	7.13 *d* (8.5)	6.89 *d* (8.8)	6.41 *dd* (8.0, 1.9)	6.94 *d* (8.7)	7.00 *d* (8.8)
2″	7.09 *d* (8.8)	7.15 *d* (8.8)	7.12 *d* (8.8)	7.14 *d* (8.8)	7.08 *brs*	7.06 *brs*	7.14 *brs*	6.99 *brs*
3″	6.77 *d* (8.8)	6.82 *d* (8.8)	6.81 *d* (8.8)	6.80 *d* (8.8)				
5″	6.77 *d* (8.8)	6.82 *d* (8.8)	6.81 *d* (8.8)	6.80 *d* (8.8)	6.99 *brs*	6.98 *brs*	7.00 *brs*	6.99 *brs*
6″	7.09 *d* (8.8)	7.15 *d* (8.8)	7.12 *d* (8.8)	7.14 *d* (8.8)				
2a	3.25 *s*	2.17 *t* (7.1)	3.49 *s*	3.51 *s*	3.18 *s*	3.23 *s*	1.57 *s*	3.43 *s*
4a	6.96 *brd* (8.2)	1.44 *tq* (7.1, 7.4)	7.06 *dd* (8.2, 1.6)	7.09 *dd* (8.2, 1.6)	6.87 *dd* (7.8, 2.2)	7.07 *dd* (8.0, 1.6)		6.87 *brd* (8.5)
5a	7.20–7.23	0.76 *t* (7.4)	7.22–7.28	7.22–7.27	7.18–7.24	7.18–7.28		7.02–7.08
6a	7.20–7.23		7.22–7.28	7.22–7.27	7.18–7.24	7.18–7.28		7.02–7.08
7a	7.20–7.23		7.22–7.28	7.22–7.27	7.18–7.24	7.18–7.28		7.02–7.08
8a	6.96 *brd* (8.2)		7.06 *dd* (8.2, 1.6)	7.09 *dd* (8.2, 1.6)	6.87 *dd* (7.8, 2.2)	7.07 *dd* (8.0, 1.6)		6.87 *brd* (8.5)
2b	3.25 *s*	2.17 *t* (7.1)	1.87 *t* (7.1)	1.62 *s*	3.71 *s*	3.75 *s*	3.95 *s*	3.77 *s*
4b	6.96 *brd* (8.2)	1.44 *tq* (7.1, 7.4)	1.30 *tq* (7.1, 7.4)		7.04 *dd* (7.8, 2.0)	6.91 *dd* (8.0, 1.6)	7.07 *dd* (7.7, 2.2)	7.09 *brd* (7.7)
5b	7.20–7.23	0.76 *t* (7.4)	0.70 *t* (7.4)		7.18–7.24	7.18–7.28	7.23–7.29	7.24–7.28
6b	7.20–7.23				7.18–7.24	7.18–7.28	7.23–7.29	7.24–7.28
7b	7.20–7.23				7.18–7.24	7.18–7.28	7.23–7.29	7.24–7.28
8b	6.96 *brd* (8.2)				7.04 *dd* (7.8, 2.0)	6.91 *dd* (8.0, 1.6)	7.07 *dd* (7.7, 2.2)	7.09 *brd* (7.7)
2c					3.89 *s*	3.81 *s*	4.08 *s*	
3c								8.05 *d*(8.8)
4c					7.41 *brd* (7.1)	7.44 *brd* (7.1)	7.47 *brd* (7.4)	6.96 *d* (8.8)
5c					7.38 *brt* (7.1)	7.41 *brt* (7.1)	7.39 *brt* (7.4)	
6c					7.31 *brt* (7.1)	7.34 *brt* (7.1)	7.32 *brt* (7.4)	6.96 *d* (8.8)
7c					7.38 *brt* (7.1)	7.41 *brt* (7.1)	7.39 *brt* (7.4)	8.05 *d* (8.8)
8c					7.41 *brd* (7.1)	7.44 *brd* (7.1)	7.47 *brd* (7.4)	

4,4″-bis(benzyloxy)-2′,5′-bis(dodecyloxy)-*p*-terphenyl and the related *p*-terphenyls have been reported (Kakali et al., 1998).

Terrestrin B (**2**), a reddish violet solid had the molecular formula of $C_{26}H_{26}O_8$ by HRFABMS. However, only 11 signals were present in its ^{13}C NMR spectrum (Table 2) that were assigned (in combination with DEPT) to an ester carbonyl (δ 173.1), two aromatic methines (δ 116.0, 132.7), five aromatic quaternary carbons including three oxygen-bearing carbons (δ 123.9, 125.0, 134.8, 142.5, 158.2), a methyl (δ 13.8), and two types of methylenes (δ 19.2, 36.4). The ^{1}H and ^{13}C NMR resonances of **2** greatly resembled those of terrestrin A (**1**), as well as to the previously isolated 2′,3′-esterfied *p*-terphenyl derivative (diacetoxy) from *Boletopsis grisea* (Hu et al., 2001c). The only difference was the identity of the acid that in case of terrestrin B was determined to be butyric acid. This was supported by the ^{1}H–^{1}H correlation between (1) H-2a/b and H-3a/b, (2) H-3a/b and H-4a/b, along with the correlation of H-2a/b and H-3a/b with C-1a/b in the HMBC spectrum. The presence of boric acid in the solution of **2** caused a similar bathochromic shift of 4 nm at the 263 nm UV maximum. Based on all of the data above, the structure of terrestrin B, a symmetrical *p*-terphenyl derivative, was determined to be 4,5′,6′,4″-tetrahydroxy-2′,3′-dibutyryloxy-[1,1′;4′,1″-terphenyl].

Terrestrin C (**3**) displayed a *quasi*-molecular ion peak at *m*/*z* 515, corresponding to $C_{30}H_{27}O_8$ by HRFABMS. The molecular formula and the comparison of ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **3** to those of terrestrin A (**1**) and B (**2**), showed that **3** is also a *p*-terphenyl derivative except for the presence of a butyryloxy and a phenylacetoxy group at C-2′ and C-3′ of the central aromatic ring. This was confirmed by the two dimensional (2D) NMR (HMBC, NOESY, etc.) and the boric acid induced bathochromic shift (3 nm) of the absorption maximum at 262 nm in its UV spectrum. It is interesting to note that the 2′,5′ position isomer of **3**, thelephantin D (Quang et al., 2003b), has almost identical spectroscopic data except for ^{13}C chemical shifts of the central ring. Thus, terrestrin C was deduced to be **3**.

Compound **4**, terrestrin D, was obtained as a grayish violet solid. High-resolution EIMS of **4** indicated a molecular formula of $C_{28}H_{22}O_8$ ($[M]^+$ at *m*/*z* 486.1317) with 18 degrees of unsaturation and showed a significant peak at *m*/*z* 352 (loss of CH_3 and $C_6H_5CH_2CO$). The IR spectrum revealed the presence of hydroxyl (3389 cm^{-1}), ester carbonyl (1757 cm^{-1}), and benzene (1612, 1525 cm^{-1}) groups. The ^{13}C NMR spectrum of **4** was very similar to that of terrestrin A (**1**), except for the presence of one acetoxy group (δ 20.0, 170.6) instead of one phenylacetoxy group. The key point in the determination of the substitution pattern of the phenylacetoxy and acetoxy groups on the *p*-terphenyl core of **4**, was the unusual high field shift of the protons of the acetoxy group (δ 1.62). These were assigned at the central ring *ortho* to the phenylacetoxy group. The anisotropic shielding caused by the carbonyl group of the phenylacetoxy group resulted in an upfield shift of the acetoxy group

Table 2
^{13}C NMR spectroscopic data for compounds **1**–**8** (150 MHz, CD_3OD)

C	1	2	3	4	5	6	7	8
1	124.8	125.0	124.9	124.9	123.5	124.1	123.7	123.7
2	132.6	132.7	132.6	132.6	132.1	118.1	132.1	132.2
3	116.1	116.0	116.1	116.0	116.2	146.2	116.1	116.2
4	158.1	158.2	158.2	158.1	158.6	146.7	158.6	158.7
5	116.1	116.0	116.1	116.0	116.2	116.2	116.1	116.2
6	132.6	132.7	132.6	132.6	132.1	122.6	132.1	132.2
1′	123.9	123.9	123.9	123.9	128.4	128.5	128.3	128.3
2′	134.7	134.8	134.7*	134.7	137.1*	137.2*	137.1*	137.5*
3′	134.7	134.8	134.8*	134.8	134.9*	134.9*	135.0*	135.2*
4′	123.9	123.9	123.9	123.9	120.4	120.4	120.4	120.8
5′	142.6	142.5	142.5†	142.6	147.2	147.2	147.2	147.3
6′	142.6	142.5	142.6†	142.6	132.1*	132.2*	132.1*	132.0*
1″	124.8	125.0	124.9	124.9	114.1	114.0	114.0	114.3
2″	132.6	132.7	132.7	132.6	107.6	107.4	107.4	107.4
3″	116.1	116.0	116.0	116.1	144.3	144.4	144.4	144.2
4″	158.1	158.2	158.2	158.2	148.8	149.1	149.1	148.8
5″	132.6	132.7	132.7	132.6	99.3	99.2	99.3	99.3
6″	116.1	116.0	116.0	116.1	152.7	152.8	152.8	152.8
1a	171.2	173.1	171.2	171.2	170.7	170.9	170.0	170.8
2a	41.1	36.4	41.5	41.5	40.8	40.9	19.8	41.2
3a	134.7	19.2	134.8	134.8	134.3	134.5		134.2
4a	130.4	13.8	130.4	130.4	130.3	130.3		130.1
5a, 7a	129.6		129.6	129.6	129.5	129.6		129.4
6a	128.1		128.2	128.2	128.1	128.1		128.0
8a	130.4		130.4		130.3	130.3		130.1
1b	171.2	173.1	173.1	170.6	170.7	170.9	170.8	171.0
2b	41.1	36.4	36.1	20.0	41.2	41.2	41.2	41.2
3b	134.7	19.2	19.0		134.4	134.5	134.5	134.5
4b	130.4	13.8	13.7		130.3	130.3	130.4	130.4
5b, 7b	129.6				129.5	129.6	129.6	129.6
6b	128.1				128.1	128.1	128.2	128.2
8b	130.4				130.3	130.3	130.4	130.4
1c					170.1	170.1	170.1	165.0
2c					41.4	41.4	41.8	120.1
3c					134.6	134.6	135.0	134.0
4c					130.7	130.8	130.7	116.9
5c					130.0	130.0	130.0	164.9
6c					128.7	128.7	128.7	116.9
7c					130.0	130.0	130.0	134.0
8c					130.7	130.8	130.7	

*† Assignments of marked values within a single column are interchangeable.

protons and is consistent with the similar shifts observed for the methylene protons H-2b of compound **3**. This was previously reported for the peracetates of ganbajunin F and G (Hu and Liu, 2001b). Also, the NOE interactions between (1) H-2a and H-2/6, and (2) H-2b and H-2″/6″ observed in the NOESY spectrum of **4**, and the 3-nm bathochromic shift (maximum at 263 nm, caused by the addition of 1% boric acid) corroborated the proposed structure of terrestrin D as 4,5′,6′,4″-tetrahydroxy-2′-phenylacetoxy-3′-acetoxy-[1,1′;4′,1″-terphenyl].

HRFABMS of the *quasi*-molecular ion peak $[M + Na]^+$ of terrestrin E (**6**) at *m/z* 733.1686 gave the molecular formula $C_{42}H_{30}O_{11}Na$ (calculated 733.1685). The ^1H NMR spectrum (Table 1) of **6** showed the presence of 18 aromatic, two singlet aromatic, and three singlet methylene protons. The spectroscopic data resembled those of ganbajunin B (**5**) (Hu et al., 2001a), also isolated in the course of this investigation, suggesting that it was also a *p*-terphenyl derivative possessing a

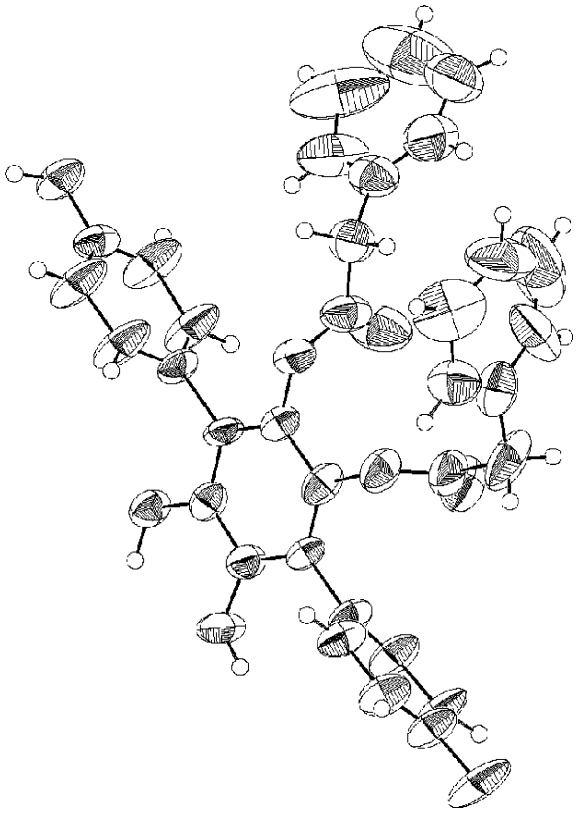

Fig. 1. The ORTEP drawing of compound **1**. Anisotropic ellipsoids are represented by a 50% probability level.

dibenzofuran unit that was also corroborated by long range correlation between (1) H-2″ and C-4′, C-1″, C-3″, C-4″, C-6″; (2) H-5″ and C-1″, C-3″, C-4″, C-6″, except for the notable difference in the NMR spectroscopic data by the presence of an aromatic *ABC* system (δ 6.41, 6.58, 6.66) corresponding to a 1,2,4-trisubstituted instead of the signals for a 1,4-disubstituted benzene ring. The location of the extra oxygen compared with ganbajunin B (**5**) was also straightforward from the HMBC correlations: (1) H-2 and C-4, C-6, C-1′; (2) H-5 and C-3, C-1; (3) H-6 and C-2, C-4, C-1′. The placement and signal assignment of three phenylacetoxy groups was achieved by comparison with the previously unreported NMR spectroscopic data of ganbajunin B (**5**) measured in CD_3OD (Table 1 and 2) with those of **6**. Thus, the structure of **6** was deduced to be 3-(3,4-dihydroxy-phenyl)-7,8-dihydroxy-1,2,4-triphenylacetoxy-dibenzofuran.

A bluish solid, terrestrin F (**7**), with a molecular formula $C_{36}H_{26}O_{10}$, determined by HRFABMS, showed positive FABMS ion peaks at *m/z* 604, 501 and 382 corresponding to $[M + H - CH_3]^+$, $[M + H - C_6H_5CH{=}CO]^+$, $[M - 2C_6H_5CH{=}CO]^+$. The IR spectrum confirmed this by the presence of absorption bands of an ester carbonyl (1772 cm^{-1}), and a monosubstituted benzene ring (1523 cm^{-1}). The ^{13}C NMR spectroscopic data of **7** (Table 1 and 2) had similarities with those of

ganbajunin B (**5**) and terrestrin C (**4**). This led to an assumtion that terrestrin F (**7**) is a dibenzofuran type of *p*-terphenyl and that the three substitution groups at the central aromatic ring are one acetoxy and two phenylacetoxy groups. The acetoxy group was located at C-2′ by the NOE cross peak between H-2a and H-2/6 and the low shift of H-2a (δ 1.57) as discussed in the case of terrestrin D (**4**) above. Hence, the structure 2-acetoxy-7,8-dihydroxy-3-(4-hydroxy-phenyl)-1,4-diphenylacetoxy-dibenzofuran was assigned to terrestrin F (**7**).

HRFABMS indicated that terrestrin G (**8**) has the molecular formula $C_{42}H_{30}O_{11}$ with one CH_2 less compared to terrestrin E (**6**). Further comparison of its NMR spectroscopic data revealed that **8** is a *p*-terphenyl derivative with a benzofuran unit with all of the substitution groups positioned at the central aromatic ring, and with the OH from C-3 moved to C-5c of an aromatic ring of the side chain group. This was also substantiated by long range correlations between (1) H-3c and C-1c, C-4c, C-5c; (2) H-4c and C-2c, C-3c, C-4c. The presence of two phenylacetoxy and one *p*-hydroxy-benzoyl was also evident from the ion peaks (positive FAB-MS) at *m/z* 578, 475 and 340 recognized as $[M - C_6H_5CH{=}CO]^+$, $[M - 2\ C_6H_5CH{=}CO]^+$, $[M + H - 2C_6H_5CH{=}CO - HOC_6H_4CO]^+$. The position of the *p*-hydroxy-benzoyl group at C-6′ was determined by the spatial proximity of 1) H-3c/7c and H-2/6 and 2) H-2a and H-5b/7b deduced from the NOESY spectrum of **8**. Furthermore, if the phenylacetoxy unit was positioned at C-6′ the chemical shifts of its protons would have to be further downfield as observed for ganbaujnin B (**5**) and terrestrins E (**6**) and F (**7**). Thus, it was established that this grayish solid, compound **8**, terrestrin G, has the structure of 3-(4-hydroxy-phenyl)-4-(4-hydroxy-benzoyloxy)-7,8-dihydroxy-1,2-diphenylacetoxy-dibenzofuran.

It should be noted that fully-aromatic polyhydroxy-*p*-terphenyls are colorless, and *p*-terphenylquinones are chemically converted to their 'leuco' peracetates through a standard reductive acetylation procedure (McMorris and Anchel, 1967). Although the purity of the isolated compounds has been repeatedly checked by HPLC, NMR and UV, and corroborated by X-ray, the coloration of the isolated materials is probably due to the presence of minor impurities of related metabolites or oxidation artefacts formed during the work-up. Terrestrin A (**1**) can serve as an example, since the crystals obtained suitable for X-ray were colorless, however all solutions of **1** possessed a violet coloration.

Naturally occurring compounds containing a *p*-terphenyl core are apparently restricted to fungi and lichens. Recently, an increasing number of metabolites based on the *p*-terphenyl nucleus and their interesting biological properties or unusual structures have been reported (Geraci et al., 2000; Hu et al., 2001a). The literature on polyhydroxy-*p*-terphenyls and *p*-terphenylquinones isolated from 35 different fungal species (both from fungal cultures and fruiting bodies of Basidiomycetes), representing a total of 115 fungal metabolites has been reviewed (Cali et al., 2003). The high concentration of simple *p*-terphenyls with a tricyclic C-18 basic skeleton, including their acyclic derivatives with the *O*-acyl *ortho*-disubstitution of the central ring, terrestrins A–D (**1**–**4**), as well as the trisubstituted *p*-terphenyls with a dibenzofuran system, terrestrins F–G (**5**, **6**), have not been reported in a single mushroom before. The capability of the mushroom enzymes to catalyze specific substitution patterns may be of chemotaxonomical significance since in previous work the most common pattern was *O*-acyl *para*-disubstituted central ring (Tsukamoto et al., 2002; Quang et al., 2003a; Quang et al., 2003b) along with some rare non-central ring substitutions (Hu and Liu, 2003). Symmetrical derivatives like terrestrins A (**1**) and B (**2**) are also uncommon in nature (Holzapfel et al., 1989; Hu et al., 2001c). For the derivatives of each species within the genus studied, reported until now, usually one acid is more frequent than the other acids that esterify the *p*-terphenyl core. For example, within the Thelephoraceae family, in genus *Thelephora*, it is phenylacetic acid (Tsukamoto et al., 2002; Hu et al., 2001a; Quang et al., 2003a), in *Hydnellum* genus it is benzoic acid (Sullivan et al., 1967; Gripenberg, 1974; Quang et al., 2004), whereas in *Boletopsis* and *Sarcodon* genera it is acetic acid (Jaegers et al., 1987; Hu et al., 2001c; Hu et al., 2002; Geraci et al., 2000). For the Paxillaceae family specifically in the *Paxillus* genus the most common acids are: 3-acetoxybutyric acid, 3-hydroxybutyric and 3-(3-methyl-oxiranyl)-acrylic (Yun et al., 2000a,b; Quang et al., 2003c,d). So, it seems that the identity of the acids, extent and pattern of the substitution of the *p*-terphenyl core could be characteristic for every fungal species. In conclusion, it can be said that the wide occurrence and differentiation of *p*-terphenyl derivatives, metabolites of the shikimate–chorismate pathway, in the mushroom kingdom and especially in the Basidiomycete could make them significant chemotaxonomical markers analogous to flavonoids in plants.

3. Experimental

3.1. General

Column chromatography (CC) was carried out on silica gel 60 (0.2–0.5 mm, 0.04–0.063 mm, Merck) and Cosmosil 75 C_{18}. Preparative medium-pressure liquid chromatography (MPLC) was performed with a Work-21 pump (Lab-Quatec Co., Ltd.) and was carried out on a Lobar column [pre-packed column size B (310-25), LiChroprep®Diol (40–63 μm), Merck]. Preparative

HPLC separations were conducted on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C 18-AR-II column. TLC was performed on silica gel plates (Kieselgel 60 F_{254}, Merck) and RP C_{18} silica gel plates (Merck). The spots on TLC were visualized by UV light (254 nm) and by spraying with 30% H_2SO_4 and Godin's reagent (Godin, 1954), followed by heating. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), using CD_3OD as the solvent. Chemical shifts are given relative to TMS δ 0.00 (ppm) as an internal standard (^1H) and δ 49.00 (ppm) from CD_3OD as a standard (^{13}C NMR). Mass spectra were obtained on a JEOL JMS AX-500 instrument or a JEOL Mstation JMS 700 instrument. X-ray crystallographic analysis was carried out on a Mac Science DIP-2020 diffractometer using Mo Kα radiation (λ = 0.71073 Å).

3.2. Material

The fruiting bodies of *T. terrestris* were collected in Aichi prefecture, Japan in October 2003 and identified by M. Nukada. A voucher specimen (KN0101) has been deposited in Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki 710-0290, Japan.

3.3. Extraction and isolation

The MeOH extract (2.98 g) of dried fruiting bodies of *T. terrestris* (10.36 g) was subjected to open column silica gel chromatography. On elution with chloroform–methanol in a gradient in order of increasing polarity (v/v, from 10:1 to 3:1 $CHCl_3$:MeOH and afterwards by washing the column with methanol) 10 fractions were obtained (fr. 1–10). Fraction 3 (429.1 mg) yielded 12 subfractions after RP C_{18}CC (aqueous MeOH, gradient, 70–100%, v/v). Subfractions 3–6 (23.1 mg), 3–7 (41.5 mg), 3–8 (89.4 mg) were subjected to MPLC with a DIOL column, eluted with $CHCl_3$:EtOAc (1:1, v/v), flow rate 0.5 ml/min, followed by prep. HPLC with a RP C_{18} column, solvent system CH_3CN:H_2O (13:7, v/v) and gave compounds: terrestrin F (**7**, 5.5 mg), terrestrin E (**6**, 6.5 mg) and ganbajunin B (**5**, 38.6 mg), respectively. Fraction 4 (289.9 mg) and fraction 5 (547.0 mg) were separated by RP C_{18}CC using the same solvent system regime as mentioned above for fr. 3 to afford further 10 and 16 subfractions. Subfraction 4–6 (19.5 mg) was purified by reversed phase prep. HPLC, CH_3CN:H_2O (13:7, v/v), to obtain terrestrin G (**8**, 9.5 mg). Additional amounts of terrestrin G (**8**) were also isolated from fr. 5–10, see below. Further separation of subfractions 5–1 (23.7 mg), 5–2 (31.3 mg), 5–3 (18.5 mg), 5–4 (27.2 mg) and 5–10 (46.6 mg) was also achieved by means of RP C_{18} prep. HPLC (CH_3CN:H_2O = 1:1 for fr. 5–1, 5–2 and 5–3 and CH_3CN:H_2O = 3:2 for fr. 5–4 and 5–10). In this way terrestrin D (**4**, 3.4 mg), was obtained from fr. 5–1, terrestrin B (**2**, 5.3 mg), from fr. 5–2, terrestrin C (**3**, 5.0 mg), and thelephantin H (**10**, 8.5 mg) from 5–3, terrestrin A (**1**, 34.4 mg) from fr. 5–4, terrestrin G (**8**, 18.8 mg) and thelephantin F (**9**, 8.7 mg) from fr. 5–10.

3.3.1. Terrestrin A (*1*)

Colorless crystals (methanol–water); m.p. 220–223 °C; Positive FABMS *m/z*: 585 $[M + Na]^+$, 563 $[M + H]^+$, 444 $[M - C_6H_5CH{=}CO]^+$, 326 $[M - 2\,C_6H_5CH{=}CO]^+$; HRFABMS *m/z* 563.1733 (calcd for $C_{34}H_{27}O_8$: 563.1706). UV λ_{max} (MeOH) nm ($\log\epsilon$): 209.8 (4.7), 220.8 (4.5), 260.6 (4.3). FTIR (KBr): 3384, 1766, 1716, 1612, 1524, 1456, 1232, 1142, 984, 831, 726, 698 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.2. Terrestrin B (*2*)

Reddish violet solid; Positive FAB-MS *m/z*: 489 $[M + Na]^+$, 467 $[M + H]^+$; HRFABMS *m/z* 467.1727 (calcd for $C_{26}H_{27}O_8$: 467.1706). UV λ_{max} (MeOH) nm ($\log\epsilon$): 205.2 (4.6), 224.6 (4.3), 262.8 (4.3). FTIR (KBr): 3375, 1763, 1612, 1524, 1457, 1237, 1175, 1104, 978, 834 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.3. Terrestrin C (*3*)

Reddish solid; Positive FABMS *m/z*: 537 $[M + Na]^+$, 515 $[M + H]^+$; HRFABMS *m/z* 515.1671 (calcd for $C_{30}H_{27}O_8$: 515.1706). UV λ_{max} (MeOH) nm ($\log\epsilon$): 205.0 (4.5), 226.3 (4.2), 262.0 (4.1). FTIR (KBr): 3419, 1747, 1612, 1525, 1456, 1230, 1173, 1106, 981, 833 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.4. Terrestrin D (*4*)

Grayish violet solid; EIMS *m/z* (rel. int.): 486 $[M]^+$ (4.9), 453 $[M - CH_3 - H_2O]^+$ (3.4), 444 (3.0), 410 $[M - C_6H_4]^+$ (11.0), 368 $[M - C_6H_5CH{=}CO]^+$ (37.8), 352 $[M - CH_3C_6H_5CH_2CO]^+$ (25.1), 326 (66.1), 310 (100), 280 (8.0), 223 (6.9), 136 (23.4), 118 $[C_6H_5CH{=}CO]^+$ (12.3), 91 $[C_6H_5CH_2]^+$ (77.9), 43 $[CH_3CO]^+$ (14.8); HREIMS *m/z* 486.1317 (calcd for $C_{28}H_{22}O_8$: 486.1315). UV λ_{max} (MeOH) nm ($\log\epsilon$): 203.8 (4.6), 225.4 (4.1), 262.6 (4.0). FTIR (KBr): 3389, 1757, 1612, 1525, 1219, 1110, 1015, 980, 834 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.5. Terrestrin E (*6*)

Greenish gray solid; Positive FABMS *m/z*: 733 $[M + Na]^+$, 711 $[M + H]^+$, 593 $[M + H - C_6H_5CH{=}CO]^+$, 475 $[M + H - 2C_6H_5CH{=}CO]^+$, 356 $[M - 3C_6H_5CH{=}CO]^+$; HRFABMS *m/z* 733.1686

(calcd for $C_{42}H_{30}O_{11}Na$: 733.1685). UV λ_{max} (MeOH) nm (log ϵ): 206.4 (4.6), 217.2 (4.0), 247.4 (4.2), 303.0 (4.2), 320.8 (4.2), 328.3 (4.2). FTIR (KBr): 3422, 1771, 1523, 1497, 1473, 1298, 1232, 1113, 997, 754, 728, 698 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

3.3.6. Terrestrin F (7)

Bluish solid; Positive FABMS *m/z*: 642 $[M + H + Na]^+$, 619 $[M + H]^+$, 604 $[M + H - CH_3]^+$, 501 $[M + H - C_6H_5CH{=}CO]^+$, 382 $[M - 2C_6H_5CH{=}CO]^+$; HRFABMS *m/z* 642.1497 (calcd for $C_{36}H_{27}O_{10}Na$: 642.1502). UV λ_{max} (MeOH) nm (log ϵ): 205.8 (4.7), 218.8 (4.5), 245.5 (4.3), 262.4 (4.3), 302.4 (4.3), 320.4 (4.3), 328.0 (4.3). FTIR (KBr): 3360, 1772, 1523, 1497, 1473, 1417, 1218, 1171, 1113, 846, 725 cm^{-1}. For ^1H and ^{13}C NMR spectra, see Tables 1 and 2.

*3.3.7. Terrestrin G (**8**)*

Grayish solid; Positive FABMS *m/z*: 719 $[M + Na]^+$, 696 $[M]^+$, 578 $[M - C_6H_5CH{=}CO]^+$, 475 $[M - 2C_6H_5CH{=}CO]^+$, 340 $[M + H - 2C_6H_5CH{=}CO - HOC_6H_4CO]^+$; HRFABMS *m/z* 696.1628 (calcd for $C_{41}H_{28}O_{11}$: 696.1632). UV λ_{max} (MeOH) nm (log ϵ): 207.0 (4.7), 263.4 (4.5), 302.4 (4.3), 321.7 (4.3), 328.0 (4.3), 345.4 (3.8). FTIR (KBr): 3385, 1752, 1609, 1515, 1496, 1472, 1253, 1166, 1121, 986, 849, 727, 696 cm^{-1}. For ^1H and ^{13} C NMR spectra, see Tables 1 and 2.

3.4. X-ray crystallographic analysis of terrestrin A (1)

A colorless single crystal of **1** having approximate dimensions $0.5 \times 0.3 \times 0.05$ mm grown from methanol water was used for the X-ray crystal analysis carried out on a Mac Science DIP-2020 diffractometer. The crystal data of **1** were as follows: $C_{34}H_{26}O_8$, MW 562.575, triclinic, space group *P*1, $a = 10.4310$ (9) Å, $b = 11.4500$ (8) Å, $c = 14.499$ (2) Å, $\alpha = 75.857$ (4)°, $\beta = 82.466$ (4)°, $\gamma = 89.689$ (7)°, $V = 1664.1$ (3) $Å^3$, $Z = 2$, $D_x = 1.171$ $Mg\,m^{-3}$, Mo Kα radiation, $\lambda = 0.71073$ Å, $\mu = 0.085$ mm^{-1}, 5844 reflections, 847 parameters; only coordinates of H atoms were refined, $R(gt) = 0.0667$, $wR(gt) = 0.1893$, $S = 1.048$. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL). Data reduction: maXus (Mackay et al., 1999). Program used to refine the structure: *SHELXL*-97 (Sheldrick, 1997); Refinement on F^2 full-matrix least-squares calculations. The atomic coordinates and equivalent isotropic displacement parameters, as well as a full list of bond distances and angles, and the structure factor table are deposited as supplementary material at the Cambridge Crystallographic Data Centre (Deposition No. CCDC 256120). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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