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Thelephantins A, B and C: three benzoyl *p*-terphenyl derivatives from the inedible mushroom *Thelephora aurantiotincta*

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Dedicated to the celebration of the 70th birthday of Professor M.H. Zenk.

Abstract

Three benzoyl *p*-terphenyl derivatives named thelephantins A, B and C were isolated from the ethyl acetate extract of fruit bodies of the Thelephoraceous Basidiomycete *Thelephora aurantiotincta*. Their structures were elucidated by analysis of high-resolution 2D NMR, MS, IR and UV spectra.
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Keywords: *Thelephora aurantiotincta*; Thelephoraceae; Fungi; Basidiomycetes; Thelephantin; Benzoyl *p*-terphenyls

1. Introduction

Mushrooms of the Thelephoraceae are widely distributed in America, Australia, New Zealand and Japan (Tsukamoto et al., 2002), and are a rich source of biologically active compounds (Jikai, 2002; Tsukamoto et al., 2002). Previously, phellodonic acid from *Phellodon melaleucus* (Stadler et al., 1993), poly(phenylacetoxy)-substituted 1,1′:4′,1″-terphenyl derivatives named ganbajunins A–G and cycloleucomelone from *Thelephora ganbajum* (Hu et al., 2001a,b) and thelephorin A from *Thelephora vialis* (Tsukamoto et al., 2002) were reported. In the course of an investigation of biologically active compounds from the Basidiomycetes fungi, cryptoporic acid A–G from *Crystoporus volvatus*, novel sesquiterpenoids from *Lentinellus ursinus*; and γ and δ-lactones and spiromentins from *Paxillus autrometosus* were isolated (Hashimoto and Asakawa, 1998). Recently, we investigated the chemical constituents of *Thelephora aurantiotincta* and isolated seven compounds (**1**–**7**). We now report the isolation and structural elucidation of those substrates, which include three new benzoyl *p*-terphenyl derivatives, named thelephantins A (**1**), B (**2**) and C (**3**) and four known compiunds (**4**–**7**) from the EtOAc extract of *T. aurantiotincta*.

2. Results and discussion

The fruiting bodies of *Thelephora aurantiotincta* were air-dried and extracted with MeOH, with the latter extract partitioned between EtOAc and water. Then, the EtOAc extract was subjected to Sephadex LH-20, diol, reversed-phase (C_{18}) column chromatography and preparative HPLC to give compounds **1**–**7**.

Compounds **4**, **5**, and **6** were determined to be thelephorin A (Tsukamoto et al., 2002), ganbajunin C (Hu et al., 2001a) and 2-O-methylatrometin (Hu et al., 2001a), respectively by comparison of the spectral data with those reported in references. *p*-Hydroxybenzoic acid (**7**) was also isolated from the EtOAc extract.

The molecular formula of thelephantin A (**1**) was found to be $C_{29}H_{24}O_9$ by HR–FABMS ($[M+H]^+$ m/z 517.1472). The IR spectrum of **1** showed absorptions at 3428, 1740 and 1614 cm^{-1} assignable to an hydroxyl, carbonyl and aromatic double bond functionalities, respectively. The UV spectrum of **1** showed UV

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absorption maxima at 210 and 260 nm attributable to an aromatic ring. The ^{1}H NMR spectrum of **1** (Table 1) revealed six aromatic proton signals (δ 6.74, 6.77, 6.83, 7.19, 7.23 and 7.76) and three aliphatic proton signals (δ 0.59, 1.30 and 1.99), whereas its ^{13}C-NMR spectrum (Table 2) showed resonances for 29 carbons including two ester carbonyls (δ 166.3 and 173.2) and five phenolic carbons (δ 142.4, 142.4, 157.9, 158.1 and 164.1). The ^{1}H and ^{13}C NMR spectral data of **1** were very similar to those of thelephorin A (**4**) (Tsukamoto et al., 2002) suggesting the presence of a *p*-terphenyl derivative. In addition, the ^{1}H–^{1}H COSY, HMBC and NOESY spectra of **1** indicated four partial structures **a**–**d**. Among them, units **a** and **c** were *para*-substituted phenyl groups attached to C-7 (δ 124.1) and C-10 (δ 123.9) of the central aromatic ring and unit **b** was a *para*-substituted benzoyl group linked to C-8 (δ 135.0) (Tsukamoto et al., 2002). Only the NMR signals for unit **d** were different from those of thelephorin A (**4**). There were ^{1}H–^{1}H correlations between (1) H-2″ and H-3″, (2) H-3″ and H-4″ in the ^{1}H–^{1}H COSY spectrum of **1**, long range correlations between H-2″, H-3″/C-1″ in the HMBC spectrum and the NOEs between H-2″ and H-18 (Fig. 1) suggesting that the partial structure for unit **d** was a butyroxyl group attached to C-11 (δ 135.0) of the central aromatic ring. Acetylation of **1** with Ac_2O and pyridine afforded the pentaacetate (**1a**), [HR-EIMS: *m*/*z* 726.1934 ($C_{39}H_{34}O_{14}$ requires *m*/*z* 726.1949); δ_H 1.98 (6H, *s*), 2.26 (3H, *s*), 2.31 (6H, *s*)] indicating the presence of five phenolic hydroxyl groups in **1**. On the basis of the previous chemical and spectral data, compound **1** was determined as depicted (Chart 1).

The molecular formula of thelephantin B (**2**) was determined to be $C_{31}H_{28}O_9$ by HR–FABMS ($[M+H]^+$ *m*/*z* 545.1858). The IR spectrum of 2 showed the presence of a hydroxyl (3356 cm^{-1}), ester (1716, 1167, 1102

Table 1
^{1}H NMR spectral data for compounds **1**, **2** and **3** (600 MHz, CD_3OD)

H	1	2	3
2, 6	6.74 *d* (8.8)	6.74 *d* (8.8)	6.74 *d* (8.8)
3, 5	7.23 *d* (8.8)	7.23 *d* (8.8)	7.23 d (8.8)
14, 18	7.19 *d* (8.8)	7.18 *d* (8.8)	7.18 *d* (8.8)
15, 17	6.83 *d* (8.8)	6.83 *d* (8.8)	6.83 *d* (8.8)
3′, 7′	7.76 *d* (8.8)	7.76 *d* (8.8)	7.76 *d* (8.8)
4′, 6′	6.77 *d* (8.8)	6.77 *d* (8.8)	6.77 *d* (8.8)
2″	1.99 *t* (7.1)	2.00 *t* (7.4)	2.03 *dd* (5.5, 15.1)
			1.77 *dd* (8.5, 15.1)
3″	1.30 *dd* (7.1, 14.6)	1.25 *m*	1.51 *m*
4″	0.59 *t* (7.1)	0.92 *m*	1.24 *m*
5″		1.02 *m*	0.61 *d* (6.9)
6″		0.71 *t* (7.4)	0.56 *d* (6.9)
7″			0.52 *d* (6.9)

Table 2
^{13}C NMR specta data for compounds **1**, **2** and **3** (150MHz, CD_3OD)

C	1	2	3
1	157.9 *s*	157.9 *s*	157.9 *s*
2, 6	116.3 *d*	116.3 *d*	116.3 *d*
3, 5	132.6 *d*	132.6 *d*	132.6 *d*
4	125.0 *s*	125.0 *s*	125.1 *s*
7	124.1 *s*	124.1 *s*	124.1 *s*
8	135.0 *s*	135.0 *s*	135.0 *s*
9	142.4 *s*	142.5 *s*	142.5 *s*
10	123.9 *s*	123.9 *s*	124.0 *s*
11	135.0 *s*	135.0 *s*	135.1 *s*
12	142.4 *s*	142.5 *s*	142.5 *s*
13	125.0 *s*	125.0 *s*	125.0 *s*
14, 18	132.7 *d*	132.7 d	132.7 *d*
15, 17	116.0 *d*	116.0 *d*	116.0 *d*
16	158.1 *s*	158.2 *s*	158.2 *s*
1′	166.3 *s*	166.3 *s*	166.3 *s*
2′	120.9 *s*	120.9 *s*	121.0 *s*
3′, 7′	133.3 *d*	133.3 *d*	133.4 *d*
4′, 6′	115.9 *d*	115.9 d	115.9 *d*
5′	164.1 *s*	164.1 *s*	164.1 *s*
1″	173.2 *s*	173.4 *s*	173.1 *s*
2″	36.4 *t*	34.6 *t*	39.6 *t*
3″	19.1 *t*	25.5 *t*	36.8 d
4″	13.6 *q*	32.0 *t*	32.6 *d*
5″		23.2 *t*	20.3 *q*
6″		14.1 q	18.0 *q*
7″			15.7 *q*

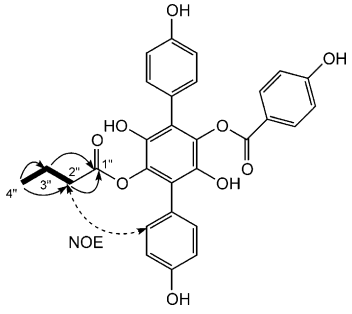

Fig. 1. Important ^{1}H–^{1}H COSY correlations (bold line), HMBC correlations (arrows) and NOESY correlations of compound **1**.

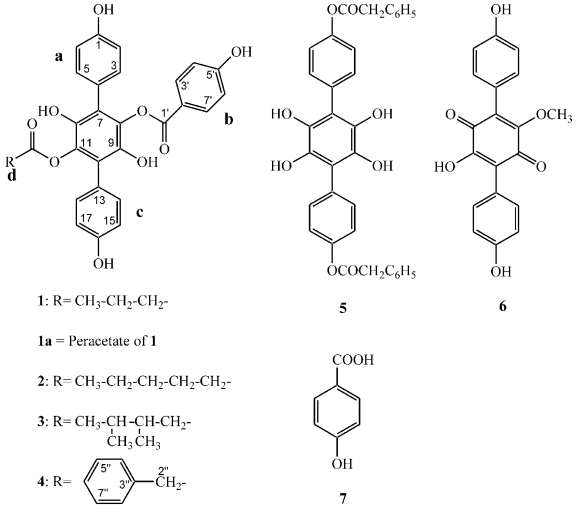

Chart 1.

cm^{-1}) and benzene ring (1608 cm^{-1}) moieties. The UV spectrum of **2** showed absorption maxima at 208 nm (log ε 4.4), 261 nm (log ε 4.6). The ^1H and ^{13}C NMR spectral data of **2** (Tables 1 and 2) also resembled those of thelephantin A (**1**) and thelephorin A (**4**) indicating a benzoyl *p*-terphenyl derivative for **2**, except for the signals of unit d. In the ^1H–^1H COSY spectrum of **2**, there were ^1H–^1H correlations between (1) H-2″ and H-3″, (2) H-3″ and H-4″, (3) H-4″ and H-5″, (4) H-5″ and H-6″. In addition, the correlations between H-2″ and H-3″/C-1″ in the HMBC spectrum, and between H-2″ and H-18 in the NOESY spectrum (Fig. 2) revealed that the partial structure for **d** was an *n*-hexanoxyl group. Based on the earlier spectral data, the structure of thelephantin B was determined and represented as **2**.

Thelephantin C (**3**) was obtained as a grayish solid. The HR–FABMS of **3** showed a molecular ion peak at *m*/*z* 559.1993 ($[M+H]^+$), suggesting the molecular formula $C_{32}H_{30}O_9$. The IR spectrum of **3** showed the presence of hydroxyl (3376 cm^{-1}), ester carbonyl (1713, 1168, 1104 cm^{-1}) and aromatic (1608 cm^{-1}) groups. The UV spectrum of **3** showed absorption maxima at 212 and 239 nm. The ^1H and ^{13}C NMR spectral data of **3** (Tables 1 and 2) were identical with those of thelephantins A (**1**), B (**2**) and thelephorin A (**4**) except for the signals of unit **d**. Unit **d** was determined to be 3″, 4″-dimethyl-pentanoxyl group. This was evident from ^1H–^1H correlations between (1) H-2″/H-3″; (2) H-3″/H-2″, H-4″ and H-7″; (3) H-4″/H-3″, H-5″ and H-6″ in ^1H–^1H COSY spectrum, and from long range correlations between (1) H-2″, H-3″ and H-7″/C-1″; (2) H-2″, H-4″, H-5″, H-6″ and H-7″/C-3″; (3) H-3″, H-5″ and H-6″/C-4″ in the HMBC spectrum (Fig. 3). Occurrence of the 3,4-dimethylpentanoic acid (unit **d**) is the first record of it as a natural product, although this acid and its reduced compound (3,4-dimethylpentanol-1) have been reported as the synthetic intermediate as well as a natural product of a forest ant pheromone, respectively (Enders and Rendenbach, 1986). Based on the earlier features, thelephantin C (**3**) was thus determined as shown. The absolute stereochemistry at C-3″ remains to be clarified.

Several *p*-terphenyl derivatives exhibit considerable bioactivities, such as HeLa cell growth inhibition (Takahashi et al., 1976), specific 5-lipoxygenase inhibitory (Takahashi et al., 1992), prolyl endopeptidase (Lee et al., 2000), lipid peroxidation inhibitory (Yun et al., 2000), antibacterial, anti-insect (Belofsky et al., 1998) and potent IgE-antibody suppressant (Kawada et al., 1998) activities. Recently, thelephorin A (**4**) was also reported as a new radical scavenger (Tsukamoto et al., 2002).

3. Experimental

3.1. General

IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. UV spectra were obtained on a Shimadzu UV-1650PC in MeOH solution. The specific optical rotations were measured on a JASCO DIP-1000 polarimeter with MeOH as solvent. NMR spectra were recorded on a Varian Unity 600 (600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR) or a Varian Mercury 300 (300 MHz), using CD_3OD or $CDCl_3$ as solvent. Chemical shifts are given with TMS (δ 0.00) used as internal standard (^1H NMR), and δ 49.00 (ppm) from CD_3OD, δ 77.03 (ppm) from $CDCl_3$ as a standard (^{13}C NMR). Mass spectra including FAB–MS and HR-FAB MS were recorded on a Jeol JMS AX-500 spectrometer. CC 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). Prep. medium-pressure liquid chromatography (MPLC) was performed with Work-21 pump (Lab-Quatec Co., Ltd) and carried out by Lobar column chromatography (Merck). HPLC was performed on a Shimadzu liquid chromatograph LC-10AS with RID-6A and SPD-10A detectors using a Waters 5C18-AR-II column. The spots on TLC were detected under UV 254 nm and by spraying with 10% H_2SO_4 or Godin reagent [Vanillin (5 g) in EtOH (500 ml) and $HClO_4$ (20 ml) in H_2O (380 ml); H_2SO_4 (30%) in H_2O] (Godin, 1954), followed by heating at 120 °C.

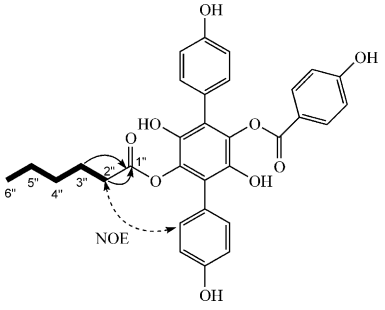

Fig. 2. Important ^1H–^1H COSY correlations (bold line), HMBC correlations (arrows) and NOESY correlations of compound **2**.

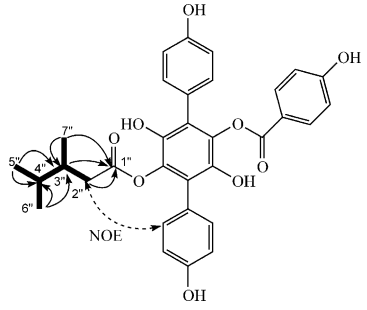

Fig. 3. Important ^1H–^1H COSY correlations (bold lines), HMBC correlations (arrows) and NOESY correlations of compound **3**.

3.2. Material

Thelephora aurantiotincta was collected in Shizenhogo-center, Saeki-cho, Wake-gun, Okayama, Japan in August 2001 and identified by Mr. Nitaro Maekawa at the Japanese mushroom center. A voucher specimen (KSU01091911) has been deposited in the Faculty of Food Culture, Kurashiki Sakuyo University, Kurashiki 710-0290, Japan.

3.3. Extraction and isolation

Fresh fruit bodies (100.0 g) of *T. aurantiotincta* were dried at room temp and extracted with MeOH. The methanolic extract was evaporated in vacuo to give a residue (3.35 g) which was partitioned between EtOAc and water. Sephadex LH-20 CC of EtOAc extract (1.28 g), using $CHCl_3$–MeOH (1:1) as eluent, gave six fractions (Fractions 1–6). Fraction 4 (179.1 mg) was purified by reversed phase prep. MPLC using MeOH–H_2O (7:3) as mobile phase, flow rate 0.5 ml/min, to give compound **5** (67.9 mg). Half of fraction 5 (242.3 mg) was divided into six sub-fractions by prep. MPLC with a diol column [(Pre-packed column size B (310–25), LiChroprep DIOL (40–63 μm), Merck] using $CHCl_3$–EtOAc (1:1) as solvent system, flow rate 0.7 ml/min. Fraction 5-1 (18.7 mg) was purified by reversed phase prep. HPLC (CH_3CN–H_2O, 7:3), flow rate 1 ml/min to give compounds **7** (3.8 mg) and **6** (4.0 mg). Fraction 5-2 contained only compound **6** (3.7 mg). Fraction 5-5 (209.3 mg) was further fractionated by reversed phase prep. MPLC using MeOH–H_2O (65:35) as solvent, flow rate 0.5 ml/min to give compounds **1** (22.8 mg), **4** (91.7 mg), **2** (10.1 mg) and **3** (11.8 mg).

3.3.1. Thelephantin A (*1*)

Grayish solid; Positive FAB–MS: 517 $[M+H]^+$; HR–FABMS m/z 517.1472 ($C_{29}H_{25}O_9$, requires m/z 517.1499). UV λ_{max} (CH_3OH) nm (log ε): 210.8 (4.7), 260.6 (4.6). IR (KBr): 3428, 2968, 2877, 1740, 1615, 1528, 1288, 1115, 1011, 973 cm^{-1}. For ^1H and ^{13}C NMR (CD_3OD) spectra see Tables 1 and 2.

3.3.2. Thelephantin B (*2*)

Grayish solid; Positive FAB-MS: 545 $[M+H]^+$; HR-FABMS m/z 545.1858 ($C_{31}H_{29}O_9$, requires m/z 545.1812). UV λ_{max} (CH_3OH) nm (log ε): 208.0 (4.6), 261.8 (4.4). IR (KBr): 3356, 2960, 1716, 1607, 1526, 1262, 1167, 1102, 974 cm^{-1}. For ^1H and ^{13}C NMR (CD_3OD) spectra see Tables 1 and 2.

3.3.3. Thelephantin C (*3*)

Grayish solid; $[\alpha]+3.75^\circ$ (c 1.01, MeOH); Positive FAB–MS: 559 $[M+H]^+$; HR-FABMS m/z 559.1993 ($C_{32}H_{31}O_9$, requires m/z 559.1968). UV λ_{max} (CH_3OH) nm (log ε): 212.0 (4.4), 261.6 (4.3). IR (KBr): 3376, 2962, 1713, 1608, 1525, 1264, 1168, 1104, 974 cm^{-1}. For ^1H and ^{13}C NMR (CD_3OD) spectra see Tables 1 and 2.

3.3.4. Acetylation of thelephantin A (*1a*)

A solution of thelephantin A (5.4 mg) in pyridine (1 ml) was treated with acetic anhydride (1 ml) and the mixture was stirred overnight at room temp. Water was added and the mixture was extracted with $CHCl_3$. The organic phase was washed with 1 N HCl, 5% $NaHCO_3$ solution and brine, dried (with $MgSO_4$) and evaporated to give a residue (6.3 mg). The residue was purified by prep. HPLC [Waters 5SL-II (SiO2); *n*-hexane/EtOAc = 1:1] to afford the pentaacetate (**1a**; 3.0 mg) as a colorless oil. EI–MS: m/z 726$[M]^+$, 614, 572 (100%), 410, 163, 121; HR-EIMS: m/z 726.1934 ($C_{39}H_{34}O_{14}$ requires m/z 726.1949); IR (KBr): 1767, 1603, 1518, 1198 cm^{-1}; UV λ_{max} (CH_3OH) nm (log ε): 244 (4.44), 207 (4.59). ^1H NMR ($CDCl_3$): δ 7.94 (2H, *d*, $J=9.1$ Hz), 7.37 (2H, *d*, $J=8.8$ Hz), 7.36 (2H, *d*, $J=8.8$ Hz), 7.13 (2H, *d*, $J=9.1$ Hz), 7.12 (2H, *d*, $J=8.8$ Hz), 7.07 (2H, *d*, $J=9.1$ Hz), 2.31 (6H, *s*), 2.26 (3H, *s*), 2.02 (2H, *t*, $J=7.1$ Hz), 1.98 (6H, *s*), 1.28 (2H, *dd*, $J=7.4$, 14.8 Hz), 0.59 (3H, *t*, $J=7.4$ Hz). ^{13}C NMR ($CDCl_3$): δ 170.4 (*s*), 169.0 (*s*), 168.7 (*s*), 167.8 (*s*), 162.8 (*s*), 154.9 (*s*), 150.6 (*s*), 139.4 (*s*), 135.4 (*s*), 131.7 (*d*), 130.8 (*d*), 130.6 (*d*), 128.8 (*s*), 128.6 (*s*), 121.8 (*d*), 121.4 (*d*), 119.2 (*s*), 115.1 (*s*), 35.3 (*t*), 21.2 (*q*), 20.1 (*q*), 18.0 (*t*), 13.2 (*q*).

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