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Anticancer Activities of Thelephantin O and Vialinin A Isolated from *Thelephora aurantiotincta*

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Supporting Information

ABSTRACT: *Thelephora aurantiotincta* is an edible mushroom belonging to the genus *Thelephora*; it grows in symbiosis with pine trees. Recently, phytochemical investigations have revealed that the genus *Thelephora* is an abundant source of *p*-terphenyl derivatives. However, their bioactivity has not yet been well characterized. In screening for natural materials with anticancer activity, a *T. aurantiotincta* ethanol extract (TAE) was found to decrease cell viability in human hepatocellular carcinoma cells (HepG2). In this study, a new *p*-terphenyl derivative, thelephantin O, and a known compound, vialinin A, were isolated as the principal bioactive components of TAE. These compounds decreased cell viability in HepG2 and human colonic carcinoma cells (Caco2), but not in noncancerous human hepatocytes. This is the first report of the isolation from *T. aurantiotincta* of selective cytotoxic agents against cancer cells.

KEYWORDS: anticancer, *p*-terphenyl derivatives, thelephantin O, vialinin A, *Thelephora aurantiotincta*

INTRODUCTION

Mushrooms, which have been used around the world as folk medicines and food for centuries, have recently been considered as a potential source of various types of chemical substances possessing characteristic pharmacological activities. *Thelephora aurantiotincta*, which belongs to the genus *Thelephora* and grows in symbiosis with pine trees, is sold in a mixture with *Thelephora ganbajun* as an edible mushroom, the unique flavor and taste of which are appreciated in the Yunnan province of China.[1] Previous phytochemical investigations conducted on the genus *Thelephora* revealed that this genus is an abundant source of *p*-terphenyl derivatives.[2] Some of these derivatives have been reported to show biological activities such as scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals,[3] neuroprotection,[4] and immunosuppression.[5,6] *T. aurantiotincta* also produces several *p*-terphenyl derivatives, such as aurantiotinin A,[1] ganbajunins C and E,[1,7] atromentin,[1,7] thelephorin A,[7,8] and thelephantins A−H,[7,9] but their bioactivities have not yet been well characterized.

The *p*-terphenyl derivatives are structurally characterized by a central hexasubstituted benzene ring and several oxygen functional groups, such as hydroxy and acyloxy groups, in various substitution modes.[2] Despite their relatively simple structure, in some *p*-terphenyl derivatives, it is difficult to determine the substitution modes because the compounds are symmetrical. Indeed, some papers have described revisions of the substitution modes with the assistance of chemical synthesis; this has also helped in investigations of the structure−bioactivity relationships of some *p*-terphenyl derivatives.[10,11]

Natural products are excellent sources of lead compounds in developing new medicaments for the treatment of diseases. This is particularly evident in the treatment of cancers, in which more than 60% of drugs are of natural origin.[12] Hence, a new natural source with anticancer activities would be a valuable tool in cancer chemoprevention. However, anticancer drugs usually have severe side effects such as hepatorenal failure. The development of new compounds that inhibit cell proliferation and/or induce cytotoxicity in tumor cells, with fewer or no side effects, is important and anticipated.

In the course of screening food materials for anticancer activity, we found that a *T. aurantiotincta* ethanol extract (TAE) decreased cell viability in human hepatocellular carcinoma cells (HepG2). In addition, we isolated a new *p*-terphenyl derivative, thelephantin O, and a known compound, vialinin A, as the principal bioactive components of TAE (Figure 1). In the present study, we report the isolation and structural elucidation of these compounds and their selective cytotoxicity against cancer cell lines.

MATERIALS AND METHODS

Materials. The fruiting bodies of *T. aurantiotincta* were collected in Aomori prefecture, Japan, in August 2008 and identified by T. Narita. A voucher specimen (AW0801) has been deposited in the Center for

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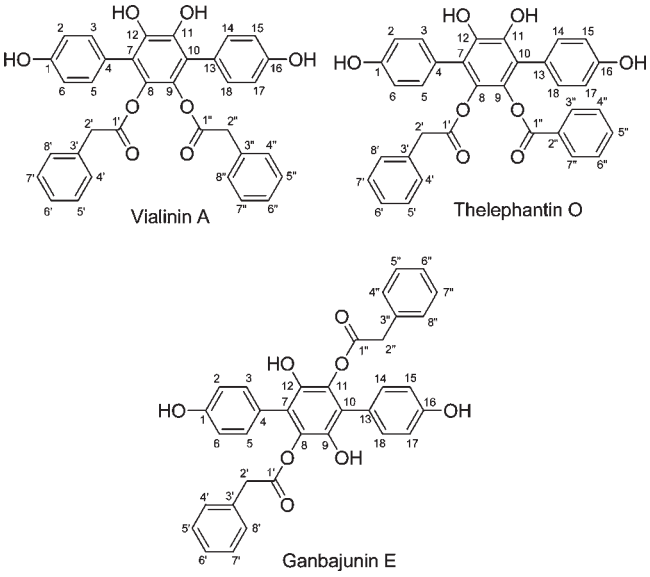

Figure 1. Structures of *p*-terphenyl derivatives.

Promotion of Research and Intellectual Property, Aomori University of Health and Welfare, Aomori, Japan. HepG2 and colonic carcinoma cells (Caco2) were provided by the Riken Cell Bank (Tsukuba, Japan). Human cryopreserved hepatocytes were purchased from Kurabo (Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM) and phosphate-buffered saline (PBS) were purchased from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from BioWest (Nuaille, France). All other chemicals were of reagent grade.

Extraction and Fractionation. The fruiting bodies of *T. aurantiotincta* were dried in the shade for 2 days. The dried bodies (100 g) were homogenized with a Waring blender and extracted with 1000 mL of 99% ethanol three times at room temperature and filtered. The resulting filtrate was evaporated in vacuo to give TAE (9.4 g). Each 300 mg portion of TAE was suspended in 750 μL of methanol and subjected to a Sep-Pak C18 cartridge (Waters Corp., Milford, MA) (20 mL, 5 g) and eluted with a stepwise gradient of 33−50, 66, and 100% methanol in water (each 60 mL), to afford four fractions: TAE1 (810 mg), TAE2 (1044 mg), TAE3 (2475 mg), and TAE4 (1890 mg). TAE3 (1740 mg), which showed the highest inhibitory activity against HepG2, was further separated into 200 fractions using a silica gel column (∅ 40 mm × 400 mm), which was eluted with 2500 mL (2:1), 2100 mL (1:1), and 900 mL (1:2) of *n*-hexane/ethyl acetate. These fractions were checked on TLC plates (silica gel 60 F254 on an aluminum sheet, Merck, Darmstadt, Germany) using a development solvent of chloroform/methanol/water (65:25:4, v/v/v) and a UV lamp (254 nm) for the visualization of spots. According to the TLC patterns, these fractions were collected in four fractions, TAE3-1 (8.7 mg), TAE3-2 (23.3 mg), TAE3-3 (571.6 mg), and TAE3-4 (520 mg).

Isolation of Bioactive Compounds by HPLC. Further separation of bioactive components from TAE3-3 was performed using a HPLC system equipped with an ODS-120T column (∅ 4.6 mm × 250 mm, Tosoh, Tokyo, Japan), an SPD-10A UV−vis detector (detection at 280 nm), an LC-10-AD pump, an SIL-10AD autoinjector (Shimadzu, Kyoto, Japan), and a fraction collector. TAE3-3 (200 mg) was dissolved in 50 mL of 0.1% formic acid/methanol (1:1). Each 50 μL portion of this solution was subjected to HPLC and eluted using 0.1% formic acid/acetonitrile (1:1) as the mobile phase at a flow rate of 0.8 mL/min, to provide compound **1** (t_R 12.15 min; total 38.8 mg) and compound **2** (t_R 13.51 min; total 80.1 mg), along with two other fractions (mixtures of unidentified compounds; fraction 1, 27.3 mg; fraction 2, 43.2 mg).

Cell Culture. HepG2 and Caco2 cells were maintained in ∅ 100 mm dishes (Iwaki Glass, Tokyo, Japan) in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 ng/mL streptomycin (Invitrogen Corp., Carlsbad, CA) at 37 °C in an atmosphere of 5% CO_2 before use. For the experiments, the cells were seeded at a concentration of 4×10^5 cells/∅ 35 mm dish (HepG2) and 2×10^5 cells/∅ 35 mm dish (Caco2) and precultured for 1 day. Human cryopreserved hepatocytes were thawed at 37 °C in a water bath within 2 min and washed with Williams medium E, containing 10% FBS, 5 mg/mL insulin, and 0.5 mM dexamethasone, to remove cell debris. The cells were then seeded at a concentration of 3.3×10^5 cells/12 well plate and precultured for 3 days. For the main culture, fractions and compounds were dissolved in DMSO and diluted in culture media immediately before use (final DMSO concentration, 0.25%). The culture media were changed to a fresh medium, and the cells were incubated for 48 h.

Measurement of Cell Viability. For the detection of the principal bioactive components from TAE, or for the evaluation of selective cytotoxicity against cancer cells, the cell viability of HepG2, Caco2, or noncancerous human hepatocytes was measured in the presence of a crude fraction or a purified component of TAE by neutral red assay, as described previously,[13] with minor modifications. This value indicated anticancer activities against cancer cell lines and cytotoxicity in noncancerous human hepatocytes. A neutral red stock solution (0.4% neutral red in water) was diluted 1:80 (v/v) in PBS. After incubation for 48 h, the cells were incubated with the neutral red solution for 1 h at 37 °C to allow the lysosomes of viable cells to take up the dye. The neutral red solution was then removed, and a mixture of 1% acetic acid and 50% ethanol was added to the cells to extract the neutral red from viable cells, and the mixture was left at room temperature for 30 min. The absorbance of each sample was then measured at 540 nm with a microplate reader. The relative cell viability (%) for control wells containing cell culture medium without samples was calculated as follows: $Abs_{540\ nm\ [sample]}/Abs_{540\ nm\ [control]} \times 100$.

Fractionation Guided by Cell Viability Assay. The dry fruiting bodies of *T. aurantiotincta* (100 g) were extracted with ethanol to give a TAE (9.4 g). As shown in Table 1, TAE showed inhibitory activity against HepG2 in a dose-dependent manner. To the best of our knowledge, this is the first demonstration of the cytotoxicity against cancer cells of *T. aurantiotincta*. For the isolation and structural determination of the causative constituents of this bioactivity, TAE was then fractionated with the guide of a cell viability assay using HepG2. TAE (9 g) was first fractionated by Sep-Pak C18 column chromatography to give TAE1 (810 mg), TAE2 (1044 mg), TAE3 (2475 mg), and TAE4 (1890 mg). As it showed the highest inhibitory activity among the fractions (Table 1), TAE3 (1740 mg) was further separated by silica gel chromatography to afford TAE3-1 (8.7 mg), TAE3-2 (23.3 mg), TAE3-3 (571.6 mg), and TAE3-4 (520 mg). As shown in Figure 2, TAE3-3 (200 mg), which exhibited the strongest inhibitory activity among TAE3-1−TAE3-4 (Table 1), was successively separated by HPLC to furnish compound **1** (38.8 mg) and compound **2** (80.1 mg) as homogeneous compounds, along with fraction 1 (27.3 mg) and fraction 2 (43.2 mg) as mixtures of unidentified compounds. Both compounds **1** and **2** displayed potent inhibitory activity against HepG2 in a dose-dependent manner (Table 1). We regarded these compounds as the principal causative constituents of the anticancer activity of *T. aurantiotincta*.

Spectrometric Analysis. NMR spectra were measured on a JEOL α-400 spectrometer (JEOL, Tokyo, Japan) (400 MHz for ^{1}H and 100 MHz for ^{13}C) in acetone-d_6 or CD_3OD at 23 °C. Chemical shifts are expressed in δ based on the residual solvent peak as a reference standard [δ (H) 2.04, δ (C) 29.8 for acetone-d_6; δ (H) 3.30, δ (C) 43.0 for CD_3OD], and coupling constants were given in hertz. To evaluate the deuterium isotope effects[14] on ^{13}C NMR chemical shifts, the spectra were also measured in CD_3OD or CD_3OH with TMS (δ 0.00) as an internal standard. HMBC was measured with an

Table 1. Effects of Various Fractions and Compounds Derived from TAE on Cell Viability against HepG2[a]

	μg/mL	cell viability (%)		μg/mL	cell viability (%)		μg/mL	cell viability (%)
control	0	100.00 ± 5.41**	control	0	100.00 ± 7.11	control	0	100.00 ± 4.61
TAE	5	88.46 ± 0.77**	TAE3-1	5	103.88 ± 7.57	fraction 1	5	83.17 ± 1.51**
	10	80.22 ± 2.37**		10	102.94 ± 0.98*		10	57.14 ± 3.06**
	20	49.38 ± 5.93**	TAE3-2	5	102.35 ± 1.52	compound **1**	5	45.89 ± 1.39**
	40	26.66 ± 0.64**		10	86.40 ± 2.40**		10	37.57 ± 2.40**
TAE1	5	92.87 ± 7.21**	TAE3-3	5	42.02 ± 5.41**	compound **2**	5	39.12 ± 1.57**
	10	91.43 ± 8.59**		10	27.78 ± 1.30**		10	23.87 ± 1.11**
TAE2	5	97.86 ± 7.13**	TAE3-4	5	105.06 ± 2.60	fraction 2	5	88.75 ± 2.65**
	10	98.38 ± 6.38**		10	92.00 ± 5.18		10	66.93 ± 3.31**
TAE3	5	72.14 ± 11.17**						
	10	34.39 ± 1.42**						
TAE4	5	101.50 ± 4.07**						
	10	97.98 ± 1.44**						

[a] Cells were treated with 0−40 μg/mL of various fractions and compounds for 48 h. Values are expressed as the mean ± SD of three independent experiments. Data were analyzed by ANOVA, followed by Tukey, to compare each group with the control. The differences between the means were significant at *, $p < 0.05$, and **, $p < 0.01$.

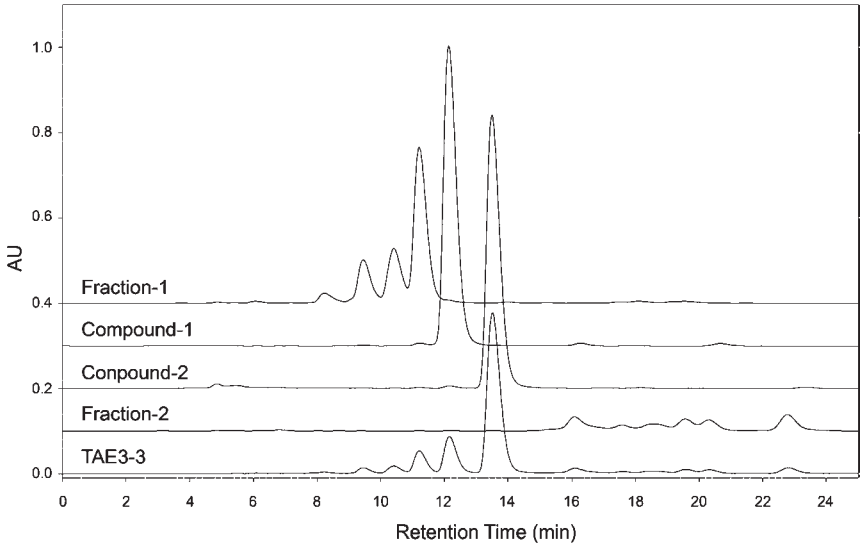

Figure 2. Representative HPLC chromatograms at 280 nm of two fractions and two compounds derived from TAE3-3.

optimized *J* value of 2.5 Hz, and NOESY was measured with a mixing time of 800 ms. IR spectra were measured on a JEOL JIR-WINSPEC 100 IR spectrometer in noted states and are reported in wavenumbers (cm^{-1}). UV spectra were measured on a UV-2550 spectrophotometer (Shimadzu, Kyoto Japan) in methanol. LC-MS/MS analysis was carried out using an API 3000 electrospray ionization mass spectrometer (Applied Biosystems, Tokyo, Japan) equipped with an 1100 series HPLC system (Agilent Technologies, Tokyo, Japan) and a TSKgel ODS-80TsQA column (∅ 2.0 mm × 150 mm, Tosoh) at 40 °C with 0.1% formic acid/acetonitrile (2:3) as the mobile phase at a flow rate of 0.3 mL/min. Compounds were detected by their UV absorption at 280 nm and by their MS in positive-ion electrospray mode (m/z 300−700). The peaks of compound **1** at 549.1 and compound **2** at 563.1 were in turn subjected to MS/MS measurements (m/z 100−700; collision energy = 10 eV). High-resolution mass spectra under fast atom bombardment (FAB) conditions were measured on a JEOL JMS-SX102A mass spectrometer using a glycerol matrix.

Compound **2** (vialinin A): violet amorphous powder; UV (MeOH) λ_{max} 261 nm; IR (KBr) ν_{max} 3487, 3088, 3066, 3031, 1745, 1614, 1526, 1455, 1298, 1253, 1173, 1136, 1121, 986, 830, 725, 698, 537, 517 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data, see Table 2; high-resolution MS (HRFABMS) m/z 563.1694 $[M + H]^+$ (calcd for $C_{34}H_{26}O_8$, 563.1706).

Compound **1** (thelephantin O): violet amorphous powder; UV (MeOH) λ_{max} 229, 262 nm; IR (KBr) ν_{max} 3430, 3064, 3032, 1740, 1612, 1526, 1452, 1430, 1264, 1173, 1107, 1059, 1024, 974, 834, 711, 535 cm^{-1}; ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectral data, see Table 2; HRFABMS m/z 549.1559 $[M + H]^+$ (calcd for $C_{33}H_{24}O_8$, 549.1549).

Synthetic Preparation of Vialinin A. Vialinin A was synthesized according to a procedure reported in the literature[11] with minor modification (see the Supporting Information).

Statistical Analysis. Data are expressed as the mean ± standard deviation (SD) of three independent experiments. Statistical differences in assay values were evaluated with a one-way analysis of variance

Table 2. ^{13}C NMR and ^{1}H NMR Spectral Data of Compound 2 in CD_3OD and Acetone-d_6 [Coupling Constants (Hertz) in Parentheses][a]

	CD_3OD				acetone-d_6	
position	^{13}C	^{1}H	HMBC (position of H)	NOESY (position of H)	^{13}C	^{1}H
1,16	158.1		H2, 3, 5, 6, 14, 15, 17, 18		157.9	
2,6,15,17	116.1	6.76 d (8.8)	H3, 5, 14, 18		115.9	6.82 d (8.8)
3,5,14,18	132.6	7.09 d (8.8)	H2, 6, 15, 17	H2′	132.3	7.11 d (8.8)
4,13	124.8		H2, 6, 15, 17		124.3	
7,10	123.9		H2, 3, 5, 6, 14, 15, 17, 18		123.1	
8,9	134.69		H3, 5, 14, 18		134.53	
11,12	142.6		H3, 5, 14, 18		141.8	
1′,1″	171.2		H2′, 2″		169.6	
2′,2″	41.1	3.25 s		H3, 5, 14, 18, 4′, 8′, 4″, 8″	40.6	3.35 s
3′,3″	134.74		H4′, 5′, 6′, 7′, 8′, 4″, 5″, 6″, 7″, 8″		134.61	
4′,8′,4″,8″	130.4	6.96 br dd (2.2, 7.8)	H5′,7′,5″,7″	H2′	130.1	7.02 d (8.0)
5′,7′,5″,7″	129.6	7.18−7.26 m	H4′,8′,4″,8″		129.2	7.18−7.29 m
6′,6″	128.1	7.18−7.26 m	H4′,8′,4″,8″		127.7	7.18−7.29 m

[a] Chemical shifts are expressed in δ based on the residual solvent peak as a reference standard [δ (H) 3.30 and δ (C) 49.0 for CD_3OD; δ (H) 2.04, and δ (C) 29.8 for acetone-d_6].

(ANOVA) followed by the Tukey multiple-comparison post hoc test. A p value of <0.05 was considered to be significant. All statistical analyses were performed with the Statcel-2 statistical package (OMS Inc., Tokorozawa, Japan).

RESULTS AND DISCUSSION

Structure Determination of Compound 2 (Vialinin A). Compound **2**, isolated as a violet material, was detected at 280 nm as a single peak (t_R 13.51 min) by reverse phase HPLC (Figure 2). The molecular formula was established as $C_{34}H_{27}O_8$ on the basis of its HRFABMS. The strong IR absorptions at 3487 and 1745 cm^{-1} suggested the presence of hydroxy and ester groups. The UV absorption at λ_{max} 261 nm indicated the presence of one or more aromatic rings. The LC-MS/MS analysis of compound **2** showed a peak at m/z 563.1 as the most abundant molecular ion ($[M + H]^+$), along with peaks at m/z 445.1 and 309.1 as product ions. These product ions indicated the elimination of phenylketene (MW 118) and phenylacetic anhydride (MW 254), respectively, from the molecular ion (m/z 563.1), which implied the presence of two phenylacetate groups in compound **2**. The ^{13}C and ^{1}H NMR spectra of compound **2** measured in CD_3OD and acetone-d_6 (Table 2) showed only 13 and 5 signals, respectively, which suggested a symmetrical structure. Finally, two possible structures, vialinin A[15] (terrestrin A[16]) and ganbajunin E[17] (Figure 1), were predicted for compound **2** by detailed 2D NMR analysis, including HMBC and NOESY.

Despite their relatively simple structures, it is difficult to determine the substitution modes in some *p*-terphenyl derivatives because they are symmetrical. Indeed, some papers have described the revision of substitution modes with the assistance of chemical synthesis.[10,11] For the complete determination of the substitution mode, the ^{13}C and ^{1}H NMR data of compound **2** were compared with those of vialinin A,[15] terrestrin A,[16] and ganbajunin E[17] (Figure 1). The identity of vialinin A with terrestrin A has already been reported, and the substitution mode of terrestrin A has been unambiguously established by X-ray crystallographic analysis. The NMR data of compound **2**, observed in acetone-d_6 and CD_3OD, were in complete agreement with those of vialinin A, obtained in acetone-d_6, and of terrestrin A, measured in CD_3OD. Consequently, it was concluded that compound **2** is identical to vialinin A and terrestrin A.

For further verification of the structure and confirmation of the presence of the anticancer effect, vialinin A was also synthesized according to a procedure reported in the literature[11] with minor modifications, in which a Suzuki−Miyaura coupling reaction[18] was employed as the key step. The synthetic vialinin A was identical to compound **2** in both spectral and biological properties, thereby establishing the anticancer effect of vialinin A.

Structure Determination of Compound 1 (Thelephantin O). Compound **1** was isolated as a violet material, which showed a single peak in HPLC analysis (t_R 12.15 min, Figure 2). The molecular weight of compound **1** was found to be 548 from the molecular ion peak of m/z 549.1 ($[M + H]^+$) in LC-MS/MS analysis. The observed fragment ion of m/z 431.1 suggested the elimination of phenylketene (MW 118) from the molecular ion, by analogy with the fragmentation of compound **2** (vialinin A). It was noted that the same fragment ion of m/z 309.1 was detected for both compounds **2** and **1**. This implied that compound **1** possessed the same *p*-terphenyl core as compound **2** and that benzoic phenylacetic anhydride (MW 240) was eliminated from the molecular ion, which would have phenylacetoxy and benzoyloxy groups. The molecular formula was established as $C_{33}H_{25}O_8$, on the basis of its HRFABMS, which gave a mass of 549.1559 $[M + H]^+$, calculated for 549.1549. The presence of hydroxy and ester groups was suggested by IR absorptions at 3430 and 1740 cm^{-1}, and the aromatic UV absorptions at λ_{max} 229 and 262 nm were in accordance with the above considerations.

The ^{1}H and ^{13}C NMR spectra of compound **1** were very similar to those of compound **2**, apart from the absence of one methylene group and the resulting slightly broken symmetry of compound **1** (Table 3).

There were two sets of ^{1}H and ^{13}C signals [6.81 ppm (d, J = 8.5 Hz)/7.17 ppm (d, J = 8.5 Hz) for ^{1}H and 116.1 ppm (CH)/124.9 ppm (C)/132.64 ppm (CH)/158.2 ppm (C) for ^{13}C; 6.70 ppm (d, J = 8.5 Hz)/7.20 ppm (d, J = 8.5 Hz) for ^{1}H

Table 3. ^{13}C NMR and ^{1}H NMR Spectral Data of Compound 1 in CD_3OD [Coupling Constants (Hz) in Parentheses][a]

position	^{13}C	^{1}H	HMBC (position of H)	NOESY (position of H)
1	158.2		H2, 3, 5, 6	
2,6	116.1	6.81 d (8.5)	H3, 5	
3,5	132.64	7.17 d (8.5)	H2, 6	H2′
4	124.9[b]		H2, 3, 5, 6[b]	
7	123.92		H3, 5	
8	134.94		H3, 5, 2″	
9	134.85		H14, 18	
10	123.98		H14, 18	
11	142.7[c]		H14, 18[c]	
12	142.7[c]		H3, 5[b]	
13	124.9[b]		H14, 15, 17, 18[b]	
14,18	132.58	7.20 d (8.5)	H15, 17	H3″, 7″
15,17	115.9	6.70 d (8.5)	H14, 18	
16	158.0		H14, 15, 17, 18	
1′	171.3		H2′	
2′	41.3	3.32 s		H3, 5, 4′, 8′, 3″, 7″
3′	134.3		H2′, 5′, 6′, 7′	
4′,8′	130.1	6.79 br d (8.0)	H2′, 5′, 6′, 7′	H2′
5′,7′	129.3	6.93−7.04 m	H4′, 6′, 8′	
6′	127.9	6.93−7.04 m	H2′, 4′, 5′, 7′, 8′	
1″	166.1		H3″, 4″, 6″, 7″	
2″	130.2		H4″, 6″	
3″,7″	131.0	7.80 br d (7.8)	H4″, 5″, 6″	H14, 18, 2′
4″,6″	129.7	7.41 br t (7.8)		
5″	134.8	7.59 br t (7.6)	H3″, 4″, 6″, 7″	

[a] Chemical shifts are expressed in δ (ppm) based on the residual solvent peak as a reference standard [δ (H) 3.30 and δ (C) 49.0 for CD_3OD]. [b] Because of the overlap of these carbon signals, HMBC correlations were observed simultaneously. [c] Because of the overlap of these carbon signals, HMBC correlations were observed simultaneously.

and 115.9 ppm (CH)/124.9 ppm (C)/132.58 ppm (CH)/158.0 ppm (C) for ^{13}C], indicating the presence of two 4-oxygenated phenyl groups. The oxygen substituents, suggested by the high-field shifted ^{1}H signals at 6.81 and 6.70 ppm, were predicted to be located on the carbons at 158.2 and 158.0 ppm by HMBC analysis.

The presence of a phenylacetoxy group was deduced as follows. A phenyl group was predicted from the ^{1}H signals at 6.79 and 6.93−7.04 ppm, which correlated with the ^{13}C signals at 127.9 ppm (CH), 129.3 ppm (CH), 130.1 ppm (CH), and 134.3 ppm (C) in the HSQC and HMBC spectra. The ^{1}H and ^{13}C signals at 3.32/41.3 ppm (CH_2) indicated a methylene group; connection of the methylene group to the ester carbonyl carbon at 171.3 ppm and to the carbon at 134.3 ppm of the phenyl group were confirmed by HMBC analysis.

The existence of an additional phenyl group was suggested by the correlations of the ^{1}H signals at 7.41, 7.59, and 7.80 ppm with the ^{13}C signals at 129.7 ppm (CH), 130.2 ppm (C), 131.0 ppm (CH), and 134.8 ppm (CH) in the HSQC and HMBC spectra, as well as by the H−H COSY correlations of the ^{1}H signals. The HMBC correlations of the ester carbonyl carbon at 166.1 ppm with the ^{1}H signals at 7.41 and 7.80 ppm, as well as the low-field shift of the protons of the phenyl group, indicated direct

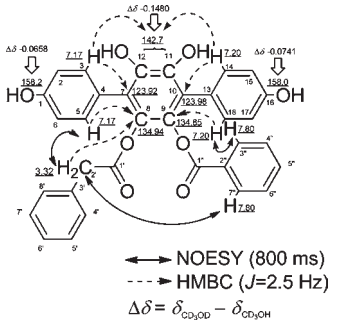

Figure 3. Important HMBC and NOESY correlations of thelephantin O (compound **1**).

connection of the carbonyl to the phenyl group, thereby confirming the presence of a benzoyloxy group in compound **1**.

The existence of a hexasubstituted benzene was suggested by the remaining six quaternary ^{13}C signals at 123.92, 134.94, 134.85, 123.98, and 142.7 ppm (two signals overlap). At this stage, four hydroxy groups remained unassigned, and they should be on the benzene rings of compound **1**. To assign the hydroxy groups, the deuterium isotope effects on ^{13}C chemical shifts were measured. When the ^{13}C NMR of compound **1** was obtained in CD_3OH, significant low-field shifts were observed only for the quaternary signals at 142.7 ppm (two signals overlap, $|\Delta\delta|$ 0.1480), 158.2 ppm ($|\Delta\delta|$ 0.0658), and 158.0 ppm ($|\Delta\delta|$ 0.0741) (the chemical shifts indicated are those obtained in CD_3OD) (Figure 3). Monohydroxy substitution in the 1,4-disubstituted benzene and dihydroxy substitution in the hexasubstituted benzene were therefore confirmed. The hexasubstituted benzene would also possess two 4-hydroxyphenyl groups, one phenylacetoxy group, and one benzoyloxy group.

The substitution mode was elucidated by HMBC with an optimized J value of 2.5 Hz and NOESY analysis with a mixing time of 800 ms (Figure 3). HMBC analysis clarified the substitution of the 4-hydroxyphenyl groups at the carbons at 123.92 and 123.98 ppm, as well as the phenylacetoxy group at the carbon at 134.94 ppm. Because the hydroxy groups were located on the carbons at 142.7 ppm (vide supra), it was deduced that the benzoyloxy group should be located on the remaining carbon at 134.85 ppm. The HMBC correlation of the proton (7.17 ppm) of the 4-hydroxyphenyl group with the carbon at 134.94 ppm, as well as the NOE correlation between the proton at 7.17 ppm and the methylene protons (3.32 ppm) of the phenylacetoxy group, predicted the ortho-relationship between the phenylacetoxy group and the 4-hydroxyphenyl group. The ortho-relationship between the benzoyloxy group and the 4-hydroxyphenyl group on the carbon at 123.98 ppm was also confirmed by the fact that the proton (7.20 ppm) of the 4-hydroxyphenyl group correlated with the carbon at 134.85 in HMBC analysis and with the proton (7.80 ppm) of the benzoyl group in NOESY analysis. The clear NOE correlation between the methylene protons (3.32 ppm) and the proton (7.80 ppm) of the benzoyl group indicated the ortho-relationship of these ester groups. Thus, the novel *p*-terphenyl structure of compound **1**, named thelephantin O, was found to be as shown in Figure 1.

Inhibitory Activity against Viability of Cancer and Noncancerous Cells. The inhibitory activity of vialinin A and thelephantin O against the cell viability of HepG2, Caco2, and noncancerous human hepatocytes was examined. As shown in Table 4, these compounds showed potent inhibitory activity

Table 4. Effects of Vialinin A and Thelephantin O on Cell Viability against HepG2, Caco2, and Hepatocytes[a]

		cell viability (%)		
	concn (μM)	HepG2	Caco2	hepatocytes
control	0	100.00 ± 4.61	100.00 ± 3.57	100.00 ± 3.42
vialinin A (isolated)	2	98.92 ± 5.14	88.78 ± 2.50**	96.79 ± 2.53
	4	77.98 ± 4.12**	80.36 ± 2.50**	99.19 ± 2.11
	8	38.85 ± 5.14**	55.41 ± 0.92**	97.18 ± 2.94
vialinin A (synthesized)	2	95.11 ± 0.51*		
	4	76.91 ± 3.97**		
	8	44.23 ± 3.54**		
thelephantin O (isolated)	2	99.71 ± 1.95	103.45 ± 2.08	96.79 ± 2.53
	4	87.96 ± 0.68**	82.54 ± 6.39**	99.19 ± 2.11
	8	53.91 ± 3.34**	67.74 ± 2.34**	97.18 ± 2.94

[a] Cells were treated with 0−8 μM vialinin A and thelephantin O for 48 h. Values are expressed as the mean ± SD of three independent experiments. Data were analyzed by ANOVA, followed by Tukey, to compare each group with the control. The differences between the means were significant at *, $p < 0.05$, and **, $p < 0.01$.

against both HepG2 and Caco2 in a dose-dependent manner. Notably, these compounds did not show cytotoxicity to noncancerous human hepatocytes. These results clarified the cancer-selective inhibitory effect of vialinin A and thelephantin O, which suggested the potential availability of vialinin A and thelephantin O for anticancer chemoprevention.

In conclusion, the results obtained in the present study demonstrate, for the first time to the best of our knowledge, that *T. aurantiotincta* induced selective cytotoxicity against cancer. In addition, we isolated a new *p*-terphenyl derivative, thelephantin O, and a known compound, vialinin A, as the principal bioactive compounds of TAE. Although the detailed mechanisms of their selective cytotoxicity against cancer cells and availability in vivo remain to be investigated, our results suggest that thelephantin O and vialinin A are attractive compounds for cancer prevention and/or treatment and will encourage further studies to increase our knowledge of the anticancer potential of *p*-terphenyl derivatives.

■ ASSOCIATED CONTENT

Supporting Information. Synthetic preparation of vialinin A. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

TAE, *Thelephora aurantiotincta* ethanol extract; HepG2, human hepatocellular carcinoma cells; Caco2, human colonic carcinoma cells.

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