Two Novel Phenylacetoxylated *p*-Terphenyls from *Thelephora ganbajun* Zang

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Two novel phenylacetoxylated *p*-terphenyl derivatives, namely ganbajunin F (6′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 5′-tetrahydroxy- *p*-terphenyl), and ganbajunin G (5′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 6′-tetrahydroxy-*p*-terphenyl), together with a known compound cycloleucomelone were isolated from the fruiting bodies of *Thelephora ganbajun* Zang. Their structures were established on the basis of spectral (MS, IR, NMR, HMBC, HMQC measurement) and chemical evidence.

Introduction

The basidiomycete *Thelephora ganbajun* Zang, also locally called 'Gan-Ba-Jun', is one of the most favorite edible mushrooms distributed in Yunnan Province, southwest of China (Mao, 1998). It grows in symbiosis with pine trees and has gastronomic interest due to its unique flavor. Despite the analysis of its essential oil (Lu *et al.*, 2000), *T. ganbajun* was poorly investigated for non-volatile constituents. In a recent study on the *T. ganbajun*, we were the first to report the presence of five unusual novel polyphenylacetoxylated *p*-terphenyl derivatives in AcOEt extracts of *T. ganbajun* (Hu *et al.*, in press). These included ganbajunin A-E. Further investigation related to chemical constituents of its MeOH extracts led to the isolation of two novel monophenylacetoxylated derivatives named ganbajunin F (6′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 5′-tetrahydroxy-*p*-terphenyl, **1**) and G (5′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 6′-tetrahydroxy-*p*-ter-phenyl, (**2**), together with a known compound cycloleucomelone (**3**). This report describes the structure elucidation of these two novel compounds.

Results and Discussion

Compounds **1** and **2** were obtained as a mixture appearing together as a single peak in normal phase and reversed phase HPLC profiles. The mixture was deduced to contain a pair of isomers (**1**, **2**) in a ratio 3:1 based on spectroscopic analysis. Their molecular formula $C_{27}H_{22}O_7$ was determined by high resolution negative FABMS ($[M-H]^+$: 457.1304, calc. 457.1287), and NMR data (Table I). Their negative FABMS exhibited a quasi-molecular ion peak at *m/z* 457 ($[M-H]^+$) and characteristic ion peaks due to loss of phenylacetoxyl and methoxyl units at *m/z* 339 ($[M-H-PhCH=C=O]^+$), 324 ($[M-H-PhCH=C=O-CH_3]^+$). Evidence for the existence of four hydroxyl groups in the molecule of both **1** and **2** was provided by the presence of four acetoxy methyl singlet signals (δ 2.35, 2.31, 2.05, 1.63 for compound **1a** and 2.34, 2.30, 2.04, 1.60, for **2a**, respectively) in the ^{1}H NMR spectra of their peracetates (**1a** and **2a**). This was confirmed by their negative FABMS which contained fragmentation ion peaks attributable to the presence of four acetate groups at *m/z* 583 ($[M-H-CH_3C=O]^+$), 541 ($[M-H-2CH_3C=O]^+$), 499 ($[M -H-3CH_3C=O]^+$), 457 ($[M-H-4CH_3C=O]^+$), except a quasi-molecular ion peak at *m/z* 625($[M-H]^+$).

In addition to signals of a phenylacetoxyl and a methoxyl group, resonances (δ 7.13, 6.88, d, J = 8.5Hz; 7.29, 6.79, d, J = 8.5Hz for **1**, and 7.35, 6.89, d, J = 8.5Hz; 7.09, 6.89, d, J = 8.5Hz for **2**, respectively) arising from the protons of AA′BB′ systems observed in their ^{1}H NMR spectra implied the existence of two 1,4-disubstituted aromatic rings which occupied 8 degrees of unsaturation in the molecular of both **1** and **2**. It was confirmed by their ^{13}C NMR spectral data (see Table I). Besides, ^{13}C NMR spectra displays six sp^2 quaternary aromatic carbons (among them, four were oxygenated), which was suggested to be represented by

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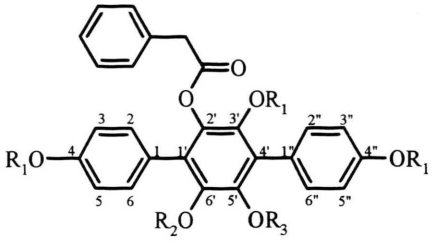
Table I. ^1H (500 MHz), and ^{13}C NMR (125 MHz) Data (δ in ppm, J in Hz) for ganbajunin F–G (**1**–**2**) in CD_3COCD_3.

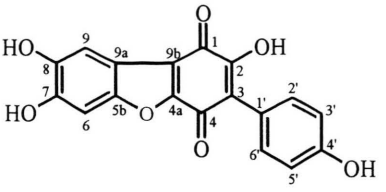
C	δ_C		HMBC (Selected)	δ_H	
	1	**2**		1	2
C (1)	125.7	125.6	H-C (3, 5)		
H-C (2, 6)	133.2	132.9		7.13 (d, J = 8.5 Hz)	7.35 (d, J = 8.5 Hz)
H-C (3, 5)	115.9	115.9		6.88 (d, J = 8.5 Hz)	6.89 (d, J = 8.5 Hz)
C (4)	158.0	157.8	H-C (2, 6)		
C (1′)	129.3	124.8	H-C (2, 6)		
C (2′)	139.1	142.1			
C (3′)	144.1	144.1			
C (4′)	118.7	124.0	H-C (2″, 6″)		
C (5′)	139.2	140.3			
C (6′)	146.9	142.2			
C (1″)	125.5	125.7	H-C (3″, 5″)		
H-C (2″, 6″)	132.3	132.6		7.29 (d, J = 8.5 Hz)	7.09 (d, J = 8.5 Hz)
H-C (3″, 5″)	115.9	115.9		6.79 (d, J = 8.5 Hz)	6.89 (d, J = 8.5 Hz)
C (4″)	157.7	157.8	H-C (2″, 6″)		
CO	172.5	172.2			
CH_2	41.3	41.3		3.62 (s)	3.62 (s)
C (Ph)	135.0	134.9			
H-Ph (*o*)	127.9	127.9		7.21–7.24 (m)	7.21–7.24 (m)
H-Ph (*m*)	130.5	130.7		6.98 (m)	6.98 (m)
H-Ph (*p*)	129.4	129.2		7.21–7.24 (m)	7.21–7.24 (m)
CH_3O	61.1	60.7		3.41 (s)	3.43 (s)

a third hexa-substituted aromatic ring taking the molecular formula and the remaining four degrees of unsaturation into account. HMBC correlations of H-2, 6 with C-1′ and of H-2″, 6″ with C-4′ permitted connection of the three aromatic rings. All these data suggested that **1** and **2** possess a *p*-terphenyl core as previously reported (Yun *et al.*, 2000; Hu *et al.*, 2001; in press) with a phenylacetoxyl, a methoxyl and four hydroxyl groups in the central rings (C-2′, 3′, 5′, 6′) and outer rings (C-4, 4″).

In order to determine the substituted position of functional groups in the structures of **1** and **2**, the ^1H NMR spectra of their peracetates (**1a** and **2a**) were carefully analyzed. Compared the signals arising from acetoxy methyl protons with those of analogues reported (Yun *et al.*, 2000; Hu *et al.*, 2001; in press; Tringali *et al.*, 1987; Elix *et al.*, 1996; Geraci *et al.*, 2000), two resonances (δ2.35, s, 2.31, s, for **1a** and 2.34, s, 2.30, s, for **2a,** respectively) appearing at decidedly lower field were assigned to protons of acetoxyl groups located at C-4 and C-4′ on the outer rings, while the remaining (2.05, s, 1.63, s, for **1a**; 2.04, s, 1.60, s, for **2a**, respectively) were assigned to those located on the central rings, in which, signals (δ 2.05, s, **1**, 2.04, s, **2**) at lower field were assigned to those adjacent to methoxyl group and resonances (δ 1.63, s, **1**; 1.60, s, **2)** at higher field to those adjacent to a phenylacetoxyl group due to anisotropic deshielding by the carbonyl of adjacent phenylacetoxyl group (Briggs *et al.*, 1976). Thus, two isomeric forms (**1a**, **2a**) were proposed for the structures of peracetates of **1** and **2**.

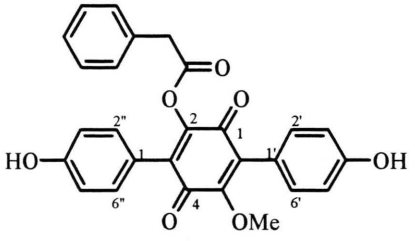
The last step is to determine which structure is consistent with the major component and which with the minor one. Based on analysis of structure itself, we suggested that the minor component represents structure **2** because *p*-terphenyls with *para*-orientated hydroxyl groups in the central ring are not stable and easily revert to correspondent 1,4-terphenylquionones by aerial oxidation (Gripenberg, 1958). In order to confirm it, the white mixture was dissolved in acetone, and its color was changed to be orange after standing overnight. The colorful mixture was purified and a pigment (**4**) was isolated. Its IR spectrum exhibited strong absorptions at 1638, 1612 cm^{-1}, indicating the existence of a quinone system (Quack *et al.*, 1982**)**. It was also abided by a quasi-molecular ion peak at





1 R_1=R_3=H, R_2=CH_3
1a R_1=R_3=$COCH_3$, R_2=CH_3
2 R_1=H, R_2=H, R_3=CH_3
2a R_1=R_2=$COCH_3$, R_3=CH_3

3



4

Fig. 1.

m/z 457([M+2-H]$^+$) displayed in its negative FABMS because a quinone frequently give [M+2]$^+$ ion due to partial hydrogenation in the ion source (Quack *et al.*, 1982). Its negative FABMS fragmentation pattern corresponds to that of **1**, **2** by losing a phenylacetoxyl group and methyl group at *m/z* 337 ([M-H-PhCH=C=O]$^+$), 322 ([M-H-PhCH=C=O-CH_3]$^+$) and its UV, IR, ^{1}H NMR spectra data were in good agreement with those of compounds with 3,6-bis(*p*-hydroxyphenyl)-1,4-benzoquinone carbon skeleton (Hu *et al.*, in press; Quack *et al.*, 1982; Besl *et al.*, 1989). Based on these analysis, **4** was identified to be 2-phenylacetoxy-5-methoxy-3,6-bis(*p*-dihydroxyphenyl)-1,4-benzoquinone, an oxidation product as previously proposed. Moreover, the ratio of **1**, **2** contained by the re-purified mixture was increased to 4.3 : 1 comparing with that of the original one (3:1) by analyzing the integral area of signals of methoxyl groups in their ^{1}H NMR spectra. All these results proposed that **2**, the minor one was really oxidated to **4** and it should be 5′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 6′-tetrahydroxy-*p*-terphenyl (**2**) and the major one, should be 6′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 5′-tetrahydroxy-*p* -terphenyl (**1**).

Experimental

General: Mp: uncorrected. UV spectral: *UV-210* spectrometer, λ_{max} (logε). IR: *Perkin-Elmer 577* spectrometer, KBr pellets ; in cm^{-1}. ^{1}H and ^{13}C NMR: *DRX-500* spectrometer; δ in ppm, J in Hz. MS: *VG Autospec-3000* spectrometer; *m/z* (rel.%). Reversed and normal phase HPLC were carried out on an Waters instrument equipped with 515 pumps, 486 tunable absorbance detector, flow rate: 1 ml/min.

Mushroom material

The fresh fruiting bodies of the basidiomycetes *Thelephora ganbajun* Zang were collected at Wudin county in Yunnan province, P. R. China, in

June, 2000. The voucher specimen (HMAS 52851) was deposited at Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation

Air-dried and finely powdered *T. ganbajun* Zang (585 g) was defatted with petroleum ether (40–60 °C) at r.t. The solvent-free powder was exhaustively extracted with MeOH (1000 ml × 5) to afford a brown gum (50 g), which was taken up in H_2O and partitioned with AcOEt. After evaporation, the aquatic fraction (32 g), was subjected to Sephadex LH-20 column chromatography (5 × 30 cm) and eluted with H_2O containing increasing amounts of MeOH. The fraction II(MeOH : H_2O, 2 : 8, v/v) afforded compound **3** (8 mg) and fraction VII (MeOH : H_2O, 6 : 4, v/v) afforded the mixture (21 mg) of compounds **1**, **2** by further RP18 column chromatography (1.5 × 20 cm) eluting with 15% MeOH and 50% MeOH solution, respectively. The mixture was analyzed by reversed phase HPLC (KNAUER Nucleosil C_{18} column, 4 mm × 20 cm; elution solvents: A: 0.1% HOAc, B: AcCN, linear gradient: 20–100% B; run time: 18 min; detective wavelength: 272 nm) and normal phase HPLC (Shimadazu Zorbox sil column, 4.6 mm × 15 cm; elution solvents: A: hexane, B: isoPrOH, linear gradient: 0–10%B; running time: 18 min; detective wavelength: 272 nm). Their profiles gave single peak at 9.76 min and 11.23 min, respectively.

Ganbajunin F (= 6′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 5′-tetrahydroxy-*p*-terphenyl, **1**), and ganbajunin G (= 5′-methoxy-2′- phenylacetoxy-3′, 4, 4″, 6′-tetrahydroxy-*p*-terphenyl, **2**) were obtained as white powder. M.p. 194–198 °C; negative FABMS, *m/z*: 549 (19, ([M-H+Gly]$^+$), 457 (77, [M-H]$^+$), 339 (100, [M-H-PhCH=C=O]$^+$), 324 (37, [M-H-PhCH=C=O-CH_3]$^+$); IR_{max}(KBr): 3200–3550 (OH), 1725 (C=O), 1609, 1523, 1455, 1423, 1252, 1127, 1099, 1021, 952, 830, 770, 728 cm^{-1}; UV(-MeOH)λ_{max}(logε): 203.5 (4.57), 274 (4.30), 373 (3.71) nm.

6′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 5′-tetracetoxy-*p*-terphenyl (**1a**) and 5′-methoxy-2′-phenylacetoxy-3′, 4, 4″, 6′-tetracetoxy-*p*-terphenyl (**2a**). 1 ml pyridine and 1 ml acetic anhydride were added to the acetone solution (0.5 ml) of the mixture (17 mg). After standing overnight, precipitates were formed as soon as poured into 20 ml cold water. The precipitates were filtered and purified by RP C-18 column chromatography. 21 mg white powder containing **1a** and **2a** was obtained. Negative FABMS, *m/z*: 625 (10, [M-H]$^+$), 583 (13, [M-H-CH_3CO]$^+$), 541 (53, [M-H-2CH_3CO]$^+$), 499 (100, [M-H-3CH_3CO]$^+$), 457 (53, [M-H-4CH_3CO]$^+$), 339 (98, [M-H-4CH_3CO-PhCH=C=O]$^+$), 323 (45, [M-2H-4CH_3CO-PhCH=C=O - CH_3]$^+$); ^{1}H NMR (500 MHz, CD_3COCD_3 , **1a**): 7.40–7.09 (m, aromatic protons), 3.53 (s, CH_2), 3.40 (s, OMe), 2.35, 2.31 (s, 1,4″-$COCH_3$), 2.05 (s, 5′-$COCH_3$), 1.63 (s, 3′-$COCH_3$); ^{1}H NMR (500 MHz, CD_3COCD_3 , **2a**): 7.40–7.09 (m, aromatic protons), 3.51 (s, CH_2Ph), 3.45 (s, OMe), 2.34, 2.30 (s, 1,4″-$COCH_3$), 2.04 (s, 5′-$COCH_3$), 1.60 (s, 3′-$COCH_3$).

Cycloleucomelone (**3**), purple crystals. M.p. 270–272 °C. UV(MeOH)λ_{max}(logε): 208.5 (4.62), 266 (4.37), 302.5 (4.45), 362 (3.76). ^{1}H NMR (500 MHz, CD_3OD): 7.25 (d, *J* = 8.6Hz), 6.79 (d, *J* = 8.6Hz). ^{13}C NMR: 99.3 (C-6), 106.5 (C-9), 115.3 (C-9a), 115.5 (C-3′, 5′), 119.2 (C-9b), 120.0 (C-3), 123.4 (C-1′), 133.4 (C-2′), 147.1 (C-8), 149.7 (C-7), 152.8 (C-5a), 154.2 (C-1), 156.3 (C-4a), 158.0 (C-4′), 178.4 (C-4), 181.04 (C-2). All spectroscopic data are consistent with literature (Jägers *et al.*, 1987).

2-Phenylacetoxy-5-methoxy-3,6-bis(*p*-dihydroxypheny)-1,4-benzoquinone (**4**). Orange powder. Negative FABMS *m/z*: 458(5, [M+2]$^+$), 457(52, [M+2-H]$^+$), 455(13, [M-H]$^+$), 337(100, [M-H-PhCH=C=O]$^+$), 322(25, [M-H-PhCH=C=O-CH_3]$^+$). IR_{max}(KBr): 3200–3525 (br.), 1754, 1638, 1612, 1601, 1512, 1442, 1282, 1229, 1176, 1076, 1089, 827 cm^{-1}. ^{1}H NMR: 7.36 (d, *J* = 8.6Hz, H-2′, 6′), 7.28 (d, *J* = 8.6Hz, H-2″, 6″), 7.18–7.24 (m, H-Ph), 6.87 (d, *J* = 8.6Hz, H-3′, 5′), 6.81 (d, *J* = 8.6Hz, H-2″, 6″), 3.90 (s, OCH_3), 3.72 (s, CH_2).

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