A new phenolic compound with antioxidant activity from the branches and leaves of Pyrus pashia

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The branches and leaves of Pyrus pashia are used to cure abdominal pain and diarrhoea in Chinese folk medicine. A new phenolic compound, 4-O-β-D-glucopyranosylbenzylbenzoate ester (1), along with 21 known ones (2–22) were isolated from the branches and leaves of this plant. Compounds 2 and 3 displayed remarkable antioxidant activities against 1,1-diphenyl-2-picrylhydrazyl radical (IC 50 = 13.26 ± 0.04 μM, 13.28 ± 0.11 μM, respectively), which were at the same grade as positive control rutin. The caffeoyl group in compounds 2 and 3 was supposed to play an important role in the antioxidant activities.

Keywords: Pyrus pashia; phenolic constituents; antioxidant activity

1. Introduction

Pyrus pashia Buch.-Ham. ex D. Don is a kind of arbor distributed in Yunnan, Sichuan and Guizhou Provinces of China growing at an elevation of 650–3000 m (Yu 1986). The flower of P. pashia is a favourite health food for lowering blood lipid in the Yunnan Province of China. Its branches and leaves are used to cure abdominal pain and diarrhoea in Chinese folk medicine. Thirteen compounds were isolated from P. pashia flowers (Liu et al. 2013) and 28 compounds were isolated from branches and leaves of this plant (Bhakuni, Gupta et al. 1971; Bhakuni, Satish et al. 1971; Zhao et al. 2013; Cai et al. 2014). Our previous work indicated that the n-butanol extract of the branches and leaves of P. pashia possesses potent antioxidant activities. Some studies have indicated that antioxidants can reduce the pain. For example, natural antioxidants such as vitamin E and C at low doses are efficient in relieving chronic pelvic pain in women with endometriosis (Santanam et al. 2013), and Combretum zeyheri and Burkea africana leaves with antioxidant activities could alleviate pain and inflammation (Dzoyem & Eloff, 2015).
To find more antioxidant constituents from the branches and leaves of *P. pashia*, a detailed phytochemical investigation on this plant was carried out, which led to the isolation of a new compound, 4-O-$\beta$-D-glucopyranosylbenzyl-benzoate ester (1), and 21 known ones (2–22) (Figure 1). The antioxidant activities of the isolated compounds were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The results showed that compounds 2 and 3 exhibited strong antioxidant activities and compounds 4, 5, 6, 9 and 16 displayed moderate antioxidant activities (Tai et al. 2011; Zhou et al. 2014) against DPPH radical (Table 1).
Table 1. DPPH radical scavenging activities of some samples.

| Sample          | IC$_{50}$ (µM)$^a$ | Sample          | IC$_{50}$ (µM)$^a$ |
|-----------------|---------------------|-----------------|---------------------|
| n-butanaol ext. | 45.54 ± 0.03$^b$    | 5               | 87.57 ± 0.02        |
| 1               | 166.51 ± 0.16       | 6               | 25.84 ± 0.11        |
| 2               | 13.26 ± 0.04        | 9               | 42.70 ± 0.15        |
| 3               | 13.28 ± 0.11        | 16              | 48.72 ± 0.08        |
| 4               | 35.92 ± 0.03        | Rutin           | 13.86 ± 0.04        |

$^a$ Each value is expressed as mean ± SD (n = 3).  
$^b$ IC$_{50}$ value is expressed as mg/mL.

This paper describes the isolation, structural elucidation of the new compound, as well as the DPPH radical scavenging activities of the isolated compounds from this plant.

2. Results and discussion

Compound 1 was obtained as amorphous powder. The molecular formula of C$_{20}$H$_{22}$O$_{8}$ was determined on the basis of its high resolution electron spray ionisation mass spectroscopy (HR-ESI-MS) [M + Na]$^+$ ion peak at m/z 413.1202 (Caled. 413.1206), as well as from nuclear magnetic resonance of $^{13}$C ($^{13}$C NMR) and distortionless enhancement by polarisation transfer (DEPT) data. The infrared spectroscopy (IR spectrum) showed the presence of hydroxyl (3428 cm$^{-1}$), carbonyl (1712 cm$^{-1}$) groups, benzene ring (and 1632 cm$^{-1}$) and ester bond (1080 cm$^{-1}$). The chemical shifts at $\delta_H$ 4.93 (d, 1H, $J = 7.2$ Hz, H-1$^\beta$) and $\delta_C$ 103.0 (d, C-1$^\beta$), 75.8 (d, C-2$^\beta$), 79.0 (d, C-3$^\beta$), 72.2 (d, C-4$^\beta$), 78.8 (d, C-5$^\beta$), 63.4 (t, C-6$^\beta$) indicated a Glc moiety presence in 1. After acid hydrolysis of 1 with 1 M HCl, D-glucose was detected by gas chromatography (GC) analysis. The anomeric proton of D-Glc moiety was determined to have the $\beta$-orientation based on their relatively large $^3$J$_{H_1,H_2}$ values of 7.2 Hz. The nuclear magnetic resonance of $^{13}$H ($^1$H NMR) spectrum of 1 exhibited signals assignable to one aromatic ABX system at $\delta_H$ 8.01 (2H, d, $J = 8.4$ Hz, H-2$^\gamma$, H-6$^\gamma$), 7.57 (1H, t, $J = 7.6$ Hz, H-4$^\gamma$), 7.46 (2H, t, $J = 7.6$ Hz, H-3$^\gamma$, H-5$^\gamma$) and one AA$'XX'$ system at $\delta_H$ 7.40 (2H, d, $J = 8.4$ Hz, H-2, H-6), 7.12 (2H, d, $J = 8.4$ Hz, H-3, H-5), and one oxygen bearing methylene group at $\delta_H$ 5.30 (2H, s, H-7). The chemical shift at $\delta_C$ 132.3 (s, C-1), 131.8 (d, C-2, C-6), 118.7 (d, C-3, C-5), 159.9 (s, C-4), 68.3 (t, C-7) and 132.3 (s, C-1$'$), 131.4 (d, C-2$'$, C-6$'$), 130.4 (d, C-3$'$, C-5$'$), 135.1 (d, C-4$'$) in DEPT and $^{13}$C NMR spectrum also suggested the presence of a 4-hydroxybenzyl alcohol moiety and a benzoyl moiety. In the HMBC spectrum, a correlation from the anomeric sugar proton at $\delta_H$ 4.93 (H-1$^\beta$) to $\delta_C$ 159.9 (C-4) of the 4-hydroxybenzyl alcohol moiety as well as a correlation from the methylene protons at $\delta_H$ 5.30 (H-7) of the benzyl alcohol moiety to the carboxylic carbon at $\delta_C$ 168.7 (H-7$'$) of the benzoxa acid moiety revealed link order of three structure units. The structure was further confirmed by $^1$H-$^1$H COSY relationships (Figure S1) as well as comparison with known compound 4-O-β-D-glucopyranosyl benzyl-4′-hydroxyl benzoate ester (Zidorn et al. 2001). Therefore, the structure of 1 was identified as 4-O-β-D-glucopyranosylbenzyl-benzoxo ester.

The known compounds, 3,5-dicaffeoylquinic acid (2) (Chen et al. 2014), methyl 3,5-dicaffeoylquinic (3) (Chen et al. 2014), methyl 5-O-cafeeylquinate (4) (Chen et al. 2014), 4-hydroxy-trans-cinnamonic acid 4-β-D-glucopyranosylobenzyl ester (5) (Zidorn et al. 2001), 4-hydroxy-cis-cinnamonic acid 4-β-D-glucopyranosyloxybenzyl ester (6) (Zidorn et al. 2001), p-hydroxyphenyl 6-O-trans-p-Coumaroyl-β-D-glucopyranoside (7) (Machida & Kikuchi, 1993), p-hydroxyphenyl 6-O-cis-p-Coumaroyl-β-D-glucopyranoside (8) (Machida & Kikuchi, 1993), 4-hydroxybenzoic acid (9) (Rukchaisirikul et al. 2010), 4-(methoxymethyl) phenyl-1-O-β-D-glucopyranoside (10) (Wang et al. 2012), 3,4-dihydroxyacetophenone (11) (Ruan et al. 2011), 3,4-dihydroxy benzaldehyde (12) (Zhang et al. 2008), p-hydroxy benzaldehyde (13) (Yang et al. 2013), (-)-3-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone-3-O-β-D-
glucopyranoside (14) (Gan et al. 2010), picein (15) (Itoh et al. 2010), caffeic acid (16) (Chai et al. 2004), trans-p-hydroxycinnamic acid (17) (Li et al. 2008), cedrusin (18) (Kizu et al. 1995), (+)-isolarisiresinol (19) (Abe & Tatsuo, 1989), (−)-lарiciresinol (20) (Zhang et al. 2010), 3-O-(β-D-glucopyranosyl)-1-(3’5’-dimethoxy-4-hydroxyphenyl-1-propanone (21) (Guo et al. 2012), myzodendrone (22) (Desmarchelier et al. 2005) were identified by comparisons with spectroscopic data of literatures.

In the DPPH radical scavenging assay, compounds 2 and 3 showed remarkable antioxidant activities (IC_{50} = 13.26 ± 0.04 μM and 13.28 ± 0.11 μM, respectively) which were at the same grade as positive control rutin (IC_{50} = 13.86 ± 0.04 μM) (Table 1). Compounds 4, 5, 6, 9 and 16 displayed moderate antioxidant activities in assay while compound 1 exhibited only weak antioxidant activity.

In the structure-activity relationships, compounds 2 and 3, both bearing two caffeic acid units, were more active than compounds 4 and 16 which both possess only one caffeic acid unit, indicating that caffeic acid moieties was likely responsible for the antioxidant activity in DPPH radical scavenging assay. The antioxidant activities of caffeic acid moieties in compounds 2, 3 and 4 were mainly due to catechol moiety in their structures (Cai et al. 2006; Kim et al. 2006; Kim et al. 2011). Compound 3, which was a methyl ester of 2, had the same strong antioxidant activity as 2, indicating that the carboxyl in 2 and 3 was not essential for the antioxidant activities. Notably, antioxidant activity of compound 6 (4-hydroxy-cis-cinnamonic acid 4-β-D-glucopyranosyloxybenzyl ester) was three times higher than 5 (4-hydroxy-trans-cinnamonic acid 4-β-D-glucopyranosyloxybenzyl ester) in the assay, implying that cis-p-hydroxy cinnamic acid was more important than trans-p-hydroxy cinnamomi acid in DPPH radical scavenging activities.

3. Experimental procedures

3.1. General procedure

Melting points were determined on an XRC-1 Melting Point Apparatus (Sichuan University Science Instrument Co., Chengdu, China) and uncorrected. Methanol (HPLC grade) was purchased from Fisher Chemicals (NJ, Fair Lawn, USA). A Shimadzu UV-VISPS 2550 spectrometer (Shimadzu, Kyota, Japan) was used for UV-VIS spectrum. NMR spectra were recorded with a Bruker DRX-600 or DRX-400 NMR spectrometer (Bruker Corporation, Llarlsruhe, Germany) using Me_{4}Si as an internal standard. GC was determined at Agilent 7890A (Agilent, Santa Clara, USA). MS was measured at an Agilent 1100 LC/MSD TOF spectrometer (Agilent, Santa Clara, USA). The water was purified with a purity water system (Chengdu YouPu Electronic Products Technology Co., Ltd., Chengdu, China and the system model name is UPTL-1-100L). Silica gel (200–300 mesh), RP-18 and Sephadex LH-20 for column chromatography were supplied by the Merck Corporation (Darmstadt, Germany). All other reagents were of analytical grade. GF_{254} plates (Qingdao Marine Chemical Corporation, Qingdao, China) were used for TLC, and Spots were visualised under UV light or by spraying with 3% FeCl_{3} in ethanol or 10% H_{2}SO_{4} in ethanol followed by heating. The 2,2-Diphenyl-1-picrylhydrazyl radical (Beijing J & K Scientific Ltd, Beijing, China) radical was used in radical scavenging assay. l-cysteine methyl ester hydrochloride (Tcishanghai Tech. Ltd, Shanghai, China), N-trimethylsilylimidazole (Beijing J & K Scientific Ltd) and silver carbonate (Beijing J & K Scientific Ltd) were used in acid hydrolysis assay of 1. Microplate reader (Bio-Tek Company, Vermont, USA) was employed to read OD date.

3.2. Plant material

The branches and leaves of P. pashia were collected in Shuangshao County, Kunming, Yunnan Province, China, in March 2008, and identified by Prof. Shu-gang Lu (School of Life Science,
3.3. Extraction and isolation

The dried branches and leaves (40 kg) were crushed into powder and extracted three times with methanol (130 L × 3) at 70°C. After removal of the solvent by evaporation, a residue (5.0 kg) was obtained. This residue was dispersed in H2O (150 L) and then extracted successively with EtOAc (150 L × 3) and n-BuOH (150 L × 3). The EtOAc and n-BuOH layers were respectively concentrated to dryness to give EtOAc ext. (1200 g) and n-BuOH ext. (2500 g). n-BuOH ext. was dissolved in 60 L of distilled water and pass through D101 macroporous resin column (150 × 24 cm i.d.) for three times, eluted with water, methanol and acetone successively. The methanol extraction (1600 g) was further chromatographed on a silica gel column (200–300 mesh), eluted with a gradient of CHCl3/MeOH (100:0, 50:1, 20:1, 9:1, 1:1, 150 L each) to afford Fractions 1-5. The CHCl3/MeOH (50:1) eluate (Fr. 2, 25.1 g) was then separated by ODS column, eluted with MeOH/H2O (50:50, 75:25, 100:0, 5.0 L each), to afford three fractions (Fr. 2a-c). Fr. 2a (1.4 g) was chromatographed on a silica gel column, eluted with CHCl3/MeOH (35:1) to afford 19 (32.7 mg). In a similar way, the CHCl3/MeOH (20:1) eluate (Fr. 3, 16.7 g) was then separated by ODS column, eluted with MeOH/H2O (50:50, 75:25, 100:0, 5.0 L each), to afford three fractions (Fr. 3a-c). Fr. 3a (2.2 g) was subjected on silica gel eluted with CHCl3/MeOH (25:1) to afford 20 (7.1 mg), 21 (5.6 mg), the mixture (20.9 mg) of 15 and 16. Fr. 4c (2.7 g) was subjected on a silica gel column eluted with CHCl3/MeOH (12:1) to obtain 11 (6.8 mg) and 12 (35.8 mg) and Fr. 4c-1. Fr. 4c-1 was subjected on sephadex LH-20 eluted with methanol to afford 9 (17.2 mg) and 13 (5.4 mg). Fr. 5 (2.0 g) was separated on a silica gel eluted with CHCl3/MeOH (7:1) to yield 2 (30.6 mg), the mixture (21.1 mg) of 7 and 8.

3.4 Characterisation results of 4-O-β-D-glucopyranosylbenzyl-benzoate ester (I)

White amorphous power; \([\alpha]_D^{20} = -19.3 (c = 1.1, \text{MeOH})\); UV (MeOH, \(\lambda_{\text{max}} 227.50 \text{nm and } 211.5 \text{nm}\); IR (KBr) \(\nu_{\text{max}} 3426, 1712, 1632, 1270, 1080 \text{cm}^{-1}\); melt point: 163.8 °C. HR-ESI-MS \(m/z 413.1209 \ [\text{M}^+ + \text{Na}]^+ \) (Calcd. for C20H22O8, 413.1212). \(^1\)H NMR (400 MHz, CDOD3) \(\delta, \text{ppm} : 7.40 (2\text{H}, d, J = 8.4 \text{Hz}, H-2, H-6), 7.12 (2\text{H}, d, J = 8.8 \text{Hz}, H-3, H-5), 5.30 (2\text{H}, s), 8.01 (1\text{H}, d, J = 8.4 \text{Hz}, H-2', H-6'), 7.46 (2\text{H}, t, J = 7.6 \text{Hz}, H-3', H-5'), 7.59 (1\text{H}, t, J = 7.6 \text{Hz}, H-4'), 4.93 (1\text{H}, d, J = 7.2 \text{Hz}, H-1'), 3.45 (2\text{H}, m, H-3', H-5'), 3.44 (1\text{H}, m, H-2'), 3.40 (1\text{H}, m, H-4'). \(^13\)C NMR (100 MHz, CDOD3) \(\delta, \text{ppm} : 132.3 (s, C-1), 131.8 (d, C-2, C-6), 118.7 (d, C-3, C-5), 159.9 (s, C-4), 68.3 (t, C-7), 132.3 (s, C-1'), 131.4 (d, C-2', C-6'), 130.4 (d, C-3', C-5'), 135.1 (d, C-4'), 168.7 (s, C-7'), 103.1 (d, C-1''), 75.8 (d, C-2''), 79.0 (d, C-3''), 72.2 (d, C-4''), 78.8 (d, C-5''), 63.4 (t, C-6'').

3.5 Acid hydrolysis of compound I and determination of sugar

The experiment was implemented using the method previously described (Li et al. 2014). A solution of compound (2.1 mg) dissolved in 1 mM HCl (dioxane/H2O, 1:1, 2.0 mL) was heated...
to 90 °C for 2 h. After cooling, the reaction mixture was neutralised with silver carbonate (5 mg), and the solvent was evaporated to dryness under reduce pressure and then extracted with CHCl3 (2.0 mL) and H2O (2.0 mL). The aqueous layer was concentrated to dryness, the residue was dissolved in dry pyridine (0.2 mL), and then L-cysteine methyl ester hydrochloride in dry pyridine (0.03 M, 0.1 mL) was added. The mixture was reacted at 60 °C for 2 h, and 0.1 mL of (trimethylsilyl)-imidazole dissolved in dry pyridine was added. After being held at 60 °C for another 2 h, the reactant was partitioned with n-hexane and H2O (0.1 mL, each). The n-hexane fraction was subjected to gas chromatographic analysis. Agilent 7890A gas chromatograph equipped with an H2 flame ionisation detector. Column: TC-1 capillary column (30 m × 0.25 mm). Column temperature: 280 °C/300 °C, programmed increase: 1°C/min, carrier gas: N2 (1 mL/min). Injector and detector temperature: 260 °C; injection volume: 1 μL; split ratio: 1/10. The sugar residue in compounds 1 was identified by comparison with the standard references: D-glucose (tR 5.78 min)

3.6. Antioxidant activities of some compounds in DPPH radical scavenging assay

The antioxidant activities of some compounds were measured in the DPPH radical scavenging assay with a slight modification of the reported method (Muhammad et al. 2015). Briefly, 150 μL of 0.2 mM DPPH prepared in MeOH were added to 96-well dishes with compounds at different concentrations. The samples were incubated for 30 min in the dark at room temperatures, and the decrease of absorbance at 515 nm was measured against MeOH using a Microplate reader (M200 PRO, Tecan Group Ltd., Switzerland). The DPPH solution was freshly prepared and used within 12 h. All determinations were performed in more than triplicate. The radical scavenging activity of the tested samples was calculated as ‘inhibition percentage’ according to the equation:

\[
\text{Inhibition percentage (\%) = } \left( \frac{A_s - A_b}{A_s - A_{\text{ref}}} \right) \times 100\%
\]

where \(A_s\) is an absorbance of test samples at 30 min; \(A_b\) is an absorbance of control DPPH solution at 30 min and \(A_{\text{ref}}\) is an absorbance of methanol at 30 min. IC50 values denote the concentration of samples required to scavenge 50% of the DPPH free radicals and were calculated from the graph plotted as inhibition percentage against the concentration (Lee et al. 2002; Kim et al. 2006).

4. Conclusion

A new phenolic compound, 4-O-β-D-glucopyranosylbenzyl-benzoate ester (1), along with twenty-one known ones (2–22) were isolated from the branches and leaves of \(P.\ pashia\). Some compounds exhibited potent antioxidant activities in the DPPH radical scavenging assay. Especially, 3,5-dicaffeoylquinic acid (2) and methyl 3,5-dicaffeoylquinate (3) displayed remarkable antioxidant activities which was at the same level as rutin in our assay. The result indicated that these compounds were responsible for the antioxidant activity of \(n\)-butanol extract of the branches and leaves of \(P.\ pashia\). The efficiency of the branches and leaves of \(P.\ pashia\) in treating abdominal pain might be related with these phenolic constituents.

Supplementary material

Experimental details relating to this paper are available online.
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Disclosure statement

No potential conflict of interest was reported by the authors.

Note

1. These authors contributed equally to this study.

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