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ScienceDirect
Phytochemistry 68 (2007) 604–608
PHYTOCHEMISTRY
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Cytotoxic farnesyl glycosides from *Pittosporum pancheri*

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Received 29 June 2006; received in revised form 11 October 2006
Available online 18 December 2006

Abstract

Bioassay guided purification of the ethanolic extract of the bark of New Caledonian *Pittosporum pancheri* Brongn. and Gris (Pittosporaceae) led to the isolation and characterization of two new farnesyl monoglycosides, pancherins A and B. The structure of these compounds were determined on the basis of spectroscopic studies. The new compounds displayed a significant activity in the *in vitro* cytotoxic assay against KB cancer cell line, and pancherin A inhibits weakly farnesyl protein transferase.
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Keywords: *Pittosporum pancheri*; Pittosporaceae; Pancherin; Farnesyl glycoside; Cytotoxicity

1. Introduction

In a systematic search for bioactive compounds in plants from New Caledonia, the ethanolic extract of the bark of *Pittosporum pancheri* Brongn. & Gris (Pittosporaceae) showed toxicity on KB cell line.

P. pancheri is a species endemic to New Caledonia. To our knowledge, there has been no phytochemical study and local uses reported for this species, but other species belonging to this genus are used in traditional medicine in Philippines and Japan (Ragasa et al., 1997; Ogihara et al., 1989).

A literature survey revealed that several species of the genus *Pittosporum* have been studied for their secondary metabolites. Triterpenoids and saponins were isolated from *P. tobira*, *P. undulatum*, *P. phylliraeoides*, *P. pentaurum*, and *P. viridiflorum* (Higuchi et al., 1983; Errington and Jefferies, 1988; Seo et al., 2002; D'Acquarica et al., 2002). These studies have shown that the genins of these compounds were often substituted by sugars (α-L-arabinose and β-D-glucose) and specific groups like angeloyl, tigloyl, senecioyl or 2-methylbutenoyl groups. The present paper reports the isolation and characterization of two new farnesyl glycosides, pancherins A (**1**) and B (**2**), as well as an evaluation of their toxic effects on KB cell line.

2. Results and discussion

Pittosporum pancheri was collected in "Parc Forestier de Montravel" in New Caledonia. The dried bark were extracted by ethanol. This extract inhibited 60% of the KB cell growth at 1 μg ml^{-1}. The extract was fractionated using a standard procedure (Bousserouel et al., 2005) and the activity of the fractions was monitored by their effect on KB cells. This bioassay-guided purification led to **1** and **2** as the most cytotoxic compounds (Fig. 1).

Structures of the new compounds were identified as 1′-*O*-farnesyl-3′-angeloyl-β-D-xylopyranose (**1**), and 1′-*O*-farnesyl-3′-senecioyl-β-D-xylopyranose **(2)** by NMR and mass spectral analysis. Pancherins A and B showed significant cytotoxicity against KB cancer cell line and pancherin A is a weak inhibitor of farnesyl protein transferase (FTase).

The molecular formula of compounds **1** and **2** was established to be $C_{25}H_{40}O_6$ by HREIMS which gave a $[M + Na]^+$ ion at *m/z* 459.2713 and 459.2712, respectively.

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0031-9422/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2006.11.008

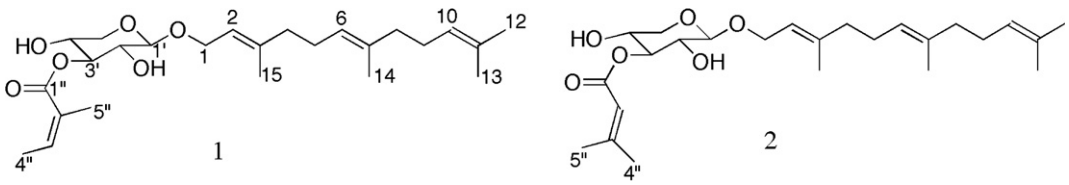

Fig. 1. Pancherins A (**1**) and B (**2**).

The structures of the two molecules were determined by ^{1}H and ^{13}C NMR spectroscopy (Table 1). Three distinct groups were observed by analyzing the correlations in the COSY, HMBC and HSQC spectra: a sugar and a farnesyl groups for both compounds and an angeloyl or a senecioyl moiety for pancherin A and B, respectively.

Compound **1** was a monoglycoside as shown by the presence in its ^{13}C NMR spectrum of one signal at δ_C 101.5 which was correlated in HSQC spectrum with one anomeric proton at δ_H 4.39. The COSY and HSQC experiments allowed complete assignment of all protons and carbons of the sugar unit. A β-D-xylopyranose was identified starting from the anomeric proton at δ_H 4.39 (*d*, $J_{1'-2'} = 6.8$ Hz), on the basis of the large coupling constants observed between H-1′ and H-2′ ($J = 6.8$ Hz); H-2′ and H-3′ ($J = 8.2$ Hz), H-3′ and H-4′ ($J = 8.2$ Hz) and H-4′ and H-5′α ($J = 8.9$ Hz). The coupling constants were within the axial-axial coupling range of 6–14 Hz. The axial positions of H-1′, H-2′, H-3′, H-4′ and H-5′α were confirmed by the presence of cross peaks between H-1′, H-3′

Table 1
^{1}H (300 MHz) and ^{13}C (75 MHz) NMR data of pancherins A (**1**) and B (**2**) in $CDCl_3$

	1		**2**	
	δ_H (m, J Hz)	δ_C	δ_H (m, J Hz)	δ_C
Farnesol				
1	4.15 (*dd*, 11.7, 7.5)		4.16 (*dd*, 7.4, 11.9)	
	4.33 (*dd*, 11.7, 6,.5)	65.5	4.34 (*m*)	65.6
2	5.34 (*t*, 7.2)	119.4	5.33 (*m*)	119.7
3	–	141.9	–	142.0
4	1.85–2.28	39.6	1.96–2.15	39.9
5	1.85–2.28	26.3	1.96–2.15	26.6
6	5.08	123.7	5.08	124.0
7	–	135.4	–	135.4
8	1.85–2.28	39.7	1.96–2.15	40.0
9	1.85–2.28	26.7	1.96–2.15	26.7
10	5.08	124.3	5.08	124.6
11	–	131.4	–	131.3
12	1.65 (*brs*)	25.8	1.66 (*brs*)	25.7
13	1.58 (*brs*)	17.7	1.58 (*brs*)	17.7
14	1.58 (*brs*)	16.0	1.58 (*brs*)	16.0
15	1.65 (*brs*)	16.5	1.66 (*brs*)	16.0
β-D-Xylopyrannose				
1′	4.39 (*d*, 6.8)	101.5	4.33 (*d*, 7.2)	101.8
2′	3.57 (*dd*, 6.8, 8.2)	71.0	3.51 (*dd*, 7.2, 8.9)	71.4
3′	4.88 (*dd*, 8.2)	77.3	4.79 (*dd*, 8.9)	77.2
4′	3.80 (*m*)	69.0	3.77 (*m*)	69.3
5′	3.34 (*dd*, 8.9, 11.9)	65.1	3.29 (*dd*, 9.5, 11.6)	65.6
	4.06 (*dd*, 4.9, 11.9)		4.04 (*dd*, 5.4, 11.6)	
Angeloyl				
1″	–	169.3		
2″	–	140.1		
3″	6.15 (*m*)	127.2		
4″	1.99	16.0		
5″	1.90	20.5		
Senecioyl				
1″			–	168.2
2″			5.76 (*m*)	115.2
3″			–	160.5
4″			1.91 (*brs*)	27.6
5″			2.17 (*brs*)	20.5

and H-5′a and between H-2′ and H-4′, observed in the NOESY spectrum. The presence of an angeloyl group was confirmed first by the resonances of two methyl groups attached on one olefinic quaternary carbon and one olefinic tertiary carbon at δ 140.1 and 127.2, respectively (Higuchi et al., 1983) and, secondly by correlations between the methyl 5″ at δ 1.90 and carbons at δ 169.3, 140.1 and 127.2, C-1″, C-2″ and C-3″, respectively, in the HMBC experiment. The presence of an α,β-unsaturated carbonyl ester was confirmed by the IR spectrum of **1** showing absorption bands at 1718 cm^{-1}. Moreover, the resonances of four methyl groups attached to three olefinic quaternary carbons were observed at δ 16.0, 16.5, 17.7 and 25.8 as well as four methylenes at δ 26.3, 26.7, 39.6, 39.7 and one oxymethylene at δ 65.5 (Table 1). Examination of the correlations of these signals in the HSQC, COSY, and HMBC spectra led to the assignment of the aglycone as farnesol. The chemical shifts of methyl carbons, C-14 and C-15 at δ 16.0 and 16.5 and those of the methylenes C-4 and C-8 at δ 39.6 and 39.7 were very similar to the values observed for (2*E*, 6*E*)-farnesol (Kasai et al., 1986; Tanaka, 1991). Only one methyl resonance for the terminal methyl C-12, appeared above δ 20 in the ^{13}C NMR spectrum of **1** indicating a *E* stereochemistry of the double bonds in the farnesyl chain (Numata et al., 1992). The absence of a signal in the region δ 30–33 in the ^{13}C NMR spectrum of **1** ruled out the other possible configuration (2*E*, 6*Z*), (2*Z*, 6*Z*), or (2*Z*, 6*E*) for the farnesyl moiety (Tanaka, 1991). The deshielding of C-1 (δ 65.5) of the farnesol unit suggested the point of linkage of the sugar unit. Finally, cross peaks between H-1′ (δ 4.39) of xylopyranose and C-1 of farnesol (δ 65.5) and between H-3′ (δ 4.88) and the carbonyl at δ 169.3 observed in the HMBC experiment, allowed us to place the farnesyl and angeloyl groups in position 1′ and 3′ of the xylopyranose, respectively.

Compound **1** was subjected to ESI-MS^n analysis. The pseudomolecular ion is observed at *m/z* 459.3 $(M + Na)^+$ and gave, in MS^2, fragments at *m/z* 359.1 and *m/z* 255.0 corresponding to the loss of $C_5H_8O_2$ (angeloic acid) and $C_{15}H_{26}O$ (farnesol) from the sugar moiety. From *m/z* (459.3 → 359.1) ion, signal is observed, in MS^3, at *m/z* 272.7 corresponding to the fragment $(C_{16}H_{26}O_2Na)^+$ obtained after characteristic glycosidic linkage ($^{1,5}X$ nomenclature proposed by Domon and Costello, 1988). Thus, compound **1**, pancherin A, was assigned the structure 1′-*O*-farnesyl-3′-angeloyl-β-D-xylopyranose.

The spectral data of compound **2** obtained from the NMR experiments were similar to those of **1** except for the $C_5H_7O_2$ groups attached on position 3′ of the sugar moiety. This group was found to be a senecioyl group according to the correlations observed in the COSY, HSQC and HMBC spectra (Higuchi et al., 1983). The presence of the senecioyl group was also confirmed by the resonances of two methyl groups attached on one olefinic quaternary carbon at δ 160.5 and one olefinic methine at δ 115.2. Compound **2**, pancherin B, was assigned the structure 1′-*O*-farnesyl-3′-senecioyl-β-D-xylopyranose.

Compounds **1** and **2** being obtained as oils, it was not possible to carry out crystallographic analysis by X-ray. However, the relative stereochemistry of **1** and **2** was determined by analysis of the coupling constants and NOESY spectra, described previously. Finally, the measurement of optical rotation of the purified sugar after acid hydrolysis and comparison with an authentic sample, confirmed that this sugar is β-D-xylopyranose.

Pancherins A and B exhibited IC_{50} at 3.5×10^{-7} M and 5.1×10^{-6} M, respectively in the *in vitro* KB cell line cytotoxic assay, indicating that a better activity is observed when the sugar unit is substituted by an angeloyl group in position 3′ instead of a senecioyl group at the same position. Taxotere (IC_{50} at 1.2×10^{-10} M) was used as positive control.

The presence of farnesyl groups associated with cytotoxicity for both compounds led us to study their possible interaction with farnesyltransferase (FTase). The Ftase is an enzyme that has been the subject of particular attention in anticancer research over the past years (Leonard, 1997; Johnston, 2001; Sousa et al., 2005). Only- pancherin A (**1**) displayed a very weak activity with 30% inhibition of the enzyme at 10^{-4} M.

3. Conclusion

The study of *Pittosporum pancheri* has led to the isolation of two new molecules: pancherins A and B. This is the first time that this type of compound bearing a farnesylated sugar is extracted from a plant of the Pittosporaceae family. Molecules showed cytotoxicity on KB cells and pancherin A inhibited weakly the protein farnesyltransferase.

4. Experimental

4.1. General experimental procedures

^1H and ^{13}C NMR spectra were recorded on a Bruker Aspect DPX 300 MHz spectrometer using $CDCl_3$ as solvent. HREI-MS experiments were performed using a MALDI-TOF spectrometer (Voyager-DeSTR; Perspective Biosystems) and ESI MS^n experiments were performed on a LCQ deca ion trap spectrometer (Thermo-Finnigan, San Jose CA, USA). The HPLC separations were performed using a Waters autopurification system equipped with a UV/vis diode array detector (190–600 nm) and a Pl-ELS 1000 ELSD detector Polymer Laboratory. IR spectra were obtained on a Perkin Elmer Spectrum BX FT-IR. The UV spectra were recorded in MeOH on a Varian Cary 100 spectrophotometer. Optical rotations were measured on a Jasco™ P1010 polarimeter. Semi-preparative HPLC was performed on a Waters PrepPak cartridge (Kromasil™ C-18, 5 μm, 250 × 10 mm) at 4.7 ml/min using a Waters 2525 Multisolvent delivery system apparatus. Analytical HPLC was performed on a Waters Alliance 2690

(Kromasil™ C-18, 5 μm, 4.6 × 250 mm) with a Waters™ 600^{E} system controller.

4.2. Plant material

The bark of *Pittosporum pancheri* were collected in New-Caledonia in Parc Forestier of Montravel, by one of us (ML) under the reference LIT 0889. A voucher specimen is kept at the herbarium of the Botanical and Tropical Ecology Department of the IRD center of Noumea, New Caledonia.

4.3. Extraction and isolation

The dried powder bark (323 g) of *P. pancheri*, was extracted by ethanol (500 ml × 3) at room temperature to give 10.7 g of crude extract. A part of the extract (1.1 g over 10.7 g) was injected on a semi-preparative column (Kromasil™ C-18, 5 μm, 250 × 10 mm). A gradient mobile phase consisting of acetonitrile/water 60:40–100:0 acetonitrile at 4.7 mL/min in 40 min was used to isolate the compounds. Pancherins A (73.6 mg, yield 0.22% w/w) and B (11.1 mg, yield 0.033% w/w) showed retention times of 31.2 and 29.8 min, respectively.

4.4. Acid hydrolysis

Two hundred milligrams of the crude ethanol extract of the bark were refluxed with 30 ml of 2 N HCl for 4 h 30 min. The mixture was extracted with EtOAc (3 × 15 ml). The acid aqueous layer was neutralised with 0.5 M NaOH and freeze-dried. One sugar was identified as xylose, by comparison with an authentic sample on TLC (R_f 0.65) in MeCOEt/*iso*PrOH/Me_2CO/H_2O (20:10:7:6). The aqueous residue was injected on a preparative LC (VersaPak C-18 spherical, 23 × 53 mm, 15 g). A gradient mobile phase consisting of MeOH/water 20:80–100:0 MeOH at 30 mL/min for 45 min was used to isolate 0.9 mg of xylose. The optical rotation of the purified sugar was measured ($[\alpha]_D$ +46° (MeOH; *c* 0.9)).

4.5. Cell culture assay for cytotoxicity activity

The human tumor cell lines KB, mouth epidermoid carcinoma were originally obtained from the ATCC. The cytotoxicity assays were performed according to a published procedure (Tempête et al., 1995).

4.6. Inhibition of farnesyltransferase protein assay

20 μl of FPP (100 μM) were added to 180 μl of a preincubated (at 30 °C) solution containing 20 μl of the enzyme per well (96 well-plate), dansyl-GCVLS-peptide (1 μM), varied concentrations of the inhibitor in 20 μL of methanol and 160 μl of buffer solution (50 mM Tris/HCl, pH 7.5; 5 mM DTT, 10 μM $ZnCl_2$, 5 mM $MgCl_2$, 0.09% CHAPS). The fluorescence development was then recorded with a fluorimeter Wallac Victor PerkinElmer. 340 and 510 nm were excitation and emission wavelengths, respectively. The reading was carried out every minute during 15 min. A positive standard is carried out with commercial (*E*,*E*)-2-(dihydroxyphosphosphinyl)-methyl-3-oxo-3-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-aminopropanoic acid, 3Na.

4.7. Pancherin A (*1*)

Yellow oil; $[\alpha]_D$ −27.7° ($CHCl_3$, *c* 0.3). UV λ_{max} MeOH (log ε): 214 nm (3.2). IR ν_{max} cm^{-1}: 3430, 2926, 2923, 2850, 2346, 1718, 1462, 1246. ^1H and ^{13}C NMR (300 MHz, $CDCl_3$) see Table 1. HRESIMS, *m*/*z* $[M + Na]^+$ 459.2713 ($C_{25}H_{40}O_6Na$ calcd. for 459.2723).

4.8. Pancherin B (*2*)

Yellow oil; $[\alpha]_D$ −22.4° ($CHCl_3$, *c* 0.4). UV λ_{max} MeOH (log ε): 214 nm (4.5). IR ν_{max} cm^{-1}: 3380, 2930, 2924, 2850, 2375, 1718, 1462. ^1H and ^{13}C NMR (300 MHz, $CDCl_3$) see Table 1. HRESIMS, *m*/*z* $[M + Na]^+$ 459.2712 ($C_{25}H_{40}O_6Na$ calcd. for 459.2723).

Acknowledgements

We express our thanks to Dr. J. Dubois and G. Aubert, who performed the FTase test and the cytotoxic assay, respectively, in the Institute of Chemistry of Natural Products, CNRS, Gif-sur-Yvette. This work was supported by a Grant given by the director of ICSN, M J.-Y Lallemand.

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