Diterpenoids and phenolics from *Pseudopanax simplex*

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Abstract: An ethanolic extract of *Pseudopanax simplex* yielded kaempferol-3,7-di-O-rhamnopyranoside, chlorogenic acid, maltol glucoside, ent-kaur-16-en-18-oic acid, and 4R,5S,9R,10S,13S-ent-pimara-7,15-dien-19-oic acid. Full NMR data for the last compound are reported for the first time.

Keywords: Araliaceae, diterpenoids, NMR, *Pseudopanax simplex*.

INTRODUCTION

The genus *Pseudopanax* encompasses 15 New Zealand endemic species. Further representatives of the genus are distributed in other parts of the Southern Hemisphere, in Southern South America, New Caledonia, and Tasmania, which were formerly part of the super-continent Gondwana. Though trees and shrubs from the genus *Pseudopanax* are widespread in New Zealand, little is known about their chemistry.¹

Fruits of *P. laetus* (Kirk) Philipson and *P. arboreus* (Murray) Philipson yielded oleanan and ursan-type saponins²,³ and from the leaves and bark, aliphatic alcohols, stigmasterol, and β-sitosterol were reported.⁴ The closely related taxon *Schefflera digitata* J. R. Forst. & G. Forst. (Araliaceae) was used by native New Zealand Maori people to treat fungal infections of the skin, which by a phytochemical investigation, has been attributed to the anti-fungal polyacetylene falcarindiol.⁵

The present communication deals with natural products isolated from dried leaves of *Pseudopanax simplex* (G. Forst.) Philipson, which was collected near Dunedin on the South Island of New Zealand.

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RESULTS AND DISCUSSION

Compounds 1–5 were isolated from ethanolic extracts of P. simplex leaves employing reversed (RP) phase column chromatography (CC), silica gel CC, and semi-preparative RP HPLC.

Compound 1 was identified as kaempferol-3,7-di-O-rhamnopyranoside by comparing its MS, 1H, 13C and 2D NMR data with literature values.6–8 Compound 2 was identified as chlorogenic acid by MS, 1H-NMR and by co-chromatography of an authentic sample.9,10 Compound 3 was identified as the γ-pyrene derivative maltol glucoside by employing mass spectrometry and 1D and 2D NMR spectroscopy.11

Mass spectrum of compound 4 indicated a molecular mass of 302 based on the [M – H]− signal at m/z = 301 in the negative mode. Taking into account the 13C NMR spectrum, which displayed twenty signals, a molecular formula of C20H30O2 was established for compound 4. Through comparison of the data obtained from 1H NMR, 13C NMR, HSQC and HMBC experiments with literature data, compound 4 was identified as ent-kaur-16-en-18-oic acid.12,13 This natural product has been reported from a number of sources, including the East Asian medicinal plant Aralia cordata Thunb. (Araliaceae).14

TABLE I. NMR data for 4R,5S,9R,10S,13S-ent-pimara-7,15-dien-19-oic acid or its enantiomer isolated from P. simplex

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<tr>
<th>Position</th>
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<sup>a</sup> Measured in CDCl<sub>3</sub> at 500 MHz and 125 MHz, respectively. Referenced to solvent residual signals and solvent signals of CDCl<sub>3</sub> (1H NMR: 7.26 ppm and 13C NMR: 77.16 ppm), respectively. <sup>b</sup> Signals might be interchangeable. <sup>c</sup> Identified via HCOSY-correlations from H<sub>7</sub>. <sup>d</sup> Overlapping signals.
Structure elucidation of compound 5 was based on MS, ¹H NMR, HCOSY, HSQC, HMBC and NOE experiments (Table I). The MS spectrum of compound 5 was nearly identical to that obtained for compound 4. This and the fact that 2D NMR data also indicated the presence of 20 carbons established that 4 and 5 were isomers with a molecular formula of C₂₀H₃₀O₂. ¹H NMR in combination with HSQC and HMBC data identified the carbon skeleton of 5 as pimara-7,15-dien-19-oic acid.¹⁵

¹³C NMR shift values for methyl groups C₁₇₁₆ and C₁₈₁₇ indicated that these groups were in equatorial position. Signals assignable to protons H-5 and H-9 showed NOE correlations and therefore had to be on the same side of the molecule. In contrast, signals for the methyl group C₂₀ showed no NOE correlations with signals of H-5 and H-9. Therefore, this methyl group had to be situated on the opposite side of the molecule. Conclusively, compound 5 was established as 4R,5S,9R,10S,13S-ent-pimara-7,15-dien-19-oic acid or its mirror image.

This compound is identical to (or the mirror image of) the one isolated in a mixture with another diterpene from a Helianthus (Asteraceae) taxon by Herz and Kulanthaivel in 1984.¹⁵ However, these authors neither isolated the compound in pure form nor did they report a full set of NMR data. Therefore, the physical data of this compound are given in the experimental part and the NMR data are summarized in Table I.

EXPERIMENTAL

Plant material
Leaves of P. simplex were collected on Swampy Summit near Dunedin/Otago/NZ in May 1999 (voucher code: 990519-11) and along the Waitati Valley Road/Dunedin/Otago/NZ in December 2002 (voucher code: 021210-01-021210-04). Voucher specimens are preserved in the herbarium of the Plant Extracts Research Unit, New Zealand Institute for Crop & Food Research at the Chemistry Department of the University of Otago in Dunedin/New Zealand.

Extraction and isolation
An ethanolic extract (10.9 g) obtained from 151 g air-dried and ground leaves of P. simplex (990519-11) was subjected to RP₁₈ column chromatography (CC) employing a gradient of H₂O,
MeCN, CHCl₃, and cyclohexane. The most polar fractions yielded 74 mg of pure compound 1. Less polar fractions contained compound 4 (220 mg). This compound was finally purified by silica gel CC using a gradient of cyclohexane and EtOAc to give 19 mg of compound 4.

A second ethanolic extract (56.4 g) obtained from 750 g of leaf material (021210-01-021210-04) was used for the isolation of compounds 2, 3 and 5. The crude extract (20.0 g) was subjected to silica gel CC employing a gradient of hexane and EtOAc. Finally, the column was eluted with EtOH. The fractions eluting with EtOH (12.9 g) contained compound 4 (220 mg). This compound was finally purified by silica gel CC using a gradient of cyclohexane and EtOAc to give 19 mg of compound 4.

A second ethanolic extract (56.4 g) obtained from 750 g of leaf material (021210-01-021210-04) was used for the isolation of compounds 2, 3 and 5. The crude extract (20.0 g) was subjected to silica gel CC employing a gradient of hexane and EtOAc. Finally, the column was eluted with EtOH. The fractions eluting with EtOH (12.9 g) contained compound 2 and 3 in a mixture with other polar compounds. Further enrichment of 2 and 3 was achieved by isocratic silica gel CC of 1.05 g of the enriched fraction using a mixture of CHCl₃/MeOH/HOAc (70/14/3, v/v/v). Fractions containing primarily 2 (119 mg) were fractionated by RP18 CC employing a gradient of H₂O, MeCN and EtOAc to give 11 mg of pure compound 2. Fractions containing 3 (196 mg) were directly used for NMR analysis.

Compound 5 was isolated from the apolar fractions (1.84 g) of the first silica gel CC. A part (1.02 g) of this fraction was subjected to isocratic silica gel CC using a mixture of EtOAc and cyclohexane (1/12, v/v). Fractions containing 5 and the above mentioned compound 4 (168 mg) were fractionated by semi-prepartive RP 18 HPLC to give 4.5 mg of 5. The following parameters were used for semi-preparative HPLC: column Waters XTerra prep MS C₁₈ (particle size 5 µm); guard column Merck Lichrospher 100 RP₁₈; oven temperature 60 ºC; injection volume 100 µl; flow rate 2.5 ml/min; detection wavelength 205 nm; solvent A H₂O, solvent B MeCN; gradient 65 % to 90 % B in 15 min.

Physical properties of the isolated diterpenes


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REFERENCES