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Taxonomically significant coumarins from three *Philotheca* species (Rutaceae)

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1. Introduction

Philotheca Rudge (Rutaceae: Boronieae) is a genus of 45 species of shrubs and undershrubs endemic to Australia. In a major taxonomic revision of the apparently closely related genera *Eriostemon* and *Philotheca*, Wilson (Wilson, 1998) transferred the majority of species once assigned to *Eriostemon* to the previously small genus *Philotheca* and divided it into four sections: *Philotheca*, *Erionema*, *Corynonema* and *Cyanochlamys*. Wilson differentiated *Philotheca sensu lato* from *Eriostemon* mainly by the presence of 1-nerved petals.

Continuing our chemotaxonomic studies of Australia rutaceous plants (Colombain et al., 2002; Girard et al., 2002, 2005a,b; Roux et al., 2006; Chlouchi et al., 2005, 2006), we report here the results of phytochemical studies of aerial parts of three *Philotheca* species: *Philotheca pachyphylla* (Paul G. Wilson) Paul G. Wilson (*P.p.*), belonging to the section *Philotheca*, and three subspecies, which are included into the section *Erionema*: *Philotheca scabra* (Paxton) Paul G. Wilson ssp. *latifolia* (Paul G. Wilson) Paul G. Wilson (*P.s.l.*), *Philotheca buxifolia* (Sm.) Paul G. Wilson ssp. *obovata* (G. Don.) Paul G. Wilson (*P.b.o.*) and *P. buxifolia* ssp. *buxifolia* (*P.b.b.*).

2. Previous works

Recently, as part of an HPLC evaluation of the petroleum ether extracts of many species of *Philotheca sensu lato*, Sultana et al. (2003) recorded the presence of dipyranocoumarins of the avicennol type in *P. pachyphylla*. To our knowledge, no previous phytochemical studies on the species have been reported.

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3. Present study

3.1. Plant material

P. pachyphylla was collected from the roadside about 30 km west of Coolgardie and a voucher P-01658360 is deposited at the West Australian Herbarium, Perth. *P. scabra* ssp. *latifolia*, (CBG-711088), *P. buxifolia* ssp. *obovata* (CBG-8001650) and *P. buxifolia* ssp. *buxifolia* (CBG-8704555) were collected from living specimens held in the Botanic Gardens of the Australian National Herbarium, Canberra.

3.2. Extraction and isolation

Dried powdered aerial parts – twigs, leaves and flowers – of each species were Soxhlet-extracted sequentially with petroleum ether (bp 40–60 °C), CH_2Cl_2, EtOAc and MeOH and the extracts concentrated under *vaccuum* by rotary evaporation.

The extracts were submitted to successive column chromatographies (silica gel 60 Merck; 0.063–0.200 mm) or fractionated by Centrifugal Partition Chromatography (CPC Kromaton® 230 mL apparatus) with upper and lower layers of the solvents system hexane/EtOH/Water (2:1:2) or heptane/EtOAc/MeOH/Water (2:3:2:3 or 1:4:1:4) or hexane/EtOAc/MeOH/Water (4:1:4:1) or EtOAc/BuOH/Water (10:1:10) or $CHCl_3$/MeOH/BuOH/Water (4:3:0.5:2), as mobile and stationary phases, respectively, using 5 ml min^{-1} flow rate, 1200 rpm rotation speed and 13–37 bar pressure. Fractions were monitored by thin layer chromatography (TLC) and then purified by successive preparative TLC.

3.2.1. P. pachyphylla (P.p.)

The petroleum ether extract (2.6 g) of the leaves (266 g) of *P.p.* yielded avicennin (25 mg), *cis*-avicennol (63 mg), *trans*-avicennol (11 mg) and avicennol ethyl ether (12 mg). The CH_2Cl_2 extract (1 g) of the leaves yielded eriostemoic acid (33 mg). The CH_2Cl_2 extract (3.5 g) of the twigs (350 g) gave *cis*-avicennol (20 mg), *trans*-avicennol (12 mg) and a mixture of tetracosyl ferulate and docosyl ferulate (10 mg). Fractionation of the EtOAc extract (2.6 g) of twigs gave the coumarin glycoside, isobaisseoside (17 mg) and two flavonoid glycosides, hespiridin (11 mg) and 3,5,7,8,3′,4′-hexahydroxyflavone-3-*O*-((6-*p*-coumaroyl)-β-glucopyranoside)-7-methyl ether (64 mg), which was also obtained from the MeOH extract (1.4 g). Finally, eriostoic acid (20 mg) was isolated from the CH_2Cl_2 extract (390 mg) of the flowers (17 g).

3.2.2. P. scabra ssp. latifolia (P.s.l.)

Fractionation of petroleum ether extract (4.7 g) of the leaves (211 g) gave eriostoic acid (24 mg), protobruceol II (3 mg), protobruceol III (4 mg) and the flavonol eriostemin (8 mg). The EtOAc extract (1.4 g) of twigs (358 g) gave scoparon (3 mg), isobaisseoside (10 mg) and scopolin (14 mg).

3.2.3. P. buxifolia ssp. obovata (P.b.o.)

The petroleum ether extract (1.7 g) of the leaves (41 g) of *P.b.o.* yielded deoxybruceol (4 mg), eriobrucinol (4 mg), eriobrucinol regioisomer A (3 mg), protobruceol II (5 mg) and eriostoic acid (2 mg). The EtOAc extract (640 mg) of the leaves yielded protobruceol II (7 mg), protobruceol III (8 mg) and isobaisseoside (97 mg).

3.2.4. P. buxifolia ssp. buxifolia (P.b.b.)

The petroleum ether extract (400 mg) of the leaves (21 g) of *P.b.b.* gave eriobrucinol (14 mg), a mixture of protobruceol II and protobruceol III (4 mg) and eriostoic acid (10 mg). The MeOH extract (100mg) of the leaves yielded isobaisseoside (41 mg).

3.3. Identification of isolated compounds

Avicennin (Sarker et al., 1994), *cis*-avicennol (Gray et al., 1977), *trans*-avicennol (Gray et al., 1975), avicennol ethyl ether (Rashid et al., 1991), eriostoic acid and eriostemoic acid (Duffield and Jefferies, 1963), tetracosyl ferulate (Addae-Mensah et al., 1992), docosyl ferulate (Bernards and Lewis, 1992), hesperidin (Markham, 1976), 3,5,7,8,3′,4′-hexahydroxyflavone-3-*O*-((6-*p*-coumaroyl)-β-glucopyranoside)-7-methyl ether (Sultana et al., 1999), isobaisseoside