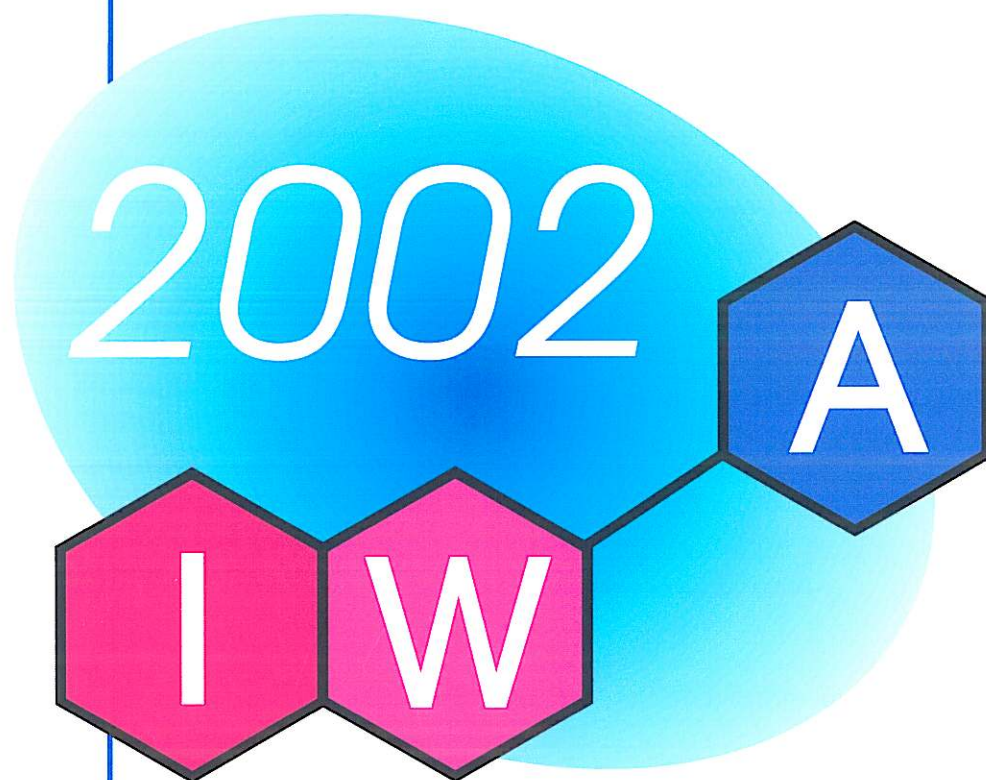


Luis Cabrera

INTERNATIONAL WORKSHOP ON ANTHOCYANINS

"Research and Development in Anthocyanins"



April 17-19, 2002
Paradise Wirrina Cove Resort
Adelaide, Australia

PROCEEDINGS

Polyacylated anthocyanins from *Hebe*

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Four acylated anthocyanins were isolated from flowers of *Hebe* spp. On the basis of LC-MS (Liquid Chromatography–Mass Spectrometry) and NMR (Nuclear Magnetic Resonance) techniques, they were identified as **(1)** cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-caffeoyl)- β -glucopyranosyl)-6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranoside]-5-*O*- β -glucopyranoside, **(2)** cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranosyl)-6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranoside]-5-*O*- β -glucopyranoside, **(3)** cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-caffeoyl)- β -glucopyranosyl)-6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranoside]-5-*O*-[6-*O*-(malonyl)- β -glucopyranoside], and **(4)** cyanidin 3-*O*-[2-*O*-(6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranosyl)-6-*O*-(*E*-*p*-coumaroyl)- β -glucopyranoside]-5-*O*-[6-*O*-(malonyl)- β -glucopyranoside]. Pigments **2** and **4** are novel compounds.

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1. Introduction

The genus *Hebe* (Scrophulariaceae) comprises approximately 250 species and cultivars. It is centered on the New Zealand archipelago comprising around 120 species, the equivalent to 5% of the native angiospermic flora (Lange, 1999). *Hebe* (fig. 1) is also represented in South America, Australia and New Guinea.

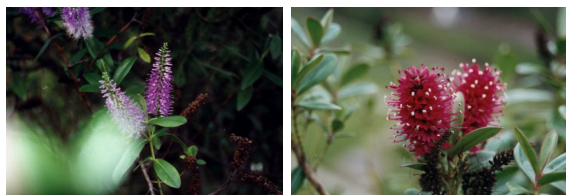


Fig. 1: *Hebe salicifolia* (right) and *Hebe speciosa* (left). Gardens of Fundação Calouste Gulbenkian, Lisboa, Portugal. Photos by Paula Pereira.

The flavonoid content in *Hebe* has been shown to contain 6- and 8-hydroxyflavone glycoside derivatives (Tomás-Barberán *et al.*, 1988; Kellam *et al.*, 1993; Mitchell *et al.*, 2001). The present work reports the anthocyanin content in *Hebe* spp (Cabrita, 1999).

2. Experimental

Isolation. Flowers from *Hebe speciosa*, *H. salicifolia* and *H. x andersonii* (200 g) were extracted with MeOH 0.1% TFA. The crude extract was concentrated, partitioned against hexane and ethyl acetate, washed on Amberlite XAD-7 and precipitated by diethyl ether. Isolation of purified anthocyanins was achieved upon combination of Sephadex LH-20 (size-exclusion) and semi-preparative HPLC (reverse phase) chromatography, affording pigments 1 - 6 (fig. 2).

It has been previously reported that at least pigment 5 was not an extraction artifact (Cabrita, 1999). However, a recent micro-extraction of *Hebe* flowers with three different solvents (methanol, ethanol and acetone with 0.1% TFA) followed by HPLC-DAD analysis did not support those previous findings.

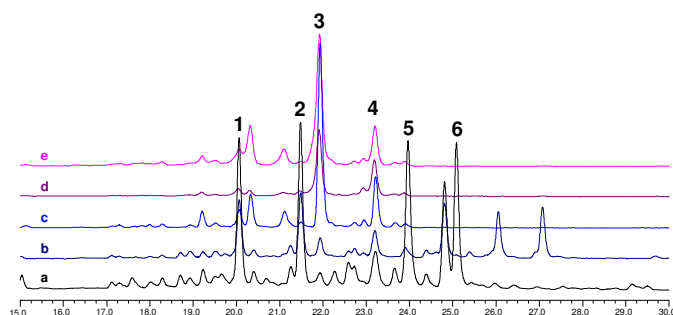


Fig. 2: HPLC-DAD analysis (520±20nm) of *Hebe speciosa* flowers extracted with different solvents during different contact periods: a) methanol - 2 years, b) ethanol - 2 years, c) methanol - 30 min, d) ethanol - 30 min, and e) acetone - 30 min. All extraction solvents were acidified with 0.1% TFA.

UV-Vis and TLC. High $A_{UV-max}/A_{Vis-max}$ ratios (140-220%) and large mobilities on microcellulose (FHW: hRf 52-89 and BAW: hRF 42-58) suggested the presence of two aromatic acylating groups.

NMR. All examined pigments were in accordance with a cyanidin 3-sophoroside-5-glucoside, where the 6^{II}-position of the 3-glucoside moiety was found to be attached to a coumaroyl group, and the 6^{IV}-position of the 2^{II}-glucosyl moiety was correlated with either a caffeoyl (pigments 1-3-5) or a coumaroyl group (pigments 2-4-6). Pigments 3-4 and 5-6 were further acylated in the 6^{III}-position of the 5-glucoside with malonyl and methyl-malonyl moieties, respectively (fig. 3).

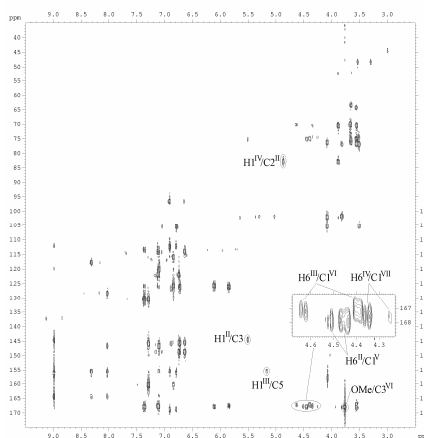


Fig. 3: HMBC spectrum of pigment 5. Inset: detail showing the cross-peaks between the protons in the 6-position of the sugar residues (II, III, IV) and the carboxyl moieties of substituent acyl groups (V, VI, VII).

LC-MS. Fragmentation patterns and molecular ions [M+]/m/z 1081 (1), 1065 (2), 1181 (5), and 1165 (6), were in accordance with the mass expected for the proposed structures and NMR data (fig. 4).

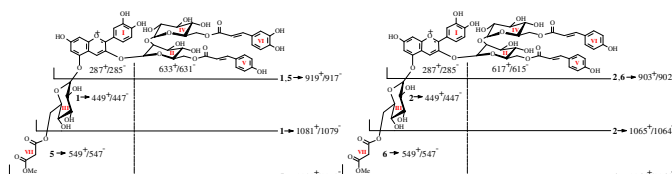


Fig. 4: fragmentation patterns obtained by LC-APCI-MS in the positive (+) and negative (-) mode for pigments 1, 5 (right) and 2, 6 (left) isolated from *Hebe* spp.

3. Conclusions

Six anthocyanins were isolated and identified from flowers of *Hebe* spp. Pigments 1-2 were found to be based on cyanidin 3-sophoroside-5-glucoside acylated with coumaroyl-caffeoyl (1) and coumaroyl-coumaroyl (2) moieties. Pigments 3-4 and 5-6 were found to be the 5-malonylglucoside and 5-(methyl)malonylglucoside derivatives of 1 and 2, respectively.

4. References

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Acknowledgments

LC gratefully acknowledges Fundação para a Ciência e Tecnologia, Portugal, for scholarships PRAXIS XXI BD/5290/95 and BPD/1639/2000. LC is very grateful to José Sena da Fonseca (Fundação Calouste Gulbenkian, Portugal) for plant material, Paula Pereira for photos of *Hebe*, and Prof. Fernando Santana (UNL/FCT) for a HPLC-DAD. The authors wish to thank Pia Knutsen (Institute of Food Research and Nutrition, Denmark) for valuable discussions.