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The chlorogenic acids of *Hemerocallis*

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Abstract

The chlorogenic acids in a methanolic extract of freeze-dried *Hemerocallis* (Chinese day lily) have been qualitatively profiled by LC–MS^3. Three caffeoylquinic acids (3-CQA (**I**), 4-CQA (**III**) and 5-CQA (**II**)), three *p*-coumaroylquinic acids (3-*p*CoQA (**IV**), 4-*p*CoQA (**VI**) and 5-*p*CoQA (**V**)) and two feruloylquinic acids (3-FQA (**VII**) and 4-FQA (**IX**)) have been identified. The dominance of the 3-acyl and 4-acyl CGA relative to the 5-acyl isomer is unusual and makes this material a convenient source of these commercially non-available chlorogenic acids. A minor revision has been made to the structure-diagnostic hierarchical key previously developed for characterising chlorogenic acids by LC–MS^n.

Keywords: Caffeoylquinic acids; Chinese day lily; Chlorogenic acids, *p*-coumaroylquinic acids; Feruloylquinic acids; *Hemerocallis* spp.; LC–MS^n

1. Introduction

Classically, chlorogenic acids (CGA) are a family of esters formed between certain *trans* cinnamic acids and (−)-quinic acid (1L-1(OH),3,4/5-tetrahydroxycyclohexane carboxylic acid) (Clifford, 1999, 2000; IUPAC, 1976). The cinnamic acids most commonly encountered are caffeic, *p*-coumaric and ferulic; 3,4-dimethoxycinnamic and sinapic are encountered less frequently (Clifford, 2000, 1999; Clifford, Knight, & Kuhnert, 2005a). CGA are widespread and any plant producing them generally contains several subgroups (defined by the number and identity of the constituent cinnamic acids), and usually several isomers within each subgroup. This complexity can make identification difficult, especially as very few authentic chlorogenic acids are commercially available (Clifford, 2003). In the absence of authenticated pure materials, the necessary chlorogenic acids must be isolated and characterised by conventional chemical methods. Alternatively, crude extracts, in association with facile TMAH *trans*-esterification (Clifford, 2003; Clifford, Kellard, & Birch, 1989a, Clifford, Kellard, & Birch, 1989b; Clifford et al., 2005a; Clifford, Knight, & Kuhnert, 2005b, 2005c), can be used as surrogates without the need for lengthy and sometimes problematic isolation of individual compounds, provided that these have distinctive chromatographic profiles. However, the list of suitable source materials is still comparatively limited (Clifford, 2003). While surveying traditional Chinese food products, we observed that Chinese day lily (*Hemerocallis* spp.) had an unexpected CGA profile that potentially made it a useful source of *p*-coumaroylquinic acids. In this paper, we report the characterisation by LC–MS^3 of the chlorogenic acids in a methanolic extract of freeze-dried *Hemerocallis*.

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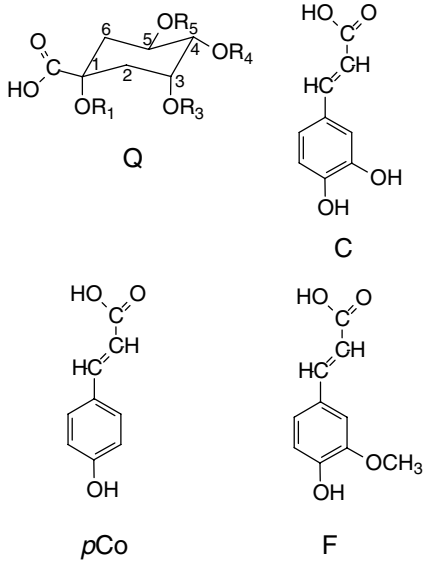
2. Materials and methods

2.1. Materials

The *Hemerocallis* was obtained from Qidong County, Hunan Province, PRC as a finely ground freeze-dried powder that is used as a constituent of certain traditional herbal medicines. This powder (1 g) was extracted (4×25 ml, 25 min each) with 70% v/v aqueous methanol using an HT1043 solid–liquid continuous extraction system (Tecator, Bristol, UK) (Clifford, Johnston, Knight, & Kuhnert, 2003). The bulked extracts were treated with Carrez reagents (1 ml reagent A plus 1 ml reagent B) (Egan, Kirk, & Sawyer, 1981) to precipitate colloidal material, diluted to 100 ml with 70% v/v aqueous methanol and filtered through a Whatman No. 1 filter paper. The methanol was removed at room temperature from an aliquot (ca. 3 ml) by evaporation with nitrogen (N-Evap-111, Organomation Associates Inc, Berlin, MA, USA) and the aqueous extracts were stored at −12 °C until required, thawed at room temperature, centrifuged at 1360×*g*, filtered through a 0.42 μm filter, and used directly for LC–MS.

2.2. LC–MS^n

The LC equipment (ThermoFinnigan, San Jose, CA, USA) comprised a Surveyor MS Pump, autosampler with 20 μl loop, and a PDA detector with a light-pipe flow cell (recording at 320, 280 and 254 nm, and scanning from 240 to 600 nm). This was interfaced with an LCQ Deca XP Plus mass spectrometer fitted with an ESI source (ThermoFinnigan, San Jose, CA, USA) and operating in data-dependent MS^n mode to obtain fragment ion *m*/*z*. As required, more sensitive targeted MS^n experiments were used to seek compounds with a particular molecular ion that might otherwise have been overlooked, e.g., *m*/*z* 353 to seek 1-CQA, and *m*/*z* 515 to seek diCQA. MS operating conditions (negative ion) had been optimised using 5-caffeoylquinic acid (**II**) (Sigma Chemical Company, Poole, Dorset, UK) with a collision energy of 35%, ionisation voltage of 3.5 kV,



Name and abbreviation	Number	R_1	R_3	R_4	R_5
3-*O*-caffeoylquinic acid (3-CQA)	I	H	C	H	H
5-*O*-caffeoylquinic acid (5-CQA)	II	H	H	H	C
4-*O*-caffeoylquinic acid (4-CQA)	III	H	H	C	H
3-*O*-*p*-coumaroylquinic acid (3-*p*CoQA)	IV	H	*p*Co	H	H
5-*O*-*p*-coumaroylquinic acid (5-*p*CoQA)	V	H	H	H	*p*Co
4-*O*-*p*-coumaroylquinic acid (4-*p*CoQA)	VI	H	H	*p*Co	H
3-*O*-feruloylquinic acid (3-FQA)	VII	H	F	H	H
5-*O*-feruloylquinic acid (5-FQA)	VIII	H	H	H	F
4-*O*-feruloylquinic acid (4-FQA)	IX	H	H	F	H

Q = quinic acid, C = caffeic acid, *p*Co = *p*-coumaric acid, F = ferulic acid

Fig. 1. The structure of chlorogenic acids found in *Hemerocallis* (IUPAC numbering) (IUPAC, 1976).