Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 95 (2006) 574–578
Food Chemistry
www.elsevier.com/locate/foodchem

# The chlorogenic acids of *Hemerocallis*

Michael N. Clifford [a,*], Weiguo Wu [b], Nikolai Kuhnert [c]

[a] *Centre for Nutrition and Food Safety, School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey GU2 7XH, UK*
[b] *College of Food Science and Technology, Hunnan Agricultural University, Changsha, Hunan 410128, PR China*
[c] *Synthetic and Biological Organic Chemistry Laboratory, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK*

Received 11 October 2004; accepted 14 January 2005

**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*.

* Corresponding author. Tel.: +44 1483 689703; fax: +44 1483 576978.
*E-mail address:* m.clifford@surrey.ac.uk (M.N. Clifford).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.01.045

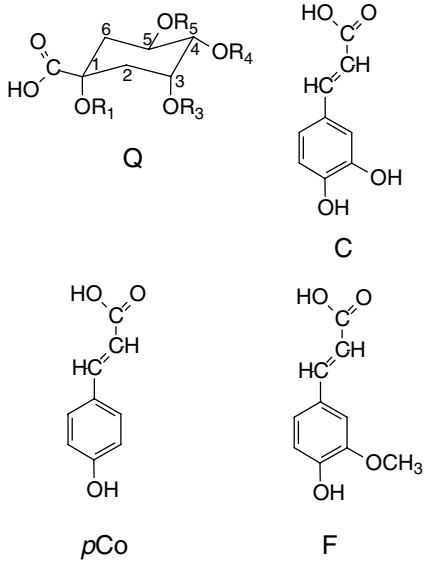
## 2. Materials and methods

### 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 ($4 \times 25$ 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).

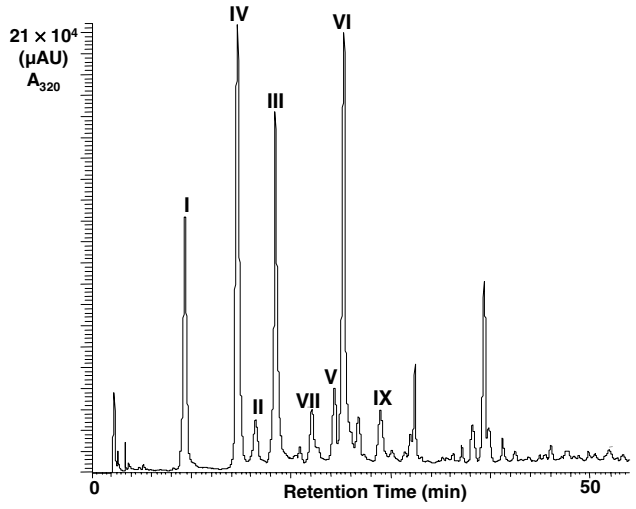



Fig. 2. Chromatogram of a *Hemerocallis* extract recorded at 320 nm.

capillary temperature 350 °C, sheath gas flow rate of 65 arbitrary units, and auxiliary gas flow rate of 10 arbitrary units (Clifford et al., 2003). All analyses were repeated a minimum of three times.

CGA separation was achieved on a 150 × 3 mm column containing Luna 5 μm phenylhexyl packing (Phenonemex, Macclesfield, UK). Solvent A was water: acetonitrile:glacial acetic acid (980:20:5 v/v, pH 2.68):solvent B was acetonitrile:glacial acetic acid (1000:5 v/v). Solvents were delivered at a total flow rate of 300 μl $min^{-1}$. The gradient profile was 4% B to 33% B linearly in 90 min, a linear increase to 100% B at 95 min, followed by 5 min isocratic, and a return to 4% B at 105 min, and 5 min isocratic to re-equilibrate (Clifford et al., 2003).

## 3. Results

All data for CGA presented in this manuscript use the recommended IUPAC numbering system (IUPAC, 1976) and specimen structures are presented in Fig. 1. A representative chromatogram and selected mass spectra are presented in Figs. 2–5.

## 4. Discussion

An initial screening of the *Hemerocallis* extract located eight chlorogenic acids. The patterns of fragmentation observed at $MS^3$ and the previously published structure-diagnostic hierarchical key (Clifford et al., 2003) enabled these to be identified as three isomers of caffeoylquinic acid (3-CQA (**I**), 4-CQA (**III**) and 5-CQA (**II**)), three isomers of *p*-coumaroylquinic acid (3-*p*CoQA (**IV**), 4-*p*CoQA (**VI**) and 5-*p*CoQA (**V**)), and two isomers of feruloylquinic acid (3-FQA (**VII**) and 4-FQA (**IX**)). The *p*-coumaroylquinic acids were present at a greater concentration than the caffeoylquinic acids, and the feruloylquinic acids were comparatively minor constituents. Unusually, for each subgroup, the

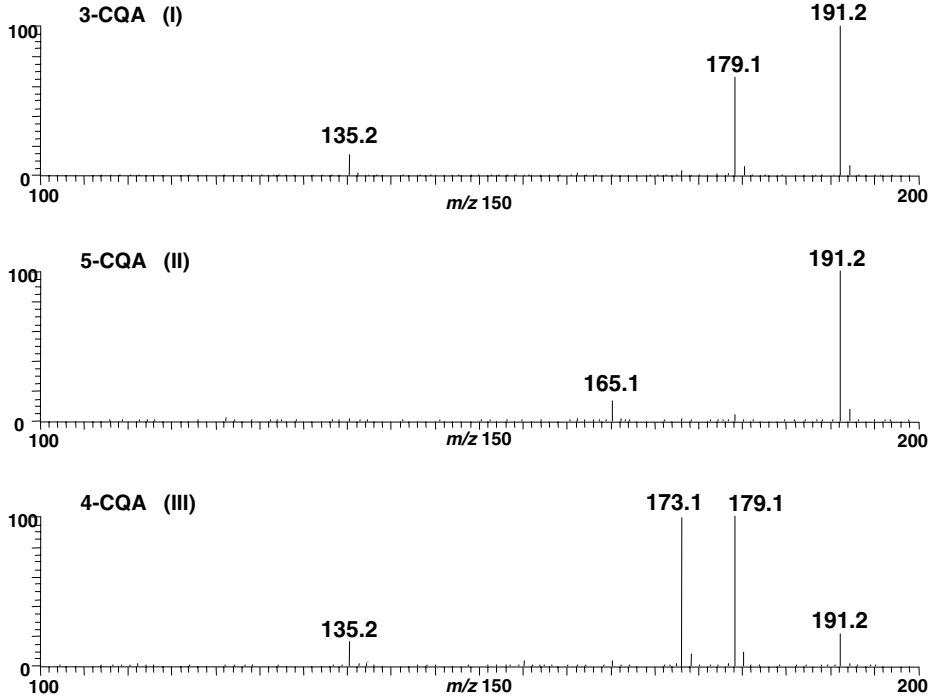



Fig. 3. $MS^2$ spectra for caffeoylquinic acids.

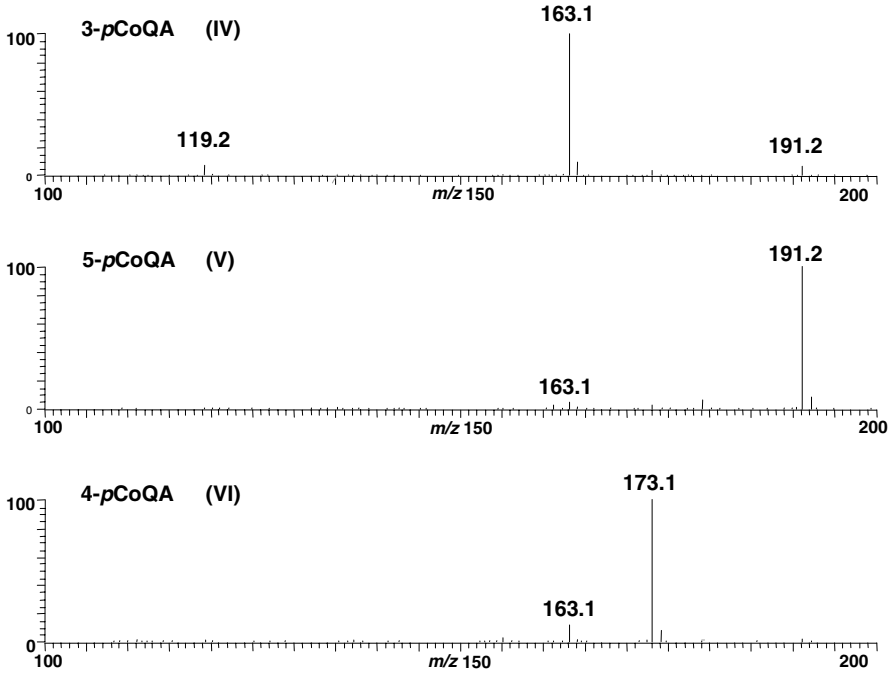



Fig. 4. $MS^2$ spectra for *p*-coumaroylquinic acids.

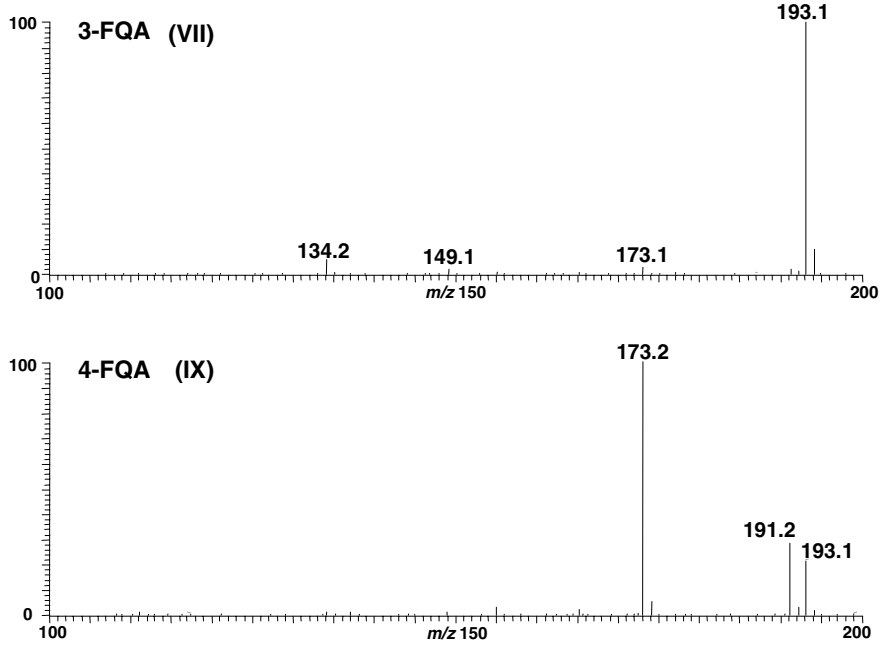



Fig. 5. $MS^2$ spectra for feruloylquinic acids.

concentrations of the 3-isomer and 4-isomer greatly exceeded the concentration of the 5-isomer (Fig. 2). Targeted $MS^2$ experiments failed to locate any diCQA, or 1-CQA. As far as we are aware, this is the first report of CGA in this species.

The use of a targeted (*m*/*z* 353) $MS^2$ experiment to seek the relatively uncommon 1-CQA demonstrated that the fragmentation of 4-CQA (**III**) under these operating conditions is slightly different from general $MS^n$, as used previously to develop the structure-diagnostic hierarchical key (Clifford et al., 2003). Originally we had reported that 4-CQA (**III**) produced an $MS^2$ base peak at *m*/*z* 173, accompanied by an *m*/*z* 179 secondary fragment ion (ca. 70% of base peak). In the targeted mode, these two ions are approximately equal in intensity, and which one forms the base peak varies from scan to scan. This

difference is not sufficient to invalidate the diagnostic key. The targeted spectra for the other seven CGA were unchanged, (see Figs. 3–5).

There are comparatively few reports of cinnamic acid conjugates in monocotyledons, the best known examples being glycerol conjugates in pineapple (Takata & Scheuer, 1976) and oats (Daniels & Martin, 1968), and shikimic acid conjugates in dates (Harborne, Williams, & Greenham, 1974; Maier, Metzler, & Huber, 1964). However, appropriate targeted $MS^2$ experiments failed to find such conjugates in this *Hemerocallis* sample.

Chlorogenic acids are widespread in dicotyledenous plants (Clifford, 2000, 1999). Usually, 5-CQA (**II**) is the dominant CGA, although, in stone fruits and maté, 3-CQA (**I**) tends to dominate, and in apples, 5-CQA (**II**) and 4-*p*CoQA (**V**) dominate their respective subgroups. However, as far as we are aware, there have not been any reports of convenient sources of 3-*p*CoQA (**IV**) and 4-CQA (**III**). Accordingly, extracts of *Hemerocallis* complement those sources of commercially non-available chlorogenic acids already identified (Clifford, 2003).

## Acknowledgements

The authors thank the China Scholarship Council for funding a postdoctoral fellowship for Dr Weiguo Wu. Technical assistance from Mr H. Roozendaal is gratefully acknowledged.

## References

Clifford, M. N. (1999). Chlorogenic acids and other cinnamates – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 79*, 362–372.

Clifford, M. N. (2000). Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture, 80*, 1033–1042.

Clifford, M. N. (2003). The analysis and characterization of chlorogenic acids and other cinnamates. In C. Santos-Buelga & G. Williamson (Eds.), *Methods in polyphenol analysis* (pp. 314–337). Cambridge: Royal Society of Chemistry.

Clifford, M. N., Johnston, K. L., Knight, S., & Kuhnert, N. (2003). A hierarchical scheme for LC–$MS^n$ identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry, 51*, 2900–2911.

Clifford, M. N., Kellard, B., & Birch, G. G. (1989a). Characterisation of caffeoylferuloylquinic acids by simultaneous isomerisation and transesterification with tetramethylammonmium hydroxide. *Food Chemistry, 34*, 81–88.

Clifford, M. N., Kellard, B., & Birch, G. G. (1989b). Characterisation of chlorogenic acids by simultaneous isomerisation and transesterification with tetramethylammonium hydroxide. *Food Chemistry, 33*, 115–123.

Clifford, M. N., Knight, S., & Kuhnert, N. (2005a). Characterization by LC–$MS^n$ of four new classes of chlorogenic acids in green coffee beans: dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids and feruloyl-dimethoxycinnamoylquinic acids. *Journal of Agricultural and Food Chemistry* (submitted).

Clifford, M. N., Knight, S., & Kuhnert, N. (2005b). Characterization by LC–$MS^n$ of four novel classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. *Journal of Agricultural and Food Chemistry* (submitted).

Clifford, M. N., Knight, S., & Kuhnert, N. (2005c). Discriminating between the six isomers of dicaffeoylquinic acid by LC–$MS^n$. *Journal of Agricultural and Food Chemistry*. (in press).

Daniels, D. G. H., & Martin, H. F. (1968). Antioxidants in oats: glyceryl esters of caffeic and ferulic acid. *Journal of the Science of Food and Agriculture, 19*, 710–712.

Egan, H., Kirk, R. S., & Sawyer, R. (1981). *Pearson's chemical analysis of foods*. London: Churchill Livingstone.

Harborne, J. B., Williams, C. A., & Greenham, J. (1974). Distribution of charged flavones and caffeoylshikimic acid in Palmae. *Phytochemistry, 13*, 1557–1559.

IUPAC. (1976). Nomenclature of cyclitols. *Biochemical Journal, 153*, 23–31.

Maier, V. P., Metzler, D. M., & Huber, A. F. (1964). 3-*O*-caffeoylshikimic acid (dactylifric acid) and its isomers, a new class of enzymic browning substrates. *Biochemical and Biophysical Research Communications, 14*, 124–128.

Takata, R. H., & Scheuer, P. J. (1976). Isolation of glyceryl esters of caffeic and *p*-coumaric acids from pineapple stems. *Lloydia, 39*, 409–411.