Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 64 (2003) 617–624
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Analysis of ellagitannins and conjugates of ellagic acid and quercetin in raspberry fruits by LC–MS^n

William Mullen[a], Takao Yokota[b], Michael E.J. Lean[c], Alan Crozier[a,*]

[a]*Plant Products and Human Nutrition Group, Graham Kerr Building, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK*
[b]*University of Glasgow Department of Human Nutrition, Queen Elizabeth Building, Royal Infirmary, Glasgow G31 2ER, UK*
[c]*Department of Bioscience, Teikyo University, Utsunomiya 320-8551, Japan*

Received 11 February 2003; received in revised form 18 March 2003

Dedicated to the memory of Professor Jeffrey B. Harborne

Abstract

The use of gradient reversed phase HPLC with diode array and MS^n detection for the analysis of ellagitannins, ellagic acid conjugates and quercetin conjugates in raspberries (*Rubus idaeus* L.) is described. MS^n is a particularly powerful tool for the analysis of trace levels of natural products in impure extracts as interpretation of fragmentation patterns, coupled in some instances with knowledge of HPLC retention properties, can facilitate the partial identification of components when reference compounds are unavailable.

Keywords: *Rubus idaeus* L.; Raspberries; Rosaceae; Ellagitannins; Ellagic acid conjugates; Quercetin conjugates; LC–MS^n; Zoom scan

1. Introduction

Raspberries (*Rubus idaeus* L.) have high levels of vitamin C and phenolic compounds and as a consequence are a rich source of dietary antioxidants (Kalt et al., 1999; de Ancos et al., 2000; Kähkönen et al., 2001). The phenolics include eleven anthocyanins the major components being cyanidin-3-sophoroside, cyanidin-3-(2^G-glucosylrutinoside) and cyanidin-3-glucoside (Francis, 1972; Torre and Barritt, 1977; Mullen et al., 2002a) and a number of ellagic acid-containing compounds (Rommel and Wrolstad 1993; Zafrilla et al., 2001) together with trace levels of quercetin-based flavonol conjugates (Henning, 1981). Ellagic acid is of particular interest from a dietary viewpoint as it has been reported to have antiviral (Corthout et al., 1991) and antioxidant activity (Kalt et al., 1999) and to provide protection against cancers of the colon (Rao et al., 1991), lung and oesophagus (Stoner and Morse, 1997). This report describes the analysis of ellagitannin, ellagic acid conjugates and quercetin conjugates (compounds **1**–**6**).

Ellagic acid has been detected in many studies with fruits, nuts and berries in which total ellagic acid was measured by analysing the ellagic acid content of extracts after acid hydrolysis (Daniel et al., 1989; Häkkinnen et al., 2000). In raspberries free ellagic acid comprises only a minor part of the total ellagic acid pool (Zafrilla et al., 2001) and ellagitannins are the primary source of ellagic acid released by acid hydrolysis (Mullen et al., 2002b). Sanguiin H-6 (**2**), the major ellagitannin (Haddock et al., 1982; Tanaka et al., 1993), accounts for over 30% of the antioxidant activity of the fruit (Mullen et al., 2002b). However, many other ellagic acid containing compounds are also present in raspberries. They range from simple sugar conjugates, such as ellagic acid-4-arabinoside (**1**) (Zafrilla et al., 2001) to tetrameric ellagitannins with molecular weights up to 3738 (Tanaka et al., 1993). To investigate the potential health benefits of ellagic acid in raspberries it is important to be able to monitor not only ellagic acid and sanguiin H-6 (**2**) but also the full complement of ellagic acid containing compounds, as following

* Corresponding author. Tel.: +44-141-330-4613; fax: +44-141-330-5394.
E-mail address: a.crozier@bio.gla.ac.uk (A. Crozier).

0031-9422/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00281-4

ingestion the absorption and bioavailability of simple sugar conjugates and the larger ellagitannins may differ markedly.

This paper reports on the analysis of quercetin and ellagic acid containing compounds in raspberries by HPLC–MS^n, which has the advantage that in the absence of reference compounds it can facilitate a degree of structural elucidation with ng quantities of sample that would not be possible with traditional single stage mass spectrometry. Previous publications have reported on the identification of anthocyanins in raspberries (Mullen et al 2002a,b). Because they have early reversed phase HPLC retention times, anthocyanins are readily separated from ellagitannins and both ellagic acid and quercetin conjugates. They can be further distinguished because when analysed by electrospray mass spectrometry, anthocyanins require positive ionisation while negative ionisation provides superior data with the ellagic acid and quercetin based compounds.

2. Results and discussion

2.1. HPLC–MS^n and diode array analysis of raspberries

HPLC coupled to an absorbance monitor has been the main method used to analyse ellagic acid. Photodiode array detectors are now more widely available and have been used to identify ellagic acid and quercetin based compounds in raspberry juice (Rommel and Wrolstad, 1993). However, distinguishing between sugar conjugates of ellagic acid and quercetin, both of which are present in raspberries, is compounded by the fact that both the conjugates and the aglycones have very similar absorbance spectra. HPLC linked to a single quadrupole mass spectrometer has been used to analyse anthocyanins in raspberries (Mullen et al., 2002a) since they can be distinguished on the basis of their mass spectra. This, however, is not the case with ellagic acid and quercetin conjugates, as both aglycones have a M_r of 302, and when linked to the same sugar both types of conjugates yield very similar negative ion mass spectra. Nevertheless, these compounds can be distinguished when an ion trap mass spectrometer is used to produce a series of daughter mass spectra (MS^n). Fragmentation of the molecular ion ($[M-H]^-$) of the conjugates yields a MS^2 fragment at m/z 301 through loss of the sugar moiety. When the m/z 301 ion is further fragmented a MS^3 spectrum is produced that can distinguish between quercetin and ellagic acid. With a collision energy of 50% the quercetin m/z 301 ion fragments producing MS^3 ions at m/z 179 and 151 while the equivalent ellagic acid ion yields MS^3 fragments at m/z 257 and 229.

Gradient reversed phase HPLC, with absorbance detection and MS^n, in the negative ion mode with an electrospray interface, was used to analyse the ellagic acid and quercetin containing compounds in an extract from freshly picked raspberries. Absorbance traces obtained at 280 and 365 nm, with 18 numbered peaks, are illustrated in Fig. 1. Identification of most compounds was achieved by MS^2 or MS^3 analysis. However, high resolution "zoom scan" analysis was required for some of the ellagitannins in order to determine their charge state. Zoom scan data are collected by using slower scans in a narrow mass range, which improves the resolution of the $^{12}C/^{13}C$ isotopes of an analyte. This allows the charge state to be determined unambiguously which in turn facilitates the correct determination of the molecular weight. Where standards were available MS and co-chromatography were carried out to confirm the identification. The identifications of peaks 1–18, based on MS^2 or MS^3 data as well as λ_{max}, are summarised below and presented in Table 1.

Peak 1 ($t_R = 21.1$ min, $\lambda_{max} = 250$ nm) had a $[M-H]^-$ at m/z 783, which was shown to be doubly charged by zoom scan analysis (Fig. 2A), giving a true mass of 1568. MS^2 of the doubly charged ion produced a sequence of singly charged fragments. The major ions were at m/z 1265 (M−302, loss of a hexahydroxydiphenoyl [HHDP] group), m/z 1103 (m/z 1265−162, loss of a glucosyl group), m/z 933 (m/z 1103−170, loss of a gallate unit), m/z 631 (m/z 933−302, cleavage of an additional HHDP group). On the basis of the MS^2 spectra this peak is tentatively identified as sanguiin H-10 (**3**) (Fig. 3), which has been isolated from *Sanguisorba officinalis* (Tanaka et al., 1985).

Peaks 2 and 3 ($t_R = 26.4$ and 27.4 min, $\lambda_{max} = 350$ nm) were not identified. Although both compounds had a $[M-H]^-$ at m/z 771 that fragmented on MS^2 to produce ions at m/z 591 (M−180), 505 (M−180−86) and 301 (M−180−86−204). MS^3 analysis of the m/z 301 ion showed fragmentation patterns different from those of quercetin and ellagic acid. The identities of peaks 2 and 3 are therefore unknown.

Peak 4 ($t_R = 28.1$ min, $\lambda_{max} = 250$ nm) had a $[M-H]^-$ at m/z 1401, which zoom scan showed to be doubly charged (Fig. 2B) giving a true mass of 2804. MS^2 of the doubly charged ion produced singly charged fragments at m/z 1869, 1567 (1869−302, loss of HHDP) and 1265 (1567−302, loss of HHDP). The remaining fragments at m/z 933 and 631 were the same as obtained with peak 1, sanguiin H-10 (**3**). On the basis of the mass spectral data, peak 4 is identified as the trimer, lambertianin C (**4**) (Fig. 3) which has previously been detected in raspberry leaves (Tanaka et al., 1993).

Peak 5 ($t_R = 28.6$ min, $\lambda_{max} = 355$ nm) had a $[M-H]^-$ at m/z 625 and the MS^2 spectrum had fragments at m/z 463 (M−162, loss of a glucosyl unit) and m/z 301 (M−324, loss of two glucosyl units). MS^3 of m/z 301 produced two major ions at m/z 179 and 151, which matches the fragmentation pattern of quercetin. On the

basis of the mass spectra data and co-chromatography, peak 5 is identified as quercetin-3,4′-diglucoside.

Peak 6 ($t_R = 29.7$ min, $\lambda_{max} = 250$ nm) had a $[M-H]^-$ at m/z 1869 which was a major MS ion in the spectrum of peak 4, lambertianin C (**4**). The MS^2 fragmentation pattern of m/z 1869 matched that of lambertianin C with ions at m/z 1567, 933 and 631. On the basis of the mass spectra and previously published data peak 6 is identified as sanguiin H-6 (**2**) (Fig. 3), the presence of which has been reported in *R. idaeus* by Haddock et al. (1982) and Tanaka et al. (1993).

Peak 7 ($t_R = 31.1$ min, $\lambda_{max} = 360$ nm) had a $[M-H]^-$ at m/z 433 and MS^2 yielded an ion at m/z 301 (M−132 loss of a pentosyl unit). The MS^3 spectrum of the m/z 301 fragment produced two major ions at m/z 257 and 229, which matches the fragmentation pattern of ellagic acid. On the basis of the mass spectra data this compound is identified as an ellagic acid pentose conjugate.

Peak 8 ($t_R = 32.5$ min, $\lambda_{max} = 250$ nm) had a $[M-H]^-$ at m/z 859, which was shown to be doubly charged by zoom scan analysis (Fig. 2C) giving a true mass of 1720. MS^2 of the doubly charged ion produced singly charged fragments at m/z 1417 (M−302, loss of HHDP), m/z 1085 (m/z 1417–332, loss of glucosyl and galloyl groups), m/z 783 (m/z 1085−302, loss of HHDP unit), m/z 631 (m/z 783–152, loss of a galloyl moiety) and m/z 301 (HHDP). On the basis of the mass spectral data peak 8 may contain either a nobotanin A or malabathrin B-like compound (Yoshida et al., 1986, 1992). Both these dimeric ellagitannins have a M_r of 1720. As with peak 1, we can find no reports on the presence of nobotanin A, malabathrin B or any of their isomers in raspberries.

Peak 9 ($t_R = 32.7$ min, $\lambda_{max} = 360$ nm) had the same mass spectrum as peak 7 indicating the presence of a second ellagic acid pentose conjugate. One of these conjugates may be an ellagic acid-4-arabinoside (Fig. 3) which has previously been detected in raspberries by Zafrilla et al. (2001).

Peak 10 ($t_R = 33.8$ min, $\lambda_{max} = 360$ nm) had a $[M-H]^-$ at m/z 301 which yielded MS^2 fragments at m/z 257 and 229. On the basis of the mass spectra data and co-chromatography, peak 10 is identified as ellagic acid.

Peaks 11 and 12 ($t_R = 36.1$ and 37.8 min, $\lambda_{max} = 355$ nm) both had a $[M-H]^-$ at m/z 609, which yielded a MS^2 fragment at m/z 301 (M−308, loss of a hexose-rhamnose sugar). In both instances the MS^3 spectrum of m/z 301 yielded quercetin-like ions at m/z 179 and 151. As no intermediate quercetin-hexose or quercetin-rhamnose MS^2 ions were detected it is concluded that the cleaved sugar is a rutinose-type fragment (i.e. glucose-rhamnose, which is known not to fragment into its constitutive sugars). The two peaks have identical mass spectra and it is concluded from the chromatographic behaviour that the earlier eluting peak 11 is probably a quercetin-galactosylrhamnoside while peak 12 is a quercetin-glucosylrhamnoside. The identity of peak 12

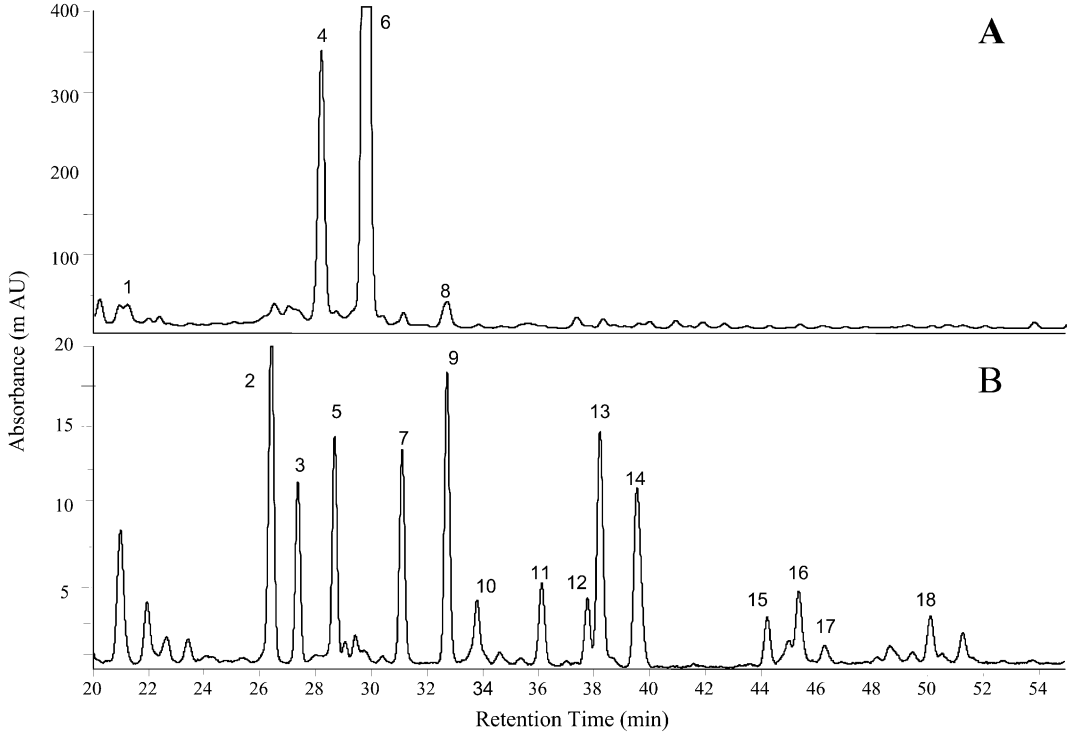

Fig. 1. Gradient reversed phase HPLC of an extract of Glen Ample raspberries. The 20–55 min segment of a 60 min gradient of 8–21% acetonitrile in water containing 1.0% formic acid. Detection at (A) 280 nm and (B) 365 nm. Numbering of peaks refers to their subsequent identification by mass spectrometry—see Table 1.

Table 1
Summary of the properties of compounds detected in an extract of raspberries by HPLC with diode array and MS^n detection[a]

Peak	R_t (min)	λ_{max} (nm)	Compound	$[M-H]^-$ (m/z)	MS^2 ions (m/z)	MS^3 ions (m/z)
1	21.1	250	Sanguiin H-10	[783]52	1265([M−H]$^-$-HHDP), 1103([M−H]$^-$-HHDP-Glc), 933([M−H]$^-$-HHDP-Glc-Galloyl), 631([M−H]$^-$-HHDP-Glc-Galloyl-HHDP)	
2	26.4	350	Unknown	771	591([M−H]$^-$−180), 505([M−H]$^-$−180−86), 301([M−H]$^-$−180−86−204)	271, 255, 179
3	27.4	350	Unknown	771	591([M−H]$^-$−180), 505([M−H]$^-$−180−86), 301([M−H]$^-$180−86−204)	271, 255, 179
4	28.1	250	Lambertianin C	[1401]52	1869, 1567(1869-HHDP), 1265(1869-HHDP-HHDP), 933(1869-HHDP-HHDP-Glc-Galloyl), 631(1869-HHDP-HHDP-Glc-Galloyl-HHDP)	
5	28.6	355	Quercetin-3,4′-diglucoside	625	463([M−H]$^-$−Glc), 301[Q]([M−H]$^-$-Glc-Glc)	179, 151
6	29.7	250	Sanguiin H-6	1869	1567([M−H]$^-$-HHDP), 1265([M−H]$^-$-HHDP-HHDP), 933([M−H]$^-$-HHDP-HHDP-Glc-Galloyl), 631([M−H]$^-$-HHDP-Glc-Galloyl-HHDP)	301[HHDP]
7	31.1	360	Ellagic acid pentose conjugate	433	301[HHDP]([M−H]$^-$-Pent)	257,229
8	32.5	250	Nobotanin A-/malabathrin B-like	[859]2	1417([M−H]$^-$-HHDP), 1085([M−H]$^-$-HHDP-Glc-Galloyl), 783([M−H]$^-$-HHDP-Glc-Galloyl-HHDP), 631([M−H]$^-$-HHDP-Glc-Galloyl-HHDP-Gallic acid), 301[HHDP]	
9	32.7	360	Ellagic acid-pentose conjugate	433	301[HHDP]([M−H]$^-$-Pent)	257, 229
10	33.8	360	Ellagic acid	301	257, 229	
11	36.1	355	Quercetin-galactosylrhamnoside	609	301[Q]([M−H]$^-$-Gal-Rham)	179, 151
12	37.8	355	Quercetin-3-rutinoside	609	301[Q]([M−H]$^-$-Glc-Rham)	179, 151
13	38.2	355	Quercetin-3-glucoside	463	301[Q](M−H]$^-$-Glc)	179, 151
14	39.5	350	Quercetin glucuronide	477	301[Q]([M−H]$^-$-GlcAC)	179, 151
15	44.2	350	Methyl ellagic acid-pentose conjugate	447	315([M−H]$^-$-Pent)	301[HHDP]
16	45.4	355	Ellagic acid-4-acetylxyloside	475	301[HHDP] (M$^-$-XylAc)	257, 229
17	46.3	355	Methyl ellagic acid pentose conjugate	447	315([M−H]$^-$-Pen)	301[HHDP]
18	50.1	355	Ellagic acid-4-acetylarabinoside	475	301 HHDP]([M−H]$^-$-AraAc)	257, 229

Q, quercetin; Glc, glucosyl; Gal, galactosyl; Rham, rhamnosyl; Pent, pentosyl; XylAc, acetylxylosyl; AraAc, acetylarabinosyl; GlcAC, glucuronoyl; HHDP, hexahydroxydiphenoyl; $[M-H]^-$, molecular ion; R_t, retention time.

[a] Peak numbers and retention times refer to HPLC traces in Fig. 1.

was confirmed as quercetin-3-*O*-glucosylrhamnoside (quercetin-3-*O*-rutinoside, alias rutin) by reference to an authentic standard (structure not shown).

Peak 13 ($t_R = 38.2$ min, $\lambda_{max} = 355$ nm) produced a $[M-H]^-$ at m/z 463 and an MS^2 spectrum with the predominant ion at m/z 301 (M−162, loss of a glucosyl unit). This yielded a quercetin MS spectrum with major fragments at m/z 179 and 151. This mass spectrum and co-chromatography with an authentic standard established that peak 13 contains quercetin-3-glucoside (structure not shown).

Peak 14 ($t_R = 39.5$ min, $\lambda_{max} = 350$ nm) had a $[M-H]^-$ at m/z 477 and MS^2 produced a major fragment at m/z 301 (M−176, loss of glucuronyl group) while MS^3 yielded major ions at m/z 179 and 151, which matches the fragmentation pattern of quercetin. On the basis of the mass spectral data and chromatographic retention peak 14 is identified as a quercetin glucuronide, the presence of which has previously been reported in raspberries (Ryan and Coffin, 1971).

Peak 15 ($t_R = 44.2$ min, $\lambda_{max} = 350$ nm) had a $[M-H]^-$ at m/z 447, MS^2 produced a major ion at 315 (M−132 loss of pentose). MS^3 yielded a major fragment at m/z 301. No MS^4 was available so this peak could either be a methyl-ellagic acid pentose conjugate or a methyl-quercetin pentose conjugate. However, as

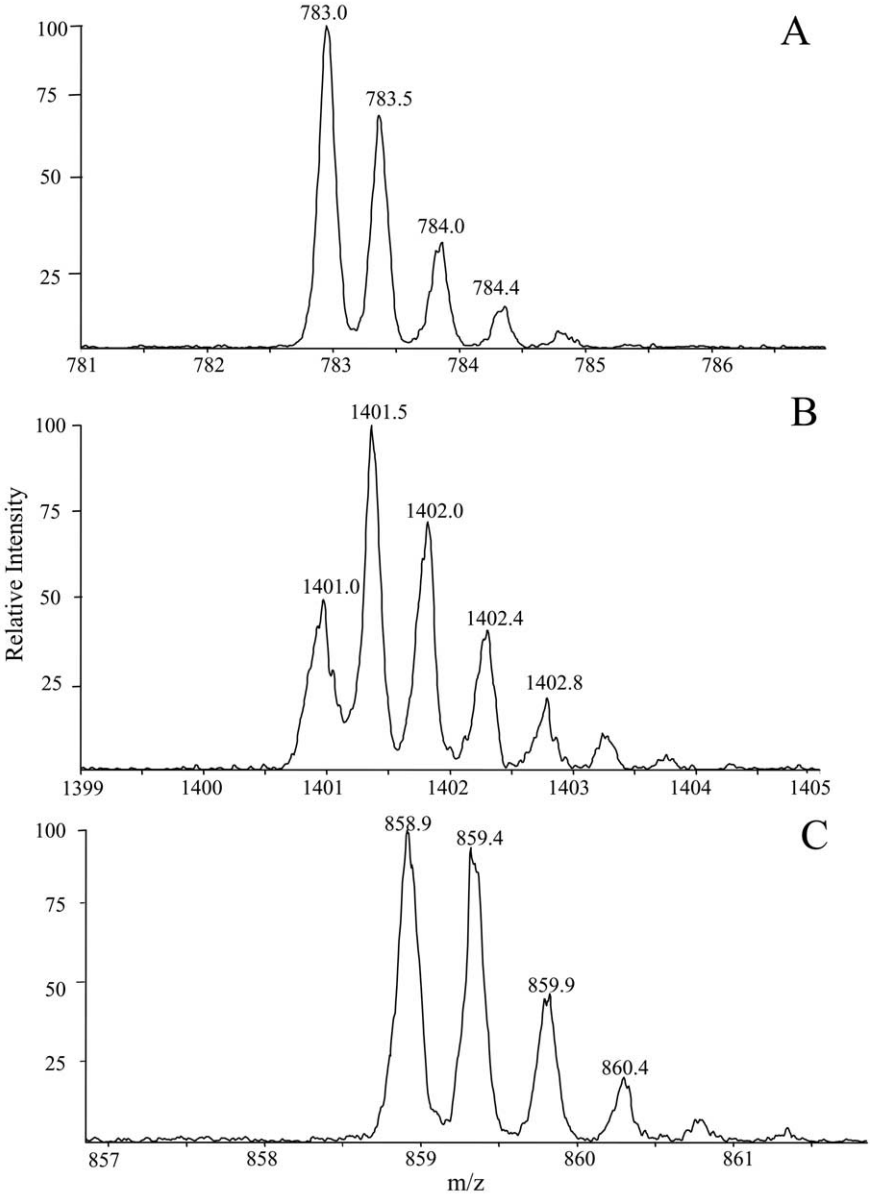

Fig. 2. Zoom scan mass spectra of (A) HPLC peak 1, (B) HPLC peak 4 and (C) HPLC peak 8. The spacing between the main peaks is equal to 0.5 amu which demonstrates the doubly charged state of the ions.

the 3′-*O*-methyl-derivative of quercetin, isorhamnetin, readily fragments to produce a range of ions at and below *m*/*z* 301, the MS data indicates, albeit tentatively, the presence of a methyl ellagic acid pentose conjugate.

Peak 16 ($t_R = 45.4$ min, $\lambda_{max} = 355$ nm) had a $[M-H]^-$ at *m*/*z* 475 and MS^2 produced a major ion at *m*/*z* 301 (M−174, loss of an acetylpentose sugar). MS^3 yielded major fragments at *m*/*z* 257 and 229. On the basis of the mass spectral data and previously published data (Zafrilla et al., 2001) peak 16 is identified as ellagic acid-4-acetylxyloside **3** (Fig. 3).

Peak 17 ($t_R = 46.3$ min, $\lambda_{max} = 355$ nm) had a $[M-H]^-$ at *m*/*z* 447 and MS^2 and MS^3 fragments the same as peak 15. It is therefore also tentatively identified as a methyl ellagic acid pentose conjugate.

Peak 18 ($t_R = 50.1$ min, $\lambda_{max} = 355$ nm) had a $[M-H]^-$ at *m*/*z* 475 which yielded a major MS^2 ion at *m*/*z* 301 (M−174, loss of acetylpentose). MS^3 produced fragments at *m*/*z* 257 and 229. On the basis of the mass spectral evidence and previously published data, peak 18 is identified as ellagic acid-4-acetylarabinoside **6** (Fig. 3) which has the same mass spectrum but elutes later than ellagic acid-4-acetylxyloside **5** (Zafrilla et al., 2001).

Our findings on ellagitannins agree with Haddock et al. (1982) that the dimer, sanguiin H-6 (**2**) is the major ellagitannin found in raspberries. However, we found

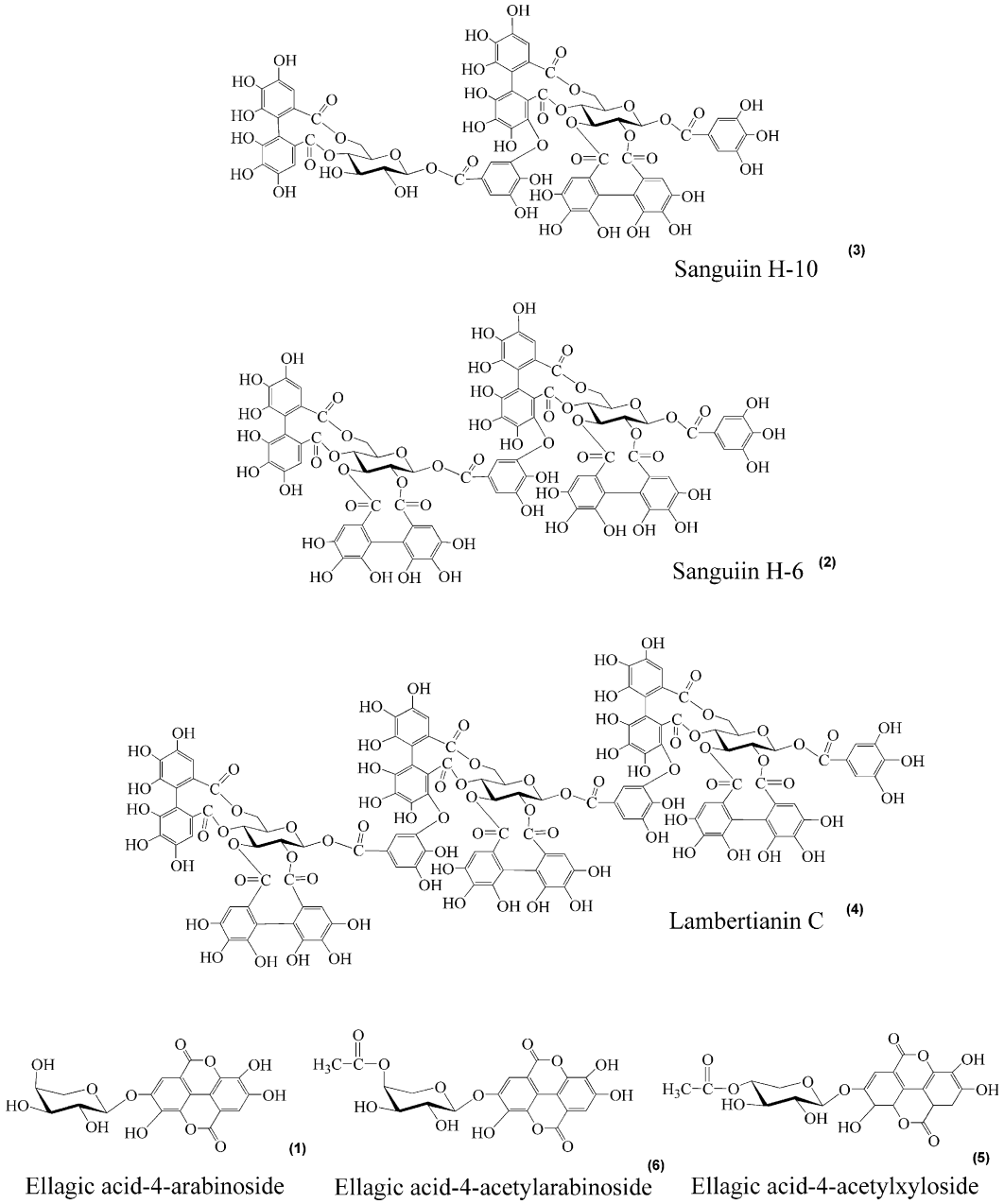

Fig. 3. Structures of the main ellagitannins and ellagic acid sugar conjugates in raspberries.

no evidence for the presence of the ellagitannin monomers casuarictin, potentillin and pedunculagin that were detected by these investigators. We did detect the trimer lambertianin C (**4**), which has been found in raspberry leaves by Tanaka et al. (1993) who also detected the tetramer, lambertianin D. There was no evidence of the presence of this compound in the raspberry fruit extract analysed in the present study.

When carrying out mass spectrometric studies on ellagitannins it is important to ensure the charge state of the $[M-H]^-$ is known. Peak 1 which has been tentatively identified as sanguiin H-10 could be mistaken for pedunculagin, which has a $[M-H]^-$ at m/z 783. However, high resolution scanning of peak 1 indicated that the m/z 783 fragment carried a double charge (Fig. 2A) making the exact mass 1568 rather than 784 amu. MS^2 of the doubly charged ion produced fragment ions with greater m/z values than that of the 'apparent' $[M-H]^-$ at m/z 783. This provided useful spectral information, which led us to believe, this peak was, in fact, sanguiin H-10 (**3**). This type of analysis would not be possible on a single quadrupole instrument.

The use of HPLC–MS–MS for the analysis of anthocyanins has been elegantly demonstrated by Guisti et al. (1999). Our study illustrates the value of MS^n analysis, compared with single stage MS and MS–MS analysis, for the partial identification of trace levels of natural products, such as quercetin glucuronide and ellagic acid arabinoside, in crude extracts when reference compounds are not available. Further information on the position of substituent groups, and the positive identification of the various ellagitannins would require the use of NMR. However, this would involve not only extensive sample purification but also a requirement for several orders of magnitude more analyte than the low ng quantities needed for HPLC–MS^n.

3. Experimental

3.1. Chemicals

Quercetin, quercetin-3-glucoside, quercetin-3,4′-diglucoside, rutin and ellagic acid were purchased from Apin Chemicals (Abingdon, Oxford, UK). Methanol and acetonitrile were obtained from Rathburn Chemicals (Walkerburn, Peebleshire UK). All other chemicals and reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK).

3.2. Plant material

Ripe field-grown raspberries (*Rubus idaeus* L.) cv. Glen Ample were hand picked at Blairgowrie, Perthshire, UK and extracted with methanol as described below within 3 h of picking.

3.3. Extraction of raspberries

Raspberries (40 g) were macerated in an ice-cold pestle and mortar and the resulting homogenate was centrifuged at 2000 *g* for 30 min at 4 °C. The supernatant was decanted and the pellet homogenised and extracted with acidified (0.1% HCl) methanol after which it was re-centrifuged. The two supernatants were combined, and made up to a known volume with acidified methanol. This was then sub-divided into 2 ml aliquots and stored in microcentrifuge tubes at −80 °C prior to analysis.

3.4. HPLC–MS^n and zoom scan analysis

Samples were analysed on a Surveyor HPLC system comprising a HPLC pump, diode array absorbance detector, scanning from 250 to 700 nm and an autosampler cooled to 4 °C (Thermo Finnigan, San Jose, USA). Separation was carried out using a 250°4.6 mm i.d. 4 μm Synergi RP-Max column (Phenomenex, Macclesfield, UK) eluted with a gradient over 60 min of 8–21% acetonitrile in 1% aqueous formic acid at a flow rate of 1 ml/min and maintained at 40 °C. After passing through the flow cell of the diode array detector the column eluate was split and 0.3 ml min was directed to a LCQ DecaXP ion trap mass spectrometer fitted with an electrospray interface (Thermo Finnigan). Analyses utilised the negative ion mode as this provided the best limits of detection for quercetin and ellagic acid. Preliminary analysis was carried out using full scan, data dependent MS–MS scanning from m/z 100–2000. Capillary temperature was 250 °C, sheath gas and auxiliary gas were 60 and 10 units/min respectively, source voltage was 2 kV. Compounds that could not be identified by MS^2 were further fragmented to produce MS^3 spectra. MS^2 fragmentation was carried out with 35% energy while MS^3 used 50% energy to ensure fragmentation of the ellagic acid ion. Zoom scan analysis was carried out to determine the charge state of three of the ellagitannin-based compounds.

Acknowledgements

This investigation was supported by a flexible funding grant to A.C. and M.E.J.L. from the Scottish Executive Rural Affairs Department. The HPLC–MS^n system was purchased with a BBSRC grant to A.C. and J.R. Coggins.

References

Corthout, J., Peiters, L.A., Claeys, M., Vanden Berghe, D.A., Vleitinck, A.J., 1991. Antiviral ellagitannins from *Spondia mombin*. Phytochemistry 30, 1129–1130.

Daniel, E.M., Krupnick, A.S., Heur, Y.-H., Blinzlewr, J.A., Nims, R.W., Stoner, G.D., 1989. Extraction, stablity and quantification of ellagic acid in various fruits and nuts. Journal of Food Composition and Analysis 2, 338–349.

de Ancos, B., González, E.M., Cano, P., 2000. Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. Journal of Agricultural and Food Chemistry 48, 4565–4570.

Francis, F.J., 1972. Anthocyanins of Durham and Heritage raspberry fruits. HortScience 7, 398.

Giusti, M., Rodríguez-Saona, L.E., Griffin, D., Wrolstad, R.E., 1999. Electrospray and tandem mass spectrometry as tools for anthocyanin analysis. Journal of Agricultural and Food Chemistry 47, 4657–4664.

Haddock, E.A., Gupta, R.K., Al-Shafi, S.M.K., Leydon, K., Haslam, E., 1982. The metabolism of gallic acid and hexahydroxydiphenic acid in plants: biogenics and molecular taxonomic considerations. Phytochemistry 21, 1049–1062.

Häkkinnen, S.H., Kärenlampi, S.O., Mykkänen, H.M., Heinonen, I.M., Törrönen, A.R., 2000. Ellagic acid content in berries: influence of domestic processing and storage. European Food Research and Technology 212, 75–80.

Henning, W., 1981. Flavonol glycoside of strawberry, raspberry and blackberry. Zeitschrift für Lebensmittel-Untersuchung und-Forschung 173, 180–187.

Kalt, W., Forney, C.F., Martin, A., Prior, R.L., 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanins after storage of small fruits. Journal of Agricultural and Food Chemistry 47, 4638–4644.

Kähkönen, M.P., Hopia, A.I., Heinonen, M., 2001. Berry phenolics and their antioxidant activity. Journal of Agricultural and Food Chemistry 49, 4076–4082.

Mullen, W., Lean, M.E.J., Crozier, A., 2002a. Rapid characterisation of anthocyanins in red raspberry fruit by HPLC coupled to a single quadrupole mass spectrometer. Journal of Chromatography A 966, 63–70.

Mullen, W., McGinn, J., Lean, M.EJ., MacLean, M.R., Gardner, P., Duthie, G.G., Crozier, A., 2002b. Ellagitannins, flavonoids and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. Journal of Agricultural and Food Chemistry 50, 5191–5196.

Rao, C.V., Tokumo, K., Rigoty, J., Zang, E., Kelloff, G., Reddy, B.S., 1991. Chemoprevention of colon carcinogenesis by dietary administration of piroxicam, difluoromethlornithine, 16β-fluor-5-androsten-17-one and ellagic acid individually and in combination. Cancer Research 51, 4528–4534.

Rommel, A., Wrolstad, R., 1993. Influence of acid and base hydrolysis on the phenolic composition of red raspberry juice. Journal of Agricultural and Food Chemistry 41, 1237–1241.

Ryan, J.J., Coffin, D.E., 1971. Flavonol glucuronides from red raspberry, *Rubus idaeus* (Rosaceae). Phytochemistry 10, 1675–1677.

Stoner, G.D., Morse, M.A., 1997. Isocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. Cancer Letters 114, 113–119.

Tanaka, T., Nonaka, G.-I., Nishioka, I., 1985. Tannins and related compounds. Part 28. Revision of the structures of sanguiins H-6, H-2 and H-3, and isolation and characterization of sanguiin H-11, a novel tetrameric hydrolysable tannin, and seven related tannins, from *Sanguisorba officinalis*. Journal of Chemistry Research (S) 176–177.

Tanaka, T., Tachibana, H., Nonaka, G., Nishioka, I., Hsu, F.-L., Kohda, H., Tanaka, O., 1993. Tannins and related compounds. CXXII. New dimeric, trimeric and tetrameric ellagitannins, lambertianins A-D, from *Rubes lambertianus* SERINGE. Chemical and Pharmaceutical Bulletin 41, 1214–1220.

Torre, L.C., Barritt, B.H., 1977. Quantitative evaluation of *Rubus* fruit anthocyanins pigments. Journal of Food Science 42, 488–490.

Yoshida, T., Ikeda, Y., Ohbayashi, H., Ishihara, K., Ohwashi, W., Shingu, T., Okuda, T., 1986. Dimeric ellagitannins in plants of Melastromataceae. Chemical and Pharmaceutical Bulletin 34, 2676–2679.

Yoshida, T., Nakata, F., Hosotani, K., Nitta, A., Okuda, T., 1992. Dimeric hydrolysable tannins from *Melastoma malabathricum*. Phytochemistry 31, 2829–2833.

Zafrilla, P., Federico, F., Tomás-Barberán, F.A., 2001. Effect of processing and storage on the antioxidant ellagic acid derivatives and flavonoids of red raspberry (*Rubus idaeus*) jams. Journal of Agricultural and Food Chemistry 49, 3651–3655.