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Generic detection of basic taxoids in wood of European Yew (*Taxus baccata*) by liquid chromatography–ion trap mass spectrometry

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ABSTRACT

The occurrence of the cardiotoxin taxine (comprising taxine B and several other basic taxoids) in leaves of *Taxus baccata* L. (European yew) is well known and has led to public concerns about the safety of eating or drinking from utensils crafted from the wood of this poisonous species. The occurrence of basic taxoids in the heartwood of *T. baccata* had not been examined in detail, although the bark is known to contain 2′β-deacetoxyaustrospicatine. Initial examination of heartwood extracts for 2′β-deacetoxyaustrospicatine by liquid chromatography–mass spectrometry (LC–MS) revealed the presence of this basic taxoid at about 0.0007% dry weight, using a standard isolated from bark. Analyses for taxine B, however, proved negative at the extract concentration analysed. Observing other basic taxoids within the heartwood extracts was facilitated by developing generic LC–MS methods that utilised a fragment arising from the *N*-containing acyl group of basic taxoids as a reporter ion. Of the various MS strategies available on a hybrid ion trap-orbitrap instrument that allowed observation of this reporter ion, combining all-ion collisions with high resolution ion filtering by the orbitrap was most effective, both in terms of the number of basic taxoids detected and sensitivity. Numerous basic taxoids, in addition to 2′β-deacetoxyaustrospicatine, were revealed by this method in heartwood extracts of *T. baccata*. Red wine readily extracted the basic taxoids from heartwood while coffee extracted them less efficiently. Contamination with basic taxoids could also be detected in soft cheese that had been spread onto wood. The generic LC–MS method for detecting basic taxoids complements specific methods for detecting taxine B when investigating yew poisoning cases in which the analysis of complex extracts may be required or taxine B has not been detected.

1. Introduction

Taxoids are a group of compounds reported mainly from species of *Taxus* (yew) comprising esters of structurally related taxane diterpenoids. Basic or alkaloidal taxoids, and other *N*-containing taxoids, have an *N*-containing acid esterified to the taxane skeleton [1–4] (Fig. 1). Although various classifications of taxoids have been proposed, basic taxoids are generally considered to be a group of alkaloids related to taxine B in having a normal taxane skeleton with a C-4(20) double bond and an *N*-containing 3-amino-3-phenylpropanoic acid (APPA) derivative esterified at C-5 [1] (usually one of the APPA derivatives **1**–**4**, see Fig. 1). Other taxoid groups also include *N*-containing taxoids such as the anticancer drug paclitaxel (Taxol™), a neutral taxoid acylated with an *N*-containing phenylisoserine derivative originally isolated from the bark of *T. brevifolia* Nutt., and taxine A, a basic taxoid that has an *abeo*-taxane skeleton acylated with an APPA derivative (henceforth APPA will be used as a generic abbreviation for 3-amino-3-phenylpropanoic acid and its derivatives that form the *N*-containing acyl groups of many *N*-containing taxoids).

The toxic principle of yew, taxine, is a crude preparation of basic taxoids, originally obtained from leaves of European yew (*Taxus baccata* L.) from which source it largely consists of taxine B (**5**) [5]. Taxine is a powerful cardiotoxic agent, and yew consumption is a frequent cause of poisoning in livestock [6,7]. Taxine A (also a component of taxine) and paclitaxel are considerably less cardiotoxic, although paclitaxel does cause heart disturbances at high doses [5,8]. The toxicological importance of taxine and the medicinal importance of paclitaxel and related taxoids has resulted in numerous analytical methods being developed, which have been reviewed by Kozuka et al. [9]. Chromatographic methods have largely focused on individual taxoids, such as the detection of known basic taxines and the quantification of taxine B in poisoning cases [10–12]. A mass spectrometric method for detecting any basic taxoid (a generic method) was developed using direct injection of samples into a triple-quadrupole instrument, and this proved effective when applied to leaf extracts of *Taxus wallichiana* Zucc. [13], although there was no separation of isomeric forms. In this paper

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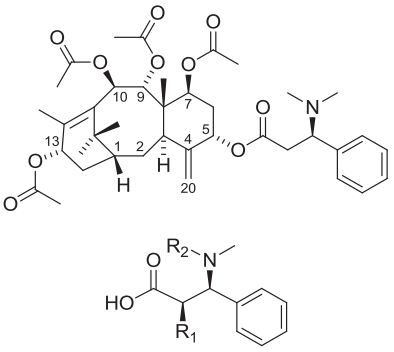

APPA

	R_1	R_2	M	Ion (*m*/*z*)
1	H	H	$C_{10}H_{13}NO_2$	180.1019
2	H	Me	$C_{11}H_{15}NO_2$	194.1176
3	OH	Me	$C_{11}H_{15}NO_3$	210.1125
4	OAc	Me	$C_{13}H_{17}NO_4$	252.1230

Fig. 1. Structures of basic taxoids. (Above) Structure of 2′β-deacetoxyaustrospicatine representing a basic taxoid with a normal taxane skeleton and a C-4(20) double bond. Related basic taxoids vary in the substituents at C-1, C-2, C-7, C-9, C-10 and C-13 and the *N*-containing acyl group at C-5 formed from 3-amino-3-phenylpropanoic acid (APPA) derivatives. (Below) Structures of APPA derivatives found in basic taxoids where M = molecular formula of the APPA derivative, and ion = *m*/*z* value of the APPA product ion observed in collision dissociation spectra of protonated basic taxoids.

we examine liquid chromatography–mass spectrometry (LC–MS) using a hybrid linear ion trap-orbitrap mass spectrometer as a generic means to detect the presence of basic taxoids, particularly in complex sample matrices without prior sample clean-up. We developed this method using the chemically complex extracts of the heartwood of *T. baccata* in order to investigate concerns over the toxicity of this wood.

The Royal Botanic Gardens Kew (RBG Kew) has received several enquiries from members of the public concerned about the safety of eating or drinking from utensils made of yew wood. These enquiries arise from the common knowledge that yew is a poisonous plant and through information in books and on the internet stating that all parts of the yew tree are poisonous, except for the red fleshy aril. However, evidence from the scientific literature on whether the heartwood of *T. baccata* causes health problems or contains basic taxoids is limited. Specialist texts and reviews on toxic woods list wood of *T. baccata* as being of concern [14,15], based on a few cases of irritation or dermatitis caused by yew wood or sawdust (primary literature cited in these reviews). Although Pliny the Elder in his Natural History made the observation on yew that 'even wine-flasks for travellers made of its wood in Gaul are known to have caused death' [16], there are no reports in the modern scientific literature of cases of poisoning from eating or drinking from yew wood utensils. Indeed a wood turner states that he has suffered no ill effects from drinking from a coffee mug he made out of yew [17] (we presume he was drinking coffee).

Evidence for the occurrence of basic taxoids in wood of *T. baccata* is limited to the visualisation of Dragendorff-positive constituents, which were assumed to be taxines [15]. The wood of *T. baccata* has yielded several new taxoids [1] but none are *N*-containing. A possible report of *N*-containing taxoids in wood of *T. baccata* comes from the detection of the neutral taxoid paclitaxel in 'twigs', determined at 0.0006% dry weight by HPLC following crude isolation of a paclitaxel fraction [18], but this study did not investigate whether the compound occurred in the bark and/or wood of the twigs; wood of *T. brevifolia* was, however, analysed and found to contain paclitaxel, again at 0.0006%. LC–MS has been used to analyse the wood of another *Taxus* species, *T. yunnanensis* W.C. Cheng & L.K. Fu (=*T. wallichiana* Zucc.), and this revealed several *N*-containing taxoids as very minor constituents [19].

The bark of *T. baccata* is reported to contain the basic taxoid 2′β-deacetoxyaustrospicatine [20,21], so an initial aim of the present study was to determine whether this basic taxoid also occurred in the heartwood. This compound and taxine from leaves provided suitable examples with which to develop a generic method for determining whether basic taxoids were present in heartwood of *T. baccata* and whether they could contaminate food or drink placed in contact with wood.

2. Materials and methods

2.1. Plant material and sample preparation

Sections of a ca. 10 cm diameter branch and a similarly sized root of *T. baccata* L. were obtained from a tree that had been felled in a private garden in Richmond, Surrey, UK, in October 2007. Part of the branch was sawn up to obtain 'chippings' of bark and blocks of softwood and heartwood. Analyses were not undertaken until December 2011; i.e. the material had been drying ('seasoning') for just over 4 years. Thin shavings (up to 100 mg) were removed from the heartwood and sapwood blocks and bark chippings with a razor blade and soaked in 1 ml of methanol overnight. After centrifugation, the supernatants were analysed directly by LC–MS. The root was separated only into root bark and wood, then sampled and extracted in the same manner.

To examine the extraction of basic taxoids into foodstuffs, shavings of heartwood were soaked in either red wine, hot instant black or hot instant white coffee for 30 min before analysing the wine or coffee directly by LC–MS. Soft cheese (1 g Camembert) was spread thinly (up to 5 mm) onto a heartwood block and left for 18 h before scraping off the cheese and extracting it in methanol. The supernatant was dried and the residue taken up in methanol such that the compounds extracted from 900 mg cheese were present in 1 ml. Before applying the cheese, the block had been wiped with a moist tissue and allowed to dry to remove any sawdust.

To examine heartwood that had been ageing for longer than 4 years, shavings were taken from two wood blocks of *T. baccata* held in the wood collection at RBG Kew. These wood blocks were donated to RBG Kew in 1980 (Kew Economic Botany Collection No. EBC 19518) and 1961 (EBC 19519) but were probably considerably older. Extracts were prepared from the shavings in the manner described above.

To obtain analytical data on the basic taxoids in 'taxine', a methanol extract was made of fresh leaves of *T. baccata* collected from a tree at RBG Kew and diluted such that the compounds extracted from 10 mg were present in 1 ml. To obtain analytical data on the basic taxoid austrospicatine, a methanol extract (1 ml) was made of a leaf (48 mg) of *Austrotaxus spicata* Compton taken from a herbarium specimen lodged at RBG Kew (sheet reference *G.McPherson 3181*).

2.2. LC–MS analysis

Samples were analysed using a LC–MS system consisting of an 'Accela' HPLC system and a 'LTQ-Orbitrap XL' hybrid linear ion trap-orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA). The instrument was controlled by XCalibur 2.0.7 software (Thermo Scientific) which was also used to analyse the data. Chromatographic separation was performed on a 150 mm × 3 mm i.d. × 3 μm Luna C18(2) column (Phenomenex, Torrance, CA, USA) using a 400 μl/min mobile phase gradient of 90:0:10–0:90:10 (water/methanol/methanol + 1% formic acid) over 20 min followed by a 5 min wash in end conditions. The column was equilibrated

in start conditions for 3 min before injection of 5 μl of samples. The column eluate was interfaced with the mass spectrometer via an 'Ion-Max' electrospray source (Thermo Scientific) operated in positive ion mode under the manufacturers' recommended conditions.

The two analysers of the hybrid mass spectrometer were programmed to perform a MS^1 survey scan and one of four types of collision induced dissociation (CID) scans: (i) standard ion trap MS^2 and MS^3; (ii) ion trap MS^2 with varying activation voltage ('pulsed Q dissociation'; $pqdMS^2$); (iii) collision cell MS^2 in the high energy collision dissociation (HCD) cell of the orbitrap ($hcdMS^2$) using nitrogen as the collision gas and performed on precursor ions isolated by the ion trap; and (iv) 'all-ion' fragmentation of the total ion beam performed in the pre-analyser quadrupole lenses (in-source induced collision dissociation; sidMS). Standard MS^2 and MS^3 fragmentations were performed in, and recorded by, the linear ion trap using a precursor ion isolation width of 4 amu and a normalised collision energy of 35% – these being the standard MS^n conditions used in our laboratory. Ion isolation widths and collision energies that most efficiently generated the APPA fragment in other methods of CID were determined by adjusting each parameter in trial LC–MS analyses. For $pqdMS^2$, again performed in and recorded by the linear ion trap, the combination of a 10 amu isolation width and 35% collision energy was selected, otherwise the default values of 0.7 for 'activation Q' and 0.1 ms for 'activation time' were set in the software. For $hcdMS^2$ a 10 amu isolation width and 15% collision energy were chosen and spectra were recorded at 7000 resolution in the orbitrap (start mass m/z 50). For sidMS fragmentation a collision energy of 60% was chosen and spectra were recorded by the orbitrap at 7000 resolution over m/z 100–1000. Precursor ion selection for all LC–MS^2 analyses was under the control of the software, which was tasked to select the most abundant ion in the preceding MS^1 survey scan, excluding M + 1 isotope peaks and any ion that had already been selected within the previous six seconds. For MS^3, the software was set to select the most abundant MS^2 fragment as the precursor ion. MS^1 survey scans (m/z 250–1000) were recorded by the orbitrap at 7000 resolution in all LC–MS analyses except for LC–$hcdMS^2$ analyses, in which the survey scans were recorded by the ion-trap at low resolution to reduce the duty cycle.

2.3. Isolation of the major basic taxoid from bark of T. baccata

Bark chippings (50 g) from the yew branch were ground to a powder and extracted twice in methanol (1 l then 500 ml) for 18 h each. The filtered extracts were combined, dried and the residue was dissolved in 500 ml 1 M HCl and partitioned twice against an equal volume of diethyl ether, recovering the aqueous phase each time. The aqueous phase was made alkaline by the addition of concentrated ammonia solution and partitioned twice against diethyl ether, recovering the ether phase each time. The combined ether phases were dried and the residue was dissolved in 15 ml of 10% aqueous methanol and subjected to flash chromatography on a 63 mm × 27 mm i.d. Isolute C18 column (International Sorbent Technology Ltd., Ystrad Mynach, UK) using a flow rate of 6 ml/min and a step gradient to 10% (60 ml) 30%, 50%, 80% and 100% (each 40 ml) aqueous methanol, collecting 20 ml fractions. The second 80% methanol fraction was dried and the residue was dissolved in $CDCl_3$. Following removal of insoluble material by centrifugation, the supernatant was taken to dryness and redissolved in $CDCl_3$ prior to analysis by NMR spectroscopy. NMR spectra were acquired on a Bruker Avance 400 MHz instrument at 30 °C. Chemical shift referencing was carried out with respect to tetramethylsilane (TMS) at 0.00 ppm. The yield of the taxoid was 4.5 mg with a purity estimated at >90% on the basis of its ^1H NMR spectrum.

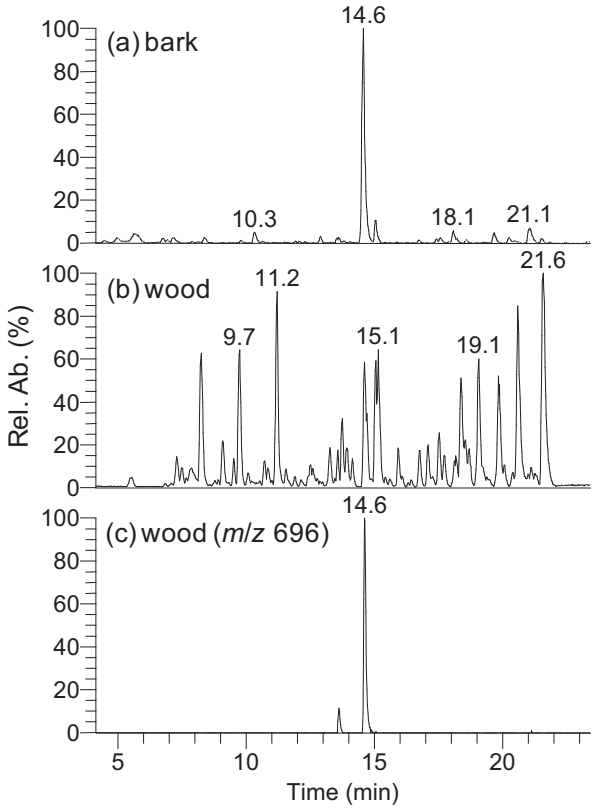

Fig. 2. LC–MS analyses of *Taxus baccata* branch extracts. (a) Base peak chromatogram of bark extract showing the major component at 14.6 min due to 2′β-deacetoxyaustrospicatine (7 mg/ml*; 100% = 4×10^8); (b) base ion chromatogram of heartwood extract (20 mg/ml*; 100% = 4×10^7); and (c) single ion chromatogram at m/z 696.3737 (±3 ppm) extracted from the heartwood analysis revealing the presence of 2′β-deacetoxyaustrospicatine (100% = 2×10^6). *Mass of sample extracted per ml of methanol.

3. Results and discussion

3.1. LC–MS analysis of T. baccata bark and characterisation of the major basic taxoid

Positive ion LC–MS analysis of a methanol extract of the stem bark of *T. baccata* revealed one major chromatographic peak at 14.6 min (Fig. 2a). For this major component, electrospray generated an ion at m/z 696.3737 which was confirmed as $[M+H]^+$ by a minor sodiated species. These ion species predicted a molecular formula of $C_{39}H_{53}NO_{10}$, which was in agreement with that of the basic taxoid 2′β-deacetoxyaustrospicatine (**6**) isolated previously from bark of *T. baccata* [20] and belonging to the same group of basic taxoids as the cardiotoxic taxine B [1]. NMR data for the major taxoid isolated from bark in this study were in good agreement with those acquired under the same solution conditions for 2′β-deacetoxyaustrospicatine (**6**) by Guo et al. [20] (these authors initially referred to **6** as the novel basic taxoid 13-deoxo-13α-acetyloxy-7β,9α-diacetyl-1,2-dideoxytaxine B, but subsequently realised it was identical to 2′β-deacetoxyaustrospicatine [21], which had been previously reported from leaves of *A. spicata* [22]). 2′β-Deacetoxyaustrospicatine was also present in the root bark of *T. baccata*, again producing the largest peak in the chromatogram, although an additional prominent peak at 12.8 min was also present. This compound (**7**) gave a protonated species at m/z 770.3745, predicting a molecular formula of $C_{41}H_{55}NO_{13}$, and showed an APPA fragment at m/z 210 (see Table 1 and later discussion). These data are in accordance with **7** being

Table 1
Nominal relative molecular masses and *m*/*z* values of protonated molecules and APPA fragment ions of the basic taxoids discussed in the text. Numbers given in parentheses with the APPA ions refer to the compound numbers given in Fig. 1.

No.	Compound	M_r	$[M+H]^+$ (*m*/*z*)	APPA ion (*m*/*z*)
5	Taxine B	583	584	194 (**2**)
6	2′β-Deacetoxyaustrospicatine	695	696	194 (**2**)
7	2α-Acetoxy-2′β-deacetylaustrospicatine (or isomer)	769	770	210 (**3**)
8	*N*-demethyl derivative of **6**	681	682	180 (**1**)
9	Deacetoxy derivative of **8**	623	624	180 (**1**)

2α-acetoxy-2′β-deacetylaustrospicatine, known from *A. spicata* leaves [22], or an isomeric form.

3.2. LC–MS analysis of T. baccata heartwood and detection of 2′β-deacetoxyaustrospicatine

LC–MS revealed numerous components in the methanol extract of heartwood of *T. baccata* obtained from the recently felled tree (Fig. 2b). Extracting an accurate mass single ion chromatogram at the calculated *m*/*z* for protonated 2′β-deacetoxyaustrospicatine (**6**) (with 3 ppm mass tolerance) revealed a component at 14.6 min which co-eluted with the standard isolated from bark and showed identical MS^n spectra (Fig. 2c). This component was also detected in sapwood samples from the branch and in wood samples from the root. Levels (% dry weight) of 2′β-deacetoxyaustrospicatine in the heartwood were estimated to be about 0.0007%. Taxine B (**5**) and its isomers, which comprised the major peaks in analyses of leaf material giving protonated molecules at *m*/*z* 584.3220 (see also Kite et al. [12]), were not reliably detected in the wood analysis: extracting an accurate mass chromatogram from the MS^1 data revealed trace signals for this ion at the expected retention time but levels were too low to confirm the ion as the monoisotopic peak of an isotope cluster or to obtain confirmation by MS^2 analysis.

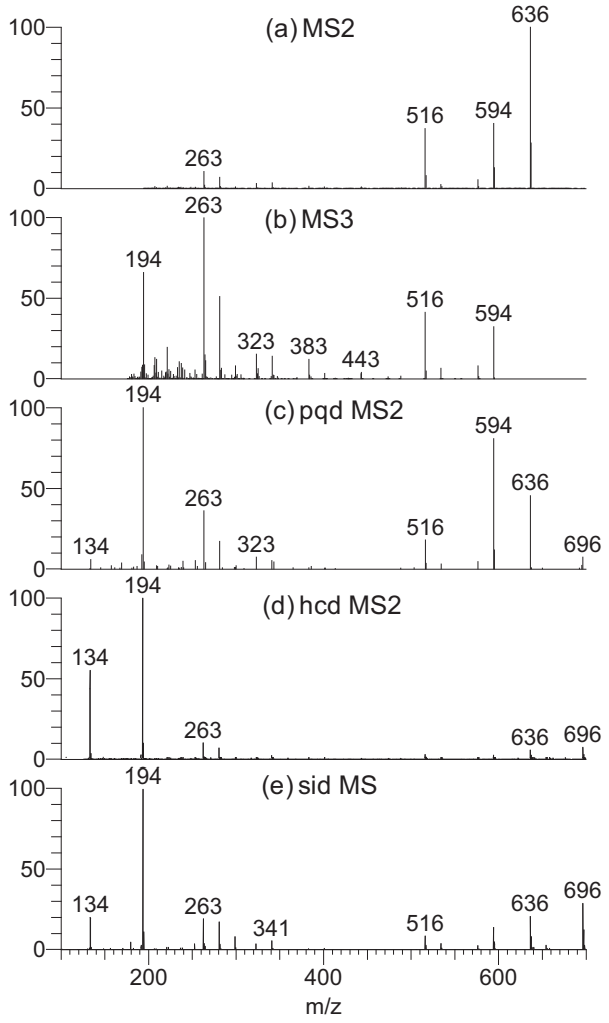

Fig. 3. Collision induced dissociation spectra of protonated 2′β-deacetoxyaustrospicatine (at *m*/*z* 696). (a) MS^2, (b) MS^3 (*m*/*z* 696 → 636), (c) pqdMS^2, (d) hcdMS^2 and (e) sidMS showing generation of the APPA ion at *m*/*z* 194.

2′β-Deacetoxyaustrospicatine (**6**) was detected in both of the heartwood block samples (EBC 19518 and 19519) obtained from the Kew Economic Botany Collections that had been in storage for a minimum of 31 and 50 years, respectively. The peak height of this component in each of the analyses was about an order of magnitude lower than that obtained from the analysis of heartwood from the recently felled tree (all analysed at 20 mg wood extracted in 1 ml). Nevertheless, this indicates that 2′β-deacetoxyaustrospicatine is likely to remain in wood that has been allowed to season for many years.

3.3. Generic detection of basic taxoids in heartwood of T. baccata

Filtering the accurate mass LC–MS^1 data obtained from the analyses of heartwood samples for components having *m*/*z* values in accordance with the protonated molecules of known basic taxoids is complicated by the large number of compounds that have been described. Thus, LC–MS methods to achieve generic detection of basic taxoids were investigated. These used the various scanning modes of the hybrid ion trap-orbitrap mass spectrometer employed in this study.

A major product ion corresponding to the protonated APPA has been shown to be generated by serial mass spectrometry of protonated basic taxoids [23], both by collision cell MS^2 [13,24] and ion trap MS^2 [10,12]. Thus filtering LC–MS^2 data for the APPA ion provides a more generic means of detecting basic taxoids which is more efficient than screening for the protonated molecules of intact taxoids, given that protonated APPAs have a more limited range of *m*/*z* values (the four APPAs reported to form the *N*-containing acyl group of most basic taxoids are listed in Fig. 1). This strategy of using the APPA fragment as a reporter ion also has the advantage of detecting novel basic taxoids that are not isobaric with any known examples. Furthermore, accurate mass filtering of high resolution data achieves greater confidence in the identification of the protonated APPA compared to $[M+H]^+$, as *m*/*z* values within a 3 ppm window of the low mass APPA ion are more likely to only agree with one ionic formula (restricting elements to C, H, N and O) than those within a 3 ppm window of *m*/*z* value of $[M+H]^+$ of basic taxoids, which are mostly within the range *m*/*z* 500–800.

Scanning for the APPA reporter ion is efficiently performed on a triple quadrupole instrument [13], although this only achieves low resolution scanning. We investigated whether the hybrid linear ion trap-orbitrap instrument, available in our laboratory, could provide alternative analytical solutions, taking advantage of high resolution scanning of the orbitrap. Following MS^2 analysis of protonated taxine B at *m*/*z* 584, observation of the small APPA product ion at *m*/*z* 194, resulting from the large neutral loss of the diterpenoid,

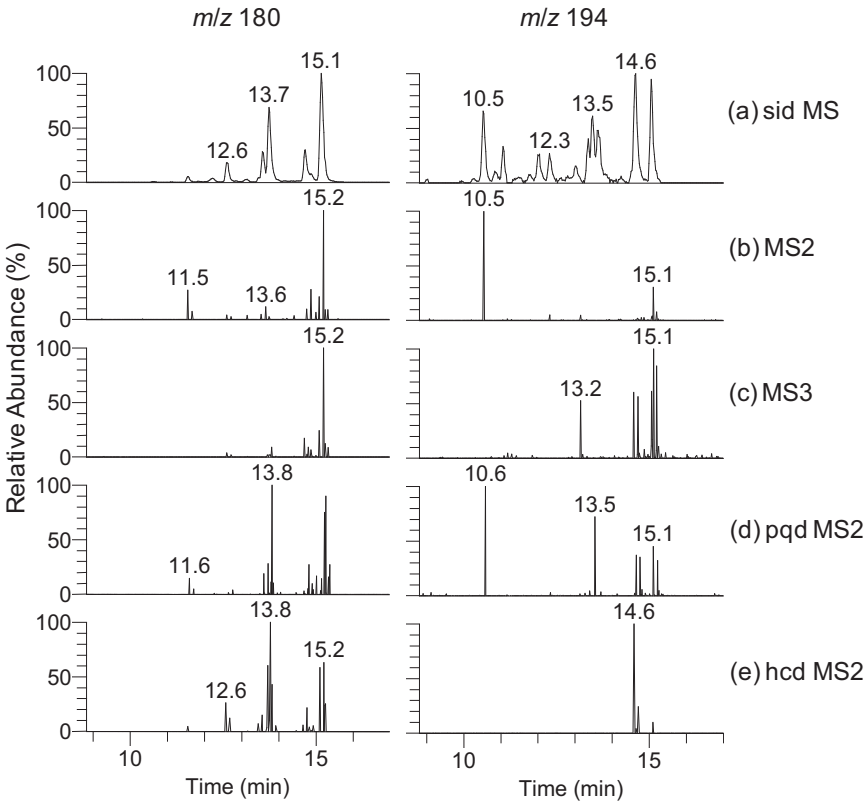

Fig. 4. Comparison of LC–MS methods for the generic detection of basic taxoids in heartwood of *Taxus baccata*. Chromatograms give responses for the APPA ions at *m*/*z* 180 (left) and 194 (right) obtained with the mass spectrometer set to record sidMS, MS^2 and MS^3, $pqdMS^2$ and $hcdMS^2$. Signal intensity is presented as a continuum for sidMS data (in which 100% = 1×10^7 for *m*/*z* 180 and 4×10^5 for *m*/*z* 194) and as 'sticks' representing single scan events for the remainder.

is just possible with an ion trap mass spectrometer using standard precursor ion activation voltages. However, for larger basic taxoids the APPA fragment is near to or below the lower limit of trapping product ions efficiently; for example, the APPA fragment at *m*/*z* 194 could not be observed following MS^2 of protonated 2′β-deacetoxyaustrospicatine (Fig. 3a). Hybrid ion trap-orbitrap mass spectrometers offer at least four strategies to circumvent this problem (see Section 2.2): (i) MS^3, (ii) $pqdMS^2$ (permitting fragment ions at lower *m*/*z* values to be observed), (iii) $hcdMS^2$ (for which there is no lower *m*/*z* limit for fragment ion observation and (iv) sidMS.

When applied to protonated 2′β-deacetoxyaustrospicatine, all four of these strategies allowed observation of the APPA ion at *m*/*z* 194 (Fig. 3) – the base peak at *m*/*z* 636 in the MS^2 spectrum (Fig. 3a) provided a suitable intermediate ion on which to perform MS^3 analysis (Fig. 3b). The efficiency of generating the *m*/*z* 194 fragment varied among these strategies: the abundance of *m*/*z* 194 as a percentage of the abundance of the precursor (*m*/*z* 696) before any isolation was 15% for the MS^3 strategy, 3% for $pqdMS^2$, 6% for $hcdMS^2$ and 40% for sidMS. The limit of quantitative detection of pure 2′β-deacetoxyaustrospicatine was 25 pg on-column by LC–sidMS (and LC–MS^1) using accurate mass filtering while LC–MS^2 methods were about 100-fold less sensitive. The most sensitive means for scanning for the diagnostic product ions of basic taxoids in LC–MS analysis of a crude extract was therefore likely to be sidMS. This method had the added advantages of a faster duty cycle (since it did not involve the isolation of precursor ions) and recording fragments from all precursor ions (being an 'all-ion MS^n' method). The other three strategies involve real-time mining of the data such that MS^n data is only obtained on the most abundant ions, thus if basic taxoids eluted amongst several more abundant components the precursor ions would not be selected.

These predictions were largely borne out by trial LC–MS analyses of *T. baccata* heartwood in which the mass spectrometer was programmed to either record sidMS at high resolution, data-dependent MS^2 and MS^3 spectra at low resolution, data-dependent $pqdMS^2$ spectra at low resolution, or data-dependent $hcdMS^2$ spectra at high resolution. Accurate mass filtering of LC–sidMS data for the various *m*/*z* values of APPA ions revealed that the most abundant of these ions, generated by subjecting the total ion beam to up-front collisions, were at *m*/*z* 180.1016, in accordance with the APPA *N*-methyl-3-amino-3-phenylpropanoic acid (calculated for $C_{10}H_{14}NO_2^+$ = *m*/*z* 180.1019) (Fig. 4a). The precursor ions responsible for generating this *m*/*z* 180 fragment constituted some of the larger peaks in the MS^1 chromatogram (without up-front collisions). As these precursor ions were of high relative abundance in the MS^1 survey scan, they were generally selected by data dependent methods; thus, after applying the product ion filter of *m*/*z* 180 at low or high resolution as appropriate, these more abundant basic taxoids were also detected by data-dependent CID methods, whether this employed normal MS^n (Fig. 4b and c), pqdMS2 (Fig. 4d) or hcdMS2 (Fig. 4e). Following LC–sidMS analysis, APPA ions at *m*/*z* 194 (measured at *m*/*z* 194.1173) expected for *N*,*N*-dimethyl-3-amino-3-phenylpropanoic acid (calculated for $C_{11}H_{16}NO_2^+$ = *m*/*z* 194.1176) were around 100-fold less abundant that those at *m*/*z* 180. Thus the data-dependent CID methods were less successful at detecting these minor components; only 2′β-deacetoxyaustrospicatine at t_R 14.6 min was revealed by all three data-dependent CID methods. LC–$hcdMS^2$ detected the fewest components as the longer duty cycle (ca. 1.8 s) of the method resulted in fewer precursor ions being selected for analysis. The best method for the generic detection of basic taxoids was therefore LC–sidMS. Another advantage of this method was that while the orbitrap was scanning for APPA ions from up-front collisions at

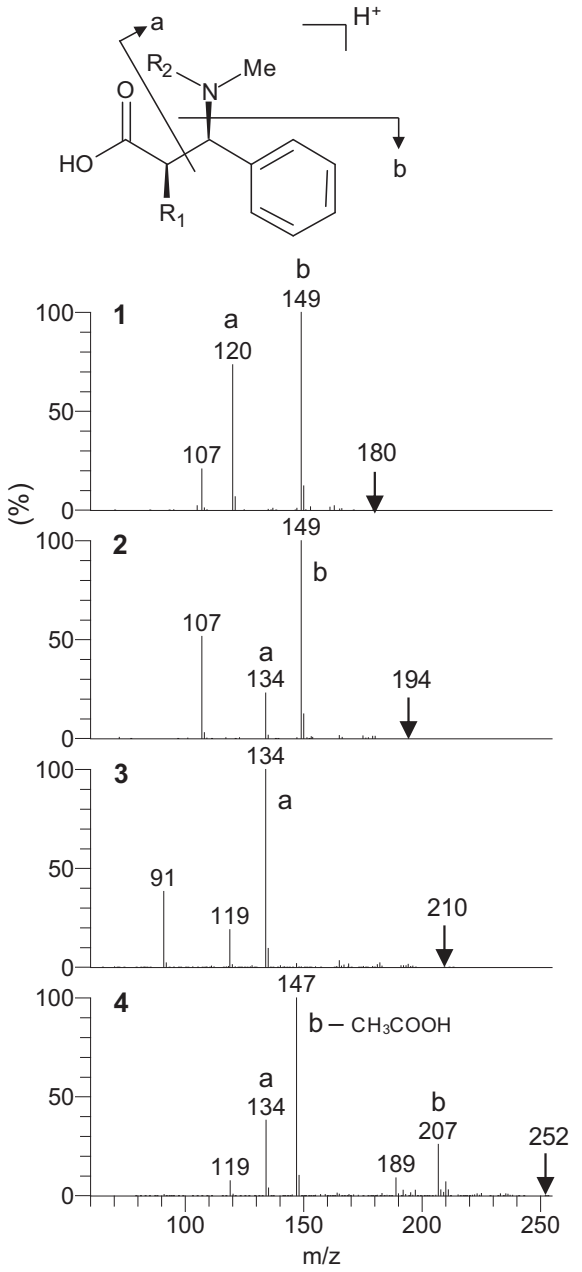

Fig. 5. Collision induced dissociation spectra of APPA ions. Compound numbers (**1–4**) and R groups are given in Fig. 1, and CID spectra were obtained by performing ion trap MS^2 on the APPA ion generated by sidMS of protonated basic taxoids. The CID spectrum of **1** was obtained from basic taxoids in *T. baccata* wood, that of **2** from 2′β-deacetoxyaustrospicatine, **3** from taxine A in a leaf extract of *T. baccata*, and the CID spectrum of **4** was obtained from austrospicatine in a leaf extract of *A. spicata*. Ionic formulae from high resolution data: *m/z* 207 = $C_{11}H_{11}O_4^+$, *m/z* 149 = $C_9H_9O_2^+$, *m/z* 147 = $C_9H_7O_2^+$, *m/z* 134 = $C_9H_{12}N^+$, *m/z* 120 = $C_8H_{10}N^+$, *m/z* 119 = $C_8H_7O^+$, *m/z* 107 = $C_7H_7O^+$, *m/z* 91 = $C_7H_7^+$.

high resolution the ion trap could be employed to record the CID spectra of these ions at low resolution, complementing the accurate mass confirmation of the APPA ion with CID spectra (Fig. 5). A disadvantage of the LC–sidMS method was that identification of the precursor of the APPA ion was ambiguous. More abundant precursor ions could be identified from the data-dependent CID methods. Of these, LC–pqdMS^2 achieved the greatest depth of ion mining due to its shorter duty cycle (ca. 0.6 s) compared to LC–MS^n (ca. 1.2 s) and LC–hcdMS2.

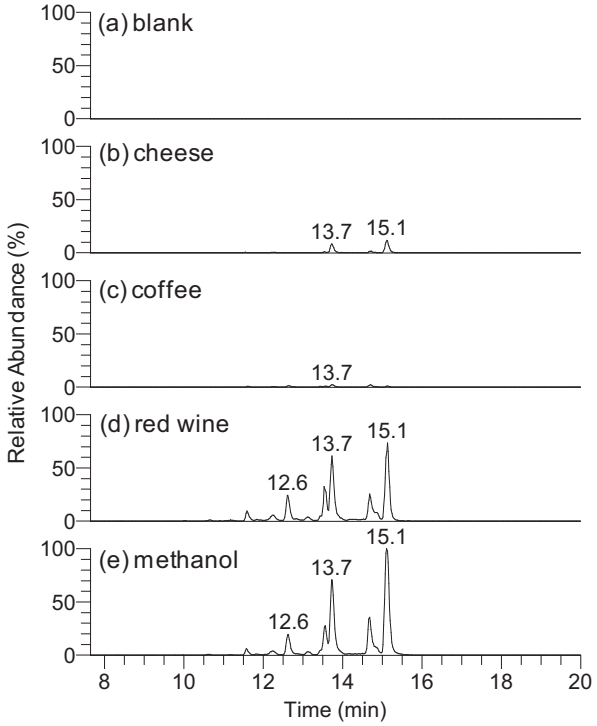

Fig. 6. Detection of basic taxoid contamination in food and drink that had contacted yew wood. (a) Red wine that had not been in contact with yew wood (blank); (b) 900 mg of Camembert cheese spread thinly on a yew wood block and left for 18 h before being scraped off and extracted in methanol (final volume 1 ml); (c–e) 30 mg of yew wood shavings placed in 1 ml of hot black coffee (c), red wine (d) or methanol (e) for 30 min; figure shows single ion chromatograms extracted at *m/z* 180 from LC–sidMS analyses (100% = 1×10^7 in all chromatograms).

Of the components in the LC–MS analyses of the wood extract generating the APPA ion at *m/z* 180, the major peaks at t_R 13.7 (**8**) and 15.1 min (**9**) (Fig. 4a) were investigated further. The *m/z* values of the protonated precursors, measured at *m/z* 682.3578 and *m/z* 624.3520, suggested molecular formulae of $C_{38}H_{51}NO_{10}$ and $C_{36}H_{49}NO_8$, respectively. Compound **8** has a molecular mass 14 Da less than 2′β-deacetoxyaustrospicatine (**6**), and given that this mass deficiency lies on the APPA substituent, it seems highly probably that this is the *N*-demethyl derivative of 2′β-deacetoxyaustrospicatine or one of its isomers – two candidate compounds have been isolated from seeds of *Taxus chinensis* var. *mairei* W.C. Cheng & L.K. Fu (=*T. wallichiana* var. *mairei* L.K. Fu & Nan Li) [25]. Compound **9**, giving $[M+H]^+$ at *m/z* 624, is 58 Da ($C_2H_2O_2$) less than **8**, suggesting it was a deacetoxy derivative, and again a candidate taxoid has been isolated from seeds of *T. chinensis* var. *mairei* [26].

3.4. Extraction of basic taxoids by wine, coffee and cheese

To examine whether wine or coffee poured into a utensil made from yew wood could become contaminated with basic taxoids, a fragment of heartwood of *T. baccata* was immersed in red wine, hot black coffee or hot white coffee for 30 min (Fig. 6). Analysis of the wine by LC–sidMS readily detected the basic taxoids from the wood, despite the presence of additional phenolic compounds from the wine. An accurate mass single ion chromatogram filtered from the MS^1 data confirmed that 2′β-deacetoxyaustrospicatine had been extracted by the wine, and it was estimated that the wine extracted 2′β-deacetoxyaustrospicatine at 20% of the efficiency of methanol. Low levels of basic taxoid contamination were also detected in the black and white coffee and cheese. The contamination of cheese may have arisen from scraping the cheese off the wood with a knife

rather than by diffusion of the basic taxoids into the cheese, but this nevertheless revealed that contamination could occur if a portion of cheese is cut from a yew wood board.

4. Conclusions

The development of a LC–MS method to detect low levels of basic taxoids with minimal sample preparation will be a useful addition to standard LC–MS methods to specifically detect taxine B in suspected yew poisoning cases, particularly those requiring the analysis of complex samples such as stomach contents. The method can be adapted for use on any low resolution MS/MS instrument although use of a high resolution instrument considerably improves the signal to noise ratio and confidence in identifying the reporter ion.

The method readily demonstrates the presence of basic taxoids in the heartwood of *T. baccata* and also that these basic taxoids can contaminate certain food and drink placed in contact with the wood. Of the basic taxoids detected, one is confirmed as 2′β-deacetoxyaustrospicatine, which belongs to the same group of taxoids as the cardiotoxic taxine B. The toxicity of 2′β-deacetoxyaustrospicatine, or other basic taxoids tentatively identified in the wood, is not known, thus it is not possible to calculate whether the quantities that might be extracted into food or drink could cause poisoning. However, given the efficiency with which wine extracted basic taxoids from untreated wood, it would seem sensible to err on the side of caution and not drink wine from a vessel made of yew wood.

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