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Negative ion electrospray mass spectrometry of neoflavonoids

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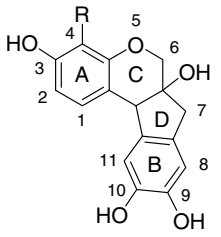
Abstract

The electrospray ionisation mass spectra of the neoflavanoids brazilin and hematoxylin are reported in both their reduced (**1** and **2**, respectively) and their oxidised forms (**3** and **4**, respectively). In the reduced forms, breakdown pathways under collision induced decomposition (CID) conditions produce fragments characteristic of rings A and C; in their oxidised forms, the fragments are characteristic of rings B and D. The structural assignments of the fragments are substantiated by recording the spectra after deuterium exchange at the hydroxyl groups.

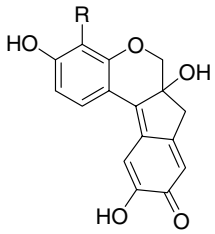
Keywords: Neoflavonoids; Mass spectrometry

1. Introduction

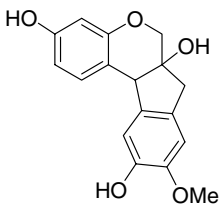
The neoflavonoid derivatives brazilin **1** and hematoxylin **2** are well-known constituents of the hard-woods *Caesalpinia* L. spp. and *Haematoxylum* L. spp., respectively. The structures of these compounds are very similar, with hematoxylin having an extra OH group in the A ring. In their oxidised forms brazilein **3** and hematein **4**, have been used for centuries as dyestuffs (Ferreira et al., 2004) and more recently as a nuclear stain for animal tissues (Bettinger and Zimmermann, 1991). The NMR spectra of brazilin have been previously reported in this journal (Kim et al., 1997) but there is little information on their mass spectra in the literature except for an isolated report of fragmentation under electron impact conditions (Bettinger and Zimmermann, 1991). However, with the advent of liquid chromatography–mass spectrometry (LC–MS), an understanding of the behaviour of neoflavonoids under electrospray conditions has become increasingly important (Cuyckens and Claeys, 2004). Accordingly, we report here an extension to neoflavonoids of our earlier work on the mass spectra of flavonoids under negative ion electrospray conditions (Ferreira et al., 1999, 2001).



1, R = H, brazilin
2, R = OH, hematoxylin



3, R = H, brazilein
4, R = OH, hematein



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In undertaking this investigation, we hoped to identify collision induced decomposition (CID) conditions which would reveal partial structural information on neoflavonoid ring systems, in either their reduced or their oxidised forms. In this way it may be possible to obtain structural information on new neoflavonoid analogues from a few micrograms of material. Such compounds are known natural products; for example, Namikoshi et al. (1987) have isolated 9-*O*-methylbrazilin **5** from Sappan Lignum, the dried heartwood of *Caesalpinia sappan* L.

2. Results and discussion

Freshly prepared methanolic solutions of the neoflavonoids **1**–**4** gave good mass spectra under negative ion electrospray conditions. Ions due to deprotonated molecules at *m/z* $[M - H]^-$ were obtained in all cases; peaks corresponding to dimers and trimers of hematoxylin **2** ($[2M - H]^-$ and $[3M - H]^-$) were sometimes observed. Samples of the reduced forms (**1** and **2**) also showed the presence of the deprotonated molecules of the corresponding oxidised species (**3** and **4**), respectively. It was confirmed by CID of the $[M - H]^-$ ions of **1** and **2** that $[M - 3H]^-$ peaks are not obtained and therefore the oxidised species are indeed present in the starting material and not formed in the mass spectrometer.

To provide further evidence of the nature of the breakdown pathways, spectra were also recorded using deuteriated methanol (MeOD) as solvent. Under these conditions, ion clusters were observed corresponding to deprotonated molecules with sequential replacement by deuterium of the hydrogen atom in each of the OH groups (with the exception of the ionised OH). In each case, the ion corresponding to the most fully deuteriated species was chosen for CID experiments (see below).

CID mass spectra of the reduced forms brazilin **1** and hematoxylin **2** show only 2 breakdown peaks (ca. >10% intensity) (Table 1). The first of these is a minor peak due to loss of H_2O; when MeOD was used as solvent, loss of HOD as well as H_2O was observed, indicating that at least one of the hydrogen atoms lost does not originate at the phenol group. It is therefore not possible to identify the position of this cleavage with any certainty; this is also commonly found in H_2O loss from the phenol groups of flavonoids (Ferreira et al., 2001).

The major peak in the spectra of both **1** and **2** is due to loss of a neutral fragment of 122 Da from the $[M - H]^-$ ion. This neutral fragment must originate from the C/D/B ring sections which are the same in both precursors. These results are best explained if the A-ring is the initial ionisation site, giving the deprotonated species **6** and **7**. Thereafter, the fragmentation can be rationalised as depicted in Scheme 1. This mechanism is supported by CID of the fully deuteriated species, which show that the major observed ion in both deuteriated samples corresponds to the A/C ring fragment with the available hydroxyl group(s) retaining their deuterium atoms.

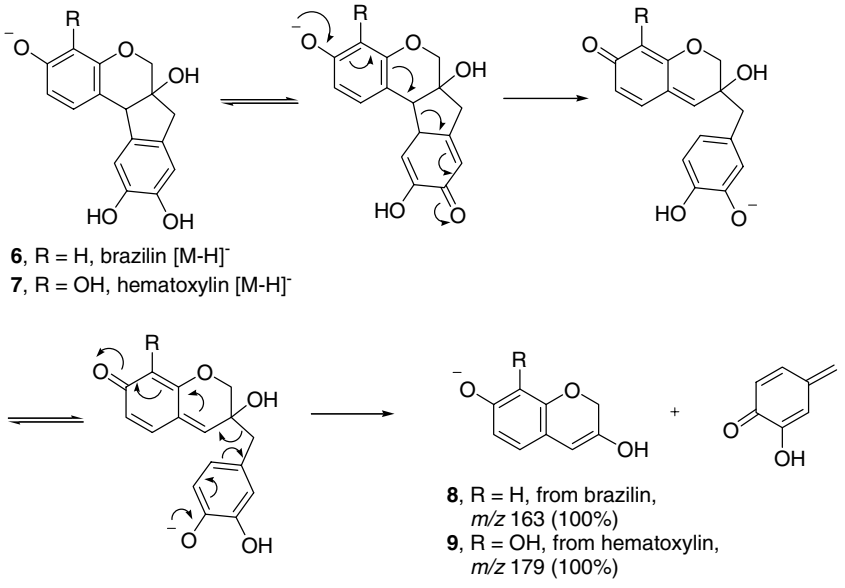
We conclude that the CID mass spectra of the reduced forms **1** and **2** of neoflavonoids provide direct information on the substituents of rings A and C; the mass of the neutral fragment lost gives supporting information on the substituents of ring B and those of the methylene group of ring D.

CID mass spectra of the oxidised forms brazilein **3** and hematein **4** show many breakdown peaks (>10% intensity) which can act as useful fingerprints for these compounds (Fig. 1 and Table 2). It is likely that that the 10-hydroxyl group of the B-ring is the initial ionisation site (Lalor and Martin, 1959) to provide the deprotonated oxidised neoflavonoid species **10** and **11**, respectively. In both cases, the highest mass fragment, though of low intensity, is due to loss of *m/z* 15 (CH_3). When the spectrum was recorded in MeOD, it is clear that these fragments correspond to loss of CH_2D; presumably the carbon atom derives from that at C(6) or C(7), together with the deuterium from the 6a-OH group. The most intense breakdown peaks were found to be due to loss of H_2O; in the deuteriated case, the major peak was due to loss of HOD, but some loss of H_2O was also observed. Some loss of CO (*m/z* 28) is also found in both cases. These are common breakdown peaks in negative ion ESI mass spectra of flavonoids (Ferreira et al., 2001; Ferriera, 2002). More unusually, a significant peak is also observed in the spectra of both **3** and **4** due to loss of a fragment of 43 Da (probably CH_3CO). In the case of **3** the deuteriated species shows peaks due to loss of both *m/z* 43 (i.e., the fragment contains no deuterium) and 44 (i.e., the fragment contains one deuterium). In the case of **4**, the situation is complicated by the presence of other peaks (*e.g.* *m/z* 255, due to loss of CO_2) so that further analysis is not possible.

Table 1
Negative ion ESI mass spectra of brazilin and hematoxylin (reduced forms) under CID conditions

Brazilin (MeOH) *m/z* (%)	Brazilin (MeOD) *m/z* (%)	Hematoxylin (MeOH) *m/z* (%)	Hematoxylin (MeOD) *m/z* (%)	Fragment lost
285 (64%) $[M - H]^-$	288 (8%) $[M_D - D]^-$	301 (100%) $[M - H]^-$	305 (14%) $[M_D - D]^-$	
267 (8%) $[M - H - 18]^-$	270 (10%) $[M_D - D - 18]^-$	283 (19%) $[M - H - 18]^-$	287 (8%) $[M_D - D - 18]^-$	H_2O
	269 (11%) $[M_D - D - 19]^-$		286 (16%) $[M_D - D - 19]^-$	HOD
163 (100%) $[M - H - 122]^-$	164 (100%) $[M_D - D - 124]^-$	179 (100%) $[M - H - 122]^-$	181 (100%) $[M_D - D - 124]^-$	See Scheme 1
	165 (40%) $[M_D - D - 123]^-$		182 (21%) $[M_D - D - 123]^-$	
	163 (16%) $[M_D - D - 125]^-$		180 (49%) $[M_D - D - 125]^-$	

M_D corresponds to the molecular mass in which all exchangeable protons are replaced by deuterium (i.e., M + 4 in the case of brazilin; M + 5 in the case of hematoxylin).



Scheme 1.

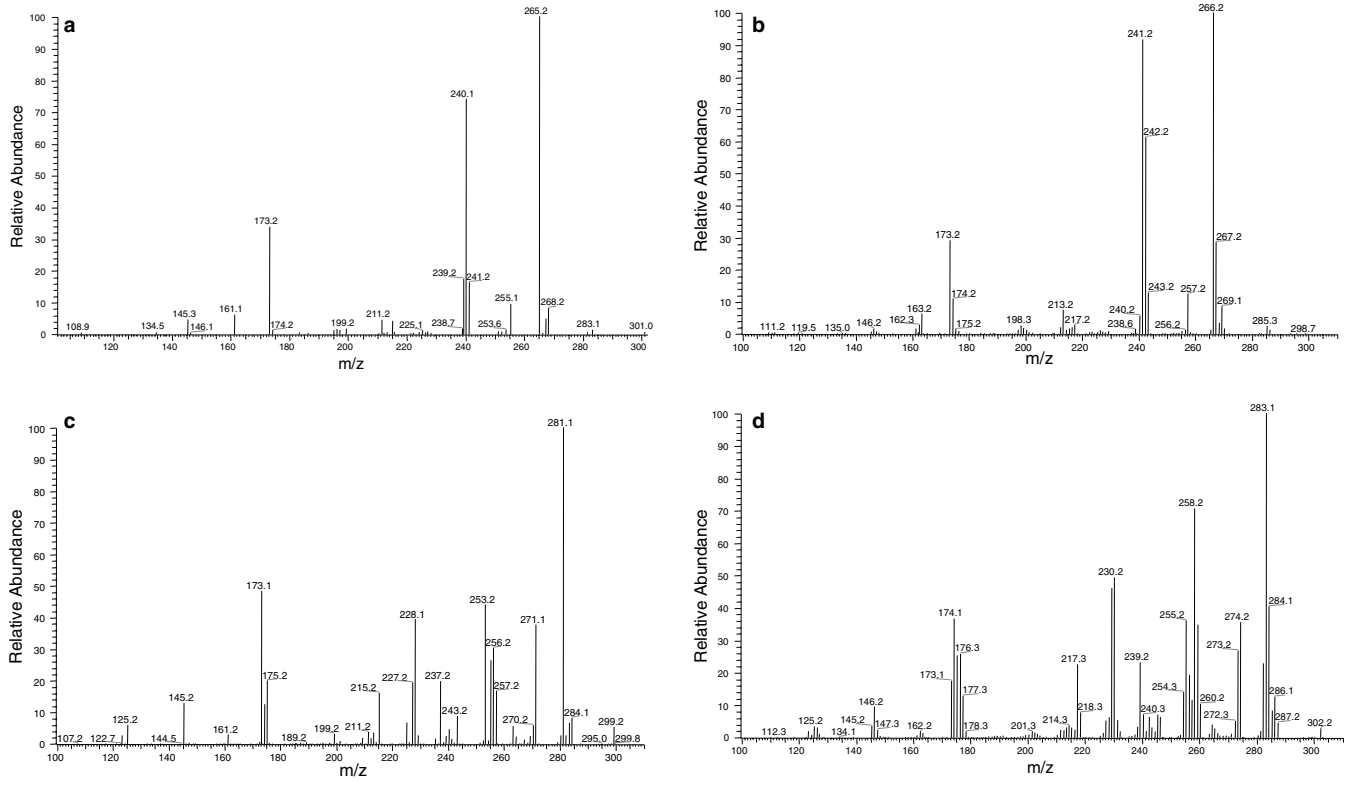


Fig. 1. (a) CID mass spectrum (MS^2) of brazilein **3** (*m*/*z* 283) in MeOH. (b) CID mass spectrum (MS^2) of deuteriated brazilein **3** (*m*/*z* 285) in MeOD. (c) CID mass spectrum (MS^2) of hematein **4** (*m*/*z* 299) in MeOH. (d) CID mass spectrum (MS^2) of deuteriated hematein **4** (*m*/*z* 302) in MeOD.

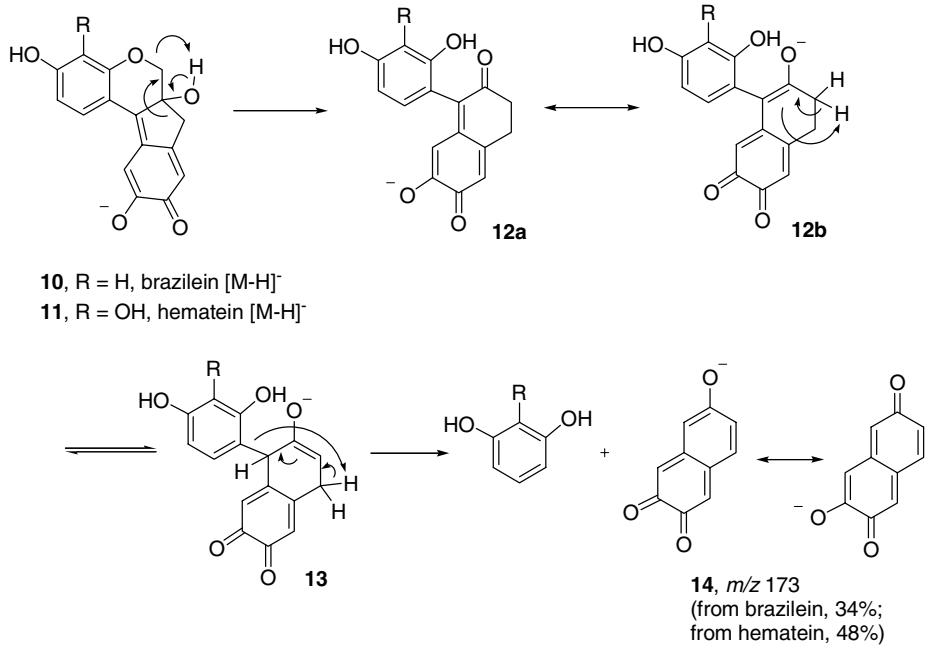
In the range *m*/*z* 180–240, the CID spectrum of hematein **4** is much more complex than that of brazilein (Fig. 1) presumably due to the juxtaposition of hydroxyl groups in ring A. Thus, peaks at *m*/*z* 253 $[M - H - H_2O - CO]^-$, 237 $[M - H - H_2O - CO_2]^-$, 228 $[M - H - C_3H_3O_2]^-$, 227 $[M - H - C_3H_4O_2]^-$ are only found in the spectrum of **4**.

The major low-mass fragment ion is found at *m*/*z* 173 in **3** and **4**. Since the same fragment is produced from both precursors, it is clear that, in contrast to the spectra of the reduced forms, the observed fragment cannot be derived from ring A. A suggested mechanism is shown in Scheme 2. Again, the mechanism is supported by the results of the MeOD deuterium labelling experiments, which,

Table 2
Negative ion ESI mass spectra of brazilein and hematein (oxidised forms) under CID conditions

Brazilein (MeOH) *m/z* (%)	Brazilein (MeOD) *m/z* (%)	Hematein (MeOH) *m/z* (%)	Hematein (MeOD) *m/z* (%)	Fragment lost
283 (77%) $[M - H]^-$	285 (100%) $[M_D - D]^-$	299 (100%) $[M - H]^-$	302 (100%) $[M_D - D]^-$	
268 (8%) $[M - H - 15]^-$	269 (8%) $[M_D - D - 16]^-$	284 (8%) $[M - H - 15]^-$	286 (12%) $[M_D - D - 16]^-$	CH_3/CH_2D
265 (100%) $[M - H - 18]^-$	267 (29%) $[M_D - D - 18]^-$	281 (100%) $[M - H - 18]^-$	284 (38%) $[M_D - D - 18]^-$	H_2O
	266 (100%) $[M_D - D - 19]^-$		283 (100%) $[M_D - D - 19]^-$	HOD
			282 (23%) $[M_D - D - 20]^-$	D_2O
255 (9%) $[M - H - 28]^-$	257 (13%) $[M_D - D - 28]^-$	271 (38%) $[M - H - 28]^-$	274 (36%) $[M_D - D - 28]^-$	CO
241 (17%) $[M - H - 42]^-$	243 (13%) $[M_D - D - 42]^-$	257 (16%) $[M - H - 42]^-$	260 (10%) $[M_D - D - 42]^-$	CH_2CO
240 (74%) $[M - H - 43]^-$	242 (62%) $[M_D - D - 43]^-$	256 (30%) $[M - H - 43]^-$	259 (35%) $[M_D - D - 43]^-$	CH_3CO
	241 (92%) $[M_D - D - 44]^-$		258 (71%) $[M_D - D - 44]^-$	CH_2DCO
239 (18%) $[M - H - 44]^-$		255 (26%) $[M - H - 44]^-$		CO_2
173 (34%) $[M - H - 110]^-$	173 (29%) $[M_D - D - 112]^-$	173 (48%) $[M - H - 126]^-$	173 (17%) $[M_D - D - 129]^-$	See Scheme 2
	174 (11%) $[M_D - D - 111]^-$			

M_D corresponds to the molecular mass in which all exchangeable protons are replaced by deuterium (i.e., M + 3 in the case of brazilein; M + 4 in the case of hematein).



Scheme 2.

in the case of brazilein **3** show relatively little evidence of deuterium incorporation in the fragment ion. The scrambling which occurs (and which is more prevalent in the spectrum of the hematein **4**) may be explained by equilibration of hydroxyl groups and the anion centre in the intermediates **12** and **13**.

In conclusion, the CID mass spectra of the oxidised forms **3** and **4** of neoflavonoids provide information on the substituents of rings B, D and the methylene group of ring C; the mass of the neutral fragment lost gives information on the substituent pattern of ring A.

3. Experimental

Hematoxylin **2** was obtained from Fluka Chemika and hematein **4** from Acros, while brazilin **1** was purchased from Pfaltz and Bauer. The oxidised form brazilein **3** was present in the sample adventitiously.

Mass spectra were recorded on a Finnigan LCQ mass spectrometer operating in negative ion electrospray mode. Samples were directly injected into the source in solution in methanol or deuteriated methanol (MeOD) as appropriate. Peaks were selected for CID with a band-width of 1.0 Da and operating parameters were optimized by tuning the detector to the $[M - H]^-$ ion peak. Data were collected and processed by LCQ Navigator software.

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