Structural Analyses of Carbon Chains in 5-Alk(en)ylresorcinols of Rye and Wheat Whole Flour by Tandem Mass Spectrometry

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The locations of double bonds and the linearity of each carbon chain in 5-alk(en)ylresorcinols present in rye and wheat whole flour were determined from collision-activated dissociation (CAD) spectra by tandem mass spectrometry. Among the determined alk(en)ylresorcinols, eleven alkylresorcinols (C₁₇ to C₂₃) and four alkadienylresorcinols (C₁₉ to C₂₅) had not been previously identified. In this study, we found that identification of a radical-anion fragment peak due to a simple allylic cleavage on the methyl side was essential for determining the locations of double bonds in positionally isomeric alkylresorcinols. In addition, an analysis of the CAD spectra of lithium-adduct cations as precursor ions was useful for determining the locations of the homoconjugated (Z,Z)-diene group in alkadienylresorcinols.

Key words: 5-alk(en)ylresorcinol; double-bond location; collision-activated dissociation; charge-remote fragmentation; tandem mass spectrometry

Bouillant et al. and our team have independently reported the identification of 5-alk(en)ylresorcinols (ARs), which are antifungal agents toward rice blast fungus, from root exudates and from etiolated rice seedlings. These rice ARs consist of five components in two homologous series: three alkylresorcinols with chain lengths of C₁₃, C₁₅, and C₁₇, and two alkadienylresorcinols with chain lengths of C₁₅ and C₁₇ and a (Z)-double bond, of which the C₁₇-alkenylresorcinol accounts for 45–55% of the total amount of ARs in rice seedlings. Collision-activated dissociation (CAD) spectra of ARs obtained by tandem mass spectrometry showed the double bonds of C₁₁, C₁₃, C₁₅, and C₁₇-alkenylresorcinols to be located on the 8′-carbon with no branching existing in the carbon chains.

A series of ARs with chain lengths of C₁₅ to C₂₃ have been isolated and identified in rye and wheat. These compounds are present during the early to mature stages of their development and are believed to act partly as antimicrobial agents. By GC and GC-EIMS analyses of these ARs, the chain lengths and the presence of pairs of isomeric alkadienylresorcinols have been revealed, but structural details such as the locations and stereochemistry of the double bonds and the linearity of the carbon chains had yet be determined. We describe here the determination of the carbon chain structures of all the known rye and wheat ARs through an analysis of CAD spectra.

Homologous series of alkyl, alkenyl, and alkadienylresorcinols were obtained by repeated column chromatography of the acetone extract of rye or wheat whole flour on silica gel, Sephadex LH-20, and again silica gel to give native ARs. The supernatant obtained by precipitation of the native ARs from n-hexane, which was partially enriched in alkyl and alkadienylresorcinol, was subjected to preparative TLC on silica gel impregnated with silver nitrate; there were three bands in the rye fraction (rye bands I to III) and two bands in the wheat fraction (wheat bands I and II). The approximate ratio of bands I, II, and III was 86%, 12%, and 2% in rye and 93% and 7% in wheat. ¹H-NMR, ¹³C-NMR, and FAB-MS data showed that each of these bands was alkylresorcinol with a saturated carbon chain (band I), alkylresorcinol with a (Z)-monoen (band II), and alkadienylresorcinol with a homoconjugated (Z,Z)-diene group (band III). The ¹H-NMR spectrum of each band in CDCl₃ (a) or CDCl₃+CD₂OD (b) gave signals characteristic of resorcinol with an alkyl substituent at the 5-position: a doublet at δ 6.25 (a) or 6.20 (b) ppm (2H, 4.6-H), a triplet at δ 6.17 (a) or 6.13 (b) ppm (1H, 2-H), and a broad singlet at around δ 4.7 ppm (phenolic OH) and triplet at δ 2.47 ppm (2H, 1'-H). The presence of a (Z)-monoen group in band II was shown from the ¹H- and ¹³C-NMR data: a multiplet at δ 5.25–5.40 ppm (2H) due to olefinic protons, and a methylene carbon at δ 27.20 ppm due to an allylic methylene carbon adjacent to a (Z)-double bond group. The presence of a homoconjugated (Z,Z)-diene group in band III was also shown from the ¹H- and ¹³C-NMR data: a multiplet at δ 5.25–5.45 ppm (4H) due to olefinic protons, and two methylene carbons at δ 27.20 and 27.24 ppm due to allylic methylene carbons adjacent to a (Z)-double bond group. Table I shows the approximate ratio of ARs in rye and wheat.

CAD of compounds that have a long hydrocarbon chain and a localized charge causes a specific and unique type of fragmentation termed “charge remote” fragmentation, and is quite useful for determining the locations of double bonds, branch points, and functional groups in a long hydrocarbon chain. As shown in Fig. 1(A), for instance, saturated fatty acids give a pattern of peaks which are evenly spaced by 14 amu with losses of C₆H₁₂₊₂ which arise from the alkyl terminus via a 1,4-elimination of H₃ (A-1). However, an unsaturated fatty acid gives a pattern of peaks with a “window” of 54 amu which is composed of abundant peaks by allylic cleavage via elimination (A-2) and of less-

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Abbreviations: CAD, collision-activated dissociation; AR(s), 5-alk(en)ylresorcinol(s).
Table I. Composition (% total) of Alk(en)yloresorcinols in Rye and Wheat

<table>
<thead>
<tr>
<th>Alkyloresorcinols (I)</th>
<th>Alkenyloresorcinols (II)</th>
<th>Alkadienylloresorcinols (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R = C₉H₂₅+₁</td>
<td>R = C₁₀H₂₅⁻₁</td>
<td>R = C₁₂H₂₅⁻₁</td>
</tr>
<tr>
<td>n = 17</td>
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<td></td>
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<tr>
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<td>AR₁₇:₁</td>
</tr>
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<td>8</td>
</tr>
<tr>
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<td>6</td>
</tr>
<tr>
<td>n = 21</td>
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</tr>
<tr>
<td>AR₂₅:₀</td>
<td>6</td>
<td>AR₂₃:₂</td>
</tr>
</tbody>
</table>

Composition is calculated from the relative intensities of the quasi-molecular ions in the negative FAB mass spectra.

(A) 1,4-Elimination of H₂

(B) Simple allylic cleavage

Fig. 1. Collision-activated Dissociation (CAD) of Fatty Acid [M-H]⁺ Ions.

abundant peaks by vinylic cleavage to and through the double bond.

i) Structures of alkylloresorcinols: rye and wheat band I

The CAD spectra of [M-H]⁺ ions of rye AR₁₇:₀ and wheat AR₁₉:₀ are shown in Fig. 2 (A and B), respectively. The CAD spectra of both AR₁₇:₀ and AR₁₉:₀ gave rise to a sequence of odd-mass series that was regularly spaced by 14 amu, after an initial loss of methane. Thus, the saturated carbon chain of AR₁₇:₀ and AR₁₉:₀ was confirmed to be linear. Linear carbon chains were also detected in other rye and wheat components (Table II).

ii) Structures of alkenyloresorcinols: rye and wheat band II

Figure 3 shows the CAD spectra of [M-H]⁺ ions of rye (A) and wheat (B) AR₁₉:₀. Neither of the peak patterns show an obvious "window" of 54 amu due to allylic cleavage via the elimination on either side of the double bond [Fig. 1(A-2)].⁹,¹⁰ Unlike the peak pattern of rice AR₁₇:₀ [Fig. 3(C)], and both are extremely complicated. This complexity was due to the presence of positional isomers of the double bond, because the presence of pairs of isomeric alkenyloresorcinols in rye and wheat has been suggested, as described in the introduction. To confirm this point, a model experiment with C₁₈:₁ fatty acids was performed. The CAD spectra of [M-H]⁺ ions of the 9(Z)-, 11(Z)-, and 13(Z)-isomers and of a mixture of two or three isomers were measured. Figure 4 shows the CAD spectra of [M-H]⁺ ions of the 9(Z)-, 11(Z)-, and 13(Z)-isomers, (A), (B), and (C), respectively, and of a 1:1 mixture of the 9(Z)- and 13(Z)-isomers (D) and of a 1:1:1 mixture of the 9(Z)-, 11(Z)-, and 13(Z)-isomers (E). Bambagioti et al.¹¹ have reported that, in negative ion CAD spectra of fatty acids and their derivatives with a (Z)-double bond, a peak corresponding to a radical-anion fragment due to simple allylic cleavage [Fig. 1(B)] was present at a position one amu higher than that due to allylic cleavage via the elimination on the methyl side [Fig. 1(A-2)/a-I]. In our CAD spectra of [M-H]⁺ ions of the fatty acids (Fig. 4), such radical-anion fragment peaks were observed at m/z 182 in the 9(Z)-isomer spectrum (A), at m/z 210 in the 11(Z)-isomer spectrum (B), and at m/z 238 in the 13(Z)-isomer spectrum (C). In the 1:1 mixture of the 9(Z)- and 13(Z)-isomers (D), two radical-anion fragments at m/z 182 and 238 were detected, and their relative intensities were comparable to the isomeric ratio. Similarly, a 1:1:1 mixture of the three isomers contained the corresponding three radical-anion fragment peaks (E). Such radical-anion fragment peaks were also present in the CAD spectra of [M-H]⁺ ions of rice AR₁₇:₁ [Fig. 3(C)] and AR₁₅:₁ (data not shown).² These results indicate that identifying the radical-anion fragment peaks in the CAD spectra of fatty acid anions and of 5-alkenyloresorcinol anions can lead directly to the determination of the double-bond locations in positionally isomeric components in a mixture without
Fig. 2. CAD Spectra of the Rye AR_{17:0} [M−H]^− Ion (A) and Wheat AR_{19:0} [M−H]^− Ion (B).

Fig. 3. CAD Spectra of the Rye AR_{18:1} [M−H]^− Ion (A), Wheat AR_{19:1} [M−H]^− Ion (B), and Rice AR_{17:1} [M−H]^− Ion (C). m/z Values written in the structures reveal fragment peaks due to allylic cleavage (Fig. 1A-2).
the need to further separate each isomer.

In fact, the radical-anion peaks in the CAD spectra of [M–H]⁻ ions of rye and wheat AR₁₉₁ were present at m/z 274 and 330 as major peaks, and at m/z 302 as minor peaks [Figs. 3(A) and (B)]. These results unambiguously indicate that the double bonds were located on the 10'-, 12',- and 14'-carbons in both cases. The approximate 10'(Z)/12(Z)/14(Z)-isomer ratio was 4/1/2 in rye and 4/1/5 in wheat from the relative intensities of the radical-anion fragment peaks. The locations of the double bonds for other rye and wheat components were determined in a similar manner (Table II).

iii) Structures of alkadienylresorcinols: rye band III

Figure 5 shows the CAD spectra of rye AR₂₁₂ with a homoconjugated (Z,Z)-diene group. Spectrum (A) was obtained by using the [M–H]⁻ ion, similarly to the cases of rice, rye, and wheat alkyl- and alkenylresorcinols. Since the peak pattern of spectrum (A) was not sufficient to deduce the location of the diene group, the CAD spectra of 9(Z),12(Z)-octadecadienoic acid as a representative compound were examined for comparison with that of rye AR₂₁₂. Figure 6 shows the CAD spectra of its [M–H]⁻ ion and [M+2Li–H]⁺ ion. In both the spectra, the expected three fragment peaks due to allylic cleavage via elimination were observed: at m/z 221, 181, and 127 for the negative ion (A) and at m/z 235, 195, and 141 for the positive ion (B). When the negative ion CAD spectrum of AR₂₁₂ [Fig. 5(A)] was directly compared with that of 9(Z),12(Z)-octadecadienoic acid [Fig. 6(A)], at least three
components of the 11',14', 12',15'-, and 13'16'-isomers were suggested to coexist. To confirm this result, the CAD spectra of the lithium-adduct cations of AR_{21,2} were measured [Figs. 5(B) to (D)] and compared with that of the C_{18:2} fatty acid [Fig. 6(B)]. Spectra (B) to (D) in Fig. 5 were obtained from the [M+Li]^+ [M+2Li−H]^+ and [M+3Li−2H]^+ ions of AR_{21,2}, respectively. The three intensive fragment peaks due to allylic cleavage in the AR_{21,2} CAD spectra (B, C, and D) by the lithium-adduct cations were observed like that in the C_{18:2} fatty acid CAD spectrum [Fig. 6(B)]. For example, in the CAD spectrum of the [M+Li]^+ ion [Fig. 5(B)], the three fragment peaks at m/z 349, 309, and 255, which correspond to allylic cleavage via elimination, were observed. This result indicates that the double bonds of the diene group were located only on the 12',15'-carbons. The CAD spectra of other adduct ions of AR_{21,2} [M+2Li−H]^+ and [M+3Li−2H]^+ [Figs. 5(C) and (D)], also show the same result as that of the [M+Li]^+ ion. Thus, the location of the diene group for AR_{21,2}, which was deduced from the [M+Li]^+ adduct ion, was also confirmed by the results from the other adduct ions [M+2Li−H]^+ and [M+3Li−2H]^+. This shows that any lithium-adduct cation of alkadienylresorcinols was applicable for determining the location of the homoconjugated diene group.

The double-bond locations in other minor components of C_{19}, C_{23}, and C_{25} were determined in a similar manner (Table II).

In conclusion, all the carbon-chain structures of rye and wheat ARs were determined from the CAD spectra by tandem mass spectrometry. Among the determined ARs, eleven alkadienylresorcinols (C_{17} to C_{23}), except the 8'(Z) and 12'(Z)-isomers of C_{17}, are from rye and wheat, and four alkadienylresorcinols (C_{19} to C_{23}) from rye not
Structures of S-Alk(en)ylresorcinols in Rye and Wheat Whole Flour

![Chemical structures](image)

**Fig. 6.** CAD Spectra of the 9(Z),12(Z)-Octadecadienoic Acid [M−H]⁻ Ion (A) and [M+2Li−H]⁻ Ion (B). m/z: Values written in the structures reveal fragment peaks due to allylic cleavage (Fig. 1(A–Z)).

| Table II. Structures of Alk(en)ylresorcinols in Rye and Wheat and Their Composition (% Total) |
|-------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|
|                                                 | C₁₅     | C₁₇     | C₁₉     | C₂₁     | C₂₃     | C₂₅     |         |
| Rye                                             |         |         |         |         |         |         |         |
| I                                               | /       | /       | Linear  | Linear  | Linear  | Linear  |         |
| II                                              | /       | /       | 8(Z) 60%* | 10(Z) 61% | 12(Z) 39% | 15(Z) 12% | /       |
| III                                             | /       | /       | /       | 10(Z) 13(Z) | 12(Z) 15(Z) | 14(Z) 17(Z) | 16(Z) 19(Z) |
| Wheat                                           |         |         |         |         |         |         |         |
| I                                               | /       | /       | 8(Z) 35% | 10(Z) 39% | 12(Z) 19% | 15(Z) 17% | /       |
| II                                              | /       | /       | 10(Z) 23% | 12(Z) 11% | 14(Z) 11% | 16(Z) 35% | /       |
| Rice                                            | I       | /       | /       | Linear  | Linear  | Linear  | Linear  |
| II                                              | /       | 8(Z) 8(Z) | /       | /       | /       | /       |         |

* Relative ratio (%). I, alkylresorcinols; II, alkylresorcinols; III, aldehydylresorcinols.

previously been identified. The saturated carbon chains were all linear. Each homolog of the alkadienylresorcinols was composed of single isomer, while each homolog of the alkylresorcinols in both rye and wheat was composed of positional isomers in double-bond locations, unlike that in rice. In addition, we found that identification of the radical-anion fragment peak due to simple allylic cleavage on the methyl side in alkylresorcinols was essential for determining the locations of double bonds in the positional isomers in a mixture. An analysis of the CAD spectra of the lithium-adduct cations as precursor ions was useful for determining the locations of the homoconjugated (Z,Z)-diene group in alkadienylresorcinols.

**Experimental**

General. NMR spectra were recorded with TMS as an internal standard, using JEOL JNM-EX 270 and GSX-500 spectrometers.

**FAB/MS and FAB/MS/MS.** A JMS HX-110/110A tandem mass spectrometer was used. Ions were produced by bombarding the sample with 6 keV Xe atoms, and these were then accelerated through a potential of 10 kV. CAD experiments were conducted by mass selection of ions with MS-I, the mass resolution for the mass-selected ions being approximately 1000. The 10 keV mass-selected ions were then activated by collision with helium in a collision chamber floated at 8 kV. The helium gas pressure was adjusted in order to attenuate the primary ion beam by 70%. Fragment ions were detected with a JEOl ADS 115 variable-dispersion array detector equipped with MS-II. Nitrobenzy alcohol (3-NBA) for negative ion MS and Magic Bullet (dithioerythritol/dithiodiglycol=1/3) saturated with lithium hydroxide for positive-ion MS were used as matrixes. All CAD spectra were obtained by using quasi-molecular anions and/or lithium-adduct cations produced by FAB as precursor ions.

**Isolation of native ARs in wheat and rye whole flour.** Rye whole flour (150 g) was extracted with acetone (200 ml). The CHCl₃-soluble portion...
(2.31 g) of the acetone extract was chromatographed in a silica gel column. Elution with 20% EtOAc-n-hexane gave crude ARs (327 mg), which were then passed through a Sephadex LH-20 column with MeOH. Partially pure ARs (238 mg) were finally purified by column chromatography on silica gel with 10% EtOAc-n-hexane to give native ARs (181 mg). The native ARs (133 mg) were obtained from wheat whole flour (200 g) in a similar manner.

Separation of alkyl-, alkenyl-, and alkyldienesresorcinols. Rye native ARs (181 mg) were dissolved in n-hexane. Forty-six mg of the supernatant (74.4 mg) was charged on silica gel plates (20 cm × 20 cm × 0.5 mm thick) impregnated with 15% silver nitrate in EtOH-H2O (1:1). The plates were developed once with CH3H2OEtOAc (85:15) up to a 10-cm height and then by a developing step up to a 15-cm height. Three bands (rye bands I, II, and III) for rye ARs and two bands (wheat bands I and II) for wheat ARs were observed upon visualization by spraying with distilled H2O. Each of the bands was scraped off and eluted with 10% MeOH-CHCl3 to give rye bands I (17.7 mg), II (11.1 mg), and III (17.7 mg). Similarly, for wheat, 36.5 mg of the supernatant gave wheat bands I (21.4 mg) and II (6.7 mg).

Rye band I (alkylresorcinol) was obtained as a pale yellow powder; negative FAB-MS m/z ([M – H]−, rel. int. %): 347 (59, AR17,0) 375 (100, AR18,0), 403 (77, AR21,0), 431 (36, AR23,0), 459 (31, AR25,0). 1H-NMR (270 MHz, CDCl3 + CD3OD) δ: 6.20 (2H, d, J = 2.3 Hz, H-4 and H-6), 6.17 (1H, t, J = 2.3 Hz, H-2), 2.47 (2H, t, J = 7.2 Hz, H-1), 1.55 (2H, m, H-2), 1.25 (approx. 25H, br s), 0.89 (5H, t, J = 6.7 Hz).

Rye band II (alkylresorcinol) was obtained as an oil; negative FAB-MS m/z ([M – H]−, rel. int. %): 345 (17, AR17,0) 373 (100, AR18,0), 401 (70, AR21,0), 429 (17, AR23,0), 457 (5, AR25,0). 1H-NMR (270 MHz, CDCl3) δ: 6.25 (2H, d, J = 2.3 Hz, H-4 and H-6), 6.17 (1H, t, J = 2.3 Hz, H-2), 2.47 (2H, t, J = 7.2 Hz, H-1), 2.00 (4H, m, CH = CH–CH2–), 1.55 (2H, m, H-2), 1.25 (approx. 25H, br s), 0.88 and 0.89 (each approx. 1.5H, t, J = 6.7 Hz). 13C-NMR (125.3 MHz, CDCl3) δ: 156.52 (s, C-1 and C-3), 146.16 (s, C-5), 129.90 and 129.87 (d), 105.03 (d, C-4 and C-6), 100.09 (d, C-2), 35.80 (t, C-1), 31.89 (t), 31.03 (t, C-2), 29.75 (t), 29.66 (t), 29.57 (t), 29.53 (t), 29.51 (t), 29.30 (t), 29.26 (t), 27.20 (t, Z-allylic CH2), 22.68 (t), 14.10 (q), minor peaks [31.96 (t), 31.77 (t), 28.97 (t), 26.20 (t), 22.33 (t), 13.99 (q)].

Rye band III (alkylresorcinol) was obtained as an oil; negative FAB-MS m/z ([M – H]−, rel. int. %): 371 (15, AR19,0) 399 (100, AR20,0), 427 (20, AR23,0), 455 (6, AR25,0); positive FAB-MS m/z (rel. int. %): 379, 405 [M + Li]+; 17, [M + 2Li–H]+; 13, AR19,0, 407, 413, 419 ([M + Li]+); 100, [M + 2Li–H]+; 70, [M + 3Li–2H]+; 19, AR21,0, 435, 441 [M + Li]+; 20, [M + 2Li–H]+; 15, AR23,0, 463 [M + Li]+; 7, AR19,0. 1H-NMR (270 MHz, CDCl3) δ: 6.25 (2H, d, J = 2.3 Hz, H-4 and H-6), 6.17 (1H, t, J = 2.3 Hz, H-2), 5.25–5.45 (4H, m, –CH = CH–), 4.65 (2H, br s, OH), 2.48 (2H, t, J = 7.2 Hz, H-1), 2.05 (4H, m, –CH = CH–), 1.55 (2H, m, H-2), 1.25 (approx. 25H, br s), 0.89 (3H, t, J = 6.7 Hz). 13C-NMR (125.3 MHz, CDCl3) δ: 156.56 (s, C-1 and C-3), 146.14 (s, C-5), 130.19 and 127.94 (d), 108.03 (d, C-4 and C-6), 100.08 (d, C-2), 35.80 (t, C-1), 31.52 (t), 31.05 (t, C-2), 29.68 (t), 29.64 (t), 29.56 (t), 29.49 (t), 29.36 (t), 29.33 (t), 29.27, 27.24, 27.20 (t, Z-allylic CH2), 22.57 (t), 14.07 (q).

Wheat band I (alkylresorcinol) was obtained as a pale yellow powder; negative FAB-MS m/z ([M – H]−, rel. int. %): 375 (30, AR19,0), 403 (100, AR21,0), 431 (29, AR23,0), 459 (10, AR25,0). 1H-NMR (270 MHz, CDCl3 + CD3OD) almost the same as that described for rye band I.

Wheat band II (alkylresorcinol) was obtained as an oil; negative FAB-MS m/z ([M – H]−, rel. int. %): 345 (10, AR17,0) 373 (100, AR18,0), 401 (4H, AR21,0), 429 (7, AR23,0). 1H-NMR (270 MHz, CDCl3) almost the same as that described for rye band II.

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References