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Detection, characterization and identification of crucifer phytoalexins using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry

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Abstract

We have analyzed 23 crucifer phytoalexins (e.g. brassinin, dioxibrassinin, cyclobrassinin, brassicanals A and C) by HPLC with diode array detection and electrospray ionization mass spectrometry (HPLC-DAD–ESI-MS) using both negative and positive ion modes. Positive ion mode ESI-MS appeared more sensitive than negative ion mode ESI-MS in detecting this group of compounds. A new HPLC separation method, new LC–MS and LC–MS^2 data and proposed fragmentation pathways, LC retention times, and UV spectra for selected compounds are reported.
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Keywords: Crucifers; Phytoalexins; HPLC-DAD–ESI-MS^2

1. Introduction

Phytoalexins are essential secondary metabolites produced de novo by plants in response to diverse forms of stress, including microbial attack [1]. The accumulation of phytoalexins is one of several induced defense responses associated with plant disease resistance [2]. Most of the crucifer phytoalexins are characterized by a substituted indole ring with nitrogen- and sulfur-containing functional groups. More than 30 indole and indole-related phytoalexins have been isolated from crucifers such as canola, rapeseed, mustard, cabbage, radish, wasabi and turnip since the first report [3] was disclosed [4]. Due to their worldwide cultivation and consumption, whether as vegetables, oilseeds or condiments, crucifers are of enormous economic importance. Therefore, not surprisingly, phytoalexins of crucifers have incited a great deal of research of their synthesis, biosynthesis, detoxification and evaluation of biological activities [5]. Interestingly, a number of epidemiological studies have shown that a diet rich in crucifer vegetables may reduce the risk of various types of cancer by modulating carcinogen metabolism and that phytoalexins appear to have a positive effect [6].

The detection of phytoalexins in extracts of elicited tissues of crucifers has been carried out by TLC with biodetection, e.g. utilizing spores of *Cladosporium* or *Bipolaris* species, and HPLC with UV or diode array detection (DAD) [4]. However, the determination of chemical structures by spectroscopic methods still requires the isolation of purified (chromatographically homogeneous material) phytoalexins from substantial amounts of plant material. For example, several kilograms of plant tissue could yield multi-grams of an extract which, after lengthy column separations, could afford 1–5 mg of a compound suitable for spectroscopic characterization by NMR, etc. [4,7]. That is, the whole isolation process is tedious, expensive and time consuming. Furthermore, considering that rather different crucifer species can produce several of the already known phytoalex-

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Table 1
Characterization of crucifer phytoalexins and related compounds **1**–**25** by HPLC-DAD–ESI-MS^2 analyses

Peak no., t_R (min)	Compound name (molecular formula)	[M]	ESI-MS: *m/z* (relative intensity, %) positive mode MS	Positive mode MS^2 of $[M+H]^{+}$[a]	Negative mode MS	UV, λ_{max} (nm)
1, 2.8	Isalexin ($C_9H_7NO_3$)	177	200 $[M+Na]^+$ (100), 178 (93), 132 (6), 105 (7)	160 (100), 132 (56), 105 (66)	ND[b]	199, 234, 335
2, 5.4	Indole-3-carboxaldehyde (C_9H_7NO)	145	168 $[M+Na]^+$ (5), 146 (70), 118 (100)	118 (100)	144 (100)	210, 245, 260, 300
3, 6.5	Dioxibrassinin ($C_{11}H_{12}N_2O_2S_2$)	268	291 $[M+Na]^+$ (80), 269 (48), 251 (15), 221 (59), 203 (100), 161 (39)	251 (19), 221 (81), 203 (100), 161 (42)	267 (12), 219 (11), 159 (100), 160 (19)	210, 255
4, 6.8	Cyclobrassinone ($C_{11}H_8N_2O_2S$)	232	255 $[M+Na]^+$ (6), 233 (100), 176 (3)	206 (17), 201 (20), 176 (100)	231 (100), 174 (13)	218, 278, 312
5, 8.8	Brassicanal C ($C_{10}H_9NO_3S$)	223	246 $[M+Na]^+$ (28), 192 (100), 174 (71), 164 (64), 148 (73), 146 (70)	–	222 (100), 192 (15), 193 (13)	215, 247, 310
6, 9.1	Camalexin ($C_{11}H_8N_2S$)	200	201 (100)	ND[b]	199 (100), 190 (6), 172 (7), 142 (3)	215, 278, 318
7, 9.3	Brassicanal A ($C_{10}H_9NOS$)	191	214 $[M+Na]^+$ (6), 192 (100), 164 (14), 117 (24)	164 (100), 117 (53)	190 (100), 175 (9)	218, 258, 269, 325
8, 10.6	Brassilexin ($C_9H_6N_2S$)	174	175 (100), 148 (5)	148 (100)	173 (100)	220, 245, 264
9, 10.6	Indolyl-3-acetonitrile ($C_{10}H_8N_2$)	156	179 $[M+Na]^+$ (2), 157 (5), 130 (100)	130 (100)	ND[b]	220, 272, 285
10, 10.9	Spirobrassinin ($C_{11}H_{12}N_2S_2$)	250	273 $[M+Na]^+$ (8), 251 (100), 203 (21), 178 (8)	203 (100), 178 (38)	249 (89), 217 (3), 201 (100)	220, 258, 296
11, 11.0	Cyclobrassinin sulfoxide ($C_{11}H_{11}N_2OS_2$)	250	273 $[M+Na]^+$ (15), 251 (3), 187 (100)	–	249 (3), 201 (4), 160 (100)	213, 226, 278, 330
12, 11.3	Brassitin ($C_{11}H_{12}N_2OS$)	220	243 $[M+Na]^+$ (28), 130 (100)	–	ND[b]	220, 270, 278
13, 12.3	Arvelexin ($C_{11}H_{10}N_2O$)	186	209 $[M+Na]^+$ (14), 187 (100), 160 (33), 147 (65), 132 (5)	160 (61), 147 (100)	ND[b]	220, 266, 280, 290
14, 12.9	Rutalexin ($C_{11}H_8N_2O_2S$)	232	255 $[M+Na]^+$ (5), 233 (100), 192 (17), 148 (17)	176 (5), 148 (100)	231 (100), 174 (4)	213, 242, 275
15, 14.4	Brassicanate A ($C_{11}H_{11}NO_2S$)	221	244 $[M+Na]^+$ (28), 222 (8), 190 (100)	–	220 (100), 205 (7)[c]	220, 238, 268, 300
16, 15.3	Caulilexin A ($C_{10}H_9NO_3S$)	223	246 $[M+Na]^+$ 224 (31), 176 (100)	176 (100)	222 (5), 176 (100)	213, 252, 318
17, 16.1	1-Methoxyspirobrassinin ($C_{12}H_{14}N_2OS_2$)	280	303 $[M+Na]^+$ (3), 281 (100), 250 (21)	250 (100), 178 (38)	ND[b]	218, 260, 295
18, 16.3	1-Methoxyindolyl-3-acetonitrile ($C_{11}H_{10}N_2O$)	186	187 (49), 160 (100), 130 (20)	171 (10), 160 (100), 146 (141)	ND[b]	220, 272
19, 18.3	Brassinin ($C_{11}H_{12}N_2S_2$)	236	259 $[M+Na]^+$ (100), 237 (2), 176 (2), 130 (51)	–	236 (100), 190 (9), 172 (7)	220, 269
20, 19.3	Sinalexin ($C_{10}H_8N_2OS$)	204	205 (39), 174 (100)	188 (8), 174 (100)	ND[b]	225, 247, 262
21, 20.4	Erucalexin ($C_{12}H_{12}N_2O_2S_2$)	280	303 $[M+Na]^+$ (2), 281 (57), 250 (100), 249 (66), 203 (35)	249 (100), 203 (26)	ND[b]	234, 262, 368
22, 20.6	Wasalexin B ($C_{13}H_{14}N_2O_2S_2$)	294	317 $[M+Na]^+$ (2), 295 (100), 263 (3), 247 (15)	263 (23), 247 (100)	ND[b]	205, 248, 285, 368
23, 22.8	Wasalexin A ($C_{13}H_{14}N_2O_2S_2$)	294	317 $[M+Na]^+$ (6), 295 (100), 263 (6), 247 (21)	263 (23), 247 (100)	ND[b]	205, 248, 285, 368
24, 23.1	Cyclobrassinin ($C_{11}H_{11}N_2S_2$)	234	235 (37), 187 (15), 162 (100)	187 (20), 162 (100)	233 (8), 190 (37), 172 (9), 161 (100)	205, 229, 285, 294
25, 23.6	1-Methoxybrassinin ($C_{12}H_{14}N_2OS_2$)	266	289 $[M+Na]^+$ (25), 267 (49), 235 (36), 219 (10), 160 (100), 146 (68), 128 (30), 117 (87)	219 (55), 160 (25), 146 (100)	265 (100), 220 (47), 190 (48), 144 (68)	218, 268

[a] ESI-MS^2 not available for $[M+H]^+$ with low intensity (<10%).
[b] ND: not detected.
[c] Negative ESI-MS^2 *m/z* 205 (100), 175 (7).

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