

# Electron Impact Mass Spectral Analysis of Flavonoids

Paul A. Hedin\* and Valeria A. Phillips

Crop Science Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture,  
Mississippi State, Mississippi 39762

Electron impact (EI) mass spectra were recorded for 43 flavones and flavonols, 7 isoflavones, 18 flavanones and dihydroflavonols, and 11 chalcones and dihydrochalcones. Chemical ionization (CH<sub>4</sub>) mass spectra were recorded for 9 flavonoid glycosides. The flavonoids gave significant fragments from the A and B rings that were classified according to the systems of Mabry and Markham (1975) and Grayer (1989). Often sufficient information from the fragmentations could be obtained to determine the molecular weight, the elemental formula, the substitution patterns in the A and B rings, and the class of flavonoid. Of the 79 flavonoids analyzed, 26 could not be located in the NIST database. With the flavonoid glycosides, CI (CH<sub>4</sub>) mass spectra gave significant fragments for the [M + 1]<sup>+</sup> of the aglycon and the sugars. However, fragments from fission of the A and B rings were very weak.

While mass spectrometry (MS) normally cannot provide sufficient information for a complete structure assignment of flavonoids, it can be used to determine the molecular weight of the aglycon and to give some information about the substituents in the A and B rings. It can also give information about the site of attachment of sugars if suitable derivatives of the flavonoids are prepared, sometimes employing chemical ionization (CI) techniques. Comprehensive information about MS of flavonoids has been published by Mabry and Markham (1975), Mabry and Ulubelen (1980), Markham (1988), and Grayer (1989) among others.

Generally predictable fragmentation patterns have been established for the major classes of flavonoid aglycons. Because each of the classes often yields one or more characteristic fragments not generated by the others, assignments may be possible. Mabry and Markham (1975) have developed a systematic nomenclature to define the various A and B ring fragments generated by EI-MS, which has been further organized by tabulation (Grayer, 1989) (Figure 1 and Table I).

The National Institute of Standards and Technology, U.S. Department of Commerce, has recently made available an affordable mass spectral database with over 50 000 spectra (Lias and Stein, 1990). However, only 20 of 46 flavonoids that had been acquired for analysis could be located in the database. Because many of the others were of specific interest, EI-MS spectra were obtained on the entire laboratory collection of flavonoids, and they were classified according to the systems of Mabry and Markham (1975) and Grayer (1989).

Also included were 31 spectra from the NIST database of structurally related flavonoids. Finally, CI-MS (CH<sub>4</sub>) data on 9 flavonoid glycosides was collected and analyzed.

## MATERIALS AND METHODS

**Experimentally Generated Flavonoid Mass Spectra.** Mass spectra were taken at 70 eV in the positive EI mode with a HP 5985B quadrupole mass spectrometer. The sample was introduced into the source of the instrument via a direct insertion probe. The source was maintained at a temperature of 200 °C, and the probe was ballistically heated from ambient temperature to 350 °C. Positive CI spectra were taken with methane as the reagent gas. The source pressure was  $3 \times 10^{-4}$  Torr, the electron energy was 235 eV, and the source temperature was 150 °C. The probe temperature was raised from ambient to 350 °C over 10 min. Spectra were obtained on solids, generally the average of three analyses in which differences were minor.

**Flavonoid Mass Spectra in NIST Database.** A search of the NIST database located 78 flavones and isoflavones, 20 flavanones, 26 chalcones, 24 aurones, and 3 catechins, a few of which were duplicates. Of this collection, 31 were selected for inclusion with the experimentally generated mass spectra because they were structurally related. It was noted that the experimentally generated spectra were essentially identical to the NIST spectra. It was initially intended to include a table of aurones, but fragments from the A and B rings were generally low in intensity and often varied by 1 or 2 mass units from that predicted from the systems of Mabry and Markham (1975) and Grayer (1989).

**Procurement.** Compounds were obtained from one of the following sources: Atomergic Chemicals Corp., Farmingdale, NY; Fluka Chemika-Bio Chemika, Ronkonkomo, NY; and Sigma Chemical Co., St. Louis, MO. The compounds were not further purified; however, they were examined by TLC before analysis.

## RESULTS AND DISCUSSION

Figure 1 gives structures for the primary A and B ring fragments generated by EI-MS. The various classes of flavonoids have specific fragmentation patterns by which they generally can be differentiated. This classification

Table I. Characteristic A and B Ring Fragments in Various Flavonoid Classes<sup>a</sup>

flavonoid class	A or B ring fragment							
	A <sub>1</sub> /A <sub>1</sub> + 1	A <sub>2</sub>	B <sub>1</sub>	B <sub>2</sub>	B <sub>3</sub>	B <sub>3</sub> - 2	B <sub>4</sub>	B <sub>5</sub>
flavanones	+	-	(+) <sup>b</sup>	-	+	-	+	-
dihydroflavonols	+	(+)	-	-	+	+	+	-
dihydrochalcones	-	+	-	-	+	-	+	-
flavones	+	-	+	(+)	-	-	-	-
isoflavones	+	-	+	-	-	-	-	-
flavonols	+	-	-	+	-	-	-	-
chalcones <sup>c</sup>	(+)	+	(+)	-	-	-	-	+

<sup>a</sup> Modified from Grayer (1989). <sup>b</sup> (+), sometimes present. <sup>c</sup> Chalcones give A<sub>1</sub><sup>+</sup> and B<sub>1</sub><sup>+</sup> on isomerization to flavones.

Table II. EI-MS Fragmentations of Selected Flavones and Flavonols<sup>a-c</sup>

compound	M <sup>+</sup>	[M - 1] <sup>+</sup>	[M - 15] <sup>+</sup>	[M - OH/ H <sub>2</sub> O] <sup>+</sup>	[M - CO/ CHO] <sup>+</sup>	[M - 43] <sup>+</sup>	[A <sub>1</sub> + 1] <sup>+</sup>	A <sub>1</sub> <sup>+</sup>	A <sub>2</sub> <sup>+</sup>	[A <sub>1</sub> - CO] <sup>+</sup>	B <sub>1</sub> <sup>+</sup>	B <sub>2</sub> <sup>+</sup>	other
flavone <sup>b</sup>	222 (100)	221 (30)			194 (42)			120 (68)		92 (52)			165 (20)
6-methylflavone <sup>a</sup>	236 (100)	235 (24)			208 (21)			134 (87)		106 (16)			105 (18)
7-hydroxyflavone <sup>a</sup>	238 (74)				210 (4)			136 (100)		108 (10)		108 (10)	105 (6)
6-methoxyflavone <sup>a</sup>	238 (100)	237 (20)			210 (78)			136 (42)		108 (36)			
4'-methoxyflavone <sup>a</sup>	252 (100)	251 (84)			222 (13)			150 (58)	135 (16)	122 (10)			223 (15), 107 (31)
5,7-dihydroxyflavone (chrysin) <sup>b</sup>	252 (80)	251 (38)			222 (18)	209 (15)		152 (18)		92 (20)	132 (100)		117 (30), 89 (34)
4,7-dihydroxyflavone <sup>a</sup>	254 (100)			238 (100)	226 (19)			137 (18)		124 (17)			255 (10)
7,8-dihydroxyflavone <sup>a</sup>	254 (62)				226 (16)			136 (42)		108 (28)			210 (66), 134 (15)
3-hydroxy-7-methoxyflavone <sup>a</sup>	268 (100)	267 (45)			226 (5)			152 (100)		124 (4)			255 (4)
7-hydroxy-4'-methoxyflavone <sup>a</sup>	268 (100)	267 (25)	253 (8)		240 (21)	225 (16)		136 (10)			132 (58)		117 (15)
3,5,7-trihydroxyflavone (galangin) <sup>b</sup>	270 (100)	269 (36)			242 (43)			153 (10)			105 (51)		271 (10), 213 (32)
4',5,7-trihydroxyflavone (pigenin) <sup>b</sup>	270 (100)	269 (17)			242 (12)			153 (22)		124 (10)	118 (9)		168 (22)
5-hydroxy-4'-methoxy-7-methylflavone <sup>a</sup>	282 (100)	281 (6)			242 (12)	239 (11)		150 (22)		122 (15)	132 (21)		283 (18)
5,7-dimethoxyflavone <sup>a</sup>	282 (100)	281 (56)		265 (26)	253 (46)			168 (5)		102 (12)	105 (14)		236 (48), 209 (31)
5,7-dihydroxy-6-methoxyflavone <sup>a</sup>	284 (100)	283 (7)	269 (71)	266 (50)	241 (62)			167 (15)		102 (10)	105 (5)		139 (26), 103 (14)
5,7-dihydroxy-4'-methoxyflavone <sup>a</sup>	284 (21)	283 (100)			256 (7)	241 (18)		152 (15)		132 (25)			
3,4',5,7-tetrahydroxyflavone (kaempferol) <sup>b</sup>	286 (100)	285 (23)			258 (20)			153 (13)			121 (48)		287 (15), 229 (27), 136 (14)
3',4',5,7-tetrahydroxyflavone (luteolin) <sup>b</sup>	286 (100)	285 (11)			258 (12)			153 (29)		134 (12)			287 (15), 129 (13), 124 (8)
3-methoxy-2-phenyl-4H-furo[2,3b]-1-benzopyran-4-one (karanjin) <sup>a</sup>	292 (52)	291 (100)		273 (9)				160 (34)		132 (8)			293 (8), 116 (5)
5-hydroxy-4',7'-dimethoxyflavone <sup>a</sup>	298 (100)	297 (10)			269 (11)	255 (12)		166 (10)			132 (22)		299 (14)
3,5,7-trihydroxy-4'-methoxyflavone (kaempferid) <sup>b</sup>	300 (100)	299 (17)		285 (14)				153 (2)		167 (15)	118 (8)		301 (22)
4',5,7-trihydroxy-3'-methoxyflavone (chrysoeriol) <sup>b</sup>	300 (100)	299 (8)			273 (12)			153 (14)		137 (16)	148 (7)		303 (12), 128 (15), 109 (8)
4',5,7-trihydroxy-6-methoxyflavone <sup>a</sup>	300 (98)	299 (8)	285 (58)	282 (60)				137 (11)					303 (15), 150 (6), 108 (4)
3',4',5,7-pentahydroxyflavone (quercetin) <sup>b</sup>	302 (100)	301 (24)						153 (33)					293 (32)
2',3',4',5,7-pentahydroxyflavone (morin) <sup>b</sup>	302 (82)			285 (100)									
5,7-dihydroxy-3-methoxy-6,8-dimethylflavone <sup>a</sup>	312 (100)			294 (30)	283 (28)	269 (12)							135 (15)
3,5-dihydroxy-4',7-dimethoxyflavone <sup>a</sup>	314 (100)	313 (5)	299 (11)	285 (8)	271 (15)			167 (15)					317 (15), 151 (5), 133 (7)
5,7-dihydroxy-3,4'-dimethoxyflavone <sup>b</sup>	314 (100)	313 (81)		296 (10)				137 (16)			121 (10)		301 (22)
3,4',5,7-tetrahydroxy-3'-methoxyflavone (isochromanin) <sup>b</sup>	316 (100)	315 (10)			287 (10)	271 (55)		153 (6)					303 (12), 128 (15), 109 (8)
3',4',5,7-tetrahydroxy-6-methoxyflavone <sup>a</sup>	316 (100)	315 (8)	301 (72)	299 (42)		273 (42)		153 (5)		167 (18)	134 (11)		317 (15), 139 (14)
3,3',4',5,6,7-hexahydroxyflavone (quercastigin) <sup>b</sup>	318 (100)	317 (26)			289 (10)			169 (12)					319 (22), 150 (11), 137 (12), 109 (6)
3,3',4',5,5',7-hexahydroxyflavone (myricetin) <sup>b</sup>	318 (100)	317 (15)			289 (6)			153 (20)					319 (14), 136 (12), 108 (6)
3,3',4',4',5,7,8-heptahydroxyflavone (gossypetin) <sup>b</sup>	318 (100)	318 (21)			289 (10)			169 (15)					319 (17), 150 (13), 137 (16), 109 (8)
3-hydroxy-4',5,7-trimethoxyflavone <sup>a</sup>	328 (72)	317 (14)		310 (24)	299 (15)	285 (20)		167 (5)					329 (17), 282 (100)
5-hydroxy-3,4',7-trimethoxyflavone <sup>a</sup>	328 (100)	327 (95)	313 (8)	309 (14)									329 (16), 150 (16), 135 (16), 119 (8)
4',5,6-trihydroxy-7,8-dimethoxyflavone <sup>a</sup>	330 (65)	329 (5)	315 (100)										297 (12), 197 (15)
3',4',5-trihydroxy-3',7-dimethoxyflavone <sup>a</sup>	330 (100)	329 (76)		312 (27)	301 (12)	287 (42)		151 (15)			164 (3)	167 (21)	331 (25)
2',3,5,7-tetramethoxyflavone <sup>a</sup>	342 (90)	341 (65)	327 (10)	323 (11)				181 (42)		152 (6)			311 (100)
3',4',5,7-tetramethoxyflavone <sup>a</sup>	342 (100)	341 (65)		325 (22)	313 (32)			167 (8)			162 (12)		296 (36), 157 (20)
5-hydroxy-3,3',4',7-tetramethoxyflavone <sup>a</sup>	358 (100)	357 (48)	343 (45)	340 (8)		315 (62)							359 (32), 165 (25)
5-hydroxy-3,3',4',7-tetramethoxyflavone <sup>a</sup>	358 (100)	357 (61)	343 (45)		327 (15)	315 (48)		167 (6)					359 (18), 165 (16), 149 (7), 119 (5)
4',5,6,7,8-pentamethoxyflavone (tangeretin) <sup>b</sup>	372 (23)		357 (100)			329 (4)		240 (1)	225 (6)		132 (3)		373 (5), 314 (11), 197 (16), 182 (13)

<sup>a</sup> Spectrum not found in NIST database. <sup>b</sup> Experimental spectrum also found in NIST database. <sup>c</sup> Spectrum from NIST database.

Table III. EI-MS Fragmentations of Isoflavones<sup>a-c</sup>

compound	M <sup>+</sup>	[M - 1] <sup>+</sup>	[M - 15] <sup>+</sup>	[A <sub>1</sub> + 1] <sup>+</sup>	A <sub>1</sub> <sup>+</sup>	B <sub>1</sub> <sup>+</sup>	other
4',7-dihydroxyisoflavone (daidzen) <sup>a</sup>	254 (100)	253 (64)		137 (78)		118 (35)	255 (14), 108 (6)
5,7-dihydroxyisoflavone <sup>c</sup>	254 (100)	253 (51)			152 (46)	102 (7)	124 (25)
7-hydroxy-4'-methoxyisoflavone (formononetin) <sup>a</sup>	268 (100)	267 (40)	253 (13)			132 (62)	269 (16), 117 (10)
4'-hydroxy-7-methoxyisoflavone <sup>c</sup>	268 (100)	267 (48)		151 (65)	150 (26)	118 (34)	268 (22), 122 (28)
4',5,7-trihydroxyisoflavone (genistein) <sup>a</sup>	270 (100)	269 (30)		153 (58)	152 (28)	118 (25)	271 (15), 126 (8)
6,7-dimethoxyisoflavone <sup>c</sup>	282 (100)	281 (78)	267 (20)		180 (24)		165 (15), 141 (21)
5,7-dihydroxy-4'-methoxyisoflavone (biochanin A) <sup>a</sup>	284 (100)	283 (18)	269 (14)		152 (16)	132 (47)	285 (15), 124 (9), 117 (11)

<sup>a</sup> Spectrum not found in NIST database. <sup>b</sup> Experimental spectrum also found in NIST database. <sup>c</sup> Spectrum from NIST database.

Table IV. EI-MS Fragmentations of Selected Flavanones and Dihydroflavonols<sup>a-c</sup>

compound	M <sup>+</sup>	[M - 1] <sup>+</sup>	[M - 29] <sup>+</sup>	[M - 43] <sup>+</sup>	[A <sub>1</sub> + 1] <sup>+</sup>	A <sub>1</sub> <sup>+</sup>	A <sub>2</sub> <sup>+</sup>	B <sub>3</sub> <sup>+</sup>	[B <sub>3</sub> - 2] <sup>+</sup>	B <sub>4</sub> <sup>+</sup>	other
flavanone <sup>b</sup>	224 (100)	223 (82)			121 (22)	120 (100)		104 (55)		92 (56)	225 (27), 147 (62)
dihydroflavonol <sup>a</sup>	240		211		121	120	105	120	118	91	241
6-hydroxyflavanone <sup>c</sup>	240 (45)	239 (17)				136 (100)		104 (5)			241 (6), 163 (16), 108 (14)
4'-methoxyflavanone <sup>c</sup>	254 (78)	253 (45)			121 (45)			134 (100)		121 (45)	255 (15), 119 (27)
5,7-dihydroxyflavanone <sup>c</sup>	256 (100)	255 (72)			153 (16)	152 (50)		104 (19)		91 (10)	179 (55), 124 (23)
4',5,7-trihydroxyflavanone (naringenin) <sup>b</sup>	272 (50)	271 (42)			153 (100)	152 (25)		120 (60)		107 (15)	273 (5), 179 (32), 166 (25), 123 (15)
5,7-dimethoxyflavanone <sup>c</sup>	284 (52)	283 (12)			181 (12)	180 (100)		104 (6)			152 (20), 137 (24)
4',5-dihydroxy-7-methoxyflavanone <sup>c</sup>	286 (85)	285 (4)			167 (100)	166 (36)		120 (52)			193 (32), 138 (28)
3',4',5,7-tetrahydroxyflavanone (eriodictol) <sup>a</sup>	288 (57)	287 (24)			153 (100)		136 (42)			123 (19)	179 (23), 166 (41)
3,3',4',7-tetrahydroxyflavone (dihydrofisetin) <sup>a</sup>	288 (10)		259 (13)		137 (100)					123 (55)	150 (8), 124 (5)
5-hydroxy-4',7-dimethoxyflavanone <sup>c</sup>	300 (73)	299 (36)			167 (15)	166 (25)		134 (100)		121 (71)	193 (20)
3',5,7-trihydroxy-4'-methoxyflavone (hesperetin) <sup>a</sup>	302 (87)	301 (30)			153 (61)			150 (66)		137 (100)	303 (12), 179 (27), 135 (59), 124 (19)
3,5,7-trihydroxy-4'-methoxyflavanone <sup>c</sup>	302 (73)	301 (22)			153 (62)	152 (10)		150 (91)		137 (100)	179 (27), 135 (52)
3',4',5-trihydroxy-7-methoxyflavanone <sup>a</sup>	302 (84)				167 (100)		136 (94)		123 (64)		
3,3',4',5,7-pentahydroxyflavanone (taxifolin) <sup>b</sup>	304 (22)		275 (31)		153 (100)	152 (23)		152 (23)	150 (26)	123 (38)	165 (16), 137 (5)
4',5,7-trimethoxyflavanone <sup>c</sup>	314 (50)	313 (36)			181 (71)	180 (29)		134 (72)		121 (100)	207 (32)
3,3',4',7-tetrahydroxy-5-methoxyflavanone <sup>c</sup>	318 (6)		289 (26)		167 (100)			152 (10)		123 (44)	
3,4',5,7-tetrahydroxy-8-methoxyflavanone <sup>a</sup>	318 (40)				183 (100)	182 (25)	167 (25)	136 (20)	134 (52)	107 (42)	

<sup>a</sup> Spectrum not found in NIST database. <sup>b</sup> Experimental spectrum also found in NIST database. <sup>c</sup> Spectrum from NIST database.

Table V. EI-MS Fragmentations of Selected Chalcones and Dihydrochalcones<sup>a-c</sup>

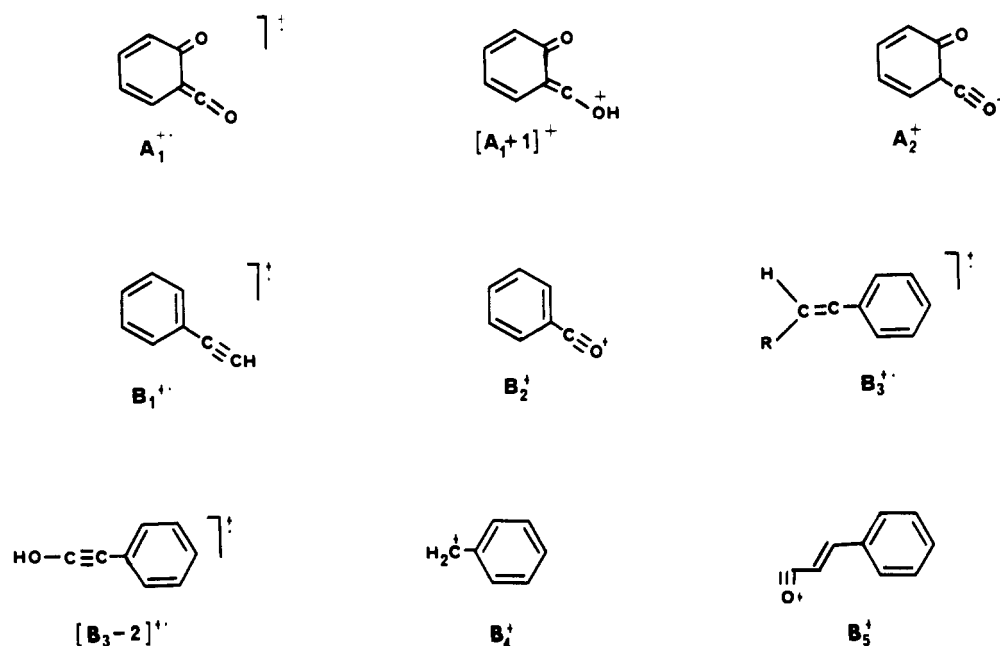
compound	M <sup>+</sup>	[M - 1] <sup>+</sup>	[M - OH] <sup>+</sup>	[M - 29] <sup>+</sup>	A <sub>1</sub> <sup>+</sup>	A <sub>2</sub> <sup>+</sup>	B <sub>1</sub> <sup>+</sup>	B <sub>2</sub> <sup>+</sup>	B <sub>3</sub> <sup>+</sup>	B <sub>4</sub> <sup>+</sup>	[B <sub>3</sub> - 28] <sup>+</sup>	other
chalcone <sup>b</sup>	208 (75)	207 (100)		179 (16)		105 (30)				131 (35)	103 (28)	
dihydrochalcone <sup>b</sup>	210					105		104	91			
2'-hydroxychalcone <sup>b</sup>	224 (100)	223 (87)				105 (10)				147 (95)	120 (55)	121 (60), 120 (70), 104 (42), 103 (51)
4'-hydroxychalcone <sup>b</sup>	224 (95)	223 (100)	207 (10)	195 (10)				121 (72)				131 (30), 121 (72)
2',4,4'-trihydroxychalcone <sup>c</sup>	256 (100)	255 (46)	239 (12)			137 (56)	120 (38)			147 (11)		257 (22), 163 (30)
2',6'-dihydroxy-4'-methoxychalcone <sup>b</sup>	270 (87)	269 (61)			166 (75)	167 (26)						271 (13), 193 (100), 138 (48)
2',6'-dihydroxy-4'-methoxydihydrochalcone <sup>a</sup>	272 (23)		255 (5)					167 (100)				273 (4), 140 (20)
2,3',4,4'-tetrahydroxychalcone (butein) <sup>a</sup>	272 (70)	271 (30)	255 (5)			135 (100)				163 (28)		273 (10), 150 (26), 136 (44), 123 (8)
2,4,4',6-tetrahydroxydihydrochalcone (phloretin) <sup>a</sup>	274 (24)		255 (9)			153 (100)		120 (48)				107 (42)
2',3,4'-trihydroxy-4'-methoxychalcone <sup>c</sup>	286 (16)				136 (42)	137 (100)	150 (51)			166 (22)		165 (24)
2',6'-dihydroxy-4,4'-dimethoxychalcone <sup>a</sup>	300 (52)	299 (37)						134 (100)		193 (30)	166 (22)	301 (8), 269 (8), 167 (14), 138 (15)

<sup>a</sup> Spectrum not found in NIST database. <sup>b</sup> Experimental spectrum also found in NIST database. <sup>c</sup> Spectrum from NIST database.

is given in Table I. Mass spectral (EI) data are listed for 43 flavones and flavonols (Table II), for 7 isoflavones (Table III), for 18 flavanones and dihydroflavonols (Table IV), and for 11 chalcones and dihydrochalcones (Table V). Also included are CI-MS fragmentations for 9 flavonoid glycosides (Table VI).

With the flavones and flavonols, the molecular ion was also the base peak in all but 11 instances, where it was still a significant peak. The loss of hydrogen was common and

of significant intensity. Methylated flavonoids produced an intense [M - 15]<sup>+</sup> ion, and this was the base peak of tangeretin which possessed 6- and 8-methoxy functions (Goudard et al., 1979). Many of the flavonoids lost [M - 28/29]<sup>+</sup>, CO or CHO, from the 4-keto function. As expected from Table I, only flavones gave A<sub>1</sub><sup>+</sup> fragments, while both flavones and flavonols gave (A<sub>1</sub> + 1)<sup>+</sup> fragments. Also, only flavones gave B<sub>1</sub><sup>+</sup> fragments, while B<sub>2</sub><sup>+</sup> fragments were more prevalent with flavonols. A feature not



R = H, for flavanones and dihydrochalcones  
R = OH, for dihydroflavonols

Figure 1. Primary EI mass spectral A and B ring fragments of flavonoids.

Table VI. CI-MS ( $\text{CH}_4$ ) Fragmentations of Selected Flavonoid Glycosides

compound	MW	fragmentations <sup>a-c</sup>
apigenin 7-apiosylglucoside	564	311 (5), 299 (15), <u>271</u> (100), 145 (10) G, 133 (61) AP, 127 (52) G, 115 (26) G, AP
naringenin 7-rhamnoglucoside	580	313 (4), 301 (8), <u>273</u> (58), 179 (12), 163 (3) G, 147 (40) R, 145 (10) G, 129 (42) R, 127 (8) G, 75 (100)
luteolin 7-glucoside	448	327 (3), 315 (18), <u>287</u> (100), 163 (3) G, 145 (18) G, 127 (18) G
isookanin 7-glucoside	450	329 (2), 317 (10), <u>289</u> (100), 163 (5) G, 153 (22) A, 145 (26) G, 127 (21) G, 115 (5) G
diosmetin 7-rhamnoglucoside	608	341 (3), 329 (25), <u>301</u> (100), 163 (4) G, 147 (22) R, 145 (12) G, 129 (17) R, 127 (6) G
quercetin 3-rhamnoglucoside	610	343 (2), 331 (12), <u>303</u> (100), 147 (10) R, 145 (15) G, 129 (24) R, 127 (12) G
tamarixetin 7-rhamnoglucoside	624	357 (2), 345 (12), <u>317</u> (100), 147 (10) R, 145 (9) G, 129 (81) R, 127 (12) G, 111 (5) R
myricetin 3-rhamnoside	464	359 (2), 347 (12), <u>319</u> (85), 47 (3) R, 129 (100) R, 111 (15) R
gossypetin 8-glucoside	480	359 (2), 347 (9), <u>319</u> (100), 145 (12) G, 127 (90) G, 115 (8) G

<sup>a</sup> The  $[M + 1]^+$  of the aglycon is underlined. <sup>b</sup> G, glucose; AP, apiose; R, rhamnose. <sup>c</sup> Experimental spectra.

noted in the earlier works (Mabry and Markham, 1975; Grayer, 1989) was the loss of CO from  $A_1^+$  with flavones but not with flavonols.

We were able to acquire only seven isoflavones for our study (Table III). However, they gave the predicted fragments including an  $M^+$  as the base peak, a strong  $[M - 1]^+$ , a noticeable  $[M - 15]^+$  for the methoxylated isoflavones, a strong  $A_1^+$  or  $[A_1 + 1]^+$ , and a strong  $B_1^+$ .

The flavanones gave strong peaks for the molecular ion ( $M^+$ ) and often a strong  $[M - 1]^+$  peak, while the dihydroflavonols gave a relatively weak  $M^+$  and the  $[M - 1]^+$  was absent (Table IV). However, the dihydroflavonols gave a  $[M - 29]^+$  ion. The base peak of both classes was the  $A_1^+$  or the  $[A_1 + 1]^+$  ion, generally the latter. Some dihydroflavonols gave an  $A_2^+$  ion. Nearly all gave prominent  $B_3^+$  and  $B_4^+$  ions, but only three dihydroflavonols gave  $[B_3 - 2]^+$  ions. The above relationships were consistent with the fragments predicted in Table I. Balza et al. (1988) reported that flavanones often produced a  $[M - 43]^+$  ion, while dihydroflavonols produced a  $[M - 57]^+$  ion; however, these ions were not found with any of the compounds studied.

Mass spectral data for 11 chalcones and dihydrochalcones are found in Table V. Dihydrochalcones gave a strong  $B_3^+$  ion, while the chalcones gave a strong  $B_5^+$  ion and a  $[B_5 - 28]^+$  ion. In two cases,  $A_1^+$  and  $B_1^+$  fragments occurred. Evidently, they appeared because chalcones are readily isomerized to flavanones and therefore give fragments characteristic of flavanones (Mabry and Markham, 1975).  $A_2^+$  ions were prominent, sometimes the base peak with both classes. All gave prominent  $M^+$  and  $[M - 1]^+$  ions. Again, the relationships were generally consistent with the fragments predicted in Table I.

Table VI gives CI-MS ( $\text{CH}_4$ ) data on nine flavonoid glycosides. The  $[M + 1]^+$  of the aglycon was the base peak with eight of the nine glycosides. The  $[M + 1]^+$  was further defined by characteristic  $[M + 29]^+$  and  $[M + 41]^+$  ions, but only with isookanin 7-glucoside was a fragment derived from fission of the A and B rings observed. The presence of the sugars was indicated by the appearance of all or some of the fragments normally observed with CI-MS of sugars: glucose 163, 145, 127; rhamnose 147, 129, 111; and apiose 151, 133, 115. The fragment intensities of the sugar attached to the flavonoid were weaker and are sometimes absent (Hedin and Phillips, 1991). While CI-MS ( $\text{CH}_4$ ) analysis of underivatized glycosides cannot provide comprehensive structural information, it does provide helpful preliminary information.

In summary, it has been confirmed that EI-MS analysis

of flavonoids can provide the molecular weights, and often the elemental formula can be inferred. Additionally, information can be obtained as to the substitution patterns of the A and B rings. In this study, the standard analyses of Mabry and Markham (1975) and Grayer (1989) were found to be generally applicable to a diverse collection of flavonoids.

## LITERATURE CITED

- Balza, F.; Crins, W. J.; Bohm, B. A.; Towers, G. H. N. Mass spectrometry in the differentiation of flavanones and dihydroflavonols. *Phytochemistry* 1988, 27, 2715-2717.
- Goudard, M.; Favre-Bonvin, J.; Strelisky, J.; Nogrđi, M.; Chopin, J. *Phytochemistry* 1979, 18, 186-187.
- Grayer, R. J. Flavonoids. In *Methods in Plant Biochemistry, Vol. I, Plant Phenolics*; Harborne, J. B., Ed.; Academic Press: San Diego, 1989; pp 283-323.
- Hedin, P. A.; Phillips, V. A. Chemical ionization (methane) mass spectrometry of sugars and their derivatives. *J. Agric. Food Chem.* 1991, 39, 1106-1109.
- Lias, S. G.; Stein, S. E. *NIST/EPA/MSDC Mass Spectral Database*, PC Version 3.0; U.S. Department of Commerce: Gaithersburg, MD, 1990.
- Mabry, T. J.; Markham, K. R. Mass spectrometry of flavonoids. In *The Flavonoids*; Harborne, J. B., Mabry, T. J., Mabry, H., Eds.; Academic Press: New York, 1975; pp 78-126.
- Mabry, T. J.; Ulubelen, A. In *Biochemical Applications of Mass Spectrometry*; Walker, G. R., Dermer, O., Eds.; Wiley: New York, 1980; pp 1131-1148.
- Markham, K. R. In *The Flavonoids—Advances in Research*; Harborne, J. B., Ed.; Chapman and Hall: London, 1988; Vol. 2, pp 427-468.

Received for review November 22, 1991. Accepted January 21, 1992.