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ELECTRON IMPACT MASS SPECTROMETRIC DIFFERENTIATION OF 5,6-DIHYDROXY-7,8-DIMETHOXY- AND 5,8-DIHYDROXY-6,7-DIMETHOXYFLAVONES

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Key Word Index—EIMS fragmentation; 5,6-dihydroxy-7,8-dimethoxyflavones; 5,8-dihydroxy-6,7-dimethoxyflavones; spectral differentiation.

Abstract—Electron Impact Mass Spectrometry provides a means of differentiating 5,6-dihydroxy-7,8-dimethoxyflavones from 5,8-dihydroxy-6,7-dimethoxyflavones. The relative abundances of $[M]^+$, $[M-H]^+$, $[M-Me]^+$ and $[M-H_2O]^+$ ions vary according to the A-ring substitution pattern. The Wessely–Moser isomers were used for comparative purposes; this acidic treatment yielded novel demethylation products.

Several naturally occurring 5,6-dihydroxy-7,8-dimethoxyflavones [1–4] and 5,8-dihydroxy-6,7-dimethoxyflavones [5–7] have been isolated and characterized in the last few years. The complete characterization of these compounds is difficult by the classical UV and NMR techniques [8]. Electron impact mass spectrometry offers an accurate and sensitive technique for the differentiation of isomeric 6- or 8-methoxylated flavonoids with three substituents in the ring A [9, 10]. We now demonstrate that definitive structural information can be gained from the EIMS of 5,6-dihydroxy-7,8-dimethoxy- and 5,8-dihydroxy-6,7-dimethoxyflavonoids; this is particularly useful when only minute amounts of compounds are available for analysis.

The presence or absence of the $[M-H_2O]^+$ peak is useful for distinguishing between these two isomers, this ion being absent in the spectra of 6-hydroxy-8-methoxy isomers and present (7% relative abundance) in 6-methoxy-8-hydroxy compounds (Table 1). However, it is not possible to differentiate on the basis of the intensity of the $[M-Me]^+$ ion relative to that of the $[M]^+$ peak, since the $[M-Me]^+$ ion constitutes the base peak in these spectra, except for isothymusin (**6**). However, the $[M]^+$ ion is of lower intensity in the 6-hydroxy-8-methoxy isomers than with the 6-methoxy-8-hydroxy isomers. The $[M-H]^+$ ion is also lower in the 6-hydroxy-8-methoxyflavones than in the isomeric 6-methoxy-8-hydroxyflavones. These results are in accordance with the more ready loss of Me and H from the 8-position than from the 6-position in 5-hydroxyflavones. This is because of the greater stability of *p*-quinonoid over *o*-quinonoid forms. The $[M-H_2O-Me]^+$ (M − 33) ion is found as a very characteristic intense peak in these spectra, this ion being of lower intensity in 6-hydroxy-8-methoxyflavones than in 6-methoxy-8-hydroxyflavones (Table 1). It has been said that the $[M-MeCO]^+$ ion is relatively intense in 6- and 8-methoxyflavonoids [11, 12], but in these substances this peak appears with low relative abundance in both types of isomer. The retro-Diels–Alder fragmentation [11, 13] yields $[A_1-Me]^+$ and $[A_1-MeCO]^+$ peaks as the most significant and abundant fragments in the low-mass range.

In the structural analysis of these compounds, the isomeric flavones were obtained by acidic treatment and the Wessely–Moser rearrangement [14] is quite useful for such purposes [2]. This acidic treatment produces demethylated products when the flavones thymusin (**1**), leucanthogenin (**7**) and thymonin (**4**) were treated. The EIMS of the demethylated flavones afforded $[M]^+$ and RDA fragments that characterize their structures. The $[M]^+$ ions were the base peaks in all spectra and important $[M-H]^+$ (30%) and $[M-H_2O]^+$ (30%) peaks were also found. The relative abundance of the fragments from these spectra are quite different of those observed in the spectrum of 5,7,8,3′,4′-pentahydroxy-6-methoxyflavone [15] ($[M-MeCO]^+$, 73%), which confirms the location of the methoxyl groups at C-7 in the demethylated compounds.

EXPERIMENTAL

Flavones. The flavones thymusin (**1**), thymonin (**4**) and pebrellin (**2**) were isolated from *Thymus membranaceus* [2], *T. vulgaris* and *T. piperella* [3] respectively, 5,6-Dihydroxy-7,8,3′,4′-tetramethoxyflavone was kindly supplied by Dr. B. Voirin isolated from *Mentha piperita* [4], and the flavone leucanthogenin (**7**) was obtained by enzymic hydrolysis from its 8-glucoside isolated from *Sideritis leucantha* [7]. Isothymusin (**6**), isoleucanthogenin (**3**), isothymonin (**8**) and the demethylated flavones (**9–11**) were obtained by acidic treatment of the naturally occurring flavones as described previously [2].

Mass spectra. These were recorded by direct inlet of the underivatized compounds (probe temp. 280–300°, ion source temp. 240°, 70 eV).

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Table 1. EIMS fragments of 5,6-dihydroxy-7,8-dimethoxyflavones, 5,8-dihydroxy-6,7-dimethoxyflavones and their demethylated products [*m/z* (relative abundance)]

Compound	Substitution pattern						$[M]^+$	$[M-H]^+$	$[M-Me]^+$	$[M-H_2O]^+$	$[M-H_2O-Me]^+$	$[M-Me-CO]^+$	$[A_1-Me]^+$	$[A_1-MeCO]^+$
	5	6	7	8	3′	4′								
1	OH	OH	OMe	OMe	—	OH	330	329	315	—	297	287	197	169
							(69)	(5)	(100)		(10)	(2)	(10)	(5)
2	OH	OH	OMe	OMe	—	OMe	344	343	329	—	311	—	197	169
							(66)	(9)	(100)		(10)		(5)	(3)
3	OH	OH	OMe	OMe	OH	OH	346	345	331	—	313	303	197	169
							(56)	(8)	(100)		(16)	(2)	(29)	(19)
4	OH	OH	OMe	OMe	OMe	OH	360	359	345	—	327	317	197	169
							(63)	(5)	(100)		(11)	(2)	(10)	(5)
5	OH	OH	OMe	OMe	OMe	OMe	374	373	359	—	341	—	197	169
							(91)	(13)	(100)		(15)		(39)	(22)
6	OH	OMe	OMe	OH	—	OH	330	329	315	312	297	287	197	169
							(100)	(9)	(97)	(7)	(23)	(5)	(21)	(10)
7	OH	OMe	OMe	OH	OH	OH	346	345	331	328	313	303	197	169
							(88)	(15)	(100)	(7)	(26)	(5)	(33)	(19)
8	OH	OMe	OMe	OH	OMe	OH	360	359	345	342	327	317	197	169
							(97)	(9)	(100)	(7)	(29)	(3)	(24)	(14)
9	OH	OH	OMe	OH	—	OH	316	315	301	298	—	273	183	155
							(100)	(26)	(18)	(27)		(3)	(10)	(8)
10	OH	OH	OMe	OH	OH	OH	332	331	317	314	—	—	183	155
11	OH	OH	OMe	OH	OMe	OH	346	345	331	328	313	—	183	155
							(100)	(29)	(30)	(30)	(4)		(9)	(9)

pentahydroxy-6-methoxyflavone, to Dr. B. Voirin for supplying a sample of 5,6-dihydroxy-7,8,3',4'-tetramethoxyflavone, and to the Spanish C.A.I.C.Y.T. for financial support of this work (grant C.S.I.C. 608/126).

REFERENCES

1. Van Den Broucke, C. O., Dommisse, R. A., Esmans, E. L. and Lemli, J. A. (1982) *Phytochemistry* **21**, 2581.
2. Ferreres, F., Barberán, F. A. T. and Tomás, F. (1985) *Phytochemistry* **24**, 1869.
3. Barberán, F. A. T., Hernández, L., Ferreres, F. and Tomás, F. (1985) *Planta Med.* 452.
4. Jullien, F., Voirin, B., Bernillon, J. and Favre-Bonvin, J. (1984) *Phytochemistry* **23**, 2972.
5. Bohlmann, F., Zdero, C. and Ziesche, J. (1979) *Phytochemistry* **18**, 1375.
6. Guerreiro, E., Kavka, J. and Giordano, O. S. (1982) *Phytochemistry* **21**, 2601.
7. Barberán, F. A. T., Tomás, F. and Ferreres, F. (1984) *Phytochemistry* **23**, 2112.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
9. Goudard, M., Favre-Bonvin, J., Lebreton, P. and Chopin, J. (1978) *Phytochemistry* **17**, 145.
10. Goudard, M., Favre-Bonvin, J., Strelisky, J., Nogradi, M. and Chopin, J. (1979) *Phytochemistry* **18**, 186.
11. Kingston, D. G. I. (1971) *Tetrahedron* **27**, 2691.
12. Wollenweber, E. (1982) *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds) p. 243. Chapman & Hall, London.
13. Audier, H. (1966) *Bull. Soc. Chim. Fr.* **9**, 2892.
14. Seshadri, T. R. (1962) *The Chemistry of Flavonoid Compounds* (Geissman, T. A., ed.) p. 184. Pergamon Press, Oxford.
15. Proksch, P., Budzikiewicz, H., Tanowitz, B. D. and Smith, D. (1984) *Phytochemistry* **23**, 679.