

NEGATIVE ION MASS SPECTRA OF FLAVONOIDS¹

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Mass spectral fragmentation patterns of flavonoids under positive ionization conditions have been well established (1-4). The dissociative +E spectra obtained by charge inversion of molecular anions of flavones and flavanones have been reported by Bowie *et al.* (5). However, the normal electron impact negative ion spectra of substituted flavonoids have not been investigated even though flavones are known to produce molecular anions and simple cleavage fragments in von Ardenne's high pressure ion source (6,7). In continuation of our work on the negative ion mass spectra of natural products (8), we have examined the electron impact negative ion mass spectra of certain flavonoids. It was also of interest to compare the hydrogen/deuterium exchange reactions in these flavonoids occurring under nci conditions with that occurring under pci conditions in the presence of D₂O (9).

The relative ion abundances in the electron impact negative ion spectra of compounds A-R are given in Table 1. The molecular anion gives rise to the base peak in all these compounds except compound L. The high electron affinity of the flavone molecule favors resonance electron capture at the carbonyl oxygen, and the resulting molecular anion is stabilized by the conjugated system and also possibly by hydrogen bonding between the 5-OH and the carbonyl oxygen. The only major fragments observed in the spectra of these compounds are (M-H)⁻ and (M-CH₃)⁻ ions presumably resulting from dissociative electron capture. The abundances of these fragment ions reflect the substitution pattern in these molecules. The nonmethylated compounds give (M-H)⁻ ions, while the

methylated flavonoids give abundant (M-CH₃)⁻ ions. In general, the negative ion spectra of these flavonoids resemble those of aflatoxins and mycotoxins, which also show three major ions, M⁻, (M-H)⁻, and (M-CH₃)⁻, in their negative ion spectra (10).

The hydrogen/deuterium exchange reactions in some of these molecules have been studied by recording their spectra under nci conditions using D₂O. The data thus obtained are presented in Table 2. Since under nci conditions, using H₂O compounds A,D,E,F,H, and I gave (M-H)⁻ and compounds K,L,M and P,Q,R gave M⁻, respectively, as the most intense ion, the deuterium exchanged (M-D)⁻ corresponds to the base peak in the former compounds, and the deuterium exchanged M⁻ gives rise to the most intense ion in the latter compounds in their D₂O nci spectra.

In the flavanones Q and R, apart from replaceable hydrogens and aromatic hydrogens, the hydrogens α to the carbonyl group also undergo exchange. This is evident from the *m/z* values of the M⁻ or (M-D)⁻ ions and the RDA fragment ions. The RDA fragment ions are shifted from *m/z* 152 to *m/z* 156 in compound Q and from *m/z* 196 to *m/z* 197 in compound R, respectively (Scheme 1).

A comparison of the H/D exchange data obtained under both positive and negative ion ci conditions indicates that the number of exchanges occurring under both the conditions are almost the same. In compounds F and Q ions corresponding to (2M-D)⁻ are also observed under D₂O nci conditions (Table 3). The distribution of the *m/z* values of these ions suggests the occurrence of these exchange reactions in the neutral molecules also. In the previous work (9),

¹CDRI Communication No. 3599.

TABLE 1. Relative Ion Abundances (%) and the $M^+/(M-CH_3)^+$ Abundance Ratios in the Electron Impact Negative Ion Spectra of Flavonoids.

Compound	Name	Ions					Ratio	
		Molecular weight	M^+	$(M-H)^+$	$(M-CH_3)^+$	Other ions	No. of OH	M^+
							No. of OMe	$(M-CH_3)^+$
A	4',5,7-Trihydroxy-flavone	270	100	7	—	—	—	—
B	3',4',5,7-Tetrahydroxy flavone	286	100	18	—	—	—	—
C	3,3',4',5,7-Penta-hydroxy flavone	302	100	11	—	—	—	—
D	4',5,7-Trihydroxy-3'-methoxy flavone	300	100	6	1	286 (2)	3	137
E	3,4',5,7-Tetrahydroxy-8-methoxy flavone	316	100	3	5	300 (1)	4	20.1
F	3,4',5,7-Tetrahydroxy-3'-methoxy flavone	316	100	6	11	—	4	9.2
G	4',5,7-Trihydroxy-3,6-dimethoxy flavone	330	100	5	9	314 (2)	1.5	11.1
H	4',5-Dihydroxy-3,6,7-trimethoxy flavone	344	100	3	11	314 (2)	0.67	9.6
I	5,7-Dihydroxy-3,4',6-trimethoxy flavone	344	100	3	41	314 (2)	0.67	2.4
J	5-Hydroxy-7,8-dimethoxy flavone	298	100	—	12	268 (3)	0.5	8.5
K	5-Hydroxy-3,4',7-trimethoxy flavone	328	100	1	33	298 (1)	0.33	3
L	5-Hydroxy-3',4',7-trimethoxy flavone	328	73	—	100	298 (1)	0.33	0.6
M	5-Hydroxy-3,4',6,7-tetramethoxy flavone	358	100	—	34	328 (1)	0.25	2.9
N	5-Hydroxy-3,3',4',7-tetramethoxy flavone	358	100	—	67	328 (3)	0.25	1.5
O	5-Hydroxy-2',3,7,8-tetramethoxy flavone	358	100	—	72	328 (5)	0.25	1.5
P	5-Hydroxy-3',4',6,7-tetramethoxy flavone	358	100	1	76	328 (1)	0.25	1.3
Q	5,7-Dihydroxy flavanone	256	100	3	—	152 (4)	—	—
R	5-Hydroxy-7,8-dimethoxy flavanone	300	100	1	22	196 (16) 181 (4)	0.5	4.6

we had pointed out that in some of these flavonoids, the H/D exchanges occurring simultaneously in both A and B rings could not be explained only on the basis of initial deuteron transfer to one of the aromatic deuterons followed by H/D exchange of the activated aromatic hydrogens on that ring in an ion/molecule collision complex and had suggested the operation of some other mechanism also for these processes. The present data indicate that one of these mechanisms

could be the H/D exchanges taking place in the neutral molecules under the high pressure conditions prevailing in the ci source.

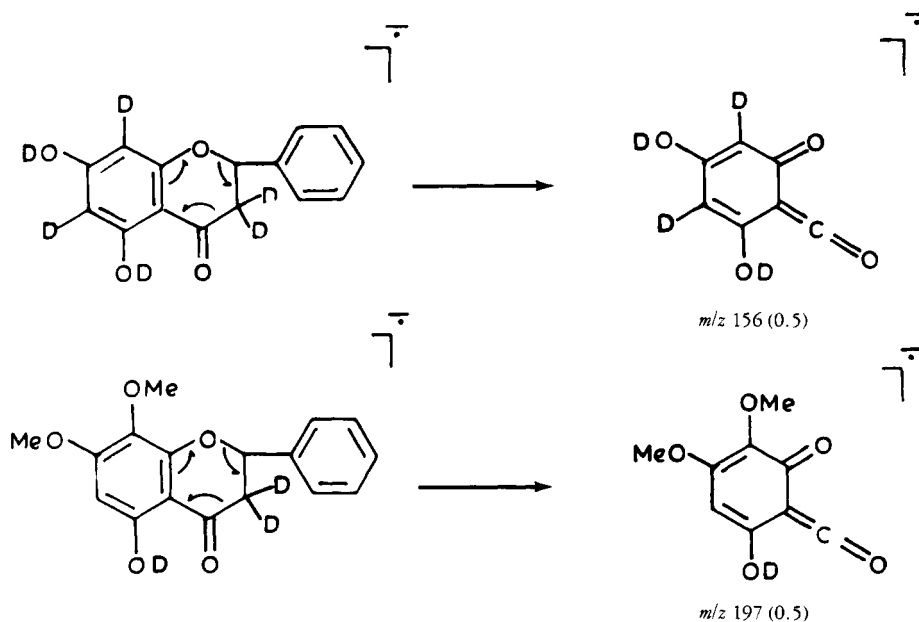
EXPERIMENTAL

FLAVONOIDS.—The flavonoids used in this study were obtained from plant sources (11,12). Methyl derivatives of some of these were prepared by treatment with CH_2N_2 . A few of them were obtained as gifts.

INSTRUMENTATION.—The negative ion mass spectra of these compounds were recorded

TABLE 2. Relative Ion Abundances (%) in the D₂O Nci Spectra

Compound	(M-1) ⁻	M ⁻	(M+1) ⁻	(M+2) ⁻	(M+3) ⁻	(M+4) ⁻	(M+5) ⁻	(M+6) ⁻	(M+7) ⁻	(M+8) ⁻
A	—	—	15.2	25.2	100	44.1	31.2	10.0	1.4	—
D	—	1.4	15.7	29.8	100	31.4	26.0	6.9	—	—
E	—	1.3	6.1	25.5	100	67.0	60.9	22.0	11.1	2.1
F	—	4.0	14.4	39.3	46.1	100	30.4	18.7	5.3	—
H	—	2.9	15.0	56.2	100	27.8	20.2	3.2	—	—
I	—	5.9	93.3	36.4	100	20.0	8.4	1.5	1.2	—
K	13.6	16.7	95.7	100	46.3	10.5	6.2	3.7	—	—
L	3.6	5.9	100	71.1	23.5	7.0	3.0	—	—	—
M	57.5	19.1	100	27.6	7.5	3.3	—	—	—	—
P	4.1	6.1	100	30.6	7.5	3.0	—	—	—	—
Q	—	—	—	8.1	14.4	100	57.7	92.3	26.1	5.6
R	10.1	35.7	91.3	100	76.4	73.3	17.8	3.1	—	—



on a JEOL D-300 spectrometer having *ei/ci* source and attached to a JMA-2000 data system. The electron impact spectra were recorded at a source pressure of 1×10^{-6} torr, while the chemical ionization spectra were recorded at a source

housing pressure of 1×10^{-5} torr. Chloride attachment spectra were obtained using a mixture of CHCl_3 and MeOH in the ratio 1:10 as a source of C1^- ions. The D_2O used was of purity >99% and was obtained from Merck, Sharp and Dohme,

TABLE 3. Relative abundances (%) of the Dimeric Ions in the D₂O Nci Spectra of Compounds F and Q. The Corresponding (M-D)⁻ Ion is 100%

Ion	Compound F	Compound Q
(2M+6) ⁻	—	1.5
(2M+7) ⁻	4.6	1.9
(2M+8) ⁻	7.1	2.7
(2M+9) ⁻	6.9	2.4
(2M+10) ⁻	4.2	2.1
(2M+11) ⁻	—	1.2

Canada. The other ion source conditions were: electron energy, 70 eV (ei) and 200 eV (ci); emission current, 300 μ A and temperature, 200°. The samples were introduced through the direct inlet system and heated to 150-200°.

ACKNOWLEDGMENTS

We are grateful to Dr. K.L. Dhar, Regional Research Laboratory, Jammu Tawi, for the generous gift of 5-hydroxy-7,8-dimethoxy flavanone, 5-hydroxy-2',3,7,8-tetramethoxy flavone, and 5-hydroxy-7,8-dimethoxy flavone and to Dr. Hiroshi Furukawa, Meijo University, Nagoya, Japan, for 8-methoxy kaempferol. Grateful acknowledgment is also made to RSIC, Lucknow, for mass spectral studies.

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Received 7 September 1984