Tetrahedron. Vol 29, pp. 4083 to 4086. Pergamon Press 1973. Printed in Great Britain

METHANE CHEMICAL IONIZATION MASS SPECTROMETRY OF FLAVONOIDS

D. G. I. KINGSTON*

Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

and

H. M. FALES

National Heart and Lung Institute and Division of Computer Research and Technology, National Institutes of Health, Bethesda, Maryland 20014

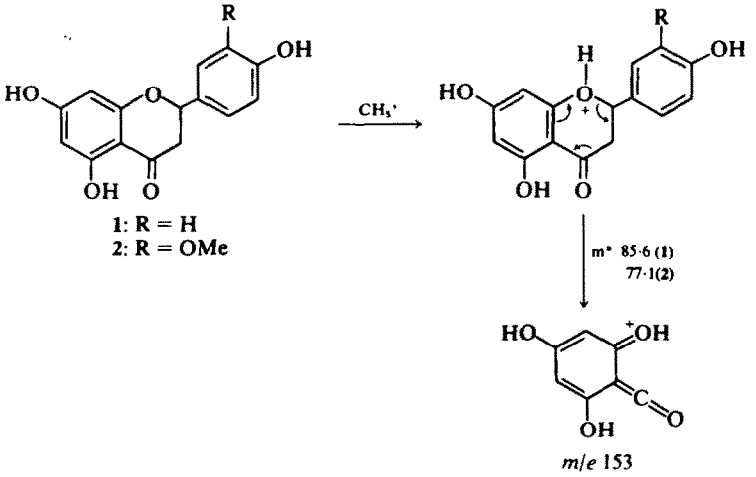
(*Received in the USA* 30 *May* 1973; *Received in the UK for publication* 31 *August* 1973)

Abstract—The chemical ionization mass spectra of a representative selection of flavones, flavonols, flavanones, and flavanols have been examined, using methane as the reagent gas. The flavones and flavonols showed no significant fragmentation under the conditions employed, but the flavanones and flavanols showed characteristic fragmentation which could be of use in structural elucidation of these compounds.

Recent applications of chemical ionization (CI) mass spectrometry to the structure elucidation of natural products have demonstrated that the technique is a powerful adjunct to electron-impact (EI) mass spectrometry. Thus, the application of CI mass spectrometry to alkaloids,[1] amino acids,[2] peptides,[3] prostaglandins,[4] and certain drugs[5] gave in each case spectra which supplied structural information complementary to that obtained by EI mass spectrometry, and a review of these and other applications has been published.[6] In a recent paper,[7] one of us discussed the application of EI mass spectrometry to structural elucidation of some flavonoids, and we now wish to present the results of our study of the methane CI mass spectra of this class of compounds.

Examination of a number of flavones, flavonols (3-hydroxyflavones) and their methyl ethers by CI mass spectrometry with methane as a reactant gas showed that these compounds give very little fragmentation under these conditions. The diagnostic fragmentations observed in the EI mass spectra of these compounds are almost entirely lacking in the CI mode, and there is, thus, no advantage in the use of CI mass spectrometry for their structural elucidation.

In contrast to the flavones, the flavanones examined did show significant diagnostic fragmentations. Taking naringenin (**1**) and hesperitin (**2**) as examples, significant peaks were observed corresponding to the fragmentations indicated in Schemes 1 and 2. It should be emphasized that the mechan-

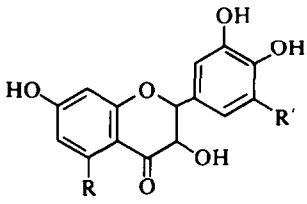


SCHEME 1

isms proposed in these schemes are rationalizations rather than experimentally determined pathways, but the parallel to known reactions in solution makes these pathways chemically reasonable. Protonation on the heterocyclic oxygen would give a protonated sample which could fragment *via* the retro Diels-Alder reaction to yield an ion of *m/e* 153 (Scheme 1). On the other hand, protonation on the A ring would yield an ion which could fragment as shown in Scheme 2 to yield ions of *m/e* 147 (from **1**) or 177 (from **2**). Protonation of the A ring in this manner is chemically reasonable, yielding a highly resonance stabilized ion, and the cleavages indicated should also proceed readily. All these transitions are supported by observation of the appropriate metastable peaks.

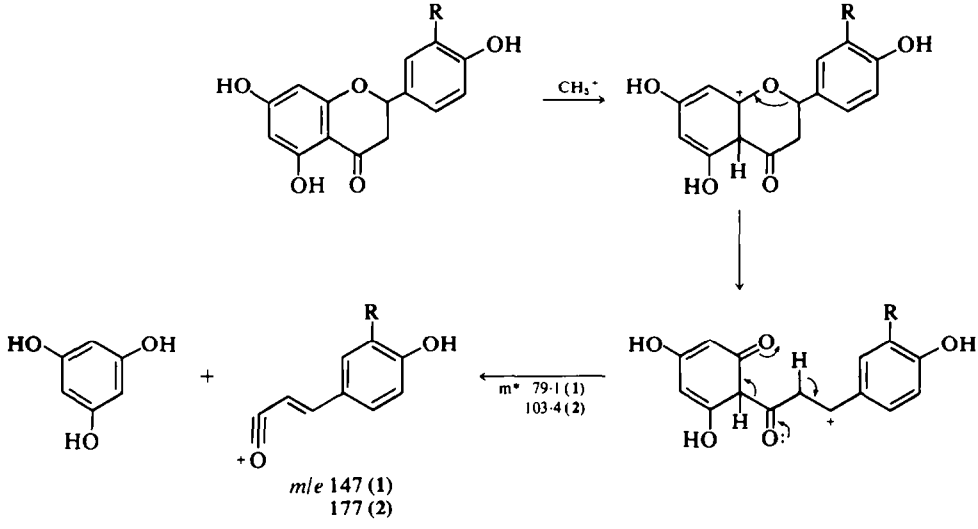
An ion whose formation is not accompanied by a metastable peak is the intense ion at *m/e* 179 in the spectra of both **1** and **2**. This ion is presumably formed by the loss of the C ring and a hydrogen atom from the protonated molecular ion, and the mechanism of Scheme 3 is suggested as a plausible rationalization of this fragmentation. This reaction could conceivably be a thermal one, but the relative stability of the flavanones suggests that it is, in fact, CI induced.

The spectra of the 3-hydroxyflavanones fustin (**3**), (+)-dihydroquercetin (**4**), and 3,3′, 5′,7-pentahydroxy flavanone (dihydrorobinetin) (**5**) were also obtained. All three compounds show relatively intense peaks at $(M—H)^+$, corresponding

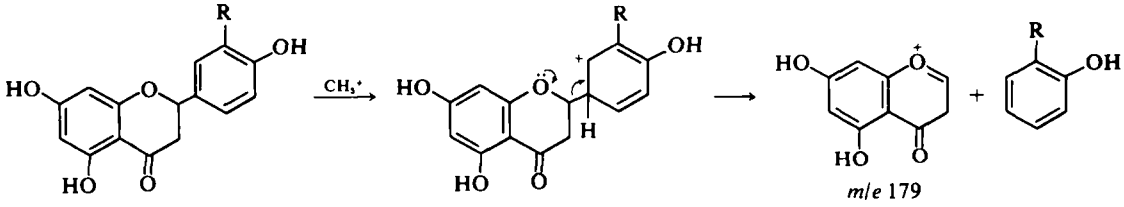


3: R = H, R′ = H
4: R = OH, R′ = H
5: R = H, R′ = OH

either to hydride abstraction from M by protonation of a C—H bond or to loss of H_2 from the protonated molecule. Loss of water from the $(M+H)^+$ ion occurs readily, but water loss from $(M—H)^+$ is not observed, suggesting that this ion is best formulated as a protonated 3-hydroxyflavone. Other major fragmentations of these compounds include the retro Diels-Alder reaction, the loss of ring C, and a new fragmentation to give an ion corresponding to a benzyl (or tropylium) ion derived from ring C plus one methylene group. The first two



SCHEME 2



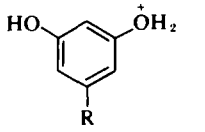
1: R = H
2: R = OMe

SCHEME 3

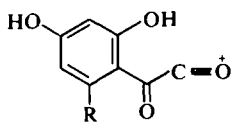
fragmentations probably occur by similar pathways to the corresponding reactions in the flavanones, (Schemes 1 and 3), and in the case of dihydrorobinetin (5) they yield ions at *m/e* 137 and 179 respectively. Formation of the benzyl ion (at *m/e* 139 in dihydrorobinetin) may be rationalized by the pathway of Scheme 4 below.

Other significant ions in the spectra of these compounds include the ions **6–8**; study of the peak shifts with different ring hydroxylation patterns indicates that ions **6** and **7** are formed from ring C. These fragmentations, taken together, thus provide a secure method for assigning substituents on the 3-hydroxyflavanone ring system to the A or C rings.

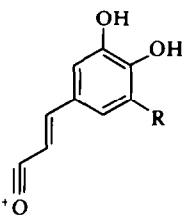
In conclusion, this work has shown that the methane chemical ionization mass spectra of the flavanones and the 3-hydroxyflavanones are valuable tools for the structure elucidation of these compounds. In the case of the flavones and flavonols, however, electron impact mass spectrometry provides more structural information and must remain the method of choice for these compounds.



6a: R = H *m/e* 111
6b: R = OH *m/e* 127



7a: R = H *m/e* 165
7b: R = OH *m/e* 181



8a: R = H *m/e* 163
8b: R = OH *m/e* 179

EXPERIMENTAL

The flavonoids used in this work were all commercially available (A. G. Fluka, Switzerland, and Aldrich Chemical Co.) or were available from earlier work.[9] Mass spectra were run on an MS-9 mass spectrometer with a chemical ionization source supplied by the Scientific Research Instruments Company, Baltimore, Maryland. Samples were introduced *via* the direct insertion probe with a methane pressure of 1 torr and a temp of 230–235°. Peaks with an intensity greater than 5% of the base peak are given below, together with certain smaller peaks associated with metastable peaks.

Chrysin (5,7-*dihydroxyflavone*). 295(5), 284(12), 256(19), 255(100, P + 1).

Apigenin (4′,5,7-*trihydroxyflavone*). 311(5), 299(15), 272(16), 271(100, P + 1).

Galangin (3,5,7-*trihydroxyflavone*). 311(5), 301(14), 299(14), 272(17), 271(100, P + 1), 270(5).

Kaempferol (3,4′5,7-*tetrahydroxyflavone*). 315(8), 288(18), 287(100, P + 1), 286(7).

3′,4′-*Dimethoxy*-7-*hydroxyflavone*. 327(8), 300(27), 299(100, P + 1).

Trimethyl apigenin (4′,5,7-*trimethoxyflavone*). 341(5), 314(19), 313(100, P + 1).

Dimethyl chrysin (5,7-*dimethoxyflavone*). 284(17), 283(100, P + 1).

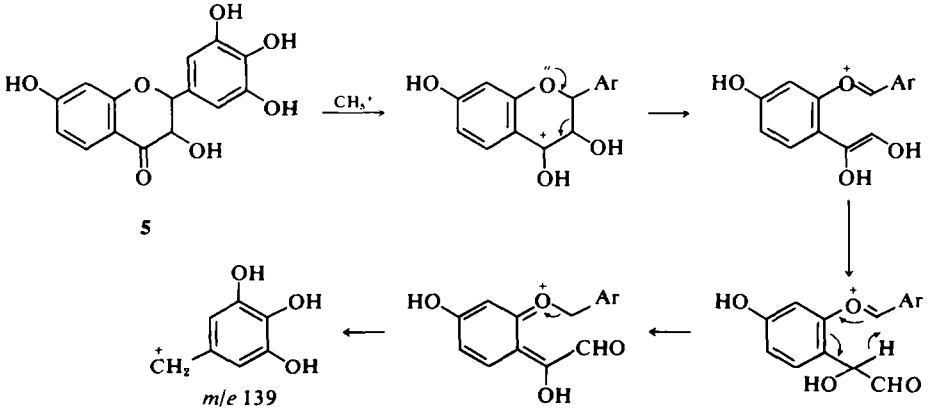
Tetramethyl kaempferol (3,4′,5,7-*tetramethoxyflavone*). 371(5), 344(21), 343(100, P + 1), 342(5).

Pentamethyl morin (2′,3,4′,6,7-*pentamethoxyflavone*). 374(22), 373(100, P + 1).

Naringenin (1) (4′,5,7-*trihydroxyflavone*). 301(10), 274(17), 273(100, P + 1), 179(24), 153(5), 147(2). m* 79·1, 85·6.

Hesperitin (2) (3′,5,7-*trihydroxy*-4′-*methoxyflavanone*). 331(9), 304(18), 303(100, P + 1), 287(6), 179(6), 177(1), 153(4). m*103·4, 77·1, 62·0.

Fustin (3) (3,3′,4′,7-*tetrahydroxyflavanone*). 317(5), 315(5), 305(14), 303(5), 299(9), 290(12), 289(70, P + 1), 288(7), 287(32), 273(20), 272(17), 271(100), 270(5), 261(7), 245(7), 243(6), 179(7), 165(9), 163(7), 151(9), 137(18), 124(6), 123(85), 111(5).



SCHEME 4

(+)-*Dihydroquercitin* (**4**) 3,3',4',5,7-*pentahydroxyflavanone*). 333(6), 331(6), 315(8), 306(17), 305(100, P + 1), 304(11), 303(48), 290(8), 289(45), 288(17), 287(100), 286(7), 261(7), 259(6), 195(8), 181(19), 179(8), 167(13), 155(6), 153(10), 151(4), 128(5), 127(77), 123(37), 111(13), 57(80).

3,3',4',5',7-*pentahydroxyflavanone* (**5**). 331(5), 315(6), 306(7), 305(44, P + 1), 304(13), 303(72), 290(8), 289(60), 288(20), 287(100), 271(6), 269(8), 261(6), 179(50), 165(16), 139(42), 137(8), 111(9).

REFERENCES

[1]H. M. Fales, H. A. Lloyd, and G. W. A. Milne, *J. Am. Chem. Soc.* **92**, 1590 (1970)

[2]G. W. A. Milne, T. Axenrod, and H. M. Fales, *Ibid.* **92**, 5170 (1970)

[3]A. A. Kiryushkin, H. M. Fales, T. Axenrod, E. J. Gilbert, and G. W. A. Milne, *Org. Mass Spectrom* **5**, 19 (1971)

[4]D. M. Desiderio, and K. Hägele, *J. Chem. Soc* (D), 1074 (1971)

[5]G. W. A. Milne, H. M. Fales, and T. Axenrod, *Analyt. Chem.* **43**, 1815 (1971)

[6]R. L. Foltz, *Lloydia* **35**, 344 (1972)

[7]D. G. I. Kingston, *Tetrahedron* **27**, 2691 (1971)

[8]T. J. Mabry, K. R. Markham, and M. B. Thomas, *The Systematic Identification of Flavonoids*, Springer-Verlag, New York, N.Y., (1970)

[9]M. M. Rao, D. G. I. Kingston, and T. D. Spitttler, *Phytochem.* **9**, 227 (1970)