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MASS SPECTROMETRY IN THE DIFFERENTIATION OF FLAVANONES AND DIHYDROFLAVONOLS*

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Key Word Index—Mass spectrometry; flavanones; dihydroflavonols.

Abstract—Flavanones and dihydroflavonols exhibit characteristic fragmentation patterns that can be utilized in their differentiation. Electron-impact and high resolution mass spectrometry are used to establish the patterns and identities of the characteristic ions.

INTRODUCTION

The utility of flavonoids as taxonomic characters has been amply demonstrated in numerous studies. Flavonoids can serve as discriminators among taxa, they can be used as markers to follow the course of hybridization in mixed populations, as well as providing indications of variability within populations [1, 2]. They are ubiquitous among angiosperms, and are also found in gymnosperms, pteridophytes, and bryophytes. In addition to their utility in systematic and evolutionary studies, flavonoids have received considerable attention for their biological and medical applications [3]. Flavanones and dihydroflavonols are obligate intermediates in the biosynthesis of two major classes of flavonoids (flavones and flavonols), and they may also be major accumulation products in their own right.

It has been established that UV analysis and colorimetric tests for these compounds are not very helpful in the elucidation of structural details which would distinguish between flavanones and dihyroflavonols [4, 5]. Because of the absence of conjugation of the chromophore through ring C in these compounds, shift reagents such as $AlCl_3$, HCl, NaOAc, and H_3BO_3 are not very useful in UV analysis. With colorimetric tests, both types of compounds behave similarly in the presence of either magnesium or zinc–hydrochloric acid. However, analyses by mass spectrometry have shown that dihydroflavonols and flavanones exhibit distinct, characteristic and diagnostic ions under electron impact. In order to confirm our previous findings, we have correlated structures as well as substituent effects on the fragmentation patterns of several of these natural products. As noticed earlier, these ions arise from the loss of a fragment involving C-3 and C-4 [6]. The usefulness of this feature in the MS analysis of these natural products lies in the fact that the ions are in the vicinity of the molecular ion region, where the presence of fragments due to the cleavage of these molecules is not very complex.

The mass fragmentation patterns of flavanones and dihydroflavonols follow the typical retro-Diels–Alder reaction, yielding fragments corresponding to rings A and B [7]. Because of the difficulty and lack of suitable procedures to distinguish between these two classes of flavonoids, further work seemed desirable to obtain more precise data for these molecules.

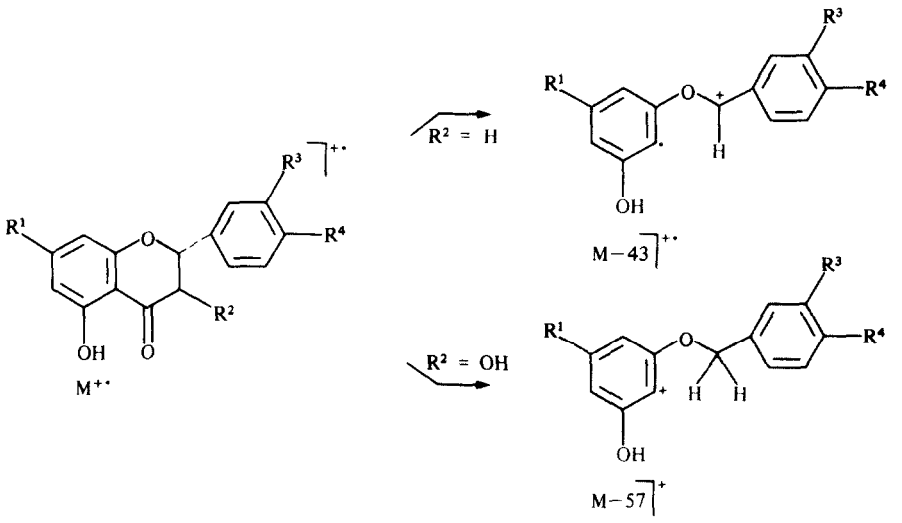
RESULTS AND DISCUSSION

We have observed that, regardless of the substituents or the degree of substitution, flavanones and dihydroflavonols produce fragments that are unique, as shown in Scheme 1. Dihydroflavonols have been readily characterized, and their mass spectra interpreted, after deuteration of their phenolic hydroxyl groups [6]. The characteristic fragment $[M-57]^+$, derived from the molecular ion after migration of a hydrogen radical from HO–C-3 to C-2 and the loss of [O=C=Ċ–OH], is consistent among different dihydroflavonols, and provides a fast and useful procedure for their identification.

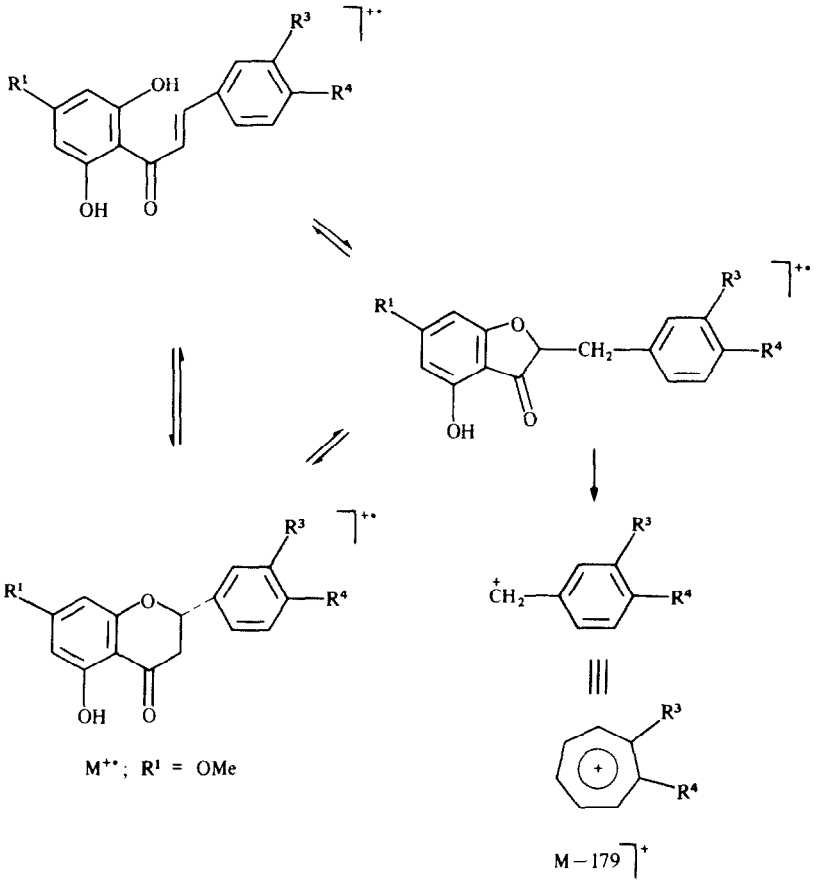
On the other hand, the fragment $[M-43]^+$ is typical of flavanones, where the loss of ketene and a hydrogen radical from the molecular ion seem to account for its composition, as shown by high-resolution mass measurements [8]. A series of flavanones have been examined which were found to exhibit similar behavior. Because of the nature of the ion at $[M-43]^+$, it might be anticipated that a substituent such as a hydroxyl group at C-4′ would stabilize the ion through resonance. Table 1 indicates a noticeable increase in the ratio of this fragment relative to the molecular ion for compounds **4**, **7** and **8**, compared to the unsubstituted or *O*-methylated compounds.

Although the ion $[M-57]^+$ is also present in the fragmentation pattern of flavanones, the intensity of this ion is much less than that of the ion at $[M-43]^+$. Thermal isomerization of chalcones to flavanones, and *vice versa*, frequently occurs in the ionization chamber [9, 10]. In addition, the presence of aurone-derived ions suggests an alternative type of chalcone isomerization [10]. Therefore, the loss of $\cdot C_2HO_2$ is presumably through the equilibrium described in Scheme 2, involving an aurone-type intermediate as well. The ion at $[M-57]^+$ supports this type of intermediate during the

*Dedicated to the memory of Dr Tony Swain.



Scheme 1.



Scheme 2.

fragmentation of flavanones, as do the very strong ions at *m/z* 121 in **2**, and at *m/z* 137 in **4** and **5** (Scheme 2).

Losses of carbon monoxide (*m/z* 28) and a methyl group (*m/z* 15) from the molecular ion in flavanones may produce the fragment $[M-43]^{+\cdot}$, as well. However, the presence of this fragment in the absence of substituents such as methyl or methoxyl groups suggests that this mechanism is very unlikely to occur.

Table 1. Characteristic fragments of flavanones (**1–8**) and dihydroflavonols (**9–11**) (% relative to molecular ion)

	R^1	R^2	R^3	R^4	$\frac{[M-43]^{+\cdot}}{[M]^{+\cdot}} \times 100$	$\frac{[M-57]^{+}}{[M]^{+\cdot}} \times 100$
1	OH	H	H	H	8.1	–
2	OMe	H	H	OMe	8.2	–
3	OMe	H	H	H	7.5	–
4	OMe	H	OMe	OH	12.6	–
5	OMe	H	OH	OMe	7.2	–
6	OH	H	H	OMe	5.8	–
7	OH	H	H	OH	9.7	–
8	OMe	H	OH	OH	9.8	–
9	OMe	OH	OH	OH	–	19.5
10	OMe	OH	H	OH	–	15.2
11	OMe	OH	OMe	OH	–	16.4

EXPERIMENTAL

Plant material. The dihyroflavonols and flavanones examined during this study were obtained mainly from the aerial parts of *Adenothamnus validus* (Brandegee) Keck [11], *Artemisia dracunculus* L. [6], *Eupatorium odoratum* L. [12], and *Holocarpha obconica* (Clausen & Keck) Keck [13].

Analytical techniques. MS were recorded on a Finnigan 1020 GC/MS, and whenever possible, on a KRATOS-AEI MS 50 high resolution mass spectrometer.

2. *5-Hydroxy-7,4'-dimethoxyflavanone.* MS *m/z* (rel. int.): 300.0991 $[M]^{+\cdot}$ (calc. for $C_{17}H_{16}O_5$: 300.0998) (65.3), 299 $[C_{17}H_{15}O_5]$ (48.9), 257 $[C_{15}H_{13}O_4]$ (5.4), 193 $[C_{10}H_9O_4]$ (48.0) 166 $[C_8H_6O_4]$ (55.9), 134 $[C_9H_{10}O]$ (100), 121 $[C_8H_9O]$ (92.3), 119 $[C_8H_7O]$ (74.6).

4. *5,4'-Dihydroxy-7,3'-dimethoxyflavanone.* MS *m/z* (rel. int.): 316.0950 $[M]^{+\cdot}$ (calc. for $C_{17}H_{16}O_6$: 316.0947 (44.4), 315 $[C_{17}H_{15}O_5]$ (26.7), 273 $[C_{15}H_{13}O_5]$ (5.6), 193 $[C_{10}H_9O_4]$ (39.9), 167 $[C_8H_7O_4]$ (58.1), 150 $[C_9H_{10}O_2]$ (55.4), 137 $[C_8H_9O_2]$ (100), 135 $[C_8H_7O_2]$ (57.5), 121 $[C_8H_9O]$ (36.5), 107 $[C_7H_7O]$ (44.1).

5. *5,3'-Dihydroxy-7,4'-dimethoxyflavanone.* MS *m/z* (rel. int.): 316.0937 $[M]^{+\cdot}$ (calc. for $C_{17}H_{16}O_6$: 316.0947) (53.3), 315 $[C_{17}H_{15}O_6]$ (21.5), 273 $[C_{15}H_{13}O_5]$ (3.8), 193 $[C_{10}H_9O_4]$ (49.5), 167 $[C_8H_7O_4]$ (93.3), 150 $[C_9H_{10}O_2]$ (80.9), 137 $[C_8H_9O_2]$ (100), 135 $[C_8H_7O_2]$ (71.0), 121 $[C_8H_9O]$ (16.1), 107 $[C_7H_7O]$ (60.9).

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