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IN-BEAM ELECTRON IMPACT, CHEMICAL IONIZATION AND NEGATIVE ION CHEMICAL IONIZATION OF FLAVONOID GLYCOSIDES

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There has been little information that the molecular weights of flavonoid glycosides were measured by conventional mass spectrometry. The application of other soft ionizations such as in-beam EI, CI and NICI except for the FD was studied, and good spectra were given by the in-beam NICI method.

The studies of the mass spectra of flavonoids have been reported by a number of groups.[1)2)] Since the electron impact (EI) mass spectrum of flavonoid glycosides yields no information about the nature of the glycosidic units, the mass spectra of glycosides as derivatives modified by acetylation,[3)] methylation,[4)] etc. have been studied.

Recent applications of field desorption (FD) mass spectrometry, a kind of soft ionization of mass spectrometry, to flavonoid glycosides have been reported by H-R. Schulten[5)] and H. Geiger.[6)] These spectra showed the molecular ion $[M]^+$ or the protonated molecular ion $[MH]^+$, however, the FD had several disadvantages (in technical dependence and reproducibility of fragment patterns, etc.), so we tried to apply other soft ionizations. It has been reported that the in-beam EI and chemical ionization (CI) techniques were used for several thermally unstable compounds,[7)] and there has been little information on the characteristics of the negative ion chemical ionization (NICI) mass spectrometry in natural products.[8)] We now present the results of the in-beam EI, CI and NICI mass spectra of twenty-one flavonoid glycosides on a Hitachi M-80 (70eV, chamber heater 200°C, sample heater 250°C).

The flavonoids of monoglycoside which have a small number of hydroxyl groups gave the $[M]^+$ and the $[MH]^+$ by the direct EI and CI, respectively, while their

relative intensities were below 0.1 percent, and it was difficult to identify them. The same results have been reported in the CI mass spectra (isobutane, ammonia) by M. Nakayama et al.[3)]

However, the in-beam EI and CI spectra exhibited higher intensities of the $[M]^+$ or $[MH]^+$ (Fig. 1) and were 1-2 percent at the best. Because the sample was closer to the electron beam in this methods, more $[M]^+$ or $[MH]^+$ ions were formed than in the conventional methods. The use of isobutane as reagent gas in CI, both the direct and the in-beam methods showed the base peak assigned to the formation of the protonated aglycone $[AH]^+$, especially the $[MH]^+$ showed the most intense ion peak in the higher mass regions of in-beam CI spectra. In the above methods, there were no information about the molecular weights of the diglycosides except those of a few special flavonoids such as hesperidin. On the other hand, the satisfactory spectra of the diglycosides were given by the in-beam NICI (isobutane). The spectrum of

Fig. 1 Spectra of direct EI, CI and in-beam EI, CI of apigenin 7-glucoside

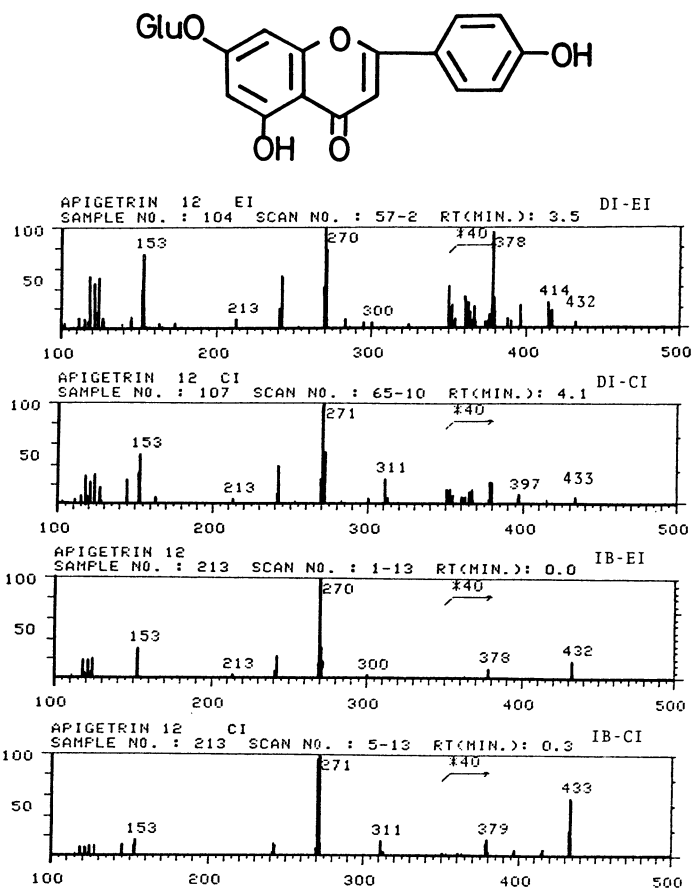


Fig. 2 In-beam NICI mass spectrum of rhamnazin 3-rutinoside

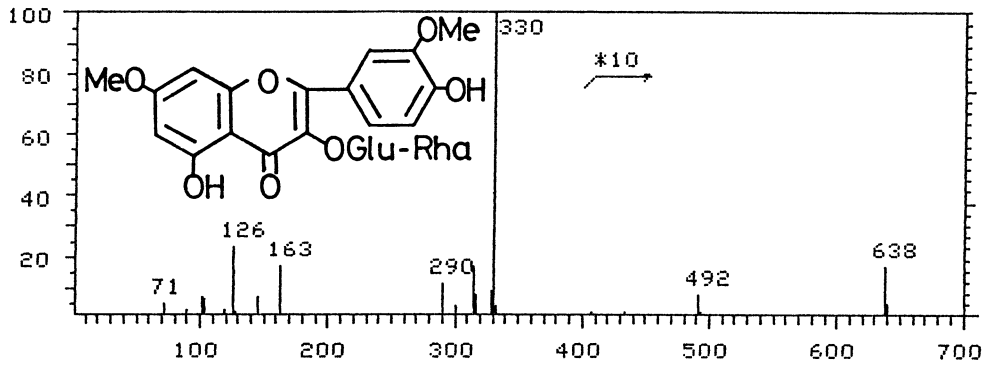


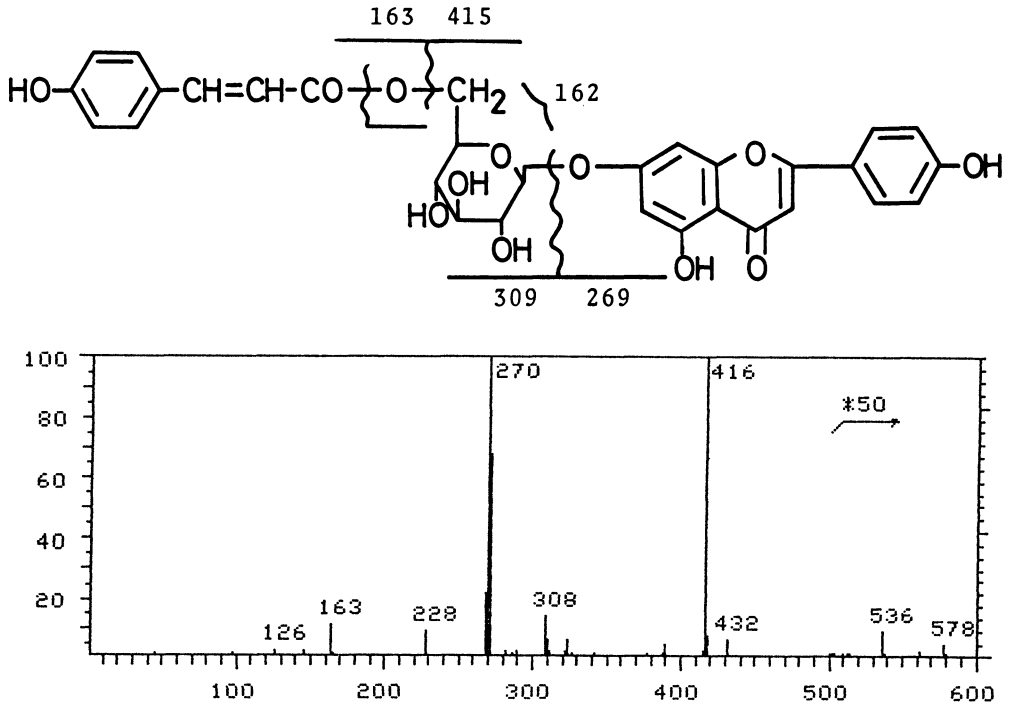
Table 1. Relative intensities of molecular ions or protonated molecular ions of flavonoid glycosides in in-beam EI, CI and NICI mass spectra

Compounds	Relative intensities (%)		
	EI $[M]^+$	CI $[MH]^+$	NICI $[M]^-$
Kaempferol-3-arabinoside	ND	ND	0.8
Apigenin-7-glucoside	1.6	1.6	9.4
Quercetin-3-xyloside	0.1	0.1	0.4
Quercetin-3-galactoside	0.06	0.04	0.7
Quercetin-3-glucoside	0.2	0.5	3.1
Irigenin-7-glucoside	1.6	1.0	0.9
Myricetin-3-rhamnoside	ND	0.04	0.8
Apigenin-6-C-glucoside (saponaretin)	0.5	ND	1.9
Daidzein-8-C-glucoside (puerarin)	16.3	10.2	0.9
Acacetin-7(-2"-O-acetyl)-glucoside	55.1	51.0	7.0
Apigenin-7(-6"-p-coumaroyl)-glucoside	ND	ND	0.08
Diosmetin-7-rutinoside	ND	ND	0.09
Naringenin-7-rutinoside	ND	ND	1.2
Hesperetin-7-rutinoside	0.2	ND	0.8
Tamarixetin-7-rutinoside	ND	ND	0.4
Quercetin-3-rutinoside	ND	ND	0.007
Rhamnazin-3-rutinoside	ND	ND	1.6
Isorhamnetin-3-rutinoside	ND	ND	0.2
Kaempferol-3,7-dirhamnoside	ND	ND	0.2
Kaempferol-7-rhamnoside-3-galactorhamnoside	ND	ND	ND
Isorhamnetin-3-rutinoside-4'-rhamnoside	ND	ND	ND

ND : not detected

rhamnazin-3-rutinoside[9] showed clearly the $[M]^-$ (m/z 638) and the fragment ion (m/z 492) which had lost one of the saccharide units, and the base peak (m/z 330) was assigned to the aglycone ion. (Fig. 2) And the molecular ion peaks of polar flavonoid glycosides (rutin, myricitrin, etc.) and a complicated one (apigenin-7(-6"-p-coumaroyl)-glucoside)[10] were also obtained. (Fig. 3) Further, the same

Fig. 3. In-beam NICI mass spectrum of apigenin-7(-6"-p-coumaroyl)-glucoside



results have been reported as to iridoide glycosides by use of the NI mass spectrometry.[11] Though in-beam NICI gave no information about the molecular weights of triglycosides of flavonoids, sometimes found in the natural sources, this method is also useful for the structural investigation of most flavonoid glycosides. The above results are showed in table 1.

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