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Analytical Separation of Isomeric Saponins by LC/MS

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Abstract—The application of LC/MS system with FRIT-FAB LC/MS interface in both positive and negative ion modes to the analytical separation of isomeric saponins was studied. The two isomeric saponins such as rosamultin and arjunetin were well separated and analyzed FRIT、FAB LC/MS system. This is another examples to structure elucidation of natural products.

Keywords—Rosamultin • Arjunetin • LC/MS • FRIT-FAB LC/MS system

Previously[1,2], we reported the isolation of (+)-catechin, sterol glucoside mixture and triterpenoid glycosides such as kaji-ichigoside F_1, rosamultin and arjunetin from the underground parts of *Rosa rugosa*.

Among them, the latter two compounds, rosamultin and arjunetin having the same molecular weight were elucidated to belong to ursane and oleanane glycoside respectively. That is, they are isomeric saponins.

In the case of numerous isomeric triterpenoid glycoside mixture as well as rosamultin and arjunetin, it is very difficult to separate by usual methods such as thin layer chromatography(TLC) and column chromatography(CC) etc.

But recently, application of high performance liquid chromatography (HPLC) could reduce the effort to separate them. Actually, we could easily separate rosamultin and arjunetin by HPLC, even though they showed one homogenous spot on TLC with several solvent systems. But direct structure elucidation by HPLC are not easy because the detectors used for HPLC are far from universal in their application. The mass spectrometer(MS) affords a more nearly universal detectors, providing an appropriate interface is available and some models have already been developed in commercial liquid chromatography/mass spectrometer(LC/MS) system. There, however, are only a few LC/MS systems.

Even though less applicability of LC/MS compared with gas chromatography/mass spectrometer(GC/MS) coupling systems which are already in wide use in the field of direct structure elucidation of natural products, useful information can be obtained with a direct liquid introduction type interface(Superfine Pressure Control LC/MS interface: SPC) and the FRIT-FAB LC/MS interface.

Very recently, Hattori *et al*[3] obtained good results to the analysis of low molecular compounds such as lignans, flavonoids and xanthones and relatively high molecular compounds such as secoiridoid glucosides and triterpene

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saponins using LC/MS system with the former or latter interface.

In this communication, we wish to report the application of LC/MS system with the FRIT-FAB LC/MS interface in both positive and negative ion modes to the analytical separation of isomeric triterpenoid glycoside.

Results and Discussion

As shown in Fig. 1 and Fig. 4, the two isomeric saponins were well separated by FRIT-FAB LC/MS system. Moreover, FAB mass spectra showed very strong $[M-1]^-$ when measured in the negative ion mode, indicating that the molecular weight can be easily determined (Fig. 2 and Fig. 3). So, the molecular weight of two saponins, rosamultin (m.w. 650) and arjunetin(m.w. 650) was measured in the

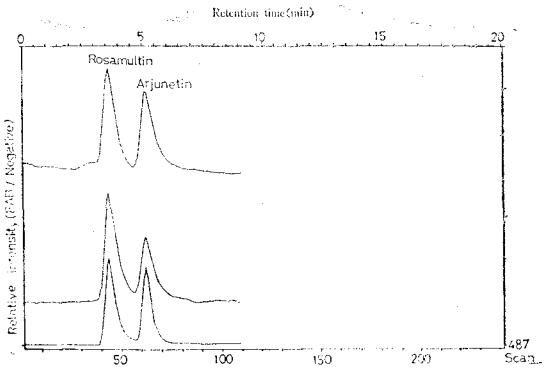

Fig. 1. Total ion current and mass chromatogram of a mixture of rosamultin(mw. 650) and arjunetin(mw. 650) measured with the FRIT-FAB LC/MS system

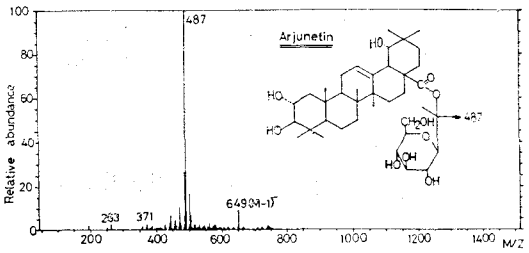

Fig. 2. Negative FAB mass spectrum of rosamultin

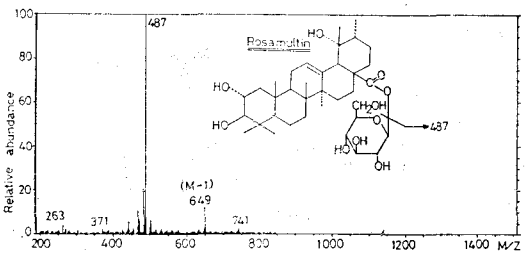

Fig. 3. Negative FAB mass spectrum of arjunetin

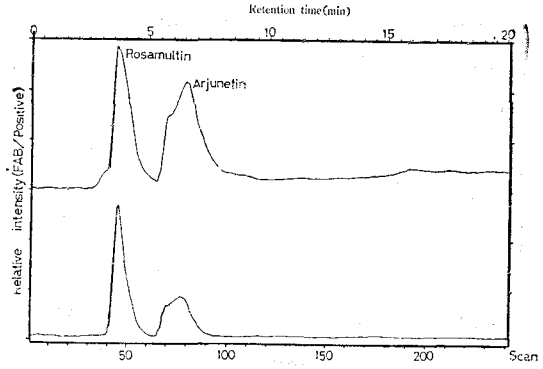

Fig. 4. Total ion current and mass chromatogram of a mixture of rosamultin(mw. 650) and arjunetin(mw. 650) measured with the FRIT-FAB LC/MS system.

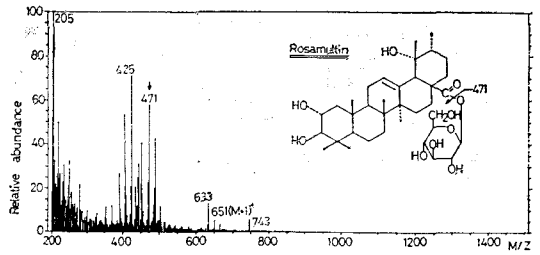

Fig. 5. Positive FAB mass spectrum of rosamultin

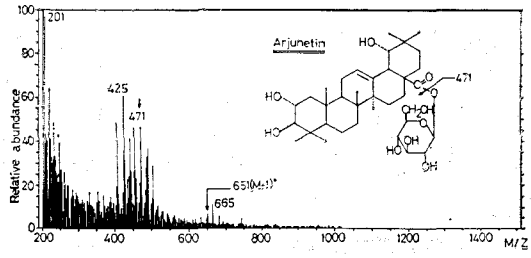

Fig. 6. Positive FAB mass spectrum of arjunetin

LC/MS negative ion mode. In addition, a strong peak originating from the aglycone, enabling us to obtain very important information for knowing the structure of compounds showed in positive or negative ion mode measurement(Fig. 2, 3, 5 and 6). The fragment ions(m/z 487 and m/z 471) originating from their aglycone moieties were observed in the positive or negative ion mode, respectively. These results indicate that a close examination of spectra meastred in the two modes will be obtained an important information on the structure elucidation from underivatized compounds.

Experimental Methods

The analysis of isomeric saponins by LC/MS was performed by a LC/MS spectrometer(Jeol, DX 300L) using the following conditions: Column; μS-Finepak SIL C_{18}, 1.5 mm i.d.×25 cm, Mobile phase; $CH_3CN:H_2O=7:3$ with 0.5% glycerin, Flowrate; 1.0 ml/min, Neutral gas; Xe, Primary beam energy; 3 Kv, Emission current; 20 mA.

Isolation of rosamultin and arjunetin mixture: This was carried out as described previously.[2]

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