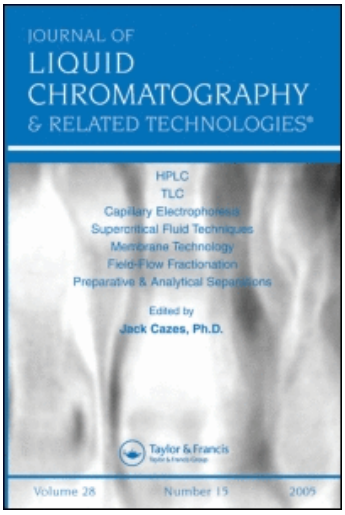

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Development of Crude Drug Analysis by Liquid Chromatography, and UV and MS Spectrometers

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DEVELOPMENT OF CRUDE DRUG ANALYSIS BY LIQUID CHROMATOGRAPHY, AND UV AND MS SPECTROMETERS

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ABSTRACT

The retention indeces of 42 standard compounds in naturally occurring drugs were studied in reversed-phase liquid chromatography. The effluent was monitores by a photodiode array detector and mass spectrometer. The spectra and the retention indeces were used for qualitative analysis of the crude extract components.

INTRODUCTION

Crude drugs and extracts from natural products have been widely used as medicines since ancient times. Many natural

substances have been isolated and purified and their structures identified. However, plant extracts are still used directly as medicines, or are chemically modified to be more effective as medicines. The analysis of such multicomponent mixtures is important in the search to find pharmacological active compounds with minimal side effect. However, their purification is difficult owing to the similar chemical structures of the byproducts.

Thus, in the present study, chromatography was carried out on standard compounds, natural products were further purified and their retention indeces determined by a reversed-phase liquid chromatograph-system. Their uv/vis spectra were on line monitored using a photodiode array spectrophotometer and their mass spectra taken with a LC-MS set-up.

EXPERIMENTAL

The liquid chromatograph system and photodiode array photometric detector (LC-PDD) were the same as used previously [1]. The liquid chromatograph equipped with a mass spectrometer (LC-MS) was a HP 1090L to which was attached a HP 5988A or a Vestec VT101 mass spectrometer. The octadecyl-bonded silica gels were 5 μm Inertsil ODS-2 and 10 μm Inertsil PREP ODS [2] obtained from Gasukuro Kogyo Inc. The column size and separation condition are given in figure and table captions.

RESULT AND DISCUSSION

The retention indeces (log k') of 42 standard compounds are listed in Table I. Their ultraviolet absorption spectra, measured by on-line LC-PDD, are given in Fig. 1. These compounds showed a wide range of polarities and the absorption intensisities of several of them were too weak to permit monitoring by the UV detector in

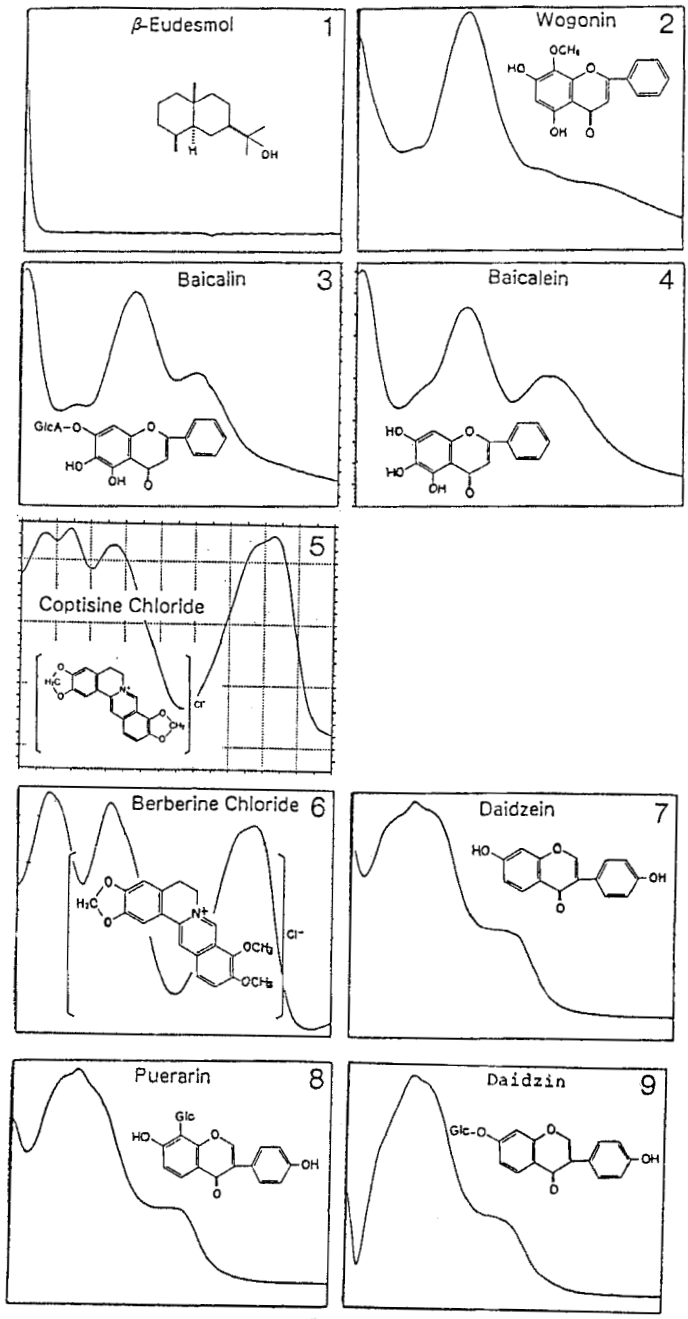


Fig. 1 Absorption spectra of crude drug standards by LC-PDD
The experimental conditions are given in Table I. The unit and wave length scale are the same as on No. 42 Shikonin. (continued)

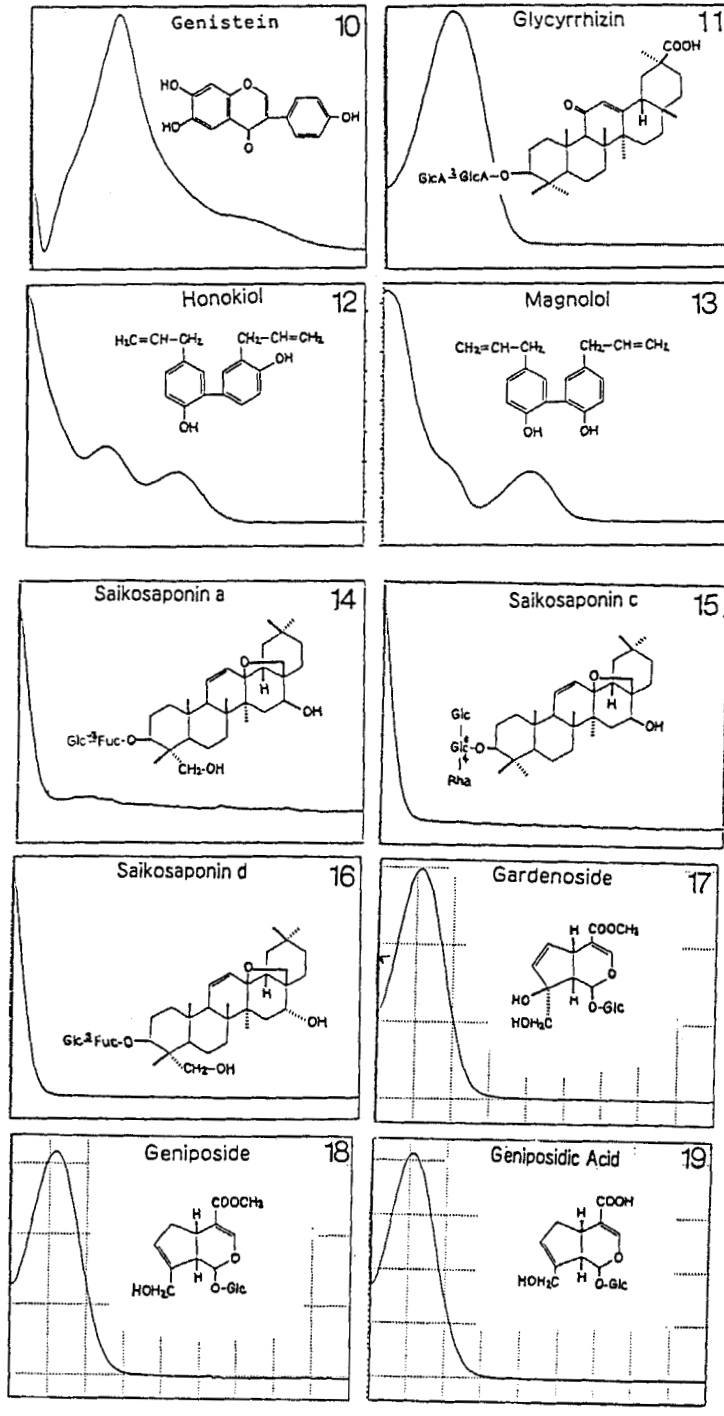
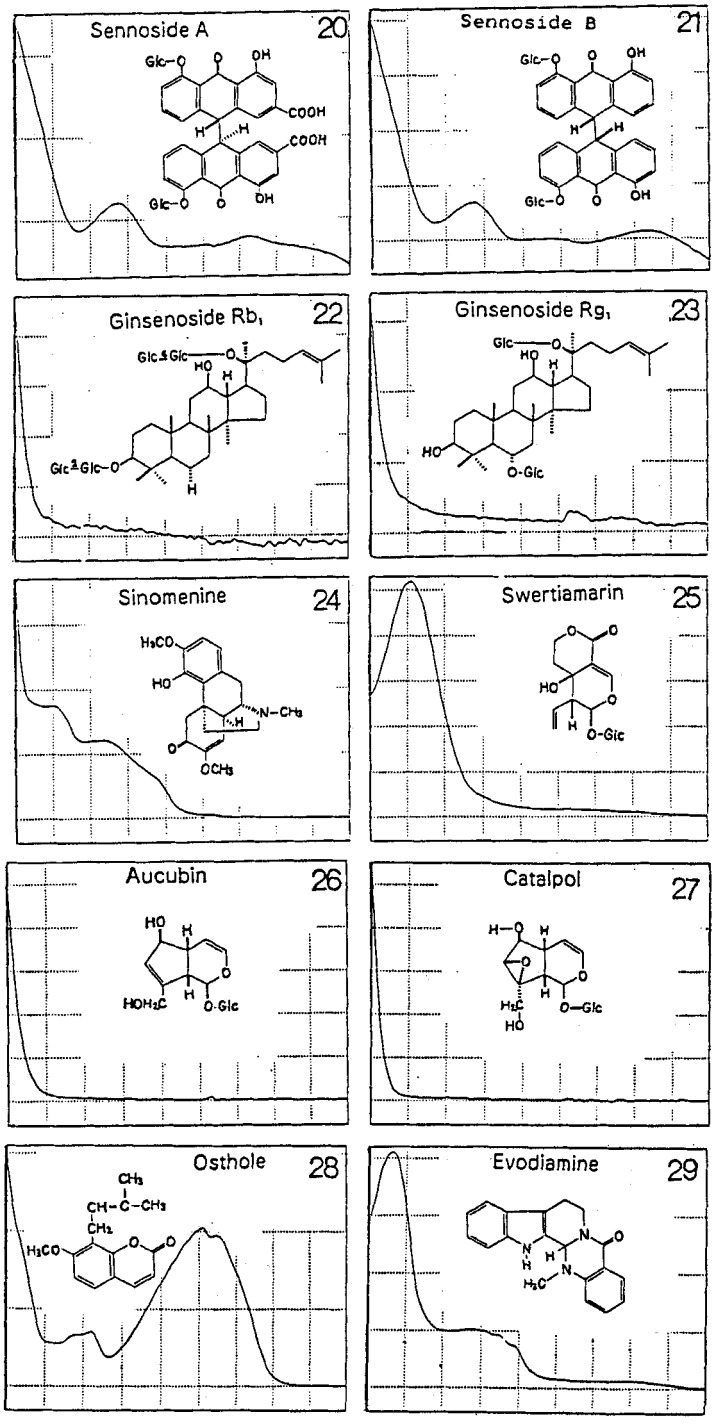


Fig. 1 (continued)



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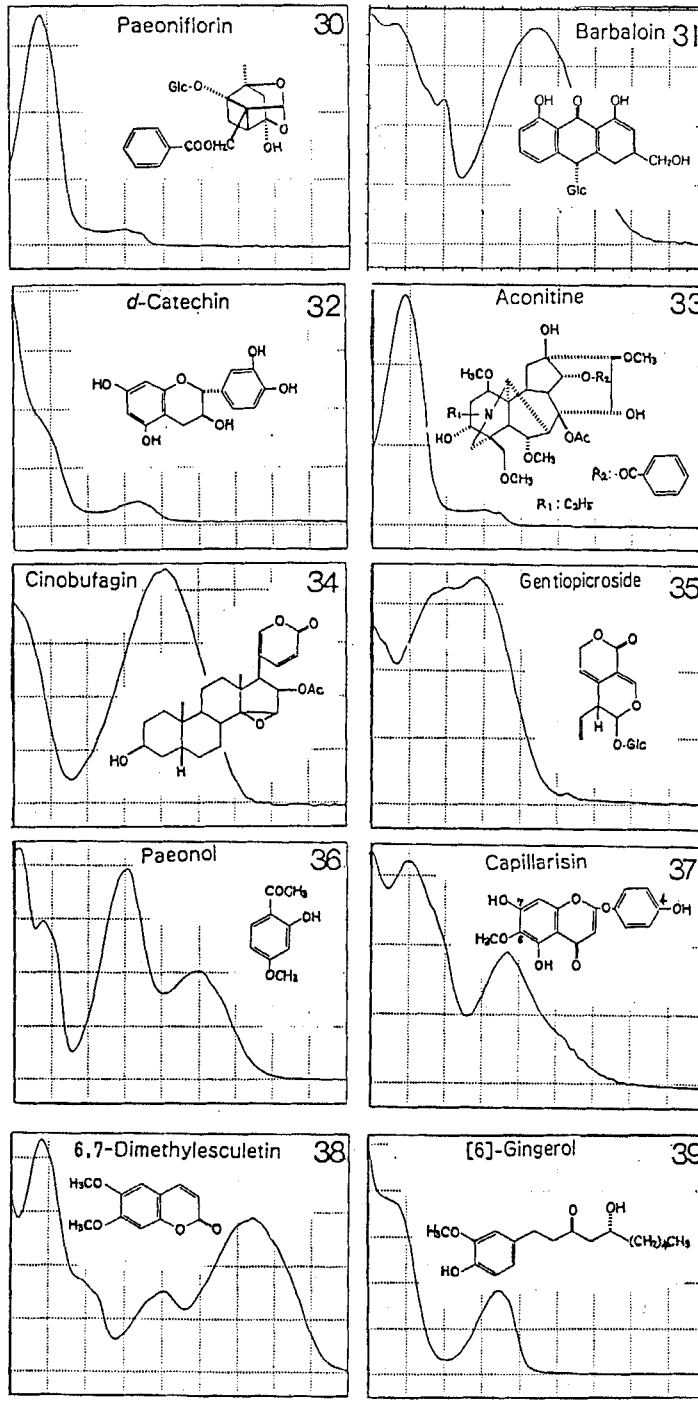

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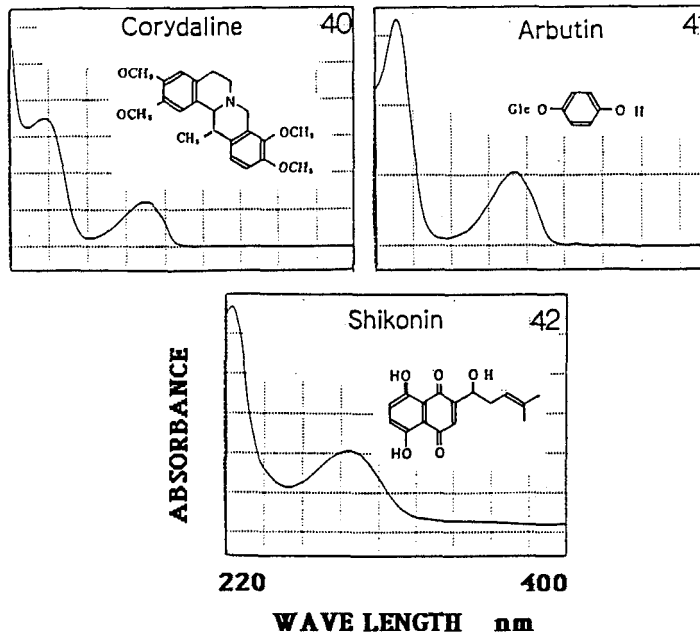

Fig. 1 (continued)

liquid chromatography, thus making their analysis difficult by a single liquid chromatographic run. Some showed essentially the same basic structures and UV spectra.

The chromatogram of a crude extract of Scutellariae Radix monitored by a LC-PDD is shown in Fig. 2. Its main components, Wogonin, Baicalin and Baicalein, were identified by a comparison of their retention times and spectra with those of standard compounds. However, several identical compounds were present in this crude extract. The chromatogram of a crude extract of Atractylodis Lanceae Rhizoma monitored by a photodiode array detector was very complicated, owing to the very weak absorption of themain component, β-Eudesmol, as shown in Fig. 3, and also,

Table 1. RETENTION INDEX OF STANDARD COMPOUNDS
Experimental condition: column 15 cm x 4.6 mm, eluent 0.05M H_3PO_4 in aq. acetonitrile at 40° C.

No.	Compounds	λmax*	λ**	MW***	log k' / % acetonitrile 60	50	40	30	20
1	β-Eudesmol	220	203	222	0.956				
2	Wogonin	220 282	254	284	0.127	0.428	0.801	1.295	
3	Baicalin	223 284 318	280	446	-0.619	-0.528	-0.278	0.199	1.003
4	Baicalein	223 282 322	280	270	-0.157	0.110	0.440	0.896	
5	Coptisine chloride	234 248 274 366	254	356			-1.852	-0.354	0.418
6	Berberline chloride	236 272 355	254	372	-0.661	-0.086	0.712		
7	Daidzein	256	254	254	-0.529	-0.283	0.008	0.372	0.991
8	Puerarin	256	254	416		-0.570	-0.221		
9	Daidzin	257	254	416			-0.590	-0.476	0.063
10	Genistein	268	254	270	-0.338	-0.023	0.303	0.738	
11	Glycylrrhizin	244	254	823	-0.614	-0.153	0.440		
12	Honokiol	220 263 300	280	266	0.433	0.836			
13	Magnolol	223 298	280	266	0.619	1.036			
14	Saikosaponin a	220	203	781	-0.255	0.137	0.707		
15	Saikosaponin c	220	203	927	-0.695	-0.292	0.255	1.080	
16	Saikosaponin d	220	203	781	-0.708	0.600	1.211		
17	Gardenoside	242	240	404				-1.161	-0.478
18	Geniposide	244	240	388			-0.598	-0.492	-0.087
19	Geniposidic acid	242	232	374				-1.361	-0.868
20	Sennoside A	220 276	254	863			-1.240	-0.502	0.459
21	Sennoside B	220 276	254	755			-1.481	-0.530	0.124
22	Ginsenoside Rb1	220	203	1109	-0.720	-0.548	-0.199	0.972	
23	Ginsenoside Rg1	220	203	801		-0.625	-0.595	-0.051	1.139
24	Sinomenine	220 241 268	203	329					-0.849
25	Swertiamarin	242	203	374	-1.439	-1.245	-1.035	-0.705	-0.235
26	Accubin	220	206	346				-1.849	-1.278
27	Catalpol	220	203	362				-2.057	-1.493
28	Osthole	220 265 322	254	244	0.577	0.909	1.315		
29	Evodiamine	234 276	254	303	0.258	0.575	0.963		
30	Paeoniflorin	238 280	254	480	-1.177	-0.920	-0.689	-0.346	0.182
31	Barbaloin	220 235 260 306	254	418	-1.038	-0.603	-0.490	-0.106	0.549
32	d-Catechin	220 287	240	290	-1.006	-0.805	-0.648	-0.455	-0.142
33	Aconitine	240 280	250	646			-1.094	-0.047	0.894
34	Cinobufagin	302	280	443	0.190	0.508	0.919		
35	Gentiopicroside	260 278	270	356	-1.236	-1.013	-0.818	-0.523	-0.043

36 Paeonol	223	236	282	320	280	166	0.164	0.396	0.657	0.992
37 Capillarisine	220	240	296		280	316	-0.226	0.045	0.364	0.843
38 6,7-Dimethyl-esculetin	238	302	348		280	206	-0.225	-0.043	0.153	0.430 0.841
39 (6)Gingerol	220	288			280	294	0.181	0.486	0.863	
40 Corydarine	220	238	290		254	369			-1.283	-0.211 0.592
41 Arbutin	230	292			280	271	-1.775	-1.446	-1.272	-1.107 -0.895
42 Shikonin	222	282			270	288	0.528	0.852	1.250	
Void volume (fructose) mL	220				210	180	1.48	1.45	1.5	1.56 1.63

*Maximum wavelength (nm), ** Measuring wavelength (nm), *** Molecular weight

other components resembled this component. This sample was analyzed by LC-MS, and its total mass-chromatogram is shown in Fig. 4-A where β-Eudesmol can be identified from its mass spectrum given in Fig. 4-B. The mass number of 222 was obtained from MW 222 - H_2O + NH_4, and 205 from 222 - H_2O + H.

Analysis was also made of Puerariae Radix. Its chromatogram, monitored by the photodiode array detector showed the presence of many compounds similar in structure (Fig. 5) and thus without standard compounds, the identification of each component by LC-PDD was quite difficult. Peaks 1, 2, 3 and 4 were identified as Puerarin, Daidzin, Daidzein and Genistein, respectively, from their retention indeces and UV spectra. These peaks were also analyzed by LC-MS. Their total ion mass chromatogram and the mass spectra of standard compounds are given in Fig. 6.

A possitive ion mass chromatogram with a mass number of 255 (mass number of Daidzin and Daidzein, 254 + H) showed three major peaks, 2,3 and 6, as shown in Fig. 6-A. That with a mass of 271 (mass number of Genistein, MW 270 + H) showed three major peaks, 4, 5 and 7 in Fig. 6-A. The one with a mass number of 417

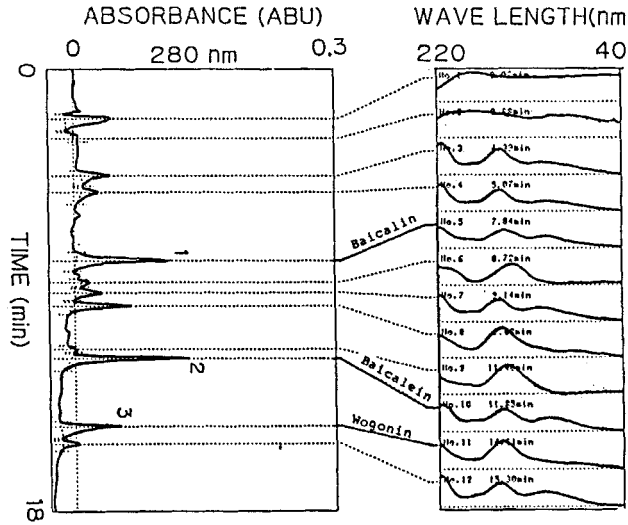

Fig. 2 Chromatogram and absorption spectra of Scutellariae Radix
column: Inertsil ODS2, 25cm x 4.6mm id, eluent: 20min gradient from 0.05% trifluoroacetic acid in 20% acetonitrile to 80% aq. acetonitrile, flow rate: 1 mL/min, column temp.: 40°C, peaks 1: Baicalin, 2: Baicalein, 3: Wogonin

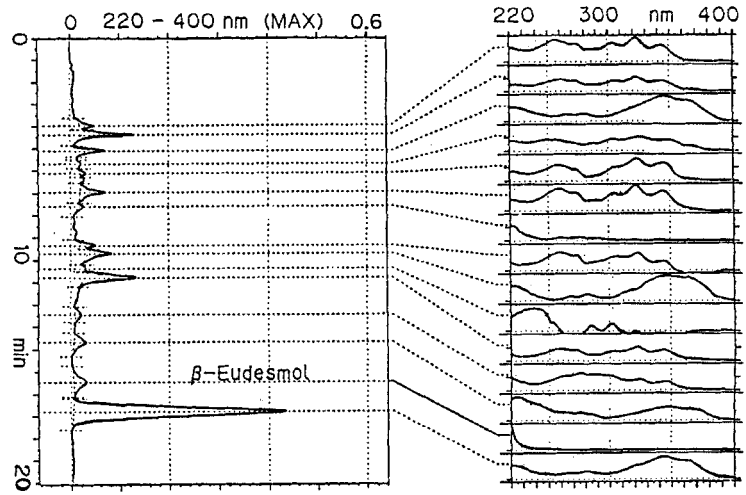

Fig. 3 Chromatogram and absorption spectra of Atractylodis Lanceae Rhizoma
column: Inertsil PREP-ODS, 25cm x 6.0mm id, eluent: 70% aq. acetonitrile, flow rate: 1.5mL/min, column temp.: 30°C, detector wave length: 220-400 nm at the maximum of each component.

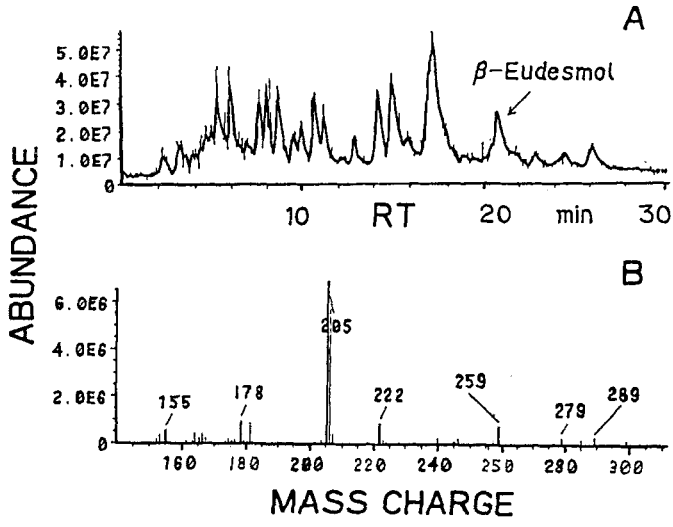

Fig. 4 Total ion mass chromatogram (A) of Atractylodis Lanceae Rhizoma and the positive mass spectrum of β-Eudesmol
Liquid chromatographic conditions are the same as in Fig. 3, except for the eluent containing 0.1M ammonium acetate. MS: HP 5988A posotive mode.

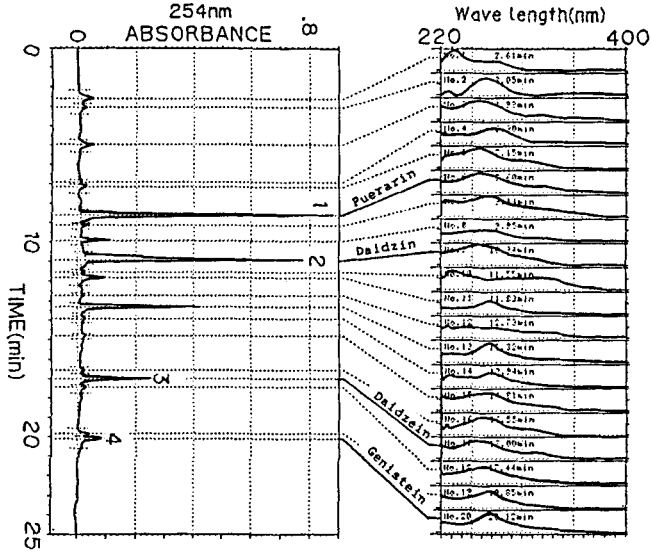

Fig. 5 Chromatogram and absorption spectra of Pueraride Radix
column: Inertsil PREP-ODS, 25cm x 6.0mm id, eluent: 25min gradient from 10% aq. acetonitrile to 80% aq. acetonitrile, column temp.: ambient, flow rate: 1.5mL/min, detector wave length: 220-400 nm at the maximum wave length of each component. peaks 1: Puerarin, 2: Daidzin, 3: Daidzein, 4: Genistein.

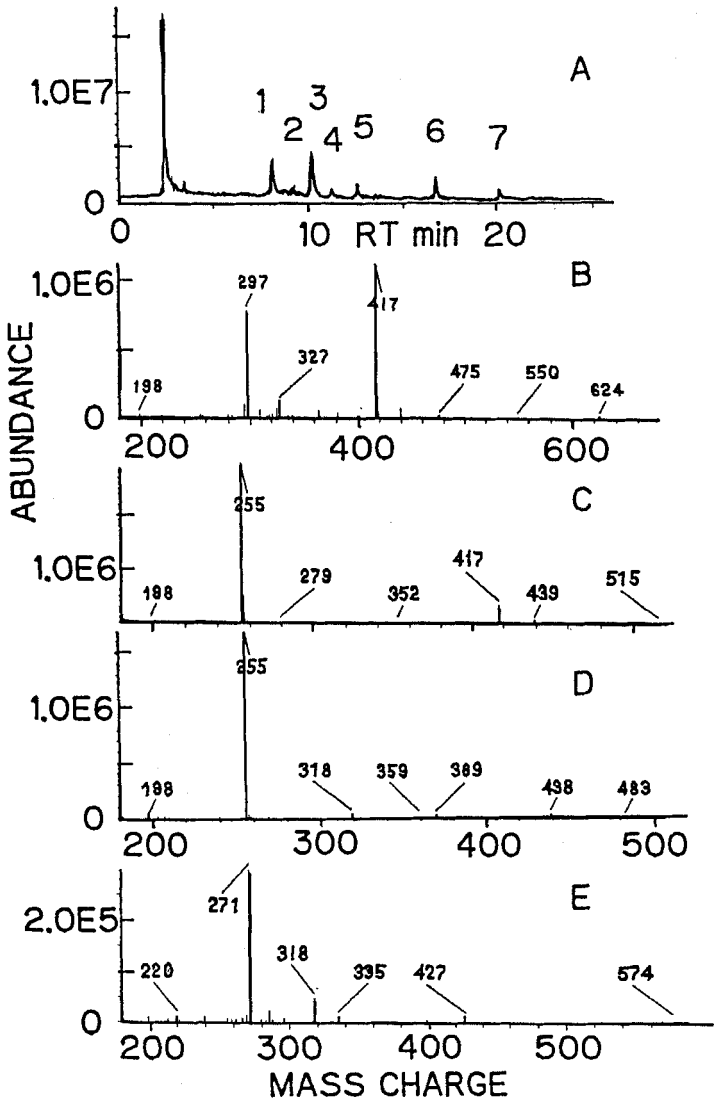

Fig. 6 Total ion mass chromatogram of Pueraride Radix and positive ion mass spectra of the major components

The chromatographic condition is the same as in Fig. 5, except for the eluent containing 0.1M ammonium acetate. MS: Vestec positive mode

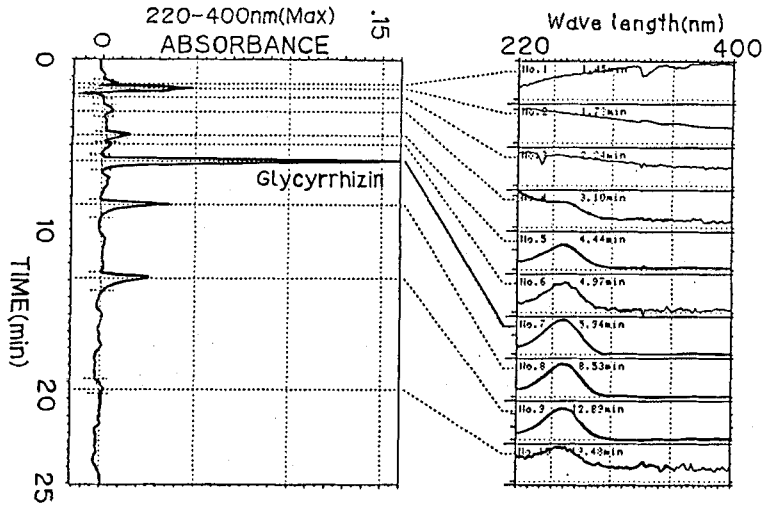

Fig. 7 LC-PDD chromatogram of Glycyrrhizae Radix
column: Inertsil ODS2, 15cm x 4.6mm id, eluent: 40% aq. acetonitrile containing 0.05M phosphoric acid, flow rate: 1.0 mL/min, column temp.: 40°C, detector wave length: 220-400 nm at the maximum of each component.

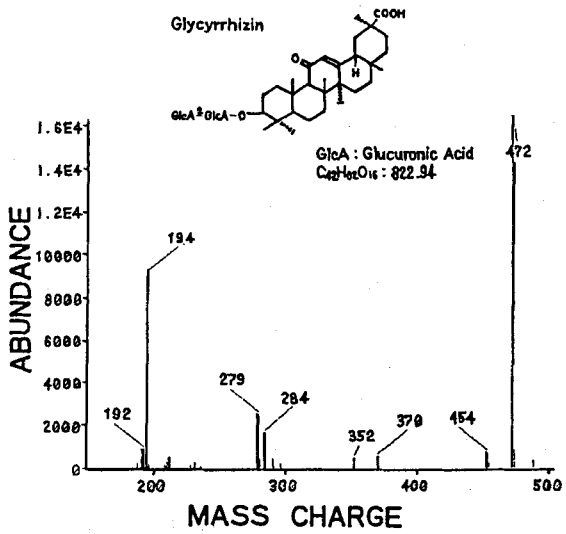

Fig. 8 Positive mass spectrum of Glycyrrhizin as measured by the flow injection method.

(mass number of Puerarin, 416 + H) gave two major peaks, 1 and 3 in Fig. 6-A. The mass spectra measured by on-line LC-MS were not exactly the same as those of standard compounds as measured by the flow injection method. The on-line mass spectra of Puerarin, Daidzin, Daidzein and Genistein are given in Fig. 6-B, C, D and E, respectively. Analysis of the mass spectra as measured by LC-MS was not simple, as also in the case of measurement by GC-MS, the reason for this possibility being that the LC-MS spectra may have been polluted by the solvent and the influence of concentration.

The LC-PDD chromatogram of Glycyrrhihizae Radix is shown in Fig. 7 and the mass spectrum of Glycyrrhizin, the major component, is given in Fig. 8. No molecular ion (823 + H) was observed, but peaks of the decomposed products, glucuronic acid and glycyrrhic acid were noted (Fig. 8). The possitive ion with a mass number of 194 was glucuronic acid (MW 194) - H2O + NH4, and that with 472, glycyrrhic acid (MW 471 + H).

These LC-PDD chromatograms of natural products showed the presence of many compounds with spectra similar to those of their major components. However, identification of the unknown components by LC-MS was difficult.

Log P values, i.e., the partition coefficients between octanol and water, have been used to estimate the solubility of drugs [3-7] and optimize reversed-phase liquid chromatography [8-15]. These values for several standard compounds were thus determined using alkylphenols as the log P standard so as to assess the retention behavior of related compounds. The standard compounds were phenol (log P = 1.54), 2-methylphenol (log P = 2.05), 2,5-dimethylphenol and 3,4-dimethylphenol (;og P = 3.02) and 2-ethylphenol (log P = 2.58) from reference 13.

From the experimental results for Inertsil PREP-ODS, 25 cm x 6.0 mm i.d., in 30 v% aq. acetonitrile containing 0.05M phosphoric acid at 40°C, the difference in the log P values of Wogonin (log P = 3.20) and Baicalein (log P = 2.47) was 0.73. Δlog P for the methyl

group was 0.780 from ref. 16. Δlog P between Baicalein and Baicalin (log P= 1.20) was 1.27. This difference must certainly be relatd to one Glucuronic acid unit. Δlog P between Daidzein (log P = 1.51) and Puerarin (log P = -0.20) was 1.71, and must arise from the presence of one Glucose unit. However, Δlog P of Glycyrrhizin (log P = 1.60) related compounds with spectra quite similar to it was 0.45 or 0.90, depending on the peak selected. This clearly demonstrated the difficulty in appling the log P calculation method for determining the structures of unknown polar compounds from retention times and spectra.

The purification and identification of naturally occuring products remain difficult processed, even using on-line LC-PDD and LC-MS. The development of better analytical techniques and theoretical approachs is thus required.

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