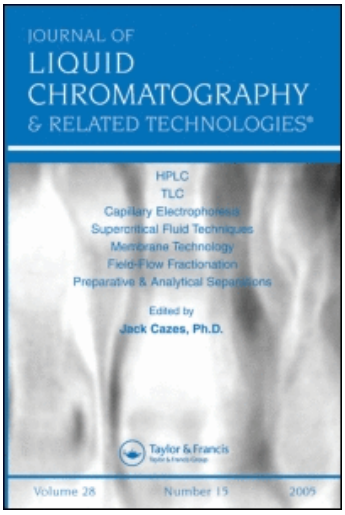

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Urushiols by Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Ion Trap Mass Spectrometry

Dal-Ho Kim[a]; Jong Oh Choi[a]; Jeong Soo Yang[b]; Dai Woon Lee[b]
[a] Organic Analysis Laboratory, Division of Chemical Metrology and Materials Evaluation, Korea Research Institute of Standards and Science, Yusong, Daejon, Republic of Korea [b] Department of Chemistry, Yonsei University, Seoul, Republic of Korea

Online publication date: 30 January 2003

To cite this Article Kim, Dal-Ho , Choi, Jong Oh , Yang, Jeong Soo and Lee, Dai Woon(2003) 'Analysis of Urushiols by Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Ion Trap Mass Spectrometry', Journal of Liquid Chromatography & Related Technologies, 26: 1, 17 — 28
To link to this Article: DOI: 10.1081/JLC-120017149
URL: http://dx.doi.org/10.1081/JLC-120017149

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 1, pp. 17–28, 2003

Analysis of Urushiols by Liquid Chromatography/Atmospheric Pressure Chemical Ionization-Ion Trap Mass Spectrometry

Dal-Ho Kim,[1,*] Jong Oh Choi,[1] Jeong Soo Yang,[2] and Dai Woon Lee[2]

[1]Organic Analysis Laboratory, Division of Chemical Metrology and Materials Evaluation, Korea Research Institute of Standards and Science, Yusong, Daejon, Republic of Korea
[2]Department of Chemistry, Yonsei University, Seoul, Republic of Korea

ABSTRACT

Urushiol derivatives in a natural polymeric paint (urushi), obtained from Korean tapping lacquer trees were separated by reverse phase liquid chromatography and analyzed by on-line atmospheric pressure chemical ionization ion trap mass spectrometry (LC/APCI-ITMS). The molecular weight and molecular structure information for each peak were obtained from full scan spectrum and collision induced dissociation

*Correspondence: Dal-Ho Kim, Organic Analysis Laboratory, Division of Chemical Metrology and Materials Evaluation, Korea Research Institute of Standards and Science, Yusong, Daejon, 305-600, Republic of Korea; E-mail: dhkim@kriss.re.kr.

DOI: 10.1081/JLC-120017149
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

(CID) spectrum, respectively. Each urushiol isomer was identified, based on the pseudomolecular ion, the pattern of product ions by CID of pseudomolecular ions, and the elution order of urushiols on the C_{18} stationary phase. The elution order was the result of other researchers by a preparative separation and NMR.

For composition analysis, the relative peak area ratios of components over total urushiol constituents were calculated from each peak area of urushiol components.

Key Words: Chemical ionization-ion trap MS; Urushiols; Compositional analysis.

INTRODUCTION

Oriental lacquers (urushi) are made from the sap of lacquer trees, Rhus vernicifera. Being produced in Korea, Japan, and China, urushi is a natural polymeric paint in the form of water/oil emulsion, consisting of water, enzymes, urushiol, polysaccharides, glycoproteins, peroxidase, and stellacyanin.[1–4] In this emulsion, the gummy substances in the water particles behave as an emulsifier, so the water particles are emulsified within the urushiol solution. The nitrogen containing substance of urushiol solution disperses the gummy substances to urushiol.[1,2] As the main constituent, taking up about 60–65% of urushi, urushiols are catechol derivatives, having carbon chains of carbon number 15 or 17 with $0 \sim 3$ double bond(s). The urushiol composition of urushi is known to be that of triene and monoene constituents, which take up about 80%, and within this content, 13′Z-catechol and 8′Z-pentadecenylcatechol take up most of it with 70%.[1,3,4]

The establishment of an efficient analysis method for urushi composition is important in the evaluation of quality, because urushiol content is one of the parameters affecting the quality of urushi.[1] Also, urushi analysis data are required in order to identify the producing areas of urushi.

For the analysis of urushiol, the barium-hydroxide titration technique was taken as the standard method in Japan.[2] However, this wet method is ineffective due to its heavy consumption of solvent and its multi-step procedure. Therefore, an alternative analysis method that can efficiently quantify the contents of urushiol, is required. In pursuing an effective alternative, there have been numerous reports of analyzing urushiol by spectrometry,[1,2,5–7] GC/FID,[1,8] SFC/FID,[1] HPLC,[5–7,9,10] and GC/MS.[1,6,7,11]

This study was focused on investigating the possibility of LC/APCI-ITMS as a simple and a new technique for urushiol analysis.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

EXPERIMENTAL

Materials

A sample of Korean urushi was obtained from a place of production (Wonju, Kangwon, Korea). As organic solvents, acetone, acetonitrile, and methanol were HPLC grade (Burdic and Jackson, Musjegonm, MI, USA). Acetic acid was an ACS grade reagent (Aldrich, St. Louis, Missouri, USA).

Sample Preparation

Two gram of the urushi sample was stirred with 50 mL of ethanol, and filtered through a 0.2 μm disposable syringe filter (nylon 66, 25 mm, catalog no. 2040; Alltech Associates, IL, USA). The filtrate was diluted 20 times with ethanol, and applied to LC/APCI-ITMS.

Instruments

Reversed-phase liquid chromatographic experiments for the separation of the urushi were performed on a chromatograph equipped with a HP 1050 autosampler and pump (Hewlett-Packard, Washington, DC, USA). Alltech Solvent miser C_{18} (250 mm × 2.5 mm × 5 μm, Alltech, 2051 Waukegan Road, Deerfield, IL, USA) was used as a stationary phase. Samples were eluted in an isocratic condition, and the eluent was methanol : 0.1% acetic acid in water (85 : 15, v/v). The HPLC conditions were as follows: volume injected, 2 μL; column pressure, 1700 psi; temperature, 25°C; and flow rate, 200 μL/min.

A Finnigan LCQ iontrap LC/MS system (Finnigan, San Jose, CA, USA), equipped with an APCI (atmospheric pressure chemical ionization) source was used. High-purity nitrogen was used for the sheath gas at 30 (an arbitrary value used in LCQ). Spectra were obtained in a positive-ion mode over the range m/z 280–400. CID experiments were performed with relative collision energy of 35%. APCI conditions were as follows: vaporizer temperature, 400°C; discharge current, 5 μA; capillary temperature, 135°C; and capillary voltage, 40 V.

RESULTS AND DISCUSSION

Optimization of Separation

As a stationary phase, C_{18} was chosen for the separation of urushiols by non-polar interaction with a benzene ring and carbon chain as an urushiol moiety. For

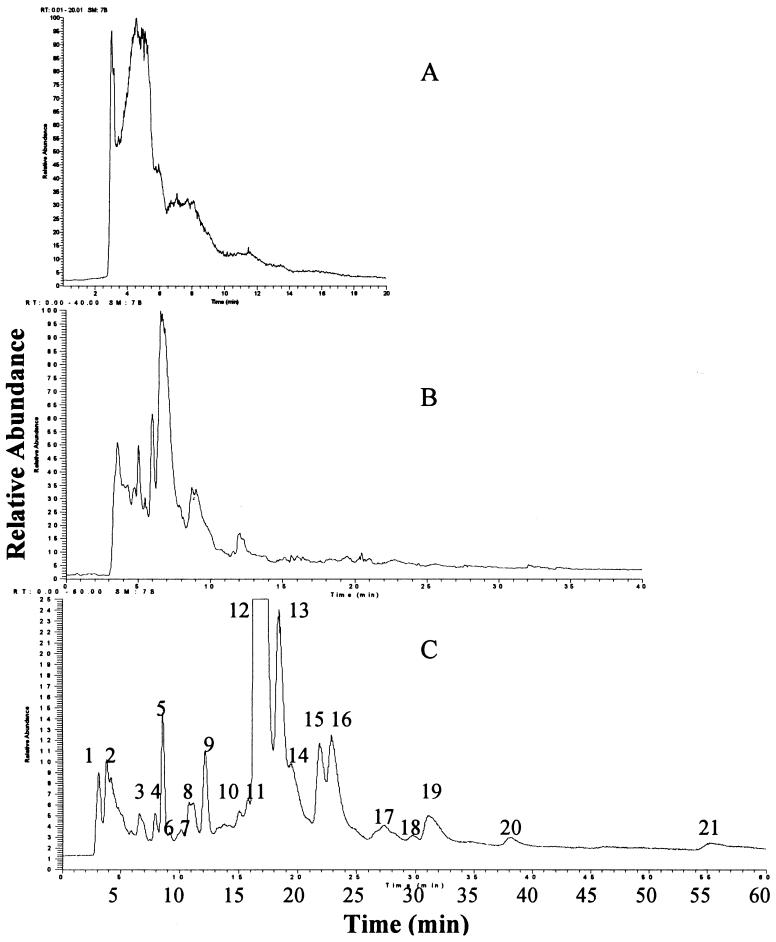

Figure 1. Total ion chromatogram of a Korean urushi sample, obtained by LC/APCI-ITMS. Methanol : water (50 : 50, v/v), A; acetonitrile : water : acetic acid (90 : 10 : 1), B; methanol : 0.1% aqueous acetic acid (85 : 15), C.

the starting point of optimization of separation, methanol : water (50 : 50, v/v) was chosen as a mobile phase. As a result, we obtained a chromatogram that was not completely separated. For a better separation condition, the mobile phase was changed to acetonitrile : water : acetic acid (90 : 10 : 1, v/v). As a result, a chromatogram as in Fig. 1-B was obtained. As shown in the figure, with this condition more peaks were separated than in the initial condition but, still, there

remained quite a lot of incompletely separated peaks. Through a series of experiments to find a better separation condition, the optimum separation condition was found with a mobile phase of methanol : aqueous 0.1% acetic acid (85 : 15, v/v), as in Fig. 1-C, and a separation time of 60 min.

Qualitative Analysis

Figure 1-C shows a TIC (total ion chromatogram) of a Korean urushi sample, obtained by a scan mode of LC/APCI-ITMS, injecting the Korean urushi sample at the optimum separation condition. More than 21 separation peaks were obtained. At the mass spectra of principal peaks on the total ion chromatogram obtained by a full scan LC/APCI-ITMS, the pseudomolecular ion $[M+H]^+$ of each constituent was the base peak. The ions of $m/z = 315$, 317, 343, 319, 345, and 321 are pseudomolecular ions of pentadecatrienyl ($m/z = 315$), pentadecadienyl ($m/z = 317$), heptadecatrienyl ($m/z = 343$), pentadecenyl ($m/z = 319$), heptadecadienyl ($m/z = 345$), and pentadecyl ($m/z = 321$), respectively.

Unlike the electron impact ionization, APCI is a soft ionization method. Therefore, in APCI, only the molecular weight of separated peaks could be determined from the pseudomolecular ion in the mass spectra. To obtain the structural information of each peak, MS/MS experiments were studied. The pseudomolecular ion ($m/z = 315$ or 317 or 313) was selected as a parent ion, then was followed by collision induced dissociation (CID), and resultant product ions were scanned.

The principal product ions of pseudomolecular ions ($m/z = 313$, 315, 317) by CID are summarized in Table 1. The spectra of separated urushiol obtained by CID of principal pseudomolecular ions can be sorted into a few groups (Table 1). The total ion chromatograms, which were obtained by CID of pseudomolecular ion of $m/z = 315$ at 35% relative collision energy, and the mass spectrum of the principal peaks in the chromatograms, are depicted in Fig. 2. The product ions by CID of the pseudomolecular ions of $m/z = 315$ (peak numbers 10–14 on the total ion chromatogram), are $m/z = 163$, 123, 149, 189, 177, 203, and 109.

In Table 2, the results of identified constituents are shown. Each urushiol is identified based on molecular weight (pseudomolecula ion), pattern of product ions by CID, and elution order of each urushiols on C_{18} stationary phase. The elution order of urushiols was the results assigned using preparative separation on C_{18} columns with a solvent mixture of acetonitrile-water–acetic acid, RI (refractive index) detection, and NMR by other researchers.[5,7] To utilize their results, we assumed that the selectivities of

Table 1. Product ions by collision induced dissociation of the principal parent ion.

	Parent ion (m/z)		
Peak no.	313	315	317
1	151, 281, 189		
3	163, 123, 149		
4			163, 149, 177, 145
5	201, 175, 187/163, 161, 147, 123, 121	163, 123, 149, 189, 177	
7		201, 163, 159, 147, 133	
10–14	163, 123, 203, 149, 175, 229	163, 123, 149, 189, 177, 203, 109	
15–16	229, 215, 173, 161, 147, 123	163, 149, 123, 189	163, 149, 177, 145
19	229, 215, 173, 161, 147, 123	149, 203, 189, 163, 217, 123	163, 149, 177
21		161,175, 147, 123, 189, 163, 149, 203	149, 163, 131

the solvent systems for our experiment (methanol–water–acetic acid), and for their experiment (acetonitrile–water–acetic acid) were the same. Later, further verification of elution orders of urushiols for methanol–water–acetic acid solvent system, identification of peaks by NMR after the fractionation of each peak using the preparative LC, is required.

Some constituents that were not previously reported (marked with an asterisk, peak numbers 3, 4, 7, 8, 10, 11 in Table 2) were additionally detected.[3] These constituents have shown the characteristic ions of urushiol, such as $m/z = 315$ (peak numbers 3, 7, 10, and 11 on the total ion chromatogram), $m/z = 317$ (peak number 4), and $m/z = 313$ (peak number 8), respectively. Only by a full scan spectrum, can we suppose the peak of number 8 in the total ion chromatogram (Rt. 11–12 min, $m/z = 313$) is a kind of urushiol. But the peak of number 8 is thought to have a different structure from urushiol because no product ions relevant to the dissociation pattern of an urushiol group were detected by the CID experiment (Table 1). On the other hand, for the peak of numbers 3, 4, 7, 10, and 11, the dissociation patterns of parent ions by CID were similar to that of urushiol's peaks (peak numbers 5, 6, $12 \sim 16$). From this, we can conclude that they are urushiol derivatives having very similar molecular

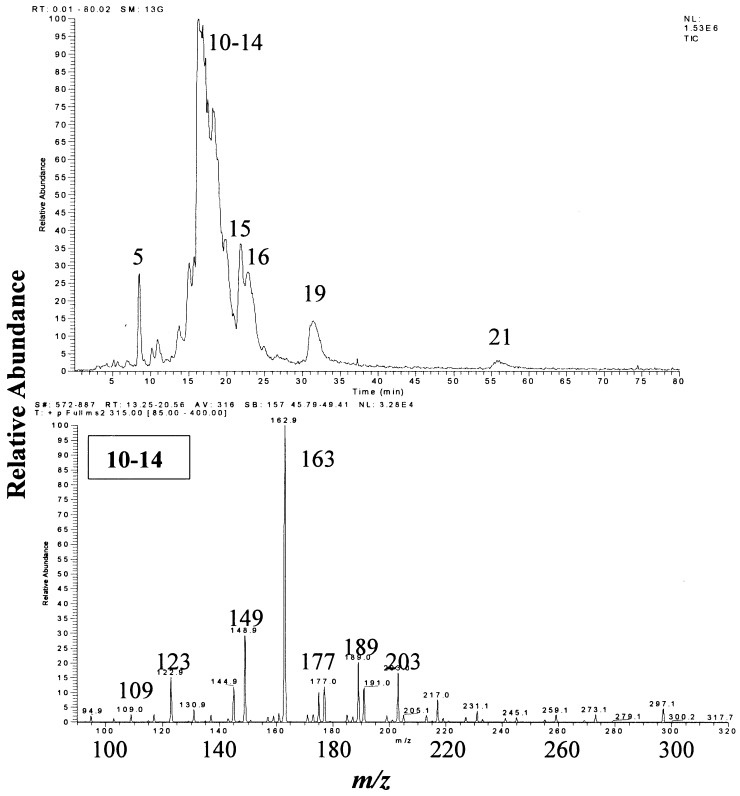

Figure 2. Total ion chromatogram and spectrum, obtained by CID for parent ion ($m/z = 315$).

structures. Later, further verification of these peaks by NMR, after the fractionation of each peak using the preparative LC, is required.

In Fig. 3, the product ions of the main constituent (pentadecatrienyl, peak number 12, $m/z = 315$), are shown. Product ions of pentadecatrienyl (Fig. 3) can be matched to the expected fragmented ions represented in Fig. 2.

Compositional Analysis

In urushi, many urushiol derivatives and isomers are included, but the standard materials are not available at the present time. Therefore, the relative

Table 2. Results of identification and relative component ratio of a Korean urushi sample.

a	R, name		MW	Peak no.[b]	Principal ilons[c]	Relative amount,[d] %
				1	331, 341	1.8
				2	345	6.5
				3[f]	329, 313	0.9
				4[f]	317	0.6
II	8′Z 11′E 13′Z-Pentadecatrienyl	15 : 3 8′c11′t13′c	314.5	5	315, 313	2.4
II	8′Z 11′Z 14′-Pentadecatrienyl	15 : 3 8′c11′c14′e	314.5	6	315, 313	0.2
				7[f]	315	0.4
				8[f]	313	1.6
I	Heptadecatetraenyl		340.5	9	341	2.7
				10[f]	315, 313	1.0
				11[f]	313, 315	1.0
I	8′Z 11′E 13′Z-Pentadecatrienyl	15 : 3 8′c11′t13′c	314.5	12	315	56.4
I	8′Z 11′Z 14′-Pentadecatrienyl	15 : 3 8′c11c14′e	314.5	13	315	6.4
I	8′Z 11′E 13′E-Pentadecatrienyl	15 : 3 8′c11′t13′t	314.5	14	315	1.3

I	8′Z 11′Z-Pentadecadienyl	15:2 8′c11′c	316.5	15	315, 317	4.5
I	8′Z 11′E-Pentadecadienyl	15:2 8′c11′t	316.5	16	317, 315	6.7
I	10′Z 13′E 15′Z-Heptadecatrienyl	17:3 10′c13′t15′c	342.5	17	343	1.1
	10′Z 13′Z 15′-Heptadecatrienyl	17:3 10′c13′c16′e	342.5	18	343	0.3
I	8′Z-Pentadecenyl	15:1 8′c	318.5	19[e]	315, 319	2.0
I	10′Z-Pentadecenyl	15:1 10′c	318.5	19[e]		
I	10′Z 13′Z-Heptadecadienyl	17:2 10′c13′c	344.5	20	345	1.0
I	Pentadecyl	15:00	320.5	21	313, 321	1.1

[a] I= II=

[b] The peak number designated on the total ion chromatogram (Fig. 1-C).
[c] Principal ions observed on the mass spectrum by full scan LC/APCI-ITMS.
[d] Relative component ratio calculated from each peak area and the total peak areas of components.
[e] Not separated.
[f] New constituents that were not reported by now.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

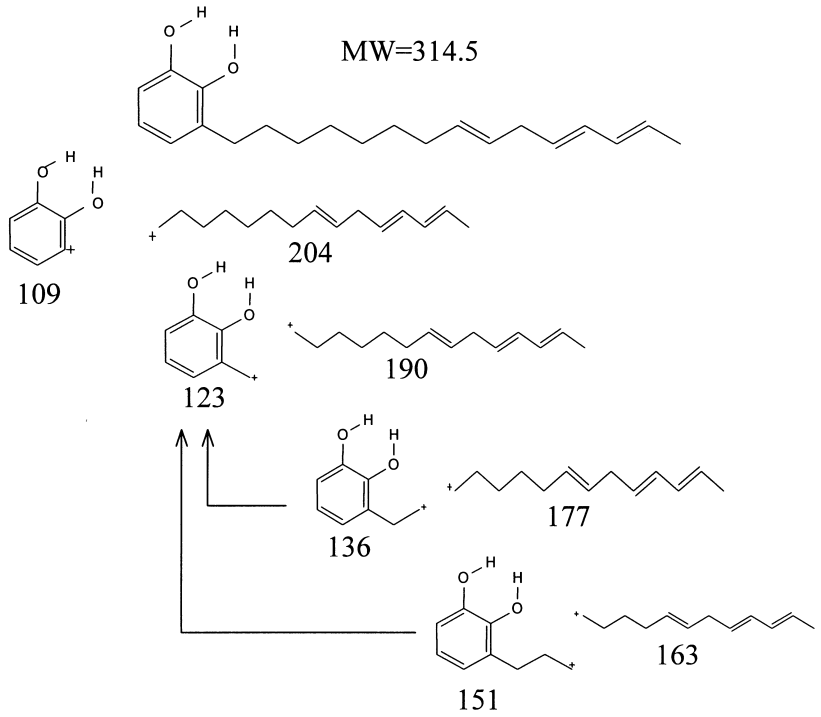

Figure 3. Product ions and their structures obtained by CID for pseudomolecular ion of pentadecatrienyl.

component ratio of urushiol constituents was calculated from each peak area and the total peak areas of components. The urushiol derivatives and isomers have similar structure and molecular weight, so we can assume that their ionization efficiencies by APCI and responses of detection are similar to one another.

Table 2 presents the results of the relative composition of urushiols for Korean urushi, analyzed by LC/APCI-ITMS. The main constituent was I-8′Z 11′E 13′Z-pentadecatrienyl, corresponding to peak number 12 in the total ion chromatogram, which showed 56.4% of component ratio. Other constituents, such as I-8′Z 11′Z 14′-pentadecatrienyl (peak number 13), I-8′Z 11′Z-pentadecadienyl (peak number 15), and I-8′Z 11′E-pentadecadienyl (peak number 16), showed 4–7% of component ratio, respectively. The rest showed 0.2–3% component ratio.

Compared to the results of the relative component ratio for Chinese and Japanese urushi analyzed by LC/RI and LC/UV by other researchers,[3,5] Korean urushi showed similar results, but certain components presented differences. These characteristics may well be utilized in the evaluation of the quality and origin of urushi.

CONCLUSION

This work has demonstrated the possibility of simple identification and quantification of urushiols using LC/APCI-ITMS. If once, we have known the elution order of urushiol derivatives using a preparative LC and NMR, we can identify and determine the urushiol derivatives by LC/APCI-ITMS without replicating laborious preparative separation and identification by NMR.

To the best of this author's knowledge, utilization of LC/APCI-ITMS technique in the analysis of urushiols was unprecedented.

The urushiols in the Korean urushi sample were identified based on the pseudomolecular ion, the pattern of product ions by CID, and the elution order of each urushiol on C_{18} stationary phase. The elution order was the results of other researchers by a preparative separation and NMR.

The relative composition of urushiol in the Korean urushi could be calculated from each peak area.

This technique can be utilized in the evaluation of the quality and origin of urushi.

REFERENCES

1. Bartus, J.; Simonsick, Jr., W.J.; Garner, C.; Nishiura, T.; Kitayma, T.; Hatada, K.; Vogl, O. Polym. J. **1994**, *26* (1), 67.
2. Nagase, K.; Miyakoshi, T. Coatings and Paints **1998**, *4* (575), 51.
3. Kumanotani, J. Progress in Organic Coatings **1995**, *26*, 163.
4. Snyder, D.M. J. of Chem. Educ. **1989**, *66* (12), 977.
5. Du, Y.; Oshima, R.; Kumanotani, J. J. of Chromatogr. **1984**, *284*, 463.
6. Oshima, R.; Yamauchi, Y.; Watanabe, C.; Kumanotani, J. J. Org. Chem. **1985**, *50*, 2613.
7. Hatada, K.; Kitayama, T.; Nishiura, T.; Nishimoto, A. Macromol. Chem. Phys. **1994**, *195*, 1865.
8. Du, Y.; Oshima, R.; Iwatsuki, H.; Kumanotani, J. J. of Chromatogr. **1984**, *295*, 179.

9. Yamauchi, Y.; Oshima, R.; Kumanotani, J. J. of Chromatogr. **1982**, *243*, 71.
10. Yamauchi, Y.; Murakami, T.; Kumanotani, J. J. of Chromatogr. **1981**, *214*, 343.
11. Rivero-Cruz, J.F.; Chavez, D.; Bautista, B.H.; Anaya, A.L.; Mata, R. Phytochemistry **1997**, *45* (5), 1003.

Received May 20, 2002
Accepted July 31, 2002
Manuscript 5868