Korean J. Food & Nutr. Vol. 19. No. 3, 267~270 (2006)

THE KOREAN JOURNAL OF
FOOD AND NUTRITION
한국식품영양학회

Analysis of the Urushiol in Korean Lacquer

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한국산 옻칠의 우루시올 성분 분석

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Abstract

In Korea, for a long time *Rhus verniciflua* has traditionally been used as an herbal medicines plants. A stem of *Rhus verniciflua* has been used to treat gastrointestinal trouble with in form of boiled chicken as a folk medicine. But it has been recognized as an extremely active allergen causing skin reactions. The chief allergenic component, urushiol, is found within the oleoresinous sap of *Rhus verniciflua*. Most components of urushiol have unsaturated side chains. These unsaturated side chains of urushiol are important to polymerization of these natural products. The urushiol components in Korean lacquer were isolated by reversed phase HPLC. The molecular weight of purified urushiol was determined as 340 from mass analysis. This compound was identified as Heptadecatetraenyl catechol (MW 340).

Key word : *Rhus verniciflua*, urushiol, ESI, FAB MS

INTRODUCTION

Saps from various kinds of lacquer tree in the family *Anacardiaceae* have been used as excellent coating materials for several thousand years in Asian countries[1]. These saps are lipophilic allergens in the *Anacardiaceae* family, include poison ivy (*Toxicodendron radicans*), eastern poison oak (*T. quercifolium*), poison sumac (*T. vernix*) and Asiatic lacquer tree. There are three kinds of oriental lacquers: that is, *Rhus verniciflua* (Korea, Japan and China), *Rhus succedanea* (North Vietnam) and *Melanorrhoes usitate* (Thailand and Burma)[2-4].

In Korea, for a long time *Rhus verniciflua* has traditionally been used as an herbal medicines plants. It is known to contain various biological activities[5]. A stem of *Rhus verniciflua* has been used to treat gastrointestinal trouble with in form of boiled chicken as a folk medicine. But it has been recognized as an extremely active allergen causing skin reactions. An allergic contact dermatitis develops usually 24 to 48 hours of exposure in previously sensitized individuals. Dermatitis affecting the face, neck and genitalia may be accompanies by severe edema[6].

These saps latex composed of urushiol (60~65%), water (20~25%), water soluble plant gums (5~7%), glycoprotein (2~5%), and small amounts of enzymes (1%) such as stellacyanin, peroxidase, and laccase[7]. The oil-soluble fraction of sap of the lacquer tree is a mixture of catechol derivatives substituted in the three position of the catechol with unsaturated (trienes, dienes, monoenes) and some saturated hydrocarbon chains - C_{15} and C_{17}

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chain lengths[8,9]. The composition of the urushiol is varied and depends on the individual botanic species of the *Rhus verniciflua* tree, and environmental condition such as geographical location, growing conditions, and season of harvesting[10].

A number of techniques have been used to analyze and characterize the components of urushiol. Analysis of urushiol was based on chemical reactions and was first characterized by GC-MS in 1975[11,12]. GC analysis requires a derivation of compound to stabilize and improve their chromatography. Development of high performance liquid chromatographic media has been progressed recently. Some of the heteroolefinic urushiol components have been resolved without chemical modification by HPLC on an ODS gel column[13~15].

The present study was conducted to isolate the urushiol of Korea lacquer. And we report here the determination of isolated urushiol using a mass spectrometry.

MATERIALS AND METHODS

1. Sample Preparation

The native saps of *Rhus verniciflua* tree was purchased from Daeanri, Wonju, Kwangwon province in Korea. It were stored in a desiccator under a nitrogen atmosphere and in a refrigerator. The saps (1 g) was dissolved with acetone and the filtered twice through a Glass fiber filter (1㎛ pores, Toyo, Japan). The filtrate was evaporated to get a crude urushiol preparation as a residue. It was dissolved in 5 ㎖ of n-hexane. The organic solvent was evaporated to dryness on a rotary evaporator (Eyela NE, Japan). The residue was dissolved in 5 ㎖ of chloroform and was concentrated(200 ㎎). The crude extract dissolved in 0.5㎖ chloroform. After filtering through a Millipore filter (0.45 ㎛), the sample was analyzed by HPLC. Plant gum, glycoprotein and protein (enzymes) were removed from the concentrate by filtration.

2. Purification

The crude extract (in chloroform) was loaded to HPLC (Gilson 321, France) with ODS silica gel preparative column (Hydrosphere C_{18}, 250×20 mm, 5 ㎛, 12 nm, YMC Co, Japan) previously equilibrated with 80% acetonitrile. A ODS prep column was used for reversed phase chromatography and was eluted with 80% acetonitrile at a flow rate 5 ㎖/min. An main peak was concentrated in vacuo and HPLC was performed using reversed phase analytical column (J'sphere ODS H-80,150×14 mm, 4 ㎛, 80Å, YMC Co, Japan). A major peak was concentrated under reduced pressure by speed vac (Hanil Modulspin 40, Korea).

3. Instrumental Analysis

The ultraviolet spectrum was monitored by a UV-160A Spectrophotometer (Shimadzu, Japan). HPLC was performed on a Gilson (France) M505 and M321 with UV-VIS detector. FAB (Fast Atom Bombardment) and ESI (Electron Spray Ionization) mass spectrometer were obtained by a Jeol JMS-700 and Micromass Q TOF2 high resolution mass spectrometer, provided by Korea Basic Science Institute (Daejon, Korea).

RESULTS AND DISCUSSION

1. Extraction and Isolation

The organic solvent extracts of native saps from RV were purified by the procedure summarized in Fig. 1.

Reverse phase chromatography was performed by HPLC with a prep-ODS column (250×20 mm) using 80% acetonitrile in 0.1% TFA (Trifluoroacetic acid) as a mobile phase at a flow rate of 5 ㎖/min for 30min. Detection was by UV absorption measurement set at 254 nm. The major peak fraction was eluted at 17.5 min (Fig. 2).

The pooled major peak was concentration *in vacuo* and

Crude Sap of RV (1 g)
↓← dissolve acetone 5 ㎖
Filtrate (glass fiber filter, 1 ㎛)
↓
Evaporation
↓← extraction with n-hexane 5 ㎖
↓← concentrate in vacuo
Crude filtrate
↓← extraction with chloroform 5 ㎖
↓← concentrate in vacuo
Preparative HPLC
↓← 80% acetonitrile in 0.1% TFA
Analytical HPLC
↓← 80% acetonitrile in 0.1% TFA
LC- MS (FAB, ESI-MS)

Fig. 1. Purification scheme of the native saps from *Rhus verniciflua*.

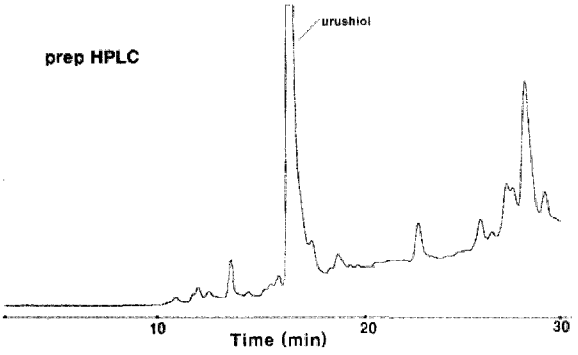

Fig. 2. Chromatograms of preparative HPLC. Column : hydrosphere C_{18}, 20×250 mm, 5 ㎛, YMC Co. Japan, Elution solvent : 80% acetonitrile in 0.1% TFA solution, Flow rate : 5 mℓ/min, Elution time : 30 min, Detection : 254 nm.

the residue was dissolved in a small of in chloroform, further purified HPLC on analytical column using 80% acetonitrile at flow rate of 1 mℓ/min. The elution time was 11.25 min in revered phase HPLC. Finally, an compound was obtained as a dark brown viscous liquid (Fig. 3).

2. Mass spectrum

Electrospray ionization was effective for urushiol, par-ticularly negative ion ESI. The instrument response in negative ESI was substantially greater than that in positive APCI (above 100 fold). Fig. 3 shows the ESI mass spectrum of purified urushiol. The negative ESI and positive FAB analysis of urushiol showed a strong mass peak $M-H^+(M-1)$= 338.9 and $M+H^+(M+1)$= 341.4 Dalton, respectively (Fig. 4, 5).

The UV-VIS spectra of the compound shown two

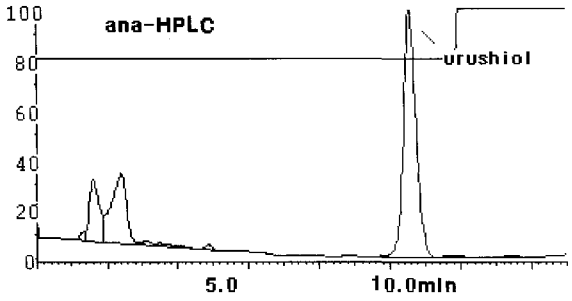

Fig. 3. Chromatograms of analysis HPLC. Column : J'sphere ODS H-80, 150×14 mm, 4 ㎛, 80 Å, YMC Co, Japan, Elution solvent : 80% acetonitrile in 0.1% TFA solution, Flow rate : 1 mℓ/min, Elution time : 15 min, Detection : 254 nm.

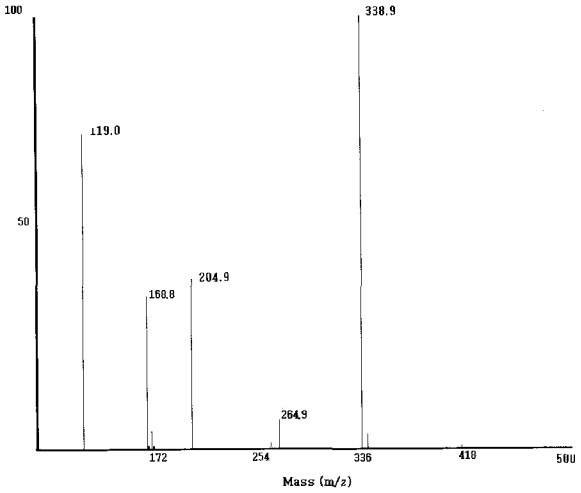

Fig. 4. Spectrum of ESI (−, Negative) MS.

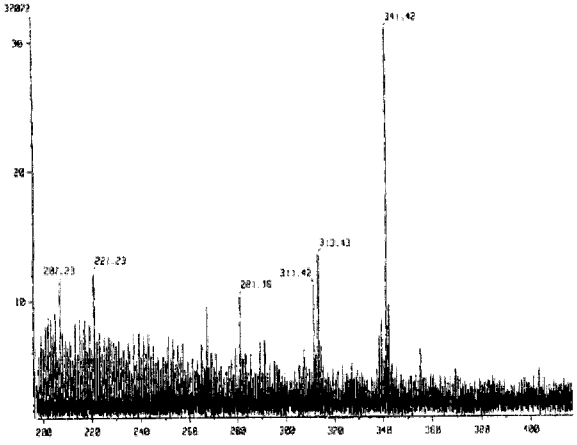

Fig. 5. Spectrum of FAB (+, positive) MS.

absorption in the range 200~800nm(date not shown : 275, 314nm). This compound was identified as m/z 340 (C_{17} tetraene, $C_{23}H_{32}O_2$, R : heptadecatetraenyl), Heptadecatetraenyl catechol and its structure was studied[17].

Compared to the relative MW for urushol analyzed by GC and HPLC by other researchers, lacquer tree(MW 314, 316, 318, 320, 332, 342, 354)[9,13] presented differences.

요 약

한국에서 옻나무(*Rhus verniciflua*)는 전통적으로 약용식물로 오랫동안 사용되어 왔다. 민간요법으로 위장병 치료를 목적으로 옻나무껍질을 닭과 함께 넣어 끓여서 백숙형태로 옻닭으로 식용하였다. 그러나 일부 사람에게서 피부발진이 발생하는 극심한 알러지

반응을 일으키는 나무로 인식되어 왔다. 알러지 발생을 유발시키는 우루시올은 옻나무의 수지상 옻칠 액의 주성분이다. 이 성분의 화학적 구조는 카테친의 기본구조에 알킬기인 불포화지방산($C_{15\sim17}$)이 곁사슬로 붙어 있다. 곁사슬의 불포화지방산 성분은 칠기의 고분자화 및 경화과정에 중요한 역할을 하며, 옻칠공예품의 색상 및 품질에 커다란 영향을 미친다. 한국에서 생산되는 원주산 옻칠액의 우루시올성분은 역상 컬럼을 사용하여 고속 액체 크로마토그라피 (HPLC)법으로 정제하여 ESI(−), FAB(+) 질량분석기(MS)로 분석한 결과 분자량 340인 Hetadecatetetraenyl catechol 이었다.

ACKNOWLEDGEMENT

This study was partially supported by Sangji-Youngseo College.

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(2006년 6월 8일 접수; 2006년 8월 28일 채택)