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SEPARATION OF URUSHIOL BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON AN 8% OCTADECYLSILANE CHEMICALLY BONDED SILICA GEL COLUMN WITH ELECTROCHEMICAL DETECTION

ANALYSIS OF URUSHIOL IN THE SAP OF LAC TREES (*RHUS VERNICIFERA*) AND THAT IN THE JAPANESE LAC-MAKING PROCESS

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SUMMARY

Urushiol, a major component of a sap of lac trees (*Rhus vernicifera*), is resolved into four components due to differences in the degree of unsaturation by high-performance liquid chromatography on Hitachi 3053 gel (an 8% octadecylsilane chemically bonded silica gel), using 1% lithium perchlorate in acetonitrile–water (7:3) as eluent and electrochemical detection. This method was applied to the analysis of the urushiol components of two samples of sap and in the lac-making process.

INTRODUCTION

It was established by Majima[1] and Sunthanker and Dawson[2] that Japanese lac urushiol is made up of four components with a 3-pentadecanylcatechol skelton with different numbers of double bonds (0–3) in the side-chain.

Urushiol is converted into either the dimethyl ether or the diacetate, which have been resolved into components with differences in the olefinic structures of the side-chains by liquid chromatography[2] and high-performance liquid chromatography (HPLC)[3]. Recent progress in developing gels for HPLC is likely to make it possible to resolve urushiol by HPLC without chemical modification. The first resolution of urushiol into its components by HPLC was performed on a μBondapak C_{18} column with UV detection[4].

While studying the resolution of urushiol by HPLC in our laboratory, the resolution was found to be dependent on the level of octadecylsilane (ODS) chemically bonded to silica gels. Urushiol can be resolved into its components on Hitachi 3053 gel (an 8% ODS chemically bonded silica gel) column using a acetonitrile–water (7:3) as the mobile phase, which can be monitered quantitatively using electrochemical detection based on voltammetry in the presence of 1% of lithium perchlorate as the supporting electrolyte in the mobile phase.

In this work, HPLC for urushiol with electrochemical detection was establish-

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ed and applied to the analysis of components of urushiol in the sap of lac trees (*Rhus vernicifera*) and in the lac-making process.

EXPERIMENTAL

Reagents

Tetrahydrofuran, chloroform and acetonitrile were commercial products of extra-pure grade.

Materials

Two samples of sap from lac trees (Chinese and Japanese) were supplied by Saito (Tokyo, Japan).

Crude urushiol, obtained as the acetone-soluble part of the sap, was dissolved in excess of *n*-hexane. The solution was filtered with a Fluoropore filter (pore size 0.1 μm, type FP-100; Sumitomo Electric Ind., Tokyo, Japan) and *n*-hexane was removed from the filtrate to give a dark-brown oily material as the residue. A solution (3 ml) of the oil (1 g) in chloroform (4 ml) was submitted to gel permeation chromatography (GPC) in 20-μl portions and urushiol monomer was separated from the oligomer and obtained by evaporating the fractions to dryness under vacuum.

In the process for making lac from the sap, the sap is stirred in a open vessel at room temperature for 30 min and then at temperatures below 45°C for 6–8 h. Samples were taken from the sap during this process after 0, 30, 70, 90, 120 and 150 min, the oily material was obtained from them as in the separation of crude urushiol monomer, and was subsequently resolved into components by HPLC.

Hydrourushiol as a standard was obtained by hydrogenation of urushiol followed by recrystallization from ethanol according to the method in the literature[5] (m.p. 57.5–58.5°C; lit.[5], 58–59°C).

Mass spectrometry

Mass spectra were obtained with an RMS-4 mass spectrometer (Hitachi, Tokyo, Japan).

GPC

The preparative system consisted of a Type KSD-P-45 pump (Kyowa Seimitsu, Tokyo, Japan), two 60 × 2.2 cm I.D. stainless-steel columns packed with TSK G2000HG (Toyo Soda, Tokyo, Japan), and a Type RI-2 refractive index (RI) detector (JAI, Tokyo, Japan). The mobile phase was chloroform.

The analytical system consisted of a Type 2396-57 pump (Milton Roy, Philadelphia, PA, U.S.A.), three 60 × 0.76 cm I.D. stainless-steel columns (packed with TSK G4000H6, G2000H8 and G10000H8 gel) and an RI detector as used in the preparative system. The mobile phase was tetrahydrofuran.

HPLC

The system consisted of a Type 2396-57 pump (Milton Roy), a 350 kg/cm^2 pressure gauge (Umetani Seiki, Osaka, Japan), a Type DAM bellows-type damper (Umetani Seiki), a Rheodyne Model 7120 20-μl syringe-loading sample injector, a Type VMD-101 electrochemical detector (Yanagimoto, Tokyo, Japan) and a 15 ×

0.4 cm I.D. stainless-steel column packed with Hitachi 3053 gel (Hitachi, Tokyo, Japan). Peak areas were calculated with a Shimazu Chromatopack E1A integrator (Shimazu Seisakusho, Kyoto, Japan). Acetonitrile–water (7:3) containing 1 % of lithium perchlorate (extra-pure grade) mixture was used as the mobile phase.

RESULTS AND DISCUSSION

A current–potential curve is shown in Fig. 1 for hydrourushiol as a standard ($15 \cdot 10^{-4}$ *M*) using 1 % lithium perchlorate in acetonitrile–water (7:3) as eluent and a rotating disk electrode (Nikko Keisoku, Yokohama, Japan).

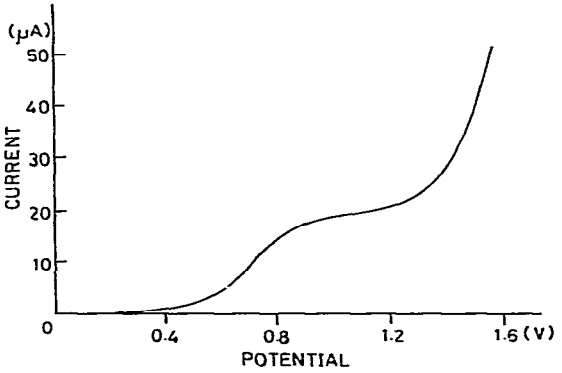

Fig. 1. Current–potential curve for hydrourushiol. Concentration, $1.52 \cdot 10^{-4}$ *M*; solvent, 1 % lithium perchlorate in acetonitrile–water (7:3); apparatus, rotating disk electrode, platinum electrode (500 rpm) and scanning speed 60 sec/V.

Based on this curve, electrochemical detection was performed at 400 or 800 mV for the resolved urushiol components, because the oxidation potential of these components is correlated with the oxidation of their catechol nucleus, and is the same regardless of the double bonds located in the side-chain far from the catechol nucleus.

As the positions and intensities of the UV absorption bands of urushiol components differ owing to the different structures of the side-chains, it is clear that a UV detector at a certain wavelength cannot detect the peaks of urushiol components quantitatively.

In Fig. 2, chromatograms obtained by HPLC with the Hitachi 3053 column are compared with those with electrochemical (at 800 mV) and UV (at 218 nm) detection for a sample of urushiol (0.72 μg, loaded in 2 μl of acetonitrile solution) using 1 % lithium perchlorate in acetonitrile–water (7:3) as eluent, indicating that electrochemical detection is much more sensitive than UV detection (the UV wavelength was selected from λ_{max} = 218 nm in *n*-hexane for hydrourushiol diacetate[3]).

Prior to assignment of the peaks, complete agreement of the peaks detected with the UV and electrochemical detectors was confirmed; peak a, both of the two separated peaks of b, peak c and peak d, detected with the UV detector (at 254 nm), were fractionated and 8 μl of each fraction were re-chromatographed on the same column using the electrochemical detector at 800 mV (see Fig. 4), using 1 % lithium perchlorate in acetonitrile–water (7:3) as eluent. It is clear that peaks a, b, c and d

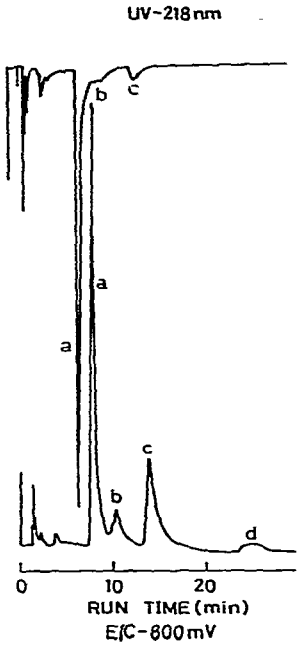

Fig. 2. Chromatogram of urushiol. HPLC conditions: column, Hitachi 3053 (5 μm), 15 × 0.4 cm I.D.; eluent, 1% lithium perchlorate in acetonitrile–water (7:3); flow-rate, 1.0 ml/min; electrochemical (EIC) detector, applied at 800 mV *vs.* Ag/AgCl, sensitivity 10 nA, UV 218 nm, 0.16 a.u.f.s.; loading, 0.72 μg.

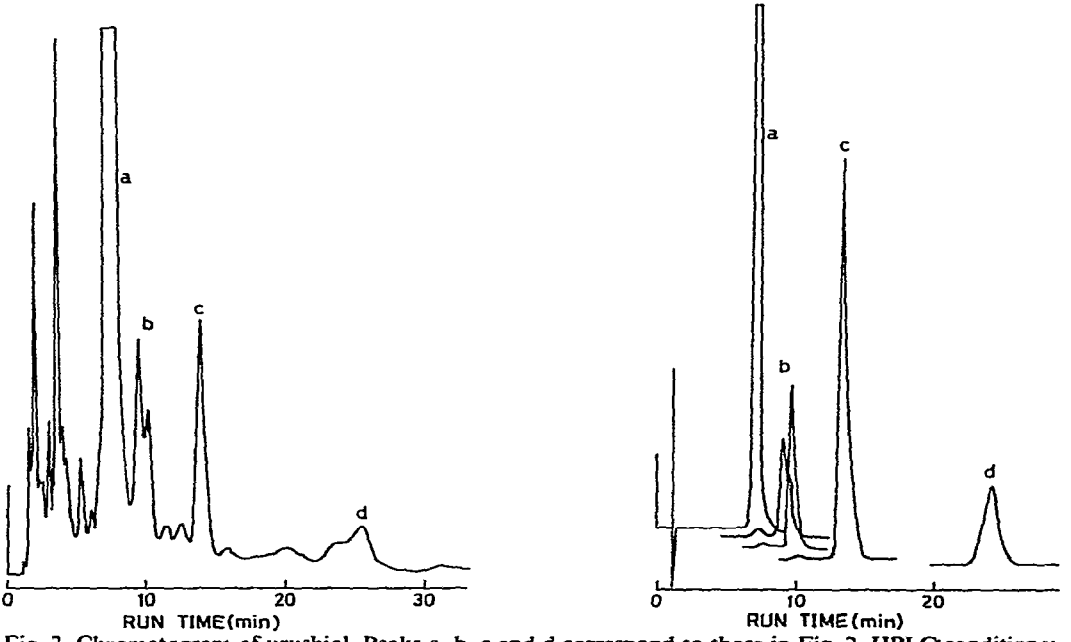

Fig. 3. Chromatogram of urushiol. Peaks a, b, c and d correspond to those in Fig. 2. HPLC conditions: column and flow-rate as in Fig. 2; eluent, acetonitrile–water (7:3); detector, UV 254 nm, 2.56 a.u.f.s.; loading, 3.0 mg.

Fig. 4. Results of re-chromatography with an electrochemical detector of fractionated peaks in Fig. 3. HPLC conditions: column, eluent and flow-rate as in Fig. 2; electrochemical detector, applied at 800 mV *vs.* Ag/AgCl, sensitivity 1 nA; loading, 8 μl of each fraction.

correspond well in the chromatograms obtained with the two types of detector (see Figs. 3 and 4).

In order to identify the peaks, peaks a, b, c and d were fractionated using acetonitrile–water (7:3) as eluent and using the UV detector (at 254 nm) (see Fig. 3), as urushiol as a substrate is oxidized in electrochemical detection. A 20-μl volume of an acetonitrile solution containing 3 mg of urushiol monomer was loaded on the column. By removal of the used eluent from the resolved peaks, fractionated materials were obtained. Mass spectrometry gave the following *m/e* values for the peaks: a, 314; b, 316; c, 318; and d, 320. These correspond to triolefinic, diolefinic, monoolefinic and saturated side-chain urushiol, respectively.

Calibration graphs for determining each of the resolved urushiol components using an electrochemical detector with measurement at 400 and 800 mV, are shown in Figs. 5 and 6, respectively. The data were obtained by injecting 20 μl of an acetonitrile solution containing a given amount of hydrourushiol ($0.125 \cdot 10^{-3}$–$3.00 \cdot 10^{-3}$ mg) on to the analytical HPLC column. The coefficient of variation of the peak area in HPLC was 1.29% at 400 mV and 0.79% at 800 mV; in each instance 20 μl of a solution of hydrourushiol (0.125 g) in 10 ml of acetonitrile was loaded using the present HPLC system.

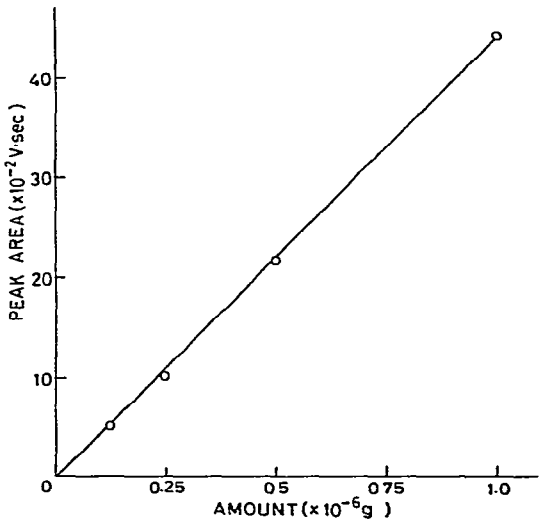

Fig. 5. Calibration graph for hydrourushiol in HPLC; correlation between peak area and amount of hydrourushiol as solute. HPLC conditions: column, eluent and flow-rate as in Fig. 2; electrochemical detector, applied at 400 mV *vs.* Ag/AgCl, sensitivity 10 nA.

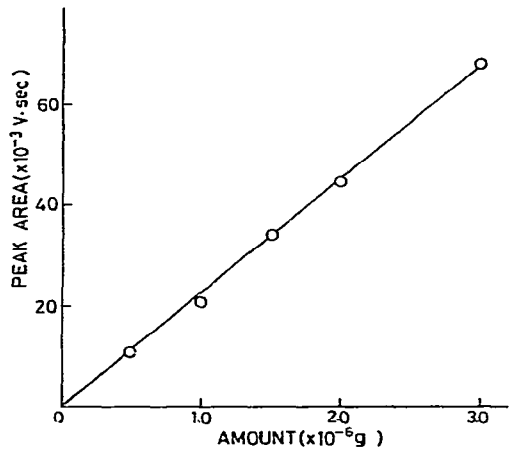

Fig. 6. Calibration graph for hydrourushiol in HPLC; correlation between peak area and amount of hydrourushiol as solute. HPLC conditions: column, eluent and flow-rate as in Fig. 2; electrochemical detector, applied at 800 mV *vs.* Ag/AgCl, sensitivity 10 nA.

Table I shows the results of analyses with electrochemical detection of urushiol obtained from two samples of the sap (Chinese and Japanese) (the constituents of urushiol do not correspond to those in the original sap of lac trees, because 20% of oligomeric urushiol was already involved in the sap used.)

From Fig. 7, it is clear that components of urushiol are oxidized in proportion

TABLE I

QUANTITATIVE ANALYSIS OF URUSHIOL COMPONENTS BY HPLC

HPLC conditions: column, eluent and flow-rate, as in Fig. 2; electrochemical detector, applied at 800 mV, sensitivity, 10 nA; loading, 1.0 μg.

Origin of sap	*Proportion (%)*			
	Triolefinic	*Diolefinic*	*Monoolefinic*	*Saturated*
Japanese	52.3	10.3	30.0	7.7
Chinese	66.0	5.1	21.5	6.7

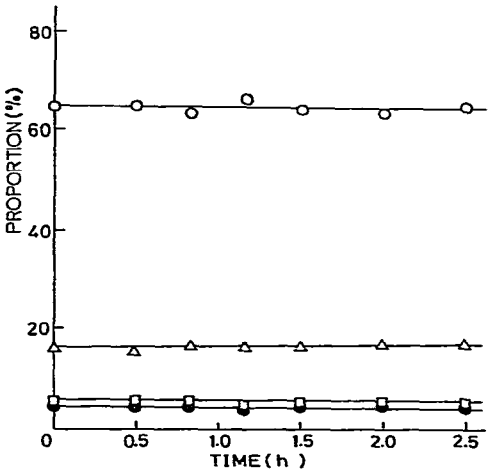

Fig. 7. Variation of urushiol components in the lac-making process, determined by HPLC: (○) triolefinic, (□) diolefinic, (△) monoolefinic and (●) saturated urushiol components, HPLC conditions as in Fig. 5; loading, 0.5 μg.

to the urushiol components in the sap used in the lac-making process. This result confirmed the previous report[6] that in the early stage of the lac-making process polymerization starts from the enzymic oxidation of urushiol into the corresponding quinone, followed by a coupling reaction with urushiol.

REFERENCES

1 R. Majima, *Chem. Ber.*, 42 (1902) 1218.
2 S. V. Sunthanker and C. R. Dawson, *J. Amer. Chem. Soc.*, 76 (1954) 5070.
3 Y. Yamauchi, R. Oshima and J. Kumanotani, *J. Chromatogr.*, 198 (1980) 49.
4 C. R. Ma, M. A. Elsohly and J. K. Baker, *J. Chromatogr.*, 200 (1980) 163.
5 R. Majima, *Toka*, 33 (1912) 655.
6 T. Kato and J. Kumanotani, *J. Polym. Sci., Part A-1*, 7 (1969) 1455.