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SEPARATION OF JAPANESE LAC URUSHIOL DIACETATE ON SILVER NITRATE-COATED SILICA GEL COLUMNS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Urushiol diacetate, derived from urushiol, a major component of the sap of Japanese lac trees (*Rhus vernicifera*), was separated into 14–16 peaks by high-performance liquid chromatography on 10% silver nitrate-coated LiChrosorb Si-60 and LiChroprep Si-60 columns (15 cm × 4 mm I.D. and 60 cm × 2.2 cm I.D., respectively) using benzene–chloroform (4:1) as eluent. Peaks were identified from UV and mass spectroscopic data, indicating that the separation of urushiol diacetate was due to the difference in the degree of unsaturation of the side-chain. Hexanol-1 was used for slurry packing the column.

INTRODUCTION

Urushiol, a major component of the sap of Japanese lac trees (*Rhus vernicifera*) is a mixture of 3-substituted catechol with pentadecyl, 8-pentadecenyl, 8,11-pentadecadienyl and 8,11,13-pentadecatrienyl groups[2,3].

Urushiol is converted into dimethylurushiol, which has been separated into four components with different degrees of unsaturation of the side-chains of urushiol by liquid chromatography on alumina[3].

The positional and geometrical isomers of some alkenes and unsaturated fatty acid esters have been separated by high-performance liquid chromatography (HPLC) on silver nitrate-coated silica gel columns[4–9], by reversed-phase HPLC with silver nitrate-containing eluents[10–15] and by HPLC on cation-exchange resins in the Ag^+ form[16,17].

Here we report the successful separation of urushiol diacetate, derived from Japanese lac urushiol, on silver nitrate-coated silica gel analytical or preparative columns by HPLC.

EXPERIMENTAL

Urushiol diacetate

Urushiol was obtained from an acetone extract of the sap of Japanese lac

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trees by evaporation at 0.08 mmHg with a molecular distillation apparatus and acetylated with acetic anhydride–phosphoric acid (catalyst) at room temperature to give urushiol diacetate. 3-Pentadecylcatechol diacetate (m.p. 50°C) was prepared according to the method in the literature[2].

Adsorbents

LiChrosorb Si-60 (5 μm; Merck, Darmstadt, G.F.R.) and LiChroprep Si-60 (15–25 μm; Merck) were dried at 100°C for 12 h. The former (1.5 g) was mixed with silver nitrate (0.15–0.3 g) in acetonitrile (50 ml), and the acetonitrile was removed at 40°C *in vacuo* to give 5, 10 and 20% silver nitrate-coated silica gels[6]. The adsorbent for preparative chromatography was also made from a mixture of LiChroprep Si-60 (200 g) and silver nitrate (20 g) in 1 l of acetonitrile as in the case for the analyticals separation.

Solvents

Commercially available chromatographic and reagent-grade solvents were used for analytical and preparative chromatography, respectively.

HPLC systems

The analytical chromatographic system consisted of a gel-packed stainless-steel column (10, 15 or 25 cm × 4 mm I.D.) (Umetani Seiki, Osaka, Japan), a plunger pump (Type SF 0396-57; Milton Roy, Philadelphia, PA, U.S.A.), a 250 kg/cm² pressure gauge (Kyowa Seimitsu, Tokyo, Japan), a bellows-type damper (Type DAM; Umetani Seiki) and a 20-μl syringe-loading sample injector (Rheodyne Model 7120). The column effluent was monitored at 280 nm with UV detector (UVIDEC-100; Jasco, Tokyo, Japan).

Preparative chromatography was performed on a stainless-steel column (60 × 2.2 cm I.D., Toyo Soda Mfg. Co., Tokyo, Japan) packed with the silver nitrate-impregnated silica gels using a 250 kg/cm² double-plunger with a linear cums (SPW-S-200; Umetani Seiki), which can generate a maximum flow-rate of 200 ml/min, and a six-way valve (Toyo Soda Mfg.) with a 1-ml loop as a sample injector. A UV detector (UVIDEC-100) with a 3-mm path length flow-through cell was used to monitor the column effluent at 280 nm.

Column packing

In the balanced density packing method, bromine is generated when tetrabromoethane is mixed with silica gel[18,19]. The viscosity packing method, using cyclohexanol or liquid paraffin[20] as a slurry medium, is not applicable to the present work, as the silver nitrate-coated silica gel is not dispersed well in either of them.

An alternative slurry packing method using hexanol-1 has been established for packing the gels, without the need for great skill, to give highly efficient columns. A mixture of 1.5 g of the silver nitrate-coated silica gel and 15 ml of silver nitrate-saturated hexanol-1 was ultrasonized in an ultrasonic cleaner (Model 8845-2; Cole-Parmer) for 10 min, and the slurry obtained was placed in a 15-ml reservoir connected to a column. The column was pressurized with benzene at a flow-rate of 7.8 ml/min at the beginning, then the flow-rate was decreased so as to keep a pressure at 650 kg/cm² by varying the stroke of an 800 kg/cm² high-pressure pump (NSP-800-9G;

Nihon Seimitsu, Tokyo, Japan). It took 7–8 min to replace the hexanol-1 completely. Then the column was conditioned by pumping benzene at a pressure of 450 kg/cm^2 for 3 h.

A preparative column was made by pouring a slurry of the silver nitrate-coated silica gel in benzene into a 500-ml reservoir connected to a column, followed by pumping benzene at a flow-rate of 120 ml/min and a pressure of 80 kg/cm^2, and then conditioned by recycling benzene for 12 h under the same conditions.

Fractionated solutes

Fractionated eluents (2–5 ml in analytical chromatography; 100–300 ml in preparative chromatography) were evaporated to dryness at room temperature *in vacuo*, and the residues were shaken with a mixture of *n*-hexane (2 ml; 10 ml) and 10% sodium chloride solution (0.5 ml; 5 ml); fractionated materials were obtained from the separated oily layer by removal of the *n*-hexane.

Spectral measurements

Mass spectra and UV spectra in *n*-hexane were obtained with an RMS-4 mass spectrometer (Hitachi, Tokyo, Japan) and an SM-401 spectrometer (Union Giken, Osaka, Japan), respectively.

RESULTS AND DISCUSSION

Preliminary experiments

Of the eluents used, benzene–chloroform mixtures, particularly a 4:1 mixture, was found to give superior resolution, reproducibility and column life.

The resolutions of the chromatograms obtained with 4:1 and 7:3 benzene–chloroform as eluents are compared in Fig. 1; the former eluent gives longer elution times but more highly resolved chromatograms.

Fig. 2 shows the effect of column length on the resolution of the chromatograms on the 5% silver nitrate-coated silica gel analytical column, indicating that a 25-cm column produced the most highly resolved chromatogram.

The capacity factor (k') and the theoretical plate number per metre (N/m) determined on the basis of the arrowed peaks in Fig. 2 are plotted against column length in Fig. 3. A maximum theoretical palte number is found with the column of length 15 cm, and the capacity factors increased considerably when a column of length 25 cm was used. Although still subject to further investigation, we believe that this increase in the capacity factor may be due to an inhomogeneous distribution of silver nitrate in the gel. Some silver nitrate was found in the effluent, which suggests that it moves with the mobile phase through the gel to some extent.

The effect of the silver nitrate coating on the resolution of the chromatograms was examined on a 15-cm column, with the results shown in Fig. 4. In addition, the apparent values of N/m and k' are plotted as a function of silver nitrate coating in Fig. 5, suggesting an optimal coating of 10% under these conditions.

It is clear that a 10% coating resolved the chromatogram better than the others, indicating a significant contribution of both silica gel sites (complexes and not complexed with silver nitrate) to a good resolution of the chromatograms.

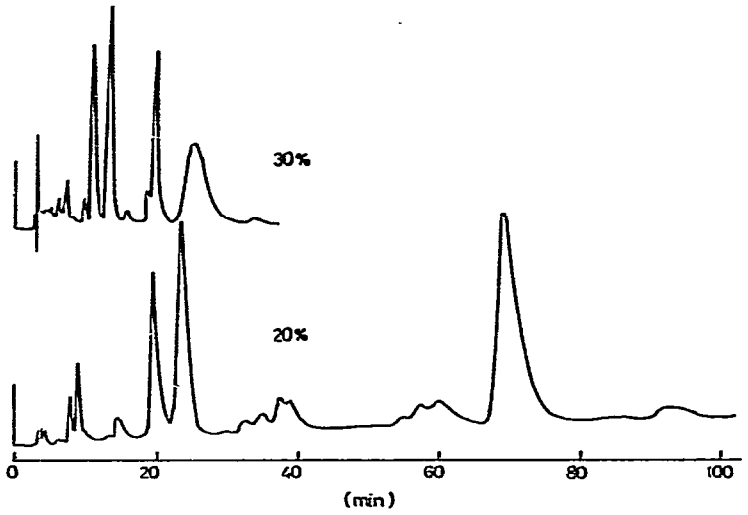

Fig. 1. Comparison of chromatograms obtained with 4:1 and 7:3 benzene–chloroform as eluents. Column, 5% silver nitrate-coated LiChrosorb Si-60, 5 μm, 25 cm × 4 mm I.D.; flow-rate, 1.0 ml/min; detector, UV, 280 nm, 0.16 a.u.f.s.

HPLC of urushiol diacetate

Analytical HPLC. As can be seen in Fig. 4, urushiol diacetate is resolved into 16 peaks with a 0.1-mg loading in 20 μl of 4:1 benzene–chloroform on a 10% silver nitrate-coated silica gel analytical column, solutes of the peaks being examined for the *m/e* value (P^+) for parent ions by mass spectrometry. From the measured P^+ values,

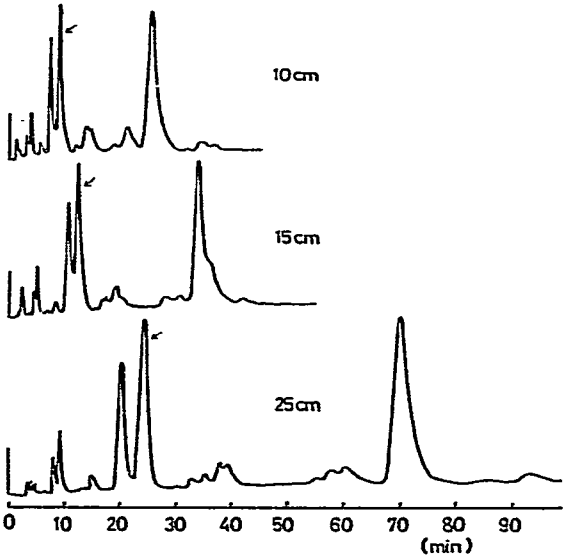

Fig. 2. Influence of column length on chromatogram. Column, 5% silver nitrate-coated LiChrosorb Si-60, 5 μm, 4 mm I.D.; eluent, benzene–chloroform (4:1); flow-rate, 1.0 ml/min; detector, UV, 280 nm, 0.16 a.u.f.s.

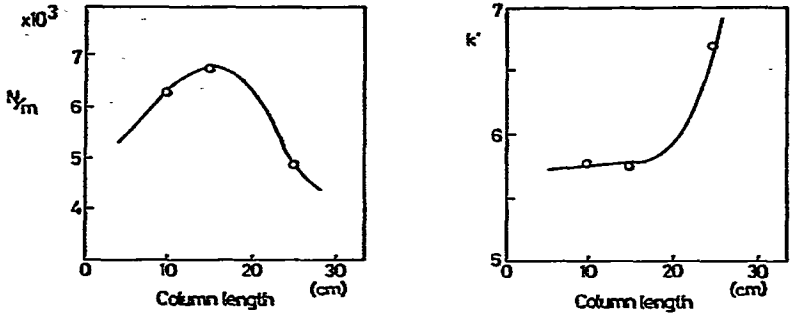

Fig. 3. Influence of column length on theoretical plate number per metre (N/m) and capacity factor (k') on the basis of the arrowed peak in Fig. 2. Conditions as in Fig. 2.

it is obvious that the solutes of peaks a (P^+, 404) and c (P^+, 402) can be ascribed to the saturated and monoolefinic side-chain urushiol diacetate, respectively, and the others (P^+, 398) to the triolefinic side-chain compound, although the unsaturated side-chain urushiol diacetate was accompanied by some very small peaks with P^+

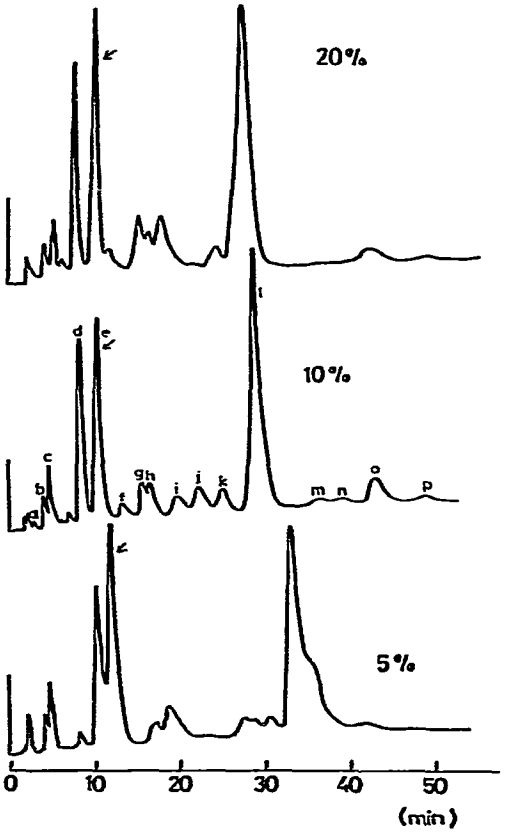

Fig. 4. Influence of silver nitrate coating on chromatogram. Column, silver nitrate-coated LiChrosorb Si-60, 5 μm, 15 cm × 4 mm I.D.; eluent, benzene–chloroform (4:1); flow-rate, 1.0 ml/min; detector, UV, 280 nm, 0.16 a.u.f.s.

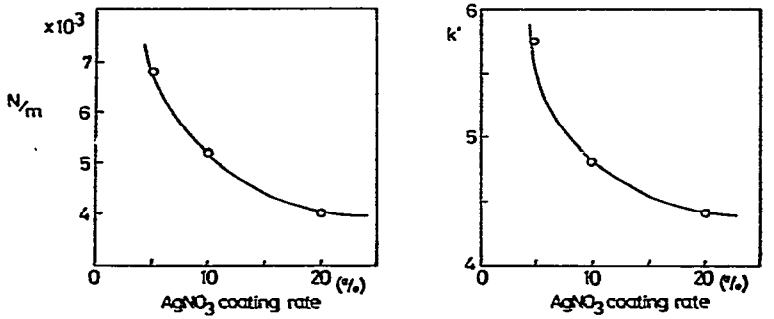

Fig. 5. Influence of silver nitrate coating on the theoretical plate number per metre (N/m) and capacity factor (k') as defined in Fig. 3. Conditions as in Fig. 4.

values larger than those expected for urushiol diacetate in mass spectrometry (see Table I), presumably owing to the occurrence of air oxidation of the unsaturated side-chains of the fractionated urushiol diacetate during the analytical procedure. The diolefinic side-chain urushiol diacetate could not be identified and might be a contaminant in the observed peaks (except peaks a and c).

TABLE I

UV AND MASS SPECTRAL DATA FOR THE SOLUTES OF THE PREPARATIVE HPLC PEAKS OF URUSHIOL DIACETATE

$\lambda_{max.}$ in nm; $\varepsilon_{max.}$ in l mol^{-1} cm^{-1}. Total yield of fractions obtained, 92.2 mg for 140 mg charged.

*Peak No.**	*Weight (mg) (%, w/w)*	*$\lambda_{max.}$ ($\varepsilon_{max.}$) in n-hexane*	*P^{+}** (P^{+} for minor peaks)*
1	5.8 (6.3)	218 ($2.0 \cdot 10^3$)	404
2	4.7 (5.1)	226 ($2.0 \cdot 10^3$)	
3	4.4 (4.8)	234 ($1.4 \cdot 10^4$)	398 (440, 446)
4	17.1 (18.5)	218 ($2.0 \cdot 10^3$)	402 (440)
5	7.4 (8.0)	217 ($1.4 \cdot 10^3$)	398 (412, 427)
		292 ($1.6 \cdot 10^3$)	
		305 ($2.1 \cdot 10^3$)	
		319 ($1.8 \cdot 10^3$)	
6	3.3 (3.6)	233 ($8.4 \cdot 10^3$)	398 (412)
		294 ($5.0 \cdot 10^3$)	
		306 ($6.9 \cdot 10^3$)	
		322 ($5.7 \cdot 10^3$)	
7	10.7 (11.6)	236 ($1.4 \cdot 10^4$)	
8	7.0 (7.6)	235 ($1.8 \cdot 10^4$)	
9	10.0 (10.8)	235 ($8.4 \cdot 10^3$)	
10	1.0 (1.1)	232 ($1.8 \cdot 10^2$)	
11	1.5 (1.6)	230 ($2.0 \cdot 10^3$)	
12	1.0 (1.1)	226 ($8.3 \cdot 10^3$)	
13	10.7 (11.6)	222 ($2.0 \cdot 10^3$)	398 (440)
		271 ($4.3 \cdot 10^3$)	
14	3.7 (4.0)	221 ($5.7 \cdot 10^3$)	
		271 ($4.5 \cdot 10^3$)	

* See Fig. 6.

** Reproduced from the P^{+} values for the corresponding peaks of the middle chromatogram in Fig. 4.

Preparative HPLC. For the quantitative and structural analysis of urushiol diacetate, preparative chromatography was performed on a 5% silver nitrate-coated silica gel column (60 × 2.2 cm I.D.) using 4:1 benzene–chloroform as eluent (see Fig. 6). A chromatogram as highly resolved as that obtained on the analytical column was obtained.

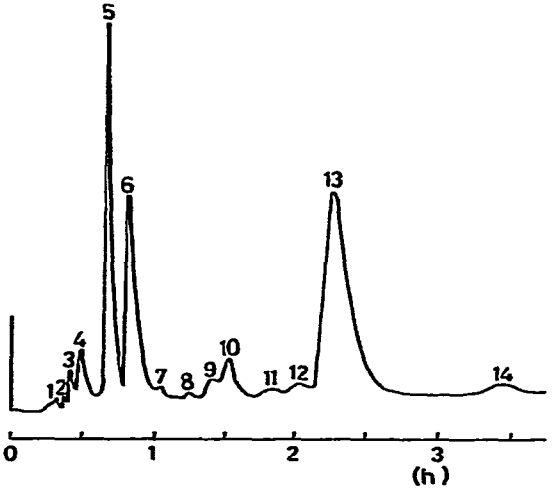

Fig. 6. Chromatogram of urushiol diacetate. Column, 5% silver nitrate-coated LiChroprep Si-60, 15–25 μm, 60 × 2.2 cm I.D.; loading, 140 mg; detector, UV, 280 nm, 0.64 a.u.f.s.

Re-chromatography on the analytical column of each of the fractions obtained revealed that each of peaks 1, 3, 4, 5 and 13 was a single peak corresponding to peaks a, b, c, d and l, respectively, in the middle chromatogram in Fig. 4, the other peaks being contaminated with neighbouring peaks. The values of P^+ for the fractionated solutes are given in Table I.

In order to establish the structural features of the olefinic side-chains of the fractionated urushiol diacetate (peaks 1–14 in Fig. 6), UV spectral data in *n*-hexane were obtained, and the results are given in Table I together with the yields of the solutes of the corresponding peaks obtained in preparative HPLC.

The solute of peak 1 crystallized and melted sharply at 50°C after being recrystallized from ethanol, and showed an infrared spectrum identical with that of an authentic sample prepared according to the literature[2]. The solute of peak 4, which crystallized partly, was ascribed to monoolefinic side-chain urushiol diacetate from the value of P^+ (402) and the UV spectral data [$\lambda_{max.}$(*n*-hexane) = 218 nm with $\varepsilon_{max.} = 2.0 \cdot 10^3$ l mol^{-1} cm^{-1}], which are the same as those for the solute of peak 1 (see Table I), characteristic of the benzene ring of urushiol diacetate.

The triolefinic structure of the side-chain of urushiol diacetate with P^+ 398 is discussed below.

The solutes of the peaks 3, 7, 8 and 9, showing $\lambda_{max.}$(*n*-hexane) 234–236 nm with $\varepsilon_{max.} = 1.4 \cdot 10^4$–$8.4 \cdot 10^3$ l mol^{-1} cm^{-1}, are likely to have a 1,4,6-triene structure, as in the 8,11,13-pentadecatrienyl group of urushiol [*cf.*, octadeca-9,11-dienoic acid or octadeca-10,12-dienoic acid, $\lambda_{max.}$ (light petroleum or ethanol) 231–235 nm with $\varepsilon_{max.} = 2.5 \cdot 10^4$–$9 \cdot 10^4$ l mol^{-1} cm^{-1}][21].

The solutes of the peaks 13 and 14, having $\lambda_{max.}$ (*n*-hexane) 271 nm with $\varepsilon_{max.} = 4.3 \cdot 10^3$ l mol^{-1} cm^{-1} and $\lambda_{max.} = 221$ and 271 nm with $\varepsilon_{max.} = 5.7 \cdot 10^3$ and $4.5 \cdot 10^3$ l mol^{-1} cm^{-1}, respectively, seem to have a conjugated triolefinic structure in the side-chain of urushiol diacetate, because octadeca-9,11,13-triolefinic acid has $\lambda_{max.}$ (light petroleum, cyclohexane or ethanol) 271–271.5 nm with $\varepsilon_{max.} = 3.6 \cdot 10^4$–$5.3 \cdot 10^4$ l mol^{-1} cm^{-1} (ref. 21), and an urushiol dimer with a conjugated triene structure in the side-chain, $\lambda_{max.}$ 272.5 nm (ethanol) with $\varepsilon_{max.} = 3.6 \cdot 10^4$ l mol^{-1} cm^{-1} (ref. 22).

UV spectral data for the solute of peak 5 or 6 is similar to those of octadeca-9, 11,13,15-tetraenoic acid, $\lambda_{max.}$ (*n*-heptane, cyclohexane or ethanol) 301–306.5 nm with $\varepsilon_{max.} = 7.7 \cdot 10^4$–$7.8 \cdot 10^4$ l mol^{-1} cm^{-1} (ref. 21), indicating a possibility that the 1,4,6-triene in the side-chain of urushiol or its diacetate shifted and conjugated with the benzene ring of urushiol diacetate, either thermally in the evaporation process or by acid catalysis in the acid-catalysed acetylation of urushiol.

CONCLUSION

From the results, it is concluded that urushiol diacetate is highly resolved according to the difference not only in the degree of unsaturation, but also in the geometrical and positional structures of the olefinic side-chain of urushiol diacetate, using the HPLC system described here.

It needs further study to establish how the various urushiol diacetate homologues may be present as components of urushiol in the present sample of urushiol acetate.

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