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# CONFIGURATION OF THE OLEFINIC BONDS IN THE HETEROOLEFINIC SIDE-CHAINS OF JAPANESE LACQUER URUSHIOL

## SEPARATION AND IDENTIFICATION OF COMPONENTS OF DIMETHYLURUSHIOL BY MEANS OF REDUCTIVE OZONOLYSIS AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The configuration of the olefinic bonds in the heteroolefinic side-chains of Japanese lacquer urushiol components has been determined. Dimethylurushiol is resolved into ten components by combined high-performance liquid chromatography (HPLC) with Unisil $QC_{18}$ and Hitachi 3043Ag gel columns due to the difference in the degree of unsaturation and in the *cis* and *trans* configuration. Each resolved di- or triolefinic dimethylurushiol component was partially reduced with hydrazine, and the monoolefinic side-chain dimethylurushiol formed was separated by HPLC on a Unisil $QC_{18}$ gel column and submitted to reductive ozonolysis, followed by derivatization of the resulting aldehydes into the 2,4-dinitrophenylhydrazones. The hydrazones were identified by HPLC using a Hewlett-Packard 1084B chromatograph with a Develosil ODS-3 gel column.

## INTRODUCTION

Since the appearance of the first paper on the constituents of the sap of lacquer trees (*Rhus vernicifera*)[1], much work has been carried out on the characterization of the urushiol not only in the sap of Japanese lacquer trees[2,3], but also in oak nuts[4] and ivy leaves[5,6]. Urushiol is now the subject of research related to its total synthesis[7–10], allergenic activity[11–13] and behaviour in the polymerization of Japanese lacquer[14–16].

After clarifying contradictory procedures and interpretations published in the past six decades, Majima[2], Sunthanker and Dawson[3] and Hashimoto and Minami[17,18] established that urushiol is composed of five components of 3-substituted catechol derivatives with pentadecyl, 8-pentadecenyl, 8,11-pentadecadienyl, 8,11,13-pentadecatrienyl and 8,11,14-pentadecatrienyl groups.

Of the possible configuration of the olefinic bonds in the side-chains of urushiol, only the *cis* configuration has been established for 3-(8′-pentadecenyl)catechol[3]. Difficulties occur in the separation of the heteroolefinic urushiol components.

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Progress has been made recently in the development of gels for use in high-performance liquid chromatography (HPLC). Some of the heteroolefinic urushiol components have since been resolved without chemical modification by HPLC on a μBondapak $C_{18}$ gel column[19] and on a Hitachi 3053 gel column[20]. Despite the resolution of urushiol diacetate into 16 components by HPLC on a silver nitrate-coated LiChrosorb Si-60 gel column[21], the acetyl group of the diacetate hindered the observation of the IR bands characteristic of *cis*- and *trans*-olefinic arrangements of the side-chain.

The Unisil $QC_{18}$ gel column is specific for the resolution of long-chain fatty acid esters owing to the differences in chain length, degree of unsaturation and position of the olefinic bond[22–24], and silver-containing silica gel is available for the separation of *cis*- and *trans*-isomers of olefins[25] by HPLC.

In this work, the configuration of the olefinic bonds in the side-chains of urushiol components has been determined by applying HPLC to the separation and identification of dimethylurushiol and related compounds. Chromatographically pure dimethylurushiol which had been obtained by gel permeation chromatography (GPC) was separated into heteroolefinic components of dimethylurushiol by HPLC on a Unisil $QC_{18}$ gel column. The separated di- and triolefinic dimethylurushiols were reduced partially with hydrazine, and the monoolefinic *cis*- and *trans*-monoolefinic dimethylurushiol components formed were separated by consecutive HPLC on Unisil $QC_{18}$ and Hitachi 3043Ag gel columns, subsequently being examined for the configuration and position of the olefinic bond by means of IR spectroscopy and reductive ozonolysis. HPLC has been applied successfully to the identification of 2,4-dinitrophenylhydrazones of the aldehydes formed in the reductive ozonolysis.

## EXPERIMENTAL

### *Reagents*

All of the solvents and reagents were of extra-pure grade, except methylene chloride used as the solvent for ozonolysis (spectrally pure grade), and were used without further purification.

### *Dimethylurushiol*

Crude urushiol, obtained as the acetone-soluble part of the sap of lacquer trees (*Rhus vernicifera*), was treated with potassium carbonate[26] and chromatographically pure dimethylurushiol was separated from the oligomeric product by GPC (two TSK G2000HG packed columns, 60 × 2.2 cm I.D.; Toyo Soda, Tokyo, Japan; eluent, chloroform; flow-rate, 3.5 ml min; loading of 0.7 g in 3 ml of chloroform).

### *Absorbents and column packing*

Unisil $QC_{18}$ gel (5 μm, 20% ODS loading; Gasukuro Kogyo, Tokyo, Japan), Hitachi 3043Ag gel (10 μm, 10% silver loading; Hitachi, Tokyo, Japan) and Develosil ODS-3 gel (3 μm, 20% loading; Nomura Chemical Co., Seto City, Aichi Prefecture, Japan) were used as received. They were packed in our laboratory into stainless-steel columns (25 cm × 8 or 7.6 mm I.D., 25 cm × 4 mm I.D. and 15 cm × 4.6 mm I.D.; Nihon Seimitsu, Tokyo, Japan) using hexanol-1–methylene chloride (1:1) as a slurry medium[27].

*HPLC system*

The preparative or analytical HPLC system for dimethylurushiol consisted of a Type SF-0369-57 pump (Milton-Roy, Philadelphia, PA, U.S.A.), gel-packed stainless-steel columns (see above), a 350 kg/cm$^2$ pressure gauge (Umetani Seiki), a Rheodyne Model 7125 20- or 500-$\mu$l syringe-loading sample injector, a Type UV log 5-III detector (Oyobunko, Tokyo, Japan) at 274 or 362 nm ($\lambda_{max}$ of 2,4-dinitrophenylhydrazones of aliphatic aldehydes in acetonitrile) and a Type RI-2 refractive index (RI) detector (JAI, Tokyo, Japan).

For HPLC analysis of 2,4-dinitrophenylhydrazones of the aldehydes prepared from dimethylurushiol, a Model 1084B liquid chromatograph (Hewlett-Packard, Washington, DC, U.S.A.) equipped with a Develosil-3 gel column (15 cm × 4.6 mm I.D.) and a Hewlett-Packard variable-wavelength UV detector (operated at 362 nm) was used under the following conditions: volume injected, 5 $\mu$l; column pressure, 146 kg/cm$^2$; column temperature, 40°C; flow-rate, 1.20 ml/min. All samples were injected on to the column automatically with a gradient eluent from acetonitrile–water (55:45) to 100% acetonitrile for 12 min, and then with 100% acetonitrile for 2 min and acetonitrile–water (55:45) for 4 min to prepare the column for the next run.

*Spectral measurements*

A Hitachi RMU-6E mass spectrometer, a High Sens SM 401 UV spectrometer (Union Giken, Osaka, Japan) and an IRA-1 grating IR spectrometer (JASCO, Tokyo, Japan) were used.

*Separation of dimethylurushiol components by HPLC*

As can be seen in Fig. 1, dimethylurushiol is resolved into peaks 1–5 with a 100–150 mg loading in 0.5 ml of acetonitrile by reversed-phase HPLC on the Unisil QC$_{18}$ gel column (25 cm × 8 mm I.D.), using acetonitrile as an eluent. The compounds in the peaks were obtained in the following yields: peak 1, 56.5 mg; 2, 9.5 mg; 3, 16 mg; 4, 1.3 mg; and 5, 4.2 mg.

By HPLC on the Hitachi 3043Ag gel column (25 cm × 7.6 mm I.D.), peak 1 in Fig. 1 was resolved into three peaks, 1-1 (1.1 mg), 1-2 (33 mg) and 1-3 (4.4 mg) (see Fig. 2a), using *n*-hexane–ethyl acetate (92.5:7.5) as the eluent at a flow-rate of 2.0 ml/min, peak 2 was resolved into peaks 2-1 (4.5 mg) and 2-2 (3.0 mg) (see Fig. 2b) and peak 3 was separated into peaks 3-1 (9.1 mg) and 3-2 (1.0 mg) (see Fig. 2c), using *n*-hexane–ethyl acetate (95:5) as the eluent.

The *m/e* values (M$^+$) for the parent ions obtained by mass spectrometry and IR and UV spectral data were obtained for the compounds in the HPLC peaks resulting from 100–150-mg loadings; the results are summarized in Table I.

*Partial reduction with hydrazine of polyolefinic dimethylurushiol components and separation of the resulting monoolefinic dimethylurushiol components*

No migration of olefinic bonds in reductive ozonolysis was established in a study with unsaturated fatty acids[28].

A mixture of the finally obtained tri- or diolefinic dimethylurushiol components (1–5 mg) and 5 ml of a 10% solution of hydrazine in ethanol, previously mixed with 0.01% of propyl gallate as an antioxidant, was stirred magnetically at 40°C until a maximum concentration of the monoolefinic dimethylurushiol component was

given by reversed-phase HPLC on the analytical Unisil $QC_{18}$ gel column (25 cm × 4 mm I.D.), using acetonitrile as the eluent and an RI detector, and the peak of the monoolefinic dimethylurushiol component formed was fractionated on the Unisil $QC_{18}$ gel column (25 cm × 8 mm I.D.) under the same conditions as for the separation of the heteroolefinic dimethylurushiol components. The compounds in the peak that had been obtained by removal of the volatile materials from the fraction was examined for IR bands in the range 900–1000 $cm^{-1}$ and a UV spectrum was also obtained.

*Reductive ozonolysis of monoolefinic side-chain dimethylurushiol and identification of 2,4-dinitrophenylhydrazones of the resulting aldehydes*

Reductive ozonolysis was performed according to the Beroza and Bierl method[29]. Ozone was passed into a solution of the fractionated monoolefinic dimethylurushiol component in 1.0 ml of methylene chloride, cooled at −70°C until excess of ozone gas passing through in 5% potassium iodide–starch solution could be detected. Nitrogen was bubbled through the ozonide solution to replace oxygen and the solution was then allowed to react with triphenylphosphine (1.0 mg). The resulting solution was mixed with 1.0 ml of 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine, previously filtered with a Sartorius membrane filter (type SM 113), and stirred magnetically for 2 h at room temperature (the above filtration is necessary to remove compounds that interfere in the HPLC of the 2,4-dinitrophenylhydrazones derived from the aldehydes). The oily layer was separated from the aqueous layer, the former was evaporated to dryness at 35°C under vacuum, and the residue obtained was shaken with a mixture of 2 *N* hydrochloric acid (1.0 ml) and *n*-hexane (2.0 ml). The oily layer was separated from the aqueous layer and the former was evaporated to dryness to give the 2,4-dinitrophenylhydrazones as the residue.

*Identification of 2,4-dinitrophenylhydrazones by HPLC*

Standard 2,4-dinitrophenylhydrazones of normal $C_1$–$C_{11}$ aliphatic aldehydes were prepared according to the method in the literature[30]. The observed melting points of these 2,4-dinitrophenylhydrazones (with literature[30,31] values in parentheses) are as follows: from formaldehyde, 168–169°C (166°C); acetaldehyde, 162–165°C (164–165°C); *n*-propionaldehyde, 148–149°C (142–148°C); *n*-butyraldehyde, 122°C (123°C); *n*-valeraldehyde, 107–110°C (104°C); *n*-caproldehyde, 107–108°C (104°C); *n*-heptaldehyde, 106–107°C (108°C); *n*-octylaldehyde, 106–107°C (106–110°C); *n*-nonylaldehyde, 108°C (106°C); *n*-capraldehyde, 103–104°C (104°C); and *n*-undecylaldehyde, 103–104°C.

Fig. 4 shows a chromatogram of a mixture of the standard 2,4-dinitrophenylhydrazones: the peaks appeared separately in order of increasing carbon number. Their retention times (minutes) and peak areas (as a percentage of the whole peak area) are indicated on each peak. HPLC was repeated three times for each standard mixture every 2 h. A high reproducibility was ascertained for the retention times, with a standard deviation of 0.01 min, and for the peak areas, with a maximum standard deviation of 1%, which is adequate for identification and quantitative analysis of the compounds in the peaks.

The 2,4-dinitrophenylhydrazones were dissolved in 1 ml of acetonitrile, and 5 µl of the solution were subjected to HPLC. In the chromatograms obtained, two

peaks with the same area should appear, corresponding to the equimolar formation of an aliphatic and a 2,3-dimethoxyphenyl-containing aldehyde from a monoolefinic side-chain dimethylurushiol. However, the experimental peak areas were not always identical, presumably owing to loss of the aldehyde or its incomplete derivatization into the corresponding 2,4-dinitrophenylhydrazone in the procedure. Further, the former peak appeared earlier than the latter, reflecting the greater hydrophilicity of the former in the gradient elution system used. These correlations are very informative for deciding whether a peak in HPLC is due to a major component or to a contaminant. In the chromatograms of the 2,4-dinitrophenylhydrazones, the retention time (minutes) and peak area (per cent) are indicated for each peak, and in the discussion, the retention time is first noted for the aliphatic aldehyde and then for its 2,3-dimethoxyphenyl-containing aldehyde in parentheses. The peaks with retention times of 0.75–0.81 and 1.42–4.45 min, which appeared in most of the chromatograms, were discarded, except for peaks 1-2-2-1 and 1-1-1, as their counter peaks did not appear. Moreover, peaks with peak areas of less than 1% were also neglected, although some of them might be meaningful.

## RESULTS AND DISCUSSION

Chromatograms of the various compounds are shown in Figs. 1–5.

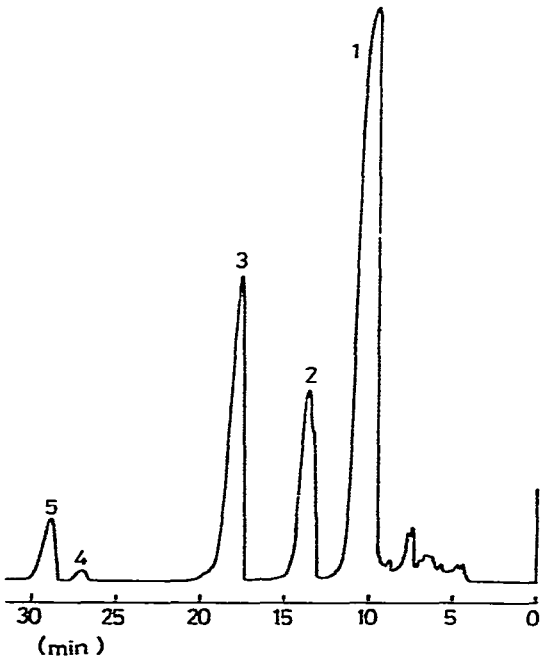


Fig. 1. Chromatograms of dimethylurushiol with acetonitrile as eluent. Column, Unisil $QC_{18}$, 5 μm, 25 cm × 8 mm I.D.); flow-rate, 2.0 ml/min; RI detector.

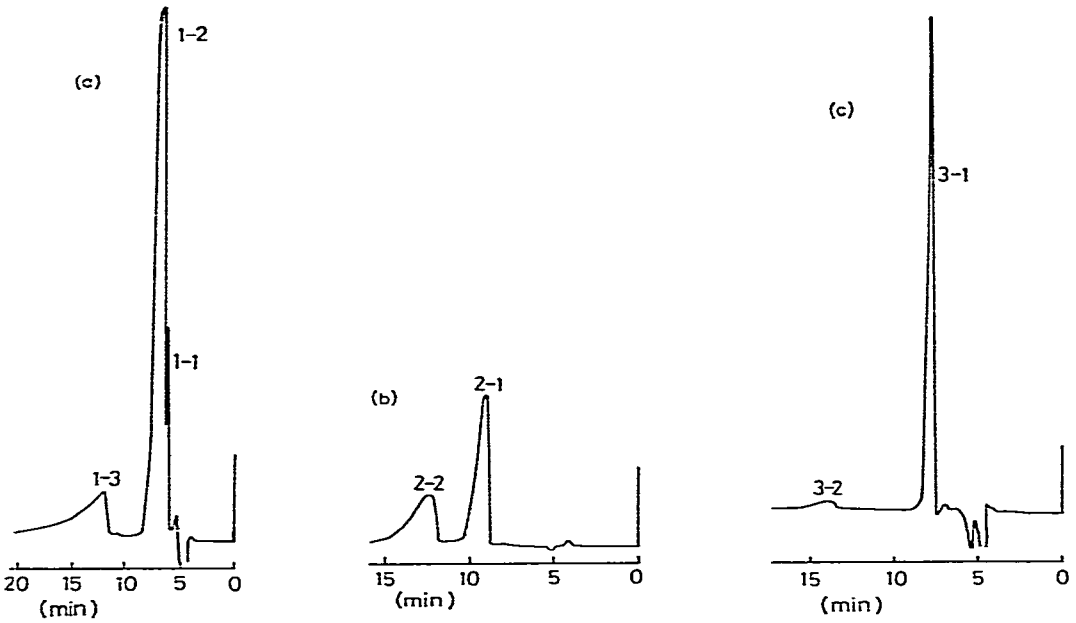


Fig. 2. Chromatograms of heteroolefinic dimethylurushiol (peak 1-3 in Fig. 1) with *n*-hexane–ethyl acetate (55:45) as eluent. Column, Hitachi 3043Ag, 10 μm, 25 cm × 7.6 mm I.D.; flow-rate, 2.0 ml/min; RI detector. (a) Peak 1; (b) peak 2; (c) peak 3.

*Resolution of dimethylurushiol by HPLC*

From the mass and IR spectral data in Table I, it is clear that, as can be seen in Fig. 1, dimethylurushiol is resolved into peaks 1–5 by HPLC on the Unisil $QC_{18}$ gel column, using acetonitrile as an eluent, appearing in the order tri-, di- and mono-olefinic $C_{15}$ side-chain dimethylurushiol components, $C_{17}$ side-chain dimethylurushiol and 3-(pentadecyl)veratrole; also, each of the di- and triolefinic peaks is further resolved into two or three peaks owing to the difference in concentration of the olefin or chain length of dimethylurushiol on the Hitachi 3043Ag gel column, using *n*-hexane–ethyl acetate (95:5) as the eluent. It should be noted that peak 3 was resolved into 3-1 (pentadecenylveratrole) and 3-2 (heptadecadienylveratrole) (see Fig. 2c).

*Constituents of Japanese lacquer urushiol*

*Carbon skeleton of Japanese lacquer urushiol*

Each peak was hydrogenated made over palladium–charcoal in ethanol and a mixed melting point determination was made using an authentic sample. In this way, peak 1-1 in Fig. 2a and peak 3-1 in Fig. 2c were identified as 3-pentadecylveratrole (m.p. 35–36°C; lit.[3], 35–36°C), peak 3-2 in Fig. 2c and peak 4 in Fig. 1 as 3-heptadecylveratrole (m.p. 41–42°C; lit.32, 43–44°C) and peak 5 also as 3-pentadecylveratrole (m.p. 35–36°C).

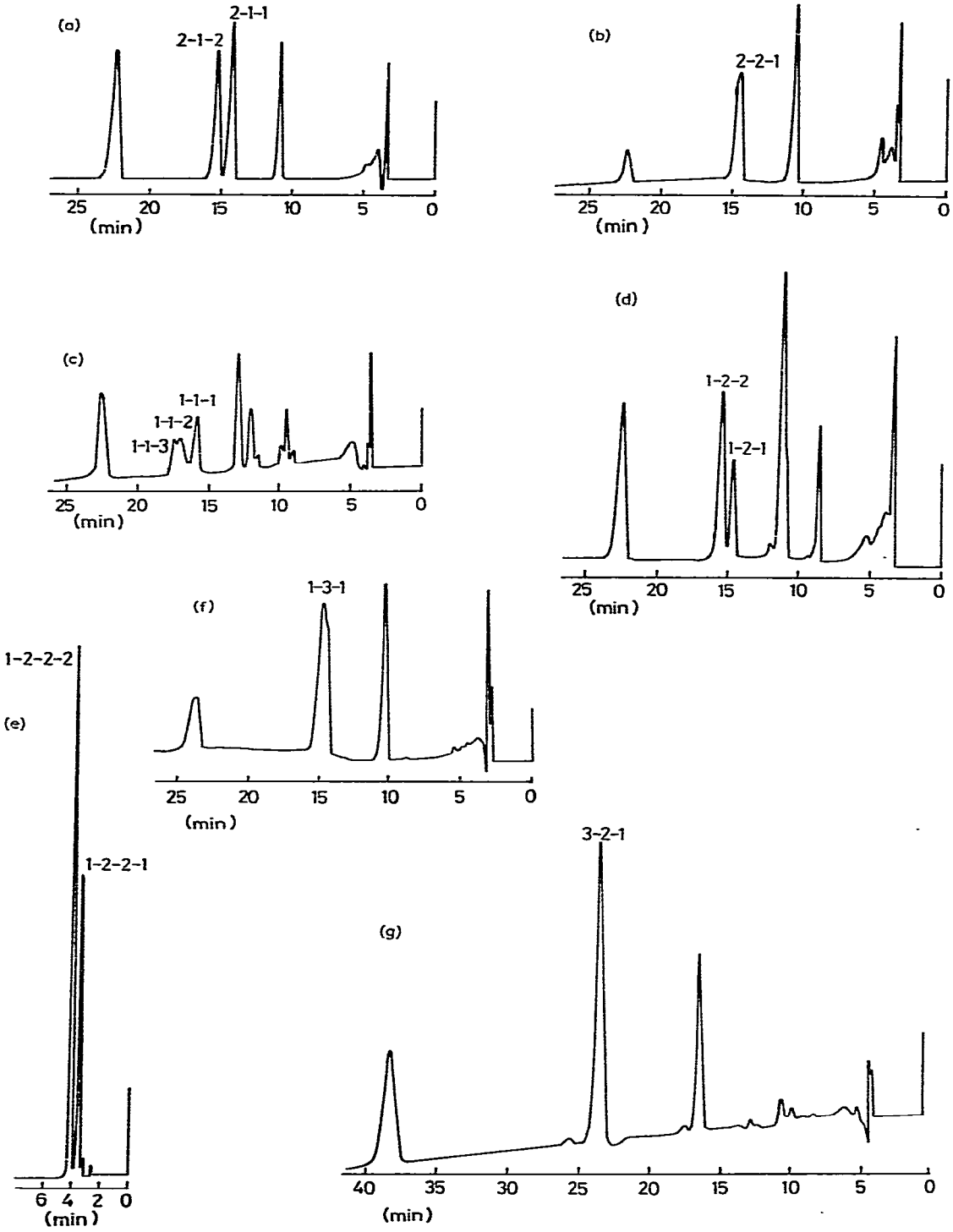


Fig. 3. Chromatograms of partially reduced di- or triolefinic dimethylurushiol of the following peaks: (a) 2-1; (b) 2-2; (c) 1-1; (d) 1-2; (e) 1-2-2; (f) 1-3; (g) 3-2. Conditions as in Fig. 1.

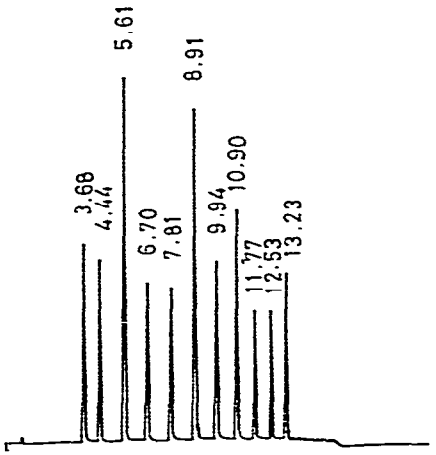


Fig. 4. Chromatograms of a mixture of standard 2,4-dinitrophenylhydrazones of $C_1$–$C_{11}$ *n*-aliphatic aldehydes with a Hewlett-Packard 1084B chromatograph with gradient elution from acetonitrile–water (55:45) to acetonitrile in 12 min. Column, Nomura Develosil ODS, 3 μm, 15 cm × 4.6 mm I.D.; flow-rate, 1.2 ml min; UV detector (362 nm). The numbers on the peaks indicate retention times in minutes.

*Identification of configuration of the olefinic bond in heteroolefinic side-chain dimethylurushiol*

*$C_{15}$ side-chain dimethylurushiol.* (a) Monoolefinic components. Peak 3-1 in Fig. 2c showed no recognizable IR band in the range 900–1000 $cm^{-1}$, and therefore it has a *cis*-olefinic bond in the side-chain. Fig. 5a, shows the chromatogram for the 2,4-dinitrophenylhydrazones of the aldehydes as the reductive ozonolysis products of peak 3-1. The major peak with a retention time of 9.94 min (11.17 min) agreed with that (9.94 min) of the standard 2,4-dinitrophenylhydrazone of heptaldehyde (see Fig. 4). Further, a minor peak with a retention time of 7.83 min (12.45 min) is in good agreement with 7.81 min for the standard valeraldehyde derivative. From these results, it is concluded that peak 3-1 is composed mainly of 3-[8′(*Z*)-pentadecenyl]veratrole and a trace of 3-[10′(*Z*)-pentadecenyl]veratrole.

(b) Diolefinic compounds. Peak 2-1 in Fig. 2b showed an IR band at 965 $cm^{-1}$, characteristic of an isolated *trans*-olefinic bond. Each of the monoolefinic peaks 2-1-1 and 2-1-2, prepared by reduction with hydrazine of peak 2-1, was fractionated by HPLC (see Fig. 3a); in the range 900–1000 $cm^{-1}$ the former showed no recognizable IR band, whereas the latter exhibited an IR band at 965 $cm^{-1}$ due to a *trans*-olefinic bond.

As can be seen in Fig. 5b, 2,4-dinitrophenylhydrazones of the aldehydes of peak 2-1-1 showed a peak with a retention time of 9.96 min (11.91 min), which can be assigned to the 2,4-dinitrophenylhydrazone of heptaldehyde in the same manner as already shown for peak 3-1. Similarly, the peak in Fig. 5c with a retention time of 6.73 min (13.03 min) can be assigned to the 2,4-dinitrophenylhydrazone of butyraldehyde, which had a retention time of 6.70 min for the standard. Therefore, it is concluded that peak 2-1 is composed of 3-[8′(*Z*),11′(*E*)-pentadecadienyl]veratrole.

Peak 2-2 in Fig. 2b showed no noticeable IR band in the range 900–1000 $cm^{-1}$, indicating that the two olefinic bonds in the side-chain have a *cis* configuration. The

peak was partially reduced with hydrazine for 3 h, and the monoolefinic peak 2-2-1 formed was fractionated (see Fig. 3b), ozonized and reduced, then derivatized into the corresponding 2,4-dinitrophenylhydrazones.

In Fig. 5d, the major peaks with retention times of 6.70 min (13.04 min) and 9.96 min (11.20 min) can be identified as the 2,4-dinitrophenylhydrazones of butyraldehyde and heptaldehyde, respectively, by comparing their retention times with those of the corresponding standards in Fig. 4. Similarly, a minor peak with a retention time of 7.82 min (12.48 min) can be ascribed to the 2,4-dinitrophenylhydrazone of valeraldehyde. Thus peak 2-2 was identified as 3-[8′(*Z*),11′(*Z*)-pentadecadienyl]-veratrole, probably contaminated with 3-[8′(*Z*),10′(*Z*)-pentadecadienyl]veratrole.

(c) Triolefinic components. Peak 1-1 in Fig. 2a showed only an IR band at 990 $cm^{-1}$, indicating the presence of a conjugated *trans–trans*-olefinic bond, isolated from the above conjugated diene, judging from the UV band at 232 nm for peak 1-1 (see Table I).

Peak 1-1 was partially reduced with hydrazine for 5 h, and a mixture of the monoolefinic component peaks 1-1-1, 1-1-2 and 1-1-3 formed (see Fig. 3c) was fractionated, ozonized and reduced. The resulted aldehydes were converted into the corresponding 2,4-dinitrophenylhydrazones, which showed three peaks with retention times of 4.45 min (14.12 min), 6.71 min (13.03 min) and 9.96 min (11.21 min), as can be seen in Fig. 5e. These peaks can be ascribed to the 2,4-dinitrophenylhydrazones of

TABLE I

MASS, UV AND IR SPECTRAL DATA FOR THE DIMETHYLURUSHIOL COMPONENTS RESOLVED BY HPLC

| *Peak No.** | *Parent peak* | $\lambda_{max}$ *(nm) in n-hexane* | *IR band* $(cm^{-1})$ | *No. of olefinic bonds* | *No. of side-chain carbon atoms* |
|---|---|---|---|---|---|
| 1-1 | 342(356**) | 232 ($1.0 \cdot 10^4$)<br>272 ($6.7 \cdot 10^3$) | 990 | 3 | 15 |
| 1-2 | 342(356**) | 231 ($2.5 \cdot 10^4$) | 990, 950 and 930 | 3 | 15 |
| 1-3 | 342 | 228 ($1.5 \cdot 10^3$)<br>274 ($5.3 \cdot 10^2$) | 920 | 3 | 15 |
| 2-1 | 344 | 228 ($8.1 \cdot 10^3$)<br>274 ($3.0 \cdot 10^3$) | 965 | 2 | 15 |
| 2-2 | 344 | 227 ($5.2 \cdot 10^3$)<br>274 ($2.3 \cdot 10^3$) | | 2 | 15 |
| 3-1 | 346 | 224 ($2.5 \cdot 10^3$)<br>274 ($7.4 \cdot 10^2$) | | 1 | 15 |
| 3-2 | 374 | 229 ($3.2 \cdot 10^3$)<br>274 ($1.2 \cdot 10^3$) | | 2 | 17 |
| 4 | 372 | 229 ($1.0 \cdot 10^3$)<br>274 ($6.0 \cdot 10^2$) | | 1 | 17 |
| 5 | 348 | 224 ($3.3 \cdot 10^3$)<br>274 ($9.6 \cdot 10^2$) | | 0 | 15 |

* Peak numbers in chromatograms in Figs. 1–5.

** A very small peak which might occur as a result of oxidation of the peak in the procedure.

acetaldehyde, butyraldehyde and heptaldehyde, respectively, as the reductive ozonolysis products of the side-chain of peak 1-1. Therefore, peak 1-1 can be identified as 3-[8′(*Z*),11′(*E*),13′(*E*)-pentadecatrienyl]veratrole.

Peak 1-2 in Fig. 2 showed IR bands at 930, 950 and 990 $cm^{-1}$, indicating the presence of a conjugated *cis–trans*-diene as well as a *cis*-olefinic bond in the side-chain. It was reduced partially with hydrazine for 3 h, and the monoolefinic dimethylurushiol components formed, peaks 1-2-1 and 1-2-2, were separated by HPLC (see Fig. 3d).

Peak 1-2-1 showed no recognizable IR band in the range 900–1000 $cm^{-1}$, indicating that it has a *cis*-olefinic bond in the side chain. On the other hand, peak 1-2-2, with a moderate IR band of a *trans*-olefinic bond at 960 $cm^{-1}$, was found to be resolved into peaks 1-2-2-1 and 1-2-2-2 by HPLC on the Hitachi 3043Ag gel column

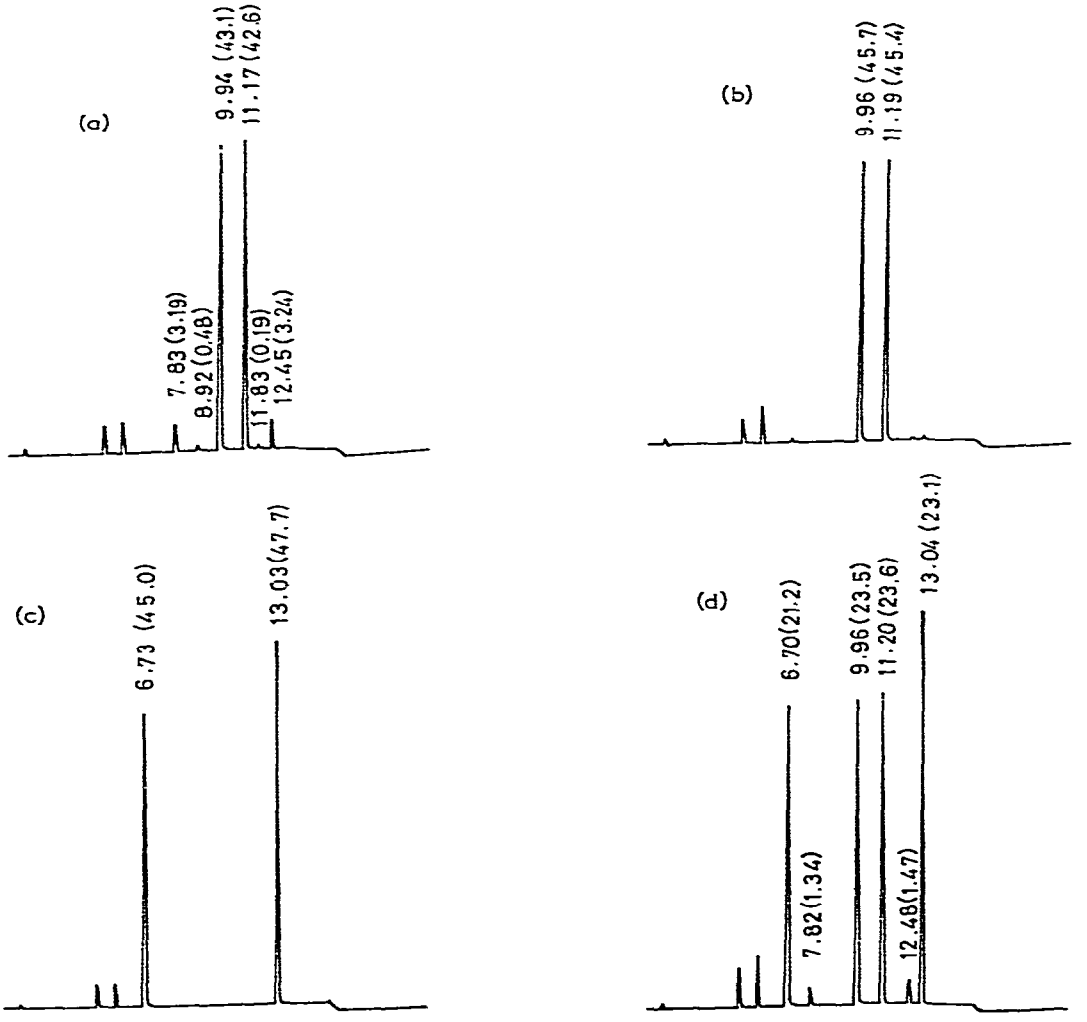


Fig. 5.

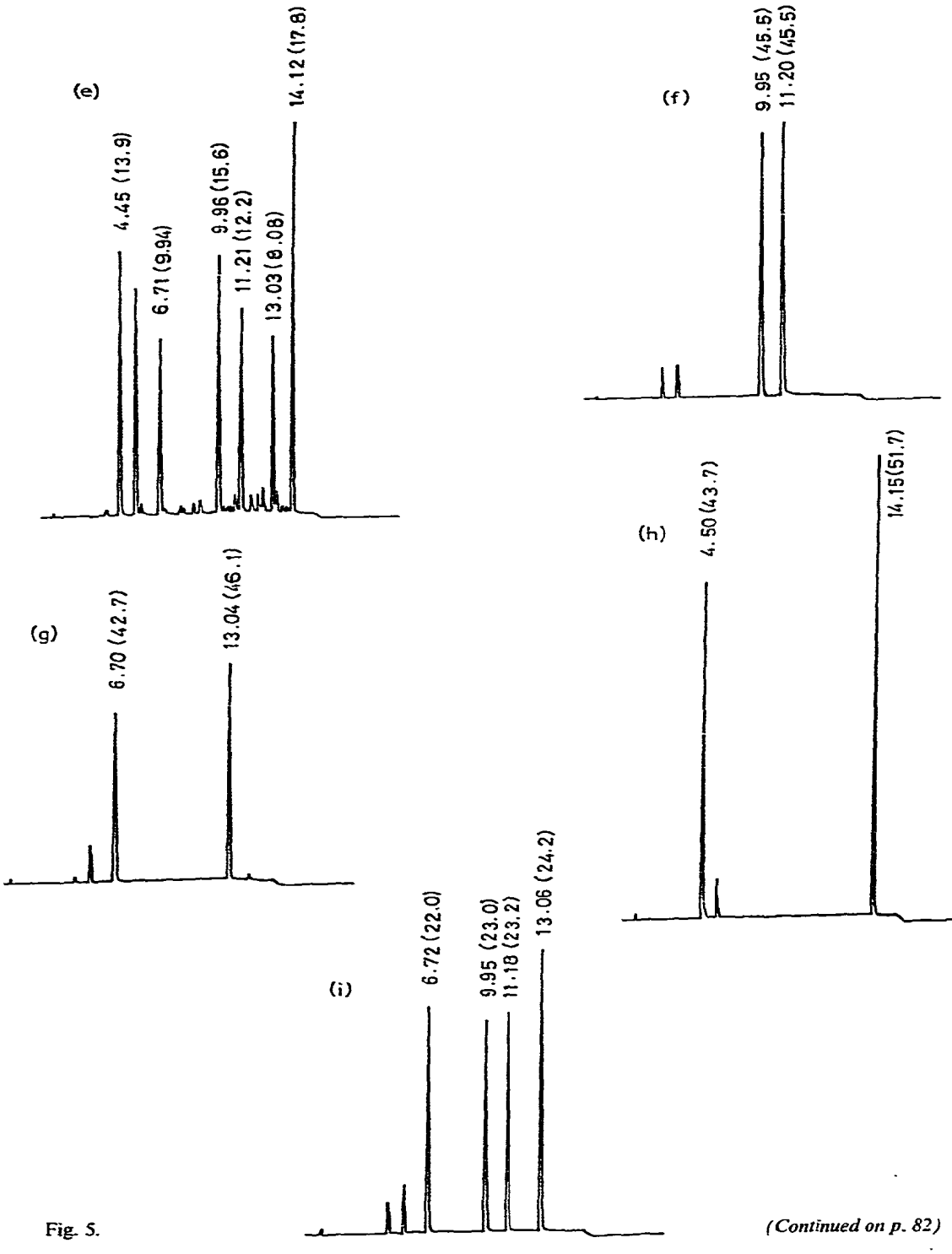


Fig. 5.

*(Continued on p. 82)*

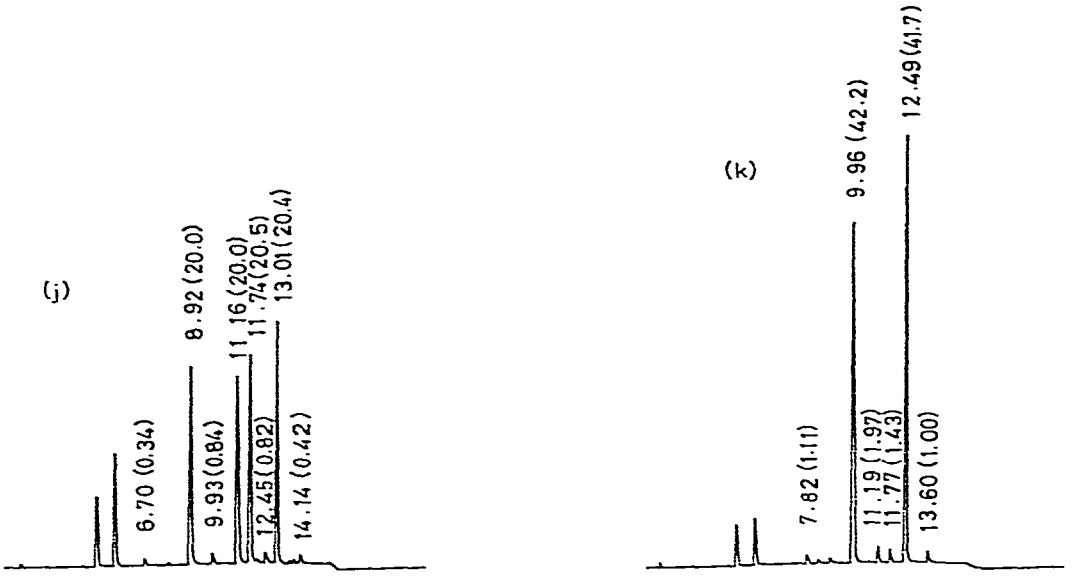


Fig. 5. Chromatograms of 2,4-dinitrophenylhydrazones of aldehydes as reductive ozonolysis products of monoolefinic dimethylurushiol of the following peaks: (a) 3-1; (b) 2-1-1; (c) 2-1-2; (d) 2-2-1; (e) 1-1-1 + 1-1-2 + 1-1-3; (f) 1-2-1; (g) 1-2-2-1; (h) 1-2-2-2; (i) 1-3-1; (j) 3-2-1; (k) 4. Conditions as in Fig. 4. Numbers with and without parentheses indicate peak area as a percentage of the whole peak area and retention time (minutes), respectively.

(25 cm × 7.6 mm I.D.) using *n*-hexane–ethyl acetate (95:5) as the eluent (see Fig. 3e); the former peak showed a strong IR band characteristic of a *trans*-olefinic bond at 960 $cm^{-1}$, whereas the latter showed no noticeable IR peak in the range 900–1000 $cm^{-1}$, indicating that the olefinic bond in the side-chain has a *cis* configuration.

Fig. 5f shows a chromatogram of the 2,4-dinitrophenylhydrazone of the reductive ozonolysis products of peak 1-2-1. The peak with a retention time of 9.95 min (11.20 min) correlates with that of the standard 2,4-dinitrophenylhydrazone of heptaldehyde (9.94 min). Similarly, the peak in Fig. 5g with a retention time of 6.70 min (13.04 min), derived from peak 1-2-2-1 in Fig. 3e, and that in Fig. 5h with a retention time of 4.50 min (14.15 min), derived from peak 1-2-2-2 in Fig. 3e, were identified as the 2,4-dinitrophenylhydrazones of heptaldehyde and acetaldehyde, respectively. From these results, it is evident that peak 1-2 is 3-[8′(*Z*),11′(*E*),13′(*Z*)-pentadecatrienyl]veratrole.

Peak 1-3 showed only a strong IR band at 920 $cm^{-1}$ characteristic of a terminal vinyl group in the range 900–1000 $cm^{-1}$, and therefore the other olefinic bonds are assigned to a *cis* configuration. The monoolefinic dimethylurushiol component peak of 1-3-1 derived from peak 1-3 by its partial reduction with hydrazine showed a doublet peak (see Fig. 3f), resulting from the equimolar formation of the 2,4-dinitrophenylhydrazones of butyraldehyde (retention time 9.95 min) and heptaldehyde (retention time 6.70 min) as the reductive ozonolysis products of the peak (see Fig. 5i). However, the peak corresponding to the 2,4-dinitrophenylhydrazone of formaldehyde derived from the terminal vinyl group could not be found, as the terminal vinyl group is preferentially reduced rather than an internal olefinic bond, as demon-

TABLE II

CONSTITUENTS OF URUSHIOL

| *Type* | *Compound* | *Concentration (%)** |
|---|---|---|
| $C_{15}$ side-chain urushiol | 3-(Pentadecyl)catechol | 4.5 |
| | 3-[8′(*Z*)-Pentadecenyl]catechol | 15.0 |
| | 3-[10′(*Z*)-Pentadecenyl]catechol | 1.5 |
| | 3-[8′(*Z*),11′(*E*)-Pentadecadienyl]catechol | 6.5 |
| | 3-[8′(*Z*),11′(*Z*)-Pentadecadienyl]catechol | 4.4 |
| | 3-[8′(*Z*),11′(*E*),13′(*E*)-Pentadectrienyl]catechol | 1.8 |
| | 3-[8′(*Z*),11′(*E*),13′(*Z*)-Pentadecatrienyl]catechol | 55.4 |
| | 3-[8′(*Z*),11′(*Z*),14′-Pentadecatrienyl]catechol | 7.4 |
| $C_{17}$ side-chain urushiol | 3-[11′(*Z*)-Heptadecenyl]catechol | 1.5 |
| | 3-[8′(*Z*),11′(*Z*)-Heptadecadienyl]catechol | 1.8 |

* These values are based tentatively on the peak heights in the chromatograms.

strated by Mori *et al.*[33]. These results indicate that peak 1-3 is identical with 3-[8′(*Z*),11′(*Z*),14-pentadecatrienyl]veratrole.

*$C_{17}$ side-chain dimethylurushiol.* Peak 3-2 in Fig. 3g, 3-(heptadecadienyl)-veratrole, showed no characteristic olefin IR band in the range 900–1000 $cm^{-1}$, indicating that the two double bonds in the side-chain have a *cis* configuration. Partial reduction with hydrazine of peak 3-2 for 3 h gave a monoolefinic dimethylurushiol, peak 3-2-1 (see Fig. 3g), which gave a mixture of 2,4-dinitrophenylhydrazones of caproldehyde and nonylaldehyde as its reductive ozonolysis products, with retention times of 8.92 min (11.61 min) and 11.74 min (13.01 min), respectively (see Fig. 5). From these results, peak 3-2 was identified as 3-[8′(*Z*),11′(*Z*)-heptadecadienyl]veratrole.

Peak 4 in Fig. 1, heptadecenylveratrole, showed no noticeable IR *trans*-band, indicating that the olefinic bonds in the side-chain have a *cis* configuration. The position of the olefinic bond in the side-chain of peak 4 was decided by assigning the peak in Fig. 5k with a retention time of 8.92 min (13.01 min) to the 2,4-dinitrophenylhydrazone of caproldehyde with a retention time of 8.91 min as a standard, concluding that peak 4 is identical with 3-[11′(*Z*)-heptadecenyl]veratrole.

## CONCLUSION

The urushiol components identified in this work are listed in Table II, together with those already reported.

3-[10′(*Z*)-Pentadecenyl]catechol and 3-[8′(*Z*),11′(*E*)-pentadecadienyl]catechol have been newly identified in addition to the already known components, 3-[8′(*Z*)-pentadecenyl]catechol and 3-[8′(*Z*),11′(*Z*)-pentadecadienyl]catechol.

Of triolefinic urushiol components, 3-[8′(*Z*),11′(*Z*),13′(*Z*)-pentadecatrienyl]-catechol has previously been believed to be a major component of urushiol without adequate evidence. However, from the present work, it became evident that this configurational structure of the side chain is erroneous, and it should be replaced by two urushiol components, 3-[8′(*Z*),11′(*E*),13′(*Z*)-pentadecatrienyl]catechol and 3-

[8′(*Z*),11′(*E*),13′(*E*)-pentadecatrienyl]catechol. 3-[8′(*Z*),11′(*Z*),14′-Pentadecatrienyl]-catechol has also been confirmed to exist in a relatively large amount in Japanese lacquer urushiol, as already reported by Hashimoto and Minami[17].

Of the components of Japanese lacquer urushiol, only $C_{15}$ side-chain urushiol has been discussed. It is clear that two urushiol homologues with a $C_{17}$ side-chain, 3-[11′(*Z*)-heptadecenyl]catechol and 3-[8′(*Z*),11′(*Z*)-heptadecadienyl]catechol should also be taken into consideration as Japanese lacquer urushiol homologues, although they are minor components. Thus the components of Japanese lacquer urushiol are seen to be similar to those of poison ivy, contrary to Sunthanker and Dawson's proposal[3].

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REFERENCES

1 S. Ishimatsu, *Mem. Manchester Library and Philosophical Society (London and Paris)*, (1879) 249.
2 R. Majima, *Chem. Ber.*, 55B (1922) 172.
3 S. V. Sunthanker and C. R. Dawson, *J. Amer. Chem. Soc.*, 76 (1954) 5070.
4 G. A. Hill, V. Mattacotti and W. D. Graham, *J. Amer. Chem. Soc.*, 76 (1954) 2959.
5 M. D. Corbett and S. Billets, *J. Pharm. Sci.*, 64 (1975) 1715.
6 S. Billets, J. C. Craig, M. D. Corbett and J. F. Vickery, *Phytochemistry*, 15 (1976) 533.
7 B. Loev and C. R. Dawson, *J. Amer. Chem. Soc.*, 78 (1956) 4083.
8 B. Loev and C. R. Dawson, *J. Org. Chem.*, 24 (1959) 980.
9 H. Halim, H. D. Locksley and J. J. Memon, *J. Chem. Soc., Perkin Trans. I*, (1980) 2331.
10 J. H. Tyman, *Chem. Soc. Rev.*, 8 (1979) 499.
11 H. Keil, D. Wasserman and C. R. Dawson, *J. Allergy*, 16 (1945) 275.
12 V. S. Byers, N. Castagnolic and W. L. Epstein, *J. Clin. Invest.*, 64 (1979) 1449.
13 E. S. Watson, J. C. Murphy, P. W. Wirth, C. W. Waller and M. A. Elsholy, *J. Invest. Dermatol.*, 76 (1981) 164.
14 T. Kato and J. Kumanotani, *J. Polym. Sci., Part C*, (1968) 519.
15 T. Kato and J. Kumanotani, *J. Polym. Sci., Part A-1*, 7 (1969) 1455.
16 J. Kumanotani, *Makromol. Chem.*, 197 (1978) 47.
17 O. Hashimoto and K. Minami, *Mokuzai Kagakukaishi*, 25 (1979) 789.
18 O. Hashimoto and K. Minami, *Mokuzai Kagakukaishi*, 26 (1980) 49.
19 C. Y. Ma, M. A. Elsohly and J. K. Baker, *J. Chromatogr.*, 200 (1980) 163.
20 Y. Yamauchi, T. Murakami and J. Kumanotani, *J. Chromatogr.*, 214 (1981) 343.
21 Y. Yamauchi, R. Oshima and J. Kumanotani, *J. Chromatogr.*, 198 (1980) 49.
22 C. R. Scholfield, *Anal. Chem.*, 47 (1975) 1417.
23 J. D. Warthen, *J. Amer. Oil Chem. Soc.*, 52 (1975) 151.
24 R. F. Borch, *Anal. Chem.*, 47 (1975) 2437.
25 M. G. M. de Rûyter and A. P. de Leenheer, *Anal. Chem.*, 51 (1978) 43.
26 P. N. Rao and L. R. Axelord, *J. Chem. Soc.*, (1961) 4769.
27 Y. Yamauchi and J. Kumanotani, *J. Chromatogr.*, 210 (1981) 512.
28 R. Viviani, *Advan. Lipid Res.*, 8 (1970) 267.
29 M. Beroza and B. A. Bierl, *Anal. Chem.*, 39 (1967) 1131.
30 N. D. Chernis and J. B. Entikin, *Identification of Organic Compounds*, Interscience, New York, London, 1963, p. 137.
31 *Dictionary of Organic Compounds*, Vols. 1–4, Eyre & Spottiswood, Great Britain, 1965.
32 R. Majima and C. Chiba, *Chem. Ber.*, 55 (1922) 195.
33 K. Mori, M. Oki, A. Sato and M. Matsui, *Tetrahedron*, 28 (1972) 3737.