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HIGH-RESOLUTION GAS–LIQUID CHROMATOGRAPHIC ANALYSIS OF URUSHIOL OF THE LAC TREE, *RHUS VERNICIFERA*, WITHOUT DERIVATIZATION

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SUMMARY

Fused-silica capillary gas–liquid chromatography has been successfully applied to the determination of urushiol congeners in the sap of the lac tree, *Rhus vernicifera*. A mixture of the urushiol congeners is satisfactorily separated into ten components on a 25 m × 0.2 mm I.D. WCOT column coated with methylsilicone (thickness of stationary phase film, 0.33 μm) without any derivatization within 13 min with temperature-programmed operation from 230 to 290°C at 5°C/min. The relative standard deviation of quantification was less than 4% for abundant components, and that of retention time was as low as 0.04%. A number of saps of different origins were analysed by this method. The performance of saps is discussed in terms of the content of the triolefinic component, which has been accurately determined here.

INTRODUCTION

In Asian countries, sap collected from various kinds of lac trees has been used as excellent coating materials for several thousand years[1]. In Japan, Korea and China *Rhus vernicifera* is grown, in Vietnam and Taiwan *Rhus succedanea* and in Burma *Melanorrhoea usitate*[2]. Urushiol is the major constituent of the sap of *Rhus vernicifera* and is also found in poison ivy, *Rhus toxicodendron radicans*. It is known to cause irritation, inflammation and blistering of the skin of sensitive individuals. The mechanism of this immunological action has been extensively examined[3,4].

Urushiol is a trivial name for 3-substituted alkylcatechols with zero, one, two and three double bonds in the C_{15} side-chain, and those identified in the sap of *Rhus vernicifera* are compounds 1–7 (refs. 5–8).

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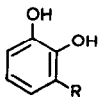
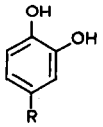


1, R = 8′*Z*,11′*E*,13′*Z*-pentadecatrienyl
2, R = 8′*Z*,11′*Z*,14′-pentadecatrienyl
3, R = 8′*Z*,11′*E*,13′*E*-pentadecatrienyl
4, R = 8′*Z*,11′*Z*-pentadecadienyl
5, R = 8′*Z*,11′*E*-pentadecadienyl
6, R = 8′*Z*-pentadecenyl
7, R = pentadecyl
8, R = 10′*Z*,13′*E*,15′*Z*-heptadecatrienyl
9, R = 10′*Z*,,13′*Z*,16′-heptadecatrienyl
10, R = 10′*Z*,13′*Z*-heptadecadienyl
11, R = 10′*Z*-heptadecenyl

In a recent paper[9], it was demonstrated that the sap of *Rhus vernicifera* originating in China contains laccols[5] 8–11 and the thitsiol derivatives 12 and 13 in addition to the above urushiol derivatives.



12, R = 8′*Z*,11′*E*,13′*Z*-pentadecatrienyl
13, R = 8′*Z*,11′*Z*,14′-pentadecatrienyl

The utilization of urushiol is increasing, so a rapid and accurate method for the determination of the ratios of urushiol congeners is desirable. Further, it is necessary to obtain each component in the underivatized form in order to examine the specificity of the immunological action of urushiol.

Because of the structural similarity of these components, their retention behaviours in gas–liquid chromatography (GLC) are very similar. In addition, they are highly sensitive to air oxidation and the isolation or determination of the each component in the underivatized form is extremely difficult by classical packed column GLC. Several derivatized forms have been employed for the determination of urushiol homologues by GLC and LC, including dimethyl ethers[7], diacetates[10] and bis-(trimethylsilyl) (TMS) ethers[11–13]. However, reversed-phase LC has recently been found to be effective for separating urushiol congeners in the intact form[14–16]. In a previous paper[9], we demonstrated that an acetic acid-containing eluent is adequate for this purpose as it prevented adsorption of the substrate on the column packing. Using this technique, we obtained several congeners in the underivatized form.

We describe here an improved method for intact urushiol congeners based on fused-silica capillary GLC. Owing to the inertness of the column wall[17], adsorption of urushiol on the column was completely eliminated. A general procedure for the analysis of urushiol is presented using this GLC technique accompanied by gel permeation chromatography (GPC). The results of the analysis of the sap of *Rhus vernicifera* of different origins by the present procedure are also reported.

EXPERIMENTAL

Materials and procedures

Sap of *Rhus vernicifera* from Hupei, Kiangsi and Shensi in China and Ibaraki, Naruko and Yamagata in Japan was analysed. Native sap (1 part) was mixed with 3 parts of acetone and the mixture was filtered. The filtrate was evaporated to give a crude urushiol preparation as a residue. Several milligrams of the residue were dissolved in 0.1 ml of chloroform and the solution was applied on to GPC columns (Gelko A110 and A120, each 50 cm × 8 mm I.D.; Hitachi Chemicals, Tokyo, Japan) and the column was eluted with chloroform. The monomeric fraction was collected and applied on to GLC column directly or after derivatization. O-Methylation was executed with dimethyl sulphate in dry acetone in the presence of potassium carbonate at 60°C for 5 h. Acetylation was effected with acetic anhydride in pyridine (100°C, 1 h) and trimethylsililation with bis(trimethylsilyl)acetamide in dry acetonitrile at room temperature for 5 min.

Instrumental

GPC was carried out on a home-built LC apparatus equipped with a refractive index detector, details of which were reported in a previous paper[8]. GLC was executed with a Hewlett-Packard 5790 instrument equipped with a flame-ionization detector, except for Fig. 1, for which a Hitachi 063 instrument with a flame-ionization detector was used. Two fused-silica WCOT columns (Hewlett-Packard, Avondale, PA, U.S.A.) were employed: silicone OV-1, 25 m × 0.2 mm I.D., thickness of liquid phase film (d_f) = 0.17 μm; and methylsilicone (Ultra 2), 25 m × 0.2 mm I.D., d_f = 0.33 μm. The carrier gas was helium and samples were injected in the split mode (splitting ratio 50:1). Peak areas and retention times were determined with a Hewlett-Packard 3990 reported integrator.

RESULTS AND DISCUSSION

Fig. 1 compares chromatograms of urushiol in different derivatized forms, *i.e.*, dimethyl ethers, diacetates and TMS ethers, and in the underivatized form on a fused-silica WCOT column coated with OV-1. The sample for dimethyl ethers had a different origin to the other derivatives. Similar results were obtained for the composition of urushiol congeners for diacetates. TMS ethers and the underivatized form; owing to the inertness of the column wall any adsorption of intact urushiol on the column was not observed. The results clearly demonstrate that urushiol can be analysed on a fused-silica WCOT column in the underivatized form both quantitatively and qualitatively.

In order to improve the separation of urushiol congeners, we tested several types of WCOT columns, and found that a WCOT column coated with methylsilicone is suitable for the present purpose. Fig. 2 is an example of such separation with temperature programming from 230 to 290°C at 5°C/min; ten components were well resolved within 13 min.

Each peak was identified by comparison of the retention times with those components that had been previously isolated by reversed-phase LC from the sap[9]. As the reproducibility of the retention times is extremely high, as will be shown later,

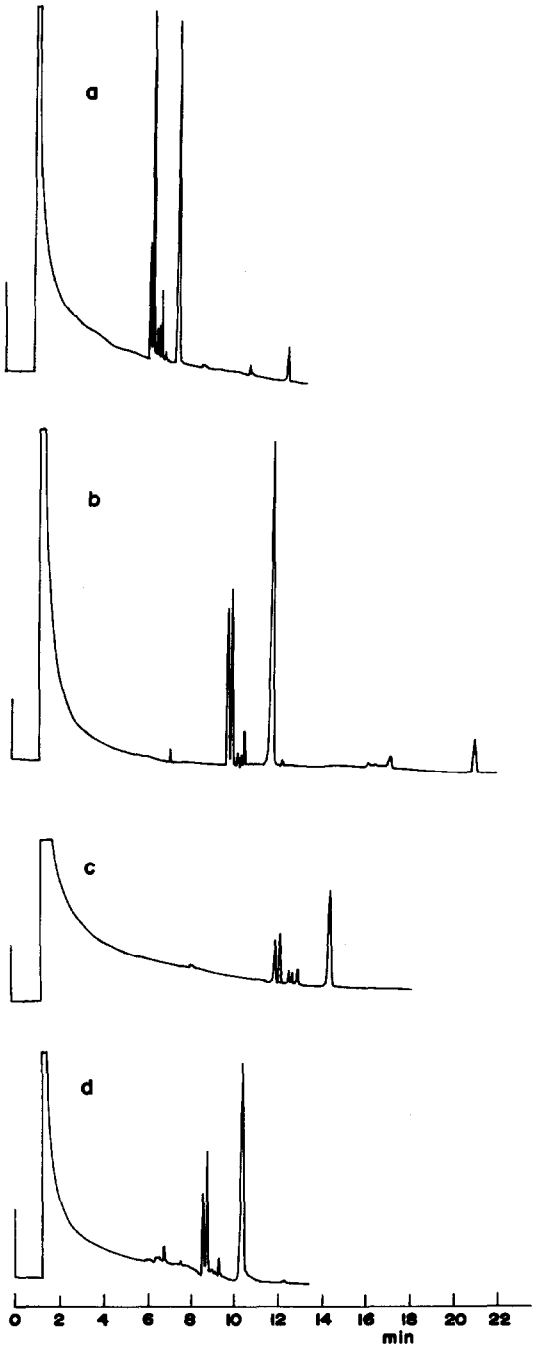


Fig. 1. Chromatograms of urushiol from sap of *Rhus vernicifera* (Japan) as (a) O-dimethyl ethers, (b) TMS ethers, (c) diacetates and (d) underivatized. Conditions: column, silicone OV-1, 25 m × 0.2 mm I.D., d_f = 0.17 μm; column temperature, 218°C; injection port temperature, 290°C; detector temperature, 290°C; carrier gas (helium) linear velocity, 32 cm/sec.

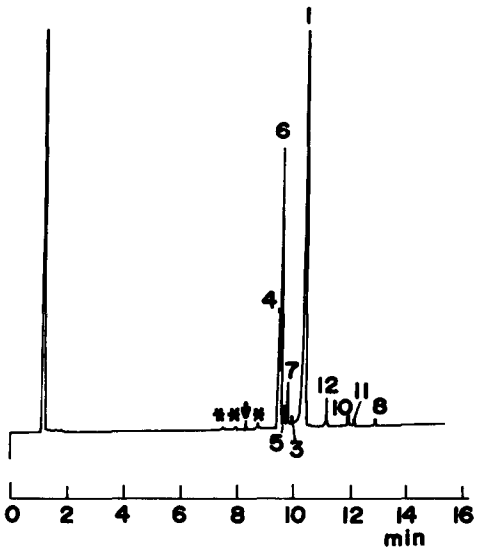


Fig. 2. Chromatogram of urushiol from sap of *Rhus vernicifera* (Hupei, China) in the underivatized form. Conditions: column, methylsilicone, 25 m × 0.2 mm I.D., d_f = 0.33 μm; column temperature, programmed from 230 to 290°C at 5°C/min; injection port temperature, 290°C; detector temperature, 290°C; carrier gas (helium) linear velocity, 40 cm/sec; splitting ratio, 50:1. Numbers on peak represent compound numbers; the peak marked with an arrow was not identified, and those marked with asterisks are phthalates from a plastic container.

the identification is accurate and reliable. It is demonstrated that all the constituents of urushiol that have been separated by reversed-phase LC are clearly distinguished by the present GLC method, depending on the number of double bonds in the side-chain and on the difference in the configuration of the double bonds. In addition, laccol and thitsiol derivatives, which are minor constituents in the sap, are discriminated.

The reproducibility of the retention time and the quality of each peak were checked. For the urushiol preparation from sap of *Rhus vernicifera* from Hupei, China, four operations were conducted to provide statistical parameters, as summarized in Tables I and II. The excellent reproducibility was confirmed from these data; the relative standard deviations for the retention time were within 0.04% and the absolute reproducibility in area counts for abundant peaks was 1–4% (relative standard deviation).

We present here a general and convenient method for quantifying each component of urushiol preparations from the sap of *Rhus vernicifera* based on the described GLC technique combined with GPC. Native sap (1 part) was mixed with 3 parts of acetone, the acetone-soluble fraction obtained by filtration was evaporated and the residue (a few milligrams were sufficient) was dissolved in chloroform (0.1 ml), then the solution was applied on to a GPC column. Usually the acetone-soluble fraction of sap includes polymeric and oligomeric substances in addition to monomeric components. Using this GPC method, the amount of the monomers was elucidated and simultaneously fractionated. The fraction of monomeric urushiol thus obtained was applied directly on to the GLC column. Using the above general pro-

TABLE I

REPRODUCIBILITY OF RETENTION TIMES OF URUSHIOL CONGENERS ON A WCOT COLUMN COATED WITH METHYLSILICONE

GLC conditions as in Fig. 2.

Compound	*Retention time (min)*					*R.S.D., %*
	Run 1	*Run 2*	*Run 3*	*Run 4*	*Average ± S.D.**	
1	10.27	10.27	10.27	10.26	10.268 ± 0.004	0.04
3	9.87	9.87	9.86	9.87	9.868 ± 0.004	0.04
4	9.38	9.37	9.37	9.37	9.372 ± 0.004	0.04
5	9.57	9.57	9.57	9.56	9.568 ± 0.004	0.04
6	9.46	9.45	9.45	9.45	9.452 ± 0.004	0.04
7	9.70	9.69	9.69	9.69	9.692 ± 0.004	0.04
8	12.80	12.79	12.79	12.80	12.795 ± 0.0055	0.05
10	11.85	11.84	11.84	11.84	11.842 ± 0.004	0.04
11	12.04	12.03	12.03	12.03	12.032 ± 0.004	0.04
12	11.09	11.09	11.08	11.08	11.085 ± 0.0055	0.05

* S.D. = Standard deviation; R.S.D. = relative standard deviation.

cedure, the oily substance of the sap of *Rhus vernicifera* can be completely analysed. Of course, this method can be extended to the analysis of oily substances of saps of other kinds of lacquer trees belonging to plant family *Anacardeacae*.

Using the general procedure based on fused-silica GLC combined with GPC, a number of saps of *Rhus vernicifera* of different origins were analysed, giving the results in Table III.

Independent of the country (China and Japan) and district of growth and the

TABLE II

REPRODUCIBILITY OF PEAK AREAS OF URUSHIOL CONGENERS

GLC conditions as in Fig. 2.

Compound	*Peak area, %*				
	Run 1	*Run 2*	*Run 3*	*Run 4*	*Average ± S.D.*
1	52.337	53.723	52.664	52.884	52.905 ± 0.592
3	0.231	0.321	0.280	0.278	0.278 ± 0.037
4	9.857	9.662	9.926	10.081	9.882 ± 0.174
5	1.599	1.472	1.498	1.524	1.523 ± 0.055
6	26.553	25.572	25.769	26.234	26.072 ± 0.396
7	3.688	3.554	3.605	3.836	3.671 ± 0.123
8	0.528	0.470	1.018	0.736	0.688 ± 0.248
10	0.654	0.722	0.746	0.754	0.719 ± 0.045
11	0.392	0.388	0.502	0.408	0.426 ± 0.054
12	2.153	2.048	2.278	1.656	2.034 ± 0.269

TABLE III

QUANTITATIVE ANALYSIS OF URUSHIOL OF *RHUS VERNICIFERA* FROM DIFFERENT DISTRICTS

GLC conditions as in Fig. 2. Results expressed as peak area (%).

Compound*	*China*			*Japan*			*Ibaraki, Japan***		
	Hupei (No. 1)	*Shensi (No. 2)*	*Kiangsi (No. 3)*	*Yamagata (No. 4)*	*Naruko (No. 5)*	*Ibaraki (No. 6)*	*July 5 (No. 7)*	*Aug. 17 (No. 8)*	*Sept. 30 (No. 9)*
1	64.13	53.72	38.49	51.30	60.75	57.24	55.92	61.96	67.03
3	–	0.32	0.42	0.19	0.23	0.35	0.29	0.38	0.26
4	2.86	9.66	2.82	14.49	10.75	5.21	7.83	2.58	2.45
5	0.85	1.47	2.80	1.03	1.23	1.50	1.54	1.56	1.16
6	23.01	25.51	47.18	25.03	19.33	25.82	25.68	21.65	20.55
7	3.06	3.55	3.90	3.13	3.25	4.35	3.95	3.63	3.42
8	0.59	0.47	0.29	0.57	0.56	1.00	0.73	0.54	0.40
0	1.32	0.72	0.28	0.71	0.33	0.27	–	0.51	0.43
1	0.18	0.39	–	0.27	–	1.25	–	0.30	–
2	1.19	2.05	0.51	2.45	1.90	0.90	2.51	3.45	3.00
Monomer content (%)	88.6			90.9	92.6		69.6	81.4	87.5

* Compounds 1, 8 and 12 contain compounds 2, 9 and 13, respectively, as trace contaminants.
** Times of harvest differed as indicated.

time when the sap was harvested, the sap of *Rhus vernicifera* is composed of identical constituents. The most abundant constituent is the trienyl compound 1 and the second one the monoenyl urushiol 6, which is in good agreement with previous reports. Compound 4 was the third most abundant constituent.

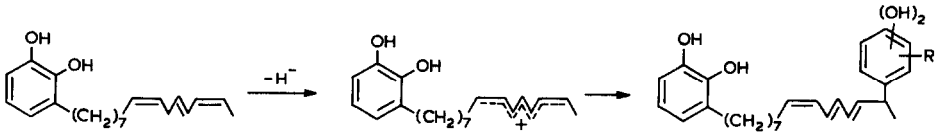
The monomer content decreased with increasing time after the harvest of the sample. This may be due to the polymerization of urushiol during storage or transportation. Samples 4 and 5 were obtained by extracting the sap with acetone soon after it had exuded from the plant in order to minimize the polymerization. The content of monomeric substances was high for these specially obtained samples, but they included several percent of polymeric materials. It needs more careful experiments to elucidate whether or not the polymeric material is produced through metabolic pathways in the plant cells or by air oxidation outside the plant organism.

In Japan, sap harvested in September gives better performance in terms of rapidity of drying and hardness of lacquer films produced therefrom, compared with those harvested in July and August. Of the Chinese saps analysed, that from Hupei was exuded from an old tree and showed a better performance than that from Kiangsi, which exuded from a young tree planted 2 years earlier and with inferior characteristics*. All the saps with good performance as a lacquer material had a high content

* This sample was a specially arranged one, and does not represent the sap of lac trees from Kiangsi.

of the trienyl compound 1 compared with the monoenyl compound 6. Hence the performance of saps is dependent on the content of 1.

Sap from lac trees has been considered to dry through oxidative coupling of urushiol mediated by the oxidoreductase laccase[18]. Recent studies confirmed that the coupling occurs by two different processes, phenol coupling to produce diphenyls and dibenzofurans and nucleus–side-chain coupling[19]. The latter unique reaction may be initiated by abstraction of hydride from the side-chain of urushiol with the urushiol quinone. A conjugated carbonium ion produced as an intermediate attacks the nucleus of urushiol electrophilically, resulting in the formation of C–C coupled dimers.



The trienyl urushiol has highest potential for this oxidative coupling reaction, as the intermediate heptatrienyl cation derived therefrom is most stable. Therefore, sap that contains a high content of the trienyl urushiol gives a performance as a coating material.

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