Journal of Chromatography, 235 (1982) 149–164
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,286

LONG-CHAIN PHENOLS

XXII*. COMPOSITIONAL STUDIES ON JAPANESE LAC (*RHUS VERNICIFERA*) BY CHROMATOGRAPHY AND MASS SPECTROMETRY

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(First received July 28th, 1981; revised manuscript received August 14th, 1981)

SUMMARY

Preparative thin-layer (TLC) and column chromatography of Japanese Lac and the hydrogenated material have been used for compositional analysis by gravimetry and the isolation of certain polar minor components. From this examination the composition was approximately 62% urushiol, 18% other monomeric/dimeric components and 20% polymeric material. By gas–liquid chromatography (GLC) of methylated or bistrimethylsilylated Japanese Lac on 2% PEGA, four unsaturated constituents the 8-monoene (19%), 8,11-diene (9%), 8,11,14-triene (6%), which is a new constituent not previously detected and the 8,11,13-triene (62%) in addition to the saturated constituent (4%) were shown to be present. The results for the two derivatives have not been corrected for different response factors of the constituents but are in good agreement. GLC–mass spectroscopy of bistrimethylsilylated Japanese Lac on 2% PEGA as stationary phase confirmed the nature of the unsaturated constituents, and on E-301 indicated the presence of C_{17} homologous urushiol and a minor polar component each comprising four constituents. Mass spectroscopy was used to determine the unsaturated composition and the semi-corrected results indicate a close similarity to those found by GLC. Bis(trimethylsilyl)urushiol is more volatile than the dimethyl derivative only on a polar stationary phase (PEGA). The dimethyl ether is more polar than the monomethyl ether (2-methoxyl-6-pentadecylphenol) by TLC but the reverse holds in GLC.

In the minor components a more polar material has been shown to be nuclear hydroxyurushiol while one of the less polar materials appears to be a diphenyl derivative isomeric with a previously described substance isolated from oxidised Japanese Lac. A mechanism for their formation is proposed.

INTRODUCTION

The quantitative composition of the major constituents and the identity of certain minor materials in Japanese Lac, *Rhus vernicifera* have been investigated.

* Part XXI, J. H. P. Tyman, V. Tychopoulos and B. A. Colenutt, *J. Chromatogr.*, 213 (1981) 287.

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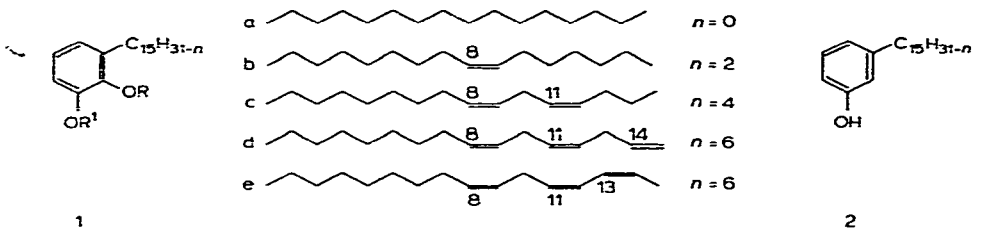

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Although this work is not yet complete it is desirable to describe the results, briefly referred to in a preliminary communication[1], in view of a recent account[2] of an analysis of urushiol diacetate by high-performance liquid chromatography (HPLC).

The heterogeneous nature of urushiol and its partial structural determination was originally investigated by Majima[3] although the column chromatographic separation of methylated urushiol constituents of Japanese Lac and of *Rhus toxicodendron* was first effected by Dawson and co-workers[4,5] and followed by structural studies which showed the former to comprise four materials (1, R = R^1 = H; a, b, c, e) and the latter three similar materials (a, b, c) and a fourth (d). The detailed chemistry has been reviewed[6].

The increasing utilisation of Japanese Lac and interest in the biosynthesis of urushiol[7] makes it of interest to have quantitative information on the component phenols and the unsaturated constituents present. No gas–liquid chromatographic (GLC), mass spectroscopic (MS) or GLC–MS studies on the phenols have previously been carried out.

As found possible for the constituents of the component phenols of *Anacardium occidentale*[8], the unsaturated composition of the urushiol constituents has been quantitatively determined by MS and confirmed by GLC on the dimethyl and bis(trimethylsilyl) ethers.

In addition to a small proportion of the less polar material cardanol[7] (2; n = 0, 2, 4, 6) several substances more polar than urushiol have been found and partially identified, one probably a diphenyl compound isomeric with an earlier described material[9] and the other a nuclear hydroxy urushiol, the *o*-quinone of which has been postulated as an intermediate in certain photochemical transformations[10].

In the methylated and methylated hydrogenated derivatives of urushiol, striking differences[11] in polarity have been found between the monomethyl (1, R = H, R^1 = CH_3) and the dimethyl ether series (1, R = R^1 = CH_3) and the former* appears to be a type of "hindered" phenol.

Our GLC work on the determination of relative molar response values of the constituents of urushiol and on HPLC analysis will be described in a subsequent paper.

EXPERIMENTAL

Materials

Japanese Lac was obtained through the help of Dr. M. Sato, National Industrial Research Institute, Sendai, Japan, and the Japanese Trade Centre, London.

* For steric reasons the methyl group has been assigned to the 1-position, the C_{15} chain being in the 3-position.

Cashew nut-shell liquid (natural) used as a reference material was obtained[12] from Mozambique cashew nuts.

Hydrogenations were carried out in a Parr apparatus with Japanese Lac (5.2455 g) in ethyl acetate (48 cm^3) with 10% palladium–carbon (0.5215 g) at 15 p.s.i., and also in a glass apparatus at atmospheric pressure. They were monitored by ^1H nuclear magnetic resonance (NMR) and argentation thin-layer chromatography (TLC). Methylations were carried out under nitrogen on Japanese Lac (1.000 g) in refluxing benzene (27 cm^3) containing anhydrous potassium carbonate and dimethyl sulphate (4.00 g) or by the phase transfer method[7]. Silylations were effected in warm anhydrous pyridine solutions with bis(trimethylsilyl)acetamide or bis(trimethylsilyl)trifluoroacetamide in five molar proportion.

TLC

TLC was carried out on silica gel G (Merck Type 60) with self-prepared microscope slides, analytical plates (10 × 8 cm, and 20 × 10 cm) with a 0.25-mm layer, and preparative plates (20 × 20 cm) with a 1-mm layer. Spots and bands were visualised with 0.1% ethanolic Rhodamine 6G. Silver nitrate plates (15% silver nitrate) were self-prepared by aqueous slurrying[13] and bands were visualised with 0.1% ethanolic dichlorofluorescein. All TLC sample applications (10% urushiol in chloroform) were made in a nitrogen-box which was also used for removal of solvent after development/visualisation. Bands were eluted overnight under nitrogen with diethyl ether, whereby selective removal of the organic material but not the fluorescein indicator occurred, the suspensions were filtered and the filtrate then concentrated. All evaporations were carried out below 60°C and samples brought back to atmospheric pressure under nitrogen.

Column chromatography

Column chromatography was effected with silica gel (MFC) in a glass column (21 × 1.25 in.) equipped with a silica disc of zero porosity and outlet tap fitted with a PTFE key. The column was wrapped with tin foil to exclude light. Japanese Lac (2.5682 g) was applied directly to the column and fractions were monitored by TLC and GLC as indicated in the results and discussion section.

GLC and GLC–MS

GLC was conducted generally with a Pye 104 dual column chromatograph equipped with a flame ionisation detector and a Vitatron recorder. Peak areas were measured by accurate triangulation and the results expressed were the average of at least six determinations. Glass columns (5 ft. × $\frac{3}{16}$ in.) with nitrogen at a flow-rate 45 cm^3/min and 20 p.s.i. were used containing acid-washed and silanised diatomite C with 3% SE-30 and (for unsaturated constituents) diatomite M (80–100 mesh) with 2% polyethyleneglycol adipate (PEGA).

GLC–MS was effected by the Physico-Chemical Measurements Unit (PCMU) (Aldermarston and Harwell, Great Britain) with a Pye 104 and 2% E-301 as stationary phase at 230°C with helium as carrier gas (40 cm^3/min). For the silylated unsaturated constituents, 2% PEGA on diatomite C at 185°C was used under similar gas flow conditions. Subsequently the low resolution D31 service was used by PCMU (Harwell) with a 6-ft. column coated with 3% OV-1 with helium (30 cm^3/min) and temperature programming from 200 to 250°C, the mass spectrometry being carried out at 70 eV with a resolution of 1000 and the ion source at 200°C.

TABLE I

TLC SEPARATION OF JAPANESE LAC AND HYDROGENATED JAPANESE LAC IN DIFFERENT SOLVENTS

Material	*Solvent*	*R_F values and observations*
Natural	Chloroform	No migration beyond R_F 0.1
Natural	Benzene	No migration beyond R_F 0.1
Natural	Acetone	All components, R_F 0.8–0.9
Natural	Chloroform–ethyl acetate (19:1)	Separation: R_F 0.49 (1, urushiol); 0.23 (2); 0.09 (3); 0.0 (4 and 5)
Natural	Chloroform–ethyl acetate (90:10)	Good separation: R_F 0.65 (1, urushiol); 0.46 (2); 0.25 (3); 0.1 (4); 0.0 (5)
Hydrogenated urushiol	Chloroform–ethyl acetate (90:10)	Good separation, sharper bands, with less tailing

Mass spectrometry for analytical purposes was carried out on an MS 902 instrument by a repeated scanning procedure[8] with direct sample insertion, through the courtesy of Mr. D. Carter, School of Pharmacy, London, and more recently by the PCMU (Harwell) low resolution D11 service (with the VG ZAB system). Accurate mass measurements were made with the D21 service.

^{1}H NMR spectra were determined on a Varian T60 instrument with tetramethylsilane as internal standard. Infrared spectra were obtained with a Unicam SP200 instrument. Elemental analyses were carried out by Mr. G. Crouch, School of Pharmacy, London, and by Butterworth Analytical Services (Teddington, Great Britain).

RESULTS AND DISCUSSION

Most separatory techniques were used in conjunction or with spectroscopic identification of materials, but it is convenient to discuss the former individually.

TLC

Solvents, chloroform, benzene, acetone, chloroform–ethyl acetate (95:5, 90:10 and 80:20) were used for the analytical TLC examination of Japanese Lac and the results are summarised in Table I. Generally chloroform–ethyl acetate (90:10) gave the best separation and the bands with R_F values 0.65 (urushiol), 0.46 and 0.25 appear to approximately correspond with those observed[9], in a different solvent, namely 0.5 (urushiol), 0.37 and 0.20 for a mildly oxidised Japanese Lac. Our MS results on the material from the two bands below urushiol have led us to different conclusions from the latter authors concerning their identity as discussed subsequently. A small band R_F 0.75, in chloroform–ethyl acetate (90:10), has been identified as cardanol[7].

Preparative TLC was used to estimate the composition and to isolate new components for structural determination. Following an initial experiment which indicated gravimetrically 61.2% urushiol to be present, four preparative separations on Japanese Lac (2.040 g) carried out with chloroform–ethyl acetate (90:10) indicated urushiol (61.9%), 3.3% of less polar material (cardanol), fraction 3 (4.7%), fraction 4 (3.0%) and fraction 5 together with polymeric material (27.1%) to be present. The streaking and manipulative problem in recovering materials sensitive to oxidation led

TABLE II

VARIATION IN R_F VALUE (TLC) OF URUSHIOL MONO- AND DIMETHYL ETHERS WITH PERCENTAGE OF CHLOROFORM IN CHLOROFORM–LIGHT PETROLEUM (B.P. 40–60°C)

Urushiol compound	*Chloroform–light petroleum (b.p. 40–60°C)*				
	0:100	*20:80*	*40:60*	*60:40*	*100:0*
Monomethyl ether	0.11	0.29	0.38	0.60	0.70
Dimethyl ether	0.06	0.17	0.24	0.48	0.65

us to use hydrogenated Japanese Lac. Hydrogenation was monitored by ^{1}H NMR examination and was continued until olefinic absorption (δ, CCl_4, 5.1–6.2) and methylenic absorption in proximity to double bonds (δ, 1.69–2.9) had disappeared*. Although less streaking was encountered, accurate analyses, particularly of smaller % components, were rendered difficult due to variations in the delineation of bands observable at the visualisation stage with level of solvent impregnation. TLC on this material revealed the main components present and was useful for the isolation of the more polar material such as fraction 3 (2.05–4.70%), fraction 4 (3.0–6.1%), fraction 5 (8.2–8.6%) and baseline polymeric material (18.7–24.8%).

Methylation of hydrogenated phenolic lipids has been useful analytically[14] and was expected as well in the present work to both aid volatilisation and stabilisation of Japanese Lac. Methylated and methylated/hydrogenated Japanese Lac were examined by TLC in the solvent system, chloroform–light petroleum (b.p. 60–80°C) and the variation in R_F values with solvent composition of the two main bands, an upper purple and a lower yellow zone, are shown in Table II. ^{1}H NMR and MS examination of the preparatively separated bands indicated that the purple band consisted of urushiol monomethyl ether (1, $n = 0$, R = H, $R^1 = CH_3$) and the yellow band was the dimethyl ether (1, $n = 0$, R = $R^1 = CH_3$), the parent molecular ions having *m/e* values 334 and 348 respectively.

The phenolic methyl ether is evidently a hindered phenol similar in TLC behaviour[11] to 2,6-di-*tert.*-butyl-4-methylphenol and a number of similar compounds in relation to the corresponding dimethyl ethers all of which had greater polarity. By GLC the monomethyl ethers were more polar than the dimethyl ethers as discussed subsequently. Methylation either of Japanese Lac or the hydrogenated material did not enable fractions 3, 4, 5 and the baseline component to be more easily characterised although it was useful for the quantitative analysis of the unsaturated constituents by GLC.

Column chromatography of Japanese Lac

Column chromatography of Japanese Lac on silica gel (MFC) was conducted with chloroform initially and then with chloroform–ethyl acetate as shown in Table III. Twenty-two fractions were collected and monitored by analytical TLC. Although the method was useful for obtaining larger quantities of minor components its pro-

* The ^{1}H NMR absorption of unsaturated constituents of urushiol in connection with synthetic studies will be described elsewhere in full.

TABLE III

COLUMN CHROMATOGRAPHIC SEPARATION OF JAPANESE LAC

EA = Ethyl acetate; C = chloroform.

Fraction no.	*Solvent*	*Wt. (g)**	*R_F (TLC) in chloroform–ethyl acetate (90:10)*
1	Chloroform	0.1160 (4.5)	0.75 (Cardanol and non-polar material)
2–12	C–EA (95:5)	1.4525 (58)	0.65 (Urushiol)
13–15	C–EA (90:10)	0.1969 (8)	0.46 *cf.*, Fraction 3
16–18	C–EA (85:15)	0.3156 (12)	0.25 *cf.*, Fraction 4
19–22	C–EA (80:20 to 75:25)	0.1068 (4)	Baseline *cf.*, Fraction 5 and baseline material.
(residue on column)		(13.5)	

* Percentages are given in parentheses.

tracted nature resulted in some deterioration of fractions, a feature observed by others[15], and only an approximate estimate of composition could be obtained. Methylated Japanese Lac was considerably more stable in column chromatographic conditions. By column chromatography of methylated Japanese Lac the phenolic monomethyl ether (purple fraction) and the dimethyl ether (yellow fraction) were effectively separated, the former being eluted first thus confirming its less polar character in adsorption conditions. The extensive range of fractions collected was monitored by analytical TLC and by GLC upon the stationary phase SE-30 and small differences both in R_F values and retention times suggested that some fractionation of the unsaturated constituents of the mono- and dimethyl ethers was taking place in column adsorption chromatographic experiments.

GLC of Japanese Lac and its hydrogenated and methylated products

GLC examination of Japanese Lac on the stationary phase 3% SE-30 at 220°C showed a series of peaks, at first thought to be due to the bands found in TLC analysis, at the (uncorrected) retention times of 6.4 min (trace %), 9.0 min (trace %), 10.8 min (5.8%), 14.2 min (26.7%), 17.2 min (57.3%), 21.0 min (10.2%) and 28.6 min (trace %). The main urushiol band from preparative TLC gave a similar chromatogram while the lower bands referred to as fractions 3, 4 and 5 were substantially non-volatile. Similar results were shown by the fractions from column chromatography. The succession of peaks was clearly due to the partial resolution of urushiol into its unsaturated constituents since hydrogenated urushiol exhibited only one peak. Subsequently this partial resolution, which does not take place with cardanol or cardol upon 3% SE-30[16] and is largely due to the triene constituent having 8,11,13- rather than 8,11,14-unsaturation, was confirmed later by GLC–MS on the bis(trimethylsilyl)ether. The uncertain level of separation of the first peak comprising the saturated and monoene constituents from the second consisting of the diene and triene constituents and the tailing encountered precluded quantitative analysis of the phenols. Methylations of Japanese Lac afforded products with symmetrical peaks but

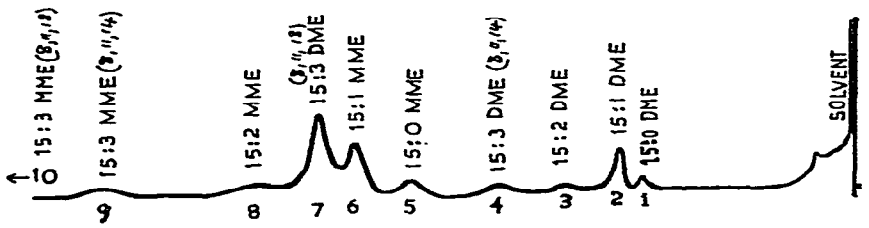


Fig. 1. GLC of methylated urushiol on 2% PEGA at 200°C. DME = Dimethyl ether; MME = monomethyl ether.

still showing resolution not only of the unsaturated constituents but also of mono- and dimethyl ethers. GLC monitoring of fractions from the column chromatographic separation of methylated Japanese Lac again confirmed these findings. Hydrogenated and methylated Japanese Lac by GLC analysis on 3% SE-30 at 220°C gave peaks at the uncorrected retention times of 9.7 min (1.9%), 13.5 min [64.7%, (15:0)-urushiol dimethyl ether], 15.2 min [27.7%, (15:0)-urushiol monomethyl ether] and 22.6 min [5.7%, (17:0)-urushiol dimethyl ether]. The identity of the two main bands was confirmed from the GLC retention times of the purple band (monomethyl ether) and yellow band (dimethyl ether), separated by preparative TLC, both of which gave single GLC peaks.

By GLC the monomethyl ether is more polar than the dimethyl ether while the reverse holds for TLC. Quantitative analysis could only be achieved by complete resolution of the unsaturated constituents for which 2% PEGA[17] appeared to be a more suitable stationary than 3% SE-30. Methylated Japanese Lac, the methylated urushiol band from preparative TLC and the hydrogenated and methylated mono- and dimethyl ethers were examined on 2% PEGA at 200°C and the retention times observed have been summarised in Table IV. Five peaks were observed in each group as seen in Fig. 1, the fourth and fifth peaks being due from MS studies to the isomeric 8,11,14- and 8,11,13-trienes respectively. Prolonged methylation resulted in a diminution of the series of peaks due to the monomethyl ether and a corresponding increase in those of the dimethyl ether as seen in the GLC trace (Fig. 2). The relative retentions[17] of the (15:0), (15:1), (15:2) and (15:3) constituents of cardanol methyl ether were similar to those for the first four peaks in each group. The greater retention of the major peak due to the 8,11,13-triene was in conformity with data for conjugated compared with non-conjugated dienes[18] and the retention time of the 8,11,14-triene was in agreement with that observed for the triene constituent of *Rhus toxico-*

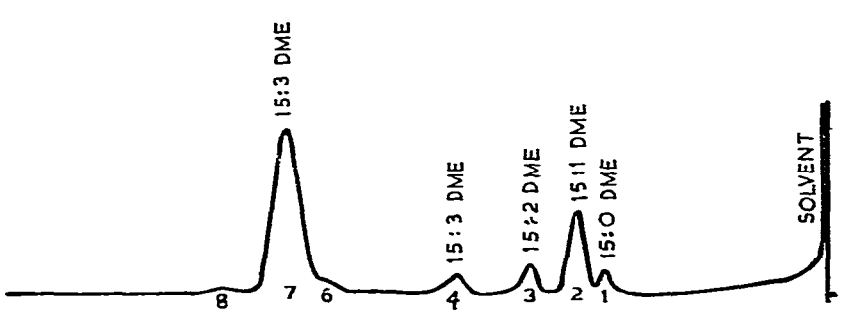


Fig. 2. GLC of methylated Japanese Lacquer (prolonged reaction) on 2% PEGA at 200°C.

TABLE IV

RETENTION DISTANCES (RD) (mm), TIMES (RT) (min) AND RELATIVE RETENTIONS (RR) OF CONSTITUENTS OF METHYLATED AND TRIMETHYLATED URUSHIOL BY GLC ON 2% PEGA AT 200°C

Parameter		*Dimethyl ether and bis-trimethylsilyl ether*					*Monomethyl ether*				
		(15:0)	*(15:1)*	*(15:2)*	*(15:3) 8, 11, 14*	*(15:3) 8, 11, 13*	*(15:0)*	*(15:1)*	*(15:2)*	*(15:3) 8, 11, 14*	*(15:3) 8, 11, 13*
Methylated urushiol (Methylated band 2 from preparative TLC)	RD	52.7 ± 0.80	58.50 ± 0.90	70.2 ± 0.70	87.8 ± 1.00	128.0 ± 1.73	108.5 ± 0.70	120.0 ± 1.40	144.5 ± 0.70	183.0	271.0
	RT*	10.54	11.70	14.04	17.56	25.60	21.7	24	28.9	36.6	54.2
	RR	1.00	1.11	1.33	1.67	2.43	2.06 (1.00)	2.28 (1.11)	2.74 (1.33)	3.47 (1.68)	5.15 (2.49)
Methylated hydrogenated urushiol	RD	54.0**	–	–	–	–	109	–	–	–	–
Trimethylsilylated urushiol	RD	62.8 ± 1.02	68.4 ± 0.83	78.3 ± 0.15	95.5 ± 1.01	138.9 ± 0.30					
	RT***	6.28	6.84	7.83	9.55	13.89	–	–	–	–	–
	RR ‡	0.59 (1.00)	0.65 (1.10)	0.74 (1.25)	0.91 (1.54)	1.32 (2.24)					

* Chart speed 5 mm/min.

** The retention distance of (15:0) urushiol was 302.3 mm, but the unsaturated constituents of the dihydric phenol had impracticably long retentions.

*** Chart speed 10 mm/min.

‡ Relative to (15:0) urushiol dimethyl ether(= 1). Values in parentheses are relative to bis- trimethylsilylurushiol.

TABLE V

COMPOSITION (%) OF UNSATURATED CONSTITUENTS OF URUSHIOL ETHERS BY GLC ON 2% PEGA

Parameter	*Constituent*				
	(15:0) Saturated	*(15:1) Monoene*	*(15:2) Diene*	*(15:3) 8, 11, 14-Triene*	*(15:3) 8, 11, 13-Triene*
Dimethyl ether					
Mol. wt.	348	346	344	342	342
GLC peak no. (Fig. 2)	1	2	3	4	7
Normalised compn. (%) (from peak area)	4.93 ± 0.19	18.45 ± 0.17	8.85 ± 0.16	5.78 ± 0.15	62.00 ± 0.37
Bis(trimethylsilyl) ether					
Mol. wt.	464	462	460	458	458
GLC peak no. (Fig. 3)	E	A	B	C	D
Normalised compn. (%) (from peak area)	3.83 ± 0.25	19.44 ± 0.82	8.79 ± 0.37	5.79 ± 0.27	62.13 ± 0.98

dendron[7,19]. Mass spectral and GLC–MS examination has confirmed these observations as described in the next section. The co-occurrence of a minor proportion of the 8,11,14-triene with the 8,11,13-triene is both of biosynthetic interest and because previous analyses by column chromatography[4,5] have not detected its presence.

Following a number of GLC analyses, summarised in Table V, a semi-quantitative* determination of the % composition of the unsaturated constituents was possible. Methylation although useful for spectral characterisation was not ideal for volatilisation purposes since it was a substantially incomplete process and the elution of the monomethyl series of peaks prolonged the procedure. Remarkably, complete trimethylsilylation was found to be relatively easily achieved by interaction of urushiol in warm pyridine solution with bis(trimethylsilyl)acetamide. The retention times of the bis(trimethylsilyl) derivatives of the constituents of urushiol on 2% PEGA at 200°C are given in Table IV, and a chromatogram shown in Fig. 3 obtained at 185°C for GLC–MS purposes. The compositional results of semi-quantitative analysis are shown in Table V and the results are in good agreement with those for the dimethyl ether series and indicate the presence of 62% of the 8,11,13-triene, 6% of the 8,11,14-triene, 9% of the 8,11-diene, 19% of the 8-monoene and 4% of the saturated constituent.

TABLE VI

% COMPOSITION OF UNSATURATED CONSTITUENTS OF URUSHIOL BY MASS SPECTROMETRY

Parameter	*Urushiol constituent*			
	(15:0) Saturated	*(15:1) Monoene*	*(15:2) Diene*	*(15:3) 8, 11, 13- and 8, 11, 14-Trienes*
Uncorrected normalised composition (from peak height)	8.53 ± 0.97	19.34 ± 1.69	10.11 ± 0.40	62.02 ± 2.59
(P + 2) peak, % of P	2.63	2.63	2.63	2.63
Normalised composition, semi-corrected	8.22	19.54	8.96	63.55

On PEGA as stationary phase urushiol bis(trimethylsilyl) ethers were more volatile than dimethyl ethers while on SE-30 the reverse applied. The relative retentions on the latter of (15:0)-urushiol bis(trimethylsilyl) ether, (15:0)-cardanol trimethylsilyl ether, (15:0)-urushiol dimethyl ether and (15:0)-urushiol monomethyl ether were 1.725, 1.00, 1.00, 1.15 respectively.

Our work on the determination of relative response factors of the unsaturated constituents is as yet incomplete. Argentation analytical TLC with the solvent, chloroform–ethyl acetate–formic acid (80:20:2) indicated the presence of five unsaturated

* The analysis is termed semi-quantitative since response factors for the unsaturated constituents were not available to correct the results. The magnitude of the correction is considered to be small however.

TABLE VII

COMPOSITION OF TRIMETHYLSILYLATED JAPANESE LAC BY GLC–MS ON NON-POLAR E-301

No.	*Rel. peak height (GLC)*	*Uncorrected composition (%), normalised**											
		C_{15} Urushiol				*C_{17} Urushiol*				*C_{15} Hydroxyurushiol*			
		MW 458 (15:3)	*460 (15:2)*	*462 (15:1)*	*464 (15:0)*	*486 (17:3)*	*488 (17:2)*	*490 (17:1)*	*492 (17:0)*	*546*	*548*	*550*	*552***
1	0.97	16.9	20.2	54.7	9.2								
2	2.35	7.8	22.3	59.7	10.4								
3	1.20	4.4	18.6	44.2	32.8								
4	0.56	37.7	20.1	18.8	23.4								
5	3.32	80.9	15.3	3.4	0.4								
6	1.14	72.5	19.3	7.7	0.5								
7	0.03					17.2	59.0	16.9	6.9				
8	0.06					13.1	29.5	59.0	10.8				
9	0.04					8.8	14.6	38.4	10.1	3.9	12.5	8.7	3.0
10	0.14					9.5	11.7	17.2	4.7	28.1	19.3	7.5	2.0
11	0.27									43.6	41.4	13.2	1.8
12	0.15									42.8	37.8	16.1	3.3

* Calculated from peak heights in the mass spectrum.

** Earlier determinations had indicated this group of peaks as having peaks in the range 548–554.

materials, in agreement with the GLC results described. Preparative separations however were unsatisfactory because of the instability of urushiol. Currently work is proceeding on the argentation TLC separation of the bis(trimethylsilyl) ethers which are readily converted to the parent phenol, rather than by column chromatography of the dibenzyl ether and reductive chemical removal of the protective group[20]. Our more recent analytical-scale results[21] suggest that preparative HPLC would be a more convenient separatory procedure for obtaining the saturated, monoene, diene and triene constituents.

We have examined the conversion of urushiol to its diacetate in pyridine solution with acetic anhydride and found it to be an inferior procedure to the formation of ether derivatives. Molecular distillation of Japanese Lac, acetylation of the distillate with acetic anhydride containing sulphuric acid followed by HPLC analysis of the diacetate has revealed a complex mixture[2], which may be partly ascribable to the acidic conditions of derivative formation. These can result in rearrangement, isomerisation and polymerisation of cashew phenols[6]. Our GLC results on bis(trimethylsilyl) derivatives have not indicated the presence of fully conjugated material at longer retention than the 8,11,13-triene although such peaks would be broad and, if minor materials, would be difficult to observe at long retentions.

MS and GLC–MS

The unsaturated composition of Japanese Lac by the direct insertion technique, as for the cashew phenols[8], has been determined by mass spectroscopy. The normalised results from peak height measurements for the saturated, monoene, diene and triene (8,11,13 and 8,11,14) constituents are summarised in Table VI, the peak heights of the diene, monoene and saturated having been corrected for the (P + 2)

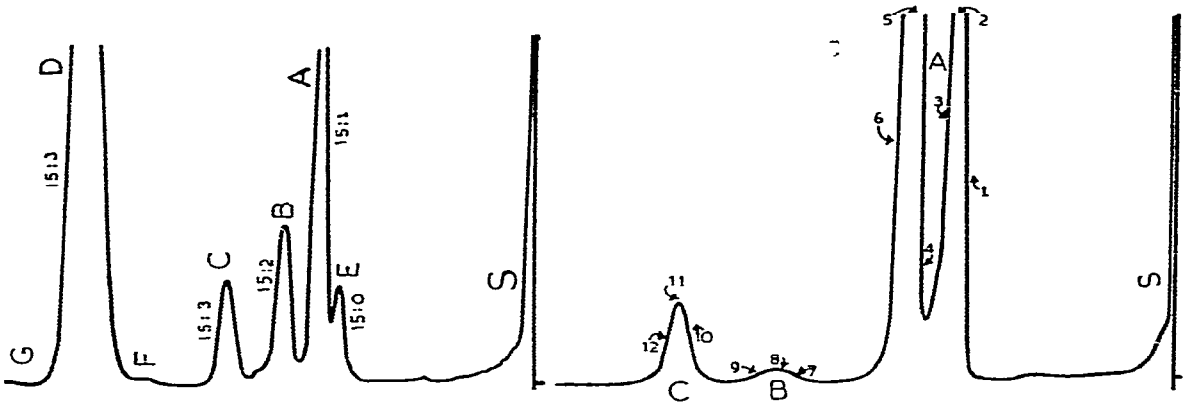


Fig. 3. GLC–MS of urushiol bis(trimethylsilyl) ether on 2% PEGA at 185°C.
Fig. 4. GLC–MS of Japanese Lacquer trimethylsilylated on 2% E-301 at 230°C.

peak contribution of the triene, diene and monoene respectively[8], but without allowance for the small difference in response factors to be expected for the unsaturated constituents. Moderately good agreement is shown with the results of GLC analysis (Tables IV and V). Methylation prior to MS did not appear necessary since the phenolic unsaturated constituents were sufficiently volatile. The unsaturated composition obtained from peak heights for the dimethyl and monomethyl ethers of the triene, diene, monoene and saturated constituents although reproducible, was different from that derived from the phenols suggesting that response factors may be intrinsically more important for the methyl derivatives, since the same sample examined by GLC gave results in agreement with those from MS on the phenols*. The unsaturated composition of C_{17} urushiol (bis-homourushiol) was also found from the series of peaks at 342, 344, 346, 348 for its triene, diene, monoene and saturated constituents respectively. Mass spectroscopy of methylated hydrogenated Japanese Lac was convenient for determining, by the repeated scanning procedure, the proportion of monomethyl and dimethyl ethers from the peak heights of the molecular ions at *m/e* 334 and *m/e* 348.

GLC–MS was used to identify the major long retention 8,11,13-triene constituent and certain minor components of greater polarity than urushiol. The % compositional results in terms of the normalised peak heights with the non-polar stationary phase E-301 which gives separations similar to SE-30 are shown in Table VII for the chromatogram of bistrimethylsilylated Japanese Lac represented by Fig. 4. Scans 1–6 show the results of the partial separation which takes place into a first peak for the saturated and monoene constituents (MW 464, 462) and a second peak for the diene and triene constituents (MW 460, 458). Scans 7 and 8 are for the C_{17} urushiol constituent (MW 486, 488, 490, 492) and scans 9–12 evidence of a minor component existing as four constituents (MW 546, 548, 550, 552) all of which were 88 mass units greater than their urushiol counterparts.

GLC–MS of the bistrimethylsilylated Japanese Lac on the semi-polar 2% PEGA at 185°C (Fig. 3) shows the peaks A, B, C, D referred to in Table VIII

* Our results on the MS of the dimethyl ether of urushiol show some agreement with those in a brief communication[22] on methylated Japanese Lac of uncertain origin, and which were not corrected in any way.

TABLE VIII

COMPOSITION OF TRIMETHYLSILYLATED JAPANESE LAC BY GLC–MS ON SEMI-POLAR PEGA

Scan no.	*Peak*	*Rel. peak height*	*Uncorrected composition (%), normalised**			
			MW 458 (15:3)	*460 (15:2)*	*462 (15:1)*	*464 (15:0)*
1	A	2.49		1.4	66.3	32.3
2	A			1.4	79.5	19.1
3	B	1.00		54.1	35.6	10.4
4	C	0.74	28.9	26.6	33.1	11.4
5	D	4.87	65.6	18.4	11.3	4.7
6	D		61.5	20.8	13.4	4.3

* Some carry over from successive scans was encountered giving in each case a higher background of the preceding constituent than expected for % (P + 2).

with major masses corresponding to the 8-monoene, 8,11-diene, 8,11,14-triene and 8,11,13-triene constituents respectively, confirming that the major material is trienoid. E represents the saturated constituent and F, G appear to represent traces of monotrimethylsilylated constituents.

From the peak height in the gas chromatogram at the particular scan number (1–6) in Table VIII an approximate composition can be calculated based on Σ (peak height_{GLC} × peak height_{MS}) for each constituent and final normalisation, the results of which show a measure of agreement with those in Tables V and VI.

Minor components of Japanese Lac

The minor components of Japanese Lac were most readily seen by TLC. By GLC–MS of the bistrimethylsilylated natural product only one minor component (*m/e* 546–552) was revealed. It seemed probable that the remaining minor materials had masses above this and since GLC was negative, MS on the trimethylsilyl derivatives of the fractions having R_F values 0.46, 0.25, 0.1, obtained by preparative TLC was investigated. The findings have been summarised in Table IX.

The main urushiol band (R_F 0.65) trimethylsilylated gave as expected from Table VII principal masses in the range 458–464 and 486–492. Material of R_F 0.46 contained some of the preceding masses and higher constituents with *m/e* 914–922 suggesting the presence of dimeric material. Bands with R_F 0.25 and 0.1 in addition to containing trimethylsilylated urushiol revealed *m/e* 546–552 corresponding to those masses found in GLC–MS examination on E-301. Different levels of side-chain unsaturation were clearly present and with the objective of obtaining the more stable saturated form and also of preventing reversion of dimeric type substances to the monomer*, hydrogenated Japanese Lac was used for the isolation of the minor materials.

MS examination of the trimethylsilylated materials gave single peaks with prominent molecular ions. The more polar material (R_F 0.25) indicated a molecular

* Diels-Alder adducts (*cf.*, 8) undergo this reversion.

TABLE IX

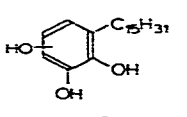
MOLECULAR IONS OF PRINCIPAL COMPONENTS FROM PREPARATIVE TLC SEPARATIONS BY MS

*Fraction no. (R_F)**	*Masses of molecular ions of trimethylsilylated material*	
	Unhydrogenated fraction	*Hydrogenated fraction*
(1) (0.75)	–	304, $C_{21}H_{36}O$ (15:0)-cardanol**
(2) Main urushiol band (0.65)	458, 460, 462, 464, (C_{15} side chain) 486, 488, 490, 492 (C_{17} side chain)	464, $C_{27}H_{52}O_2Si_2$ 492, $C_{29}H_{56}O_2Si_2$
(3) (0.46)	914–926 (and 458–464)	926, $C_{54}H_{102}O_4Si_4$
(4) (0.25)	546–582 (and 458–464)	552, $C_{30}H_{60}Si_3$; 926 (small)
(5) (0.1)	546–552 (and 458–464)	552, $C_{30}H_{60}O_3Si_3$
(6) Baseline (0)	458, 460, 462, 464 (small)	348, $C_{21}H_{34}O_2$

* Solvent, chloroform–ethyl acetate (90:10).
** Ref. 7.

ion *m/e* 552 and the molecular weight by accurate mass measurement was 552.3858 ($C_{30}H_{60}O_3Si_3$ requires 552.3850). The ^1H NMR spectrum, in particular the ratio of aromatic to benzylic protons, the polarity, the similar unsaturation to that of urushiol and the molecular weight [88 mass units more than for bis(trimethylsilyl)urushiol] suggest that this component is an hydroxyurushiol (3) in the tristrimethylsilylated form. From limited spectral evidence compound 3 appears to be 2,3,5-trihydroxy- rather than 1,3,4-trihydroxy-substituted but further work is required with model compounds to substantiate this. Although this compound has not been isolated previously from Japanese Lac its transient formation in the photochemical decomposition of dilute solutions of Japanese Lac has been postulated[10]. The less polar component (R_F 0.46) exhibited a molecular ion at *m/e* 926 in which region accurate mass measurement was not possible. Its structure is most probably 4 or 5, in the form of the tetrakis (trimethylsilyl) derivative, rather than 6 which has been described as a product of oxidised Japanese Lac[23] having R_F 0.2. Our own spectral work is incomplete at the present and we have reservations on this group of diphenyls pending the preparation of certain reference compounds. A greater R_F value and reduced polarity would be expected for the sterically hindered structures 4 and 5 in comparison with 6. For the pairs of compounds, 2,2′,4,4′,6,6′- and 3,3′,4,4′,5,5′-hexachlorodiphenyl, 2,2′,4,4′-tetrachloro- and 4,4′-dichlorodiphenyl, the R_F values reported[24] support this.

Structures such as 7 have been postulated[9] as minor components of oxidised



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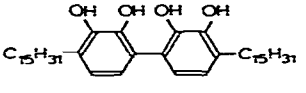

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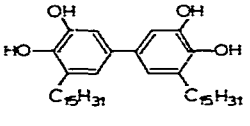

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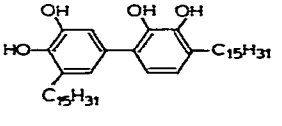

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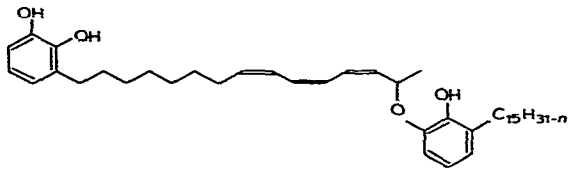

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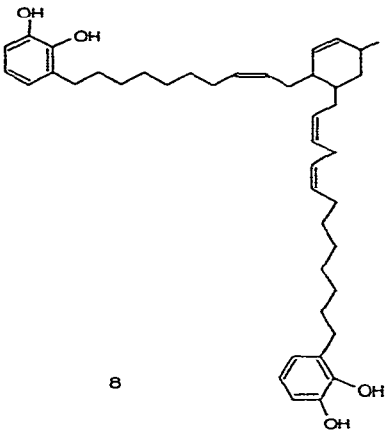
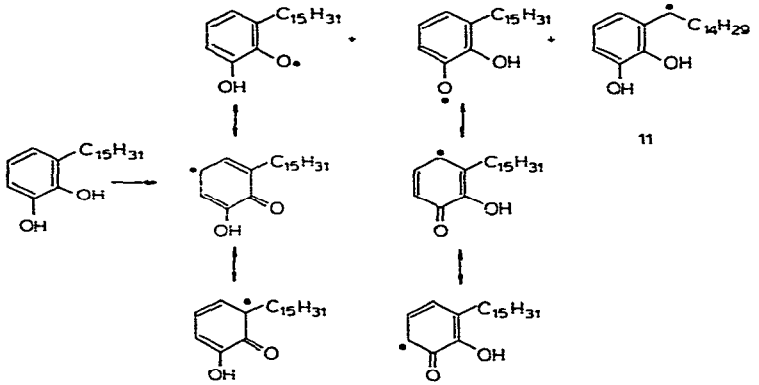


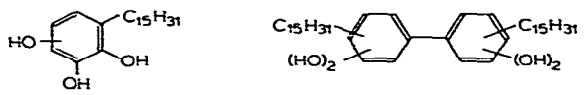
8

Japanese Lac on the basis of model reactions. Trimethylsilylation of the fully saturated material (7, $n = 0$) would afford a derivative with *m/e* 854, a mass not observed however in our work as a major peak. A Diels-Alder type product such as 8, reminiscent of kitol from vitamin A[25,26], after saturation and trimethylsilylation would give a mass at *m/e* 924. It would be expected to be chromatographically similar to urushiol itself but our experiments have not revealed its presence.

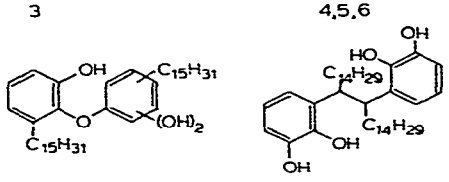
It seems likely that minor materials arise from a common precursor of a reac-



9 10 11



3 4,5,6



12 13

tive intermediate type which can then yield diverse products. An ionic mechanism has been proposed[10] to explain the ethoxylation of urushiol in dilute ethanol solution in the presence of light and moisture. In hexane, carbon tetrachloride and chloroform solutions of urushiol were observed to be stable. The conditions in our work of preparative TLC and the subsequent processing of separated bands under essentially anhydrous conditions and in the absence of light are therefore unlikely to have contributed to the formation of minor materials. Their origin must lie with Lac in its natural state and at the extraction stage.

A scheme proceeding by a radical participation is shown involving attack at either oxygen of saturated urushiol to give the resonant radicals 9, 10 or to a less extent at benzylic carbon to give 11 which could then by the action of water or by coupling reactions lead to the observed products (3) and others (4, 5, 6) believed to be present. In the natural product the side-chain allylic methylene system would be likely to be involved also and it is clear that other modes of reaction such as the formation of 12 and 13 could occur. A radical such as 9 would be more sterically stabilised[27] than 10 as well as through the contribution of a tertiary C radical and thus the formation of 2,3,5-trihydroxypentadecylbenzene would appear to be favoured. From preparative TLC it is clear that many minor polar products are present in urushiol and a more complex oxidative situation is apparent than has perhaps hitherto been appreciated.

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