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REVERSED-PHASE LIQUID CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF CONSTITUENTS OF URUSHIOL IN THE SAP OF THE LAC TREE, *RHUS VERNICIFERA*

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SUMMARY

Urushiol in the sap of lac tree, *Rhus vernicifera*, is directly separated into more than ten components by reversed-phase liquid chromatography using acetonitrile–water–acetic acid (80:20:2 of 90:10:2) as the eluent. The ^{1}H NMR parameters, especially those for the triolefinic components, are presented. New substances, 3-(10′*Z*, 13′*E*, 15′*Z*-heptadecatrienyl)catechol and 4-(8′*Z*, 11′*E*, 13′*Z*-pentadecatrienyl)catechol, were found, although they were contaminated with 3-(10′*Z*, 13′*Z*, 16′-heptadecatrienyl)catechol and 4-(8′*Z*, 11′*Z*, 14′-pentadecatrienyl)catechol, respectively. The quantitation by this separation system with refractive index detection nearly coincides with the weight content of each component. Samples from two different sources, Hupei and Kiangsi in China, were analysed by this method.

INTRODUCTION

Urushiol is a mixture of 3-substituted catechols with C_{15}-alkyl or -alkenyl groups[1–3]. Recently, Yamauchi *et al.*[4] determined the structure of the main component of urushiol in lac trees, *Rhus vernicifera*, to be 3-(8′*Z*, 11′*E*, 13′*Z*-pentadecatrienyl)catechol (1) by liquid chromatographic (LC) separation of O-dimethyl derivatives followed by ozonolysis, and identified nine other components.

Analysis by gas–liquid chromatography as trimethylsilyl ethers[5–7] and LC as diacetyl[8] and dimethyl derivatives[4,9] has been used, but the derivatization was complicated and quantification was difficult[10,11]. Further, intact samples are required in order to examine contact allerginicity[12].

Recent papers have described the direct LC separation of urushiol. Elsohly and co-workers[13,14] reported the separation on 10-μm ODS-silica gel columns of urushiol in poison oak and poison ivy. Yamauchi *et al.*[15] examined the separation

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of lac urushiol on several types of ODS-silica gel columns and found low carbon-content gel columns to be suitable. Octylsilylated silica gel columns were also found to be effective for the separation of urushiol[16]. However, they could discriminate only four components, depending on the number of olefinic groups in the side-chain, despite the presence of more than ten components in lac urushiol[4], and considerable peak broadening occurred on ODS columns of higher efficiency.

In examining the chromatographic behaviour of urushiol, we found that the use of acetic acid-containing eluents prevented the peak broadening usually observed for acidic substances. Here we demonstrate the applicability of reversed-phase LC with an acidic eluent to urushiol analysis. The 400-MHz ^{1}H NMR spectra were analysed for the triolefinic components of urushiol given by this method, confirming the previously presented unique side-chain structures. Further, new components found by this method were identified by comparing the ^{1}H NMR spectra with those of compound 1.

EXPERIMENTAL

Materials and procedure

The sap of lac trees (*Rhus vernicifera*) (Hupei or Kiangsi, China) was stirred with 3 parts of acetone and filtered through a filter-paper. The filtrate was evaporated to give a crude urushiol preparation, which was subjected to preparative gel-permeation chromatography (GPC). Monomeric urushiol was obtained in a yield of more than 90%. It was applied to LC columns and the fractions obtained were evaporated at 35°C to give aqueous urushiol dispersions, which were extracted with distilled *n*-hexane. The hexane solution was evaporated at 35°C, yielding the components of urushiol.

Instruments

The chromatographic apparatus used was as described previously[4]. Preparative GPC was carried out on two columns of TSK-gel G2000HG (60 × 2.2 cm I.D.) using chloroform as eluent and a refractive index (RI) monitor. Preparative reversed-phase LC was effected on a 30 × 2.2 cm I.D. column packed with ODS-silica gel (Hitachi 3053, 5 μm) or on a 25 × 0.8 cm I.D. column packed with ODS-silica gel (TSK-gel LS410, 5 μm). For analytical purposes, ODS-silica columns (Develosil ODS-5, 5 μm, 25 × 0.45 cm I.D.; or ODS-3, 3 μm, 15 × 0.45 cm I.D.; Nomura Chemicals, Seto, Aichi, Japan) were used.

Electron-impact mass spectra were obtained with a Hitachi RMU-6E mass spectrometer. IR spectra were recorded with a JASCO IRA-102 instrument. ^{1}H NMR spectra were obtained on a Varian EM-390 spectrometer (resonance frequency 90 MHz) or a JEOL GX-400 Fourier transform (FT) spectrometer (400 MHz) in C^2HCl_3; chemical shifts were determined from internal tetramethylsilane (for 90 MHz) or chloroform (δ7.26 for 400 MHz).

RESULTS AND DISCUSSION

Separation

Monomeric urushiol was prepared by GPC of the sap of lac tree from Hupei,

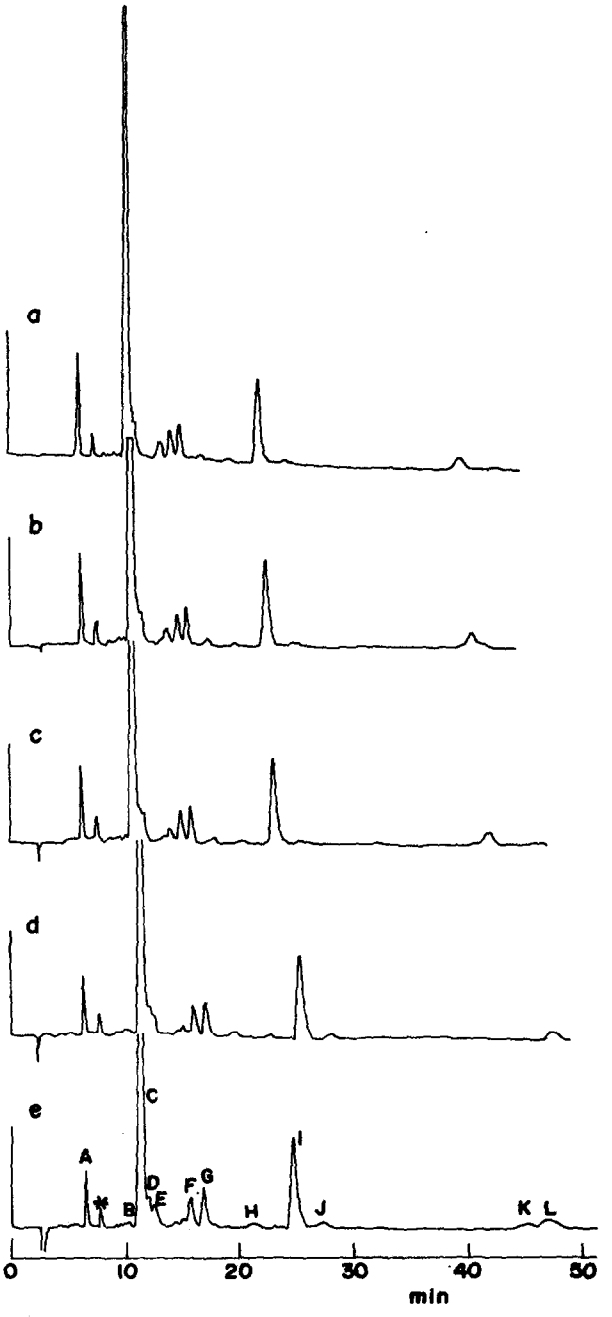


Fig. 1. Analytical chromatograms of urushiol in the sap of *Rhus vernicifera* from Hupei, China. Conditions: column, Develosil ODS-5 (5 μm), 25 × 0.45 cm I.D.; flow-rate, 1.25 ml/min; detector, 254 nm, 0.32 a.u.f.s.; eluent, acetonitrile–water–acetic acid (90:10:2) containing silver nitrate: (a) 5.8, (b) 11.6, (c) 29, (d) 58 and (e) 116 m*M*.

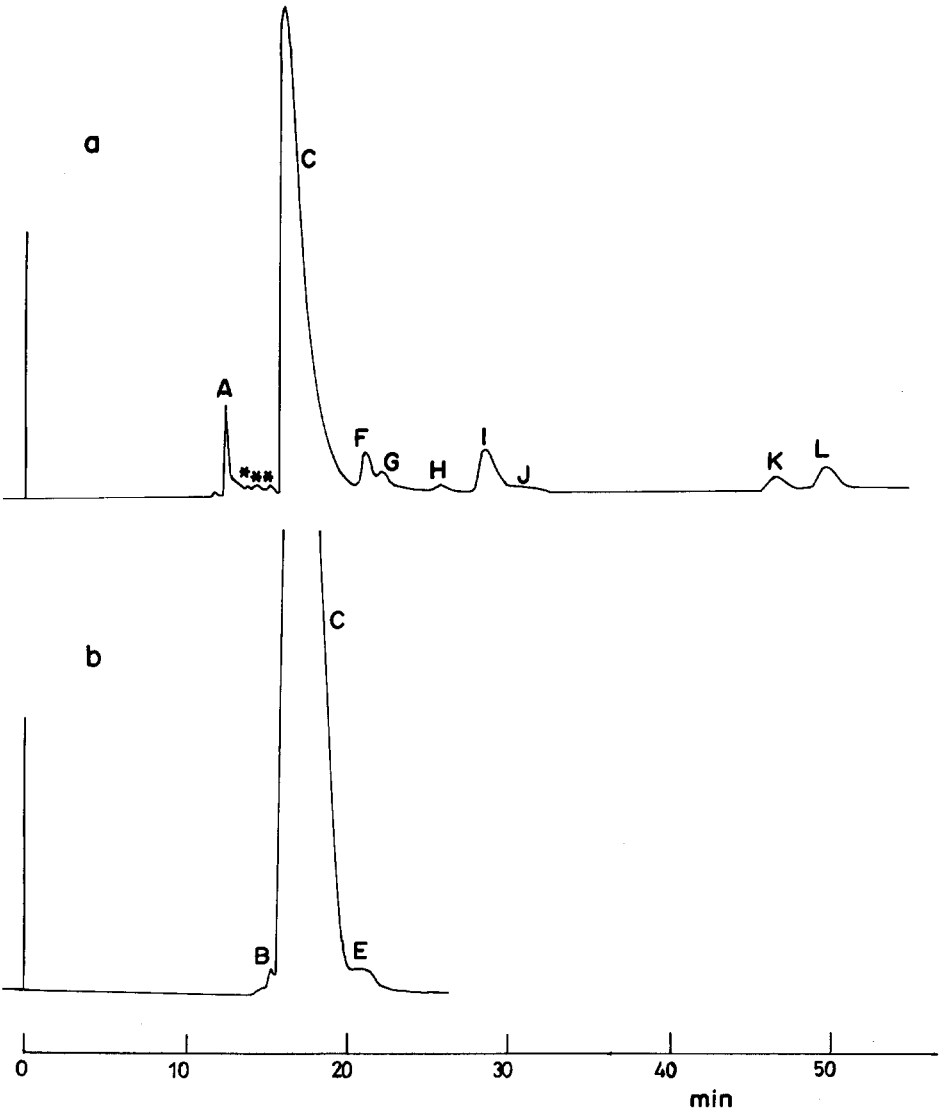


Fig. 2. Preparative chromatogram of urushiol in the sap of *Rhus vernicifera* from Hupei, China, using acetonitrile–water–acetic acid (80:20:2) as the eluent and a UV detector at 254 nm. Other conditions: (a) column, Hitachi 3053 (5 μm), 30 × 2.2 cm I.D.; flow-rate, 11 ml/min; loading, 140 mg; (b) column, TSK-gel LS-410 (5 μm), 25 × 0.8 cm I.D.; flow-rate, 2.5 ml/min; loading, 15 mg.

China, as described by Yamauchi *et al.*[4]. The elution behaviour of intact urushiol was examined on a 150 × 4.5 mm I.D. ODS-silica column, with acetonitrile–water (90:10) containing 2% of acetic acid as eluent. A slightly acidic eluent is preferred from the viewpoint of the stability of urushiol and the separation efficiency.

It was reported[17] that the addition of silver ions to the mobile phase was effective in resolving retinyl esters in reversed-phase LC. For the separation of urushiol, a substantial improvement could not be achieved by addition of silver nitrate

TABLE I

CHARACTERISTICS OF CONSTITUENTS OF URUSHIOL IN *RHUS VERNICEFERA*

Compound	*Peak in Fig. 1*	*Mass spectrum:*		*IR shift (cm^{-1})*	
		M^+	*Base peak*	*Olefin*	*Phenyl*
1*	C	314	123	980, 945, 920	770, 730
3	E	314	123	985	770, 730
4	F	316	123	–	770, 730
5	G	316	123	965	770, 730
6	I	318	123	–	775, 730
7	L	320	123	–	775, 735
8*	H	342	123	980, 945, 920	770, 730
10	J	344	123	–	770, 730
11	K	346	123	–	770, 730
12*	A	314	123	980, 943, 920	860, 810

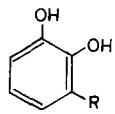
* Compounds 1, 8 and 12 were obtained contaminated with compounds 2, 9 and 13, respectively.

at concentrations up to 290 m*M*, although a slight enhancement in the resolution of peaks C–E (Fig. 1) was observed. We subsequently used eluents without silver ions.

Urushiol was first applied to an ODS-silica preparative column (30 × 2.2 cm) with acetonitrile–water–acetic acid (80:20:2) as the eluent, giving the chromatogram shown in Fig. 2a; fraction C was rechromatographed on a semi-preparative column (25 × 0.8 cm I.D.) (Fig. 2b). After these operations, eleven fractions were separated, although peaks C and D could not be resolved; the peaks marked with asterisks in Fig. 2 were present in only very small amounts and were not analysed here.

Identification

The spectroscopic characteristics of each fraction are summarized in Tables I and II. In each mass spectrum a base peak was observed at *m/z* 123, which corresponds to the dihydroxytropylium ion, $C_7H_5(OH)_2^+$. All of the components showed common IR absorption bands of a catechol nucleus (3400, 1640, 1620 and 1280 cm^{-1}) and, except for fraction A, exhibited out-of-plane bending vibrations of a 1,2,3-trisubstituted phenyl group (770 and 730 cm^{-1}), indicating they are 3-alkylcatechol derivatives, *i.e.* urushiol or laccol homologues[18].



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

TABLE II

^{1}H NMR FOR URUSHIOL CONSTITUENTS IN *RHUS VERNICIFERA**

Parameter	*Position*	*Compound*											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*11*	*12*	*13*
Chemical shift δ	OH	5.18		5.10			5.15		5.13		5.16	4.40	
		5.23		5.01								4.46	
	H-3											6.62	
	H-4-6	6.72		6.72	6.70	6.68	6.71	6.68	6.72		6.71		
	H-5											6.54	
	H-6											6.64	
	H-1′	2.61		2.62	2.61	2.62	2.60	2.55	2.61		2.62	2.61	
	H-2′	1.62		1.62	1.59	1.59	1.58	1.60	1.62		1.56	1.62	
	H-3′–6′	1.34		1.33	1.31	1.32	1.29					1.34	
	H-7′	2.05		2.04	2.02	1.99	1.99		1.34		1.31	2.06	
	H-8′	5.41		5.42	5.35	5.38	5.35					5.42	
	H-9′	5.40		5.38	5.35	5.38	5.35		2.05		2.05	5.43	
	H-10′	2.86		2.80	2.77	2.71	1.99	1.22	5.42		5.34	2.86	
	H-11′	5.64		5.53	5.35	5.38	1.29		5.42		5.34	5.64	
	H-12′	6.35		6.02	5.35	5.38	1.29		2.86		2.03	6.36	
	H-13′	5.99		6.02	2.02	1.99	1.29		5.64		1.31	5.99	
	H-14′	5.44	5.83	5.61	1.31	1.32	1.29		6.36		1.31	5.44	5.83
	H-15′	1.74	4.99*c***	1.73	0.89	0.88	0.87	0.87	5.99		1.31	1.74	4.99*c*
			5.06*t*										5.06*t*
	H-16′								5.44	5.83	1.31		
	H-17′								1.74	4.99*c*	0.87		
										5.06*t*			

Coupling constant (Hz)	3, 5										2.7	
	5, 6										8.4	
	1′, 2′	8.2			7.6	8.0	7.5	7.0	8.2		8.2	
	2′, 3′	8.2							8.7		8.2	
	7′, 8′	7.1		7.1							6.4	
	8′, 9′	10.5		10.4							6.4	
	9′, 10′	7.1		7.1	5.9	5.5			7.1		6.1	
	10′, 11′	6.1		7.1	5.9	5.5			10.5		6.1	
	11′, 12′	14.0		14.7					7.1		14.0	
	12′, 13′	9.7		–					6.1		9.7	
	13′, 14′	9.7	6.0	14.7					14.0		9.7	6.0
	14′, 15′	7.4	16.0*t****	7.1	7.6	6.6			9.7		7.4	16.0*t*
			9.5*c*									9.5*c*
	15′, 15′		1.8									1.8
	15′, 16′								9.7	6.0		
	16′, 17′								7.4	16.0*t*		
										9.5*c*		
	17′, 17′									1.8		1.8
	10′, 12′	1.2									1.2	
	11′, 13′	1.8									1.8	
	12′, 14′	1.2								1.2	1.2	
	13′, 15′	1.1	1.8							1.8	1.1	1.8
	14′, 16′									1.2		
	15′, 17′									1.1		

* The resonance frequency for compounds 1, 2, 3, 8, 9, 12 and 13 was 400 MHz and for the other compounds 90 MHz.

** *c* and *t* represent protons *cis* and *trans* to H-14′.

*** *c* and *t* represent *cis* and *trans* olefinic coupling.

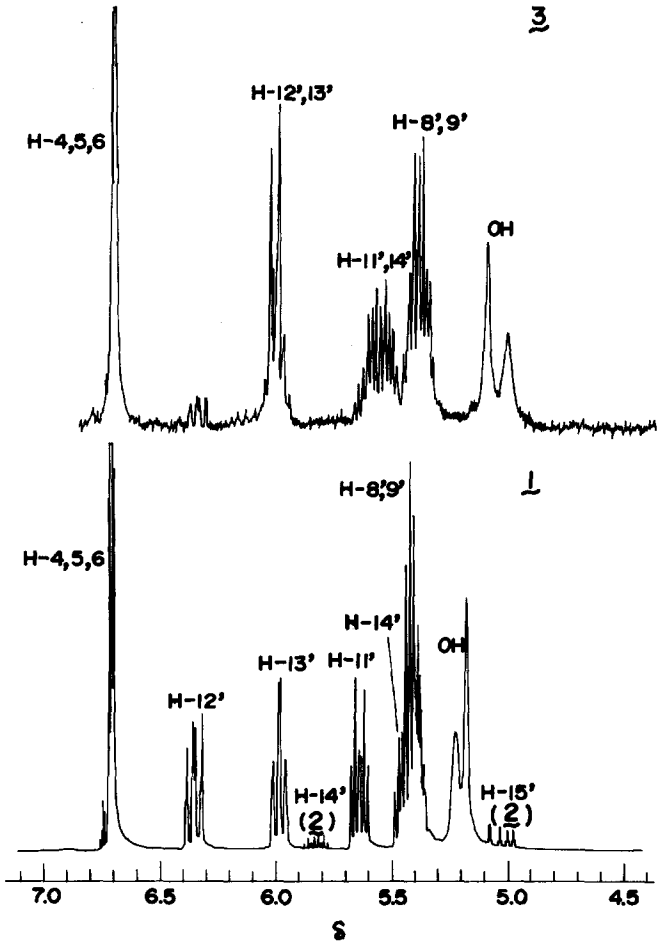


Fig. 3. Partial 400-MHz ^{1}H NMR spectra of triolefinic components of urushiol (1 and 3).

The M^+ values indicated that fractions C and E have a pentadecatrienyl side-chain, F and G a pentadecadienyl side-chain and I and L a pentadecenyl and a pentadecyl side-chain.

Fraction C (contaminated with D) has IR bands at 980 and 945 cm^{-1} with equal intensities due to a *cis–trans* conjugated diene[19] and identified as compound 1. The 400-MHz ^{1}H NMR spectrum (Fig. 3) was analysed as summarized in Table II after extensive decoupling experiments, which confirmed the unique side-chain structure of compound 1. In the spectrum, very small resonances of vinyl protons occurred at δ4.99 (*cis*-H-15'), 5.06 (*trans*-H-15') and 5.83 (H-14'). This may be interpreted as given from compound 2 in peak D.

In the IR spectrum of E, an intense absorption band of a *trans–trans* conjugated diene was observed at 985 cm^{-1}. It was identified with compound 3 after analysis of the ^{1}H NMR spectrum (Table II). The olefinic region of the ^{1}H NMR spectrum is compared with that of 1 in Fig. 3. H-12' and H-13' in compound 3 are

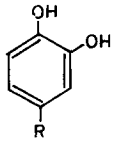
equivalent in chemical shift and an apparent doublet with J = 14.7 Hz is given[20].

Fraction G exhibited an intense IR band of a *trans* double bond at 965 cm^{-1}, in contrast to fraction F, which showed no such signal. Although the exact structures of fractions G and F, especially the locations of double bonds, could not be determined in this study, they were identified as compounds 4 and 5, respectively, referring to previous work[4]. Similarly, fractions I and L were identified as compounds 6 and 7. Compounds 1–7 have already been reported by Yamauchi *et al.*[4] as O-dimethyl derivatives.

From the M^+ values, fractions B, H, J and K were elucidated to have a heptadecatetraenyl, a heptadecatrienyl, a heptadecadienyl and a heptadecenyl side-chain, respectively. Fraction H showed IR and 400-MHz ^1H NMR spectra completely identical with those of fraction C, indicating that it is compound 8 contaminated with compound 9. These compounds were found in the sap of *Rhus vernicifera* for the first time in this investigation.

Fractions J and K exhibited no IR signal of a *trans* double bond and were identified as compounds 10 and 11, respectively, referring to previous work[4]. Fraction B has a heptadecatetraenyl side-chain, as judged from the M^+ value (*m/z* 340). However, its content was as low as 0.1% and a further detailed structural determination could not be carried out here.

Fraction A showed IR bands due to a 1,2,4-trisubstituted phenyl group (860 and 810 cm^{-1}) and those of a *cis–trans* conjugated diene (980 and 943 cm^{-1}). The olefinic region of the ^1H NMR spectrum (Fig. 3a) was completely identical with that of fraction C or H. However, the resonances of phenyl protons were different from those of C and H and are interpreted as resulting from 4-alkylcatechol (Table II). The main constituent of this fraction was identified as a thitsiol derivative[16], compound 12; it may be contaminated with compound 13 as in fractions C and H



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

Compounds 12 and 13 were also found for the first time in this investigation. Analysis: found, C 79.61, H 9.65; calculated for $C_{21}H_{30}O_2$, C 80.21, H 9.62%.

It should be noted that the series of laccol and thitsiol homologues are present in the sap of *Rhus vernicifera* in addition to the urushiol homologues, and that the unique configuration of the side-cain of the main component of urushiol (1) also prevails in triolefinic components of laccol (8) and thitsiol (12). The main triolefinic component of laccol in the sap of the lac tree from Formosa, *Rhus succedanea*, has a side-chain with a similar structure to that of compound 3, *viz.*, 3-(10′*Z*, 13′*E*, 15′*E*-heptadecatrienyl)catechol[21]. These findings are significant to the biosynthetic pathway of urushiol.

Quantification

The amounts of each component of urushiol in the sap of lac trees from Hupei

TABLE III

QUANTITIZATION OF URUSHIOL IN *RHUS VERNICIFERA*

Compound	*Peak in Fig. 1*	R_t* *(min)*	*Hupei*		*Kiangsi by LC*
			By weight	*By LC*	
C_{15}:					
1(2)	C	5.72	70.6	77.5	53.1
3	E	7.56	0.3	–	–
4	F	8.20	3.2	2.3	3.3
5	G	8.68	4.3	3.4	7.2
6	I	12.67	9.6	11.1	32.4
7	L	23.80	3.8	2.1	3.0
C_{17}:					
8(9)	H	9.79	–	0.6	1.1
10	J	14.04	1.6	0.9	Trace
11	K	23.24	2.2	0.8	Trace
C_{15} (1, 2, 4-):					
12(13)	A	3.49	1.0	0.8	0.8

* Retention times in reversed-phase LC. Conditions: column, Develosil ODS-3, 15 × 0.45 cm I.D.; eluent, acetonitrile–water–acetic acid (90:10:2); detection, RI; flow-rate, 1.25 ml/min.

were determined on 0.2–0.5 mg of samples by the proposed LC system on a 3-μm ODS column (15 × 0.45 cm I.D.) with RI detection within 25 min; the baseline separation of peaks K and L could not be obtained. The results are compared with those estimated from their weights after fractionation of *ca.* 200 mg of a sample on a 25 × 0.8 cm I.D. ODS column (Table III). The two sets of values are nearly identical, indicating that the proposed LC method is reliable for the quantification of urushiol components. As an example, urushiol from the sap of lac tree of different origins (Hupei and Kiangsi, China) were analysed, giving the results shown in Table III.

Further detailed analyses of urushiol of different origins (places, time of harvest and ages of trees) are now in progress.

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