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LONG-CHAIN PHENOLS

URUSHIOL, LACCOL, THITSIOL AND PHENYLALKYL CATECHOL COMPOUNDS IN BURMESE LAC FROM *MELANORRHOEA USITATA*

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SUMMARY

Fused-silica capillary gas–liquid chromatography has been used for the separation of both the trimethylsilyl and methylene ether derivatives of some long-chain substituted benzene-1,2-diol (catechol) lipids from *Melanorrhoea usitata*. The sap contains congeners of urushiol (8%), laccol (57%), thitsiol (26%), 3-(phenylalkyl) catechols (8%) and 4-(phenylalkyl)catechols (1%), and many of these metabolites were identified by gas chromatography–mass spectrometry. Reversed-phase liquid chromatography was used to isolate the main chemical component present in both the laccol and thitsiol. These were characterised as (8′*Z*,11′*Z*)-3-(heptadeca-8′,11′-dienyl)catechol and (8′*Z*,11′*Z*)-4-(heptadeca-8′,11′-dienyl)catechol, respectively, by a sequential oxidation scheme whereby methyl esters of the degradation products were obtained and subsequently analysed by gas chromatography–mass spectrometry.

INTRODUCTION

Saps from various kinds of lac tree in the family *Anacardiacae* have been used as excellent decorative coating materials for several thousand years in Asian countries[1]. *Melanorrhoea usitata*, which is also called the Burmese lac tree, has been used as a source of lacquer in Burma and Thailand[2].

Earlier investigations established that a main constituent in Burmese lac, known as thitsiol, consisted of a mixture of 4-substituted benzene-1,2-diol (catechol) compounds possessing a C_{17} *n*-alkyl or alkenyl group at this position[3]. However, the position of the double bond in the side-chain of the thitsiol congeners was not determined[4]. Du *et al.*[5] recently reported that the 3-(phenylalkyl) compounds (Ih) and (Ij) are the main components in Burmese lac, whereas thitsiol, whose structure remained undefined, was present in relatively lower concentrations.

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We have used fused-silica gas–liquid chromatography in conjunction with mass spectrometry (GC–MS) to analyse Burmese lac. An attempt was also made to separate the main components from the lac using reversed-phase liquid chromatography (LC) in order to determine the position of the double bond in thitsiol, and also in laccol, which is the 3-substituted catechol fraction. A scheme was evolved whereby the position of the double bond in the side-chain could be established by cleavage to give readily identifiable fragments.

This relatively simple procedure involves methylation of the phenolic hydroxyl groups, hydroxylation of the double bond, oxidation with Jones reagent, and methylation of the carboxylic acids so that the resultant methyl esters may be identified by GC–MS. It can be applied to other long-chain alkenyl phenols, such as occur in poison oak and poison ivy lipids from the family *Anacardiacae*, in a manner similar to the sodium borohydride reduction procedure described previously[6].

EXPERIMENTAL

Lacquer

Sap of *M. usitata* from near Chiang Mai, Thailand, was analysed. Native sap (65 g) was dissolved in acetone and filtered. The filtrate was evaporated to give the crude lac as a viscous black oil (60 g).

Purification of the laccol or thitsiol fractions produced by column chromatography of the crude lac was achieved by solvent–solvent extraction with hexane and acetonitrile[7].

Derivatives for GC–MS

Trimethylsilylation of the phenolic groups was achieved by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 70°C for 20 min to give bis-O-(trimethylsilyl) derivatives (bis-O-TMS derivatives).

Methylene ethers were made with diiodomethane in dry acetone in the presence of potassium carbonate at 60°C for 5 h. Dimethyl ethers were made from dimethyl sulphate[8]. Methyl esters in benzene solution were prepared with boron trifluoride in methanol.

Glycol was condensed with acetone in the presence of copper sulphate and heated at 50°C for 1 h to yield acetonide derivatives[9].

Hydrogenations were carried out in a Parr apparatus using 5% palladium–carbon at 35 p.s.i., according to the method of Majima[3].

Hydroxylation[10] and oxidative cleavage[11]

A solution of osmium tetroxide (9.6 mg) in anhydrous ether (5 ml) was added to a solution of the dimethyl ether derivative (4.3 mg) in anhydrous ether (10 ml) containing pyridine (6.2 mg). After 24 h standing at room temperature, 10% potassium hydroxide (4 ml) and mannitol (19.9 mg) were added. The mixture was extracted with ethyl acetate (10 ml) and washed with dilute hydrochloric acid and water and dried with magnesium sulphate. Evaporation of the solvent gave the glycol as a colourless solid.

The glycol (2 mg) in acetone (2 ml) was treated with Jones reagent (0.01 ml), and the solution was stirred for 3 min and diluted with water (3 ml). Small amounts

of sodium bisulphite were added. The solution was extracted with ether (10 ml), washed with water and dried with magnesium sulphate. Evaporation of the solvent gave a mixture of carboxylic acids.

GC and GC–MS

GC was conducted using a Hewlett-Packard 5890A chromatograph equipped with a flame-ionisation detector and a Shimadzu C-R3A chromatopac integrator. A 25 m × 0.2 mm I.D. fused-silica cross-linked methyl silicone column (Hewlett-Packard) was used with a temperature programme from 50°C to 280°C at 4°C/min.

GC–MS was carried out using a Hewlett-Packard 5985B GC–MS computer data system. The same type of capillary column (50 m × 0.2 mm I.D.) was used connected directly to the ion source. Samples were introduced onto the chromatograph column using an on-column injector (OCI-3, SGE, Australia). The oven temperature was programmed from 30°C to 300°C at 4 or 8°C/min, with hydrogen as carrier gas at a linear gas velocity of 30 cm/s.

Column chromatography

Column chromatography was effected with silica gel (grade 923, 100–200 mesh) in a glass column (24 × 1.2 in. I.D.) using aluminium foil to exclude light. Burmese lac (20 g) was applied directly to the column, and then eluted with ethyl acetate in light petroleum (b.p. 40–70°C); the percentage of ethyl acetate was gradually increased from 2 to 15%.

Thin-layer chromatography (TLC) and LC

TLC was performed on silica gel (Kieselgel 60G) with 30% ethyl acetate in light petroleum (b.p. 40–70°C). The crude laccol and thitsiol fractions had R_F values of 0.5 and 0.4, respectively.

The liquid chromatograph consisted of a pump Model 6000A and an absorbance detector Model 440 (Waters Assoc., Milford, MA, U.S.A.) connected with an integrating recorder (Hewlett-Packard 3390A). An analytical Resolve C_{18} column (10 × 0.8 cm I.D., 10 μm particle size) with a RCM-100 compression module (Waters Assoc.) and a 20-μl loop injector were used. The mobile phase was acetonitrile–water–acetic acid (90:10:2, v/v/v) at 0.50 ml/min, and the monitoring wavelength was 280 nm.

Spectroscopic analysis

Infrared (IR) spectra were recorded on a Perkin-Elmer 680 spectrometer, ultraviolet (UV) spectra (in ethanol) were recorded on a Pye-Unicam SP8-250 spectrometer, and NMR spectra were recorded on a Hitachi Perkin-Elmer R20B spectrometer (resonance frequency 60 MHz) or a Bruker Fourier transform (FT) spectrometer (400 MHz) in [^{2}H]trichloromethane; chemical shifts were determined from internal tetramethylsilane (for 60 MHz) or trichloromethane (δ 7.26 for 400 MHz).

RESULTS AND DISCUSSION

Analysis of crude Burmese lac

Urushiol congeners from *Rhus vernicifera* have recently been successfully sep-

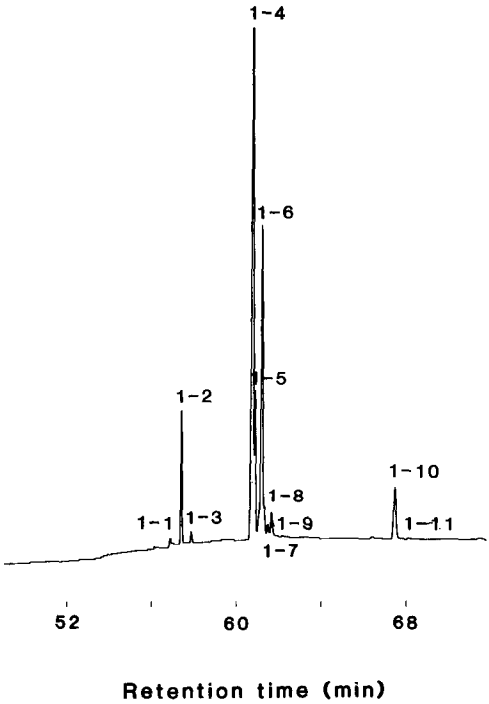


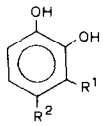
Fig. 1. Gas chromatogram of the bis-O-(trimethylsilyl) derivatives of Burmese lac on a 25 m × 0.2 mm I.D. capillary fused-silica cross-linked methyl silicone column; temperature programme from 50°C to 280°C at 4°C/min.

arated and identified by GC–MS using both packed[12] and capillary[13] columns. Using capillary columnn GC–MS we found that the trimethylsilyl derivatives of phenols from crude Burmese lac could be successfully separated into many components as shown in Fig. 1. The concentration of the minor components in the lac, such as 1-9 and 1-11, could be enhanced by column chromatography, as shown by GC–MS.

The structures of most of these bis-O-TMS derivatives could be inferred from mass spectral data (Table I). The *m/e* of the parent peak gave the length of the aliphatic side-chain, and the degree of unsaturation. The only unknown factors were the aromatic substitution pattern and the position of the double bonds in the side-chain of the monoene or diene components.

All of the bis-O-TMS derivatives showed a prominent molecular ion together with fragments at *m/e* 267 and 179, which were formed by benzylic cleavage of the side-chain to give a bis-O-TMS tropylium ion and the loss of tetramethylsilane[7,14]. In all cases it was found that benzylic cleavage to produce a strong bis-O-TMS tropylium ion was more prominent for the 1,2,4-substituted aromatic system than for the isomeric 1,2,3-substituted products in agreement with our previous work[15], and the former compounds had slightly higher retention times on GC.

The saturated hydrourushiol (Ib) together with the previously unknown isomer (Ic), and hydrolaccol (Ig) were readily identified by the *m/e* of the parent ion, and the intensity of the bis-O-TMS tropylium ion.



I

	R^1	R^2
Ia	Pentadecenyl	H
Ib	Pentadecyl	H
Ic	H	Pentadecyl
Id	Heptadeca-8′,11′-dienyl	H
Ie	Heptadecenyl	H
If	H	Heptadeca-8′,11′-dienyl
Ig	Heptadecyl	H
Ih	10′-Phenyldecyl	H
Ii	H	10′-Phenyldecyl
Ij	12′-Phenyldodecyl	H
Ik	H	12′-Phenyldodecyl
Il	H	Heptadecyl

Similar considerations gave the length of the side-chain and the substitution pattern in the phenylalkyl catechol lipids I(h–k). These components were easily identified by the tropylium ion at *m/e* 91, which is derived from the alkylbenzene part of the side chain. Du *et al.*[5] reported that (Ij) was the main component (53%) together with (Ih) (11%) in the sap of *M. usitata*, but (Ii) and (Ik) are now reported to be present for the first time. Any differences in the composition of Burmese lac that have been reported[3,5] are probably due to polymerisation reactions occurring during storage.

Chromatographic analysis of the bis-O-TMS derivatives of hydrogenated Burmese lac reduced the number of components present in the mixture as shown in Fig. 2. Peak assignments are shown in Table II, and this also gives the relative amounts of urushiol, laccol, thitsiol, and minor constituents in the lac. The retention time of the lipids (Ih) and (Ii) appeared to overlap with the hydrolaccol (Ig) and hydrothitsiol (Il) respectively, but they could be detected by the presence of the tropylium ion (*m/e* 91) on close examination of the mass spectra of the peaks.

Further confirmation of these structures was obtained by GC–MS analysis of the methylene ethers of crude Burmese lac. Table III shows that there was fair agreement between the analyses of the methylene ether and bis-O-TMS derivatives of Burmese lac. All of the methylene ether derivatives showed a prominent parent ion and a strong fragment at *m/e* 135, which arises from benzylic cleavage of the side-chain.

Characterisation of alkenyl side-chain

An attempt was then made to characterise the two main components in the lac, (Id) and (If), by location of the position of the double bonds in the dienes. These compounds are very sensitive to aerial oxidation and they readily polymerise. A modified extraction procedure using hexane and acetonitrile was used to separate the phenolic lipids from the crude lac, and the components (Id) and (If) were isolated by reversed-phase LC.

TABLE I

GC–MS ANALYSIS AND COMPOSITION OF TRIMETHYLSILYLATED BURMESE LAC

Bis-O-TMS ether of	*Peak in Fig. 1*	*Retention time (min)*	*Approximate relative concentration**	*Mass spectrum (m/e)*
Ia	1-1	57.0	0.4	462(M,1%), 281(25), 267(1), 179(12), 73(100)
Ib	1-2	57.5	7.4	464(M,1%), 281(1), 267(1), 179(7), 73(100)
Ic	1-3	58.0	0.7	464(M,10%), 281(7), 267(25), 179(2), 73(100)
Id	1-4	60.8	47.6	488(M,7%), 473(1), 267(5), 179(2), 73(100)
Ie	1-5	61.0	8.8	490(M,2%), 267(11), 179(14), 73(100)
If	1-6	61.3	25.9	488(M,8%), 473(1), 267(20), 179(25), 73(100)
Ig	1-7	61.6	1.0	492(M,5%), 477(1), 267(3), 73(100)
Ih	1-8	61.8	1.9	470(M,5%), 455(1), 267(1), 179(10), 91(18), 73(100)
Ii	1-9	62.3	0.3	470(M,10%), 455(1), 267(25), 179(25), 91(25), 73(100)
Ij	1-10	67.6	5.7	498(M,2%), 483(1), 267(1), 179(7), 91(17), 73(100)
Ik	1-11	68.2	0.3	498(M,10%), 483(1), 267(27), 179(17), 91(22), 73(100)

* Calculated from peak area in chromatogram.

TABLE II

GC–MS ANALYSIS AND COMPOSITION OF TRIMETHYLSILYLATED HYDROGENATED BURMESE LAC

Bis-O-TMS ether of	*Peak in Fig. 2*	*Retention time (min)*	*Approximate relative concentration**	*Mass spectrum (m/e)*
Ib	2-1	57.5	6.4	464(M, 1%), 281(1), 267(1), 179(7), 73(100)
Ic	2-2	58.0	0.6	464(M, 7%), 281(7), 267(25), 179(20), 73(100)
Ig	2-3	61.7	61.1	492(M, 5%), 477(1), 267(3), 179(3), 73(100)
Il	2-4	62.1	24.7	492(M, 15%), 477(1), 267(3), 179(30), 73(100)
Ij	2-5	67.6	6.9	498(M, 2%), 483(1), 267(1), 179(7), 91(17), 73(100)
Ik	2-6	68.2	0.3	498(M, 10%), 483(1), 267(27), 179(17), 91(22), 73(100)

* Calculated from peak area in chromatogram.

The UV spectrum of (Id) corresponded closely to that of (*E*)-3-(octadec-9′-enyl) catechol, confirming a 1,2,3-substitution pattern[15]. The IR spectrum had bands at 780 and 735 cm^{-1}, and both olefinic bonds appear to have the *cis* configuration, although there was slight absorption at 960 cm^{-1}. Hydrogenation of (Id) gave a colourless solid having the same melting point as hydrolaccol (Ig)[3]. The ^1H NMR spectrum of the diene (Id) was identical with that of (8′*Z*,11′*Z*)-3-(heptadeca-8′,11′-dienyl)catechol, which has previously been found in poison oak[7]. An oxidative degradation procedure was developed, which may be applied to determine the position of the double bonds in alkenyl-substituted catechol compounds, and this was applied to the laccol (Id).

Methylation of the laccol component (Id) gave the dimethyl ether (IIa), which was subsequently treated with osmium tetroxide to give the glycols (IIb) as a colourless solid. The structure of the glycols (IIb) was confirmed by condensation with acetone to give an acetonide (IIg), which showed a characteristic fragment at *m/e* 171 due to α-cleavage to the terminal dioxolane ring, and also small fragments at *m/e* 235 and 335, which were assigned to fragmentation at the second dioxolane system.

The glycols (IIb) were then oxidised with Jones reagent and the carboxylic acids were methylated and subjected to GC–MS. The products showed two main components, which corresponded to methyl-8-(2′3′-dimethoxyphenyl)octanoate (IIc) and methyl hexanoate[16]. The aromatic ester (IIc) had a molecular ion at 294 (M^+,

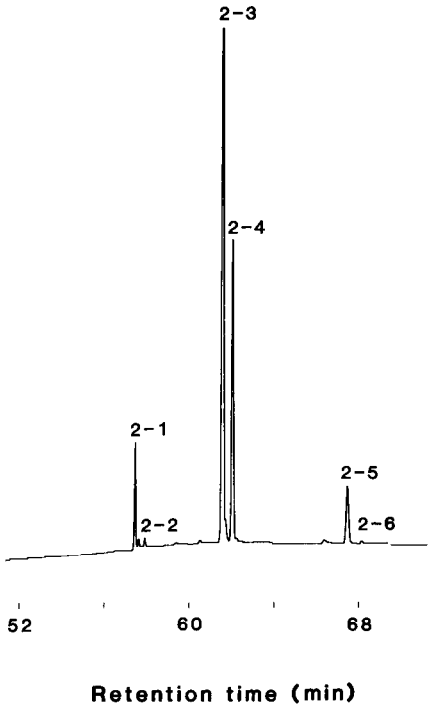
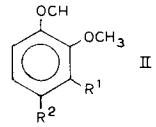


Fig. 2. Gas chromatogram of bis-O-(trimethylsilyl) derivatives of hydrogenated Burmese lac on a 25 m × 0.2 mm I.D. capillary fused-silica cross-linked methyl silicone column; temperature programme from 50°C to 280°C at 4°C/min.

20%), and showed fragmentary ions at *m/e* 151 and 136. Attempts to isolate dimethyl malonate were unsuccessful because the malondialdehyde intermediate is unstable[17]. Scission of the diene must have occurred at positions 8 and 11 so the structure of the laccol is (8′*Z*,11′*Z*)-3-(heptadeca-8′11′-dienyl)catechol (Id).

The thitsiol component (If) was also isolated in a pure state by preparative LC. The retention time of the bis-O-TMS derivative was higher than that of the corresponding *ortho*-product, and the bis-O-TMS tropylium ion was more prominent.



II

	R^1	R^2
IIa	$-(CH_2)_7-CH=CH-CH_2-CH=CH-(CH_2)_4-CH_3$	H
IIb	$-(CH_2)_7-CH(OH)-CH(OH)-CH_2-CH(OH)-CH(OH)-(CH_2)_4-CH_3$	H
IIc	$-(CH_2)_7-COOCH_3$	H
IId	H	$-(CH_2)_7-CH=CH-CH_2-CH=CH-(CH_2)_4-CH_3$
IIe	H	$-(CH_2)_7-CH(OH)-CH(OH)-CH_2-CH(OH)-CH(OH)-(CH_2)_4-CH_3$
IIf	H	$-(CH_2)_7-COOCH_3$
IIg	$-(CH_2)_7-CH(O-C(CH_3)_2-O)-CH(O-C(CH_3)_2-O)-CH_2-CH(O-C(CH_3)_2-O)-CH(O-C(CH_3)_2-O)-(CH_2)_4-CH_3$	H
IIh	H	$-(CH_2)_7-CH(O-C(CH_3)_2-O)-CH(O-C(CH_3)_2-O)-CH_2-CH(O-C(CH_3)_2-O)-CH(O-C(CH_3)_2-O)-(CH_2)_4-CH_3$

The UV spectrum of (If) was similar to that of (*E*)-4-(octadec-9′-enyl) catechol[15], and the IR spectrum showed absorption bands at 845 and 805 cm^{-1} corresponding to a 1,2,4-trisubstituted benzene derivative. IR absorption bands were absent at 965 cm^{-1}, which suggests that the double bonds have a *cis*-configuration. The

TABLE III

GC–MS ANALYSIS AND COMPOSITION OF METHYLENE ETHERS OF BURMESE LAC

50 m × 0.2 mm I.D. capillary fused-silica cross-linked methyl silicone column; temperature programme from 35°C to 300°C at 8°C/min.

Methylene ether of	*Retention time (min)*	*Approximate relative concentration**	*Mass spectrum (m/e)*
Ib	32.8	10	332(M, 10%), 136(45), 135(40)
Id	34.8	51	356(M, 10%), 341(1), 135(100), 81(38), 67(55)
Ie	34.9	14	358(M, 5%), 341(1), 135(3)
Ie**	35.0	4	358(M, 1%), 135(5)
If	35.5	17	356(M, 2%), 135(67), 67(14)
Ii	38.7	4	366(M, 2%), 135(10), 91(7)

* Calculated from peak area in the chromatogram, excluding minor peaks.
** *cis–trans* isomers of the olefinic bond in the side-chain.

NMR spectrum of the thitsiol (If) was similar to that of the corresponding *ortho*-product (Id), which has been reported previously[7], but the aromatic system showed complex spin–spin coupling and there were slight shifts in the position of some of the methylene groups.

When the oxidative degradation procedure was applied to the dimethyl ether (IId) of thitsiol it gave methyl-8-(3′,4′-dimethoxyphenyl)octanoate (IIf) and methyl hexanoate as the main esters. The retention time of (IIf) was longer than that of (IIc), and the mass spectrum showed the parent molecular ion at 294(M^+, 8%) with the main fragmentary ion at *m/e* 152 (100%).

The glycol (IIe) gave an acetonide (IIh) on condensation with acetone, and this derivative again showed a characteristic fragment at *m/e* 171 due to α-cleavage. The structure of the main component in thitsiol is therefore (8′*Z*,11′*Z*)-4-(heptadeca-8′,11′-dienyl)catechol (If). An attempt is being made to isolate enough of components (Ia) and (Ie) to elucidate the position of the double bonds in the monoenes, and it also remains to determine the structures of some other minor substituents.

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