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Note

Analysis of long-chain phenols in the sap of the Burmese lac tree, *Melanorrhoea usitate* by capillary gas–liquid chromatography

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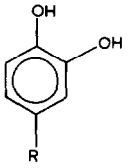
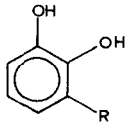
The main constituent of the sap of the Burmese lac tree, *Melanorrhoea usitate*, has been regarded as thitsiol, a trivial name for catechol 4-substituted with a normal C_{17} or C_{15} alkyl or alkenyl side chain, since its discovery by Majima[1,2]. However, we have recently elucidated that, in addition to thitsiol derivatives (1a–1f), the sap contains homologues of laccol (2a–2e), urushiol (2f and 2g), 4-substituted catechol (3a and 3b), 5-substituted resorcinol (4a and 4b) and catechol 3-substituted with 10-phenyldecyl or 12-phenyldodecyl (5a and 5b) and *m*-substituted phenol with a characteristic side chain (6a–6h)[3].



1a, R = $C_{17}H_{29}$ (all *cis*)
1b, R = $C_{17}H_{29}$ (includes at least one *trans* double bond)
1c, R = $C_{17}H_{31}$ (all *cis*)
1d, R = $C_{17}H_{33}$ (*trans*)
1e, R = $C_{15}H_{29}$ (*cis*)
1f, R = $C_{15}H_{31}$



2a, R = $C_{17}H_{29}$ (all *cis*)
2b, R = $C_{17}H_{29}$ includes at least one *trans* double bond)
2c, R = $C_{17}H_{31}$ (all *cis*)

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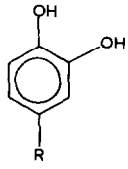
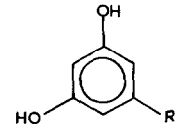
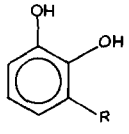
2d, R = $C_{17}H_{33}$ (*cis*)
2e, R = $C_{17}H_{33}$ (possibly *trans*)
2f, R = $C_{15}H_{29}$ (*cis*)
2g, R = $C_{15}H_{31}$



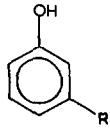
3a, R = $-(CH_2)_{10}C_6H_5$
3b, R = $-(CH_2)_{12}C_6H_5$



4a, R = $-(CH_2)_{10}C_6H_5$
4b, R = $-(CH_2)_{12}C_6H_5$



5a, R = $-(CH_2)_{10}C_6H_5$
5b, R = $-(CH_2)_{12}C_6H_5$



6a, R = $C_{17}H_{31}$
6b, R = $-CH_2CH(OH)C_{17}H_{31}$
6c, R = $-CH_2CH(OH)C_{17}H_{29}$
6d, R = $-(CH_2)_{10}C_6H_5$
6e, R = $-(CH_2)_{12}C_6H_5$
6f, R = $-CH_2CO(CH_2)_{10}C_6H_5$
6g, R = $-CH_2CO(CH_2)_{12}C_6H_5$
6h, R = $-(CH_2)CH(OH)\,(CH_2)_{12}C_6H_5$

Among the compounds depicted above, 1e, 2e, 2f, 6a–6c, 6g and 6h were obtained as a mixture and identified by gas–liquid chromatography–mass spectrometry (GLC–MS).

These characteristic secondary plant metabolites of the lac tree are now very important for coating technology and considered to be promising for future applications. A convenient analytical procedure for this class of natural plant products is required for the quality control of sap.

In a previous paper[4] it was reported that more than thirteen congeners of urushiol from the Japanese or Chinese lac tree, *Rhus vernicifera* were successfully analyzed in the intact form by capillary GLC with high reproducibility of retention

times and relative peak areas. We attempted to apply this facile technique to the analysis of the oily component of the sap of the Burmese lac tree. In this article, the GLC separation of intact phenolic lipids in the sap of *Melanorrhoea usitate* is reported. The optimization of temperature programs and an examination of the reproducibility of the retention time and peak area of each constituent are also described.

EXPERIMENTAL

Native sap of the Burmese lac tree, *Melanorrhoea usitate*, supplied by courtesy of The Southsea Association (Tokyo) and The Burmese Embassy in Tokyo, was mixed with three parts of acetone and filtered. The filtrate was subjected to gel permeation chromatography (TSK-gel G2000H_6, 60 × 2.2 cm × 2; eluent, chloroform; flow-rate, 5 ml/min; detector, RI) in order to collect a monomeric fraction (49 wt. %), which was evaporated and diluted in chloroform (to *ca.* 1 mg/ml) and directly injected onto a fused-silica WCOT column.

GLC was carried out with a Hewlett-Packard 3790 instrument equipped with a flame ionization detector in a split mode (splitting ratio, 50/1) using helium as the carrier gas. Retention times and peak areas were determined with an Hewlett-Packard 3390 reporting integrator. A fused-silica WCOT column (methylsilicone, 12.5 m × 0.2 mm; thickness of liquid film, 0.33 μm; Hewlett-Packard, Avondale, PA, U.S.A.) was employed.

RESULTS AND DISCUSSION

As in the case of urushiol from the lac tree, *Rhus vernicifera*[4], the present fraction was satisfactorily eluted intact from a WCOT column containing non-polar stationary phase, without adsorption despite the higher molecular weights compared with the urushiol congeners. This is due to the highly inert nature of the column wall[5] and again demonstrates the usefulness of the modern fused-silica capillary GLC technique.

Fig. 1 shows chromatograms of the oily component of the sap of the Burmese lac tree, *Melanorrhoea usitate* obtained under various temperature programs. Each peak was assigned by comparing the retention times of pure constituents separated from the sap by reversed-phase liquid chromatography[3]. Since the reproducibility of retention times is extremely high as will be described later, the assignment of each peak in the chromatogram is unambiguous. Compounds 6b and 6g were obtained as a mixture and identified by GLC–MS.

The elution order of substrates having the same substituent was as follows: 3-substituted phenol < 3-substituted catechol < 4-substituted catechol < 5-substituted resorcinol. For example, among compounds with a 10-phenyldecyl group, 6d emerged first followed by 5a, 3a and 4a.

For urushiol congeners[4] the elution order depended on the number and geometry of double bonds in the side chain; the 8*Z*,11*Z*-dienylurushiol appeared first followed by 8*Z*-monoenyl-, 8*Z*,11*E*-dienyl-,saturated-, 8*Z*,11*E*,13*E*-trienyl- and 8*Z*,11*E*,13*Z*-trienylurushiols. Laccol congeners in the sap of the Burmese lac tree were eluted in the order *cis*-dienyl (2c) < *cis*-monoenyl (2d) < *cis*-trienyl (2a), and

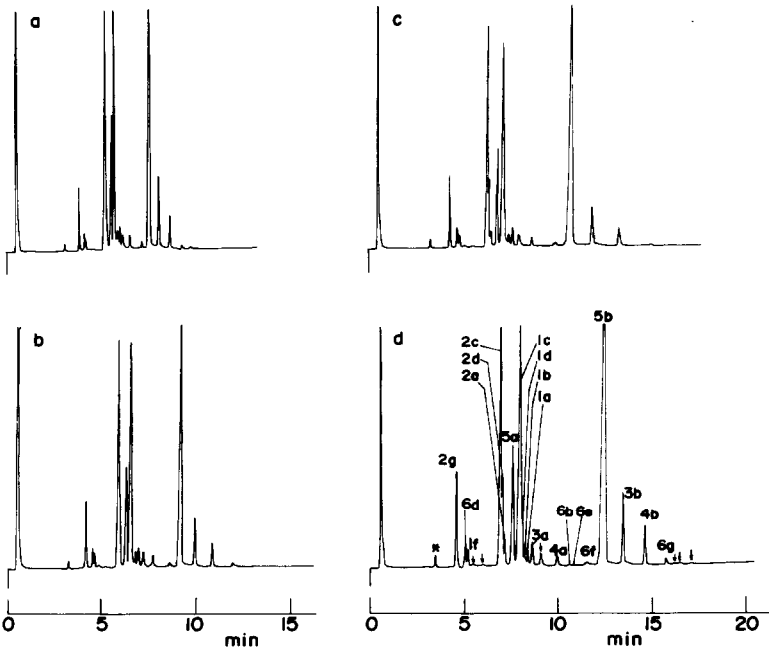


Fig. 1. Chromatograms of the oily component of the sap from the Burmese lac tree, *Melanorrhoea usitate* in the underivatized form. Conditions: column, methylsilicone, 12.5 m × 0.2 mm I.D., d_f = 0.33 μm; column programmed from 250°C (maintained for 1 min) to 300°C at 5°C/min (a), 2.5°C/min (b) and 1°C/min (c) and from 250°C (maintained for 10 min) to 300°C at 5°C/min (d); injection-port and detector temperatures, 300°C; carrier gas (helium) linear velocity, 36 cm/sec; splitting ratio, 50/1. Numbers represent compounds depicted in the text; peaks with an arrow were not identified, and that with an asterisk is due to phthalate from a plastic container.

for thitsiol congeners in the order *cis*-dienyl (1c) < *trans*-monoenyl (1d) < trienyl with at least one *trans* double bond (1b) *cis*-trienyl (1a). The position and geometry of the double bonds in the latter congeners are not explicitly determined at present. Nonetheless, there exists a general trend that a *cis*-dienyl congener emerges before a *cis*-monoenyl one, and the latter before a trienyl congener.

With a temperature program from 250 to 300°C at 5°C/min, all the constituents emerged within 10 min (Fig. 1a). The main features of the composition of the sap can be seen from this rapid chromatography, whereas the separation of the individual series of laccol or thitsiol compounds was somewhat difficult within reasonable analysis times. To improve the separation of these congeners, the rate of increase in column temperature was decreased (Fig. 1b and c). At 1°C/min, some homologues of laccol and thitsiol were separated from the peaks of the most abundant $C_{17:2}$ laccol (2c) and $C_{17:2}$ thitsiol (1c), respectively. Under isothermal operation at 250°C, the separation of these minor constituents was still incomplete. However, for practical routine analysis in the quality control of natural sap, the described separation may be satisfactory.

After six analyses using the temperature program from 250 to 300°C at 5°C/min, statistical parameters were estimated for representative constituents (Tables I and II). It is seen that the reproducibility of the retention time and the area of each

TABLE I

REPRODUCIBILITY OF RETENTION TIMES (min) OF PHENOLIC LIPIDS IN THE SAP OF THE BURMESE LAC TREE, *MELANORRHOEA USITATE*

GLC conditions as in Fig. 1a. S.D. = Standard deviation; R.S.D. = relative standard deviation.

No.	*Compound*	*Analysis*						*Average ± S.D.*	*R.S.D. (%)*
		1	*2*	*3*	*4*	*5*	*6*		
1	2g	3.85	3.82	3.83	3.83	3.84	3.83	3.833 ± 0.010	0.27
2	6d	4.15	4.12	4.13	4.13	4.13	4.13	4.132 ± 0.010	0.24
3	1f	4.22	4.20	4.21	4.21	4.21	4.20	4.208 ± 0.008	0.18
4	2c	5.16	5.14	5.15	5.14	5.15	5.14	5.147 ± 0.008	0.16
5	5a	5.49	5.47	5.48	5.47	5.48	5.47	5.477 ± 0.008	0.15
6	1c	5.63	5.60	5.62	5.61	5.62	5.61	5.615 ± 0.010	0.19
7	1a	5.84	5.82	5.83	5.82	5.83	5.82	5.827 ± 0.008	0.14
8	3a	5.95	5.93	5.94	5.93	5.94	5.93	5.937 ± 0.008	0.14
9	4a	6.50	6.47	6.48	6.48	6.49	6.47	6.482 ± 0.012	0.18
10	6f	7.12	7.10	7.11	7.10	7.11	7.10	7.107 ± 0.008	0.11
11	5b	7.50	7.46	7.49	7.48	7.49	7.47	7.482 ± 0.015	0.20
12	3b	7.98	7.95	7.96	7.96	7.97	7.95	7.962 ± 0.012	0.15
13	4b	8.57	8.55	8.56	8.55	8.56	8.55	8.557 ± 0.008	0.10

peak are excellent; the relative standard deviations of retention times are 0.1–0.27% and the absolute reproducibility in peak area counts for abundant peaks is 2–7% (relative standard deviation).

From the results of the present analysis, one can see that the major constituents of the oily component of the sap from the Burmese lac tree are 3-(12-phenyldode-

TABLE II

REPRODUCIBILITY OF PEAK AREAS (%) OF PHENOLIC LIPIDS IN THE SAP OF THE BURMESE LAC TREE, *MELANORRHOE USITATE*

GLC conditions as in Fig. 1a.

No.	*Compound*	*Analysis*						*Average ± S.D.*
		1	*2*	*3*	*4*	*5*	*6*	
1	2g	3.773	4.101	3.983	3.968	3.863	3.768	3.909 ± 0.132
2	6d	1.120	1.638	1.124	1.364	1.511	1.493	1.375 ± 0.214
3	1f	0.620	0.879	0.632	0.663	0.759	0.841	0.734 ± 0.110
4	2c	21.051	20.585	20.688	17.699	17.701	20.550	19.712 ± 1.568
5	5a	7.173	7.178	8.292	7.467	7.863	7.212	7.531 ± 0.458
6	1c	20.111	20.162	20.441	20.560	21.210	20.259	20.457 ± 0.406
7	1a	0.638	0.616	0.899	0.647	0.969	0.600	0.728 ± 0.162
8	3a	1.062	1.076	1.197	1.130	1.244	1.059	1.128 ± 0.078
9	4a	0.622	0.705	0.675	0.729	0.705	0.708	0.691 ± 0.004
10	6f	0.346	0.361	0.342	0.400	0.326	0.334	0.352 ± 0.003
11	5b	36.305	35.025	34.795	37.777	36.181	35.689	35.962 ± 1.075
12	3b	3.562	3.638	3.531	3.733	3.684	3.633	3.630 ± 0.007
13	4b	2.035	2.124	2.066	2.178	2.151	2.110	2.104 ± 0.005

cyl)catechol (5b) (35%), $C_{17:2}$ thitsiol (1c) (20%), $C_{17:2}$ laccol (2c) (20%), 3-(10-phenyldecyl)catechol (5a) (7.5%), hydrourushiol (2g) (3.9%) and 4-(12-phenyldodecyl)catechol (3b) (3.6%). However, it should be noted that the present sample contained only 49% of the monomeric fraction. This may lead to underestimation of the content of olefinic thitsiol and laccol in the intact sap exuded from the plant, since these compounds have a high tendency to autooxidative polymerization which may occur during storage and result in a decrease in the monomer content.

In summary, we have developed a convenient and accurate analytical procedure for a highly complicated mixture of natural phenolic lipids in the sap of the Burmese lac tree.

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