obtained for the acetic acid procedure were observed with these compounds.

Registry No. 1, 29943-42-8; 2, 2081-44-9; 3, 72250-03-4; 4, 96728-48-2; 5, 96728-52-8; 6, 96728-49-3; 7, 96728-50-6; 8, 96728-51-7; 9, 96728-53-9; 10 (isomer 1), 96728-54-0; 10 (isomer 2), 96728-55-1; 11 (isomer 1), 96728-56-2; 11 (isomer 2), 96728-57-3; 12 (isomer 1), 96728-58-4; 12 (isomer 2), 96728-59-5; 13 (isomer 1), 96728-60-8; 13 (isomer 2), 96728-61-9; 14, 96728-63-1; 15, 96728-62-0; 15 (thioketal), 96728-67-5; 16 (isomer 1), 96728-65-3; 16 (isomer 2), 96728-66-4; 19, 96728-68-6; morpholine, 110-91-8; methyl vinyl ketone, 78-94-4; (methoxymethyl)triphenylphosphonium chloride, 4009-98-7; dimethyl oxalate, 553-90-2; ethyl cyclohexanecarboxylate, 3289-28-9; 2-carbethoxycyclohexanone, 1655-07-8; tosylhydrazine, 1576-35-8; 7-carbomethoxy-2-oxadecal-6-one tosylhydrazone, 96728-64-2; ethanedithiol, 540-63-6.

Enzymic Oxidative Coupling of Urushiol in Sap of the Lac Tree, *Rhus vernicifera*

Ryuichi Oshima,*† Yoshio Yamauchi,‡ Chuichi Watanabe,§ and Ju Kumanotani⊥

Institute of Industrial Science, University of Tokyo, 7-22-1, Roppongi, Minatoku, Tokyo 106, Japan, and Yokogawa-Hewlett-Packard, Takaidohigashi, Suginamiku, Tokyo 153, Japan

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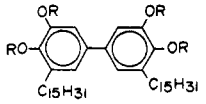
Dimerization of urushiol is a significant initial step of the laccase-catalyzed polymerization of natural lacquer. Urushiol dimers produced during the physiological oxidation of urushiol were thoroughly separated by liquid chromatography. Obtained dimers have been classified into four types of compounds, viz., biphenyls, dibenzofurans, nucleus–side chain bound dimers, and their side chain oxidized products. Dibenzofurans are derived by successive oxidation of biphenyls and the last type of compounds by oxidation of parent dimers. Then it has been established that the dimerization of urushiol in natural sap proceeds through two predominant reaction routes, i.e., phenol coupling and nucleus–side chain coupling. For the first and third types of compounds, product distribution with special regard to the orientation of reactions has been compared to frontier electron densities of possible reaction species. It has been inferred that an attack of urushiol–semiquinone on the urushiol nucleus affords biphenyls. Urushiol–quinone produced by disproportionation of the semiquinone abstracts hydride from the side chain of triolefinic urushiol, 3-[8′(*Z*),11′(*E*),13′(*Z*)-pentadecatrienyl]catechol (**3**), to give the 1,7-disubstituted heptatrienyl cation **24**. Electrophilic substitution of the cation to the urushiol nucleus yields nucleus–side chain bound dimers. In this reaction, C–C coupling occurs exclusively, and derivatives of dihydric phenols are given as dimers of urushiol. This regiospecificity may be due to the slightly acidic reaction medium of natural sap and facilitates subsequent oxidation mediated by polyphenol oxidase laccase.

Sap of the lac tree, *Rhus vernicifera*, dries into a tough and brilliant film and has been used as naturally occuring coating material for thousands of years in the Orient.[1] It is a latex composed of urushiol[2] (60%), water (30%), plant gum[3] (7%), water-insoluble glycoprotein (2%), and copper glycoproteins (laccase[4] and stellacyanin[5]) (ca. 0.1%).

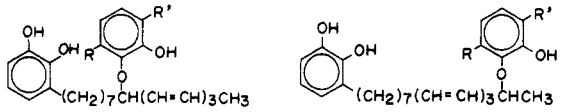
The principal reaction of the film-making process is believed to be oxidative coupling of urushiol under the catalytic action of the oxidoreductase laccase.[6] Quinoid compounds were detected as an intermediate in the course of this process.[7] Symmetric biphenyl was identified in a mildly oxidized sap as the tetraacetate **1**,[8] which was



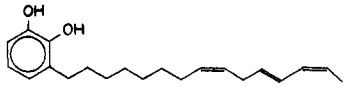
1, R = CH_3CO

5, R = CH_3O

derived by phenol coupling of urushiol. In addition, from studies of model reactions between 4-*tert*-butyl-*o*-benzoquinone and certain olefinic compounds, it was speculated[9] that nucleus–side chain C–O coupling compounds (**2a** or **2b**) were given through the reaction between urushiol–



2a, R/R′ = H/$C_{15}H_{31}$ **2b**, R/R′ = H/$C_{15}H_{31}$



3

quinone and triolefinic urushiol 3-[8′(*Z*),11′(*E*),13′(*Z*)-

† University of Tokyo.

‡ University of Tokyo. Current address: Japan Spectroscopic Industries Co. Ltd., Ishikawacyo, Hachioji, Tokyo 192, Japan.

§ Yokogawa-Hewlett-Packard. Current address: Research and Development Laboratory, Dow Chemical Japan, Ltd., 92-2 Ogurano, Gotemba, Shizuoka 412, Japan.

⊥ University of Tokyo. Current address: Faculty of Engineering, Ehime University, 3 Bunkyocyo, Matsuyama, Ehime 790, Japan.

(1) Kumanotani, J. In "Polymer Application of Renewable-Resource Materials"; Carraher, C. E., Jr., Sperling, L. H., Eds.; Plenum Press: New York, 1983; pp 225–248.

(2) (a) Majima, R. *Ber. Dtsch. Chem. Ges. B* **1922**, *55B*, 172–191. (b) Sunthanker, S. V.; Dawson, C. R. *J. Am. Chem. Soc.* **1954**, *76*, 5070–5074. (c) Yamauchi, Y.; Oshima, R.; Kumanotani, J. *J. Chromatogr.* **1982**, *243*, 71–84. (d) Du, Y.; Oshima, R.; Kumanotani, J. *J. Chromatogr.* **1984**, *284*, 463–470.

(3) Oshima, R.; Kumanotani, J. *Carbohydr. Res.* **1984**, *127*, 43–57.

(4) Reinhammer, B. In "The Coordination Chemistry of Metalloenzymes"; Bertini, I., Drago, R. S., Luchinat, C., Eds.; D. Reidel: Dordrecht, The Netherlands, 1983; pp 177–200.

(5) Peisach, J.; Levine, W. G.; Blumberg, W. E. *J. Biol. Chem.* **1967**, *242*, 2847–2858.

(6) Kumanotani, J. *Makromol. Chem.* **1978**, *179*, 47–61.

(7) Kumanotani, J.; Kato, T.; Hikosaka, A. *J. Polym. Sci., Part C* **1968**, *23*, 519–531.

(8) Kato, T.; Kumanotani, J. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 2375–2376.

(9) Kato, T.; Kumanotani, J. *J. Polym. Sci., Polym. Chem. Ed.* **1969**, *7*, 1455–1465.

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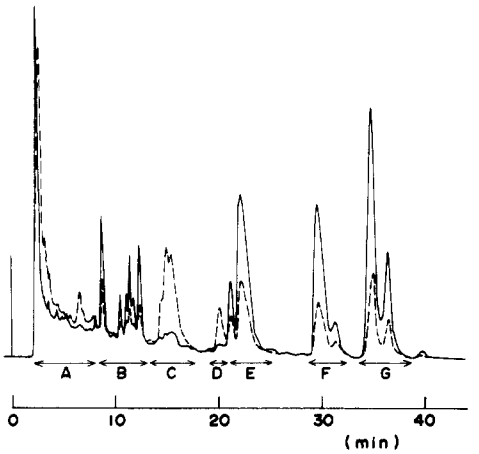


Figure 1. Separation of methylated and hydrogenated dimers of urushiol by reversed-phase liquid chromatography. Conditions: column, Develosil ODS-3 (3 μm, 0.8 × 15 cm); eluent, 7/3 CH_3CN/CH_2Cl_2 v/v; flow rate, 2.5 mL/min; detector, UV 254 nm (bold line) and RI (broken line); sample charge, 20 mg.

pentadecatrienyl]catechol (**3**), which is a major constituent of urushiol of *Rhus vernicifera*.[2c]

However, accurate and precise data regarding the product distribution in the physiological urushiol dimerization are indispensable for deeper understanding of the film-making process of the Japanese lacquer.

On the other hand, phenol coupling has been recognized as an important biosynthetic route for natural products and has been used to synthesize a number of significant compounds.[10] It is generally accepted that the in vitro oxidative dimerization of phenols mediated by oxidating reagents is the result of a coupling of two radicals, but rather it is the result of an attack of one radical on another phenol.[10a] Whereas, as to the mechanism of physiological phenol coupling catalyzed by enzyme oxidases no convincing conclusion is available, because this reaction usually gave a complicated mixture of products hard to be separated.

In this paper, we report some 20 dimeric substances of urushiol isolated from mildly oxidized sap of the lac tree, *Rhus vernicifera*, by the use of liquid chromatography (LC). It has been revealed that the laccase-catalyzed dimerization of urushiol proceeds through two different routes, i.e., phenol coupling and nucleus–side chain C–C coupling. The orientation of these two reactions differs to a great extent, and the former route is inferred to occur by radical substitution of urushiol–semiquinone on the nucleus of urushiol apart from the radical coupling mechanism accepted for in vitro phenol coupling.

Results

Separation. Sap of the lac tree, *Rhus vernicifera*, was oxidized by the traditional treatment ("sugurome" process), i.e., stirring the sap for 4–6 h at 40 °C under ambient atmosphere. After this treatment, the content of water decreased to 2–3%, about 20% of urushiol was converted to dimers and higher oligomeric substances, and the apparently homogeneous dispersion was given. The oxidized product was extracted with acetone, and the extract was subjected to GPC to collect a dimeric portion. The methylated and hydrogenated dimeric substance was first resolved by reversed-phase LC (Figure 1). Each fraction was further separated by liquid–solid chromatography (LSC). Consequently, 20 compounds were obtained and identified. They were classified into four types of compounds, viz., biphenyls, dibenzofurans, nucleus–side chain bound dimers, and their side chain oxidized compounds.

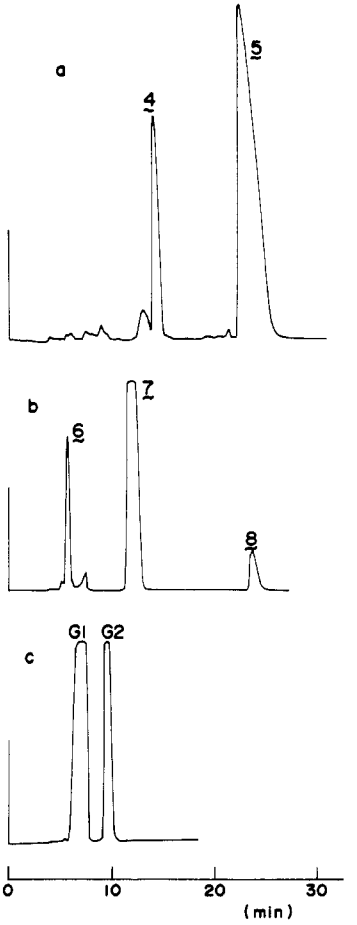
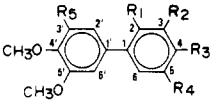


Figure 2. Separation of fractions E (a), F (b), and G (c) of Figure 1 by liquid–solid chromatography. Conditions: column, Develosil 60-3 (3 μm, 0.8 × 25 cm); eluent, 96/4 *n*-hexane/ethyl acetate; flow rate, 2.5 mL/min; detector, UV 254 nm.

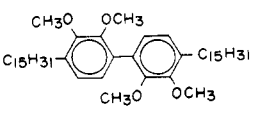
Biphenyls. From fractions E and F in Figure 1, biphenyl derivatives **4–7** were obtained through preparative LSC operation (Figure 2a,b).



4, $R_1 = C_{15}H_{31}$, $R_2 = R_3 = OCH_3$, $R_4 = H$, $R_5 = C_{15}H_{31}$

7, $R_1 = R_2 = OCH_3$, $R_3 = C_{15}H_{31}$, $R_4 = H$, $R_5 = C_{15}H_{31}$

8, $R_1 = H$, $R_2 = C_{17}H_{35}$, $R_3 = R_4 = OCH_3$, $R_5 = C_{15}H_{31}$



6

In EI-MS diagrams, they showed a base peak corresponding to the M^+ ion (m/z 694 for **4–7** and m/z 722 for **8**) and a weak fragment of the $M^+ - 197$ ($C_{14}H_{29}$) ion; other

(10) (a) Barton, D. H. R. *Proc. Chem. Soc.* **1963**, 293–298. (b) Mihaukivuc, H. L.; Cekovic, Z. In "The Chemistry of the Hydroxyl Group"; Patai, S., Ed.; Interscience: London, 1970; pp 505–592. (c) McDonald, E.; Suksamrarn, A. *J. Chem. Soc., Perkin Trans. 1* **1978**, 440–446. (d) Barrett, A. G. M.; Barton, D. H. R.; Franckowiak, G.; Papaioannou, D.; Widdowson, D. A. *J. Chem. Soc., Perkin Trans. 1* **1979**, 662–668. (e) Battersby, A. R.; Bhatnager, A. K.; Hackett, P.; Thornber, C. W.; Staunton, J. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2002–2009. (f) Feringa, B.; Wynberg, H. *J. Org. Chem.* **1981**, *46*, 2547–2557. (g) Torssell, K. B. "Natural Product Chemistry"; Wiley: Chichester, England, 1983; pp 146–154.

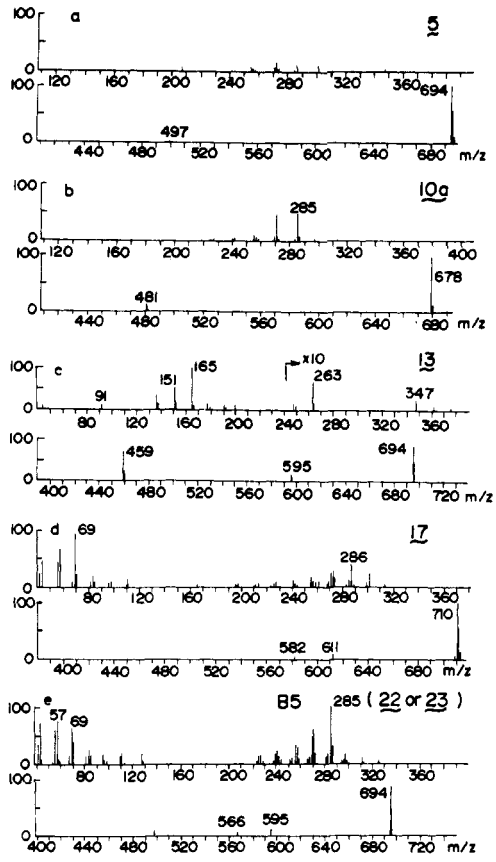


Figure 3. EI-MS diagrams of representative urushiol dimers. Numbers stand for compounds in the text.

fragmentation was negligible (Figure 3a). Compound **4** showed IR bands due to out-of-plane bending deformation of 1,2,3,4- and 1,2,3,5-tetrasubstituted benzene rings at 840, 800, and 720 cm^{-1}. 90-MHz ^1H NMR signals due to four OCH_3 groups were recognized as an apparent broad singlet at δ 3.83, and two singlets (δ 6.77, 6.75) of H-2′,6′ and a multiplet (δ 6.61) due to H-5,6 were observed. Compound **5** showed IR data ascribed to a 1,2,3,5-tetrasubstituted benzene ring and two singlets from OCH_3 at δ 3.77 and 3.89 and a singlet of apparently equivalent four aromatic protons at δ 6.80 in the ^1H NMR spectrum. Compound **6** exhibited two singlets of OCH_3 at δ 3.60 and 3.85; the former resonance suffered from the ring-current effect and was assigned to the OCH_3 group at 2 (or 2′)-position. This showed a multiplet of aromatic protons at δ 6.82. IR data of compound **7** indicated that it consists of 1,2,3,4- and 1,2,3,5-tetrasubstituted benzenes. It showed a multiplet of aromatic protons at δ 6.77 and four distinct ^1H NMR peaks of OCH_3 at δ 3.57, 3.82, 3.87, and 3.90, the first of which was upfield shifted as a result of the ring-current effect. Compound **8** exhibited the same spectroscopic data as compound **5** except the M^+ value. This is derived by the reaction between urushiol and laccol (catechol substituted with a C_{17} alkyl or alkenyl side chain at the 3-position), which is included in the sap as a minor component.[2c,d]

Electronic spectra of the biphenyl dimers were consistent with the proposed structures; 2 (or 2′)-substituted compounds are reluctant to assume coplanar conformation because of the steric constraint and showed absorption maxima at shorter wavelength[11] (Table I).

Dibenzofurans. From fraction G of Figure 1, two dibenzofuran isomers were given (Figure 2c). The base peak

(11) Suzuki, H. *Bull. Chem. Soc. Jpn.* **1959**, *32*, 1350–1356.

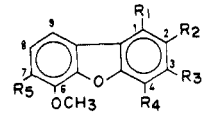
Table I. Electronic Spectra of Biphenyls and Dibenzofurans Derived by Dimerization of Urushiol

compd	λ_{max},[a] nm (ϵ)
4	252 (7100), 282 (sh, 4000)
5	268 (13 500), 290 (sh, 10 100)
6	249 (6000)
7	260 (12 900), 288 (sh)
8	268, 290 (sh)
9a or **9b**	243 (15 100), 260 (11 900), 296 (12 500), 320 (9300)
10a	240 (15 000), 261 (10 700), 295 (11 900), 310 (sh, 10 000)
17	269 (8000), 290 (sh)
B6 (**18** or **19**)	261, 288 (sh)
B7 (**18** or **19**)	261 (12 000), 288 (sh)
B3 (**20** or **21**)	243, 261, 296.5, 320
B4 (**20** or **21**)	242 (16 000), 260 (13 000), 296 (12 800), 320 (9600)
B5 (**22** or **23**)	241 (17 600), 261 (13 500), 297 (15 900), 312 (sh, 10 200)
B8 (**22** or **23**)	240 (9300), 261 (7000), 297 (8200), 311 (sh, 5000)

[a] In CH_2Cl_2.

in each EI-MS diagram is the M^+ ion (m/z 678) (Figure 3b). They showed characteristic electronic spectra (Table I). In the ^1H NMR spectra, an OCH_3 resonance at δ 3.93 (singlet) and AB-type doublet of doublets at δ 6.98 and 7.33 with J = 9 Hz are common to both compounds, and they were assigned to 6-OCH_3, H-8, and H-9, respectively.

A resonance of H-9 is slightly downfield shifted for compound G2 in Figure 2c (δ 7.35) compared with that of compound G1 (δ 7.32), indicating the 1-position of compound G2 is substituted. Remaining aromatic signals were observed at δ 7.15 for compound G1 and δ 6.93 for compound G2, and they were assigned to H-1 and H-4, respectively. Consequently, compound G1 was identified with **9a** or **9b** and compound G2 with **10a** or **10b**.

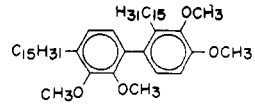


9a, R_1 = H, R_2 = $C_{15}H_{31}$, R_3 = R_4 = OCH_3, R_5 = $C_{15}H_{31}$

9b, R_1 = H, R_2 = R_3 = OCH_3, R_4 = R_5 = $C_{15}H_{31}$

10a, R_1 = R_2 = OCH_3, R_3 = $C_{15}H_{31}$, R_4 = H, R_5 = $C_{15}H_{31}$

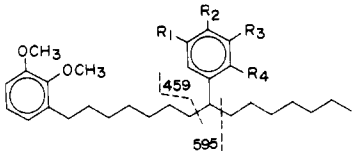
10b, R_1 = $C_{15}H_{31}$, R_2 = R_3 = OCH_3, R_4 = H, R_5 = $C_{15}H_{31}$



6′

These dibenzofuran derivatives were considered to be derived from biphenyls through subsequent oxidation; **10a** is from **6**, **10b** is from **6′**, and both **9a** and **9b** are from **7**. Since compound **6′** was not found, compound G2 was identified with **10a**. However, at present, we cannot distinguish between the two possibilities, i.e., **9a** and **9b**, for the structure of compound G1.

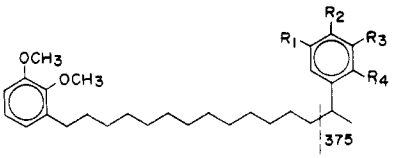
Nucleus–Side Chain C–C Bound Dimers. After two LSC separations of fraction C of Figure 1 on a silica gel column using 96/4 *n*-hexane/ethyl acetate (for the first operation) and 99/1 *n*-hexane/dioxane (for the second) as eluents, compounds **12** and **13** were obtained in the pure form. Compound **11** was obtained as a mixture with **12** in the ratio of 62/38 and **14** as a mixture with **11** in the ratio of 71/29. Compounds **15** and **16** were obtained from fraction D of Figure 1 with respective GLC purity of 71% and 94%. In the IR spectra of compounds **13**–**16**, absorption bands of 1,2,3- (710, 750 cm^{-1}) and 1,2,3,4-substituted benzene rings (810 cm^{-1}) were observed, and in



11, $R_1 = R_2 = OCH_3$, $R_3 = C_{15}H_{31}$, $R_4 = H$

13, $R_1 = H$, $R_2 = R_3 = OCH_3$, $R_4 = C_{15}H_{31}$

15, $R_1 = H$, $R_2 = C_{15}H_{31}$, $R_3 = R_4 = OCH_3$



12, $R_1 = R_2 = OCH_3$, $R_3 = C_{15}H_{31}$, $R_4 = H$

14, $R_1 = H$, $R_2 = R_3 = OCH_3$, $R_4 = C_{15}H_{31}$

16, $R_1 = H$, $R_2 = C_{15}H_{31}$, $R_3 = R_4 = OCH_3$

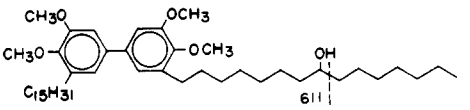
those of compounds **11** and **12**, peaks due to 1,2,3- and 1,2,3,5-substituted benzene rings (840 cm^{-1}) were recognized.

Since the GLC column temperature for these compounds was as high as 330 °C on a fused-silica capillary column with nonpolar liquid phase (silicone OV-1), a GLC–MS technique could not be employed to measure mass spectra of **11**, **14**, and **15**. However, we succeeded to obtain the MS diagram of each compound by measuring mass spectrum every 2 s at increasing ion-source temperatures. In the total-ion trace for a mixture of these compounds (e.g., Figure 4), two peaks were observed, and they exhibited different mass spectra. They were regarded as those of pure **11**, **14**, and **15**.

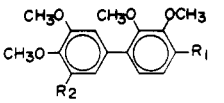
In the EI-MS diagram for each of the above six compounds, the M^+ ion was observed at m/z 694, though it was not intense (Figure 3c). This was confirmed by the fact that the $M^+ + 1$ ion (m/z 695) was detected as a base peak in the CI-MS diagrams (ionizing gas, isobutane) of compounds **12** and **13**. Base peaks in the EI-MS curves were observed at m/z 151, corresponding to a dimethoxytropyllium ion, $C_7H_5(OCH_3)_2^+$, or at m/z 165 ($CH_2C_7H_5(OCH_3)_2^+$). For compounds **11**, **13**, and **15**, the m/z 459 and 595 ions were remarkable, whereas the m/z 375 ion was characteristic for **12**, **14**, and **16**. These fragment ions were derived by β-elimination[12] and clearly indicate the coupled position in side chains. In LSC curves (column, 3-μm silica gel (4.6 mm i.d. × 15 cm); eluent, 97.5/2.5 *n*-hexane/ethyl acetate v/v; flow rate, 1.25 mL/min), compounds **15** and **16** showed much shortened retention times (3.93 min) as compared with those of **13** (8.70 min) and **14** (7.95 min), leading to the conclusion that all of the OCH_3 groups in the former compounds are hindered.

200-MHz ^1H NMR spectrum of **13** showed a quintet (J = 8 Hz) at δ 3.745 due to a phenyl-substituted methine proton.

Side Chain Oxidized Dimers. These compounds were obtained from the fraction B of Figure 1, one from the front peak and six from the rear part, through subsequent LSC operation (Figure 5). In the FD-MS diagrams, compounds **17–19** showed a single peak at m/z 710 and **20–23** at m/z 694. These were also intense in the EI-MS diagrams and then explicitly assigned to M^+ ion. They have a hydroxyl group as revealed by the IR spectra. In

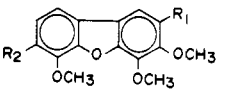


17



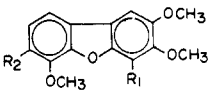
18, $R_1 = -(CH_2)_7CH(OH)C_7H_{15}$, $R_2 = C_{15}H_{31}$

19, $R_1 = C_{15}H_{31}$, $R_2 = -(CH_2)_7CH(OH)C_7H_{15}$



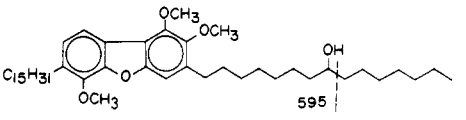
20a, $R_1 = C_{15}H_{31}$, $R_2 = -(CH_2)_7CH(OH)C_7H_{15}$

21a, $R_1 = -(CH_2)_7CH(OH)C_7H_{15}$, $R_2 = C_{15}H_{31}$



20b, $R_1 = C_{15}H_{31}$, $R_2 = -(CH_2)_7CH(OH)C_7H_{15}$

21b, $R_1 = -(CH_2)_7CH(OH)C_7H_{15}$, $R_2 = C_{15}H_{31}$



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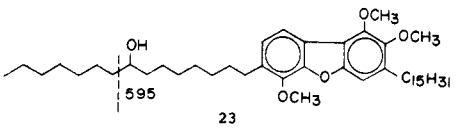
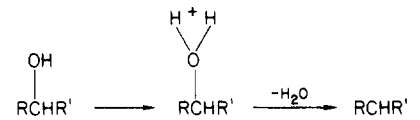


23

EI-MS diagrams, these compounds exhibited a series of fragment ions (m/z 43, 57, 69, 240, 255, 270, 285, 301) derived from alcohol derivatives (Figure 3d,e). Further, in CI-MS diagrams, intense $M^+ + 1 - 18$ (H_2O) ions were observed, which were given by dehydration of oxonium ions derived by protonation of the parent compounds.[13]



Electronic spectra (Table I) clearly indicated compounds **17–19** are derivatives of biphenyl and compounds **20–23** are those of dibenzofuran. Comparing these with the spectra of **4–10**, substituted positions in nuclei for **17–23** were unambiguously determined.

The former compounds exhibited the weak MS peak of m/z 611 and the latter that of m/z 595 (Figure 3d,e). These are given by the α-cleavage. Consequently the hydroxyl-substituted position in side chains was determined to be C-8. ^1H NMR resonances due to a hydroxyl-substituted methine proton and a hydroxyl proton were detected at δ 3.715 (quintet) and 5.164 (singlet) for compound B6 (**19** or **20**).

Compound **17** is symmetric, has no isomer due to the difference in the position of a hydroxyl group and was explicitly identified. However, respective pairs of compounds **18**/**19**, **20**/**21**, and **22**/**23** are isomers that differ

(12) Budzikiewicz, H.; Djerassi, C.; Williams, D. H. "Mass Spectroscopy of Organic Compounds"; Holden-Day: San Francisco, 1967.

(13) Nakata, H. *Shitsuryo Bunseki* **1980**, *28*, 293–316.

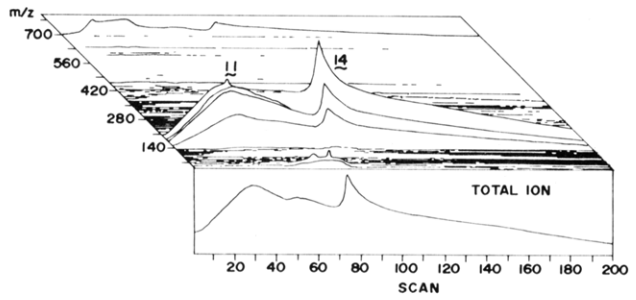


Figure 4. Three-dimensional representation of the EI-MS diagram of a mixture of **11** and **14**. Spectra were measured every 2 s at increasing ion-source temperatures from 100 to 250 °C at 30 °C/min.

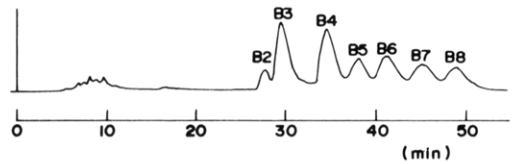


Figure 5. Separation of the rear part of fraction B of Figure 1 by liquid–solid chromatography. Conditions: column, Develosil 60-3 (3 μm, 0.8 × 25 cm); eluent, 92.5/7.5 *n*-hexane/ethyl acetate; flow rate, 2.5 mL/min; detection, UV 254 nm.

from each other only on the substituted position of an alcoholic hydroxyl group. We are unable to discriminate between the two compounds in each pair.

Fraction A of Figure 1 contained many components as revealed by GLC and was not further resolved. The EI-MS measurement showed that the main component was a nucleus–side chain C–C bound dimer with a hydroxyl group at the side chain.

Discussion

In this study, four types of dimeric compounds were found in mildly oxidized sap of the lac tree, *Rhus vernicifera*, which may reflect the fundamental reaction of the polymerization of urushiol in the sap. The apparent yield of each type of compounds is listed in Table II. Among these compounds, dibenzofurans may be derived by the subsequent oxidation of biphenyls as in the case of the condensation of 3-isopropylcatechol and orcinol.[14] Compounds in fractions A and B of Figure 1 may be found by oxidation of the side chain olefinic moiety of parent dimers or urushiol as will be discussed later. Accordingly, the dimerization of urushiol proceeds through two different routes, i.e., phenol coupling and nucleus–side chain coupling. The former route has already been revealed by the identification of **1**.[8] Although the latter route has also been speculated to exist in previous papers,[1,6-9,15] it is first established in the present study. Further, this coupling has been clarified to occur exclusively through the formation of a C–C bond; C–O bond formation was not observed in the physiological dimerization of urushiol.

It is believed that in vivo polymerization of urushiol is carried out under the action of laccase.[1] Since no definite specificity was apparent on the orientation of the coupling products (both diphenyls and nucleus–side chain coupling dimers), enzymatic reaction may not be operative in the coupling process directly.[16] It is possible that only the initial oxidation of urushiol to urushiol–quinone[17,18] is catalyzed by laccase.

Table II. Yields of Urushiol Dimers

compd class	ratio wt %	compd	coupling position	yield, mg
biphenyls	26.6	**4**	4–5	2
		5	5–5	9
		6	6–6	1
		7	5–6	6
		8	5–5	1
dibenzofurans	10.8	**9a** or **9b**		7
		10a		2
nucleus–side chain bound dimers	18.3	**11**	5–8′	1.7
		12	5–14′	2.8
		13	4–8′	2.0
		14	4–14′	3.6
		15	6–8′	1.0
		16	6–14′	2.0
oxidized biphenyls and dibenzofurans	12.5			
unknown (oxidized nucleus–side chain coupling dimers)	33.3			

For the oxidized sap analyzed here, the conversion was relatively low, and trimers and higher oligomers were found in less than several per cent of the initial urushiol. Consequently, the relative yields of dimeric compounds may provide a certain measure of the reactivity of respective carbon atoms in the nucleus of urushiol. Inspecting yields of compounds **4**, **5**, **7**, and **11**–**16** (Table II), one can recognize that the orientation of the two reaction routes differs to a great extent from each other. In the phenol coupling the reactivity varied in the order of C-5 > C-6 > C-4, whereas, in the nucleus–side chain coupling the order of reactivity was C-4 = C-5 > C-6. Since compounds **6** and **7** may be converted into dibenzofurans, the reactivity at C-6 in the phenol coupling should be considered much higher than that assumed from their yields.

It has been generally accepted that dihydric phenols are converted into corresponding quinones by the laccase-mediated oxidation. Actually, in the course of polymerization of the sap, a quinoid compound was previously detected.[7] Recently it has been revealed that when oxidized with laccase in microemulsion, 3-pentadecylcatechol (hydrourushiol) changes to 3-pentadecyl-*o*-benzoquinone. This quinone is given by disproportionation of semiquinone radicals.[17] Accordingly, the following substances should be taken as reacting species in the sap, i.e., urushiol (and its anion), the semiquinone, and the quinone.

Molecular orbital calculations were carried out for 3-methylcatechol and its anion, 3-methyl-*o*-benzoquinone, and 3-methyl-*o*-benzosemiquinone by the MINDO/2 method[19] as model compounds of urushiol, urushiol–quinone, and urushiol–semiquinone, respectively. The dimensions of these compounds were assumed to refer to those of catechol[21] and *o*-benzoquinone.[21] Frontier orbital energies of respective species are compared in Figure 6. For neutral 3-methylcatechol the next highest occupied molecular orbital (NHOMO) is in close proximity to the highest occupied molecular orbital (HOMO) in energy, and the extent of contribution of the former orbital to the frontier

(14) Waiss, A. C.; Kuhnle, J. A.; Windle, J. J.; Wiersema, A. K. *Tetrahedron Lett.* **1966**, 6251–6255.
(15) Kato, T.; Taniai, M.; Yokoo, K.; Kumanotani, J. *Can. J. Chem.* **1969**, *47*, 2106–2107.
(16) Hamilton, G. A. *Adv. Enzymol.* **1969**, *32*, 55–96.
(17) Nakamura, T. *Biochem. Biophys. Res. Commun.* **1960**, *2*, 111–113.
(18) Nakamura, T.; Ogura, Y. *J. Biochem. (Tokyo)* **1968**, *64*, 267–270.
(19) Dewar, M. J. S.; Haselbach, E. *J. Am. Chem. Soc.* **1970**, *92*, 590–598. Bodor, N.; Dewar, M. J. S.; Harget, A.; Haselbach, E. *J. Am. Chem. Soc.* **1970**, *92*, 3854–3859. The MO calculation program was coded by Dr. T. D. Tuong.
(20) Brown, C. J. *Acta Crystallogr.* **1966**, *21*, 170–174.
(21) Macdonald, A. L.; Trotter, J. *J. Chem. Soc., Perkin Trans. 2* **1973**, 476–480.

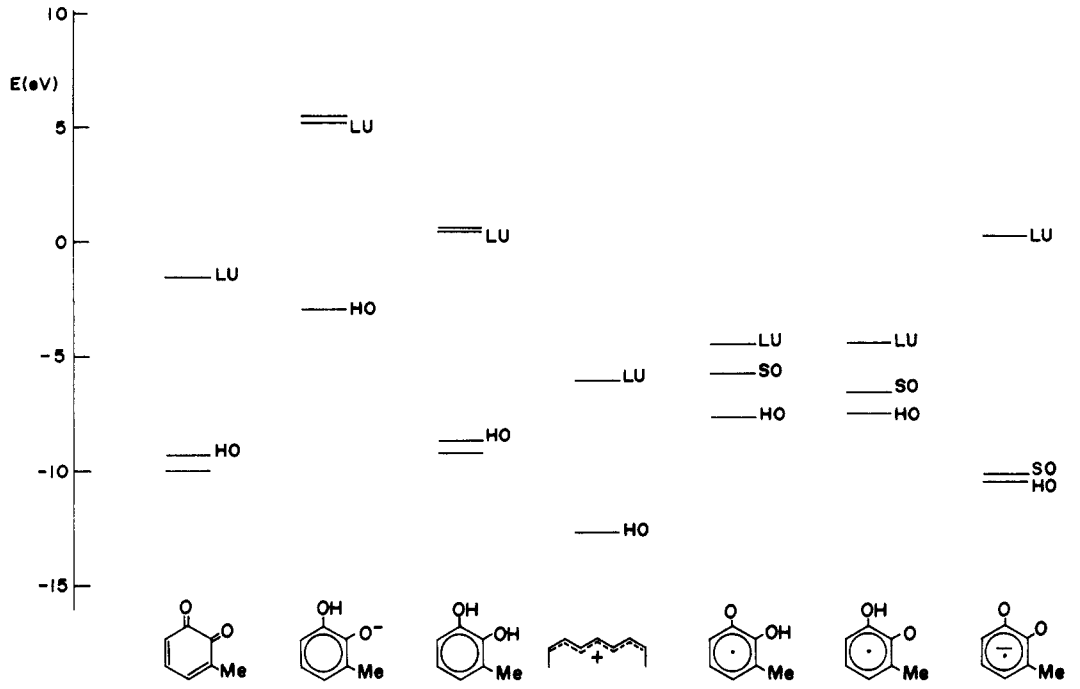
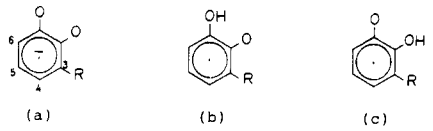


Figure 6. Frontier orbitals of model compounds of possible reaction species in oxidation of urushiol.

Table III. Spin-Density Distribution in *o*-Benzosemiquinones



R	form	position 3	4	5	6	ref
H	anion (a)	0.029	0.139	0.139	0.029	26
		0.028	0.139	0.139	0.028	27
		0.032	0.135	0.135	0.032	28
		0.022	0.141	0.141	0.022	28
		0.026	0.127	0.127	0.026	29
	neutral (b)	0.159	0.071	0.322	0.071	26
		0.143	0.006	0.297	0.057	30
	neutral (c)	0.071	0.322	0.071	0.159	26
		0.057	0.297	0.006	0.173	30
	neutral (av)	0.035	0.152	0.152	0.035	31
CH_3	anion (a)		0.107	0.137	0.029	32
C_3H_7	anion (a)		0.100	0.146	0.025	32

electron density[22] at the rth atom (f_r) was evaluated following the equation[23]

$$f_r = 2[C_r(1)^2 + C_r(2)^2 \exp(-DE)]/[1 + \exp(-DE)]$$

where E is the energy difference between two orbitals (in the units of β (ca. −3 eV)[24]), $C_r(1)$ and $C_r(2)$ are the coefficients of LCAO MO at the rth atom in HOMO and NHOMO, respectively, and D is a constant that determines the degree of contribution of the next MO and assumed to be 3. Similarly, the contribution of the next lowest unoccupied orbital was taken into consideration for the estimation of f_r for nucleophilic and radical substitution. Figure 7 summarizes frontier electron densities of compounds under consideration.

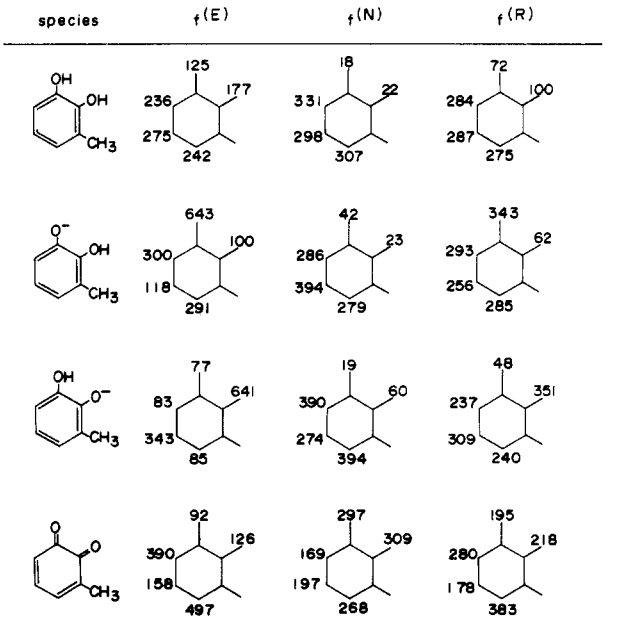


Figure 7. Frontier electron densities (×10³) of 3-methylcatechol and 3-methyl-*o*-benzoquinone calculated by the MINDO/2 method.

Spin densities (ρ) in *o*-benzosemiquinone and 3-alkyl-*o*-benzosemiquinone anions were calculated from proton hyperfine splittings (a^H) by using the McConell equation,[25] $a^H = Q\rho$ with $Q = -27$ G, and are listed in Table III, where those of protonated *o*-benzosemiquinone are also included. In the *o*-benzosemiquinone anion radical, the spin density

(22) Fukui, K.; Yonezawa, T.; Shingu, H. *J. Chem. Phys.* **1952**, *20*, 722–725. Fleming, I. "Frontier Orbitals and Organic Chemical Reactions"; Wiley: London, 1976.
(23) Fukui, K.; Yonezawa, T.; Nagata, C.; Shingu, H. *J. Chem. Phys.* **1954**, *21*, 1433–1442.
(24) Yonezawa, T.; Nagata, C.; Kato, H.; Imamura, S.; Morokuma, K. "Ryoshi Kagaku Nyumon"; Kagaku Dojin: Kyoto, Japan, 1968; p 145.
(25) McConnell, H. M. *J. Chem. Phys.* **1956**, *24*, 632.
(26) Smith, I. C. P.; Carrington, A. *Mol. Phys.* **1967**, *12*, 439–448.
(27) Dixon, W. T.; Moghimi, M.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1974**, *70*, 1713–1720.
(28) Loth, K.; Graf, F.; Gunthard, Hs. H. *Chem. Phys. Lett.* **1977**, *45*, 191–195.
(29) Felix, C. C.; Sealy, R. C. *J. Am. Chem. Soc.* **1982**, *104*, 1555–1560.
(30) Loth, K.; Graf, F.; Gunthard, Hs. H. *Chem. Phys.* **1976**, *13*, 95–118.
(31) Dixon, W. T.; Murphy, D. *J. Chem. Soc., Faraday Trans. 2* **1976**, *72*, 135–142.
(32) Stone, T. J.; Waters, W. A. *J. Chem. Soc.* **1965**, 1488–1494.

at C-4 (ρ_4) or C-5 (ρ_5) is extremely high compared with that at C-3 (ρ_3) or C-6 (ρ_6). Also in neutral *o*-benzosemiquinone, this trend is preserved. Alkyl substitution at C-3 decreases ρ_4 in the anion radical to a certain extent; however, the low value of ρ_6 is unaltered, leading to $|\rho_5| > |\rho_4| > |\rho_6|$. Although there is no data for the proton hyperfine splittings in protonated (or neutral) 3-alkyl-*o*-benzosemiquinone, it may be assumed that the order of spin densities at the respective carbon atoms is same as those in the anion radicals.

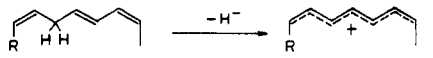
The sap is a W/O-type emulsion, laccase is believed to be present in the aqueous phase, and initial oxidation may occur at the interface between the two phases. The sap is slightly acidic (pH 4.2–5.3),[33] presumably due to the buffering action of plant gum which has uronic acid units (4-*O*-methyl-D-glucuronic acid) at the nonreducing ends and exists as the salt with counter cations of Na^+, K^+, and Mg^{2+}.[3] In this circumstance, urushiol exists preferentially in the neutral form since its pK_a value is considered to be higher than that of catechol (9.85). It may be adequate to assume that *o*-benzosemiquinone is in either anionic or neutral (protonated) form because its pK_a value is 3–4.[34]

After the argument of Musso,[35] phenol coupling could proceed through three different routes, viz., (1) radical coupling,[10a] (2) radical substitution, and (3) nucleophilic addition of phenolate to quinone. The first route has generally been accepted as a reasonable mechanism for the reaction of most phenols oxidized by one-electron oxidants such as ferricyanate and ferrous chloride, where radical species (phenoxy radicals) are present in a great amount. However, this route is inconsistent with the product distribution of biphenyls produced by the dimerization of urushiol; the spin density at C-6 is very low in both neutral and anionic semiquinones in contrast to the higher reactivity of C-6 than that of C-4 for the phenol coupling reaction in sap (Table II). The last route was also excluded because (1) either urushiolate or the quinone is expected to exist in only a small amount and (2) the molecular orbital consideration predicts the highest yield of the C-4-bound compound for this reaction.

The radical substitution may be rationalized for the mechanism of the present phenol coupling from the consideration of the frontier electron population of 3-methylcatechol, where that on C-6 is almost same as that on C-5. Further, the facts that radical concentration may be low and that the reaction is slow in the sap are in agreement with the criterion of Musso for radical substitution.

The frontier orbital (SOMO) of neutral 3-methyl-*o*-benzosemiquinone lies between the LUMO and the HOMO of 3-methylcatechol (Figure 6). This is consistent with the necessary condition for the reaction to occur.[36] Whereas, the SOMO of the anion radical is below the HOMO of 3-methylcatechol. It is known that the spin densities in the *o*-benzosemiquinone anion radical are affected by either solvation or the presence of the metal ions[28,29] and then the molecular orbital energy levels may vary depending on the environment. It is noted that there exist Mg^{2+} ions in the sap which can interact with the anion radical.[29]

The nucleus–side chain coupling is a unique alkylation. Radical substitution of 1,7-disubstituted heptatrienyl radical produced by autooxidation onto the urushiol nucleus seems inadequate because the orientation of this reaction was different from that of the phenol coupling. An alternative mechanism appropriate to this reaction is the electrophilic substitution of 1,7-disubstituted heptatrienyl cation **24** on the urushiol nucleus. This cation may be produced from triolefinic urushiol **3** by dehydrogenation with urushiol–quinone. Quinoid compounds (especially



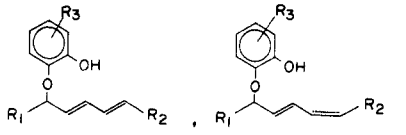
3, R = $Ph(OH)_2$-$(CH_2)_7$- 24

o-benzoquinones) are known to be dehydrogenating agents,[37] and 4-*tert*-butyl-*o*-benzoquinone dehydrogenates from methyl linoleate to give C–O coupling products **25**.[15]

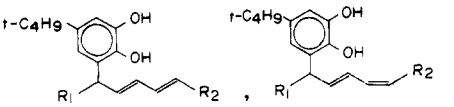
Molecular orbital calculation was executed on the 1,7-dimethylheptatrienyl cation, and the energy levels of the LUMO and the HOMO are shown in Figure 6. In the calculation, the coplanar conformation was assumed with the C–C bond length of 1.44 Å for the conjugated part, and rotation angles were settled as shown in the structure depicted in Figure 6 referring the structure of trienyl urushiol **3**. The frontier orbital (LUMO) of the cation is close to the HOMO of 3-methylcatechol.

From the frontier electron distribution, the reactivity of 3-methylcatechol against electrophiles is expected to vary in the order of C-5 > C-4 > C-6. This can explain the low yield of C-6-bound compounds **15** and **16**, whereas yields of C-4-bound compounds **13** and **14** seem somewhat higher than those predicted from frontier electron population. The origin of this discrepancy is unclear at present.

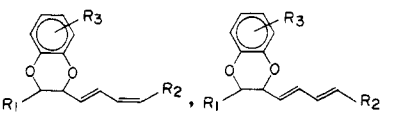
In a bulk reaction between 4-*tert*-butyl-*o*-benzoquinone and methyl linoleate under nitrogen atmosphere, C–O-bound products **25** were previously identified.[15] Recently, C–C-bound products **26** and benzodioxins **27** were found in addition to **25** in the ratio of **26/27/25** = 2/1/1.[38]



25, R_1/R_2 = C_5H_{11}/-$(CH_2)_7COOCH_3$, R_3 = t-C_4H_9 at 4 or 5-position



26, R_1/R_2 = C_5H_{11}/-$(CH_2)_7COOCH_3$

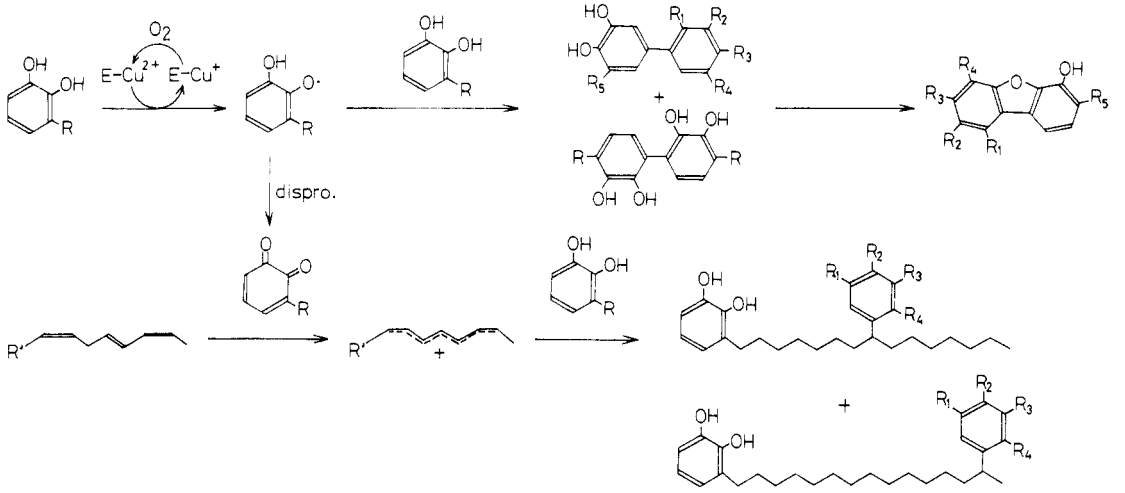


27, R_1/R_2 = C_5H_{11}/-$(CH_2)_7COOCH_3$, R_3 = t-C_4H_9 at 4 or 5-position

Further, a bulk reaction between 4-*tert*-butyl-*o*-benzoquinone and 3-[8′(*Z*),11′(*E*),13′(*Z*)-pentadecyl]veratrole afforded C–C- and C–O-coupled nucleus–side chain bound products in the ratio of 3/14.[39] In contrast, urushiol dimerization in the natural sap yielded exclusively C–C-coupled products, which facilitates subsequent oxidation by a polyphenol oxidase, laccase. This precise regiospecificity may be controlled by the acidity of the reaction medium (pH 4.2–5.3). In basic medium, urushiol would

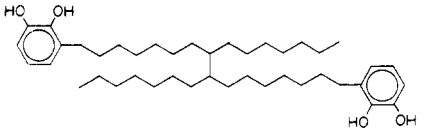
(33) Matsui, E. "Urushikagaku"; Nikkankogyo-shinbunsha: Tokyo, 1963; p 30.
(34) Rao, P. S.; Hayon, E. *J. Phys. Chem.* **1973**, *77*, 2274–2276.
(35) Musso, H. In "Oxidative Coupling of Phenols"; Taylor, W. I., Battersby, A. R., Eds.; Marcel Dekker: New York, 1967; pp 1–94.
(36) Nagakura, S.; Tanaka, J. *J. Chem. Phys.* **1954**, *22*, 236.
(37) Becker, H.-D. In "The Chemistry of the Quinoid Compounds"; Patai, S., Ed.; Wiley: London, 1974; pp 335–423.
(38) Yamauchi, Y.; Kumanotani, J. *Polym. Prepr. Jpn.* **1982**, *31*, 2G01.
(39) Takada, M. Master's Thesis, Department of Synthetic Chemistry, University of Tokyo, 1983.

Scheme I



exist as the anion that has the highest reactivity to electrophiles at oxygen atoms (Figure 7).

Side chain oxidized dimers **17–23** may be given by oxidation of side chains in urushiol or parent dimers. The usual autoxidation mechanism[40] may be applicable as the interaction of molecular oxygen with metal complexes (laccase etc.) may produce reactive oxygen species such as superoxide and peroxide as precursors in this mechanism.[41] On the other hand, the nucleophilic addition of water to the heptatrienyl cation **24** may be considered as the alternative mechanism for this process since water is a stronger nucleophile than phenol (urushiol), and an autoxidation mechanism may not be appropriate to describe the oxidation of the side chain of urushiol in sap where antioxidants (urushiol) exist in a great amount. Further, the fact that none of the side chain–side chain bound products like compound **28** were found in the mildly oxidized sap favors the latter mechanism. In this stage of investigation, however, we have no convincing evidence that can discriminate between the above two possibilities.



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As a summary, Scheme I has been proposed as an overall mechanism that describes the laccase-mediated oxidative coupling of urushiol in sap of the lac tree, *Rhus vernicifera*.

Experimental Section

IR spectra were recorded on an IRA-1 instrument (JASCO, Tokyo). ^{1}H NMR spectra were obtained with a Varian EM-390 spectrometer at a resonance frequency of 90 MHz. FT-NMR data were obtained with a JEOL FX-200S instrument by the coutersy of Dr. K. Kanoda at 200 MHz for ^{1}H resonance and 50 MHz for ^{13}C resonance. EI- and CI-MS (ionizing gas, isobutane) diagrams were obtained with a 5985B GLC–MS system (Hewlett-Packard, Avondale, PA) at 70 eV and FD-MS data with a Hitachi M-80 spectrometer.

GLC analysis was carried out on a 5880A gas chromatograph equipped with a flame ionization detector on a fused-silica capillary column (silicone OV-1, 12.5 m × 0.20 mm i.d., thickness of liquid phase = 0.11 μm, Hewlett-Packard) using He as the carrier gas in a split mode (split ratio, 100/1). The column temperature was 330 °C, the injection-port temperature was 370 °C, and the detector temperature was 300 °C.

The liquid chromatograph was constructed with a Milton-Roy minipump, a bellows-type damper (type DAM, Umetani Seiki, Osaka), and a syringe-loading sample injection valve (type 7125, Rheodyne, CA). The effluent was monitored with a UV detector (UVIDEC 100II, JASCO) or a differential refractive index monitor (RI-2, Japan Analytical Industries Co., Tokyo). Stainless steel columns packed with ODS silica (Develosil ODS-3, 3 μm, 150 × 8 mm i.d., Nomura Chemicals Co., Seto, Japan) and silica gel (Develosil 60-3, 250 × 8 mm i.d.) were used for semipreparative purpose. Analytical LC operation was done on 150 × 4.5 mm i.d. columns packed with the above gels. Column packing was carried out by the slurry method.[42]

Sap of the lac tree (*Rhus vernicifera*, Japan) was slowly stirred at 40 °C for 4 h in a wooden vessel. The obtained darkened material was poured into acetone (3 parts), and the mixture was filtered with filter paper. The filtrate was evaporated, and the residue (1 g) was subjected to preparative GPC (column, TSK-gel G2000HG, 60 × 2.2 cm × 2; eluent, chloroform; detector, RI; flow rate, 5 mL/min), and a dimeric portion was collected. It was then methylated with dimethyl sulfate in dry acetone in the presence of K_2CO_3 (for 5 h at 60 °C) and hydrogenated with hydrazine hydrate in methanol at room temperature for 5 h to yield 178 mg of sample. It was resolved by reversed-phase LC and LSC.

3,4,3′,4′-Tetramethoxy-2,5′-dipentadecylbiphenyl (4): mp 51–53 °C; IR (KBr) 2920, 2850, 1580, 1480, 1280, 1080, 1015, 880, 840, 800, 720 cm^{-1}; ^{1}H NMR (90 MHz, CCl_4) δ 6.77 (s, 1 H), 6.75 (s, 1 H), 6.61 (m, 2 H, H-5,6), 3.83 (m, 12 H, OCH_3), 2.54 (t, 4 H, $PhCH_2$), 1.60 (m, 4 H, CH_3CH_2), 1.25 (m, 48 H, CH_2), 0.88 (t, 6 H, CH_3); EI-MS, *m/z* (relative intensity) 694 (100, M^+), 497 (5, $M^+ - 197$). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.21. Found: C, 78.69; H, 11.30.

3,4,3′,4′-Tetramethoxy-5,5′-dipentadecylbiphenyl (5): mp 59–60 °C; IR (KBr) 2920, 2850, 1580, 1480, 1280, 1220, 1120, 1080, 1005, 840, 720 cm^{-1}; ^{1}H NMR (90 MHz, CCl_4) δ 6.80 (s, 4 H, aromatic), 3.89 (s, 6 H, OCH_3), 3.77 (s, 6 H, OCH_3), 2.60 (t, 4 H, $PhCH_2$), 1.60 (m, 4 H, CH_3CH_2), 1.26 (m, 48 H, CH_2), 0.87 (t, 6 H, CH_3); EI-MS, *m/z* (relative intensity) 694 (100, M^+), 497 (5, $M^+ - 197$). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.21. Found: C, 79.50; H, 11.24.

2,3,2′,3′-Tetramethoxy-4,4′-dipentadecylbiphenyl (6): mp 63–65 °C; IR (KBr) 2920, 2850, 1580, 1480, 1280, 1120, 1005, 810, 720 cm^{-1}; ^{1}H NMR (90 MHz, CCl_4) δ 6.82 (m, 4 H, aromatic), 3.85 (s, 6 H, OCH_3 at 3- and 3′-position), 3.60 (s, 6 H, OCH_3 at 2- and 2′-position), 2.62 (t, 4 H, $PhCH_2$), 1.6 (m, 4 H, CH_3CH_2), 1.26 (m, 48 H, CH_2), 0.88 (t, 6 H, CH_3); EI-MS, *m/z* (relative intensity) 694 (100, M^+), 497 (3, $M^+ - 197$). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.21. Found: C, 79.30; H, 11.26.

2,3,3′,4′-Tetramethoxy-4,5′-dipentadecylbiphenyl (7): IR (neat) 2920, 2850, 1480, 1390, 1250, 1120, 1010, 840, 820, 720 cm^{-1}; ^{1}H NMR (90 MHz, CCl_4) δ 6.77 (m, 4 H, aromatic), 3.90 (s, 3 H,

(40) Porter, N. A.; Weber, B. A.; Weenen, H.; Khan, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 5597–5601.

(41) Sheldon, R. A.; Kochi, J. K. "Metal-Catalyzed Oxidation of Organic Compounds"; Academic Press: New York, 1981; pp 71–119.

(42) Yamauchi, Y.; Kumanotani, J. *J. Chromatogr.* **1981**, *210*, 512–515.

OCH_3), 3.87 (s, 3 H, OCH_3), 3.82 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3 at 2-position), 2.59 (t, 4 H, $PhCH_2$), 1.60 (m, 4 H, CH_3CH_2), 1.26 (m, 48 H, CH_2), 0.89 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 694 (100, M^+), 497 (3, $M^+ - 197$). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.21. Found: C, 79.32; H, 11.26.

3,4,3′,4′-Tetramethoxy-5-pentadecyl-5′-heptadecylbiphenyl (8): IR (neat) 2920, 2850, 1580, 1470, 1270, 1090, 1010, 840, 720 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 6.79 (m, 4 H, aromatic), 3.91 (s, 6 H, OCH_3), 3.79 (s, 6 H, OCH_3), 2.65 (t, 4 H, $PhCH_2$), 1.6 (m, 4 H, CH_3CH_2), 1.24 (m, 52 H, CH_2), 0.87 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 722 (100, M^+).

3,4,6-Trimethoxy-2,7-dipentadecyldibenzofuran (9a) or 2,3,6-trimethoxy-4,7-dipentadecyldibenzofuran (9b): mp 47–49 °C; IR (KBr) 2920, 2850, 1600, 1460, 1260, 1190, 1060, 1030, 790, 720 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 7.32 (d, 1 H, H-9, J = 9 Hz), 7.15 (s, 1 H, H-1), 6.98 (d, 1 H, H-8, J = 9 Hz), 4.22 (s, 3 H, OCH_3 at 3-position), 3.92 (s, 3 H, OCH_3 at 6-position), 3.83 (s, 3 H, OCH_3 at 2- or 4-position), 2.98 (t, 2 H, $PhCH_2$), 2.71 (t, 2 H, $PhCH_2$), 1.6 (m, 4 H, CH_3CH_2), 1.26 (m, 52 H, CH_2), 0.88 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 678 (100, M^+), 481 (15, $M^+ - 197$), 285 (50), 271 (50). Anal. Calcd for $C_{45}H_{74}O_4$: C, 79.59; H, 10.98. Found: C, 79.53; H, 10.92.

1,2,6-Trimethoxy-3,7-dipentadecyldibenzofuran (10a): mp 36–38 °C; IR (KBr) 2920, 2850, 1600, 1460, 1310, 1220, 1020, 810, 720 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 7.35 (d, 1 H, H-9, J = 9 Hz), 6.98 (d, 1 H, H-8, J = 9 Hz), 6.93 (s, 1 H, H-4), 4.03 (s, 3 H, OCH_3 at 1-position), 3.93 (s, 3 H, OCH_3 at 6-position), 3.79 (s, 3 H, OCH_3 at 2-position), 3.01 (t, 2 H, $PhCH_2$), 2.71 (t, 2 H, $PhCH_2$), 1.6 (m, 4 H, CH_3CH_2), 1.25 (m, 48 H, CH_2), 0.89 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 678 (100, M^+), 481 (15, $M^+ - 197$), 285 (50), 271 (50). Anal. Calcd for $C_{45}H_{74}O_4$: C, 79.59; H, 10.98. Found: C, 78.90; H, 10.67.

3-[8-(3,4-Dimethoxy-5-pentadecylphenyl)pentadecyl]veratrole (11) was obtained as a mixture with the isomer **12** in the ratio of 62/38: IR (neat) 2920, 2850, 1590, 1460, 1270, 1230, 1080, 1010, 840, 800, 740 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 6.8–6.4 (m, 5 H, aromatic), 3.82 (br s, 6 H, OCH_3), 3.72 (br s, 6 H, OCH_3), 2.52 (m, 4 H, $PhCH_2$), 1.6–1.2 (m, 50 H, CH_2), 0.87 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 694 (38, M^+), 595 (9), 459 (15), 347 (8), 165 (48), 151 (100). Anal. Calcd for $C_{46}H_{78}O_4$: C, 78.83; H, 11.31. Found: C, 78.83; H, 11.31.

3-[14-(3,4-Dimethoxy-5-pentadecylphenyl)pentadecyl]veratrole (12): IR (KBr) 2920, 2850, 1590, 1470, 1270, 1220, 1090, 1010, 840, 800, 740 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 6.75–6.4 (m, 5 H, aromatic), 3.83 (s, 6 H, OCH_3), 3.79 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 2.54 (m, 4 H, $PhCH_2$), 1.6 (m, 4 H, CH_3CH_2 and $CHCH_2$), 1.25 (m, 46 H, CH_2), 1.10 (d, 3 H, $CHCH_3$), 0.87 (t, 3 H, CH_3); EI-MS, m/z (relative intensity) 694 (45, M^+), 375 (33), 179 (40), 165 (45), 151 (100), 137 (60). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.31. Found: C, 78.83; H, 11.15.

3-[8-(3,4-Dimethoxy-2-pentadecylphenyl)pentadecyl]veratrole (13): IR (KBr) 2920, 2850, 1580, 1470, 1275, 1220, 1080, 1020, 800, 740, 710 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 6.953 (dd, 1 H, H-5, J = 8 Hz), 6.828 (d, 1 H, H-6″, J = 9 Hz), 6.750 (d, 2 H, H-6 and H-4, J = 8 Hz), 6.731 (d, 1 H, H-5″), 3.847 (s, 3 H, OCH_3), 3.829 (s, 3 H, OCH_3), 3.807 (s, 3 H, OCH_3), 3.796 (s, 3 H, OCH_3), 3.745 (q, 1 H, CH, J = 8 Hz), 2.60 (m, 4 H, $PhCH_2$), 1.50 (m, 8 H, $CHCH_2$ and CH_2CH_3), 1.25 (m, 42 H, CH_2), 0.878 (t, 3 H, CH_3, J = 8 Hz), 0.856 (t, 3 H, CH_3, J = 8 Hz); EI-MS, m/z (relative intensity) 694 (9, M^+), 595 (2), 459 (8), 263 (7), 165 (100), 151 (60), 137 (38). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.31. Found: C, 79.56; H, 11.53.

3-[14-(3,4-Dimethoxy-2-pentadecylphenyl)pentadecyl]veratrole (14) was obtained as a mixture with **11** in the ratio of 71/29: IR (KBr) 2920, 2850, 1580, 1470, 1275, 1220, 1085, 1020, 800, 740, 710 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 6.93–6.57 (m, 5 H, aromatic), 3.82 (s, 3 H, OCH_3), 3.80 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 2.56 (m, 4 H, $PhCH_2$), 1.60–1.20 (m, 50 H, CH_2), 1.09 (d, 3 H, $CHCH_3$), 0.87 (t, 3 H, CH_3); EI-MS, m/z (relative intensity) 694 (8, M^+), 375 (52), 179 (100), 165 (43), 151 (61), 137 (43), 91 (22). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.31. Found: C, 78.95; H, 11.13.

3-[8-(2,3-Dimethoxy-4-pentadecylphenyl)pentadecyl]veratrole (15) was obtained as a mixture with **16** in the ratio of 74/26: IR (KBr) 2920, 2850, 1580, 1460, 1275, 1220, 1090, 1020, 810, 740, 710 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 7–6.8 (m, 5 H, aromatic), 3.9–3.7 (m, 12 H, OCH_3), 1.6–1.2 (m, 48 H, CH_2), 0.87 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 694 (28, M^+), 595 (2), 459 (14), 165 (95), 151 (100), 137 (43). Anal. Calcd for $C_{46}H_{78}O_4$: C, 79.48; H, 11.31. Found: C, 79.38; H, 11.18.

3-[8-(2,3-Dimethoxy-4-pentadecylphenyl)pentadecyl]veratrole (16): IR (KBr) 2920, 2850, 1580, 1470, 1275, 1220, 1085, 1020, 810, 780, 740, 710 cm^{-1}; ^1H NMR (90 MHz, CCl_4) δ 7–6.8 (m, 5 H, aromatic), 3.9–3.7 (m, 12 H, OCH_3), 1.6–1.2 (m, 48 H, CH_2), 0.87 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 695 (34), 375 (51), 179 (70), 165 (45), 151 (100), 137 (53).

3,4,3′,4′-Tetramethoxy-5-pentadecyl-5′-(8-hydroxypentadecyl)biphenyl (17): IR (KBr) 3400, 2920, 2850, 1580, 1480, 1270, 1220, 1090, 1010, 840 cm^{-1}; EI-MS, m/z (relative intensity) 710 (100, M^+), 611 (7), 313 (23), 286 (40), 69 (90), 57 (70), 44 (48); CI-MS, m/z (relative intensity) 710 (13, $M^+ + 1$), 693 (100, $M^+ + 1 - 18$); FD-MS, m/z (relative intensity) 711 (100, $M^+ + 1$).

Compound B6. 2,3,3′,4′-Tetramethoxy-4-(8-hydroxypentadecyl)-5′-pentadecylbiphenyl (18) or 2,3,3′,4′-tetramethoxy-4-pentadecyl-5′-(8-hydroxypentadecyl)biphenyl (19): IR (KBr) 2920, 2850, 1580, 1460, 1390, 1260, 1220, 1110, 1020, 820 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.0–6.8 (m, 4 H, aromatic), 5.164 (s, 1 H, OH), 3.894 (s, 3 H, OCH_3), 3.877 (s, 3 H, OCH_3), 3.852 (s, 3 H, OCH_3), 3.715 (q, 1 H, CH), 2.634 (m, 4 H, $PhCH_2$), 1.6–1.25 (m, 48 H, CH_2), 0.879 (t, 6 H, CH_3); EI-MS, m/z (relative intensity) 710 (40, M^+), 611 (8), 301 (20), 285 (21), 270 (25), 255 (20), 69 (90), 57 (55), 42 (40); FD-MS, m/z (relative intensity) 710 (100, M^+).

Compound B7. 19 or 20: IR (KBr) 2920, 2850, 1580, 1460, 1390, 1260, 1220, 1120, 1020, 820, 720 cm^{-1}; EI-MS, m/z (relative intensity) 710 (100, M^+), 611 (8), 301 (30), 285 (25), 270 (35), 255 (32), 69 (90), 57 (53), 43 (40); FD-MS, m/z (relative intensity) 710 (100, M^+).

Compound B3. 3,4,6-Trimethoxy-2-pentadecyl-7-(8-hydroxypentadecyl)dibenzofuran (20a), 3,4,6-trimethoxy-2-(8-hydroxypentadecyl)-7-pentadecyldibenzofuran (21a), 2,3,6-trimethoxy-4-pentadecyl-7-(8-hydroxypentadecyl)dibenzofuran (20b), or 2,3,6-trimethoxy-4-(8-hydroxypentadecyl)-7-pentadecyldibenzofuran (21b): IR (KBr) 2920, 2850, 1600, 1460, 1260, 1220, 1190, 1120, 1050, 800 cm^{-1}; EI-MS, m/z (relative intensity) 694 (78, M^+), 595 (7), 481 (10), 285 (75), 270 (93), 69 (100), 55 (55), 42 (50); FD-MS, m/z (relative intensity) 694 (100, M^+).

Compound B4. 20a, 21a, 20b, or 21b: IR (KBr) 2920, 2850, 1600, 1460, 1260, 1190, 1050, 800 cm^{-1}; EI-MS, m/z (relative intensity) 694 (100, M^+), 595 (10), 481 (7), 285 (90), 270 (82), 255 (54), 69 (94), 57 (73), 42 (67); FD-MS, m/z (relative intensity) 694 (100, M^+).

Compound B5. 1,2,6-Trimethoxy-3-pentadecyl-7-(8-hydroxypentadecyl)dibenzofuran (22) or 1,2,6-trimethoxy-3-(8-hydroxypentadecyl)-7-pentadecyldibenzofuran (23): IR (KBr) 2920, 2850, 1600, 1460, 1310, 1220, 1195, 1165, 1120, 1060, 1020, 810 cm^{-1}; EI-MS, m/z (relative intensity) 694 (100, M^+), 595 (6.5), 481 (12), 285 (61), 270 (73), 69 (100), 57 (67), 42 (55); FD-MS, m/z (relative intensity) 694 (100, M^+).

Compound B8. 22 or 23: IR (KBr) 2920, 2850, 1630, 1600, 1460, 1425, 1305, 1220, 1195, 1165, 1120, 1020, 810 cm^{-1}; EI-MS, m/z (relative intensity) 694 (83, M^+), 595 (8), 481 (8), 285 (100), 270 (68), 255 (30), 69 (89), 57 (73), 55 (60), 42 (72); FD-MS, m/z (relative intensity) 694 (100, M^+).

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