Artificial Urushi: Design, Synthesis, and Enzymatic Curing of New Urushiol Analogues

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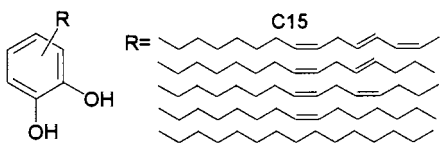
"Artificial urushi" was prepared by laccase-catalyzed curing of new urushiol analogues. The curing of the catechol derivative having a linolenoyl group proceeded in the presence of acetone powder, yielding the crosslinked film (artificial urushi) with high hardness and gloss surface, which are comparable with those of natural urushi coating.

Urushi is a typical Japanese traditional coating showing excellent toughness and brilliance for a long period.[1,2] In the early days of this century, pioneering works by Majima revealed that main important components of urushi are "urushiols", whose structure is a catechol derivative with unsaturated hydrocarbon chains consisting of a mixture of monoenes, dienes, and trienes at 3- or 4-position of catechol.[3,4] Typical urushiols are shown as follows.

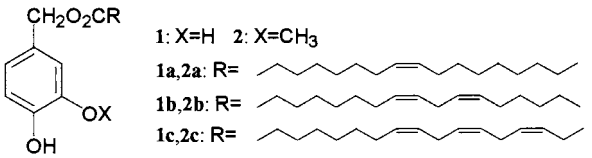


Crosslinking of urushiols takes place slowly with laccase catalysis under air to produce an insoluble polymeric film of urushi. The film formation is supposed to be accomplished mainly by a laccase-catalyzed oxidative coupling of the phenol moiety of the urushiol to give an oligophenol and a subsequent aerobic oxidation of unsaturated alkyl chains in the oligomer.[5,6] Urushi can be regarded as the sole example of practical natural paints utilizing in vitro enzymatic catalysis for hardening. The film-forming of urushiol proceeds under air at room temperature without organic solvents, and hence, urushi seems very desirable for coating materials from the environmental standpoint. However, modeling study of urushi has been scarcely attempted.[7] This is mainly due to the difficulty in preparation of urushiol.[8]

Recently, enzymatic syntheses of phenolic polymers[9,10] have received much attention as an alternative process for preparation of conventional phenolic resins without using toxic formaldehyde, a monomer for production of conventional phenolic resins (phenol–formaldehyde resins). Oxidative polymerization of various phenol derivatives proceeded using a peroxidase or laccase as catalyst under mild reaction conditions, yielding a new class of polyphenols showing high thermal stability.

In the present study, we have created a novel system of enzymatic polymerization, i.e., a laccase-catalyzed crosslinking reaction of new "urushiol analogues" (**1** and **2**) for the preparation of "artificial urushi". This modeling is the first example on the single-step synthesis of urushi-like cured film from monomeric phenol derivatives (urushiol analogues). The urushiol analogues were readily synthesized using lipase as catalyst and enzymatically cured under mild reaction conditions without using an organic solvent, therefore, the present multi-enzymatic processes are highly significant as a fundamental study for an alternative of conventional commercial coatings utilizing much organic solvents and severe hardening conditions.

For urushiol synthesis, multi-step reaction pathways were required since the direct introduction of the unsaturated group onto the phenolic aromatics is very difficult. To overcome this barrier, we have designed novel urushiol analogues (**1** and **2**), in which the unsaturated group is connected with the phenolic group through an ester linkage. The analogues were synthesized by a lipase-catalyzed esterification of the corresponding phenols having a primary alcohol with unsaturated fatty acids having different number of double bonds.[11,12]



Enzymatic crosslinking reaction of **1** and **2** was carried out by *Pycnoporus coccineus* laccase catalyst (2.3×10^5 units per 1g of **1** or **2**) in the presence of acetone powder (AP, an acetone-insoluble part of the urushi sap containing mainly polysaccharides and glycoproteins) with 80% humidity at 30 °C for 24 h. AP is a third component of the sap and believed to act as emulsifier of oily urushiol and aqueous laccase solution. AP had no laccase activity. The film formation was observed from urushiol analogues, **1b**, **1c**, **2b**, and **2c**, having more than 2 carbon–carbon double bonds and the resulting film was insoluble in organic solvents and water. On the other hand, **1a** and **2a** were not cured. The control experiment (without using laccase) did not afford the polymeric film. These data indicate that the present curing took place through the enzymatic catalysis and two or three unsaturated groups in the side chain were required for the hardening. The hardening of **1** proceeded faster than that of **2**.

The curing of **1** was monitored by using a dynamic microhardness tester (Figure 1). At the initial stage of the curing of **1c**, the hardness value was very small and the sudden increase was observed after two weeks. Later, the value gradually increased to reach ca. 150 N/mm^2 after 5 weeks. The gloss value of the film surface was more than 100. The pencil scratch hardness reached H after 15 days, which is hard enough for industrial uses. The hardness and gloss values of the present cured film are comparable to those of natural urushi coating; the curing of the urushiol analogues produced the brilliant film with the high gloss surface. In the curing of **1b**, on the other

hand, the hardness value was less than 5 N/mm^2 after 6 weeks, yielding the soft film. The reaction monitoring of **1** by FT-IR revealed that the crosslinking mechanism of the present polymer was similar to that of natural urushi.

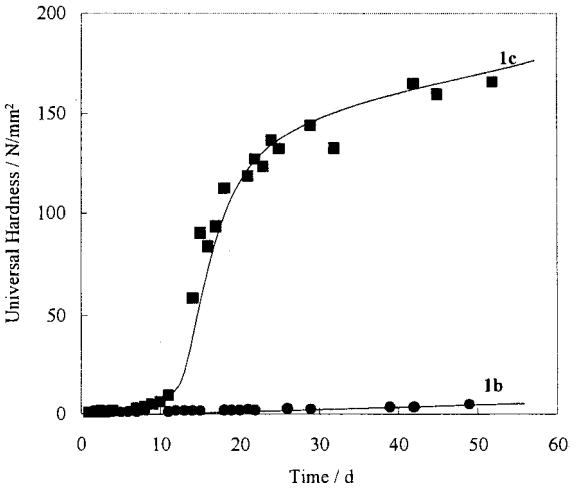


Figure 1. Time course in hardening of artificial urushi films from **1b** and **1c** by using Fischer microhardness tester.

Recently, starch–urea phosphate (SP), a synthetic material, has been reported to be highly effective as the third component for in vitro enzymatic curing of urushiols.[13] The curing of **1b** and **1c** in the presence of SP took place, however, the hardness was much smaller than that using AP as the third component. Interestingly, the laccase-curing of **1b** produced the crosslinked film with relatively good hardness (30 N/mm^2 after 10 weeks).

Figure 2 shows dynamic elasticity of the cured film after drying for 5 months, which was prepared from **1c** in the presence of AP. The glass transition temperature was observed at 102 ºC. From the increase of storage modulus (E') in the region at high temperatures, it was found that the unreacted unsaturated carbon–carbon double bonds remained in the measured sample. The smooth trace of dissipation factor (tan δ) means the homogeneous structure of the present cured film, suggesting the

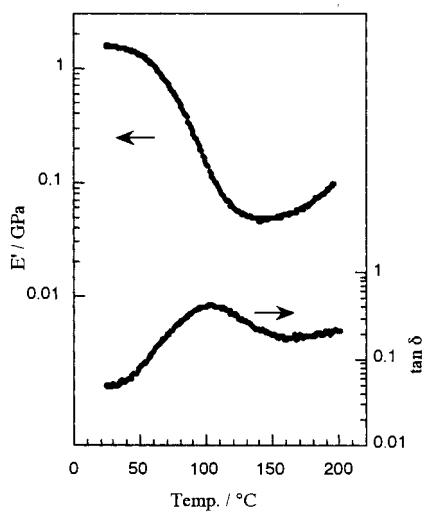


Figure 2. Dynamic viscoelasticity of artificial urushi from **1c**.

good miscibility between the urushiol analogue and AP. These dynamic elastic behaviors of the artificial urushi were very similar to those of natural urushi.

In conclusion, "artificial urushi" has been developed by enzymatic crosslinking of new urushiol analogues, which were designed and synthesized by lipase-catalyzed regioselective acylation with facile procedures. These compounds were cured in the presence of commercially available laccase catalyst under mild reaction conditions without use of organic solvents to produce the crosslinked polymeric film with high gloss surface and good elastic properties. In case of the combination of the urushiol analogue and starch–urea phosphate, the artificial urushi was prepared only from synthetic compounds. Therefore, the present method has large potential for a future environmentally-benign process of polymer coating, giving an example system of "green polymer chemistry".[14]

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11 Very recently, we have reported a lipase-catalyzed regioselective acylation of 4-(2-hydroxyethyl)phenol in the side chain with unsaturated fatty acids: T. Tsujimoto, R. Ikeda, H. Uyama, and S. Kobayashi, *Chem. Lett.*, **2000**, 1122. Urushiol analogues **1** and **2** were synthesized according to the modified procedure of this literature.
12 A mixture of vanillyl alcohol (3.1 g, 20 mmol), linoleic acid (2.8 g, 10 mmol), and crude *Pseudomonas cepacia* lipase (10 g) in a mixture of 90 mL of *i*-propyl ether and 10 mL of tetrahydrofuran was heated at 60 °C under gentle stirring. After 240 h, the enzyme was removed by filtration and the filtrate was poured into water. The organic layer was separated and further washed twice with water. The organic solution was dried over sodium sulfate and the solvent was evaporated under reduced pressure. The remaining vanillyl alcohol was removed by recrystallization to give 3.4 g of 2-methoxy-[4-(*cis*, *cis*-9,12-octadecadienoyloxy)methyl]phenol (**2b**) (yield 81%). ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, CH_3), 1.26 (14H, br, –CCH_2C–), 1.52 (2H, m, –C(=O)CH_2CH_2C–), 2.01 (4H, m, –CH=CHCH_2C–), 2.29 (2H, t, –C(=O)CH_2C), 2.73 (2H, m, –CH=CHCH_2CH=CH–), 3.75 (3H, s, OCH_3), 4.95 (2H, s, ArCH_2O), 5.32 (4H, m, –CH=CH–), 6.73–6.91 (3H, m, Ar), 9.06(1H, br, ArOH).
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