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Enzymatic epoxidation and polymerization of cardanol obtained from a renewable resource and curing of epoxide-containing polycardanol

Yong Hwan Kim [b,*], Eun Suk An [a], Seung Young Park [a], Bong Keun Song [a,*]

[a] *Korea Research Institute of Chemical Technology, PO Box 107, Yusong, Taejon 305-600, Republic of Korea*
[b] *Department of Chemical Engineering, Kwangwoon University, Seoul 139-701, Republic of Korea*

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Abstract

Epoxide-containing polycardanol was enzymatically synthesized via two routes using two different enzymes, viz. lipase and peroxidase. Lipase catalysis was used for the epoxidation of the unsaturated alkyl chains of both cardanol and polycardanol. Peroxidase catalysis was used for the polymerization of both cardanol and epoxide-containing cardanol. One route was the synthesis of epoxide-containing cardanol from cardanol, hydrogen peroxide and an organic acid in the presence of lipase, followed by the polymerization of the phenolic functional groups of cardanol using peroxidase. In the other route, polymerized cardanol was prepared from cardanol and hydrogen peroxide in the presence of peroxidase and, subsequently, the epoxide-containing polycardanol was synthesized from polycardanol, hydrogen peroxide and an organic acid in the presence of lipase. NMR and IR spectroscopy confirmed the polymer structure, and the former route yielded epoxide-containing polycardanol in a higher yield of over 90%. The curing of the resulting polymers proceeded thermally at 150 °C, yielding transparent polymeric films with a high gloss surface within 3 h. The pencil scratch hardness of the present films was improved compared with that of polycardanol. Owing to the epoxide contained in the polymerized cardanol, the film cured with phenalkamine showed a higher hardness value after a relatively short curing time.

Keywords: Lipase; Peroxidase; Cardanol; Epoxide-containing polycardanol

1. Introduction

The worldwide demand for replacing the petroleum derived raw materials used in the production of polymeric materials with renewable plant-based ones is quite significant in terms of its potential social and environmental impact [1]. Using such plant-based raw materials would contribute to global sustainability without the depletion of scarce resources. Furthermore, these materials are sometimes cheaper than petrochemicals.

We previously reported a method of producing new functional polymers from plant oil under mild reaction conditions [2–4]. Cashew nut shell liquid (CNSL) constitutes nearly one-third of the total nut weight; thus, a large amount of CNSL is formed as a by-product of the mechanical processes used to render the cashew kernel edible and its total production approaches one million tons annually. Thermally treated CNSL, whose main component is cardanol, a phenol derivative mainly having a *meta* substituent of a C15 unsaturated hydrocarbon chain with one to three double bonds, has various potential industrial utilizations, such as resins, friction lining materials, and surface coatings; however, only a small part of the CNSL that is produced is used in the industrial field.

Recently, we reported that the oxidative polymerization of thermally treated CNSL using peroxidase secreted from the basidiomycete fungus, *Coprinus cinereus*, produced the oily soluble polymer very efficiently, and that this polymer can be used as a glossy coating material [4].

Lipase is known to catalyze the epoxidation of unsaturated groups in the presence of a catalytic amount of carboxylic acids under mild reaction conditions [5,6]. The conventional epoxidation process utilizes peracetic or performic acid to elicit oxygen transfer to double bonds, resulting in low yields due to side reactions such as the acid-catalyzed ring opening of oxiranes. In contrast, the enzymatic epoxidation provides a mild and simple alternative, especially for the production of sensitive epoxides.

* Corresponding authors. Tel.: +82 2 940 5675; fax: +82 2 941 1785.
E-mail addresses: metalkim@kw.ac.kr (Y.H. Kim), bksong@krict.re.kr (B.K. Song).

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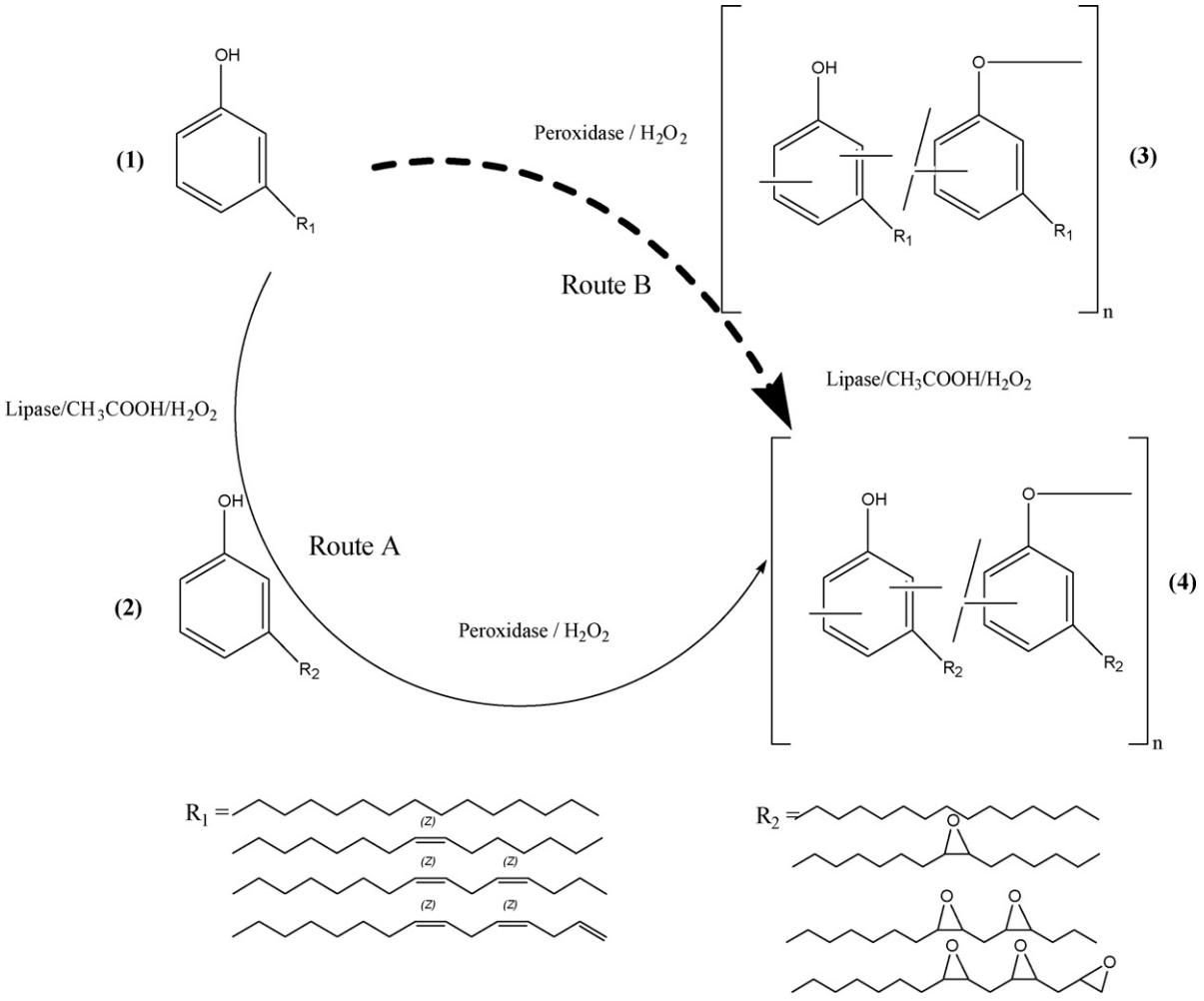

Fig. 1. Reaction scheme of cardanol and polycardanol. Solid line: reaction route A; dotted line: reaction route B.

This study deals with the enzymatic synthesis and curing of polycardanols having an epoxide group in their side chain. The epoxide-containing polycardanols were synthesized using lipase catalyst via two routes (Fig. 1). One involves the synthesis of polycardanol from cardanol using peroxidase, followed by the epoxidation of the unsaturated groups in the side chain (route A). In the other route, epoxide-containing cardanol is prepared from cardanol in the presence of lipase and, subsequently, the epoxide-containing cardanol is polymerized with peroxidase (route B). To our knowledge, this is the first example of the enzymatic synthesis of epoxide-containing polyaromatics such as polycardanol.

2. Experimental

2.1. Production and purification of the fungal peroxidase

C. cinereus IFO 8371 was used as the peroxidase-producing strain. The medium used for peroxidase production contained 30 g/l glucose, 5 g/l peptone (Difco Lab., USA), and 3 g/l yeast extract (Difco Lab., USA). The culture was started by inoculating a spore suspension, which was prepared by adding 5 ml of the above medium to a solid culture and vibrating this for 30 s in a 500 ml Erlenmeyer flask containing 70 ml of culture medium, and then incubating it on a shaking incubator at 120 rpm and 30 °C. The culture medium was filtered through a membrane filter (pore size: 0.45 μm, Whatman) and the filtrate was assayed for peroxidase activity, glucose concentration, and pH. The cells retained by the filter were dried in an oven for 2 days and then weighed.

The supernatant from the culture broth was concentrated by ultrafiltration (Amicon Ultra-4 centrifugal filter, 10 kDa MWCO) and desalted with 0.1 M phosphate buffer (pH 5.0). The desalted CiP solution was purified by size exclusion chromatography (SEC) using a silica-based column (Bio-Sil SEC 125, Bio-Rad) and 0.1 M phosphate buffer (pH 5.0) as the eluent. The CiP fraction collected was concentrated by ultrafiltration (Amicon Ultra-4 centrifugal filter, 10 kDa MWCO).

2.2. Enzymatic epoxidation of cardanol or polycardanol having unsaturated double bonds in the side chain

A typical run was as follows. A mixture of cardanol (1.5 g, 5 mmol, Palmer International, USA) or polycardanol (1.5 g, polymerized by peroxidase, as shown in the following section) having unsaturated double bonds in the side chain, lipase

(200 mg, Novozyme 435, Novozyme, Denmark) and acetic acid (287 mg, 5 mmol, Sigma, USA) in 10 ml of toluene was placed in a 25 ml three neck flask. Hydrogen peroxide (30%, 0.85 ml, 7.5 mmol) was added continuously to the mixture for 6 h using a perfusion pump. After the removal of the Novozyme 435 beads by filtration, the filtrate was collected. The organic solvent was removed under reduced pressure, and the residue was dried in vacuo to give 1.42 g of the cardanol containing epoxide (yield 95%).

2.3. Enzymatic polymerization of cardanol or epoxide-containing cardanol using peroxidase

About 0.6 g (2 mmol) of cardanol or the epoxide-containing cardanol which was synthesized with lipase was dissolved in a mixture of 12.5 ml 2-propanol and 12.5 ml phosphate buffer (100 mM, pH 7.0). About 6000 units of CiP were added to the reaction mixture, which was then stirred for 5 min and the reaction started by the continuous addition of H_2O_2 (30%, 300 μl, 2 mmol) for 5 h at room temperature with gentle stirring. After 24 h, the reaction mixture was concentrated under reduced pressure. Ethyl acetate (20 ml) was added to the residue, and the organic top layer was separated, followed by the removal of the solvent under reduced pressure. Methanol was added to the oily residue to remove any unreacted cardanol. The methanol-insoluble material was separated by centrifugation and dried in a vacuum to give the epoxide-containing polycardanol.

2.4. Analytical methods

Gel permeation chromatography (GPC) analysis was carried out using a refractive index detector under the following conditions: PL4 mixed BB columns (TOSOH, Japan) and tetrahydrofuran as the solvent at 1.0 ml min^{-1}. The calibration curves for the GPC analysis were obtained using polystyrene standards. FT-IR spectra were recorded on a Perkin-Elmer FT-IR 2000 to confirm the polymer structure and ^{1}H NMR spectra were recorded on a Bruker AMX-500 FT-NMR Spectrometer (Bruker Co., Germany). The epoxide content was measured by the titration of the consumed hydrogen bromide according to ASTMD1652-97.

The sample film was prepared on a glass slide by using an applicator to obtain a thickness of 50 μm. One gram of phenalkamine (NC540, Cardolite Co., USA), a kind of aliphatic polyamine was added to 10 g of epoxide-containing polycardanol solution dissolved in ethyl acetate. Thermal treatment at 150 °C was performed to make cured coating film on slide glass. The pencil scratch hardness was evaluated by means of Mitsubishi Uni pencils with different hardness and a pencil scratch apparatus by applying a constant force.

3. Results and discussion

3.1. Lipase-catalyzed synthesis of epoxide-containing cardanol or polycardanol

Candida antarctica lipase immobilized on macroporous acrylic resin is used industrially for the modification of triglyceride oils [7]. In this study, lipase was used as a catalyst for the epoxidation of the unsaturated groups of cardanol or polycardanol. As the first step of route A (solid line), cardanol was epoxidized with the help of immobilized lipase. Table 1 shows the effect of the solvents on the epoxidation of cardanol. As shown in Table 1, the highest level of epoxidation was achieved in toluene, whereas the lowest level was achieved in methylethylketone. Generally, solvents with a high log *P* value (water immiscible solvents) have a beneficial effect on the stability of enzyme proteins, because their hydration layer can be maintained [8]. Since methylethylketone has a low log *P* value compared with the other solvents shown in Table 1, the hydration layer around the lipase protein can easily be disrupted, resulting in a low yield and epoxidation ratio. This solvent effect was also observed for the epoxidation of polycardanol (route B, dotted line) as shown in Table 1. Similar results can be found in a previous report about the epoxidation of unsaturated carboxylic acids, where epoxidation-using lipase (CalB) was carried out in toluene [5]. However, the epoxide contents ranged from 0.51 to 0.57 mequiv./mmol, which were lower than expected. Cardanol is known to have 1.5 mequiv./mmol of unsaturated double bonds in its *meta*-substituted alkyl groups [9]. Only 38% of these unsaturated double bonds were supposed to be converted to epoxide groups.

The enzymatic epoxidation of the unsaturated groups in (**1**) and (**3**) was performed using hydrogen peroxide as the oxidizing agent in the presence of acetic acid at room temperature for 6 h. The structures of products (**2**) and (**4**) were confirmed by NMR and IR spectroscopy. Fig. 2 shows the ^{1}H NMR spectra of (**1**) and (**3**) and their epoxidized products, (**2**) and (**4**), respectively. In the ^{1}H NMR spectrum of (**1**), the characteristic peaks for the CH=CH protons were observed at δ 5.8, 5.4 and 5.0 (peaks b, c, and d, respectively) (Fig. 2). On the other hand, while these

Table 1
Epoxidation of cardanol and polycardanol by lipase (CalB)

	Solvent for epoxidation	Yield (%)	Log *P* value	Epoxide content (mequiv./mmol)
Cardanol (**1**)	Toluene	95.3	2.38	0.52
Cardanol (**1**)	*n*-Hexane	86.0	3.65	0.52
Cardanol (**1**)	Tetrahydrofuran	82.7	0.70	0.51
Cardanol (**1**)	Methylethylketone	81.3	0.737	0.51
Polycardanol (**3**)	Toluene	93.9	2.38	0.57

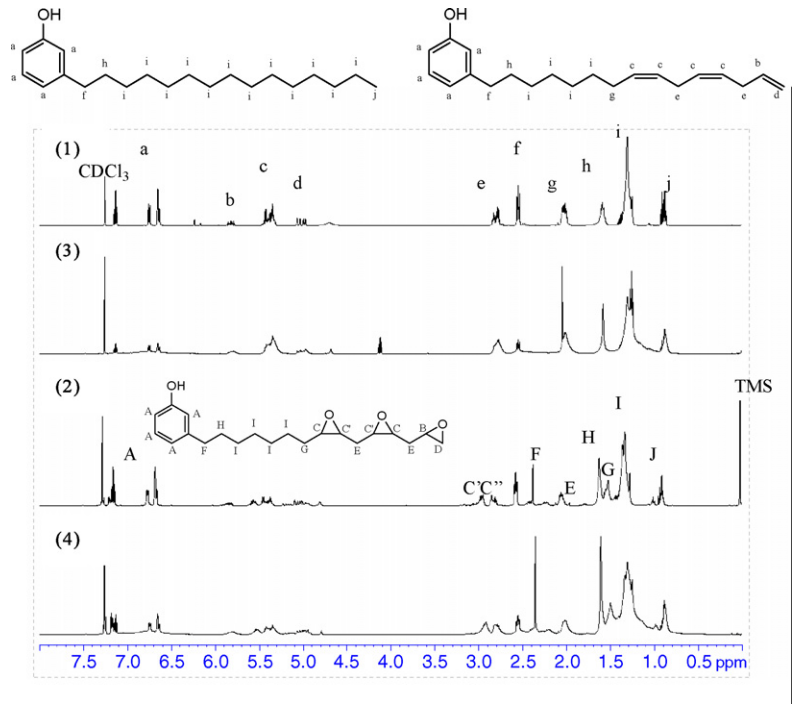

Fig. 2. 1H NMR spectra of (**1**) cardanol and (**2**) epoxide-containing cardanol (**3**) polycardanol and (**4**) epoxide-containing polycardanol.

peaks disappeared in the spectra of the epoxidized products, (**2**) and (**4**), new peaks (peaks C and C′) were seen at δ 2.9 and 2.8, which were ascribed to the methine protons of the oxirane ring (Fig. 2). The neighboring methylene protons (Fig. 2, g and e) appeared to be down shifted by the epoxidation, as shown in Fig. 2(G and E). All of the peaks of (**3**) and (**4**) became broader than those of (**1**) and (**2**), respectively (Fig. 2). The polymerization of cardanol is known to make all of the peaks broader, since this phenomenon was already observed in another study [11]. However, unsaturated double bonds can be observed even after the polymerization of cardanol (Fig. 2, c, b, and d). This means that the unsaturated groups did not react and only the phenolic moiety was polymerized during the polymerization catalyzed by CiP. In the FT-IR spectra of (**1**) and (**3**), there was a characteristic peak at 3010 cm^{-1} which was ascribed to the C–H stretching of the inner unsaturated moiety. Peaks were observed at 3400 cm^{-1} due to the vibration of the O–H linkage of the phenolic group, and three characteristic peaks were also observed at 1240, 1190, 1155 cm^{-1}, respectively, which were ascribed to the vibrations of the C–O–C and/or C–OH linkages because of the polymerization of cardanol and the epoxide-containing cardanol (Fig. 3(**3**) and (**4**)). After the epoxidation, this peak disappeared, as shown in Fig. 3. This characteristic peak at 3010 cm^{-1} also disappeared when polycardanol was cured, either thermally at 150 °C or chemically by adding a curing agent such as cobalt naphthenate [2,3].

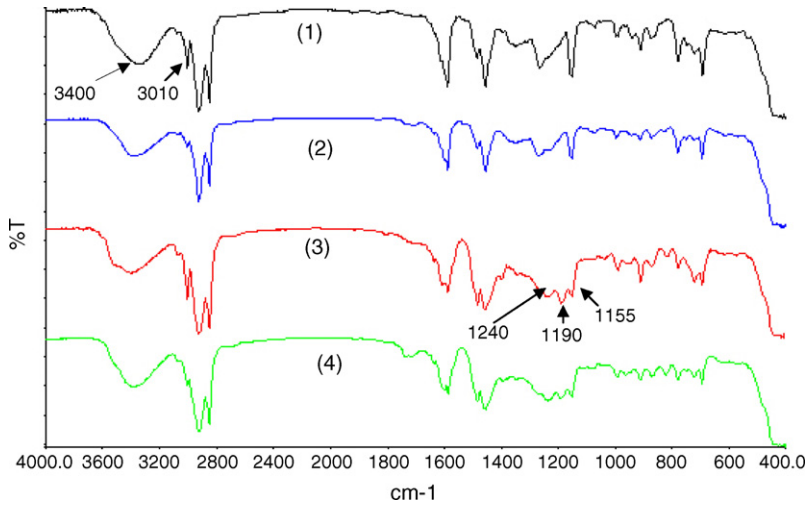

Fig. 3. FT-IR spectra of (**1**) cardanol and (**2**) epoxide-containing cardanol (**3**) polycardanol and (**4**) epoxide-containing polycardanol.

Table 2
Polymerization of cardanol and epoxide-containing cardanol by *Coprinus cinereus* peroxidase

	Solvent for epoxidation	Polymerization yield (%)	Mw	Mn
Cardanol (**1**)	2-Propanol	74	5,221	3411
Cardanol (**1**)	Methanol	55	10,808	3540
Cardanol (**1**)	Ethanol	62	8,974	4096
Cardanol (**1**)	1,4-Dioxane	0	–	–
Epoxide-containing cardanol (**2**)	2-Propanol	96	5,830	4310
Epoxide-containing cardanol (**2**)	Methanol	71	7,800	3200
Epoxide-containing cardanol (**2**)	Ethanol	82	8,602	3620
Epoxide-containing cardanol (**2**)	1,4-Dioxane	0	–	–

3.2. Peroxidase-catalyzed polymerization of cardanol or epoxide-containing cardanol

Peroxidase from fungi *C. cinereus* (CiP) can polymerize *meta*-substituted phenols such as cardanol with chemical selectivity [4]. CiP was used to obtain polycardanol (**3**) and epoxide-containing polycardanol (**4**). When the CiP-catalyzed polymerization of cardanol was performed in various water-miscible organic solvent/phosphate buffer (50:50 w/w) mixtures, 2-propanol produced the highest yield of 74% (cardanol) and polymerization was not achieved in aqueous *t*-butanol or 1,4-dioxane. The molecular weight of the polycardanol formed was also affected by the nature of the solvent. The molecular weight increased from 5221 to 10,808 when 2-propanol was replaced by methanol. A similar tendency was found for SBP in the polymerization of cardanol [2]. CiP catalyzed the polymerization of epoxide-containing cardanol, as shown in Table 2. Compared with the polymerization of cardanol, epoxide-containing cardanol was more easily polymerized and its yield approached 96%. While two groups (the phenolic moiety and the unsaturated hydrocarbon group) are subjected to polymerization in the case of cardanol [8], only one group (the phenolic moiety) is subjected to polymerization in the case of the epoxide-containing cardanol, because most of the unsaturated hydrocarbon groups were converted to oxirane groups. The phenoxy radicals produced by CiP in the case of the epoxide-containing cardanol would be expected to combine with each other and result in a polymer product without the loss of the unsaturated hydrocarbon groups. This may be the reason for the higher yield of the polymerized product which can be obtained in the case of the epoxide-containing cardanol. Fig. 2 indicates that no ring-opening phenomenon occurs during polymerization, because of the oxidative environment produced by the presence of hydrogen peroxide. However, a significant difference was observed in the case of the epoxide-containing polycardanol (**4**) depending on the synthesis pathway. While the epoxide-containing polycardanol synthesized by route A showed a viscous liquid state, that produced by route B showed a gel-like appearance. In addition, this gel-like polymer showed very poor solubility in most solvents, which is very problematic for its application as a coating material. Polycardanol (**3**) having unsaturated hydrocarbons is known to be susceptible to oxidation and the formation of cross-linkages between the polymer chains. During its epoxidation by lipase/H_2O_2/acetic acid, it was expected that some of the polycardanol would be cross-linked, due to the highly oxidative environment. The amount of cross-linkage formation in unsaturated hydrocarbons tends to be higher for the polymer than for the monomer. Based on these results, route A rather than route B is recommended to produce epoxide-containing polycardanol.

3.3. Curing of epoxide-containing polycardanol

The curing of cardanol (**1**), epoxide-containing cardanol (**2**), polycardanol (**3**), epoxide-containing polycardanol (**4**) obtained via route A, and epoxide-containing polycardanol (**4**) with curing agent such as phenalkamine was carried out by thermal treatment at 150 °C for various times. The sample film was prepared on a glass slide by using an applicator to obtain a thickness of 50 μm. The curing behavior was monitored by measuring the pencil scratch hardness. Polycardanol (**3**) and epoxide-containing polycardanol (**4**) were cured to give transparent films insoluble in organic solvents. Their pencil scratch hardness was in the order, (**1**) < (**2**) < (**3**) < (**4**), suggesting that the epoxy group plays a major role in the hardness of the cured film. In the cured film of (**4**), the hardness (9H) was much higher than that of a commercially available coating (general alkyd resin, 4H) [10].

Table 3
Curing of epoxide-containing polycardanol and its hardness

	1 h	2 h	3 h	5 h
Cardanol (**1**)	–	–	–	–
Epoxide-containing cardanol (**2**)	–	–	–	–
Polycardanol (**3**)	–	TF	2B	H
Epoxide-containing polycardanol (**4**)	TF	5B	H	9H
Epoxide-containing polycardanol (**4**) + phenalkamine (Cardolite NC540)	2H	5H	7H	>9H

Curing at 150 °C; TF: touch free hardness.

This implies that the aromatic group of the epoxide-containing polycardanol endows it with strong mechanical properties, as would be expected. By the addition of a curing agent such as phenalkamine, which is commonly used in the shipbuilding industry, the curing time could be significantly reduced, as shown in Table 3. The hardness of the cured film approached 5H within 2 h, which is sufficiently high for use as a commercial coating. For applications requiring strong hardness and mechanical durability this epoxide-containing polycardanol constitutes a possible alternative to the existing phenol-formaldehyde epoxy resin.

4. Conclusions

Epoxide-containing cardanol (**2**) and polycardanol (**4**) were synthesized via two routes using lipase as a catalyst. *C. antarctica* lipase efficiently catalyzed the epoxidation of the unsaturated alkenyl groups of cardanol and polycardanol. The enzymatic epoxidation proceeded under mild reaction conditions, leading to a high epoxidation ratio. The resulting epoxide-containing polycardanol was thermally cured to give transparent films with a high gloss surface. The hardness of the films exceeded 9H, which is the upper measurement limit of the pencil scratch test.

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