Synthesis, Characterization, and Bioactivity of Rosin Quaternary Ammonium Salt Derivatives

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Two series of rosin quaternary ammonium salts (QAS) were synthesized using the same path. The structure of the target products was characterized by HPLC, MS, IR, and ^{1}HNMR, and the bioactivity was determined by filter paper method using *Trametes versicolor* (white-rot fungus) and *Gloeophyllum trabeum* (brown-rot fungus), which are two kinds of general wood decay fungi in nature. The results showed that all compounds tested had a satisfactory anti-fungal effect at the molarity of 0.025 mmol/mL. Hereinto, acrylpimaric Gemini QAS had better bioactivity than dehydrogenated or tetrahydrogenated rosin QAS against *Trametes versicolor*. To this fungus, quaternary ammonium groups, which wraps up the membrane of microorganism and disrupts the balance in cell membrane, plays the leading role for its bioactivity. To *Gloeophyllum trabeum*, the inhibition activity of acrylpimaric QAS and dehydrogenated rosin QAS are almost at the same level and larger than tetrahydrogenated rosin QAS, so we conclude that both quaternary ammonium group and aromatic group play important roles. Compared with dodecyl dimethyl benzyl ammonium chloride (1227), which is a commercially available quaternary ammonium salt type fungicide, acrylpimaric acid quaternary ammonium salts have approximate bioactivity against *Gloeophyllum trabeum*. In conclusion, rosin derivatives with functional groups would do well in wood preservative applications.

Keywords: Rosin; Quaternary ammonium salt; Anti-fungal activity; Filter paper method

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INTRODUCTION

Rosin is a mixture of compounds, of which 90% are acidic with the formula of $C_{19}H_{29}COOH$ (called resin acids) and the remaining 10% are neutral compounds. Resin acids have a characteristic bulky hydrophenanthrene structure that provides them with excellent chemical and thermal stabilities (Wang *et al.* 2009) and two areas for potential interactions with other species – a double-bond conjugate system and a carboxyl group (Maiti *et al.* 1989). After their modification various rosin derivatives have been used as curing agents (Liu *et al.* 2009, 2010; Wang *et al.* 2008), film-forming materials (Pathak *et al.* 1985; Sheorey and Dorle 1991), tablets (Nande *et al.* 2006), composites (Bicu and Mustata 2007; Yao *et al.* 2011), varnishes (Maiti *et al.* 1989), hardeners (Atta *et al.* 2004), and fungicide (Li *et al.* 2012). Dehydrogenated rosin (DHR) and tetrahydrogenated rosin (THR) are widely used in many fields such as medicine (Wilbon *et al.* 2010; Zheng *et al.* 2010). Furthermore, rosin cationic quaternary ammonium salts were reported to be antistatic (Yasuyoshi *et al.* 1987), bactericidal (Dong *et al.* 2011), and abstergent (Zhang 2004).

As a terpenoid, the resin acid framework plays a crucial role as a hydrophobic group. Kubo *et al.* (1995) proposed a hypothesis of "head-tail structure chemicals" affecting antifungal activity. It was noted that the chemicals are composed of one hydrophilic head and one hydrophobic tail. The intermolecular hydrogen bond of the hydrophilic head wraps up the membrane of microorganism while the hydrophobic tail disturbs the lipid-protein interface of integral proteins and disrupts the balance in the cell membrane. As a hydrophilic functional group, quaternary ammonium had robust and broad spectrum anti-fungal activity (Menger and Littau 1991). In addition, Chen *et al.* (2012) reported that dehydroabietic quaternary ammonium salt polymers had satisfactory bioactivity and better efficacy, as well as reduced residual toxicity, increased selectivity, and prolonged lifetime. However the bioactivity decreased markedly relative to that of the monomer. Within limits, the higher degree of quaternization, the lower bioactivity. A series of Diels-Alder adducts of resin acid with acrylic acid were synthesized, and these showed great antimicrobial activity against different kinds of bacteria by filter paper method (Li *et al.* 2012; Wang *et al.* 2012). It was also reported that aromatic constituents of compounds had broad spectrum anti-fungal activity (Cockroft *et al.* 2007; Pattnaik *et al.* 1997). As an inexpensive natural product, fungicides related to rosin have superiority.

In this paper, two series of rosin quaternary ammonium salts were synthesized and characterized by HPLC, IR, MS, and ^{1}HNMR. The goal was to analyze the different anti-fungal activities of compounds with different functional groups. The bioactivity against wood decay fungi was determined by filter paper method using *Trametes versicolor* and *Gloeophyllum trabeum*, and the observed bioactivities of the products corresponded with earlier findings. Rosin derivatives with antifungal-functional-group have potential market as wood preservatives, so more attention will be paid to them.

EXPERIMENTAL

Materials and Instruments

Acrylpimaric acid (APA) was synthesized and purified according to the literature (Li *et al.* 2012), and the purity was 84% according to GC analysis. Dehydrogenated rosin (DHR) and tetrahydrogenated rosin (THR) showed purity of 89% and 84%, respectively, when tested by GC analysis. Dodecyl dimethyl benzyl ammonium chloride (1227) is commercially available in China with more than 90% cationic-active matter content. Others, such as dichloromethane, oxalyl chloride, tetrabutyl ammonium bromide (TBAB), alcohol, acetone, epichlorohydrin, diethyl ether, 30% trimethylamine aqueous solution, and triethylamine were reagents of analytical grade. HPLC analysis was detected with an Agilent 1100 spectrometer with a separation column of C18 at 30°C. Its mobile phase was methyl alcohol and water (95:5, v/v). IR analysis was performed on a Magna 560 FT-IR spectrometer, which was made by the Nicolet company, and a KBr press method was used. ^{1}HNMR (400 MHz) spectra were recorded on a Bruker 400MHz Advance spectrometer.

Synthesis of APE (3), DHRE(7a) or THRE (7b)

APA (18.7 g, 0.05 mol)/DHR (30 g, 0.1mol)/THR (30 g, 0.1mol) was dissolved in dichloromethane (40 mL) in a three-neck glass reactor equipped with a condenser, a temperature control, and a magnetic stirrer device. Then, oxalyl chloride (13.8 g, 0.11 mol) was added dropwise and reacted 3 h at room temperature. Dichloromethane and

excessive oxalyl chloride were excluded by distillation. Then, epichlorohydrin (18.5 g, 0.2 mol) and TBAB (0.32 g, 0.001 mol) were added dropwise and the reaction temperature was heated to 50°C and kept for 4 h. A high-viscosity liquid was obtained after reduced pressure distillation was used to remove the excess epichlorohydrin. The yields of APE, DHRE, and THRE were 83%, 89%, and 88%, respectively.

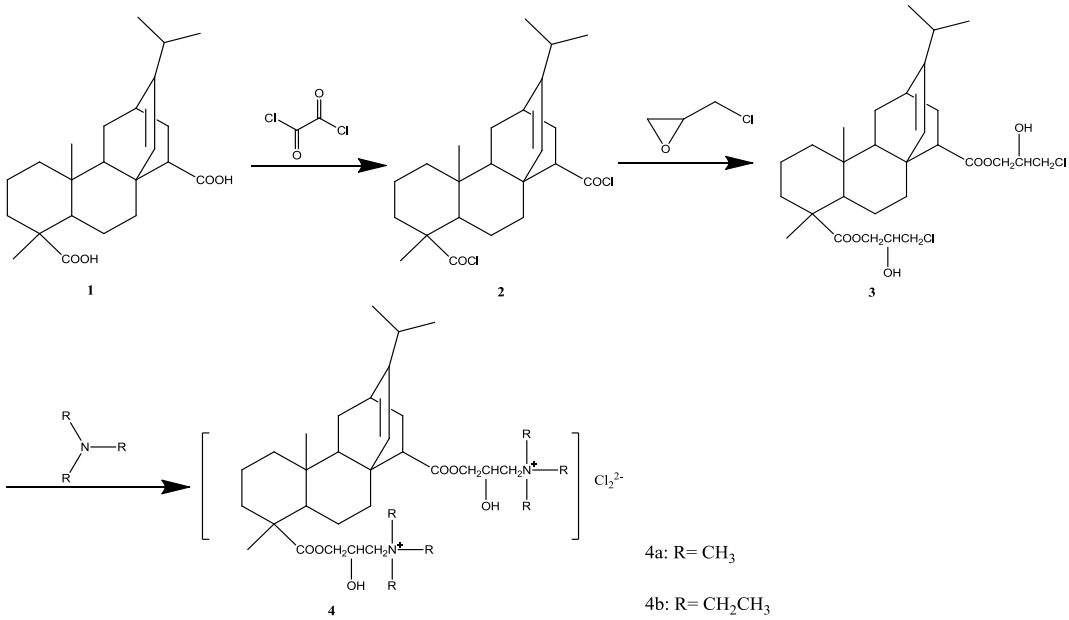

Fig. 1. Synthetic routes of APQAS

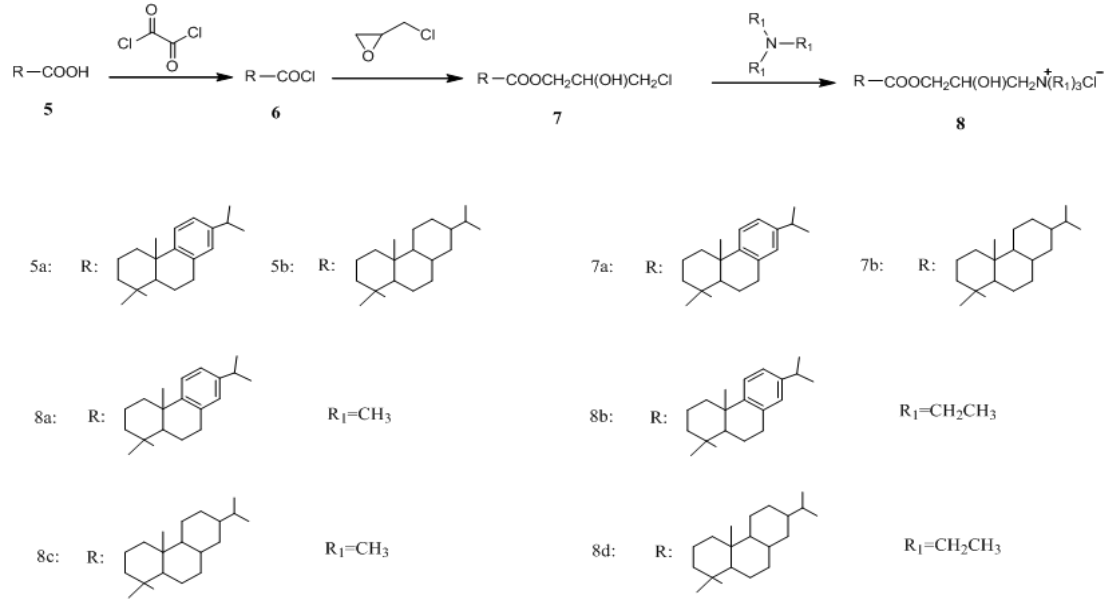

Fig. 2. Synthetic routes of DHQAS and THQAS

Synthesis of APQAS (4a, 4b) /DHQAS (8a, 8b)/THQAS (8c, 8d)

APE (15.8 g, 0.03 mol)/DHRE (22.6 g, 0.06 mol)/THRE (22.6 g, 0.06 mol) and triethylamine (9.1 g, 0.09 mol)/30% trimethylamine aqueous solution (15.9 g, 0.09 mol) were dissolved in 50 mL of alcohol in a three-neck glass reactor equipped with a

condenser, a temperature control, and a magnet stirrer device. The temperature was kept at 85°C for 3.5 h (Zhou *et al.* 2007; Shi 2003). Alcohol was removed after distilled under reduced pressure. Then the mixture was extracted by ether and water three times. Aqueous phase remained and distilled under reduced pressure for recrystallization from alcohol and acetone.

Bioactivity

The bioactivity of compounds was tested by filter paper method with the fungi of *Trametes versicolor* and *Gloeophyllum trabeum*. The raw materials and products were dissolved separately in 75% ethanol to obtain the molarity of 0.025 mmol/mL. Then, discs were dipped in these solutions for 10 min and air-dried. Additional discs were dipped in 75% ethanol without chemicals served as controls and the discs in 0.025 mmol/mL dodecyl dimethyl benzyl ammonium chloride (1227) served as positive, antifungal comparators. Meanwhile, potato dextrose agar (PDA) was decanted into petri dishes with actively growing mycelium of *Trametes versicolor* or *Gloeophyllum trabeum* when it was cool enough but still liquid. After set into concretion, all discs were placed separately onto the middle surface of the PDA, then sealed up. Each chemical condition was repeated 3 times, and all plates were cultured at 28°C for 3 to 5 days (Li *et al.* 2010).

$$\text{Diameter of inhibition zone (mm)} = A - A_0 \quad (1)$$

where A_0 is diameter of inhibition zone of control, and A is diameter of inhibition zone in the presence of chemicals tested.

Table 1. Average Diameter of Inhibition Zone of 4a, 4b, 8a, and 8b against *Trametes versicolor* and *Gloeophyllum trabeum* in 0.025 mmol/mL *

compound	*Trametes versicolor*	*Gloeophyllum trabeum*
4a	13	10
4b	12	9
8a	10	10
8b	9	10
8c	8	5
8d	9	5
1227	22	14

* Average diameter values are the mean of three independent trials

RESULTS AND DISCUSSION

The synthetic routes of APQAS, DHQAS, and THQAS are shown in Figs. 1 and 2, respectively. The first reactions of both routes are to activate the reactivity of the raw materials of APA, DHR, and THR. Compared with APA, dehydrogenated rosin has an additional aromatic group, which provides fungistasis against many fungi, and after reaction with tertiary amine in the last reactions, quaternary ammonium bioactive groups are brought in.

IR spectra of compounds were collected on a Magna 560 FT-IR spectrometer. Compared with the raw material 1, the absorbance at 3430 cm^{-1} in the other three

spectrograms heightens as the three-membered ring of epichlorohydrin opened and -OH is produced. The absorbance at 1700 cm^{-1} of C=O stretching vibration in spectrogram of 1 converts to 1720 cm^{-1} of 3, 4a, and 4b after the esterification, and so is the respective absorbance of the compounds in Fig. 2.

^{1}HNMR spectrograms were recorded on a Bruker 400MHz Advance spectrometer. As shown in the spectrograms, chemical shifts between 7.36 ppm and 6.58 ppm can be assigned to the hydrogen belonging to benzene in the phenanthrene ring. Chemical shifts between 4.35 ppm and 3.74 ppm belong to the hydrogen of chlorohydrin after reacted with APA, HR or DHR. Chemical shifts between 3.59 ppm and 2.95 ppm are the hydrogen belong to the carbon next to nitrogen atom.

Bioactivity was observed for all target compounds derived from acrylpimaric acid, tetrahydrogenated rosin, or dehydrogenated rosin, and the raw material themselves except 2 and 6, which are stable and likely to regress to 1 and 5, respectively. These were measured by the filter paper method using two general wood-decay fungi with PDA culture medium in this paper. Although Rao (2007) reported that rosin has a little bioactivity toward Gram-positive bacteria, it showed no anti-fungal activity toward either *Trametes versicolor* or *Gloeophyllum trabeum* in the present work. Moreover, compounds synthesized with quaternary ammonium group had satisfactory anti-fungal activity, as Table 1 and Fig. 3 show. At the same molarity of 0.025 mmol/mL, they all showed an obvious inhibition zone against *Trametes versicolor* and *Gloeophyllum trabeum*. Toward *Gloeophyllum trabeum*, 4a, 4b, 8a, and 8b exhibited almost the same bioactivity, but the activity of 8c and 8d was weaker. As Gemini quaternary ammonium salts, 4a and 4b had a double equivalent of quaternary ammonium groups. And compared with 8a and 8c, the only difference is that there is an aromatic group in the hydrophobic group of 8a, and the same difference applies to 8b and 8d. So we concluded that both quaternary ammonium group and aromatic group play important roles against *Gloeophyllum trabeum.* As reported, compounds with quaternary ammonium group had more probability to wrap up the membrane of the microorganism (Kubo *et al.* 1995) and with aromatic substructures have remarkable influence on their antifungal activity (Uchiro *et al.* 2002). The result of this work is in good agreement with these previous studies. Product 4a and 4b had better bioactivity than 8a, 8b, 8c, and 8d against *Trametes versicolor*, and comparing 8a and 8b with 8c and 8d, there was only a little difference among their bioactivity. This showed that quaternary ammonium groups played the leading role against this kind of wood-decay fungus, and the effect of aromatic groups was not significant. Moreover, compared with the commercially available fungicide 1227, the positive antifungal comparator, inhibition ability of 4a and 4b is not far behind, especially against *Gloeophyllum trabeum*.

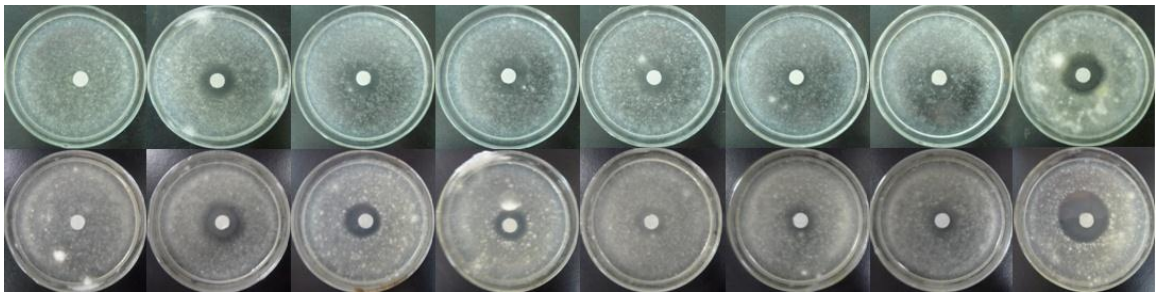

Fig. 3 Inhibition of the samples to the fungi;
From left to right: control, 4a, 4b, 8a, 8b, 8c, 8d and 1227. From top to bottom: *Trametes versicolor* and *Gloeophyllum trabeum*.

Bis-N-(3-hydroabietoxy-2-hydroxy) propyl-trimethyl ammonium chloride(4a)

Light yellow powder, yield: 71%. HPLC: 90.5% (2.720min). IR (cm^{-1}): 3423 (-OH), 2935, 2865, 1720 (-COOC-), 1567, 1457, 1390, 1245, 1135 and 1052. ^{1}HNMR (400 MHz, D_2O) δ = 5.35 (s, 1H), 4.71-4.33 (m, 2H, -C*H*-OH), 4.30-3.74 (m, 4H, -CH_2-), 3.55-3.44 (m, 4H, -CH_2-N-), 3.35-3.28 (m, 18H, -N-CH_3), 2.50-2.07 (m, 4H), 2.04-1.08 (m, 15H), 1.07-0.31 (m, 12H). ESI-MS m/z = 303 $[M]^{2+}$

Bis-N-(3-hydroabietoxy-2-hydroxy) propyl-triethyl ammonium chloride(4b)

Light yellow powder, yield: 72%. HPLC: 86.2% (2.775min). IR (cm^{-1}): 3415 (-OH), 2933, 2867, 1720 (-COOC-), 1635, 1575, 1475, 1386, 1245, 1151, 1105, 1049, 979 and 910. ^{1}HNMR (400 MHz, D_2O) δ = 5.39 (s, 1H), 4.25- 4.08 (m, 4H,-CH_2-), 3.99-3.85 (m, 2H, -C*H*-OH), 3.59-3.30 (m, 16H, -CH_2-N-), 2.50-2.37 (m, 2H), 2.22-2.10 (m, 2H), 1.95-1.39 (m, 17H), 1.38-1.24 (m, 18H,-N-CH_3), 1.21-1.01 (m, 10H). ESI-MS m/z = 345 $[M]^{2+}$

N-(3-dehydroabietoxy-2-hydroxy) propyl-trimethyl ammonium chloride (8a)

White powder, yield: 82%. HPLC: 86.8% (2.923 min). IR (cm^{-1}): 3417 (-OH), 2946, 1720 (-COOC-), 1635, 1473, 1110 and 1056. ^{1}HNMR (400 MHz, D_2O) δ = 7.36-6.58 (m, 3H, Ar-H), 4.42 (s, 1H, -C*H*-OH), 4.28-3.95 (m, 2H, -CH_2-), 3.49-3.28 (m, 2H, -CH_2-N-), 3.25-2.98 (m, 9H, -N-CH_3), 2.90-2.39 (m, 3H), 2.09-0.92 (m, 21H). ESI-MS m/z = 416 $[M]^+$

N-(3-dehydroabietoxy-2-hydroxy) propyl-triethyl ammonium chloride (8b)

White powder, yield: 79%. HPLC: 90.3% (3.116 min). IR (cm^{-1}): 3416 (-OH), 2940, 1720 (-COOC-), 1677, 1457, 1394, 1240, 1172, 1124 and 1108. ^{1}HNMR (400 MHz, D_2O) δ = 7.29-6.73 (m, 3H, Ar-H), 4.34 (s, 1H, -C*H*-OH), 4.13-3.92 (m, 2H, -CH_2-), 3.45-2.98 (m, 8H, -CH_2-N-), 2.85-2.42 (m, 3H), 2.03-0.68 (m, 30H). ESI-MS m/z = 458 $[M]^+$

N-(3-hydroabietoxy-2-hydroxy) propyl-trimethyl ammonium chloride (8c)

White powder, yield: 70%. HPLC: 89.2% (3.344 min). IR (cm^{-1}): 3374 (-OH), 3237, 2927, 2865, 1720 (-COOC-), 1455, 1384, 1236, 1132 and 1101. ^{1}HNMR (400 MHz, D_2O) δ = 4.50-4.32 (m, 1H, -C*H*-OH), 4.25-4.05 (m, 2H, -CH_2-), 3.70-3.24 (m, 9H, -N-CH_3), 3.16-2.84 (m, 2H), 2.35-0.33 (m, 33H). ESI-MS m/z = 422 $[M]^+$

N-(3-hydroabietoxy-2-hydroxy) propyl-triethyl ammonium chloride (8d)

White powder, yield: 73%. HPLC: 87.5% (3.529 min). IR (cm^{-1}): 3365 (-OH), 3226, 2927, 2869, 1720 (-COOC-), 1457, 1396, 1243 and 1091. ^{1}HNMR (400 MHz, D_2O) δ = 4.47-4.36 (m, 1H, -C*H*-OH), 4.12-3.97 (m, 2H, -CH_2-), 3.51-3.13 (m, 8H, -CH_2-N-), 2.11-0.51 (m, 42H). ESI-MS m/z = 464 $[M]^+$

CONCLUSIONS

1. Two series of rosin quaternary ammonium salt derivatives were synthesized by three steps with two kinds of rosin derivatives as raw materials. The structures of target products were characterized by HPLC, IR, MS, and ^{1}H NMR.

2. All of the rosin quaternary ammonium salts synthesized in this work exhibited obvious anti-fungal activity at the molarity of 0.025 mmol/mL against *Trametes versicolor* and *Gloeophyllum trabeum*. Rosin Gemini quaternary ammonium salts 4a and 4b showed better bioactivity in this test than 8a, 8b, 8c, and 8d against *Trametes versicolor*. This demonstrates that the quaternary ammonium group plays the leading role to this fungus. To *Gloeophyllum trabeum*, the inhibition zone diameters of 4a, 4b, 8a, and 8b were almost the same and larger than 8c and 8d. Thus it can be concluded that both quaternary ammonium group and aromatic group play important roles.

ACKNOWLEDGMENTS

The authors are grateful for the support of National Science Foundation (31070487), Foundation for University Young Core Teachers of Heilongjiang Province of China (1154G49), and the Paper Fund Project for Graduate Student of Northeast Forestry University (SPIP10).

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Article submitted: October 9, 2012; Peer review completed: Nov. 1, 2012; Revised version received and accepted: Dec; 12, 2102; Published: December 14, 2012.