

Preparation and Antimicrobial Activity of Rosin-based Carbamate Group-containing Quaternary Ammonium Salt Derivatives

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A novel rosin-based carbamate was prepared by the reaction of *N,N*-dimethylaminopropylamine with rosin-based cyclic carbonate. Using LiBr in conjunction with ethylene glycol as a catalyst, carbon dioxide was treated with triglycidyl ester of maleopimaric acid to prepare the rosin-based cyclic carbonate. The carbamate was then quaternized to form three rosin-based carbamate group-containing quaternary ammonium salt derivatives. The chemical structures of all new compounds were characterized by IR, ¹H NMR, and ¹³C NMR. The antimicrobial activities of the carbamate and quaternary ammonium salt derivatives were investigated. The bioassay test results showed that all derivatives exhibited strong inhibition against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Streptomyces microflavus*. However, no visible antifungal activity was found against *Saccharomyces cerevisiae* or *Aspergillus niger*, except for the activity of the carbamate derivative against *S. cerevisiae*.

Keywords: Rosin; Cyclic carbonate; Carbamate; Quaternary ammonium salt; Antibacterial activity

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INTRODUCTION

The alarming prevalence of drug-resistant bacteria has posed serious threats to human health. Therefore, numerous efforts have been devoted to the development of new antibacterial agents (Wilson 2009; Kumagai *et al.* 2012; Lin *et al.* 2013; Fosso *et al.* 2012). Among them, quaternary ammonium salt-containing cationic compounds have attracted considerable attention due to their robust and broad-spectrum antibacterial activities (Wynne *et al.* 2011; Fouda *et al.* 2013; Chanawanno *et al.* 2010). However, increasing usage of these compounds has caused environmental problems due to their poor biodegradability. As a result, the development of compounds that are environmentally friendly and biodegradable has gained much attention (Lim *et al.* 2012). Furthermore, it has been reported that the water-solubility and biodegradability of compounds could be improved by adding new polar groups, such as ethers, esters, and amides, into the molecular structure (Liu *et al.* 2012a).

Rosin, an abundantly available natural resource obtained from pine and conifer trees, has been widely used as an antimicrobial agent and polymer due to its characteristic properties such as biocompatibility, biodegradability, and its low cost (Wang *et al.* 2012; Li *et al.* 2012; Liang *et al.* 2013; Xu *et al.* 2011; Yao *et al.* 2011). Although compounds

with carbamate linkages have demonstrated antibacterial activity (Tan *et al.* 2011; Kaneko *et al.* 2007; Selvakumar *et al.* 2008; Adam *et al.* 2013; Galán *et al.* 2013), there have not been any reports on rosin-based quaternary ammonium salt-containing cationic antibacterial compounds with carbamate groups.

In this work, a series of novel rosin-based quaternary ammonium salt derivatives containing carbamate groups were synthesized by the quaternization of rosin-based carbamate with alkyl bromides (Fig. 1). The chemical structures of the resulting products were characterized by FT-IR, ^1H NMR, and ^{13}C NMR. The antimicrobial activities of the carbamate and quaternary ammonium salt derivatives were determined against five bacteria and two fungi using a paper disc diffusion assay. By introducing the carbamate polar groups into the molecular structure, the biodegradability and antibacterial activity of the compounds were expected to improve.

EXPERIMENTAL

Materials and Instruments

Rosin, which was obtained from a commercial source (Wu Zhou Pine Chemicals Ltd. Guangxi, China), consists primarily of resin acids with the formula of $\text{C}_{19}\text{H}_{29}\text{COOH}$. It was used without further purification. Carbon dioxide (99.99%) was commercially available. All other chemical reagents were chemically pure and were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). A triglycidyl ester of maleopimaric acid with the epoxy equivalent weight of 312.5 g/eq was prepared according to the literature (Kong *et al.* 1994, 1995; Liu *et al.* 2012b). The NMR measurements were performed on a Bruker DRX 500 spectrometer (Bruker, Switzerland). Chemical shifts are given with respect to $\text{CHCl}_3/\text{CDCl}_3$ (δ ^1H = 7.24, δ ^{13}C = 77.0 ppm). The IR spectra were recorded on a Nicolet IS10 FT-IR spectrophotometer (Nicolet, Madison, WI, USA).

The synthetic routes of rosin-based carbamate and quaternary ammonium salt derivatives are shown in Fig.1.

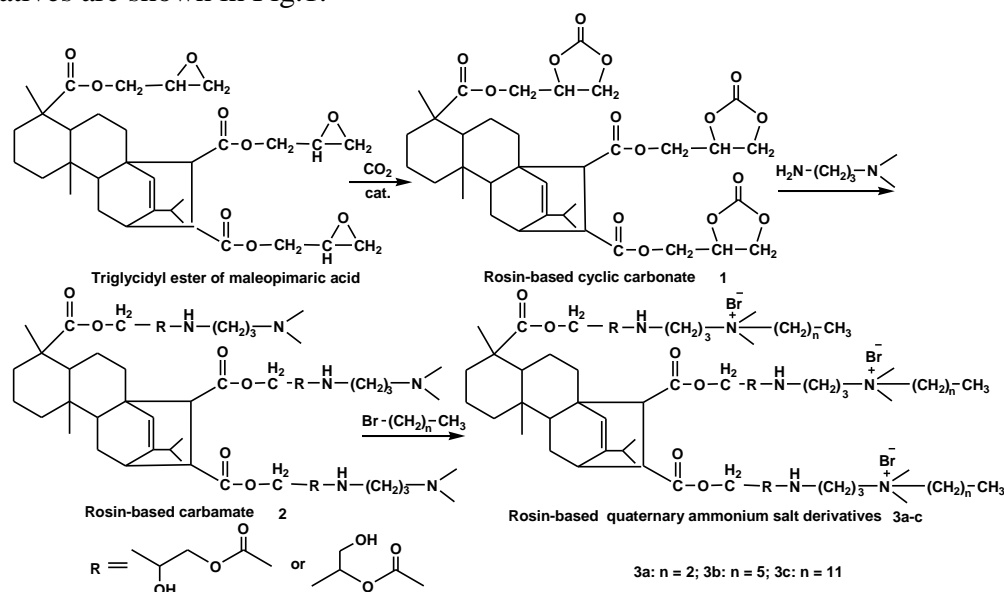


Fig. 1. Synthetic routes of the rosin-based carbamate and quaternary ammonium salt derivatives

General Procedure for the Formation of Rosin-based Cyclic Carbonate 1

Triglycidyl ester of maleopimaric acid (200 g, 0.64 mol), LiBr (2.0 g, 0.02 mol), ethyleneglycol (1.0 g, 0.016 mol), and *N*-methyl pyrrolidone (70 mL) were placed in an autoclave equipped with a magnetic stirrer. The reaction mixture was heated to 110 °C, the atmosphere was replaced with carbon dioxide, and the pressure was maintained at 2 MPa.

The level of reaction was monitored by IR spectroscopy. As the absorbance band due to formation of the cyclic carbonate moieties appeared and increased in intensity at 1791 cm⁻¹, the characteristic absorption peaks of epoxy groups at 910 cm⁻¹ decreased and disappeared. The reaction was completed in about 7 h. The catalyst and *N*-methyl pyrrolidone were completely removed by washing with water. A brown solid of compound **1** was obtained after drying by vacuum.

Rosin-based cyclic carbonate 1

Brown solid, yield: 89%. IR (cm⁻¹): 2932, 2868 (CH₃, CH₂); 1791 (C=O); 1724 (C=O); 1457, 1388 (CH₃, CH₂); 1161, 1095, 1049 (C–O–C). ¹H NMR (500 MHz, CDCl₃, δ): 5.37 (–C=CH–); 4.87-5.08 (methine hydrogens of cyclic carbonate group); 4.02-4.23 (methylene hydrogens of cyclic carbonate group); 4.26-4.65 (O–CH₂–); 2.95-3.00 (CH–C=); 2.87-2.91 (CH–C=O); 2.52-2.65 (CH–CH₃); 1.05-1.86 (–CH₂, –CH₃). ¹³C NMR (500 MHz, CDCl₃, δ): 177.9, 172.1, 171.9 (C=O); 154.7 (cyclicarbonate carbon); 147.3, 123.3 (–C=C–); 73.9 (methine carbons of cyclic carbonate); 66.0 (O–CH₂–); 63.3 (methylene carbons of cyclic carbonate); 55.7, 53.8, 49.3, 47.3, 40.9, 37.5, 36.5, 34.5, 32.3, 30.5, 28.2, 21.6, 21.1, 20.2, 19.5, 17.4, 16.7, 16.5, 15.5 (carbons of the rosin skeleton structure).

General Procedure for the Formation of Rosin-based Carbamate 2

N,N-dimethylaminopropylamine (11.5 g, 0.112 mol) was added to the solution of compound **1** (40.0 g, 0.112 mol) in ethanol (40 mL). The mixture was stirred at 75 °C, and the extent of reaction was followed by IR spectroscopy.

While the absorbance band due to cyclic carbonate moieties at 1791 cm⁻¹ diminished and finally disappeared, new bands due to the carbamate C=O and N–H groups appeared at 1716 cm⁻¹ and 1530 cm⁻¹, respectively. The reaction was completed after 2 h. The solution was concentrated *in vacuo* to give the mixture as a yellow solid. Purification was accomplished by recrystallization with ethanol and ethyl acetate to give compound **2**.

Rosin-based carbamate 2

Yellow solid, yield: 92%. IR (cm⁻¹): 3338 (–OH); 2943, 2866 (CH₃, CH₂); 1716 (C=O); 1530 (N–H); 1461, 1385 (CH₃, CH₂); 1140 (N–C); 1101, 1038 (C–O–C). ¹H NMR (500 MHz, CDCl₃, δ): 5.37 (–C=CH–); 3.64-4.39 (O–CH₂–, CH–OH, CH₂–OH, O–CH); 3.14-3.36 (N–CH₂–); 2.85-2.95 (CH–C=O); 2.54-2.65 (CH–CH₃); 2.21 (N–CH₃); 1.04-1.80 (–CH₂, –CH₃). ¹³C NMR (500 MHz, CDCl₃, δ): 178.5, 173.3, 172.1 (C=O); 156.8 (NH–CO); 148.3, 123.6 (–C=C–); 62.5-72.5 (O–CH₂–, CH–OH, CH₂–OH); 56.7 (N–CH₂–); 45.1 (N–CH₃); 53.8, 52.1, 49.2, 47.1, 39.4, 37.5, 36.3, 34.9, 32.4, 30.5, 29.4, 26.9, 21.6, 21.1, 20.2, 19.5, 17.4, 16.7, 16.5, 15.5 (carbons of the rosin skeleton structure).

General Procedure for the Formation of Rosin-based Carbamate Group-containing Quaternary Ammonium Salt Derivatives 3 (3a-c)

Compound 2 (20.0 g, 0.04 mol) and alkyl bromide (0.4 mol) were dissolved in ethanol (40 mL). The reaction mixture was heated to 70 °C, and the extent of reaction was followed by ¹H NMR spectroscopy while the signal at 2.21 ppm, which was assigned to the N-CH₃ unit, finally disappeared. The reaction was completed after 15 h. The solution was concentrated *in vacuo* to give the mixture as a brown solid; purification was accomplished by recrystallization with ethanol and ethyl acetate to give compound 3 (3a-c).

Rosin-based carbamate group-containing quaternary ammonium salt derivative 3a

Brown solid, yield: 78%. IR (cm⁻¹): 3324 (–OH); 2944, 2869 (CH₃, CH₂); 1712 (C=O); 1528 (N–H); 1461, 1386 (CH₃, CH₂); 1177 (N⁺–C); 1150 (N–C); 1103, 1042 (C–O–C). ¹H NMR (500 MHz, CDCl₃, δ): 5.34 (–C=CH–); 3.45–4.30 (O–CH₂–, CH–OH, CH₂–OH, O–CH); 3.18–3.32 (NH–CH₂–); 3.05 (N⁺–CH₃); 2.87–2.91 (N⁺–CH₂); 2.79–2.85 (CH–C=O); 2.60–2.69 (N⁺–CH₂); 2.48–2.58 (CH–CH₃); 0.90–1.98 (–CH₂, –CH₃). ¹³C NMR (500 MHz, CDCl₃, δ): 178.2, 173.6, 172.7 (C=O); 156.9 (NH–CO); 147.7, 123.9 (–C=C–); 61.6–72.5 (O–CH₂–, CH–OH, CH₂–OH, N⁺–CH₂); 49.8 (N⁺–CH₃); 55.3, 49.4, 48.2, 42.8, 37.4, 36.6, 35.4, 34.5, 32.3, 30.2, 28.4, 25.7, 22.9, 21.5, 20.2, 19.4, 17.1, 16.5, 16.0, 15.6, 9.6 (carbons of the rosin skeleton structure and carbon chain).

Rosin-based carbamate group-containing quaternary ammonium salt derivative 3b

Brown solid, yield: 85%. IR (cm⁻¹): 3338 (–OH); 2930, 2868 (CH₃, CH₂); 1713 (C=O); 1531 (N–H); 1464, 1386 (CH₃, CH₂); 1177 (N⁺–C); 1149 (N–C); 1104, 1039 (C–O–C). ¹H NMR (500 MHz, CDCl₃, δ): 5.37 (–C=CH–); 3.48–4.27 (O–CH₂–, CH–OH, CH₂–OH, O–CH); 3.17–3.35 (NH–CH₂–); 3.08 (N⁺–CH₃); 2.87–2.93 (N⁺–CH₂); 2.75–2.83 (CH–C=O); 2.54–2.65 (N⁺–CH₂); 2.36–2.43 (CH–CH₃); 0.91–1.99 (–CH₂, –CH₃). ¹³C NMR (500 MHz, CDCl₃, δ): 178.2, 173.4, 173.2 (C=O); 156.8 (NH–CO); 147.7, 123.7 (–C=C–); 61.5–72.2 (O–CH₂–, CH–OH, CH₂–OH, N⁺–CH₂); 49.6 (N⁺–CH₃); 52.0, 48.8, 48.2, 41.9, 40.5, 37.1, 36.9, 36.0, 35.3, 32.0, 31.7, 30.5, 25.2, 22.9, 22.5, 21.7, 21.1, 19.2, 16.2, 15.0, 12.5 (carbons of the rosin skeleton structure and carbon chain).

Rosin-based carbamate group-containing quaternary ammonium salt derivative 3c

Brown solid, yield: 80%. IR (cm⁻¹): 3329 (–OH); 2923, 2853 (CH₃, CH₂); 1715 (C=O); 1530 (N–H); 1465, 1386 (CH₃, CH₂); 1177 (N⁺–C); 1150 (N–C); 1103, 1038 (C–O–C). ¹H NMR (500 MHz, CDCl₃, δ): 5.39 (–C=CH–); 3.58–4.33 (O–CH₂–, CH–OH, CH₂–OH, O–CH); 3.24–3.37 (NH–CH₂–); 3.13 (N⁺–CH₃); 2.85–2.94 (N⁺–CH₂); 2.73–2.83 (CH–C=O); 2.51–2.62 (N⁺–CH₂); 2.36–2.42 (CH–CH₃); 0.89–2.00 (–CH₂, –CH₃). ¹³C NMR (500 MHz, CDCl₃, δ): 178.2, 173.4, 172.8 (C=O); 156.8 (NH–CO); 147.6, 124.0 (–C=C–); 61.5–72.5 (O–CH₂–, CH–OH, CH₂–OH, N⁺–CH₂); 49.8 (N⁺–CH₃); 52.1, 48.8, 48.2, 42.0, 37.1, 36.2, 35.3, 34.2, 32.1, 31.3, 29.0, 28.9, 28.7, 28.5, 25.7, 22.6, 22.0, 21.8, 21.2, 20.1, 19.3, 16.4, 15.7, 15.0, 12.8 (carbons of the rosin skeleton structure and carbon chain).

Antimicrobial Activity Testing

The antimicrobial activities of rosin-based carbamate and quaternary ammonium salt derivatives were determined against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Streptomyces microflavus*, *Saccharomyces*

cerevisiae, and *Aspergillus niger* using a paper disc diffusion assay. Tests were performed according to the literature (Choudhary *et al.* 2013) with some modifications. The fresh microbial strains from the culture medium were diluted with sterilized physiological saline. Then, 0.1 mL of each microbial suspension (10^5 – 10^6 CFU/mL) was spread onto the surface of the culture medium to create a microbial lawn. Sterile filter paper discs of 5 mm in diameter were wetted with a solution of the compounds (5 mg/mL) and were left to dry before being placed on the microbial lawn. The plates of *S. aureus*, *E. coli*, *B. subtilis*, *B. thuringiensis*, and *S. microflavus* were incubated at 37 °C for 24 h, and the plates of *S. cerevisiae* and *A. niger* were incubated at 37 °C for 3 days. The zones of inhibition were measured in mm with a transparent ruler, and the average was calculated as the mean of triplicate experiments.

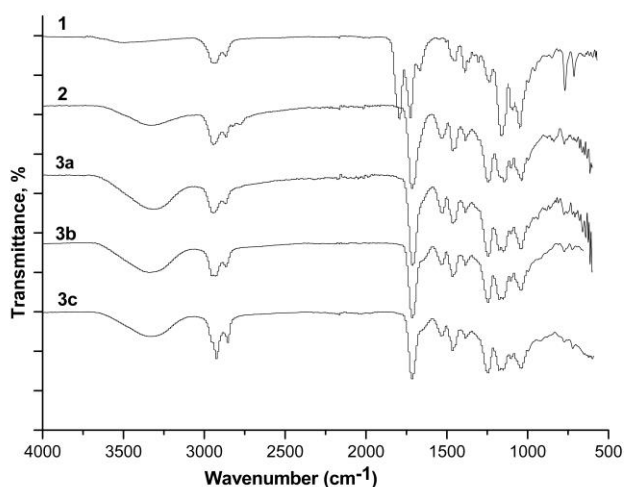


Fig. 2. FT-IR spectra of 1, 2 and 3a-c

RESULTS AND DISCUSSION

Synthesis of Rosin-based Cyclic Carbonate

The coupling reaction of carbon dioxide with epoxides to form cyclic carbonates has been described extensively from the viewpoint of environmental preservation (Sakakura *et al.* 2007). Furthermore, compounds with two hydroxyl groups on the vicinal carbons have been reported to effectively promote the reaction (Liang *et al.* 2011; Ma *et al.* 2012). As illustrated in Fig. 1, rosin-based cyclic carbonate was prepared by converting the epoxy groups of triglycidyl ester of maleopimaric acid with carbon dioxide in the presence of LiBr and ethylene glycol. When ethylene glycol was added to the reaction, the new catalytic system exhibited higher activity (Miyata *et al.* 2012). The extent of the reaction was followed using IR spectroscopy by focusing on the appearance of a new peak at 1791 cm^{-1} due to the carbonyl of the cyclic carbonate moieties and the disappearance of the characteristic absorption peak of epoxy groups at 910 cm^{-1} . Another confirmation of the formation of cyclic carbonate groups was the appearance of new signals at 4.87 to 5.08 and 4.02 to 4.23 ppm in the ^1H NMR of the product, which were

assigned to the CH and CH₂ of cyclic carbonate, respectively, as well as a signal at 154.7 ppm in the ¹³C NMR of the product, which was due to the C=O of the cyclic carbonate.

Reaction of Rosin-based Cyclic Carbonate with *N,N*-Dimethylamino-propylamine

It has been reported that the cleavage of five-membered carbonate rings using amines leads to the formation of carbamate derivatives. The reaction yields two types of products that contain primary or secondary alcohol groups, and the main product is always the one with a secondary alcohol (Garipov *et al.* 2003; Vilkauskaitė *et al.* 2013). The reaction of rosin-based cyclic carbonate with *N,N*-dimethylaminopropylamine was performed to show the ring opening of cyclic carbonate groups and the formation of carbamate groups. Compared with the IR spectrum of rosin-based cyclic carbonate, the disappearance of the carbonate bands at 1791 cm⁻¹ and the appearance of new bands at 3338, 1716, and 1530 cm⁻¹, which were assigned to the hydroxyl group, carbonyl group, and N–H deformation of the carbamate groups, respectively, confirmed that the carbonate groups were completely converted into carbamate groups. Additionally, the appearance of a new signal at 156.8 ppm in the ¹³C NMR of the product, which was assigned to the C=O of the carbamate groups, also confirmed the formation of the rosin-based carbamate. Absorbance bands due to the ester and carbamate groups overlap to some extent, and no amide band was observed, which implied that there were no reactions between amine and ester groups.

Formation of Rosin-based Carbamate Group-containing Quaternary Ammonium Salt Derivatives

A series of novel rosin-based quaternary ammonium salt derivatives containing carbamate groups was synthesized by the reaction of rosin-based carbamate with alkyl bromides. When compared with the NMR of rosin-based carbamate, the signals of N⁺–CH₃ at 3.05, 3.08, and 3.13 ppm in the ¹H NMR of **3a**, **3b**, and **3c**, respectively, were shifted to a lower field due to the electron-withdrawing effect of N⁺. Furthermore, the values of the carbon atoms (N⁺–CH₃) also shifted to a lower field for the same reason. These results suggest that the reactions of rosin-based carbamate with alkyl bromides took place as expected and that the quaternary ammonium salt derivatives were synthesized successfully.

Antimicrobial Activity of Compounds

The antimicrobial activities of rosin-based carbamate and its quaternary ammonium salt derivatives were determined against different kinds of microorganisms five bacteria and two fungi (Table 1). It was shown that compounds **2** and **3a-c** had antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis*, *B. thuringiensis*, and *S. microflavus*. Notably, compound **2** exhibited excellent antibacterial activity against *S. aureus*, with an inhibitory zone diameter of 27.3 mm (with a concentration of 5 mg/mL). Compared to compounds **2**, **3a**, and **3c**, **3b** had the highest antibacterial activity against *B. subtilis*, *B. thuringiensis*, and *S. microflavus*, with inhibitory zone diameters of 20.75, 13.25, and 14.5 mm, respectively. Although non-modified rosin (Goodson *et al.* 1999) quaternary ammonium salt cationic compounds as well as compounds with carbamate groups have demonstrated antibacterial activity, the synthesized compounds show no visible antifungal activity against *S. cerevisiae* and *A. niger*, even when incubated for 3

days, with the exception of the carbamate derivative against *S. cerevisiae*, with an inhibitory zone diameter 6.75 mm. Interestingly, when compared to rosin-based carbamate, compounds **3a-c** have lower antimicrobial activity against *S. aureus* and *S. cerevisiae*, in spite of the addition of quaternary ammonium salt cationic groups into the molecular structure.

Table 1. Antimicrobial Activities (Inhibition Zone Diameter in mm) of Compounds

Compound	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>B. thuringiensis</i>	<i>S. microflavus</i>	<i>S. cerevisiae</i>	<i>A. niger</i>
2	27.3	13.13	18	13	11.5	6.75	0
3a	12.75	19.25	17.25	8	9.5	0	0
3b	12.5	13.5	20.75	13.25	14.5	0	0
3c	9.25	15.63	9.25	7.25	8	0	0

Compounds with a concentration of 5 mg/mL were tested against the microbial strains; Inhibitory zone diameters were measured in mm; Values are mean of triplicate.

CONCLUSIONS

1. A novel rosin-based cyclic carbonate was synthesized successfully by treatment of carbon dioxide with triglycidyl ester of maleopimaric acid using LiBr in conjunction with ethylene glycol as a catalyst. Notably, the addition of ethylene glycol promoted the reaction efficiently and made the reaction complete within 7 h.
2. A series of rosin-based carbamate group-containing quaternary ammonium salt derivatives were prepared via the intermediate rosin-based carbamate. Their antimicrobial activities were investigated using a paper disc diffusion assay. The results show that all derivatives exhibited strong inhibition against *S. aureus*, *E. coli*, *B. subtilis*, *B. thuringiensis*, and *S. microflavus* and had no visible antifungal activity against *S. cerevisiae* and *A. niger* with concentrations of 5 mg/mL.

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