Journal of Molecular Liquids 294 (2019) 111669

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Synthesis, physicochemical and antimicrobial properties of cardanol-derived quaternary ammonium compounds (QACs) with heterocyclic polar head

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ARTICLE INFO

Article history:
Received 10 May 2019
Received in revised form 29 August 2019
Accepted 31 August 2019
Available online 02 September 2019

Keywords:
New biomass raw material
Cardanol
Quaternary ammonium compounds (QACs)
Surface activities
Antimicrobial activity
Scanning electron microscopy (SEM)

ABSTRACT

As new biomass derived raw material, cardanol has attracted the attention of numerous academic and industrial groups due to its renewability and unique structural features. In this work, seven cardanol-derived quaternary ammonium compounds (QACs) were synthetized with their physicochemical properties and antimicrobial ability evaluated. The surface tension was measured in aqueous medium by the platinum ring method, **2b** has the optimal surface activities, which is considerably better than commercialized QAC product cetyltrimethylammonium bromide. Both broth dilution method and agar dilution method were used to obtain the MIC and MBC values of the targeted QACs. **1a**, **1b**, **2b** inhibit the tested bacterial at a concentration of 32 μg/mL. Scanning electron microscopy (SEM) results intuitively showed that the QACs could interact with the bacterial cell membrane, for disrupting the integrity of the membrane. Meanwhile, it was found that the antimicrobial activity depended not only on the alkyl chain length, but also on the CMC value. To sum, seven cardanol-derived QACs were synthetized easily, which showed excellent surface activities and antimicrobial activity, as a promising alternative to the existing fossil fuel derived cationic surfactants and antiseptics.

1. Introduction

With the diminishing of fossil fuel resources, surfactants synthetized from renewable raw materials are gaining increasing attention from the academic and industrial fields. Up to now, surfactants derived from sustainable resources such as amino acids [1,2], glucose [3], rosin [4] etc. have been well studied. Meanwhile, agro-industry waste like cardanol could be adopted as an abundant bio-renewable source for further chemical derivatization.

CNSL (cashew nut shell liquid) is a by-product of the cashew nut processing industry [5], accounting for 25% of the total cashew nut weight [6]. The global yield of CNSL almost approaches one million tons per year [7,8]. Cardanol could be obtained after distillation of CNSL [9,10]. There are 3 types of reactive sites on the chemical structure of cardanol: the phenolic hydroxyl group, aromatic ring and the unsaturated position of the olefin in the side chain [11–14]. The unique chemical structure makes cardanol easy for chemical derivatization. Many research groups have synthetized a variety of functional products based on cardanol, such as epoxy compounds [15,16], acrylic monomers [17,18], plasticizers [19], coatings [20] and surfactants [21,22]. Faye et al. [23] synthesized three ionic surfactants: a cationic, an anionic and a zwitterionic surfactant, derived from cardanol, which exhibited good surface activities and solubility. They could be sustainable alternatives to alkylbenzene surfactants. Peungjitton et al. [24] synthesized sodium cardanol sulfonate surfactants, of which surface activities and detergency properties were measured. Compared with commercialized dodecylbenzene sulfonate, the results showed that the sodium cardanol sulfonate could be used as an alternative to anionic surfactant. Atta et al. [25] studied syntheses of new nonionic surfactants derived from cardanol. The good adsorption of as-synthetized surfactants at water/oil surface were evaluated as asphaltene dispersants and emulsion breakers for heavy crude oil. Godoy et al. [26] reported syntheses of cardanol-based cationic surfactants. Wang et al. [27] carried out ether and quaternization reactions based on cardanol to obtain cardanol-derived QACs and studied their applications in emulsion polymerization. The majority of reports regarding cardanol-derived QACs were involved in physicochemical applications. Meanwhile,

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https://doi.org/10.1016/j.molliq.2019.111669
0167-7322/

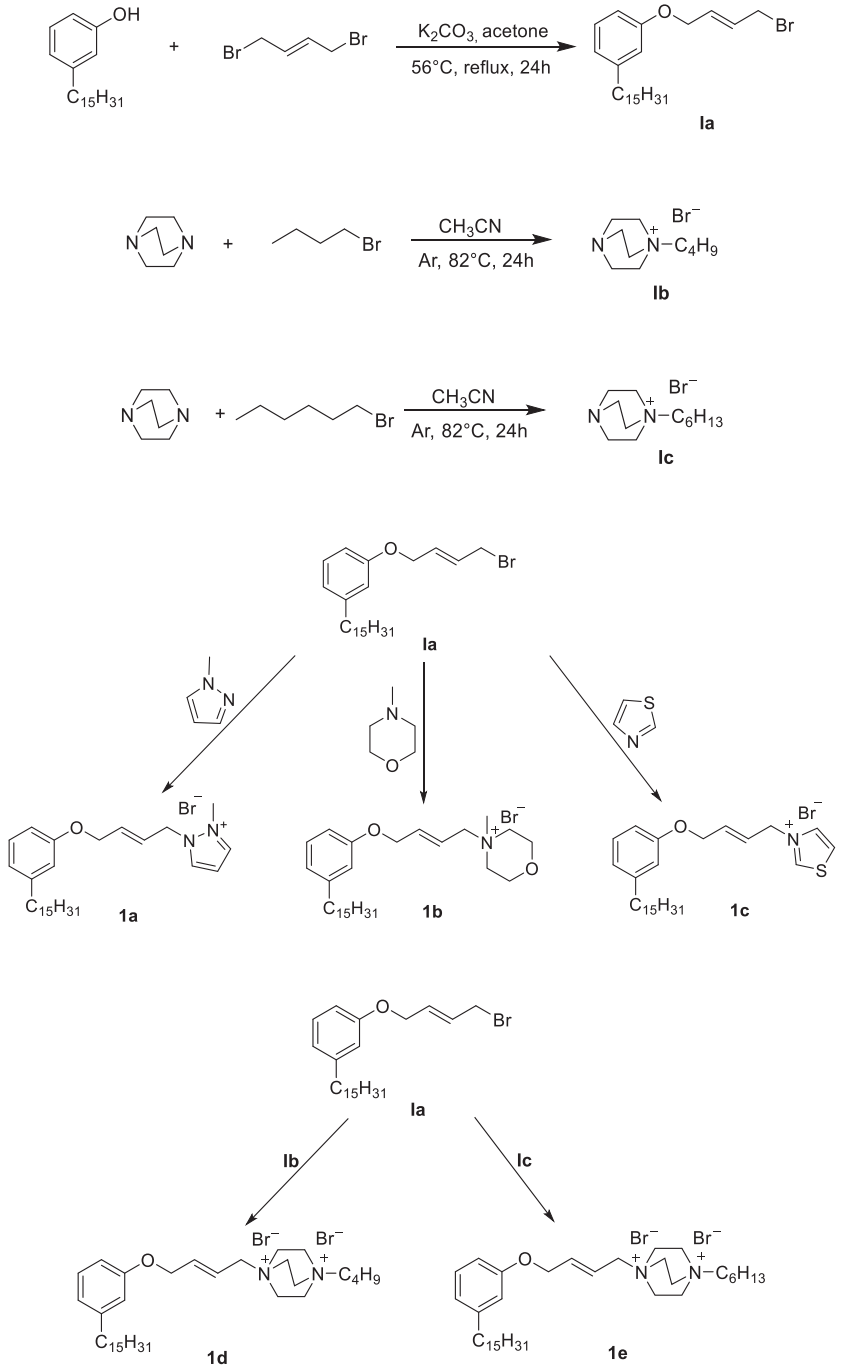
few papers paid attention to the antimicrobial application of cardanol-derived QACs.

Here, we synthesized mono-heterocyclic quaternary ammonium compounds (QACs) and asymmetric Gemini quaternary ammonium compounds with double bond or carbon chain containing hydroxyl linked group derived from cardanol. Meanwhile, the physicochemical properties of cardanol-derived QACs, such as surface tension, Krafft temperature (T_k), wettability, were studied. In addition, we investigated antimicrobial activity by broth microdilution method, agar dilution method and scanning electron microscopy (SEM) monitoring. As-synthetized cardanol-derived QACs in this work possess excellent physicochemical properties and antimicrobial efficacy, making them as highly desirable alternative of fossil fuel derived counterparts.

2. Materials and methods

2.1. Materials

Cardanol (3-pentadecylphenol) (industrial grade 90%), SIGMA-ALDRICH, Co., Ltd.; (E)-1,4-Dibromobut-2-Ene (≥98%), Shanghai Titan Scientific Co., Ltd.; (±)-Epichlorohydrin (AR), Shanghai Aladdin Biochemical Technology Co., Ltd.; 1,4-Diazabicyclo[2.2.2]Octane (99%), Adamas Reagent Co., Ltd.; 1-Methylpyrazole (≥98%), Shanghai Dibo Chemical Technology Co., Ltd.; N-Methylmorpholine (99%), Saan Chemical Technology (Shanghai) Co., Ltd.; Thiazole (99%), Beijing Bailingwei Technology Co., Ltd.; n-Butyl bromide (>99%), Shanghai Macklin Biochemical Co., Ltd.; Hexyl Bromide (99%), Adamas Reagent Co., Ltd.; Hydrobromic acid (AR), Shanghai Lingfeng Chemical Reagent Co., Ltd.;



Scheme 1. Syntheses of cardanol-derived mono-heterocyclic QACs and asymmetric Gemini QACs with double bond linked group.

Yeast extract powder (BR), Shanghai Macklin Biochemical Co., Ltd.; Tryptone (OXIDE), Shanghai Macklin Biochemical Co., Ltd.; Sodium Chloride (AR, ≥99.5%), Shanghai Titan Scientific Co., Ltd.

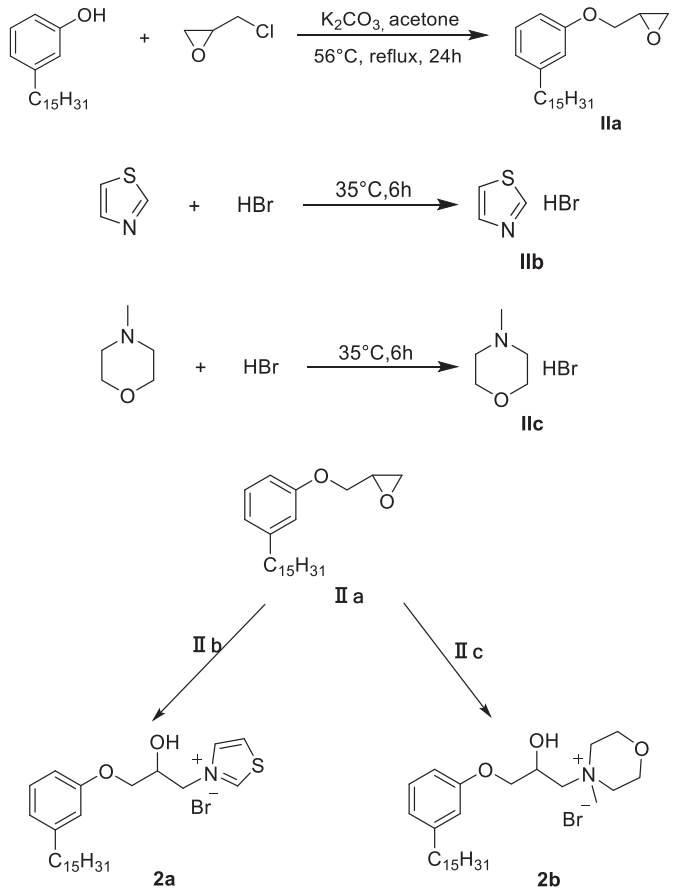
2.2. Synthesis

2.2.1. Syntheses of cardanol-derived mono-heterocyclic QACs and asymmetric Gemini QACs with double bond linked group (Scheme 1)

2.2.2. Syntheses of cardanol-derived mono-heterocyclic QACs with carbon chain contained hydroxyl linked group (Scheme 2)

2.3. Interface properties measurement

The surface tension of cardanol-derived QACs was measured by the platinum ring method using JK99C automatic tension meter at 20 (± 2) °C. 20 mL of deionized water solution was placed in a clean cup crystallizing dish with a diameter of 60 mm. By using the step-by-step dilution-extraction method [28], an aqueous solution (0.001 mol/L) of QAC was dropwise added to crystallizing dish, and then the surface tension was measured. The platinum was thoroughly cleaned and dried before measurement, and the surface tension was considered as an average of three replicates [29]. The critical micelle concentration (CMC) is determined by the intersection of the falling line and the steady line after CMC on the γ-c curve [30].



Scheme 2. Syntheses of cardanol-derived mono-heterocyclic QACs with carbon chain contained hydroxyl linked group.

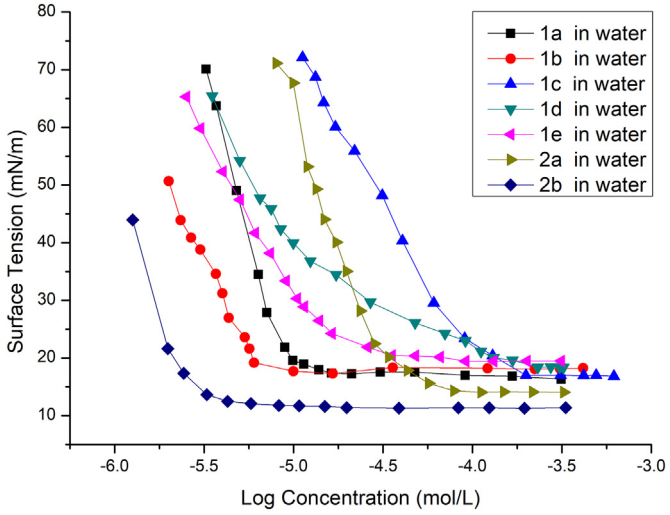


Fig. 1. Surface tension of cardanol-derived QACs at 20 (± 2) °C.

2.4. Krafft temperature (T_k) measurement

The Krafft temperature is the critical solution temperature of the surfactant. Surfactants do not dissolve appreciably in aqueous solutions below T_k. The concentration of the surfactant solution was set to 1.0 wt%, when the temperature rose to T_k, solubility of the surfactant sharply rose. After heating the surfactant solution until the solution was clarified, the clear solution was gradually cooled in ice-bath. The temperature at which the clarified surfactant solution appeared turbid was recorded as the T_k. The experiment was repeated three times and averaged [31].

2.5. Contact angle measurement

The wettability of cardanol-derived QACs was determined by using a Biolin contact angle meter to measure the contact angle of the film of surfactant with water molecule. The experiment was repeated three times and averaged. The smaller contact angle signifies greater wettability with water molecule [32].

2.6. Antimicrobial activity measurement

The tested microorganisms were Gram-positive bacterium, *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633); Gram-negative bacterium, *Escherichia coli* (ATCC 25922).

2.6.1. Determination of minimum inhibitory concentration (MIC) - broth dilution method

Prepared a 1280 μg/mL aqueous solution of cardanol-derived QAC solution using sterile water, aqueous solution was filtered with a 0.45 μm membrane, and then diluted to get QAC solution concentrations:

Table 1
Adsorption parameters of cardanol-derived QACs at 20 (± 2) °C.

Compound	CMC (mM)	γ_{CMC} (mN/m)	C_{20} (10^{-4} mol/L)	pC_{20}	CMC/C_{20}	Γ_{max} (μmol/m^2)	A_{min} (nm^2)
1a	0.009	17.12	0.045	5.350	2.01	10.90	0.15
1b	0.005	17.74	0.018	5.728	2.74	6.82	0.24
1c	0.090	17.54	0.256	4.592	3.52	4.32	0.38
1d	0.027	19.00	0.054	5.265	4.88	1.22	1.36
1e	0.022	19.43	0.040	5.402	5.65	3.06	0.54
2a	0.032	13.98	0.121	4.916	2.69	7.90	0.21
2b	0.002	11.32	0.010	6.005	2.47	10.12	0.16

Table 2
Krafft temperature (T_k) and the contact angles of cardanol-derived QACs.

Compound	T_k (°C)	Average angle (°)
1a	<0	7.59
1b	3	18.89
1c	8	27.30
1d	3	38.06
1e	>90	48.79
2a	<0	26.19
2b	<0	5.23

640, 320, 160, 80, 40, 20, 10, 5, 2.5, 1.25 μg/mL. Different concentrations of cardanol-derived QACs (10 μL) were regularly added to the sterilized a 96-well plate. Meanwhile, 90 μL of 10^5 cfu/mL bacterial suspension was added to each well. After the 96-well plate was cultured in a 37 °C incubator for 24 h [33,34], it was observed whether or not the bacteria grew to obtain the minimum concentration at which no bacterial growth was observed by analyzing the absorbance of bacteria suspension at 600 nm, which was considered as the MIC.

2.6.2. Determination of minimum bactericidal concentration (MBC) - agar dilution method

Prepared a 2048 μg/mL aqueous solution of cardanol-derived QAC solution using sterile water, aqueous solution was filtered with a 0.45 μm membrane, and then diluted to get antimicrobial solution concentrations: 1028, 512, 256, 128, 64, 32, 16 μg/mL. Sterilizing the liquid agar media by autoclave at temperature of 120 °C for 20 min [35], followed 15 mL of liquid agar medium at high temperature mixed with different concentrations of cardanol-derived QAC solution (1 mL). And then, the mixture was poured into the culture dish to be solidified to obtain antibacterial agar medium as samples, the agar medium without cardanol-derived QAC as control group. 100 μL of a bacterial suspension of 10^5 cfu/mL was added to the culture dish of above agar medium and spread it with an applicator [36]. The culture medium was cultured in a 37 °C incubator for 24 h. The number of bacterial strains in all samples and control groups were recorded. The minimum bactericidal concentration (MBC) is the minimum concentration at which the growth of live bacteria is reduced by ≥99.9% [37].

2.6.3. Scanning electron microscope (SEM)

Scanning electron microscopic study of the influence of MIC concentration cardanol-derived QAC on the microorganisms of *S. aureus*, *E. coli* have been performed. The mixture of 100 μL of cardanol-derived QAC solution with good antibacterial effect and 900 μL of 10^5 cfu/mL microorganisms suspension was placed in a 2 mL centrifuge tube, which was used to obtain experimental sample. The mixture of 100 μL of sterilized deionized water and 900 μL of 10^5 cfu/mL microorganisms suspension was used as a control group. The mixture was placed in a 37 °C incubator for 24 h. Then, the mixture was fixed with 2.5% glutaraldehyde, washed three times with phosphate buffered saline, dehydrated with 20%, 40%, 60%, 80%, 95%, 100% alcohol. After that, the as-treated mixture (10 μL) was dropped on the glass and dried at clean room, finally, sputtered coated. The SEM images of the bacteria were recorded on a scanning electron microscope [38].

3. Results and discussion

3.1. Evaluation of surface activity by surface tension measurement

In this work, the surface tension of aqueous solution of as-synthesized cardanol-derived QACs was measured to evaluate surface activities. At 20 (± 2) °C, the relationship between the surface tension (γ) and the logarithm concentration (log C) of the aqueous solution of cardanol-derived QACs are shown in Fig. 1. With the concentration of the aqueous solution of QACs increased, the surface tension decreased, reached at a steady value, finally. The critical micelle concentration (CMC) is determined by the intersection of the falling line and the steady line on the γ-log C curve. When the critical micelle concentration is exceeded, the solution is dissolved to saturation by the amphiphilic molecules. There is no longer any change in surface tension [39–41].

According to the graph of relationship between the surface tension γ and log C, adsorption parameters, such as CMC, γ_{CMC}, C_{20}, pC_{20}, Γ_{max}, A_{min} and CMC/C_{20}, were obtained by calculating. The surface tension of CMC is γ_{CMC}, which is the minimum surface tension value that can be reached by increasing the concentration of the aqueous solution of QAC; C_{20} is the concentration of QAC at which the surface tension of deionized water is reduced by 20 mN/m. The adsorption efficiency pC_{20}, is the negative logarithm of C_{20} [42].

$$pC_{20} = -\log C_{20} \quad (1)$$

Γ_{max} is the maximum excess surface concentration, the difference between the amount of the substance containing the solute in the surface layer per unit surface area and the amount of the substance containing the solute in the bulk of the solution. A_{min} is the smallest area occupied by adsorbed amphiphilic molecule at the air/water interface when the gas-liquid interface reaches saturation. Γ and A reflect the arrangement of amphiphilic molecules at the air/water interface. Γ_{max} and

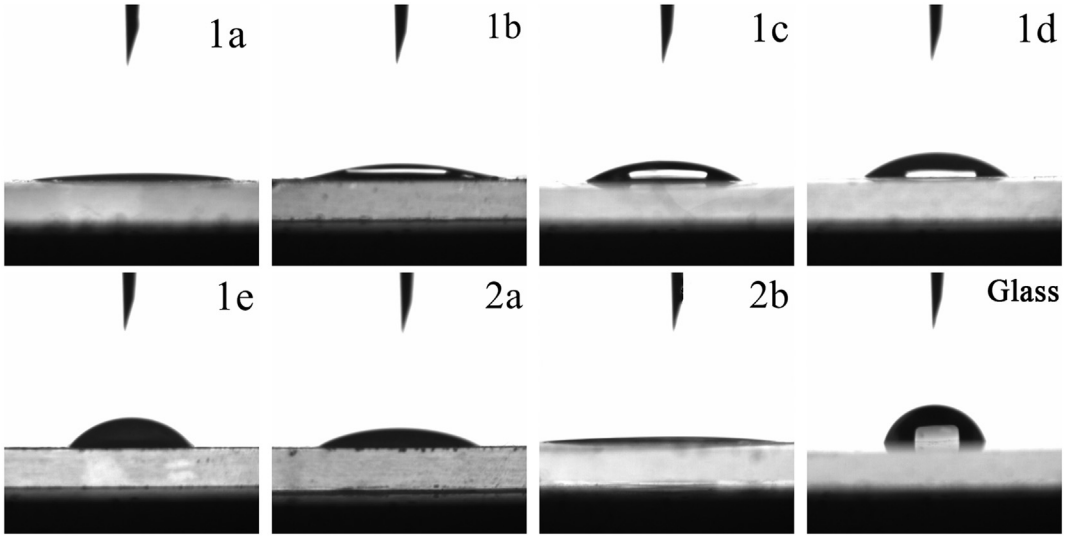


Fig. 2. The contact angles of the film of cardanol-derived QACs (on glass sheet) with water molecules.

A_{min} reflect the denser arrangement of amphiphilic molecules in solution. The value of CMC/C_{20} reflects the relative adsorption activity of the surfactant at the interface and the ability to form micelles in aqueous solution.

$$\Gamma_{max} = -(\partial\gamma/\partial \ln C)/2.3nRT \quad (2)$$

$$A_{min} = 10^{24}/(N_A\Gamma_{max}) \quad (3)$$

$$CMC/C_{20} = CMC/C_{20} \quad (4)$$

where γ is the surface tension of cardanol-derived QACs. R is the gas constant, R is equal to 8.315×10^7. T is the absolute temperature of gas. $(\partial\gamma/\partial \ln C)$ is the slope of the linear simulation diagram of the surface tension before CMC. The parameter n is the number of ionic species. N_A is the Avogadro constant (6.02×10^{23}) [43].

As is indicated in Table 1, the CMC values of cardanol-derived QACs are lower 2–3 orders of magnitude than commercialized QAC product cetyltrimethylammonium bromide (CMC = 1 mmol/L), the γ_{CMC} values are lower than that of cetyltrimethylammonium bromide (γ_{CMC} = 35.3 mN/m) [44]. **2b** has the optimal the surface activities, the critical micelle concentration = 0.002 mmol/L, the surface tension = 11.31mN/m. Khadidja et al. [45] investigated a phenylene on the hydrophobic chain contributed to micellization. Due to hydrophobic effect and π-π interactions among the amphiphilic molecules, which may be one reason for better surface activities of cardanol-derived QACs. Yao et al. [46] adding double bond into surfactants confirmed that, the surface activities such as CMC value, emulsifying property, foaming property and foam stability would improve a lot. Pei et al. [47] studied the adsorption and micellization behaviors of QACs containing hydroxyl group in aqueous medium. The results clearly exhibited that QACs can form intermolecular hydrogen bonds in aqueous medium, which could boost interfacial adsorption and the formation of micelle directly. Grosmaire et al. [48,49] mentioned that surfactants with heterocyclic head groups have higher surface activities and more abundant aggregation behaviors. Therefore, introducing the double bond, hydroxyl, heterocyclic polar head group into QACs could reinforce the solubility, surface activities and aggregation behaviors in the aqueous medium, resulting a rise in CMC value, which may be the other reason for better surface activities of cardanol-derived QACs. For cardanol-derived asymmetric Gemini QACs, as the alkyl chain increased, the CMC values decreased and the γ_{CMC} rose. Since the longer alkyl chain lead to the stronger hydrophobic effect between the QAC molecules, the easier micellization, the smaller CMC value [50]. At the same time, γ_{CMC} showed an opposite trend. Geng et al. [51] investigated that more methylene groups are exposed due to the longer free hydrophobic chains and the possibility of free rotation and curling, resulting in an increase in surface energy and γ_{CMC}.

The low flexibility of short spacer chains [52] of cardanol-derived asymmetric Gemini QACs lead to a larger value of area per adsorbed amphiphilic molecule than cardanol-derived mono-heterocyclic QACs, higher A_{min} can be anticipated. The data conformed that the adsorption efficiency pC_{20} values are inversely related to the CMC values as mentioned by Pinazo et al. [52]. The adsorption efficiency increased with a higher hydrophobic effect. Lower C_{20} and higher pC_{20} indicate higher adsorption efficiency of QAC, **2b** is optimum, having highest pC_{20}. Meanwhile, the larger values of Γ_{max} and smaller values of A_{min} and CMC/C_{20} indicate that **1a**, **2b** have a more dense alignment of molecules and better micellization tendency.

3.2. Krafft temperature (T_k) measurement

For surfactants, in the lower temperature range, the solubility rises very slowly with increasing temperature. When the temperature rises to a certain value, the solubility increases rapidly, which is regarded as Krafft temperature (T_k) [53]. A lower T_k results in the better solubility of the surfactant at low temperature. As is seen from Table 2, the T_k of **1a**, **2a**, **2b** show excellent solubility at 0 °C, and enable them to be used in low temperature, which have a wide range of applications. The T_k of **1b**, **1c**, **1d** are all below 10 °C, being known as good water soluble surfactant. As mentioned by Mahantesh et al. [54], incorporating hydroxyl, heterocyclic polar head group into QACs could lower the solubility. However, the T_k of **1e** is above 90 °C. S. Ban et al. [55] found that the presence of hydrophobic chain led to the aggregation of molecules and increased hydrophobicity, thereby resulting in the decrease of solubility and the increase of T_k value. Therefore, as the alkyl chain increased, the T_k of cardanol-derived asymmetric Gemini QACs were increased, solubility decreased as shown in Table 2.

Table 3
Antimicrobial activity results of cardanol-derived QACs (μg/mL) on Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli*).

Compound	*S. aureus*		*B. subtilis*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
1a	32	64	8	32	16	32
1b	32	64	16	64	32	128
1c	128	>128	64	128	>128	>128
1d	64	>128	16	64	16	64
1e	64	128	16	32	16	64
2a	128	>128	64	>128	>128	>128
2b	32	64	8	32	16	32

3.3. The contact angles of the film of cardanol-derived QACs (on glass sheet) with water molecules

Good wettability of cardanol-derived QACs enriches the application as surfactants. The contact angle clearly reflects the wetting ability of QAC and the surface affinity of water molecules and the amphiphilic molecules shown in Fig. 2. A smaller contact angle means more excellent wettability and spreadability on the film of cardanol-derived QACs. As can be seen in Table 2, the contact angle of **2b** almost reached to 0°, which is lower than the contact angle of glass with water molecules, indicating that water molecules have excellent spreading properties on the film of **2b**, and it also shows that **2b** has good wettability.

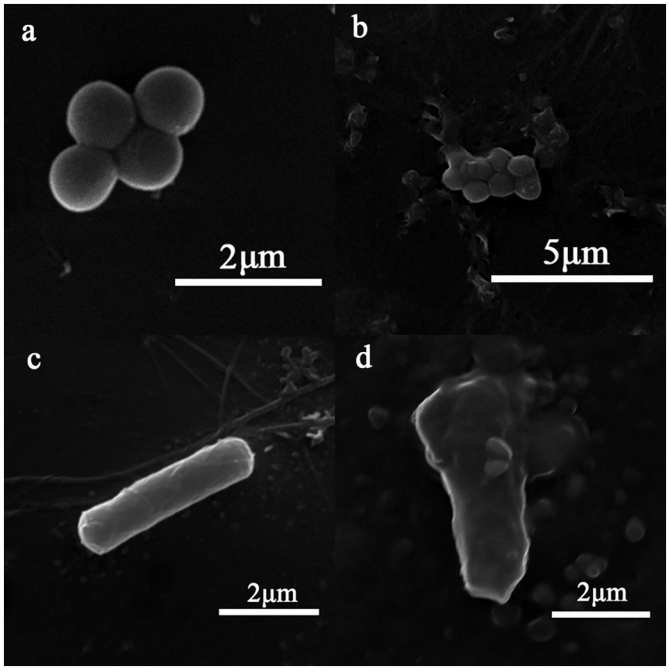


Fig. 3. SEM images for: (a) *S. aureus* untreated; (b) *S. aureus* treated with 32 μg/mL **2b**; (c) *E. coli* untreated; (d) *E. coli* treated with 16 μg/mL **2b**. The scale bar is marked in the figures.

According to Table 2, other cardanol-derived QACs also have certain wettability and spreadability. As the increasing of alkyl chain of cardanol-derived asymmetric Gemini QACs, the contact angle increased and wettability decreased [37].

3.4. Antimicrobial activity

By using both broth dilution method and agar dilution method, the MIC and MBC values of cardanol-derived QACs were determined. The MIC and MBC values were measured to evaluate antimicrobial activity in Table 3, which revealed that most of cardanol-derived QACs exhibited inhibition activities against the tested bacterial. Sharma et al. [56] found out that a rise in polarity of the head group in surfactants promoted antimicrobial property. The polarity of head group can be improved by adding the double bond or hydroxyl. It can be seen from the Table 3 that the antimicrobial effect of QACs **1a**, **1b**, **2b** are found to be more prominent and superior to the traditional single surfactant, which can inhibit or even kill the tested bacterial at a concentration of 32 μg/mL or lower. The bacterial inhibition effects are found to be remarkable when the concentrations of QAC **2b** are in the range from 32 to 64 μg/mL for *S. aureus*, from 8 to 32 μg/mL for *B. subtilis*, and from 16 to 32 μg/mL for *E. coli*, respectively.

It is known from the literature [58,61] that the length of hydrophobic chain has a prominent effect on the antimicrobial activity of cationic surfactants. Too long or too short of alkyl chain adverse to antimicrobial activity of cationic surfactants. As the increasing of alkyl chain of cardanol-derived asymmetric Gemini QACs, the antimicrobial property increased due to the more hydrophobic environment. Hence, the antimicrobial activity of **1e** is stronger than **1d**. At concentrations below CMC values, QAC molecules are evenly dissolved in the culture medium, easily exhibit antimicrobial behaviors by electrostatic and hydrophobic interactions with bacterial cell membranes. Hydrophobicity of alkyl chain and electron density of ammonium nitrogen atom are the key factors affecting the antimicrobial activity of surfactants. Hence, Asadov et al. [57] mentioned that the antimicrobial activity depend not only on the length of alkyl chain but also on the CMC value. As the CMC values decrease, the antimicrobial activities are improved [57]. The experimental data show that QACs **1a**, **1b**, **2b** exhibited significant inhibitory effect against three pathogenic bacteria, whose CMC values exactly are also lowest. **1c** and **2a** showed poor antimicrobial properties, probably, due to the smaller thiazole heterocycle molecule than 1-methylpyrazole and n-methylmorpholine, and the higher CMC value. For cardanol-derived asymmetric Gemini QACs **1d** and **1e**, the CMC values are higher, thus, the antimicrobial properties is relatively weaker.

Scanning electron microscopy (SEM) as an intuitive means was used to obtain a visual insight into the bacterial killing of cardanol-derive QAC. SEM images of *S. aureus* and *E. coli* treated with QAC **2b** (16 and 32 μg/mL, respectively) for 12 h and without treatment with QAC (as a control) were dis-played in Fig. 3.

Both untreated samples showed the presence of the integrative spherical and rod shaped cells (Fig. 3, a and c for *S. aureus* and *E. coli*, respectively) with preserved cell membranes. However, irregular-shaped bacteria were observed after treatment with **2b** (Fig. 3, b and d for *S. aureus* and d for *E. coli*, respectively) [59,60]. Therefore, as mentioned in most of the literature [61,62], the patterns of antimicrobial behaviors are that QACs molecules adsorb on the cell membranes by opposite charges electrostatic attraction and interact with bacterial cell membranes. And then the cell membranes become disorganized. Finally, the leakage of autolysin from the cytoplasm causes the cell membranes to dissolve. As a result, destructive effects occur in bacterial cells, including structural disintegration and the formation of cell membranes pores. Cardanol-derived QACs interacted with bacterial cell membranes to form pores and destroy cell membranes, which may result in structural changes and the loss of cytoplasmic components and ultimately cell death.

4. Conclusion

Two series of seven quaternary ammonium compounds (QACs) derived from renewable biomass raw material cardanol have been synthesized with excellent surface activity, lower critical micelle concentration (CMC), Krafft temperature (T_k) and contact angle, compared with traditional surfactants. Furthermore, cardanol-derived QACs are found to have better antimicrobial efficacy for varieties of bacteria at a lower concentration. The damaging cell images of SEM were clearly observed that cardanol-derived QACs have effects on pathogenic bacteria. Undoubtedly, the syntheses of cardanol-derived QACs promote development of cationic surfactants.

Acknowledgment

This research was financially supported by the National Natural Science Foundation of China (21772039, 21272069) and the Fundamental Research Funds for the Central Universities and Key Laboratory of Organofluorine Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2019.111669.

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