

Synthesis and Properties of A Series of Bio-based Surfactants with Different Hydrophobic Chain Length

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ABSTRACT

We synthesized a series of diacyl D-glyceric acid (D-GA) sodium salts [dihexanoyl D-GA sodium salt (diC6GA-Na), dioctanoyl D-GA sodium salt (diC8GA-Na), didecanoyl D-GA sodium salt (diC10GA-Na), and dilauroyl D-GA sodium salt (diC12GA-Na)] from D-GA and respective acyl chlorides, and investigated some properties. Synthesized diC_nGAs were not water-soluble, whereas diC6GA-Na, diC8GA-Na and diC10GA-Na were. Surface tension measurements of diC6GA-Na, diC8GA-Na and diC10GA-Na in water revealed that the critical micelle concentration (CMC) was 2.92 mM, 0.82 mM and 0.23 mM, and surface tension at the CMC was 33.9 mN/m, 25.5 mN/m and 27.9 mN/m, respectively. These three diC_nGA-Na showed stability in the temperature range of 5-60 °C. The data support the use of these GA derivatives as green surfactants.

Keywords: D-glyceric acid, diacyl glycerate, glycerol, green surfactant, interfacial property

1 INTRODUCTION

The sustainable development of society needs the use of safe and renewable resources. Much attention has been paid to applying both chemical and biological processes to convert biomass to commodity chemicals as well as clean fuels. Glycerol can be produced from biomass (e.g., vesitable oils, animal fats) *via* the hydrolysis or methanolysis of triglycerides. Due to the increase in biodiesel production, the production of glycerol has increased, and it has become a promising and abundant carbon source for industrial microbiology.

Glyceric acid (2,3-dihydroxypropanoic acid, GA) is a natural organic compound found in specific plants as a minor constituent [1, 2], but can be mass-produced biotechnologically from glycerol [3–7]. In particular, the acetic acid bacteria *Acetobacter tropicalis* induces chirality at C-2 of GA and produces enantiopure D-GA with a 99% enantiomer excess (ee) [3]. Multiple biological activities of D-GA as well as GA derivatives such as diacyl GA and glucosyl GA have been reported [8–14] (Figure 1).

Among GA derivatives, diacyl GAs are suggested to have biological activities. For example, Lesová et al. reported that GAs esterified with long acyl chains (> C16) exhibit antitrypsin activity [15]. Also, we synthesized diacyl GAs with acyl chain lengths of C16 and C18 and investigated their biological properties [10–12]. However, their hydrophobic nature, which derives from their long acyl chains, has limited the number of studies on their biological and physical properties.

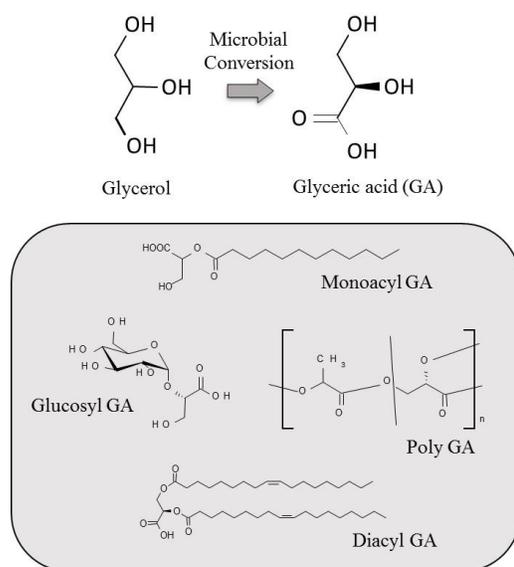


Figure 1: GA derivatives

In this study, to improve the water solubility of diacyl GAs, we synthesized diacyl D-GAs with shorter acyl lengths of hexanoyl (C6) to lauroyl (C12) groups, and investigated their interfacial properties.

2 EXPERIMENTAL

2.1 Materials

D-GA with an ee of 99% was prepared from glycerol by oxidative fermentation using *Acetobacter tropicalis* NBRC

16470 [3, 4]. The D-GA calcium salts were converted to free acids using a DOWEX (Dow Chemicals, Midland, MI) cation exchange resin. Acyl chlorides (hexanoyl chloride, C6; octanoyl chloride, C8; decanoyl chloride, C10; lauroyl chloride, C12) were purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents and solvents were obtained from Wako Pure Chemicals.

2.2 Synthesis of diC_nGAs and their sodium salts

Acylation of D-GA was performed in anhydrous acetone in the presence of dimethylaminopyridine and triethylamine, as described previously [16] (Figure 2). Acylation was initiated by the drop-wise addition of acyl chlorides over 30 min at 0 °C. The solution continued to be incubated at 0°C for an additional 30 min. Reaction progress was monitored by thin layer chromatography (TLC). After the disappearance of the TLC spot corresponding to GA, the reaction mixture was filtered using No. 40 filter paper (GE Healthcare UK Ltd., Little Chalfont, UK) and filtrates were evaporated *in vacuo*. Then, 1 M HCl and ethyl acetate were added to the resulting material and the organic layer was collected. The fraction was dried using anhydrous Na₂SO₄ and solvents were removed by evaporation. The resulting crude oil was purified using silica gel chromatography with hexane, hexane/ethyl acetate (8:2, v/v), and ethyl acetate eluents. Fractions containing diC_nGA were collected, dried using anhydrous Na₂SO₄, and concentrated *in vacuo*. The resulting compound was characterized by nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS).

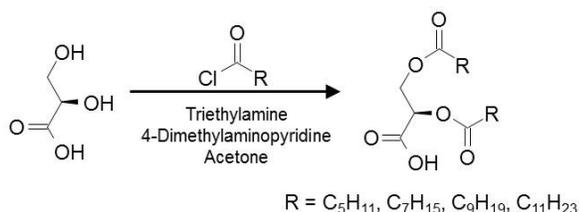


Figure 2: Synthesis of diacyl GAs

The synthesized diC_nGAs were dissolved in a methanol/water (1:1) solution and converted to sodium salts by neutralization with an equivalent volume of 1 M NaOH. After neutralization, the methanol was evaporated, and the resulting diC_nGA sodium salt (diC_nGA-Na) was recovered by lyophilization. The structures of these sodium salts were confirmed by ¹H NMR.

2.3 Hydrophilic-lipophilic balance

Molecular weights of diC₆GA, diC₈GA, diC₁₀GA, diC₁₂GA, and their sodium salts were calculated from their chemical structures. Hydrophilic-lipophilic balance (HLB)

values were derived directly from the molecular structures according to Griffin's expression: $HLB = 20 \times [1 - (\text{mass of hydrophobic part} / \text{total molecular mass})]$.

2.4 Measurement of surface tension

The surface tension of aqueous solutions containing diC_nGA-Na were measured at 25 °C using the pendant-drop method with an automatic interfacial tensiometer (DM500, Kyowa Interface Science, Niiza, Japan) and Drop Shape Analysis software (FAMAS v2.01, Kyowa Interface Science). The CMC of each synthesized compound was calculated from the crosspoint on each respective surface tension curve. Surface tensions at CMC (γ_{CMC}) were also determined.

2.5 Measurement of conductivity

The electrical conductivity of diC₆GA-Na, diC₈GA-Na, diC₁₀GA-Na and diC₁₂GA-Na (10 mM each, above the CMC) were measured using an electric conductivity meter (DKK-TOA Corporation, Tokyo, Japan) to investigate whether the Krafft point of these diacyl glycerate solutions, indicated by a prominent transition in conductivity as a function of temperature, could be defined. Conductivity measurements were performed from 5-60 °C.

2.6 Analytical procedure

TLC plates were developed with a chloroform/methanol mixture (8:2), and the organic compounds were visualized by heating at 120°C for 5 min with a 5% (w/v) phosphoric acid solution in ethanol containing 5% (v/v) sulfuric acid and 0.6% phosphoric acid. ¹H and ¹³C NMR spectra in CDCl₃ were recorded on a Bruker AV-400 spectrometer at 27°C (Bruker, Karlsruhe, Germany). LC-MS was performed on a Shimadzu LC-MS 2020 system (Shimadzu, Otsu, Japan) equipped with a reverse-phase Synergi 4U column (150 × 2.0 mm, Phenomenex, Torrance, CA). Samples were eluted in 0.1% (v/v) formic acid/acetonitrile (15:85, v/v) at 0.2 mL/min and the column was kept at 40 °C during analysis. Effluents were ionized by electrospray-ionization (ESI) and detected in negative ion mode with a *m/z* range of 50–2000.

3 RESULTS AND DISCUSSION

We synthesized diC₆GA, diC₈GA, diC₁₀GA, diC₁₂GA, and their sodium salts using the free acid form of GA in acetone (Figure 2). The obtained diC₆GA diC₈GA diC₁₀GA and diC₁₂GA were purified with yields of 58.3%, 51.0%, 68.3% and 71.4%, respectively. The structure of diC_nGA was confirmed by NMR and LC-MS. Collectively, NMR data confirmed the synthesis of respective diC_nGAs (data not shown). LC-MS analyses revealed that [M-H]⁻ (*m/z* = 301), [M-H]⁻ (*m/z*=357), [M]⁻ (*m/z*=414) and [M-H]⁻

($m/z=469$) were the main ion forms of diC6GA, diC8GA, diC10GA and diC12GA, respectively, with additional adduct ions (formate and proton adducts).

While diC8GA was obtained as a yellow oil [15], diC10GA and diC12GA were obtained as white solids and their free acid forms were not water soluble. All diC_nGAs were converted to their corresponding sodium salts by neutralization with NaOH. The 10 mM solutions of diC6GA-Na, diC8GA-Na and diC10GA-Na were soluble in water, whereas a solution of 10 mM diC12GA-Na precipitated at room temperature (Figure 3).

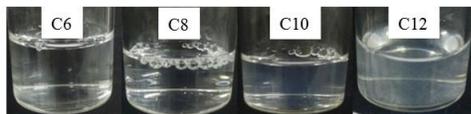


Figure 3: Aqueous solutions of 10 mM diC_nGA-Na

The MW and HLB data for these diacyl D-GA sodium salts calculated from their chemical structures are summarized in Table 1.

diC _n GA	Functional group	HLB
diC6GA	-COOH	10.59
diC6GA	-COONa	11.17
diC8GA	-COOH	8.94
diC8GA	-COONa	9.52
diC10GA	-COOH	7.73
diC10GA	-COONa	8.30
diC12GA	-COOH	6.81
diC12GA	-COONa	7.36

Table 1: HLB values of diC_nGA and diC_nGA-Na

The surface tension of diC6GA-Na, diC8GA-Na and diC10GA-Na in water was evaluated using the pendant drop method. As shown in Figure 4, a decrease in the surface tension of respective diC_nGA-Na was observed with increasing concentration. The position of the crosspoint in the surface tension plot in Figure 4 indicates the CMC values of diC6GA-Na, diC8GA-Na and diC10GA-Na were 2.92 mM, 0.82 mM [16] and 0.23 mM, respectively. Generally, water solubility increases as the chain length of the acyl group becomes shorter. It is therefore not surprising that the CMC of diC10GA-Na is lower than that of diC8GA-Na and that the CMC of diC8GA-Na is lower than that of diC6GA-Na. The CMCs of the three acyl D-GA sodium salts follow the well-known Klevens equation, which predicts a linear decrease in CMC with increasing hydrocarbon chain length (data not shown).

The surface tension at the CMC was 33.9 mN/m, 25.5 mN/m [16] and 27.9 mN/m for diC6GA-Na, diC8GA-Na and diC10GA-Na, respectively. Thus, the observed surface-

tension-lowering activity around 30 mN/m demonstrates the potential of diC_nGA-Na as useful surfactants.

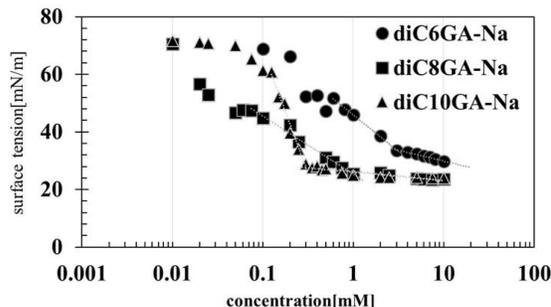


Figure 4: Surface tension vs. concentration plot of diC_nGA-Na.

The conductivity measurements were carried out at 10 mM of diC_nGA sodium salts in the temperature range from 5 to 60 °C. No significant changes in conductivity of diC6GA-Na, diC8GA-Na and diC10GA-Na were observed (data not shown). The observed monotonic and featureless increase of conductivity within the temperature range suggested that the Krafft point is below 5 °C.

By contrast, we found in diC12GA-Na an inflection point at 42 °C, suggesting that it is the Krafft point of diC12GA-Na (Figure 5).

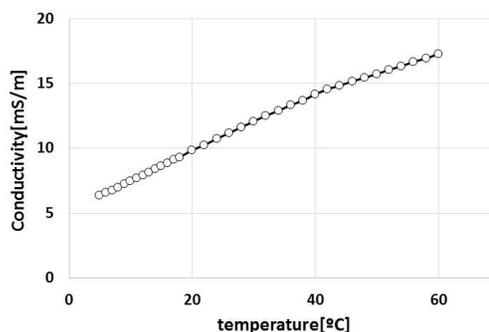


Figure 5: Conductivity-temperature plots of 10 mM diC12GA-Na.

4 CONCLUSION

In this study, we synthesized dihexanoyl D-GA, dioctanoyl D-GA, didecanoyl D-GA, dilauroyl D-GA, and their sodium salts from D-GA and respective acyl chlorides. Although diC_nGAs were not water-soluble, their sodium salts had superior surface properties in water compared to those of commercially available synthetic surfactants such as SDS (γ CMC, ca. 38 mN/m) [13]. We are now investigating some applications suitable for respective green surfactants.

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