Comparative analysis of bio-based amino acid surfactants obtained via Diels–Alder reaction of cyclic anhydrides

Abstract: Current changes in environmental legislation and customer demands set an urge for the development of more sustainable surfactants. Thus, the objective of this work was the development of novel environmentally friendly amino acid surfactants. Combining Diels–Alder cyclization of myrcene with maleic or citraconic anhydride followed by ring opening with amino acids enabled a synthesis route with a principal 100% atom economy. Variation of amino acids resulted in a large structural variety of anionic and amphoteric surfactants. Lysine gave access to either a mono-acylated product bearing a cationic side chain or a bi-acylated gemini surfactant. First, anhydride precursors were synthesized in yields of >90% in a Diels–Alder reaction under microwave radiation and subsequent amino acid coupling in aqueous environment gave fully bio-based surfactants in good yields and purity. Physicochemical characterization showed an enhanced decrease in surface tension upon addition of amino acids to the myrcene–anhydride backbone, resulting in a minimal value of 31 mN·m⁻¹ for gemini–lysine. Foamability and foam stability were significantly increased at skin-friendly pH 5.5 by incorporation of amino acids. The carboxylic groups of surfactants with arginine were esterified with ethanol to access cationic compounds. Comparative analysis revealed moderate antimicrobial effects against yeast, Gram-positive bacteria, and Gram-negative bacteria.

Keywords: green chemistry, bio-based surfactants, amino acids, antimicrobial activity, surface tension

1 Introduction

In recent years, a general trend towards “greener” products has emerged. Following this trend, research efforts are shifting to the development of new environmentally friendly methods to produce greener chemicals [1,2]. For example, petroleum-derived linear alkylbenzene sulfonates are excellent surfactants but have inherent toxicity to aquatic life [3], while alkylphenol polyethoxylates are reported to cause major environmental problems due to their bioaccumulation and estrogenic effects [4,5]. The use of renewable resources enables the production of more environmentally friendly surfactants with improved biological degradation profiles [6,7]. In addition to sugars such as glucose, amino acids can also be readily used as hydrophilic head group. Amino acid-based surfactants are generally considered to be milder, biocompatible, and ecological substitutes for petrochemical manufactured surfactants [8–10]. Research has shown that amino acid surfactants can be prepared by reductive amination using fatty alcohols or aldehydes, resulting in the formation of N-acyl amino acids. However, the linkage usually relies on the application of reducing agents such as boron hydride or its derivatives to reduce the imine moiety to the more stable amine function [11–13]. In industrial scale synthesis, amino acids or protein hydrolysates are usually converted to N-acyl amino acids by acylation with fatty acid chlorides using the Schotten–Baumann method [14,15]. Alongside the major product, a stoichiometric amount of HCl is formed as an unavoidable by-product. The need to neutralize the by-product proves to be the major drawback of the Schotten–Baumann reaction and leads to an overall lower atom economy [16–18]. In recent approaches, the biocatalytic synthesis of acylamino acids with novel aminoacylases has been successfully carried out [19–21].

In the search for alternative environmentally friendly and sustainable routes to new amino acid surfactants, the Diels–Alder reaction of the monoterpenic myrcene and anhydrides was identified as a viable method for the conversion of amino acids in a reaction with high atom
economy and without stoichiometric additives. First reports of the Diels–Alder reaction by Otto Diels and Kurt Alder date back 90 years and the combination of the terpene myrcene with maleic anhydride (MA) was discovered soon after [22–24]. Myrcene is derived from the turpentine oil obtained from pine trees and has recently been produced by modified microorganisms [25–27]. MA, on the other hand, is regularly produced by oxidation of 1,3-butadiene, but has been shown to be easily obtained from biomass and is well suited as a green and renewable platform substrate [28,29]. Citraconic anhydride (CA), along with its isomer itaconic anhydride, can be accessed by dehydration of citric acid, forming a mixture of both isomers with the thermodynamically more stable CA strongly preferred [30,31]. In recent developments, the reaction of myrcene with MA has been mainly used to produce monomers with unsaturated backbone for the production and further modification of polyesters and polyamides [32,33]. The possibility of application for the synthesis of surfactants by ring-opening addition with organic alcohols, amines, or water has been described only in the patent literature [34,35] and the combination with amino acids as potential new bio-based surfactants has not yet been tested.

In this work, the combination of the Diels–Alder reaction with MA and CA followed by ring-opening coupling to amino acids is presented, which gives fully bio-based products with high atomic economy (Figure 1). The structurally diverse products exhibited surfactant properties, which were analyzed based on their surface tension and foaming capacity. Subsequent esterification of the carboxylic groups resulted in novel cationic lipids with antimicrobial activity.

2 Materials and methods

2.1 Materials

Myrcene (90%) was purchased from Acros Organics, MA (≥98%), CA (98%), acetonitrile (99.5%), heptane (98%), formic acid (99%), and citric acid (99%) were purchased from Fisher Scientific. Tetrahydrofuran (THF) (99.5%, not stabilized), EtOH (99.5%), NaOH (99%), and amino acids (≥98.5%) were purchased from CarlRoth. Trimethylsilyl chloride (TMS-Cl) (≥98%) was obtained from Sigma–Aldrich and NMR-solvents (CDCl₃, CD₃OD, and D₂O, each 99.9%) were from TCI.

2.2 General microwave assisted Diels–Alder reaction procedure

MA (5.003.0 mg, 50.0 mmol) was dissolved in 10 mL THF and mixed with myrcene (9.52 ml, 7.52 g, 50.0 mmol, 90% purity) in a microwave reaction vessel. The reaction was conducted in a CEM Discover microwave synthesizer at 110°C with P_max = 80 W for a reaction time of 15 min. A transparent yellow solution was obtained, from which conversion was determined by GC-FID analysis. For CA, no solvent was added, and the reaction time was prolonged to 30 min.

2.3 Coupling of cyclic anhydride intermediates with amino acids

Condensation reactions with 50 mmol of amino acid in its deprotonated form were carried out in an acetone–water mixture following the Schotten–Baumann approach described by Takehara et al. [14]. Formation of a turbid mixture allowed filtration without the necessity for precipitation by HCl. Solvent was evaporated and the raw product was washed twice with petrol ether. The product was lyophilized and analyzed by HPLC, LC-MS, and NMR as described in supplementary data. Lysine–gemini surfactants were synthesized by application of reduced amount of amino acid (25.0 mmol, 0.5 eq.) and NaOH (0.99 g, 0.25 mmol, 0.5 eq.), while myrcene–anhydride amount was kept at 50 mmol. Selectivity was raised by further prolongation of the anhydride precursor addition time. Investigation of the possible coupling mechanism of lysine and anhydride-precursors was done by application of Nα- or Nε-tert-butyloxycarbonyl (boc)-protected lysine as a substrate.

2.4 General esterification protocol for amino acid surfactants

Esterification of carboxylic acid groups was mediated by TMS-Cl using methods according to Takaishi et al. [36].
Prior synthesized amino acid surfactant was used as a substrate (1.0 mmol) and dissolved in EtOH (5 ml, 86.0 mmol) and an excess of TMS-Cl (0.63 ml, 5.0 mmol) was added. The mixture was heated to 50°C for a period of 5 h. After cooling to room temperature, the solvent and volatile by-products were removed in vacuo and the product was obtained as solid.

### 2.5 Product purification

Raw product mixtures were purified using a preparative Interchim Inc. Puriflash 450-LC system, equipped with a preparative Kromasil column (C18, 5 µm, 250 mm × 200 mm). Raw products were dissolved in a 1:1 mixture of acetonitrile and water, chromatographic separation was conducted in a gradient method starting at 20% acetonitrile and ending at 90% acetonitrile with 0.1 vol% formic acid added to both phases. Chromatographic samples were collected and lyophilized.

### 2.6 Wilhelmy plate and pendant drop surface tension analysis

Surface tension analysis was done with a DCAT tensiometer using a Wilhelmy Plate PT 11 (10 mm × 19.9 mm × 0.2 mm) and analyzed with the DCATS software from DataPhysics as a mean of 50 measured values after the standard deviation was below a threshold of ±0.05 mN·m⁻¹. For a concentration series, the measurements were conducted from the lowest to the highest concentration. The critical micelle concentration (CMC) was determined graphically from a plot of the surface tension against the log of the surfactant concentration.

For surface tension determination with the pendant drop method a DataPhysics OCA contact angle system with SCA22 software for analysis was used. The volume of the pendant drop was dosed at a rate of 1 µl·s⁻¹ to the maximum size in a cuvette partially filled with water to minimize evaporation during the measurement over a period of 10 min. A surfactant concentration of 4 mmol·l⁻¹ related to the major active product was used. The pH was set to 5.5 with citric acid or 1 M NaOH solution before the measurements began. About 40 µl of test substance was spread on the LB- or YM plates; supernatant liquid was removed and the cultures incubated for 45 min at 37°C and 75 min at 30°C, respectively. After incubation, plates were further prepared by punching holes with the wider end of Pasteur pipettes (d = 5.5 mm). About 40 µl of test substance was placed in four different dilutions (40, 20, 10, and 5 mmol·l⁻¹ based on active compound in 100 mmol·l⁻¹ Tris-buffer pH 7.5) in the cavities and the plates were incubated for 24 h at 37°C and 30°C. About 100 mM Tris-buffer (pH 7.5) and the water-hydrolyzed Diels–Alder adducts were tested as negative controls. Commercially available cationic N-lauroyl arginine ethylester (LAE), ampicillin, and zeocin (C. viswanathii) were used as antimicrobial positive control. All tests were run in triplicate setup.

### 3 Results and discussion

#### 3.1 Diels–Alder catalyzed coupling of myrcene to anhydrides

Starting from the cyclic, unsaturated anhydrides, the Diels–Alder reaction offers an attractive possibility to extend the hydrophobic residue. In particular, the absence of additional coupling reagents, catalysts, and additives leads to a high atom economy of the reaction. The basic lipophilic tail of the surfactants was synthesized following the method of Hornung et al. [32] using microwave irradiation to mediate the [4 + 2]-cycloaddition reaction (Figure 2a). The method was applied to myrcene and MA using THF as a solvent. As described in the literature [32], MA conversion went nearly quantitative (96%) within 5 min (Figure 2b). Furthermore, the reaction was transferred successfully to the fully bio-based CA. The liquid compound enabled a solvent-free synthesis, which makes the overall reaction more sustainable. The cycloaddition reaction with CA required 30 min observations...
to reach a maximum yield of up to 90%, which may be attributed to the additional steric hindrance of the methylene group or the solvent-free conditions during synthesis. Further investigation employing LC-MS confirmed synthesis of both target molecules in ring-closed anhydride form (Figure 2c and d).

3.2 Synthesis of amino acid-based surfactants by ring-opening condensation

The N-terminal ring-opening addition of amino acids to the cyclic anhydrides generates amphiphilic products with great structural variety (Figure 3a). Depending on the charge of the amino acid side chain, either anionic surfactants with two or three acid groups or amphoteric amphiphiles with two carboxyl and one cationic side chain are accessible. The raw anhydride reaction mixture was added slowly to a solution of target amino acid dissolved in water. The pH of the amino acid solution was adjusted to an alkaline pH of >9 to deprotonate the amine group, enabling nucleophilic attack of the anhydride. Similarly, the Schotten–Baumann reaction is conducted for the synthesis of acylamino acids from the corresponding acyl chlorides [14]. Addition of the anhydride to the aqueous amino acid solution gives complete conversion of the anhydride, yielding the desired surfactant molecules in the form of two isomers, which could not be separated by HPLC or

Figure 2: (a) Reaction scheme for conversion of myrcene with cyclic anhydrides MA and CA, (b) time-dependent yield of microwave-assisted synthesis of MA (blue) and CA (green) precursors, (c + d) GC-chromatograms of MSA (c) and CA (d) after 30 min reaction time.
GC analysis. Due to the necessity of using aqueous solutions to dissolve the amino acid, the hydrolyzed by-product from ring-opening condensation of the anhydride with water was observed as well (Figure 3b). Success of the amino acid coupling was confirmed by comparing the $^1$H-NMR spectra of the hydrolyzed and the amino acid-opened product (Figure 3c). Due to the structural and electron-density differences, a chemical shift of one of the former anhydride ring protons is observable. In the hydrolyzed by-product, both protons of the connection between myrcene and anhydride moieties are chemically nearly identical, whereas the protons in the amino acid-opened product lack this equivalency, since one of the acid groups is transformed into an amide function.

Except for the cyclic, secondary amine function of proline, all amino acids were successfully coupled to the anhydride function of the MA intermediate. Best results were obtained for histidine, phenylalanine, and glycine with yields of 85–90% (Table 1). Glutamic acid and aspartic acid could be isolated in 83% and 46% yield, respectively, while the corresponding amides, glutamine and asparagine, performed significantly worse. The reaction was successfully transferred and repeated for selected amino acids, applying CA as a linker molecule. CA possesses an additional methyl group at position 3 of the cyclic anhydride, which results in two different isomeric products after Diels–Alder coupling (Figure 3, structures 2a and b). This leads to the formation of four possible isomeric surfactant products in the
Figure 3: (a) Reaction scheme of Diels–Alder product conversion in condensation reactions with amino acids, (b) evaporative light scattering detector chromatogram obtained by ring opening of the myrcene–MA intermediate with phenylalanine and (c) comparison of the $^1$H-NMR spectra of the hydrolysis product (top) and the phenylalanine-coupling product (complete $^1$H-NMR and $^1$H–$^1$H-COSY-NMR spectra of the coupling product are shown in Figures S1 and S2).
consecutive ring-opening reaction, which could not be separated in HPLC analysis. Arginine and phenylalanine were converted in yields up to 95% (Table 1).

Lysine was successfully converted to the desired N-acylated product, but HPLC- and LC-MS analyses showed the formation of the bi-acylated product as well (Figure 4). In contrast, the alcohol groups in the side chains of tyrosine, threonine, serine, and the thiol group of cysteine did not act as a nucleophile to catalyze ring opening of the anhydride under the chosen reaction conditions and thus no two-fold acylations were observed in these cases. Therefore, only lysine shows the capability to form a gemini-type surfactant with two hydrophobic chains coupled to a single amino acid group, giving access to interesting surfactants with a larger hydrophobic tail [37,38]. As outlined in Table 1, high yields of 93% and 99% were obtained with the MA and CA, when a two-fold excess of lysine was added to the coupling reaction. Further investigations with tert-butyloxycarbonyl (boc) protection groups, starting from Nα- and Nε-boc-lysine, implicate that only Nα-boc-lysine was acylated successfully by the addition of the myrcene–MA precursor at the Nε group. This observation indicates that the production of the lysine–gemini surfactant probably proceeds via the initial addition of the anhydride to the epsilon-amine function and a subsequent addition of a second anhydride moiety to the remaining alpha-amine.

Table 1: Isolated yields, purities, and physicochemical data of the myrcene–anhydride–amino acid surfactants (amino acids are outlined in 3-letter code)

<table>
<thead>
<tr>
<th>Diels–Alder adduct</th>
<th>Amino acid</th>
<th>Yisol (%)</th>
<th>Purity (%)</th>
<th>σ (mN·m⁻¹)</th>
<th>h_foam initial (mm)</th>
<th>h_foam at 5 min (mm)</th>
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<tr>
<td>(A) Products leading to dicarboxylic surfactants</td>
<td>Myr-MA</td>
<td>H₂Oᵃ</td>
<td>99</td>
<td>99</td>
<td>60.9 ± 0.4</td>
<td>110</td>
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<tr>
<td></td>
<td>Myr-CA</td>
<td>H₂Oᵃ</td>
<td>99</td>
<td>99</td>
<td>56.9 ± 0.2</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Myr-MA</td>
<td>Ala</td>
<td>69</td>
<td>53</td>
<td>54.0 ± 0.4</td>
<td>116</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Asn</td>
<td>46</td>
<td>63</td>
<td>45.7 ± 0.3</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>Myr-MA</td>
<td>Cys</td>
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<td>34.0 ± 0.6</td>
<td>120</td>
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<td>Myr-MA</td>
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<td>20</td>
<td>56.4 ± 1.2</td>
<td>130</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Gly</td>
<td>91</td>
<td>61</td>
<td>56.4 ± 1.2</td>
<td>117</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Ile</td>
<td>32</td>
<td>67</td>
<td>54.0 ± 1.3</td>
<td>105</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Leu</td>
<td>37</td>
<td>71</td>
<td>44.3 ± 0.2</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Myr-MA</td>
<td>Met</td>
<td>94</td>
<td>88</td>
<td>53.4 ± 2.6</td>
<td>124</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Phe</td>
<td>86</td>
<td>84</td>
<td>43.3 ± 0.5</td>
<td>133</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Phe</td>
<td>78</td>
<td>68</td>
<td>44.5 ± 0.8</td>
<td>134</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Ser</td>
<td>71</td>
<td>93</td>
<td>40.7 ± 1.1</td>
<td>140</td>
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<tr>
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<td>Myr-MA</td>
<td>Thr</td>
<td>94</td>
<td>92</td>
<td>37.1 ± 1.0</td>
<td>130</td>
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<td>Myr-MA</td>
<td>Trp</td>
<td>46</td>
<td>90</td>
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<td>133</td>
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<td></td>
<td>Myr-MA</td>
<td>Tyr</td>
<td>95</td>
<td>68</td>
<td>42.4 ± 0.6</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Val</td>
<td>95</td>
<td>59</td>
<td>45.2 ± 0.5</td>
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<td>(B) Products leading to tricarboxylic surfactants</td>
<td>Myr-MA</td>
<td>Asp</td>
<td>88</td>
<td>50</td>
<td>53.7 ± 0.4</td>
<td>124</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Glu</td>
<td>99</td>
<td>81</td>
<td>52.5 ± 4.4</td>
<td>125</td>
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<tr>
<td></td>
<td>Myr-CA</td>
<td>Glu</td>
<td>25</td>
<td>82</td>
<td>56.5 ± 0.4</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Myr-MA</td>
<td>R₂Lysᵇ</td>
<td>93</td>
<td>93</td>
<td>32.5 ± 0.1</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>Myr-CA</td>
<td>R₂Lysᵇ</td>
<td>99</td>
<td>75</td>
<td>31.5 ± 0.8</td>
<td>122</td>
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<td>(C) Products leading to surfactants with two carboxylic and one basic group</td>
<td>Myr-MA</td>
<td>Arg</td>
<td>68</td>
<td>93</td>
<td>38.0 ± 0.3</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>Myr-CA</td>
<td>Arg</td>
<td>95</td>
<td>50</td>
<td>31.8 ± 0.2</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>Myr-MA</td>
<td>His</td>
<td>91</td>
<td>89</td>
<td>56.7 ± 0.6</td>
<td>128</td>
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<tr>
<td></td>
<td>Myr-MA</td>
<td>Lysᶜ</td>
<td>89</td>
<td>31/42ᵃ</td>
<td>36.1 ± 0.1</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>Myr-CA</td>
<td>Lysᶜ</td>
<td>51</td>
<td>91</td>
<td>33.4 ± 0.8</td>
<td>139</td>
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</table>

The corresponding MS spectra can be found in supplementary data Figures S3–S29. Surface tension was analyzed at a surfactant concentration of 4 mmol·l⁻¹ and a pH of 5.5 and foam comparison was done after stirring (h_foam initial) and after 5 min without stirring. a = ring opening of Diels–Alder adduct with water; b = a twofold excess of Diels–Alder precursor was applied to synthesize the bi-acylated product and c = in an equimolar approach, lysine-based surfactants were obtained as a mixture of mono- and bi-acylated product.
Figure 4: (a) HPLC chromatogram of the product mixture obtained from lysine acylation, (b) + (c) MS spectra of mono-acylated and bi-acylated lysine.
3.3 Physicochemical properties of the Diels–Alder surfactants

Concentration dependent surface tension measurements were conducted with the Wilhelmy plate method for evaluation of the CMC of N-(myrcene-MA)-tryptophan and N-(myrcene-MA)-serine at pH 7 (Figure 5a). Similar values of around 2 mmol·l⁻¹ for the serine and 3 mmol·l⁻¹ for the tryptophan surfactant were obtained, which are close to their maximum solubility. Hence, a surfactant concentration of 4 mmol·l⁻¹, slightly above the CMC, was chosen for surface tension comparison of all amino acid surfactants with the pendant drop method. Analysis of the pH dependence was done at skin-friendly pH 5.5 instead of the
initially used pH 7 with the pendent drop method over a period of 10 min (Figure 5b). In dependence of the amino acid head group, different effects were observed (Figures S31–S33). While the arginine surfactant was not influenced by pH, surface tension was lower at pH 5.5 for tryptophan, but higher at pH 5.5 for lysine. The lysine-based surfactant gave the lowest surface tension of around 30 mN·m$^{-1}$ at pH 7.

Interference of the adjacent carboxylic groups was proven for the water hydrolyzed myrcene–maleic acid product by titration resulting in pK$_S$ values of around 3.8 and 6.8 (Figure S34). Titration of the amino acid surfactants could not resolve the individual pK$_S$ values; nevertheless, a partial protonation at a pH of 5.5 can be expected. The protonation state of the carboxylic groups will result in mixtures of anionic and nonionic surfactant molecules, which influence the surface properties in a pH-dependent manner. The exact ratios of protonated and non-protonated forms and how they influence the surface properties need further investigation.

Figure 6: Comparison of MS spectra of (a) N-(myrcene-CA)-arginine and (b) N-(myrcene-CA)-arginine-OEt after esterification. The spectra for the corresponding MA-based surfactants are shown in Figures S5 and S30.
For comparison of all MA- and CA-based surfactants at a concentration of 4 mmol·L$^{-1}$, a pH of 5.5 was chosen, which is a typical value for shampoo and cosmetic preparations (Table 1). A minimal decrease of the surface tensions to final values of 57 and 61 mN·m$^{-1}$ was observed, when the water hydrolyzed Diels–Alder adducts were applied. All amino acid surfactants exhibited at least slightly better surface activities than the water hydrolyzed products and their surface tensions ranged between 31 and 56 mN·m$^{-1}$. The acidic amino acids, aspartate and glutamate, gave a minimal improvement in comparison to the hydrolysis product, resulting in minimal surface tensions of 52–57 mN·m$^{-1}$. This leads to the assumption that the hydrophobic tail is too small for a large hydrophilic section bearing a total of three negative charges within the surfactant head group. The finding can be substantiated by the fact that the bi-acylated lysine, which possesses three carboxylic acid groups but a larger hydrophobic moiety due to its gemini-type structure, exhibited the lowest surface tension with values of 31.5 and 32.5 mN·m$^{-1}$ for the CA and MA adducts.

Application of the non-polar and polar amino acids usually produces surfactants with two negative charges and reach mediocre minimal surface tensions of around 40–50 mN·m$^{-1}$. Only cysteine and threonine, bearing a thionyl- or a hydroxyl side chain, gave better results of 34.0 and 38.7 mN·m$^{-1}$. Particularly, the cysteine-based product may be interesting for e.g. cosmetic applications due to its potential antioxidative properties [39]. Upon addition of the basic amino acids, arginine, histidine, and mono-acylated lysine, amphoterically surfactants were produced, each with two negative and one positive charge. Good surface activity of 31–38 mN·m$^{-1}$ was obtained for the arginine and lysine derivatives, whereas histidine exhibited a significantly lower surface activity reaching 56.7 mN·m$^{-1}$. According to Tabohashi et al. [40], comparable amino acid surfactants show similar results with surface tensions around 30 mN·m$^{-1}$. The slightly lower activity in comparison to lauric acid-based surfactants might be explained by the fact that the Diels–Alder products contain a cyclic moiety, which shortens the length and reduces the flexibility of the hydrophobic tail at comparable carbon count.

Foamability was analyzed with a Krüss DFA100 foam analyzer and foam stability was observed over time after an initial foaming induced by aeration (Table 1). Initial foam build-up was detectable with the water hydrolyzed Diels–Alder adducts, but after stopping the air feed, the foam immediately began to collapse, resulting in an almost complete degradation within 30 s. Coupling of an amino acid to the myrcene–anhydride motive increases the initial foamability by around 30%, yielding initial foam heights between 100 and 140 mm. The amino acid surfactant foam showed better stability in comparison to the hydrolysis product, resulting in good stability over a period.

**Table 2: Microbial inhibition of B. subtilis, C. glutamicum, E. coli, and C. viswanathii in agar plate tests applying surfactants in a concentration range from 5 to 40 mmol·L$^{-1}$**

<table>
<thead>
<tr>
<th>Diels–Alder adduct</th>
<th>Amino acid</th>
<th>B. subtilis</th>
<th>C. glutamicum</th>
<th>E. coli</th>
<th>C. viswanathii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myr-MA</td>
<td>Phe</td>
<td>40+/20–</td>
<td>40+/20–</td>
<td>40x/20–</td>
<td>nd</td>
</tr>
<tr>
<td>Myr-MA</td>
<td>Arg</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Myr-MA-OEt</td>
<td>Arg-OEt</td>
<td>40+/20–</td>
<td>40+/20+10/5–</td>
<td>40x/20–</td>
<td>40+/20+10/5–</td>
</tr>
<tr>
<td>Myr-CA</td>
<td>Arg</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Myr-CA-OEt</td>
<td>Arg-OEt</td>
<td>40+/20–</td>
<td>40+/20+10+/5–</td>
<td>40x/20–</td>
<td>40+/20–</td>
</tr>
</tbody>
</table>

Note: nd = no inhibition detected at highest concentration, ++ = large inhibition area, + = clearly visible inhibition area, ○ = small inhibition area, X = weak inhibition, ambiguous test results and − = no inhibition detected.
of 5 min, especially for the polar amino acids. For arginine, serine, and tryptophan as well as the mono- and bi-acylated lysine foam heights of around 120 mm were detected, while threonine and tyrosine had a residual foam height of 80 mm after 5 min, respectively. Among the non-polar amino acids, only valine shows a moderate foam stability resulting in 40 mm after 5 min. Prolonged foam stability tests for some promising candidates were conducted over a period of 30 min (Figure 5b). Hardly any foam decrease from 10 min onwards was observed for the arginine, lysine, and serine derivatives making them interesting product candidates for applications in shampoo formulations, where foam is a desired feature [41]. As expected from their similar structure, the citraconic-anhydride-based derivatives gave similar foaming results. In all cases low surface tension values correlated with good foam stability.

3.4 Synthesis of cationic surfactants and evaluation of antimicrobial properties

Surfactants with the cationic amino acid arginine are known to exhibit antimicrobial activity and LAE is applied in industrially relevant scale [42,43]. Since the Diels–Alder-based products exhibit similar structural motives, esterification was performed with ethanol and TMS-Cl as a catalyst to obtain the fully esterified cationic surfactants. After evaporation of ethanol and trimethyl silyl derivatives, mass spectrometric analyses revealed the successful transformation as outlined in Figure 6 for N-(myrcene-CA)-arginine and its ethyl ester. Following a greener pathway, future syntheses may be conducted by application of esterified amino acids or by esterification of the surfactant product following the protocol of Turhanen et al. for both approaches [44].

Antimicrobial activity was analyzed against the yeast C. viswanathii, E. coli as Gram-negative and C. glutamicum and B. subtilis as Gram-positive bacteria. C. glutamicum was chosen, because its cell wall structure differs significantly from that of B. subtilis containing a mycolate outer membrane similar to the pathogenic bacterium Mycobacterium tuberculosis [45]. The series of MA-based surfactants shown in Table 1 was tested in preliminary assays against the bacterial cultures. Here, only N-(myrcene-MA)-phenylalanine exhibited some antimicrobial activity (Table 2). Similarly, a weak inhibition of several microorganisms was observed with the structurally related lauroyl-phenylalanine [46]. As expected from literature results [43,44,46], the cationic compounds N-(myrcene-CA)-arginine-OEt and N-(myrcene-MA)-arginine-OEt exhibited a better antimicrobial activity than the non-esterified counterparts (Figure 7 and Table 2). In comparison, the positive control LAE showed stronger antimicrobial effects, which may be attributed to the chain length differences of the surfactants. Tailoring of the hydrophobic chain, either by exchanging the diene component or choosing a different alcohol for esterification, may enhance the antimicrobial properties of the Diels–Alder-based surfactants. In accordance with Joondan et al. [47], the esterified catonic compounds were more active against Gram-positive, especially against C. glutamicum than Gram-negative E. coli. Interestingly, the cationic esters also showed activity against C. viswanathii and upon structural optimization, this class of compound might be utilized as antifungal agents.

4 Conclusions

Novel bio-based amino acid surfactants were synthesized in good yields and with high atom efficiency in a two- or three-step approach combining Diels–Alder cyclization, ring-opening condensation with amino acids, and esterification of the remaining carboxylic groups. Variation of the renewable diene, anhydride intermediate, amino acid, and alcohol moiety opens up a large structural space for specific tailoring of surfactant properties. In this study, the β-pinene-derived monoterpenes myrcene was selected as an example diene for the synthesis of a variety of anionic, amphoteric, and cationic surfactants. In particular, arginine-, lysine-, and cysteine-based products showed promising surfactant behavior, and the cationic arginine derivatives exhibited antimicrobial properties. In summary, this new class of surfactant has the potential to expand the spectrum of mild and environmentally benign amino acid surfactants for skin and hair care applications. Focusing future research on renewable dienes could lead to more hydrophobic surfactants with potentially interesting application profiles.

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References


