Synthesis and Antimicrobial Activity of the Essential Oil Compounds (E)- and (Z)-3-Hexenyl Nonanoate and Two Analogues

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ABSTRACT

The synthesis of (E)- and (Z)-3-hexenyl nonanoate, known constituents of essential oil containing plants, and two related compounds is reported. These compounds were assembled from nonanoyl chloride or nonanoic acid and the respective alcohols. In particular, it was found that the use of triethylamine as a co-solvent was necessary to avoid acid-induced isomerization of the alkenes, which resulted in an inseparable mixture of products. The antimicrobial activity of the four hexenyl and hexyl nonanoate compounds was undertaken using microdilution minimum inhibitory concentration (MIC) analysis against eight test microorganisms. All four compounds demonstrated activity, with (E)-3-hexenyl nonanoate (1b) having the highest inhibition (MIC value of 0.45 mg mL–1) against Pseudomonas aeruginosa ATCC 27858. Furthermore, this compound demonstrated the highest broad-spectrum activity (mean MIC value of 1.24 ± 0.50 mg mL–1) with noteworthy activity against all pathogens tested.

KEYWORDS

Essential oil constituent, (E)- and (Z)-3-hexenyl nonanoate, antimicrobial, ester synthesis, acid-induced alkene isomerizations.

Numerous studies and reviews on the subject matter of compounds isolated from plants have demonstrated that essential oil compounds display antimicrobial activity. Of particular interest to this work is a study where it was found that (Z)-3-hexenyl nonanoate (1a) (Figure 1) was a major essential oil component (16 %) from Heteropyxis natalensis, a deciduous tree, from the family Heteropyxidaceae, having strongly aromatic foliage and being specific to the geographical location of Lagalametse in the Waterberg (Northern Province, South Africa). In addition, the (E)-isomer (1b) has also been observed in plants, albeit in much lower amounts – see for instance the 0.2 % of this compound isolated from Cinnamomum tamala (Ham.) Nees & Eberm.a The authors of the South African study on Heteropyxis natalensis postulated that the particular (Z)-compound could be responsible for the overall antimicrobial properties of the essential oil. This hypothesis was based on a geographical variation study, whereby differences of antimicrobial activity were observed between varied regions. The most active (mean MIC value of 2.1 mg mL–1 against five test pathogens) of the Heteropyxis natalensis oil was evident where the major constituent comprised of (Z)-3-hexenyl nonanoate (1a). This compound was not present in any of the other geographical samples which had poorer activity (mean MIC value of between 4.0–7.4 mg mL–1 against the five test pathogens). Of interest is that compound 1a which has been associated with food additives; however, little else is known concerning the biological properties of these particular class of compounds. It was thus decided to synthesize compounds 1a and 1b, as well as two structural analogues 1c and 1d and test them for their putative antimicrobial efficacy.

Firstly, hexyl nonanoate (1c) was readily synthesized from nonanoic acid (2) and hexanol, using sulfuric acid as a catalyst, to afford the fully saturated ester (Scheme 1). The formation of the ester functionality was confirmed by the 13C NMR spectrum which showed a signal at δ 173.6. This synthesis was followed by the preparation of the unsaturated esters, performed by firstly converting nonanoic acid into its acid chloride derivative by the use of thionyl chloride (Scheme 1). Initial attempts of synthesizing the hexenyl waxes with the commercially available unsaturated alcohol components cis-3-hexen-1-ol, trans-3-hexen-1-ol and cis-2-hexen-1-ol, and utilizing between 2 and 10 equivalents of Et3N as a base, gave acceptable yields of the corresponding esters. Unfortunately, the 13C NMR spectroscopic analysis indicated that the products were mixtures of geometric isomers, despite the use of excess base, and purification by distillation proved problematic. The formation of a product mixture would suggest that the alkene intercepts the proximal acidic proton of the intermediate by way of an intramolecular process, despite base being present in solution.

Figure 1 Structures of (Z)- 1a and (E)-3-hexenyl nonanoate 1b.
Fortunately, the utilization of Et₃N as a co-solvent, rather than just a reagent, suppressed the acid-mediated isomerizations observed previously and gave the desired esters 1a, 1b and 1d as single compounds and in good yield after purification by silica gel column chromatography using ethyl acetate-hexane mixtures as eluent.

In terms of analysis, the compounds were characterized by ¹H NMR and ¹³C NMR spectroscopy (see supplementary files for NMR spectra associated with the compounds synthesized). Of note was that in all four esters the expected ester carbonyl signal was seen, as before, in the ¹³C NMR spectrum at ~173. Finally, all the compounds gave satisfactory high-resolution mass spectroscopic analyses.

The four synthesized compounds were then evaluated against eight test microorganisms, where the antimicrobial minimum inhibitory concentration (MIC) results are presented in Table 1. It was observed that all four compounds demonstrated interesting activity, with the (E)-isomer 1b having the highest inhibition against Pseudomonas aeruginosa ATCC 27858 (MIC value of 0.45 mg mL⁻¹). Furthermore, this compound demonstrated the highest broad-spectrum activity against all pathogens tested with a mean MIC value of 1.24 ± 0.50 mg mL⁻¹. It has been observed before that the antimicrobial activity of essential oil compounds generally have much poorer activities than bioactive compounds isolated from the less volatile fractions in plants. This is not unexpected, as in previous studies from one of our team, where both essential oils and extracts from the same plant species were examined for antimicrobial activity, the essential oils were generally less active. In addition, it is also known that the inhibitory efficacies of essential oil compounds have a wide range of activity - for example, van Zyl has shown for a range of essential oils that (-)-menthone was the most active compound, having noteworthy activity of 6.48 mM (1.25 mg mL⁻¹) against C. albicans. In comparison, the hexenyl-type compounds studied here are comparable in activity to the most active compounds found in the previous study. Furthermore, perusal of the literature reveals that to the best of our knowledge, the antimicrobial activities for these related hexyl and hexenynanoates are reported here for the first time. It should also be pointed out that a previous study has established important relationships between waxes and other lipids and essential oils found in plant membranes, and the microbes that feed on these plants. We trust that this paper will add to the knowledge in this interesting area of research.

In conclusion, this paper describes the development of a versatile approach to synthesize wax-like esters containing unsaturations in the alcohol portion, with the base being utilized as a co-solvent to prevent scrambling of the proximal alkene groups. Finally, based on the antimicrobial activities found for the small library of synthesized compounds, and in comparison with antimicrobial data for other essential oil compounds, it can be concluded that the antimicrobial activity for these synthesized hexenyl-type compounds demonstrate some potential.

### Table 1

<table>
<thead>
<tr>
<th>Microbes tested</th>
<th>Synthesized compounds</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1a</td>
<td>1b</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>P. aeruginosa ATCC 27858</td>
<td>0.85</td>
<td>0.45</td>
</tr>
<tr>
<td>M. catarrhalis ATCC 23246</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>S. aureus ATCC 25923</td>
<td>3.13</td>
<td>1.56</td>
</tr>
<tr>
<td>B. cereus ATCC 11778</td>
<td>1.56</td>
<td>1.56</td>
</tr>
<tr>
<td>E. faecalis ATCC 29212</td>
<td>3.10</td>
<td>1.21</td>
</tr>
<tr>
<td>C. albicans ATCC 10251</td>
<td>1.53</td>
<td>1.09</td>
</tr>
<tr>
<td>C. neoformans ATCC 90112</td>
<td>0.50</td>
<td>0.89</td>
</tr>
<tr>
<td>Mean MIC</td>
<td>1.73 ± 0.50</td>
<td>1.24 ± 0.50</td>
</tr>
</tbody>
</table>

* Ciprofloxacin for bacteria or amphotericin B for yeasts given in µg mL⁻¹.
SEQ mass spectrometer, a Waters API Q-TOF Ultima or Waters GCT Premier mass spectrometer. All reactions were monitored by TLC carried out on 0.2 mm aluminium silica gel (60 F<sub>254</sub>) pre-coated plates using UV light. Macherey-Nagel kieselgel 60 (particle size 0.063–0.200 mm) was used for conventional silica gel chromatography. Commercially available reagents and solvents were purified and dried when necessary by conventional techniques<sup>15</sup>. Dichloromethane was dried by distillation from calcium hydride. Unless otherwise mentioned, all other reagents were purchased from commercial sources and were used without further purification. Reactions were performed under a blanket of inert gas (Ar or N<sub>2</sub>) unless specified.

**Hxyl nonanoate 1c**: Nonanoic acid 2 (1.00 g, 6.32 mmol) and 1-hexanol (0.646 g, 6.32 mmol) and two drops of concentrated H<sub>2</sub>SO<sub>4</sub> were dissolved in toluene (5 mL), after which the reaction mixture was heated at reflux overnight. The reaction was then sequentially washed with concentrated Na<sub>2</sub>CO<sub>3</sub>, solution (10 mL) and distilled water (3 × 20 mL). The toluene was then removed under reduced pressure and the resultant oil purified by silica gel column chromatography (eluent: 20/1 hexane/ethyl acetate) to give the nonanoate esters (0.500 g, 2.84 mmol) in 98% yield. 1H NMR (300 MHz, CDCl<sub>3</sub>): δ 5.57–5.48 (m, 1H, CH=), 5.38–5.27 (m, 1H, CH=), 4.02 (t, 2H, J = 7.1 Hz, CH<sub>2</sub>), 3.83 (t, 2H, J = 7.8 Hz, CH), 2.31–2.23 (m, 4H, 2 × CH<sub>2</sub>), 2.03–1.93 (m, 2H, CH<sub>2</sub>), 1.54 (br quartet, 2H, J = 7.2 Hz, CH<sub>2</sub>), 1.20–1.25 (br s, 10H, 5 × CH<sub>3</sub>), 0.91 (t, 3H, J = 7.5 Hz, CH<sub>3</sub>), 0.83 (br t, 3H, J = 7.0 Hz, CH<sub>3</sub>); 1<sup>3</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 173.6 (C=O), 134.9 (C=C), 124.1 (C=C), 63.8 (CH<sub>3</sub>), 34.3 (CH<sub>2</sub>), 32.0 (CH<sub>3</sub>), 31.8 (CH<sub>3</sub>), 29.2 (CH<sub>3</sub>), 29.1 (CH<sub>3</sub>), 25.5 (CH<sub>2</sub>), 25.0 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 14.0 (CH<sub>3</sub>), 13.6 (CH<sub>3</sub>); HRMS: calculated for C<sub>15</sub>H<sub>28</sub>O<sub>2</sub> 241.2168 (M + H)<sup>+</sup>, found 241.2169 (M + H)<sup>+</sup>.

(Z)-2-Hexynyl nonanoate 1d: This ester was obtained as a light yellow liquid (0.511 g, 75 %). 1H NMR (300 MHz, CDCl<sub>3</sub>): δ 5.65–5.45 (m, 2H, 2H, J = 6.5 Hz, CH=), 4.56 (d, 2H, J = 6.5 Hz, CH=), 2.22 (t, 2H, J = 7.7 Hz, CH<sub>2</sub>CH<sub>2</sub>), 2.08–1.98 (m, 2H, CH<sub>2</sub>), 1.53 (br quintet, 2H, J = 7.0 Hz, CH<sub>2</sub>CH<sub>2</sub>), 1.42–1.32 (m, 2H, CH<sub>2</sub>), 1.30–1.20 (br s, 10H, 5 × CH<sub>3</sub>), 0.89–0.81 (m, 6H, 2 × CH<sub>3</sub>); 1<sup>3</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 173.4 (C=O), 134.8 (C=C), 123.7 (C=C), 60.0 (CH<sub>3</sub>), 34.2 (CH<sub>3</sub>), 31.7 (CH<sub>3</sub>), 29.4 (CH<sub>3</sub>), 29.1 (CH<sub>3</sub>), 29.0 (CH<sub>3</sub>), 24.9 (CH<sub>2</sub>), 22.5 (CH<sub>2</sub>), 22.5 (CH<sub>3</sub>), 13.9 (CH<sub>3</sub>), 13.5 (CH<sub>3</sub>); HRMS: calculated for C<sub>15</sub>H<sub>28</sub>O<sub>2</sub> 241.2168 (M + H)<sup>+</sup>, found 241.2168 (M + H)<sup>+</sup>.

**Antimicrobial Activity**

The MIC determination of the four synthesized hexyl nonanoate-type oil based compounds was undertaken to determine the antimicrobial activity using the MIC microtitre plate method<sup>20</sup>. The MIC assays were undertaken on Gram-negative test organisms Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27858 and Morexella catarrhalis ATCC 23246. Gram-positive test organisms were Staphylococcus aureus ATCC 29523, Bacillus cereus ATCC 11778 and Enterococcus faecalis ATCC 29212. Two yeast strains (Candida albicans ATCC 10231 and Cryptococcus neoforans ATCC 90112) were included in the antifungal analysis. All bacterial cultures were subcultured from stock agar plates and then grown in Tryptone Soy broth for 18 h. The yeasts were incubated for an additional 24 h. Test samples were transferred into the first row of a microtitre plate, at starting stock concentrations of 25 mg mL<sup>–1</sup>. Serial dilutions were performed and the cultures introduced, yielding an approximate inoculum size of 1 × 10<sup>6</sup> colony forming units (CFU) mL<sup>–1</sup>. Optimal incubation conditions followed; 37 °C for 24 h for bacteria and 48 h for the yeasts. Ciprofloxacin or amphotericin B at starting stock concentrations of 0.01 mg mL<sup>–1</sup> were used as positive controls for bacteria and yeasts respectively. A 0.4 mg mL<sup>–1</sup>-iodonitrotetrazolium violet solution was prepared and 40 μL transferred to all inoculated wells after incubation. The microtitre plates were examined after 6 h to determine a colour change in relation to the concentration of microbial growth<sup>2</sup>. The yeasts were examined after 24 h. All MIC assays were undertaken at least in triplicate.

**Supplementary Data**

<sup>1</sup>H and 1<sup>3</sup>C NMR spectra for all synthesized compounds are available online.

**Acknowledgements**

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**Z-3-Hexenyl nonanoate 1a**: This ester was obtained as a light yellow liquid (0.491 g, 72 %). 1H NMR (300 MHz, CDCl<sub>3</sub>): δ 5.46–5.37 (m, 1H, CH=), 5.28–5.19 (m, 1H, CH=), 3.96 (br t, 2H, J = 7.0 Hz, CH<sub>2</sub>), 2.29 (q, 2H, J = 7.0 Hz, CH<sub>2</sub>CH<sub>3</sub>), 2.18 (t, 2H, J = 7.6 Hz, CH<sub>2</sub>), 2.03–1.93 (m, 2H, CH<sub>2</sub>CH<sub>2</sub>), 1.49 (br quartet, 2H, J = 7.0 Hz, CH<sub>2</sub>CH<sub>2</sub>), 1.15–1.25 (br s, 10H, 5 × CH<sub>3</sub>), 0.87 (t, 3H, J = 7.6 Hz, CH<sub>3</sub>), 0.78 (br t, 3H, J = 7.1 Hz, CH), 1<sup>3</sup>C NMR (75 MHz, CDCl<sub>3</sub>): δ 173.4 (C=O), 134.2 (C=C), 123.7 (C=C), 63.5 (CH<sub>2</sub>), 34.1 (CH<sub>3</sub>), 31.7 (CH<sub>3</sub>), 29.1 (CH<sub>3</sub>), 29.0 (CH<sub>3</sub>), 26.7 (CH<sub>3</sub>), 24.9 (CH<sub>3</sub>), 22.5 (CH<sub>3</sub>), 20.5 (CH<sub>3</sub>), 14.0 (CH<sub>3</sub>), 13.9 (CH<sub>3</sub>); HRMS: calculated for C<sub>15</sub>H<sub>28</sub>O<sub>2</sub> 241.2168 (M + H)<sup>+</sup>, found 241.2169 (M + H)<sup>+</sup>.
References and Notes


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Supplementary information – NMR Spectra of compounds synthesized
Hexyl nonanoate 1c:
(Z)-3-Hexenyl nonanoate 1a:
(E)-3-Hexyl nonanoate 1b:
(Z)-2-Hexenyl nonanoate 1d: