S. Afr. J. Chem., 2012, **65**, 202–205,
<http://journals.sabinet.co.za/sajchem/>.

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

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Received 9 August 2012, revised 26 August 2012, accepted 2 September 2012.

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-mediated 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 micro-organisms. 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[1–7]. Of particular interest to this work is a study[8] 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.[9] 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)[8]. 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.

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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 ^{13}C 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 **2** into its acid chloride derivative **3** by the use of thionyl chloride (Scheme 1)[10].

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 Et_3N as a base, gave acceptable yields of the corresponding esters. Unfortunately, the ^{13}C 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.[11]

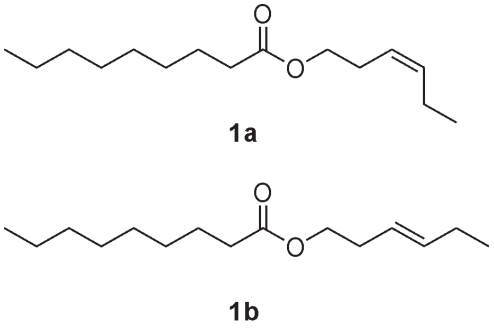
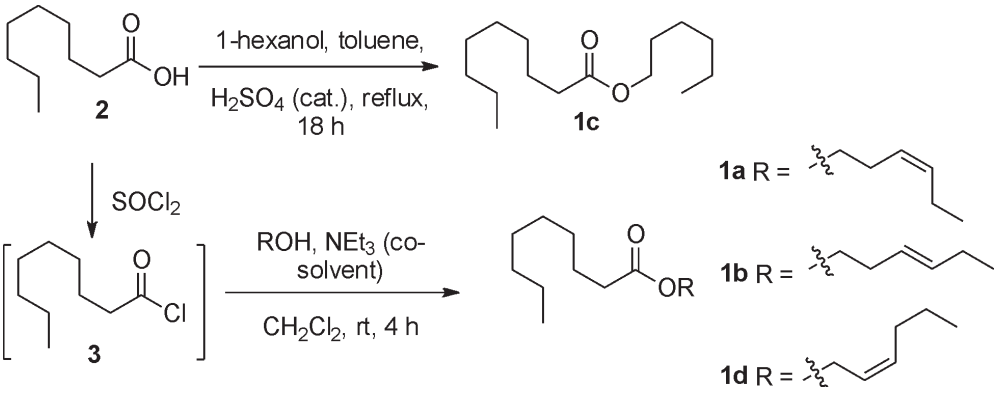


Figure 1 Structures of (*Z*)- **1a** and (*E*)-3-hexenyl nonanoate **1b**.



Scheme 1
Synthetic scheme for compounds **1a–d**.

Fortunately, the utilization of Et_3N as a co-solvent, rather than a 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 ^{1}H NMR and ^{13}C 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 ^{13}C NMR spectrum at ~δ 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[12]. 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[13–16]. 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*[4]. In comparison, the hexenyl-type compounds studied here are comparable in activity to the most active compounds found in the previous study[4]. Furthermore, perusal of the literature reveals that to the best of our knowledge, the antimicrobial activities for these related hexyl and hexenylnonanoates are reported here for the first time. It should also be pointed out that a previous study[17] 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[18].

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.

Experimental

General Experimental Details: ^{1}H NMR and ^{13}C NMR spectra were recorded either on a Bruker 300 or Bruker DRX 500 spectrometer at the frequency indicated. Infrared spectra were recorded on either a Bruker IFS 25 Fourier Transform spectrometer or on a Bruker Vector 22 Fourier Transform spectrometer. Mass spectra were recorded on a Kratos MS 9/50, VG 70E MS or VG 70

Table 1 Antimicrobial activity (MIC values in mg mL^{-1}) of the four hexyl nonanoate-essential oil type compounds synthesized.

Microbes tested	Synthesized compounds				Control [a]
	1a	1b	1c	1d	
E. coli ATCC 25922	1.56	1.56	1.56	1.56	0.03
P. aeruginosa ATCC 27858	0.85	0.45	0.49	1.19	0.25
M. cattarhalis ATCC 23246	1.56	1.56	2.61	2.08	0.63
S. aureus ATCC 25923	3.13	1.56	1.56	2.35	0.83
B. cereus ATCC 11778	1.56	1.56	1.56	1.56	0.80
E. faecalis ATCC 29212	3.10	1.21	3.50	1.06	1.25
C. albicans ATCC 10231	1.53	1.09	1.09	1.50	1.25
C. neoformans ATCC 90112	0.50	0.89	0.89	0.89	1.25
Mean MIC	1.73 ± 0.50	1.24 ± 0.50	1.66 ± 0.50	1.52 ± 0.50	

[a] 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_{254}) pre-coated plates using UV light. Machereye-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[19]. 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_2) unless specified.

Hexyl nonanoate 1c: Nonanoic acid **2** (1.00 g, 6.32 mmol), 1-hexanol (0.646 g, 6.32 mmol) and two drops of concentrated H_2SO_4 were dissolved in toluene (5 mL), after which the reaction mixture was heated at reflux overnight. The reaction mixture was then sequentially washed with concentrated Na_2CO_3 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 afford the product **1c** as a colorless oil (1.12 g, 73 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.05 (2H, t, J = 6.5, OCH_2), 2.28 (2H, t, J = 7.4, CH_2CO_2), 1.61 (4H, br m, $CH_2CH_2CO_2$ and OCH_2CH_2), 1.30 (16H, br m, 8 × CH_2), 0.88–0.80 (6H, br m, 2 × CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.6 (C=O), 64.0 (CH_2CO_2), 34.0 (OCH_2), 31.5 (CH_2), 31.1 (CH_2), 28.9 (CH_2), 28.83 (CH_2), 28.81 (CH_2), 28.3 (CH_2), 25.3 (CH_2), 24.7 (CH_2), 22.3 (CH_2), 22.2 (CH_2), 13.7 (CH_3), 13.6 (CH_3);HRMS: calculated for $C_{15}H_{28}O_2$ 243.2324 $(M + H)^+$, found 243.2328 $(M + H)^+$.

Nonanoyl chloride 3: Nonanoic acid **2** (1.00 g, 6.32 mmol) was dissolved in dry toluene (20 mL) and the solution heated to 85 °C. $SOCl_2$ (1.13 g, 9.48 mmol) was then added drop-wise over 1 h and the solution was stirred at 85 °C for a further 6 h. The toluene and excess $SOCl_2$ were then removed on a rotary evaporator, leaving nonanoyl chloride **3** as an amber-coloured oil, which was utilized in further reactions without purification. See reference 10 for more details.

General procedure for the synthesis of unsaturated nonanoate esters 1a, **1b** and **1d**: To a well stirred solution of nonanoyl chloride **3** (0.500 g, 2.84 mmol) in CH_2Cl_2 (15 mL), a solution of appropriate hexenol (0.290 g, 2.84 mmol, 1 equiv.) and NEt_3 (5 mL) in CH_2Cl_2 (10 mL) was added drop-wise. After the addition was complete (10 min.) the reaction mixture was stirred for a further 4 h at rt. The reaction mixture was subsequently extracted with water (3 × 10 mL) and the organic layer was dried (Na_2SO_4) and evaporated under vacuum. The pure product was obtained from the crude residue by silica gel column chromatography (eluent: 20/1 hexane/ethyl acetate) to give the nonanoate esters **1a**, **1b** and **1d** as pale colourless to yellow oils.

(*Z*)-3-Hexenyl nonanoate 1a: This ester was obtained as a light yellow liquid (0.491 g, 72 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.46–5.37 (m, 1H, CH=), 5.28–5.19 (m, 1H, CH=), 3.96 (br t, 2H, J = 7.0 Hz, OCH_2), 2.25 (q, 2H, J = 7.0 Hz, CH_2CH_3), 2.18 (t, 2H, J = 7.6 Hz, CH_2CH_2), 2.03–1.93 (m, 2H, CH_2CH_2), 1.49 (br quintet, 2H, J = 7.0 Hz, $CH_2CH_2CH_2$), 1.15–1.25 (br s, 10H, 5 × CH_2), 0.87 (t, 3H, J = 7.6 Hz, CH_3), 0.78 (br t, 3H, J = 7.1 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4 (C=O), 134.2 (C=C), 123.7 (C=C), 63.5 (CH_2), 34.1 (CH_2), 31.7 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 26.7 (CH_2), 24.9 (CH_2), 22.5 (CH_2), 20.5 (CH_2), 14.0 (CH_3), 13.9 (CH_3); HRMS: calculated for $C_{15}H_{28}O_2$ 241.2168 $(M + H)^+$, found 241.2169 $(M + H)^+$.

(*E*)-3-Hexenyl nonanoate 1b: This ester was obtained as a pale yellow liquid (0.505 g, 74 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.57–5.48 (m, 1H, CH=), 5.38–5.27 (m, 1H, CH=), 4.02 (t, 2H, J = 6.9 Hz, OCH_2), 2.31–2.23 (m, 4H, 2 × CH_2), 2.03–1.93 (m, 2H, CH_2), 1.54 (br quintet, 2H, J = 7.2 Hz, CH_2CH_3), 1.20–1.25 (br s, 10H, 5 × CH_2), 0.91 (t, 3H, J = 7.5 Hz, CH_3), 0.83 (br t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.6 (C=O), 134.9 (C=C), 124.1 (C=C), 63.8 (CH_2), 34.3 (CH_2), 32.0 (CH_2), 31.8 (CH_2), 29.2 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 25.5 (CH_2) 25.0 (CH_2), 22.6 (CH_2), 14.0 (CH_3), 13.6 (CH_3); HRMS: calculated for $C_{15}H_{28}O_2$ 241.2168 $(M + H)^+$, found 241.2169 $(M + H)^+$.

(*Z*)-2-Hexenyl nonanoate 1d: This ester was obtained as a light yellow liquid (0.511 g, 75 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.60–5.45 (m, 2H, CH=), 4.56 (d, 2H, J = 6.5 Hz, CH=), 2.22 (t, 2H, J = 7.7 Hz, CH_2CH_2), 2.08–1.98 (m, 2H, CH_2), 1.53 (br quintet, 2H, J = 7.0 Hz, $CH_2CH_2CH_2$), 1.42–1.32 (m, 2H, CH_2), 1.30–1.20 (br s, 10 H, 5 × CH_2), 0.89–0.81 (m, 6H, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4 (C=O), 134.8 (C=C), 123.7 (C=C), 60.0 (CH_2), 34.2 (CH_2), 31.7 (CH_2), 29.4 (CH_2), 29.1 (CH_2), 29.1 (CH_2), 29.0 (CH_2), 24.9 (CH_2), 22.5 (CH_2), 22.5 (CH_2), 13.9 (CH_3), 13.5 (CH_3); HRMS: calculated for $C_{15}H_{28}O_2$ 241.2168 $(M + H)^+$, found 241.2168 $(M + H)^+$.

Antimicrobial Activity

The MIC determination of the four synthesized hexyl nonanoate-type oil based compounds were undertaken to determine the antimicrobial activity using the MIC microtitre plate method[20]. The MIC assays were undertaken on Gram-negative test organisms *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27858 and *Morexella cattarhalis* ATCC 23246. Gram-positive test organisms tested were *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778 and *Enterococcus facealis* ATCC 29212. Two yeast strains (*Candida albicans* ATCC 10231 and *Cryptococcus neoformans* ATCC 90112) were included in the antimicrobial analysis. All bacterial cultures were subcultured from stock agar plates and then grown in Tryptone Soya 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^{-1}. Serial dilutions were performed and the cultures introduced, yielding an approximate inoculum size of 1 × 10^8 colony forming units (CFU) mL^{-1}. 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^{-1} were used as positive controls for bacteria and yeasts respectively. A 0.4 mg mL^{-1} *p*-iodonitrotetrazoliumviolet 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[21]. The yeasts were examined after 24 h. All MIC assays were undertaken at least in triplicate.

Supplementary Data

^{1}H and ^{13}C NMR spectra for all synthesized compounds are available online.

Acknowledgements

This work was supported by the National Research Foundation (NRF), Pretoria, The University of the Witwatersrand (University and Science Faculty Research Councils) and Stellenbosch University (Faculty and Departmental funding). Thanks go to the NRF for a postdoctoral Innovations Fellowship. We also gratefully acknowledge Mr. R. Mampa for the NMR spectroscopy service. Finally, Mr. B. Moolman, Mr. F. Hiten and Dr. M. Stander (Stellenbosch University) are acknowledged for providing MS spectroscopy services.

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