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Short Note

N-(((1*S*,5*R*)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl)-3-((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-ene/ane-2-carboxamido)-*N*,*N*-dimethylpropan-1-aminium Bromide

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Abstract: The synthesis of the title compounds was performed from (-)-cis-myrtanic and (-)-myrtenic acids. The compounds obtained were characterized using ^{1}H- and ^{13}C-NMR, IR, and high-resolution mass spectrometry. Despite the presence of quaternary ammonium moiety, both compounds had moderate antimicrobial activity with a MIC of 128 μg/mL on *S. aureus* and 512 μg/mL on *E. coli*. The antifungal activity was low on *Candida* isolates, while also comparable with conventional antimycotic (Fluconazole) on filamentous fungi. These data suggest that two bulky bicyclic terpene fragments apparently both increase lipophilicity and close the quaternary ammonium moiety located in the center of molecules and thus drastically decrease the antimicrobial potential of bipharmacophore.

Keywords: cis-myrtanic and myrtenic acids; myrtenol

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1. Introduction

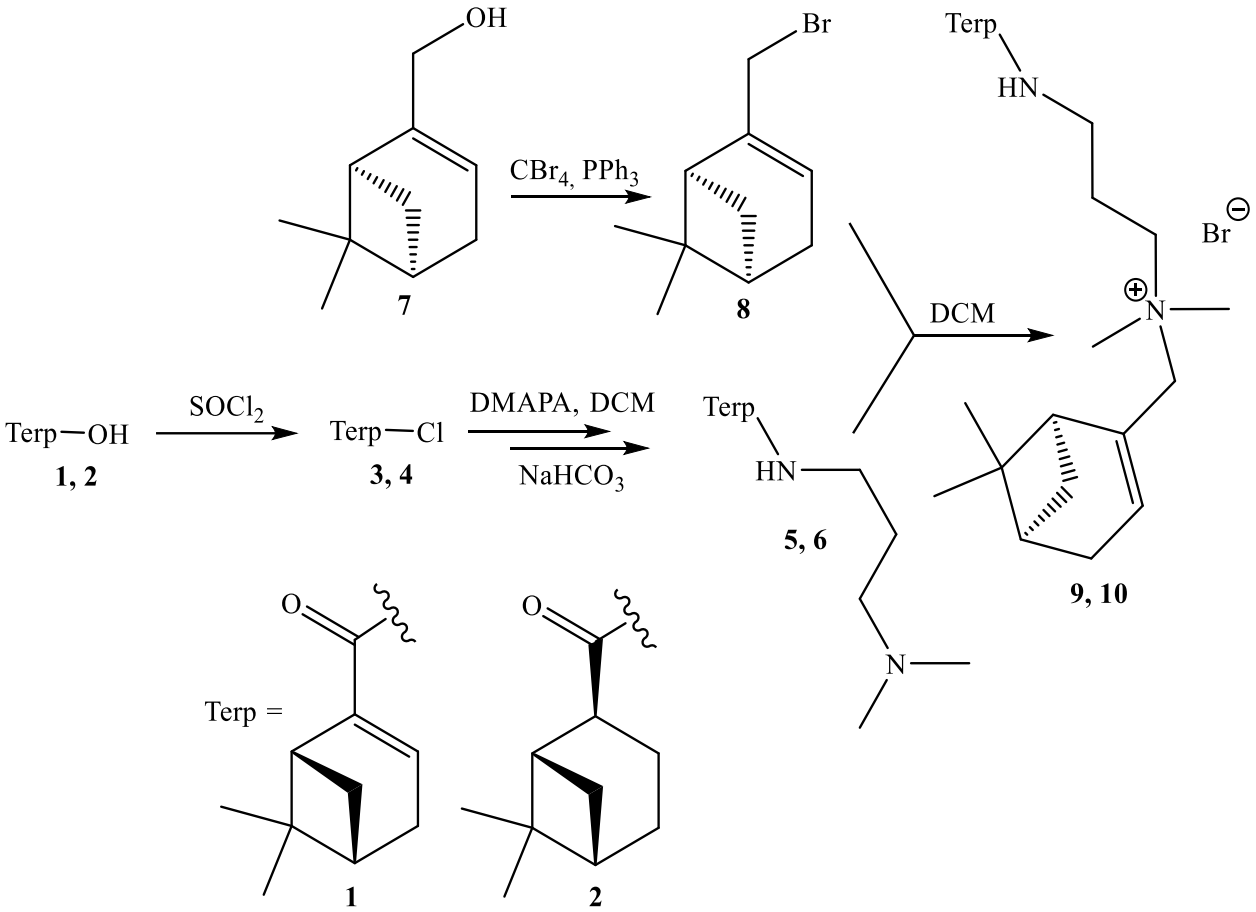
Owing to the large-scale industrial production of terpenes, their importance as a starting material in organic synthesis, and the general use of their derivatives in medicine, cosmetics, and perfumery, there exists continuous interest in this field of natural compound chemistry.

Nitrogen-containing derivatives of cis-myrtanic and myrtenic acids are known to have fungicidal activity against yeast and mycelial fungi, including phytopathogens [1,2]. We have recently shown that terpene alcohol (+)-myrtenol demonstrated a synergistic antimicrobial effect with amikacin, fluconazole, and benzalkonium chloride on several clinical isolates of *Staphylococcus aureus* and *Candida albicans*, while (-)-myrtenol increased the properties of amikacin and fluconazole to repress biofilm formation in half of the *S. aureus* and *C. albicans* isolates [3]. It is known that quaternary ammonium compounds, in particular, benzyldimethyl[3-(myristoylamino)propyl]ammonium chloride monohydrate, which is commercially available as Miramistin®, exhibit high antibacterial and fungicidal activity [4]. In this regard, the combination of several pharmacophore fragments (miramistin and terpenoid) in one molecule allowed the increase in the antimicrobial properties of obtained compounds in comparison with initial ones.

The present paper aims to synthesize promising antibacterial and antifungal agents along with combined fragments of miramistin, myrtenol, and myrtan(ene) acids.

2. Results and Discussion

The synthesis of terpenoids **9** and **10** was performed in three steps (Scheme 1).



Scheme 1. Synthesis of terpenoids **9**, **10**.

The first stage included the reaction of monoterpenoid acids **1**, **2** with thionyl chloride. Further, an equimolar amount of dimethylaminopropylamine (DMAPA) was added to the resulting acid chlorides **3**, **4**, followed by the treatment of the mixtures with $NaHCO_3$. As a result of the reactions of amides **5**, **6** with an excess of bromide **8**, followed by washing with hexane, target compounds **9**, **10** were obtained in 80% and 79% yields, respectively.

The structures of compounds **9** and **10** were confirmed by spectroscopic methods (^{1}H- and ^{13}C-NMR, IR, and high-resolution mass spectrometry, see Supplementary Materials).

Both compounds were tested for antibacterial and antifungal activities. Surprisingly, despite the presence of quaternary ammonium moiety, both **9** and **10** had moderate antimicrobial activity with a MIC of 128 μg/mL on *S. aureus* and 512 μg/mL on *E. coli* (Table 1). The antifungal activity was low on *Candida* isolates, while also comparable with conventional antimycotic (Fluconazole) on filamentous fungi. Apparently, this could be a consequence of the too-high lipophilicity of structures containing two bulky bicyclic terpene fragments, which, in turn, also close the quaternary ammonium moiety located in the center of molecules.

Table 1. Antibacterial and antifungal activities of **9** and **10**.

Strains	MIC, μg/mL				
	9	**10**	**Miramistin**	**Ciprofloxacin**	**Fluconazole**
S. aureus ATCC29213	128	128	4	0.25	ND
E. coli MG1655	512	512	8	2	ND
Candida albicans NCTC- 885-653	750	1500	>750	ND	46
Candida tropicalis Y-1513/784	750	1500	>1500	ND	94
Aspergillus niger F-1119	750	>1500	>1500	ND	>1500
Rhizopus nigricans F-1537/1722	>1500	>1500	>1500	ND	>1500
Fusarium oxysporum (clinical isolate)	>1500	>1500	>1500	ND	>1500
Trichophyton rubrum (clinical isolate)	>1500	>1500	>1500	ND	>1500

* ND–not determined.

3. Material and Methods

3.1. General

Dichloromethane (DCM), hexane, and thionyl chloride were reagent-grade and used without purification. DMAPA, tetrabromomethane, and triphenylphosphine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Myrtenic **1** and myrtanic **2** acids were obtained from corresponding alcohols according to [1,5.]. (+)-Myrtenol **7** was prepared by a known procedure [5]. Acyl chlorides **3**, **4** were obtained in accordance with [2]. The preparation of (+)-myrtenyl bromide **8** was performed according to [6].

The reaction progress and purity of compounds were monitored by TLC on Sorbfil PTLC-AF-A-UF plates (developer–anisaldehyde and sulfuric acid in ethanol 5:5:90).

The IR spectra were recorded on a Spectrum two PERKIN ELMER FT-IR spectrometer with attachment UATR (Single Reflection Diamond). Samples were applied to the attachment and pressed with a hand press until maximum absorption was obtained. For analyzing optical rotation "ADP 440" (B + S), a polarimeter was used.

HRMS mass spectra were obtained on a quadrupole time-of-flight (t, qTOF) AB Sciex Triple TOF 5600 mass spectrometer (AB SCIEX PTE. Ltd., Singapore) using a turbo-ion spray source (nebulizer gas nitrogen, a positive ionization polarity, needle voltage 5500 V). The recording of the spectra was performed in "TOF MS" mode with collision energy 10 eV, declustering potential 100 eV, and a resolution of more than 30 000 full-width half-maximum. Samples with the analyte concentration of 5 μmol/L were prepared by dissolving the test compounds in methanol (hypergrade for LC-MS, Merck, Darmstadt, Germany).

NMR spectra were recorded on a Bruker AVANCE-II-500 spectrometer with operating frequencies of 500 MHz (for ^{1}H) and 125 MHz (for ^{13}C) in a $CDCl_3$ solvent using standard Bruker pulse programs. The numbering of atoms in the description of the NMR spectra differs from the numbering in the names of compounds.

3.2. General Procedure for Synthesis of (1R,5S)-N-(3-(dimethylamino)propyl)-6,6-dimethylbicyclohept-2-ene/ane-2-carboxamide **5**, **6**

To a round bottom flask containing corresponding acid chloride (0,55 mmol) in DCM (25 mL), an equimolar amount of DMAPA was added and stirred for 30 min at room temperature. Then, $NaHCO_3$ was added until the gassing stopped. After filtration and evaporation in vacuo of the reaction mixture compounds, **5**, **6** as impure pale yellow amorphous solids were obtained with yields of 91% and 88%, respectively.

3.3. General Procedure for Synthesis of N-(((1S,5R)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)-methyl)-3-((1R,5S)-6,6-dimethylbicyclo[3.1.1]hept-2-ene/ane-2-carboxamido)-N,N-dimethylpropan-1-aminium bromide **9**, **10**

Compound **8** (0.6 mmol) was added to the DCM (20 mL) solution of amide (**5** or **6**) (0.47 mmol). The reaction mixture was left for 40 min at room temperature. After solvent evaporation, the in-vacuo product was washed with hexane, filtrated, and dried to provide target compounds **9, 10**.

N-(((1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl)-3-((1*R*,5*S*)-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-carboxamido)-*N*,*N*-dimethylpropan-1-aminium bromide (**9**). Whitish cream amorphous solid. Yield: 80%. IR, v, cm^{-1}: 3439 (NH), 1643, 1612 (C=C), 1557 (NH-C=O). $[\alpha]^{23}_D = -9.45°$ (c 0.811; MeOH).

NMR ^1H ($CDCl_3$) δ, ppm: 0.90 (s, 6H, C$\underline{H}_3$-1,2), 0.92 (s, 6H, C$\underline{H}_3$-3,4), 1.25 (dd, 2H, C$\underline{H}_2$-7′,17′, J = 26.0;8.9), 1.35 (d, 4H, C$\underline{H}_2$-7,17, J = 8.9), 2.14 (s, 2H, C$\underline{H}_2$-11), 2.20 (m, 4H, C$\underline{H}_2$-8,15), 2.45 (m, 2H, C$\underline{H}$-5,16), 2.73 (m, 2H, C$\underline{H}$-6,18), 3.18 (d, 6H, N^+(C$\underline{H}_3$)$_2$-20, J = 9.7), 3.47 (d, 2H, C$\underline{H}_2$-10, J = 6.5), 3.73 (t, 2H, C$\underline{H}_2$-12, J = 15.7;8.3), 4.00 (q, 2H, C$\underline{H}_2$-13, J = 102.7;12.0), 6.21 (s, 1H, C$\underline{H}$-14), 6.72 (s, 1H, C$\underline{H}$-9), 7.71 (s, 1H, N$\underline{H}$-19).

NMR $^{13}C\{^1H\}$ ($CDCl_3$) δ, ppm: 21.0 (C$\underline{H}_3$-1,2), 21.2 (C$\underline{H}_3$-3,4), 23.0 (C$\underline{H}_2$-21), 26.0 (C$\underline{H}_2$-13), 32.1 (C$\underline{H}_2$-14), 32.9 (C$\underline{H}_2$-12), 33.0 (C$\underline{H}_2$-11), 37.5 (C$\underline{-}$6), 38.0 (C$\underline{-}$5), 39.8 (C$\underline{H}_2$-20), 40.5 (C$\underline{H}$-8,10), 41.8 (C$\underline{H}$-7), 46.7 (C$\underline{H}$-9), 50.2 (N^+(C$\underline{H}_3$)$_2$-24), 63.0 (C$\underline{H}_2$-22), 60.9 (C$\underline{H}_2$-23), 130.8 (C$\underline{H}$-16), 136.0 (C$\underline{-}$18), 136.8 (C$\underline{H}$-15), 143.0 (C$\underline{-}$17), 168.2 (C$\underline{O}$-19).

HRMS: m/z $[M]^+$ calcd for $C_{25}H_{41}N_2O^+$: 385.3219; found: 385.3219.

N-(((1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl)-3-((1S,2S,5S)-6,6-dimethylbicyclo[3.1.1]heptane-2-carboxamido)-*N*,*N*-dimethylpropan-1-aminium bromide (**10**). Whitish cream amorphous solid. Yield: 79%. IR, v, cm^{-1}: 3401 (NH), 1633 (C=C), 1531 (NH-C=O). $[\alpha]^{23}_D = -12.83°$ (c 0.149; MeOH).

NMR ^1H ($CDCl_3$) δ, ppm: 0.86 (s, 6H, C$\underline{H}_3$-1,2), 0.87 (s, 6H, C$\underline{H}_3$-3,4), 1.25 (t, 2H, C$\underline{H}_2$-7′, J = 20.8;10.1), 1.30 (s, 3H, C$\underline{H}_2$-17,17′), 1.40 (s, 3H, C$\underline{H}_2$-7), 1.82 (s, 2H, C$\underline{H}_2$-11), 1.99 (s, 2H, C$\underline{H}_2$-8), 2.10, 2.15 (m, 4H, C$\underline{H}_2$-15,15′), 2.35 (m, 2H, C$\underline{H}_2$-8′), 2.35 (s, 1H, C$\underline{H}$-5), 2.52 (m, 2H, C$\underline{H}$-6,18), 2.80 (s, 1H, C$\underline{H}$-16), 2.96 (m, 1H, C$\underline{H}$-21), 3.18 (d, 6H, N^+(C$\underline{H}_3$)$_2$-20, J = 11.0), 3.49, 3.31 (m, 2H, C$\underline{H}_2$-9,9′), 3.72 (t, 2H, C$\underline{H}_2$-10, J = 14.7;7.7), 4.00 (q, 2H, C$\underline{H}_2$-12, J = 104.0;12.7), 5.36 (s, 2H, C$\underline{H}_2$-13), 6.17 (s, 1H, C$\underline{H}$-14), 7.35 (s, 1H, N$\underline{H}$-19).

NMR $^{13}C\{^1H\}$ ($CDCl_3$) δ, ppm: 21.5 (C$\underline{H}_2$-21), 22.0 (C$\underline{H}_3$-1,2), 22.8 (C$\underline{H}_3$-3,4), 26.6 (C$\underline{H}_2$-13,15), 30.3 (C$\underline{H}_2$-11), 31.5 (C$\underline{H}_2$-12,14), 36.6 (C$\underline{-}$6), 38.5 (C$\underline{H}_2$-20), 39.5 (C$\underline{H}$-7), 40.5 (C$\underline{H}$-17), 43.8 (C$\underline{-}$5), 45.0 (C$\underline{H}$-10), 46.0 (C$\underline{H}$-9), 49.9 (N^+(C$\underline{H}_3$)$_2$-24), 49.9 (C$\underline{H}$-8), 63.0 (C$\underline{H}_2$-22), 69.8 (C$\underline{H}_2$-23), 136.0 (C$\underline{H}$-16), 136.5 (C$\underline{-}$18), 173.6 (C$\underline{O}$-19).

HRMS: m/z $[M]^+$ calcd for $C_{25}H_{43}N_2O^+$: 387.3375; found: 387.3375.

3.4. Antibacterial and Antifungal Activities

Staphylococcus aureus ATCC® 29213™ and *Escherichia coli* MG1655 were used as test organisms and grown on a Mueller–Hinton broth (MH, BD Difco) during experiments. The fungal strains *Candida albicans* NCTC-885-653, *Candida tropicalis* Y-1513/784, *Aspergillus niger* F-1119, and *Rhizopus nigricans* F-1537/1722 were obtained from an all-Russian collection of microorganisms, Moscow, Russia. *Trichophyton rubrum* C1 (clinical isolate from skin) and *Fusarium oxysporum* C2611-17 (clinical isolate from skin) were obtained from a collection of clinical isolates at the Kazan Institute of Microbiology and Epidemiology (Kazan, Russia). Fungi were identified by morphological characteristics and by using an AuxaColor 2 Colorimetric sugar-assimilation yeast-identification kit (Bio-Rad, Hercules, CA, USA). All strains were grown in RPMI or Sabouraud broth.

Minimum inhibitory concentrations (MICs) of the compounds were determined using the broth microdilution method in 96-well plates (Eppendorf) in MH broth (for bacteria) or in tubes with RPMI (for yeast fungi) and Sabouraud broth (for filamentous fungi) in accordance with EUCAST guidelines for antimicrobial susceptibility testing [7]. Bacteria were incubated at 37 °C for 24 h. Yeasts and filamentous fungi were incubated at 30 °C

for 2 and 5 days, respectively. The MIC was defined as the lowest concentration of the compound at which no visible growth could be seen.

Supplementary Materials: The following supporting information can be downloaded online. Compound **9**: Copies of ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$-d_1; Copies of HRMS spectra; Copy of IR spectrum. Compound **10**: Copies of ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$-d_1; Copies of HRMS spectra; Copy of IR spectrum. Raw NMR files.

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