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The first total synthesis of (±)-4-methoxydecanoic acid: a novel antifungal fatty acid

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ABSTRACT

The hitherto unknown (±)-4-methoxydecanoic acid was synthesized in six steps and in 25% overall yield starting from commercially available 4-penten-1-ol. The title compound demonstrated 17-fold higher antifungal activity (MIC = 1.5 mM) against *Candida albicans* ATCC 60193 and *Cryptococcus neoformans* ATCC 66031 when compared to unsubstituted *n*-decanoic acid. Our results demonstrate that mid-chain methoxylation appears to be a viable strategy for increasing the fungitoxicity of fatty acids.

The (±)-4-hydroxydecanoic acid is an elusive fatty acid to isolate from either a natural or synthetic source because of its tendency to easily cyclize to the well-known γ-decalactone.[1] In fact, the spectral data for 4-hydroxydecanoic acid were first reported in 1996 by G. Feron and collaborators, attesting to the difficulty in isolating the pure hydroxylated fatty acid from the γ-decalactone.[1] The γ-decalactone is an important compound for the aroma and food industries since the compound imparts a peach–apricot flavor to foods[2,3] and is also responsible for the fruity flower odor of gardenia perfumes.[1] The γ-decalactone has also been used in <5 ppm concentrations in a selected number of cigarette brands.[4]

There are just a few scattered reports on the toxicity of (±)-4-hydroxydecanoic acid but it has been reported that the presence of a hydroxyl group in the acyl chain greatly decreases toxicity. However, the γ-decalactone has been reported to have higher antibacterial activity than (±)-4-hydroxydecanoic acid since it inhibits the growth of some bacteria such as *Bacillus subtilis* and some fungi such as *Fusarium oxysporum* and *Trichothecium roseum* at concentrations between 0.1 and 0.7 mM.[1] It should also be noted here that the 3-(*R*)-hydroxydecanoic acid has been isolated from *Lactobacillus plantarum* and displayed considerable antifungal activity (MIC = 10–100 μg/mL) against different molds and yeasts.[5]

We have previously shown that α-methoxylation increases the antifungal activity of fatty acids,[6,7] but nothing is known as to the effect of mid-chain methoxylation on the antifungal activity of these compounds. We envisioned that a compound such as the (±)-4-methoxydecanoic acid (**1**) could be a good starting template to study such an effect. In addition, we were expecting this methoxylated fatty acid to be more fungitoxic than the corresponding compounds, unsubstituted *n*-decanoic acid (capric acid) or isolated (±)-4-hydroxydecanoic acid. The choice of a 10-carbon chain length for this study is justified by the fact that capric acid kills *Candida albicans* at 10 mM by the postulated mechanism of the fungal plasma membrane disintegration while other saturated fatty acids are not effective.[8]

Furthermore, unlike (±)-4-hydroxydecanoic acid, (±)-4-methoxydecanoic acid (**1**) cannot be cyclized to the corresponding γ-lactone thus allowing the facile study of its fungitoxicity. This investigation was also designed to determine whether mid-chain methoxylation is a viable substitution to α-methoxylation for enhancing the antifungal activity of fatty acids such as capric acid. Previous studies by our group with the (±)-2-methoxydecanoic acid have shown that the 2-OMe-10:0 acid is only 1.5-fold more fungitoxic than capric acid (10:0) against *C. albicans* (ATCC 14053). However, against *Cryptococcus neoformans* (ATCC 66031) the 2-OMe-10:0 acid was not more antifungal than capric acid.[6]

Our six-step synthesis started with the protection of the primary alcohol of commercially available 4-penten-1-ol (**2**) with dihydropyran (DHP) and catalytic amounts of *p*-toluenesulfonic acid (PTSA) in $CHCl_3$ at rt for 5 h to afford the 1-[(tetrahydropyran-2-yl)oxy]-2-pentene in an 88% yield (Scheme 1). The double bond was effectively epoxidized in the presence of magnesium monoperoxyphthalate (MMPP) in EtOH as solvent for 48 h, to yield

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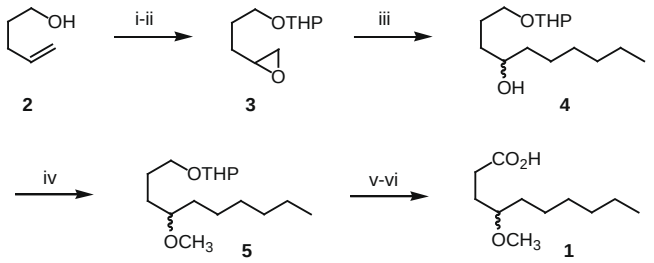

Scheme 1. Reagents and conditions: (i) DHP/PTSA,$CHCl_3$, rt, 5 h, 88%; (ii) MMPP/EtOH, 48 h, 89%; (iii) $CH_3(CH_2)_3CH_2MgBr$, Cu(I)/THF, −78 °C to −30 °C, 81%; (iv) NaH/CH_3I, THF, 0 °C to rt, 2 h, 88%; (v) PTSA, $CHCl_3$, 45 °C, 2 h, 73%; (vi) PDC/DMF, 24 h, 63%.

the 4,5-epoxy-1-[(tetrahydropyran-2-yl)oxy]pentane (**3**) in 89% yield after purification of the crude product using silica gel column chromatography (60–200 mesh) and eluting with hexane/diethyl ether (8:2). MMPP turned out to be more efficient than the classical *m*-chloroperoxybenzoic acid (*m*-CPBA) in epoxidizing these alkenes, since the latter reagent only afforded moderate to low yields even after long reaction times. The THP-protected epoxide **3** was then opened with 1-pentylmagnesium bromide assisted by catalytic amounts of copper(I) chloride in THF at a reaction temperature range of −78 °C to −30 °C, which afforded the desired 4-hydroxy-1-[(tetrahydropyran-2-yl)oxy]decane (**4**) in an 81% yield after silica gel (60–200 mesh) column chromatographic purification. The free hydroxyl group in **4** was then readily methylated with methyl iodide in the presence of sodium hydride in THF, which afforded the 4-methoxy-1-[(tetrahydropyran-2-yl)oxy]decane (**5**) in an 88% yield. Deprotection of the primary alcohol was effectively accomplished with PTSA in $CHCl_3$ at 45 °C for 2 h, which afforded the (±)-4-methoxydecan-1-ol in a 73% yield (Scheme 1). Final oxidation to the acid was accomplished by reaction of the alcohol with pyridinium dichromate (PDC) in DMF for 24 h, which resulted in a 63% yield of **1**.[9] The overall yield for this six-step synthesis was 25%.

The most significant absorption in the NMR spectrum of **1** was observed for the carbons and hydrogens bearing the methoxy functionality. For example, the methoxy protons resonated at δ 3.32 ppm and the methoxy carbon was observed at δ 56.5 ppm, while the methine hydrogen (*CH*OCH_3) resonated at δ 3.20 ppm and the methine carbon (*C*$HOCH_3$) at δ 79.9 ppm. These ^{1}H NMR and ^{13}C NMR displacements seem to be characteristic for saturated mid-chain methoxylated fatty acids and useful as a future reference for other similar analogs. It is also interesting to mention that in the 70 eV electron impact (EI) mass spectrum of **1** the typical McLafferty rearrangement of fatty acids at m/z = 60 was greatly reduced (1% relative abundance) by the presence of the methoxy functionality at C-4. In the mass spectrum of **1** the α-fragmentation at both sides of the methoxylated carbon predominated, but the fragments containing the carboxyl end (at m/z = 117 corresponding to $C_5H_9O_3^+$ and at m/z = 85 corresponding to $C_4H_5O_2^+$) were the most abundant.

The antifungal activity of **1** was determined against a fluconazole-resistant strain of *C. albicans* (ATCC 60193) and against *C. neoformans* (ATCC 66031) following our previously published protocol (Table 1).[6,7] *n*-Decanoic acid was also tested as a control. As can be seen from the data given in Table 1 the (±)-4-methoxydecanoic acid (**1**) was approximately 17-fold more antifungal against both fungal strains (MIC = 1457 μM) when compared to *n*-decanoic acid (MIC = 25,478 μM). Therefore, the antifungal results clearly show

Table 1
Antifungal activity (MIC values, μM) against *Candida albicans* (SDB) and *Cryptococcus neoformans* (SDB) at 35–37 °C after 24–48 h[a]

Compound	*C. albicans* ATCC 60193	*C. neoformans* ATCC 66031
(±)-4-Methoxydecanoic acid (**1**)	1457	1457
Decanoic acid	25,478	25,478
Fluconazole	>500	<0.9
Amphotericin B	<0.3	<0.3
DMSO	>5000	>5000

[a] The results are the average of three separate experiments. The upper limit of the standard error of the mean (SEM) was ±10%.

that C-4 methoxylation increased the antifungal activity of the parent *n*-decanoic acid. In fact, for *n*-decanoic acid C-4 methoxy substitution seems to be more effective than C-2 methoxy substitution in increasing the antifungal activity of *n*-decanoic acid.[6]

As to the reasons for the better antifungal activity of **1** over that of *n*-decanoic acid we can only speculate at this stage and more mechanistic studies are required. The addition of the C-4 methoxy functionality possibly makes the fatty acid more soluble than unsubstituted *n*-decanoic acid thus facilitating its interaction with the target sites. In addition, based on the previously published antifungal mechanism of decanoic acid[8] we can also speculate that acid **1** seems to be able to more efficiently disrupt the fungal membranes due to the mid-chain methoxy substitution. Moreover, the title compound **1** may also inhibit fatty acid biosynthesis within the fungi interacting with some key enzymes. In summary, our results clearly demonstrate that mid-chain methoxylated fatty acids are valuable compounds that can be optimized for developing more potent antifungal agents and thus merit further scrutiny in the search for better antifungal analogs.

Acknowledgments

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9. *Spectral data for the (±)-4-methoxydecanoic acid* (**1**): Transparent oil; IR (neat): ν_{max} 3500–2500, 2928, 1712, 1462, 1377, 1282, 1096, 936 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.32 (s, 3H, –OCH_3), 3.20 (m, 1H, H-4), 2.43 (t, J = 7.5 Hz, 2H, H-2), 1.97–1.67 (m, 2H, H-3), 1.52 (m, 2H, H-5), 1.27 (m, 8H, –CH_2–), 0.87 (t, J = 6.7 Hz, 3H, –CH_3); ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 179.6, 79.9, 56.5, 33.1, 31.8, 29.9, 29.4, 28.2, 25.1, 22.6, 14.0; GC–MS (70 eV) m/z (rel. intensity) 201(M^+−1, 0.1), 187(2), 170(1), 169(2), 129(18), 116(54), 97(16), 85(100), 71(14), 60(1), 57(7), 55(24); HRMS (APCI): calcd for $C_{11}H_{23}O_3$ $(M+H)^+$ 203.1642, found: 203.1639.