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SHORT COMMUNICATION

A Straightforward Method for Synthesizing Bioactive Resorcinolic Lipid Analogues

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Abstract:

Resorcinolic lipids, a class of bioactive amphiphilic molecules found widely in nature, hold potential for a variety of biological and industrial applications. This report describes the synthesis of three bioactive structural analogues of resorcinolic lipids, obtained by subjecting ethyl (*E*)-2-undecenoate and ethyl acetoacetate to a Michael reaction in the presence of sodium ethoxide to generate a Michael adduct, followed by cyclization in the reaction medium. Ethyl 2-octyl-4,6-dioxocyclohexanecarboxylate (**7**) was thus produced with a 60% yield. To perform an aromatization step, **7** was subsequently treated with I_2 in methanol under reflux, producing a combined 80% yield of 2,4-dimethoxy-6-octyl-ethyl benzoate (**1**) and 2-hydroxy-4-methoxy-6-octyl-ethyl benzoate (**2**) at a 7:3 ratio, respectively. 2-Hydroxy-4-methoxy-6-octyl-benzoic acid was obtained with a 60% yield by treating **1** with $BBr_3/CHCl_3$. The structures of the synthesized compounds and intermediates were elucidated by ^1H and ^{13}C NMR spectroscopy, employing two-dimensional techniques (HSQC and HMBC).

Keywords: resorcinolic lipids; cytosporones; phomopsin C; cladosporin

1. Introduction

Resorcinolic lipids are abundant in nature, exhibiting notable biological properties [1]. Octaketidic cytosporones isolated from endophytic fungi stand out among these compounds, exhibiting important biological properties, including fungicidal, allelopathic, bactericidal, and cytotoxic activities [2]. Cytosporone A (Figure 1), the earliest to be isolated, was obtained from *Phoma* sp., a phytopathogenic fungus [3]. In 2000, Clardy et al. isolated both cytosporones A and B from the two endophytic fungi *Cytospora* sp. and *Diaporthe* sp. independently [4]. Cytosporone B proved cytotoxic against a number of tumor cell strains [5]. Notably, cytosporone B interacts directly with the binding domain of nuclear orphan receptor 77 (Nur77) [6, 7], a feature that drew considerable attention, making this lipid class widely known. Phomopsin C, derived from the endophytic mangrove fungus *Phomopsin* sp. [8], was isolated as the methoxylated form of cytosporone B. This compound, like cytosporone B, proved active against H460 and LCCAP cancer cells. Cladosporin (Figure 1), another noteworthy octaketide, is synthesized by several fungal genera, including *Cladosporium*, *Chaetomium*, *Penicillium*, *Eurotium*, and *Aspergillus* [9]. Cladosporin exhibits useful antifungal, antibiotic, and plant growth inhibitory properties, in addition to eliciting anti-inflammatory responses in mouse lung tissue [10] and acting as a potent antimalarial agent [11].

Several natural resorcinolic lipids and analogues have been synthesized, including cytosporones [2, 12, 13], cladosporin, and their respective analogues [9]. AMS35AA, AMS35BB, and AMS049 (Figure 1), synthetic analogues of cytosporones and cladosporin, have been

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recently shown to potentiate the mutagenic effect of cyclophosphamide and to induce apoptosis in mice. Neither genotoxic nor mutagenic, these analogues do not interfere with biochemical parameters. These traits suggest potential therapeutic utility as chemotherapeutic adjuvants in cancer treatment [14-17]. 2-[2,3,4-Trimethoxy-6-(1-octanoyl)phenyl]acetate (TMPA) (Figure 1), another synthetic analogue of cytosporones and cladosporin has proven a powerful antidiabetic agent [7].

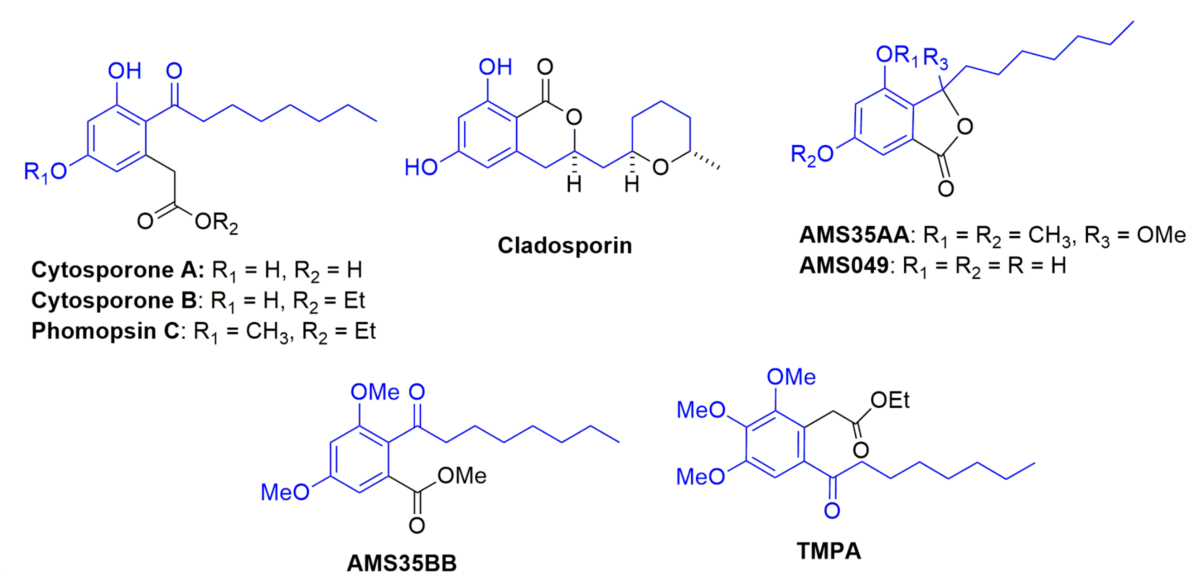
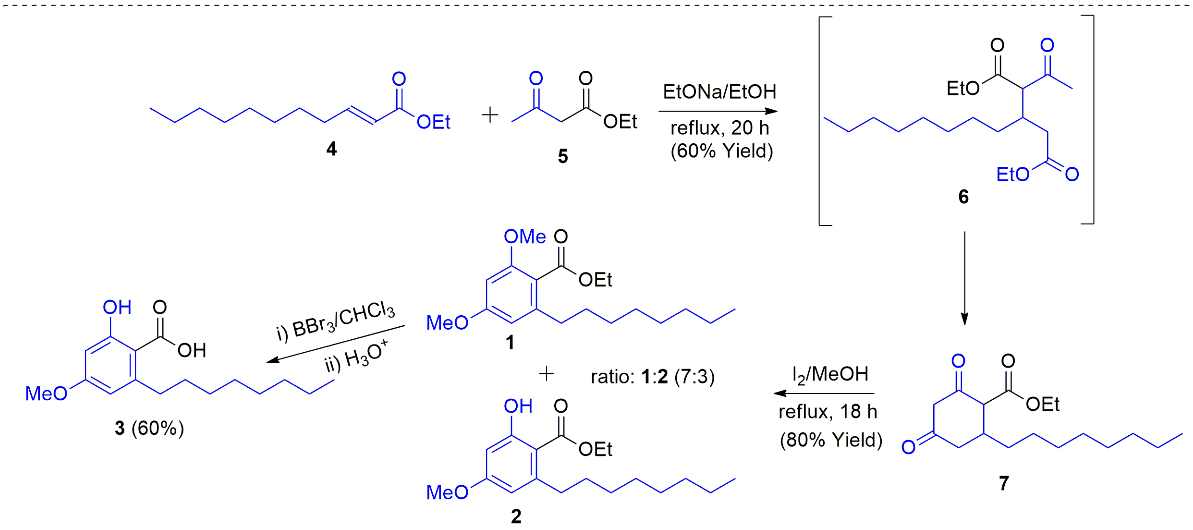


Figure 1. Examples of natural resorcinolic lipids and synthetic analogues.

As part of our ongoing interest in the synthesis of resorcinolic lipids for biological use, we report the preparation of three novel analogues of the octaketides cytosporone A, cytosporone B, phomopsin C, and cladosporin, obtained by running a classical Michael addition reaction followed by a crucial cycling reaction.

2. Results and Discussion

Scheme 1 depicts the sequence of reactions selected for synthesizing resorcinolic lipids **1-3**. The α,β-unsaturated ester **4** was prepared in three steps from 1-nonanol, with an overall yield of 48% (see Supplementary Material).



Scheme 1. Synthesis of resorcinolic lipids **1-3**.

Ester **4** was treated with ethyl acetoacetate (**5**) and sodium ethoxide in ethanol, producing compound **7** with a 60% yield. Compound **7** was then treated with iodine in methanol under reflux, giving a combined 80% yield of aromatic compounds **1** and **2** at a 7:3 ratio, respectively. Compound **3** was obtained with a 60% yield after treatment of **1** with BBr_3 in chloroform. All compounds were confirmed by ^1H and ^{13}C NMR spectrometry analysis. Methoxyl positions in the aromatic rings of **2** and **3** were confirmed using two-dimensional NMR techniques (HSQC and HMBC).

Resorcinolic acid **3** is homologous to olivetolic acid, as well as to depsides isolated from lichens [18-21] and to defense secretions of *Crematogaster* sp. ants [22].

3. Material and Methods

General methods

All the starting materials were obtained commercially and used as purchased. TLC was performed on glass plates coated with silica gel 60 F254. The plates were visualized using UV radiation (254 nm), iodine, or both. Column chromatography was performed on silica gel (60 × 120 mesh) in a glass column. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 apparatus using tetramethylsilane (TMS) as the internal standard. Chemical shifts (δ) were recorded in ppm with respect to TMS, with coupling constants (J) given in hertz.

Experimental procedure

*Ethyl 2-octyl-4,6-dioxocyclohexanecarboxylate (**7**)*: Under a nitrogen atmosphere, metallic sodium (0.696 g, 32.4 mmol) and superdry ethanol (63 mL) were employed to prepare an ethanol solution of sodium ethoxide, to which, under the same atmosphere, ethyl acetoacetate (4.21 g, 37.23 mmol) was added. The mixture was refluxed for 30 min. Ethyl (*E*)-ethyl undec-2-enoate (**4**) was then added (6.9 g, 32.4 mmol) via an addition funnel over a 30 min period, and the resulting mixture was refluxed for another 20 h. At this point, the reaction medium was cooled to 8 °C and 3 M sulfuric acid added until pH 7 was attained. The sodium sulfate precipitate was removed by filtration and the filtrate treated with 3 M aqueous HCl solution until reaching pH 4. The product was extracted with $CHCl_3$ (3 × 20 mL). The organic phases were combined and dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford ethyl 2-octyl-4,6-dioxo-1-cyclohexanecarboxylate (**7**) and isomers, with a 60% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 0.83 (m), 1.21 (m), 2.42-2.50 (m), 3.09 (m), 3.41 (s), 3.67 (m), 4.19 (m), 12.26 (s, 1H).

*Ethyl 2,4-dimethoxy-6-octylbenzoate (**1**) and ethyl 2-hydroxy-4-methoxy-6-octylbenzoate (**2**):* A solution of compound **7** (4.72 g, 15.9 mmol) and molecular iodine (8.09 g, 31.8 mmol) in methanol (70 mL) was heated to reflux for 20 h. The reaction mixture was diluted with dichloromethane and washed with aqueous $NaHSO_3$ and brine. After solvent removal under reduced pressure, the residue was purified by silica gel column chromatography using hexane:ethyl acetate (3:1) as the eluent, yielding a combined 80% of compounds **1** and **2** at a 7:3 ratio, respectively.

Compound 1: Yellowish oil. ^1H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, 3 H, J = 6.8 Hz), 1.19-1.32 (m, 10H), 1.35 (t, 3H, J = 7.1 Hz), 1.56 (m, 2H), 2.53 (m, 2H), 3.78 (s, 3H), 3.79 (s, 3H), 4.35 (q, 2H, J = 7.1 Hz), 6.30 (d, 1H, J = 1.9 Hz), 6.31 (dt, 1H, J = 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_3), 143 (CH_3), 22.7 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.6 (CH_2), 31.3 (CH_2), 31.9 (CH_2), 33.9 (CH_2), 55.3 (CH_2), 55.8 (CH_2), 60.9 (CH_2), 96.2 (CH), 105.7 (CH), 116.7 (C), 149.9 (C), 157.9 (C), 161.3 (C), 168.4 (C).

Compound 2: Yellowish oil. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.9 Hz), 1.17-1.34 (m, 12H), 1.40 (t, 3H, J = 7.1 Hz), 1.53 (m, 2H), 2.85 (m, 2H), 3.79 (s, 3H), 4.38 (q, 2H, J = 7.1 Hz), 6.27 (d, 1H, J = 2.64 Hz), 6.31 (d, 1H, J = 2.64 Hz), 11.83 (s 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_3), 14.1 (CH_3), 22.5 (CH_2), 29.3 (CH_2), 29.6 (CH_2), 29.9 (CH_2), 31.9 (CH_2), 32.0 (CH_2), 37.1 (CH_2), 55.2 (CH_2), 61.2 (CH_2), 98.8 (CH), 104.7 (C), 110.6 (CH), 148.1 (C), 163.8 (C), 165.4 (C), 171.6 (C).

*2-Hydroxy-4-methoxy-6-octylbenzoic acid (**3**):* Compound **1** was dissolved in 20 mL of anhydrous chloroform (100 mg, 0.31 mmol) in an ice and NaCl bath under magnetic stirring and a nitrogen atmosphere. After 30 min stirring, 1.2 mL (6.8 mmol) of boron tribromide was added and the reaction medium was stirred for a further 20 h at room temperature. A 1 mL volume of distilled water was then added, and the product extracted with chloroform (3 × 10 mL). The organic phase was dried with $MgSO_4$ and concentrated in a rotary evaporator. The product was purified by silica gel column chromatography using hexane:ethyl acetate mixtures as eluents, starting with pure hexane, then with gradients of 10%, 20%, 30%, 50%, and 70% ethyl acetate in hexane. Yield of a brown solid was 60%. ^1H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 6.9 Hz), 1.11-1,41 (m, 12H), 1.53 (m, 2 H), 2.91 (m, 2 H), 3.81 (s, 3H), 6.33 (d, 1H, J = 2.56 Hz), 6.34 (d, 1H, J = 2.56 Hz), 11.59 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1 (CH_3), 22.7 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.8 (CH_2), 31.7 (CH_2), 31.8 (CH_2), 36.7 (CH_2), 55.4 (CH_2), 98.8 (CH), 103.2 (C), 111.6 (CH), 149.6 (C), 164.9 (C), 166.6 (C), 175.2 (C).

4. Conclusions

The protocol described for preparation of bioactive resorcinolic lipid analogues proved to be simple and efficient and is expected to help expand the repertory of this class of small molecules.

Supporting Information

Supplementary data (NMR spectra and procedure to prepare compound **4**) are available at http://www.orbital.ufms.br/index.php/Chemistry/article/downloadSuppFile/237/407 as a PDF file.

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