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Synthesis and antimicrobial activity of geranyloxy- and farnesyloxy-acetophenone derivatives against oral pathogens

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ABSTRACT

The compounds 2′,6′-dihydroxy-4′-geranyloxyacetophenone (**1**) and 2′,6′-dihydroxy-4′-farnesyloxy-acetophenone (**2**) are oxyprenylated secondary metabolites extracted from plants belonging to the Rutaceae family. In this study, **1** and **2** were synthesized and tested for their antimicrobial activity toward major oral pathogens. Compounds **1** and **2** were synthesized by selective prenylation of 2,4,6-trihydroxyacetophenone at the 4′ position with geranyl and farnesyl bromide, respectively. Compound **1** showed stronger antimicrobial activity than **2** against major oral pathogens, including Gram positive bacteria (*Streptococcus mutans, Streptococcus sobrinus*), Gram negative bacteria (*Prevotella intermedia, Porphyromonas gingivalis*) and *Candida albicans*. Evidences were obtained that the mode of action of **1** and **2** may be related to their iron-chelating property. This study suggests that **1** and **2** may represent potential natural molecules for the prevention/treatment of common oral infections, including dental caries, periodontal disease, and candidiasis.

1. Introduction

Oxyprenylated secondary metabolites have been considered for decades merely as biosynthetic intermediates of C-prenylated compounds and only in the last ten years have been characterized as phytochemicals exerting valuable biological activities. Oxyprenylated acetophenones represent a rare class of natural products in which a terpenyl chain, more frequently a geranyl (C_{10}) or a farnesyl (C_{15}) one, is attached to the aromatic ring by a carbon–carbon or a carbon–oxygen link. In this context the compounds 2′,6′-dihydroxy-4′-geranyloxyacetophenone (**1**) and 2′,6′-dihydroxy-4′-farnesyloxyacetophenone (**2**) have been previously extracted from the fruits of *Evodia merrillii* Kanehira & Sasaki [1] from the aerial parts of *Boronia ramosa* Benth. [2], from the fruits and aerial parts of *Melicope obscura* (Coode) T.G. Hartley, *Melicope obtusifolia* sp. *obtusifolia* var. *arborea* (Coode) T.G. Hartley [3], and *Melicope semecarpifolia* (Merr.) T. G. Hartley [4], all plants belonging to the Rutaceae family. Since these secondary metabolites are found in relatively low amounts in nature, the development of an efficient chemical synthesis procedure is required to better characterize their pharmacological properties.

Oral diseases, including dental caries, periodontal disease and candidiasis, are major public health problems because of their high prevalence and incidence in all regions of the world. The etiology of both dental caries and periodontal disease has long been known to be related to colonization of oral sites by specific bacteria. *Streptococcus mutans* and *Streptococcus sobrinus*, the primary acidogenic components of dental biofilm, are able to metabolize exogenous dietary carbohydrates and to generate lactic acid, resulting in demineralization of tooth enamel [5]. As dental biofilm matures, colonization shifts toward facultative and anaerobic bacterial species, leading to supra-gingival plaque accumulation and gingival inflammation which are the first clinical signs of periodontal disease. While gingivitis is a mild and reversible form of periodontal disease, periodontitis causes permanent damages to tooth-supporting tissues and may lead to tooth loss [6]. A group of about ten bacterial species, mostly Gram negative anaerobic bacteria, have been associated to

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periodontal disease [7]. Oral candidiasis is an opportunistic infection of the oral cavity caused by an overgrowth of *Candida* species, the most common being *Candida albicans* [8]. The most prevalent forms of oral candidiasis are pseudomembranous candidiasis (also called thrush) and erythematous candidiasis, which includes denture stomatitis [8].

Controlling the above pathogens is the primary action for maintaining oral health. Active compounds endowed with a capacity to exert antimicrobial activity toward oral pathogens have received considerable attention as they may represent potential new therapeutic agents for the prevention/treatment of oral infections. In this study, we synthesized compounds **1** and **2** and evaluated the antimicrobial properties toward major pathogens associated with dental caries, periodontal disease, and candidiasis.

2. Materials and methods

2.1. Chemical synthesis

Compounds **1** and **2** were synthesized by selective prenylation of 2,4,6-trihydroxyacetophenone at the 4′ position with geranyl and farnesyl bromide, respectively, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the base in acetone. Briefly, to a solution of 2,4,6-trihydroxyacetophenone (1.0 mM) in acetone (5.0 ml), DBU (1.0 mM) and geranyl/farnesyl bromide (1.02 mM) were added in sequence and the resulting mixture was left to react for 2 h at room temperature. The solution was then diluted with water (50 ml) and extracted with *n*-hexane (3×10 ml). The collected organic phases were dried over anhydrous Na_2SO_4 and evaporated to dryness. The desired compound was obtained by crystallization (*n*-hexane). The resulting compounds were characterized by ^{1}H-nuclear magnetic resonance (NMR), ^{13}C NMR, infrared (IR) and mass spectroscopy, as routinely performed in our laboratory.

2.2. Microorganisms

Aggregatibacter actinomycetemcomitans ATCC 29522 (serotype 2), *Porphyromonas gingivalis* ATCC 33277, *Prevotella intermedia* 5W2, *S. mutans* ATCC 25175, *S. sobrinus* ATCC 33478, and *C. albicans* ATCC 28366 were used. Bacteria were grown in Todd–Hewitt broth (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 0.001% hemin and 0.0001% vitamin K (THB-HK). *A. actinomycetemcomitans, P. gingivalis,* and *P. intermedia* were incubated under anaerobic conditions ($N_2-H_2-CO_2$/80:10:10), while *S. mutans* and *S. sobrinus* were grown aerobically, all at 37 °C. *C. albicans* was cultivated in Yeast Nitrogen Base (YNB; BBL Microbiology Systems) medium supplemented with 0.5% glucose and incubated aerobically at 37 °C.

2.3. Determination of minimal inhibitory concentrations and minimal microbicidal concentrations

Cultures of microorganisms were diluted in fresh broth medium to obtain an optical density at 660 nm (OD_{660}) of 0.2. Samples (100 µl) were added to the wells of a 96-well tissue culture plate containing 100 µl of serial dilutions (100 µg ml^{-1} to 6.25 µg ml^{-1}) of **1** or **2**. Control wells with no substance but with substance vehicle were also inoculated. Penicillin G (Sigma-Aldrich Canada, Oakville, ON, CANADA) and Nystatin (EMD Biosciences Inc., San Diego, CA, USA) were used as reference controls for growth inhibition. After incubation for 48 h at 37 °C (aerobic or anaerobic according to the microorganism), the OD_{660} was recorded using a microplate reader. The concentration of compounds that caused complete growth inhibition was recorded as the minimal inhibitory concentration (MIC). Ten-µl samples obtained from the wells showing no visible growth was spread on agar plates to determine the minimal microbicidal concentration (MMC) of the compounds. All the above assays were run in triplicate.

2.4. Influence of iron content on growth inhibition of P. gingivalis

The effect of **1** and **2** on growth of *P. gingivalis* was further investigated under iron-limiting conditions. Mycoplasma Broth Base (MBB; BBL Microbiology Systems) (iron content ≤8 nM) medium supplemented with 10 µM hemin was used as a medium with iron-restricted conditions. The growth inhibitory effect was determined as above.

2.5. Analysis of complex formation between iron and compounds **1** and **2**

UV/Vis spectroscopic analysis was performed as previously reported [9] to monitor the formation of complexes between iron and compounds **1** and **2**.

3. Results and discussion

Compounds **1** and **2** were synthesized by selective prenylation of 2,4,6-trihydroxyacetophenone at the 4′ position with geranyl and farnesyl bromide, respectively, in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as the

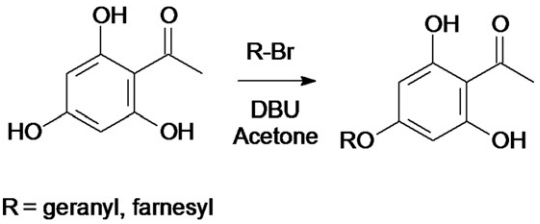

Fig. 1. Synthesis of compounds **1** and **2**.

Table 1
Minimal inhibitory concentrations (MICs) and minimal microbicidal concentrations (MMCs) of compounds **1** and **2** toward *S. sobrinus, S. mutans, A. actinomycetemcomitans, P. intermedia, P. gingivalis,* and *C. albicans.*

Microorganism	Compound **1** (μg ml^{-1})		Compound **2** (μg ml^{-1})		Reference control (μg ml^{-1})[a]	
	MIC	MMC	MIC	MMC	MIC	MMC
S. sobrinus	100	100	>100	>100	0.098	1.56
S. mutans	100	100	>100	>100	0.049	1.56
A. actinomycetemcomitans	>100	>100	>100	>100	1.56	6.25
P. intermedia	25	50	>100	>100	0.392	3.125
P. gingivalis	12.5	12.5	12.5	12.5	0.098	0.78
C. albicans	25	50	>100	>100	3.125	6.25

[a] Penicillin G was used for bacteria and Nystatin for *C. albicans*.

base in acetone (Fig. 1). The yields of **1** and **2** were 55% and 62%, respectively. The selectivity in geranylation/farnesylation of the parent acetophenone may be explained by a preferential hydrogen abscission by the bulky amine DBU from the more sterically accessible 4-OH. Both products were characterized by IR, 1H NMR, 13C NMR and GC–MS. The recorded data were in full agreement with those already reported for the same compounds [1–4].

MICs and MMCs of compounds **1** and **2** as well as of reference controls (penicillin G and Nystatin) are reported in Table 1. The MIC of compound **1** for both Gram positive bacteria (*S. mutans, S. sobrinus*) tested was 100 μg ml^{-1}. This concentration also represented the MMC. At this concentration, compound **2** only caused a 40% and 36% growth inhibition of *S. sobrinus* and *S. mutans*, respectively (data not shown). The effect of **1** and **2** on growth of three Gram negative oral pathogens was also investigated. While **1** and **2** had no significant effect on growth of *A. actinomycetemcomitans*, compound **1** was highly active on *P. intermedia* and *P. gingivalis* showing MIC of 25 and 12.5 μg ml^{-1} and MMC of 50 and 12.5 μg ml^{-1}, respectively. Although compound **2** only caused a 32% inhibition of growth of *P. intermedia* at the highest concentration tested (100 μg ml^{-1}), it showed MIC and MMC of 12.5 μg ml^{-1} on *P. gingivalis*. Lastly, the effect of **1** and **2** on growth of the pathogenic yeast *C. albicans* was tested. As it was the case for bacteria, compound **1** was more effective than **2** with a MIC of 25 μg ml^{-1} and a MMC of 50 μg ml^{-1}. At the highest concentration tested (100 μg ml^{-1}), compound **2** caused a 33% inhibition of the growth of *C. albicans*. The reference controls penicillin G and Nystatin were found to be more active than **1** and **2** (Table 1). The above results on antimicrobial activity of **1** and **2** suggest that increasing the length of the side chain from C_{10} (geranyl) to C_{15} (farnesyl) resulted in a marked decrease of the antimicrobial properties of the oxyprenylated acetophones. To the best of our knowledge, our study is the first to report the antimicrobial activity of **1** and **2**. However, the antimicrobial activity of oxyprenyloxy secondary metabolites having a benzophenone, cinnamic acid, or xanthone core has been previously reported [10].

Since hydroxyacetophenones such as **1** and **2** have been shown to possess metal chelating activity [11], we evaluated their growth inhibitory effect on *P. gingivalis* cultivated in the medium MBB + 10 μM hemin, which represents an iron-restricted condition. Under this iron poor condition, both **1** and **2** completely prevented the growth of *P. gingivalis* at the lowest concentration tested (6.25 μg ml^{-1}) that also corresponded to the MMC. The structural features of **1** and **2** (e.g. the presence of a double OH function in ortho position to an acetyl group) could allow an efficient metal chelation. To validate this hypothesis we performed UV/Vis spectroscopic analysis to monitor the formation of complexes between iron and compounds **1** and **2**. This analysis showed the formation of iron complexes with **1** and **2** (data not shown). Since iron is an essential nutrient to support microbial growth, iron chelation by **1** and **2** likely contributes to the antimicrobial properties of the compounds.

Very few studies have investigated the pharmacological properties of **1** and **2**. In a recent study, Bruyère et al. [12] reported the capacity of these two oxyprenylated acetophenones to inhibit proliferation of cancer cell lines. On the basis of the data reported in this study, compounds **1** and **2** may represent potential natural molecules for the prevention/treatment of common oral infections, including dental caries, periodontal disease, and candidiasis.

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