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Total syntheses of the aromatase inhibitors, mammeasins C and D, from Thai medicinal plant *Mammea siamensis*

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ABSTRACT

The first total syntheses of the geranylated pyranocoumarins, mameasins C (**1**) and D (**2**), aromatase inhibitors isolated from the flowers of *Mammea siamensis*, were accomplished in five steps, starting from phloroglucinol **3**. In this strategy, the characteristic pyran ring-fused coumarin core of **1** and **2** was effectively constructed by Friedel-Crafts acylation of **3**, followed by Reformatsky reaction of the resultant ketone to give a key coumarin intermediate **9**. Compound **9** was converted to targets **1** and **2** in a stepwise manner by successive *C*-acylation and *O*-geranylation, followed by a [1,3]-sigmatropic geranyl shift. Furthermore, screening of intermediates obtained in the synthetic pathway to **1** and **2** revealed that de-geranylated pyranocoumarins (**10** and **11**) show superior aromatase inhibitory activity as compared to the natural products **1** and **2**.

1. Introduction

Coumarins constitute an important class of heterocyclic compounds that are known as benzo-α-pyrones, wherein a pyran ring is fused with a benzene ring. Natural and synthetic coumarins have attracted considerable interest because they exhibit a diverse range of biological activities, which depend on the substitution pattern on the coumarin ring. A number of studies focusing on a broad array of pharmacological and biochemical properties such as anti-coagulant,[1] anti-alzheimer,[2] anti-viral,[3] anti-bacterial,[4] anti-fungal,[4b,4c,5] anti-inflammatory,[6] and anti-oxidant[4e,7] properties have been reported. In addition, the anti-proliferative and anti-tumor activities of various coumarins have been extensively investigated.[8] Some coumarins have also been used in clinical trials to demonstrate activity against breast cancer, prostate cancer, malignant melanoma, and metastatic renal cell carcinoma.[8k,8l,9] Furthermore, coumarins are used as additives in food and cosmetics, and as optical brightening agents.[10] Coumarins also find application in photochemotherapy for the treatment of certain skin diseases such as psoriasis, vitiligo, eczema, and mycosis.[11]

In the course of our characterization studies on bioactive constituents in Thai natural medicine,[12,13,14] we reported that a methanol extract of the flowers of *Mammea siamensis*, which have been used for preparing a heart tonic in Thai traditional medicine ("Sarapi" in Thai).[13,14] The coumarin constituents showed inhibitory effects on nitric oxide production in lipopolysaccharide-activated RAW264.7 cells.[13] Moreover, our continuing studies revealed that the methanol extract shows inhibitory activity against aromatase. The enzyme is responsible for a key step in the biosynthesis of estrogens, which plays a crucial role in the pathogenesis of breast cancer and is known to express itself at higher levels in breast cancer cells than in non-cancerous breast cells. Consequently, aromatase is a key therapeutic target in the treatment and prevention of estrogen-dependent breast cancer.[8m,8n] Based on bioassay-guided separation, two new geranylated coumarins, mammeasins C and D (**1** and **2**), were isolated together with 20 known coumarins[14] (Fig. 1). Compounds **1** and **2** are rare coumarins, wherein a dioxaphenalene type framework is constructed by fusing a pyran ring to the coumarin unit. Both these compounds

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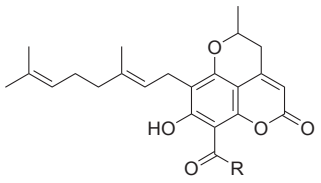


mammeasin C (**1**): R = ^{i}Pr, mammeasin D (**2**): R = ^{n}Pr

Fig. 1. The structure of Mammeasin C (**1**) and D (**2**).

showed potent aromatase inhibitory activity [IC_{50} (μM): **1** = 2.7, **2** = 3.6] comparable to that of aminoglutethimide (IC_{50} = 2.0 μM), which was used as a reference standard.[14] Compounds **1** and **2** find potential application as seeds for the development of therapeutic agents against breast cancer; hence, ensuring ready availability of analogues for structure-activity relationship (SAR) studies is an important research goal. In this regard, establishing a practical and short-step approach for the construction of the three-ring-fused coumarin core in **1** and **2** is imperative. Therefore, beginning with an SAR study on mammeasins C and D (**1** and **2**), we report herein the first total syntheses of **1** and **2** in five steps starting from commercially available phloroglucinol **3**. Furthermore, we compared the aromatase inhibitory activity of synthetic intermediates (**15** and **16**) to **1** and **2** with those of the parent coumarins, demonstrating that de-geranylation effectively enhanced the inhibitory activity against aromatase.

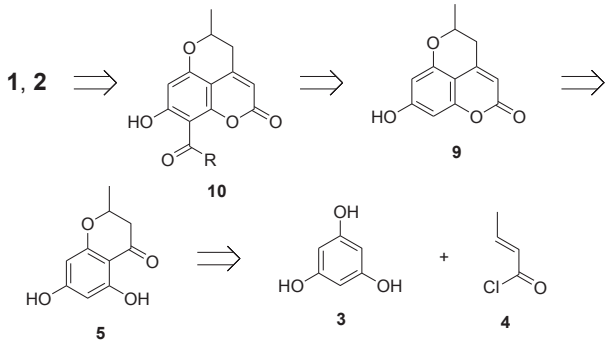
2. Results and discussion

2.1. Syntheses of mammeasins C (1) and D (2)

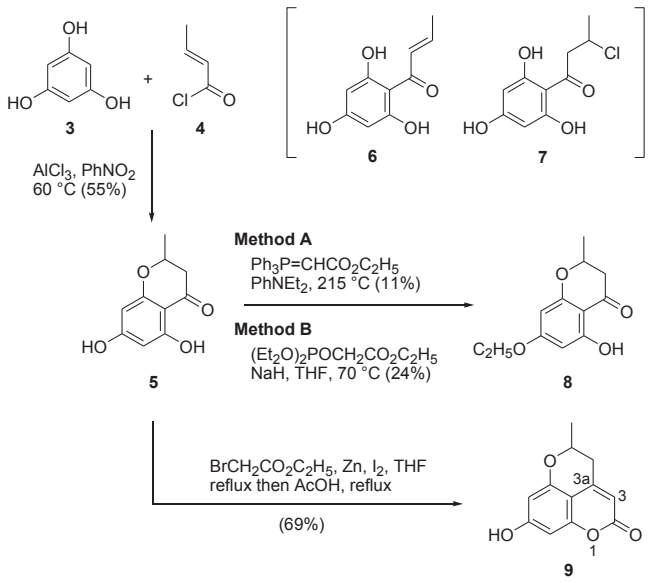
As shown in the retrosynthetic analysis, compound **9**, the key pyranocoumarin motif of **1** and **2**, was constructed by coumarin synthesis from an known ketone **5** prepared by Friedel-Crafts acylation of phloroglucinol **3**, and subsequent internal conjugated addition of the resultant enone intermediate. Compound **9** was then converted to targets **1** and **2** via Friedel-Crafts acylation and subsequent geranylation of ketone **10** (Scheme 1).

Based on the modified version of a published method,[15] phloroglucinol **3** was subjected to Friedel-Crafts acylation with crotonyl chloride, as an alternative to crotonic anhydride. The reaction afforded the desired chromanone 5,7-dihydroxy-2-methylchroman-4-one **5**, albeit in a disappointing yield of 31%. This yield was close to the reported value (36%)[15] and did not improve despite several attempts. Careful examination of the crude mixture revealed the formation of (2*E*)-(2,4,6-trihydroxyphenyl)-but-2-en-1-one **6** (an intermediate of **5**) and its HCl adduct, 3-chloro-(2,4,6-trihydroxyphenyl)-butan-1-one **7** (a by-product), which significantly decreased the yield of **5**. Fortunately, both **6** and **7** could be converted to the desired ketone **5** by NaOH treatment of the crude mixture prior to purification, and chemical yield of **5** was successfully improved up to 51%. Compound **5** was sequentially subjected to a Wittig reaction with $Ph_3P = CHCO_2C_2H_5$ as per a reported to a coumarin synthesis[16]; however, the reaction afforded 7-*O*-ethylated compound **8**, which is the ester-exchange product between the non-hydrogen-bonded hydroxyl of **5** and the ethoxy moiety of the Wittig reagent, in 11% yield. There was no evidence for the formation of the desired pyranocoumarin, 4,5-dihydro-8-hydroxy-5-methyl-2*H*-pyrano[4,3,2-*de*]coumarin **9**, despite careful chromatographic examination of the reaction mixture. Horner-Emmons reaction with $(Et_2O)_2POCH_2CO_2C_2H_5$[17] also resulted in the formation of **8**. On the other hand, the coumarin ring core was successfully constructed by Reformatsky[18] reaction with bromoacetate, followed by treatment with AcOH, to furnish the desired pyranocoumarin **9** in 69% yield. The structural motif of **9** was evidenced by the ^{13}C NMR spectrum, which displayed three kinds of sp^2-carbon signals at δ_{C3} 105.6, δ_{C3a} 152.0, and $\delta_{C=O}$ 164.0, attributed to the α,β-unsaturated enone system. No signal due to the ketonic carbonyl carbon was observed in the case of **5** ($\delta_{C=O}$ 196.4). The ^1H NMR spectrum also showed a signal due to an olefin proton at the α−position to the carbonyl at δ_H 5.86. No signal due to the hydrogen-bonded hydroxyl proton was observed at around 12 ppm (Scheme 2).

With the key pyranocoumarin core in hand, we attempted the functionalization of the benzene ring from **9** via a three-step sequence. First, Friedel-Crafts acylation of **9** with butyryl chloride or isobutyryl chloride was carried out under the standard conditions for the acylation of phenolic compounds,[19] and two regioisomers (**10**/**11**) in 4:1 and 5:1 ratio were obtained. The fact acylation of phenol ethers tends to occur at the para position[20] and the difference in electron density between the C6a- and C9a-oxygens of **9** suggested that the major isomers were 9-butyryl-4,5-dihydro-8-hydroxy-5-methyl-2*H*-pyrano[4,3,2-*de*]coumarin **10a** and 9-isobutyryl-4,5-dihydro-8-hydroxy-5-methyl-2*H*-pyrano[4,3,2-*de*]coumarin **10b**, while the minor isomers were the corresponding C7 regioisomers **11a** and **11b**. However, these products were not easily distinguishable from each other on the basis of their ^1H and ^{13}C NMR spectra. For example, the ^1H NMR spectrum of the



Scheme 1. Retrosynthetic analysis of Mammeasins C (**1**) and D (**2**).



Scheme 2. Construction of pyranocoumarin framework (**9**) from phloroglucinol (**3**).