Fall 2016

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Mark F. Mechelke
St. Cloud State University, mfmechelke@stcloudstate.edu

Katy Platt
St. Cloud State University

Connor Pribula
St. Cloud State University

CHEMOTHERAPEUTIC DRUG DESIGN: AN EFFICIENT SYNTHESIS OF 4-SUBSTITUTED ALPHA-METHYLENE-GAMMA-LACTONES

Mark F. Mechelke†, Katy Platt*, and Connor Pribula*

Department of Chemistry and Biochemistry, St. Cloud State University, Robert H. Wick Science Building, 825 1st Avenue South, St. Cloud, MN 56301, mmechelke@stcloudstate.edu

Abstract

Sesquiterpene lactones are plant-derived compounds that have been shown to possess significant activity against inflammation and cancer. Comparative studies of sesquiterpene lactone structure and tumor cytotoxicity indicate that the presence of an α-methylene-γ-lactone moiety is necessary for bioactivity. This observation has led to the hypothesis that simple compounds containing this pharmacophore may also exhibit similar anti-cancer properties. To test this theory, an efficient synthesis of 4-substituted α-methylene-γ-lactones has been developed. The model compound in this study, α-methylene-γ-dodecalactone, has been prepared from commercially available 1-decene in six steps. The synthesis features a nucleophilic epoxide ring-opening reaction followed by an intramolecular cyclization to prepare a key lactone intermediate. The described sequence should provide access to a large number of 4-substituted α-methylene-γ-lactone analogues that can be used to better understand the role alkyl substituents play in the bioactivity of this class of compounds.

Keywords: Cancer, Nuclear factor–kappa B, Glutathione, Sesquiterpene lactones, Costunolide, Alpha-methylene-gamma-lactones

Introduction

Throughout history, plants have been used for their medicinal properties. In 1960, the National Cancer Institute initiated a large-scale screening program of approximately 35,000 plant samples in an effort to identify new anti-tumor agents. As a direct result of this and related programs, 67% of anti-cancer drugs used today are natural products or natural product derivatives (1). Sesquiterpene lactones are a class of compounds that were discovered early on during this screening process. Sesquiterpene lactones are naturally occurring plant terpenoids that are composed of three isoprene units and a lactone group. Isolated in highest quantity from plants of the *Compositae* family, sesquiterpene lactones exhibit a broad spectrum of biological activity, ranging from anti-inflammatory to anti-cancer properties (2). In 1969, a study was performed that compared the structure of more than 50 sesquiterpene lactones to their cytotoxicity (3). This research suggested that the α-methylene-γ-lactone moiety, found in a large number of sesquiterpene lactones, was required for bioactivity. Subsequent studies confirmed that the presence of an α,β-unsaturated carbonyl system is essential for tumor inhibition (4). An example of a sesquiterpene lactone that exhibits activity against cancer is costunolide **[1]** (Figure 1). Isolated from the roots of the *Saussurea lappa* plant, costunolide **[1]** has demonstrated potent activity against a variety of cancer cell lines (5,6).

Natural products, like costunolide, that contain an α-methylene-γ-lactone functional group have been shown to react as Michael-type acceptors with intracellular thiols. By forming covalent bonds with thiols, sesquiterpene lactones can inhibit cancer by inducing apoptosis and preventing unregulated cell growth (2). For example, glutathione (GSH) is a natural antioxidant found in all cells. By forming a covalent, thioether bond with the cysteine amino acid residue found in GSH, costunolide effectively removes GSH from the cell (7,8). This decrease in GSH triggers the build-up of oxygen free radicals within the cancer cell, thus initiating apoptosis. Through a similar mechanism, costuonlide can inhibit nuclear factor-kappa B (NF-κB). NF-κB is a protein complex that controls the transcription of DNA during cell division. Over-activation of NF-κB has been found in a wide variety of tumor types (9). Sesquiterpene lactones have been shown to react with cysteine sulfhydryl groups on the NF-κB p65 subunit (10). This direct alkylation of the transcription factor prevents DNA from binding to active NF-κB and therefore inhibits cell proliferation (10).

The crucial role that the α-methylene lactone moiety plays in sesquiterpene lactone biological activity has led to the development of many synthetic methods to prepare this structural feature (11-14). A number of different compounds containing this structural motif have been synthesized and tested (15-20). The goal of this project was to synthesize an analogue of a parent sesquiterpene lactone, conserving the α-methylene-γ-lactone moiety, but removing the complex carbo-cyclic skeleton. The compound, α-methylene-γ-dodecalactone **[2]**, was targeted for synthesis due to its resemblance to the natural product costunolide (Figure 1). It is anticipated that the synthetic sequence used to make compound **[2]** can be used to make a large number of 4-substituted α-methylene-γ-lactone analogues by simply changing the alkene starting material (Scheme 1). It is hoped that these simple analogues will retain the potent bioactivity observed by the more complex, sesquiterpene lactone natural products.

Experimental

Column chromatography was performed on 230-400 mesh silica gel. NMR spectroscopy was recorded on a JEOL 400

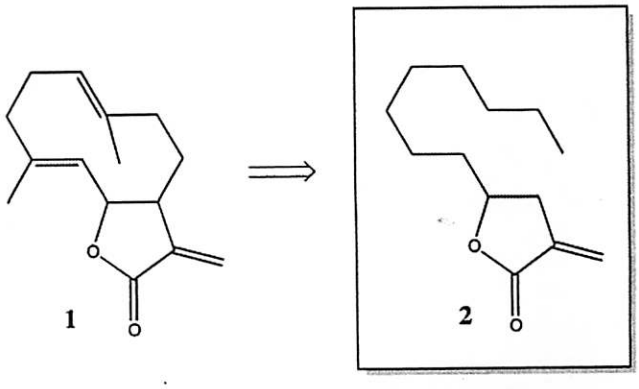
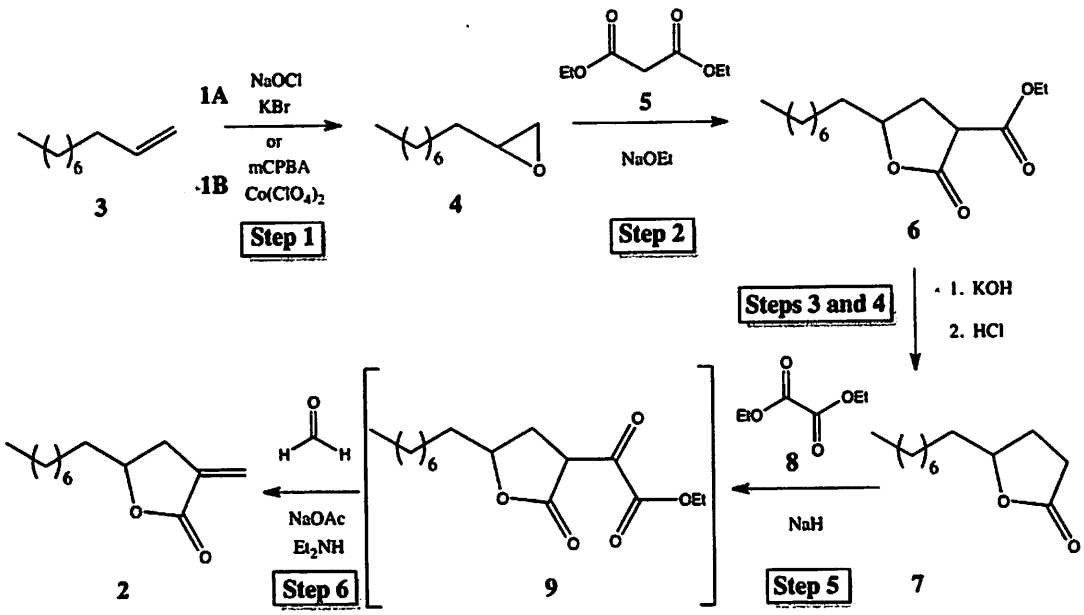


Figure 1. Costunolide [1] and Synthetic Analogue [2].



Scheme 1. Synthesis of Costunolide Analogue [2].

MHz NMR spectrometer using deuterated chloroform solvent. Gas chromatography-mass spectrometry was carried out on an Agilent Technologies 7890/5975C gas chromatograph-mass spectrometer. The GC column (30m x 0.25mm) has a 0.25 μm thick polydimethyl-siloxane (PDMS) with 5% phenyl substitution stationary phase. The oven conditions were: injection port temperature = 250°C; oven starting temperature = 70°C for 5 minutes; ramp of 20°C/minute up to 250°C; the temperature was held for 10 minutes at 250°C. All starting chemicals were purchased from Sigma-Aldrich, Inc. (Atlanta, GA) and used as received.

Step 1A: Bleach Epoxidation (21)

Commercially available 1-decene **[3]** (1.50 mL, 7.92 mmol) was dissolved in acetonitrile (40 mL) and water (40 mL). The biphasic solution was treated with sodium carbonate (1.602 g, 12.92 mmol), potassium bromide (1.519 g, 12.76 mmol), and sodium hypochlorite (8.25% NaOCl in water, 20.0 mL, 24.6 mmol). The reaction was allowed to stir at room temperature for 24 hours. At this time, a second portion of sodium hypochlorite (8.25% NaOCl in water, 20.0 mL, 24.6 mmol) was added and the reaction mixture was allowed to stir for an additional 24 hours. Water (50 mL) was added to the resulting mixture and an ethyl acetate extraction was performed (2 x 75 mL). The organic layers were dried over magnesium sulfate, filtered, and concentrated to provide crude epoxide **[4]** as a yellow oil. There were four impurities present in the crude epoxide product: 1-decene (RT = 5.803 min) and three minor products at RT = 11.101 min (Cl present), 11.132 min (Br present), and 11.651 min (Br present). All three of these products were shown by MS to contain halides and were most likely formed by epoxide opening. The crude epoxide was characterized by gas chromatography-mass spectrometry (GC-MS) and carried on crude into the next step. **GC-MS** RT = 8.902 min, M^+-H_2O = 138.

Step 1B: Cobalt(II) Perchlorate/ mCPBA Epoxidation (22)

Commercially available 1-decene **[3]** (1.00 mL, 5.28 mmol) was dissolved in acetonitrile (50 mL) and treated with cobalt(II) perchlorate (0.065 g, 0.18 mmol) and 3-chloro-peroxybenzoic acid or mCPBA (1.820 g, 10.55 mmol). The reaction was allowed to stir at room temperature. At 30 minutes, an aliquot was removed and GC-MS analysis showed 86% conversion to the epoxide product. Other than 1-decene, the only other compound observed on the GC-MS was 3-chlorobenzoic acid (RT = 10.099 min). After stirring for 1 hour, the reaction mixture was diluted with saturated sodium bicarbonate(aq) (50 mL) and an ethyl acetate extraction was performed (2 x 50 mL). GC-MS analysis after the extraction illustrated only trace amounts of 3-chlorobenzoic acid present in the ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated to provide crude epoxide **[4]** as a reddish-brown oil. The epoxide was characterized by GC-MS and carried on crude into the next step. **GC-MS** RT = 8.902 min, M^+-H_2O = 138.

Step 2: Epoxide Opening and Intramolecular Cyclization (23-25)

A 0.74 M solution of sodium ethoxide was prepared by dissolving sodium metal (0.425 g, 18.5 mmol) in absolute ethanol (25 mL). Diethyl malonate **[5]** (2.40 mL, 15.8 mmol) was subsequently added to the sodium ethoxide solution. In a separate round bottom flask, crude epoxide **[4]** (because it was crude, the theoretical yield from Step 1A was used: 7.92 mmol) was dissolved in absolute ethanol (20 mL). The epoxide solution was transferred via cannula into the reaction flask containing sodium ethoxide and diethyl malonate. The resulting reaction mixture was heated at reflux for 24 hours. The reaction was cooled to room temperature and treated with diethyl ether (20 mL) and 1.0 M HCl (20 mL). An extraction was performed with diethyl ether (2 x 50 mL) and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum. Crude ester **[6]** was isolated as a yellow oil. GC-MS analysis of the crude product showed two major peaks representing the two diastereomers of ester **[6]**. Impurities observed in the product were diethyl malonate (RT = 7.229 min), ethyl hydrogen malonate (RT = 7.718 min), 1-ethoxy-2-decanol (RT = 10.805 min), and lactone **[7]** (12.298 min). The two diastereomers were carried on crude into the next step with these impurities. **GC-MS** RT = 14.074 min, M^+-H_2O = 252; RT = 14.116 min, M^+-H_2O = 252.

The same procedure was followed for crude epoxide **[4]** (theoretical yield from Step 1B: 5.28 mmol) prepared from the cobalt(II) perchlorate/mCPBA procedure. Crude ester **[6]** was isolated as an orange oil. GC-MS analysis of the crude product showed two major peaks representing the two diastereomers of ester **[6]**. Impurities observed in the product were diethyl malonate (RT = 7.229 min), ethyl hydrogen malonate (RT = 7.718 min), 3-chlorobenzoic acid (RT = 10.099 min), and lactone **[7]** (12.298 min). The two diastereomers were carried on crude into the next step.

Steps 3 and 4: Saponification and Decarboxylation (23,24,26)

Crude ester **[6]** (theoretical yield from Step 2A: 7.92 mmol) was treated with a 10.0 M potassium hydroxide solution (16.0 mL, 160 mmol). The resulting mixture was heated to reflux. After 2 hours, the reaction was cooled to room temperature and diluted with diethyl ether (10 mL). A 1.0 M potassium hydroxide(aq)/ diethyl ether extraction was performed (2 x 10 mL KOH(aq)). The carboxylic acid salt intermediate remained in the aqueous layer. Non-acidic impurities were removed in the diethyl ether layer. This was desirable because the impurity 1-ethoxy-2-decanol had very similar R_f values to lactone **[7]** and could only be removed in this manner. The aqueous layer was acidified with concentrated HCl until a pH of 1 was achieved. A diethyl ether extraction was performed on the aqueous layer (2 x 75 mL) and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The resulting reddish-brown oil was treated with 1.0 M HCl(aq) (10.0 mL) and heated at reflux for 24 hours. The mixture was diluted with water (20 mL) and extracted with diethyl ether (2 x 75 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. Purification by column chromatography (25% ethyl acetate in hexanes; R_f = 0.218) afforded lactone **[7]** as a light yellow oil (0.608 g, 38.8% yield over the four steps). **^{1}H NMR** (400 MHz) δ 4.49 – 4.42 (m, 1H), 2.50 (dd, *J* = 9.6, 7.2 Hz, 2H), 2.33-2.25 (m, 1H), 1.87-1.77 (m, 1H), 1.75-1.66 (m, 1H), 1.61-1.51 (m, 1H), 1.47-1.18 (m, 12H), 0.85 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR** (400 MHz) δ 177.4, 81.1, 35.7, 31.9, 29.5, 29.4, 29.2, 28.9, 28.1, 25.3, 22.7, 14.2. **GC-MS** RT = 12.298 min, M^+-H_2O = 180.

The same procedure was followed for crude ester **[6]** (theoretical yield from Step 2B: 5.28 mmol) prepared from the cobalt(II) perchlorate/mCPBA procedure. Purification by column chromatography afforded lactone **[7]** (0.347 g, 33.2% yield over the four steps).

Steps 5 and 6: Alpha-Methylene Formation (11,12)

Lactone **[7]** (0.270 g, 1.36 mmol) was dissolved in anhydrous diethyl ether (20 mL) and treated with degreased sodium hydride (0.163 g, 6.80 mmol). Diethyl oxalate **[8]** (0.92 mL, 6.80 mmol) was subsequently added to the flask and the reaction was heated at reflux for 20 hours. The resulting mixture was quenched with 3.0 M sulfuric acid (10.0 mL) and a diethyl ether extraction (2 x 40 mL) was performed. The combined ether fractions were dried over magnesium sulfate, filtered, and concentrated to afford the desired intermediate **[9]** which was carried on crude into the next step. **GC-MS** RT = 15.059 min, M^+ = 298.

Intermediate **[9]** was dissolved in 1,4-dioxane (4.0 mL) and treated with sodium acetate (0.010 g, 0.12 mmol), aqueous formaldehyde solution (1.0 mL, 37 wt. % in water, 13 mmol), and diethylamine (0.50 mL, 4.8 mmol). The resulting solution was stirred at room temperature. After 48 hours, the reaction mixture was acidified to a pH of 1 with 1.0 M HCl and diluted with water (15 mL). An ethyl acetate extraction was performed (2 x 40 mL). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated to afford crude product **[2]**. The crude product was purified by flash column chromatography (25% ethyl acetate in hexanes; R_f = 0.333) to afford α-methylene-γ-lactone **[2]** as a clear oil (0.196 g, 68.6% over the two steps). **^{1}H NMR** (400 MHz) δ 6.20 (t, *J* = 2.8 Hz, 1H), 5.60 (t, *J* = 2.4 Hz, 1H), 4.54-4.45 (m, 1H), 3.03 (dddd, *J* = 17.0, 7.6, 2.8, 2.4 Hz, 1H), 2.55 (dddd, *J* = 17.0, 6.0, 2.8, 2.4 Hz, 1H), 1.76-1.66 (m, 1H), 1.64-1.53 (m, 1H), 1.50-1.20 (m, 12H), 0.86 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR** (400 MHz) δ 170.4, 134.9, 121.8, 77.7, 36.3, 33.6, 31.9, 29.5, 29.3, 29.2, 24.9, 22.7, 14.1. **GC-MS** RT = 12.594 min, M^+ = 210.

Results and Discussion

The first step in the synthetic sequence was an epoxidation of 1-decene **[3]**. Epoxidation of terminal alkenes is often difficult due to their poor nucleophilicity (22). Two methods were used to prepare the epoxide. The first method (Scheme 1, Step 1A) involved using bleach, a very inexpensive and convenient oxidant. 1-Decene was treated with sodium hypochlorite and bromide ion and the reaction was monitored by gas chromatography (21). After 48 hours, the reaction provided a good yield of epoxide **[4]**. The problems with this reaction were its long reaction times and its reproducibility. Because of these issues, an alternative route to epoxide **[4]** was developed. Treatment of 1-decene **[3]** with 3-chloroperoxybenzoic acid and cobalt(II) perchlorate catalyst (Figure 1, Step 1B) afforded epoxide **[4]** quickly and in high yield (22). This step was very reproducible and very clean. The only by-product observed was 3-chlorobenzoic acid which was easily removed via basic extraction.

Crude epoxide **[4]** was treated with the carbanion of diethyl malonate **[5]**. Due to sterics, the malonic ester anion preferentially attacked the terminal carbon atom of the epoxide, forming an alkoxide anion that subsequently underwent an intramolecular cyclization reaction to form ethyl ester **[6]** (23-25). The product was characterized by gas chromatography-mass spectrometry. Two peaks were observed on the GC spectrum representing the two diastereomers of ester **[6]**.

A saponification reaction was performed on ester **[6]**. Treatment with potassium hydroxide afforded the carboxylic acid salt. The solubility of the salt in water was used to remove some of the impurities formed during the epoxide-opening step via acid/base extraction. The carboxylic acid salt was acidified and heated at reflux for 24 hours. It is important to note that this reaction was difficult to monitor via gas chromatography-mass spectrometry. The carboxylic acid intermediate was observed to decarboxylate into lactone **[7]** under the high temperature conditions of the GC injection port. Therefore, the carboxylic acid and lactone **[7]** exhibited identical GC retention times and mass spectra. Confirmation of lactone **[7]** formation could only be observed by ^{1}H and ^{13}C NMR. Lactone **[7]** was purified by column chromatography. This was the first step in the synthetic sequence that a pure yield was obtained. Both the bleach and 3-chloroperoxybenzoic acid epoxidation sequences provided similar yields, 38.8% and 33.2% respectively. This correlates

to a 76-79% yield per step in the four-step sequence used to prepare lactone **[7]**.

A previously reported, two-step α-methylenation process was used to complete the synthesis of costunolide analogue **[2]** (11,12). In step 5, the enolate anion of lactone **[7]** was treated with diethyl oxalate to form the ethyloxalyl derivative **[9]** in excellent yield based on GC analysis. Upon treatment with aqueous formaldehyde and diethylamine, intermediate **[9]** underwent a Mannich-type reaction followed by a β-dicarbonyl cleavage to afford α-methylene-γ-lactone **[2]** in 68.6% yield over the two steps.

Conclusion

In conclusion, the target compound, α-methylene-γ-lactone **[2]**, was prepared in six steps in a 26.6% overall yield. This correlates to approximately an 80% yield per step. Other methods have been developed to prepare this same compound. These sequences, however, are much more complex involving tin-mediated reactions, manganese(III) lactone annulations, and electrolysis of α-carboxy-α-phenylthiomethyl-γ-butyrolactones (27-30). The sequence outlined in this publication is simple and effective, requiring the use of only two column chromatography purification steps. The simplicity of this methodology should allow for facile production of a wide variety of 4-substituted α-methylene-γ-lactone analogues by merely using different terminal alkene starting materials. It is anticipated that these analogues will help illustrate the relevance of the alkyl substituent in the bioactivity of this class of compounds.

Acknowledgment

The Agilent 7890/5975C gas chromatograph-mass spectrometer and the JEOL 400 MHz NMR used to analyze the compounds in this research project were acquired with funds provided by NSF-MRI grant 1229165 and NSF-MRI grant 1428657 respectively awarded to the Department of Chemistry and Biochemistry at St. Cloud State University.

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