Short Report

Total Synthesis of Altissimacoumarin D, a Small Molecule Sirtuin1 Activator

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The total synthesis of the plant natural product altissimacoumarin D was achieved by the Mitsunobu alkylation of isofraxidin by geraniol. Isofraxidin was prepared from 2,4-dihydroxybenzaldehyde in five steps. The key reaction was the Knoevenagel condensation of an *ortho*-hydroxybenzaldehyde with Meldrum's acid under neutral conditions in water and one-pot acid catalyzed cyclization to the coumarin.

Keywords: natural products, epigenetics, sirtuins, coumarins

Introduction

Historically, natural products are a rich source of biologically active compounds including many of our current therapeutic agents.[1-5] In the present day, natural products continue to play a significant role in the development of small molecule chemical probes for the new science of epigenetics.[6] Numerous natural products that inhibit specific epigenetic enzyme-catalyzed structural modifications of nucleic acids and histone proteins are known, such as laccaic acid (targeting deoxyribonucleic acid (DNA) methyltransferase),[7] anacardic acid (targeting histone acetyltransferase),[8] trichostatin A (targeting histone deacetylase),[9] nauhoic acid (targeting lysine methyltransferase)[10] and tripartin (targeting lysine demethylase).[11] The depsipeptide romidepsin, a histone deacetylase inhibitor of bacterial origin, is a particularly impressive case as it has progressed from discovery to an approved epigenetic drug for the treatment of T-cell lymphoma.[12,13]

The zinc-dependent histone deacetylases (HDACs) are enzymes that hydrolyze acyl-lysine (chiefly acetyl-lysine) residues in proteins and revert them to native lysine, usually with a gene silencing effect as a consequence.[14] An additional class of sirtuin histone deacylases catalyze the same reaction through an entirely different nicotinamide adenine dinucleotide (NAD⁺)-dependent catalytic mechanism. The seven human sirtuins, Sirt1-7, are implicated in a number of disease conditions and there is much interest in both activators and inhibitors of sirtuin enzymes.[15] Although some dietary natural products such as resveratrol, quercetin or epigallocatechin gallate are reported as sirtuin modulators, their promiscuous nature is non-ideal for use as chemical probes and more selective molecules are needed. A recent lead from natural product screening comes from *Ailanthus altissima* (the tree of heaven), a plant used in Chinese and Korean traditional medicine. From the bark extract, four coumarins were identified that are Sirt1 activators (Figure 1).[16] At a concentration of 10 μM, the most active compound altissimacoumarin D significantly increased Sirt1 catalyzed deacetylation of a p53 peptide Sirt1 substrate *in vitro* and decreased the transcriptional activity of p53 in a cell-based reporter assay.

Due to the interesting biological activity, we embarked on the total synthesis of altissimacoumarin D. Our biogenetic retrosynthesis (Figure 2) assumed that the likely origin of altissimacoumarin D (**1**) is through the geranylation of another coumarin natural product, isofraxidin (**2**). There are several total syntheses of isofraxidin in the literature, by Spath and Jerzmanowska-Sienkiewiczowa,[17] Spath and Dobrovolny,[18] Rouessac and Leclerc,[19] Chen[20] and Gao *et al*.[21] from which we selected the Chen route as the starting point for our studies due to its concise and simple nature. In Chen's four step synthesis, isofraxidin arose
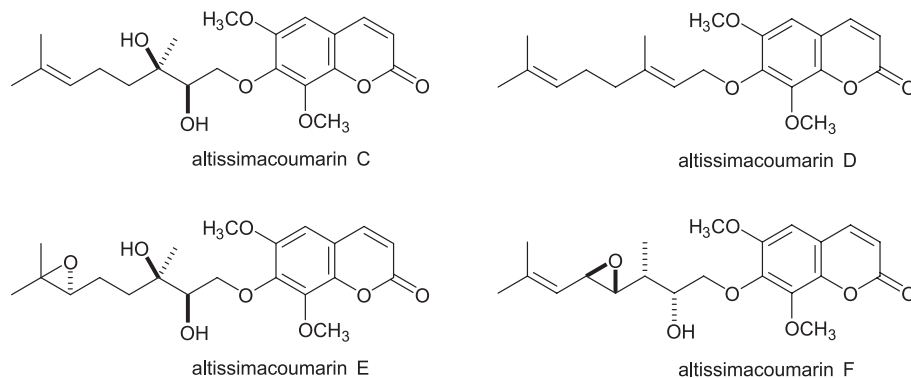
**Figure 1.** The structures of altissimacoumarin Sirt1 activators.

from the formation of a coumarin from an *o*-hydroxy benzaldehyde derivative, which is in turn prepared by the functionalization of resorcinol. Nevertheless, we have substantially altered the reaction conditions for the Knoevenagel reaction and subsequent coumarin cyclization compared to the published procedure.

Results and Discussion

Our synthesis began with the halogenation of commercially available 2,4-dihydroxybenzaldehyde (**3**, Scheme 1) to give the dibrominated derivative **4**. Halogen displacement under van Koten's conditions with copper(I) chloride and sodium methoxide afforded 2,4-dihydroxy-3,5-dimethoxybenzaldehyde (**5**) in good yield.[22] At this stage, Chen's Knoevenagel condensation with diethyl malonate was attempted but resulted in poor yields. Furthermore, the lack of experimental detail made an effective reproduction difficult. The method used in Rouessac's synthesis,[19] whereby a trimethoxylated benzaldehyde was condensed with Meldrum's acid and zinc oxide, was also unsatisfactory when applied to our aldehyde **5**. After investigating alternative reaction

conditions, we achieved a high yield of the Knoevenagel adduct **6** with Bigi's procedure.[23] This mild protocol with Meldrum's acid in the absence of extraneous acid or base catalysis has the benefit of being environmentally green as it is conducted in water without organic co-solvents. While intermediate **6** was cyclized to coumarin **7** in 90% yield under acid catalysis, we discovered that telescoping the Knoevenagel condensation and cyclization in a one-pot operation from aldehyde **5** to coumarin **7** was more efficient. The final step in the isofraxidin synthesis was the coumarin decarboxylation, for which our best yields were obtained by refluxing **7** in pyridine and ethylene glycol.[24]

With isofraxidin in hand through a five step sequence (58% overall yield), we introduced the geranyl sidechain. Under Mitsunobu conditions at room temperature, the reaction between isofraxidin and geraniol (Scheme 2) provided altissimacoumarin D (**1**). Although the reaction proceeded in a modest 54% yield, there was no attempt at optimization as we obtained a sufficient quantity (100 mg) of the natural product for biological studies. Our synthetic material had an excellent match with the ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopic data reported for the natural product (Table 1).
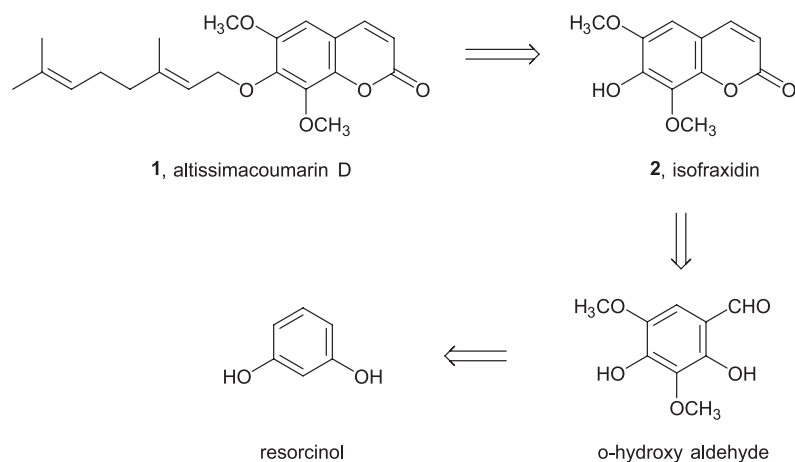
Figure 2. Retrosynthesis of altissimacoumarin D (**1**) from resorcinol.
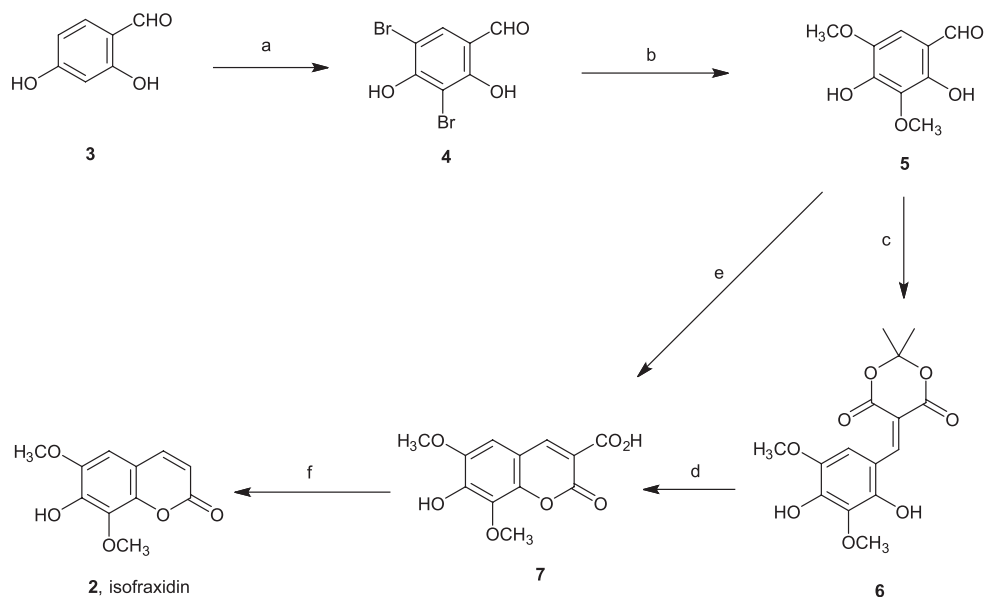
Scheme 1. (a) Br$_2$, EtOH, 30 min (98%); (b) NaOMe, CuCl, MeOH/DMF, reflux, 4 h (70%); (c) Meldrum's acid, H$_2$O, 80 °C, 2 h, (94%); (d) H$_2$SO$_4$, 90 min (90%); (e) one-pot combination of (c) and (d) (90%); (f) Py, ethylene glycol, reflux, 3.5 h (94%).
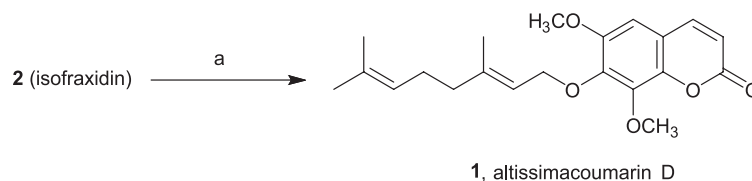
Scheme 2. (a) Geraniol, Ph$_3$P, diisopropyl azodicarboxylate (DIAD), tetrahydrofuran (THF), 24 h (54%).

Table 1. Comparison of altissimacoumarin D ^{1}H and ^{13}C NMR chemical shifts between the naturally isolated material and the synthetic sample

δ_H, natural product	δ_H, synthetic sample	δ_C, natural product	δ_C, synthetic sample
1.59 (s)	1.52 (s)	16.4	16.4
1.67 (s)	1.60 (s)	17.6	17.6
1.69 (s)	1.63 (s)	25.6	25.6
2.05, 2.08 (m)	1.99 (m)	26.3	26.3
3.89 (s)	3.82 (s)	39.6	39.6
4.03 (s)	3.96 (s)	56.3	56.2
4.68 (d, *J* 7 Hz)	4.62 (d, *J* 7 Hz)	61.7	61.7
5.06 (t, *J* 7 Hz)	5.00 (t, *J* 8 Hz)	70.3	70.2
5.56 (t, *J* 7 Hz)	5.49 (t, *J* 8 Hz)	103.5	103.5
6.34 (d, *J* 10 Hz)	6.28 (d, *J* 8 Hz)	114.4	114.4
6.66 (s)	6.58 (s)	115.1	115.1
7.61 (d, *J* 10 Hz)	7.54 (d, *J* 12 Hz)	119.6	119.6
		123.8	123.8
		131.7	131.7
		141.8	141.8
		142.5	142.5
		142.9	143.0
		143.5	143.4
		144.9	144.9
		150.7	150.7
		160.6	160.5

Conclusions

In summary, we report the total synthesis of the Sirt1 activator altissimacoumarin D in six steps from 2,4-dihydroxybenzaldehyde. A noteworthy feature is the new high-yielding route towards the intermediary isofraxidin via Knoevenagel condensation in water followed by cyclization to the coumarin carboxylic acid **7** in one-pot. Thermal decomposition of the pyridinium salt of **7** effected decarboxylation to give isofraxidin in high yield, after which geranylation afforded altissimacoumarin D. The ready access to altissimacoumarin D will facilitate its use as a starting material for the synthesis of the more heavily oxygenated natural products in this family, while the modular route facilitates the synthesis of analogues for structure-activity relationship studies.

Experimental

2,4-Dihydroxybenzaldehyde was obtained commercially (Sigma-Aldrich Co., St. Louis, MO, USA) and used without further purification. All reactions involving a dry atmosphere were performed with a slight positive pressure of argon. Analytical thin-layer chromatography

was done on 0.25 mm silica gel 60 F254 plates, and compounds visualized using UV irradiation or $KMnO_4$. Flash chromatography was carried out on columns packed with silica gel 60 (0.040-0.063 mm particle sizes). Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Avance-III spectrometer. The splitting patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet).

3,5-Dibromo-2,4-dihydroxybenzaldehyde (**4**, CAS 116096-91-4)

To a stirred solution of 2,4-dihydroxybenzaldehyde (2.8 g, 20 mmol) in ethanol (40 mL) was added bromine (6.4 g, 40 mmol) dropwise at room temperature. After stirring for 30 min the reaction mixture was poured into distilled water (100 mL) and filtered. The filtrate was washed with water and the residual bromine present in the aqueous phase was quenched with a saturated solution of sodium thiosulfate. Compound **4** was obtained as a white powder (5.9 g, 98%), and the physical and spectroscopic data are in accordance with those previously reported.[25] mp 188-189 ºC (Lit.[25] mp 200 ºC); ^{1}H NMR (400 MHz, methanol-d_4) δ 7.88 (s, 1H), 9.71 (s, 1H); ^{13}C NMR (100 MHz, methanol-d_4) δ 103.1, 119.9, 132.0, 138.5, 154.1, 154.7, 196.2.

2,4-Dihydroxy-3,5-dimethoxybenzaldehyde (**5**, CAS 182427-46-9)

To a stirred solution of **4** (11.8 g, 40 mmol) in a 2:5 solution of anhydrous methanol in anhydrous dimethylformamide (70 mL) was added CuCl (0.5 g, 5 mmol) and NaOMe (21.6 g, 400 mmol). The reaction mixture was heated under reflux at 100 ºC for 4 h. The solvent was then evaporated *in vacuo*, distilled water (50 mL) was added and the pH adjusted to 2 using concentrated HCl. After 15 min the reaction mixture was transferred into a separation funnel and the product extracted using chloroform (5 × 20 mL). The organic layer was washed with brine, dried with $MgSO_4$, filtered and the solvent evaporated *in vacuo*. Compound **5** was obtained as a brown solid (5.5 g, 70%), and the physical and spectroscopic data are in accordance with those previously reported.[21] mp 88-89 ºC (Lit.[21] mp 87.5-89.8 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (s, 6H), 7.29 (s, 1H), 8.68 (s, 1H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 56.6, 60.9, 109.0, 112.9, 134.6, 141.3, 147.1, 151.7, 194.6.

5-(2,4-Dihydroxy-3,5-dimethoxybenzylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**6**)

Meldrum's acid (0.16 g, 1 mmol) was added to a stirred solution of aldehyde **5** (0.210 g, 1 mmol) in 2 mL of water at 80 ºC and the reaction mixture left for 2 h to the point where a precipitate was formed. The solid was filtered and dried to give compound **6** as a white solid (0.323 g, 94%). mp 259-261 ºC; ^{1}H NMR (400 MHz, MeOD-d_1) δ 2.05 (s, 6H), 3.85 (s, 3H), 3.88 (s, 3H), 7.05 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (100 MHz, MeOD-d_1) δ 56.2, 60.8, 105.8, 109.5, 112.8, 134.2, 144.7, 145.9, 146.9, 149.6, 157.5, 164.2 (some carbon signals are coincident).

7-Hydroxy-6,8-dimethoxy-2-oxo-2*H*-chromene-3-carboxylic acid (**7**, CAS 149143-82-8)

Meldrum's acid (0.16 g, 1 mmol) was added to a stirred solution of aldehyde **5** (0.210 g, 1 mmol) in 2 mL of water at 80 ºC. After two hours, the reaction mixture was cooled and acidified at 0 ºC to pH 1 with concentrated sulfuric acid. After stirring for 1.5 h, the solution was poured onto crushed ice and warmed to room temperature. The crystals were filtered, washed with water, and dried to give coumarin **7** as white crystals (0.25 g, 90%), and the physical and spectroscopic data are in accordance with those previously reported.[19] mp 227-228 ºC (Lit.[19] mp 227-228 ºC); ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 6H), 4.63 (br s, 2H), 7.26 (s, 1H), 8.66 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.2, 60.8, 105.9, 109.5, 112.8, 134.2, 144.7, 145.9, 146.9, 149.6, 157.5, 164.2.

7-Hydroxy-6,8-dimethoxy-2*H*-chromen-2-one (**2**, isofraxidin, CAS 486-21-5)

Coumarin **7** (0.32 g, 1.4 mmol) was refluxed for 3.5 h in a solution of pyridine/ethylene glycol (1.35 mL/1.21 mL). The solution was cooled, acidified with 2 M HCl and extracted with dichloromethane. Solvent evaporation afforded isofraxidin (**2**) as white crystals (0.25 g, 94%), and the physical and spectroscopic data are in accordance with those previously reported.[19] mp 148-149 ºC (Lit.[19] mp 144 ºC); ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.86 (s, 3H), 4.01 (s,3H), 6.21 (d, *J* 9.0 Hz, 1H), 6.59 (s, 1H), 7.54 (d, *J* 9.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 56.2, 60.8, 105.9, 109.5,112.8, 134.2, 144.5, 145.9, 146.9, 149.6, 164.2.

Altissimacoumarin D (**1**, CAS 1388627-67-5)

To a stirring solution of isofraxidin (0.110 g, 0.31 mmol) in anhydrous tetrahydrofuran (THF, 1.0 mL) was added triphenylphosphine (0.081 g, 0.31 mmol) and geraniol (0.05 mL, 0.29 mmol). DIAD (0.05 g, 0.25 mmol) in THF (0.5 mL) was added dropwise and the reaction mixture left stirring for 24 hours. The solvent was removed and the crude product purified by preparative thin layer chromatography with an eluent of 6% $MeOH/CH_2Cl_2$. The product was extracted from the silica with a solution

of 10% MeOH/CH$_2$Cl$_2$, filtered and the solvent evaporated *in vacuo* to afford altissimacoumarin D as a colourless solid (0.10 g, 54%); mp 225-226 °C; ^{1}H NMR (400 MHz, CDCl$_3$) δ 1.52 (s, 3H), 1.60 (s, 3H), 1.63 (s, 3H), 1.99 (m, 4H), 3.82 (s, 3H), 3.96 (s, 3H), 4.62 (d, *J* 7 Hz, 2H), 5.00 (t, *J* 8 Hz, 1H), 5.49 (t, *J* 8 Hz, 1H), 6.28 (d, *J* 8 Hz, 1H), 6.58 (s, 1H), 7.54 (d, *J* 12 Hz); ^{13}C NMR (100 MHz, CDCl$_3$) δ 16.4, 17.6, 25.6, 26.3, 39.6, 56.2, 61.7, 70.2, 103.5, 114.4, 115.1, 119.6, 123.8, 131.7, 141.8, 142.5, 143.0, 143.4, 144.9, 150.7, 160.5.

Supplementary Information

Supplementary information (^{1}H and ^{13}C NMR spectra) is available free of charge at http://jbcs.org.br as a PDF file.

Acknowledgments

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