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Total Synthesis of (+)-Angelmarin

Jakob Magolan and Mark J. Coster*

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan 4111, Queensland, Australia

m.coster@griffith.edu.au

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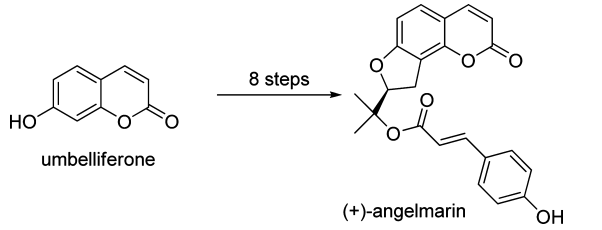
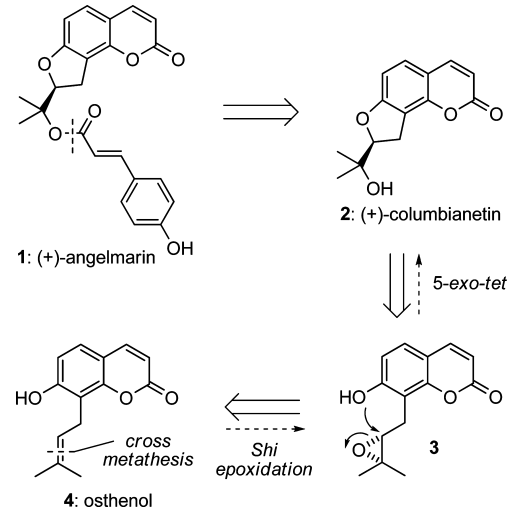


An efficient 8-step enantioselective total synthesis of (+)-angelmarin, starting from commercially available umbelliferone, has been achieved. Key reactions include olefin cross-metathesis and a Shi epoxidation−cyclization sequence.

Cancer cells within rapidly growing tumors are often subject to low oxygen and nutrient supply,[1] and show remarkable tolerance to such starvation conditions.[2] Pancreatic cancer is the most deadly of human malignancies, with the lowest 5-year survival rates of all cancers (generally <5%). It is unresponsive to most current chemotherapeutic agents[3] and displays an astonishing tolerance to extreme nutrient-deprivation over prolonged periods of time.[2a]

An anti-austerity therapeutic strategy, targeting this metabolic adaptation of cancer cells, was proposed in 2000 by Esumi and co-workers.[2a] Through the development of an assay method for antiausterity activity,[4] several natural products have been identified with preferential cytotoxicity to PANC-1 cells in nutrient-deprived medium while showing no activity in nutrient-sufficient medium.[5] Two of these compounds, kigamicin D[5a] and arctigenin,[5c] were further demonstrated to suppress tumor growth of pancreatic cancer cell lines in nude mice.

SCHEME 1. Retrosynthetic Analysis for (+)-Angelmarin



In an effort to identify new antiausterity natural products, Kadota and co-workers reported the structure of (+)-angelmarin (**1**, Scheme 1), isolated by bioassay-guided fractionation of the CH_2Cl_2-extract of *Angelica pubescens*.[5b] Angelmarin (**1**) shows 100% preferential cytotoxicity (PC_{100}) against PANC-1 cells at 0.01 μg/mL. The absolute configuration of this new natural product was assigned through analysis of its circular dichroism spectrum and comparison of specific rotation values for its saponification product to that reported for columbianetin (**2**), a compound identified in 1964 from hydrolysis of two related natural products from *Lomatium columbianum*.[6]

The potent bioactivity of **1** in this unique therapeutic area motivated us to develop an efficient and scalable total synthesis that would allow ready access to structural analogues for biological evaluation and the elucidation of structure−activity relationships (SAR). Our retrosynthetic approach relies on initial disconnection of **1** across the ester to give columbianetin (**2**) and *p*-hydroxycinnamic acid (Scheme 1). In turn, **2** would be derived from epoxide **3**, via base-mediated 5-*exo-tet* cyclization, and the latter compound would be obtained by asymmetric epoxidation of osthenol (**4**), using the Shi protocol.[7]

Racemic columbianetin has previously been prepared by Shipchandler (1970),[8] Steck (1971),[9] and Franke (1971).[10] The syntheses of Steck and Franke utilized a base-mediated 5-*exo-tet* cyclization of a phenolic epoxide (**3**) to achieve the dihydrobenzofuran framework. Franke's synthesis of *rac*-columbianetin was the most efficient to date, providing **2** in 5 steps from commercially available substrates, cf. Shipchandler

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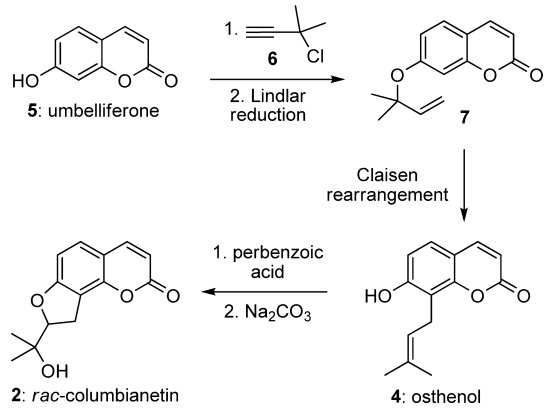
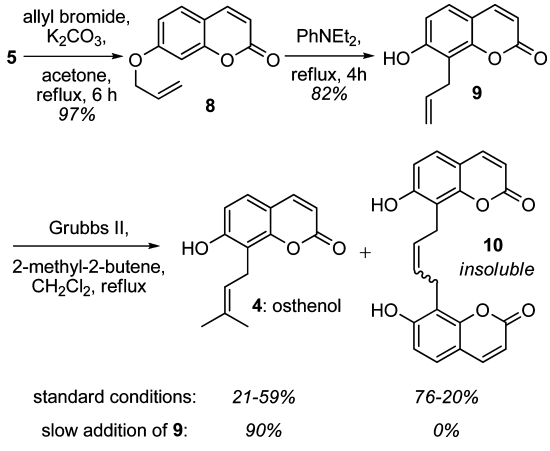
SCHEME 2. Franke's Synthesis of Racemic Columbianetin



SCHEME 3. Synthesis of Osthenol via Cross-Metathesis



(ca. 2% over 10 steps) and Steck (ca. 1% over 4 steps). As illustrated in Scheme 2, Franke converted commercially available umbelliferone (**5**) to osthenol (**4**),[11] via phenol alkylation with 3-chloro-3-methyl-1-butyne (**6**), alkyne reduction using Lindlar's catalyst, and a notably regioselective Claisen rearrangement of **7**, according to a three-step procedure previously developed by Taylor.[12,13] Epoxidation of osthenol (**4**), followed by cyclization in the presence of sodium carbonate, provided *rac*-columbianetin (**2**) in ca. 46% yield over the two steps.

We noted an opportunity to modify the Franke−Taylor strategy by avoiding the use of relatively expensive 3-chloro-3-methyl-1-butyne (**6**),[14] in favor of a simpler allyl ether Claisen rearrangement, followed by olefin cross-metathesis to convert the allyl substituent to the desired isoprenyl moiety. This approach is cost-effective, highly scalable, and has the advantage that analogues of the natural product may be readily accessed through the use of different cross-metathesis partners, greatly facilitating the future extension of this work to explore structure−activity relationships for **1**. Furthermore, compared to previous work, our approach to the key intermediate,

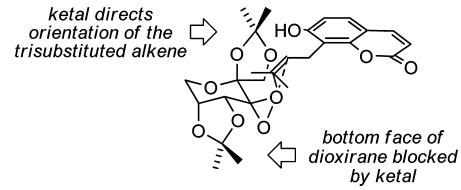


FIGURE 1. Anticipated selectivity for the Shi epoxidation.

columbianetin, via Shi epoxidation allows enantiodivergent access through the use of either enantiomer of the chiral ketone catalyst.

Our synthetic sequence began with allylation of umbelliferone, followed by Claisen rearrangement of allyl ether **8** in refluxing *N*,*N*-diethylaniline to yield **9** (Scheme 3). These steps utilized precedented, highly scalable procedures,[15] requiring no chromatographic purification steps. Cross-metathesis of terminal alkene **9** with 2-methyl-2-butene, in the presence of Grubbs' second generation catalyst[16] was successful on small scales (<200 mg of substrate) under typical reaction conditions (15 mol % of catalyst, 0.015 M in 1:1 v/v 2-methyl-2-butene−CH_2Cl_2).[17] Upon scaling up (>1 g), where the use of large quantities of 2-methyl-2-butene became uneconomical, we observed the formation of significant quantities of the insoluble homodimerization product **10** when the reaction was performed at higher concentration and/or with lower quantities of 2-methyl-2-butene, with concomitant low yields of **4**.[18] The formation of **10** was circumvented, and the quantity of 2-methyl-2-butene minimized, by slow addition of a dilute solution of **9** (0.02 M in CH_2Cl_2) to a refluxing solution of Grubbs II (5 mol %) in 1:1 v/v 2-methyl-2-butene−CH_2Cl_2 (see the Experimental Section for details).

We next sought to exploit the Shi asymmetric epoxidation procedure[7] for the enantioselective conversion of osthenol (**4**) to (+)-columbianetin, via epoxide **3**. Applying the model offered by Shi to our system (Figure 1), we anticipated approach of the dioxirane catalyst from the bottom face of osthenol (as shown). Following olefin epoxidation, 5-*exo-tet* cyclization of the corresponding phenoxide onto the resulting epoxide was thought likely to occur in situ under the basic conditions, to yield (*S*)-columbianetin, corresponding to the natural (+)-enantiomer.

Epoxidation of osthenol with *m*-CPBA in the presence of K_2CO_3 readily yielded *rac*-columbianetin (**2**) in 82% yield (Table 1, entry 1). Application of commonly used Shi epoxidation conditions,[19] which involve simultaneous syringe pump addition of aqueous K_2CO_3 and Oxone solutions to a buffered, biphasic reaction mixture of substrate and D-fructose-derived catalyst (**11**) in DMM−CH_3CN−H_2O, resulted in very slow epoxidation of osthenol (**4**) providing low yields of **2**, with negligible enantioselectivity (Table 1, entries 2 and 3).[20] Attempts to optimize this reaction by varying concentrations and ratios of reagents were not successful. Similarly, little

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(14) 3-Chloro-3-methyl-1-butyne (**6**) is available for $96.20 for 5 g.
(15) Clarke, D. J.; Robinson, R. S. *Tetrahedron* **2002**, *58*, 2831.
(16) Scholl, M.; Ding, S.; Lee, C. W.; Grubbs, R. H. *Org. Lett.* **1999**, *1*, 953.
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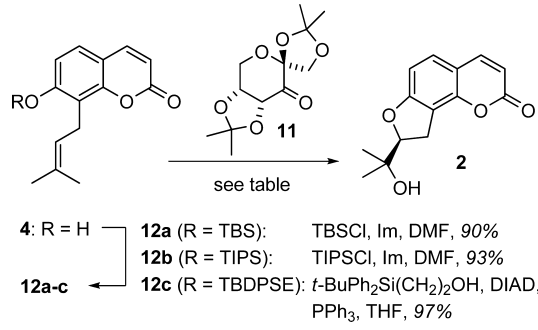
(18) Olefin **10** precipitated from the reaction mixture was isolated by filtration and identified by NMR analysis in d_6-DMSO.
(19) Wang, Z.-X.; Tu, Y.; Frohn, M.; Zhang, J.-R.; Shi, Y. *J. Am. Chem. Soc.* **1997**, *119*, 11224.
(20) The enantiomeric excess of **2** could not be determined by using chiral HPLC under standard conditions; however, polarimetry proved satisfactory, due to the large specific rotation reported for (+)-columbianetin, see ref 5b.

TABLE 1. Shi Epoxidation Study: Synthesis of (+)-Columbianetin



entry	substrate	method and conditions[d]	yield,[a] %	ee,[b] %
1	**4**	*m*-CPBA, K_2CO_3, CH_2Cl_2, rt	82	
2	**4**	A (0.2 equiv of **11**, rt)	35	5
3	**4**	A (1 equiv of **11**, rt)	39	5
4	**4**	B (1 equiv of **11**, 0 °C)	42	9
5	**12a**	A (0.2 equiv of **11**, rt), then TBAF, THF, rt, 2 h	49	70
6	**12b**	A (0.2 equiv of **11**, rt), then TBAF, THF, rt, 2 h	65	68
7	**12b**	A (1 equiv of **11**, rt), then TBAF, THF, rt, 2 h	66	70
8	**12b**	A (0.2 equiv of **11**, 0 °C), then TBAF, THF, rt, 2 h	68	71 (75)[c]
9	**12b**	B (1 equiv of **11**, rt), then TBAF, THF, rt, 2 h	31	59
10	**12c**	A (1 equiv of **11**, rt), then TBAF, THF, 50 °C, 16 h	71	75

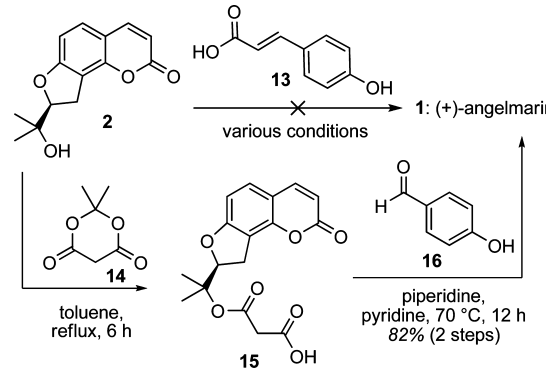
[a] Isolated yields. [b] Approximate ee values were determined by polarimetric analysis in comparison to literature specific rotation values for (+)-**2**.[5b] The correlation between optical purity and ee for **2** was validated with material of 75% ee, see below and ref 25. [c] Product from entry 8 was recrystallized from EtOAc/hexanes to afford material with slightly improved 75% ee. [d] Method A: Ketone **11** (0.2−1 equiv), TBAS (0.2 equiv), Oxone (1.8 equiv), K_2CO_3 (7.2 equiv), MeCN−DMM−0.05 M $Na_2B_4O_7$ in 0.004 M aq Na_2EDTA (1:2:2 v/v). Method B: Ketone **11** (1 equiv) and H_2O_2 (4.0 equiv) in CH_3CN−2.0 M K_2CO_3 in 0.0040 M Na_2EDTA (1:1 v/v), 0 °C.

improvement in ee was observed when Oxone was replaced with H_2O_2 as described in an alternative procedure published by Shu and Shi[21] (Table 1, entry 4). We reasoned that the observed lack of enantioselectivity in these epoxidations is due to the presence of the free phenol functionality in the substrate. To the best of our knowledge, there are no reported examples of Shi epoxidations in the presence of a phenol. Low enantioselectivities have previously been reported for some aliphatic alcohol substrates (at pH <10),[22] with epoxidation by Oxone itself being implicated. However, unlike these reported examples, careful control of pH did not improve selectivity in our system. We propose that the low enantioselectivities we observed are due to significant water solubility of the phenol substrate at the high pH (ca. 10) required for efficient oxidation of **11** by Oxone, resulting in direct epoxidation by Oxone representing the major oxidation pathway.

In an effort to improve the enantioselectivity of the above procedure, we next sought to mask the phenol of osthenol, prior to epoxidation. Our choice of protecting group was restricted by both the alkaline epoxidation conditions and the knowledge that subsequent deprotection conditions would need to be nonacidic, in order to avoid an undesired, acid-promoted, 6-*endo-tet* cyclization of the phenol onto the epoxide **3**. With this in mind, we prepared *tert*-butyldimethylsilyl (TBS) ether derivative **12a**. Gratifyingly, Shi epoxidation of **12a** using standard conditions, followed by treatment of the crude product with TBAF in THF, yielded (+)-columbianetin (**2**) in 49% yield and 70% ee (Table 1, entry 5).

We noted the TBS ether was somewhat labile under the basic epoxidation conditions, as a small amount of columbianetin was observable by ^{1}H NMR prior to TBAF treatment. Thus, the significantly more base-stable triisopropylsilyl (TIPS) ether **12b** was prepared. Shi epoxidation of **12b**, followed by fluoride treatment, resulted in considerably improved yields but similar ee values (Table 1, entries 6−8). Unfortunately, although columbianetin (**2**) could be recrystallized from EtOAc/hexane, this process yielded no improvement in ee beyond 75%. Application of the alternative, H_2O_2-mediated, Shi epoxidation conditions led to decreased yield and ee (Table 1, entry 9). On the basis of the transition state model illustrated in Figure 1, we considered the possibility that the silyl protection of the phenol led to unfavorable steric interaction between this bulky group and the upper acetonide. With this in mind, we prepared a more flexible, and potentially less sterically demanding, derivative **12c** bearing a 2-(*tert*-butyldiphenylsilyl)ethyl (TBDPSE) protecting group.[23] Epoxidation of **12c** and deprotection/cyclization of the crude product yielded (+)-columbianetin with a slight improvement in yield and enantioselectivity (Table 1, entry 10).

SCHEME 4. Completion of the Total Synthesis



To complete the total synthesis of angelmarin, we sought to esterify the tertiary alcohol of (+)-columbianetin (**2**) directly with coumeric acid (**13**, Scheme 4). Not surprisingly, the presence of the nucleophilic phenol of coumeric acid made standard esterification procedures untenable. Furthermore, our efforts to achieve acid-promoted esterification of columbianetin, via a tertiary carbocation, were hampered by lack of reactivity under mild conditions,[24] while more forceful acidic conditions, e.g., *p*-toluenesulfonic acid (TsOH) in refluxing toluene, resulted in elimination/isomerization to give the corresponding benzofuran. A more lucrative, and ultimately successful, approach involved treatment of (+)-columbianetin with Meldrum's acid (**14**) to afford carboxylic acid **15**. Crude **15**, when heated with 4-hydroxybenzaldehyde (**16**) in pyridine in the presence of piperidine, underwent a Doebner−Knoevenagel condensation to yield (+)-angelmarin (**1**) in 82% over the 2 steps. Synthetic **1** provided spectroscopic data in full accordance with that

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(24) Srinivas, K. V. N. S.; Mahender, I.; Das, B. *Synthesis* **2003**, 2479.
(25) Synthetic **1** was obtained from **2** of 75% ee by polarimetry (Table 1, entry 8). The specific rotation for synthetic **1** is consistent with 75% ee and this value was confirmed by chiral HPLC (see the Supporting Information).

reported for the natural product.[5b] The measured specific rotation $[\alpha]^{25}_{D}$ +163 (*c* 0.2, $CHCl_3$) [cf. +218.7 (*c* 0.025, $CHCl_3$)[5b]] matched well in both sign and magnitude, validating the absolute configuration assigned for the natural product.[25]

In conclusion, we have completed the first total synthesis of the antiausterity agent (+)-angelmarin, in 8 steps with an overall yield of 37%. Furthermore, our sequence is well suited to the generation of structural analogues, e.g., by substitution of 4-hydroxybenzaldehyde (**16**) with alternate aldehydes in the final step of the synthesis. The synthesis and biological evaluation of analogues of **1**, providing valuable SAR data, will be reported in due course.

Experimental Section

Osthenol (4). A solution of 2-methyl-2-butene (50 mL) and dichloromethane (50 mL) was briefly deoxygenated by purging with argon, before Grubbs' second generation catalyst (0.367 g, 0.433 mmol) was added. The reaction mixture was heated to reflux (oil bath temperature of 45 °C) and a warm (40 °C) solution of **9** (1.75 g, 8.66 mmol) in deoxygenated CH_2Cl_2 (400 mL) was added via cannula over 2 h. The reaction was heated for an additional 2 h, then cooled to room temperature, absorbed onto silica, and purified via column chromatography (gradient elution with ethyl acetate in petroleum spirits) to yield osthenol (**4**) (1.79 g, 7.80 mmol, 90%) as a pale green solid. Mp 89−91 °C; R_f 0.57 (50% ethyl acetate in petroleum spirits); IR (neat) ν 3270, 1694, 1603, 1572, 1308, 1248, 832 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.62 (d, J = 9.5 Hz, 1H), 7.22 (d, J = 8.5 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 6.30 (s, 1H), 6.24 (d, J = 9.5 Hz, 1H), 5.27 (t, J = 7.0 Hz, 1H), 3.62 (d, J = 7.0 Hz, 2H), 1.86 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 161.8, 158.4, 153.1, 144.3, 135.7, 126.6, 120.3, 114.9, 113.2, 112.7, 112.3, 25.8, 22.1, 18.0; HRMS (ESI-TOF) calcd for $C_{14}H_{14}O_3Na$ $[M + Na]^+$ 253.0835, found 253.0830.

(+)-Angelmarin (1). To a solution of (+)-columbianetin (**2**, 103 mg, 0.418 mmol) in toluene (5 mL) was added Meldrum's acid (**14**, 67 mg, 0.465 mmol). The resulting solution was heated to reflux for 7 h, at which point TLC analysis indicated complete consumption of starting material. The reaction mixture was cooled to room temperature and the solvent was removed in vacuo to yield crude carboxylic acid **15** as a yellow oil that was immediately dissolved in pyridine (5 mL). Piperidine (4 drops) and *p*-hydroxybenzaldehyde (**16**, 56.1 mg, 0.501 mmol) were added and the reaction mixture was heated at 70 °C for 16 h. After being cooled to room temperature, the reaction mixture was diluted with ethyl acetate and washed with 5% HCl, water, and brine, then dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (gradient elution with ethyl acetate in petroleum spirits) to yield (+)-angelmarin (**1**, 134 mg, 82% yield). R_f 0.21 (30% ethyl acetate in petroleum spirits); $[\alpha]^{25}_{D}$ + 163 (*c* 0.2, $CHCl_3$), 75% ee by chiral HPLC, see the Supporting Information. IR (neat) ν 3284, 1733, 1706, 1616, 1606, 1586, 1514, 1489, 1457, 1387, 1330, 1263, 1169, 1136, 1008, 980, 833 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.67 (d, J = 9.5 Hz, 1H), 7.34 (d, J = 16.0 Hz, 1H), 7.29 (d, J = 8.5 Hz, 1H), 7.26 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 6.78 (d, J = 8.5 Hz, 1H), 6.19 (d, J = 9.5 Hz, 1H), 6.12 (d, J = 16.0 Hz, 1H), 5.19 (dd, J = 9.5, 7.5 Hz, 1H), 3.32−3.42 (m, 2H), 1.64 (s, 3H), 1.60 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 164.1, 161.6, 158.4, 151.2, 144.6, 144.3, 129.9, 128.9, 126.6, 116.1, 115.9, 113.6, 113.1, 112.0, 106.9, 89.2, 82.2, 27.5, 22.1, 21.2; HRMS (ESI-TOF) calcd for $C_{23}H_{20}O_6Na$ $[M + Na]^+$ 415.1152, found 415.1171.

Acknowledgment. We thank Professor Shigetoshi Kadota and Dr. Suresh Awale (University of Toyama) for copies of authentic spectra for **1**, the Australian Research Council (ARC) for funding (DP0986795) and a fellowship to M.J.C., and Drs. Joshua Fischer and Hoan Vu (Griffith University) for preparation of *t*-$BuPh_2Si(CH_2)_2OH$ and mass spectrometry, respectively.

Supporting Information Available: General experimental, experimental procedures, characterization data for compounds **8**, **9**, **12**, **13**, **14**, (+)-**2**, and *rac*-**2**, and ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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