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A Study on the Synthesis of Antiangiogenic (+)-Coronarin A and Congeners from (+)-Sclareolide

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Abstract—Coronarin A **1**, *epi*-coronarin A **2** and some synthetic intermediates **14a** and **14b** synthesized from sclareolide exhibit good growth inhibition activities on HUVEC proliferation. In particular, coronarin A **1** and *epi*-coronarin A **2** effectively suppressed the growth factor induced tube formation of HUVEC at the concentration of 10 μg/mL.
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Introduction

Angiogenesis, the development process of the formation of new blood vessels from existing host capillaries, in normal vascular system is involved in wound healing, embryonic development, and the female reproductive cycle under elaborate regulations. On the other hand, in abnormal systems, angiogenesis is believed to be responsible for rheumatoid arthritis, ocular retinopathy and tumors.[1] In particular, tumor angiogenesis caused by angiogenic inducers, such as the fibroblast growth factor (FGF), the vascular endothelial growth factor (VEGF), angiogenin, transforming growth factors (TGF-α and TGF-β), the platelet-derived growth factor (PDGF), the tumor necrosis factor α (TNF-α), interleukins, chemokines, and angiopectins.[2] Angiogenesis plays a key role in the growth of the solid tumors, their invasion, and metastasis. Therefore, the control of angiogenesis may be a promising therapeutic strategy for the related diseases.[3]

Strategies for regulating angiogenesis have been carried out mainly in molecular biology, such as the isolation and identification of the endogenous inhibitor,[4] as well as gene[5] and antibody therapy.[6] However, it has been insufficiently carried out to develop small molecule *antiangiogenic agents despite the settlement of bioavail-*ability, biostability and effectiveness. Therefore, it is very important to discover the antiangiogenic small molecules that might be suitable as clinical therapies.

The initial and key steps in tumor angiogenesis are mainly the roles of the endothelial cells (ECs), the migration, differentiation and tube formation of the ECs.[7] Therefore, in order to search for a novel small angiogenesis inhibitor, this study tested the inhibitory effects of promising natural products and their related compounds against the proliferation of human umbilical vein endothelial cells (HUVEC) in response to the various growth factors.

The furanolabdane diterpenoids, coronarin A (**1**) and coronarin B-F, are isolated from rhizomes of the Brazilian antirheumatic medicinal plant, *Hedychium Coronarium* (Zingiberaceae).[8,9] These compounds exhibit a significant cytotoxic effect against V-79 cells and sarcoma 180 ascites in mice.[8] Because rheumatoid arthritis, complex chronic inflammatory disease in the joints, are basically caused by angiogenesis, the components in antirheumatic *Hedychium C.* are predicted to show antiangiogenic activity.

In the process of synthesizing those natural products, Jung and Lee reported the synthesis[10] and biological properties[11] of 7-*epi*-coronarin A (**2**), coronarin E (**3**)[12] and cytotoxic intermediates from (−)-sclareol (**4**). However they could not synthesize coronarin A (**1**). Therefore, this study reports the first synthesis of

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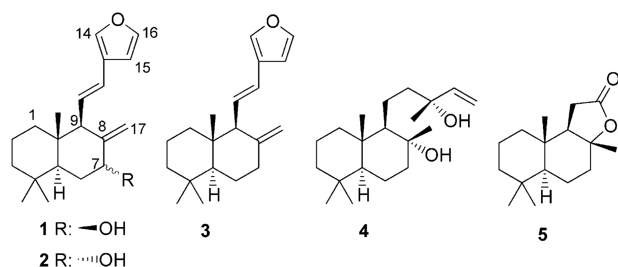
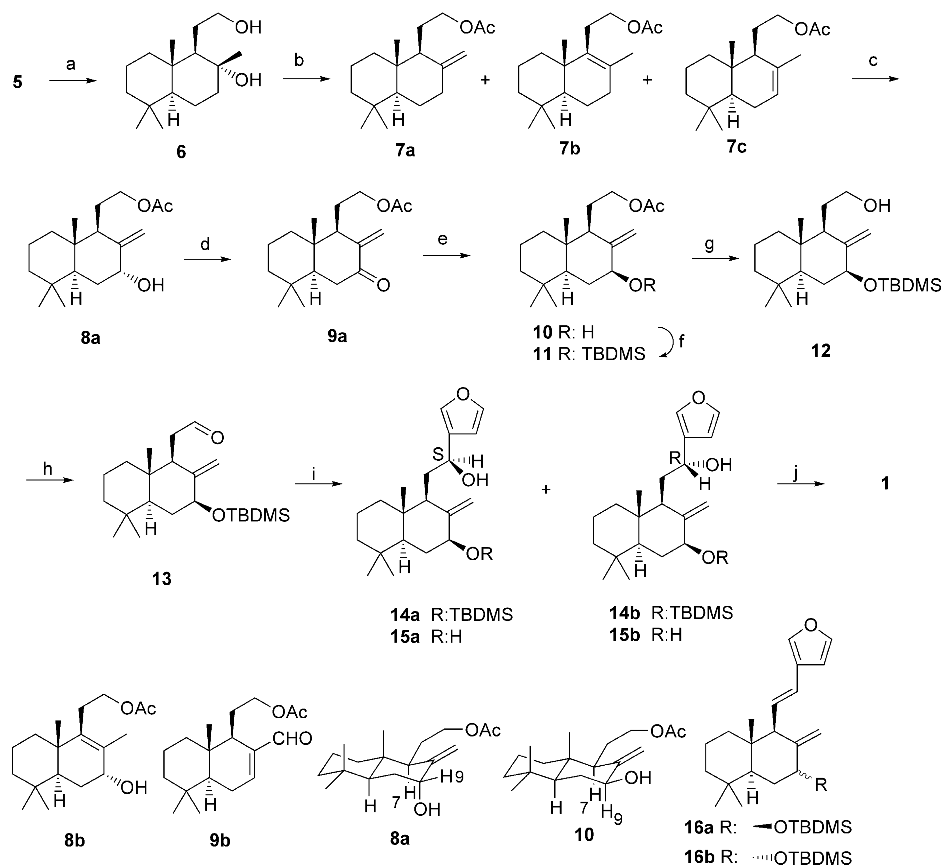


Figure 1.

coronarin (**1**) with development of more efficient synthetic pathway from (+)-sclareolide (**5**), which is a cheap, commercially available and potentially good chiral starting material,[13] and is particularly useful to construct the C-7 stereochemistry of target molecule **1** (Fig. 1).

Chemistry

Hydride reduction of **5** with LAH in THF gave diol (**6**) in a quantitative yield.[14] The treatment of **6** with excess acetic anhydride in collidine afforded an inseparable mixture of exo methylene isomer (**7a**), which is an antifeedant albicanyl acetate homologue,[15] and two endo methylene isomers (**7b** and **7c**), respectively, in the ratio of 3:1:1 in an 85% yield. The allylic hydroxylation of the mixture of **7a**, **7b** and **7c** with SeO_2 and *t*-BuOOH in a methylene chloride (MC) for 2 h gave **8a**, the 7-α-hydroxy isomer as the only product in 45% yields. However after 24 h, it gave a separable mixture of **8a** (47%) and **8b** (12%), which is the allylic hydroxylation product of **7b**. The treatment of **8a** with PCC in MC afforded a α, β-unsaturated ketone (**9a**) and an α, β-unsaturated aldehyde (**9b**), which is an oxidative rearranged product, in a 75% yield, respectively, in the ratio of 8:1. The 7-β-hydroxy isomer (**10**) having a natural stereochemistry of the target compound (**1**) was gained by the reduction of **9a** with $NaBH_4$ in MeOH in a 98% yield. The stereochemistry of the 7-position in **8a** and **10** could be conformed in comparison with the lower NOESY effect of the 9-axial and 7-equitorial hydrogens in **8a** and the larger effect the 9-axial and 7-axial hydrogens in **10**.[8] Deacetylation of **11**, which was a TBDMS protecting compound of **10**, gave **12**, which was transformed by PCC oxidation to the aldehyde molecule (**13**) in an 80% overall yield from **10**. The unstable aldehyde (**13**) was immediately reacted with 3-furyllithium at −78 °C, which was generated 3-bromofuran with *n*-butyllithium at −78 °C under afforded the separable diastereomeric mixture **14a**, which is a sterically less hindered major product, and **14b** in 72% yield. The stereochemistry of the C-12 of



Scheme 1. Reagents and conditions: (a) LAH, THF, reflux, 5 h, 97%; (b) Ac_2O, collidine, reflux, 16 h, 85%; (c) SeO_2, *t*-BuOOH, MC, rt, 2 h, 45%; (d) PCC, MC, rt, 3 h, 75%; (e) $NaBH_4$, MeOH, rt, 1 h, 98%; (f) TBDMSCl, $AgNO_3$, DMF, rt, 1 h, 89%; (g) Na_2CO_3, MeOH, rt, 2 h, 92%; (h) PCC, MC, rt, 3 h, 98%; (i) 3-bromofuran, *n*-BuLi, THF, −78 °C to rt, 72%, **14a**: **14b** = 3:1; (j) 2,6-lutidine, MsCl, MC, rt, 24 h and then gently warming to evaporate solvent, 68%.

14a and **14b** could not be conformed by the NOSEY spectra because of the flexibility of the C-9 furan side chain. The dehydration of the diastereomeric mixture **14** with 2,6-lutidine (10 equiv) in the presence of methanesulfonyl chloride and then the subsequent desilyation afforded the coronarin A (**1**) exclusively in 68% yield via **16a** (Scheme 1).

Biology

Initially, the antiangiogenic effect of coronarin A **1** and its related molecules were examined on a HUVEC[16] proliferation assay using the MTT colorimetric method.[17] The results are listed in Table 1. Coronarin A (**1**) and its epimer (**2**) showed potent inhibition activities whereas sclareol (**3**) and sclareolide (**4**), a *trans*-decalin structure with no furan moiety, exhibit no activity. Compounds **14a** and **14b**, the precursors of coronarin A with a furan group, exhibited a similar activity to **1** and **2**. Although **15a**, **15b** and **16a**, **16b** have a similar structure to coronarin A (**1**) and its related antiangiogenic molecules (**2**, **14a** and **14b**) they have no significant inhibition effect on HUVEC growth. So, when assuming the growth inhibition effects in Table 1, it was hypothesized that the proper hydrophilic *trans*-decalin with a conjugated furan group might be a lead compound for use as angiogenic inhibitors.

Next, their ability to suppress the growth factor induced tube formation by HUVEC was assessed at the concentration of 10 μg/mL.[18] As shown in Table 2, *epi*-coronarin A (**2**) effectively inhibited the tube formation by 90% while coronarin A exhibited mild inhibition activity by 55%. Compounds (**14a**, **14b**, **15a**, **15b**, **16a** and **16b**) failed to inhibit the blood tube formation to some degree.

Table 1. HUVEC proliferation inhibition assay results for selected compounds[a]

Compd	Growth inhibition IC_{50} (μg/mL)	Compd	Growth inhibition IC_{50} (μg/mL)
1	4.22	**14b**	3.76
2	3.12	**15a**	29.67
4	> 50	**15b**	15.03
5	> 50	**16a**	> 50
14a	7.71	**16b**	> 50

[a]IC_{50} was calculated from nonlinear regression by GraphPad Prism software.

Table 2. HUVEC tube formation assay results for selected compounds[a]

Compd	Inhibition percentage at 10 μg/mL	Compd	Inhibition percentage at 10 μg/mL
1	55	**14b**	10
2	90	**15a**	10
4	Not tested	**15b**	0
5	Not tested	**16a**	22
14a	20	**16b**	5

[a]Values expressed in percentage of HUVEC tube branches/well as compared to untreated control.

In conclusion, coronarin A (**1**), a constituent of the Brazilian antirheumatic medicinal plant, its epimer (**2**) and some intermediates (**14a** and **14b**) have a high growth inhibition effect on HUVEC. In particular, coronarin A (**1**) and *epi*-coronarin A (**2**) have shown an inhibition effect on the endothelial tube formation. With the early studies on the structure–activity relationship of the coronarin analogues, this study confirmed that the furanoladane-type natural product would be excellent lead compound for use as an antiangiogenic inhibitor. Currently, detailed studies on the structure–activity relationships coupled with molecular modeling aimed at developing new and efficient angiogenic inhibitors are underway.

Acknowledgements

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15. Spectral data of selected compounds. **14a**: white crystal, mp 109–111 °C; $[\alpha]_D^{20}$ −32.0 (c = 0.08, $CHCl_3$); IR (KBr pellet) ν_{max} 3363, 3098, 1463, 836 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ7.41 (1H, t, J = 1.56 Hz, H16), 7.29 (1H, t, J = 0.93 Hz, H15), 6.41 (1H, dd, J = 1.65 Hz, 0.63 Hz, H14), 5.32 (1H, d, J = 1.35 Hz, H17), 4.85 (1H, d, J = 1.35 Hz, H17), 4.73 (1H, dd, J = 9.99 Hz, 4.59 Hz, H12), 3.77 (1H, dd, J = 10.89 Hz, 5.13 Hz, H7), 2.03 (1H, td, J = 13.3 Hz, 4.50 Hz, H9), 1.89 (2H, m, H11), 0.93 (9H, s, *t*-Butyl), 0.84, 0.79, 0.69 (each 3H, s, H20, H19, H20) 0.07 (6H, d, J = 6.57 Hz, Si-$(CH_3)_2$) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ150.1, 143.6, 139.7, 128.6, 108.2, 104.5, 74.9, 65.7, 53.1, 51.2, 41.9, 39.1, 38.7, 34.5, 33.3, 31.5, 25.9, 21.6, 19.3, 18.6, 14.6, −5.0 ppm; GC/MSD (m/z) reten-

tion time 23.2 (min) 432 (M^+), 414, 399, 375, 357, 320, 300, 263, 141, 97, 75 (100); **14b**: white crystal, mp 104–106 °C; $[\alpha]_D^{20}$ −190 (c = 0.02, $CHCl_3$); IR (KBr pellet) ν_{max} 3421, 3098, 1462, 837cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ7.39 (2H, br s, H15, H16), 6.42 (1H, br s, H14), 5.30 (1H, d, J = 1.38 Hz, H17), 4.66 (1H, t, J = 5.67 Hz, H12), 4.62 (1H, d, J = 1.38 Hz, H17), 3.98 (1H, dd, J = 10.62 Hz, 5.22 Hz, H7), 0.94 (9H, s, t-Butyl), 0.90, 0.81, 0.67 (each 3H, s, H20, H19, H18) 0.09 (6H, d, J = 4.32 Hz, Si-$(CH_3)_2$) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ150.2, 143.3, 138.5, 1 30.2, 108.5, 104.2, 74.9, 65.2, 53.2, 50.6, 42.0, 39.0, 38.9, 34.6, 33.4, 32.5, 26.0, 21.6, 19.3, 18.5, 14.6, −4.9 ppm; GC/MSD (m/z) retention time 22.8 (min) 432 (M^+), 414, 399, 375, 357, 320, 300, 263, 141, 97, 75(100); **1**: $[\alpha]_D^{20}$ +24.5 (c = 0.28, $CHCl_3$) ($[\alpha]_D^{20}$ +25.4 (c = 0.28, $CHCl_3$) in ref 8) ^{1}H NMR (300 MHz; $CDCl_3$) δ7.37 (1H, br s, H16), 7.36 (1H, br s, H15), 6.56 (1H, d, J = 0.84 Hz, H14), 6.21 (1H, d, J = 15.7 Hz, H12), 5.99 (1H, dd, J = 15.7, 9.72 Hz, H11), 5.13 (1H, s, H17), 4.74 (1H, s, H17), 4.08 (1H, dd, J = 10.72 Hz, 5.28 Hz, H7), 2.35 (1H, d, J = 9.63 Hz, H9), 0.92, 0.85, 0.84 (each 3H, s, H20, H19, H18) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ151.8, 143.4, 139.8, 127.2, 124.4, 121.6, 108.5, 104.9, 73.4, 59.5, 52.8, 42.2, 40.4, 39.2, 33.6, 33.5, 33.2, 21.6, 19.8, 14.5 ppm.

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