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FIRST SYNTHESIS OF RACEMIC METHYLOPHIOPOGONANONE B AND ITS INHIBITORY ACTIVITY OF HYPOXIA-INDUCIBLE FACTOR-1α

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Abstract – Methylophiopogonanone B, a constituent of Ophiopogonis tuber, found to be a potent inhibitor of hypoxia induced factor-1α (HIF-1α) activity, was synthesized from 2,4,6-trihydroxy-3,5-dimethylacetophenone in 57% yield. The synthetic methylophiopogonanone B inhibited the reporter activity at 3 to 10 μg/mL.

Methylophiopogonanone B (**1**) (Figure 1) is a homoisoflavanone, found in Ophiopogonis tuber (*Ophiopogon japonicus* KER-GAWLER var. genuinus Maxim. or *Ophiopogon japonicus* KER-GAWLER cv. NANUS, Liliaceae) that was used as a crude drug.[1-5]

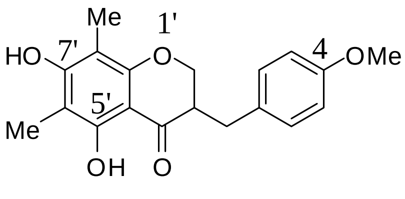
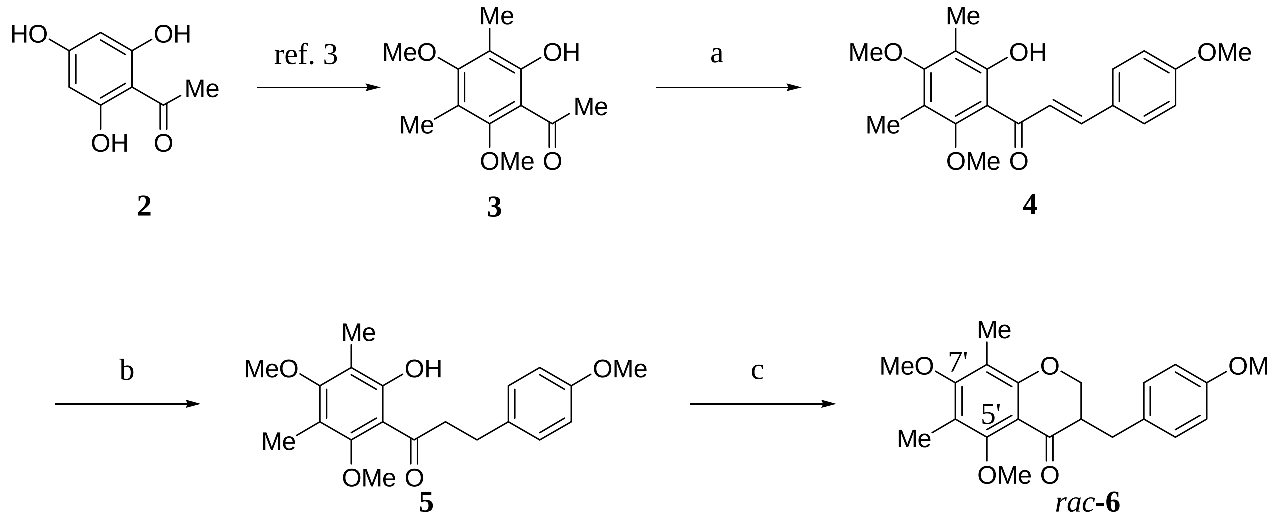


Figure 1. Structure of methylophiopogonanone B (**1**)

In a previous study, it was found that **1** inhibited hypoxia-inducible facter-1α (HIF-1α) activity *in vitro*.[6] Hif-1α is a key transcription factor which regulates expression of several genes involved in the hypoxia-responses and angiogenesis during carcinogenesis, and **1** can be a potentially angiogenic inhibitor. As **1** was a minor constituent in Ophiopogonis tubers (0.0002%), we could not continue *in vivo* experiment. For further biological and pharmaceutical studies, the suppliment of **1** by the organic synthesis was demanded. In this paper, we report the synthesis of *rac*-**1** and its HIF-1α inhibitory activity.

2,4-Dimethoxy-3,5-dimethyl-6-hydroxyacetophenone (**3**) was obtained from 2,4,6-trihydroxyacetophenone (**2**).[7] Compound **3** was condensed with 4-methoxybenzaldehyde to afford chalcone **4** in 84% yield. Then, hydrogenation of **4** gave dihydrochalcone **5** in 94% yield. Construction of C-ring of the homoisoflanvone by the use of sodium and formaldehyde,[3] or Kirkiacharian's method[8] was not achieved. Instead, Jaspal's method using formaldehyde and diethylamine was applied, and homoisoflavanone *rac*-**6** was obtained.[9]

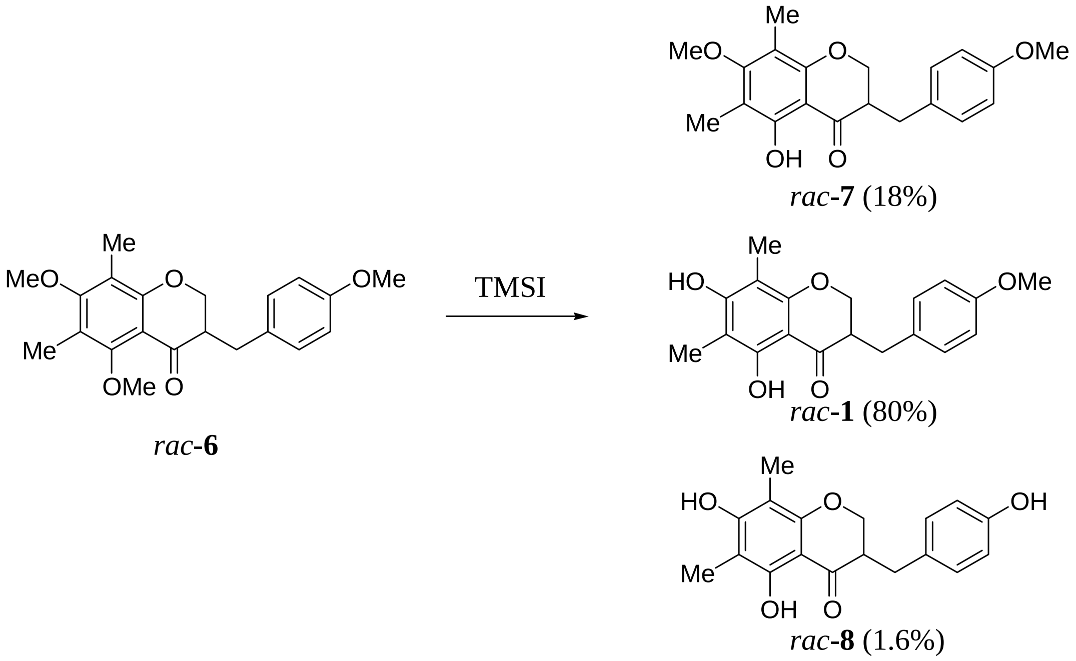


Scheme 1. Conditions: a) 4-methoxybenzaldehyde, potassium hydroxide, MeOH - water, 84%. b) H2, 5% Pd/C, 94%. c) formaldehyde, diethylamine, 94%

The regioselective cleavages of methyl ether at 5' and 7' position of *rac*-**6** are recommended for the synthesis of **1**. We tested several acidic conditions to obtain *rac*-**1**, and iodotrimethylsilane (TMSI) was found to be effective to the selective cleavage. On treatment of *rac*-**6** with 2.0 equivalents of TMSI at rt for 8 h, demethylation was occurred selectively at 7' position to afford *rac*-**7** in 84% yield. While, treatment of *rac*-**6** with 4.0 equivalent of TMSI at 50 ˚C for 7 days gave *rac*-**1** in 80% yield accompanied with *rac*-**7** (18%) and *rac*-**8**[10] (1.6%). This selectivity of TMSI against two methoxy groups can be considered that the acidity of hydroxy group at 7' of *rac*-**1** is higher than that of normal phenol since the C-7' hydroxyl group is affected by the conjugation of C-4 carbonyl group and the steric effects by two *o*-methyl groups.

The ^{1}H and ^{13}C NMR of synthetic *rac*-**1** was identical with the authentic sample of natural **1**, and this is the first synthesis of *rac*-**1**.

Next, we examined HIF-1α inhibitory activity of synthetic *rac*-**1** by the reporter assay.[6,11] A4-4 cells were cultured in the presence of various concentrations of synthetic *rac*-**1** for 16 h under hypoxic condition, and then luciferase activity (filled bar) and cell viability (open squares) were measured. As can be seen in Figure 2, synthetic *rac*-**1** was effective at 3 - 10 μg/mL in inhibiting the reporter activity without affecting cellular survival significantly.



Scheme 2

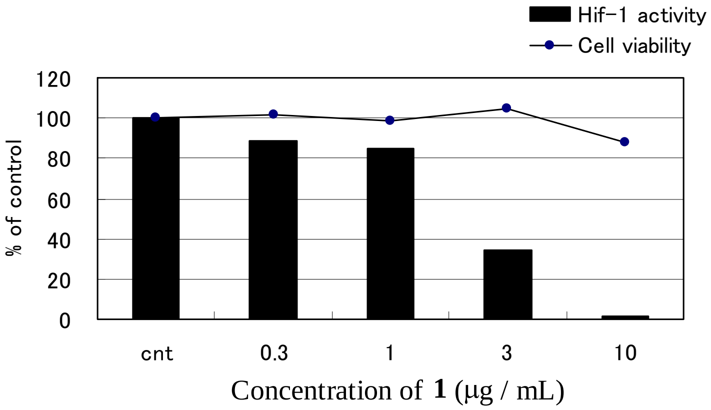


Figure 2. Effect of synthetic **1** on reporter activity induced by hypoxia.

We succeeded in synthesizing *rac*-methylophiopogonanone B (*rac*-**1**) from **3** in 57% overall yield, and the synthetic *rac*-**1** showed HIF-1α inhibitory activity in the same concentration range as natural **1**.[6]

In addition, it was reported that **1** promoted Rho activation and tublin depolymerization.[12] Our synthetic route is important to investigate the anticancer activity of **1** and its derivatives. Study of structure and the activity relationship of **1** are on progress.

EXPERIMENTAL

General

The NMR spectra were recorded in $CDCl_3$ on JEOL-AL-400 spectrometer. Mass spectra were measured by JEOL JMS-AM II 50. Elemental analysis was achieved by EA-1108 Elemental Analyzer (Fisons Inc.). Compound **6** was synthesized as following the reported procedure.[3]

5',7'-Dimethoxy-6',8'-dimethyl-3'-(4-methoxybenzyl)chroman-4'-one (*rac*-6)

To the solution of **5** (1.00 g, 2.91 mmol) in EtOH (75 mL) were added paraformaldehyde (0.30 g, 0.01 mol) and diethylamine (3.00 mL, 2.11 g, 28.8 mmol) and the reaction mixture was refluxed for 24 h. The mixture was evaporated under reduced pressure, the residue was dissolved in Et_2O and washed with water. The ethereal solution was dried over $MgSO_4$ and evaporated under reduced pressure to afford 5,7-dimethoxy-6,8-dimethyl-3-(4-methoxybenzyl)chroman-4-one (**6**) (0.97 g, 2.72 mmol, 94%) as a colorless oil. ^{1}H NMR (400 MHz,$CDCl_3$) δ: 2.08 (3H, s), 2.13 (3H, s), 2.63 (1H, dd, *J* = 10.8, 14 Hz), 2.71-2.77 (1H, m), 3.17 (1H, dd, *J* = 4.0, 14 Hz), 3.71 (3H, s), 3.77 (3H, s), 3.78 (3H, s), 4.10 (1H, dd, *J* = 8.0, 11.2 Hz), 4.30 (1H, dd, *J* = 4.4, 11.2 Hz), 6.83 (2H, d, *J* = 8.4 Hz), 7.13 (2H, d, *J* = 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 8.9, 9.1, 32.1, 48.8, 55.6, 60.4, 61.4, 69.1, 111.7, 114.3, 114.3, 115.5, 118.8, 130.4, 130.4, 130.8, 158.2, 158.6, 160.0, 163.4, 192.6. EI-HR-MS calcd for $C_{21}H_{24}O_5$: 356.1624. Found: 356.1628.

Selective cleavage of methoxy group of 6 by TMSI for the synthesis of *rac*-1

To the solution of *rac*-**6** (234 mg, 0.659 mmol) in $CHCl_3$ (1.0 mL) under N_2 atmosphere was added TMSI (0.80 mL, 2.6 mmol), and the resultant mixture was stirred at 50 ˚C. The course of the reaction was monitored by TLC and the reaction proceeded slowly and stopped after 7 d. The resulting mixture was added to aq. NaCl (20 mL) and extracted by Et_2O (20 mL) three times. The ethereal solution was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography (silica gel (10 g), hexane - AcOEt (20:1, v/v)) to afford *rac*-**7** (43.3 mg, 0.126 mmol, 18%), *rac*-**1** (174 mg, 0.530 mmol, 80%) and 5,7-dihydroxy-6,8-dimethyl-3-(4-hydroxybenzyl)-chroman-4-one (*rac*-**8**) (3.3 mg, 0.110 mmol, 1.6%).

rac-**7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.03 (3H, s), 2.08 (3H, s), 2.69 (1H, dd, *J* = 10, 13.6 Hz), 2.78-2.83 (1H, m), 3.16 (1H, dd, *J* = 4.0, 13.6 Hz), 3.71 (3H, s), 3.78 (3H, s), 4.10 (1H, dd, *J* = 7.6, 11.4

Hz), 4.28 (1H, dd, *J* = 4.4, 11.4 Hz), 6.84 (2H, d, *J* = 8.8 Hz), 7.13 (1H, d, *J* = 8.8 Hz), 12.12 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 7.8, 8.2, 31.8, 47.0, 55.2, 60.2, 68.8, 104.4, 109.1, 111.0, 114.0, 114.0, 129.8, 130.0, 130.0, 157.8, 158.4, 159.5, 165.1, 199.3. *Anal.*Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48. Found: C, 70.00; H, 6.50 %. The ^{1}H and ^{13}C NMR spectra of *rac*-**8** were superimposable with those reported value.[10]

rac-**1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.01 (3H, s), 2.05 (3H, s), 2.68 (1H, dd, *J* = 10.8, 13.8 Hz), 2.75-2.80 (1H, m), 3.16 (1H, dd, *J* = 4.4, 13.8 Hz), 3.78 (3H, s), 4.10 (1H, dd, *J* = 7.2, 11.6 Hz), 4.26 (1H, dd, *J* = 4.4, 11.6 Hz), 5.38 (1H, s), 6.84 (2H, d, *J* = 8.8 Hz), 7.13 (2H, d, *J* = 8.8 Hz), 12.36 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 6.8, 6.8, 7.3, 32.0, 46.8, 55.3, 68.9, 101.5, 102.3, 114.1, 120.5, 129.6, 130.1, 130.1, 156.5, 158.4, 159.6, 160.6, 198.5. EI-HR-MS calcd for $C_{19}H_{20}O_5$: 328.1311. Found: 328.1315.

Measurement for HIF-1α inhibitory activiy of *rac*-1.

Cell Culture: A stable transformant of CHO cells (clone A4-4) was established by the transfection of HIF-1-dependent luciferease (5XHRE/pGL3/VEGF/E1b) and neomycin-resistant genes as described previously.[13]

Luciferase assay: Cells were plated into 96-well tissue culture plates (Falcon) at a density of 1 X 10^4 cells/well, and treated with synthetic methylophiopgonanone (**1**) 16 h later. They were incubated further 48 h under hypoxic conditions, and harvested for the determination of luciferase activity. The assay was carried out using a kit provided by Promega Corp. (Madison, WI, USA) following the manufacturer's manual. The cytotoxicity was estimated by the MTT method as previously reported.[13]

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