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SYNTHESIS OF METHYLOPHIOPOGONANONE A

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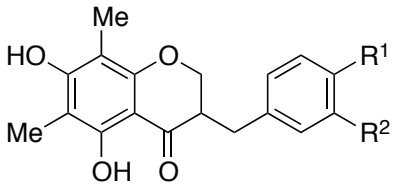
Abstract – Ophiopogon Root (root of *Ophiopogon japonicus* Ker-Gawler, Liliaceae) is a crude drug used as expectorant, anti-cough and tonic in Kampo medicine (traditional Japanese medicine) as well as other traditional medicines of Asian countries. It contains characteristic homoisoflavonoids with methylated ring A. We synthesized methylophiopogonanone A (**1**), which is a candidate marker compound for identification test of Ophiopogon Root, from phloroglucinol in 9 steps with overall yield of 11.1%.

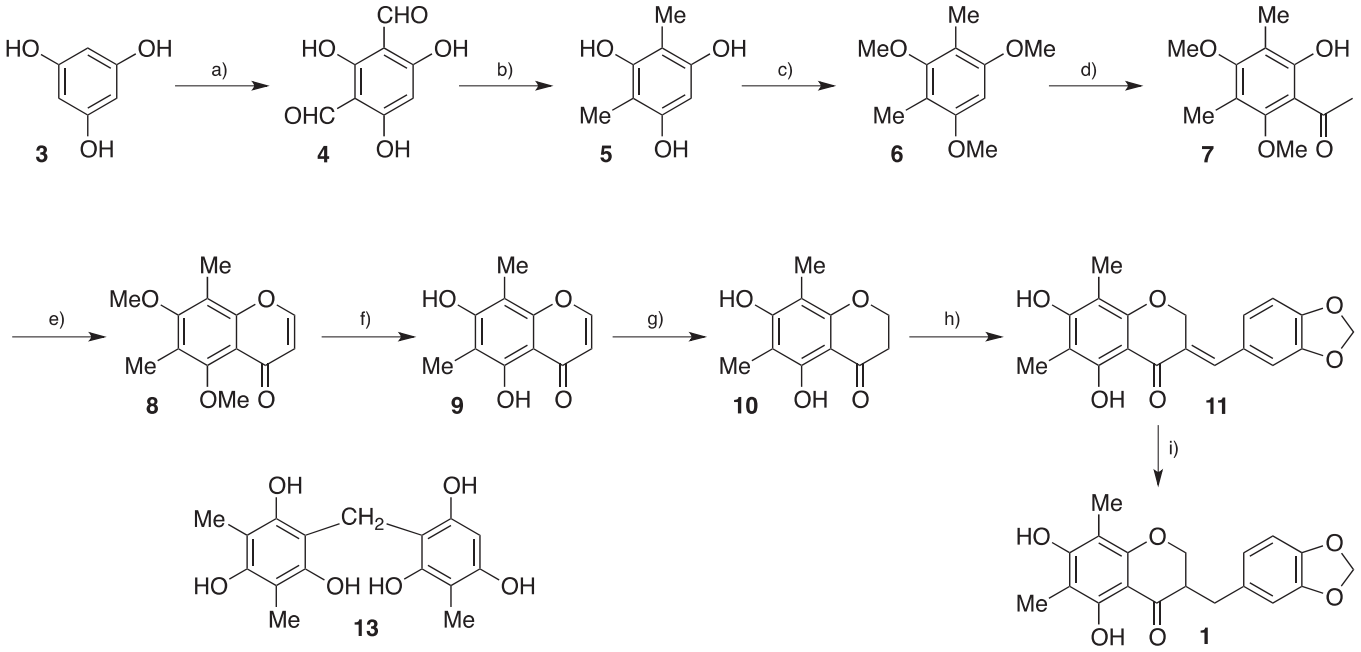
Ophiopogon Root (enlarged part of the root of *Ophiopogon japonicus* Ker-Gawler, Liliaceae)[1] is a crude drug used in Kampo medicine (traditional Japanese medicine) as well as other traditional medicines of Asian countries. It is used as expectorant, anti-cough and tonic. It contains characteristic homoisoflavonoids with methylated ring A.[2] One of the methylated homoisoflavonoids, methylophiopogonanone (MOPN) A (**1**), serves as a marker compound in identification of this crude drug by TLC. Although a standard compound is necessary for identification by TLC, purification of this compound from natural sources is not cost-effective and synthesis of MOPN A has not been reported.



methylophiopogonanone A (**1**): R^1,R^2=-OCH_2O-
methylophiopogonanone B (**2**): R^1=OMe, R^2=H

Synthesis and inhibitory activity against hypoxia-inducible factor-1a was reported for MOPN B (**2**),[3] which differs the substituents on ring B from MOPN A. In the reported synthesis of MOPN B, the methylated ring A part was synthesized from 2,4,6-trihydroxyacetophenone (phloroacetophenone). However, the yields of *C*-methylation products were very low and the yield of desired 3,5-dimethyl derivative (**7**) was only 6.6%.[4] Recently, Jung et al. reported an efficient preparation method of 3,5-dimethylated phloroacetophenone derivative starting from phloroglucinol (**3**) in the synthesis of

6,8-dimethylated isoflavones.[7] In this paper, we applied this method to the synthesis of MOPN A (Scheme 1).



Scheme 1. Synthesis of methylophiopogonanone A (**1**). a) i) $POCl_3$/DMF, 1,4-dioxane, ii) H_2O, rt, 3 h (86%); b) $NaBH_3CN$/HCl, THF, rt, 3 h (58%); c) Me_2SO_4/K_2CO_3, acetone, overnight (82%); d) $Ac_2O/BF_3 \cdot Et_2O$, Ar, 90 ºC, 1.5 h (83%); e) i) HCO_2Et, NaH, Ar, 0 ºC, 3 h, ii) HCl/MeOH, rt, overnight (93%); f) i) $AlCl_3$, benzene, Ar, reflux, 3 h, ii) HCl, 0 ºC (97%); g) H_2/Pd-C, acetone/MeOH, rt, overnight (99%); h) piperonal, HCl/EtOH, 90 ºC, 1 h (61%); i) H_2/Pd-C, acetone/MeOH, rt, overnight (60%).

2-Hydroxy-4,6-dimethoxy-3,5-dimethylacetophenone (**7**) was prepared according to the procedure reported by Jung et al.[7] Phloroglucinol (1,3,5-trihydroxybenzene, **3**) was formylated with the Vilsmeyer reagent to give a di-formyl compound **4** in 86% yield. The formyl groups of **4** were reduced by $NaBH_3CN$ to give 1,3,5-trihydroxy-2,4-dimethylbenzene **5** in 58% yield. In this reaction, a dimeric product **13** (y. 10%) was also obtained. The dimethylated phloroglucinol **5** was methylated with dimethyl sulfate (y. 82%) followed by acylation with acetic anhydride/BF_3-Et_2O (y. 83%) to give 2-hydroxy-4,6-dimethoxy-3,5-dimethylacetophenone (**7**). Preparation of the chromone from **7** followed the report of Mukerjee et al.[9] Condensation of the acetophenone **7** and ethyl formate with NaH followed by an acid treatment gave chromene **8** in 93% yield. Demethylation of the chromene **8** by $AlCl_3$ (y. 97%) followed by a catalytic hydrogenation of the double bond (y. 99%) gave the chromone **10**. Condensation of the chromone **10** with piperonal (y. 61%)[10] followed by catalytic hydrogenation (y. 60%) gave MOPN A (**1**). The ^{1}H- and ^{13}C-NMR spectral data of the synthetic compound were in good agreement with the reported data.[2a] Thus, we synthesized MOPN A from phloroglucinol in 9 steps with the overall yield of 11.1%.

EXPERIMENTAL

All melting points are uncorrected. The IR spectra were recorded on a JASCO FT/IR-4700 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a Varian 400-MR spectrometer at 400 and 100 MHz, respectively, Varian Unity Inova 500-MR spectrometer at 500 and 125 MHz, or with a JEOL JNM-ECP-600 spectrometer at 600 and 150 MHz. The ^{1}H and ^{13}C chemical shifts (δ) are reported in parts per million (ppm) relative to TMS as internal standard. EMI-MS were obtained on a JEOL MS-T100LPAccuTOF LC-plus.

2,4,6-Trihydroxyisophthalaldehyde (4)[7]

Phosphorous oxychloride (1.92 mL, 20 mmol) was added dropwise to *N*,*N*-dimethylformamide (DMF, 1.56 mL, 20 mmol) at 0 ºC with stirring under argon, and the mixture was stirred at room temperature for 30 min to form Vilsmeyer reagent. The Vilsmeyer reagent diluted with anhydrous 1,4-dioxane (3 mL) was added dropwise to a stirred solution of phloroglucinol **3** (1.0 g, 7.9 mmol) in anhydrous 1,4-dioxane (5 mL) under argon at 0 ºC. The mixture was slowly warmed to room temperature and the stirring was continued overnight. The mixture was added water and stirred for 3 h. The precipitates were collected by filtration and refluxed with water for 5 min, collected by filtration and dried to give **4** (1.17 g, 81%). Combined filtrates were extracted with AcOEt and the AcOEt layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography (hexane:AcOEt=1:1) to give additional **4** (70 mg, 4.8%).

4: pale pink crystalline powder (hexane-AcOEt), mp 218–220 ºC [lit. 218–220 ºC].[7] ^{1}H-NMR (400 MHz, DMSO-d_6): 10.02 (s, 2H), 5.91 (s, 1H). ^{13}C-NMR (150 MHz, DMSO-d_6): 191.3, 169.3, 168.9, 103.7, 94.0.

2,4-Dimethylbenzene-1,3,5-triol (5)[7]

To a stirred mixture of **4** (1.5 g, 8.2 mmol) and sodium cyanoborohydride (2.6 g, 41 mmol) in THF (80 mL) containing methyl orange as an indicator, 3N HCl (14 mL) was added slowly to keep the orange color of the mixture over 3 h. After completion of the reaction, water was added and extracted with AcOEt. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography (hexane:AcOEt=7:3) to give **5** (734 mg, 58%) together with a dimeric product **13** (120 mg, brown solid, mp 122–123 ºC).

5: orange crystalline powder (hexane-AcOEt), mp 162–163 ºC [lit. 158–160 ºC].[7] ^{1}H-NMR (400 MHz, CD_3OD): 5.95 (s, 1H), 1.98 (s, 6H). ^{13}C-NMR (125 MHz, CD_3OD): 155.3, 154.5, 104.1, 96.0, 8.6.

13: ^{1}H-NMR (500 MHz, CD_3OD): 6.01 (1H, s), 3.72 (2H, s), 2.04 (6H, s), 1.98 (3H, s). ^{13}C-NMR (125 MHz, CD_3OD): 155.6, 154.8, 153.2, 152.9, 151.7, 109.1, 107.8, 105.8, 105.0, 95.8, 18.9, 9.2, 8.6.

1,3,5-Trimethoxy-2,4-dimethylbenzene (6)[7]

To a mixture of **5** (0.5 g, 3.25 mmol), K_2CO_3 (2.7 g, 20 mmol) in anhydrous acetone (10 mL), dimethyl sulfate (2.0 mL, 21 mmol) was added dropwise and the mixture was stirred under argon overnight. The mixture was filtered and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (hexane:AcOEt=9:1) to give **6** (0.52 g, 82%).

6: colorless needles (EtOH-H_2O), mp 46–47 ºC [lit. 46–48 ºC].[7] ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.28 (s, 1H), 3.82 (s, 6H), 3.68 (s, 3H), 2.10 (s, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.8, 156.6, 111.5, 91.6, 60.1, 55.8, 8.5.

1-(2-Hydroxy-4,6-dimethoxy-3,5-dimethylphenyl)ethanone (7)[7]

To a mixture of **6** (0.36 g, 1.8 mmol) and acetic anhydride (1.0 mL, 11 mmol), boron trifluoride diethyl etherate (0.50 mL, 4.0 mmol) was added dropwise at 0 ºC under argon. The mixture was stirred at 90 ºC for 1.5 h, then cooled to room temperature and stirred overnight. Water (12 mL) was added to the mixture and stirred for 1 h. The mixture was extracted with AcOEt and the organic layer was washed with, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography (hexane:AcOEt=9:1) to afford **7** (0.34 g, 83%).

7: yellow needles (EtOH-H_2O), mp 48–49 ºC [lit. 49–51 ºC].[7] ^{1}H-NMR (400 MHz, $CDCl_3$): δ 13.19 (s, 1H), 3.74 (s, 3H), 3.73 (s, 3H), 2.72 (s, 3H), 2.16 (s, 3H), 2.13 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 204.5, 163.7, 161.4, 159.1, 115.6, 115.3, 111.6, 61.6, 60.1, 31.6, 9.2, 8.6.

5,7-Dimethoxy-6,8-dimethylchromone (8)

To a solution of **7** (0.44 g, 2.0 mmol) in ethyl formate (16 mL), sodium hydride (60% dispersion in mineral oil, 0.70 g, 18 mmol) was added dropwise at 0 ºC over a period of 80 min under nitrogen, and the mixture was stirred for 1.5 h. The reaction was quenched with MeOH (1.0 mL) and conc. HCl (3.2 mL) was added. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was added water and extracted with $CHCl_3$. The organic extract was washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel column chromatography (hexane:AcOEt=3:2) to afford **8** (0.43 g, 93%).

8: colorless needles (hexane-AcOEt), mp 92–94 ºC. HR-MS: *m/z* 257.09143 $[M+Na]^+$ (calcd for $C_{13}H_{14}NaO_4$, 257.07898); IR: 1656 cm^{-1} (C=O); ^{1}H-NMR (400 MHz, $CDCl_3$): 7.76 (d, *J*=6.0 Hz, 1H), 6.21 (d, *J*=6.0 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 2.32 (s, 3H), 2.27 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): 176.9, 161.3, 156.1, 155.2, 153.2, 122.7, 116.2, 115.9, 113.9, 61.5, 60.4, 9.0, 8.9.

5,7-Dihydroxy-6,8-dimethylchromone (9)[11]

A solution of **8** (0.42 g, 1.8 mmol) in dry benzene was heated at 90 ºC under reflux with anhydrous aluminium chloride (2.0 g, 15 mmol) for 3 h under argon. The mixture was concentrated and added 2M

HCl at 0 ºC, then stirred for 2 h. The precipitates were filtered and recrystallized from MeOH-H_2O to give **9**.

9: pale yellow needles (MeOH-H_2O), mp 253–255 ºC [lit. 254 ºC].[11] ^{1}H-NMR (400 MHz, DMSO-d_6): 12.92 (s, 1H), 8.26 (d, *J*=6.0 Hz, 1H), 6.28 (d, *J*=6.0 Hz, 1H), 2.15 (s, 3H), 2.05 (s, 3H); ^{13}C-NMR (100 MHz, DMSO-d_6): 181.6, 159.8, 157.2, 156.1, 152.9, 109.9, 107.0, 104.7, 101.8, 8.0, 7.9.

5,7-Dihydroxy-6,8-dimethylchromanone (10)[9]

A mixture of **9** (0.11 g, 0.5 mmol) and Pd-C (Pd 10%, 80 mg) in acetone (15 mL) and MeOH (5 mL), was stirred overnight under hydrogen. The mixture was filtered and the filtrate was concentrated to dryness to obtain **10** (109.7 mg, 99%).

10: pale yellow crystalline powder (dil. EtOH), mp 213–214 ºC [lit. 214–215 ºC]. [9] ^{1}H-NMR (400 MHz, DMSO-d_6): 12.42 (s, 1H), 4.43 (t, *J*=6.4 Hz, 2H), 2.75 (t, *J*=6.6 Hz, 2H), 1.94 (s, 6H); ^{13}C-NMR (125 MHz, DMSO-d_6): 196.8, 162.2, 158.4, 157.5, 103.0, 102.2, 101.9, 66.2, 35.8, 8.0, 7.5.

Dehydromethylophiopogonanone A (11)

To a solution of **10** (0.10 mg, 0.5 mmol) and piperonal (0.20 mg, 1.3 mmol) in EtOH (10 mL), HCl gas was bubbled at 90 ºC for 1 h. The mixture was concentrated and the residue was purified by silica gel column chromatography (hexane:acetone=9:1) to obtain **11** (0.10 g, 61%).

11: yellow needles ($CHCl_3$-hexane), mp 198–200 ºC. HR-MS: *m/z* 339.08020 $[M-H]^-$ (calcd for $C_{19}H_{15}O_6$, 339.08686); IR: 1626 cm^{-1} (C=O), 3334 cm^{-1} (OH); ^{1}H-NMR (500 MHz, DMSO-d_6): 13.01 (s, 1H), 7.67 (brs, 1H), 7.07 (d, *J*=1.5 Hz, 1H), 7.04 (d, *J*=8.0 Hz, 1H), 6.99 (dd, *J*=1.5, 8.0, 1H), 6.12 (s, 2H), 5.33 (d, *J*=2.0 Hz, 2H), 1.97 (s, 3H), 1.94 (s, 3H); ^{13}C-NMR (125 MHz, DMSO-d_6): 184.6, 162.7, 159.3, 156.3, 148.6, 147.7, 135.9, 128.1, 127.8, 125.8, 109.9, 108.5, 103.6, 102.4, 101.7, 101.6, 66.9, 7.9, 7.6.

Methylophiopogonanone A (1)[2a]

A mixture of **11** (50 mg, 0.15 mmol) and Pd-C (Pd 10%, 50 mg) in acetone (10 mL) was stirred overnight under hydrogen. The mixture was filtered and the filtrate was concentrated to dryness to obtain **1** (31 mg, 60%).

1: colorless amorphous powder (AcOEt-hexane), mp 142–143 ºC.[12] ^{1}H-NMR (500 MHz, $CDCl_3$): 12.36 (s, 1H), 6.76 (d, *J*=8.0 Hz, 1H), 6.73 (d, *J*=1.5 Hz, 1H), 6.67 (dd, *J*=1.5, 8.0 Hz, 1H), 5.95 (s, 2H), 5.41 (s, 1H), 4.29 (dd, *J*=4.5, 11.5 Hz, 1H), 4.12 (dd, *J*=7.0, 11.5 Hz, 1H), 3.15 (dd, *J*=4.5, 14.0 Hz, 1H), 2.76-2.81 (m, 1H), 2.68 (dd, *J*=10.5 14.0 Hz, 1H), 2.07 (s, 3H), 2.03 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): 198.3, 160.6, 159.6, 157.7, 147.8, 146.3, 131.8, 122.1, 109.4, 108.4, 102.9, 102.2, 101.6, 101.0, 68.8, 46.8, 32.6, 7.4, 6.9; ^{13}C-NMR (125 MHz, DMSO-d_6): 198.0, 162.4, 158.6, 157.2, 147.2, 145.6, 131.9, 122.0, 109.1, 108.0, 103.2, 102.2, 101.0, 100.7, 68.8, 45.2, 31.6, 8.0, 7.5.

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12. Softening began at 120 ºC. Although melting point of methylophiopogonanone A crystallized from CCl_4 was reported to be 166–167 ºC (needles),[2a] crystallization of the synthetic **1** from CCl_4 also gave amorphous powder, which showed the same melting point with that from AcOEt-hexane.