Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. VII.   Studies on the Homoisoflavonoids of the Subterranean Part of *Ophiopogon japonicus* Ker-Gawler cv. Nanus. (1)[1]

Takayuki Asano, Tetsuya Murayama, Yasuaki Hirai, and Junzo Shoji*

School of Pharmaceutical Sciences, Showa University, Hatanodai, Shinagawa-ku, Tokyo 142, Japan.
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Six known homoisoflavonoidal compounds (1—6) and three new homoisoflavonoidal compounds (7—9) were isolated from the ether-soluble fraction of the subterranean part of *Ophiopogon japonicus* Ker-Gawler cv. Nanus. Among them, 1, 2, 3, 4, 5 and 6 were identified as methylophiopogonone A (1), ophiopogonone A (2), methylophiopogonanone A (3), ophiopogonanone A (4), JE-III (5) and desmethylisoophiopogonone B (6), respectively. The structures of compounds 7—9 were elucidated as 5,7,2'-trihydroxy-6-methyl-3-(3',4'-methylenedioxybenzyl)chromone (7), 5,7,2'-trihydroxy-8-methyl-3-(3',4'-methylenedioxybenzyl)chromone (8), and a racemate of 5-hydroxy-7,8-dimethoxy-6-methyl-3-(3',4'-dihydroxybenzyl)chroman-4-one (9), respectively.

In a preceding paper,[2] the chemical constituents of the subterranean part of *Liriope spicata* var. *prolifera* and *L. muscari* (Liliaceae) were reported. The present paper deals with the isolation and structure elucidation of homoisoflavonoidal compounds of the subterranean part of *Ophiopogon japonicus* Ker-Gawler cv. Nanus.

The subterranean part of *O. japonicus* Ker-Gawler cv. Nanus were extracted with hot methanol, and the methanol extract was treated by the method described in the Experimental section. Six homoisoflavonoidal compounds, 1—6 and three new homoisoflavonoidal compounds, 7—9 were isolated. Based on the infrared (IR), ultraviolet (UV), ^{13}C-nuclear magnetic resonance (^{13}C-NMR), ^{1}H-NMR and circular dichroism (CD) spectra of compounds 1, 2, 3, 4, 5 and 6, the compound structures were deduced to be methylophiopogonone A [5,7-dihydroxy-6,8-dimethyl-3-(3',4'-methylenedioxybenzyl)chromone] (1),[3] ophiopogonone A [5,7-dihydroxy-6-methyl-3-(3',4'-methylenedioxybenzyl)chromone (2),[4] methylophiopogonanone A [5,7-dihydroxy-6,8-dimethyl-(3*R*)-(3',4'-methylenedioxybenzyl)-chroman-4-one] (3),[3] ophiopogonanone A [5,7-dihydroxy-6-methyl-(3*R*)-(3',4'-methylenedioxybenzyl)chroman-4-one] (4),[4] JE-III [5,7-dihydroxy-3-(4'-hydroxybenzyl)-6-methylchromone] (5)[5] and desmethylisoophiopogonone B [5,7-dihydroxy-3-(4'-hydroxybenzyl)-8-methylchromone] (6),[4] respectively. Finally, the compounds were identified by direct comparison with an authentic sample by a mixed fusion.

Compounds 7, 8 and 9 were positive in ferric chloride reaction, and based on UV and IR spectrometric studies, compounds 7 and 8 were believed to be homoisoflavone derivatives, while compound 9 was assumed to be a homoisoflavanone derivative. The molecular weight and the number of carbon atoms of each compound were determined by mass spectrometry (MS) and by ^{13}C-NMR spectrometry.

Compounds 7 and 8 showed the presence of hydroxyl, α,β-unsaturated carbonyl at 1660 cm^{-1} and a methylenedioxy group at 924 cm^{-1} in IR spectra, and the MS of compounds 7 and 8 showed the same molecular ion peak (M+) at *m/z* 342, respectively.^{13}C-NMR spectra suggested

that both compounds were composed of eighteen carbon atoms. The ^{1}H-NMR spectra of compounds 7 and 8 showed the presence of three hydroxyl groups at δ 12.72, 9.69, 8.17 and at δ 12.37, 9.62, 8.18, one aromatic methyl group at δ 2.05 (6-CH$_3$) and at δ 2.16 (8-CH$_3$), one methylenedioxy group at δ 5.91 and at δ 5.91, one olefinic proton of C-2 at δ 8.08 (H-2) and at δ 8.05 (H-2), one methylene proton of C-9 at δ 3.69 and at δ 3.70 and two *ortho*-coupled aromatic protons at δ 6.37 (1H, d, *J*=7.9 Hz, H-5'), 6.76 (1H, d, *J*=7.9 Hz, H-6') and at δ 6.38 (1H, d, *J*=8.3 Hz, H-5'), 6.77 (1H, d, *J*=8.3 Hz, H-6'), respectively. Consequently, compounds 7 and 8 were suggested to be the positional isomers of homoisoflavone, whose one aromatic methyl group is located at either C-6 or C-8 of A ring. Based on the chemical shifts of the aromatic proton and aromatic methyl group,[5] the structures of compounds 7 and 8 were established as 5,7,2'-trihydroxy-6-methyl-3-(3',4'-methylenedioxybenzyl)chromone (7) and 5,7,2'-trihydroxy-8-methyl-3-(3',4'-methylenedioxybenzyl)chromone (8), respectively.
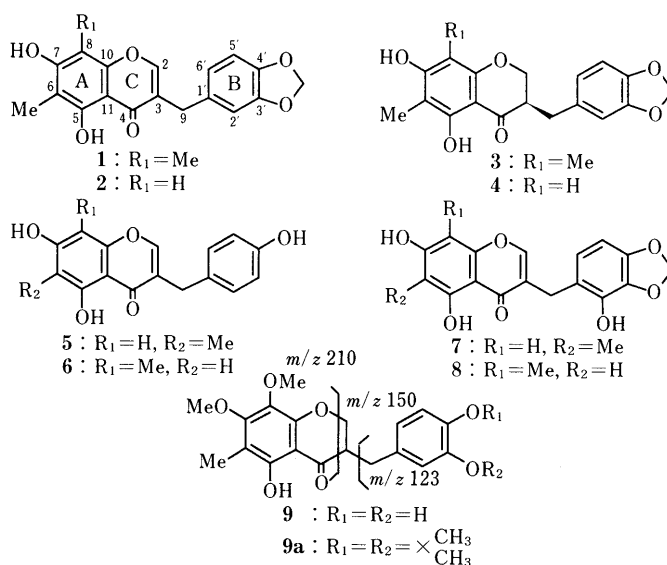

1 : R$_1$=Me
2 : R$_1$=H

3 : R$_1$=Me
4 : R$_1$=H

5 : R$_1$=H, R$_2$=Me
6 : R$_1$=Me, R$_2$=H

7 : R$_1$=H, R$_2$=Me
8 : R$_1$=Me, R$_2$=H

m/z 210
m/z 150
m/z 123

9 : R$_1$=R$_2$=H
9a : R$_1$=R$_2$=$\times$CH$_3$CH$_3$

Chart 1

The IR spectrum of compound **9** showed the hydroxyl and carbonyl absorption bands, while the MS of **9** showed the molecular ion peak (M$^+$) at m/z 360, which was suggested to have nineteen carbon atoms by ^{13}C-NMR spectrum. The ^{1}H-NMR and ^{1}H-^{1}H correlation spectroscopy (COSY) spectra of **9** showed benzylmethylene proton signals at δ 2.65 and 3.21, methylene proton signals at δ 4.25 and 4.42, methine proton signal at δ 3.09, ABX type aromatic protons of B ring at δ 6.32, 6.44 and 6.96, one aromatic methyl proton signal at δ 1.98, three hydroxyl proton signals at δ 8.19, 8.51 and 12.12 and two methoxy proton signals at δ 3.75 and 4.00.

MS of compound **9** showed a dihydroxytropyrium fragment peak due to the B-ring at m/z 150 and 123 and two fragment peaks due to the A-ring at m/z 211 and 210 occurred by the retro Diels–Alder cleavage reaction. Based on the MS of **9**, this compound has one hydroxyl, one aromatic methyl and two methoxyl groups in A-ring and two hydroxyl groups in B-ring. Three partial structures of B ring, 3,4-dihydroxy, 2,5-dihydroxy and 2,4-dihydroxy patterns, were suggested and the substitution pattern of B-ring was finally proved by chemical reaction to form a isopropylidene ketal ring with p-toluenesulfonic acid and dimethoxypropane in benzene. On the other hand, the substitution pattern of the A ring of compound **9** was determined as follows. It was pointed out that the signal of C-5 carbon atom is generally shifted to the high field by about 1.5 ppm with the addition of deuteromethanol.[6] Based on this report, the carbon signal at δ 157 ppm of ^{13}C-NMR spectrum of compound **9** was assigned to C-5 carbon atom. The long range ^{13}C–^{1}H COSY spectrum of compound **9** revealed cross peaks due to $^3J_{C-H}$ (C-5/6-CH$_3$, C-7/6-CH$_3$) to confirm the substitution pattern of A ring as shown in Chart 1. Furthermore, the CD spectrum of **9** did not show any Cotton effect between 250 and 400 nm.[7]

Consequently, the structure of **9** was concluded to be a racemate of 5-hydroxy-7,8-dimethoxy-6-methyl-3-(3′,4′-dihydroxybenzyl)chroman-4-one.

In this paper we have described a study on the homoisoflavonoidal constituents of *Ophiopogon japonicus* KER-GAWLER cv. Nanus and the isolation of six known compounds (**1**, **2**, **3**, **4**, **5**, and **6**) and three new compounds (**7**, **8** and **9**). Among the former, compounds **1**, **3**, **4**, **5** and **6** were isolated from Ophiopogonis tuber and **2** was isolated from *O. jaburan* (KUNTH) LODD. Further investigation on glycosidic constituents of this plant will be reported in a later paper.

Experimental

Extraction and Isolation The fresh subterranean part of *Ophiopogon japonicus* KER-GAWLER cv. Nanus (36.6 kg) was crushed and extracted with hot MeOH (30 l × 3). The extract was combined and evaporated to dryness *in vacuo*. The residue (1.36 kg) was dissolved in water and extracted with ether to afford ether-soluble fraction (159.0 g). The aqueous layer was extracted with 1-BuOH saturated with water, and BuOH-soluble fraction was concentrated *in vacuo* to afford a brown residue (310.0 g). The ether soluble fraction was subjected to column chromatography on Sephadex LH-20 with CHCl$_3$–MeOH–H$_2$O (7:3:0.4, v/v) to afford six fractions (frs. A—F). Fraction D was subjected to column chromatography on silicic acid with benzene–acetone (9:1, v/v) to give five fractions (frs. D-1—D-5). Fraction D-1 was subjected to column chromatography on silicic acid with benzene and the eluate was purified by recrystallization to give compounds **1** (115 mg), **3** (1100 mg) and **9** (90 mg). Fraction D-2 was subjected to column chromatography on silicic acid with hexane–acetone (8:2, v/v) and each eluate was recrystallized to afford compounds **2** (90 mg), **4** (43 mg) and **5** (90 mg). Fraction D-5 was subjected to column chromatography on silicic acid with CHCl$_3$–MeOH–H$_2$O (7:3:0.4, v/v) to give five fractions (frs. D-5-1—D-5-5). Fraction D-5-2 was subjected to HPLC (column; Fuji-Gel Chromatorex-ODS, 5 μm, i.d. 20 × 250 mm, solvent; 50% MeCN) to give compounds **6** (40 mg), **7** (385 mg) and **8** (50 mg).

Properties Compound **1**: Pale yellow needles (from MeOH), mp 208—210 °C. IR ν_{max}^{KBr} cm^{-1}: 3520, 1700, 1620, 900. UV λ_{max}^{MeOH} nm (log ε): 292 (3.99), 266 (3.95). ^{13}C-NMR: see Table I. EIMS m/z: 340 (M$^+$), 181, 135.

Compound **2**: Pale yellow needles (from MeOH), mp 199—201 °C. IR ν_{max}^{KBr} cm^{-1}: 3300, 1660, 1640, 940. UV λ_{max}^{MeOH} nm (log ε): 294 (3.98), 262 (3.97). ^{13}C-NMR: see Table I. EIMS m/z: 326 (M$^+$), 167, 135.

Compound **3**: Colorless needles (from MeOH), mp 165—166 °C, [α]$_D^{23}$

TABLE I. ^{13}C-NMR Chemical Shifts of Homoisoflavonoidal Compounds

Carbon	1	2	3	4	5	6	7	8	9
2	152.6	153.8	68.8	68.3	153.5	155.1	154.3	154.6	70.0
3	120.7$^{a)}$	121.1$^{c)}$	44.8	44.9	121.4	121.1	122.0	121.3$^{e)}$	45.9
4	180.6	180.3	197.6	197.2	181.4	181.0	181.0	181.4	200.3
5	159.2	161.8	161.9	160.5	161.8	161.3	162.3	162.0	157.0
6	103.7	103.5	102.8	100.4	103.4	97.6	104.0	98.3	103.9$^{f)}$
7	154.0	155.0	156.8	164.0	155.1$^{d)}$	155.1	155.6	155.3	160.0
8	101.1	92.3	101.7	93.4	92.2	101.2	92.8	101.8	132.9
9	29.2	29.3	31.1	29.2	28.6	28.5	24.4	24.2	29.6
10	155.0	158.0	158.1	159.7	158.0	158.3	158.7	159.0	152.8
11	106.4	106.3	100.6	102.8	106.2	103.7	107.0	104.2	104.1$^{f)}$
6-Me	7.48$^{b)}$	6.9	7.1	6.9	6.8	—	7.5	—	7.6
8-Me	7.51$^{b)}$	—	7.5	—	—	7.8	—	7.3	—
1′	132.6	132.6	131.4	131.4	128.7	128.6	121.3	120.8$^{e)}$	131.9
2′	108.5	108.6	108.7	108.6	128.9	128.9	147.0	147.0	111.0
3′	146.6	146.7	146.8	146.8	114.5	114.5	135.0	134.9	156.0$^{g)}$
4′	145.0	145.1	145.2	145.2	155.0$^{d)}$	155.1	138.9	138.7	155.2$^{g)}$
5′	107.5	107.6	107.6	107.6	114.5	114.5	100.2	100.1	107.8
6′	120.9$^{a)}$	120.9$^{c)}$	121.5	121.5	128.9	128.9	121.4	122.2	116.1
O–CH$_2$–O	100.2	100.2	100.2	100.3	—	—	100.7	100.7	—
OMe	—	—	—	—	—	—	—	—	60.9
OMe	—	—	—	—	—	—	—	—	61.4

Chemical shifts measured in DMSO-d_6 (**1**—**8**) and CDCl$_3$ (**9**). a—g) Assignment may be interchangeable within the same column.

$-72°$ ($c = 1.00$, CHCl$_3$). IR ν_{max}^{KBr} cm^{-1}: 3420, 1640, 1630, 940. UV λ_{max}^{MeOH} nm (log ε): 296 (4.00), 214 (3.86). ^{13}C-NMR: see Table I. EIMS m/z: 342 (M$^+$), 135. CD ($c = 5.85 \times 10^{-5}$, MeOH) $[\theta]_\lambda$ (nm): $+3400$ (315), -12000 (295) (negative maximum), $+5100$ (270).

Compound 4: Colorless needles (from MeOH), mp 174—175 °C, $[\alpha]_D^{23} - 63°$ ($c = 1.00$, CHCl$_3$). IR ν_{max}^{KBr} cm^{-1}: 3420, 1640, 1600, 920. UV λ_{max}^{MeOH} nm (log ε): 294 (3.98), 213 (3.84). ^{13}C-NMR: see Table I. EIMS m/z: 328 (M$^+$), 135. CD ($c = 6.11 \times 10^{-5}$, MeOH) $[\theta]_\lambda$ (nm): $+3300$ (315), -13000 (295) (negative maximum), $+4900$ (270).

Compound 5: Pale yellow needles (from MeOH), mp 250—252 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1627. UV λ_{max}^{MeOH} nm (log ε): 294 (3.94), 262 (3.89). ^{13}C-NMR: see Table I. EIMS m/z: 298 (M$^+$), 167.

Compound 6: Pale yellow needles (from MeOH), mp 203—205 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1627. UV λ_{max}^{MeOH} nm (log ε): 299 (4.01), 260 (3.95). ^{13}C-NMR: see Table I. EIMS m/z: 298 (M$^+$), 167, 107.

Compound 7: Pale yellow needles (from MeOH), mp 275—276 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1625, 924. UV λ_{max}^{MeOH} nm (log ε): 299 (4.01), 260 (3.95). ^{1}H-NMR (CDCl$_3$) δ: 12.72 (1H, s, 5-OH), 9.69 (1H, s, 2'-OH), 8.17 (1H, s, 7-OH), 8.08 (1H, s, H-2), 6.76 (1H, d, $J = 7.9$ Hz, H-6'), 6.48 (1H, s, H-8), 6.37 (1H, d, $J = 7.9$ Hz, H-5'), 5.91 (2H, s, –O–CH$_2$–O–), 3.69 (2H, s, H-9), 2.05 (3H, s, 6-CH$_3$). ^{13}C-NMR: see Table I. EIMS m/z: 342 (M$^+$): 167, 151, 107. *Anal.* Calcd for C$_{18}$H$_{14}$O$_7$: C, 63.16; H, 4.12. Found: C, 62.88; H, 4.09.

Compound 8: Pale yellow needles (from MeOH), mp 226—227 °C. IR ν_{max}^{KBr} cm^{-1}: 3400, 1660, 1624, 924. UV λ_{max}^{MeOH} nm (log ε): 300 (4.01), 262 (3.96). ^{1}H-NMR (CDCl$_3$) δ: 12.37 (1H, s, 5-OH), 9.62 (1H, s, 2'-OH), 8.18 (1H, s, 7-OH), 8.05 (1H, s, H-2), 6.77 (1H, d, $J = 8.3$ Hz, H-6'), 6.38 (1H, d, $J = 8.3$ Hz, H-5'), 6.36 (1H, s, H-6), 5.91 (2H, s, –O–CH$_2$–O–), 3.70 (2H, s, H-9), 2.16 (3H, s, 8-CH$_3$). ^{13}C-NMR: see Table I. EIMS m/z: 342 (M$^+$): 167, 151, 107. *Anal.* Calcd for C$_{18}$H$_{14}$O$_7$: C, 63.16; H, 4.12. Found: C, 63.34; H 4.07.

Compound 9: Yellow syrup, $[\alpha]_D^{23} \pm 0°$ ($c = 0.50$, CHCl$_3$). IR ν_{max}^{KBr} cm^{-1}: 3420, 1620. UV λ_{max}^{MeOH} nm (log ε): 286 (4.01), 216 (3.86). ^{1}H-NMR (CDCl$_3$) δ: 12.12 (1H, s, 5-OH), 8.51, 8.19 (each 1H, s, 3'-OH or 4'-OH), 6.96 (1H, d, $J = 7.9$ Hz, H-5'), 6.44 (1H, d, $J = 2.0$ Hz, H-2'), 6.32 (1H, dd, $J = 7.9$, 2.0 Hz, H-6'), 4.42 (1H, dd, $J = 12.3$, 4.1 Hz, H-2α), 4.25 (1H, dd, $J = 12.3$, 8.2 Hz, H-2β), 4.00 (3H, s, 7-OCH$_3$), 3.75 (3H, s, 8-OCH$_3$), 3.21 (1H, dd,

$J = 13.3$, 4.8 Hz, H-9α), 3.09 (1H, m, H-3), 2.65 (1H, dd, $J = 13.3$, 9.6 Hz, H-9β), 1.98 (3H, s, 6-CH$_3$). ^{13}C-NMR: see Table I. EIMS m/z: 360 (M$^+$), 211, 210, 150, 123. HRMS m/z: 360.1257 [M$^+$] (Calcd for C$_{19}$H$_{20}$O$_7$: 360.1209).

Isopropylidene Derivative of 9 (9a) A mixture of compound 9 (10 mg), 4-dimethoxypropane (0.5 ml) and a catalytic amount of *p*-toluenesulfonic acid in dry benzene was allowed to stand at room temperature for 30 min. After neutralization with sat. NaHCO$_3$ solution the resulting mixture was extracted with ether. The organic layer was washed with water, dried over anhydrous Na$_2$SO$_4$ and evaporated. The residue was subjected to column chromatography on Sephadex LH-20 (eluted by MeOH) to afforded compound 9a (3.0 mg).

Compound 9a: Colorless syrup. ^{1}H-NMR (CDCl$_3$) δ: 11.80 (1H, s, 5-OH), 3.97 (3H, s, 7-OCH$_3$), 3.75 (3H, s, 8-OCH$_3$), 2.02 (3H, s, 6-CH$_3$), 1.21 (6H, s, *gem*-dimethyl).

References
1) A part of this work was presented at the 110th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1990.
2) B. Y. Yu, Y. Hirai, J. Shoji, and G. J. Xu, *Chem. Pharm. Bull.*, **38**, 1931 (1990).
3) A. Tada, R. Kasai, T. Saitoh, and J. Shoji, *Chem. Pharm. Bull.*, **28**, 1477 (1980).
4) A. Tada, R. Kasai, T. Saitoh, and J. Shoji, *Chem. Pharm. Bull.*, **28**, 2039 (1980).
5) Y. Watanabe, S. Sanada, Y. Ida, and J. Shoji, *Chem. Pharm. Bull.*, **33**, 5358 (1985).
6) Y. Terui and K. Tori, Abstracts of Papers, 17th Symposium Papers on NMR, Tokyo, 1978, p. 37.
7) M. Namikoshi and T. Saitoh, *Chem. Pharm. Bull.*, **35**, 3597 (1987).