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Coumarins from Bark of *Fraxinus japonica* and *F. mandshurica* var. *japonica*

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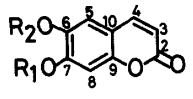
Two biologically active coumarins, scopoletin (**1**) and isofraxidin (**2**), along with known coumarins, esculetin (**3**), fraxetin (**4**), esculin (**5**) and fraxin (**6**), were newly isolated from the bark of *Fraxinus japonica* BLUME (Oleaceae). On the other hand, the bark of *F. mandshurica* RUPR. var. *japonica* MAXIM gave only known coumarins, fraxetin (**4**), fraxinol (**7**) and mandshurin (**8**).

Keywords——*Fraxinus japonica*; *Fraxinus mandshurica* var. *japonica*; Oleaceae; coumarin; scopoletin; isofraxidin; antihypertensive activity; ^{13}C-NMR spectra

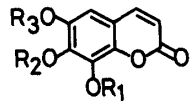
The dried bark of *Fraxinus japonica* BLUME (Oleaceae), which is on the market as the oriental medicine "shinpi" (秦皮) in Japan, has been used since olden times as a diuretic, an antifebrile, an analgesic and an anti-rheumatic,[1] while *F. mandshurica* RUPR. var. *japonica* MAXIM, widely distributed in Hokkaido, the northern part of Japan, is not used. In China, the dried bark of *F. mandshurica* (水曲柳皮) is sometimes used as a substitute for the Chinese crude drug "qinpi" (Cortex Fraxini, 秦皮).[2]

So far, the isolation of various coumarins, *i.e.*, esculetin (**3**), fraxetin (**4**), esculin (**5**) and fraxin (**6**) from *F. japonica*,[3a-c] and fraxetin (**4**), fraxinol (**7**) and mandshurin (**8**) from *F. mandshurica* var. *japonica*,[4a,b] from these barks has been reported. Among them, coumarins **3**, **5** and **6** are known to be active principles of "shinpi" for diuretic and anti-inflammatory action.[5a-d]

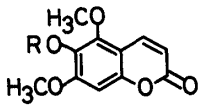
In the previous paper,[6] we reported the isolation of lignans from these barks. As a continuation of our studies on the constituents of *Fraxinus* bark, this paper deals with the isolation of two biologically active coumarins, scopoletin (**1**) and isofraxidin (**2**), along with known coumarins **3**—**6**, from *F. japonica*. On the other hand, the bark of *F. mandshurica* var. *japonica* gave coumarins **4**, **7** and **8**, but did not yield coumarins **1**—**3**, **5** and **6**. The extraction and separation were carried out as described in Experimental.



1: $R_1=H$, $R_2=CH_3$
3: $R_1=R_2=H$
5: $R_1=H$, $R_2=$glucose
9: $R_1=CH_3$, $R_2=H$
9a: $R_1=R_2=CH_3$



2: $R_1=R_3=CH_3$, $R_2=H$
4: $R_1=R_2=H$, $R_3=CH_3$
4a: $R_1=R_2=R_3=CH_3$
6: $R_1=$glucose, $R_2=H$, $R_3=CH_3$
6a: $R_1=$glucose, $R_2=R_3=CH_3$
6b: $R_1=H$, $R_2=R_3=CH_3$



7: R=H
7a: $R=CH_3$
8: R=glucose

Chart 1

The ultraviolet (UV), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data of **1** were in good agreement

TABLE I. ^{13}C-NMR Chemical Shifts[a)]

	2	4	4a	6	6a	6b
C-2	160.0	160.4	159.6	160.2	159.7	160.0
C-3 (d)	111.9	111.8	114.5	112.2	114.6	114.4
C-4 (d)	144.7	145.0	144.2	144.7	144.3	144.6
C-5 (d)	104.4	100.3	104.9	105.1	104.9	100.2
C-6	145.5	145.3	149.6	145.4	149.8	149.6
C-7	143.9	139.2	145.1	143.7	144.8	140.1
C-8	134.6	132.8	140.1	131.6	136.4	138.2
C-9	142.9	139.4	142.0	142.7	142.0	138.5
C-10	110.1	110.2	114.3	110.2	114.2	114.2
OCH_3	56.1	56.0	56.0	56.1	56.0	55.9
	60.6		60.8		60.8	60.4
			61.2			

a) The spectra were taken in DMSO-d_6 in micro cells with a JNM-FX 60 spectrometer (15.00 MHz), with TMS as an internal reference.

TABLE II. *O*-Methylation and *O*-Glucosylation Shift Values in DMSO-d_6

	7-*O*-Methylation ($\Delta\delta$)			8-*O*-Methylation ($\Delta\delta$)		8-*O*-Glucosylation ($\Delta\delta$)	
	4a−2	6b−4	6a−6	2−4	4a−6b	6−4	6a−6b
C-2	−0.4	−0.4	−0.5	−0.4	−0.4	−0.2	−0.3
C-3	+2.6	+2.6	+2.4	+0.1	+0.1	+0.4	+0.2
C-4	−0.5	−0.4	−0.4	−0.3	−0.4	−0.3	−0.3
C-5	+0.5	−0.1	−0.2	+4.1	+4.7	+4.8	+4.7
C-6	+4.1	+4.3	+4.4	+0.2	0	+0.1	+0.2
C-7	+1.2	+0.9	+1.1	+4.7	+5.0	+4.5	+4.7
C-8	+5.5	+5.4	+4.8	+1.8	+1.9	−1.2	−1.8
C-9	−0.9	−0.9	−0.7	+3.5	+3.5	+3.3	+3.5
C-10	+4.2	+4.0	+4.0	−0.1	+0.1	0	0

TABLE III. ^{13}C-NMR Chemical Shifts[a)]

	9	9a	7	7a	5-Methoxylation ($\Delta\delta$)		6-*O*-Methylation ($\Delta\delta$)
					7−9	7a−9a	7a−7
C-2	160.7	160.4	160.4	160.1	−0.3	−0.3	−0.3
C-3	112.5 (d)	112.5 (d)	111.9 (d)	112.1 (d)	−0.6	−0.4	+0.2
C-4	144.1 (d)	144.2 (d)	139.0 (d)	139.0 (d)	−5.1	−5.2	0
C-5	111.9 (d)	109.0 (d)	142.8	148.8	+30.9	+39.8	+6.0
C-6	143.6	145.8	135.5	137.7	−8.1	−8.1	+2.2
C-7	151.8	152.5	152.9	157.0	+1.1	+4.5	+4.1
C-8	99.9 (d)	99.9 (d)	95.3 (d)	96.0 (d)	−4.6	−3.9	+0.7
C-9	148.4	149.4	147.7	150.9	−0.7	+1.5	+3.2
C-10	111.5	111.1	106.6	106.4	−4.9	−4.7	−0.2
OCH_3	56.0	55.8	56.3	56.4			
		56.0	61.0	60.7			
				61.8			

a) The spectra were taken in DMSO-d_6 in micro cells with a JNM-FX 60 spectrometer (15.00 MHz), with TMS as an internal reference.

with those of authentic scopoletin from *Olea europaea* L. subsp. *africana* (MILL) GREEN (Oleaceae).[7]

The UV, IR and ^{1}H-NMR spectral data of **2** were in good agreement with those of authentic isofraxidin from *Acanthopanax senticosus* HARMS (*Eleutherococcus senticosus* MAXIM) (Araliaceae, Ciwujia, 刺五加).[8]

The physical and spectral data of **3**—**8** were in good agreement with those given in the literature.[3a-c,4a,b] Furthermore, the ^{13}C-NMR spectra of **2**, fraxin methyl ether (**6a**), fraxidin (**6b**), fraxinol (**7**) and fraxinol methyl ether (**7a**) were assigned on the basis of the established assignments for **4**,[9] fraxetin dimethyl ether (**4a**),[10] fraxin (**6**),[9] isoscopoletin (**9**) and scoparone (**9a**),[7] respectively, as well as the signal multiplicity in off-resonance decoupled spectra, the additivity rule of substituent effects and *O*-methylation and *O*-glucosylation shift values,[11a-d] as shown in Tables I, II and III.

The occurrence of **1** in Oleaceae is already known in *Olea* barks,[7] which have been used as an antifebrile and an anti-rheumatic agent, and as a tonic in Europe and South Africa,[12a,b] and in bark of *Fraxinus excelsior* L,[13] which has been used as a folk medicine in Europe.[14] The occurrence of **2** is also known in bark of *F. excelsior*.[15a,b]

In regard to the biological activity of the coumarins isolated, coumarins **1** and **2** showed high inhibitory activity against cyclic adenosine monophosphate(cAMP)-phosphodiesterase *in vitro* (IC_{50}($\times 10^{-5}$ M): 4.9 and 6.4, respectively).[16] Weinryb *et al.* reported that a considerable number of therapeutic agents used as antipsychotics, antianxiety agents, antihypertensives and so on showed inhibitory effects against phosphodiesterase.[17] In fact, antihypertensive[18] and analgesic[19] activities of **1**, and sedative[8] and anticancer[20] activities of **2** have been reported. We also observed antihypertensive action of **1** in spontaneously hypertensive rats (SHR) (20 mmHg, 10 mg/kg, *i.v.*).[21]

It is noteworthy from the medicinal viewpoint that the biologically active coumarins **1** and **2**, in addition to anti-inflammatory coumarins **3** and **5**, have been isolated from the bark of *F. japonica* used as a crude drug, while the bark of *F. mandshurica* var. *japonica*, which is not used as a medicine, did not contain these biologically active coumarins.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: UV spectra, Shimadzu UV-210; IR spectra, Hitachi 270-30; ^{1}H-NMR spectra, Hitachi R-40; ^{13}C-NMR spectra, JEOL JNM-FX 60 equipped with a JEC-980 computer; mass spectrum (MS), Hitachi RMU-7L. The ^{1}H-NMR and ^{13}C-NMR spectra were taken in dimethylsulfoxide-d_6 (DMSO-d_6) with tetramethylsilane ($\delta=0$) as an internal reference. The abbreviations used are as follows: s, singlet; d, doublet; sh, shoulder.

Precoated thin-layer chromatography (TLC) plates, Silica gel 60 F_{254} (Merck), were used for TLC. The spots were detected under UV (254 nm) as dark spots and under UV (360 nm) as bright fluorescent spots. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation——Dry powdered bark (3.5 kg) of *F. japonica* was extracted four times with hot MeOH. The MeOH solution was concentrated to a small volume under reduced pressure, diluted with water and filtered. The filtrate was extracted successively with ether, $CHCl_3$ and BuOH. The ether layer was evaporated to dryness, and the ether extract (16.7 g) was chromatographed on a silica gel column with a $CHCl_3$–EtOAc gradient. The fractions were monitored by TLC developed with toluene–ether (1 : 2, saturated with 10% AcOH soln.). The fractions showing TLC spots at *Rf* 0.31, 0.28, 0.25 and 0.19 gave 153.6 mg of **1**, 259.1 mg of **2**, 322.1 mg of **3** and 744.4 mg of **4**, respectively. The BuOH layer was evaporated to dryness, and the BuOH extract (40.0 g) was chromatographed on a silica gel column with a $CHCl_3$–EtOH gradient. The fractions were monitored by TLC developed with $CH_3COC_2H_5$–EtOAc–HCOOH–H_2O–benzene (4 : 3 : 1 : 1 : 2, upper layer). The fractions showing TLC spots at *Rf* 0.28 and 0.19 gave 573.6 mg of **5** and 1.3 g of **6**, respectively.

Dry powdered bark (4.3 kg) of *F. mandshurica* var. *japonica* was treated in the same manner as described for *F. japonica*. The ether extract (15.7 g) gave 1.1 g of **7** and 43.3 mg of **4**. The BuOH extract (170.7 g) gave 103.9 mg of **8**.

Scopoletin (1)——Colorless needles from MeOH, $C_{10}H_8O_4$, mp 205—207 °C. This compound was identified by direct comparison with an authentic sample.

Isofraxidin (2)——Colorless needles from MeOH, $C_{11}H_{10}O_5$, mp 144—146 °C. This compound was identified by direct comparison with an authentic sample.

Esculetin (3)——Pale yellow prisms from MeOH, $C_9H_6O_4$, mp 272—274 °C. This compound was identified by direct comparison with an authentic sample.

Fraxetin (4)——Yellow needles from MeOH, $C_{10}H_8O_5$, mp 235—237 °C. The physical and spectral data of **4** were in good agreement with those given in the literature.[3a-c,4a,b]

Fraxetin Dimethyl Ether (4a)——**4** (50.3 mg) in MeOH was methylated with diazomethane in the usual way. The crude product was crystallized from MeOH to give **4a** (43.7 mg) as pale yellow needles, mp 102—104 °C. UV λ_{max}^{EtOH} nm (log ε): 229.3 (4.31), 295.5 (4.07), 338.0 (3.90). IR ν_{max}^{KBr} cm^{-1}: 1722 (CO), 1600 (C=C), 1568, 1492 (arom. C=C). MS: Calcd for $C_{12}H_{12}O_5$, 236.0683. Obsd., 236.0677. ^{1}H-NMR δ: 3.81, 3.84, 3.88 (9H, each s, 3×OCH_3), 6.31 (1H, d, J=10 Hz, C_3-H), 7.03 (1H, s, C_5-H), 7.87 (1H, d, J=10 Hz, C_4-H).

Methylation of **2** with diazomethane also gave **4a**.

Esculin (5)——Colorless crystalline powder from EtOH, $C_{15}H_{16}O_9 \cdot 1/2H_2O$, mp 152—154 °C. $[\alpha]_D^{22}$ −88.5° (c=0.33 in MeOH). Acid hydrolysis of **5** with 1% H_2SO_4 soln. gave **3** and D-glucose. Compound **5** was identified by direct comparison with an authentic sample.

Fraxin (6)——Pale yellow needles from EtOH, $C_{16}H_{18}O_{10} \cdot 1/2H_2O$, mp 208—209 °C. $[\alpha]_D^{25}$ +26.3° (c=0.89 in MeOH). Acid hydrolysis of **6** with 1% H_2SO_4 soln. gave **4** and D-glucose. The physical and spectral data of **6** were in good agreement with those given in the literature.[3a-c,4a,b]

Fraxin Methyl Ether (6a)——**6** (130 mg) in MeOH was methylated with diazomethane in the usual way. The crude product was crystallized from MeOH to give **6a** (91 mg) as a colorless crystalline powder, mp 191—193 °C. $[\alpha]_D^{25}$ −22.1° (c=0.44 in MeOH). UV λ_{max}^{EtOH} nm (log ε): 229.0 (4.30), 294.7 (4.00), 339.5 (3.85). IR ν_{max}^{KBr} cm^{-1}: 3396 (OH), 1716 (CO), 1604 (C=C), 1568, 1494 (arom. C=C). ^{1}H-NMR δ: 3.82 (6H, s, 2×OCH_3), 4.96 (1H, d, J=6 Hz, anomeric H), 6.33 (1H, d, J=10 Hz, C_3-H), 7.03 (1H, s, C_5-H), 7.86 (1H, d, J=10 Hz, C_4-H). *Anal.* Calcd for $C_{17}H_{20}O_{10} \cdot 1/4H_2O$: C, 52.51; H, 5.31. Found: C, 52.52; H, 5.26.

Fraxidin (6b)——A solution of **6a** (58 mg) in 1% H_2SO_4 soln. (70 ml) was heated on a boiling water bath for 1 h. The hydrolysis product was extracted with ether. The ether extract was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from MeOH to give **6b** (31 mg) as colorless prisms, mp 198—200 °C. UV λ_{max}^{EtOH} nm (log ε): 225.4 (4.17), 256.3 (3.63), 309.5 (4.01). IR ν_{max}^{KBr} cm^{-1}: 3230 (OH), 1690 (CO), 1610 (C=C), 1572, 1502 (arom. C=C). MS: Calcd for $C_{11}H_{10}O_5$, 222.0527. Obsd., 222.0514. ^{1}H-NMR δ: 3.74, 3.78 (6H, each s, 2×OCH_3), 6.28 (1H, d, J=10 Hz, C_3-H), 6.75 (1H, s, C_5-H), 7.83 (1H, d, J=10 Hz, C_4-H).

Fraxinol (7)——Pale yellow pillars from EtOH, $C_{11}H_{10}O_5$, mp 171—174 °C. The physical and spectral data of **7** were in good agreement with those given in the literature.[4a,b]

Fraxinol Methyl Ether (7a)——**7** (72 mg) in MeOH was methylated with diazomethane in the usual way. The crude product was crystallized from MeOH to give **7a** (61.3 mg) as colorless plates, mp 70.5—72 °C. UV λ_{max}^{EtOH} nm (log ε): 224.5 (4.15) sh, 251.8 (3.62) sh, 321.2 (4.00). IR ν_{max}^{KBr} cm^{-1}: 1736 (CO), 1616 (C=C), 1562, 1494 (arom. C=C). MS: Calcd for $C_{12}H_{12}O_5$, 236.0683. Obsd., 236.0671. ^{1}H-NMR δ: 3.73, 3.86, 3.91 (9H, each s, 3×OCH_3), 6.17 (1H, d, J=10 Hz, C_3-H), 6.79 (1H, s, C_8-H), 7.88 (1H, d, J=10 Hz, C_4-H).

Mandshurin (8)——Colorless crystalline powder from EtOH, $C_{17}H_{20}O_{10} \cdot H_2O$, mp 128—130 °C. $[\alpha]_D^{25}$ −27.7° (c=0.79 in MeOH). Acid hydrolysis of **8** with 1% H_2SO_4 soln. gave **7** and D-glucose. The physical and spectral data of **8** were in good agreement with those given in the literature.[4a,b]

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21) SHR were anesthetized with pentobarbital sodium, 50 mg/kg, *i.p.* and urethane, 1.75 g/kg, *s.c.* Scopoletin (**1**) dissolved in physiological saline soln. was administered intravenously to these rats through the femoral vein and tested for antihypertensive activity. Blood pressure was directly measured by carotid artery cannulation.