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Phenolic Compounds from *Forsythia* Leaves. II[1)]

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The phenolic compounds in leaves of four *Forsythia* species were investigated. Phillygenin (**1**), (+)-pinoresinol (**2**), phillyrin (**3**), (+)-pinoresinol-β-D-glucoside (**4**), forsythiaside (**11**) and rutin (**13**) were isolated from *Forsythia europaea* DEGEN *et* BALD, arctigenin (**5**), matairesinol (**6**), arctiin (**7**), matairesinoside (**8**), acteoside (**12**) and **13** from *F. ovata* NAKAI, and (+)-pinoresinol monomethyl ether (**9**), (+)-pinoresinol monomethyl ether-β-D-glucoside (**10**), compounds **1**—**4**, **12** and **13** from *F. japonica* MAKINO and *F. giraldiana* LIGEL.

The distributions of lignans, lignan glucosides, phenylpropanoids and flavonoids in seven *Forsythia* species investigated so far (*F. suspensa*, *F. viridissima*, *F. koreana*, *F. europaea*, *F. japonica*, *F. ovata* and *F. giraldiana*) were compared.

Keywords——*Forsythia* leaves; *Forsythia europaea*; *Forsythia japonica*; *Forsythia ovata*; *Forsythia giraldiana*; Oleaceae; phenolic compound; lignan; phenylpropanoid; distribution

In a continuation of our studies on the constituents of *Forsythia* fruits, we noted that there are differences in the occurrence of phenolic compounds depending on the species.[2)] In the previous paper,[1)] we dealt with phenolic compounds from the leaves of *Forsythia suspensa* VAHL, *F. viridissima* LINDLEY and *F. koreana* NAKAI, and reported that the constituent patterns in leaves of these species were broadly similar to those reported earlier for the fruits. Furthermore, our interest has been directed to the investigation of the phenolic compounds of other *Forsythia* plants.

This paper describes the isolation of phenolic compounds from the leaves of *F. europaea* DEGEN *et* BALD, *F. japonica* MAKINO, *F. ovata* NAKAI and *F. giraldiana* LIGEL. The leaves of each species were extracted and treated as described in the experimental section. The leaves of *F. europaea* gave phillygenin (**1**), (+)-pinoresinol (**2**), phillyrin (**3**), (+)-pinoresinol-β-D-glucoside (**4**), forsythiaside (**11**) and rutin (**13**). The leaves of *F. ovata* gave arctigenin (**5**), matairesinol (**6**), arctiin (**7**), matairesinoside (**8**), acteoside (**12**) and **13**. The leaves of *F. japonica* and *F. giraldiana* gave (+)-pinoresinol monomethyl ether (**9**), (+)-pinoresinol monomethyl ether-β-D-glucoside (**10**), **1**—**4**, **12** and **13**.

This is the first isolation of **10** as a natural product, though it has been synthesized from **4** by methylation with diazomethane.[3)]

Eight *Forsythia* species have been described in the world.[4)] The profiles of phenolic compounds isolated from leaves of the seven *Forsythia* species investigated so far are summarized in Table I. Based on the distributions of phenolic compounds isolated, the constituents may be tentatively classified into four groups: firstly phillyrin (**3**) and forsythiaside (**11**) [sugar moiety, α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside], secondly arctiin (**7**) and acteoside (**12**) [sugar moiety, α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranoside], thirdly phillyrin (**3**), (+)-pinoresinol monomethyl ether-β-D-glucoside (**10**) and

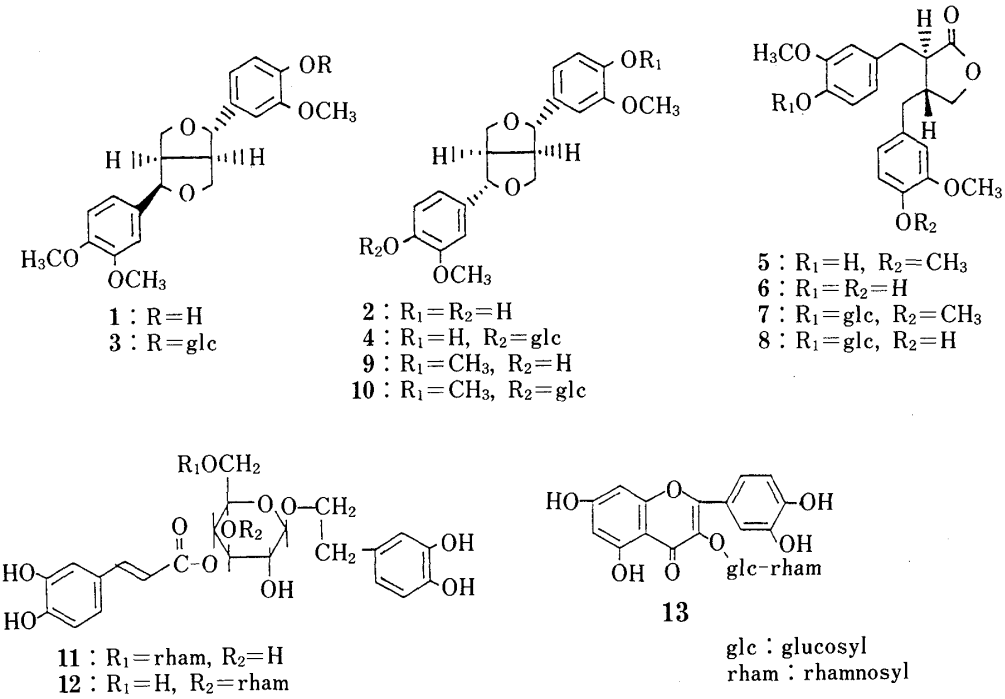

Chart 1

TABLE I. Phenolic Compounds Isolated from *Forsythia* Leaves

Species	Compounds		
	Lignans and lignan glucosides	Phenylpropanoids	Flavonoid
F. suspensa *F. europaea*	Phillygenin (**1**) (+)-Pinoresinol (**2**) Phillyrin (**3**)[a] (+)-Pinoresinol-β-D-glucoside (**4**)	Forsythiaside (**11**)[a]	Rutin (**13**)
F. viridissima *F. ovata*	Arctigenin (**5**) Matairesinol (**6**) Arctiin (**7**)[a] Matairesinoside (**8**)		
F. japonica *F. giraldiana*	Phillygenin (**1**) (+)-Pinoresinol monomethyl ether (**9**) (+)-Pinoresinol (**2**) Phillyrin (**3**)[a] (+)-Pinoresinol monomethyl ether-β-D-glucoside (**10**)[a] (+)-Pinoresinol-β-D-glucoside (**4**)	Acteoside (**12**)[a]	
F. koreana	Phillygenin (**1**) Arctigenin (**5**) (+)-Pinoresinol (**2**) Matairesinol (**6**) Phillyrin (**3**)[a] Arctiin (**7**)[a] (+)-Pinoresinol-β-D-glucoside (**4**) Matairesinoside (**8**)	Forsythiaside (**11**)[a] Acteoside (**12**)[a]	

a) The major constituents.

acteoside (**12**), and fourthly phillyrin (**3**), arctiin (**7**), forsythiaside (**11**) and acteoside (**12**). The occurrence of rutin (**13**) was common to all species.

This result is noteworthy from the chemotaxonomical viewpoint. Further, analysis of leaf constituents in these species by high-performance liquid chromatography (HPLC) showed the presence of several small peaks due to the unidentified compounds in addition to the large peaks due to the compounds isolated in this paper. Therefore, in order to establish the chemotaxonomical classification of *Forsythia* species on the basis of phenolic compounds, it is necessary to identify the minor compounds as well. More detailed studies are in progress.

Experimental

All melting points were determined on Yanagimoto micro-melting point apparatus and are uncorrected. The following instruments were used: optical rotations, Yanagimoto OR-10; ultraviolet (UV) spectra, Shimadzu UV-210; infrared (IR) spectra, Hitachi 270-30; proton nuclear magnetic resonance (^{1}H-NMR) spectra, JEOL JNM-FX 90Q with tetramethylsilane (TMS, $\delta=0$) as an internal reference; carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra, JEOL JNM-FX 60 equipped with a JEC-980 computer; mass spectra (MS), Shimadzu LKB-9000 and JEOL JMS-DX 303. The abbreviations used are as follows: s, singlet; d, doublet; m, multiplet. Precoated thin-layer chromatography (TLC) plates, Silica gel $60F_{254}$ (Merck), were used for TLC and preparative TLC. The spots were detected under UV (254 nm) illumination as dark absorbing spots, or by spraying the plates with 10% H_2SO_4 solution and heating. A high-performance liquid chromatography (HPLC) column, Erma ERC-ODS-2122 (8 × 250 mm), was used for HPLC. Silica gel (100 mesh, Mallinckrodt) was used for column chromatography.

Isolation——Dry leaves of *F. europaea* (17.5 g), collected in August 1986 at Kyoto Herbal Garden, Pharmacognosy Laboratories, Central Research Division, Takeda Chemical Industries, Ltd., Japan, were 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. The ether extract (0.2 g) was subjected to column chromatography on silica gel; elution was carried out with a $CHCl_3$–AcOEt solvent system with gradually increasing proportions of AcOEt. The fractions showing a TLC spot at *Rf* 0.64 were concentrated, and the residue was purified by preparative TLC using $CHCl_3$–AcOEt (4 : 1) to give 27.2 mg of **1**. When treated in the same way as described for **1**, the fractions showing a TLC spot at *Rf* 0.46 gave 14.9 mg of **2**. The $CHCl_3$ layer was evaporated to dryness. The $CHCl_3$ extract (0.3 g) was subjected to column chromatography on silica gel; elution was carried out with a $CHCl_3$–EtOH solvent system with gradually increasing proportions of EtOH. The fractions were monitored by TLC developed with $CHCl_3$–EtOH (4 : 1). The fractions showing a TLC spot at *Rf* 0.37 were concentrated, and the residue was purified by preparative TLC using $CHCl_3$–EtOH (4 : 1) to give 178.0 mg of **3**. When treated in the same way as described for **3**, the fractions showing TLC spot at *Rf* 0.27 gave 9.0 mg of **4**. The BuOH layer was evaporated to dryness. The BuOH extract (1.6 g) was subjected to column chromatography on Sephadex LH-20, eluting with H_2O. Repeated re-chromatography on Sephadex LH-20 gave 220.1 mg of **11** and 184.1 mg of **13**.

Dry leaves of *F. ovata* (19.4 g), collected in August 1985 at the Botanic Garden, Karl-Marx-Universität, Leipzig, DDR, were treated in the same manner as described for *F. europaea*. The ether extract (0.3 g) gave 74.9 mg of **5** and 22.8 mg of **6**. The $CHCl_3$ extract (1.1 g) gave 833.7 mg of **7** and 106.3 mg of **8**. The BuOH extract (1.6 g) gave 309.8 mg of **12** and 45.8 mg of **13**.

Dry leaves of *F. japonica* (26.2 g), collected in August 1977 at Medicinal Botanic Garden, Nagoya City University, Nagoya, Japan, were treated in the same manner as described for *F. europaea*. The ether extract (0.4 g) gave 39.1 mg of **1**, 16.9 mg of **2** and 28.2 mg of **9**. The $CHCl_3$ extract (0.9 g) gave a mixture of **3** and **10**, and 16.8 mg of **4**. The mixture was separated by HPLC (conditions: eluent, CH_3CN–water (15 : 85); flow rate, 4 ml/min; detector, UV detector (280 nm)) to give 268.2 mg of **3** and 164.2 mg of **10**. The BuOH extract (2.1 g) gave 481.2 mg of **12** and 69.0 mg of **13**.

Dry leaves of *F. giraldiana* (30 g), collected in August 1985 at Botanic Garden, Karl-Marx-Universität, Leipzig, DDR, were treated in the same manner as described for *F. europaea*. The ether extract (0.8 g) gave 39.0 mg of **1**, 28.0 mg of **2** and 202.5 mg of **9**. The $CHCl_3$ extract (0.9 g) gave a mixture of **3** and **10**, and 7.1 mg of **4**. The mixture was separated by HPLC to give 144.4 mg of **3** and 505.4 mg of **10**. The BuOH extract (1.3 g) gave 177.9 mg of **12** and 18.2 mg of **13**.

(+)-Pinoresinol Monomethyl Ether (9)——An amorphous powder. $[\alpha]_D^{23}$ +65.6° ($c=1.9$, $CHCl_3$). TLC *Rf* 0.57 (solvent system, $CHCl_3$: AcOEt = 1 : 1). UV λ_{max}^{EtOH} nm (log ε): 232 (4.16), 280 (3.73). IR ν_{max}^{KBr} cm^{-1}: 3404 (OH), 1610, 1518 (arom. C=C). Electron impact-mass spectra (EI-MS) *m/z*: 372 (M^+, $C_{21}H_{24}O_6$). ^{1}H-NMR (in $CDCl_3$) δ: 3.00—3.30 (2H, m, $C_{1,5}$-H), 3.86, 3.87, 3.88 (9H, each s, 3 × OCH_3), 3.70—4.35 (4H, m, $C_{4,8}$-H), 4.73 (2H, d, $J=5$ Hz, $C_{2,6}$-H), 6.70—7.00 (6H, m, arom. H). ^{13}C-NMR (in $CDCl_3$) δ: 54.0 (C-1, 5), 71.5 (C-4, 8), 85.6 (C-2, 6), 132.7 (C-1′), 133.6 (C-1″), 109.3 (C-2′), 108.6 (C-2″), 146.6 (C-3′), 149.2 (C-3″), 145.2 (C-4′), 148.6 (C-4″), 114.3 (C-5′), 111.2 (C-

5″), 118.8 (C-6′), 118.1 (C-6″), 55.7 (OCH_3).

(+)-Pinoresinol Monomethyl Ether-β-D-glucoside (10)——Colorless needles from EtOH. mp 148—150 °C. $[\alpha]_D^{23}$ +8.7° (c=1.1, MeOH). TLC Rf 0.39 (solvent system, $CHCl_3$: EtOH=4 : 1). UV λ_{max}^{MeOH} nm (log ε): 229 (4.23), 2.78 (3.74). IR ν_{max}^{KBr} cm^{-1}: 1590, 1518 (arom. C=C). Fast atom bombardment mass spectra (FAB-MS) m/z: 557 $[M(C_{27}H_{34}O_{11})+Na]^+$. ^{1}H-NMR (in CD_3OD) δ: 2.95—3.25 (2H, m, $C_{1,5}$-H), 3.80, 3.82, 3.86 (9H, each s, 3×OCH_3), 3.70—4.40 (4H, m, $C_{4,8}$-H), 6.80—7.25 (6H, m, arom. H). ^{13}C-NMR (in DMSO-d_6) δ: 53.5 (C-1, 5), 71.0 (C-4, 8), 84.8 (C-2,6), 135.1 (C-1′), 133.8 (C-1″), 110.5 (C-2′), 109.9 (C-2″), 148.9 (C-3′), 148.7 (C-3″), 145.8 (C-4′), 148.1 (C-4″), 115.2 (C-5′), 111.6 (C-5″), 118.1 (C-6′, 6″), 55.4, 55.6 (OCH_3), 100.1 (glc-l), 73.1 (glc-2), 76.8 (glc-3), 69.6 (glc-4), 76.8 (glc-5), 60.6 (glc-6).

Identification of Phenolic Compounds——Compounds **1** [colorless needles, mp 133—134 °C, $[\alpha]_D^{22}$ +120° (MeOH)], **2** [amorphous powder, $[\alpha]_D^{22}$ +61.6° ($CHCl_3$)], **3** [colorless needles, mp 146—148 °C, $[\alpha]_D^{22}$ +46.9° (MeOH)], **4** [amorphous powder, $[\alpha]_D^{20}$ +8.6° (EtOH)], **5** [colorless prisms, mp 90—92 °C, $[\alpha]_D^{23}$ −34.6° (EtOH)], **6** [colorless prisms, mp 89—91 °C, $[\alpha]_D^{22}$ −41.8° (EtOH)], **7** [colorless fine needles, mp 104—107 °C, $[\alpha]_D^{23}$ −45.1° (EtOH)], **8** [amorphous powder, mp 100—102 °C, $[\alpha]_D^{23}$ −44.1° (EtOH)], **11** [amorphous powder, mp 144—150 °C, $[\alpha]_D^{20}$ −18.6° (EtOH)], **12** [amorphous powder, mp 145—150 °C, $[\alpha]_D^{20}$ −66.5° (MeOH)] and **13** [yellow fine needles, mp 190—193 °C] were identified as phillygenin, (+)-pinoresinol, phillyrin, (+)-pinoresinol-β-D-glucoside, arctigenin, matairesinol, arctiin, matairesinoside, forsythiaside, acteoside and rutin by direct comparison with the respective authentic samples.[2)]

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