Spiro-biflavonoids from *Larix olgensis* HENRY var. *koreana* NAKAI

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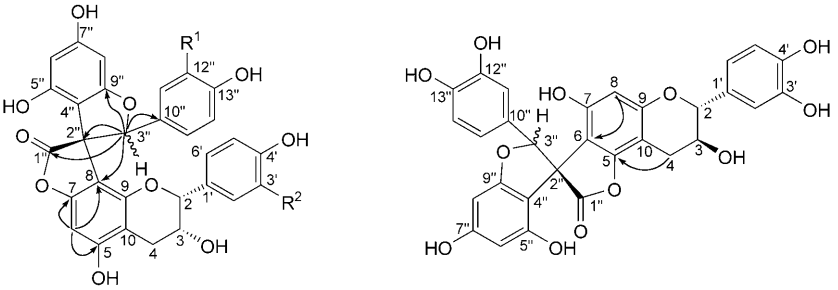
Four new spiro-biflavonoids, named olgensisinols A–D (**1**–**4**), along with a known one, vitisinol (**5**), were isolated from the stem bark of *Larix olgensis* HENRY var. *koreana* NAKAI. Their structures were established on the basis of spectroscopic analysis and molecular-model studies.

Introduction. – *Larix olgensis* HENRY var. *koreana* NAKAI (Pinaceae) is distributed in the Changbai Mountain area of Jilin Province and the drainage area of the Mudan River of Heilongjiang Province in China. Its stem bark has been used to produce tannin extract for China's indigenous leather industry for a long time [1]. Recently, we reported diterpenoids and lignans isolated from the $CHCl_3$-soluble fraction of the 80% EtOH extract of the bark of the title plant [2][3]. By reason of our continuous interest in the title plant, we examined the AcOEt-soluble fraction, leading to the isolation of five spiro-biflavonoids, olgensisinols A–D (**1**–**4**) and vitisinol (**5**) (*Fig. 1*)[1]).



1 R^1 = OH, R^2 = H, 3"β-H
2 R^1 = R^2 = OH, 3"α-H
5 R^1 = R^2 = OH, 3"β-H
6 R^1 = R^2 = H, 3"β-H

3 3"α-H
4 3"β-H

Fig. 1. *Structures and significant* $^1H,^{13}C$*-HMBC correlations* (H → C) *of* **1**–**4**[1])

[1]) Arbitrary atom numberings; for systematic names, see *Exper. Part.*

This paper describes the isolation and structure elucidation of the new spiro-biflavonoids **1**–**4**.

Results and Discussion. – Compound **5** ($[\alpha]_D^{20} = -126.5$ ($c = 1.72$, MeOH); CD ($c = 1.69$, MeOH): λ ([θ]) 232 (+1983), 243 (−39599), 286 (−18927)) was identified to be vitisinol on the basis of its physical and spectroscopic data which were well in agreement with those reported [4].

Compound **1** was obtained as yellow powder. The HR-ESI-MS of **1** gave the empirical molecular formula $C_{30}H_{22}O_{11}$ ($[M-1]^-$ at m/z 557.1089), and the IR spectrum showed absorption bands due to OH (3421 cm^{-1}) and aromatic groups (1623 and 1515 cm^{-1}) as well as to a γ-lactone carbonyl group (1782 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of **5**. Further spectroscopic data established the structure of olgensisinol A (**1**) as 2-(3,4-dihydroxyphenyl)-3′,4′-dihydro-3′,4,5′,6-tetrahydroxy-2′-(4-hydroxyphenyl)spiro[benzofuran-3(2*H*),9′(8′*H*)-[2*H*]furo-[2,3-*h*][1]benzopyran]-8′-one. The CD absorptions of **1** (λ[θ]) 230 (+10267), 237 (0), 243 (−26947), 284 (−14876)) were identical with those of **5** and of larixinol (**6**; (λ[θ]) 224 (+411185), 234 (0), 241 (−385871), 283 (−37594)), disclosing that they had the same absolute configuration. The absolute configuration of **6** had been tentatively assigned as (2*R*,2′*R*,3*R*,3′*R*) by *Shen et al.* according to its X-ray diffraction and possible biogenetic pathway [5] (systematic numbering). Thus, the configuration of **1** is *rel*-(2*R*,2′*R*,3*R*,3′*R*).

The ^{1}H-NMR spectrum of **1** (*Table* 1) displayed signals of two *meta*-coupled aromatic protons (δ 5.91 and 5.98 (each *d*, $J = 2.0$ Hz), aromatic protons with an *ABC*-type pattern (δ 6.60 (*d*, $J = 2.0$ Hz, 1 H), 6.53 (*d*, $J = 8.0$, 1 H), and 6.46 (*dd*, $J = 8.0$, 2.0 Hz, 1 H) and characteristic signals attributed to H−C(2), H−C(3), and CH_2(4)[1]) of a 2,3-*cis* flavan-3-ol moiety (δ 4.74 (*s*, 1 H), 4.10 (*m*, 1 H), and 2.67 (*d*, $J = 4.0$ Hz, 2 H). An aromatic-proton *s* at δ 6.08 (*s*, 1 H) suggested that C(6) or C(8)[1]) of the flavan-3-ol unit was substituted. The ^{13}C-NMR spectrum (*Table* 2) showed 30 signals, which were assigned to 1 CH_2, 13 CH, and 16 quaternary C-atoms based on the ^{13}C-DEPT experiments. With the exception of the signals of a *p*-substituted aromatic ring (δ(H) 6.77 and 6.99 (each *d*, $J = 9.0$ Hz, each 2 H); δ(C) 115.7(2 C) and 128.7(2 C) in **1** instead of the signals ascribed to ring B of **5**, the NMR spectra of **1** and **5** were very similar, suggesting a 3′-deoxyvitisinol[1]) structure for **1**. The ROESY (*Fig.* 2) correlations H−C(2)/H−C(11″) and H−C(15″)[1]) confirmed that **1** had the same spacial orientation as **5**.

The empirical molecular formula of compound **2** was determined to be $C_{30}H_{22}O_{12}$ by its HR-ESI-MS $[M-1]^-$ signal at m/z 573.1039, indicating an isomer of **5**. The ^{1}H-NMR spectrum of **2** (*Table 1*) showed signals similar to those of **5**, such as an epiafzelechin moiety, two benzene rings, and a downfield proton (δ(H) 6.25 (*s*)). The ^{13}C-NMR (*Table* 2) data of **2** were almost superimposed with those of **5**, except that the δ values of C(1″) and C(3″)[1]) were shifted upfield ($\Delta\delta = -4$ and -3.5, resp.) for **2** as compared to **5**, suggesting a different configuration, at C(3″). The ROESY data revealed a 2″,3″-*cis* relation for **2** (NOEs H−C(3″)/H−C(2′) and H−C(6′)[1]), see *Fig.* 2). Correspondingly, the CD curve of **2** was obviously different from those of **1** and **5**, and olgensisinol B (**2**) was thus formulated as *rel*-(2*R*,2′*S*,3*S*,3′*S*)-2,2′-bis(3,4-dihydroxyphenyl)-3′,4′-dihydro-3′,4,5′,6-tetrahydroxy-spiro[benzofuran-3(2*H*),9′(8′*H*)-[2*H*]furo[2,3-*h*][1]benzopyran]-8′-one.

Compounds **3** and **4** had the same molecular formula $C_{30}H_{22}O_{12}$, deduced from their HR-ESI-MS $[M-1]^-$ peaks at m/z 573.1031 and 573.1030, respectively. Their MS, IR, and NMR spectra showed that they were also spiro-biflavonoids by comparison with

Table 1. *^{1}H-NMR Data of Compounds* **1**–**4** *at 500 MHz in CD_3OD*[1]). δ in ppm, J in Hz.

	1	**2**	**3**	**4**
H–C(2)	4.74 (*s*)	4.90 (*s*)	4.58 (*d*, *J* = 7.3)	4.68 (*d*, *J* = 7.5)
H–C(3)	4.09–4.10 (*m*)	4.35 (*dd*, *J* = 3.0, 4.0)	3.97–4.00 (*m*)	4.00–4.02 (*m*)
CH_2(4)	2.67 (*d*, *J* = 4.0)	2.95 (*dd*, *J* = 4.0, 17.0, H_α)	2.85 (*dd*, *J* = 5.0, 16.0, H_α)	2.80 (*dd*, *J* = 5.0, 16.0, H_α)
		2.87 (*dd*, *J* = 3.0, 17.0, H_β)	2.55 (*dd*, *J* = 8.0, 16.0, H_β)	2.57 (*dd*, *J* = 8.0, 16.0, H_β)
H–C(6)	6.08 (*s*)	6.17 (*s*)		
H–C(8)			5.88 (*s*)	6.19 (*s*)
H–C(2′)	6.99 (*d*, *J* = 9.0)	6.86 (*d*, *J* = 2.0)	6.78 (*d*, *J* = 1.7)	6.88 (*d*, *J* = 1.6)
H–C(3′)	6.77 (*d*, *J* = 9.0)			
H–C(5′)	6.77 (*d*, *J* = 9.0)	6.71 (*d*, *J* = 8.0)	6.73 (*d*, *J* = 8.0)	6.80 (*d*, *J* = 8.1)
H–C(6′)	6.99 (*d*, *J* = 9.0)	6.66 (*dd*, *J* = 2.0, 8.0)	6.58 (*dd*, *J* = 1.7, 8.0)	6.75 (*dd*, *J* = 1.6, 8.1)
H–C(3″)	5.79 (*s*)	6.25 (*s*)	5.72 (*s*)	6.07 (*s*)
H–C(6″)	5.91 (*d*, *J* = 2.0)	5.80 (*d*, *J* = 2.0)	5.83 (*d*, *J* = 1.8)	5.85 (*d*, *J* = 2.0)
H–C(8″)	5.98 (*d*, *J* = 2.0)	5.96 (*d*, *J* = 2.0)	5.95 (*d*, *J* = 1.8)	5.98 (*d*, *J* = 2.0)
H–C(11″)	6.60 (*d*, *J* = 2.,0)	6.73 (*d*, *J* = 2.0)	6.85 (*d*, *J* = 2.0)	6.67 (*d*, *J* = 1.5)
H–C(14″)	6.53 (*d*, *J* = 8.0)	6.67 (*d*, *J* = 8.0)	6.58 (*d*, *J* = 8.4)	6.64 (*d*, *J* = 8.2)
H–C(15″)	6.46 (*dd*, *J* = 2.0, 8.0)	6.58 (*dd*, *J* = 2.0, 8.0)	6.71 (*dd*, *J* = 2.0, 8.4)	6.42 (*dd*, *J* = 1.5, 8.2)

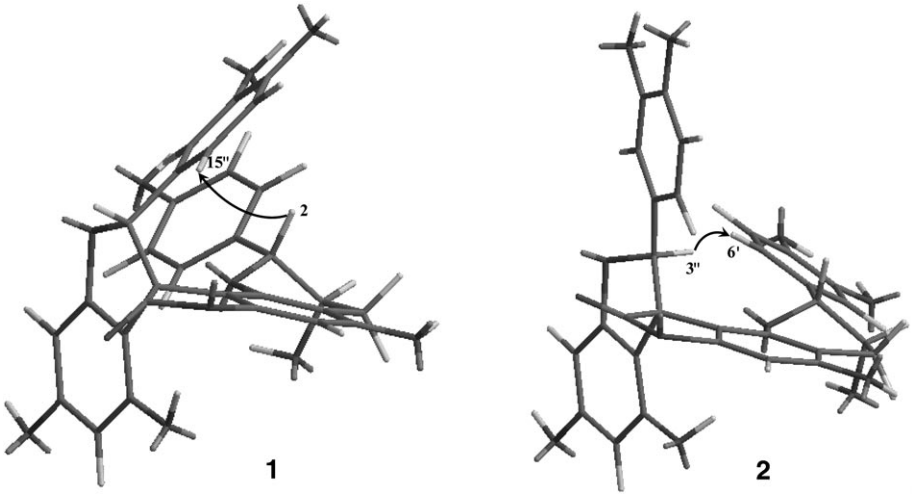


Fig. 2. *Key ROESY correlations of* **1** *and* **2**[1]) (The models were generated by Chem3D Ultra 8.0, (*CambridgeSoft*), calculated minimize energy using MM2 with Mininum *RMS* = 0.100)

those of **1**, **2**, and **5**. Departing from the latter compounds, the flavan-3-ol unit of **3** and **4** were elucidated to have a 2,3-*trans* configuration based on the diagnostic H–C(2)[1]) signals in the ^{1}H-NMR spectra (*Table 1*; δ 4.58 (*d*, *J* = 7.3 Hz) and 4.68 (*d*, *J* = 7.5 Hz), resp.) [6]. Furthermore, the linkage of the two flavonoid units in **3** and **4** were determined as C(2″)–C(6) and C-(1″)–O–C(5)[1]) by comparison of their ^{13}C-NMR data (*Table 2*) with those of cinchonain Ic [7] and by the HMBC correlations (*Fig. 1*). Concerning the relative configuration at C(2″) and C(3″), an inspection of the δ(C) of the γ-lacatone carbonyl group of some reported spiro-biflavones and related structures, such

Table 2. *^{13}C-NMR Data of Compounds* **1**–**4** *at 125 MHz in CD_3OD*[1]). δ in ppm.

	1	**2**	**3**	**4**
C(2)	79.1	79.6	83.5	83.7
C(3)	67.0	66.2	68.4	68.4
C(4)	29.0	29.3	28.4	28.4
C(5)	158.0	158.3	152.9	154.2
C(6)	91.2	91.6	107.6	107.5
C(7)	152.8	153.9	155.2	154.7
C(8)	106.1	106.5	100.7	100.2
C(9)	153.2	153.0	157.3	157.6
C(10)	104.1	104.7	97.5	98.2
C(1′)	130.9	131.5	132.2	132.2
C(2′)	128.7	114.7	115.8	115.8
C(3′)	115.7	145.9	146.8[a])	146.7
C(4′)	157.6	145.6	146.8[a])	146.8
C(5′)	115.7	116.0	116.6	116.7
C(6′)	128.7	119.3	120.5	120.6
C(1″)	181.0	177.1	181.1	177.3
C(2″)	61.8	61.5	62.5	62.4
C(3″)	94.5	90.9	95.6	90.6
C(4″)	106.6	105.8	107.1	106.3
C(5″)	155.4	156.0	155.8	156.7
C(6″)	96.8	96.7	97.2	97.3
C(7″)	161.4	161.1	161.7	161.7
C(8″)	91.1	90.7	91.1	91.3
C(9″)	163.8	164.6	164.9	165.0
C(10″)	129.1	129.0	129.1	129.0
C(11″)	114.0	114.3	115.0	114.8
C(12″)	145.8	145.9	146.2[a])	146.5
C(13″)	145.9	146.4	146.7	147.0
C(14″)	115.5	115.9	116.3	116.5
C(15″)	118.1	118.7	119.6	119.0

[a]) May be interchangeable.

as yuccaols A–E [8][9], of **5** and **6**, as well as of **1** and **2** showed that this δ(C) was shifted downfield by *ca.* 4 ppm for the *trans*-dihydrofurans (δ(C) *ca.* 181) as compared to the *cis*-dihydrofurans (δ(C) *ca.* 177). Thus the benzodihydrofuran moiety of **3** and **4** was established to be *trans* and *cis*, respectively. Compounds **3** and **4** were, therefore, elucidated to be *rel*-(2*R*,3*S*,7′*R*,8′*S*)- and *rel*-(2*R*,3*R*,7′*S*,8′*R*)-2,7′-bis(3,4-dihydroxyphenyl)-8′,9′-dihydro-4,4′,6,8-tetrahydroxy-spiro[benzofuran-3(2*H*),3′(2′*H*)-[7*H*]furo-[2,3-*f*][1]benzopyran]-2′-one, respectively.

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Experimental Part

General. Column chromatography (CC): *Sephadex LH-20* (*Pharmacia Fine Chemicals*, Piscataway, NJ, USA); *ODS* (25–40 μ, *Merck*); *XAD-7HP* (*Rohm and Haas*, USA); *MCI* gel *CHP-20P* (75–150 μm; *Mitsubishi*

Chemical Industries, Tokyo, Japan). M.p.: *RY-2* apparatus (*Analytical Instruments Co*., Tianjin, China); uncorrected. Optical rotations: *Perkin-Elmer-343* polarimeter. Circular dichroism (CD): *Jasco-J-810* spectropolarimeter; $\lambda([\theta])$ in nm. IR Spectra: *Bruker-Vector-22* spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *DRX-500* spectrometer (500 and 125 MHz); in CD_3OD; δ in ppm rel. to $SiMe_4$, J in Hz. HR-ESI-MS: *Q-Tof* micro mass spectrometer; in m/z.

Plant Material. The stem barks of *Larix olgensis* HENRY var. *koreana* NAKAI were collected in Jilin Province, China, in September, 2002, and identified by Professor *Han-chen Zheng*, Department of Pharmacognosy of this college. A voucher specimen (20020910) is deposited at our laboratory.

Extraction and Isolation. The air-dried and powdered stem bark (10 kg) of *L. olegensis* was extracted with 80% EtOH (30 l). After evaporation of EtOH, the remaining aq. soln. (3 l) was partitioned successively with $CHCl_3$ (3×3 l) and AcOEt (3×3 l). A part (300 g) of the AcOEt extract (total 670 g) was subjected to CC (*XAD-7 HP* resin, H_2O, 20% EtOH, 40% EtOH, 60% EtOH, and EtOH): *Fractions I–V. Fr. III* (88 g) was subjected to CC (*Sephadex LH-20*, 20%, 40%, 60% MeOH/H_2O): *Fr. III.a–c. Fr. III.b* (25 g) was further purified by CC (*MCI* gel *CHP-20P*; gradient $H_2O \rightarrow$ EtOH): **5** (300 mg; with 20% EtOH) and **1** (34 mg; with 35% EtOH). *Fr. III.c* (30 g) was separated into *Fr. III.c.1* (12 g) and *III.c.2* (14 g) by CC (*Sephadex LH-20*; 50% MeOH). *Fr. II.c.1* was purified by CC (*ODS*, 35% MeOH): **2** (88 mg). *Fr. III.c.2* was separated by repeated CC (*ODS*, 40% MeOH): **3** (21 mg) and **4** (14 mg).

rel-*(2*R*,2′*R*,3*R*,3′*R*)-2-(3,4-Dihydroxyphenyl)-3′,4′-dihydro-3′,4,5′,6-tetrahydroxy-2′-(4-hydroxyphenyl)-spiro[benzofuran-3(2*H*),9′(8′*H*)-[2*H*]furo[2,3-*h*][1]benzopyran]-8′-one* (= *Olgensisin A*; **1**): Yellow powder. M.p. 180° (dec.). $[\alpha]_D^{20} = -113.3$ ($c = 0.60$, MeOH). CD ($c = 1.36$ mM, MeOH): 230 (+10267), 243 (−26947), 284 (−14876). IR (KBr): 3421(OH), 2925, 1782, 1623, 1515, 1467, 1383, 1173, 1138, 1087, 1064, 1008, 817. ^{1}H- and ^{13}C-NMR (CD_3OD); *Tables 1* and *2*. ESI-MS: 581.09 ($[M+Na]^+$), 1137.24 ($[2M+Na]^+$). HR-ESI-MS: 557.1089 ($[M-1]^-$, $C_{30}H_{21}O_{11}^-$; calc. 557.1084).

*(2*R*,2′*S*,3*S*,3′*S*)-2,2′-Bis(3,4-dihydroxyphenyl)-3′,4′-dihydro-3′,4,5′,6-tetrahydroxyspiro[benzofuran-3(2*H*),9′(8′*H*)-[2*H*]furo[2,3-*h*][1]benzopyran]-8′-one* (= *Olgensisin B*; **2**): Yellow powder. M.p. 170° (dec.). $[\alpha]_D^{20} = -26.1$ ($c = 2.98$, MeOH). CD ($c = 0.627$ mM, MeOH): 214 (−16251), 230 (−5823), 239 (+9885), 281 (+9987). IR (KBr): 3480, 2925, 1785, 1625, 1520, 1467, 1359, 1283, 1190, 1135, 1078, 1015, 926, 875, 816, 634. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. ESI-MS: 597.09 ($[M+Na]^+$), 1171.27 ($[2M+Na]^+$). HR-EI-MS: 573.1039 ($[M-1]^-$, $C_{30}H_{21}O_{12}^-$; calc. 573.1033).

rel-*(2*R*,3*S*,7′*R*,8′*S*)-2,7′-Bis(3,4-dihydroxyphenyl-8′,9′-dihydro-4,4′,6,8′-tetrahydroxyspiro[benzofuran-3(2*H*),3′(2′*H*)-[7*H*]furo[2,3-f][1]benzopyran]-2′-one* (= *Olgensisin C*; **3**): Yellow powder. M.p. 160° (dec.). $[\alpha]_D^{20} = -19.5$ ($c = 1.03$, MeOH). CD ($c = 1.45$ mM, MeOH): 226 (+13880), 243 (−12383), 290 (−9739). IR (KBr): 3422, 2924, 1785, 1621, 1512, 1447, 1383, 1284, 1143, 1077, 1035, 873, 817, 779. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. ESI-MS: 575.12 ($[M+1]^+$), 597.11 ($[M+Na]^+$). HR-EI-MS: 573.1031 ($[M-1]^-$, $C_{30}H_{21}O_{12}^-$; calc. 573.1033).

rel-*(2*R*,3*R*,7′*S*,8′*R*)-2,7′-Bis(3,4-dihydroxyphenyl)-8′,9′-dihydro-4,4′,6,8′-tetrahydroxyspiro[benzofuran-3(2*H*),3′(2′*H*)-[7*H*]furo[2,3-f][1]benzopyran]-2′-one* (= *Olgensisin D*; **4**): Yellow powder. M.p. 160° (dec.). $[\alpha]_D^{20} = +109.2°$ ($c = 1.91$, MeOH). CD ($c = 1.71$ mM, MeOH): 222 (+2346), 257 (+1583), 288 (+16187). IR (KBr): 3419, 1786, 1623, 1513, 1449, 1382, 1284, 1140, 1076, 1033, 872, 818, 634. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. ESI-MS: 597.03 ($[M+Na]^+$), 1171.11 ($[2M+Na]^+$). HR-EI-MS: 573.1030 ($[M-1]^-$, $C_{30}H_{21}O_{12}^-$; calc. 573.1033).

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