Apocynins A—D: New Phenylpropanoid-substituted Flavan-3-ols Isolated from Leaves of *Apocynum venetum* (*Luobuma-Ye*)

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Four new phenylpropanoid-substituted flavan-3-ols called apocynins A—D (1—4) have been isolated from the leaves of *Apocynum venetum* (Apocynaceae), together with two known phenylpropanoid-substituted flavan-3-ols, catechin-[8,7-e]-4α-(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone (5) and cinchonain Ia (6), and four known flavan-3-ols, (−)-epicatechin, (+)-catechin, (−)-epigallocatechin, and (+)-gallocatechin. Their structures were elucidated on the basis of spectral analysis, including 2D NMR and CD spectra. They showed hepatoprotective activity against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

Key words apocynin; *Apocynum venetum*; phenylpropanoid-substituted flavan-3-ol; *Luobuma-Ye* (羅布麻葉); hepatoprotective activity; Apocynaceae

Apocynum venetum is a perennial plant belonging to the family Apocynaceae, and its leaves have been used, under the name *Luobuma-Ye* (羅布麻葉), to treat cardiac disease, hypertension, nephritis, neurasthenia, influenza, etc.[1,2] Recently, we have reported that an extract of the leaves has antilipid peroxidation, antihypercholesteremic, and antihypertensive activities and preventive activity against atherosclerosis.[3] In our continued study of *Luobuma-Ye*, a water extract of the leaves of *A. venetum* was found to exhibit hepatoprotective activity against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes (inhibition rate at 50 μg/ml, 55.8%).[4] Thus we separated the water extract with a combination of Sephadex LH-20 column chromatography and reversed-phase preparative TLC and obtained four new phenylpropanoid-substituted flavan-3-ols called apocynins A—D (**1**—**4**, yields from water extract were 0.0024%, 0.0097%, 0.0035%, and 0.0031%, respectively), along with two known phenylpropanoid-substituted flavan-3-ols, catechin-[8,7-e]-4α-(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone[5,6] (**5**, 0.0020%) and cinchonain Ia[5—7] (**6**, 0.0020%), and four known flavan-3-ols, (−)-epicatechin (0.0488%), (+)-catechin (0.0015%), (−)-epigallocatechin (0.0258%), and (+)-gallocatechin (0.0078%). In this communication, we report the structure elucidation of apocynins A—D (**1**—**4**) using spectroscopic methods.

Apocynins A (**1**)[8] and B (**2**)[9] were obtained as brown solids and showed the same $[M+H]^+$ ion at *m/z* 469, 16 amu more than **5** and **6**, in positive-ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **1** and **2** were similar to each other (Table 1) and to those of catechin-[8,7-e]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone[5,6] (**7**) and **5**, respectively. Their ^{1}H- and ^{13}C-NMR data, however, showed the signals of a 3,4,5-trisubstituted phenyl group, instead of those of a catechin B-ring in **7** and **5**, indicating that **1** and **2** should contain a gallocatechin unit, not a catechin unit. This was confirmed by the field gradient-pulsed (FG-pulsed) ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) and difference NOE spectra. In the FG-pulsed HMBC spectrum of **1**, H-2 and H-2′,6′ showed the correlations with C-2′,6′ and C-2, respectively, while H-7″ was correlated with C-2″ and C-6″ (Fig. 1, left). On the other hand, in the difference NOE experiments, **1** showed NOEs from H-2 to H-2′,6′ (9.8%) and from H-2′,6′ to H-2 (7.2%) and H-3 (3.8%). Similarly, **2** revealed NOEs from H-2 to H-2′,6′ (9.7%), from H-2′,6′ to H-2 (7.2%) and H-3 (5.8%), from H-2″ to H-7″ (3.4%), from H-6″ to H-7″ (3.6%), and from H-7″ to H-2″ (3.3%) and H-6″ (3.1%). Then the absolute configurations of **1** and **2** were determined by comparing the $[\alpha]_D$ and CD data with those of **7** and **5**. The $[\alpha]_D^{25}$ values of **1** and **2** were −46.9 °C and +49.9 °C, respectively, while they showed CD bands of opposite signs at 233 nm (**1**, $[\theta]_{233}$ −13200; **2**, $[\theta]_{233}$ +7590). The same relationship occurred between **7** ($[\alpha]_D^{25}$ −159.2 °C, $[\theta]_{234}$ −94000)[6] and **5** ($[\alpha]_D^{25}$ +93.7 °C, $[\theta]_{233}$ +60000).[6] Thus it was concluded that the structures of apocynins A and B were **1** and **2**, respectively.

Apocynin C (**3**) was also obtained as a brown solid and showed the same $[M+H]^+$ ion as **1** and **2** in positive-ion FAB-MS. The ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **1** and **2**, but the very small (nearly zero) coupling constant between H-2 and H-3 suggested that the flavan-3-ol unit was an epigallocatechin unit. The presence of an epigallocatechin unit was confirmed by the correlations observed in the FG-pulsed HMBC spectrum (Fig. 1, right). The configuration at C-7″ was determined to be β based on the sign of the CD band at 233 nm ($\Delta\varepsilon$ −3.0; $[\theta]_{233}$ −9900), which was the same (minus) as that of **6** ($\Delta\varepsilon$ −37.0)[5—7] but not of its 7″ α-epimer ($\Delta\varepsilon$ +10.9).[5—7]

Apocynin D (**4**) was also obtained as a brown solid and showed the same pseudomolecular ion (*m/z* 469). The ^{1}H- and ^{13}C-NMR spectra of **4** resembled those of **1** and **2**, suggesting that **4** should also be a phenylpropanoid-substituted

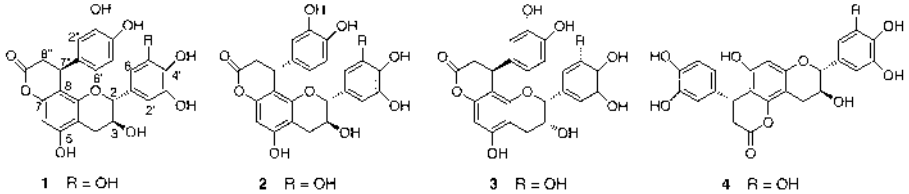

Chart 1

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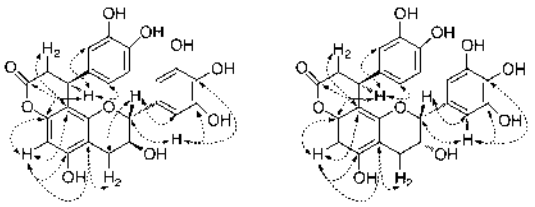
Table 1. ^{1}H- and ^{13}C-NMR Data for Apocynins A—D (**1**—**4**) in CD_3OD

	1		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	4.52 d (7.5)	83.6	4.64 d (6.3)	82.5	4.79 s	79.7	4.67 d (6.5)	82.7
3	3.92 ddd (8.2, 7.5, 5.6)	68.5	4.02 ddd (7.0, 6.3, 5.0)	68.0	4.22 dd (4.5, 3.0)	66.7	4.06 ddd (7.0, 6.5, 5.6)	68.0
4	2.91 dd (16.5, 5.6)	28.8	2.80 dd (16.5, 5.0)	27.5	2.89 dd (16.0, 4.5)	29.2	2.84 dd (16.5, 5.6)	27.3
	2.58 dd (16.5, 8.2)		2.59 dd (16.5, 7.0)		2.82 dd (16.0, 3.0)		2.68 dd (16.5, 7.0)	
5		156.8		156.9		157.2		154.8
6	6.20 s	96.4	6.19 s	96.3	6.19 s	96.2		106.8
7		152.1		152.2		152.0		155.6
8		105.7		105.7		106.0	6.22 s	99.7
4a		106.1		106.7		105.1		101.3
8a		153.2		152.9		153.3		151.9
1′		130.9		131.4		131.2		131.1
2′, 6′	6.25 s	107.3	6.35 s	107.0	6.50 s	106.7	6.37 s	107.1
3′, 5′		146.7		146.9		146.6		146.3
4′		134.1		134.0		133.7		134.0
1″		135.2		135.1		135.3		134.8
2″	6.53 d (2.0)	115.1	6.51 d (2.0)	115.1	6.53 d (2.0)	115.0	6.54 d (2.0)	115.0
3″		146.5		145.3		146.2		146.9
4″		146.2		145.9		145.1		146.3
5″	6.65 d (8.0)	116.6	6.60 d (8.0)	116.3	6.60 d (8.0)	116.4	6.65 d (8.0)	116.4
6″	6.44 dd (8.0, 2.0)	119.5	6.41 dd (8.0, 2.0)	119.2	6.46 dd (8.0, 2.0)	119.2	6.46 dd (8.0, 2.0)	119.2
7″	4.38 dd (7.0, 1.6)	35.2	4.45 dd (6.7, 1.6)	35.2	4.45 dd (6.7, 1.9)	35.3	4.44 dd (7.0, 1.6)	35.1
8″	3.02 dd (15.7, 7.0)	38.6	3.08 dd (15.7, 6.7)	38.5	3.05 dd (15.7, 6.7)	38.5	3.02 dd (15.7, 7.0)	38.4
	2.81 dd (15.7, 1.6)		2.86 dd (15.7, 1.6)		2.87 dd (15.7, 1.9)		2.87 dd (15.7, 1.6)	
9″		170.6		170.7		170.7		170.3

δ in ppm from TMS (coupling constants in Hz are given in parentheses).



1 3

Fig. 1. Long-range Correlations Observed in the FG-pulsed HMBC Spectra of **1** and **3**

gallocatechin. This was confirmed by the NOEs observed in the difference NOE experiments: H-2 to H-2′,6′ (10.2%), H-2′,6′ to H-2 (6.5%) and H-3 (4.8%), H-7″ to H-2″ (6.7%) and H-6″ (5.0%). The ^{13}C-NMR chemical shift of the A-ring methine (δ 99.7) in **4** was, however, in accord with those of C-6 phenylpropanoid-substituted catechins (δ 99.8)[6] and epicatechins (δ 99.4),[7] rather than those of **1** (δ 96.4), **2** (δ 96.3) and C-8 phenylpropanoid-substituted catechins (δ 96.3—96.6)[5,6] or epicatechins (δ 95.8—96.9).[5—7] The configuration at C-7″ was determined to be α, by comparing the sign of the CD band at 233 nm ($[\alpha]_{233}$ +6930) with those of the 7″α-type catechin derivative **8** ($[\theta]_{225}$ +24000)[6] and its 7″β-epimer ($[\theta]_{230}$ −33000).[6] From these data, it was concluded that the structure of apocynin D was **4**.

Compounds **1**—**6** showed hepatoprotective activity against D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes at 20—80 μM in a concentration-dependent manner.[8—12] At 80 μM concentration, **1**—**6** showed 60.0, 39.6, 46.1, 36.8, 44.5, and 95.1% inhibition against cell death, while (+)-catechin, (−)-epicatechin, (+)-gallocatechin, and (−)-epigallocatechin showed weaker inhibitions (*ca*. 15—30%). Thus the presence of the phenylpropanoid unit could be important for improving the inhibitory activity.

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8) Apocynin A (**1**): $[\alpha]_D^{25}$ −46.9° (*c* 0.19, MeOH). UV λ_{max} (MeOH) nm (log ε): 210 (4.74), 230sh, 270 (4.02). FAB-MS *m*/*z*: 469 $[M+H]^+$. CD $[\theta]$ (c=2.91×10^{-5}, MeOH, nm): −13200 (233). Inhibition rates (%) at 20, 40, and 80 μM: 15.6, 24.4, 60.0.
9) Apocynin B (**2**): $[\alpha]_D^{25}$ +49.9° (*c* 0.46, MeOH). UV λ_{max} (MeOH) nm (log ε): 218 (4.59), 230sh, 270 (3.86). FAB-MS *m*/*z*: 469 $[M+H]^+$. CD $[\theta]$ (c=5.2×10^{-5}, MeOH, nm): +7590 (233). Inhibition rates (%) at 20, 40, and 80 μM: 2.7, 13.0, 39.6.
10) Apocynin C (**3**): $[\alpha]_D^{25}$ −57.6° (*c* 0.28, MeOH). UV λ_{max} (MeOH) nm (log ε): 210 (4.84), 230sh, 270 (4.00). FAB-MS *m*/*z*: 469 $[M+H]^+$. CD $[\theta]$ (c=2.0×10^{-5}, MeOH, nm): −9900 (233). Inhibition rates (%) at 20, 40, and 80 μM: 9.5, 14.2, 46.1.
11) Apocynin D (**4**): $[\alpha]_D^{25}$ +45.5° (*c* 0.23, MeOH). UV λ_{max} (MeOH) nm (log ε): 210 (4.52), 230sh, 270 (3.75). FAB-MS *m*/*z*: 469 $[M+H]^+$. CD $[\theta]$ (c=5.3×10^{-5}, MeOH, nm): +6930 (233). Inhibition rates (%) at 20, 40, and 80 μM: 12.3, 13.4, 36.8.
12) Inhibition rates (%) of **5**, **6**, and silibinin, a positive control, at 20, 40, and 80 μM: **5**, 10.7, 13.6, 44.5; **6**, 26.6, 47.3, 95.1; silibinin, 14.8, 39.4, 86.4.