Medicinal Foodstuffs. XXIV.[1] Chemical Constituents of the Processed Leaves of *Apocynum venetum* L.: Absolute Stereostructures of Apocynosides I and II

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Two new ionone glucosides, named apocynosides I and II, were isolated from the roasted leaves of *Apocynum venetum* L. together with nine known compounds. The absolute stereostructures of apocynosides I and II were determined by chemical and physicochemical evidence, which included the application of a modified Mosher's method and the circular dichroism helicity rule.

Key words apocynoside; *Apocynum venetum*; ionone glucoside; absolute stereostructure; modified Mosher's method; circular dichroism helicity rule

The Apocynaceae plant *Apocynum* (*A.*) *venetum* L. (Chinese name "**羅布麻**") is used for the treatment of cardiac diseases, hypertension, and hepatitis. Recently, the roasted leaves of this plant have been used as a health food in Japan. As chemical constituents of this plant, many phenolic compounds have been isolated from the leaves of *A. venetum*.[2]

In the course of our continuing studies on the bioactive constituents of medicinal foodstuffs,[1,3] we have isolated two new ionone glucosides, called apocynosides I (**1**) and II (**2**), together with nine known compounds from the roasted leaves of *A. venetum* L. This paper describes the isolation and structural elucidation of apocynosides I (**1**) and II (**2**).

The roasted leaves of *A. venetum* L. were extracted with methanol under reflux. The methanol extract was partitioned into an ethyl acetate–water mixture to furnish the ethyl acetate-soluble portion and water phase. The water phase was further extracted with 1-butanol to give a 1-butanol-soluble portion and a water-soluble portion. The ethyl acetate-soluble portion was subjected to normal- and reversed-phase silica gel column chromatography and HPLC to give quercetin (0.014%), phytol[4] (0.0003%), and lupeol[5] (0.012%). The 1-butanol-soluble portion was subjected to normal-phase silica gel column chromatography to give seven fractions. Fraction 4 was separated by reversed-phase silica gel column chromatography and repeated HPLC to give apocynosides I (**1**, 0.0002%) and II (**2**, 0.0004%), together with phenethyl alcohol xylopyranosyl(1→6)glucopyranoside[6] (0.0006%), *Z*-hex-3-en-1-ol xylopyranosyl(1→6)glucopyranoside[7] (0.0008%), isoquercitrin[8] (0.0005%), and kaempferol 3-*O*-β-D-galactopyranoside[9] (0.0001%). Fraction 5 was subjected to reversed-phase silica gel column chromatography and purified by HPLC to give benzyl alcohol xylopyranosyl(1→6)glucopyranoside[6] (0.0007%) and hyperoside[9] (0.0002%).

Apocynoside I (**1**) was isolated as an amorphous powder with positive optical rotation ($[\alpha]_D^{27}$ +79.2°). The IR spectrum of **1** showed absorption bands assignable to hydroxyl and enone functions at 3432, 1655, 1619, and 1076 cm^{-1}. In the negative- and positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 385 $(M-H)^-$ and *m/z* 387 $(M+H)^+$, and the molecular formula $C_{19}H_{30}O_8$ of **1** was determined by high-resolution MS measurement. Furthermore, a fragment ion peak at *m/z* 223 $(M-C_6H_{11}O_5)^-$, which was derived by cleavage of the glycoside linkage at the 1′-position, was observed in the negative-ion FAB-MS. By acid hydrolysis with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane, **1** liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolidine derivatives.[10] Enzymatic hydrolysis of **1** with β-glucosidase yielded an aglycone called apocynol A (**3**), whose molecular formula, $C_{13}H_{20}O_3$, was determined from the positive-ion FAB-MS [*m/z* 225 $(M+H)^+$] and by high-resolution MS measurement. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3**, which were assigned by various NMR analytical methods,[11] showed the presence of two singlet methyls [δ 0.99, 1.04 (both s, 13, 12-H_3)], a doublet methyl [δ 1.28 (d, J=6.4 Hz, 10-H_3)], a methine and methylene bearing a hydroxyl group [δ 4.34 (dd-like, 9-H), 4.17, 4.23 (both d, J=17.1 Hz, 11-H_2)], a disubstituted olefin [δ 5.67 (dd, J=8.5, 15.6 Hz, 7-H), 5.60 (dd, J=6.5, 15.6 Hz, 8-H)], and an enone function [δ 6.18 (s, 4-H)] together with a methine [δ 2.59 (d, J=8.5 Hz, 6-H)], a methylene [δ 2.13, 2.41 (both d, J=16.8 Hz, 2-H_2)], and a quaternary carbon [δ_C 36.2 (1-C)]. On the other hand, the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1** showed signals due to an apocynol A moiety, together with a β-D-glucopyranosyl part [δ 4.84 (d, J=7.6 Hz, 1′-H)]. In the HMBC experiment on **1**, long-range correlations were observed between the following protons and carbons: 2-H_2 and 1, 3-C, 11-H_2 and 4, 5, 6-C, 12, 13-H_3 and 1, 2, 6-C, 10-H_3 and 8, 9-C, 8-H and 6, 7, 9-C, 1′-H and 11-C (Fig. 1). By comparison of the ^{13}C-NMR data for **1** with those for **3**, a

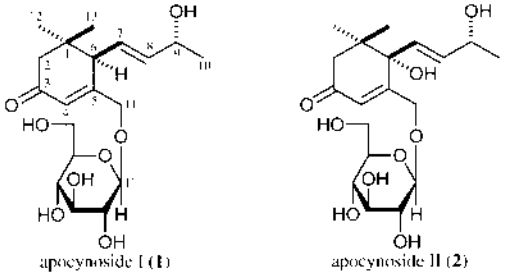

Chart 1

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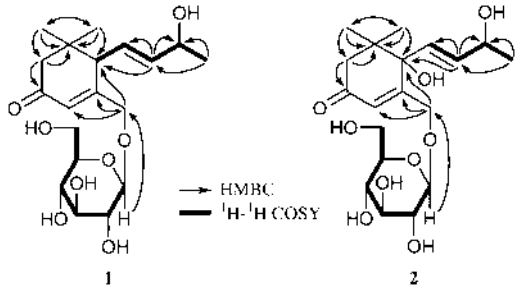

Fig. 1. HMBC and ^{1}H–^{1}H COSY Correlations of **1** and **2**

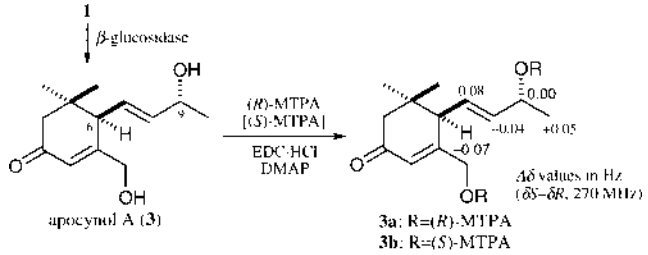

Fig. 2

Table 1. ^{13}C-NMR Data of Apocynosides I (**1**) and II (**2**) and Apocynols A (**3**) and B (**4**)

	1[a]	**2**[a]	**3**[b]	**4**[a]
1	36.1	42.0	36.2	42.0
2	48.6	50.3	48.1	50.5
3	198.2	198.1	199.3	198.2
4	124.0	124.1	122.4	122.8
5	160.9	163.3	163.7	168.5
6	51.0	78.0	50.8	78.7
7	141.0	129.1	138.6	129.6
8	125.4	137.6	125.4	135.8
9	62.4	67.4	68.1	67.4
10	24.5	24.6	13.5	24.6
11	69.3	69.3	63.9	61.0
12	27.6	24.2	27.7	24.1
13	27.1	23.5	27.1	23.6
Glc-1′	103.8	103.9		
2′	75.2	75.1		
3′	78.6	78.5		
4′	71.7	71.7		
5′	78.6	78.4		
6′	62.8	62.6		

a) 68 MHz, pyridine-d_5, *b*) 125 MHz, $CDCl_3$.

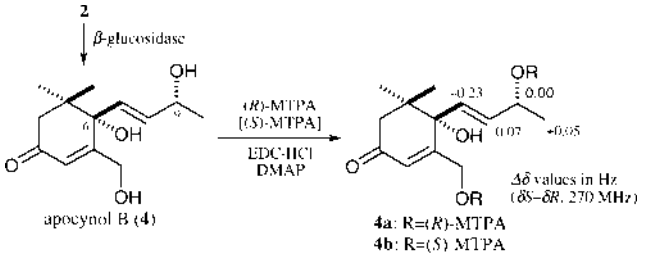

Fig. 3

glycosidation shift was observed around the 11-carbon in **1**. These findings and comparisons of the ^{1}H-NMR and ^{13}C-NMR spectra for **1** with those for known related ionone glucosides[12] led us to formulate the 11-*O*-β-D-glucopyranosyl-9,11-dihydroxyionol structure of **1**. The relative stereostructure of **1** was characterized by ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY). Namely, NOE correlations were observed between the 12-protons and the 2α and 6-protons and between the 13-protons and the 2β and 7-protons.

In order to clarify the absolute stereostructure of the 9-position in **1**, the aglycone (**3**) was subjected to a modified Mosher's method.[13] Namely, **3** was treated with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenyl acetate (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)cardoiimide (EDC·HCl) and 4-dimethylaminopyridine (DMAP) to give the 9,11-di-(*R*)-MTPA ester (**3a**) and the 9,11-di-(*S*)-MTPA ester (**3b**), respectively. As shown in Fig. 2, signals due to protons attached to the 7 and 8-positions in **3a** were observed at lower fields ($\Delta\delta$: negative) compared to those of **3b**, while the signal due to the 10-position in **3a** was observed at a higher field ($\Delta\delta$: positive) compared to that of **3b**. Consequently, the absolute configuration at the 9-position was elucidated as *R*. Finally, the absolute stereostructure of the 6-position in **1** was determined by application of the circular dichroism (CD) helicity rule.[14] That is, since the CD spectrum of **1** showed a positive Cotton effect at 239 nm, the 6-position was determined to be an *R* configuration. On the basis of the above evidence, the absolute stereostructure of apocynoside I (**1**) was elucidated.

Apocynoside II (**2**), obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{26}$ +25.1°), showed absorption bands due to hydroxyl and enone functions in its IR spectrum. The molecular formula $C_{19}H_{30}O_9$ of **2** was determined from the negative- and positive-ion FAB-MS [*m/z* 401 $(M-H)^-$, *m/z* 425 $(M+Na)^+$] and by high-resolution MS measurement. Acid hydrolysis of **2** liberated D-glucose,[11] while an aglycone, apocynol B (**4**), was obtained by enzymatic hydrolysis. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[11] of **4** showed signals assignable to two singlet methyls [δ 1.15, 1.34 (both s, 13, 12-H_3)], a doublet methyl [δ 1.39 (d, J=6.4 Hz, 10-H_3)], an olefin [δ 6.38 (dd, J=3.1, 12.8 Hz, 8-H), 6.39 (d, J=12.8 Hz, 7-H)], a methylene and methine bearing a hydroxyl group [δ 4.67 (dd, J=3.1, 6.4 Hz, 9-H), 4.81, 5.07 (both d, J=18.6 Hz, 11-H_2)], and an enone function [δ 7.00 (s, 4-H)], together with a methylene [δ 2.50, 2.74 (both d, J=16.8 Hz, 2-H_2)] and two quaternary carbons (δ_C 42.0, 67.4). On the other hand, the ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[11] of **2** indicated the presence of an apocynol B moiety and a β-D-glucopyranosyl part [δ 4.85 (d, J=7.6 Hz, 1′-H)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** and **4** were superimposable on those of **1** and **3**, respectively, except for same signals around the 6-position. The connectivities of the quaternary carbons at the 1 and 10-positions and the position of the glucosidic linkage were clarified by an HMBC experiment with **2**. Namely, HMBC correlations were observed between the following protons and carbons: 2-H_2 and 1, 3-C, 11-H_2 and 4, 5, 6-C, 12, 13-H_3 and 1, 2, 6-C, 10-H_3 and 8, 9-C, 8-H and 6, 7, 9-C, 1′-H and 11-C (Fig. 1). To clarify the absolute configuration of the 6 and 9-positions, **2** and **4** were subjected to the application of a modified Mosher's method and the CD helicity rule. As shown in Fig. 3, signals due to the 7 and 8-protons in the ^{1}H-NMR spec-

trum of the (*S*)-MTPA ester (**4b**) were observed at higher fields compared to those for the (*R*)-MTPA ester (**4a**) ($\Delta\delta$: negative), while the signal due to the 10-protons of the (*S*)-MTPA was observed at a lower field than that for the (*R*)-MTPA ($\Delta\delta$: positive). Furthermore, the CD spectrum of **2** showed a positive Cotton effect at 237 nm. On the basis of the above evidence, the 6 and 9-positions were determined to be 6*S* and 9*R* configurations, and the absolute stereostructure of apocynoside II (**2**) was characterized as shown.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1)]

Isolation of Apocynosides I (1) and II (2) and Known Compounds from the Processed Leaves of *A. venetum* L. The roasted leaves of *A. venetum* L. (17 kg, provided from Toyama Chemical Co., Ltd. and cultivated and processed in China) were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided an MeOH extract (2.6 kg, 15.3%), and the extract (2.6 kg) was partitioned into the AcOEt–H_2O (1 : 1) mixture. The H_2O phase was further extracted with *n*-BuOH. Removal of the solvent under reduced pressure from the AcOEt and *n*-BuOH-soluble portion yielded 1.1 kg and 328 g of residue, respectively. The AcOEt-soluble portion (374.3 g) was subjected to normal-phase silica gel column chromatography [3.6 kg, *n*-hexane–AcOEt (10 : 1→5 : 1→2 : 1→1 : 1, v/v)→$CHCl_3$–MeOH (5 : 1→1 : 1, v/v)→MeOH] to give twelve fractions [Fr. 1 (33.8 g), Fr. 2 (12.5 g), Fr. 3 (10.5 g), Fr. 4 (22.7 g), Fr. 5 (22.4 g), Fr. 6 (44.5 g), Fr. 7 (11.9 g), Fr. 8 (6.7 g), Fr. 9 (12.4 g), Fr. 10 (50.5 g), Fr. 11 (64.4 g), Fr. 12 (81.8 g)]. Fraction 4 (21 g) was further separated by reversed-phase silica gel column chromatography [630 g, MeOH–H_2O (80 : 20→90 : 10→95 : 5, v/v)→MeOH] and HPLC [YMC-Pack ODS-A (250×20 mm i.d., YMC Co., Ltd.), MeOH–H_2O (90 : 10, v/v)] to give phytol (47.8 mg, 0.0003%), and lupeol (2010 mg, 0.012%). Fraction 9 (12.4 g) was separated by reversed-phase silica gel column chromatography [380 g, MeOH–H_2O (50 : 50→60 : 40→70 : 30→95 : 5, v/v)→MeOH] to give quercetin (2400 mg, 0.014%). The *n*-BuOH-soluble portion (300 g) was subjected to normal-phase silica gel column chromatography [BW-200 (Fuji Silysia, Ltd., 3 kg), $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer→7 : 3 : 0.5→6 : 4 : 1, v/v)→MeOH] to give seven fractions [Fr. 1 (78.8 g), Fr. 2 (32.7 g), Fr. 3 (36.2 g), Fr. 4 (27.6 g), Fr. 5 (35.8 g), Fr. 6 (82.9 g), Fr. 7 (40.5 g)]. Fraction 4 (25 g) was further separated by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia, Ltd., 750 g), MeOH–H_2O (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] and repeated HPLC [MeOH–H_2O (30 : 70, 50 : 50, v/v), CH_3CN–H_2O (15 : 85, 20 : 80, v/v)] to give apocynosides I (**1**, 41 mg, 0.0002%) and II (**2**, 73 mg, 0.0004%), phenethyl alcohol xylopyranosyl(1→6)glucopyranoside (104 mg, 0.0006%), *Z*-hex-3-en-1-ol xylopyranosyl(1→6)glucopyranoside (133 mg, 0.0008%), isoquercitrin (83 mg, 0.0005%), and kaempferol 3-*O*-β-D-galactopyranoside (10 mg, 0.0001%). Fraction 5 (75 g) was separated by reversed-phase silica gel column chromatography [750 g, MeOH–H_2O (30 : 70→50 : 50→70 : 30, v/v)→MeOH] and purified by HPLC [MeOH–H_2O (45 : 55, v/v), CH_3CN–H_2O (20 : 80, v/v)] to give benzyl alcohol xylopyranosyl(1→6)glucopyranoside (116 mg, 0.0007%) and hyperoside (30 mg, 0.0002%). The known compounds were identified by comparison of their physical data ($[\alpha]_D$, ^{1}H-NMR, ^{13}C-NMR) with reported values.[4—9)] Quercetin was identified by comparison of an authentic sample.

Apocynoside I (**1**): An amorphous powder, $[\alpha]_D^{27}$ +79.2° (*c*=1.2, MeOH), High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{31}O_8$ $(M+H)^+$: 387.2019. Found: 387.2036. CD (*c*=0.036, MeOH) $\Delta\varepsilon$ (nm): +20.0 (239) (positive max.), −1.4 (314) (negative max). UV λ_{max}^{MeOH} nm (log ε): 236 (4.0). IR (KBr): 3432, 1655, 1619, 1076 cm^{-1}. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 0.87 (3H, s, 13-H_3), 0.94 (3H, s, 12-H_3), 1.42 (3H, d, *J*=6.4 Hz, 10-H_3), 2.15 (1H, d, *J*=16.5 Hz, 2α-H), 2.51 (1H, d, *J*=16.5 Hz, 2β-H), 2.69 (1H, d, *J*=8.9 Hz, 6-H), 3.90 (1H, m, 5′-H), 4.06 (1H, t-like, 2′-H), 4.18 (1H, m, 3′-H), 4.20 (1H, m, 4′-H), 4.34 (1H, dd, *J*=5.2, 11.6 Hz), 4.48 (1H, m) (6′-H_2), 4.51 (1H, m), 4.67 (1H, d, *J*=14.9 Hz) (11-H_2), 4.53 (1H, m, 9-H), 4.84 (1H, d, *J*=7.6 Hz, 1′-H), 5.80 (1H, dd, *J*=8.9, 15.3 Hz, 7-H), 5.91 (1H, dd, *J*=5.2, 15.3 Hz, 8-H), 6.70 (1H, s, 4-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 385 $(M-H)^-$, 223 $(M-C_6H_{11}O_5)^-$. Positive-ion FAB-MS: *m/z* 387 $(M+H)^+$.

Apocynoside II (**2**): An amorphous powder, $[\alpha]_D^{26}$ +25.1° (*c*=0.4, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{19}H_{30}O_9Na$ $(M+Na)^+$: 425.1788. Found: 425.1793. CD (*c*=0.028, MeOH) $\Delta\varepsilon$ (nm): +10.0 (234) (positive max.), −0.68 (326) (negative max). UV λ_{max}^{MeOH} nm (log ε): 325 (3.1), 234 (3.8). IR (KBr): 3432, 1655, 1638, 1076 cm^{-1}. ^{1}H-NMR (270 MHz, pyridine-d_5) δ: 1.11 (3H, s, 13-H_3), 1.24 (3H, s, 12-H_3), 1.42 (3H, d, *J*=6.3 Hz, 10-H_3), 2.41 (1H, d, *J*=16.5 Hz, 2α-H), 2.68 (1H, d, *J*=16.5 Hz, 2β-H), 3.84 (1H, m, 5′-H), 4.04 (1H, t-like, 2′-H), 4.17 (1H, m, 3′-H), 4.19 (1H, m, 4′-H), 4.28 (1H, dd, *J*=5.0, 11.8 Hz), 4.42 (1H, dd, *J*=5.0, 11.8 Hz) (6′-H_2), 4.64 (1H, dd, *J*=4.7, 6.3 Hz, 9-H), 4.85 (1H, d, *J*=7.6 Hz, 1′-H), 4.83, 5.20 (1H each, both d, *J*=16.5 Hz, 11-H_2), 6.36 (1H, dd, *J*=4.7, 15.5 Hz, 8-H), 6.27 (1H, d, *J*=15.5 Hz, 7-H), 6.93 (1H, s, 4-H). ^{13}C-NMR (68 MHz, pyridine-d_5) δ_C: given in Table 1. Negative-ion FAB-MS: *m/z* 401 $(M-H)^-$. Positive-ion FAB-MS: *m/z* 425 $(M+Na)^+$.

Acid Hydrolysis of Apocynosides I (1) and II (2) A solution of **1** and **2** (3 mg each) in 5% aq. H_2SO_4–1,4-dioxane (1 : 1, v/v, 2 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form), and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose; GLC conditions: column: Supeluco STB™-1, 30 m×0.25 mm (i.d.) capillary column, column temperature: 230 °C, He flow rate: 15 ml/min, t_R: 24.2 min.

Enzymatic Hydrolysis of Apocynosides I (1) and II (2) Give Apocynols A (3) and B (4) A solution of **1** or **2** (10 mg each) in 0.2 M acetate buffer (pH 4.4, 2 ml) was treated with β-glucosidase (Oriental Yeast Co., Ltd., 10 mg) and stirred at 38 °C for 24 h. After EtOH was added to the reaction mixture, the solvent was removed *in vacuo*. The crude product was purified by normal-phase silica gel column chromatography [1 g, $CHCl_3$–MeOH–H_2O (15 : 3 : 1, lower layer)] to give **3** (5.7 mg, 98%) and **4** (4.6 mg, 74%).

Apocynol A (**3**): An amorphous powder, $[\alpha]_D^{27}$ +289.9° (*c*=0.2, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{13}H_{21}O_3$ $(M+H)^+$: 225.1525. Found: 225.1492. CD (*c*=0.0028, MeOH) $\Delta\varepsilon$ (nm): +20.4 (243) (positive max.), −1.0 (319) (negative max.). UV λ_{max}^{MeOH} nm (log ε): 236 (4.2). IR (KBr): 3401, 1655, 1608, 1055 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.99 (3H, s, 13-H_3), 1.04 (3H, s, 12-H_3), 1.28 (3H, d, *J*=6.4 Hz, 10-H_3), 2.13 (1H, d, *J*=16.8 Hz, 2α-H), 2.41 (1H, d, *J*=16.8 Hz, 2β-H), 2.59 (1H, d, *J*=8.5 Hz, 6-H), 4.17, 4.23 (1H each, both d, *J*=17.1 Hz, 11-H_2), 4.34 (1H, dd-like, 9-H), 5.60 (1H, dd, *J*=6.5, 15.6 Hz, 8-H), 5.67 (1H, dd, *J*=8.5, 15.6 Hz, 7-H), 6.18 (1H, s, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS: *m/z* 225 $(M+H)^+$.

Apocynol B (**4**): An amorphous powder, $[\alpha]_D^{27}$ +104.7° (*c*=0.8, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{13}H_{20}O_4Na$ $(M+Na)^+$: 263.1294. Found: 263.1272. CD (*c*=0.0018, MeOH) $\Delta\varepsilon$ (nm): +9.2 (232) (positive max.), −0.4 (322) (negative max.). UV λ_{max}^{MeOH} nm (log ε): 236 (3.9). IR (KBr): 3401, 1655, 1611, 1064 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 1.15 (3H, s, 13-H_3), 1.34 (3H, s, 12-H_3), 1.39 (3H, d, *J*=6.4 Hz, 10-H_3), 2.50 (1H, d, *J*=16.8 Hz, 2α-H), 2.74 (1H, d, *J*=16.8 Hz, 2β-H), 4.67 (1H, dd, *J*=3.1, 6.4 Hz, 9-H), 4.81, 5.07 (1H each, both d, *J*=18.6 Hz, 11-H_2), 6.38 (1H, dd, *J*=3.1, 12.8 Hz, 8-H), 6.39 (1H, d, *J*=12.8 Hz, 7-H), 7.00 (1H, s, 4-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: *m/z* 263 $(M+Na)^+$.

Preparation of the (*R*)-(+)-MTPA Esters (3a, 4a) from Apocynols A (3) and B (4) A solution of **3** (1.0 mg) in dry CH_2Cl_2 (1 ml) was treated with (*R*)-MTPA (12 mg), EDC HCl (10 mg), and DMAP (10 mg), and the whole mixture was stirred at room temperature (25 °C) for 30 min. The reaction mixture was poured into brine and the whole was extracted with AcOEt. The AcOEt extract was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent from the extract gave a residue, which was purified by normal-phase silica gel column chromatography [1 g, *n*-hexane–AcOEt (2 : 1, v/v)] to furnish **3a** (1.2 mg).

(*R*)-MTPA Ester of Apocynol A (**3a**): An amorphous powder. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.91 (3H, s, 13-H_3), 0.97 (3H, s, 12-H_3), 1.37 (3H, d, *J*=6.3 Hz, 10-H_3), 2.12 (1H, d, *J*=16.9 Hz, 2α-H), 2.33 (1H, d, *J*=16.9 Hz, 2β-H), 2.57 (1H, d, *J*=7.9 Hz, 6-H), 4.73, 4.82 (1H each, both d, *J*=18.6 Hz, 11-H_2), 5.61 (1H, m, 7-H), 5.54 (1H, m, 9-H), 5.56 (1H, m, 8-H), 6.00 (1H, s, 4-H).

The (*R*)-MTPA ester (**4a**, 1.5 mg) was also prepared form **4** (2.0 mg) by the procedure described above.

(*R*)-MTPA Ester of Apocynol B (**4a**): An amorphous powder. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.92 (3H, s, 13-H_3), 1.01 (3H, s, 12-H_3), 1.38 (3H, d, *J*=6.3 Hz, 10-H_3), 2.27 (1H, d, *J*=17.1 Hz, 2α-H), 2.37 (1H, d, *J*=17.1 Hz,

2β-H), 4.83, 4.97 (1H each, both d, J=15.9 Hz, 11-H_2), 5.60 (1H, dq-like, 9-H), 5.90 (1H, m, 7-H), 5.93 (1H, m, 8-H), 5.93 (1H, s, 4-H).

Preparation of the (*S*)-(−)-MTPA Esters (3b, 4b) from Apocynols A (3) and B (4) A solution of **3** (1.0 mg) in CH_2Cl_2 (1 ml) was treated with (*S*)-MTPA (12 mg), EDC·HCl (10 mg), and DMAP (10 mg), and the whole mixture was stirred at room temperature (25 °C) for 30 min. It was worked up as described for the above AcOEt extract, which was purified by normal-phase silica gel column chromatography [1 g, *n*-hexane–AcOEt (2 : 1, v/v)] to furnish **3b** (1.4 mg).

(*S*)-MTPA Ester of Apocynol A (**3b**): An amorphous powder. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.87 (3H, s, 13-H_3), 0.93 (3H, s, 12-H_3), 1.42 (3H, d, J=6.3 Hz, 10-H_3), 2.09 (1H, d, J=16.8 Hz, 2α-H), 2.25 (1H, d, J=16.8 Hz, 2β-H), 2.50 (1H, d-like, 6-H), 4.63, 4.82 (1H each, both d, J=13.8 Hz, 11-H_2), 5.52 (1H, m, 8-H), 5.53 (1H, m, 7-H), 5.54 (1H, m, 9-H), 5.96 (1H, s, 4-H).

The (*S*)-MTPA ester (**4b**, 1.8 mg) was also prepared from **4** (2.0 mg).

(*S*)-MTPA Ester of Apocynol B (**4b**): An amorphous powder. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.89 (3H, s, 13-H_3), 1.01 (3H, s, 12-H_3), 1.43 (3H, d, J=6.4 Hz, 10-H_3), 2.13 (1H, br s, 2α-H), 2.26 (1H, br s, 2β-H), 4.67, 5.05 (1H each, both d, J=15.9 Hz, 11-H_2), 5.60 (1H, dq-like, 9-H), 5.67 (1H, m, 7-H), 5.86 (1H, m, 8-H), 5.88 (1H, s, 4-H).

References and Notes

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