# NEW CATHARANTHUSOPIMARANOSIDE A AND B FROM HAIRY ROOT CULTURES OF *Catharanthus roseus*

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*Two new compounds catharanthusopimaranoside A (**1**) and catharanthusopimaranoside B (**2**) along with three known compounds have been isolated from the hairy root cultures of* Catharanthus roseus*. Their structures have been elucidated with the help of 500 MHz NMR using 1D and 2D spectral methods viz: NMR, $^{1}H$–$^{1}H$ COSY, $^{1}H$–$^{13}C$ HETCOR, etc., and DEPT aided by EIMS, FABMS, and IR spectroscopy.*

**Key words**: *Catharanthus roseus*, Apocynaceae, hairy root cultures, new constituents; catharanthusopimaranoside A, catharanthusopimaranoside B.

The periwinkle, *Catharanthus roseus* (Apocynaceae), is widely used as an ornamental plant as well as a medicinal plant. *C. roseus* is a herbaceous shrub [1] and has been extensively studied due to its production of two valuable alkaloids, vincristine and vinblastine, which are used in the treatment of human neoplasm, and an alkaloid from the root, ajmalicine, which is used in the treatment of circulatory disorders and hypertension. Biologically, indole alkaloids produced by plants are believed to play a role as antimicrobial and antifeeding compounds [2, 3]. This Madagascan periwinkle produces numerous indole alkaloids which have important therapeutic activities [4]. Only few phenolic compounds have been reported in this genus [5, 6]. Recently [7], two flavonols, trisaccharides of kaempferol and quercetin, have been isolated and identified. Several indole alkaloids have been isolated from the *C. roseus* cell suspension cultures [8, 9]. However, the production of the most valuable compounds reported from this plant, vincristine and vinblastine, which are terpenoid indole alkaloids [10], has not yet been achieved in these cultures. Besides indole alkaloids, the presence of anthocyanidins [11], phenolics [9, 12], and terpenoid compounds [8, 9] in the cultures of *C. roseus* has been reported. As part of its secondary metabolism, this plant produces pharmaceutically valuable terpenoid indole alkaloids such as vincristine and vinblastine which are used as anticancer drugs. A very low yield of these compounds is a major motivation of the research interest in this plant. Although the hairy root cultures do not produce these two bisindole alkaloids, which consist of catharanthine and vindoline, they have been shown to produce catharanthine and tabersonine.

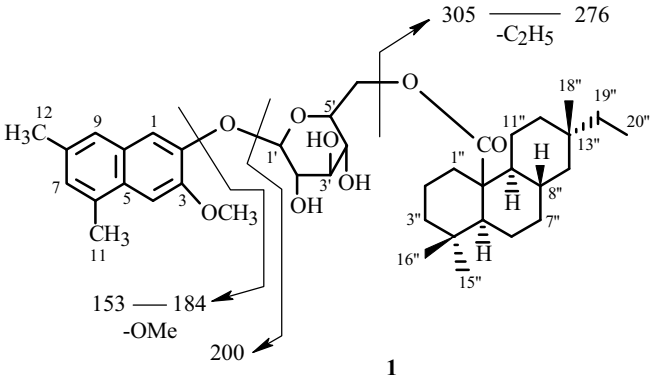


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TABLE 1. $^{1}H$ (500 MHz) and $^{13}C$ (125 MHz) NMR Data for Compound **1** and **2** in MeOD (J/Hz in parenthesis)

| C atom | Compound | | | |
|---|---|---|---|---|
| | **1** | | **2** | |
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
| 1 | 7.18 s | 112.86 | - | 154.43 |
| 2 | - | 155.41 | - | 150.75 |
| 3 | - | 152.61 | 7.97 d (J = 7.5) | 129.71 |
| 4 | 6.92 s | 119.57 | 6.80 d (J = 7.5) | 111.85 |
| 5 | - | 138.66 | - | 131.77 |
| 6 | - | 134.26 | 7.12 s | 135.37 |
| 7 | 6.74 d (J = 2.5) | 104.20 | - | 128.25 |
| 8 | - | 133.63 | - | 128.79 |
| 9 | 6.72 d (J = 2.5) | 112.42 | 6.90 s | 124.36 |
| 10 | - | 129.03 | - | 137.81 |
| 11 | 1.66 br.s | 30.89 | 1.64 s | 29.15 |
| 12 | 1.62 br.s | 31.01 | 1.63 s | 26.87 |
| 1′ | 5.51 d (J = 7.0) | 101.35 | 5.74 d (J = 5.5) | 100.82 |
| 2′ | 4.34 d (J = 9.0) | 72.54 | 3.89 m | 77.50 |
| 3′ | 3.64 m | 71.63 | 3.76 m | 77.24 |
| 4′ | 3.68 m | 70.79 | 3.86 m | 84.46 |
| 5′ | 3.73 m | 75.16 | 3.20 d (J = 9.5); 3.18 d (J = 9.5) | 61.23 |
| 6′ | 3.51 d (J = 7.0), 3.49 d (J = 7.0) | 64.98 | | |
| 1″ | 2.18 ddd (J = 2.5, 2, 8.5) | 34.22 | 2.08 m, 2.72 m | 32.16 |
| | 2.92 m | | | |
| 2″ | 2.53 m, 2.31 m | 31.20 | 2.61 m, 2.69 m | 26.37 |
| 3″ | 1.92 dd (J = 6.0, 1.5) | 28.55 | 1.90 m, 1.85 m | 28.47 |
| | 2.02 m | | | |
| 4″ | - | 32.89 | - | 46.09 |
| 5″ | 3.08 dd (J = 5.0, 5.5) | 56.41 | 3.18 m | 56.48 |
| 6″ | 1.60 m, 1.62 m | 23.88 | 1.83 m, 1.81 m | 22.86 |
| 7″ | 1.82 m, 1.79 m | 28.33 | 1.88 m, 1.74 m | 32.09 |
| 8″ | 2.77 br.s | 45.28 | 2.69 m | 50.77 |
| 9″ | 2.48 m | 52.35 | 2.45 m | 52.01 |
| 10″ | - | 35.36 | - | 32.11 |
| 11″ | 2.03 m, 1.94 m | 32.63 | 1.74 m, 1.96 m | 42.87 |
| 12″ | 1.84 dd (J = 2.5, 8.5) | 26.18 | 1.88 m, 2.02 m | 39.86 |
| | 1.62 m | | | |
| 13″ | - | 33.21 | - | 48.17 |
| 14″ | 1.40 m, 1.36 m | 32.17 | 1.70 m, 1.72 m | 31.28 |
| 15″ | 1.28 br.s | 30.61 | 1.29 br.s | 29.93 |
| 16″ | 1.32 br.s | 30.51 | 1.25 br.s | 29.59 |
| 17″ | - | 173.11 | - | 171.61 |
| 18″ | 1.28 br.s | 14.57 | 1.25 br.s | 26.59 |
| 19″ | 1.45 m, 1.24 m | 27.61 | 1.74 m, 1.22 m | 23.21 |
| 20″ | 0.89 t (J = 7.0) | 13.20 | 0.87 t (J = 6.5) | 13.31 |
| OMe | 3.30 br.s | 56.95 | 3.66 s | 56.15 |

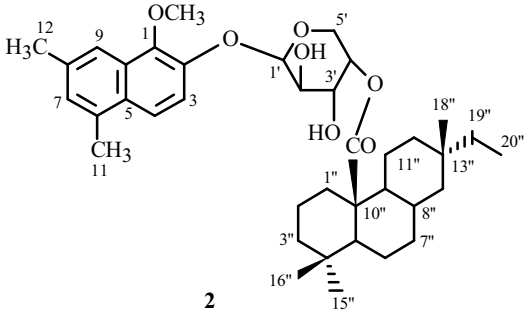
This paper deals with the isolation and structural elucidation of two new compounds **1** and **2** on the basis of spectral data and chemical reactions, and also the isolation of three known compounds. Due to the high significance of medicinal natural products of this plant roots, work in this area has already been done. The aim of the present investigation is to report some of the new findings in the form of natural product from culture roots of *C. roseus*.

Compound **1**, designated as catharanthusopimaranoside A, showed charactetristic IR absorption bands for hydroxyl groups (3412, 3365, 3260 $cm^{-1}$) and ester group (1730 $cm^{-1}$). Its molecular ion peak was established at *m/z* 652 on the basis of mass and $^{13}C$ NMR spectral data corresponding to the *β*-naphthol glucoside attached with a tricyclic diterpene $C_{39}H_{56}O_8$. The important ion fragments generated at *m/z* 184 $[C_2–O\ fission]^+$, 162 $[C_6H_{10}O_5]^+$, 153 $[184–OMe]^+$, 200 $[C_1'–O\ fission]^+$, 305 $[C_6'–O\ fission]^+$, and 276 $[305–C_2H_5]^+$, indicated that the dimethyl methoxy substituted naphthol glycoside was esterified with a pimaric acid-type moiety.

The $^1H$ NMR spectrum of **1** exhibited two one-proton signals at δ 7.18 and 6.92 assigned to *para*-coupled H-1 and H-4, respectively. Two one-proton doublets at δ 6.74 (J = 2.5 Hz) and 6.72 (J = 2.5 Hz) were attributed to meta-coupled H-7 and H-9, respectively. Four one-proton doublets at δ 5.51 (J = 5.0 Hz), 4.34 (J = 9.0 Hz) and at δ 3.51 (J = 7.0 Hz), and δ 3.49 (J = 7.0 Hz) were ascribed correspondingly to anomeric H-1′, hydroxymethine H-2′, and oxygenated methylene $H_2$-6a and $H_2$-6b protons. The remaining sugar protons appeared as one-proton multiplets at δ 3.64 (H-3′), 3.68 (H-4′), and 3.73 (H-5′). A three-proton broad signal at δ 3.30 was attributed to methoxy protons. Five three-proton broad signals at δ 1.66, 1.62, 1.28, 1.32, and 1.28 were associated with the methyl protons Me-11, Me-12, Me-15″, Me-16″, and Me-18″, respectively.

The remaining methylene and methine protons resonated in the range δ 3.08–1.40. A three-proton triplet at δ 0.89 (J = 7.0 Hz) was assigned to C-20″ primary methyl protons. The $^{13}C$ NMR spectrum of **1** displayed aromatic carbon signals between δ 155.00–104.20, anomeric carbon signals at δ 101.35, sugar carbon signals between δ 75.16–64.98, ester carbon at δ 173.11, methoxy carbon signal at δ 56.95, and methyl carbons at δ 30.89 (C-11), 31.01 (C-12), 30.61 (C-15″), 30.51 (C-16″), 14.57 (C-18″), and 13.20 (C-20″). The remaining methylene and methine carbon signals were in the range δ 52.35–27.61. The multiplicity of each carbon was determined by analysis of the DEPT spectrum. The $^1H$ and $^{13}C$ NMR chemical shifts of compound **1** were compared with the related pimarane-type diterpenes [13–17]. The $^1H$–$^1H$ COSY spectrum of **1** showed correlation of H-9 with H-1 and H-7; $H_3$-18″ with $H_2$-12″; $H_2$-19″ with $H_2$-14″; and $H_3$-15″ with $H_2$-3″, H-5″, and $H_3$-16″. The $^1H$–$^{13}C$ HETCOR spectrum of **1** exhibited correlation of C-11 with H-7; C-12 with H-7 and H-9; C-2 with H-1 and H-4; C-1′ with H-2′, H-3′, and H-5′; C-6′ with H-5′ and H-4′; C-17 with $H_2$-6′ and $H_2$-1″; C-18″ with $H_2$-12″, $H_2$-19″, and $H_2$-14″. In the HSQC spectrum correlations were observed as C-1 with H-7 and C-1′ with H-4′. Acid hydrolysis of **1** yielded glucose (TLC comparable). On the basis of spectral data analysis and chemical reaction the structure of **1** has been established as 3-methoxy-6, 8-dimethyl-*β*-naphthyl-*β*-*D*-glucopyranosyl-6′-pimaran-17″-oic acid ester. This is an unreported naphthalene glucoside.

Compound **2**, named catharanthusopimaranoside B, showed characteristic IR absorption bands for hydroxyl groups (3445, 3419 $cm^{-1}$) and ester group (1724 $cm^{-1}$). Its molecular ion peak was established at *m/z* 622 on the basis of mass and $^{13}C$ NMR spectra corresponding to a *β*-naphthol glycoside attached with a tricyclic diterpenic moiety $C_{38}H_{54}O_7$. The important ion fragments arose at *m/z* 184 $[C_2–O\ fission]^+$, 148 $[C_5H_8O_5$, sugar moiety$]^+$, 153 $[184–Me]^+$, 200 $[C\text{-}1'–O\ fission]^+$, 289 $[C\text{-}17''–O\ fission]^+$ and 273 $[289–Me]^+$ indicated that in compound **2** the *α*-naphthyl and C-20 diterpenic moieties were attached to the arabinose glycone part.




**2**

The $^1H$ NMR spectrum of **2** exhibited two one-proton doublets at δ 7.97 (J = 7.5 Hz) and 6.80 (J = 7.5 Hz), assigned to *ortho*-coupled aromatic H-3 and H-4 protons, respectively. Two one-proton broad signals at δ 7.12 and 6.90 were ascribed to *para*-coupled H-6 and H-9 respectively. Three one-proton doublets at δ 5.74 (J = 5.5 Hz) and at d 3.20 (J = 9.5 Hz) and 3.18 (J = 9.5 Hz) were attributed corresponding to anomeric H-1′ and oxygenated methylene protons $H_2$-5′. Four one-proton multiplets between δ 4.54 and 3.76 were associated with the remaining sugar protons. A three-proton broad signal at δ 3.66 was due to methoxy protons. Five three-proton broad signals at δ 1.64, 1.63, 1.29,1.27, and 1.25 were attributed to methyl protons C-11 and C-12 attached to the aromatic ring and tertiary methyl C-15″, C-16″, and C-18″, respectively. A three-proton triplets at δ 0.87 (J = 6.5 Hz) was due to C-20″ primary methyl protons. The remaining methylene and methine protons resonated

between δ 3.18–1.22. The $^{13}$C NMR spectrum of **2** exhibited aromatic carbon signals between δ 154.43–111.85, anomeric carbon at δ 100.8 (C-1′), sugar carbon signals between δ 84.46–61.23, ester carbon at δ 171.61 (C-17″), methoxy carbon at δ 56.15, methyl carbons at δ 29.15 (C-11), 26.87 (C-12), 29.93 (C-15″), 29.59 (C-16″), 26.59 (C-18″), and 13.31 (C-20″), and the remaining methylene and methine carbons in the range δ 56.48–22.86. The shifting of the C-4′ signal to the downfield range suggested attachment of the diterpenic moiety at C-4′. The $^{1}$H and $^{13}$C NMR chemical shifts of compound **2** were compared with the related pimarane-type diterpenes [13–17]. The multiplicity of each carbon was determined by analysis of DEPT spectrum. The $^{1}$H–$^{1}$H COSY spectrum of **2** showed correlation of H-3 with H-4; H-1′ with H-2′ and H-3′; H-15″ with $H_2$-3″ and H-16″; and $H_3$-18″ with $H_2$-12″, $H_2$-14″, and $H_2$-19″. The $^{1}$H–$^{13}$C HETCOR spectrum of **2** exhibited correlation of $H_3$-11 with C-6; $H_3$-12 with C-9; H-3 with C-4; H-2′ with C-3′ and C-4′; and H-4′ with C-17″. The HSQC spectrum of **2** showed long-range correlations observed as H-1′ with C-4 and H-15″ with C-9″. Acid hydrolysis of **2** yielded β-L-arabinose as a glycone moiety (TLC comparable). The structure of **2** has been formulated as 1-methoxy-6, 8-dimethyl-β-naphthyl-β-L-arabinopyranosyl-4′-pimaran-17″-oic acid ester. This is a new phytoconstituent isolated from *Catharanthus roseus* roots.

## EXPERIMENTAL

**Chemicals**. All chemicals used were of analytical grade: Hexane, ethyl acetate, methanol, ethanol, sulfuric acid, and vanillin were purchased from Daejung Chemicals and Metals Co. Ltd, Korea. Pre-coated TLC plates (layer thickness 0.25 mm), silica gel for column chromatography (70–230 mesh ASTM), and LiChroprep RP-18 (40–63 μm) were from Merck (Darmstadt, Germany). Authentic standards of β-sitosterol and D-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Instrumentation**. Melting points were determined on an Electrochemical Engineering melting point apparatus (Electrochemical Engineering Ltd., model No. IA9100 Electrothermal, Seoul, South Korea). Optical rotation was measured on an AA-10 model polarimeter (Instruments Ltd., Seoul, South Korea). Both $^{1}$H and $^{13}$C-NMR spectra were obtained with a Bruker Avance model DRX-500 spectrometer operating at 500 and 125 MHz, respectively. This NMR machine is available at Seoul National University (SNU), Seoul, South Korea, and all NMR spectra were obtained at SNU. NMR spectra were obtained in deuterated chloroform, pyridine, and methanol using tetramethylsilane (TMS) as internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (J) in Hz. EIMS were recorded on a JEOL JMS-SX 102 A spectrometer and FAB/MS on a JEOL JMS-AX 505 WA. This mass machine is available at Seoul National University, Seoul, South Korea, and all mass spectra were obtained at SNU. IR spectra were recorded on a Thermo Mattson, Infinity Gold FT-IR (German) model 60-AR spectrophotometer. This IR machine is available at the Korea Institute of Science and Technology (KIST) Seoul, South Korea.

**Culture Conditions**. The hairy root line used in this study was previously generated by infection of *C. roseus* seedling with *Agrobacterium rhizogenes* 15834 [18]. The culture media consisted of a filter-sterilized solution of 3% sucrose, half-strength Gamborg's B5 salts, and full-strength Gamborg's vitamins with the pH adjusted to 5.7. The 50-mL cultures were grown in 250-mL Erlenmeyer flasks to the late exponential phase in the dark at 26°C at 100 rpm.

**Extraction of Hairy Roots**. The powdered hairy roots of *C. roseus* (200 g) were immersed in methanol (1.5 liters) for three days at room temperature and then the supernatant was concentrated under vacuum to yield 22.5 g of the extract. This material was suspended in water and extracted with ethyl acetate and *n*-butanol successively to produce 11.2 g of ethyl acetate extract and 7.4 g of *n*-butanol extract.

**Isolation of the Compounds from Ethyl Acetate Extract**. The entire ethyl acetate extract was subjected to normal phase CC over silica gel (400 g) to yield 26 fractions (each of 250 mL) with the following eluants: fractions 1–2 with *n*-hexane, fractions 3–4 with *n*-hexane:ethyl acetate (9:1), fractions 5–6 with *n*-hexane:ethyl acetate (8:2), fractions 7–8 with *n*-hexane:ethyl acetate (7:3), fractions 9–10 with *n*-hexane:ethyl acetate (1:1), fractions 11–12 with hexane : ethyl acetate (3:7), fraction 13–14 with ethyl acetate, fractions 15–16 with ethyl acetate:methanol (9.5:0.5), fraction 17–18 with ethyl acetate:methanol (9:1), fractions 19–20 with ethyl acetate:methanol (7:3), fractions 21–22 with ethyl acetate:methanol (1:1), fractions 23–24 with ethyl acetate:methanol (3:7), and fractions 25–26 in methanol. All fractions were examined by TLC. Fractions 1–4 were not further separated due to the low amount of substance. Fractions 5–6 (0.8 g) were crystallized after purification by CC to yielded β-sitosterol (20 mg), whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fractions 7–8 (0.6 g) were further purified by CC over silica gel (100 g; each fraction of 100 mL) eluting with chloroform and chloroform:methanol mixtures (99:1, 98.5:1.5, 98.2, 97.5:2.5 and 97:3) to afford 3-epibetulinic acid (120 mg). Fractions 11–12 with hexane:ethyl acetate (3:7), after re-separation with

chloroform:methanol (99:1, 99:2, 97:3, 96:4 and 95:5), afforded five fractions. Fraction 4 (from the eluant of $CHCl_3$:MeOH (96:4) was re-chromatographed over Lichroprep RP18 ODS (50 g; each fraction of 50 mL). The elution was sequentially performed with methanol containing 80, 60, 40 20, 10, and 0% of water to yield *n*-pentadecanyl octa-dec-19-en-oate (15 mg). Fractions 15–16 with EtOAc–MeOH (9:1) were rechromatographed over Lichroprep RP18 ODS (50 g; each fraction of 50 mL). The elution was sequentially performed with methanol containing 80, 60, 40 20, 10, and 0 % of water to yield two new compounds (**1**) and (**2**).

**3-Methoxy-6,8-dimethyl-β-naphthyl-β-D-glucopyranosyl-6′-pimaran-17″-oic Acid Ester (1)**. Colorless solid; mp 198–200°C; $[\alpha]_D^{22}$ +26.1° (*c* 0.15, MeOH); IR ($\nu_{max}$, KBr, $cm^{-1}$): 3412, 3365, 3260, 2925, 2854, 1730, 1628, 1596, 1458, 1432, 1365, 1213, 1185, 1115, 1031; EIMS *m/z* (rel. int.) 652 $[M]^+$ ($C_{39}H_{56}O_8$), 305 (10.6), 276 (4.0), 200 (10.8), 184 (9.6), 162 (3.6), 153 (3.3). $^1$H NMR (MeOD, 500 MHz) and $^{13}$C NMR (MeOD, 125 MHz), δ: see Table 1.

**Acid Hydrolysis of 1**. Compound **1** (7 mg) was refluxed with 2 mL of 1 M hydrochloric acid:dioxane (1:1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness and partitioned with chloroform and water four times, and each extract was concentrated. The chloroform extract contained the aglycone portion, while the water extract contained D-glucose (co-chromatographed on TLC with authentic sample).

**1-Methoxy-6,8-dimethyl-β-naphthyl-β-L-arabinopyranosyl-4′-pimaran-17″-oic Acid Ester (2).** Colorless solid; mp 202–202°C; $[\alpha]_D^{22}$ + 28.1° (*c* 0.14, MeOH); IR ($\nu_{max}$, KBr, $cm^{-1}$): 3445, 3419, 2926, 2853, 1724, 1628, 1458, 1270, 1216, 1121, 1032; EIMS *m/z* (rel. int.) 622 $[M]^+$ ($C_{38}H_{54}O_7$) (1.1), 443(6.4), 368 (65.5), 339 (9.8), 305 (3.3), 289 (2.7), 273 (1.2), 213 (13.8), 200 (100), 184 (10.5). $^1$H NMR (MeOD, 500 MHz) and $^{13}$C NMR (MeOD, 125 MHz), δ: see Table 1.

**Acid Hydrolysis 2**. Compound **2** (5 mg) was refluxed with 2 mL of 1 M hydrochloric acid:dioxane (1:1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness and partitioned with chloroform and water four times, and each extract was concentrated. The chloroform extract contained the aglycone portion, while the water extract contained β-L-arabinose (co-chromatographed on TLC with authentic sample).

## ACKNOWLEDGMENT

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