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A New Aliphatic Glycoside Constituent from the Hairy Root Cultures of *Catharanthus roseus*

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A new long chain aliphatic glycoside (*n*-heptacosan-13α-ol-13β-D-glucopyrasnoside) along with known compounds, *n*-hentetracont-36-en-5β-ol and β-sitosterol were isolated from the methanolic extract of the cultured hairy roots of *Catharanthus roseus*. The structures of these compounds were elucidated by a combination of spectral methods (IR, EIMS, FABMS, ^{1}H and ^{13}C NMR). To the best of our knowledge, *n*-hentetracont-36-en-5β-ol was identified for the first time from the hairy roots of *C. roseus*.

Key Words: *Catharanthus roseus*, Apocynaceae, *n*-Heptacosan-13α-ol-13β-D-glucopyrasnoside, *n*-Hentetracont-36-en-5β-ol.

INTRODUCTION

The periwinkle, *Catharanthus roseus* (Apocynaceae), is widely used an ornamental plant as well as 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 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 that 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* have 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 low

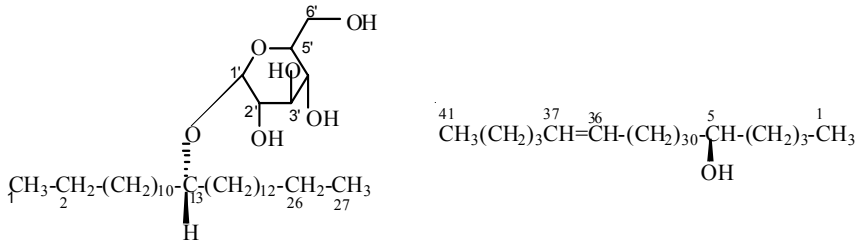
yield of these compounds is a major motivation of the research interest in this plant. Although the hairy root cultures do not produce these two bisindole alkaloids that consist of catharanthine and vindoline, they have been shown to produce catharanthine and tabersonine. This paper deals with the isolation and structural elucidation of one new (**1**) and one known (**2**) compounds from the cultured hairy roots of *C. roseus* on the basis of spectral data and chemical reactions. This is the first report of the isolation of chemical compounds from the hairy root cultures of *C. roseus*. Due to high significance of medicinal natural products of this plant roots the 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 roots along with some known compounds. Further investigations and other isolated fractions are in progress.

EXPERIMENTAL

Melting points were determined using Electrochemical Engineering (Electrothermal, Seoul, South Korea) model IA9100 melting point apparatus. Specific rotation was measured with an instruments Ltd (Seoul, South Korea) model AA-10 polarimeter. ^{1}H- and ^{13}C -NMR spectra were obtained at 500 and 125 MHz, respectively, using a Bruker Avance model DRX-500 spectrometer at the Seoul National University (SNU), Seoul, South Korea. NMR spectra were obtained in deuterated chloroform and methanol using tetramethylsilane (TMS) as an internal standard, with chemical shifts expressed in ppm (δ) and coupling constants (*J*) in Hz. EIMS and FABMS were recorded on Jeol JMS-SX 102A and Jeol JMS-AX 505WA spectrophotometers, respectively, at the Seoul National University. IR spectra were recorded on a Thermo Mattson Infinity Gold FT-IR model 60-AR spectrophotometer at the Korea Institute of Science and Technology (KIST) Seoul, South Korea.

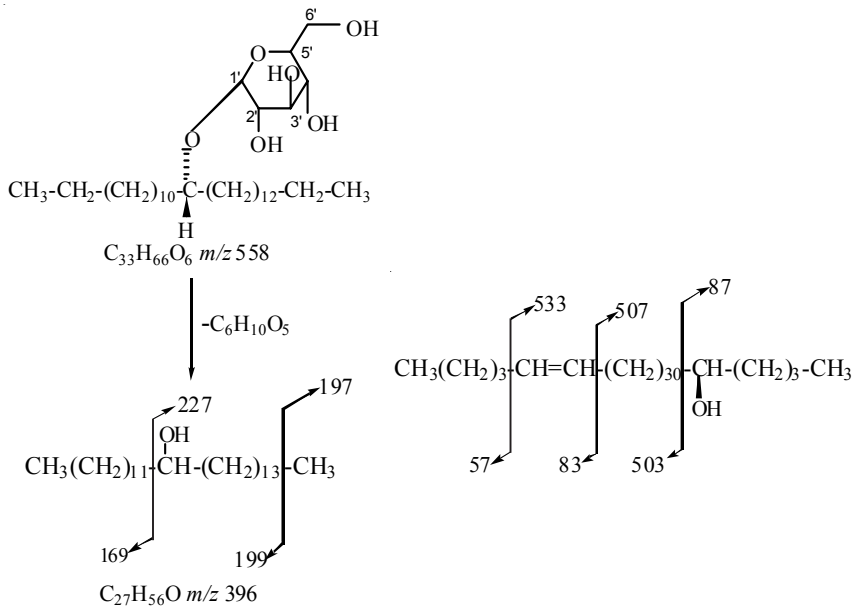
Culture conditions: The hairy root line used in this study was previously generated by infection of *C. roseus* seedling with *Agrobacterium rhizogenes* 15834[13]. The culture media consisted of a filter-sterilized solution of 3 % sucrose, half-strength Gamborg's B5 salts and full-strength Gamborg's vitamins with the pH adjusted to 5.7. The 50 mL cultures were grown in 250 mL Erlenmeyer flasks to late exponential phase in the dark at 26ºC at 100 rpm.

Extraction of hairy roots: The powdered hairy roots of *C. roseus* (200 g) were immersed in methanol (1.5 L) for 3 d 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 and 7.4 g of *n*-butanol extract.



(1) **(2)**

Structure of compounds (**1**) and (**2**)



(1) **(2)**

Mass fragmentation pattern of (**1**) and (**2**)

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 fraction 250 mL) with the following eluants: fractions 1-2 with *n*-hexane, fractions 3-4 with *n*-hexane:ethyl acetate (9:1, v/v), fractions 5-6 with *n*-hexane:ethyl acetate (8:2, v/v), fractions 7-8 with *n*-hexane:ethyl acetate (7:3, v/v), fractions 9-10 with *n*-hexane:ethyl acetate (1:1, v/v), fractions 11-12 with hexane:ethyl acetate (3:7, v/v), fractions 13-14 with ethyl acetate, fractions 15-16 with ethyl acetate:methanol (9.5:0.5, v/v), fractions 17-18 with ethyl acetate:methanol (9:1, v/v), fractions 19-20 with ethyl acetate:methanol (7:3, v/v), fractions 21-22 with ethyl acetate:methanol (1:1, v/v), fractions 23-24 with ethyl

acetate: methanol (3:7, v/v) and fractions 25-26 in methanol. All fractions were examined by TLC. Fractions 1-4 was not further separated due to the low amount. Fractions 5-6 (0.8 g) was crystallized after the purification by CC and then yielded β-sitosterol (20 mg) whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fractions 17-18 (0.6 g) was further purified by CC over silica gel (100 g; each fraction of 100 mL) eluting with dichloromethane and chloroform: methanol mixtures (98:2, 96:4, 94:6, 92:8 and 9:1). Fraction 4 [from the eluent of $CHCl_3$:MeOH (94:6, v/v)] was rechromatographed over Lichroprep RP-18 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 compounds **1** (35 mg) and **2** (23 mg). The separation of compounds of other fractions is in progress.

***n*-Heptacosan-13α-ol-13β-D-glucopyrasnoside (1):** IR (KBr, ν_{max}): 3460, 3431, 3390, 2925, 2854, 1630, 1460, 1215, 1075, 722 cm^{-1}; ^1H NMR (500 MHz; MeOD) δ: 5.31 (1H, d, *J* = 7.1 Hz, H-1'), 3.85 (1H, m, H-5'), 3.81 (1H, dd, *J* = 3.5, 7.0 Hz, H-2'), 3.68 (1H, dd, *J* = 4.5, 5.0 Hz, H-4'), 3.63 (1H, br m, $W_{½}$ = 7.0 Hz, H-13β), 3.55 (1H, dd, *J* = 3.5, 5.0 Hz, H-3'), 3.34 (2H, br s, H_2-6'), 2.03 (2H, m, CH_2), 1.68 (2H, m, CH_2), 1.40 (2H, m, CH_2), 1.28 (42H, br s, 21 × CH_2), 0.91 (3H, t, *J* = 6.85 Hz, Me-1), 0.88 (3H, t, *J* = 7.0 Hz, Me-27); ^{13}C NMR (125 MHz; MeOD) δ: 101.23 (C-1'), 73.41 (C-5'), 71.62 (C-13), 71.35 (C-2'), 67.17 (C-3'), 66.09 (C-4'), 62.32 (C-6'), 37.16 (CH_2), 32.37 (CH_2), 30.81 (22 × CH_2), 14.72 (Me-1), 14.69 (Me-27); EIMS m/z (rel. int.) 558 $[M]^+$($C_{33}H_{66}O_6$). 395 (3.9), 382 (4.3), 368 (21.5), 354 (15.0), 338 (19.1), 322 (48.9), 293 (9.7), 267 (13.1), 251 (9.7), 227 (12.7), 222 (46.4), 199 (100), 197 (18.2), 186 (43.0), 172 (7.7), 169 (20.5), 155 (25.2), 129 (14.9), 97 (14.6), 85 (13.9), 71 (17.4), 57 (25.5).

Acid hydrolysis of 1: Compound **1** (6 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 an authentic sample).

***n*-Hentetracont-36-en-5β-ol (2):** IR (KBr, ν_{max}): 3435, 2922, 2852, 1634, 1463, 1390, 1078, 722 cm^{-1}; ^1H NMR (500 MHz; $CDCl_3$) δ: 5.38 (1H, m, H-37), 5.35 (1H, m, H-36), 4.19 (1H, br s, $W_{½}$ = 18.5 Hz, H-5α), 2.04 (2H, m, H_2-38), 2.00 (2H, m, H_2-35), 1.80 (4H, br s, 2 × CH_2), 1.25 (64 H, br s, 32 × CH_2), 0.89 (3H, t, *J* = 6.5 Hz, Me-1), 0.86 (3H, t, *J* = 7.0 Hz, Me-41); ^{13}C NMR (125 MHz; MeOD) δ: 124.31 (C-36), 124.03 (C-37), 76.98 (C-5), 32.72 (CH_2), 30.03 (CH_2), 30.01 (CH_2), 29.92 (29 × CH_2), 29.64 (CH_2, 29.62 (CH_2), 27.59 (CH_2), 26.94 (CH_2), 22.92 (CH_2), 14.34 (Me-1, Me-41); EIMS m/z (rel. int.) 590 $[M]^+$($C_{41}H_{82}O$) (6.3), 562

(14.1), 533 (17.0), 507 (5.7), 503 (18.0), 423 (4.7), 367 (14.5), 339 (6.4), 325 (22.1), 297 (60.8), 255 (8.7), 127 (25.5), 111(38.1), 97 (70.6), 87 (18.0), 83 (77.5), 69 (87.2), 57 (100).

RESULTS AND DISCUSSION

Compound **1** was obtained as a colourless crystalline mass and gave positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3460, 3431, 3390 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its molecular formula was established at m/z 558 corresponding to molecular formula of an aliphatic glycoside, $C_{33}H_{66}O_6$ on the basis of positive ion FABMS and EI mass spectrometries. It indicated one double bond equivalents which was adjusted in the glycosidic moiety. The prominent ions generated at m/z 197 $[C_{13}\text{-}C_{14}$ fission$]^+$, 199 $[M\text{-}197\text{-}C_6H_{11}O_5]^+$, 169 $[C_{12}\text{-}C_{13}$ fission$]^+$ and 227 $[M\text{-}169\text{-}C_6H_{10}O_5]^+$ suggested the presence of glycosidic moiety at C-13. The mass spectrum of **1** displayed ion fragments relating to C_nH_{2n+1}, C_nH_{2n} and C_nH_{2n-1} ions in higher abundance for lower fragments. Most of the ions were separated by 14 mass units and decrease in abundance with increasing the molecular weight of long chain straight hydrocarbons. The absence of $[M\text{-}Me]^+$ ion suggested its straight chain nature. Elimination of $[C_5H_{10}O_2]^+$ moiety formed an ion peak at m/z 396 The ion peak at m/z 382, 368, 354, 338, *etc.* were arosed due to subsequent removal of the methylene groups from the mass unit 396.

The ^1H NMR spectrum of **1** exhibited a one-proton doublet at δ 5.31 (J = 7.1 Hz), assigned to anomeric H-1'. A one-proton broad signal as multiplet at δ 3.63 with half-width of 7.0 Hz was attributed to β-oriented H-13 oxygentated methine proton. A two-proton broad signal at δ 3.55 was ascribed to hydroxymethylene H_2-6' protons. The remaining methine protons of the sugar moiety appeared between δ 3.85-3.68. Two three-proton triplets at δ 0.91 (J = 6.85 Hz) and 0.88 (J = 7.0 Hz), were accounted to terminal C-1 and C-27 primary methyl protons. Three two-protons multiplets at δ 2.03, 1.68, 1.40 and a 42 proton broad signal at δ 1.28 were associated with the methylene protons of the molecule. The ^{13}C NMR spectrum of **1** exhibted signals for anomeric carbon at δ 101.23 (C-1'), carbinol carbons δ 73.41 (C-5'), 71.62 (C-13), 71.35 (C-2'), 67.17 (C-3') and 66.09 (C-4'), hydroxymethylene carbon at δ 62.32 (C-6'), methylene carbons at δ 37.16, 32.37 and 30.81 and methyl carbons at δ 14.72 (C-1) and 14.69 (C-27). The absence of any signal beyond δ 5.31 in the ^1H NMR spectrum and beyond δ 101.23 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. The multiplicity of each carbon signal was determined by analysis DEPT spectrum. The $^1H\text{-}^1H$ COSY spectrum of **1** showed correlation of H-1' with H-2', H-3', H-5' and H-13; and

H_3-1 and H_3-27 with neighbouring methylene protons. ^1H-^{13}C NMR HETCOR spectrum of **1** displayed correlation of H-1' with C-13, C-2', C-3' and C-5'. In HSQC spectrum correlation of H_2-6' was observed with C-13. Acid hydrolysis of **1** yielded β-D-glucose (TLC comparable) as a glycone moiety. On the basis of spectral data analysis and chemical reactions the structure of **1** has been elucidated as *n*-heptacosan-13α-ol-13β-D-glucopyranoside.

Compound **2** was obtained as a colourless product and it decolourized bromine water indicating unsaturated nature of the molecule. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3435 cm^{-1}), unsaturation (1634 cm^{-1}) and long aliphatic chain (722 cm^{-1}). Its molecular weight has been established as m/z 590 corresponding to molecular formula of an unsaturated aliphatic alcohol $C_{41}H_{82}O$ on the basis of FAB and EI mass spectrometries. It indicated one double bond equivalent adjustable in the vinylic linkage. The mass spectrum of **2** displayed ion fragments relating to C_nH_{2n-1}, C_nH_{2n} and C_nH_{2n+1} ion in higher abundance for lower fragments. Most of the ion fragments were separated by 14 mass units and decresed in abundance with increasing weight of long straight chain hydrocarbon. The absence of $[M\text{-}Me]^+$ ion suggested its straight chain nature. More intense clusters of ion peaks corresponding to C_nH_{2n-1} (m/z 69, 83, 97, 111, 125, 153, *etc.*) in comparison to that corresponding to C_nH_{2n+1} (m/z 71, 85, 99, 113, 127, 141, *etc.*) supported olefinic nature of the molecule[14,15]. The prominent ion fragments generated at m/z 87, 503 $[C_5\text{-}C_6$ fission$]^+$, 83, 507 $[C_{35}\text{-}C_{36}$ fission$]^+$ and 57, 533 $[C_{37}\text{-}C_{38}$ fission$]^+$ suggested the location of the hydroxyl group at C-5 and vinylic linkage at C_{36}.

The ^1H NMR spectrum of **2** displayed two one-proton multiplets at δ 5.38 and 5.35 assigned to vinylic H-37 and H-36, respectively. A one-proton broad signal at δ 4.19 with half-width of 18.5 Hz was attributed to a-oriented C-5 carbinol proton. Two multiplets at δ 2.04 and 2.00, both integrated for two-protons each were ascribed to C-38 and C-35 methylene protons, respectively, attached to the vinylic carbons. Two three-proton triplets at δ 0.89 (J = 6.5 Hz) and 0.86 (J = 7.0 Hz) were accounted to C-1 and C-41, primary methyl protons respectively. The remaining methylene protons resonated at δ 1.80 (4H) and δ 1.25 (64H). ^{13}C NMR spectrum of **2** exhibited important signals for vinylic carbons at δ 123.3 (C-36) and 124.03 (C-37), carbinol carbon at δ 76.98 (C-5), methyl carbon at δ 14.31 (C-1, C-41) and methylene carbons between δ 32.72-22.92. The multiplicity of the carbon signals was determined by analysis of DEPT spectrum. The ^1H-^1H COSY spectrum of **2** showed correlation of the carbinol protons with the adjacent methylene carbons H_2-4, H_2-3, H_2-2, H_2-5, H_2-6 and H_2-7; and vinylic proton H_2-36 with H-37, H_2-38, H_2-39,

H_2-35 and H_2-34. In ^1H-^{13}C HETCOR spectrum correlation of **2** H_3-1 and H_3-41 were observed with carbon signals of the adjacent methylene groups. In HSQC spectrum Me-41 carbon signal interacted with C-37 proton signal. On the basis of the foregoing account the structure of **2** has been established as *n*-hentetracont-36-en-5β-ol.

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