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

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One new compound, 3,7,11,19,23,27-hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside (**1**), along with the three known compounds, 3-epibetulinic acid (**2**), *n*-pentadecanyl octa-dec-19-en-oate (**3**) and β-sitosterol (**4**) were isolated from the methanolic extract of the cultured *Catharanthus roseus* hairy roots. The structures of the one new and three known compounds were elucidated using one- and two-dimensional NMR in combination with IR, EI/MS, FAB/MS. To the best of our knowledge, 3,7,11,19,23,27-hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside, 3-epibetulinic acid and *n*-pentadecanyl octa-dec-19-en-oate were identified for the first time from the hairy roots of *C. roseus*.

Key Words : *Catharanthus roseus*, Apocynaceae, Culture hairy root composition, New and known compounds

Introduction

The periwinkle, *Catharanthus roseus* (Apocynaceae), is widely used 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, two flavonols trisaccharides of kaempferol and quercetin have isolated and identified.[7] 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* 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 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 compound (**1**) and three known compounds (**2-4**) from the cultured hairy roots of *C. roseus* on the basis of spectral data and chemical reactions. Previously compound **2** and **3** are not reported with the complete spectral data and these compounds have reported for the first time from this cultured

[a]These two authors are equally contributed to this study.

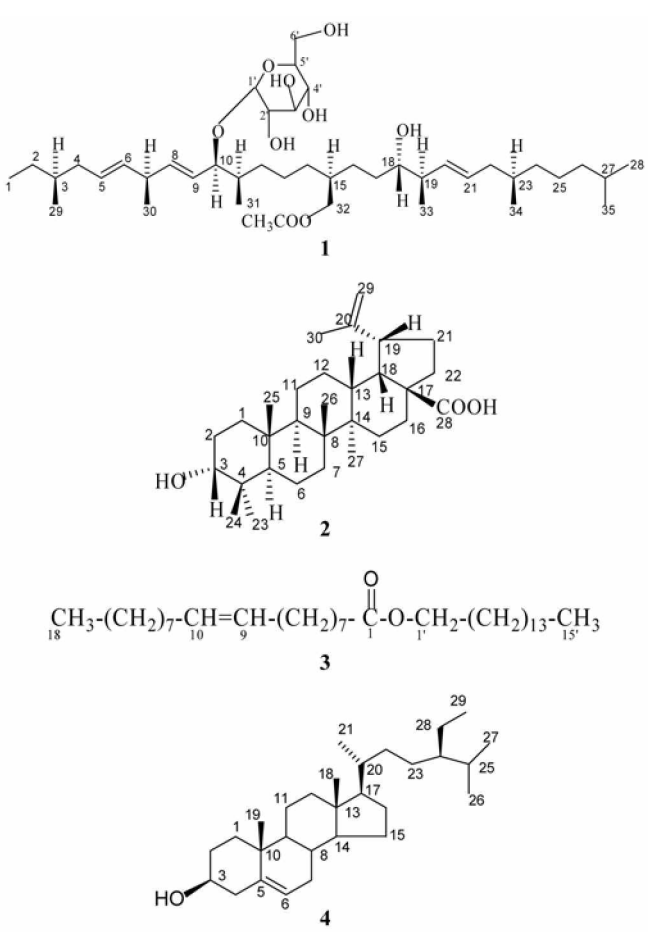

Figure 1. Chemical structures of **1-4**.

roots. Also, 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.

Results and Discussion

Compound **1** was obtained as a light yellow semisolid crystalline mass. It responded positive tests for glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3420, 3390, 3285 cm^{-1}), ester group (1736 cm^{-1}), and unsaturation (1640 cm^{-1}). Its FAB mass spectrum displayed a molecular ion peak at *m/z* 738 corresponding to $C_{43}H_{78}O_9$. Elimination of glycosidic unit from the $[M]^+$ generated an aglycone at *m/z* 576 $[C_{37}H_{68}O_4]^+$ and removal of the acetyl group from this unit produced a mass ion at *m/z* 533. The prominent ion peaks arose at *m/z* 476, 57 $[C_3-C_4$ fission$]^-$, 462, 71 $[C_4-C_5$ fission$]^+$, 436, 97 $[C_6-C_7$ fission$]^-$, 151, 382 $[C_9-C_{10}$ fission$]^+$, 181, and 352 $[C_{10}-C_{11}$ fission$]^+$ supported the existence of vinylic linkages at C-5 and C-8 and one of the hydroxyl groups at C-10. The ion peak at *m/z* 251 and 282 $[C_{14}-C_{15}$ fission$]^+$, 295 and 238 $[C_{15}-C_{16}$ fission$]^-$, 210 and 323 $[C_{17}-C_{18}$ fission$]^-$, and 180 and 353 $[C_{18}-C_{19}$ fission$]^+$ suggested the location of the hydroxyl group at C-18 and the *O*-acetyl group at C-32. The ion peaks yielded at *m/z* 381 and 152 $[C_{19}-C_{20}$ fission$]^-$, 127 and 407 $[C_{21}-C_{22}$ fission$]^+$, 113 and 420 $[C_{22}-C_{23}$ fission$]^+$, and 85 and 448 $[C_{23}-C_{24}$ fission$]^+$ indicated the existence of the remaining vinylic linkage at C-20. The fragmentation pattern of **1** is shown in Figure 2.

The ^{1}H-NMR spectrum of **1** exhibited three multiplet signals at δ 5.34, 5.35, and 5.36 were integrated for two protons each, assigned to vinylic H-5 and H-9, H-6 and H-20, and H-8 and H-21, respectively. A doublet of doublet signal at δ3.51 (1H, J = 9.0, 9.0 Hz) and a doublet of double doublet signal at δ 3.46 (1H, J = 3.5, 5.5, 4.0 Hz) were ascribed to H-10 and H-18, respectively. Two 1H doublet signals at δ 3.84 (J = 6.5 Hz) and 3.81 (J = 6.5 Hz) were attributed to the two protons attached to C-32. Four 1H doublet signals at δ4.36 (J = 8.0 Hz), 4.32 (J = 3.5 Hz), 3.02 (J = 6.5 Hz), and 3.00 (J = 6.5 Hz), two 1H double doublet signals at δ3.44 (J = 4.0, 3.5 Hz) and 3.46 (J = 3.5, 5.5 Hz), and a 1H multiplet signal at δ3.54 were accounted to the sugar protons. A broad 3H signal at δ 2.40 was associated with the acetyl group. A 3H triplet at δ0.76 (J = 6.0 Hz) was assigned to primary methyl protons attached to C-1. A broad signal at δ0.67 was ascribed to six protons attached to C-28 and C-35. Five 3H doublets at δ0.79 (J = 6.0 Hz), 0.91 (J = 6.0 Hz), 0.80 (J = 6.5 Hz), 0.89 (J = 6.5 Hz), and 0.86 (J =7.0 Hz) were associated with the methyl protons attached to C-29, C-30, C-31, C-33 and C-34, respectively. The remaining methylene and methine protons resonated between δ 2.33-1.25. The ^{13}C-NMR spectrum of **1** showed

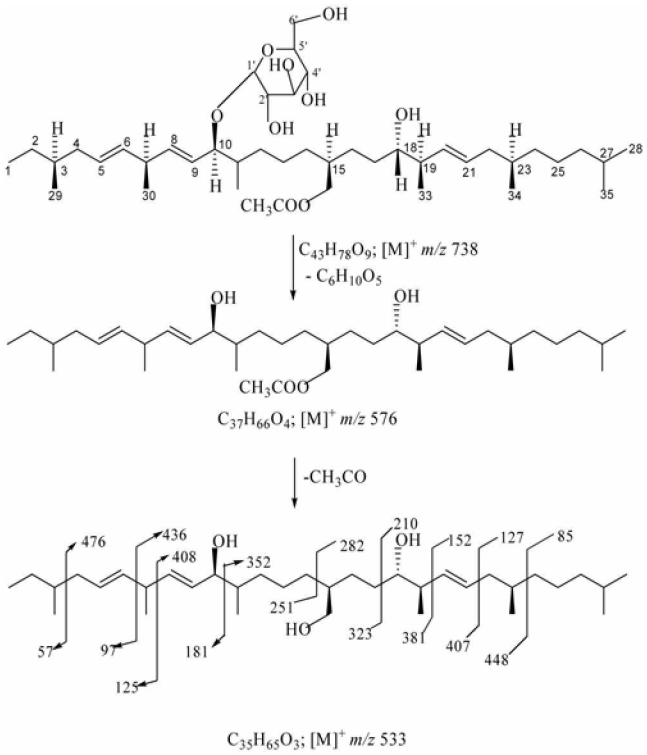

Figure 2. Fragmentation patterns of compound **1**.

important signals for vinylic carbons at δ 130.21 (C-5), 129.91 (C-6), 138.47 (C-8), 150.15 (C-9), 128.10 (C-20), and 122.26 (C-21), for tertiary carbons at δ70.57 (C-10) and 70.45 (C-18), for two methylene carbons attached to oxygen atoms at δ63.73 (C-32) and 63.10 (C-6'), for the anomeric carbon at δ101.45 (C-1'), for the remaining sugar carbons at δ76.46 (C-2'), 74.03 (C-3'), 73.74 (C-4'), and 79.83 (C-5'), for the methyl carbons at δ 19.54 (C-28), 14.31 (C-29), 21.43 (C-30), 19.09 (C-31), 18.44 (C-33), 18.90 (C-34), and 15.07 (C-35), and for the primary methyl carbon at δ12.05 (C-1). The remaining methylene and methine carbons resonatated between δ56.97-72.88. Two carbon signals at δ 174.55 and 34.43 were attributed to the acetyl group. The multiplicity of each carbon was determined by DEPT experiments. There were nine primary, 13 methylene, 20 methine, and one quaternary carbon in the molecule. The HMBC shows the correlation between the anomeric proton of the glucose and C-10. This indicates that the sugar moiety is attached to C-10. Also, H-32 and methyl protons in the *O*-acetyl group showed the correlation with the carbonyl carbon. These correlations presented the attached position (C-32) of the O-acetyl group. The carbon signals of C-9 correlated with H-8, H-7, and H-10 and of C-18 correlated with H-19 and H-20 were found in the HMBC spectrum. The ^{1}H-^{1}H COSY spectrum of **1** exhibited the linear connection patterns between H-1', H-2', H-3', and H-5', between H-30, H-7, H-6, and H-5, between H-30, H-8, and H-9, between H-18, H-17, and H-16 , and between H-18, H-19, and H-20. Acid hydrolysis of **1** yielded D-glucose which was determined by co-TLC with an authentic sample of D-glucose. On the basis of the foregoing account the structure

of **1** has been established as 3,7,11,19,23,27-hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10,18-diol-10-β-D-glucopyranoside. This is a new compound isolated from the cultured hairy roots of *C. roseus*.

Compound **2**, 3-epibetulinic acid, was obtained as a colorless crystalline mass from the $CHCl_3$-MeOH (95 : 5) eluent system. It responded positively to Liebermann-Burchard test for triterpnes. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3448 cm^{-1}), carboxylic group (1688 cm^{-1}), and unsaturation (1640 cm^{-1}). The EI and positive ion FAB mass spectra established the molecular ion peak at *m/z* 456 which is corresponding to a molecular formula $C_{30}H_{48}O_3$. It indicated seven double bond equivalents; five of them were adjusted in the pentacyclic carbon frame-work of triterpene and one each in the vinylic linkage and carboxylic group. The prominent ion peaks arose at *m/z* 100, 356 $[C_1-C_{10}$ fission$]^+$, 82 $[100-H_2O]^-$, 113 $[C_{1,10}-C_{5,10}-C_{5,6}$ fission$]^-$, 95 $[113-H_2O]^+$, 109 $[C_{1,10}-C_{5,10}-C_{6,7}$ fission-$H_2O]^-$, 55 $[C_{3,4}-C_{5,10}-C_{5,6}$ fission$]^+$, 69 $[C_{3,4}-C_{5,10}-C_{6,7}$ fission$]^-$, 83 $[C_{3,4}-C_{5,10}-C_{7,8}$ fission$]^+$, and 123 $[C_{1,10}-C_{5,10}-C_{7,8}$ fission-$H_2O]^-$. This suggested the location of hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic grounds. The ion peaks generated at *m/z* 208, 248 $[C_{8,14}-C_{9,11}$ fission$]^+$, 190 $[208-H_2O]^-$, 203 $[248-COOH]^-$, 207 $[248-C_3H_5]^+$, 234, 222 $[C_{8,14}-C_{11,12}$ fission$]^+$, 189 $[234-COOH]^+$, 204 $[222-H_2O]^+$, 220, 236 $[C_{8,14}-C_{12,13}$ fission$]^-$, 175 $[220-COOH]^+$, 218 $[236-H_2O]^-$, and 221 $[236-Me]^+$ which suggested the saturated nature of ring C. The ion peaks arose at *m/z* 304, 152 $[C_{16,17}-C_{13,18}$ fission$]^+$, 107 $[152-COOH]^+$, 286 $[304-H_2O]^+$, 272 $[286-CH_2]^+$, 258 $[272-CH_2]^-$, 121 $[C_{15,16}-C_{13,18}$ fission-COOH$]^-$, 135 $[C_{14,15}-C_{13,18}$ fission-COOH$]^-$, 441 $[M-Me]^+$, 438 $[M-H_2O]^-$, 423 $[438-H_2O]^+$, 411 $[M-COOH]^-$, 395 $[411-Me]^+$, and 410 $[M-HCOOH]^+$ indicated the saturated nature of ring D and attachment of the carboxylic group at C-28. The fragmentation pattern of **2** is shown in Figure 3.

The ^{1}H-NMR spectrum of **2** displayed two 1H doublet signals at δ 4.72 and 4.59 with coupling constant (J) of 1.5 Hz each that are assigned to the methylene protons (H-29). A 1H doublet of doublet signal at δ 3.15 with coupling constants (J) of 5.5 and 5.5 Hz was attributed to H-3. A 1H multiplet signal at δ 3.01 was ascribed to H-19. A 1H doublet of doublet signal at δ 1.91 with J values of 5.0 and 6.5 Hz was accounted to 18-H having interaction with H-19 and H-13. A 3H broad signal was associated with H-30 methyl protons. Three broad signals at δ 0.99, 0.84, and 0.75 were accounted as the methyl protons attached to C-23, C-25, and C-27, respectively. A 6H broad signal at δ 0.95 was assigned to the methyl protons attached to C-24 and C-26. A 1H multiplet signal at δ 2.25 was ascribed to H-5. The remaining methylene and methine protons resonated between δ 1.58-0.93.

More compelling evidence for the structure of **2** was provided by the analysis of its ^{13}C-NMR spectrum, which showed the presence of 30 carbon atoms. The assignment of the carbon chemical shifts were made by comparison of the δ values of the corresponding carbon atoms in the structurally similar compounds of lupene series.[13] The important signals appeared for carboxylic group at δ 179.80 (C-28), vinylic carbons at δ 150.43 (C-20), and δ 110.04 (C-29), carbinol carbon at δ 79.36 (C-3), and methyl carbons at δ 28.46 (C-23), 15.94 (C-24), 16.49 (C-25), 16.64 (C-26), 15.20 (C-27), and 19.72 (C-30). The multiplicity of each carbon was determined by the analysis of DEPT spectrum. There were six methyl, eleven methylene, six methine, and seven quaternary carbons in the molecule. The ^{1}H-^{1}H COSY spectrum of **2** showed the connection patterns of H-19 with H-18 and H-21, of H-3 with H-2, of H-2 with H-1. The HMBC spectrum of **2** exhibited ^{1}H-^{13}C correlations of C-28 with H-16 and H-22, of C-20 with H-30 and H-18, of C-29 with H_3-30 and H-19, and of C-3 with H-2, H-23 and H-24. On the basis of spectral data analysis and chemical reactions, the structure of **2** has elucidated as lup-20(29)-en-18-βH-

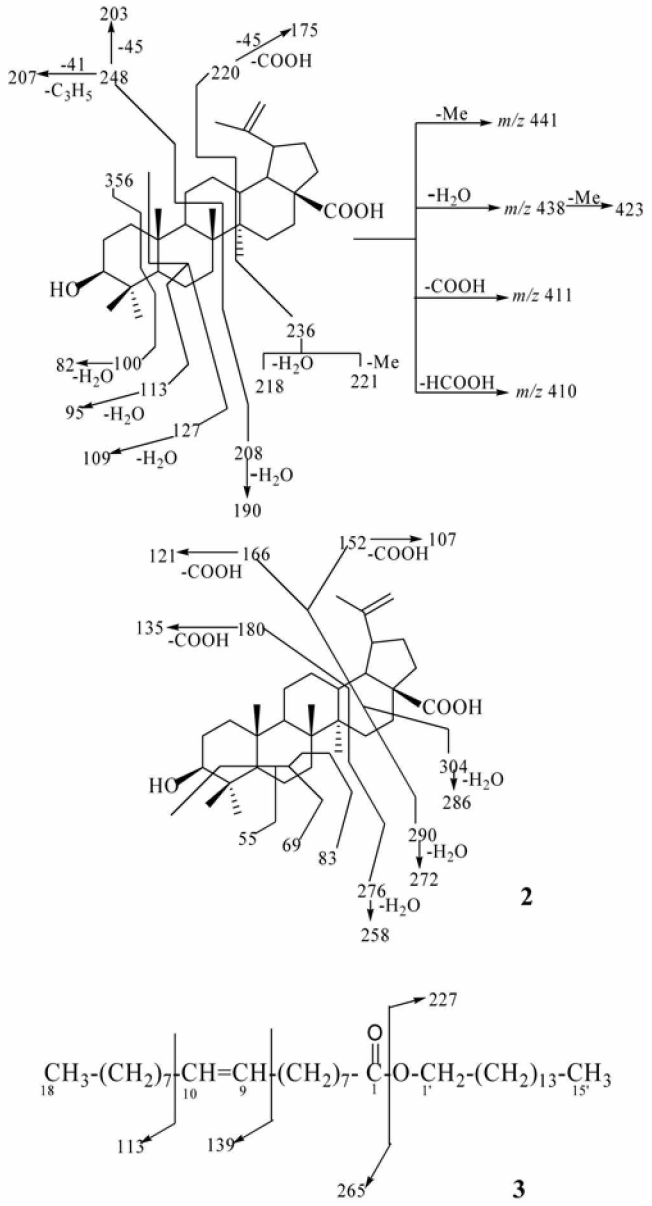

Figure 3. Fragmentation patterns of compound **2** and **3**.

3α-ol which is a previously reported compound.[14]

Compound **3**, an aliphatic ester, was obtained as a yellow gum mass from the eluent system of $CHCl_3$-MeOH (95 : 5). It decolorized bromine water which indicates the presence of unsaturated linkage(s) in the molecule. Its IR spectrum displayed characteristic absorption bands for a carbonyl group of ester (1721 cm^{-1}) and unsaturation (1640 cm^{-1}). Its EI and FABMS spectra displayed a molecular ion peak at m/z 492 that provides a molecular formula of $C_{33}H_{64}O_2$. The prominent ion peaks generated at m/z 113 [C_{11}-C_{11} fission]$^-$, 139 [C_8-C_9 fission]$^-$, and 265 and 227 [CO-O fission]$^+$. This suggested that an oleic acid group is esterified with a 15C aliphatic moiety. The fragmentation pattern of **3** is shown in Figure 3.

The ^{1}H-NMR spectrum of **3** displayed two 1H multiplet signals at δ 5.41 and 5.36 which were assigned to the vinylic H-9 and H-10, respectively. Two 1H doublet signals at δ 3.68 (J = 4.5 Hz) and 3.65 (J = 4.5 Hz) were attributed to H-1' methylene protons. Another set of two 1H doublet signals at δ 2.30 (J = 7.5 Hz) and 2.22 (J = 7.5 Hz) was accounted to H-2 methylene protons adjacent to the ester group. Two 2H multiplet signals at δ 2.17 and 2.05 were ascribed to H-8 and H-11 methylene protons. Two 3H triplet signals at δ 0.85 (J = 5.5 Hz) and 0.82 (J = 6.5 Hz) were associated with the terminal H-18 and H-15', respectively. The ^{13}C-NMR spectrum of **3** exhibited important signals for a carbonyl carbon at δ 171.37 (C-1), vinylic carbons at δ 130.25 (C-9) and 129.02 (C-10), an oxygenated methylene carbon at δ 70.79 (C-1'), and methyl carbons at δ 14.33 (C-18) and 14.26 (C-15). The multiplicity of each carbon signal was determined by DEPT experiments. The ^{1}H-^{1}H COSY spectrum of **3** showed correlation of vinylic H-9 and H-10 with H-8 and H-11, respectively. Acid hydrolysis of **3** yielded oleic acid which was confirmed by co-TLC with an authentic sample of oleic acid. On the basis of spectral data analysis and chemical reactions, the structure of **3** has been established as *n*-pentadecanyl octa-dec-19-en-oate.

Experimental Section

Chemicals. All chemicals were of an analytical grade: hexane, ethyl acetate, methanol, ethanol, sulphuric acid and vanillin were purchased from Daejung Chemicals and Metals (Seoul, South Korea). Pre-coated TLC plates (layer thickness 0.5 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, oleic acid, and D-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Instrumentation. 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. EI/MS and FAB/MS 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.[15] 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 26 °C at 100 rpm.

Extraction of hairy roots. The powdered hairy roots of *C. roseus* (200 g) were immersed in methanol (1.5 litre) 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 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 fraction 250 mL) with the following eluents: fraction 1-2 with *n*-hexane, fractions 3-4 with *n*-hexane:ethyl acetate (9:1, v/v), fraction 5-6 with *n*-hexane:ethyl acetate (8:2, v/v), fraction 7-8 with *n*-hexane:ethyl acetate (7:3, v/v), fraction 9-10 with *n*-hexane:ethyl acetate (1:1, v/v), fraction 11-12 with hexane:ethyl acetate (3:7, v/v), fraction 13-14 with ethyl acetate, fraction 15-16 with ethyl acetate:methanol (9.5:0.5, v/v), fraction 17-18 with ethyl acetate:methanol (9:1, v/v), fraction 19-20 with ethyl acetate:methanol (7:3, v/v), fraction 21-22 with ethyl acetate:methanol (1:1, v/v), fraction 23-24 with ethyl acetate:methanol (3:7, v/v), and fraction 25-26 in methanol. All fractions were examined by TLC. Fraction 1-4 was not further separated due to the low amount. Fraction 5-6 (0.8 g) was crystallized after the purification by CC, and then yielded β-sitosterol (**4**, 20 mg) whose identity was confirmed through the comparison of TLC and spectroscopic data with those of an authentic sample. Fraction 7-8 (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 (99:1, 98.5:1.5, 98.2, 97.5:2.5 and 97:3, v/v) to afford one pure compound (120 mg, **2**). Fraction 11-12 with hexane:ethyl acetate (3:7, v/v), after re-separation with chloroform:methanol (99:1, 99:2, 97:3, 96:4 and 95:5, v/v), afforded five fractions. Fraction 4 (from the eluent of $CHCl_3$:MeOH (96:4, v/v)) and fraction 5 (from the eluent of $CHCl_3$:MeOH (95:5, v/v)) were re-chromatographed over Lichroprep RP18 ODS (50 g; each fraction of 50 mL). The eluting was sequentially performed with methanol containing 80, 60, 40 20, 10, and 0% of water to yield compounds **1** (35 mg) and **3**

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz)-NMR spectroscopic data of **1** in MeOD

Position	^{1}H-NMR	^{13}C-NMR
1	0.76 t (6.0, 3H)	12.05
2	1.29 br s (2H)	29.88
3	1.58 m (1H)	39.09
4	2.30 dd (7.5, 7.0, 1H)	39.97
	2.33 dd (7.5, 7.0, 1H)	
5	5.34 m (1H)	130.21
6	5.35 m (1H)	129.91
7	2.04 dd (7.0, 7.0, 1H)	56.97
8	5.36 m (1H)	138.47
9	5.34 m (1H)	150.15
10	3.51 dd (9.0, 9.0, 1H)	70.57
11	1.52 m (1H)	29.88
12	1.29 br s (2H)	22.88
13	1.34 br s (2H)	24.55
14	1.25 br s (2H)	33.91
15	1.60 m (1H)	42.53
16	1.29 br s (2H)	34.48
17	1.60 m (1H)	36.91
	1.48 m (1H)	
18	3.46 ddd (3.5, 5.5, 4.0, 1H)	70.45
19	2.00 dd (5.5, 6.6, 1H)	50.37
20	5.35 m (1H)	128.10
21	5.36 m (1H)	122.26
22	2.05 dd (7.0, 7.0, 1H)	37.48
	2.02 dd (7.0, 5.5, 1H)	
23	1.56 m (1H)	29.95
24	1.25 br s (2H)	27.43
25	1.25 br s (2H)	32.07
26	1.25 br s (2H)	32.13
27	1.48 m (1H)	39.03
28	0.67 br s (3H)	19.54
29	0.78 d (6.0) (3H)	14.31
30	0.91 d (6.0) (3H)	21.43
31	0.80 d (6.5) (3H)	19.09
32	3.84 d (6.5) (1H)	63.73
	3.81 d (6.5) (1H)	
33	0.89 d (6.5) (3H)	18.44
34	0.86 d (7.0) (3H)	18.90
35	0.67 br s (3H)	15.07
1'	4.36 d (8.0, 1H)	101.45
2'	4.32 d (3.5, 1H)	76.46
3'	3.44 dd (4.0, 3.5, 1H)	74.03
4'	3.46 dd (3.5, 5.5, 1H)	73.74
5'	3.54 m (1H)	79.83
6'	3.02 d (6.5, 1H)	63.10
	3.00 d (6.5, 1H)	
CH_3COO	2.40 br s (3H)	174.55 (CO)
		34.43 (Me-C)

J values (in Hz) are in parenthesis

(23 mg). The separation of compounds of other fractions is in progress.

3,7,11,19,23,27-Hexamethyl-15-hydroxymethylene-*n*-octacos-5,8,20-triene-10β,18α-diol-10β-D-glucopyranoside (1). Light yellow semisolid. $[\alpha]_D^{25}$ +21.5° (*c* 0.12, MeOH); IR (KBr) ν_{max}: 3420, 3390, 3285, 2926, 2853, 1736, 1640, 1463, 1376, 1174, 1082, 1053, 1021 cm^{-1}; EIMS *m/z* (rel. int.): 532 [M-$C_6H_{11}O_5$-CH_3CO] (1.7), 476 (2.5), 462 (2.6), 448 (2.7), 436 (4.1), 420 (2.7), 408 (3.6), 407 (3.8), 382 (14.5), 381 (19.2), 353 (100), 352 (75.9), 324 (14.9), 323 (18.3), 295 (12.3), 282 (5.7), 251 (48.3), 238 (15.0), 210 (38.3), 209 (16.2), 181 (13.8), 180 (17.3), 152 (18.2), 151 (17.9), 127 (21.2), 125 (34.8), 113 (28.6), 97 (57.0), 85 (47.7), 71 (65.3), 57 (83.9); FAB-MS (positive mode) $[M+H]^-$ *m/z*: 739 $[M]^+$ ($C_{43}H_{79}O_9$);]$^+$: ^{1}H and ^{13}C-NMR (Table 1).

3-Epibetulinic acid (2). Colourless solid; IR (KBr) ν_{max}: 3448, 2940, 2852, 1688, 1640, 1452, 1378, 1237, 1038, 884 cm^{-1}; ^{1}H-NMR (500 MHz; MeOD): δ 4.72 (1H, d, *J* = 1.5 Hz, H_2-29a), 4.59 (1H, d, *J* =1.5 Hz, H_2-29b), 3.15 (1H, dd, *J* = 5.5, 5.5 Hz, H-3β), 3.01 (1H, m, H-19β), 2.25 (1H, m, H-5α), 1.91 (1H, dd, *J* = 5.0, 6.5 Hz, H-18α), 1.58 (1H, m, H-13β), 1.69 (3H, br s, Me-30), 0.99 (3H, br s, Me-23), 0.95 (6H, br s, Me-24, Me-26), 0.84 (3H, br s, Me-25), 0.75 (3H, br s, Me-27); ^{13}C-NMR (125 MHz; MeOD): δ 39.14 (C-1), 27.61 (C-2), 79.36 (C-3), 39.66 (C-4), 56.30 (C-5), 19.05 (C-6), 35.15 (C-7), 41.47 (C-8), 51.43 (C-9), 37.92 (C-10), 21.67 (C-11), 26.36 (C-12), 39.56 (C-13), 43.19 (C-14), 31.33 (C-15), 33.03 (C-16), 57.03 (C-17), 47.85 (C-18), 50.03 (C-19), 151.43 (C-20), 30.43 (C-21), 37.85 (C-22), 28.46 (C-23), 15.94 (C-24), 16.49 (C-25), 16.64 (C-26), 15.20 (C-27), 179.80 (C-28), 110.04 (C-29), 19.72 (C-30); EIMS m/z (rel. int.): 456 $[M]^-$ ($C_{30}H_{48}O_3$) (64.8), 441 (7.4), 438 (30.2), 423 (14.9), 411 (9.1), 395 (13.9), 369 (4.5), 356 (2.9), 327 (3.4), 317 (6.8), 302 (9.7), 287 (4.7), 259 (9.9), 348 (54.6), 233 (30.4) , 220 (32.2), 207 (61.7), 203 (33.0), 189 (100), 175 (29.4), 161 (17.1), 147 (20.1), 135(37.8), 121 (29.2), 119 (27.7), 95 (31.0), 81 (27.8), 69 (22.1), 55 (18.8); Positive ion FABMS *m/z* 457 $[C_{30}H_{49}O_3]^-$; negative ion FABMS *m/z* 455 $[C_{30}H_{47}O_3]^+$.

***n*-Pentadecanyl octa-dec-19-en-oate (3).** Yellow gum; IR (KBr) ν_{max}: 2922, 2853, 1721, 1640, 1462, 1254, 1106, 748 cm^{-1}; ^{1}H-NMR (500 MHz; $CDCl_3$): δ 5.41 (1H, m, H-9), 5.36 (1H, m, H-10), 3.68 (1H, d, *J* = 4.5 Hz, H2-1'a), 3.65 (1H, d, *J* = 4.5 Hz, H-1'b), 2.33 (1H, d, *J* = 7.5 Hz, H-2a), 2.22 (1H, d, *J* = 7.5 Hz, H_2-2b), 2.17 (2H, m, H_2-8), 2.05 (2H, m, H_2-11), 1.61 (2H, m, H_2-3), 1.25 (46 H, br s, 23 x CH_2), 0.85 (3H, t, *J* = 5.5 Hz), Me-18), 0.82 (3H, t, *J* = 6.5 Hz, Me-15'); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 171.37 (C-1), 130.25 (C-9), 129.02 (C-10), 70.79 (C-1'), 38.96 (CH_2), 36.16 (CH_2), 33.94 (CH_2), 32.14 (CH_2), 30.59 (CH_2), 30.38 (CH_2), 29.92 (CH_2), 29.82 (CH_2), 29.67 (7 x CH_2), 29.58 (CH_2), 29.54 (CH_2), 29.48 (CH_2), 29.33 (CH_2), 29.15 (CH_2), 28.94 (CH_2), 27.44 (CH_2), 26.93 (CH_2), 25.85 (CH_2), 24.94 (CH_2), 23.20 (CH_2), 22.91 (CH_2), 14.33 (Me-18), 14.26 (Me-15); EIMS: *m/z* (rel. int.): 492 $[M]^-$ ($C_{33}H_{64}O_2$] (1.2), 463 (5.3), 435 (10.1), 393 (13.2), 281 (15.9), 265 (7.8), 251 (16.1), 227 (5.3), 223 (14.3), 167 (46.9), 149 (40.3), 139 (26.3), 113 (40.7), 111 (59.7), 97 (64.9), 83 (74.7), 69 (70.0), 57 (100).

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).

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