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Studies on the Chemical Constituents of the New Zealand Deer Velvet Antler *Cervus elaphus var. scoticus*-(I)

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Abstract – 44 compounds and 9 minerals were isolated from and detected in the New Zealand deer velvet antler *Cervus elaphus var. scoticus* Lönnberg. The chemical structures of (**1 - 26**) were identified on the basis of the spectroscopic methods and comparisons with literature, respectively. The structures were identified as cholesterol (CS, **6**), 7-keto-CS (**7**), 7β-hydroxy-CS (**8**), and 7α-hydroxy-CS (**9**), and included 12 steroid 3β-*O*-(palmitic/stearic/myristic acid esters; PM/SA/MS) [CS-3β-*O*-PM (**1 - 1**), CS-3β-*O*-SA (**1 - 2**), CS-3β-*O*-MR (**1 - 3**), 7-keto-CS-3β-*O*-PM (**2 - 1**), 7-keto-CS-3β-*O*-SA (**2 - 2**), 7-keto-CS-3β-*O*-MR (**2 - 3**), 7β-hydroxy-CS-3β-*O*-SA (**3 -1**), 7β-hydroxy-CS-3β-*O*-PM (**3 - 2**), 7β-hydroxy-CS-3β-*O*-MR (**3 - 3**), 7α-hydroxy-CS-3β-*O*-SA (**4 - 1**), 7α-hydroxy-CS-3β-*O*-PM (**4 - 2**), and 7α-hydroxy-CS-3β-*O*-MR (**4 - 3**)], dinonyl phthalate (**5**), 8 nucleic acids analogues [uracil (**10**), deoxyguanosine (**11**), deoxyuridine (**12**), uridine (**13**), deoxyadenosine (**14**), adenosine (**15**), inosine (**16**), and guanosine (**17**)], and the 9 free amino acids [L-phenylalanine (**18**), L-isoleucine (**19**), L-leucine (**20**), L-tyrosine (**21**), L-valine (**22**), L-proline (**23**), L-threonine (**24**), L-alanine (**25**), and L-hydroxyproline (**26**)]. Also, there are 8 kinds of amino acids [asparagine, serine, glutamine, glycine, histidine, arginine, methionine, and lysine], 2 sialic acids [N-acetylneuraminic acid (**27**), ketodeoxynonulosonic acid (**28**)], and 9 minerals [Na > K > Ca > Mg > Fe > Zn > B > Al > Cu] were detected from the autoaminoacid analyzer and ICP spectrometer, HPAEC-PAD/HPLC-FLD, respectively. 9 kinds of oxycholesterol-3β-*O*-fatty acid ester (**2 - 1, 2 - 2, 2 - 3, 3 - 1, 3 - 2, 3 - 3, 4 - 1, 4 - 2**, and **4 - 3**) and 3 nucleic acids (**12**, **14**, and **15**) were isolated from the velvet antler for the first time. 6 kinds of steroids (**7**, **8**, **9**, **2 - 1**, **3 - 1**, and **4 - 1**) were examined for their anti-proliferative effects against L1210, P388D1, K562, MEG-01, KG-1, MOLT-4, A549, HepG2, MCF-7, SK-OV-3, and SW-620 cancer cell lines. They showed anti-proliferative effects with IC_{50} values of 0.06, 2.16, 2.42, > 50.0, 1.66 and 8.31 μM against L1210, while the values were 24.05, 9.44, 5.22, 0.25. 9.48 and 49.77 μM against P388D1, respectively. The others were inactive.

Keywords – New Zealand deer velvet antler, *Cervus elaphus var. scoticus,* 7-Ketocholesteryl 3β-*O*-palmitate, 7β-Hydroxycholesteryl 3β-*O*-stearate, 7α-Hydroxycholesteryl 3β-*O*-stearate, Anti-proliferative effects of steroids analogues

Introduction

Velvet antler refers to the whole cartilaginous antler in a precalcified stage on growing antlers. It is an ingredient in traditional medicine and is used as a growth tonic for children, as well as for body strengthening, blood cell production, the immune system, cardiovascular health and geriatric therapies.[5]

Deer velvet antler is composed of proteins and growth factors, amino acids, and insulin-like growth factors (IGF) which are precursors for the growth hormone production, and promotes muscle growth, tissue growth and organ health, collagen, glycosaminoglycans (GAG's), glucosamine, chondroitin sulphate, hyaluronic, lipids, phospholipids, prostaglandins, minerals production.[20,22]

In previous papers, cholesterol, cholest-5-en-3β-ol-7-one, cholest-5-en-3β, 7β-diol, cholesterol esters, *p*-hydoxybenzaldehyde, uracil, uridine, hypoxanthine, nicotinic acid, creatine and urea were isolated from *Cervus nippon* var. *mantchuricus,*[10] phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, phosphatidic acid, lysophosphatidyl ethanolamine, lysophosphatidyl choline were isolated from C. *nippon* var. *taiouans*,[26] cholesteryl myristate, cholesteryl oleate, cholesteryl palmitate, and cholesteryl stearate were isolated from C. *elaphus*.[25]

The present paper deals with the isolation and identification of the compounds from the New Zealand deer velvet antler *Cervus elaphus var. scoticus* which is the

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world's largest producer, producing 450 tons of deer velvet antler per year[11] and includes an evaluation of its their anti-proliferative effects on 11 kinds of cancer cell lines.

Experimental

Instrument & reagents – The nuclear magnetic resonance (NMR) spectra were obtained on a Bruker Biospin Avance II 400 MHz spectrometer. FAB-MS and LC-MSMS spectrometric data were acquired with a JMS-600W/JEOL, JMS-700 (JEOL) and AQUITY TQD (Waters) mass spectrometer. CD (circular dichroism) spectra were taken by a Jasco J-715 spectropolarimeter. Silica Gel (230 - 400 mesh, Merck) was used for column chromatography. TLC was carried out using Merck silica Gel 60 F_{254}. HPLC (high performance liquid chromatography) was performed using a Waters 510 pump, Sodex RI71 detector [Porasil™ Silica 1~20 μm, 19 mm ID × 300 mm] and Jasco pump PU-1580, UV 2075 plus [ODS (octadeoxysilane, C18) 5 μm, 10 mm ID × 250 mm (Daiso Co. Ltd.)]. HPLC solvents were from JT Baker, USA. Standard reagents for comparison experiments were N-acetylneuraminic acid (NANA, Sigma, A0812, St. Louis, MO, USA), ketodeoxynonulosonic acid (KDN, Sigma, 60714, St. Louis, MO, USA). Also, others were 1, 2-diamino-4, 5-methyleneoxybenzene (DMB, Takara Bio inc., 4400, Shiga, Japan). Amino acids autoanalysis was performed using a ODS column (Waters Discovery, 4.6 mm ID × 250 mm, 5 μm), Alliance 2695 HPLC/Waters 2475 fluorescence detector (AccQ-Tag method). Elemental analysis was performed using an ICP (Inductively Coupled Plasma Spectrometer)/Perkin-Elmer Optima 7300DV, cyclonic chamber in a dual view method. Sialic acid analysis was performed using a HPAEC-PAD (high performance anion exchange chromatography, pulsed amperometric detection), CarboPac™ PA10 column, 0.4 mmID × 250 mm, 50 mM sodium hydroxide, and HPLC/FLD (fluorescence). RPMI 1640 was purchased from Gibco (Lifetech, New York, USA). A Premix WST-1 cell proliferation assay system was purchased from Takara (Takara Bio Inc., Otsu, Japan). K562, MEG-01, KG-1, MOLT-4 (leukemia), A549 (lung), HepG2 (liver), MCF-7 (breast), SK-OV-3 (ovary), SW-620 (colon), and L1210, P388D1 (leukemia, Rodent) were obtained from KCLB (Korean Cell Line Bank, Seoul, Korea).

Material – The dried New Zealand deer velvet antler *Cervus elaphus var. scoticus* was purchased from New Zealand in May 2012. The animal identification was performed by KGC raw materials headquarters, Korea Ginseng Corp., Daejeon, Korea and the voucher specimen NY-2012 was deposited at the R&D headquarters, Korea Ginseng Corp., Daejeon, Korea.

Extraction and isolation (Fig. 1) – In the year 2012, collected velvet antler (1 piece, 520 g: upper including tip (U&T) 130 g, mid (M) 160 g, base (B) 230 g) was sliced into 3 mm thick pieces, after hair removal. Each piece [U&T 100 g, M 100 g, B 100 g] was extracted 3 times with acetone at 25 ºC for 1 day which yielded the acetone extract (U&T 1.12 g, M 1.00 g, B 0.81 g). The pieces were extracted with 3 times methanol at 25 ºC for 1 day which yielded the methanol extract (U&T 3.80 g, M 3.62 g, B 3.39 g). Finally, the pieces were extracted 3 times with water 25 ºC for 1 day which yielded the water extract (U&T 5.93 g, M 4.51 g, B 3.28 g).

The methanol extract (U&T 3.80 g) was suspended in water and partitioned with chloroform. The resulting fractions were concentrated to give the chloroform extracts (2.64 g) and water extracts (1.16 g), respectively. In succession, the chloroform extract (2.64 g) was chromatographed on a silica gel column chromatography and eluted with a gradient of n-hexane → n-hexane : ethyl acetate = 50 : 1 → 20 : 1 → 10 : 1 → 5 : 1 → 2 : 1 → 1 : 1 → 1 : 2 → 1 : 5 → ethyl acetate → methanol in 4 fractions (N-1, N-2, N-3, and N-4).

Fraction N-1 (105 mg) was subjected on normal phase HPLC eluting with a silica gel column (n-hexane : ethyl acetate = 50 : 1) to create 5 subfractions which are cholesteryl 3*β*-*O*-fatty acid esters (**1 - 1**, **1 - 2**, **1 - 3**, 20 mg), 7-ketocholesteryl 3*β*-*O*-fatty acid esters (**2 - 1**, **2 - 2**, **2 - 3**, 8 mg), 7*β*-hydroxycholesteryl 3*β*-*O*-fatty acid esters (**3 - 1**, **3 - 2**, **3 - 3**, 6 mg), 7*α*-hydroxycholesteryl 3*β*-*O*-fatty acid esters (**4 - 1**, **4 - 2**, **4 - 3**, 5 mg), and dinonyl phthalate (**5**, 20 mg), respectively. Continuously, one of the subfractions which was a mixture of cholesteryl fatty acid 3*β*-*O*-esters (20 mg) was subjected to reverse phase HPLC eluting with an ODS column (isopropanol - acetonitrile = 1 : 1, 205 nm) to create cholesteryl 3*β*-*O*-palmitate **(1 - 1)**, cholesteryl 3*β*-*O*-stearate (**1 - 2)**, and cholesteryl 3*β*-*O*-myristate **(1 - 3)**, at a ratio of 42 : 21 : 15, respectively. Similar to the above, the mixtures of 7-ketocholesteryl 3*β*-*O*-fatty acid esters, 7*β*-hydroxycholesterol 3*β*-*O*-fatty acid esters, and 7*α*-hydroxycholesterol 3*β*-*O*-fatty acid esters were subjected to reverse phase HPLC eluting with an ODS column (isopropanol - acetonitrile = 4 : 5, 205 nm) to create [7-ketocholesteryl 3*β*-*O*-palmitate **(2 - 1)**, 7-ketocholesteryl 3*β*-*O*-stearate (**2 - 2)**, 7-ketocholesteryl 3*β*-*O*-myristate **(2 - 3)**, at a ratio of 20 : 13 : 11], [7*β*-hydroxycholesterol 3*β*-*O*-stearate (**3 - 1**), 7*β*-hydroxycholesterol 3*β*-*O*-palmitate (**3 - 2**), 7*β*-

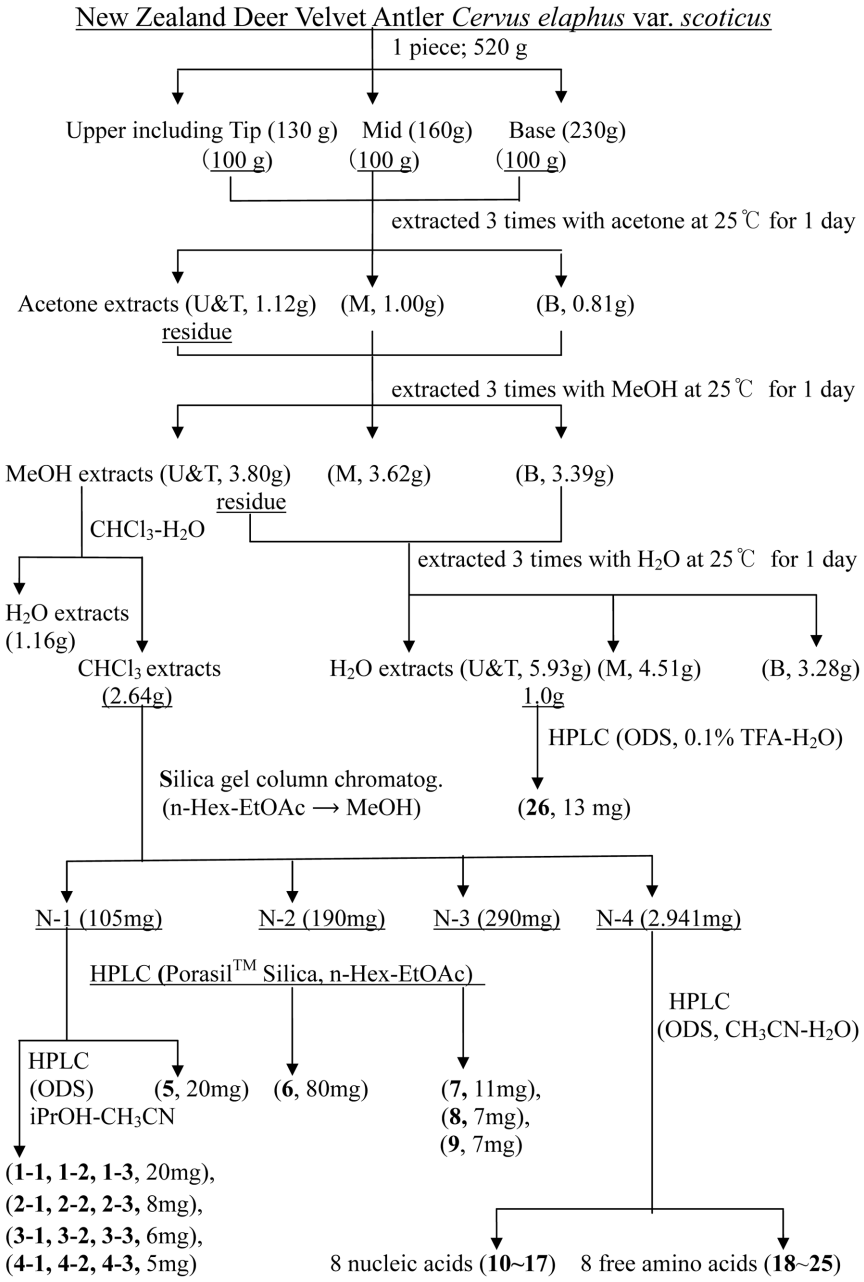


Fig. 1. Extraction and isolation of compounds (**1~26**) from New Zealand Deer Velvet Antler *Cervus elaphus* var. *scoticus*.

hydroxycholesterol 3*β*-*O*-myristate (**3 - 3**) at a ratio of 41 : 22 : 8], [7*α*-hydroxycholesterol 3*β*-*O*-stearate (**4 - 1**), 7*α*-hydroxycholesterol 3*β*-*O*-palmitate (**4 - 2**), 7*α*-hydroxycholesterol 3*β*-*O*-myristate (**4 - 3**) at a ratio of 12 : 7 : 2], respectively. Also, the fraction N-2 (190 mg) was subjected to normal phase HPLC eluting with a silica gel column (n-hexane : ethyl acetate = 10 : 1, RI detector) to create cholesterol (**6**, 80 mg). N-3 (290 mg) was subjected to normal phase HPLC eluting with a silica gel column (n-hexane : ethyl acetate = 5 : 1, RI detector) to create 7-ketocholesterol (**7**, 11 mg), 7*β*-hydroxycholesterol (**8**, 7 mg), 7*α*-hydroxycholesterol (**9**, 7 mg), respectively.

Next, N-4 including nucleic acids and free amino acids, 2.941 g, was subjected to reverse phase HPLC (ODS, 205 nm), eluting with a gradient of acetonitrile /water (0 : 100 → 60 : 40) to reveal 8 nucleic acids (uracil (**10**, 7 mg), deoxyguanosine (**11**, 3 mg), deoxyuridine (**12**, 3 mg), uridine (**13**, 21 mg), deoxyadenosine (**14**, 2 mg), adenosine (**15**, 13 mg), inosine (**16**, 6 mg), guanosine (**17**, 15 mg) and 8 free amino acids (L-phenylalanine (**18**, 15 mg), L-isoleucine

(**19**, 3 mg), L-leucine (**20**, 9 mg), L-tyrosine (**21**, 9 mg), L-valine (**22**, 5 mg), L-proline (**23**, 11 mg), L-threonine (**24**, 8 mg), L-alanine (**25**, 25 mg), respectively. Also, the water extract (U&T 1.0 g) was subjected to reverse phase HPLC (ODS, 205 nm), eluting with a 0.1% trifluoroacetic acid to reveal L-hydroxyproline (**26**, 13 mg).

Measurments of the CD spectropolarimeter – The free amino acids **18**~**26** were measured for the CD spectrum of each amino acids at 210nm in 0.5N aqueous hydrochloric acid.[13]

Amino acids autoanalysis – The water extract of the velvet antler was performed using an amino acids autoanalyzer which included 17 kinds of amino acids: phenylalanine, isoleucine, leucine, tyrosine, valine, proline, threonine, alanine, cystine, asparagine, serine, glutamine, glycine, histidine, arginine, methionine, lysine, and cystine.

Sialic acids analysis – 1) The 70% (v/v) ethanol extract of the velvet antler (100 mg) was treated with 50 mM hydrochloric acid 35 mL at 80 ºC, for 3 hours. The hydrolysate which included (**27 and 28**), was then analyzed using a HPAEC-PAD analysis. 2) The same hydrolysate as above, was performed on fluorescence derivatives of (**27**) by using a DMB reagent, then analyzed using a HPLC-FLD analysis.

Elemental Analysis – The water extract of the velvet antler including several minerals was analyzed using an ICP which revealed by 25 kinds of minerals: Na, K, Ca, Mg, Fe, Zn, B, Al, Cu, Pb, Cd, As, Hg, Be, Cr, Mn, Co, Ni, ga, Se, In, Bi, U, Ba, and Li.

Cell Proliferation Assays – All cancer cell lines (K562, MEG-01, KG-1, MOLT-4, L1210, P388D1, A549, HepG2, MCF-7, SK-OV-3, and SW-620) were maintained in RPMI 1640 which included 10% FBS and 1% penicillin-streptomycin. Cells were cultured at 37 ºC in a 5% CO_2 incubator. Each cell line was plated in 96-well plates at 1×10^4 cells/well. Cells were incubated with serial dilutions of each reagent for 48 hours. Cell proliferation was measured using the WST-1 assay with the Premix WST-1 cell proliferation assay system. And IC_{50} values were calculated using CalcuSyn software (Biosoft, Cambridge, UK).[6]

Cholesteryl 3*β*-*O*-palmitate (1 - 1) – Yellow oil; FAB-MS, *m/z* 369 [cholesterol – H_2O + H]$^+$, 255 [$C_{15}H_{31}CO_2$.]$^+$, 239 [$C_{15}H_{31}CO$]$^+$. This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,25]

Cholesteryl 3*β*-*O*-stearate (1 - 2) – Yellow oil; FAB-MS, *m/z* 369 [cholesterol – H_2O + H]$^+$, 283 [$C_{17}H_{35}CO_2$]$^+$ This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,25]

Cholesteryl 3*β*-*O*-myristate (1 - 3) Yellow oil; FAB-MS, *m/z* 369 [cholesterol – H_2O + H]$^+$, 227 [$C_{13}H_{27}CO_2$]$^+$. This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,25]

7-Ketocholesteryl 3*β*-*O*-palmitate (2 - 1) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.70 (1H, d, *J* = 1.0 Hz, H-6), 4.73 (1H, m, H-3), 2.44 (1H, m, H-4), 2.30 (2H, m, H-15), 2.25 (1H, m, H-8), 0.68 (3H, s, 18-CH_3), 0.92 (3H, d, *J* = 6.5 Hz, 21-CH_3), 0.87 (3H, d, *J* = 6.5 Hz, 26-CH_3), 0.86 (3H, d, *J* = 6.5 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; LC-MSMS (ES^-), *m/z* 381 [7-ketocholesterol – H_2O – H]$^-$, 255 [$C_{15}H_{31}CO_2$]$^-$, 239 [$C_{15}H_{31}CO$]$^-$.

7-Ketocholesteryl 3*β*-*O*-stearate (2 - 2) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.70 (1H, d, *J* = 1.0 Hz, H-6), 4.73 (1H, m, H-3), 2.44 (1H, m, H-4), 2.30 (2H, m, H-15), 2.25 (1H, m, H-8), 0.68 (3H, s, 18-CH_3), 0.92 (3H, d, *J* = 6.5 Hz, 21-CH_3), 0.87 (3H, d, *J* = 6.5 Hz, 26-CH_3), 0.86 (3H, d, *J* = 6.5 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; LC-MSMS (ES^-), *m/z* 381 [7-ketocholesterol – H_2O – H]$^-$, 283 [$C_{17}H_{35}CO_2$,]$^-$.

7-Ketocholesteryl 3*β*-*O*-myristate (2 - 3) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.70 (1H, d, *J* = 1.0 Hz, H-6), 4.73 (1H, m, H-3), 2.44 (1H, m, H-4), 2.30 (2H, m, H-15), 2.25 (1H, m, H-8), 0.68 (3H, s, 18-CH_3), 0.92 (3H, d, *J* = 6.5 Hz, 21-CH_3), 0.87 (3H, d, *J* = 6.5 Hz, 26-CH_3), 0.86 (3H, d, *J* = 6.5 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; LC-MSMS (ES^-), *m/z* 381 [7-ketocholesterol – H_2O – H]$^-$, 227 [$C_{13}H_{27}CO_2$]$^-$.

7*β*-Hydroxycholesteryl 3*β*-*O*-stearate (3 - 1) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.31 (1H, br s, H-6), 3.85 (1H, d, *J* = 7.5 Hz, H-7), 4.67 (1H, m, H-3), 0.68 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 0.92 (3H, d, *J* = 7.0 Hz, 21-CH_3), 0.87 (3H, d, *J* = 7.0 Hz, 26-CH_3), 0.86 (3H, d, *J* = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; FAB-MS, *m/z* 651 [M-H2O]$^+$, 403 [7*β*-hydroxycholesterol + H]$^+$, 384 [7*β*-hydroxycholesterol – H_2O]$^+$, 367 [7*β*-hydroxycholesterol – 2H_2O + H]$^+$, 283 [$C_{17}H_{35}CO_2$,]$^+$.

7*β*-Hydroxycholesteryl 3*β*-*O*-palmitate (3 - 2) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.31 (1H, br s, H-6), 3.85 (1H, d, *J* = 7.6 Hz, H-7), 4.67 (1H, m, H-3), 0.68 (3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 0.92 (3H, d, *J* = 7.0 Hz, 21-CH_3), 0.87 (3H, d, *J* = 7.0 Hz, 26-CH_3), 0.86 (3H, d, *J* = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; LC-MSMS (ES^-), *m/z* 383 [7*β*-hydroxycholesterol – H_2O – H]$^-$, 255 [$C_{15}H_{31}CO_2$]$^-$, 239 [$C_{15}H_{31}CO$]$^-$.

7*β*-Hydroxycholesteryl 3*β*-*O*-myristate (3 - 3) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.31 (1H, br s, H-6), 3.85 (1H, d, *J* = 7.6 Hz, H-7), 4.67 (1H, m, H-3), 0.68

Table 1. ^{13}C NMR (100 MHz, $CDCl_3$, δ) data of oxycholesterol-3β-O-fatty acid esters: (**2 - 1**, **2 - 2**, **2 - 3**, **3 - 1**, **3 - 2**, **3 - 3**, **4 - 1**, **4 - 2**, and **4 - 3**)

No.	**2 - 1**	**2 - 2**	**2 - 3**	**3 - 1**	**3 - 2**	**3 - 3**	**4 - 1**	**4 - 2**	**4 - 3**
1	36.0	36.0	36.0	36.7	36.7	36.7	36.2	36.2	36.2
2	27.4	27.4	27.4	27.8	27.8	27.8	27.6	27.6	27.6
3	71.9	71.9	71.9	73.2	73.2	73.2	73.1	73.1	73.1
4	37.8	37.8	37.8	37.6	37.6	37.6	38.0	38.0	38.0
5	164.0	164.0	164.0	142.5	142.5	142.5	145.3	145.3	145.3
6	126.7	126.7	126.7	126.2	126.2	126.2	124.7	124.7	124.7
7	202.1	202.1	202.1	73.7	73.7	73.7	65.4	65.4	65.4
8	45.4	45.4	45.4	40.8	40.8	40.8	37.5	37.5	37.5
9	50.0	50.0	50.0	48.2	48.2	48.2	42.2	42.2	42.2
10	38.3	38.3	38.3	36.5	36.5	36.5	37.5	37.5	37.5
11	21.2	21.2	21.2	21.0	21.0	21.0	20.7	20.7	20.7
12	38.7	38.7	38.7	39.5	39.5	39.5	39.1	39.1	39.1
13	43.1	43.1	43.1	42.9	42.9	42.9	42.1	42.1	42.1
14	49.8	49.8	49.8	55.9	55.9	55.9	49.4	49.4	49.4
15	26.3	26.3	26.3	26.4	26.4	26.4	24.3	24.3	24.3
16	28.6	28.6	28.6	28.6	28.6	28.6	28.3	28.3	28.3
17	54.8	54.8	54.8	55.4	55.4	55.4	55.8	55.8	55.8
18	12.0	12.0	12.0	11.8	11.8	11.8	11.6	11.6	11.6
19	17.3	17.3	17.3	19.2	19.2	19.2	18.2	18.2	18.2
20	35.7	35.7	35.7	35.8	35.8	35.8	35.8	35.8	35.8
21	18.9	18.9	18.9	18.8	18.8	18.8	18.7	18.7	18.7
22	36.2	36.2	36.2	36.2	36.2	36.2	36.2	36.2	36.2
23	23.8	23.8	23.8	23.8	23.8	23.8	23.7	23.7	23.7
24	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5	39.5
25	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0	28.0
26	22.6	22.6	22.6	22.8	22.8	22.8	22.8	22.8	22.8
27	22.8	22.8	22.8	22.6	22.6	22.6	22.6	22.6	22.6
1'	173.2	173.3	172.6	173.3	173.1	172.5	172.7	173.3	173.2
2'	34.4	34.7	34.0	34.7	34.4	34.0	34.7	34.4	34.0
3'	24.9	29.9	25.2	30.0	24.9	25.2	30.0	24.9	25.2
4'	29.9	29.9	29.1	30.0	29.9	29.1	30.0	29.9	29.1
5'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
6'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
7'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
8'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
9'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
10'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
11'	29.9	29.9	29.9	30.0	29.9	29.9	30.0	29.9	29.9
12'	29.9	29.9	31.9	30.0	29.9	31.9	30.0	29.9	31.9
13'	29.9	29.9	29.1	30.0	29.9	29.1	30.0	29.9	29.1
14'	31.9	24.9	14.1	30.0	31.9	14.1	30.0	31.9	14.1
15'	22.9	29.9		30.0	22.7		30.0	22.7	
16'	14.2	31.9		32.0	14.2		32.0	14.2	
17'		22.7		22.7			22.7		
18'		14.1		14.1			14.1		

(3H, s, 18-CH_3), 1.06 (3H, s, 19-CH_3), 0.92 (3H, d, J = 7.0 Hz, 21-CH_3), 0.87 (3H, d, J = 7.0 Hz, 26-CH_3), 0.86 (3H, d, J = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; LC-MSMS (ES^-), *m/z* 383 [7*β*-hydroxycholesterol – H_2O – H]$^-$, 227 [$C_{13}H_{27}CO_2$]$^-$.

7*α*-Hydroxycholesteryl 3*β*-*O*-stearate (4 - 1) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.65 (1H, br s, H-6), 3.87 (1H, br s, H-7), 4.68 (1H, m, H-3), 0.70 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 0.93 (3H, d, J = 7.0 Hz, 21-CH_3), 0.87 (3H, d, J = 7.0 Hz, 26-CH_3), 0.86 (3H, d, J = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; FAB-MS, *m/z* 401 [7*α*-hydroxycholesterol – H]$^+$, 369 [cholesterol – H_2O + H]$^+$, 283 [$C_{17}H_{35}CO_2$]$^+$.

7*α*-Hydroxycholesteryl 3*β*-*O*-palmitate (4 - 2) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.65 (1H, br.s, H-6), 3.87 (1H, br s, H-7), 4.68 (1H, m, H-3), 0.70 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 0.93 (3H, d, J = 7.0 Hz, 21-CH_3), 0.87 (3H, d, J = 7.0 Hz, 26-CH_3), 0.86 (3H, d, J = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; FAB-MS, *m/z* 367 [7*α*-hydroxycholesterol-$2H_2O$ + H]$^+$, 255 [$C_{15}H_{31}CO_2$.]$^+$, 239 [$C_{15}H_{31}CO$]$^+$.

7*α*-Hydroxycholesteryl 3*β*-*O*-myristate (4 - 3) – Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.65 (1H, br. s, H-6), 3.87 (1H, br s, H-7), 4.68 (1H, m, H-3), 0.70 (3H, s, 18-CH_3), 1.03 (3H, s, 19-CH_3), 0.93 (3H, d, J = 7.0 Hz, 21-CH_3), 0.87 (3H, d, J = 7.0 Hz, 26-CH_3), 0.86 (3H, d, J = 7.0 Hz, 27-CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: Table 1; FAB-MS, *m/z* 401 [7*α*-hydroxycholesterol – H]$^+$, 367 [7*α*-hydroxycholesterol – $2H_2O$ + H]$^+$, 227 [$C_{13}H_{27}CO_2$]$^-$.

Dinonyl phthalate (5) – Yellow oil; FAB-MS, *m/z* 419 [M + H]$^+$, 293 [M – C_9H_{19} + H]$^+$ + H$^+$, 275 [M – $C_9H_{19}O_1$]$^+$ This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[17]

Cholesterol (6) – Amorphous white powder; FAB-MS, *m/z* 386 [M]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[2]

7-Ketocholesterol (7) – Amorphous white powder; FAB-MS, *m/z* 401[M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,24,25]

7*β*-Hydroxycholesterol (8) – Amorphous white powder; FAB-MS, *m/z* 402 [M]$^+$, 401 [M – H]$^+$, 367 [M –$2H_2O$ + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,25]

7*α*-Hydroxycholesterol (9) – Amorphous white powder; FAB-MS, *m/z* 367 [M – $2H_2O$ + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[10,25]

Uracil (10) – Amorphous white powder; FAB-MS, *m/z* 113 [M+H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[9,16,25]

Deoxyguanosine (11) – Amorphous white powder; FAB-MS, *m/z* 268 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[19]

Deoxyuridine (12) – Amorphous white powder; FAB-MS, *m/z* 229 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[1]

Uridine (13) – Amorphous white powder; FAB-MS, *m/z* 245 [M + H]$^+$, 185, 149, 113, 93, 75; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[4,17]

Deoxyadenosine (14) – amorphous white powder; FAB-MS, *m/z* 252 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[7]

Adenosine (15) – Amorphous white powder; FAB-MS, *m/z* 268 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[7]

Inosine (16) – Amorphous white powder; FAB-MS, *m/z* 269 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[23]

Guanosine (17) – Amorphous white powder; FAB-MS, *m/z* 306 [M + Na]$^+$, 284 [M + H]$^+$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[8]

L-Phenylalanine (18) – Amorphous white powder; LC-MSMS, *m/z* 164 [M – H]$^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[18]

L-Isoleucine (19) – Amorphous white powder; LC-MSMS, *m/z* 130 [M – H]$^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[3]

L-Leucine (20) – Amorphous white powder; LC-MSMS, *m/z* 130 [M – H]$^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[18]

L-Tyrosine (21) – Amorphous white powder; LC-MSMS, *m/z* 180 [M – H]$^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[18]

L-Valine (22) – Amorphous white powder; LC-MSMS, *m/z* 116 [M – H]$^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[18]

L-Proline (23) – Amorphous white powder; LC-MSMS, *m/z* 114 [M – H]$^-$; This compound exhibited

comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values values.[18]

L-Threonine (24) – Amorphous white powder; LC-MSMS, *m/z* 118 $[M-H]^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values values.[18]

L-Alanine (25) – Amorphous white powder; LC-MSMS, *m/z* 88 $[M-H]^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values values.[18]

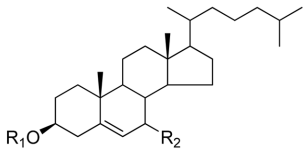
L-Hydroxyproline (**26**) – Amorphous white powder; LC-MSMS, *m/z* 130 $[M-H]^-$; This compound exhibited comparable spectroscopic data (^{1}H and ^{13}C-NMR) to published values.[21]

Results and Discussion

The 16 steroid analogues (**1 - 1**, **1 - 2**, **1 - 3**, **2 - 1**, **2 - 2**, **2 - 3**, **3 - 1**, **3 - 2**, **3 - 3**, **4 - 1**, **4 - 2**, **4 - 3**, **6**, **7**, **8**, **and 9**) together were isolated from the New Zealand deer velvet antler *Cervus elaphus var. scoticus* (Fig. 2). Four of them (**6**, **7**, **8**, and **9**) were cholesterol and its analogues. The ^{1}H-NMR spectrum of (**6~9**) showed the signals assignable to two tertiary methyls (18, 19-CH_3), three secondary methyls (21, 26, 27-CH_3), one hydroxyl (H-3), and an olefinic proton (H-6). This was due to the sterol part were closely similar to those of cholesterol and its derivatives, respectively. Based on the evidence, compound (**6~9**) were determined as cholesterol as shown in Fig. 2. These structures were elucidated through comparison of the FAB-MS (LC-MSMS), ^{1}H- and ^{13}C-NMR data and being compared with related data from the cited literatures[(**6**),[2] (**7**),[10,24,25] (**8**),[10,24,25] (**9**),[10,25]] respectively. The compounds (**7** and **8**) showed cytotoxic activity.[12,15] 12 compounds (**1 - 1**, **1 - 2**, **1 - 3**, **2 - 1**, **2 - 2**, **2 - 3**, **3 - 1**, **3 - 2**, **3 - 3**, **4 - 1**, **4 - 2**, and **4 - 3**) were identified as steroid analogues of 3β-O-fatty acid esters.

Those 3 compounds (**1 - 1**, **1 - 2**, and **1 - 3**) were identified as cholesteryl 3β-O-fatty acid esters, isolated from the deer velvet antler *C. nippon var. mantchuricus,*[10] *C. elaphus*.[25]

The ^{1}H-NMR spectrums of (**2 - 1**, **2 - 2**, and **2 - 3**) all showed the signals due to the 7-ketocholesteryl moiety, the ester linkages branching at the 3β-hydroxy moiety in (**2 - 1~2 - 3**) were further confirmed by the ^{1}H NMR signals due to the 3α-H of 7-ketocholesterol residue. They were observed at δ: 4.73 (1H, m, H-3) in (**2 - 1**), 4.73 (1H, m, H-3) in (**2 - 2**), 4.73 (1H, m, H-3) in (**2 - 3**); whereas the signals due to the 3α-H of 7-ketocholesterol residue (**7**) were δ: 3.60 (1H. m, H-3). The ^{13}C-NMR



Compounds	R_1	R_2	
		α	β
CS-3β-O-PM (1-1)	$-COC_{15}H_{31}$	-H	-H
CS-3β-O-SA (1-2)	$-COC_{17}H_{35}$	-H	-H
CS-3β-O-MR (1-3)	$-COC_{13}H_{27}$	-H	-H
7-Keto-CS-3β-O-PM (2-1)	$-COC_{15}H_{31}$	=O	
7-Keto-CS-3β-O-SA (2-2)	$-COC_{17}H_{35}$	=O	
7-Keto-CS-3β-O-MR (2-3)	$-COC_{13}H_{27}$	=O	
7β-Hydroxy-CS-3β-O-SA (3-1)	$-COC_{17}H_{35}$	-H	-OH
7β-Hydroxy-CS-3β-O-PM (3-2)	$-COC_{15}H_{31}$	-H	-OH
7β-Hydroxy-CS-3β-O-MR (3-3)	$-COC_{13}H_{27}$	-H	-OH
7α-Hydroxy-CS-3β-O-SA (4-1)	$-COC_{17}H_{35}$	-OH	-H
7α-Hydroxy-CS-3β-O-PM (4-2)	$-COC_{15}H_{31}$	-OH	-H
7α-Hydroxy-CS-3β-O-MR (4-3)	$-COC_{13}H_{27}$	-OH	-H
Cholesterol (CS: 6)	-H	-H	-H
7-Keto-CS (7)	-H	=O	
7β-Hydroxy-CS (8)	-H	-H	-OH
7α-Hydroxy-CS (9)	-H	-OH	-H

Fig. 2. Structures of cholesterol (CS), oxycholesterols and oxycholesterol-3β-O-fatty acid esters: (**1 - 1**, **1 - 2**, **1 - 3**, **2 - 1**, **2 - 2**, **2 - 3**, **3 - 1**, **3 - 2**, **3 - 3**, **4 - 1**, **4 - 2**, and **4 - 3**).

signals due to the 7-ketocholesterol part were very similar to those of the 7-ketocholesteryl 3β-*O*-palmitate (**2 - 1**), 7-ketocholesteryl 3β-*O*-stearate (**2-2**), and 7-ketocholesteryl 3β-*O*-myristate (**2 - 3**), except for one more fresh lactone moiety carbon (δ: 173.2, 173.3, 172.6), respectively. Based on this evidence, (**2 - 1~2 - 3**) were determined as 7-ketocholesteryl 3β-*O*-palmitate (**2 - 1**), 7-ketocholesteryl 3β-*O*-stearate (**2 -2**), and 7-ketocholesteryl 3β-*O*-myristate (**2 - 3**). The fatty acid residues of the compound (**2 - 1~2 - 3**) were determined as 255 $[C_{15}H_{31}CO_2.]^+$, 283 $[C_{17}H_{35}CO_2]^+$, 227 $[C_{13}H_{27}CO_2]^+$ by FAB-MS analysis, respectively. The compounds (**2 - 1~2 - 3**) were isolated from the velvet antler for the first time.

The ^{1}H-NMR spectrums of (**3 - 1**, **3 - 2**, and **3 - 3**) all showed the signals due to those of 7β-hydroxycholesterol, similar to the above, the ester linkages branching at 3β-hydroxy moiety in the compound (**3 - 1~3 - 3**) were further confirmed by the ^{1}H NMR signals due to the 3α-H of 7β-hydroxycholesterol residues. They were observed at δ: 4.67 (1H, m, H-3) in (**3 - 1**), 4.67 (1H, m, H-3) in (**3 - 2**), 4.67 (1H, m, H-3) in (**3 - 3**); whereas the signals due to the 3α-H of 3β-hydroxyl residues (**8**) were δ: 3.59 (1H. m, H-3). The ^{13}C-NMR signals due to the 7β-hydroxycholesterol part were very similar to those of 7β-

Table 2. The anti-proliferative effects of oxycholesterol analogues *in vitro*, respectively: (**7**, **8**, **9**, **2 - 1**, **3 - 1**, and **4 - 1**)

Cell lines	IC_{50} (μM)						
	7-Keto-CS	**7**	**8**	**9**	**2 - 1**	**3 - 1**	**4 - 1**
K562	60.03	126.92	73.08	97.88	> 50.00	> 500.00	> 250.00
MEG-01	65.73	106.42	65.27	80.08	> 50.00	377.62	> 250.00
KG-1	30.52	84.19	24.13	83.55	> 50.00	442.05	> 250.00
MOLT-4	37.85	57.45	22.35	65.12	> 50.00	131.31	> 250.00
L1210	4.24	0.06	2.16	2.42	> 50.00	1.66	8.31
P388D1	6.68	24.05	9.44	5.22	0.25	9.48	49.77
A549	26.33	59.41	37.2	81.38	> 50.00	> 500.00	> 250.00
HepG2	74.27	162.8	60.8	> 500.00	> 50.00	> 500.00	> 250.00
MCF-7	200.69	381.65	217.57	> 500.00	> 50.00	> 500.00	> 250.00
SK-OV-3	37.05	148.32	67.81	309.49	> 50.00	> 500.00	> 250.00
SW-620	81.38	289.48	92.24	422.72	> 50.00	> 500.00	> 250.00

hydroxycholesteryl 3*β*-*O*-stearate (**3 - 1**), 7*β*-hydroxycholesteryl 3*β*-*O*-stearate palmitate (**3 - 2**), and 7*β*-hydroxycholesteryl 3*β*-*O*-myristate (**3 - 3**), except for one more fresh lactone group carbon (δ: 173.3, 173.1, 172.5), respectively. Based on the evidence, (**3 - 1~3 - 3**) were determined as 7*β*-hydroxycholesteryl 3*β*-*O*-stearate (**3 - 1**), 7*β*-hydroxycholesteryl 3*β*-*O*-palmitate (**3 - 2**), and 7*β*-hydroxycholesteryl 3*β*-*O*-myristate (**3 - 3**). The fatty acid residues of the (**3 - 1~3 - 3**) were determined as 283 $[C_{17}H_{35}CO_2]^+$, 255 $[C_{15}H_{31}CO_2.]^+$, 227 $[C_{13}H_{27}CO_2]^+$ by FAB-MS analysis, respectively. The compounds (**3 - 1~3 - 3**) were isolated from the velvet antler for the first time.

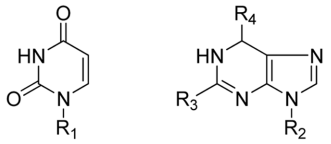
The ^{1}H-NMR spectrums of (**4 - 1**, **4 - 2**, and **4 - 3**) all showed the signals due to those of 7*α*-hydroxycholesterol. Similar to the above, the ester linkages branching at the 3*β*-hydroxy moiety in (**4 - 1~4 - 3**) were further confirmed by the ^{1}H NMR signals due to the 3*α*-H of 7*α*-hydroxycholesterol residues. They were observed at δ: 4.68 (1H, m, H-3) in (**4 - 1**), 4.68 (1H, m, H-3) in (**4 - 2**), 4.68 (1H, m, H-3) in (**4 - 3**); whereas the signals due to the 3*α*-H of 7*α*-hydroxycholesterol residues (**9**) was δ: 3.59 (1H. m, H-3). The ^{13}C-NMR signals due to the 7*α*-hydroxycholesterol part were very similar to those of 7*α*-hydroxycholesteryl 3*β*-*O*-palmitate (**4 - 1**), 7*α*-hydroxycholesteryl 3*β*-*O*-stearate (**4 - 2**), and 7*α*-hydroxycholesteryl 3*β*-*O*-myristate (**4 - 3**), except for one more fresh lactone group carbon (δ: 172.7, 173.3, 173.2), respectively. Based on these evidences, (**4 - 1~4 - 3**) were determined as 7*α*-hydroxycholesteryl 3*β*-*O*-stearate (**4-1**), 7*α*-hydroxycholesteryl 3*β*-*O*-palmitate (**4-2**), 7*α*-hydroxycholesteryl 3*β*-*O*-myristate (**4 - 3**). The fatty acid residues of the (**4 - 1~4 - 3**) were determined as 283 $[C_{17}H_{35}CO_2]^+$, 255 $[C_{15}H_{31}CO_2.]^+$, and 227 $[C_{13}H_{27}CO_2]^+$ by FAB-MS analysis, respectively. The compounds (**4 - 1~4 - 3**) were isolated from antler velvet for the first time.

The 6 kind steroid analogues (**7**, **8**, **9**, **2 - 1**, **3 - 1**, and **4 - 1**) exhibited moderate anti-proliferative effects towards for K562, MEG-01, KG-1, MOLT-4, L1210, P388D1, A549, HepG2, MCF-7, SK-OV-3, and SW-620 *in vitro*, respectively (Table 2).[6]

The molecular formula of the compound, dinonyl phthalate (**5**) was determined as $C_{26}H_{42}O_4$ by FAB-MS analysis. The ^{1}H-NMR spectrum of **5** showed the signals assignable to the two nonyl moiety (δ: 4.21, 4H, m, H-1'; 1.8~1.1, 32H, m, H-2', 3', 4', 5', 6', 7', 8'; 0.89, 6H, m, 9'-CH_3) and the The ^{1}H and ^{13}C-NMR signals due to the nonyl alcohol part and phthalic acid residues were closely similar to those of the phthalic acid ester of nonyl alcohol residues, respectively. Based on the evidence, compound 5 was determined as dinonyl phthalate (**5**).[17]

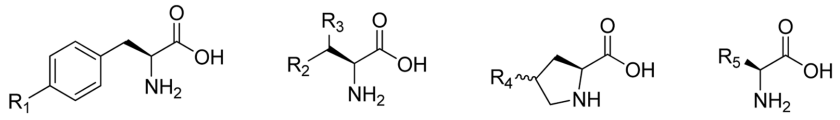
The 8 compounds (**10**, **11**, **12**, **13**, **14**, **15**, **16**, and **17**) were identified as nucleic acid analogues on the basis of spectroscopic methods and the comparisons with literature (Fig. 3). The compound **10** was determined as uracil (**10**),[9,16,25] 2'-deoxyguanosine (**11**),[5,19] 1-*β*-D-deoxyribofuranosyluracil (**12**),[1] uridine (**13**),[17] 2'-deoxyadenosine (**14**),[5,7] adenosine (**15**),[5,7] inosine (**16**),[23] guanosine (**17**),[5,8,9] respectively. Based on these evidence of above sources, 2'-deoxyguanosine (**11**), 1-*β*-D-deoxyribofuranosyluracil (**12**), and 2'-deoxyadenosine (**14**) were isolated from antler velvet for the first time.

The 9 compounds (**18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, and **26**) were identified as free amino acids on the basis of spectroscopic methods and the comparisons with the literature (Fig. 4). The compound **18** was determined as phenylalanine (**18**),[18] isoleucine (**19**),[3] leucine (**20**),[18] tyrosine (**21**),[18] leucine (**22**),[18] proline (**23**),[18] threonine (**24**),[18] alanine (**25**),[18] and hydroxyproline (**26**),[21] respectively.



Compounds	R_1	R_2	R_3	R_4
Uracil (**10**)	-H			
deoxyguanosine (**11**)		2'-deoxyribosyl	$-NH_2$	=O
Deoxyuridine (**12**)	2'-deoxyribosyl			
Uridine (**13**)	ribosyl			
Deoxyadenosine (**14**)		2'-deoxyribosyl	-H	$-NH_2$
Adenosine (**15**)		ribosyl	-H	$-NH_2$
Inosine (**16**)		ribosyl	-H	=O
Guanosine (**17**)		ribosyl	$-NH_2$	=O

Fig. 3. Structures of nucleic acid analogues: (**10**, **11**, **12**, **13**, **14**, **15**, **16**, and **17**).



Compounds	R_1	R_2	R_3	R_4	R_5
L-phenylalanine (**18**)	-H				
L-isoleucine (**19**)		$-CH_2CH_3$	$-CH_3$		
L-leucine (**20**)		$-CH(CH_3)_2$	**-** H_2		
L-tyrosine (**21**)	-OH				
L-valine (**22**)		- CH_3	$-CH_3$		
L-proline (**23**)				- H	
L-threonine (**24**)					- $CH(OH)CH_3$
L-alanine (**25**)					$-CH_3$
L-hydroxyproline (**26**)				-OH	

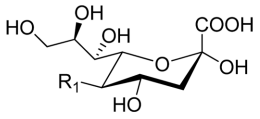
Fig. 4. Structures of free amino acids: (**18**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, and **26**).

All free amino acids of compound **18**~**26** were determined as all L-types by exihiting positive cotton effects by measuring the CD spectrum at 210 nm.[13] Also, the 8 kinds of free amino acids were also detected as asparagine (12.98 mg/g), serine (12.59 mg/g), glutamine (34.29 mg/g), glycine (1.73 mg/g), histidine (65.63 mg/g), arginine (4.98 mg/g), methionine (1.95 mg/g), and lysine (14.93 mg/g) from using an amino acids autoanalyzer.

Sialic acids (NANA, **27**: 6.25 mg/g, KDN, **28**: 5.93 mg/g) from the 70% (v/v) ethanol extract including the velvet antler of the gangliosides were detected using an HPAEC-PAD quantitatively and DMB derivative of (NANA, **27**): 2.052, 2.458, 3.258, and 3.853 mg/g from the water extract of being extracted at 75 °C, 85 °C, 95 °C and 121 °C, were then analyzed using an HPLC/FLD, quantitatively (Fig. 5).[14]

The water extract of the velvet antler included several minerals detected (mg/Kg); Na (58,531.19) > K (40,444.06) > Ca (670.76) > Mg (547.19) > Fe (64.26) > Zn (13.15) > B (11.83) > Al (10.69) > Cu (6.55); the following were not detected using the ICP: Pb, Cd, As, Hg, Be, Cr, Mn, Co, Ni, ga, Se, In, Bi, U, Ba, and Li.

In conclusion, in the present study, forty-four compounds and nine minerals were isolated and detected; whereas there were sixteen heavy metals not detected in the 2012 year New Zealand deer velvet antler *Cervus*



Compounds	R_1
N-acetylneuraminic acid (**27**)	-*N*-acetyl
ketodeoxynonulosonic acid (**28**)	-OH

Fig. 5. Structures of sialic acids: (**27** and **28**).

elaphus var. scoticus. The 9 steroid analogues (**2 - 1**, **2 - 2**, **2 - 3**, **3 - 1**, **3 - 2**, **3 - 3**, **4 - 1**, **4 - 2**, and **4 - 3**) and 3 nucleic acid analogues (**12**, **14**, and **15**) were isolated from the antler velvet for the first time. Also, 6 kinds of steroid analogues (**7**, **8**, **9**, **2 - 1**, **3 - 1**, and **4 - 1**) were examined for their anti-proliferative effects against L1210, P388D1, K562, MEG-01, KG-1, MOLT-4, A549, HepG2, MCF-7, SK-OV-3, and SW-620 cancer cell lines. They showed anti-proliferative effects with IC_{50} values of 0.06, 2.16, 2.42, > 50.0, 1.66 and 8.31 μM against L1210, while the values were 24.05, 9.44, 5.22, 0.25. 9.48 and 49.77 μM against P388D1, respectively. The others were mostly inactive.

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