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Lanostane Triterpenoids from *Ganoderma tropicum* Collected in Vietnam and Their Nitroblue Tetrazolium Reductive Activity *In Vitro*

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Abstract – A new compound, 3*β*-acetoxylanosta-7,9(11),24-triene-26-al (**3**), and seven known compounds (**1** – **2** and **4** – **8**) were isolated from *Ganoderma tropicum* (Jung.) Bres. collected in Tay Nguyen, Vietnam. The structures of these compounds were determined by one- and two-dimensional nuclear magnetic resonance spectroscopy, electrospray ionization mass spectrometry (ESI-MS), and high-resolution ESI-MS, and by comparison with literature data. All of the isolated compounds were tested for nitroblue tetrazolium (NBT) reduction activity in *Saccharomyces cerevisiae*-stimulated RAW 246.7 cells. Among them, compounds **2** – **4** and **6** – **8** enhanced the NBT reduction in a dose-dependent manner.
Keywords – *Ganoderma tropicum*, 3*β*-acetoxylanosta-7,9(11),24-triene-26-al, nitroblue tetrazolium (NBT) reduction assay

Introduction

Ganoderma tropicum belongs to the family Ganodermataceae. However, despite being used as a medicine in China and some regions of Vietnam, it is one of the least studied species of the genus. Early studies using thin-layer chromatography (TLC) to screen the chemical contents of *G. tropicum* and *G. lucidum* revealed some similarities in terms of triterpenoids and polysaccharides.[1] This suggests that chemical compounds isolated from *G. tropicum* may have potent pharmacological activities like those of *G. lucidum*, which has already been established as a valuable medicinal fungus. Lanostane triterpenes,[2-6] ergosterols,[2] and xanthones[2] have been reported as chemical constituents of *G. tropicum.* Certain lanostane triterpenes do not display significant cytotoxic activity or acetylcholinesterase inhibition.[2,5]

In this work, nitroblue tetrazolium (NBT) reduction assay on macrophages *in vitro* was used to screen our isolated compounds for primary immunomodulatory activity. This reagent reacts with reducing agents inside the cell during an oxidative burst event due to various stimuli, such as lipopolysaccharides or inactivated bacteria (the heat-inactivated yeast as *Saccharomyces cerevisiae*),[7] and is a colorimetric indicator of the reaction. An NBT reduction test was conducted to verify the enhancement or suppression effect of the isolated compounds on the phagocytic activity of macrophages *in vitro*. We isolated eight compounds from *G. tropicum*, including a new compound **3** and seven known ones (**1** – **2** and **4** – **8**). Six of the isolated compounds (**2** – **5**, **7**, and **8**) were evaluated for their ability to enhance NBT reduction in *Saccharomyces cerevisiae*-stimulated RAW 246.7 cells.

Experimental

General experimental procedures – Nuclear magnetic resonance (NMR) (^{1}H: 500 MHz; ^{13}C: 125 MHz) experiments were performed on a Bruker Advance 500 spectrometer (Bruker, Billerica, MA, USA). The chemical shift is reported as ppm downfield from tetramethylsilane (TMS), with coupling constants (*J*) in Hz. Mass spectra were obtained using an AGILENT 1200 series LC/MSD ion trap (Agilent Technologies, Palo Alto, CA, USA). Analytical TLC was performed on Kieselgel 60 F_{254} and RP-18 F_{254} TLC plates (silica gel, 0.25-mm layer thickness;

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Merck, Darmstadt, Germany). Spots were visualized using ultraviolet irradiation (254 – 366 nm), and by spraying with 10% H_2SO_4 followed by heating with a heat gun. Column chromatography (CC) was performed on silica gel (70 – 230; 230 – 400 mesh; Merck) and reversed-phase (RP) C_{18} resin (230 – 400 mesh; Merck) columns. The experiments involved RAW 264.7 macrophage cell line (provided by Prof. D.V. Delfino of Perugia University, Italy), inactivated *S. cerevisiae*, fetal bovine serum (FBS) and RPMI medium (GIBCO), other standard chemicals for bio-assays [ethanol (EtOH), potassium hydroxide (KOH), and dimethyl sulfoxide (DMSO)], 96-well plates (Corning Inc., Corning, NY, USA), and an ELISA microplate reader (BioTek, Winooski, VT, USA).

Mushroom materials – The fruiting bodies of *G. tropicum* were collected in Tay Nguyen, Vietnam in December 2018 and identified by professor Nguyen Phuong Dai Nguyen of Tay Nguyen University. A voucher specimen (number DL02) was deposited.

Extraction and isolation – The dried fruiting bodies of *G. tropicum* (4.2 kg) were extracted three times with 80% EtOH at 60 ºC. After solvent evaporation under reduced pressure, 255 g of total extract was collected (yield: 6%), dissolved in distilled water, and extracted to provide extracts of dichloromethane (DGT, 33.3 g), ethyl acetate (EGT, 58.7 g), and water-soluble residue (163.0 g). The DGT (30 g) was purified by silica gel CC using a gradient elution solvent system of *n*-hexane:ethyl acetate:methanol (*n*-hexane:EtOAc:MeOH; 100:1:1 to 5:1:1 and 0:2:1, v/v/v) to yield five fractions (D1 – D7). Fraction D1 (120 mg) was separated by RP-C_{18} CC through isocratic elution with acetone-water (20:1, v/v) to provide three fractions (D1.1 – D1.3). Compound **1** (15 mg) was obtained by filter-washing D1.2 (35 mg) with MeOH. Fraction D2 (250 mg) was subjected to silica gel CC, and elution with *n*-hexane:acetone (10:1, v/v) for fractionation into two subfractions (D2.1 – D2.2). Fraction D2.1 (35 mg), purified by filter-washing with *n*-hexane, yielded compound **2** (10 mg). Compound **3** (6 mg) was purified from fraction D2.2 (65 mg) by RP-C_{18} CC by eluting with acetone:water (3:1, v/v). Extract EGT (50 g) was isolated by silica gel CC by eluting with a stepwise gradient of dichloromethane:methanol (DCM:MeOH; 100:1 to 1:1, v/v) to yield six fractions (E1 – E6). Compound **4** (12 mg) was isolated from fraction E1 (189 mg) by silica gel CC, through elution with DCM:MeOH (20:1, v/v). Fraction E2 (120 mg) was continuously separated on an RP-C_{18} column by isocratic elution with MeOH:H_2O (5:1, v/v), to provide three fractions (E2.1 – E2.3). Compounds **5** (7 mg) and **6** (9 mg) were isolated from fraction E2.3 (131 mg) by silica gel CC, eluting with DCM:MeOH (15:1, v/v). Fraction E3 (5.56 g) was separated by silica gel CC and eluted using a gradient of DCM:MeOH (15:1 to 1:1, v/v) to obtain compound **7** (13 mg) and four fractions (E3.1 – E3.4). Compound **8** (10 mg) was purified from fraction E3.3 (95 mg) on an RP-C_{18} column by isocratic elution with MeOH:H_2O (3:1, v/v).

3*β*-Hydroxylanosta-7,9(11),24-triene (1) – white powder; ESI-MS: *m/z* 425.5 $[M+H]^+$ (Calcd. $C_{30}H_{48}O$, M = 424.4); ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 3.25 (1H, dd, J = 4.5, 11.5 Hz, H-3), 5.47 (1H, brd, J = 6.0 Hz, H-7), 5.32 (1H, brd, J = 6.0 Hz, H-11), 0.57 (3H, s, H-18), 0.99 (3H, s, H-19), 0.91 (3H, d, J = 6.5 Hz, H-21), 5.11 (1H, t, J = 6.0 Hz, H-24), 1.69 (3H, s, H-26), 1.61 (3H, s, H-27), 1.01 (3H, s, H-29), 0.88 (6H, s, H-28, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 35.8 (C-1), 27.9 (C-2), 79.0 (C-3), 38.7 (C-4), 49.2 (C-5), 23.0 (C-6), 120.2 (C-7), 142.8 (C-8), 146.0 (C-9), 37.4 (C-10), 116.4 (C-11), 37.9 (C-12), 43.8 (C-13), 50.4 (C-14), 31.6 (C-15), 27.9 (C-16), 51.0 (C-17), 15.7 (C-18), 22.8 (C-19), 36.1 (C-20), 18.5 (C-21), 36.3 (C-22), 25.0 (C-23), 125.2 (C-24), 131.0 (C-25), 25.7 (C-26), 17.6 (C-27), 28.2 (C-28), 15.8 (C-29), 25.6 (C-30).

Methyl 2-hydroxynonacosanoate (2) – white powder; ESI-MS: *m/z* 485.4 $[M+OH]^-$ (Calcd. $C_{30}H_{60}O_3$, M = 468.5); ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 4.19 (1H, dd, J = 4.0, 7.5 Hz, H-2), 1.26-1.80 (52xH), 0.88 (3H, t, J = 14.0 Hz, H-29), 3.79 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$): δ_C 175.9 (C-1), 70.5 (C-2), 34.5-22.7 (26xCH_2), 14.1 (C-29), 52.5 (OCH_3).

3*β*-Acetoxylanosta-7,9(11),24-triene-26-al (3) – pale yellow powder; HR-ESI-MS: *m/z* 503.3517 $[M+Na]^+$ (Calcd. $C_{32}H_{48}O_3$, 480.3603); ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

Ganoderic acid Y (4) – needle crystals; ESI-MS: *m/z* 453.40 $[M-H]^-$ (Calcd. $C_{30}H_{46}O_3$, M=454.3); ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.18 (1H, dd, J = 5.0, 10.0 Hz, H-3), 5.52 (1H, brd, J = 5.5 Hz, H-7), 5.37 (1H, brd, J = 6.0 Hz, H-11), 0.62 (3H, s, H-18), 1.01 (3H, s, H-19), 0.98 (3H, d, J = 6.0 Hz, H-21), 6.80 (1H, t, J = 7.5 Hz, H-24), 1.83 (3H, s, H-27), 0.86 (3H, s, H-28), 1.00 (3H, s, H-29), 0.92 (3H, s, H-30); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 37.0 (C-1), 28.9 (C-2), 79.5 (C-3), 39.7 (C-4), 50.6 (C-5), 24.0 (C-6), 121.5 (C-7), 143.8 (C-8), 147.3 (C-9), 38.4 (C-10), 117.2 (C-11), 38.9 (C-12), 44.9 (C-13), 51.4 (C-14), 32.5 (C-15), 28.4 (C-16), 52.1 (C-17), 16.3 (C-18), 23.2 (C-19), 37.3 (C-20), 18.8 (C-21), 35.9 (C-22), 26.5 (C-23), 144.4 (C-24), 128.5 (C-25), 171.6 (C-26), 12.4 (C-27), 28.8 (C-28), 16.4 (C-29), 26.1 (C-30).

Resinacein R (5) – white power; ESI-MS *m/z*: 571.5

Table 1. NMR data of compound **3**

Positions	δ_H[a,b] (J in Hz)	δ_C[a,c], type	HMBC
1	1.24 (1H, m) 1.61 (1H, m)	34.7, CH_2	
2	1.29 (1H, m) 2.40 (1H, m)	26.1, CH_2	
3	4.51 (1H, dd, 4.5, 11.5)	80.8, CH	C-4, C-28, C-29, OAc
4	-	37.8, C	
5	1.19 (1H, m)	49.3, CH	
6	2.29 (2H, m)	22.9, CH_2	
7	5.47 (1H, br s)	120.1, CH	
8	-	142.6, C	
9	-	145.7, C	
10	-	37.3, C	
11	5.32 (1H, d, 6.0)	116.4, CH	
12	2.10 (1H, m) 2.24 (1H, m)	37.6, CH_2	
13	-	43.8, C	
14	-	50.3, C	
15	1.40 (1H, m) 1.61 (1H, m)	31.5, CH_2	
16	1.30 (1H, m) 2.00 (1H, m)	27.9, CH_2	
17	1.60 (1H, m)	50.9, CH	
18	0.57 (3H, s)	15.7, CH_3	C-12, C-13, C-14, C-17
19	1.01 (3H, s)	22.8, CH_3	C-1, C-5, C-9, C-10
20	1.41 (1H, m)	36.2, CH	
21	0.94 (3H, d, 6.5)	18.3, CH_3	C-17, C-20, C-22
22	1.51 (1H, m) 2.00 (1H, m)	35.4, CH_2	
23	1.72 (2H, m)	24.3, CH_2	
24	6.49 (1H, t, 6.5)	155.5, CH	C-26, C-27
25	-	139.2, C	
26	9.40 (1H, s)	195.4, CHO	C-24, C-25, C-27
27	1.77 (3H, s)	9.2, CH_3	C-24, C-25, C-26
28	0.91 (3H, s)	28.1, CH_3	C-3, C-4, C-5, C-29
29	0.95 (3H, s)	16.9, CH_3	C-3, C-4, C-5, C-28
30	0.91 (3H, s)	25.5, CH_3	C-8, C-13, C-14, C-15
$COOCH_3$	- 2.06 (3H, s)	171.0, C 21.3, CH_3	 COO

[a] 500 MHz, [b] $CDCl_3$, [c] 125 MHz

$[M+Na]^+$ (Calcd. $C_{31}H_{48}O_8$, M=548.3); ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.17 (1H, dd, J = 5.0, 12.0 Hz, H-3), 4.54 (1H, dd, J = 7.5, 10.0 Hz, H-7), 4.79 (1H, m, H-15), 1.00 (3H, s, H-18), 1.28 (3H, s, H-19), 0.91 (3H, d, J = 6.5 Hz, H-21), 4.38 (1H, d, J = 5.0 Hz, H-24), 1.11 (3H, d, J = 7.0 Hz, H-27), 1.04 (3H, s, H-28), 0.86 (3H, s, H-29), 1.27 (3H, s, H-30), 3.71 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 35.9 (C-1), 28.4 (C-2), 79.0 (C-3), 39.7 (C-4), 50.5 (C-5), 29.0 (C-6), 70.2 (C-7), 161.3 (C-8), 143.1 (C-9), 39.7 (C-10), 202.3 (C-11), 53.2 (C-12), 48.5 (C-13), 55.4 (C-14), 73.2 (C-15), 37.1 (C-16), 49.2 (C-17), 17.6 (C-18), 19.8 (C-19), 33.3 (C-20), 20.2 (C-21), 46.6 (C-22), 212.9 (C-23), 78.8 (C-24), 43.5 (C-25), 176.1 (C-26), 11.5 (C-27), 28.7 (C-28), 16.4 (C-29), 20.0 (C-30), 52.4 (OCH_3).

3*β*,7*β*-Dihydroxy-11,15,23-trioxolanost-8,16-dien-26-oic acid (6) – yellow needles; ESI-MS: *m/z* 497.35 $[M-H_2O+H]^+$ (Calcd. $C_{30}H_{42}O_7$, M=514.3); ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.19 (1H, dd, *J* = 5.0, 12.0 Hz, H-3), 4.84 (1H, overlap, H-7), 5.78 (1H, s, H-16), 1.25 (3H, s, H-18), 1.24 (3H, s, H-19), 1.15 (3H, d, *J* = 7.0 Hz, H-21), 1.13 (3H, d, *J* = 7.0 Hz, H-27), 1.06 (3H, s, H-28), 0.87 (3H, s, H-29), 1.57 (3H, s, H-30); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 35.9 (C-1), 28.3 (C-2), 78.9 (C-3), 39.8 (C-4), 50.6 (C-5), 27.4 (C-6), 68.4 (C-7), 159.4 (C-8), 143.7 (C-9), 40.3 (C-10), 199.6 (C-11), 45.5 (C-12), 52.8 (C-13), 59.6 (C-14), 212.2 (C-15), 123.5 (C-16), 189.9 (C-17), 31.5 (C-18), 19.4 (C-19), 29.9 (C-20), 19.8 (C-21), 48.7 (C-22), 210.9 (C-23), 47.6 (C-24), 39.3 (C-25), 183.7 (C-26), 18.7 (C-27), 28.7 (C-28), 16.3 (C-29), 33.7 (C-30).

3*β*,7*β*,15*α*,24-Tetrahydroxy-11,23-dioxolanost-8-en-26-oic acid (7) – yellow oil; HR-ESI-MS: *m/z* 533.3142 $[M-H]^-$ (Calcd. $C_{30}H_{46}O_8$, M=534.3193); ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.18 (1H, dd, *J* = 4.5, 11.5 Hz, H-3), 4.55 (1H, dd, *J* = 7.5, 10.0 Hz, H-7), 4.78 (1H, overlap, H-15), 1.00 (3H, s, H-18), 1.26 (3H, s, H-19), 0.91 (3H, d, *J* = 6.0 Hz, H-21), 4.44 (1H, d, *J* = 5.0 Hz, H-24), 1.09 (3H, d, *J* = 7.0 Hz, H-27), 1.03 (3H, s, H-28), 0.86 (3H, s, H-29), 1.27 (3H, s, H-30); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 35.9 (C-1), 28.3 (C-2), 79.1 (C-3), 39.6 (C-4), 50.4 (C-5), 29.0 (C-6), 70.1 (C-7), 161.4 (C-8), 143.1 (C-9), 39.6 (C-10), 202.6 (C-11), 53.1 (C-12), 48.3 (C-13), 55.3 (C-14), 73.2 (C-15), 37.0 (C-16), 49.0 (C-17), 19.8 (C-18), 17.5 (C-19), 33.3 (C-20), 20.0 (C-21), 46.7 (C-22), 212.9 (C-23), 79.0 (C-24), 44.3 (C-25), 180.0 (C-26), 12.0 (C-27), 28.7 (C-28), 16.3 (C-29), 20.2 (C-30).

Resinacein S (8) – white power; ESI-MS *m/z*: 533.5 $[M+H]^+$ (Calcd. $C_{30}H_{46}O_8$, M=534.3). ^{1}H-NMR (500 MHz, CD_3OD): δ_H 3.17 (1H, dd, *J* = 5.0, 12.0 Hz, H-3), 4.86 (1H, t, *J* = 9.0 Hz, H-7), 1.03 (3H, s, H-18), 1.24 (3H, s, H-19), 0.97 (3H, d, *J* = 7.5 Hz, H-21), 4.39 (1H, d, *J* = 5.0 Hz, H-24), 1.13 (3H, d, *J* = 7.0 Hz, H-27), 1.05 (3H, s, H-28), 0.86 (3H, s, H-29), 1.39 (3H, s, H-30); ^{13}C-NMR (125 MHz, CD_3OD): δ_C 36.0 (C-1), 28.3 (C-2), 79.0 (C-3), 39.7 (C-4), 50.3 (C-5), 28.0 (C-6), 67.9 (C-7), 158.9 (C-8), 144.1 (C-9), 39.9 (C-10), 200.4 (C-11), 51.5 (C-12), 46.9 (C-13), 60.4 (C-14), 218.3 (C-15), 41.8 (C-16), 46.7 (C-17), 17.8 (C-18), 18.9 (C-19), 32.6 (C-20), 20.2 (C-21), 46.4 (C-22), 212.5 (C-23), 78.7 (C-24), 43.3 (C-25), 177.6 (C-26), 11.7 (C-27), 28.7 (C-28), 16.2 (C-29), 24.9 (C-30).

Nitroblue tetrazolium dye reduction assay – The NBT reduction assay was carried out according to previously reported methods with slight modification.[7] In brief, RAW 264.7 cells were precultured using RPMI 1640 in a 96-well plate at a seeding concentration of 2.5×10^5 cells/well. The cells were then incubated with *G. tropicum* products, using different concentrations or concanavalin A at 20 ng/ml as a positive control, for a further 24 h. Cell cultures that were not incubated were used as controls. After incubating for 24 h at 37 ºC in a 5% CO_2 humidified atmosphere, 20 µL of heat-inactivated *S. cerevisiae* solution (5×10^7 particles/mL) and 20 µL of NBT solution in phosphate-buffered saline were added and further incubated at room temperature. After 1 h, wells containing macrophages were washed using basic RPMI-1640 medium (without FBS or other supplements), and then a further four times with 200 µL of MeOH. After drying in the open air, 120 µL of 2 M KOH and 140 µL of DMSO were added. The optical density (OD) was measured using a 96-well plate reader at 570 nm and the NBT reductive activities were calculated according to the following formula:

$$\text{NBT reduction } (\%) = (OD_{sample} - OD_{reference\ control})/(OD_{reference\ control}) \times 100$$

The EC_{50} value represents the effective concentration required for a 50% enhancement of oxidative burst reduction activity.

Statistical analysis – Value is expressed as mean ± S.E.M.

Result and Discussion

Eight lanostane triterpenes were isolated from DCM and EtOAc extracts of the fruiting bodies of *G. tropicum*, including a new compound (**3**) and seven known ones (**1**, **2**, and **4** – **8**). These compounds were isolated by silica gel and reverse-phase silica gel (RP-C_{18}) CC. The structures of the known compounds were identified by ^{1}H- and ^{13}C-NMR spectroscopy, distortionless enhancement by polarization transfer (DEPT) and heteronuclear single-quantum coherence (HSQC) measurements, heteronuclear multiple bond correlations (HMBCs), and comparison with literature data, as follows: 3*β*-hydroxylanosta-7,9(11),24-triene (**1**),[8] methyl 2-hydroxynonacosanoate (**2**),[9] ganoderic acid Y (**4**),[2] resinacein R (**5**),[10] 3*β*,7*β*-dihydroxy-11,15,23-trioxolanost-8,16-dien-26-oic acid (**6**),[11] 3*β*,7*β*,15*α*,24-tetrahydroxy-11,23-dioxolanost-8-en-26-oic acid (**7**),[5] and resinacein S (**8**)[10] (Fig. 1).

Compound **3** was isolated as a pale yellow powder. The molecular formula of **3** was determined as $C_{32}H_{48}O_3$ by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) according to the $[M+Na]^+$ molecular ion

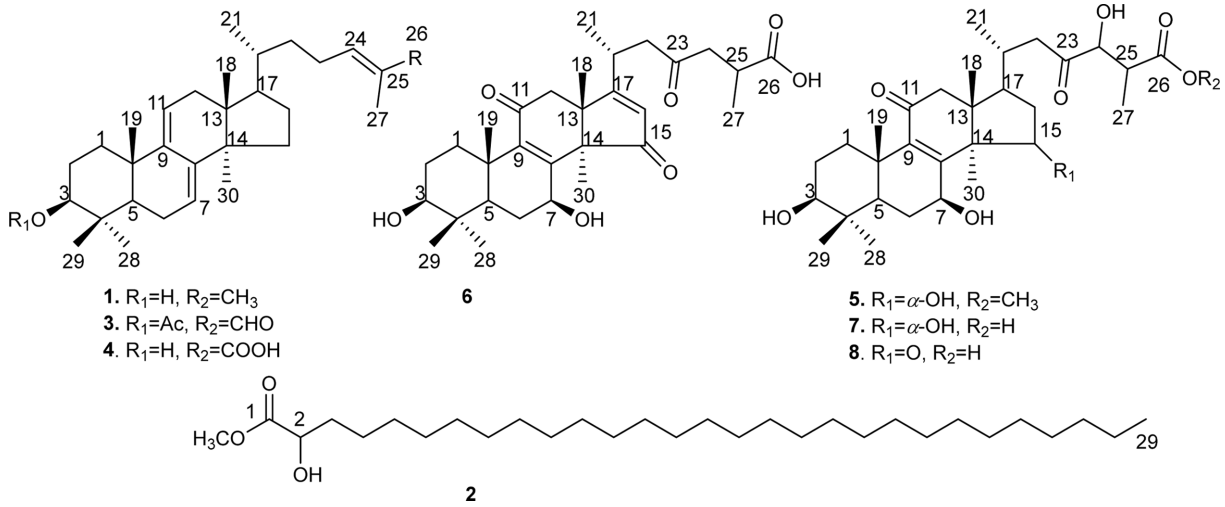

Fig. 1. The structures of compounds **1 - 8**.

observed at *m/z* 503.3517 (Calcd. for $C_{32}H_{48}O_3Na$, 503.3501). The one-dimensional NMR spectra of **3** were typical of a lanostane derivative.[8] The ^{1}H-NMR spectrum of **3** showed signals corresponding to seven methyl protons at δ_H 0.57 (3H, s, H-18), 1.01 (3H, s, H-19), 0.94 (3H, d, J = 6.5 Hz, H-21), 1.77 (3H, s, H-27), 0.91 (3H, s, H-28), 0.95 (3H, s, H-29), 0.91 (3H, s, H-30), one acetoxyl proton at δ_H 2.06 (3H, s, $COOCH_3$), one oxymethine proton at δ_H 4.51 (1H, dd, J = 4.5, 11.5 Hz, H-3), three olefinic protons at δ_H 5.47 (1H, br s, H-7), 5.32 (1H, d, J = 6.0 Hz, H-11), and 6.49 (1H, t, J = 6.5 Hz, H-24), and one aldehyde proton at δ_H 9.40 (1H, s). Furthermore, the ^{13}C-NMR and DEPT spectra of **3** also showed the presence of 32 carbons, including 8 methyls, 8 methylenes, 7 methines (including 3 olefins and 1 oxymethine), and 9 quaternary carbons (including 1 acetoxyl). The carbon signals were resolved in the ^{13}C-NMR spectrum of **3** with the aid of HSQC (Table 1). The ^{1}H and ^{13}C-NMR spectra of **3** were closely similar to those of 3β-hydroxylanosta-7,9(11),24-triene (**1**) except for the presence of acetyl (δ_H 2.06 and δ_C 21.3, 171.0) and aldehyde (δ_H 9.40 and δ_C 195.4) groups and the absence of one methyl group. The HMBC correlations (Fig. 2) of H-3 (δ_H 4.51) with C-4 (δ_C 37.8), C-28 (δ_C 28.1), C-29 (δ_C 16.9), and acetyl (δ_C 171.0) suggested acetylation at C-3. The position of an aldehyde group at C-26 in **3** was confirmed by the HMBC correlations of H-26 (δ_H 9.40) with C-24 (δ_C 155.5), C-25 (δ_C 139.2), and C-27 (δ_C 9.2), and H-27 (δ_H 1.77) with C-24, C-25, and C-26 (δ_C 195.4). Moreover, the chemical shift of C-24 (δ_C 155.5), C-25 (δ_C 139.2), and C-27 (δ_C 9.2) in **3** differed from that of **1** [δ_C 125.2 (C-24), δ_C 131.0 (C-25), and δ_C 17.6 (C-27)], indicating that

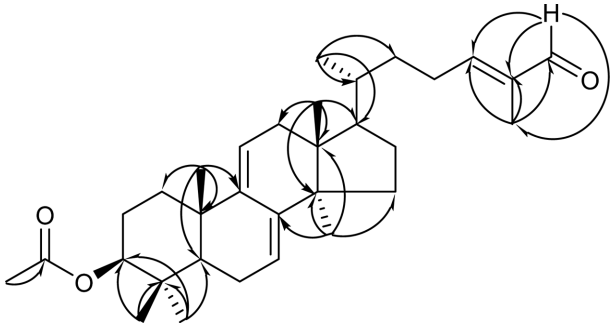

Fig. 2. Key HMBC correlations of compound **3**.

acetyl group was located at the C-26 position. The relative configuration of 3-OH was determined as β due to the coupling constant between H-3 and H-2 ($J_{3\text{-}2}$ 4.5 and 11.5 Hz).[12] The configurations of the remaining positions were determined as in Figures 1 and 2 based on a natural lanostane skeleton and compared with the reference.[2,8] Based on the above results, the structure of **3** was elucidated as 3β-acetoxylanosta-7,9(11),24-triene-26-al.

Compounds (**1** – **8**) were tested for NBT reduction activity at concentrations of 0.8, 4, 20, 100, and 200 μM. Most of the isolated compounds enhanced NBT reduction in a dose-dependent manner (Table 2). The EC_{50} value could not be determined due to cell death at concentrations > 100 μM. Compound **3** showed the best NBT reduction activity, which may have been due to the presence of an aldehyde group at C-26.[13] Compound **7** showed a greater NBT reduction than **8**, in part because of the –OH group at C-15 in **7** showing stronger activity than the ketone group at C-15 in **8**. Additionally, compound **7** had stronger activity than **5**, which indicated the involvement of methylation at C-26. Overall, these

Table 2. The NBT reduction activity of compounds **1** - **8**

Concentration (μM)	NBT reduction (%)								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Concanavalin A[a]
200	-[b]	+[c]	+[c]	+[c]	+[c]	5.22 ± 0.2	+[c]	+[c]	
100	-[b]	29.71 ± 1.26	+[c]	+[c]	10.52 ± 1.51	2.42 ± 0.3	32.59 ± 1.23	17.62 ± 2.10	
20	-[b]	25.98 ± 0.68	46.52 ± 1.35	29.71 ± 1.85	8.63 ± 0.84	1.49 ± 0.2	16.43 ± 1.12	8.36 ± 0.69	20.82 ± 2.52
4	-[b]	5.50 ± 0.87	29.25 ± 2.01	-[b]	7.21 ± 1.03	0.75 ± 0.1	11.84 ± 0.89	7.73 ± 0.45	-[b]
0.8	-[b]	2.73 ± 0.21	15.11 ± 1.03	-[b]	3.03 ± 0.63	0.93 ± 0.1	0.08 ± 0.001	2.02 ± 0.17	-[b]

The data are the average values of three repeated experiments (mean ± standard error of the mean). [a)] Control, [b)] negative, and [c)] cell death.

results suggest the involvement of positions C-15 and C-26, and the replacement group, in NBT reduction activity.

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