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SYNTHESIS AND ANTITUMOR ACTIVITY OF A NEW ERGOSTEROL DERIVATIVE

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*Ergosterol-3-*O*-(*β*-D-galactopyranosyl-(1→4)-*β*-D-glucopyranoside) was efficiently synthesized and evaluated for its inhibitory activities against S180 cell lines compared with ergosterol. The structures of all synthesized compounds were fully characterized by spectroscopic data (NMR, MS). The antitumor activity of the new derivative was significantly enhanced compared with ergosterol.*

Keywords: ergosterol, glycosylation, derivative, antitumor activity.

Saponins are natural compounds present in various higher plants that are usually found in roots, tubers, leaves, blooms, or seeds. Saponins were classified into triterpenes and steroids based on the carbon skeleton. Their sugar parts are mostly oligosaccharides, arranged either in linear or branched fashion, attached to hydroxyl groups through an acetal linkage. Modern research has found that saponins have antitumor effects on many cancer cells. Several saponins inhibit tumor cell growth by cell cycle arrest and apoptosis with IC_{50} values up to 0.2 mM. Meanwhile, saponins in combination with conventional tumor treatment strategies have improved the success rate of therapeutics [1].

Ergosterol is one of the phytosterols that are widely distributed in fungal cultures and also the most important component in membranes. It is involved in numerous biological functions such as membrane fluidity regulation, activity and distribution of integral proteins, and control of the cellular cycle [2, 3]. Moreover, it posseses different pharmacological activities such as antibacterial, antiviral, and anticancer [4].

Although a small quantity of ergosterol glucoside derivatives has been extracted from *Cordyceps gunnii*, nevertheless, it was difficult to study it due to its low content and complicated composition. Chemical synthesis is one of the prospective routes for obtaining homogeneous saponins, thus affording an opportunity to understand and apply this important branch of natural products [5].

In our previous study [6], we successfully synthesized two new ergosterol glycosides, ergosterol-3-*O*-*β*-D-glucopyranoside and ergosterol-3-*O*-*β*-D-galactopyranoside. The antitumor activities of these compounds have been investigated, and the result indicate that both of them have significant cytotoxic and antitumor activity on the S180 cell line [7].

In order to further understand the effect of the sugar part of the ergosterol glycosides on the biological activity, we selected D-lactose as the sugar part, then used benzoyl protection for the lactose hydroxyl groups; then we used the trichloroacetimidate method [8] for the glycosylation procedure (Scheme 1). In this study, the glycosylation of the ergosterol with the donor (compound **3**) was achieved under the conditions described above, promoted by TMSOTf (trimethylsilyl trifluoromethanesulfonate), to give compound **4** in 78.0% yield. The stereochemistry of the newly introduced glycosidic linkage was determined to be *β* on the basis of the Glc H-1, H-2 coupling constant ($J_{1,2}$ = 8.0 Hz) [9]. De-*O*-benzoylation of compound **4** in methanol-dichloromethane quantitatively offered the target compound **5** in 81.0% yield (Scheme 2).

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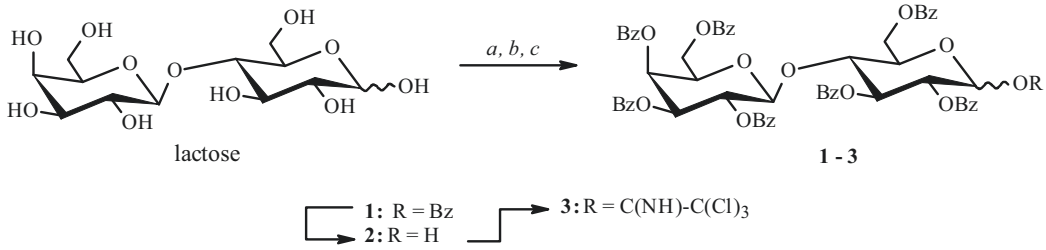
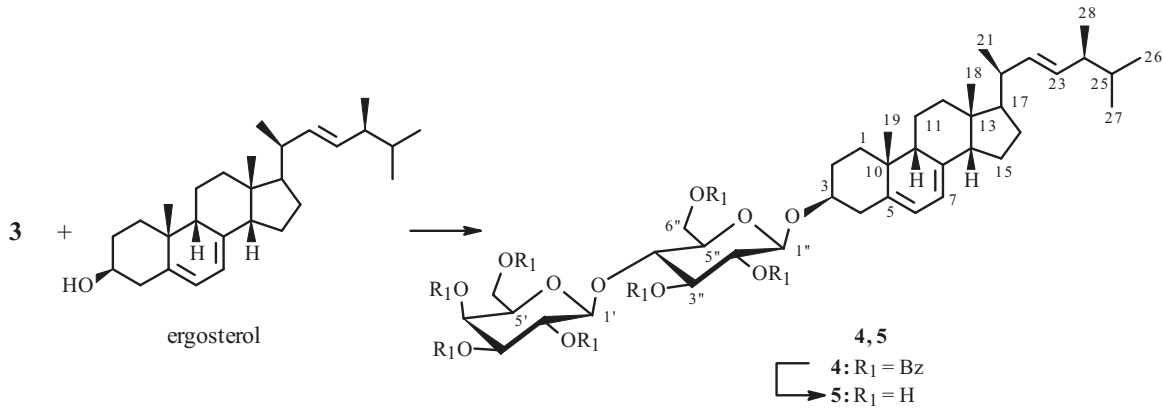


a. BzCl, Py, 0°C to r.t., 16 h; *b*. THF, methylamine, r.t., 2h; *c*. CH_2Cl_2, DBU, CCl_3CN r.t., 3 h

Scheme 1



Scheme 2

The antitumor activity of the new derivative *in vitro* was evaluated using the MTT assay [10].

The new glycoside was tested for its anticancer activity against S108 cell lines by the MTT-based assay *in vitro*. The data showed that the antitumor activity of the new derivative was higher than that of ergosterol. In 48 h, the new derivative displayed the best inhibition rate of 93.4% at the concentration of 200 μg/mL (Fig. 1). We found that the antitumor activity of this new derivative was higher than that of ergosterol-3-*O*-(*β*-D-galactopyranoside) in 24 h compared with our previous study, and the antitumor activity of the new derivative was higher than that of ergosterol-3-*O*-(*β*-D-glucopyranoside) in 48 and 72 h [6]. Thus, the sugar part of the ergosterol glycosides plays an important role in the improvement of its biological activity, so further study is currently being carried out in our laboratory.

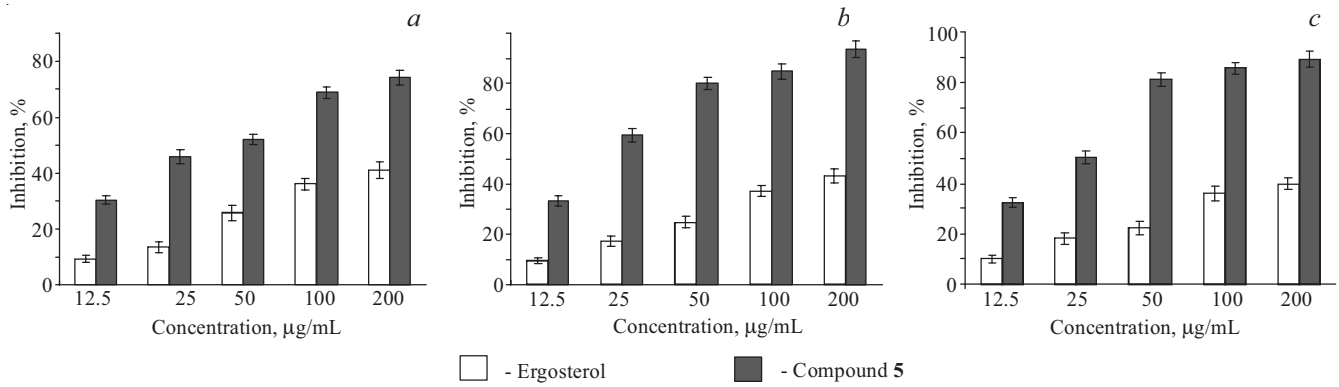


Fig. 1. Growth inhibition activities of compound **5** and ergosterol on S180 cell lines at 24 h (*a*), 48 h (*b*), and 72 h (*c*).

EXPERIMENTAL

Ergosterol-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-*β*-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranoside) (4). To a stirred solution of 2,3,4,6-tetra-*O*-benzoyl-*β*-D-galactopyranoyl-(1→4)-2,3,6-tri-*O*-benzoyl-*β*-D-glucopyranosyl, trichloroacetimidate (**3**, 2.84 g, 2.34 mmol) in 200 mL of dry CH_2Cl_2 4Å molecular sieves (1.5 g) was added, followed by ergosterol (0.90 g, 2.27 mmol) as a solid powder. The mixture was stirred for 0.5 h at 0°C, then TMSOTf (0.17 mL, 1.0 mmol) was added under Ar protection. The mixture was continuously stirred at 0°C for 1 h and room temperature for another 0.5 h,

then neutralized by Et_3N. The yellow solution was diluted with CH_2Cl_2 and filtered to remove the molecular sieves. The organic layer was concentrated under reduced pressure to give a yellow syrup, which was purified by column chromatography (EtOAc–petroleum ether, 1:4, v/v) to give compound **4** (2.57 g, 1.77 mmol, 78.0%) as a white powder with R_f 0.61 (EtOAc–petroleum ether, 1:4, v/v); $[\alpha]_D^{25}$ +20° (*c* 0.1, CH_2Cl_2).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.10–8.03 (35H, m, ArH), 5.84 (1H, d, J = 5.6, H-6), 5.63 (1H, d, J = 8.0, H-1″), 5.78–5.69 (3H, m, H-7, 22, 23), 5.47 (1H, dd, J = 3.1, H-4′), 5.43 (1H, t, J = 9.5, H-3″), 5.36 (1H, dd, J = 10.1, 8.2, H-2′), 5.21 (1H, dd, J = 9.5, 8.0, H-2″), 5.11 (1H, dd, J = 10.1, 3.1, H-3′), 4.79 (1H, d, J = 8.1, H-1′), 4.63 (1H, dd, J = 12.3, 2.1, H-6″), 4.38–4.27 (3H, m, H-6″, H-6a′, 6b′), 4.11–4.04 (2H, m, H-5′, 4″), 3.96–3.87 (1H, m, H-5″), 3.82–3.74 (1H, m, H-3), 1.55 (3H, d, J = 6.4, H-21), 1.47 (3H, d, J = 3.4, H-27), 1.35 (3H, d, J = 8.9, H-25), 1.08 (3H, s, H-19), 0.96 (3H, t, J = 6.1, H-28), 0.89 (3H, s, H-18). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 165.84, 165.81, 165.59, 165.45, 165.41, 165.24, 165.18 (C=O, Bz), 158.53 (C-8), 147.49 (C-5), 140.63 (C-23), 135.51 (C-22), 135.37–123.01 (CH, Ar), 117.95 (C-6), 99.69 (C-1″), 99.24 (C-1′), 86.97 (C-7), 77.95 (C-5″), 76.75 (C-4″), 72.55 (C-2″), 71.11 (C-2′), 70.99 (C-5′), 69.26 (C-4′), 68.71 (C-3″), 67.12 (C-3′), 63.07, 62.69 (C-6′, 6″), 57.62 (C-3), 56.63 (C-17), 55.23 (C-14), 48.52 (C-9), 48.41 (C-13), 46.31 (C-24), 43.49 (C-20), 41.58 (C-12), 40.08 (C-4), 39.43 (C-1), 35.82 (C-25), 34.69 (C-2), 30.81 (C-10), 29.92 (C-16), 28.22 (C-15), 24.89 (C-26), 23.84 (C-11), 20.98 (C-21), 20.88 (C-27), 20.64 (C-28), 20.25 (C-19), 20.07 (C-18), 19.14 (C-9). HR-ESI-MS *m/z* 1471.6234, $[M + Na]^+$ (calcd for $C_{89}H_{92}O_{18}Na$, 1471.6182).

Ergosterol-3-*O*-(β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside) (5). Compound **4** (2.04 g, 1.41 mmol) was dissolved in MeOH–CH_2Cl_2 (1:1, 80 mL), and then 172 mg NaOMe was added. After stirring at room temperature for 3.5 h, the solution was neutralized by ion-exchange resin (H^+), and then filtered and concentrated. The white residue was purified by column chromatography (CH_2Cl_2–MeOH, 8:1→5:1) to give **5** as a white solid (0.82 g, 1.14 mmol, 81.0%) with R_f 0.28 (CH_2Cl_2–MeOH, 5:1, v/v) $[\alpha]_D^{25}$ –15° (*c* 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 5.71 (1H, d, J = 5.7, H-6), 5.68–5.42 (3H, m, H-7, 22, 23), 5.35 (1H, d, J = 8.0, H-1″), 5.26 (1H, dd, J = 3.2, H-4′), 5.24 (1H, t, J = 9.2, H-3″), 5.11 (1H, dd, J = 10.4, 8.0, H-2′), 5.05 (1H, dd, J = 9.2, 8.4, H-2″), 4.96 (1H, dd, J = 10.4, 3.2, H-3′), 4.57 (1H, d, J = 8.0, H-1′), 4.47 (1H, dd, J = 12.5, 2.0, H-6″), 4.16–4.05 (3H, m, H-6″, H-6a′, 6b′), 3.89–3.82 (2H, m, H-5′, 4″), 3.77–3.74 (1H, m, H-5″), 3.71–3.63 (1H, m, H-3), 1.25 (3H, d, J = 6.7, H-21), 1.21 (3H, d, J = 3.5, H-27), 0.95 (3H, d, J = 8.6, H-25), 0.91 (3H, s, H-19), 0.86 (3H, t, J = 6.4, H-28), 0.73 (3H, s, H-18). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 158.93 (C-8), 147.29 (C-5), 134.83 (C-23), 130.57 (C-22), 118.35 (C-6), 117.62 (C-7), 102.99 (C-1″), 102.47 (C-1′), 77.71 (C-2′), 76.65 (C-4″), 75.85 (C-2″), 74.48 (C-5″), 74.13 (C-5′), 73.42 (C-3′), 71.58 (C-3″), 71.56 (C-4′), 67.62 (C-3), 62.50, 62.19 (C-6′, 6″), 56.53 (C-17), 55.23 (C-14), 49.52 (C-9), 48.41 (C-13), 46.31 (C-24), 41.59 (C-20), 40.38 (C-12), 35.78 (C-4), 34.47 (C-1), 30.98 (C-25), 29.69 (C-2), 28.76 (C-10), 27.92 (C-16), 25.32 (C-15), 24.69 (C-26), 23.84 (C-11), 22.68 (C-21), 20.98 (C-27), 20.64 (C-28), 20.26 (C-19), 19.10 (C-18), 17.14 (C-9). HR-ESI-MS *m/z* 743.4386 $[M + Na]^+$ (calcd for $C_{40}H_{64}O_{11}Na$, 743.4347).

Antitumor Activity. Growth inhibition against S180 from the laboratory of Tianjin University of Science and Technology caused by compound **5** and ergosterol was studied to compare their antitumor activities. The inhibitory effects at 24 h (*a*) and 48 h (*b*) were assessed by the colorimetric-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assays after measuring the number of viable cells that survived through treatment with 25, 50, 100, 200, and 400 mg/mL of tested compounds for 1–3 days.

The cell lines were maintained in a medium consisting of 10% RPMI 1640/FCS at 37°C in an atmosphere of 5% CO_2. Malignant cell lines, 10^6cells/mL in 96-well round bottom plates (Costar Corporation, Cambridge, MA, USA), were cultured with various concentrations at 24 (*a*) and 48 h (*b*) in a 5% CO_2-air humidified atmosphere at 37°C for 20, 44, and 68 h, then MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well. MTT uptake was measured after 4 h of incubation using a scintillation counter. The inhibitory effect of each compound on cell proliferation was calculated using the following equation:

Inhibition persent (%) = (Control group (cpm) – Experimental group (cpm)/Control group (cpm)) × 100.

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REFERENCES

1. J. P. Vincken, L. Heng, and H. Gruppen, *Phytochemistry*, **68**, 275 (2007).
2. An-Ling Zhang, La-Ping Liu, Min Wang, and Jin-Ming Gao, *Chem. Nat. Compd*., **43**, 637 (2007).
3. N. V. Kovganko, *Chem. Nat. Compd*., **35**, 597 (1999).
4. N. H. Georgopapadakou and T. J. Walsh, *Science*, **264**, 371 (1994).
5. J. W. Bok, L. Lermer, J. Chilton, H. G. Klingeman, and G. H. N. Towers, *Phytochemistry*, **51**, 891 (1999).
6. Z. Y. Zhu, Q. Yao, Y. Liu, C. L. Si, J. Chen, N. Liu, H. Y. Lian, L. N. Ding, and Y. M. Zhang, *J. Asian Nat. Prod. Res*., **14**, 429 (2012).
7. Z. Y. Zhu, S. F. Li, R. Q. Liu, J. Yuan, H. B. Wang, Y. M. Zhang, and Y. Liu, *Chin. J. Chem*., **28**, 2245 (2010).
8. F. Qu, Y. X. Li, and Y. C. Zhang, *Chin. J. Org. Chem*., **23**, 249 (2003).
9. S. Bregant, Y. Zhang, J. M. Mallet, A. Brodzki, and P. Sinay, *Glycoconjugate J*., **16**, 757 (1999).
10. R. P. Schmidt, *Pure Appl. Chem.*, **61**, 1257 (1989).