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Semisynthesis and bioactive evaluation of oxidized products from 20(S)-ginsenoside Rg_3, Rh_2, protopanaxadiol (PPD) and their 20(R)-epimers as cytotoxic agents

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ABSTRACT

A series of oxidized products have been systematically semisynthesized from 20(S)-ginsenoside Rg_3, Rh_2, 20(S)-protopanaxadiol (PPD) and their 20(R)-epimers and the majority of these products were evaluated for their cytotoxic activity against HeLa cells and HepG2 cells by MTT assay for the first time. Twenty-two products were obtained and elucidated based on comprehensive ^{1}H NMR, ^{13}C NMR, two-dimensional (2D) NMR, and mass spectral data and the results reported in previous literature. All the four ocotillol type saponins (20S,24R(δ86, δ85); 20S,24S(δ87, δ88); 20R,24R(δ86, δ86); 20R,24S(δ86, δ87) were obtained. In addition, eight compounds (**3**, **8**, **9**, **10**, **15**, **16**, **19** and **22**) with the cyclized side chain were firstly identified. Most of the tested compounds possessed cytotoxicity to a certain degree against the two types of cells which implied these oxidized products could play a certain role on anti-cancer functions of the raw materials *in vivo*. Meanwhile, the results proved that the configurations at C-20 or C-24 and the number of glycosyl at C-3 have important influence on the cytotoxicity. The products **1**, **2**, **11–17**, **20** and **22** should possess great activities and deserved further investigation as potential cytotoxic agents.

1. Introduction

Ginsenosides, as the major active constituents in rare medicinal herbs *Panax ginseng* C.A. Meyer and *Panax quinquefolius* L., etc, belong to the triterpenoid saponins. According to their sapogenins, ginsenosides are classified into four types (Fig. 1), including protopanaxadiol (PPD), protopanaxatriol (PPT), oleanane, and ocotillol [1,2]. Many studies indicated that ginsenosides have pharmacological activities such as anti-cancer, improving cardiovascular health, protecting central nervous system, and immunoregulation [3–5]. In addition, it has been proved that the secondary ginsenosides and aglycone obtained from ginsenosides by hydrolysis and dehydration processes *in vivo* or *in vitro* have pronounced anti-cancer activity [6,7]. Moreover, 20(R)-ginsenoside Rg_3 as basis of Shen-yi capsule has been approved by the State Drug Administration as the first-class new anti-cancer drug in China [8,9]. However, oral administration of these secondary ginsenosides bought about poor absorption and low bioavailability [10–12]. Hence, numerous scientific researchers devoted themselves to not only efficiently transforming ginsenosides to secondary ginsenosides or aglycone, but also further making the modification or transformation from the perspective of chemistry and biology with the purpose of obtaining derivative products with great properties [13–16].

As we known, secondary ginsenoside Rg_3, Rh_2, and their aglycone protopanaxdiol (PPD) possess great bioactivities, and the characterization of the metabolism of ginsenosides is important for explaining its pharmacological effects. Pharmacokinetic studies indicated oxygenation and deglycosylation were found to be the major metabolic path ways of ginsenosides after oral administration [17,18]. The double bond between C-24 and C-25 was identified as one of the oxygenation sites to produce additional hydroxyl or epoxy groups in the side chain [19], and the metabolites with 20,24-epoxy groups may be generated. Based on the above analysis, we speculated that the research on oxidized products may provide valuable information for clarifying pharmacological activity mechanism. In light of this, we employed chemical methods and systematically semisynthesized a series of oxidized products by using 20(S)-ginsenoside Rg_3, Rh_2, PPD, and their 20(R)-epimers as raw materials, and further we assessed the cytotoxic activity to investigate structure–activity relationship. Moreover, we expected to investigate pharmacological activity of these raw materials and find novel compounds with great bioactivity.

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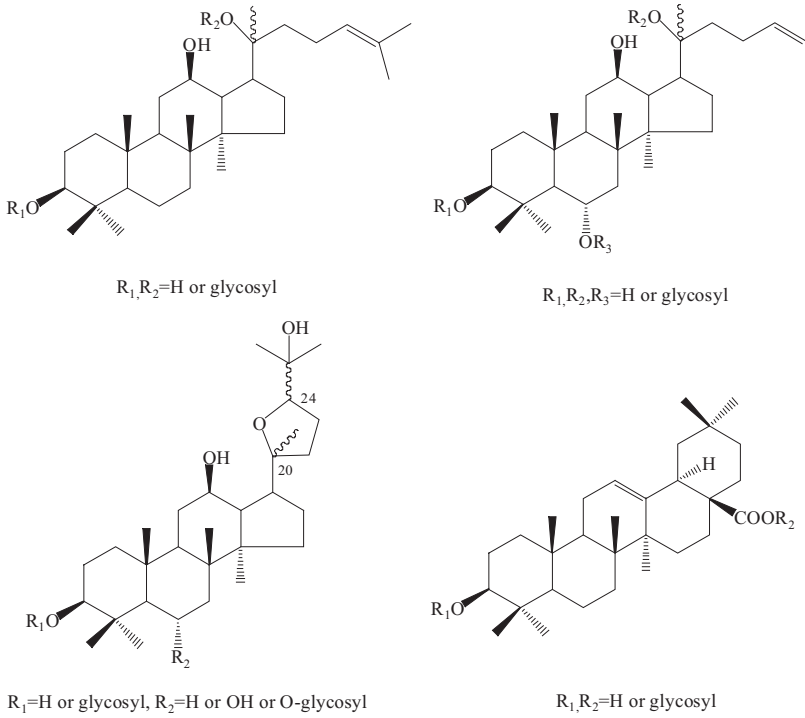

Fig. 1. Structures of four types of ginsenosides.

2. Experimental

2.1. General experimental procedures

Melting points were recorded on an XT-4 micro melting point apparatus and uncorrected (Beijing Teck Instrument Co., Ltd., People's Republic of China). HR-MS (ESI) data were obtained on an Agilent1290-micrOTOF Q II LC–MS instrument (Brucker, Germany). The ^{1}H NMR, ^{13}C NMR, and two-dimensional (2D) NMR spectra were obtained on a Bruker Avance-600 spectrometer using TMS as an internal standard (Brucker, Swiss). The chemical shift (δ) and coupling constant (J) were expressed in ppm and Hz, respectively. Signals were described as singlet (s), doublet (d), triplet (t), quartet (q) as well as multiplet (m). For column chromatographic separation, silica gel 60 (100–200 mesh, Qingdao Marine Chemical Ltd., People's Republic of China) and DAISOGEL ODS-AP (200 Å, Daiso Co., Ltd., Japan) were used. Thin-layer chromatography (TLC) were carried out on silica gel 60F_{254} (Merck, Germany) and RP_{18} $F_{254}S$ (Merck, Germany) and stained by spraying 10% H_2SO_4 solution. Preparative chromatographic separation was performed with a Shimadzu LC-6A pump and a Shimadzu RID-10A refractive index detector using a COSMOSIL C18 column (5 μm, 10ID × 250 mm). Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethy lthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, USA) and Infinite F200 Pro microplate reader (Tecan, Swiss) were used in cytotoxic experiment. Ginsenoside 20(S)-Rg_3, 20(S)-Rh_2, 20(S)-PPD and their 20(R)-epimers were prepared in our own laboratory, and their purities were more than 97% (Scheme 1). All other chemicals and solvents were of analytical grade without further purification and commercially available.

2.2. Semisynthesis and separation

Semisynthetic method referred to previous report [20] with hydrogen peroxide as the oxidant. Ginsenoside was dissolved in 1,4-dioxane and concentrated sulfuric acid was used to adjust pH to 3.0–5.0. After the oxidant was added dropwise with stirring, the reaction mixture was refluxed at 80 °C until the raw material disappeared. Then the reaction mixture was neutralized with 0.1 mol L^{-1} sodium hydroxide solution and subsequently filtered to remove the insoluble substances. The filtrate was dried under reduced pressure and finally we got the residues.

All the compounds were purified from the corresponding residues by column chromatography, crystallization or preparing chromatographic separation. Compounds **1**–**4** were obtained from 20 (S)-PPD, while compounds **5**–**10** from 20(R)-PPD, compounds **11**–**12** from 20(S)-ginsenoside Rh_2, compounds **13**–**16** from 20(R)-ginsenoside Rh_2, compounds **17**–**19** from 20(S)-ginsenoside Rg_3, compounds **20**–**22** from 20(R)-ginsenoside Rg_3, respectively. The structures were confirmed based on ^{1}H NMR, ^{13}C NMR, 2D NMR, and MS data and by comparison with results reported in literature.

2.2.1. Compounds *1*–*4* from 20(S)-PPD

Compound **1**, white needle-like crystals, m.p. 229–231 °C. ESI-MS: m/z 477.3 $[M+H]^+$. ^{1}H NMR (C_5D_5N, 600 MHz) δ: 3.84 (t, J = 7.8 Hz, 1H), 3.61 (td, J = 10.2, 4.8 Hz, 1H), 3.31 (dd, J = 10.8, 4.8 Hz, 1H), 1.35 (s, 3H), 1.16 (s, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 0.90 (s, 3H), 0.89 (s, 3H), 0.77 (s, 3H), 0.74 (s, 3H). ^{13}C NMR (C_5D_5N, 150 MHz) δ: 86.8, 85.7, 78.1, 71.2, 70.4, 56.5, 52.3, 50.9, 49.9, 48.5, 40.1, 39.6, 39.5, 37.5, 35.3, 32.9, 32.5, 31.7, 28.9, 28.7, 28.3, 27.8, 27.3, 27.0, 25.6, 18.9, 18.4, 16.7, 16.3, 15.7.

Compound **2**, white power, m.p. 133–135 °C. ESI-MS: m/z 477.3 $[M+H]^+$. ^{1}H NMR (C_5D_5N, 600 MHz) δ: 4.08 (q, J = 5.4 Hz, 1H), 3.68 (td, J = 10.2, 4.8 Hz, 1H), 3.34 (dd, J = 11.4, 4.8 Hz, 1H), 1.34 (s, 3H), 1.20 (s, 6H), 1.12 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.82 (s, 3H), 0.80 (s, 3H). ^{13}C NMR (C_5D_5N, 150 MHz) δ: 88.5, 87.1, 78.1, 70.9, 70.0, 56.6, 52.4, 50.8, 49.7, 49.6, 40.1, 39.7, 39.6, 37.5, 35.3, 32.8, 32.7, 32.3, 29.1, 28.7, 28.4, 27.1, 26.7, 25.9, 18.9, 18.2, 16.8, 16.4, 15.8.

Compound **3**, white powder, m.p. 139–141 °C. HR-MS (ESI): calcd for $C_{30}H_{53}O_4$ $[M+H]^+$ 477.3938; found 477.3932. The NMR data were in Table 1.