Synthesis, Biological Activity of Salidroside and Its Analogues

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Salidroside is a phenylpropanoid glycoside isolated from Rhodiola rosea L., a traditional Chinese medicinal plant, and has displayed a broad spectrum of pharmacological properties. In this paper, about 18 novel salidroside analogues were prepared through Koenigs–Knorr method, the effects of these compounds over PC12 was assessed with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The novel compounds differ in the substituents attached to the benzene ring or in the glycosyl donor. According to the data, compounds (3,5-dimethoxyphenyl)methyl β-D-glucopyranoside and (3,5-dimethoxyphenyl)methyl β-D-galactopyranoside with methoxy group at 3 and 5-positions of the benzene ring were the most viability at concentration of 300 μmol/l and 60 μmol/l, respectively.

Key words salidroside; analogue; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method; hypoglycemia and serum limitation; PC12 cell

Salidroside, a traditional Chinese medicine, was known as the main bioactive component in the root of Rhodiola sachalinensis A. Bor., and was reported to have many pharmacological properties, such as resisting anoxia, anti-radiation and antifatigue, improving oxygen lack and postponing ageing. In recent years, salidroside has been used in such special posts as diver, astronaut, pilot and mountaineer to enhance the ability for survival in adverse environment. Furthermore, many researches revealed that it has the activities of preventing cardiovascular disease and anti-tumor. How- ever, the resource of wild R. sachalinensis A. Bor. was on the edge of exhaustion.

Salidroside is a 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside (Fig. 1, 5e) which may be synthesized by glucosylation of tyrosol. Considerable effort has been devoted to the isolation and assess of pharmacological properties, synthesis and structure modification of salidroside is very few. In order to search for more activity compounds, 20 compounds (Fig. 1, 4a—d, 5e—f, 4g—t) were successfully produced by Koenigs–Knorr method.

The synthesis of the compounds 3a—t was completed, which starting from the corresponding acetobromo-sugars (α-D-glucose, α-D-galactose) with the suitable substituted aromatic alcohol. The reaction was conducted in dry ethyl ether and dichloromethane for 10 h under dark with the presence of Ag2CO3. The process was optimized in the presence of 4 Å MS. Powdered MS 4 Å was dried at 140 °C under reduced pressure for 1 d before use. The designed target compounds 4a—t were obtained from 3a—t by direct deacetyl-ation with CH3ONa/CH3OH in good yields (Chart 1, over 90% to almost in quantitative). The catalytic hydrogenation of 4e and f to remove the protection of benzyl in the phenol hydroxy group is operated by refluxed with 5% Pd/C and HCOONH4 in anhydrous methanol (Chart 1).

According to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, after the PC12 cells were exposed to hypoglycemia and serum limitation for 24 h, it was found that hypoglycemia and serum limitation induced significant decreases in cell viability of cultured PC12 cells, as compared to that of control group. Exposure to hypoglycemia and serum limitation for 24 h was used to induce PC12 cell injury in all experiments unless otherwise stated. The cell viability of PC12 for each compound provide a quantitative comparison of salidroside and its analogues. MTT assay confirmed that the exposure to hypoglycemia and serum limitation caused the cell viability loss in cultured PC12 cells, as compared to that of control group. Exposure to hypoglycemia and serum limitation for 24 h was used to induce PC12 cell injury in all experiments unless otherwise stated. The cell viability of PC12 for each compound provide a quantitative comparison of salidroside and its analogues.

Experimental

General Commercial reagents were used without further purification unless otherwise stated. All melting points are uncorrected. 1H-NMR spectra...
were recorded on a Bruker AC 400 instrument at 500 MHz, and the following abbreviations are used for the description of the patterns: s = singlet, d = doublet, t = triplet, m = multiplet. The 1H-NMR spectra were recorded with tetramethylsilane (TMS, 0.00) as the internal standard and were run in dimethyl sulfoxide (DMSO) or D2O. J values are expressed in Hz. Flash chromatography was performed on silica gel (200—300 mesh) (Hai Yang Chemical Factory, Qingdao, Shangdong, P.R. China). Compounds 2a and b were prepared through literature.10) General Procedure for Compounds 4a—t A mixture of 2a or 2b (2.05 g, 5 mmol) and alcohol (4 mmol) was dissolved in dry dichloromethane—ether (2:1, 15 ml) and 2 g 4 Å MS was added, then stirred at room temperature under N2 atmosphere for 20 min. After that Ag2CO3 (1.4 g, 10 mmol) was added rapidly to the mixture and stirring continued for 10 h without light. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography using 60, 300, or 1000 mmol/l compounds synthesized above for 24 h, the cells were subjected to hypoglycemia and serum limitation by replacing the culture medium with the glucose-free DMEM supplement with 10% horse serum, 5% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin in 5% CO2/air at 37 °C. After being pretreated with 60, 300, or 1000 mmol/l compounds synthesized above for 24 h, the cells were subjected to hypoglycemia and serum limitation by replacing the culture medium with the glucose-free DMEM supplement with 1% horse serum and 1% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin, and in the presence of compounds at original concentrations for another 24 h incubation.

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References and Notes
1) Chen Y. M., Chen J., Mao G. M., Fu J. Y., Zhejiang Prev. Med., 19, 52 (2009). 2) Zhejiang Prev. Med. Vol. 58, No. 12, 1628—1630 (2010). 3) 4-(4-Hydroxyphenyl)ethyl β-D-Glucopyranoside (5e): A mixture of 4e (180 mg, 460 mmol), 5% Pd/C (208 mg) and ammonium formate (0.15 g, 2.3 mmol) in MeOH (6 ml) was stirred at refluxing for 6 h. After that diluted with MeOH and acidified with Amberlite IRA-120 (H+ form) to pH 3.32 (3H, m), 2.70—2.74 (2H, m). IR (KBr) cm−1: 1079, 1020. Anal. Calcd for C14H20O7: C, 55.99; H, 6.71. Found: C, 56.08; H, 6.77.

Cell Culture Rat PC-12 cells, obtained from the American Type Culture Collection (Manassas, VA, U.S.A.), were plated and maintained in high-glucose Dulbecco’s modiﬁed Eagle’s medium (DMEM) supplemented with 10% horse serum, 5% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin in 5% CO2/air at 37 °C. After being pretreated with 60, 300, or 1000 mmol/l compounds synthesized above for 24 h, the cells were subjected to hypoglycemia and serum limitation by replacing the culture medium with the glucose-free DMEM supplement with 1% horse serum and 1% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin, and in the presence of compounds at original concentrations for another 24 h incubation. At the end of incubation, the MTT solution was added and further incubated at 37 °C for 4 h followed by the addition of DMSO to dissolve the resulting formazan. The absorbance (optical density (OD)) values were measured by spectrophotometry at 570 nm with an EIX-800 MicroeLa reader (Bio-Tek Inc., U.S.A.).

Table 1. Experiment Data of Compounds

<table>
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<tr>
<th>Compound No.</th>
<th>Yield (%)</th>
<th>Compound No.</th>
<th>Yield (%)</th>
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<th>δ (1H)</th>
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<td>4a</td>
<td>60</td>
<td>120—121</td>
<td>4.18 (J = 7.8 Hz)</td>
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<tr>
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<td>4b</td>
<td>96</td>
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<td>4d</td>
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<td>97—98</td>
<td>4.13 (J = 7.2 Hz)</td>
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<td>5j</td>
<td>92</td>
<td>138—140</td>
<td>4.45 (J = 6.85 Hz)</td>
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</table>

References and Notes


11) Benzyl β-α-galactopyranoside (4b): 1H-NMR (DMSO) δ: 7.38—7.40 (2H, d, J=6.95 Hz), 7.30—7.35 (2H, m), 7.25—7.28 (1H, m), 4.89 (1H, d, J=4.85 Hz), 4.79 (1H, d, J=12.30 Hz), 4.65 (1H, d, J=5.45 Hz), 4.53—4.58 (2H, m), 4.33 (1H, d, J=4.70 Hz), 4.18 (1H, d, J=7.70 Hz), 3.64 (1H, t, J=3.65 Hz), 3.49—3.57 (2H, m), 3.25—3.27 (1H, m). IR (KBr) cm⁻¹: 3412, 2943, 1650, 1544, 1418, 1211, 1141, 1077, 1040. Anal. Calcd for C₁₃H₁₈O₆: C, 57.77; H, 6.71. Found: C, 57.68; H, 6.80.

12) (3,5-Dimethoxyphenyl)methyl β-α-glucopyranoside (4e): 1H-NMR (DMSO) δ: 7.25—7.29 (4H, m), 7.17—7.20 (1H, m), 3.91—3.96 (1H, m), 3.62—3.68 (2H, m), 3.40—3.45 (1H, m), 4.91 (1H, d, J=4.95 Hz), 4.86 (1H, d, J=4.86 Hz), 4.83 (1H, d, J=5.10 Hz), 4.43 (1H, t, J=6.01 Hz), 4.17 (1H, d, J=7.80 Hz), 3.11—3.15 (1H, m), 3.07—3.10 (1H, m), 3.01—3.05 (1H, m), 2.93—2.98 (1H, m), 2.83—2.87 (2H, m). IR (KBr) cm⁻¹: 3362, 1610, 1515, 1210, 1072, 1030. Anal. Calcd for C₁₅H₂₂O₈: C, 54.54; H, 6.71. Found: C, 54.62; H, 6.80. (3,5-Dimethoxyphenyl)methyl β-α-glucopyranoside (4s): 1H-NMR (D₂O) δ: 6.73 (2H, s), 6.58 (1H, s), 4.89 (1H, d, J=12 Hz), 4.74 (1H, d, J=12.5 Hz), 4.51 (1H, d, J=8 Hz), 3.94 (1H, d, J=12 Hz), 3.84 (6H, s), 3.70—3.75 (1H, m), 3.38—3.49 (3H, m), 3.31 (1H, t, J=8.5 Hz). IR (KBr) cm⁻¹: 3377, 2937, 2836, 1596, 1472, 1210, 1160, 1038, 1012. Anal. Calcd for C₁₅H₂₂O₈: C, 54.54; H, 6.71. Found: C, 54.72; H, 6.80. (3,5-Dimethoxyphenyl)methyl β-α-galactopyranoside (4t): 1H-NMR (D₂O) δ: 6.74 (2H, s), 6.59 (1H, s), 4.90 (1H, d, J=12 Hz), 4.75 (1H, d, J=12.5 Hz), 4.45 (1H, d, J=8 Hz), 3.92 (1H, s), 3.85 (6H, s), 3.77—3.81 (2H, m), 3.61—3.69 (2H, m), 3.59 (1H, t, J=8.5 Hz). IR (KBr) cm⁻¹: 3384, 2937, 2878, 1601, 1473, 1208, 1157, 1048, 1017. Anal. Calcd for C₁₅H₂₂O₈: C, 54.54; H, 6.71. Found: C, 54.43; H, 6.58.
