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Disciplines
Medicinal-Pharmaceutical Chemistry | Veterinary Microbiology and Immunobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

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Heterocycles from Wine: Synthesis and Biological Evaluation of Salidrosides

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Abstract

Wine is composed of a variety of tannins, of which a sub-class includes salidrosides, which are largely uninvestigated compounds. The first syntheses of galloylated salidrosides are reported in 7 steps from commercially available starting materials through a platform approach. The antimicrobial activity of the salidrosides against *E. coli* strains is described.

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1. Introduction

Wine has a complex and varied chemical composition from which numerous health benefits arise upon moderate consumption. Advances in analytical instrumentation have revealed potent bioactive components that contribute to wine’s positive effects despite their presence in low levels.\(^1\) Natural heterocycles in wine were featured in an extensive review by Wamhoff and Gribble\(^2,3\) which are associated with color, astringency, and oxidation rate during the aging process.\(^3\) Another class of biologically-active compounds in red wine includes polyphenols like resveratrol (Figure 1). As a class, polyphenols broadly exhibit antimicrobial and antioxidant activity and prevent cardiovascular disorders; *in vitro* studies also support their inhibition of cancer cell growth.\(^4-7\) The polyphenols termed tannins become incorporated into wine when it is stored in tannin-rich oak barrels.\(^8,9\) As astringents, tannins not only enhance the perceived taste and smell of the wine, but they also decrease blood pressure and facilitate blood clotting.\(^10\)

![Structures of selected natural polyphenols found in wine](image)

Figure 1. Structures of selected natural polyphenols found in wine – resveratrol (1), quercetin (2), catechin (3), and tyrosol (4)

Hydrolyzable tannins include gallotannin and the emerging class of ellagitannin natural products, including penta-*O*-galloyl-D-glucopyranoside (5) and tellimagrandin I (6), respectively, which are based on their defining core structures (Figure 2). While ellagitannins are characterized by galloyl groups connected by oxidation to become hexahydroxydiphenoyl units, gallotannins generally have galloyl moieties in free rotation. The structures of over a thousand naturally-occurring ellagitannins have been elucidated; their challenging molecular architecture and biological activity have made them popular synthetic targets for organic chemists.\(^11-14\)
Salidrosides contain a \( p \)-hydroxyphenethyl alcohol unit connected through an ether linkage at C-1” to form a tyrosol glycoside. In China, naturally-occurring salidrosides have been commonly added to wines to produce “health wines” which are claimed to promote immunity and additionally have anti-fatigue and antioxidant properties. The simplest salidroside (7), the most well-studied example, could potentially treat neurodegenerative diseases since it ameliorates cell apoptosis triggered by oxidative stress.

While the scientific literature has detailed studies of the biological activity of salidroside (7), the structure-activity relationship of more substituted analogues have remained largely uninvestigated mainly due to the inadequate availability of comprehensive libraries. Based on the structural trend observed with other tannins, we predicted that galloylated salidrosides would influence the resulting biological activity. We created a platform approach to access galloyl-substituted salidroside members, for which syntheses have never been reported. We envisioned that providing unique polyphenol compounds would allow researchers to systematically and more thoroughly analyze the structure-activity relationship.

The more complex salidroside derivatives we targeted contain galloyl units attached to the glucose moiety, which include \( 2'',3'',4'',6'' \)-tetra-\( O \)-galloyl salidroside (8) and the \( 6''-O \)-galloyl salidroside (9) which were isolated from the bark of *Quercus stenophylla* Makino (Figure 3).

**Figure 2.** Representative gallotannin, ellagitannin, and salidroside structures.

**Figure 3.** Galloyl-substituted salidrosides from *Quercus stenophylla* Makino.
2. Results and Discussion

We first began by synthesizing salidroside (7) to obtain a synthetic sample to use as a comparison for the biological activity analyses. We designed a synthetic strategy to take advantage of the common intermediate 2-(4-benzyloxyphenyl)ethyl β-D-glucopyranoside (11) which could readily be manipulated to generate diverse analogues. Our synthesis towards intermediate 11 commenced by coupling 2-(4-benzyloxyphenyl)ethanol22 and commercially available 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide 10 mediated by Ag₂CO₃. The resultant crude mixture was directly deacetylated using sodium methoxide to generate 11. Hydrogenation with palladium on carbon generated salidroside 7 in 83% yield, where all characterization data matched those previously reported in the literature (Scheme 1).23

After achieving salidroside (7) as a benchmark for comparison, the synthesis of 8 began by treating 11 with 3,4,5-tribenzyloxybenzoyl chloride, which in turn was prepared in three steps from methyl gallate.24 Tribenzylgalloyl groups were installed in the presence of DMAP, generating salidroside 12 in 79% yield, which was followed by global deprotection to furnish 8 in 72% yield.

Finally, the synthesis of 9 required selective esterification of 11. We initially attempted to facilitate the controlled addition of the tribenzyloxybenzoyl group to 11 with Me₂SnCl₂ and DIPEA; however, the reaction conditions were not selective and only provided complex mixtures. To improve the selective esterification on the C-6” position, we modified the conditions previously used to obtain 12 and reduced the equivalents of DMAP relative to the 3,4,5-tribenzyloxybenzoyl chloride to provide 13 in 24% yield after a period of 72 hours. Salidroside 9 was achieved in 80% yield after hydrogenation.

Scheme 1. Reaction conditions: (a) 2-(4-Benzylxyloxyphenyl)ethanol, Ag₂CO₃, CH₂Cl₂, rt, 24 h; (b) MeONa/MeOH, rt, 16 h, 55% over two steps; (c) H₂, Pd/C, THF, 40 °C, 24 h, 83%; (d) 3,4,5-Tribenzyloxybenzoyl chloride, DMAP, MeCN, rt, 24 h, 79%; (e) 3,4,5-Tribenzyloxybenzoyl chloride,
DMAP, pyridine, rt, 72 h, 24%; (f) H₂, Pd/C, THF, 40 °C, 24 h, 72%; (g) H₂, Pd/C, THF, 40 °C, 24 h, 80%.

Once the salidroside analogues were prepared, they were ready for biological testing. The antimicrobial activity of salidrosides 7, 8, and 9 was tested using a disc diffusion assay as described by us previously using wild-type *Escherichia coli* K-12 (strain MG1655). Tests were repeated three times to check for reproducibility. The diameter measurements of the zones of inhibition for *E. coli* MG1655 were as follows: salidroside 7: 0 mm; salidroside 8: 18 mm; salidroside 9: 22 mm. The antibiotic ampicillin (100 µg/ml) gave a zone of inhibition of 35 mm while the DMSO solvent control gave 0 mm.

### 3. Conclusion

In summary, wine is comprised of a complex portfolio of compounds that include polyphenols. Among the polyphenols, the class of tannins has emerged as an attractive research topic to discover potential benefits to human health. Specifically, the biological activity of salidrosides has remained uninvestigated. We report the first synthesis of galloyl-substituted salidrosides in 7 steps from commercially available starting materials. The synthetic strategy utilizes a platform intermediate from which additional diversity can be generated. Galloylated salidrosides 8 and 9 revealed that they exhibit effective antimicrobial activity which could be applied toward the synthesis of additional analogues to investigate the structure-activity relationship.

### 4. Experimental Section

#### 4.1. General Methods

All starting materials were purchased from Sigma-Aldrich; solvents were purchased from Fisher Scientific and used without further purification. Palladium, 5% on activated carbon was purchased from Strem Chemicals (product number: 46-1747). All reactions were carried out in flame-dried glassware under argon with dry solvents under anhydrous conditions unless otherwise specified. All yields refer to chromatographically isolated products. Reactions were monitored by thin-layer chromatography (TLC) and carried out on 0.20 mm silica gel plates using UV light as a visualizing agent and either potassium permanganate or 5% sulfuric acid in methanol with heat as developing agents. Silica gel 60A, particle size 0.032 – 0.063 mm, was used for flash column chromatography. ¹H and ¹³C NMR spectra were acquired on a Varian MR-400 or Bruker Avance III 600 MHz spectrometer. ¹H and ¹³C chemical shifts (δ) are given in ppm relative to the residual protonated solvent peak (CDCl₃: δH = 7.26 ppm, δC = 77.0 ppm; CD₃OD: δH = 3.31 ppm, δC = 49.0 ppm; (CD₃)₂SO: δH = 2.50 ppm, δC = 39.52 ppm; (CD₃)₂CO: δH = 2.05 ppm, δC = 29.84 ppm) as an internal reference. High-resolution mass spectra (HRMS) were recorded on an Agilent 6540 QTOF (quadrupole time of flight) mass spectrometer using ESI (electrospray ionization).

#### 4.2. 2-(4-benzyloxyphenyl)ethyl β-D-glucopyranoside (11)

Over the course of the reaction, the flask was protected from light. To a solution of 2-(4-benzyloxyphenyl)ethanol (1.37 g, 6.0 mmol) in dichloromethane (7.5 mL) was added molecular sieves (4Å, ~1.0 g) and Ag₂CO₃ (1.38 g, 5.0 mmol). The resulting mixture was stirred for 15 minutes at room temperature, then the solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (2.06 g, 5.0 mmol) in dichloromethane (7.5 mL) was added dropwise over 5 min. The resulting mixture was stirred at room temperature for 24 h then filtered through a pad of Celite. The filtrate was then washed
with aqueous NaHCO₃ (20 mL) and brine (20 mL), dried over sodium sulfate, and concentrated in vacuo. The crude product was subjected to the next reaction without purification. The crude solid was dissolved in methanol (25 mL) after which NaOMe (0.40 g, 7.5 mmol) was added. The resulting mixture was stirred for 16 h at room temperature. Concentrated HCl was added dropwise to adjust the pH to 6-7 after which the neutralized mixture was then concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, MeOH:CH₂Cl₂ 1% to 10%) to give 11 (1.11 g, 55%) as a white solid; m.p. 88-89 °C; R_f (10% MeOH/CH₂Cl₂) 0.16; dH (400 MHz CD₃OD) 7.42 (2H, d, J 6.9 Hz), 7.36 (2H, t, J 7.3 Hz), 7.29 (1H, t, J 7.2 Hz), 7.17 (2H, d, J 8.5 Hz), 6.90 (2H, d, J 8.6 Hz), 5.05 (2H, s), 4.29 (1H, d, J 7.8 Hz), 4.05 (1H, dt, J 9.5, 7.4 Hz), 3.89 – 3.83 (1H, m), 3.75 – 3.69 (1H, m), 3.66 (1H, dd, J 11.9, 5.3 Hz), 3.37 – 3.33 (1H, m), 3.28 – 3.24 (2H, m), 3.18 (1H, t, J 7.6 Hz), 2.87 (2H, t, J 7.8 Hz); dC (100 MHz, CD₃OD): 158.5, 138.6, 132.1, 130.9, 129.3, 128.4, 115.7, 104.1, 77.9, 77.7, 74.9, 71.8, 71.4, 70.8, 62.6, 36.2; HRMS (ESI -TOF): [M-H] -, found 389.1607. C₂₁H₂₅O₇ requires 389.1606.

4.3. 2-(4-hydroxyphenyl)ethyl β-D-glucopyranoside or salidroside (7)

A suspension of 11 (0.23 g, 0.60 mmol) and palladium (100 mg, 0.047 mmol, 5 wt % on activated carbon) in dry THF (10 mL) was stirred at 40 °C under a hydrogen gas atmosphere for 24 h. After cooling, the reaction mixture was filtered through a pad of Celite. The filtrate was then concentrated in vacuo. The crude product was crystallized from water to give 7 (0.15 g, 83%) as a white solid; m.p. 156-158 °C; dH (400 MHz CD₃OD) 7.06 (2H, d, J 8.4 Hz), 6.69 (2H, d, J 8.4 Hz), 4.29 (1H, d, J 7.8 Hz), 4.07 – 3.99 (1H, m), 3.92 – 3.81 (1H, m), 3.74 – 3.61 (2H, m), 3.38 – 3.32 (1H, m), 3.29 – 3.25 (2H, m), 3.18 (1H, dd, J 11.2 Hz), 2.87 – 2.79 (2H, m); dC (100 MHz, CD₃OD): 156.8, 130.9, 130.7, 116.1, 104.3, 78.1, 77.9, 75.1, 72.1, 71.6, 62.7, 36.4; HRMS (ESI -TOF): [M-H] -, found 299.1138. C₁₄H₁₉O₇ requires 299.1136. (All characterization data matched those previously reported in the literature.)

4.4. 2-(4-benzyloxyphenyl)ethyl 2″,3″,4″,6″-tetra-O-(3,4,5-tribenzyloxybenzoyl)-β-D-glucopyranoside (12)

A suspension of 11 (0.39 g, 1.0 mmol) and 3,4,5-tribenzyloxybenzoyl chloride (1.84 g, 4.0 mmol) in acetonitrile (50 mL) was stirred at room temperature. After 15 minutes, DMAP (0.51 g, 4.2 mmol) was added and the mixture continued stirring for an additional 24 h at room temperature. The reaction mixture was then concentrated in vacuo. The crude product was dissolved in toluene (20 mL) at 60 °C. After cooling, the solution was decanted and purified by flash column chromatography (silica gel, MeOH:CH₂Cl₂ 1% to 10%) to give 12 (1.64 g, 79%) as a white solid; m.p. 150-152 °C; dH (400 MHz CDCl₃) 7.43 – 7.15 (73H, m), 6.97 (2H, d, J 8.4 Hz), 6.64 (2H, d, J 8.5 Hz), 5.86 (1H, t, J 9.9 Hz), 5.63 (1H, t, J 9.7 Hz), 5.54 (1H, dd, J 10.0, 7.8 Hz), 5.10 – 4.98 (18H, m), 4.96 (2H, s), 4.91 (4H, s), 4.84 (2H, s), 4.82 (1H, s), 4.76 (1H, dd, J 12.1, 3.5 Hz), 4.39 (1H, dd, J 12.1, 5.4 Hz), 4.21 – 4.07 (2H, m), 3.74 – 3.65 (1H, m), 2.89 – 2.71 (2H, m); HRMS (ESI-TOF): [M+K] +, found 2117.7392. C₁₃₃H₁₁₄O₂₃K requires 2117.7382.

4.5. 2-(4-hydroxyphenyl)ethyl 2″,3″,4″,6″-tetra-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside (8)

A suspension of 12 (0.60 g, 0.29 mmol) and palladium (1.10 g, 0.52 mmol, 5 wt % on activated carbon) in dry THF (60 mL) was stirred at 40 °C under hydrogen gas atmosphere for 24 h. After cooling, the reaction mixture was filtered through a pad of Celite. The filtrate was then concentrated
in vacuo. The crude product was dissolved in ethyl acetate (100 mL) and washed with a saturated aqueous solution of (NH₄)₂SO₄ (2x20 mL). The organic layer was then dried over Na₂SO₄, and concentrated in vacuo. The crude product was crystallized from water to give 8 (0.19 g, 72%) as a white solid; m.p. 190-192 °C; dH (400 MHz (CD₃)₂CO) 7.20 (2H, s), 7.09 (2H, s), 7.03 (2H, s), 6.96 (2H, d, J 8.4 Hz), 6.94 (2H, s), 6.61 (2H, d, J 8.2 Hz), 6.52 (1H, t, J 9.7 Hz), 5.34 (1H, dd, J 9.9, 8.2 Hz), 5.08 (1H, d, J 8.0 Hz), 4.52 (1H, dd, J 12.3, 2.1 Hz), 4.38 (1H, dd, J 12.2, 5.1 Hz), 4.34 – 4.28 (1H, m), 3.98 (1H, dt, J 10.2, 7.1), 3.75 (1H, dt, J 10.0, 7.3 Hz), 2.78 – 2.67 (2H, m).

dC (100 MHz, (CD₃)₂CO): 166.4, 165.9, 165.7, 165.5, 165.3, 146.0, 145.9, 145.8, 139.3, 139.1, 139.0, 130.7, 130.0, 121.5, 121.1, 120.5, 115.9, 110.2, 110.1, 110.0, 110.0, 101.6, 73.6, 73.0, 72.5, 71.7, 69.8, 63.1, 35.9; HRMS (ESI-TOF): [M+Na]+, found 931.1546. C₄₂H₃₆O₂₃Na requires 931.1546.

4.6. 2-(4-benzyloxyphenyl)ethyl 6″-O-(3,4,5-tribenzyloxybenzoyl)-β-D-glucopyranoside (13)

To a solution of 11 (100 mg, 0.26 mmol) and DMAP (15.6 mg, 0.13 mmol) in anhydrous pyridine (5.0 mL) was added 3,4,5-tribenzyloxybenzoyl chloride²⁴ (344 mg, 0.75 mmol) in small portions over 1 h at room temperature. After stirring the reaction for 72 h at room temperature, the reaction mixture was concentrated in vacuo. The crude product was dissolved in dichloromethane (50 mL), washed with HCl (0.1M, 2x25 mL), aqueous NaHCO₃ (25 mL), and brine (2x25 mL). After drying over Na₂SO₄, the organic layer was concentrated in vacuo. The crude product was purified by preparative TLC (silica gel, MeOH:CH₂Cl₂ 1:9) to give 13 (54 mg, 24%) as a yellow solid; m.p. 123-125 °C; Rf (10% MeOH/CH₂Cl₂) 0.55; dH (600 MHz (CD₃)₂CO) δ 7.54 – 7.25 (22H), 7.08 (2H, d, J 8.3 Hz), 6.82 (2H, d, J 8.4 Hz), 4.10 (1H, d, J 7.7 Hz), 3.93 (1H, q, J 8.3 Hz), 3.71 (1H, q, J 8.4 Hz), 3.66 (1H, t, J 6.9 Hz), 3.47 (2H, dd, J 5.2, 3.3 Hz), 3.28 (1H, t, J 8.0 Hz), 2.85 – 2.76 (2H, m); dC (150 MHz, (CD₃)₂CO) 166.2, 158.2, 153.5, 143.2, 138.8, 138.5, 137.9, 131.9, 130.7, 129.3, 129.2, 128.9, 128.8, 128.6, 128.5, 128.3, 126.4, 115.4, 109.6, 104.3, 78.0, 75.5, 74.9, 74.8, 71.7, 71.6, 71.5, 70.3, 65.2, 36.2; HRMS (ESI-TOF): [M+Na]+ found 835.3108. C₄₉H₄₈O₁₁Na requires 835.3089.

4.7. 2-(4-hydroxyphenyl)ethyl 6″-O-(3,4,5-trihydroxybenzoyl)-β-D-glucopyranoside (9)

A suspension of 13 (20 mg, 0.025 mmol) and palladium (50 mg, 0.023 mmol, 5 wt % on activated carbon) in dry THF (3 mL) was stirred at 40 °C under a hydrogen gas atmosphere for 24 h. After cooling, the reaction mixture was filtered through a pad of Celite. The filtrate was then concentrated in vacuo. The crude product was purified by preparative TLC (silica gel, MeOH:CH₂Cl₂: 1:9) to give 9 (9 mg, 80%) as a yellow oil; Rf (10% MeOH/CH₂Cl₂) 0.1; dH (400 MHz (CD₃)₂CO) 7.15 (2H, s), 7.04 (2H, d, J 7.7 Hz), 6.71 (2H, d, J 7.4 Hz), 4.54 (1H, d, J 11.8 Hz), 4.39 (2H, dd, J 18.1, 6.2 Hz), 3.92 (1H, q, J 9.0, 6.5 Hz), 3.71 – 3.56 (2H, m), 3.51 – 3.41 (2H, m), 3.23 (1H, t, J 7.8 Hz), 2.77 (2H, t, J 7.3 Hz). dC (100 MHz, (CD₃)₂CO) 166.7, 156.5, 146.0, 138.7, 130.7, 130.2, 130.0, 121.8, 115.9, 109.9, 104.3, 77.8, 74.9, 74.8, 71.5, 71.2, 64.4, 36.1; HRMS (ESI-TOF): [M-H]- found 451.1237. C₂₉H₂₉O₁₁Na requires 451.1246. (All characterization data matched those previously reported in the literature.)

References and notes