SUPPLEMENTARY MATERIAL

Three new resin glycosides compounds from *Argyreia acuta* and their α-glucosidase inhibitory activity

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**Abstract**

Three new phenolic compounds, acutacoside C (1), acutacoside D (2), and acutacoside E (3) were isolated from the airial part of *Argyreia acuta*. The oligosaccharide chain was composed with two glucose and three rhamnoses, and the aglycone was (11S)-hydroxyhexadecanoic acid (jalapinolic acid). The core of the three compounds was operculinic acid B, which was rare in resin glycosides. Their structures were established by a combination of spectroscopic and chemical methods. Compounds 1–3 have been evaluated for inhibitory activity against $\alpha$-glucosidase, which all showed weak inhibitory activities.

**Keywords:** *Argyreia acuta*, resin glycosides, structural identification, $\alpha$-glucosidase
| Position | 1          | 2          | 3          |
|----------|------------|------------|------------|
|          | $^{13}$C   | $^1$H      | $^{13}$C   | $^1$H      | $^{13}$C   | $^1$H      |
| Glu-1    | 104.2      | 5.03 d (7.5) | 104.4      | 5.12 d (8.0) | 104.      | 4.93 d (6.5) |
| 2        | 81.7       | 3.93 *      | 82.0       | 3.89 *      | 82.0      | 3.93 *      |
| 3        | 76.3       | 4.29 *      | 76.6       | 4.19 *      | 76.6      | 4.20 *      |
| 4        | 71.6       | 4.24 *      | 71.8       | 4.14 *      | 71.8      | 4.14 *      |
| 5        | 77.8       | 3.97 *      | 78.0       | 3.87 *      | 78.0      | 3.87 *      |
| 6        | 62.6       | 4.42 *      | 62.8       | 4.31 *      | 62.8      | 4.33 *      |
| Rha-1    | 98.3       | 5.71 br s   | 98.6       | 5.61 br s   | 98.5      | 5.62 br s   |
| 2        | 73.3       | 6.15 br s   | 73.5       | 6.06 br s   | 73.5      | 6.05 br s   |
| 3        | 69.1       | 5.18 dd (9.5, 3.5) | 69.5 | 5.07 dd (9.0, 3.5) | 69.3 | 5.08 dd (9.5, 3.5) |
| 4        | 81.7       | 4.31 dd (9.5, 9.5) | 81.0 | 4.21 dd (9.5, 9.0) | 82.0 | 4.21 dd (9.5, 9.5) |
| 5        | 68.8       | 4.49 *      | 69.0       | 4.37 *      | 69.1      | 4.39 *      |
| 6        | 18.9       | 1.72 d (6.0) | 19.2       | 1.62 d (6.0) | 19.1      | 1.62 d (6.0) |
| Rha'-1   | 100.0      | 5.99 br s   | 99.7       | 6.34 br s   | 100.      | 5.87 br s   |
| 2        | 72.8       | 6.44 br s   | 72.7       | 6.32 br s   | 73.0      | 6.36 br s   |
| 3        | 79.7       | 4.93 dd (9.0, 3.0) | 80.0 | 4.84 dd (9.5, 3.5) | 79.9 | 4.83 dd (8.5, 3.0) |
| 4        | 78.2       | 4.26 dd (9.0, 9.0) | 78.3 | 4.17 dd (9.5, 9.5) | 78.4 | 4.16 dd (8.5, 8.5) |
| 5        | 68.3       | 4.62 *      | 68.4       | 4.44 *      | 68.5      | 4.50 *      |
| 6        | 18.8       | 1.76 d (5.5) | 19.1       | 1.69 d (6.5) | 19.0      | 1.67 d (5.5) |
| Rha''-1  | 103.2      | 6.41 br s   | 99.8       | 5.96 br s   | 103.      | 6.31 br s   |
| 2        | 69.7       | 5.38 br s   | 74.1       | 6.29 br s   | 69.9      | 5.29 br s   |
| 3        | 73.1       | 6.11 dd (3.0, 10.0) | 68.2 | 4.79 dd (3.5, 10.0) | 73.3 | 6.02 dd (3.0, 10.0) |
| 4        | 71.5       | 6.19 t (10.0) | 74.7      | 5.82 t (10.0) | 71.7      | 6.11 t (10.0) |
| 5        | 67.9       | 4.56 *      | 68.2       | 4.49 dd (10.0, 6.5) | 68.2 | 4.44 *      |
| 6        | 17.7       | 1.55 d (6.0) | 18.0       | 1.54 d (6.5) | 17.9      | 1.45 d (6.5) |
| Glu'-1   | 105.3      | 5.24 d (7.2) | 105.0      | 4.92 d (7.5) | 105.      | 5.14 d (7.5) |
| 2        | 75.0       | 4.08 *      | 75.0       | 3.89 *      | 75.2      | 3.94 *      |
| 3        | 78.7       | 4.19 *      | 78.4       | 4.19 *      | 79.0      | 4.07 *      |
| 4        | 71.3       | 4.05 *      | 71.4       | 4.14 *      | 71.5      | 3.97 *      |
| 5        | 77.9       | 3.92 *      | 78.3       | 3.82 *      | 78.1      | 3.82 *      |
| 6        | 62.7       | 4.54 *      | 63.0       | 4.40 *      | 63.0      | 4.44 *      |
| Ag-1     | 173.0      |            | 173.4      |            | 173.      |            |
| 2        | 34.0       | 2.46 m, 2.39 m | 34.3 | 2.47 m, 2.33 m | 34.3      | 2.37 m, 2.30 m |
| 11       | 82.5       | 4.00 *      | 82.8       | 3.99 *      | 82.7      | 3.90 *      |
| 16       | 14.1       | 0.83 t (7.5) | 14.3       | 0.86 t (7.0) | 14.3      | 0.83 t (7.5) |
| Cna-1    | 166.2      |            | 167.0      |            | 166.      |            |
| 2        | 118.3      | 6.70 d (16.0) | 118.7      | 6.37 d (16.0) | 118.      | 6.60 d (16.0) |
| 3        | 145.3      | 7.97 d (16.0) | 145.2      | 7.66 d (16.0) | 145.      | 7.87 d (16.0) |
| 1''      | 134.6      |            | 134.6      |            | 134.      |            |
| 2' and 6' | 128.3      | 7.56 m     | 128.5      | 7.26 m     | 128.      | 7.46 m     |
| 3' and 5' | 129.0      | 7.45 m     | 129.0      | 7.17 m     | 129.      | 7.35 m     |
| 4'       | 130.5      | 7.45 m     | 130.5      | 7.17 m     | 130.      | 7.35 m     |
| Mba-1    | 175.8      |            | 176.4      |            | 176.      |            |
| 2        | 41.3       | 2.59 m     | 41.2       | 2.53 m     | 41.6      | 2.49 m     |
| 2-CH₃ | 16.7 | 1.25 d (7.0) | 17.0 | 1.22 d (7.0) | 17.0 | 1.15 d (7.0) |
| 4    | 11.6 | 0.95 t (7.0) | 11.7 | 0.92 t (7.0) | 11.8 | 0.87 t (7.0) |
|      |       |           |      |              |      |              |
| Deca-1 | 173.4 |            |      |              |      |              |
| 2    | 34.2 | 2.43 m     |      |              |      |              |
| 12   | 14.1 | 0.93 t (5.5)|      |              |      |              |
| Dodeca-1 | 173.9 | 173. |      |              |      |              |
| 2    | 34.4 | 2.37 m     | 34.4 | 2.35 m      |      |              |
| 12   | 14.3 | 0.86 t (7.0)| 14.3 | 0.83 t (7.5)|      |              |

Chemical shifts (δ) are in ppm relative to TMS. The spin coupling (J) is given in parentheses (Hz). Chemical shifts marked with an asterisk (*) indicate overlapped signals. Spin-coupled patterns are designated as follows: br s = broad singlet, d = doublet, t = triplet, m = multiplet.

Abbreviations: Glc = glucose; Rha = rhamnose; Ag = 11-hydroxyhexadecanoyl; Mba = 2S-methylbutanoyl; Cna = trans-cinnamoyl; Deca = n-decanoyl; Dodeca = n-dodecanoyl.

### Table S2 α-Glucosidase inhibition of compounds 1–3 and acarbose

| Compound | α-Glucosidase Inhibition Contstant (IC₅₀) [μM] |
|----------|-----------------------------------------------|
| 1        | 188.6 ± 5.2                                   |
| 2        | 157.8 ± 4.6                                   |
| 3        | 174.4 ± 3.9                                   |
| acarbose | 388.0 ± 8.5                                   |

*IC₅₀ is defined as the concentration that resulted in a 50% α-glucosidase inhibition and the results are means ± standard deviation of three independent replicates; ° Positive control substance.*

Figure S1. Key HMBCs from H to C for Acutacoside C (1)
The identification procedures of organic acids, sugars, and aglycone:

Compounds 1–3 (7 mg each) in 5% KOH (3 mL) were refluxed at 90 °C for 2 h, respectively. The reaction mixture was acidified to pH 4.0 with 2 mol/L HCl and extracted with hexane (3 mL × 2) and n-BuOH (3 mL × 2). The organic layer was washed with H2O, dried over anhydrous Na2SO4, then methylated following. The hexane extract, was combined with 0.1 mL 0.5 M CH3ONa solution, then shaken for 5 min at room temperature, before adding 5 μL CH3COOH and 1 g anhydrous CaCl2 powder, heating for 1 h, followed by centrifugation for 2–3 min at 2000–3000 rpm.min⁻¹. The supernatant was analyzed by GC-MS on a TRACE GC ULTRA DSQ II instrument under the following conditions: 30 m × 0.25 mm × 0.25 μm, TG-5MS (Thermo) column; He, 0.8 mL/min; 40 °C, 3min; 50–310 °C, Δ0 °C/min, 70 eV. 2-Methylbutyric acid methyl ester (tR 4.39 min) m/z [M+H]+ 117 (5), 101 (23), 88 (96), 57 (100), 41 (55), 29 (45), 27 (19), and trans-cinnamic acid methyl ester (tR 13.29 min) m/z [M]+ m/z162 (40), 131 (100), 103 (66), 77 (32), from 1-3 was identified. n-decanoic acid methyl ester (tR 12.37 min): m/z 172 [M]+ (4), 155 (5), 143 (30), 129 (5), 87 (59), 74 (100), 55 (18) from 1 was identified. n-dodecanoyl acid methyl ester (tR15.17 min) m/z [M]+ 200 (1), 172 (1), 168 (10), 157 (15), 143(18), 129 (7), 87 (64), 74 (100), 55 (25), 43 (20), 41 (18) from 2-3 was identified. The 2-methylbutanoic acid as proved to be S configuration by comparing the specific rotation with that of authentic 2S-methylbutanoic acid (Yin, Y.Q., Wang, J.S., Luo, J.G., Kong, L.Y., 2009). Acidic hydrolysis of operculinic acid B liberated the aglycone, 11-hydroxyhexadecanoic acid, which was identifidey S-configuration (Yin, Y.Q., et al., 2008) and the monosaccharides mixture was derivatized and detected with GC-MS by comparison with those of authentic samples to improve as D-fucose, L-rhamnose and D-glucose (Luo, J.G., Ma, L., Kong, L.Y., 2008).
Figure S2. The HR-TOF-MS spectrum of compound 1

Figure S3. The $^1$H-NMR spectrum of compound 1
Figure S4. The $^{13}$C-NMR spectrum of compound 1
Figure S5. The TOCSY spectrum of compound 1

Figure S6. The HSQC spectrum of compound 1

Figure S7. The HMBC spectrum of compound 1
Figure S8. The enlarged HMBC spectrum of compound 1

Figure S9. The HR-TOF-MS spectrum of compound 2
Figure S10. The $^1$H-NMR spectrum of compound 2

Figure S11. The $^{13}$C-NMR spectrum of compound 2
Figure S12. The TOCSY spectrum of compound 2

Figure S13. The HSQC spectrum of compound 2
Figure S14. The HMBC spectrum of compound 2

Figure S15. The enlarged HMBC spectrum of compound 2
Figure S16. The HR-TOF-MS spectrum of compound 3

Figure S17. The $^1$H-NMR spectrum of compound 3
Figure S18. The $^{13}$C-NMR spectrum of compound 3

Figure S19. The TOCSY spectrum of compound 3
Figure S20. The HSQC spectrum of compound 3

Figure S21. The HMBC spectrum of compound 3
Figure S22. The enlarged HMBC spectrum of compound 3

Butanoic acid, 2-methyl-, methyl ester
Formula C6H12O2, MW 116, CAS # 868-57-5, Entry# 23242

Butyric acid, 2-methyl-, methyl ester

Decanoic acid, methyl ester
Formula C11H22O2, MW 186, CAS # 110-42-9, Entry# 38299
Capric acid methyl ester
Fig S23. The GC-MS spectral of organic acid and sugar