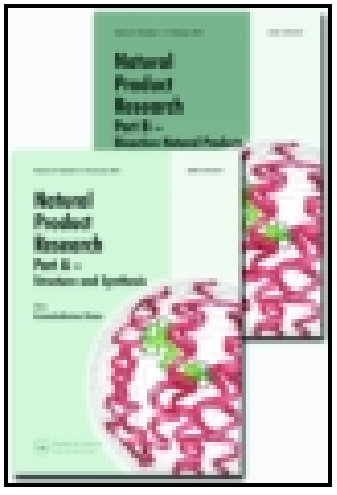

Natural Product Research: Formerly Natural Product Letters

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

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Published online: 30 Apr 2015.

Natural Product Research, 2015
http://dx.doi.org/10.1080/14786419.2015.1030739

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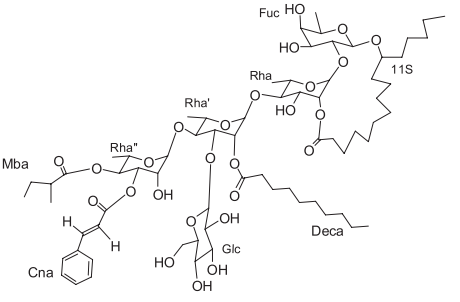
(*Received 28 November 2014; final version received 13 March 2015*)



Two new compounds of acutacosides **1** and **2**, pentasaccharide resin glycosides were isolated from the aerial parts of *Argyreia acuta*. The core of the two compounds was operculinic acid A, and they were esterfied at the same position, just one substituent group was linked at C-2 of Rha. The absolute configuration of the aglycone in the two compounds was established by Mosher's method, which was (11*S*)-hydroxyhexadecanoic acid (jalapinolic acid). Their structures were established by a combination of spectroscopic and chemical methods.

Keywords: resin glycoside; operculinic acid A; *Argyreia acuta*

1. Introduction

Argyreia acuta L. is a member of the genus *Argyreia* (Convolvulaceae; Editorial Committee of China flora of Chinese Academy of Sciences 1997). *A. acuta* is a common folk herbal medicine, with the efficacies of dispelling wind and eliminating dampness, relieving cough and reducing sputum, stopping the bleeding and promoting tissue regeneration, relaxing and activating the tendons, removing toxicity for eliminating carbuncles. Now a few compounds were found, and recent research found it has hemostasis properties (Cai & Xu 2013). The resin glycoside showed characteristics of Convolvulaceae; however, so far no resin glycoside has been reported from *A. acuta*. In this study, two new pentasaccharide resin glycosides, designated as acutacosides A (**1**) and acutacosides B (**2**), were isolated from the aerial parts of *A. acuta*. The new compounds are maclactones of operculinic acid A, partially esterified with Mba, Cna, Dodeca and Deca of different fatty acids. The lactone esterification site of the aglycone, jalpinolic acid, was attached to the second saccharide at C-2 in **1** and **2** (Figure 1). Their structures were elucidated on the basis of extensive spectroscopic data interpretation and chemical degradation.

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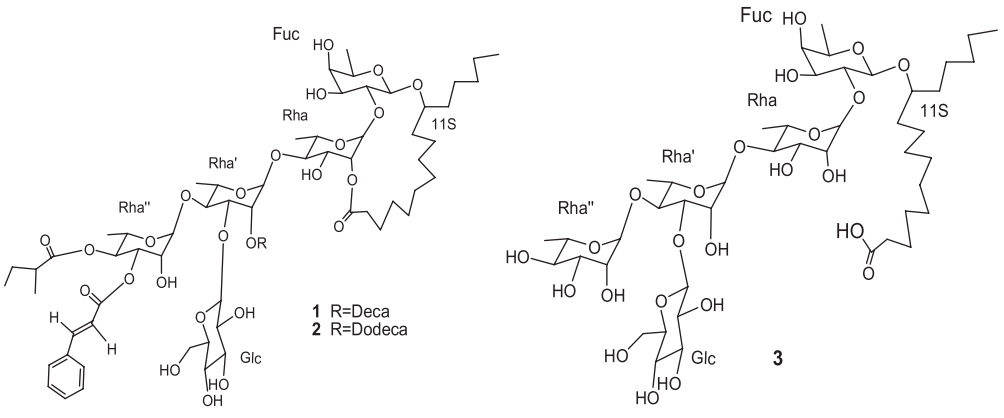

Figure 1. The structures of compounds **1**–**3**.

2. Results and discussion

A 95% EtOH extract of the dried aerial parts of *A. acuta* was partitioned between $CHCl_3$ and H_2O to afford a resinous fraction which was chromatographed over Si gel, Rp-18, Sephadex LH-20 and purified by preparative HPLC to afford compounds **1** and **2**, which were hydrolysed with alkaline and acid to afford organic acid and operculinic acid A (**3**). Peaks in the chromatograms were detected from the alkaline hydrolysis mixtures and identified by comparison with authentic samples as 2-methylbutyric acid methyl ester (t_R 4.39 min) *m/z* $[M + H]^+$ 117 (5), 101 (23), 88 (96), 57 (100), 41 (55), 29 (45), 27 (19), and transcinnamic acid methyl ester (t_R 13.29 min) *m/z* $[M]^+$ 162 (40), 131 (100), 103 (66), 77 (32), from **1** to **2** was identified. *n*-Decanoic acid (t_R 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 (t_R 15.17 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** was identified. The 2-methylbutanoic acid was proved to be *S*-configuration by comparing the specific rotation with that of authentic 2*S*-methylbutanoic acid (Yin et al. 2009). Acidic hydrolysis of operculinic acid A liberated the aglycone, 11-hydroxyhexadecanoic acid, which was identified *S*-configuration (Yin et al. 2008) and the monosaccharides mixture was derivatised and detected with GC–MS (gas chromatography-mass spectrometer) by comparison with those of authentic samples to improve as D-fucose, L-rhamnose and D-glucose (Luo et al. 2008).

Acutacosides A (**1**) and B (**2**), obtained as a white, amorphous powder. In HR-TOF-MS, a pseudomolecular ion peak at *m/z* 1391.7322 (100%) $[M + Na]^+$ (calculated for 1391.7339) was corresponding to the molecular formula $C_{70}H_{112}O_{26}$ (**1**) and a pseudomolecular ion peak at *m/z* 1419.7686 (100%) $[M + Na]^+$ (calculated for 1419.7652) was determined as the molecular formula $C_{72}H_{116}O_{26}$ (**2**). Its IR spectrum gave peaks of hydroxyl (3443 cm^{-1}), carbonyl (1723 cm^{-1}) and aromatic (1637 cm^{-1}) groups from **1** and peaks of hydroxyl (3442 cm^{-1}), carbonyl (1722 cm^{-1}) and aromatic (1637 cm^{-1}) groups from **2**. Alkaline hydrolysis of **1** and **2** afforded operculinic acid A (**3**) and dodecanoic, decanoic, 2-methylbutyric and *trans*-cinnamic acids. 2-Methylbutyric acid was found to have the *S*-configuration by comparison of its optical rotation value with that of an authentic sample. Acid hydrolysis afforded the monosaccharides mixture, which was derivatised and detected with GC–MS by comparing with those of authentic samples to improve as D-fucose, L-rhamnose and D-glucose. The ^{13}C NMR data of **1** and **2** were all the same from 177 to 41 ppm. All these evidences suggested that **1** and **2** were the substituents attaching the same sites, the only difference was in the decanoic group in **1** and dodecanoic group in **2**. The ^1H NMR data of **1** (Table S1) exhibited two *trans*-coupled olefinic protons at δ_H 6.60 (d, $J = 16.0$ Hz, H-2 of Cna) and 7.87 (d, $J = 16.0$ Hz, H-3 of Cna), a multiplet due to five

protons at δ_H 7.27–7.45 (m, C_6H_5 of Cna) and at δ_H 0.81 (t, $J = 7.0$ Hz, H-4 of Mba), 1.15 (d, $J = 7.4$ Hz, CH_3-2 of Mba) and 2.48 (m, H-2 of Mba). Also a methyl triplet signal at 0.83 ppm and a triplet-like signal in a methylene group at CH_2-2 (2.32 ppm) of a decanoyl group, and two signals at 2.28 (1H, m) and 2.44 (1H, m) ppm of the non-equivalent protons of the methylene group at C-2 in the aglycone moiety were observed, suggesting a macrocyclic lactone-type structure.

The ^{13}C NMR spectrum of **1** exhibited five signals at δ_C 105.8, 104.6, 103.6, 100.6 and 98.9 assigned to anomeric carbons of five sugar units and δ_C 176.3, 173.8, 173.4, and 166.8 for four ester carbonyl carbons. Then the anomeric protons at 5.09 (d, $J = 7.5$ Hz), 4.74 (d, $J = 7.4$ Hz), 6.27 (br s), 5.84 (br s) and 5.52 (br s) by HSQC data, respectively. All protons in each saccharide system were assigned by 2D NMR TOCSY, HMBC and HSQC experiments, leading to the identification of one glucopyranosyl unit, one fucopyranosyl unit and three rhamnopyranosyl units as the monosaccharides present in **1**. The interglycosidic connectivities were determined from the following HMBC (Figure S1): C-2 (80.2 ppm) of fucose with H-1 (5.52 ppm) of rhamnose; C-4 (82.5 ppm) of rhamose with H-1 (5.84 ppm) of rhamnose′; C-4 (80.1 ppm) of rhamnose′ with H-1 (6.27 ppm) of rhamnose″ and C-3 (79.3 ppm) of rhamnose′ with H-1 (5.09 ppm) of glucose. Esterification positions was determined by HMBC data, between H-4 of rhamnose″ (δ_H 6.09) and δ_C 176.0 (C-1 of Mba); H-3 of rhamnose″ (δ_H 6.01) and δ_C 166.4 (C-1 of Cna); H-2 of rhamnose′ (δ_H 6.33) and δ_C 173.8 (C-1 of Deca) and H-2 of rhamnose (δ_H 5.93) and δ_C 173.4 (C-1 of aglycone), respectively. The position of the jalapinolic acid unit was finally determined by HMBC between jalapinolic acid H-11 (3.83 ppm) and fucose C-1 (104.6 ppm), with which correlations established the structure of **1** (Figure S1). From these observations, the structure of acutacoside A (**1**) was elucidated as (*S*)-jalapinolic acid 11-*O*-a-L-rhamnopyranosyl-(1 → 3)-*O*-[3-*O*-*trans*-cinnamoyl-4-*O*-(*S*)-2-methylbutyryl-a-L-rhamnopyranosyl-(1 → 4)]-*O*-[2-*O*-*n*-decanoy]-a-L-rhamnopyranosyl-(1 → 4)-*O*-a-L-rhamnopyranosyl-(1 → 2)-*O*-*β*-D-fucopyranoside, intramolecular 1,2″-ester (Figure 1).

The NMR spectra (Table S1) of **2** were similar to those of **1**, the interglycosidic connectivities and esterification sites were all the same though by 2D NMR TOCSY, HMBC and HSQC, just one group of decanoyl was different by GC–MS experiments. Accordingly, the structure of **2** was elucidated as (*S*)-jalapinolic acid 11-*O*-a-L-rhamnopyranosyl-(1 → 3)-*O*-[3-*O*-*trans*-cinnamoyl-4-*O*-(*S*)-2-methylbutyryl-a-L-rhamnopyranosyl-(1 → 4)]-*O*-[2-*O*-*n*-dodecanoy]-a-L-rhamnopyranosyl(1 → 4)-*O*-a-L-rhamnopyranosyl-(1 → 2)-*O*-*β*-D-fucopyranoside, intramolecular 1,2″-ester (Figure 1).

3. Experimental

3.1. *General*

NMR spectra were recorded on INOVA 500 spectrometers (^1H NMR, HSQC and HMBC at 500 MHz; ^{13}C NMR at 125 MHz) using C_5D_5N as solvent with tetramethylsilane as internal reference. The chemical shifts were given in δ (ppm) and coupling constants in Hz. HR-TOF-MS experiments were performed on AB SCIEX Triple TOF 5600 plus MS spectrometer. UV on a Shimadzu UV-2550 spectrophotometer and IR spectra were measured on a Shimadzu FTIR Bruker-TENSOR 37 spectrophotometer. GC–MS experiment was performed on a TRACE GC ULTRA DSQII intrument. Optical rotations were measured with an Anton Paar-MCP600 polarimeter in MeOH solution. The centrifugation was applied with D05 (Hunan Hexi Instrument Co., Ltd, Changsha, China). Adsorbents for column chromatography were silica gel (200–300 μm, Qingdao Marine Chemical Co., Ltd, Qingdao, China), Sephadex LH-20 (75–150 μm, Pharmacia, Uppsala, Sweden), ODS (octa decylsilyl silicion) (40–63 μm, FuJi, Tokyo, Japan). Preparative HPLC was performed using a Shimadzu LC-6AD series instrument equipped with a UV detector at 280 nm and Shim-Park RP-C_{18} column (20 × 200 mm i.d.). Thin-layer

chromatography was performed on pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd) and detected by spraying with 10% H_2SO_4–EtOH.

3.2. *Plant material*

The aerial parts of *A. acuta* L. were collected at Guilin City, Guangxi Province, China, in August 2013, and identified by Prof. Jizhu Liu. A voucher specimen (no. 2013-2) is deposited at Department of Traditional Chinese Medicinal Chemistry, Guangdong Pharmaceutical University.

3.3. *Extraction and isolation*

The aerial parts (15 kg) of *A. acuta* were cut to pieces and were extracted two times with 95% EtOH under reflux for 2 h. The extract (1.2 kg) was partitioned into $CHCl_3$ and H_2O-soluble fractions. The $CHCl_3$ extract (0.3 kg) was subjected to column chromatography on silica gel and eluted with CH_2Cl_2–MeOH (20:1, 5:1) to give two subfractions. The first subfraction (20.1 g) was purified by Sephadex LH-20 column with MeOH as eluent, factions of 6–27 (4.8 g) was purified by RPHPLC and were eluted with 97% MeOH/H_2O, two peaks were collected from fraction 1, to afford acutacoside A (**1**, 35 mg, t_R 48.25 min) and acutacosid B (**2**, 13.8 mg, t_R 38.41 min).

3.4. *Spectral data*

*Acutacoside A (**1**)*: A white powder; m.p. 140.1–141.1°C; $[\alpha]_D^{25}$ 50 (*c* 0.01, MeOH). UV λ_{max} (MeOH) nm (log *e*): 279.5 (0.97), 217 (0.67) nm; IR (KBr) ν_{max} cm^{-1}: 3443 (hydroxyl), 1724 (carbonyl group), 1637 (aromatic moiety). For ^1H (C_5D_5N, 500 MHz) and ^{13}C (C_5D_5N, 125 MHz) NMR spectral data, see Table S1. HR-TOF-MS: *m/z* 1391.7322 $[M + Na]^+$ (calculated for 1391.7339, $C_{70}H_{112}O_{26}Na$).

*Acutacoside B (**2**)*: A white powder; m.p. 137.5–140.0°C; $[\alpha]_D^{25}$ 17.3 (*c* 0.02, MeOH). UV λ_{max} (MeOH) nm (log *e*): 279.5 (1.21), 217.5 (0.82) nm; IR (KBr) ν_{max} cm^{-1}: 3443 (hydroxyl), 1722 (carbonyl group), 1637 (aromatic moiety). For ^1H (C_5D_5N, 500 MHz) and ^{13}C (C_5D_5N, 125 MHz) NMR spectral data, see Table S1. HR-TOF-MS: *m/z* 1419.7686 $[M + Na]^+$ (calculated for 1419.7652, $C_{72}H_{116}O_{26}Na$).

3.5. *Hydrolysis*

3.5.1. *Alkaline hydrolysis of **1** and **2***

Compounds **1** and **2** (10 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 N HCl and extracted with hexane (3 mL × 2) and *n*-BuOH (3 mL × 2). The organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, then methylated by following method (She 2004): the hexane extracted was mixed with 0.1 mL 0.5 mol CH_3ONa solution, rocked for 5 min at room temperature, then 5 μL CH_3COOH and 1 g anhydrous $CaCl_2$ powder were added, stewed for 1 h, centrifuged for 2–3 min at 2000–3000 rpm min^{-1}, then the supernatant was analysed by GC–MS. GC–MS on a TRACE GC ULTRA DSQII instrument under the following conditions: 30 m × 0.25 mm × 0.25 μm, TG-5MS (Thermo) column; He, 0.8 mL min^{-1}; 40°C, 3 min; 50–310°C, Δ10°C min^{-1}, 70 eV.

3.5.2. *Acid hydrolysis and sugar analysis*

The glycosidic acid (**3**, 3 mg, from alkaline hydrolysis) was methylated with MeOH and catalysed with 0.5 N H_2SO_4 to give operculinic acid A methyl ester (**4**). Compound **4** was hydrolysed with

1 N H_2SO_4 and extracted with ether to obtain 11-hydroxyhexadecanoic acid methyl ester (**5**). The aqueous layer of acidic hydrolysis was concentrated under reduced pressure to give a residue of the sugars. The residue was dissolved in pyridine (0.1 mL), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 mL) was added. The mixture was kept at 60°C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 mL each) and then the hexane extract was analysed by GC–MS on a TRACE GC ULTRA DSQII instrument under the following conditions: 30 m × 0.25 mm × 0.25 μm, TG-5MS (Thermo) column; He, 0.8 mL min^{-1}; 60°C, 3 min; 60–180°C, Δ10°C min^{-1} keep 3 min, 180–205°C, Δ3°C min^{-1} keep 5 min, 205–300°C, Δ20°C min^{-1} keep 5 min, 70 eV. In the acid hydrolysate of operculinic acid A methyl ester, D-fucose, L-rhamnose and D-glucose were confirmed by comparing the retention times of their derivatives with those of authentic D-fucose (t_R 30.35 min), L-rhamnose (t_R 30.14 min) and D-glucose (t_R 31.65 min) derivatives prepared in the same way, respectively.

3.5.3. *Preparation of Mosher's esters*

The procedures for the preparation of Mosher's esters, which was determined the absolute configuration of 11*S* of the aglycone, were same as described previously for resin glycosides from *Ipomoea batatas*.

4. Conclusion

In conclusion, investigation of the aerial parts of *A. acuta* afforded two new compounds. Their structures were established by different spectroscopic analyses.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S17.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Natural Science Foundation of China [grant number 81202885]; Natural Science Foundation of Guangdong province of China [grant number S2013040013819].

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