A New Brominated Norsesquiterpene Glycoside From the Rhizomes of Acorus tatarinowii Schott

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Abstract

A new brominated norsesquiterpene glycoside, acoruside (1), has been isolated from the rhizomes of Acorus tatarinowii Schott, together with 8 known compounds (2-9). Their structures were elucidated mainly based on 1-dimensional (1D) and 2D nuclear magnetic resonance spectra. The absolute configuration of compound 1 was determined by comparing its experimental and calculated electronic circular dichroism spectra. The in vitro tests indicated that at 10 µM, compounds 2, 3, and 4 aggravated serum deprivation injuries of PC12 cells, compound 2 aggravated rotenone-induced injuries of PC12 cells, and compounds 3 and 4 aggravated the oxygen-glucose deprivation-induced injuries of PC12 cells.

Keywords

Acorus tatarinowii Schott, acoruside, brominated norsesquiterpene glycoside, structural elucidation, bioactivities

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Acorus tatarinowii Schott (a synonym of Acorus calamus var. angustatus Besser), a perennial herb, is widely distributed in Asia. In China, it is mainly distributed in the southern area of Yellow River. It is also distributed in India, Thailand, Japan, the Philippines, Indonesia, and South Korea. In the ancient literature of China, it is always cultivated by refined scholars as a kind of ornamental plant, and, through the ages, many poems about this plant were composed by great names, such as Du Fu of the Tang Dynasty and Lu You of the Song Dynasty. So, it is endowed with a strong cultural meaning. As a traditional Chinese medicine, the dried rhizomes of A. tatarinowii have been used for more than 2000 years in China to treat epilepsy, forgetfulness, stomachache, and rheumatism. Previous pharmacological studies have indicated that the species possesses extensive bioactivities, such as sedation, hypnosis, anticonvulsant, antidepressant, nootropic, and antibacterial. Previous chemical investigations afforded a wide variety of chemical constituents, including phenylpropanoids, lignans, diterpenes, alkaloids, and sesquiterpenes. As part of our ongoing search focused on the genus Acorus, a new brominated norsesquiterpene glycoside, acoruside (1), and 8 known compounds have been isolated and identified from the 95% ethanol (EtOH) extract of the rhizomes of A. tatarinowii (Figure 1). Their neuroprotective activities were also evaluated.

Results and Discussion

Compound 1 was obtained as colorless needle-type crystals (methanol [MeOH]). Its molecular formula was determined as C₂₀H₃₁BrO₈ on the basis of high-resolution electrospray ionisation mass spectrometry (HRESIMS), which displayed quasi-molecular ion peaks at m/z 501.1114 ([M + Na]⁺, calculated for C₂₀H₃₁BrNaO₈, 501.1095) and 503.1091 (calculated for C₂₀H₃₁BrNaO₈, 503.1080). The ultraviolet (UV) spectrum (257 nm) suggested the existence of a conjugated diene...
The infrared (IR) spectrum (3394 cm⁻¹) revealed the presence of hydroxyl groups. The ¹H-nuclear magnetic resonance (NMR) spectrum of 1 showed 3 tertiary methyl groups at δ 1.96 (3H, s, Me-5), 1.16 (3H, s, Me-9′), and 0.93 (3H, s, Me-7′); a pair of trans-oriented olefinic protons at δ 7.73 (1H, d, J = 15.5 Hz, H-3) and 6.25 (1H, d, J = 15.5 Hz, H-4); and a trisubstituted double bond at δ 5.84 (1H, s, H-1). The proton signals at δ 3.0-4.5 implied that compound 1 possessed a sugar moiety, which showed an anomeric proton at δ 4.36 (1H, d, J = 8.0 Hz, H-1″). After acid hydrolysis, the sugar moiety was confirmed to be d-glucose, which was identified by gas chromatographic (GC) analysis of its trimethylsilyl L-cysteine derivative. The ¹³C-NMR and distortionless enhancement by polarization transfer spectra of 1 showed 20 carbon signals, of which a glucosyl resonated at δ 103.1 (C-1″), 78.0 (C-3″ or 5″), 77.9 (C-3″ or 5″), 75.1 (C-2″), 71.6 (C-4″), and 62.7 (C-6″). Apart from the sugar moiety, an olefinic quaternary carbon at δ 141.3 (C-2); 2 oxygen-bearing quaternary carbons at δ 87.6 (C-2′) and 83.2 (C-1′); an aliphatic quaternary carbon at about δ 49.0 (overlapped by solvent signals, C-6′); 3 olefinic methine groups at δ 133.1 (C-3), 130.4 (C-4), and 127.8 (C-1); an oxygen-bearing methine group at δ 74.0 (C-4′); 3 methylene groups at δ 77.1 (C-8′) and 42.8 (C-3′ and 5′); and 3 methyl groups at δ 20.5 (C-5), 19.7 (C-9′), and 16.4 (C-7′) were also shown. The above data of the aglycone were very similar to those of compound 2 (Table 1 and Supplemental Figure S13), except for C-1, C-2, and C-4′. More significantly, no carboxyl signal was displayed in the ¹³C-NMR spectrum of 1, indicating that the bromine atom was linked with C-1. This conjecture was confirmed by the 2D NMR spectra. The ¹H-¹H correlation spectroscopy spectrum showed correlations between H-3′ and H-4′, and H-4′ and H-5′, corresponding to the structural segment of C-3′-C-4′-C-5′. The heteronuclear multiple bond correlation (HMBC) spectrum showed a long-range correlation between H-3 and C-1′, which indicated that C-4 was linked with C-1′. The HMBC from H-8′ to C-2′ and the chemical shifts implied that an oxygen atom is present between C-2′ and C-8′. The HMBC between H-1″ and C-4′, H-4′, and C-1″ indicated that the sugar moiety was linked with the aglycone at C-4′. Based on the above data, the planar structure of compound 1 was determined (Figure 2).

The relative configuration of 1 was determined by combined analyses of coupling constants and rotating frame Overhauser effect spectroscopy (ROESY) spectrum data. The glucose unit was determined to be the β-anomer on the basis of the ³JH₁″-H₂″ value (8.0 Hz). Differently, the α-D-glucosyl unit typically shows the anomeric proton as a doublet with J = 2-4 Hz. The ROESY correlation between H-4′ and H-8′ indicated that they were both located on the same side (α-oriented) of the ring, so Me-7′ and Me-9′ were located on the other side (β-oriented). The ROESY correlations between H-4 and Me-7′/Me-9′ suggested that the conjugated diene segment was β-oriented and OH-1′ was α-oriented. The geometry of the C-1/C-2 double bond was assigned as Z, based on the ROESY correlations between Me-5 and H-1/H-4. The absolute configuration of 1 was determined by comparing experimental and calculated electronic circular dichroism (ECD) spectra. The
conformers of compound 1 were obtained using the GMMX package implemented in Gaussian 16W (Gaussian 2016) with the MMFF94 force field. Next, they were optimized with density functional theory (DFT) calculations at the B97-3c level in MeOH. Based on the DFT energies, conformers with a Boltzmann distribution ≥1% were imported into the ORCA 4.1.1 package. Calculations of the ECD spectra were performed by the time-dependent DFT (TDDFT) method at the PB0/def2-SV(P) level. The input files of optimization and TDDFT calculations were generated by the Multiwfn 3.7 package. The results showed that the experimental and calculated spectra were in good agreement. Thus, the absolute configuration of 1 was determined to be 1′S,2′R,4′S,6′R. From these data, compound 1 was determined as (1R,3S,5R,8S)-8-((1E,3Z)-4-bromo-3-methylbuta-1,3-dien-1-yl)-1,5-dimethyl-6-oxabicyclo[3.2.1]octane-8-ol-3-O-β-D-glucopyranoside and was named as acoruside (Figure 1).

Table 1. 1H-NMR (500 MHz) and 13C-NMR (125 MHz) Spectroscopic Data of Compounds 1 and 2 in Deuterated Methanol.

| No. | δC  | δH (J in Hz) | δC  | δH (J in Hz) |
|-----|-----|--------------|-----|--------------|
| 1   | 127.8 | 5.84 (1H, s) | 119.2 | 5.79 (1H, s) |
| 2   | 141.3 |              | 151.5 |              |
| 3   | 133.1 | 7.73 (1H, d, J = 15.5) | 131.8 | 8.01 (1H, d, J = 16.0) |
| 4   | 130.4 | 6.25 (1H, d, J = 15.5) | 135.2 | 6.52 (1H, d, J = 16.0) |
| 5   | 20.5  | 1.96 (3H, s) | 21.3  | 2.11 (3H, d, J = 0.7) |
| 1′  | 83.2  |              | 83.3  |              |
| 2′  | 87.6  |              | 87.8  |              |
| 3′  | 42.8  | 1.82 (2H, m) | 46.0  | 2.06 (1H, dd, J = 13.8, 7.2) |
| 4′  | 74.0  | 4.24 (1H, m) | 64.6  | 4.14 (1H, m) |
| 5′  | 42.8  | 2.16 (1H, dd, J = 13.5, 6.5) | 44.6  | 1.87 (1H, m) |
| 6′  | 49.0a |              | 49.5  |              |
| 7′  | 16.4  | 0.93 (3H, s) | 16.4  | 0.96 (3H, s) |
| 8′  | 77.1  | 3.79 (1H, d, J = 7.3) | 77.3  | 3.83 (1H, d, J = 7.1) |
| 9′  | 19.7  | 1.16 (3H, s) | 19.7  | 1.18 (3H, s) |
| 1″  | 83.3  |              | 169.6 |              |
| 2″  | 75.1  | 3.15 (1H, t-like, J = 8.0) | 78.0b | 3.36 (1H, t-like, J = 9.0) |
| 3″  | 78.0b | 3.36 (1H, t-like, J = 9.0) | 71.6  | 3.30 (1H, overlap) |
| 4″  | 77.9b | 3.28 (1H, m) | 56.7  | 3.86 (1H, dd, J = 12.0, 1.5) |
| 5″  | 62.7  | 3.67 (1H, dd, J = 12.0, 5.0) | 66.0  | 4.14 (1H, m) |

Abbreviation: NMR, nuclear magnetic resonance.

aOverlapped by solvent signals. C-6′ was proved by the existence of heteronuclear multiple bond correlation signals.
bMay be reversed in the same column.

Figure 2. Key heteronuclear multiple bond correlation (HMBC), rotating frame Overhauser effect spectroscopy (ROESY), and 1H-1H correlation spectroscopy (COSY) correlations of compound 1.
By comparison of the NMR spectroscopic and MS data with those reported in the literature, 8 known compounds were identified as dihydrophaseic acid (2), 5-hydroxymethyl furan (3), (E)-4-[5-(hydroxymethyl)furan-2-yl]but-3-en-2-one (4), tatarine C (5), β-n-butyl-β-D-tagatopyranoside (6), 6-C-β-D-glycopyranosyl-8-C-α-L-arabinosyl apigenin (7), spinosin (8), and zivulgarin (9) (Figure 1).

All the isolated compounds were tested for their protective effects to PC12 cell lines. As a result, compounds 2, 3, and 4 aggravated serum-deprivation-induced injuries of PC12 cells, compounds 2 and 4 aggravated rotenone-induced injuries of PC12 cells, and compounds 3 and 4 aggravated the OGD-induced injuries of PC12 cells at 10 µM (Table 2). However, it must be noted that previous investigations indicated that A. tatarinowii possesses protective effects to the injured PC12 cell lines, which is opposite to this paper’s results. It is suggested that neuroprotective materials such as β-asarone contained in A. tatarinowii neutralize the injuring effects of some compounds on PC12 cells. Hence, as a whole, A. tatarinowii still possesses neuroprotection properties.

### Table 2. Effects of Compounds 1-9 on Serum Deprivation, Rotenone, and OGD-Induced Injuries of PC12 Cells In Vitro (means ± SD, N = 3).

| No. | Serum deprivation | Rotenone | OGD |
|-----|-------------------|----------|-----|
| Control | 100.0 ± 7.2 | 100.0 ± 5.9 | 100.0 ± 6.2 |
| Model | 51.0 ± 5.6*** | 71.0 ± 6.1*** | 58.2 ± 7.7*** |
| 1 | 46.78 ± 4.7 | 71.4 ± 2.8 | 55.9 ± 6.5 |
| 2 | 41.6 ± 8.1* | 60.3 ± 10.2* | 54.5 ± 7.0 |
| 3 | 36.3 ± 4.9** | 72.5 ± 8.7 | 48.9 ± 7.2* |
| 4 | 42.9 ± 5.4* | 68.6 ± 5.4 | 51.3 ± 7.8* |
| 5 | 50.1 ± 4.6 | 71.6 ± 4.8 | 58.2 ± 7.7 |
| 6 | 46.6 ± 5.0 | 70.1 ± 5.2 | 55.4 ± 1.9 |
| 7 | 47.4 ± 7.9 | 72.6 ± 5.3 | 53.7 ± 7.9 |
| 8/9 | 46.6 ± 8.9 | 65.0 ± 9.1 | 58.8 ± 9.6 |

Abbreviation: OGD, oxygen-glucose deprivation.

***p < 0.01 versus control; *p < 0.05, **p < 0.01 versus model.

Experimental

**General Experimental Procedures**

Optical rotations were measured with a JASCO P-2000 polarimeter. UV spectra were recorded on a JASCO V-650 spectrophotometer, IR spectra on a Nicolet 5700 spectrometer using the Fourier-transform infrared microscope transmission method, and NMR spectra with a Bruker AV500-III spectrometer. HRESIMS were obtained on an Agilent 1100 series LC/MSD ion trap mass spectrometer. Melting points were measured on an XT5B micromelting point apparatus and were uncorrected. GC was conducted on an Agilent 7890A instrument. Analytical high-performance liquid chromatography (HPLC) was carried out on an Agilent 1200 INFINITY system with an Agilent ZORBAX SB-C18 column (5 µm, 4.6 × 150 mm). Semipreparative HPLC utilized a Shimadzu LC-6AD pump with a Shimadzu SPD-M20A detector and an Agilent ZORBAX SB-C18 column (5 µm, 9.4 × 250 mm). Column chromatography was performed on Diaion HP-20 resin (Mitsubishi Chemical Corporation, Japan), silica gel (200-300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 (GE Healthcare, America), and ODS (50 µm, YMC, Japan). Thin-layer chromatography was carried out with GF254 plates (Qingdao Marine Chemical Factory, China). Compounds were visualized by spraying with 10% sulfuric acid in EtOH followed by heating.

**Plant Material**

The rhizomes of A. tatarinowii were purchased from Anguo country, Hebei Province, China, and authenticated by Professor Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (No. S-2286) was deposited at the Herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

**Extraction and Isolation**

The dried rhizomes of A. tatarinowii were powdered (18 kg), and extracted with 95% EtOH under reflux (each time 1 hour, 5 L × 3 times). The mixed solvent was evaporated under decompression to give a residue (1.9 kg), which was suspended in water and partitioned with light petroleum (1 L × 3), ethyl acetate (1 L × 3), and n-butanol (1 L × 3), successively. The organic solvents were evaporated under reduced pressure to provide extracts of light petroleum (360 g), ethyl acetate (400 g), and n-butanol (320 g). The n-butanol portion was dissolved in water and filtered, then the filtrate was added to a macro-absorption Diaion HP-20 resin column and eluted with a gradient of MeOH-water (H2O) (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 10:0) to give 10 fractions, named F1-F10. F4 was chromatographed on a Sephadex LH-20 column eluting with a...
gradient of MeOH-H₂O (2:8, 4:6, 6:4, 8:2, and 10:0) to give 16 fractions, named F4a-F4 p. Fractions F4g-F4j were combined and chromatographed on a medium pressure reversed-phase C₁₈ silica gel column to yield 1 (16 mg). F3 was chromatographed on a medium pressure reversed-phase C₁₈ silica gel column to yield 2 (25 mg). F2 was chromatographed repeatedly on Sephadex LH-20 and silica gel columns and finally purified by preparative HPLC (MeOH:H₂O = 22:78, 4 mL/min, 210 nm) to yield 3 (150 mg, tₚ = 43 minutes). F8 was chromatographed on a silica gel column eluting with a gradient of dichloromethane (CH₂Cl₂)-MeOH (8:1, 6:1, 4:1, 2:1, 0:1) to give 9 fractions, named F5a-F5 i. F5d was purified by semipreparative HPLC (MeOH:H₂O = 29:71, 2.5 mL/min, 210 nm) to yield 4 (10 mg, tₚ = 37 minutes) and 5 (29 mg, tₚ = 26 mg). F5e was purified with a Sephadex LH-20 column to yield 6 (12 mg). F5f was purified by semipreparative HPLC (acetonitrile:H₂O = 14:86, 2.5 mL/min, 210 nm) to yield 7 (8 mg, tₚ = 31 minutes). F5g was purified by semipreparative HPLC (acetonitrile:H₂O = 27:73, 2.5 mL/min, 210 nm) to yield a mixture of 8 and 9 (11 mg, tₚ = 43 minutes; compounds 8 and 9 could not be isolated by HPLC, and their ¹H and ¹³C NMR resonance signals were assigned by 2D NMR spectra combined with literature data).

Acoruside (1). Colorless needle type crystals (MeOH): mp: 249-251 °C; [α]D = -24.7 ± 0.21 (c 0.21, MeOH); UV (MeOH) λmax (log ε) 201 nm (2.70), 257 nm (2.65); IR νmax 3394, 2933, 1679, 1379, 1076 cm⁻¹; ECD (MeOH) λmax (Δε) 266 nm (-1.24), 244 nm (-0.95); ¹H-NMR (500 MHz, CD₃OD) and ¹³C-NMR (125 MHz, CD₃OD) data see Table 1; HRESIMS (positive ion mode) m/z 501.1114 [M + Na]+ (calculated for C₂₀H₃₁³¹BrO₈Na, 501.1095), 503.1091 [M + Na]+ (calculated for C₂₀H₃₁³¹BrNaO₈, 503.1080).

Acid Hydrolysis of Acoruside (1) and Determination of the Absolute Configuration of Sugar

Compound 1 (1 mg) was dissolved in 2 M hydrochloric acid-H₂O (2 mL) and heated under reflux for 4 hours. The reaction mixture was extracted with EtOAc after cooling. The aqueous layer was evaporated under vacuum, diluted repeatedly with H₂O, and evaporated in vacuo to furnish a neutral residue. This was dissolved in anhydrous pyridine (1 mL), to which 2 mg of l-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 hours, and after evaporation in vacuo to dryness, 0.2 mL of N-trimethylsilylimidazole was added; the mixture was kept at 60 °C for another 2 hours. The reaction mixture was partitioned between n-hexane and H₂O (2 mL each), and the n-hexane extract analyzed by GC under the following conditions: capillary column, HP-5 (30 m × 0.25 mm, with a 0.25-µm film, Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 160 °C, then raised to 280 at 5 °C/min, final temperature maintained for 10 minutes; carrier, N₂ gas. From the acid hydrolysate of 1, n-glucose was confirmed by comparison of the retention time of its derivative with that of authentic sugar derivatized in the same way, which showed a retention time of 19.16 minutes.

Bioactive Assays

The in vitro neuroprotective activities were tested by the method described previously.

Conclusion

A new brominated norsesquiterpene glycoside, acoruside (1), and 8 known compounds were separated from the dried rhizomes of A. tatarinowii. Their chemical structures were elucidated from extensive spectroscopic data and by comparison with literature. Furthermore, in vitro tests suggested compounds 2, 3, and 4 could aggravate serum-deprivation-injuries of PC12 cells, compound 2 aggravated rotenone-induced injuries of PC12 cells, and compounds 3 and 4 aggravated the OGD-induced injuries of PC12 cells at 10 µM.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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