Acortatarins A and B, Two Novel Antioxidative Spiroalkaloids with a Naturally Unusual Morpholine Motif from *Acorus tatarinowii*

Xiao-Gang Tong, Li-Li Zhou, Yue-Hu Wang, Cheng-Feng Xia, Ye Wang, Min Liang,
Fan-Fan Hou,^{*} and Yong-Xian Cheng^{*}

State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, Yunnan, PRC, Graduate School of Chinese Academy of Sciences, Beijing 100039, PRC, Division of Nephrology, Nanfang Hospital, Southern Medical University, Key Laboratory for Organ Failure Research, Education Ministry, Guangzhou, 510515, Guangdong, PRC, and The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Guiyang 550002, Guizhou, PRC

yxcheng@mail.kib.ac.cn (Y.X.C); ffhou@pub.guangzhou.gd.cn (F.F.H)

Supporting Information

- S1. Experimental Section
- S2. ¹H NMR spectrum of acortatarin A (**1**) in CD₃OD
- S3. ¹³C NMR spectrum of acortatarin A (**1**) in CD₃OD
- S4. ¹H-¹H COSY spectrum of acortatarin A (**1**) in CD₃OD
- S5. HSQC spectrum of acortatarin A (**1**) in CD₃OD
- S6. HMBC spectrum of acortatarin A (**1**) in CD₃OD
- S7. FABMS spectrum of acortatarin A (**1**)
- S8. IR spectrum of acortatarin A (**1**)
- S9. ¹H NMR spectrum of acortatarin B (**2**) in CD₃OD
- S10. ¹³C NMR spectrum of acortatarin B (**2**) in CD₃OD
- S11. ¹H-¹H COSY spectrum of acortatarin B (**2**) in CD₃OD

- S12. HSQC spectrum of acortatarin B (**2**) in CD₃OD
- S13. HMBC spectrum of acortatarin B (**2**) in CD₃OD
- S14. ROESY spectrum of acortatarin B (**2**) in CD₃OD
- S15. FABMS spectrum of acortatarin B (**2**)
- S16. IR spectrum of acortatarin B (**2**)
- S17. ¹H NMR spectrum of **1a** in CDCl₃
- S18. ¹H NMR spectrum of (*S*)-MTPA ester of **1a** in CDCl₃
- S19. ¹H-¹H COSY spectrum of (*S*)-MTPA ester of **1a** in CDCl₃
- S20. ¹H NMR spectrum of (*R*)-MTPA ester of **1a** in CDCl₃
- S21. ¹H-¹H COSY spectrum of (*R*)-MTPA ester of **1a** in CDCl₃

S1. Experimental Section

General Experimental Procedures. Melting points were measured on an SGW X-4 melting instrument and uncorrected. Optical rotations were measured using a Horiba SEPA-300 polarimeter. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. IR spectra were obtained on a Tensor 27 with KBr pellets. NMR spectra were recorded on a Bruker AV-400 or a DRX-500 spectrometer with TMS as an internal standard. FABMS were measured on a VG Autospec-3000 spectrometer. HRESIMS were determined with an API QSTAR Pulsar 1 spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Co. Ltd., P.R. China), MCI gel CHP 20P, 75–150 μ M, Mitsubishi Chemical Industries Ltd.), RP-18 (40-63 μ m, Daiso Co., Japan), and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Semi-preparative HPLC (Agilent 1100 HPLC system, Zorbax SB-C-18, Agilent, 9.4 mm $\times$ 25 cm). Spots on TLC plates were detected under UV radiation and by heating after spraying with 10% H₂SO₄ in EtOH.

Plant Material: The rhizome of *A. tatarinowii* was purchased from Yunnan Corporation of Materia Medica (YCMM), Yunnan Province, P.R. China, and identified by Mr. Hong-Yan Sun, at YCMM. A voucher specimen (CHYX0001) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS, P.R. China.

Extraction and Isolation: The air-dried powders of *A. tatarinowii* rhizome (50 kg)

were extracted with boiling water (2×100 L) and then EtOH (1×100 L) under reflux to give an extract (4.7 kg), which was suspended in water (6 L) followed by exhaustive partition with EtOAc and *n*-BuOH. The *n*-BuOH soluble extract (315 g) was separated by silica gel column chromatography eluted with gradient CHCl_3 - MeOH - H_2O to supply eight fractions (I - VIII). The fraction IV (9.3 g) was passed through a MCI gel CHP 20P column with gradient aqueous MeOH to yield four sub-fractions (IV-A - IV-D). The sub-fraction IV-B (1.6 g) was purified by C_{18} silica gel, Sephadex LH-20 and semi-preparative HPLC (Agilent 1100 HPLC system, Zorbax SB-C-18, Agilent, 9.4 mm $\times$ 25 cm, MeOH - H_2O , 20:80) to afford compounds **1** (7.3 mg) and **2** (3.4 mg).

Preparation of (*S*)- and (*R*)-Mosher esters of **1**:

(a) BuMe_2SiCl , Et_3N , DMAP, CH_2Cl_2 ; (b) MTPA-Cl, Et_3N , DMAP, CH_2Cl_2 .

(a) To a solution of acortatarin A (**1**) (5.3 mg, 0.021 mmol) and anhydrous Et_3N (4 μL , 0.03 mmol) in dry CH_2Cl_2 (1 mL) cooled at -10°C was added BuMe_2SiCl (3.8 mg, 0.025 mmol). The reaction mixture was stirred for 8 h and then was allowed to be warmed to room temperature over 6 h. MeOH (10 μL) was added to quench the reaction and mixture was continued stirring for additional 30 min. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (petroleum/ethyl acetate, 4:1) to give 2.9 mg of product, and recover

1.9 mg of the acortatarin A.

(b) The above product (2.9 mg, 0.0079 mmol) was divided into two portions, which were dissolved in dry CH₂Cl₂ (0.5 mL). Et₃N (2 μ L, 0.016 mmol) and catalytic amount of DMAP were added, followed by (*R*)- or (*S*)- α -methoxy- α -(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) (1.2 μ L, 0.0063 mmol). The reaction mixture was stirred at room temperature until TLC showed all the starting material was consumed. The mixture was evaporated to removed the organic solvent and the residue was purified by silica gel column chromatography (petroleum/ethyl acetate, 10:1) to afford 0.8 mg (*R*)-MTPA and 1.2 mg (*S*)-MTPA esters, respectively.

X-ray crystal data and structure refinement for acortatarin A (1):

Empirical formula	C ₁₂ H ₁₅ NO ₅
Formula weight	253.25
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P2(1)
Unit cell dimensions	a=7.2060 (11) Å, α = 90 ° b=7.0551(11) Å, β = 97.86 (3) ° c=11.5524 (17) Å, γ = 90 °
Volume	581.80 (15) Å ³

Z	2
Calculated density	1.446 Mg/m ³
Absorption coefficient	0.113 mm ⁻¹
F (000)	268
Crystal size	0.299 × 0.187 × 0.101 mm ³
Theta range for data collection	2.85 to 25.99 °
Limiting indices	-7 ≤ h ≤ 8, -8 ≤ k ≤ 8, -12 ≤ l ≤ 14
Reflections collected / unique	3200/1237 [R(int) = 0.0458]
Completeness to theta = 26.00	99.4 %
Absorption correction	Empirical
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	1237 / 1 / 166
Goodness-of-fit on F ²	1.046
Final R indices [I > 2σ(I)]	R1 = 0.0403, wR2 = 0.1057
R indices (all data)	R1 = 0.0417, wR2 = 0.1068
Absolute structure parameter	10(10)
Largest diff. peak and hole	0.195 and -0.169 e. Å ⁻³

Colorless crystals of **1** were obtained in MeOH. Crystal data were collected on a Bruker SMART CCD detector employing graphite monochromated Mo-K α radiation ($\lambda=0.71073$ Å) at 293(2) K and operating in the ϕ - ω scan mode. The structure was solved by direct methods SHELXS-97¹ and refined with full-matrix least-squares calculations on F^2 using SHELX-97.² All non-hydrogen atoms were refined

anisotropically. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. Crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-760186). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; email: deposit@ccdc.cam.ac.uk).

References:

- (1) Sheldrick, G. M. *SHELXS-97: Program for X-ray Crystal Structure Solution*; University of Göttingen: Göttingen, Germany, 1997.
- (2) Sheldrick, G. M. *SHELX-97: Program for X-ray Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.

S2. ¹H NMR spectrum of acortatarin A (1) in CD₃OD

S3. ^{13}C NMR spectrum of acortatarin A (1) in CD_3OD

1

1

S6. HMBC spectrum of acortatarin A (1) in CD₃OD

S7. FABMS spectrum of acortatarin A (1)

S8. IR spectrum of acortatarin A (1)

Sample : Scp-88	Frequency Range : 399.271 - 3996.57	Measured on : 28/12/2009
Technique : KBr压片	Resolution : 4	Instrument : Tensor27
Customer : 091228IR	Zerofilling : 2	Sample Scans : 16
Acquisition : Double Sided, For		

S9. ^1H NMR spectrum of acortatarin B (2) in CD_3OD

S10. ¹³C NMR spectrum of acortatarin B (2) in CD₃OD

qscp92 c13

S11. ^1H - ^1H COSY spectrum of acortatarin B (2) in CD_3OD

qscd92 hsqc

qscd92 hmbc

S15. FABMS spectrum of acortatarin B (2)

S16. IR spectrum of acortatarin B (2)

Sample : Scp-92	Frequency Range : 399.271 - 3996.57	Measured on : 29/12/2009
Technique : KBr压片	Resolution : 4	Instrument : Tensor27
Customer : 091229IR	ZeroFilling : 2	Acquisition : Double Sided, For
		Sample Scans : 16

S17. ^1H NMR spectrum of **1a** in CDCl_3

S18. ^1H NMR spectrum of (*S*)-MTPA ester of **1a** in CDCl_3

S19. ^1H - ^1H COSY spectrum of (*S*)-MTPA ester of **1a** in CDCl_3

S20. ^1H NMR spectrum of (*R*)-MTPA ester of **1a** in CDCl_3

S21. ^1H - ^1H COSY spectrum of (*R*)-MTPA ester of **1a** in CDCl_3