ORGANIC LETTERS

2013
Vol. 15, No. 8
1970–1973

Ternatusine A, a New Pyrrole Derivative with an Epoxyoxepino Ring from *Ranunculus ternatus*

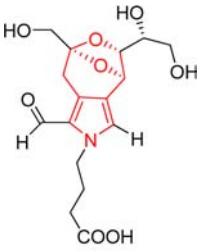
Zhilai Zhan, Ziming Feng, Yanan Yang, Li Li, Jianshuang Jiang, and Peicheng Zhang*

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, P. R. China

pczhang@imm.ac.cn

Received March 11, 2013

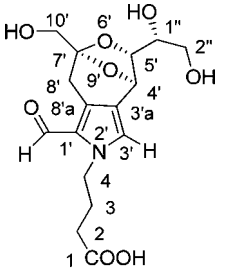
ABSTRACT



Ternatusine A
4′*R*,5′*R*,7′*S*,1″*R*

Ternatusine A (1), a novel alkaloid with an unprecedented epoxyoxepino[4,5-*c*] pyrrole ring, was isolated from the roots of *Ranunculus ternatus* Thunb. Its unusual structure, including its absolute stereochemistry, was determined using UV, IR, HRESIMS, and 1D and 2D NMR data and through comparison of the experimental and calculated electronic circular dichroism (ECD) spectra. A possible biosynthetic pathway for ternatusine A was postulated.

Ranunculus ternatus Thunb (Ranunculaceae) is mainly distributed in the Henan and Shandong provinces in China. Its root is used in traditional Chinese Medicine for the treatment of faucitis, tuberculosis, neck scrofula, and breast cancer, among other ailments.[1] Some pharmacology experiments have indicated that extracts of the root of this plant have remarkable antitumor[2] and antituberculosis activity.[3] Previous investigations of this plant have revealed the presence of triterpenes,[4] biflavonoids,[5] indolopyridoquinazoline alkaloidal glycosides,[6] and other glycosides.[7] Recently, a known pyrrole alkaloid, 4-[2-formyl-5-(hydroxymethyl)-1*H*-pyrrol-1-yl]butanoic acid was isolated and showed potent inhibitory activity against *Mycobacterium tuberculosis* H37Rv *in vitro*.[8] In our search for additional constituents from this plant, a new pyrrole alkaloid with a rare epoxyoxepino[4,5-*c*]pyrrole ring named ternatusine A (**1**) was isolated from the root of *R. ternatus*. Its structure was elucidated on the basis of spectroscopic data, chemical methods, and electronic circular dichroism (ECD).



Ternatusine A
4′*R*,5′*R*,7′*S*,1″*R*

(1) Liao, H.; Tong, Z.-P. *China Pharmacy* **2008**, *19*, 955–957.
(2) Wang, A.-W.; Wang, M.; Yuan, R.-J.; Tian, J.-K.; Wu, L.-M.; Geng, H. *Nat. Prod. Res. Dev.* **2004**, *16*, 529–531.
(3) He, K.-X.; Lv, S.-J. *Lab. Med. Clin.* **2008**, *5*, 354–356.
(4) Zhao, Y.; Ruan, J.-L.; Wang, J.-H.; Cong, Y.; Song, S.; Cai, Y.-L.; Fang, W.; Zhou, D.-N. *Nat. Prod. Res.* **2008**, *22*, 233–240.
(5) Xiong, Y.; Deng, K.-Z.; Guo, Y.-Q.; Gao, W.-Y. *Chin. Tradit. Herb. Drugs* **2008**, *39*, 1449–1452.
(6) Zang, L.; Yang, Z.; Tian, J.-K. *Chem. Pharm. Bull.* **2007**, *55*, 1267–1269.
(7) (a) Tian, J.-K.; Sun, F.; Cheng, Y.-Y. *J. Asian Nat. Prod. Res.* **2006**, *8*, 35–39. (b) Tian, J.-K.; Sun, F.; Cheng, Y.-Y. *Chin. Chem. Lett.* **2005**, *16*, 928–930.
(8) (a) Qi, Z.-J.; Ying, H.-M.; Li, J.-J.; Zhang, L.; Tian, J.-K. *Chin. Pharm. J.* **2012**, *47*, 101–103. (b) Zhang, L.; Tian, J.-K.; Qi, Z.-J.; Ying, H.-M.; Rong, Y.-M.; Chen, R.-Z. C.N. Patent 102028764 A, 2011.

10.1021/ol400643q
Published on Web 04/04/2013

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula, $C_{16}H_{21}NO_8$, was established using HRESIMS with m/z 356.1345 $[M + H]^+$ (calcd 356.1340) and m/z 378.1166 $[M + Na]^+$ (calcd 378.1159), which requires 7 degrees of unsaturation. The UV spectrum of **1** exhibited an absorption maximum at 296 nm, which is characteristic of a pyrrole-2-aldehyde.[9] Absorption bands at 1720 and 1640 cm^{-1} in the IR spectrum of **1** were assigned to a carboxylic acid and an aldehyde group, respectively.

The ^1H NMR data (Table 1) revealed the presence of one aldehyde hydrogen at δ_H 9.46 (s) and an isolated aromatic proton at δ_H 7.20 (1H, s, H-3′). Additionally, four methylenes at δ_H 2.16 (2H, t, J = 7.0 Hz, H-2), 2.01 (2H, m, H-3), 4.29 (2H, t, J = 7.0 Hz, H-4), 2.98 (1H, d, J = 17.5 Hz, H-8′), and 3.16 (1H, d, J = 17.5 Hz, H-8′); two oxymethylenes at δ_H 3.87 (2H, s, H-10′), 3.83 (1H, dd, J = 2.5; 11.5 Hz, H-2″), and 3.64 (1H, dd, J = 6.0 Hz, 11.5, H-2″); and three oxymethines at δ_H 5.53 (1H, s, H-4′), 4.01 (1H, d, J = 8.5, H-5′), and 3.71 (1H, m, H-1″) were observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum (Table 1) showed resonances of 16 carbons that could be attributed by the DEPT spectrum to six methylene, five methine, and five quaternary carbons. The ^{13}C NMR resonances at δ_C 129.8 (C), 129.9 (CH), 124.3 (C), 132.9 (C), and 182.7 (CHO) suggested the presence of a pyrrole-2-aldehyde skeleton.[10]

In the $^1H-^1H$ COSY experiment, the correlations of H-2 with H-3, H-3 with H-4 combined with the HMBC correlations from H-2 and H-3 to C-1 at δ_C 184.8 confirmed the existence of a butanoic acid moiety. The connection between the pyrrole-2-aldehyde skeleton and the butanoic acid moiety was established from an HMBC experiment. In the HMBC spectrum (Figure 1), the correlation between H-4 and C-1′/C-3′ was observed, suggesting that the butanoic acid moiety is connected to the nitrogen atom of the 2-formyl-pyrrole fragment. Furthermore, the $^1H-^1H$ COSY correlations of H-2″ with H-1″ and H-1″ with H-5′ and the HMBC correlations of H-2″ with C-1″/C-5′, H-1″ with C-2″/C-5′/C-4′, H-5′ with C-1″/C-4′/C-3′a, and H-4′ with C-8′a/C-3′a/C-3′/C-5′ suggested that a four-carbon chain fragment existed and was connected to C-3′a of the pyrrole-2-aldehyde skeleton. Additionally, the key HMBC correlations of H-10′ with C-7′/C-8′ and H-8′ with C-7′/C-8′a/C-3′a/C-1′ indicated that a three-carbon chain fragment was connected to C-8′a. These observations showed that **1** was a 3,4-disubstituted pyrrole-2-aldehyde derivative. The absence of other sp or sp^2 carbon signals and the remaining 2 degrees of unsaturation implied that **1** contained two more rings. The presence of a quaternary carbon (C-7′) at δ_C 110.6 in the ^{13}C NMR spectrum and the key HMBC correlations of H-5′ with C-7′ and H-4′ with C-7′ suggested that a dioxolane was formed between two side chains and that an epoxyoxepino moiety existed in **1**.

Table 1. NMR Spectroscopic Data of Ternatusine A (**1**)[a]

no.	δ_H (J in Hz)	δ_C	HMBC[b]
1		184.8	
2	2.16 (t, 7.0)	36.8	1, 3, 4
3	2.01 (m)	30.4	1, 2, 4
4	4.29 (t, 7.0)	51.1	3, 1′, 3′
1′		129.8	
3′	7.20 (s)	129.9	4, 1′, 3′a, 8′a
3′a		124.3	
4′	5.53 (s)	75.9	3′, 3′a, 8′a, 5′, 7′, 1″
5′	4.01 (d, 8.5)	87.0	3′a, 4′, 7′, 1″, 2″
7′		110.6	
8′	2.98 (d, 17.5)	33.1	1′, 3′a, 5′, 7′, 8′a
	3.16 (d, 17.5)		1′, 3′a, 5′, 7′, 8′a, 10′
8′a		132.9	
10′	3.87 (s)	66.3	5′, 7′, 8′
1″	3.71 (m)	74.1	4′, 5′, 2″
2″	3.83 (dd, 11.5, 2.5)	65.3	5′, 1″
	3.64 (dd, 11.5, 6.0)		5′, 1″
-CHO	9.46 (s)	182.7	1′

[a] Data acquired in D_2O at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR. [b] HMBC correlations are from the proton(s) to the indicated carbon(s).

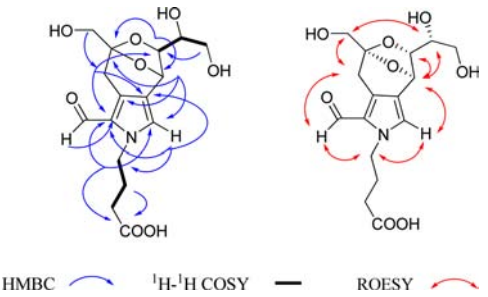


Figure 1. Key HMBC, $^1H-^1H$ COS Y and ROESY correlations of **1**.

The relative configuration of **1** was deduced through the ^1H NMR coupling constants, the ROESY experiment, and systematic conformational analysis. With the chiral carbon of the 1,2-dihydroxyethyl moiety discounted, the epoxyoxepino ring, with three chiral carbon atoms, has a total of four pairs of enantiomers (**1a**: 4′*R*,5′*R*,7′*S* and **1b**: 4′*S*,5′*S*,7′*R*; **1c**: 4′*R*,5′*S*,7′*S* and **1d**: 4′*S*,5′*R*,7′*R*; **1e**: 4′*S*,5′*R*,7′*S* and **1f**: 4′*R*,5′*S*,7′*R*; and **1g**: 4′*S*,5′*S*,7′*S* and **1h**: 4′*R*,5′*R*,7′*R*). Due to the rigidity of the epoxyoxepino ring, two pairs of enantiomers (**1e** and **1f**, **1g** and **1h**) were nonexistent and could be excluded. Furthermore, the conformationally flexible butanoic acid side chain had an insignificant effect on the optimized conformations of the epoxyoxepino ring. Simplified structures of **2** and **3** were used for conformational analysis (Figure 2). A systematic conformational analysis was performed for **2a**, **2c**, **3a**, and **3c** using MMFF94 molecular mechanics force field calculations to obtain the lowest-energy conformers (Figure 3). The dihedral angles of $H_4'-C_4'-C_5'-H_5'$ were nearly 90° in the optimized conformations of **2a** and **3a** and nearly 40° in the optimized conformations of **2c** and **3c**, indicating

(9) Chin, Y. W.; Lim, S. W.; Kim, S. H.; Shin, D. Y.; Suh, Y. G.; Kim, Y. B.; Kim, Y. C.; Kim, J. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 79–81.
(10) Jeon, K. O.; Yu, J. S.; Lee, C. K. *Heterocycles* **2003**, *60*, 2685–2705.

that the conformational effect of the side chain chiral carbon atom on the epoxyoxepino ring in **1** can be negated (Supporting Information (SI)). The facts that no coupling between H-4′ and H-5′ was observed in the ^{1}H NMR and that the correlation between H-4′ and H-5′ existed in the ROESY experiment (Figure 2) favored a pair of enantiomers, 4′*R*,5′*R*,7′*S* and 4′*S*,5′*S*,7′*R*, as the conformations for the epoxyoxepino ring in **1**.

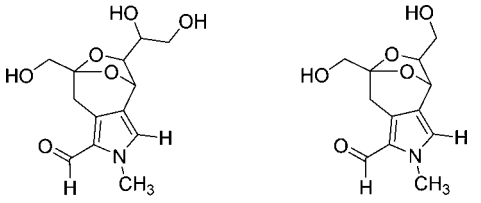


Figure 2. Structures of **2** and **3**.

The absolute configuration of the epoxyoxepino ring in **1** was established by comparison of the experimental ECD spectrum and calculated ECD data using the time-dependent density functional theory (TD-DFT) method at the B3LYP/6-31G(d) level. With the absolute configuration of C-1″ noted to have no effect on the CD spectrum of the epoxyoxepino ring in **1** (Supporting Information), the ECD calculation was performed after optimization of the selected conformers **3a**: 4′*R*,5′*R*,7′*S* and **3b**: 4′*S*,5′*S*,7′*R*. Comparison of theoretically calculated and experimental ECD curves (Figure 4) permitted the assignment of the absolute configuration of **1** as 4′*R*,5′*R*,7′*S*. The absolute configuration of C-1″ in **1** was determined using Snatzke′s method, which employs dimolybdenum tetraacetate [$Mo_2(AcO)_4$] as an auxiliary chromophore.[11] The positive CD band observed at ~305 nm ("band IV") in the induced CD spectrum of **1** (Supporting Information) led to the assignment of the *R*-configuration for C-1″ in the 1,2-dihydroxyethyl moiety.

On the basis of the above evidence, the structure of **1** was determined to be 4′*R*,5′*R*,7′*S*,1″*R*-4-[5′-(1″,2″-dihydroxyethyl)-1′-formyl-7′-(hydroxymethyl)-4′,5′,7′,8′-tetrahydro-2*H*-4′,7′-epoxyoxepino[4,5-*c*]pyrrol-2-yl] butanoic acid and was named ternatusine A.

Ternatusine A (**1**) represents the first example of a new epoxyoxepino[4,5-*c*] pyrrole ring framework. Recently, several 2-formyl-pyrrole derivatives were isolated from *Lycium chinense*,[9] *Acorus tatarinowii*,[12] and Bee-collected

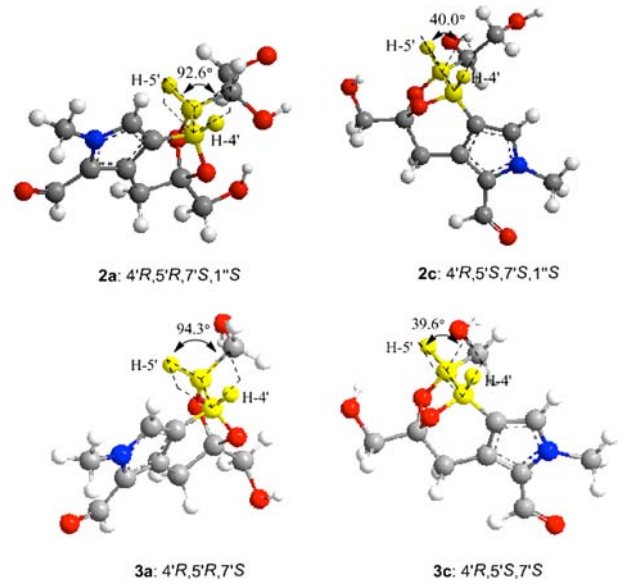


Figure 3. Optimized conformations of **2a**, **2c**, **3a**, and **3c**.

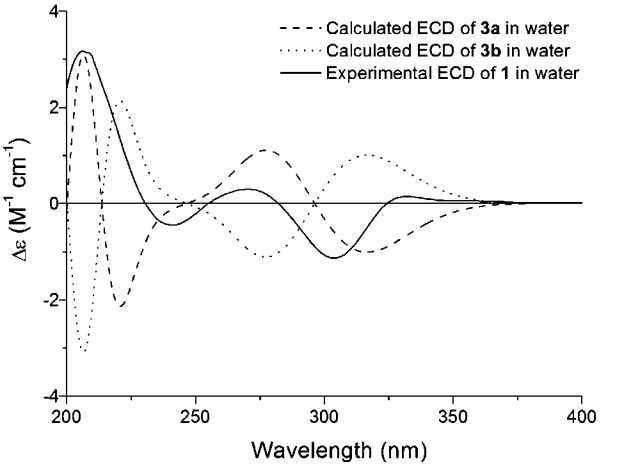


Figure 4. Experimental ECD spectrum of **1** and calculated ECD spectra of **3a** and **3b** in H_2O.

Brassica campestris pollen.[13] A plausible biogenetic pathway suggests that the 2-formyl-pyrrole skeleton carbons in these derivatives orginated from one molecule of glucose. In contrast, the 2-formyl-pyrrole skeleton in **1** should be formed by two molecules of glucose. Thus, 3-deoxyglucosone (3-DG)[14] and γ-*N*-(1-deoxy-D-fructosyl)-aminobutyric acid were supposed to be the precursors in the plausible biogenetic pathway of **1** (Scheme 1). γ-*N*-(1-Deoxy-D-fructosyl)aminobutyric acid is an Amadori rearrangement

(11) (a) Frelek, J.; Snatzke, G.; Szczepek, W. J. *Fresenius. J. Anal. Chem.* **1993**, *345*, 683–687. (b) Frelek, J.; Ikekawa, N.; Takatstuto, S.; Snatzke, G. *Chirality* **1997**, *9*, 578–582. (c) Bari, L. D.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. *J. Org. Chem.* **2001**, *66*, 4819–4825. (d) Liu, J.; Liu, Y.-B.; Si, Y.-K.; Yu, S.-S.; Qu, J.; Xu, S.; Hu, Y.-C.; Ma, S.-G. *Steroids* **2009**, *74*, 51–61.

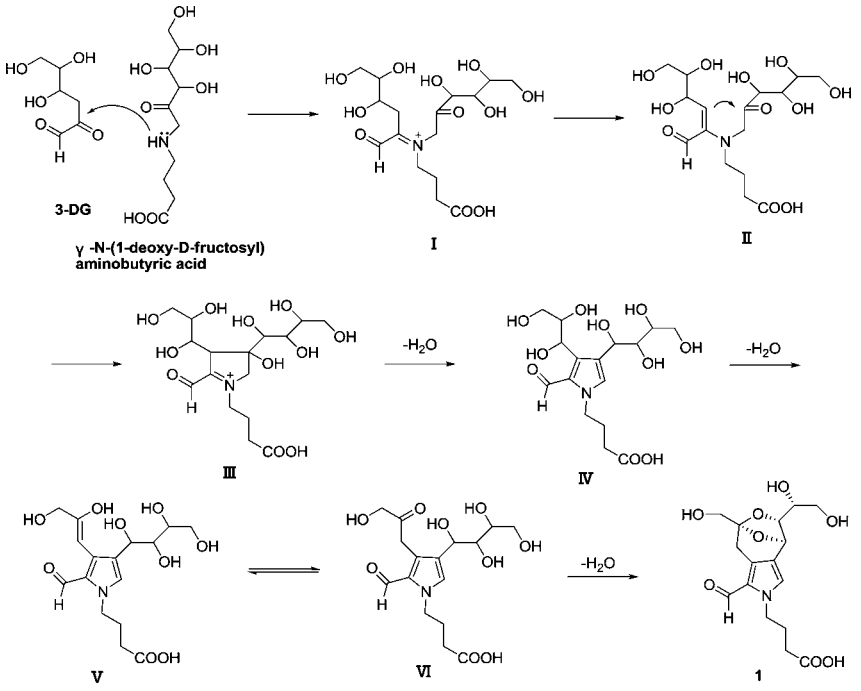
(12) Tong, X.-G.; Zhou, L.-L.; Wang, Y.-H.; Xia, C.; Wang, Y.; Liang, M.; Hou, F.-F.; Cheng, Y.-X. *Org. Lett.* **2010**, *12*, 1844–1847.

(13) Guo, J.-L.; Feng, Z.-M.; Yang, Y.-J.; Zhang, Z.-W.; Zhang, P.-C. *Chem. Pharm. Bull.* **2010**, *58*, 983–985.

(14) Arlene, F. G.; Birgit, Q.; Thomas, P. M.; Milton, S. F. *J. Agric. Food Chem.* **1988**, *36*, 677–680.

(15) Tomita, H.; Noguchi, M.; Tamaki, E. *Agric. Biol. Chem.* **1965**, *29*, 959–961.

Scheme 1. Hypothetical Biogenetic Pathway of **1**



product formed from the reaction between D-glucose and γ-aminobutyric acid and was isolated in 1965 from cured tobacco leaves.[15] 3-DG can readily react with the amino group of γ-*N*-(1-deoxy-D-fructosyl)-aminobutyric acid to give I, followed by intramolecular nucleophilic addition and dehydration to give IV.[16] Finally, the cyclic ketal in **1** was formed by dehydration and an intramolecular nucleophilic addition.

In bioassay experiments using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, **1** exhibited no cytotoxicity against A549, Bel7420, BGC-823, HCT-8, and A2780 at 10 μM.

Acknowledgment. The authors are grateful to Ms. Yinghong Wang of Analytical and Testing Center, Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, for conducting NMR experiments. This project was supported by the National Science and Technology Project of China (2012ZX09301002-001003), the Graduate Innovation Foundation of Peking Union Medical College (2011-1007-020), and the Fundamental Research Funds for the Central Institutes (No. 2012ZD03).

Supporting Information Available. Detailed experimental procedures, physicochemical properties, 1D and 2D NMR, MS and IR spectra, and related original ECD calculation data for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Farmar, J. G.; Ulrich, P. C.; Cerami, A. *J. Org. Chem.* **1988**, *53*, 2346–2349.

The authors declare no competing financial interest.