



Turtschamide, a cytotoxic putrescine bisamide from *Corydalis turtschaninovii*

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ABSTRACT

A putrescine bisamide with a unique cyclic structure derived from L-tyrosine, turtschamide (**1**), was isolated from the tubers of *Corydalis turtschaninovii*. The structure of **1** was established by extensive spectroscopic study, and its absolute configuration was determined by a combination of NOE experiment and application of the Marfey's method. Turtschamide (**1**) exhibited cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cells.

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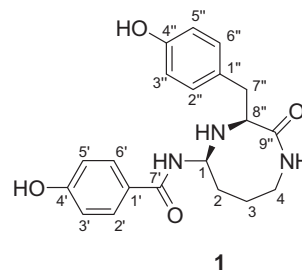
The plants of genus *Corydalis* (family Papaveraceae) have been used as analgetic drugs and anticancer medicines in East Asia.¹ The tubers of *Corydalis* sp. are known to contain various active alkaloids (e.g., bulbocapnine, corydaline, corydine, berberine, coptisine, columbamine, and protopine).^{1–4} During our search for structurally interesting compounds from *Corydalis* sp., we have reported the isolation of some benzyloisoquinoline alkaloids⁵ and triterpenoids from *Corydalis ternata*.⁶ Recently, we have reported the isolation of two tetrahydroprotoberberine–aporphine dimeric alkaloids, corydaturschines A and B, and a new aporphine derivative, ethyl glausuccinate, from the tubers of *Corydalis turtschaninovii*.⁷ In Korea, the tubers of this plant have been used as a traditional medicine for an analgesic, an antispasmodic, and a treatment of gastric ulcers.⁸ Further investigation of the extracts from the tubers of *C. turtschaninovii* resulted in the isolation of a new putrescine bisamide with a unique cyclic structure derived from L-tyrosine, turtschamide (**1**). In this Letter, we describe the isolation, structure elucidation, and biological activity of turtschamide (**1**) (Fig. 1).

The tubers of *C. turtschaninovii* (10 kg, dry) were extracted with 50% EtOH, and the extracts were successively partitioned with *n*-hexane, CHCl₃, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction was subjected to repeated silica gel column (CHCl₃/MeOH) chromatography and was purified by C₁₈ HPLC (Econosil RP-18 10 μ column; 250 × 10 mm, 50% MeOH, Shodex refractive index detector) to give **1**.

Turtschamide (**1**) was isolated as an optically active amorphous gum, [α]_D²⁵ –15.7 (c 0.25, MeOH).⁹ Its HRESIMS indicated a [M+H]⁺ ion peak at *m/z* 370.1770, suggesting a molecular formula of

C₂₀H₂₃N₃O₄ (calcd for C₂₀H₂₄N₃O₄, 370.1767) from which 11 degrees of unsaturation can be deduced. The IR spectrum showed absorption bands for hydroxy (3336 cm⁻¹) and carbonyl functionalities (1690 cm⁻¹). The ¹³C (Table 1) and DEPT-135 NMR spectra exhibited 16 signals for 4 methylene, 6 methine, and 6 quaternary carbons. Full ¹H and ¹³C NMR assignments (Table 1) were established by ¹H–¹H COSY, ¹H–¹³C HMQC, and ¹H–¹³C HMBC experiments.

The analysis of the ¹H–¹H COSY and HMBC spectra (Fig. 2) suggested the gross structures of three partial units (units A–C) as follows. A set of a 1,4-disubstituted benzene moiety was suggested by signals at δ_{H} 6.82 (2H, dd, *J* = 8.5, 2.0 Hz, H-3', 5') and 7.88 (2H, dd, *J* = 8.5, 2.0 Hz, H-2', 6') observed in the ¹H NMR spectrum, which was supported by the analysis of HMBC correlations (Fig. 2). The HMBC correlation from H-2' and H-6' (δ_{H} 7.88) to C-7' (δ_{C} 169.6) suggested the existence of a carbonyl group at C-7'. Thus, the gross structure of unit A was proposed as shown. In unit B, the ¹H–¹H



1

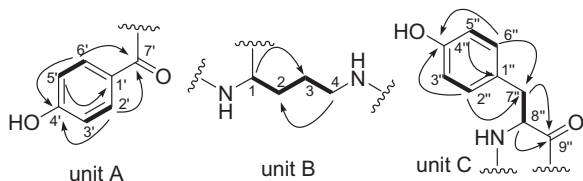
Figure 1. Structure of turtschamide (**1**).

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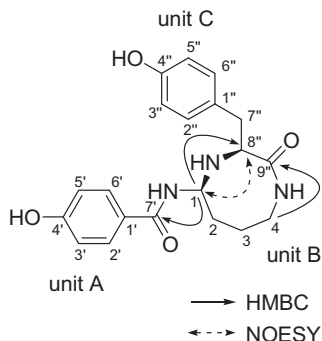
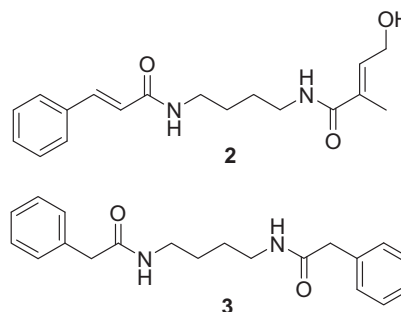
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Table 1
¹H and ¹³C NMR spectroscopic data of turtschamide in CD₃OD

Position	$\delta_{\text{H}}^{\text{a}}$	mult (<i>J</i> in Hz)	$\delta_{\text{C}}^{\text{b}}$	
1	4.05	dd (10.5, 6.0)	58.8	CH
2	1.28	m	28.2	CH ₂
3	1.83	m	21.5	CH ₂
4	3.57	m	44.7	CH ₂
4'	3.36	m		
1''			121.9	C
2''	7.88	dd (8.5, 2.0)	131.7	CH
3''	6.82	dd (8.5, 2.0)	114.8	CH
4''			162.0	C
5''	6.82	dd (8.5, 2.0)	114.8	CH
6''	7.88	dd (8.5, 2.0)	131.7	CH
7''			169.6	C
1'''			126.4	C
2'''	7.05	dd (8.5, 2.0)	130.9	CH
3'''	6.71	dd (8.5, 2.0)	115.0	CH
4'''			156.4	C
5'''	6.71	dd (8.5, 2.0)	115.0	CH
6'''	7.05	dd (8.5, 2.0)	130.9	CH
7'''	3.04	dd (14.0, 4.5)	36.5	CH ₂
8'''	3.08	dd (14.0, 5.0)		
9'''	4.37	dd (5.0, 4.5)	56.7	CH
			165.7	C

^a 500 MHz.^b 125 MHz; Based on HMQC and HMBC spectra.**Figure 2.** Selected COSY (–) and HMBC (→) correlations for units A (C-1' to C-7'), B (C-1 to C-4), and C (C-1'' to C-9'') of turtschamide (1).

COSY spectrum revealed the connectivities of C-1 to C-4 (Fig. 2). Therefore, the structure of 1-substituted putrescine (unit B) was elucidated as shown. Similarly, another set of a 1,4-disubstituted benzene moiety was assigned by signals at δ_{H} 6.71 (2H, dd, $J = 8.5, 2.0$ Hz, H-3'', 5'') and 7.05 (2H, dd, $J = 8.5, 2.0$ Hz, H-2'', 6''). The ¹H–¹H COSY spectrum suggested the connectivity of C-7'' to C-8'', while the connectivities of C-1'' to C-7'' and C-8'' to C-9'' were determined by the analysis of HMBC correlations for H-2''/C-7'', H-6''/C-7'', H-7''/C-9'', and H-8''/C-9'' (Fig. 2). From these data, the structure of unit C was established as shown. The connectivity of unit A to unit B was implied based on an HMBC correlation from H-1 to C-7' (Fig. 3). Similarly, the connectivities between unit B and unit C were deduced by HMBC cross peaks of H-1/C-8'' and

**Figure 3.** Gross structure of turtschamide (1).**Figure 4.** Structure of related putrescine bisamides (2 and 3).

H-4/C-9''. Accordingly, the gross structure of turtschamide (1) was elucidated as shown in Figure 3.

The assignment of the relative configuration of 1 was determined on the basis of NOE experiment. A strong NOE was observed between H-1 and H-8'', which supported that they were on the same side of the eight-membered 1,4-diazocane ring. To determine the absolute configuration of the chiral centers of 1, Marfey's method for HPLC was used (Supplementary data).^{10,11} The hydrolyzate of 1 was derivatized with *N* α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide (FDAA) and analyzed by HPLC using an ODS column.¹² The HPLC chromatogram (UV 340 nm) of the FDAA-derivatized hydrolyzate exhibited a peak for L-Tyr with a close peak area, which indicated that turtschamide (1) possesses a L-Tyr residue. On the basis of analyzing the information from Marfey's method and the observed NOE data, the absolute configurations of 1 were assigned as 1*R* and 8'*S*.

A small family of related putrescine bisamides has been reported as natural products from several plants,^{13–16} and the most closely related ones are dasyclamide (2) from the leaves of *Aglaia dasyclada*¹³ and aglaiduline (3) from the leaves of *Aglaia edulis* (Fig. 4).¹⁶ They have two substituents on both sides of the putrescine bisamide, respectively, including a hydroxytyglic acid, a cinnamic acid, and a phenylacetic acid. This is the first report of the isolation of the cyclized putrescine bisamide with two substituents forming a 1,4-diazocane ring. Turtschamide (1) contains a putrescine unit, which plays important roles in the biosynthesis of pyrrolidine, tropane, and pyrrolizidine alkaloids. As shown in related putrescine bisamides, putrescine tends to be substituted at both ends of the molecule easily.^{13–16} Turtschamide (1) might be derived from a combination of 4-hydroxybenzoic acid and L-tyrosine at both ends of putrescine to form an intermediate, which is followed by hydroxylation and cyclization to generate 1.

Turtschamide (1) was evaluated for potential antitumoral cytotoxicity against A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT15 (colon cancer) cell lines using the sulforhodamine B (SRB) bioassay.¹⁷ The results demonstrated that 1 exhibited cytotoxicity against all of the cell lines tested with IC₅₀ values of 4.53 ± 0.7, 5.54 ± 1.3, 3.28 ± 0.5, and 6.92 ± 2.1 μM, respectively, for A549, SK-OV-3, SK-MEL-2, and HCT-15 cells. Turtschamide (1) thus possesses moderate cytotoxicity that is slightly weaker than that of the clinically used anticancer drug, etoposide (IC₅₀ values of 0.74, 1.37, 0.81, and 1.83 μM against A549, SK-OV-3, SK-MEL-2, and HCT-15 cells, respectively).

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2012.01.043.

References and notes

1. Nawrot, R.; Wolun-Cholewa, M.; Bialas, W.; Wyrzykowska, D.; Balcerkiewicz, S.; Gozdzička-Jozefiak, A. *BMC Complement. Altern. Med.* **2010**, *10*, 78.
2. Lee, K. H.; Huh, J. W.; Choi, M. M.; Yoon, S. Y.; Yang, S. J.; Hong, H. N.; Cho, S. W. *Exp. Mol. Med.* **2005**, *37*, 371.
3. Hung, T. M.; Na, M. K.; Dat, N. T.; Ngoc, T. M.; Youn, U. J.; Kim, H. J.; Min, B. S.; Lee, J. P.; Bae, K. H. *J. Ethnopharmacol.* **2008**, *119*, 74.
4. Matsuda, H.; Shiimoto, H.; Naruto, S.; Namba, K.; Kubo, M. *Planta Med.* **1988**, *54*, 27.
5. Kim, K. H.; Lee, I. K.; Piao, C. J.; Choi, S. U.; Lee, J. H.; Kim, Y. S.; Lee, K. R. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4487.
6. Kim, K. H.; Lee, I. K.; Choi, S. U.; Lee, J. H.; Moon, E.; Kim, S. Y.; Lee, K. R. *Planta Med.* **2011**, *77*, 1555.
7. Kim, K. H.; Piao, C. J.; Choi, S. U.; Son, M. W.; Lee, K. R. *Planta Med.* **2010**, *76*, 1732.
8. Lee, H. Y.; Kim, C. W. *Kor. J. Pharmacogn.* **1999**, *30*, 332.
9. *Turtschamide (1)*: amorphous gum; $[\alpha]_D^{25}$: -15.7 (c 0.25, MeOH); UV λ_{\max} (MeOH) nm (log ϵ): 204 (4.18), 245 (4.12), 286 (2.78), 313 (1.71); IR (KBr): ν_{\max} = 3336, 2944, 1690, 1456, 1278, 1056, 675 cm^{-1} ; ^1H (CD_3OD , 500 MHz) and ^{13}C NMR (CD_3OD , 125 MHz): see Table 1; ESI-MS m/z 370 $[\text{M}+\text{H}]^+$; positive HR-ESI-MS m/z 370.1770 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_{20}\text{H}_{24}\text{N}_3\text{O}_4$, 370.1767).
10. Marfey, P. *Carsberg Res. Commun.* **1984**, *49*, 591.
11. Isaka, M.; Palasarn, S.; Lapanun, S.; Sriklung, K. *J. Nat. Prod.* **2007**, *70*, 675.
12. First, to break the connectivity of C-1 and –NH–C-8, following reaction was performed. To a solution of turtschamide (**1**, 2.0 mg) in CH_3CN (9.0 mL) and glacial acetic acid (3.0 mL) was added NaCNBH_3 (3.0 mmol) and the reaction mixture was heated at 55 °C for 12 h, cooled to room temperature, evaporated to dryness and redissolved in CH_2Cl_2 . The organic layer was washed with 1 N NaOH, brine and filtered through Na_2SO_4 , concentrated. Then, the reaction mixture was hydrolyzed by heating in 6 N HCl (1 mL) at 110 °C for 15 h. After cooling, the solution was evaporated to dryness and dissolved in H_2O (150 μL). To this solution was added 1% (w/v) FDAA (Marfey's reagent, N_α -(2,4-dinitro-5-fluorophenyl)-L-alaninamide) in acetone (300 μL) and 1 M NaHCO_3 solution (70 μL) and the mixture was incubated at 40 °C for 1 h. The reaction was quenched by the addition of 1 M HCl (70 μL) and the resulting homogeneous solution was diluted with MeOH (1.0 mL). Standard L- and D-Tyr were also derivatized with FDAA in the same manner as that of the hydrolyzate of **1**. Five microliters of the FDAA derivatives were analyzed by HPLC using an ODS column. HPLC analysis was performed with the following conditions: INNO Column (INNOPIA Co., 10.0 \times 250 mm, 5 μm , iP-ODS-N-120-5 μm), mobile phase MeCN/(0.05% TFA in H_2O), 25:75, flow rate 1.0 mL/min, UV detection at 340 nm. Retention times (min) of the FDAA amino acid derivatives used as standards were as follows: L-Tyr (19.9 min), and D-Tyr (27.4 min). The HPLC chromatogram of the derivatized hydrolyzate of **1** contained a peak of L-Tyr (19.9 min).
13. Chaidir, Lin, W. H.; Ebel, R.; Edrada, R. A.; Wray, V.; Nimitz, M.; Sumaryono, W.; Proksch, P. *J. Nat. Prod.* **2001**, *64*, 1216.
14. Duong, T. N.; Edrada, R.; Ebel, R.; Wray, V.; Frank, W.; Duong, A. T.; Lin, W. H.; Proksch, P. *J. Nat. Prod.* **2007**, *70*, 1640.
15. Abdelfattah, M. S.; Toume, K.; Ahmed, F.; Sadhu, S. K.; Ishibashi, M. *Chem. Pharm. Bull.* **2010**, *58*, 1116.
16. Saifah, E.; Suttisri, R.; Shamsub, S.; Pengsuparp, T.; Lipipun, V. *Phytochemistry* **1999**, *52*, 1085.
17. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.