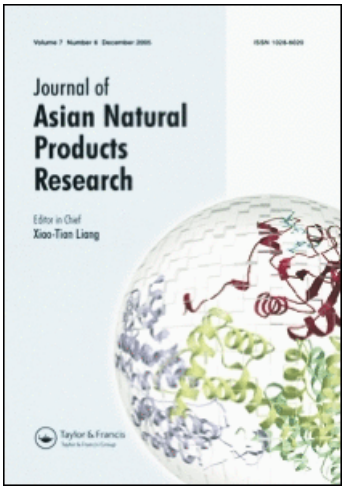

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Tan, Ni , Cai, Xiao-Ling , Wang, San-Yong , Pan, Jia-Hui , Tao, Yi-Wen , She, Zhi-Gang , Zhou, Shi-Ning , Lin, Yong-Cheng and Vrijmoed, Lilian L. P.(2008) 'A new hTopo I isomerase inhibitor produced by a mangrove endophytic fungus no. 2240', Journal of Asian Natural Products Research, 10: 7, 607 — 610
To link to this Article: DOI: 10.1080/10286020802133167
URL: http://dx.doi.org/10.1080/10286020802133167

Journal of Asian Natural Products Research
Vol. 10, No. 7, July 2008, 607–610

A new hTopo I isomerase inhibitor produced by a mangrove endophytic fungus no. 2240

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(*Received 13 September 2006; final version received 23 April 2007*)

A new hTopo I isomerase inhibitor, (+)-3,3′,7,7′,8,8′-hexahydroxy-5,5′-dimethylbianthraquinone (2240A), was isolated from the mangrove endophytic fungus no. 2240 collected from an estuarine mangrove at the South China Sea coast. Its structure was elucidated by spectral analyses including two-dimensional NMR, HR-EI-MS, IR, and UV. The hTopo I isomerase inhibition experiment showed that 2240A (**1**) possessed strong inhibiting activity. When its inhibition concentration was 4.65 μmol/l, its percent inhibition rate was 59.1%, while the lowest inhibition concentration of the positive control camptothecin was 1.00×10^3 μmol/l.

Keywords: mangrove endophytic fungus; (+)-3,3′,7,7′,8,8′-hexahydroxy-5,5′-dimethylbianthraquinone; 2240A; hTopo I isomerase inhibitor

1. Introduction

The mangrove habitat has been proved to be a rich source of new fungal species that form the second largest ecological subgroup of marine fungi [1]. As the novel and active secondary metabolites were found, the mangrove endophytic fungi have gained increased attention in the last decade. In our search for secondary metabolites of endophytic fungi, many bioactive and/or novel compounds were isolated [2–7]. In this paper, we report the isolation, structural elucidation, and bioactivity of a new hTopo I isomerase inhibitor, 2240A (**1**) (Figure 1), from the fungus (strain no. 2240).

2. Results and discussion

A 200 l fermentation broth was concentrated and extracted with ethyl acetate. The extract was repeatedly chromatographed on silica gel. An orange-red compound was obtained, $[\alpha]_D^{20} + 62.50$ (*c* 0.08, dioxin), UV λ_{max} (MeOH) 220.40 nm (log ε, 1.72) and mp 300°C. The molecular formula of the compound was determined to be $C_{30}H_{18}O_{10}$ by HR-EI-MS at *m/z* 538.0886. The numbers of protons and carbons observed in the ^1H and ^{13}C NMR spectra were half of the molecular formula, which indicated that **1** was a symmetrical molecule. In the EI-MS spectra, there were two main fragmentations (*m/z* 326 and 269). The fragmentation at *m/z* 326 was probably caused by the double RDA pyrolysis in rings **B** and **B′**, including the fractures of the bonds C_9—C_{12}, C_{10}—C_{11}, C_9'—C_{12}', and C_{10}'—C_{11}'. Another fragmentation at *m/z* 269 was produced by the cleavage of the C_1—C_1' bond. The IR spectrum exhibited the presence of hydroxyl groups at 3464 and 3251 cm^{-1}; carbonyl groups at 1674 cm^{-1}; aryl groups

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802133167
http://www.informaworld.com

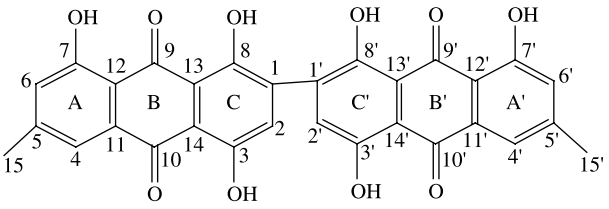

Figure 1. Structure of 2240A (**1**).

at 1624, 1570, and 1550 cm^{-1}; and methyls at 1393 cm^{-1}. In the ^{1}H NMR spectrum, the presence of three chelated hydroxyl groups in the molecule was proved by downfield chemical shifts (δ 11.17, 12.03, and 12.76). In the ^{13}C NMR spectrum (Table 2), signals of two carbonyl groups (δ 189.3 and 181.9), 12 olefinic carbons (δ 123.3, 107.0, 164.2, 160.9, 123.3, 147.9, 120.2, 160.9, 181.9, 189.3, 133.1, 113.0, 108.7, and 131.1) and one methyl group (δ 21.3) were observed. The 22 unsaturation equivalents required by the molecular formula and more than 20 olefinic carbon atoms indicated that 2240A belonged to the anthraquinone-type compound. The ^{1}H and ^{13}C NMR spectral data were assigned by HMQC. The HMBC spectral data established the overall molecular structure (Figure 3). The correlations between C-6, C-7, C-12, and 7-OH showed that 7-OH was connected to ring **A**, especially the correlation between H-4 and C-10 defined the connection between rings **A** and **B**. Similarly, the correlations between C-2, C-3, C-14, and 3-OH defined that 3-OH was connected to ring **C**. According to the related literature [8–11], the attributions of chemical shifts for the chelated hydroxyl groups and the carbonyl groups could be easily found out. From the above information, it could be concluded that the connection points between two structural fragmentations of the symmetrical compound (**1**) were C-1 and C-1′, not C-2 and C-2′ or C-1 and C-2′. 2240A (**1**) was similar to skyrin (**2**) (Figure 4), expect for the presence of three chelated hydroxyls instead of two in skyrin; C-12 resonated at δ 113.0 in **1**, while it was at δ 131.1 in skyrin [8].

The hTopo I isomerase inhibition experiment showed that 2240A possessed strong inhibiting activity. When its inhibition concentration was 4.65×10^{-3} μmol/ml, its percent inhibition rate was 59.1% (Table 1). The lowest inhibiting concentration of **1** (2.35×10^{2} μmol/l) was less than the positive control camptothecin (1.00×10^{3} μmol/l).

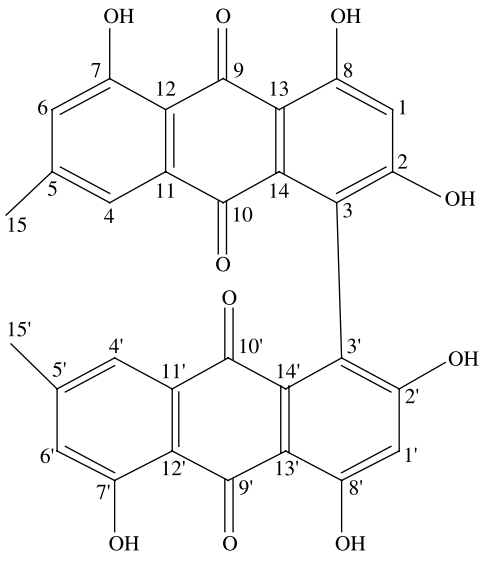

Figure 2. Structure of skyrin (**2**).

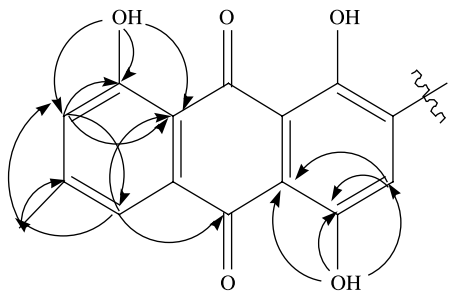

Figure 3. The selective HMBC correlations of 2240A.

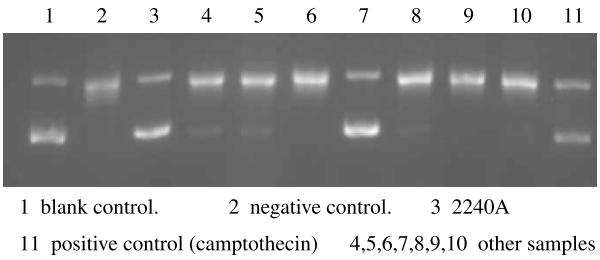

Figure 4. Fluor-S multimager gel photocopy of hTopo I isomerase inhibition experiment.

3. Experimental

3.1 General experimental procedures

The melting points were measured on X-4 melting point apparatus from Beijing Tac Co. Ltd and are uncorrected. Optical rotations were measured in methanol on Schmidt + Haensch Polartronic HH W5 using 1 dm cell at 20°C. IR spectra were measured in the region 400–4000 cm^{-1} on Equinox 55 FT-IR analyzer. The sample was prepared either as a KBr plate or pellet. ^{1}H and ^{13}C NMR spectra were measured in deuterated DMSO at Mercury Plus 500 from Varian Co. Ltd. UV spectra were from Shimadzu UV-2501 PC UV–Vis spectrophotometer. Mass spectra were obtained on MAT95XP high-resolution mass spectrometer from Thermo Co. Ltd.

3.2 Material

A fungus strain (no. 2240) was isolated from an estuarine mangrove at the South China Sea coast. It is deposited in the Department of Applied Chemistry, Zhongshan University, Guangzhou, China, and the Department of Biology and Chemistry, City University of Hong Kong, China. This fungus has no spore and its species was unidentified.

3.3 Culture conditions

Starter cultures (from Professor E.B. Gareth and Dr L.L.P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250 ml Erlenmeyer flask containing 100 ml of liquid medium GYP (5 g/l glucose, 1 g/l peptone, 0.5 g/l yeast extract, 0.5 g/l beef extract, and 3 g/l NaCl). The flask was incubated at 30°C for 40 days.

3.4 Extraction and isolation

The cultures (20 l) were filtered through cheesecloth. The filtrate was concentrated to 5 l *in vacuo* below 50°C and extracted three times by shaking with an equal volume of ethyl acetate. The combined organic extracts (20.8 g) were applied to a silica gel column, eluting with a gradient of petroleum ether to ethyl acetate to offer **1** (25 mg).

3.4.1 2240A (**1**)

An orange-red compound, mp 300°C, $[\alpha]_D^{20} + 62.50$ (*c* 0.08, dioxin); UV λ_{max} (MeOH): 220.40 nm ($\log \varepsilon$ 1.72); IR (KBr) ν_{max} (cm^{-1}): 3464, 3251, 1674, 1624, 1570, 1550, 1482, 1457, 1393, 1365, 1250, 1209; NMR spectral data: see Table 2; EI-MS *m/z*: 538 $[M]^+$, 326, and 269; HR-EI-MS *m/z*: 538.0886 (calcd for $C_{30}H_{18}O_{10}$, 538.0894).

3.5 hTopo I isomerase inhibition experiment

Four solution systems were firstly completed, including blank control, negative control, positive control, and the tested sample. Their compositions were shown in Table 1. After they had been kept for 30 min at 37°C, the reactions were ended. Then, the solution systems were electrophoresed for 35 min at room temperature under a voltage of 80 v/cm in TBE containing 1% agarose, dyed for 30 min in 0.5% EB, and washed three times with distilled water. Finally,

Table 1. Inhibition activity of 2240A to hTopo I isomerase.

System	Buffer solution (μl)	pBR322 (μg)	hTopoI (U)	Sample concentration	Percent inhibition rate
Blank control	20	0.5			
Negative control	20	0.5	1		0
Positive control	20	0.5	1	10^3 μmol/l camptothecin	100%
Tested sample	20	0.5	1	4.65 μmol/l 2240A	59.1%

Table 2. NMR spectral data of compound **1** (DMSO-d_6).

Position	δ_c (125 Hz)	δ_H (500 Hz)	HMBC
1, 1′	123.3 (C)		
2, 2′	107.0 (CH)	6.7 (s)	C-1, 1′, 3, 3′, 14, 14′
3, 3′	164.2 (C)		
4, 4′	120.2 (CH)	7.27 (d, 1.5 Hz)	C-6, 6′, 10, 10′, 12, 12′, 15, 15′
5, 5′	147.9 (C)		
6, 6′	123.3 (CH)	7.13 (d, 1.5 Hz)	C-4, 4′, 7, 7′, 12, 12′, 15, 15′
7, 7′	160.9 (C)		
8, 8′	160.9 (C)		
9, 9′	189.3 (C)		
10, 10′	181.9 (C)		
11, 11′	133.1 (C)		
12, 12′	113.0 (C)		
13, 13′	131.8 (C)		
14, 14′	108.7 (C)		
15, 15′	21.3 (CH_3)	2.33 (s)	C-4, 4′, 5, 5′, 6, 6′
3, 3′-OH		12.76	C-2, 2′, 3, 3′, 14, 14′
7, 7′-OH		12.03	C-6, 6′, 7, 7′, 12, 12′
8, 8′-OH		11.17	

the test results were analyzed by Fluor-S multimager gel photocopy system (Figure 4).

Acknowledgements

We wish to acknowledge the financial support from the National Natural Science Foundation of China (20072058, 20572136), the 863 Foundation of China (2003AA624010, 2006AA09Z422), the Natural Science Foundation of Guangdong Province, China (021732,2003A3050401), and the Star Lake Biotechnology Co. Inc., Zhaoqing, Guangdong, China.

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