

Two New 2(1*H*)-Pyrazinone Derivatives from the Plant Endophyte *Streptomyces* sp. KIB-H1992

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Abstract: Two new 2(1*H*)-pyrazinone derivatives, 3,6-diisopropyl-5-methylpyrazin-2(1*H*)-one (**1**) and 5-(hydroxymethyl)-3,6-diisopropylpyrazin-2(1*H*)-one (**2**), were isolated from the fermentation broth of endophytic actinomycete *Streptomyces* sp. KIB-H1992. Their structures were established based on the detailed spectroscopic analyses, including ESI-MS, HR-ESI-MS, 1D and 2D NMR spectra.

Keywords: 2(1*H*)-pyrazinone derivatives; endophytic actinomycete; *Streptomyces* sp.; spectroscopic analyses.

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1. Microorganism Material

The strain designated KIB-H1992 was isolated from *Cassia tora* Linn., which was collected in Yuanjiang, Yunnan Province, China, in 2016. It was identified as *Streptomyces* sp. by a 16S rRNA gene sequence (GenBank accession no. MN180858), and showed a 100.0% identity to the *Streptomyces mobaraensis* strain NRRL B-3729 (GenBank accession no. NR_043830.1) and *Streptomyces mobaraensis* strain NBRC 13819 (GenBank accession no. NR_112524.1).

2. Previous Studies

Endophytes, ranging from fungi, bacteria and actinomycetes, are extremely diverse and source of functional metabolites with chemical and bioactive diversity [1-3]. More recently, Endophytes have attracted increasing attention in the discovery of natural products with potential physiological activity [4,5]. As part of our continuous efforts in screening for more secondary metabolites from endophytic microorganisms [6,7], two new 2(1*H*)-pyrazinone derivatives were obtained from the fermentation

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broth of endophytic actinomycete *Streptomyces* sp. KIB-H1992. To our best knowledge, there are a variety of 2(1H)-Pyrazinone derivatives with diverse structures have hitherto been isolated and identified, and some of them possess potent biological activities [8-14].

3. Present Study

The strain KIB-H1992 was cultivated on ISP2 agar plates (malt extract 10 g, agar 20 g, glucose 4 g and yeast extract 4 g in 1 L of water, pH 7.2) at 30 °C for five days. After that, it was transferred into 250 mL baffled Erlenmeyer flasks, each flask was filled with 50 mL of Tryptone Soy Broth (30 g/L, pH 7.3 ± 0.2), and cultivated on a rotary shaker (200 rpm) at 30 °C for one day. Fermentations were carried out in 1000 mL baffle Erlenmeyer flasks with liquots (12.5 mL) of the culture. Each flask was filled with 250 mL of medium consisting of 1% soluble starch (w/v), 1% glucose (w/v), 1% glycerol (w/v), 0.5% tryptone (w/v), 0.5% yeast extract (w/v) and 0.3% CaCO₃ (w/v) in deionized water (pH 7.0), and cultured on rotary shaker (200 rpm) at 30 °C for seven days.

The fermentation broth (20 L) was centrifuged (4000 rpm, 15 min), the supernatant liquid was extracted with ethyl acetate (6 × 5 L) and concentrated in vacuo to afford 2.5 g crude extract. The mycelium was extracted with acetone (4 × 0.5 L), and the acetone was removed in vacuo to yield the aqueous concentrate, which was then extracted with ethyl acetate (1 L × 3) and concentrated to give 1.1 g crude extract. Both extracts were combined for further purification. The crude extract (3.6 g) was fractionated by flash column chromatography on silica gel eluting with petroleum ether/ethyl acetate (20 : 1 to 0 : 1) as the eluent to afford five fractions (Fr1–Fr5). Fr4 was sequentially separated using Sephadex LH-20 column with methanol as eluent into four subfractions (Fr4A–Fr4D). Fr4C was then subjected to semipreparative HPLC (Hitachi HPLC system, YMC–Triart C18 column, 250 × 10 mm, DAD detector), elution with 55% methanol containing 0.1% acetic acid at a flow rate of 3 mL min⁻¹ gave **1** (*t_R* = 24.1, 9.1 mg) and **2** (*t_R* = 13.5 min, 3.1 mg).

3,6-diisopropyl-5-methylpyrazin-2(1H)-one (1): white powder. IR ν_{\max} (KBr): 3447, 2964, 2876, 1636, 1601, 1468, 1129, 501 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ_{H} 12.33 (1H, brs, NH), 3.37 (1H, m, H-9), 3.02 (1H, m, H-7), 2.30 (3H, s, H₃-5'), 1.32 (6H, d, *J* = 7.2 Hz, H₃-8 and H₃-10), 1.24 (6H, d, *J* = 6.6 Hz, H₃-11 and H₃-12); ¹³C NMR (150 MHz, CDCl₃): δ_{C} 159.9 (C-3), 156.7 (C-2), 138.4 (C-6), 127.1 (C-5), 30.6 (C-10), 28.5 (C-7), 20.2x2 (C-8 and C-9), 19.9x2 (C-11 and C-12), 18.8 (C-5'). HR-ESI-MS: C₁₁H₁₈N₂O at *m/z* 195.1497 [M+H]⁺, (calcd for C₁₁H₁₈N₂O *m/z*: 195.1492).

5-(hydroxymethyl)-3,6-diisopropylpyrazin-2(1H)-one (2): white powder. IR ν_{\max} (KBr): 3447, 2964, 2876, 1636, 1601, 1468, 1129, 501 cm⁻¹. ¹H NMR (600 MHz, CDCl₃): δ_{H} 12.06 12.06 (1H, brs, NH), 4.58 (2H, s, H₂-5'), 3.41 (1H, m, H-9), 3.01 (1H, m, H-7), 1.33 (6H, d, *J* = 7.2 Hz, H₃-8 and H₃-10), 1.26 (6H, d, *J* = 6.6 Hz, H₃-11 and H₃-12); ¹³C NMR (150 MHz, CDCl₃): δ_{C} 160.2 (C-3), 157.1 (C-2), 139.3 (C-6), 128.1 (C-5), 60.1 (C-5'), 30.3 (C-10), 27.4 (C-7), 20.5x2 (C-8 and C-9), 19.9x2 (C-11 and C-12). HRESI-MS: C₁₁H₁₈N₂O₂ at *m/z* 211.1439 [M+H]⁺, (calcd for C₁₁H₁₈N₂O₂, *m/z*: 211.1441).

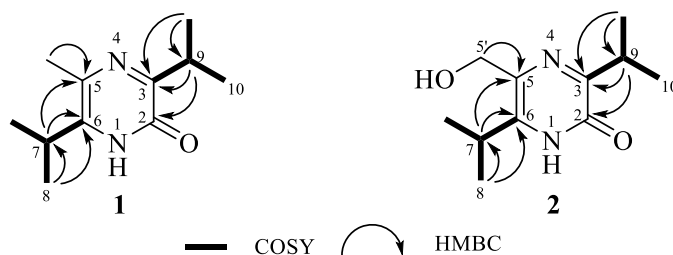


Figure 1. Structures of compounds **1-2**, and their key 2D NMR correlations.

Compound **1** was obtained as white powder. The molecular formula was assigned as C₁₁H₁₈N₂O

with four degrees of unsaturation by ESI-MS (m/z 195 $[M+H]^+$, m/z 217 $[M+Na]^+$) and HR-ESI-MS (m/z 195.1497 $[M+H]^+$, calcd 195.1492). Its 1H NMR spectrum (Table 1) showed feature signals for two isopropyls at 3.02 (1H, hept, $J = 7.2$ Hz), 1.32 (6H, d, $J = 7.2$ Hz) and 3.37 (1H, hept, $J = 6.6$ Hz), 1.24 (6H, d, $J = 6.6$ Hz), which was further identified by 1H - 1H COSY spectrum. The ^{13}C NMR spectrum (Table 1), combined with the HSQC spectra, displayed the presence of 9 carbons (two carbon signal for CH_3 of two isopropyls overlapped), including five methyl groups (δ_c 18.8, 20.0×2 and 20.2×2), two methines (δ_c 28.5 and 30.6), and four quaternary carbons including two olefin carbons (δ_c 127.1 and 138.4). The spectroscopic data indicated that the structure of **1** was highly similar to that of 3-isobutyl-6-isopropyl-5-methylpyrazin-2(1H)-one [15] with the major difference that an additional isopropyl group was appeared in **1** instead of the isobutyl group. The HMBC correlations from δ_H 3.02 (H-7) to δ_c 127.1 (C-5) and δ_c 138.4 (C-6), and from δ_H 1.32 (H-8) to δ_c 138.4 (C-6) indicated that an isopropyl was attached to C6, and suggested the presence of C5-C6 olefin. The methyl at C5 was assigned on the basis of the key HMBC correlations from δ_H 2.30 (CH_3 -5) to δ_c 127.1 (C-5) and δ_c 138.4 (C-6), and the 1H NMR data comparison of compound **1** at δ_H 2.30 (3H, s) with 3-isobutyl-6-isopropyl-5-methylpyrazin-2(1H)-one at δ_H 2.28 (3H, s), 3,6-diisobutyl-5-methylpyrazin-2(1H)-one at δ_H 2.29 (3H, s) [15] and Sorazinone B [11] at δ_H 2.26 (3H, s). The remaining isopropyl group was deduced to be attached to C3 by HMBC correlations from δ_H 1.26 (H-10) to δ_c 159.9 (C-3) and δ_c 30.6 (C-9), and from δ_H 3.37 (H-9) to δ_c 156.7 (C-2) and 159.9 (C-3). Additionally, there is suggestive of an amido carbonyl group at C2 position and an imine carbon at C3 position from the above HMBC correlations. From the foregoing evidences, compound **1** was determined to be 3,6-diisopropyl-5-methylpyrazin-2(1H)-one (Figure 1). The structures of 3-isobutyl-6-isopropyl-5-methylpyrazin-2(1H)-one, 3,6-diisobutyl-5-methylpyrazin-2(1H)-one and Sorazinone B were shown in the supporting information, and data comparison of compound **1** with these most similar compounds was also listed in supporting information.

Compound **2** was obtained as a white powder. Its ESI-MS spectrum showed a pseudo-molecular ion peak at m/z 211 $[M+H]^+$ and a m/z 233 $[M+Na]^+$, and molecular formula was determined as $C_{11}H_{18}N_2O_2$ by HRESI-MS at m/z 211.1439 $[M+H]^+$ (calcd for $C_{11}H_{18}N_2O_2$, 211.1441). The 1H and ^{13}C NMR spectra (Table 1) of compound **2** was very similar to those of compound **1**; however, **2** showed the presence of an additional methylene (δ_H 4.58, δ_c 60.1) instead of methyl (δ_H 2.30, δ_c 18.8) in **1**. In addition, comparing that the HR-ESI-MS with **1**, there is 16 mass units more than that of it and suggestive of a hydroxymethyl at C5 position of **2**. Thus, **2** was elucidated to be 5-(hydroxymethyl)-3,6-diisopropylpyrazin-2(1H)-one (Figure 1).

Table 1. 1H (600 MHz) and ^{13}C NMR (150MHz) data of compounds **1** and **2** in $CDCl_3$.

Position	1		2	
	δ_c (ppm)	δ_H (ppm, J in Hz)	δ_c (ppm)	δ_H (ppm, J in Hz)
1		12.33 (1H, brs)		12.06 (1H, brs)
2	156.7		157.1	
3	159.9		160.2	
5	127.1		128.1	
5-Me/5 \wedge	18.8	2.30 (3H, s)	60.1	4.58 (2H, s)
6	138.4		139.3	
7	28.5	3.02 (1H, hept, $J = 7.2$ Hz)	27.4	3.01 (1H, hept, $J = 7.2$ Hz)
8	20.2	1.32 (6H, d, $J = 7.2$ Hz)	20.5	1.33 (6H, d, $J = 7.2$ Hz)
9	30.6	3.37 (1H, hept, $J = 6.6$ Hz)	30.3	3.41 (1H, hept, $J = 6.6$ Hz)
10	19.9	1.24 (6H, d, $J = 6.6$ Hz)	19.9	1.26 (6H, d, $J = 6.6$ Hz)

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Supporting Information

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