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NOTE

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New Cyclopentenone Derivatives from an Endophytic *Streptomyces* sp. Isolated from the Mangrove Plant *Aegiceras comiculatum*

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Abstract From the mangrove plant *Aegiceras comiculatum* collected in South China an endophytic *Streptomyces* sp. was isolated. Following cultivation in a seawater-based medium, four new cyclopentenone derivatives, namely (5*R*) 3-amino-5-hydroxy-5-vinyl-2-cyclopenten-1-one (**1**), (5*R*) 5-hydroxy-3-[(methoxy-carbonyl)amino]-5-vinyl-2-cyclopenten-1-one (**2**), (5*R*) 5-hydroxy-3-[[2-(4-hydroxyphenyl)ethyl]amino]-5-vinyl-2-cyclopenten-1-one (**3**), and 3-isobutylpropanamide-2-cyclopenten-1-one (**4**), were obtained from the fermentation broth. Their structures were elucidated by extensive spectroscopic (UV, IR, ESIMS, and 2D NMR) data analyses.

Keywords endophyte, *Streptomyces* sp., *Aegiceras comiculatum*, (5*R*) 3-amino-5-hydroxy-5-vinyl-2-cyclo-penten-1-one, cyclopentenone derivatives

Introduction

Endophytes are microorganisms that invade the healthy tissues of living plants without causing disease symptoms. The endophytic association with the hosts results in advantages, such as increased plant growth, deterrence of fungal pathogens, prevention of herbivory, and drought tolerance. Endophytic microorganisms from mangrove plants have become an important source of pharmacologically active metabolites [1~7]. In continuation of our investigation on chemical diversity from marine microorganisms, an endophytic *Streptomyces* sp. was isolated from the mangrove plant *Aegiceras comiculatum*, which has been examined chemically [8]. From the cultivation in a seawater-based medium, four new cyclopentenone derivatives (**1**~**4**) were isolated. This paper reports a structure elucidation of the obtained compounds.

Results and Discussion

Compound **1** was isolated as a yellowish oil, and its molecular formula was established as $C_7H_9NO_2$ on the basis of HRESIMS data (*m/z* 162.0540 $[M+Na]^+$, calcd. 162.0531) and NMR data, indicating four degrees of unsaturation. The IR absorptions at 3308, 3181, 1617, and 1531 cm^{-1} suggested the presence of hydroxyl or/and amino, and olefinic groups. The ^1H NMR spectrum exhibited the signals for a mono-substituted vinyl group at δ_H 5.15 (1H, dd, J=1.2, 10.5 Hz), 5.37 (1H, dd, J=1.2, 17.1 Hz), and 5.89 (1H, dd, J=10.5, 17.1 Hz), a tri-substituted vinyl proton at δ_H 4.98 (1H, br s), and aliphatic methylene signals at δ_H 2.85 (1H, d, J=17.4 Hz) and 2.70 (1H, d, J=17.4 Hz). The ^{13}C NMR and DEPT spectra displayed seven carbon signals involving a keto carbon at δ_C 204.67 (s, C-1), four olefinic carbons at δ_C 114.2 (t, C-7), 141.2 (d, C-6), 179.2 (s, C-3), and 97.8 (d, C-2), one oxygenated quaternary carbon at δ_C 80.0 (s, C-5), and a methylene at δ 44.2 (t, C-4). The HMQC spectrum

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supported the assignment of the chemical shifts for the protonated carbons. The above NMR data were very similar with those reported for 3-dimethylamino-5-hydroxy-5-vinyl-2-cyclopenten-1-one [9], in which the carbon signals of β-dimethylamino substituted propenone unit were assigned to 200.9 (s), 174.1 (s), and 96.1 (d), respectively [9]. The HMBC and COSY correlations of **1** further supported the existence of a cyclopenten-1-one unit. The HMBC correlations from the vinyl methylene protons at δ_H 5.15 (dd) and 5.37 (dd) to C-5, and from δ_H 5.89 (dd) to C-4, C-5, and C-1, deduced the location of the terminal double bond and a hydroxyl group at the same position C-5, and confirmed the partial structure of 5-hydroxy-5-vinyl-2-cyclopenten-1-one. The remaining elements of NH_2 was obviously consistent with an amino group which was linked to C-3 rather than to C-2 of the cyclopenten-1-one. This fact was deduced by the singlet olefinic proton at δ_H 4.98 (1H, br s, H-2) showing no NOE correlation with the methylene protons at C-4 and the vinyl signal C-3 (δ_C 179.2, s) resonated at extremely downfield. In regard to the stereochemistry of chiral carbon C-5, the value of optical rotation ($[\alpha]_D$ +127.1°) mainly induced by the substituents at C-5 was in positive, the same phase as that of 3-methoxy-5-hydroxy-5-vinyl-2-cyclopenten-1-one ($[\alpha]_D$ +63°) [11], implying that both compounds shared the same configuration. Since the CD spectrum of latter showed a positive Cotton effect at 281 nm ($n \rightarrow \pi^*$ transition) [10], analysis of the geometrical arrangement of the molecule in the eight octants formed by the symmetry planes of the orbital of the keto group indicated the absolute configuration as 5*R*. Accordingly, the configuration of C-5 in **1** was supposed to be 5*R*. The structure of **1** was thus determined as (5*R*) 3-amino-5-hydroxy-5-vinyl-2-cyclopenten-1-one.

The molecular formula of **2** was established as $C_9H_{11}NO_4$ on the basis of HRESIMS (*m*/*z* 220.0590, calcd. for $C_9H_{11}NO_4Na$, 220.0586) and NMR data, indicating a $C_2H_2O_2$ unit more than that of **1**. The comparable NMR spectral data between **1** and **2** indicated that **2** possessed the same basic skeleton of 5-hydroxy-5-vinyl-2-cyclopenten-1-one. However, The NMR data of **2** differed from those of **1** in the substituent at C-3 where presence of an additional methoxy group (δ_H 3.78, δ_C 53.5) and a quaternary carbon (δ_C 154.8, s, C-1′) were determined to form a methylcarbamate on the basis of the HMBC correlation between the methoxy protons and C-1′ in association with the evidence of the significant highfield value of the carbonyl carbon, a similar resonance as the same group in romucosine [11]. The stereo-configuration at C-5 was supposed to be the same as that of **1** due to the positive value of optical rotation of **2**. The structure of **2** was thus determined as (5*R*) 5-hydroxy-3-[(methoxycarbonyl)-amino]-5-vinyl-2-cyclopenten-1-one.

The HRESIMS and NMR data of **3** indicated its molecular formula to be $C_{15}H_{17}NO_3$. Comparison of the ^1H and ^{13}C NMR data of **3** with those of **1** and **2** (Table 1) revealed a 5-hydroxy-5-vinyl-2-cyclopenten-1-one moiety, the same unit as that of **1** and **2**. However, the NMR spectra indicated that **3** contained an additional *p*-hydroxyphenylethyl moiety from the signals at δ_H 7.01 (2H, d, J=8.5 Hz), 6.70 (2H, d, J=8.5 Hz), 3.42 (2H, t, J=7.2 Hz), and 2.81 (2H, t, J=7.2 Hz), and the carbon signals at δ_C 157.2 (s), 130.8 (d, 2×C), 116.4 (d, 2×C), 47.2 (t), and 34.9 (t). This moiety was further confirmed by HMBC and COSY correlations, and it was deduced to form a *p*-hydroxyphenylethylamine at C-3 of the cyclopentenone core on the basis of the HMBC correlation of the methylene protons at δ_H 3.40 (2H, t, J=7.2 Hz) with a carbon at δ_C 176.2 (s, C-3). The stereochemistry of **3** was regarded to be the same as that of **1** and **2** due to the positive optical rotation. Therefore, the structure of **3** was determined as 5-hydroxy-3-[[2-(4-hydroxyphenyl)ethyl]-amino]-5-vinyl-2-cyclopenten-1-one.

Compound **4** was isolated as white amorphous powder, the molecular formula of **4** was established as $C_{12}H_{19}NO_2$ on the basis of HRESIMS and NMR data. The ^{13}C NMR and DEPT spectra of **4** displayed 12 carbon signals including two overlapping methyl resonances at δ_C 20.1 (q), five methylene at δ_C 47.0 (t), 35.3 (t), 33.7 (t), 31.8 (t), and 28.9 (t), one sp^3 methine at δ_C 28.5 (d), one olefinic methine at δ_C 129.3 (d), and three quaternary carbons at δ_C 209.6 (s), 181.2 (s), and 170.9 (s). Analyses of ^1H and ^{13}C NMR data in association with 2D NMR correlations and comparison of its NMR data with those of **1** to **3** resulted in a basic skeleton of a cyclopentenone nucleus, in which the HMBC correlations between the methylene protons at δ_H 2.38 (m, H_2-5) and the carbons at δ_C 209.6 (s, C-1), 129.3 (d, C-2) and 181.2 (s, C-3), and between the other methylene protons at δ_H 2.58 (m, H_2-4) and C-1, C-2, and C-3, as well as COSY correlation between H_2-4 and H_2-5 were observed. Moreover, a propionyl unit was established due to the COSY correlation between δ_H 2.45 (t, J=7.2, H_2-7) and 2.75 (t, J=7.2, H_2-6), and the HMBC correlations from H-6 and H-7 to a carbonyl carbon at δ_C 170.9 (s, C-8). This moiety was deduced to link to C-3 through C–C bond according to the HMBC correlations of H-2 (δ_H 5.91, s) and H_2-4 (δ_H 2.58, m) with a methylene carbon at δ_C 28.9 (t, C-6). The remaining four carbons were in constitute with a isobutyl group due to COSY correlations from δ_H 1.76 (m, H-2′) to δ_H 3.07 (dd, J=6.3, 6.6 Hz, H_2-1′) and overlapping methyl protons at δ_H 0.90 (d, J=6.6 Hz, H_3-3′, H_3-4′), and the HMBC corrections

Table 1 The ^{1}H and ^{13}C NMR data of compounds **1** to **4**

	1[a]		**2**[a]		**3**[a]		**4**[b]	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	204.7, s		207.8, s		204.1, s		209.6, s	
2	97.8, d	4.98 br s	108.2, d	6.03 br s	95.3, d	4.93 br s	129.3, d	5.91 s
3	179.2, s		169.6, s		176.2, s		181.2, s	
4	44.2, t	2.70 d (17.4)	44.6, t	3.01 d (18.0)	44.2, t	2.67 d (17.1)	31.8, t	2.58 m
		2.85 d (17.4)		2.84 d (18.0)		2.78 d (17.1)		
5	80.0, s		78.3, s		79.4, s		35.3, t	2.38 m
6	141.2, d	5.89 dd (10.5, 17.1)	140.0, d	5.88 dd (10.5, 17.1)	141.2, d	5.85 dd (10.8, 17.1)	28.9, t	2.75 t (7.2)
7	114.2, t	5.15 dd (1.2, 10.5)	114.9, t	5.19 dd (1.2, 10.5)	114.2, t	5.14 dd (0.9, 10.8)	33.7, t	2.45 t (7.2)
		5.37 dd (1.2, 17.1)		5.39 dd (1.2, 17.1)		5.34 dd (0.9, 10.8)		
8							170.9, d	
1′			154.8, s		47.2, t	3.42 t (7.2)	47.0, t	3.07 dd (6.3, 6.6)
2′			53.5, q	3.78 s	34.9, t	2.81 t (7.2)	28.5, d	1.76 m
3′					130.6, s		20.1, q	0.90 d (6.6)
4′					130.8, d	7.01 d (8.5)	20.1, q	0.90 d (6.6)
5′					116.4, d	6.70 d (8.5)		
6′					157.2, s			
7′					116.4, d	6.70 d (8.5)		
8′					130.6, d	7.01 d (8.5)		

[a] Measured in CD_3OD, [b] Measured in $CDCl_3$

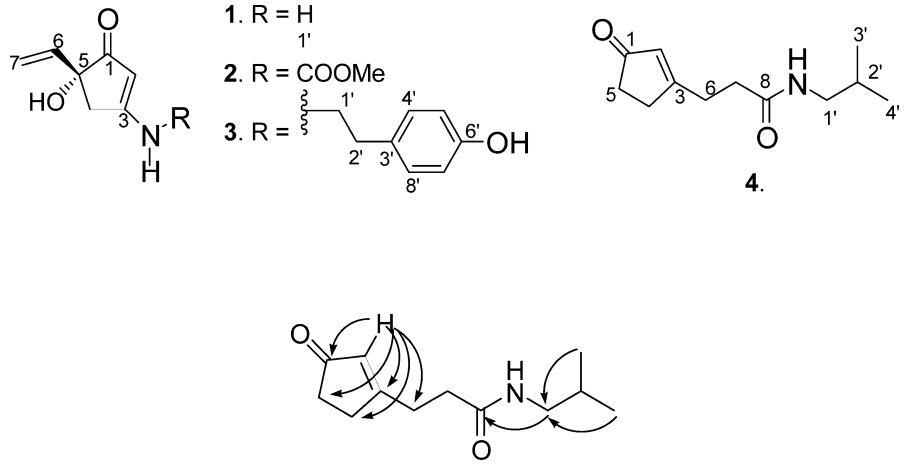

Fig. 1 Main HMBC correlations of **4**.

from H_3-3′ and H_3-4′ to C-2′ (δ_C 28.5, d) and C-1′ (δ_C 47.0, t) and the methyl carbon of each other. The isobutyl unit was proved to form an amide with carbonyl carbon C-8 (δ_C 170.9, s) through the HMBC correlation between H-1′ and C-8 (Figure 1) and IR absorptions at 1674 and 3294 nm. The structure of **4** was thus determined as 3-isobutylpropanamide-2-cyclopenten-1-one.

It is interesting to note that the rare 3-amino-5-hydroxy-5-vinyl-2-cyclopenten-1-one derivatives have previously been isolated from fungi (*Trichoderma*) [9, 10]. This is the first time to discover new compounds of the same type from an actinomycete. It was reported that *Trichoderma* could convert ciprofloxacin and norfloxacin to those conjugates [12]. All the compounds were tested for antibiotic against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium smegmatis*, *M. vaccae*,

Penicillium notatum, *Phoma destructive*, *Sporobolomyces salmonicolor*, and *Candida albicans*, and antiviral activity against Coxsackie B3, Influenza A, and Herpes simplex 1, but showed no activity.

Experimental

Gerneral

Optical rotations were measured on a DR. KERNCHEN digital automatic polarimeter. IR spectra were recorded on a Bruker IFS 55. NMR spectra were measured on a Bruker Avance dpx 300 (1D) and Bruker Avance drx 500 (2D). ESIMS including HRMS were obtained by triple quadrupole mass spectrometer Quattro (VG Biotech, England).

Streptomyces Strain

The endophytic *Streptomyces* sp. (GT-20026114) was isolated from the leaves of mangrove plant *Aegiceras comiculatum* which was collected from Xia'men, Fujian province of China in August 2002, and incubated on ISP2 medium (artificial sea salt solution 800 ml/liter, malidixic acid 20 mg/liter and cycloheximide 30 mg/liter). Based on morphological and chemotaxonomic characteristics, the strain GT-20026114 can be tentatively assigned to the genus *Streptomuces* by Hans-Knoell-Institute. The strain was deposited in HKI, Germany.

Culture Conditions

The spores of GT-20026114 growing on agar slants (ISP2 culture medium) was transferred to a flask (20 ml) and was inoculated on liquid medium GYT (glucose 5 g/liter, peptone 1 g/liter, yeast extract 0.5 g/liter, beef extract 0.5 g/liter, NaCl 3 g/liter). The flask was incubated at 28°C on a rotary shaker for 48 hours, and the mycelium was aseptically transferred to a 500 ml Erlenmeyer flasks containing culture liquid (200 ml). The fermentation culture was then incubated at 28°C under shaking condition for 5 days.

Extraction and Isolation

The fermented supernatant of GT-20026114 (200 liters) was passed through a XAD-161M resin column (20×120 cm) by eluting with a gradient MeOH - H_2O (from 40 : 60 to 90 : 10 in 38 minutes) to collect seven fractions (FA∼FG). The fractions were lyophilized, and were screened on TLC (chemical screening) to combine fractions FE∼FG, which was desalted by extracting with methanol. The MeOH extract (19 g) was subjected to a silica gel column by eluting with $CHCl_3$ - MeOH (10 : 1) to yield **2** (10 mg), **3** (5 mg) and **4** (17 mg), respectively. The remaining fractions were combined (2.0 g) and chromatographied on silica gel column ($CHCl_3$ - MeOH 9 : 1) firstly, and then purified on Sephadex LH-20 by using MeOH as an eluting solvent to afford **1** (90 mg).

3-Amino-5-hydroxy-5-vinyl-2-cyclopenten-1-one (**1**), yellowish oil, $[\alpha]_D^{20}$ +127.1° (*c* 1.54, MeOH), UV λ_{max} nm (MeOH): 270, IR ν_{max} (KBr) cm^{-1}: 3308, 3181, 2360, 2340, 1617, 1531, 1418, 1215, 1069, 926. ^1H and ^{13}C NMR data, see Table 1. ESIMS *m/z*: 140 $[M+H]^+$, 162 $[M+Na]^+$. HRESIMS *m/z* 162.0540 (calcd for $C_7H_9NO_2Na$, 162.0531).

5-Hydroxy-3-[(methoxycarbonyl)amino]-5-vinyl-2-cyclopenten-1-one (**2**), yellowish oil, $[\alpha]_D^{20}$ +67.6° (*c* 1.16, MeOH), UV λ_{max} nm (MeOH): 261, IR ν_{max} (KBr) cm^{-1}: 3710, 3245, 1540, 1522, 1507, 1222, 784. ^1H and ^{13}C NMR data, see Table 1. ESIMS *m/z*: 198 $[M+H]^+$. HRESIMS *m/z* 220.0590 (calcd for $C_9H_{11}NO_4Na$, 220.0586).

5-Hydroxy-3-[[2-(4-hydroxyphenyl)ethyl]amino]-5-vinyl-2-cyclopenten-1-one (**3**), yellowish oil, $[\alpha]_D^{20}$ +59.3° (*c* 1.73, MeOH), UV λ_{max} nm (MeOH): 232, IR λ_{max} (KBr) cm^{-1}: 3274, 1559, 1515, 1456, 1220, 774. ^1H and ^{13}C NMR data, see Table 1. ESIMS *m/z*: 260 $[M+H]^+$. HRESIMS *m/z* 260.1279 (calcd for $C_{15}H_{18}NO_3$, 260.1287).

3-Isobutylpropanamide-2-cyclopenten-1-one (**4**), white amorphous powder, UV λ_{max} nm (MeOH) 242, IR λ_{max} (KBr) cm^{-1}: 3294, 2924, 1674,1643, 1612, 1556, 1507, 1224, 849, 778. ^1H and ^{13}C NMR data, see Table 1. ESIMS *m/z*: 210 $[M+H]^+$, 232 $[M+Na]^+$. HRESIMS *m/z*: 210.1500 (calcd for $C_{12}H_{20}NO_2$, 210.1494).

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