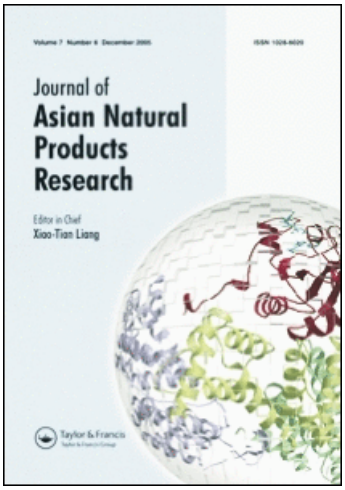

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A new chloro-monoterpene from the mangrove endophytic fungus *Tryblidiopycnis* sp. (4275)

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A new chloro-monoterpene (compound **1**) and three known compounds, peroxyergosterol, uracil and methylisocoumarin, were isolated from the ethyl acetate extract of the fermentation broth of the mangrove endophytic fungus *Tryblidiopycnis* sp. (4275) obtained from Kandelia woody tissue from Mai Po, Hong Kong. Its structure was determined spectroscopically and by X-ray crystallographic analysis.

Keywords: Mangrove endophytic fungus; Marine fungus; Monoterpene

1. Introduction

Marine microorganisms are potentially prolific sources of highly bioactive secondary metabolites that may prove to be very helpful in the development of new pharmaceutical agents [1,2]. As part of our ongoing search for new antitumor metabolites from marine microorganisms [3–6], we investigated the fungus *Tryblidiopycnis* sp. (# 4275). This paper describes a novel chloro-monoterpene, (1*S*,2*S*,3*S*,4*R*)-3-chloro-4-(2-hydroxypropa-n-2-yl)-1-methylcyclohexane-1,2-diol (**1**), together with three known compounds, peroxyergosterol, uracil and methylisocoumarin, isolated from the fermentation broth of the mangrove endophytic fungus *Tryblidiopycnis* sp. (4275).

2. Results and discussion

One hundred and fifty litres of fermentation broth were concentrated and extracted with ethyl acetate. The extract was repeatedly chromatographed on silica gel. Compound **1** was obtained as colorless black crystals with mp 146–148°C and $[\alpha]_D^{20} = +43.3$. The molecular formula, $C_{10}H_{19}O_3Cl$, was determined by HRESI-MS (*m/z* $[M + Na]^+$245.0934). EI-MS

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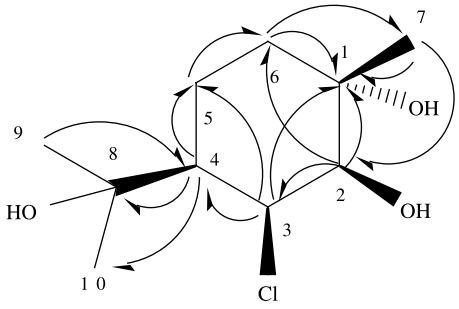

Figure 1. HMBC correlations of compound **1**.

showed characteristic $[M + 2 + Na]^+$ peaks ($M + Na$: $M + 2 + Na = 3:1$) indicative of the presence of one chlorine atom, and a prominent fragment ion peak at *m/z* 209.16 ($C_{10}H_{19}O_3$), revealing the presence of a chlorine atom that was facilely lost from the molecular ion.

The IR spectrum showed characteristic absorption bands at 3411, 3365, 3242, 2964, 2879, 1471, 1382, and 1040 cm^{-1}, ascribable to hydroxyl and methyl groups. The ^{13}C-NMR spectrum of **1** contained 10 signals that could be classified from the DEPT spectrum as two quaternary carbons (δ_C 71.2, 70.6), two methylenes (δ_C 37.1, 20.4), three methines (δ_C 78.4, 63.4, 46.9) and three methyls (δ_C 28.6, 28.3, 27.3). The assignment of the protons was accomplished by HMQC. Since one unsaturation was accounted for, compound **1** was inferred to contain one ring.

The ^{1}H–^{1}H COSY spectrum of **1** revealed the presence of a $CHCH_2CH_2$ moiety, assigned to H-4/H-5/H-6. In the HMBC spectrum, the ^{1}H signal at δ_H 4.11 (H-2) was correlated with the ^{13}C resonance at δ_C 70.6, 63.4, 37.1 (C-1, C-3 and C-6), the ^{1}H signal at δ_H 5.02 (H-3) with the proton resonance at δ_C 70.6, 46.9 and 20.4 (C-1, C-4 and C-5), the ^{1}H signal at δ_H 2.74 (H-4) with the ^{13}C resonance at δ_C 20.4, 71.2, 28.6, 28.3 (C-5, C-8, C-9 and C-10), and the ^{1}H signal at δ_H 1.75 (H-7) with the proton resonance at δ_C 70.6, 78.4, 37.1 (C-1, C-2 and C-6). Taking all the data into account, we established the structure of the new compound as formula **1** (figure 1 and table 1), and the absolute configuration was determined by X-ray diffraction analysis (figure 2) as 1*S*, 2*S*, 3*S*, and 4*R*.

Table 1. NMR data for **1** (500 MHz, pyridine-d_5, δ in ppm, *J* in Hz).

No.	*^{1}H*	*^{13}C (DEPT)*	*H-HCOSY*	*HMBC*
1		70.6 (C)		
2	4.11 (dd, 3.0, 1.0)	78.4 (CH)	H-3	C-1, 3, 6
3	5.02 (m)	63.4 (CH)	H-2, 4	C-1, 2, 4, 5
4	2.74 (ddd, 12.0, 5.5, 3.0)	46.9 (CH)	H-3, 5, 6	C-5, 8, 9, 10
5	1.98 (m), 1.90 (m)	20.4 (CH_2)	H-4, 5	C-4, 6
6	2.14 (ddd, 13.0, 13.0, 3.0) 1.83 (ddd, 13.0, 3.0, 3.0)	37.1 (CH_2)	H-5, 7	C-1, 5, 7
7	1.75 (s)	27.3 (CH_3)	H-6	C-1, 2, 6
8		71.2 (C)		
9	1.52 (s)	28.6 (CH_3)		C-4, 8, 10
10	1.52 (s)	28.3 (CH_3)		C-8, 9

500 MHz for ^{1}H and 125 MHz for ^{13}C.

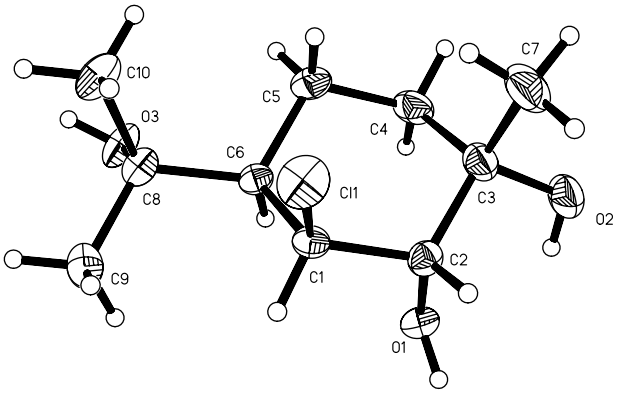

Figure 2. X-ray structure of **1** drawn by ORTEP.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Polartronic HHW5 digital polarimeter. IR spectra were determined on a Nicolet 5DX-FTIR infrared spectrometer using KBr pellets. NMR spectra were taken on a Varian Inova-500 spectrometer, using pyridine-d_5 as solvent and TMS as internal standard. EIMS and HRESIMS were measured on a VG Autospec-500 mass spectrometer. X-ray diffraction was measured on a Brucker Smart 1000 CCD. Silica GF254 for TLC and silica gel (200–300 mesh) for CC were produced by Qingdao Marine Chemical Company (Qingdao, China). Solvents and chemicals were of analytical grade and purchased from Guangzhou Chemical Company (Guangdong, China).

3.2 Fungus 4275

Strain 4275 was isolated from Kandelia woody tissue Mai Po, Hong Kong. The strain was characterized as belonging to the mangrove endophytic fungus *Tryblidiopycnis* sp. It has been deposited at the Department of Applied Chemistry, Zhongshan University, Guangzhou, China.

3.3 Culture conditions

Starter cultures (from Professor E.B. Gareth Jones and Professor L.L.P. Vrimjoed) 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 (glucose 10 g/l, peptone 2 g/l, yeast extract 1 g/l, NaCl 3 g/l, pH 7.0). The flask was incubated at 28°C on a rotary shaker for 3–5 days, and the mycelium was transferred aseptically to a 500 ml Erlenmeyer flask containing culture liquid (200 ml). The flask was then incubated at 28°C for 25 days.

3.4 Extraction and separation of metabolites

The cultures (150 l) were filtered through cheesecloth. The filtrate was concentrated to 5l below 50°C and extracted three times by shaking with an equal volume of ethyl acetate.

The combined organic extracts were subjected to a silica gel column, eluting with a gradient of petroleum ether to ethyl acetate to yield compound **1** (10 mg), peroxyergosterol (15 mg), uracil (25 mg) and methylisocoumarin (30 mg).

3.4.1 Compound 1. Colorless block, mp 146–148°C, $[\alpha]_D^{20} = +43.3$ (c 0.0015, MeOH). IR (KBr) ν_{max} (cm^{-1}): 1708, 1605, 1497, 1240, 915, 698; HRESI MS m/z $[M + Na]^+$245.0934 (calcd for $C_{10}H_{19}O_3Cl$, 245.0922); ESIMS m/z 245 $[M + Na]^+$, 247 $[M + Na + 2]^+$, 209 $[M + Na\text{-}Cl]^+$; ^{1}H-NMR, ^{13}C-NMR and 2D-NMR, see table 1.

3.4.2 X-ray structure of compound 1. Colorless orthorhombic crystal, 0.50 × 0.41 × 0.21 mm, obtained from EtOAc/petroleum ether. Space group $P2(1)2(1)2(1)$; unit cell dimensions $a = 7.092(3)$ Å, $b = 9.649(4)$ Å, $c = 16.890(7)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 1155.7(8)$ Å^3, $Z = 4$, $D_{calcd} = 1.280$ mg/m^3, $\mu = 0.312$ mm^{-1}, $F(000) = 480$; absolute structure parameter 0.00 (7). All single-crystal data were collected using the hemisphere technique on a Bruker AXS SMART 1000 CCD diffractometer with graphite-monochromated Mo Kα radiation $\lambda = 0.71073$ Å at 293(2) K. The structures were solved by direct methods using SHELXTL V6.10 (Bruker AXS, Madison, WI, USA) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied with the Siemens Area Detector Absorption Program (SADABS). The final value of R was 0.0361, $wR2 = 0.0794$ [$I > 2\sigma(I)$]. The CIF file of the X-ray data of compound **1** was deposited in the CCDC (deposit number 256198).

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References

[1] P.G. Williams, W.Y. Yoshida, M.K. Quon, R.E. Moore, V.J. Paul. *J. Nat. Prod.*, **66**, 1545 (2003).
[2] G. Schlingmann, L. Milne, G.T. Carter. *Tetrahedron.*, **58**, 6825 (2002).
[3] Y.C. Lin, X.Y. Wu, S. Feng, G.C. Jiang, S.N. Zhou, L.L.P. Vrijmoed, E.B.G. Jones. *Tetrahedron Lett.*, **42**, 449 (2001).
[4] Y.C. Lin, X.Y. Wu, S. Feng, G.C. Jiang, S.N. Zhou, L.L.P. Vrijmoed, E.B.G. Jones, K. Krohn, K. Steingrover, F. Zsila. *J. Org. Chem.*, **66**, 6252 (2001).
[5] Y.C. Lin, X.Y. Wu, Z.J. Deng, J. Wang, S.N. Zhou, L.L.P. Vrijmoed, E.B.G. Jones. *Phytochemistry.*, **59**, 469 (2002).
[6] G.Y. Chen, Y.C. Lin, L. Wen, L.L.P. Vrijmoed, E.B.G. Jones. *Tetrahedron*, **59**, 4907 (2003).
[7] F. Kiuchi, Y. Ttano, N. Uchiyama, C. Honda, A. Tsubouchi, J. Nakajima-Shimada, T. Aoki. *J. Nat. Prod.*, **65**, 509 (2002).
[8] A.A. Ahmed. *J. Nat. Prod.*, **63**, 989 (2000).
[9] A. Numata, M. Iritani, T. Yamada, K. Minoura, E. Matsumura, T. Yamori, T. Tsuruo. *Tetrahedron Lett.*, **38**, 8215 (1997).