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THREE NEW ISOCHROMANS FROM THE MYCELIAL CULTURE OF A *CYLINDROCARPON* FUNGUS

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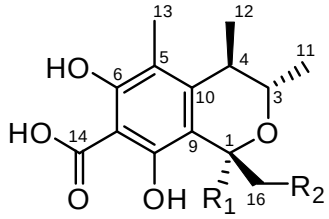
Abstract – Three new isochroman derivatives, 1-acetonyl-7-carboxyl-6,8-dihydroxy-3,4,5-trimethylisochroman (**1**), 7-carboxyl-6,8-dihydroxy-1,1,3,4,5-pentamethylisochroman (**2**), and 6,8-dihydroxy-3,4,5-trimethylisochroman (**3**), together with decarboxydihydrocitrinone (**4**), were isolated from mycelial solid culture of a *Cylindrocarpon* fungus. Their structures were established by spectroscopic methods.

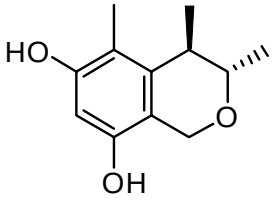
In the course of our continual search for antibacterial and antifungal natural products produced by filamentous fungi collected in South China, we found that the EtOH extract from mycelial solid culture of an Ascomycetes, *Cylindrocarpon* sp. SC0537, showed potent antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Peronophythora litchii*, one of the main pathogens causing litchi (*Litchi chinensis* Sonn.) fruit rot. We therefore investigated the secondary metabolites of this fungus, and obtained three new isochroman derivatives (**1–3**) along with a known isochroman, decarboxydihydrocitrinone (**4**).[1] We herein report the isolation and structure elucidation of these new compounds.

The mycelia of the fungus, *Cylindrocarpon* sp. SC0537, were grown on solid culture for 15 days at 28°C in the dark. The EtOH extract of the mycelial culture was sequentially fractionated with petroleum ether, $CHCl_3$ and EtOAc. The $CHCl_3$-soluble fraction was separated by SiO_2 and Sephadex LH-20 column chromatography to afford the compounds (**1**–**4**).



1 R_1 = H, R_2 = Ac

2 R_1 = Me, R_2 = H



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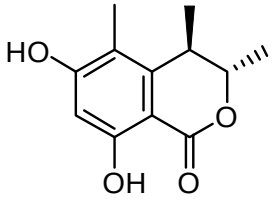

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1β-Acetonyl-7-carboxyl-6,8-dihydroxy-3α,4β,5-trimethylisochroman (**1**) had the molecular formula, $C_{16}H_{20}O_6$, by combined analysis of its HRTOFMS, ^{13}C NMR and DEPT data. The ^1H NMR spectrum (Table 1) exhibited signals for 17 nonexchangeable protons, including two singlets at δ 2.03 (3H, H-13) and δ 2.20 (3H, 16-COC*H*$_3$), and two doublets at δ 1.11 (3H, H-11) and δ 1.26 (3H, H-12) for four methyl groups, two multiplets at δ 3.94 (1H, H-3) and δ 2.65 (1H, H-4), and a double doublet at δ 5.24 (1H, H-1) for three methines, and two double doublets at δ 2.59 (1H, H-16a) and δ 3.38 (1H, H-16b) for a methylene. The ^{13}C NMR (Table 1) and DEPT spectra indicated the presence of four methyl groups, a methylene (δ 50.6, C-16), a ketone carbonyl group (δ 211.6, 16-*C*OCH$_3$), a carboxyl group (δ 179.8, C-14), three methines, two oxygenated (δ 67.1, C-1; δ 74.1, C-3), as well as a fully substituted benzene ring in which two carbons were oxygenated (δ 159.7, C-6; δ 156.7, C-8). The connectivities among these groups and carbons were deduced from the COSY and HMBC spectra (Figure 1). The presence of NOE correlations between H-3/H-12, H-4/H-11, H-4/H-13, H-3/H-4, H-12/H-13, and H-1/H-11 in the NOESY spectrum indicated that H-1, H-4, and 3-Me were at the same side and in α-orientation while 1-acetonyl and 4-Me were in β-orientation. These NOE interactions in combination of the ^1H NMR small coupling constant between H-3 and H-4 (1.4 Hz) showed that the pyran ring was in a half chair form with C-1, C-4, C-9, and C-10 held at the same plane, while C-3 oriented down and O-2 up the plane.[2] Therefore, the structure of **1** was elucidated as shown.

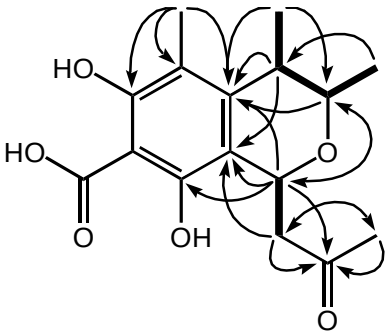

Figure 1. ^1H–^1H COSY (bold line) and main HMBC (arrow) correlations of **1**

7-Carboxyl-6,8-dihydroxy-1,1,3α,4β,5-pentamethylisochroman (**2**) was established as having a molecular formula of $C_{15}H_{20}O_5$ by its HRTOFMS, ESIMS, and NMR (^1H, ^{13}C, and DEPT) data. The ^1H and ^{13}C NMR spectra of **2** (Table 1) were closely similar to those of **1** except that the proton and carbon signals for the acetonyl group and the oxygenated methine assigned to C-1 in **1** were absent. Instead, signals for two methyl groups [δ_H 1.57 (3H, s), δ_C 30.2; δ_H 1.56 (3H, s), δ_C 28.7] and an oxygenated quaternary carbon (δ_C 74.9) were present. Based on evidence above as well as the ^1H-^1H COSY, HSQC, and HMBC

data, 7-carboxyl-6,8-dihydroxy-1,1,3,4,5-pentamethylisochroman could be readily derived for **2**. The NOE interactions observed in the NOESY spectrum and the proton coupling constant, $J_{3,4}$ = 3.6 Hz,[2] in the ^{1}H NMR spectrum indicated the same relative stereochemistry of **2** as that of **1**. Compound (**2**) was thus elucidated as depicted.

Table 1. ^{1}H and ^{13}C NMR data for **1**–**4**[a]

	1[b]		**2**[b]		**3**[c]		**4**[b]	
position	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1α	5.24 dd (2.8, 9.2)	67.1		74.9	4.56 d (15.2)	59.7		175.8
1β					4.49 d (15.2)			
3	3.94 dq (1.4, 6.3)	74.1	3.92 dq (3.6, 6.8)	73.5	3.82 dq (2.5, 6.6)	74.6	3.68 m	69.2
4	2.65 dq (1.4, 7.0)	37.0	2.72 dq (3.6, 6.5)	37.8	2.59 dq (2.5, 6.9)	35.9	2.91 m	41.6
5		112.8		112.5		113.4		111.2
6		159.7		159.3		155.0		159.6
7		101.9		101.9	6.29 s	100.5	6.03 s	102.8
8		156.7		157.4		151.6		159.2
9		112.9		118.8		112.3		111.2
10		143.1		143.4		138.5		147.3
11	1.11 d (6.0)	20.6	1.22 d (6.0)	22.1	1.15 d (6.0)	20.7	0.95 d (6.0)	19.6
12	1.26 d (6.8)	18.6	1.17 d (6.8)	20.5	1.16 d (6.8)	18.1	1.03 d (7.0)	15.4
13	2.03 s	9.8	2.05 s	10.9	2.01 s	10.3	1.95 s	10.4
14		179.8		180.2				
15			1.57 s	30.2				
16a	2.59 dd (9.2, 15.1)	50.6	1.56 s	28.7				
16b	3.38 dd (2.7, 15.1)							
*C*OCH$_3$		211.6						
CO*CH*$_3$	2.20 s	30.2						

[a]Chemical shifts (δ) in ppm, coupling constants (parentheses) given in Hz.

[b]Methanol-d_4 as solvent.

[c]Acetone-d_6 as solvent.

6,8-Dihydroxy-3α,4β,5-trimethylisochroman (**3**) was assigned a molecular formula of $C_{12}H_{16}O_3$, which was also derived from HRTOFMS, ^{13}C NMR, and DEPT data. Its ^{1}H and ^{13}C NMR spectra (Table 1) indicated that the structure of **3** is closely related to the known isocoumarin, decarboxydihydrocitrinone (**4**) [1], except C-1 in **3** was a methylene [δ_H 4.56 and 4.49 (each 1H, d, J = 15.2 Hz), δ_C 59.7] rather than a

carbonyl in **4**. The α-orientation of 3-Me and β-orientation of 4-Me were evidently indicated by the ^{1}H NMR coupling constant, $J_{3,4}$ = 2.5 Hz,[2] as well as the NOESY spectrum.

The antibacterial and antifungal activities of the isolated isochromans were assessed by the agar diffusion method with paper disks [3,4] using *Peronophythora litchii, Staphylococcus aureus, Escherichia coli*, *Saccharouyces cerevisiae*, and *Aspergillus fumigantus* as the test microorganisms. The assessments showed that they all were inactive to all tested microorganisms at the dosages of 100—600 μg. It is noted that the antibacterial and antifungal activities of some isochromans have been previously reported.[5-9]

EXPERIMENTAL

General Experimental Procedures. Optical rotations were obtained on a Perkin-Elmer 341 Polarimeter with MeOH as solvent. UV spectra were recorded on a Perkin-Elmer Delta 35 UV-vis spectrophotometer. The ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker DRX-400 instrument using TMS as an internal reference. HRTOFMS data were obtained on an API QSTAR TOF mass spectrometer. ESIMS were collected on an API-2000 LC/MS/MS system by direct inlet. For column chromatography, SiO_2 (100-200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China) and Sephadex LH-20 were used. TLC was performed on precoated plates (Kieselgel 60 GF254, Merck) with detection effected by exposure to I_2 vapor and spraying with H_2SO_4 (10 %) in EtOH followed by heating.

Producing Fungus and Fermentation. Fruiting bodies of *Psathyrella* DH0235 were collected in Dinghu Mountain Biosphere, Guangdong, China, in May 2002. From a young fruiting body of the mushroom, the fungus *Cylindrocarpon* sp. SC0537 was isolated by tissue culture. The mushroom and the fungal mycelia were authenticated by Prof. Xingzhong Liu, one of co-authors. The mycelial culture of *Cylindrocarpon* sp. SC0537 is deposited in the culture collection of China General Microbiological Culture Collection Center (**CGMCC**), Beijing, China. For maintenance on glycerol and submerged culture, the fungus was grown on PDA medium. Fermentation of the fungus was performed by the previously described procedure.[3]

Extraction and Isolation. The mycelial culture of *Cylindrocarpon* sp. SC0537 were extracted with 95% EtOH three times at room temperature. The EtOH solution, after concentration in vacuo, was suspended in H_2O, and this aqueous suspension was sequentially extracted three times each with petroleum ether, $CHCl_3$, and EtOAc. The combined $CHCl_3$ solution, upon evaporation, yielded the deep brown syrup (4.35 g). This syrup was subjected to SiO_2 column chromatography, eluted with $CHCl_3$-MeOH mixtures of increasing polarities (100:0 to 80:20), to obtain seven fractions (I-VII). Further separation by SiO_2 column chromatography eluted with petroleum ether-acetone (75:25) followed by Sephadex LH-20 column chromatography eluted with MeOH afforded **3** (30 mg) from fraction IV, **1** (40 mg) and **2** (50 mg) from fraction VI, and **4** (80 mg) from fraction VII.

1-Acetonyl-7-carboxyl-6,8-dihydroxy-3,4,5-trimethylisochroman (**1**): yellow amorphous solid, $[\alpha]^{20}_{D}$ +27.8° (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 211.8 (4.17), 252.4 (3.63), 318.9 (3.19); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; ESIMS *m/z* 307 $[M - H]^-$; HRTOFMS *m/z* 307.1182 $[M - H]^-$ (calcd for $C_{16}H_{19}O_6$, 307.1181).

7-Carboxyl-6,8-dihydroxy-1,1,3,4,5-pentamethylisochroman (**2**): yellow amorphous solid, $[\alpha]^{20}_{D}$ −31.4° (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ε) 216.8 (4.32), 255.7 (3.98), 282.2 (3.55); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; ESIMS: *m/z* 279 $[M - H]^-$; HRTOFMS *m/z* 279.1233 $[M - H]^-$ (calcd for $C_{15}H_{19}O_5$, 279.1232).

6,8-Dihydroxy-3,4,5-trimethylisochroman (**3**): yellow amorphous solid; $[\alpha]^{20}_{D}$ −15.5° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 205.3 (4.25), 226.5(3.85), 282.5(3.56); ^{1}H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6), see Table 1; ESIMS *m/z* 209 $[M + H]^+$; HRTOFMS *m/z* 209.1178 $[M + H]^+$ (calcd for $C_{12}H_{17}O_3$, 209.1177).

Decarboxydihydrocitrinone (**4**): yellow amorphous solid, $[\alpha]^{20}_{D}$ −13.5° (*c* 0.26, MeOH); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD), see Table 1; ESIMS *m/z* 221.0 $[M - H]^-$.

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