Paecilospirone, a Unique Spiro[chroman-2,1'(3'*H*)-isobenzofuran] Derivative Isolated from Tropical Marine Fungus *Paecilomyces* sp.[1]

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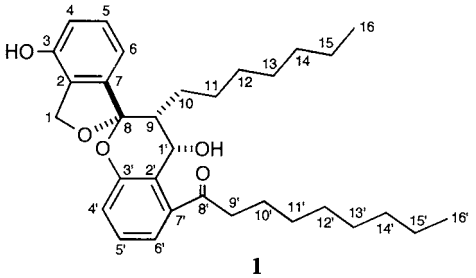
A spiroacetal compound named paecilospirone was isolated from a marine fungus *Paecilomyces* sp. collected at Yap Island. Paecilospirone was found by the screening assay for detecting inhibitors of microtubule assembly using conidia of *Pyricularia oryzae*. The structure was assigned based on the spectroscopic data. This is the first report of the isolation and characterization of spiro[chroman-2,1'(3'*H*)-isobenzofuran] derivative as a natural product.

Marine organisms such as sponges, tunicates, corals, and macroalgae are rich sources of many potent bioactive and structurally interesting compounds.[2] Recently, marine microorganisms such as bacteria, fungi, and microalgae have been utilized for the sources of bioactive secondary metabolites.[3] In the course of our studies on inhibitors of microtubule assembly, we have isolated a new compound named paecilospirone (**1**) from a fungus *Paecilomyces* sp. isolated from the coral reef at Yap Island, Federated States of Micronesia. Inhibitors of microtubules are important compounds as antitumor agents[4] and also as biochemical tools to study the structures and functions of microtubules and tubulins.[5] Paecilospirone (**1**) was found during our systematic screening program on marine fungi by the bioassay method we have recently developed for searching of antimitotic compounds[6,7] and had a unique spiroacetal structure, spiro[chroman-2,1'(3'*H*)-isobenzofuran] derivative. This is the first report of the isolation and characterization of this skeletal structure as a biosynthetic product.



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Paecilomyces sp. was cultured in a petri dish (15 mL).[8] MeOH (8 mL) was added to the culture broth and filtered. The filtrate showed a curling effect on mycelia germinated from conidia of *Pyricularia oryzae* P-2b, which is the characteristic deformation caused by antimitotic agents.[6] The bioactivity was reproduced by the MeOH extract of mycelia obtained from larger scale culture by filtration of the culture broths (1.5 L).[8] The bioassay guided separation of the extract by an ODS column (100% MeOH eluate) followed by successive silica gel column chromatography (twice, benzene-acetone = 11:1 and 98:2) gave **1**[9] ($[\alpha]_D^{25}$ = +202.5° (*c* 0.37, MeOH), 5.6 μg/mL culture broth).

The molecular formula of **1**, $C_{32}H_{44}O_5$, was deduced from HRFABMS and NMR data. ^{1}H and ^{13}C NMR data for **1** showed the presence of two benzene rings, each one ketone and secondary alcohol, and two hydrocarbon chains. The ^{1}H-^{1}H COSY spectra of **1** revealed the connection of carbon bonds at 4-5-6, 1'-9-10-11, 15-16, 4'-5', 9'-10'-11', and 15'-16'. The connectivity of carbons 1 through 11 and 1' through 11' were elucidated by HMBC experiments. The length of two hydrocarbon chains were assigned from ^{13}C chemical shifts of C-10 to C-16 and C-10' to C-16' and confirmed by ESIMS/CID/mass spectra of **1** (Figure 1).

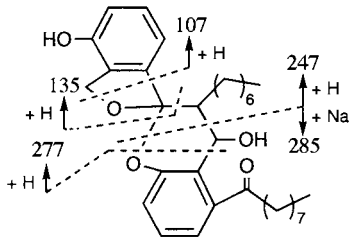

Figure 1. Fragment ions detected in the ESIMS/CID/mass spectra of paecilospirone (**1**).

The relative stereochemistry of **1** was assigned from ^{1}H NMR and ROESY data. The coupling constants between H-1' and H-9, H-9 and H-10a, and H-9 and H-10b were 4.0, 4.0, and 12.0 Hz, respectively, which showed H-9 and 1'-OH group are pseudaxial and H-1' is pseudequatorial orientation. The ROESY experiment revealed the NOE correlations (Figure 2) and confirmed the relative stereochemistry of **1**. The absolute configuration is now under investigation and will be reported elsewhere.

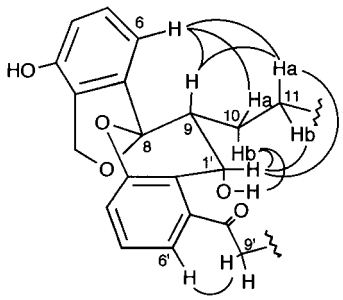

Figure 2. NOE correlations assigned from the ROESY spectrum of paecilospirone (**1**).

Paecilospirone (**1**) has the unique skeletal structure, spiro[chroman-2,1'(3'*H*)-isobenzofuran], which has only been reported as chemical reaction products. Two hemiacetal derivatives of spiro[chroman-2,1'(3'*H*)-isobenzofuran]-3,4-dione were synthesized by oxidation with periodic acid from 2'-hydroxymethylflavonol.[10] Two diastereomers of 4-hydroxy derivatives were prepared from phthalide with the mixture of metallic samarium and bromine (4:3).[11] Paecilospirone (**1**) is, therefore, the first example of this unique skeletal structure obtained from natural sources.

Paecilospirone (**1**) may be biosynthesized from two units of an octaketide, 2-hydroxy-6-(1-oxononyl)benzaldehyde via aldol condensation. This would be a new route to dimeric derivatives biosynthesized from two polyketide units.[12] The inhibitory activity of **1** to purified porcine brain microtubule proteins[13] was weak (20% inhibition at 50 μM), and accordingly, **1** was not antifungal at 25 μg/disc (disc diffusion assay) nor cytotoxic at 20 μg/mL to several tumor cell lines. The biosynthesis and biological activity of **1** are the interesting subjects for future studies.

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References and Notes

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1 This paper is dedicated to Professor Kenneth L. Rinehart on the occasion of his 70th birthday.

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8 The fungus was cultured in a half nutrient potato dextrose medium for three weeks at 20 ˚C. For the isolation of paecilospirone (**1**), ten 500-mL flasks (contained each 150 mL of broth) were used for culture, and the mycelia were separated from culture broths by filtration. The bioactivity was reproduced by MeOH extracts from mycelia, but not by the broth filtrates. Details of isolation, identification, and culture of *Paecilomyces* sp. will be published elsewhere.

9 Paecilospirone (**1**), $[\alpha]_D^{25}$ = +202.5˚ (*c* 0.37, MeOH); HRFABMS: $[M + Na]^+$ *m/z* = 531.3087, calcd for $C_{32}H_{44}O_5Na$, 531.3086; UV λ_{max} nm (MeOH): 206, 218, 251, 278, and 299; IR (KBr): 3340, 2930, 2860, 1666, 1607, 1582, 1452, 1096, 1060, 1000, and 840 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 5.11 (d, 13.0 Hz, H-1a), 5.21 (d, 13.0 Hz, H-1b), 6.80 (d, 8.0 Hz, H-4), 7.24 (dd, 7.5 and 8.0 Hz, H-5), 6.87 (d, 7.5 Hz, H-6), 2.49 (ddd, 12.0, 4.0, and 4.0 Hz, H-9), 1.07 (m, H-10a), 1.80 (m, H-10b), 1.20 (m, H-11a), 1.57 (m, H-11b), 1.2-1.35 (18H, m, H_2-12-15 and 11'-15'), 0.84 (t, 7.0 Hz, H_3-16), 5.11 (dd, 7.0 and 4.0 Hz, H-1'), 6.97 (dd, 7.0 and 2.0 Hz, H-4'), 7.28 (m, H-5'), 7.27 (m, H-6'), 2.97 (m, H_2-9'), 1.74 (m, H_2-10'), 0.88 (t, 7.0 Hz, H_3-16'), 4.36 (d, 7.0 Hz, 1'-OH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 71.3 (C-1), 126.4 (C-2), 140.8 (C-3), 115.9 (C-4), 129.8 (C-5), 113.9 (C-6), 150.5 (C-7), 112.2 (C-8), 42.4 (C-9), 25.2 (C-10), 26.7 (C-11), 29.2- 29.6 (5t, C-12, 13, 11', 12', and 13'), 31.8 (2t, C-14 and 14'), 22.6 (2t, C-15 and 15'), 14.1 (2q, C-16 and 16'), 60.7 (C-1'), 123.5 (C-2'), 139.7 (C-3'), 121.4 (C-4'), 128.9 (C-5'), 121.5 (C-6'), 152.2 (C-7'), 206.9 (C-8'), 42.3 (C-9'), 24.4 (C-10').

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