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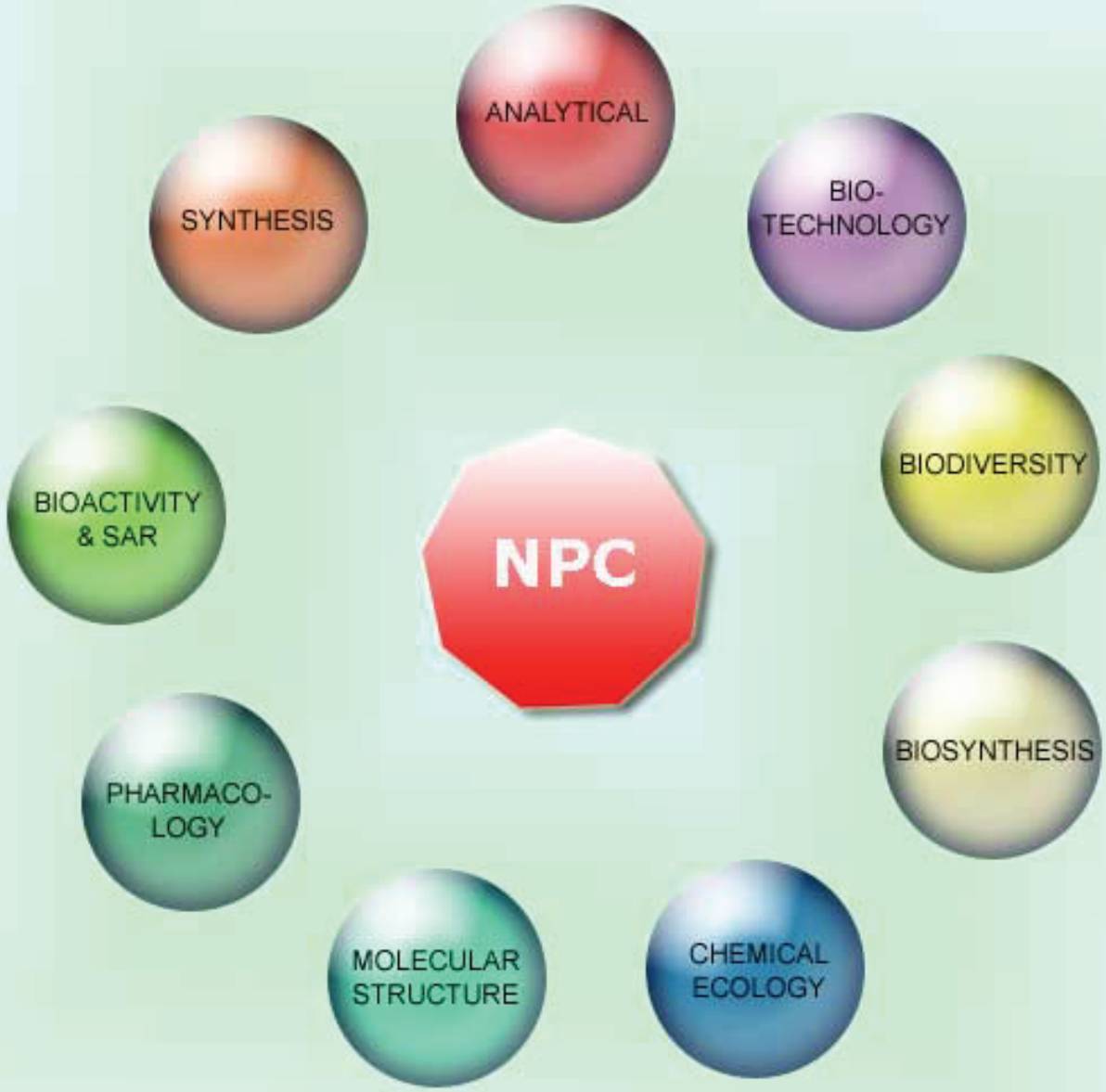

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Talarofuranone, a New Talaroconvolutin Analog from the Endophytic Fungus *Talaromyces purpurogenus* from *Pouteria campechiana* Seeds

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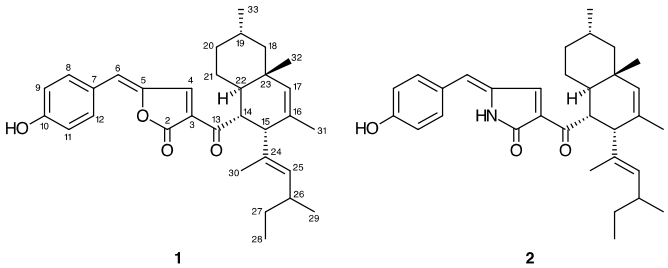
An endophytic fungus *Talaromyces purpurogenus* was isolated from the seeds of the popular edible fruit *Pouteria campechiana*. The fungus was fermented in potato dextrose agar and the fungal media were extracted with EtOAc. Chromatographic separation of the EtOAc extracts over silica gel, Sephadex LH-20 and preparative thin layer chromatography furnished a furanone analogue of talaroconvolutin A, named talarofuranone (**1**), along with talaroconvolutin A (**2**), 4-hydroxyacetophenone, tyrosol and ergosterol. The structure of **1** was determined by comparing the NMR data with that of **2** and by HRFABMS.

Keywords: Talarofuranone, *Talaromyces purpurogenus*, *Pouteria campechiana*, Endophytic fungi, Talaroconvolutin A.

Fungi play an important role in our lives and some, such as mushrooms, have been used by humans as food from time immemorial. Endophytes are microorganisms that live in the intercellular spaces of stems, petioles, roots and leaves of plants causing no discernible manifestation of their presence and have typically remained unnoticed [1]. In a continuation of our studies on bioactive secondary metabolites produced by fungal endophytes associated with Sri Lankan plants [2-6], we investigated metabolites of an endophytic fungus isolated from the seeds of a popular edible fruit *Pouteria campechiana* of the family Sapotaceae. In this paper we report the isolation and structure elucidation, by comparison of their NMR data with literature values, of a new furanone analog (**1**) of talaroconvolutin A (**2**) [7], along with **2**, 4-hydroxyacetophenone (**3**) [8], tyrosol (**4**) [9] and ergosterol (**5**).

The structure of talarofuranone (**1**) was determined on the basis of the following spectral evidence. The ^{1}H NMR spectrum of **1** resembled that of **2** except for considerable differences in the chemical shifts of H-4, H-6 and H-8/H-12, as well as the lack of the exchangeable NH proton (δ 8.50) found in **1** (Table 1). The COSY spectrum of **1** revealed that the coupling networks in the C-14 to C-33 moiety and ortho-coupling in the benzene ring (C-7 to C-12) were identical to those found in compound **2**. The ^{13}C NMR spectrum indicated the presence of 32 carbons in the molecule (C-8/C-12 and C-9/C-11 were counted as two signals each). The ^{13}C NMR data of **1** were also in good agreement with those of **2** except for the diagnostic difference of C-5 (15.4 ppm upfield-shifted from that of **2**) and C-8/C-12 (2.4 ppm downfield-shifted from that of **2**). Replacement of the tetramic acid five-membered ring with a furanone ring was suggested from the above findings. This was supported by the HR-FABMS data of **1**, which gave the molecular formula, $C_{32}H_{40}O_4$, for **1** instead of $C_{32}H_{41}NO_3$ for **2**. The relative configuration of the stereogenic centers at C-14/C-15/C-19/C-22/C-23 could be identical to that of **2** because the appearance and shift of the ^{1}H signals in this moiety were very similar to those of **2** and the ^{13}C shifts of **1** were virtually identical to the respective signals of **2**. The (*E*)-configuration of the C-5/C-6-double bond was secured by an NOE experiment, in which irradiation of H-6 caused an enhancement of the signal intensity of H-4 and H-8/H-12.



1 **2**

Figure 1: Structures of compounds **1** and **2**.

The absolute stereochemistry of **1** is assumed to be identical to that of **2**, based on the negative molecular rotation values of **1** and **2** ($[\alpha]_D^{24}$ –103 and –111 [7], respectively), yet it is not established. The configuration at the C-26 stereogenic center of **1** could be *R*, since L-isoleucine is expected to be a biosynthetic precursor for the C-25–C-29 moiety.

Preliminary study of the EtOAc extracts of culture broth and mycelium did not show antifungal activity against *Cladosporium cladosporioides* in spite of the reported antifungal activity of compound **2** [7]. Both EtOAc extracts showed antioxidant properties (IC_{50} values, 160 and 480 ppm, respectively) when examined for DPPH radical scavenging activity. Furthermore, the EtOAc extract of the culture broth showed phytotoxicic activity, inhibiting lettuce seed germination by 100% at 125 ppm and the growth of shoot and root of *Lactuca sativa* seeds (61 and 63 % inhibition, respectively) at 200 ppm. The broth extract exhibited a weak toxicity (LD_{50} value, 481 ppm) in the *Artemia salina* lethality assay.

Experimental

***General**:* NMR, Bruker DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer; HRMS-FAB, JEOL JMS-700 spectrometer; Silica gel (Art No. 7734 and 9385, Merck), Sephadex LH-20 (Art No. 20100, Fluka) and silica gel 60F254 pre-coated aluminum plates (Merck Art No. 1.05554.001) were used for chromatography.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data in $CDCl_3$ for compounds **1** and **2** (mult. *J* in Hz).

	1		**2**	
No	δ_C	δ_H	δ_C	δ_H
2	167.2	—	169.9	—
3	127.4	—	131.8	—
4	145.0	7.94 (s)	144.7	7.62 (s)
5	148.2	—	132.8	—
6	119.8	6.22 (s)	120.4	6.31 (s)
7	125.8	—	126.9	—
8/12	133.9	7.79 (d, 8.7)	131.5	7.42 (d, 8.7)
9/11	116.3	6.91 (d, 8.7)	116.6	6.94 (d, 8.7)
10	158.0	—	157.3	—
13	194.6	—	196.3	—
14	49.5	3.83 (dd, 12.3, 7.7)	49.6	3.93 (dd, 12.4, 7.7)
15	50.9	3.20 (d, 7.7)	50.8	3.22 (d, 7.6)
16	129.8	—	130.2	—
17	136.3	5.39 (s)	136.5	5.41 (s)
18	45.8	0.95 (m)	48.6	0.92 (m)
		1.48 (m)		1.47 (m)
19	27.5	1.69 (m)	27.5	1.69 (m)
20	35.8	0.97 (m)	35.8	0.97 (m)
		1.71 (m)		1.71 (m)
21	24.4	0.97 (m)	24.4	0.97 (m)
		1.71 (m)		1.71 (m)
22	40.1	1.86 (td, 12.3, 2.4)	40.1	1.88 (m)
23	35.1	—	35.2	—
24	133.9	—	133.1	—
25	136.5	4.74 (d, 9.5)	136.6	4.74 (d, 9.1)
26	34.1	2.14 (m)	34.1	2.13 (m)
27	30.3	1.07 (m)	30.3	1.05 (m)
		1.23 (m)		1.23 (m)
28	12.0	0.75 (t, 7.4)	12.0	0.75 (t, 7.4)
29	20.7	0.67 (d, 6.7)	20.7	0.68 (d, 6.7)
30	14.0[a]	1.45 (s)	14.0[a]	1.47 (s)
31	22.1	1.56 (s)	22.3	1.52 (s)
32	20.6	0.95 (s)	20.5	1.91 (s)
33	22.8	0.86 (d, 6.2)	22.8	0.86 (d, 6.2)
1-NH				8.50 (s)
10-OH		5.43 (br)		6.06 (br)

[a]Very weak signal.

Isolation and identification of endophytic fungus: Seeds of *P. campechiana* were obtained from the ripe fruits collected from the Central Province of Sri Lanka in May 2012. Seeds were washed with running tap water to remove surface dust and debris. After triple sterilization of seeds with ethanol, 2.5% NaOCl and distilled water, a few segments of the inner part of the seeds were placed on PDA media in a Petri dish (9 cm) and incubated at room temperature. Emerging fungi were isolated after 5 days. To obtain a pure culture, the reddish colored pigment releasing endophytic fungus was serially transferred into PDA media. An endophytic fungus thus isolated was identified as *Talaromyces purpurogenus* through molecular means using the internal transcriber spacer (ITS) region of the rDNA gene. DNA was extracted using a Promega, Wizard Genomic DNA purification kit (A1120) and the ITS region was amplified using the universal eukaryotic primers of ITS1 and ITS4. These experiments were performed by the Dept. of Molecular Biology and Biotechnology, University of Peradeniya, Sri Lanka. BLAST search indicated that the sequence of the ITS region had 99% similarity to that of *T. purpurogenus* strain FRR 1061 (GenBank Acc. No. AY373926.1). Photographic evidence of the seeds of *P. campechiana* and the fungal strain *T. purpurogenus* IFS/GP1 were deposited at the National Institute of Fundamental Studies.

Extraction and isolation of compounds: Large scale culturing of the fungus was carried out by inoculating *T. purpurogenus* culture grown on PDA medium in 1L conical flasks (x 20), each containing 400 mL of PDB medium, which were allowed to stand still at room temperature for 10 days, and then incubated while shaking every other day on a laboratory shaker for another 18 days. The medium was filtered and the filtrate extracted with EtOAc 3 times. Concentration of the EtOAc layer furnished the EtOAc extract (616 mg). The residual mycelium was crushed and extracted with EtOAc to give the EtOAc extract (1.16 g). Both EtOAc extracts were subjected to bioassays for antifungal activity against *Cladosporium cladosporioides* [10], for DPPH radical scavenging activity, brine shrimp lethality and phytotoxicity assayed by lettuce seed germination [5]. TLC analysis indicated the close similarity of the EtOAc extract from the PDB medium and that from the mycelium. Hence, the two EtOAc extracts were combined (1.5 g) and chromatographed over silica, Sephadex LH-20 and preparative TLC to furnished compounds **1** (12 mg), **2** (32 mg), **3** (13 mg), **4** (6 mg) and **5** (100 mg).

Talarofuranone (**1**)
Yellow amorphous solid.
$[\alpha]_D^{24}$: –103 (*c*, 0.1, $CHCl_3$).
UV (MeOH) λ max: 205, 214, 229, 235, 416 nm.
^{1}H and ^{13}C NMR ($CDCl_3$): Table 1.
HRFABMS *m/z*: 489.3007 $[M+H]^+$ (489.3005; calculated for $C_{32}H_{41}O_4$).

Supplementary data: ^{1}H and ^{13}C NMR spectra of **1** and **2** are available.

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