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Pyrofomins A-D, polyoxygenated sesquiterpenoids from *Pyrofomes demidoffii*

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ABSTRACT

Pyrofomins A-D, four polyoxygenated sesquiterpenoids have been isolated from the methanolic extract of the fruit bodies of *Pyrofomes demidoffii*. Their structures are elucidated by IR, HR-FTICR-MS, and 2D NMR spectroscopy. Furthermore, the cedrane carbon skeleton of pyrofomin A (**1**) is confirmed by X-ray crystallographic analysis. The sesquiterpenoids **1**–**4** show neither cytotoxicity against KB cells nor antimicrobial activity.

1. Introduction

The genus *Pyrofomes* Kotl. & Pouzar belongs to the family Polyporaceae in Basidiomycetes and forms mainly perennial basidiocarps. The genus can easily recognized by its reddish basidiocarps and the truncate spores. The type species of the genus, *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar (Juniper Pocket Rot), grows exclusively on *Juniperus* spp. and causes a white trunk rot of living trees. *P. demidoffii* has been recorded on junipers in Central Europe, Africa, North America and China [1,2]. To our knowledge no chemical investigation is reported for *P. demidoffii*. The species is described as a rare medicinal mushroom in China [2] and shows in a bioactivity screening that the culture filtrate of this fungus contains biologically active substances that inhibit *in vitro* grows of a wide range of bacteria and fungi of medical importance, whereas the tested crude extracts was more active against gram positive bacteria than gram negative bacteria [3,4]. Echinotinctone (2,6-dihydroxy-1,8-dimethyl-3H-xanthen-3-one), an orange pigment was isolated from the wood-rotting polypore *Echinodontium tinctorium* (Ell. & Ev.) Ell. & Ev. and *Pyrofomes albomarginatus* (Lév.) Ryv. [5]. The latter species related to *P. demidoffii* occurring widespread in the tropics from Japan to Zaire. During our field trip in Ethiopia, we collected *P. demidoffii* that allowed us to investigate its chemical constituents. This paper describes the isolation and structural elucidation of four polyoxygenated sesquiterpenoids (**1**–**4**) and preliminary results on their biological activity.

2. Experimental

2.1. General

Determinations of melting points were accomplished with a Leica DM LS2 microscope (without correction). Optical rotation was determined using a Jasco DIP-1000 Digital Polarimeter. IR spectra were measured on a Thermo Nicolet 5700 FT-IR spectrometer, on an ATR crystal (diamond). TLC was carried out on precoated aluminum TLC plates with RP-18 F_{254} (Merck, 0.25 mm). Column chromatography (CC) was carried out on silica gel 60 (Merck), Sephadex LH-20 (Fluka) and Lichrospher RP-18 (Merck). All ^{1}H and ^{13}C NMR spectra were recorded on a Varian MERCURY-VX 400 system at 399.929 MHz (^{1}H and 2D) and 100.567 MHz (^{13}C). Chemical shifts are referenced to the internal standards tetramethylsilane (TMS) as ($\delta = 0$ ppm, 1H) and CD_3OD ($\delta =$ 49.0, ^{13}C) or pyridine-d_5 ($\delta = 123.5$ ppm, ^{13}C). For NOESY spectra a mixing time of 0.6 s was used.

The high resolution positive ion ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). The sample solutions

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were introduced continuously *via* a syringe pump with a flow rate of 120 μl h^{-1}.

Data for X-ray diffraction analyses of single crystals were collected on a Stoe-IPDS diffractometer at 200 K using Mo Kα radiation (λ = 0.71073 Å, graphite monochromator).

2.2. Fungus material

Pyrofomes demidoffii (Lév.) Kotl. & Pouzar was collected from living specimen of *Juniperus procera* Hochst. ex A. Rich (leg./det. N Arnold & D. Abate, 18.05.2008) in Menagesha State Forest located about 30 km southwest of Addis Ababa (Ethiopia). A voucher specimen is deposited at IPB (number DQD026).

2.3. Extraction and isolation

Dried fruit bodies of *Pyrofomes demidoffii* (24 g) were grinded and exhaustively extracted with methanol. The resulting yellowish solution was concentrated *in vacuo* to afford a dark brown residue (3.2 g), which was separated by size exclusion chromatography on Sephadex LH-20 using MeOH as mobile phase followed by silica gel column chromatography (solvent system $CHCl_3$-MeOH 21:1 v/v) to afford nine fractions.

Fraction 4 (233.0 mg) was further separated by stationary RP-18 column chromatography (solvent MeOH-H_2O, 4:6 v/v) to yield compound **4** (11.9 mg). Fraction 6 (315.0 mg) was chromatographed on RP-18 column chromatography (solvent MeOH-H_2O, 2:8 v/v) to yield compound **1** (35.3 mg) and compound **2** (101.2 mg). Fraction 7 (68.1 mg) was purified by RP-18 column (solvent MeOH-H_2O 1:4 v/v) to give compound **3** (11.6 mg).

0.5 g dried wood of *Juniperus procera* were extracted with methanol. The resulting crude extract was evaporated to dryness and directly used for TLC comparison with isolated compounds **1**–**4**.

2.3.1. Pyrofomin A (*1*)

Colorless crystals (EtOAc-acetone: 4–1), mp: 242–245 °C. $[\alpha]_D = -16.5$ (MeOH, 1.00). Rf 0.69, RP-18 TLC, H_2O-MeOH (1:1). CD (MeOH): −293.8 (−1.54). ESI-FTICR-MS: 291.1572, calculated for $C_{15}H_{24}O_4Na^+$ 291.1567. IR (KBr): 3343, 3200, 2971, 2880, 1662, 1616, 1569, 1394, 1108, 1053, 958. ^1H and ^{13}C NMR spectra (CD_3OD) (Tables 1 and 2).

2.3.2. Pyrofomin B (*2*)

Colorless oil $[\alpha]_D = +23.3$ (MeOH, 0.74). Rf 0.64, RP-18 TLC, H_2O-MeOH (1:1). ESI-FTICR-MS: 293.1729, calculated for $C_{15}H_{26}O_4Na^+$ 293.1723. IR (KBr): 3344, 3205, 2971, 2941, 1468, 1443, 1392, 1255, 1114, 1048, 941. ^1H and ^{13}C NMR spectra (CD_3OD) (Tables 1 and 2).

Table 2
^{13}C NMR spectra of compounds **1**–**4** (CD_3OD).

Position	Compound **1**	Compound **2**	Compound **3**	Compound **4**
1	52.6	55.1	58.7	49.0
2	77.6	77.8	78.0	77.2
3	50.4	50.9	51.6	51.1
4	72.2	72.1	70.2	71.0
5	67.3	65.8	66.2	60.5
6	46.1	46.5	48.7	39.5
7	83.9	86.4	55.1	210.2[a]
8	53.8	78.2	74.6	72.6
9	216.8	35.9	35.2	210.4[a]
10	50.1	33.7	33.4	46.5
11	35.8	39.6	35.7	42.3
12	25.2	25.2	25.7	27.5
13	22.1	24.6	70.4	21.6
14	24.6	26.4	23.5	29.6
15	14.6	26.5	30.8	6.9

[a] May be interchanged.

2.3.3. Pyrofomin C (*3*)

Colorless oil; $[\alpha]_D = +12.40$ (MeOH, 0.56). Rf 0.40, RP-18 TLC, H_2O-MeOH (8:2). ESI-FTICR-MS: 293.1729, calculated for $C_{15}H_{26}O_4Na^+$ 293.1723. IR (KBr): 3339, 3200, 2964, 2932, 1393, 1113, 1050, 934. ^1H and ^{13}C NMR spectra (CD_3OD) (Tables 1 and 2).

2.3.4. Pyrofomin D (*4*)

Colorless oil; $[\alpha]_D = +14.2$ (MeOH, 0.60). Rf 0.38, RP-18 TLC, H_2O-MeOH (6:4). ESI-FTICR-MS: 289.1416, calculated for $C_{15}H_{22}O_4Na^+$ 289.1410. IR (KBr): 3345, 3200, 2973, 1733, 1699, 1663, 1616, 1568, 1396, 1394, 1088, 949. ^1H and ^{13}C NMR spectra (CD_3OD) (Tables 1 and 2). ^1H NMR (pyridine, d_5) δ 6.59 (1H, d, J = 4.8 Hz, 4-OH), 6.29 (1H, s, 2-OH), 4.83 (1H, dddd, J = 9.5, 8.9, 5.0, 4.8 Hz, H-4), 3.02 (1H, dd, J = 19.9, 3.1 Hz, H-11), 2.98 (1H, dd, J = 19.9, 1.5 Hz, H-11), 2.92 (1H, dd, J = 14.1, 9.5 Hz, H-3), 2.79 (1H, dd, J = 18.5, 3.1 Hz, H-10), 2.66 (1H, d, J = 18.5 Hz, H-10), 2.36 (1H, dd, J = 8.9, 1.5 Hz, H-5), 2.30 (1H, dd, J = 14.1, 5.0 Hz, H-3), 1.66 (3H, s, H-12), 1.36 (3H, s, H-15), 1.26 (3H, s, H-13), 1.18 (3H, s, H-14); ^{13}C NMR (pyridine, d_5) δ 209.1 (C-7), 209.1 (C-9), 76.2 (C-2), 71.8 (C-8), 70.1 (C-4), 60.1 (C-5), 52.0 (C-3), 48.4 (C-1), 46.3 (C-10), 42.4 (C-11), 39.0 (C-6), 29.5 (C-14), 28.1 (C-12), 21.5 (C-13), 7.2 (C-15).

2.4. Crystal data for *1*

Colorless crystals of **1** were obtained from a solvent mixture of EtOAc and acetone. Intensity data were collected at 220 K on a STOE IPDS II diffractometer, using Mo-*K*α (λ = 1.5418 Å) radiation. Data collection, cell refinement, and data reduction were performed with the STOE X-Area

Table 1
^1H NMR spectra of compounds **1**–**4** (CD_3OD).

Position	Compound **1**	Compound **2**	Compound **3**	Compound **4**
3	2.01, dd (12.7, 5.7)	1.98, dd (12.5, 5.6)	2.03, dd (12.6, 5.7)	2.43, dd (14.6, 9.7)
	1.67, dd (12.7, 8.0)	1.66, dd (12.5, 8.1)	1.69, dd (12.6, 7.4)	1.78, dd (14.6, 5.0)
4	4.29, ddd (8.0, 6.6, 5.7)	4.28, ddd (8.1, 6.9, 5.6)	4.25, ddd (7.4, 6.6, 5.7)	4.36, ddd (9.7, 9.1, 5.0)
5	1.36, br d (6.6)	1.72, m	1.76, dd (6.6, 1.1)	1.90, dd (9.1, 2.1)
7			1.89, br d (5.5)	
8	2.64, br q (7.3)			
9		2.00, m	1.94, m	
		1.72, m	1.66, m	
10	2.59, br d (15.2)	1.58, ddd(12.7, 12.7, 5.6)	1.61, m	2.44, dd (18.6, 3.6)
	2.12, ddd (15.2, 2.5, 1.2)	1.45, m	1.55, m	2.32, d (18.6)
11	2.25, ddd (12.3, 2.5, 2.3)	2.12, dd (12.1, 2.7)	2.05, ddd (12.9, 5.5, 3.0)	2.46, dd (19.4, 3.6)
	1.69, dd (12.3, 1.2)	1.20, dd (12.1, 1.2)	1.21, br d (12.9)	2.35, dd (19.4, 2.1)
12	1.23, s	1.23, s	1.24, s	1.36, s
13	1.06, s	1.08, s	3.57, d (10.6); 3.55, d (10.6)	1.00, s
14	0.94, s	1.26, s	1.40, s	0.96, s
15	1.19, d (7.3)	1.32, s	1.27, s	0.99, s