Spirobenzofuran, a New Bioactive Metabolite from *Acremonium* sp. HKI 0230

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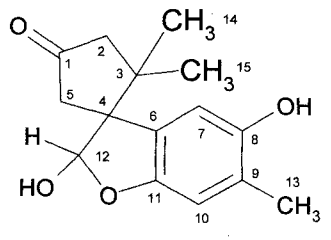
In a screening for bioactive metabolites from fungi we disclosed recently the strain *Acremonium* sp. HKI 0230 as producer of a narrow spectrum antibacterial compound. Here we report isolation and biological activity of spirobenzofuran (**1**) as a new bioactive fungal metabolite. The strain *Acremonium* sp. HKI 0230 was isolated from a soil sample collected at Pismo Beach, California, USA. It was deposited in the strain collection of the Hans-Knöll-Institute of Natural Products Rsearch Jena, Germany. During cultivation on malt agar the pale pink colonies reached 1.5~2.0 cm diameter after 10 days cultivation at 23°C, the hyphae were 1~2 μm in diameter. Phialoconidia in very long chains showed a elongate-cylindrical, elongate-fusiform or small dacryoid form (1.5~2 μm).

For submerged cultivation small pieces (1~2 cm^2) of a slant agar culture of the strain HKI 0230 grown on malt agar composed of (g/liter): malt extract 40, yeast extract 4, deionized water (pH 6.0) were inoculated into 500 ml Erlenmeyer flasks containing 100 ml of a culture medium composed of (g/liter): D-glucose 10, maltose 20, soytone 2, yeast extract 1, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, $ZnSO_4 \cdot 7H_2O$ 0.008, (pH 6.0). Cultivation occurred for 14 days at 25°C whereby a dense lawn of mycelium developed. Thereafter, 20 liters of the culture broth were extracted twice with 20 liters of ethyl acetate. The dried extract was evaporated *in vacuo*. The residue (1.8 g) was subjected to column chromatography on Sephadex LH-20 (MeOH). Bioactive fractions as tested by agar plate diffusion assay using *Bacillus subtilis* ATCC 6633[3] were pooled, evaporated to dryness and chromatographed on a silica gel column (5×40 cm) which was eluted by $CHCl_3$ and subsequently $CHCl_3$/MeOH (95 : 5, v/v). The bioactive fractions were finally purified by preparative TLC on silica gel aluminium sheets (Merck, $CHCl_3$/MeOH 95 : 5, v/v). 15 mg of the waxy bioactive fraction (**1**) were thus obtained staining reddish with 1% vanillin/conc. H_2SO_4. The molecular weight and the elemental composition of spirobenzofuran (**1**) was determined by HREI-MS (*m/z* 262.1196 (M^+); calcd. 262.1184 for $C_{15}H_{18}O_4$; high-resolution sector-field mass spectrometer AMD 402, AMD Intectra Harpstedt, Germany) suggesting the occurrence of seven double bonds and rings, respectively. The IR spectrum showed the presence of a keto group due to λ_{max} 1729 cm^{-1}. Conclusive evidence for the structure of **1** as shown in Fig. 1 was furnished by 1D and 2D NMR measurements (^1H, ^{13}C, DEPT 135, COSY, NOESY, HSQC, HMBC; Bruker Avance DRX 500). The ^1H NMR spectrum suggested the presence of three methyl groups (0.84, 0.97, 2.05 ppm), four methylene protons (2.23, 2.32, 2.42, 2.82 ppm) and two aromatic singlet protons (6.51 and 6.53 ppm). The ^{13}C NMR and DEPT spectra showed 15 carbons whereby a keto group (C-1, 216.2 ppm) and six aromatic carbons of a tetrasubstituted benzene ring with two attached protons (H-7, H-10) in para position were suggested. The signal at 102.1 ppm (C-12) was attributable to a cyclic hemiacetal structure.

Assignment of structure of **1** was based on instructive C, H long-range couplings (HMBC). Thus, cross-peaks between H_A-5/H_B-5 and C-1, C-2, C-3, C-4, C-6 and C-12 as well as between H-12 and C-3, C-4, C-5 and C-6 were of pivotal importance. Spirobenzofuran (**1**) thus appears as a new sesquiterpene structure which seems to be related biosynthetically to the lagopodin family of fungal metabolites[1,2]. However it is distinguishable by the presence of an unusual tricyclic spiro ring system. In the NOE spectrum of **1** weak correlations of H-12 with both H-

Fig. 1. Chemical constitution of spirobenzofuran (**1**).



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3 and H-5 protons were visible. Hence the relative positions of substituents at C-4 can not be assigned.

1 displayed moderate antimicrobial activity against few Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC 25 μg/ml)[3]. No activity was found against Gram-negative bacteria and fungi.

Experimental

Spirobenzofuran (**1**): appearance: wax, molecular weight: 262.1196 (M^+; calcd. 262.1184 for $C_{15}H_{18}O_4$), ESI-MS: *m/z* 285.0 ($[M+Na]^+$); *m/z* 261.2 ($[M-H]^-$); *m/z* 297.3 ($[M+Cl]^-$); *m/z* 559.3 ($[2M+Cl]^-$). IR (λ_{max}, cm^{-1}, KBr): 773, 866, 921, 982, 1018, 1072, 1149, 1180, 1203, 1273, 1300, 1381, 1418, 1458, 1487, 1633, 1691, 1729, 3370. Optical rotation ($[\alpha]_D$; 22°C, 9.3 mg/ml, MeOH): −23.9°. TLC ($CHCl_3$/MeOH 9 : 1, silica gel Merck): Rf 0.75. ^1H NMR (500 MHz, DMSO-d_6; δ in ppm, *J* in Hz; s: singlet, d: doublet): 0.84 (H-15; s; 3H), 0.97 (H-14; s; 3H), 2.05 (H-13 s; 3H), 2.23 (H_A-2; d; 18.3; 1H), 2.32 (H_B-2; d; 18.3; 1H), 2.42 (H_A-5; d; 19.0; 1H), 2.82 (H_B-5; d; 19.0, 1H), 5.78 (H-12; d; 5.6; 1H), 6.51 (H-10; s; 1H), 6.53 (H-7; s; 1H), 7.14 (HO-12; d; 5.6; 1H), 8.65 (HO-8; s; 1H). ^{13}C NMR (125 MHz, DMSO-d_6, δ in ppm): 16.3 (C-13), 23.0 (C-14), 24.5 (C-15), 41.1 (C-3), 42.7 (C-5), 51.9 (C-2), 59.1 (C-4), 102.1 (C-12), 111.0 (C-10), 111.2 (C-7), 123.7 (C-9), 126.5 (C-6), 148.9 (C-8), 150.3 (C-11), 216.2 (C-1).

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