VOL. 56 NO. 9, SEPT. 2003 THE JOURNAL OF ANTIBIOTICS pp. 792 — 794

7-Methoxy-2,3-dimethylbenzofuran-5-ol, a New Antioxidant from *Malbranchea cinnamomea* HKI 0286

B. SCHLEGEL*, A. HÄRTL, F. A. GOLLMICK and U. GRÄFE†

Hans-Knöll-Institute for Natural Products Research, Beutenbergstrasse 11a, D-07745 Jena, Germany

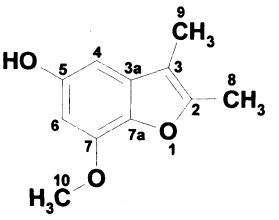
(Received for publication February 4, 2003)

In the course of our continuing screening for inhibitors of xanthine oxidase and peroxidase as key enzymes of purine metabolism and oxygen radical generation[1,2] we found the culture extract of the fungus *Malbranchea cinnamomea* HKI 0286 to contain an inhibitory principle. Here we report the isolation and properties of 7-methoxy-2,3-dimethylbenzofuran-5-ol (**1**; Figure 1) as a new natural antioxidant.

The fungal strain *Malbranchea cinnamomea* HKI 0286 from the strain collection of Hans-Knöll-Institute for Natural Products Research (Jena, Germany) was cultivated in 1 liter Erlenmeyer flasks containing 250 ml of a medium composed as follows (g/liter): dextrose 10, malt extract 20, soybean meal 2, yeast extract 1, KH_2PO_4 1, and $MgSO_4 \times 7H_2O$ 0.5, pH 6.0. The cultivation was done on rotary shakers (110 r.p.m.) for 14 days at 35°C.

After 14 days the culture broth (14 liters) was extracted three times with ethyl acetate, and the combined extracts were dried and evaporated to yield 2.1 g of a crude product. Isolation of **1** was carried out by serveral subsequent chromatographic steps such as column chromatography on silica gel 60 (Merck, 0.063~0.1 mm, $CHCl_3$/MeOH, 95 : 5, v/v) and preparative TLC on silica gel aluminium sheets (Merck) using the same eluent. Compounds on the chromatograms were detected by their staining behaviour in the presence of 1% vanillin in conc. H_2SO_4. Finally 25 mg of **1** with Rf 0.9 on TLC staining bluish were obtained as a solid compound. After some days standing at ambient

Fig. 1. Structure of 7-methoxy-2,3-dimethylbenzofuran-5-ol (**1**) from *Malbranchea cinnamomea* HKI 0286.



1

Table 1. Physico-chemical properties of **1**.

	1
Appearance	Semicrystalline solid
M.P.	108 – 110 °C
Molecular weight (M^+; HREI-MS)	192.0780 (calcd. 192.0786)
Chemical formula	$C_{11}H_{12}O_3$
UV λ^{MeOH}_{max}; nm (ε)	215 (6.300), 312 (2.835)
IR (λ_{max}; cm^{-1})	751, 836, 966, 1049, 1118, 1126, 1188, 1216, 1248, 1292, 1364, 1399, 1439, 1493, 1555, 1599, 1633, 2920, 3428
R_f on TLC Silica gel ($CHCl_3$/MeOH, 95:5)	0.9

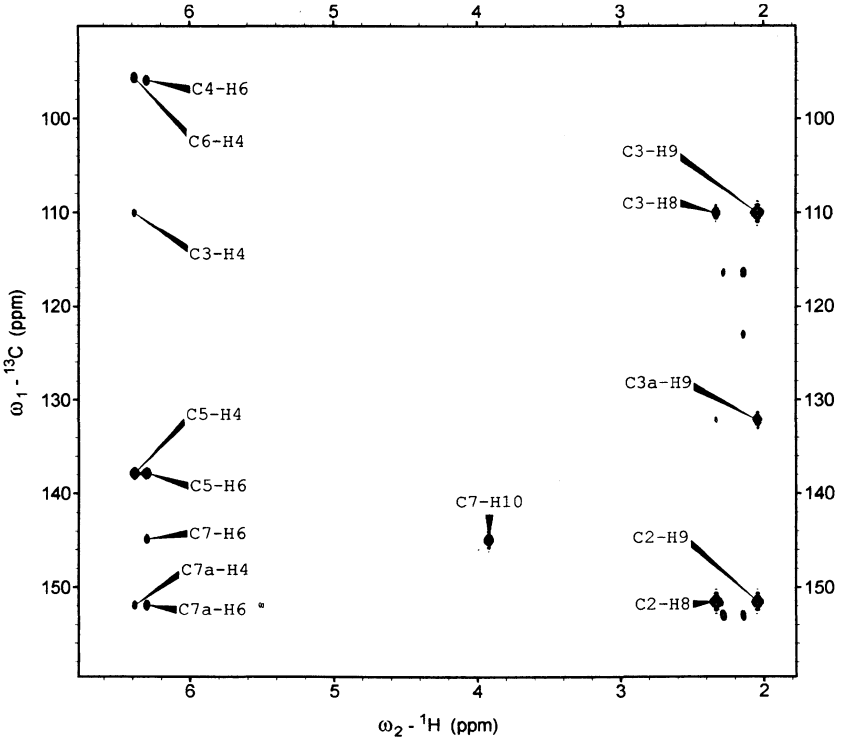
† Deceased February 14, 2003.

* Corresponding author: Brigitte.Schlegel@hki-jena.de

temperature the spot of **1** on the chromatogram turned reddish due to autoxidation and formation of quinoide products. The physico-chemical properties of **1** are shown in Table 1. The structure of **1** as shown in Figure 1 was assigned on the basis of optical spectroscopy (UV, FTIR, polarimetry), mass spectrometry (ESI-MS, HREI-MS) and 1D/2D NMR spectroscopy (^{1}H, ^{13}C, DEPT, ^{1}H,^{1}H-COSY, HMQC, HMBC). **1** was optically inactive. The FTIR spectrum suggested the occurrence of aromatic structures (1555, 1599, 1633 cm^{-1}) and a hydroxyl group (3428 cm^{-1}). The ESI-MS showed *m/z* 193.2 ($[M+H]^+$), and in the HREI-MS *m/z* 192.0780 (M^+; 100 %, calcd. 192.0786 for $C_{11}H_{12}O_3$) was visible. The ^{13}C NMR spectrum ($CDCl_3$) displayed 11 carbons which were assigned on the basis of the DEPT spectrum as two methyls (11.8 ppm (C-8), 8.0 ppm (C-9)), one methoxyl (56.0 ppm (C-10)), four oxygenated olefinic or aromatic carbons (151.5 ppm (C-2), 151.8 ppm (C-7a), 144.8 ppm (C-7), 137.8 ppm (C-5)), two protonated aromatic carbons (95.7 ppm (C-6), 97.0 ppm (C-4)) and two additional quaternary carbons at 132.1 ppm (C-3a) and 110.0 ppm (C-3). In the ^{1}H NMR spectrum 12 protons appeared at 2.33 ppm (H-8, s, 3H), 2.04 ppm (H-9, s, 3H), 3.92 (H-10, s, 3H), 6.39 ppm (H-4, d, 1.8 Hz, 1H), 6.30 ppm (H-6, d, 1.8 Hz, 1H), and one broad proton signal at 4.92 ppm (OH-5, br).

The COSY spectrum and $^4J_{H\text{-}4,H\text{-}6}$=1.8 Hz indicated the presence of meta-coupled protons. Conclusive evidence for the structure of **1** was furnished by C,H long-range coupled NMR spectra (HMBC, Fig. 2) showing a series of instructive $^2J_{C,H}$ and $^3J_{C,H}$ couplings which enabled us together with the three couplings from the NOESY spectrum doubtlessly the assignment of carbon and proton signals (Table 2). Especially the NOE between H-9 (methyl) and H-4 gives us the evidence for the position of C-4. The couplings from the HMBC and the NOESY spectrum are shown in Fig. 3. 7-Methoxy-2,3-dimethylbenzofuran-5-ol (**1**) thus appears as a new representative of the benzofuran family of natural products. It displayed strong inhibition of xanthine oxidase[1] with IC_{50} 9.8 μM and horse radish peroxidase with IC_{50} 7.5 μM. Moreover, **1** inhibited the polymorphonuclear lymphocyte reaction[2] (preliminary results). The inhibitory properties of **1** may be due to the presence of a cryptic hydroquinone structure similar as occurring in the tocopherol analogue Trolox®[3] which can be oxidized easily to the quinone.

Fig. 2. HMBC spectrum of **1**.



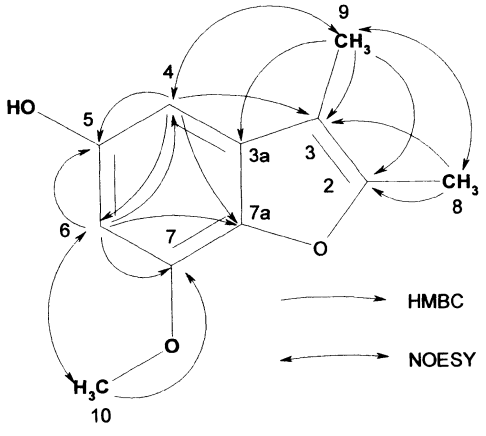
The peaks are marked with the corresponding atom numbers. Unmarked peaks are due to minor impurities.

Table 2. Assignment of ^{1}H and ^{13}C NMR spectra of **1** (500 MHz; in $CDCl_3$; chemical shifts (δ) in ppm; coupling constants in Hz).

	1	
Position	δ_C	δ_H
2	151.5 (s)	-
3	110.0 (s)	-
3a	132.1 (s)	-
4	97.0 (d)	6.39 d, 1.8
5	137.8 (s)	4.92 br (OH)
6	95.7 (d)	6.30 d, 1.8
7	144.8 (s)	-
7a	151.8 (s)	-
8	11.8 (q)	2.33 (s)
9	8.0 (q)	2.04 (s)
10	56.0 (q)	3.92 (s)

Abbreviations: s=singlet, d: doublet, br: broad, q=quartet.

Fig. 3. Long range couplings from HMBC and NOESY spectra.



Experimental

UV spectra were recorded on a Specord double-beam 2000 spectrometer (Analytik Jena, Germany), FTIR on a Mattson FTIR spectrometer satellite equipped with an ATR device (Mattson, Chicago, USA), ESI mass spectra on a triple quadrupole instrument Quattro (VG Biotech, Altrincham, England), HREI-MS on a Finnigan MAT 95 XL (Finnigan, Bremen, Germany), and NMR spectra on a 500 MHz Bruker Avance DRX 500 spectrometer (Bruker, Karlsruhe, Germany). Measurements of xanthine oxidase[1] activity and polymorphonuclear lymphocyte reaction[2] were done as was described in the literature. Horse radish peroxidase was measured by formation of 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol) radicals and endoperoxides in presence of H_2O_2. Their decomposition yielded 3-aminophthalate dianion which was excitable to emit visible light. This chemiluminescence was measured as relative light units. The addition of **1** to the reaction mixture of a single assay in a total volume of 250 μl per well of a 96 well microplate (50 μl luminol (0.4 mg/ml PBS buffer), 50 μl horse radish peroxidase (Sigma; 0.066 U/ml, **1**: 0.1~10 μg in 50 μl PBS buffer, 50 μl H_2O_2 (30%) and PBS buffer ad 250 μl) resulted in continuous suppression of light emission until the antioxidant (**1**) was consumed. Chemiluminescence was measured by a labsystem Luminoskan RS instrument.

Acknowledgement

Support of this work by FCI Frankfurt (Germany) is gratefully acknowledged.

References

1) PIERCE, L. A.; W. O. TARNOW-MORDI & I. A. CREE: Antibiotics effects on phagocyte chemoluminescence *in vitro*. Int. J. Clin. Lab. Res. 25: 93~98, 1995
2) YU, PEI-WEN & C. J. CZUPRYNSKI: Regulation of luminol-dependent chemiluminescence and degranulation by bovine neutrophils stimulated with opsonized zymosan. Vet. Immunol. Immunopathol. 50: 29~42, 1996
3) MILLER, N. J.; C. RICE-EVANS, M. J. DAVIES, V. GOPINATHAN & A. MILNER: A novel method für measuring antioxidant capacity and ist application to monitoring the antioxidant status in premature neonates. Clinical Science 84: 407~412, 1993