COMMUNICATIONS TO THE EDITOR

5-Hydroxy-3,4,7-triphenyl-2,6-benzofurandione, a New Xanthine Oxidase Inhibitor from *Peniophora sanguinea*

Sir:

In the course of our screening for new inhibitors of xanthine oxidase (XO) we found the extract of a wood-rotting fungus, *Peniophora sanguinea*[1~3] to contain an active principle during the common lucigenin-coupled assay of this enzyme.[4] Xanthine oxidase was selected as a screening target due to its role in nucleic acid metabolism, hyperuricemia (gout), acute pancreatitis, cholecystitis, adult respiratory distress syndrome, its important contribution to superoxide anion radical formation and related disorders such as mycocardidal damage during ischemia and/or reperfusion injury, bacterial translocation from the gastrointestinal tract and several other diseases as well[5~8]. Due to severe adverse effects of the therapeutically used inhibitor allopurinol there is an urgent demand for new natural products as XO inhibitors having non-purine structures[8]. In this paper we describe the isolation, physico-chemical properties, structure elucidation and biological activities of 5-hydroxy-3,4,7-triphenyl-2,6-benzofurandione (**1**; $C_{27}H_{18}O_4$) from the fruiting bodies of this fungus coproducing xylerythrin (**2**)[1,2,4].

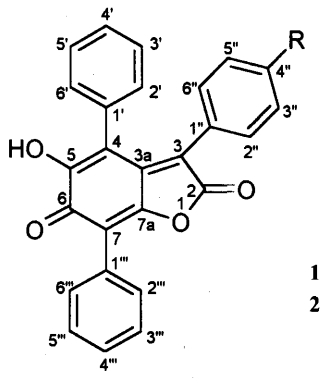
350 g (wet weight) of fruiting bodies of *Peniophora sanguinea* collected in the forest of Seitenbrück near Jena were extracted for 3 days with 3 liters methanol. The extract was evaporated *in vacuo* to 300 ml, diluted with 500 ml water and reextracted with 2 liters ethyl acetate. The dried ethyl acetate extract was evaporated to dryness *in vacuo*. The residue (2.4 g oily product) was dissolved in $CHCl_3$/MeOH (90:10; 30 ml) and applied to the top of a silica gel column (silica gel 60 0.04~0.065 mm, Merck, 6 × 40 cm, $CHCl_3$). Stepwise elution by a gradient of chloroform to methanol (100:0; 95:5; 9:1; 8:2) afforded two fractions containing greenish pigments. Further purification was carried out by preparative TLC (silica gel 60, aluminium sheets, Merck, $CHCl_3$/MeOH, 95:5) whereby **1** was eluted from a zone with Rf 0.9 and **2** from a zone with Rf 0.6 by $CHCl_3$/MeOH (1:1). Further purification was achieved by preparative HPLC on silica gel RP_{18} (Spherisorb ODS-2; 2.5 cm × 25 cm; binary gradient acetonitrile to water 95:5 to 5:95; 30 minutes). Yield: 25 mg **1** and 15 mg **2**. The physico-chemical properties of the new XO inhibitor **1** are summarized in Table 1.

The structure of the new metabolite **1** was settled by mass spectrometric and NMR spectroscopic investigations. The UV-VIS spectrum of **1** was identical with that of coproduced **2**. In the IR spectrum the diagnostic carbonyl band was visible at 1680 cm^{-1}. The electrospray mass spectrum of **1** displayed *m/z* 393.2 ($[M+H]^+$) in the positive mode and *m/z* 391.4 ($[M-H]^-$) in the negative ion mode. By collision-induced decomposition of *m/z* 391.0 $[M-H]^-$ diagnostic fragments with *m/z* 315.2 $[M-C_6H_5]$ and *m/z* 116.6 $[M-CO_2; -3C_6H_5]$

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

Appearance	Greenish microcrystals
Melting point	137~138°C
Chemical formula	$C_{26}H_{16}O_4$
Molecular weight	392
HREI-MS ($[M]^+$)	*m/z* calcd. 392.1089; found. 392.1092
UV λ_{max} nm (ε) in MeOH	385 (530); 240 (8960)
IR ν_{max} cm^{-1}	695, 889, 921, 1027, 1139, 1179, 1218, 1267, 1300, 1341, 1379, 1406, 1435, 1488, 1515, 1592, 1627 (aromate), 1719 (CO), 1768 (CO), 2950, 3425 (OH)
Retention time (minute) during HPLC: (column 2.5 × 250 mm, gradient 99% acetonitrile to 99% water, 20 minutes Rf, (TlC, Silica gel, $CHCl_3$/MeOH 95:5)	14.5 0.90

Fig. 1. Chemical structure of 5-hydroxy-3,4,7-triphenyl-2,6-benzofurandione (**1**) and xylerythrin (**2**) from *Peniophora sanguinea*.



1 R=H
2 R=OH

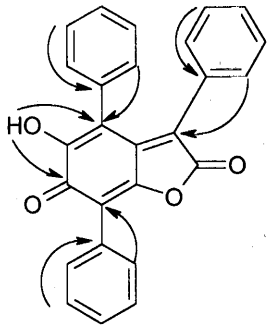
Table 2. ^{1}H and ^{13}C chemical shifts of **1** (in $CDCl_3$; δ in ppm relative to internal TMS).

Position	δ_C	δ_H
2	167.0	—
3	131.1	—
3a	138.9	—
4	114.0	—
5	157.5	—
5-OH	—	—
6	180.8	—
7	114.9	—
7a	146.9	—
1'	131.4	—
2'	129.5	7.1
3'	127.8	7.06
4'	128.1	7.09
5'	127.8	7.06
6'	129.5	7.1
1''	118.9	—
2''	129.8	6.99
3''	127.5	7.01
4''	129.4	7.15
5''	127.5	7.01
6''	129.8	6.99
1'''	128.6	—
2'''	130.5	7.65
3'''	128.3	7.49
4'''	129.1	7.43
5'''	128.3	7.49
6'''	130.5	7.65

were generated. The elemental composition of **1** was determined as $C_{26}H_{16}O_4$ by high-resolution electron impact (HREI) mass spectrometry due to *m/z* 393.1081 ($[M+H]^+$, calcd. 393.1092).

Conclusive evidence of the chemical structure of **1** (Fig. 1) was obtained by one- and two-dimensional NMR

Fig. 2. Instructive C, H long range couplings in the HMBC spectrum of **1**.



experiments (COSY, TOCSY, NOESY, HSQC and HMBC; Table 1). The ^{1}H chemical shift and coupling data of the three aromatic substituents are compatible with the diagnostic pattern of mono-substituted benzene rings. Due to the presence of six pairs of equivalent carbons, only twenty carbon signals were observable in the ^{13}C NMR spectrum of **1**. Analysis of the DEPT spectrum revealed only nine methine signals of which six showed doubled intensity. The observation of a cross peak in the HMBC spectrum between the 2'-H/6'-H hydrogens and C-4, the 2''-H/6''-H hydrogens and C-3 and the 2'''-H/6'''-H hydrogens and C-7 supplied diagnostic information about the triphenyl substitution of the 2,6-benzofurandione skeleton. The observed $^{3}J_{C,H}$ long-range couplings in the HMBC spectrum between the 5-OH and carbons Nos. 4 and 6 further settled the substitution pattern. The remaining four quarternary carbons Nos. 3a and 7a were readily ascribed to a second ring structure due to the chemical formula (see HRFAB-MS) suggesting the presence of not less than 19 double bond equivalents in the molecule. This conclusion was supported by the close similarity of the proton and carbon chemical shift data of **1** with those of the coproduced xylerythrin (**2**)[1,2]. The only exception was the carbon chemical shift C-4'' in **1** which appeared at 129.4 ppm instead of 159.0 ppm as was found in the ^{13}C NMR spectrum of **2**.

1 and **2** inhibited xanthine oxidase with an efficiency comparable to allopurinol as a known and therapeutically useful inhibitor of this enzyme. Thus in the cell-free system both metabolites suppressed chemiluminescence (CL) induced by xanthine oxidase and enhanced by lucigenin. Concentration-dependent suppression of chemiluminescence was observed with $IC_{50}=0.50\,\mu g/ml$ (1.276 μM) for **1** and, respectively, 0.73 $\mu g/ml$ (1.79 μM)

Table 3. Suppression of chemiluminescence generated by the hypoxanthine-xanthine oxidase/lucigenin-coupled production of superoxide anion radicals in presence of **1**, **2**, and allopurinol (expressed as percentage decrease of relative light units (RLU) in comparison to the chemiluminescence of the nontreated control (=100%).

Inhibitory concentration (μg/ml)	Suppression of chemiluminescence[a]		
	1	**2**	Allopurinol
0.2	63.1±2.6	80.8±1.5	58.4±2.8
0.4	54.6±0.9	69.0±0.7	44.0±3.4
0.8	37.0±2.7	47.5±7.1	31.9±2.7

[a] Mean values of three measurements±standard deviations.

for **2** with an efficiency comparable to allpurinol (IC_{50} = 0.32 μg/ml (2.35 μM); Table 3). Moreover, **1** displays moderate cytopathic effect against L-929 mouse fibroblast cells, K-562 human leukaemia cells and HeLa cells with IC_{50} 2.8, 3.8 and 14.1 μg/ml, respectively[5].

Thus phenyl-substituted 2,6-benzofuranediones such as **1** and **2** appear as new inhibitors of xanthine oxidase and the generation of superoxide anion radicals with potential applications in the treatment of gout, stroke and cardiovascular diseases. But it seems unclear at present whether compounds **1** and **2** inhibit the enzyme activity of xanthine oxidase or prevent lucigenin reaction by scavenging superoxide anion radicals.

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