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Bioorganic & Medicinal Chemistry Letters 17 (2007) 2558–2560
Bioorganic & Medicinal Chemistry Letters

Quercinol, an anti-inflammatory chromene from the wood-rotting fungus *Daedalea quercina* (Oak Mazegill)

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Received 2 January 2007; revised 1 February 2007; accepted 3 February 2007
Available online 7 February 2007

Abstract—The fungus *Daedalea quercina* (oak mazegill) was examined for its capability of producing antioxidative and anti-inflammatory compounds. Bioactivity guided fractionation of the extract from a mycelial culture led to the isolation of quercinol, which was identified as (−)-(2*S*)-2-hydroxymethyl-2-methyl-6-hydroxychromene **1** by NMR and X-ray analyses. The cryptic hydroquinone **1** shows a broad anti-inflammatory activity against cyclooxygenase 2 (COX-2), xanthine oxidase (XO), and horseradish peroxidase (HRP) at micromolar concentrations.

Reactive oxygen species (ROS), such as hydroperoxide and superoxide radicals, and singlet oxygen, are involved in various processes triggered by biotic or abiotic stresses.[1,2] In many organisms ROS represent a first line of defense that is usually set up immediately or shortly after contact with a pathogen.[3] Such hypersensitive response is a common induced plant defense mechanism to combat invasion by pathogens like saprotrophic fungi.[4] In humans, however, ROS not only play a role in immune response in macrophages, but also in unwanted deleterious reactions. In fact, various diseases such as inflammation, arteriosclerosis, allergies, and neuropathological destruction are also initiated by reactive oxygen species and oxygenated signaling molecules. Enzymes involved in the formation of inflammation mediators have thus emerged as important targets for drug development.[5]

In the course of our search for novel anti-inflammatory and antioxidative compounds from fungi[6,7] we were intrigued by the metabolic capabilities of the wood-rotting basidiomycete *Daedalea quercina* (commonly known as Oak Mazegill). This wood decay fungus, which generally grows on living oaks, appeared to be well equipped to neutralize first line defense chemicals of the plant. We actually noticed that culture extracts show strong inhibition of enzymes generating or utilizing ROS. Here we report the isolation and full structure elucidation of the active principle, quercinol, and its evaluation as potent anti-inflammatory agent.

The producing strain, *D. quercina* HKI 0319, was collected from an oak tree in a forest near Jena, Germany. Mycelial cultures of the strain HKI 0319 were derived from tissue plugs of the fruiting body. The crude extract from an up-scaled fermentation (20 L) was subjected to bioassay-guided fractionation. Open column chromatography on Amberlite XAD 1180 and silica with subsequent recrystallization yielded 1.0 g of quercinol as a colorless solid. The structure of **1** (Fig. 1) was assigned on the basis of optical spectroscopy, mass spectrometry (ESI-MS, HREI-MS), and 1D/2D NMR spectroscopy, and X-ray crystallography.

The molecular formula of **1** ($C_{11}H_{12}O_3$) was established by HRESI-MS and ^{13}C NMR, suggesting the presence of six double bond or ring equivalents in the molecule. Conclusive evidence for the structure of **1** was obtained from NMR data (^1H, ^{13}C, DEPT, COSY, HMQC, HMBC, and NOESY). The ^1H- and $^1H,^1H$-COSY NMR spectra indicated an olefin structure (H-3; H-4) and the occurrence of *ortho*- and *meta*-coupled protons (H-5; H-7; H-8). The partial structures of **1** (Fig. 1) were strongly supported by ^{13}C NMR data. 11 carbons were

Keywords: Chromenes; Natural products; Fungal metabolites; Anti-inflammatory agents; Oxidative burst.
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doi:10.1016/j.bmcl.2007.02.008

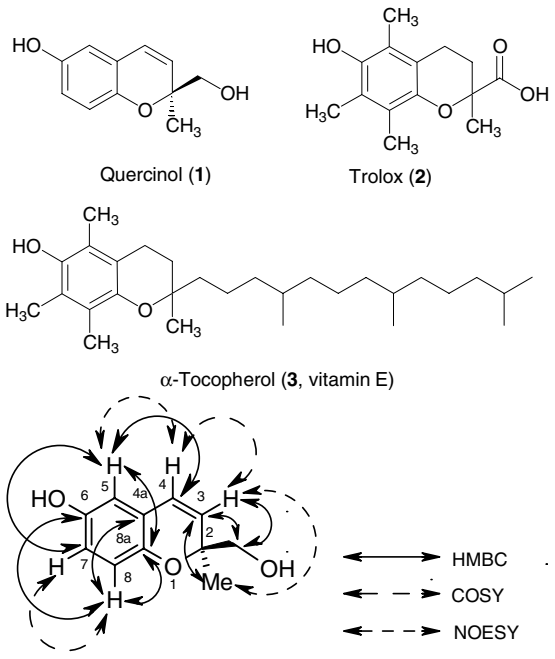


Figure 1. Structures of quercinol (**1**) with selected connectivities, trolox® and α-tocopherol.

visible. The signal at 149.9 ppm (C-6) was attributed to a phenolic carbon, while the signal at 145.8 ppm (C-8a) indicated a heteroatom substituted aromatic carbon. Carbons resonating at 127.6 (C-3), 124.8 (C-4), 121.6 (C-4a), 116.7 (C-8), 115.8 (C-7), and 113.2 (C-5) ppm indicated aromatic and olefinic protons. In addition, the data implied the presence of a fourfold substituted ether carbon (δ 79.0, C-2), a secondary carbinol (δ 68.3, 2-CH_2O), and a methyl (δ 22.2, 2-CH_3). HMBC correlations were observed for C-3 and the 2-methyl as well as the 2-hydroxymethyl protons, respectively, between C-4 and H-5, between C-4a and H-8, between C-6 and H-8, between C-7 and H-5, and between C-8a and H-8 and H-5.

All 1D and 2D NMR data unequivocally established the structure of **1** as 2-hydroxymethyl-2-methyl-2*H*-chromen-6-ol, named quercinol, which represents a new member in the chromene family of natural products.[8] **1** features a single chiral center and shows optical activity ($[\alpha]_D^{22}$ (MeOH, $c = 0.66$), −9.9°). In order to solve the absolute configuration of (−)-**1**, crystals were raised by slow recrystallization from diethyl ether/hexane and examined by X-ray (Fig. 2). By this way the structure determined by 2D NMR experiments was confirmed, and the absolute configuration at the C-2-position was established as (2*S*).[9]

1 was subjected to a panel of assays using enzymes involved in inflammatory processes and oxidative burst. In xanthine oxidase (XO)[10] and horseradish peroxidase (HRP) assays[11] the hydroxychromene showed moderate enzyme inhibition similar to the standard agents allopurinol and acetylcysteine (see Table 1).

Furthermore, **1** efficiently blocked 3α-hydroxysteroid dehydrogenase (3α-HSD) and proved to be a potent inhibitor of COX-1 and COX-2. In the COX-2 and HRP assays, the bioactivity profile of **1** is moderate and similar to Trolox® (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, **2**), a water-soluble synthetic vitamin E analogue (**3**), albeit structural differences are obvious. Nonetheless, these cryptic hydroquinones may act as potent radical and ROS scavengers.[12] In analogy to tocopherol the mode of antioxidant activity can be rationalized as shown in Scheme 1.

It should be noted that **1** has already been prepared in the course of synthetic studies, but its potent antioxidative and anti-inflammatory effects have been overlooked.[13,14] A few naturally occurring 2-methyl-2-hydroxymethylchromene derivatives have been reported, albeit only from plant sources, such as *Pteris longipinna*,[15] *Ageratina riparia*,[16] *Blepharispermum subsessile*,[17] and *Garcinia kola*.[18] 2,2-Dialkyl substituted chromenes were isolated from the fungal *Crucibulum, Lactarius, Aspergillus silvaticus*, and *Cylindrocarpon* species.[19–22]

It is tempting to speculate about the role of quercinol in its natural context. Obviously the fungus, which infects pruning wounds of oak trees, can cope with the hypersensitive response. Considering the high potency against ROS quercinol might serve the fungus to dampen the plant defense mechanism.

Table 1. In vitro inhibitory potencies (IC_{50} in μmol L^{-1}) of **1** and standard substances to cyclooxygenase **1** and **2** (COX-1, COX-2), 3α-hydroxysteroid dehydrogenase (3α-HSD), xanthine oxidase (XO), and horseradish peroxidase (HRP)

Compound	COX-1	COX-2	3α-HSD	XO	HRP
(-)-**1**	4.7	0.63	114	21	68
Trolox®	0.13	0.15	>1000	>1000	150
Indomethacin	5.6	77.1	14	—	—
Allopurinol	—	—	—	2.2	—
NAC	—	—	—	—	31

NAC, *N*-acetylcysteine. Values are means of three experiments.

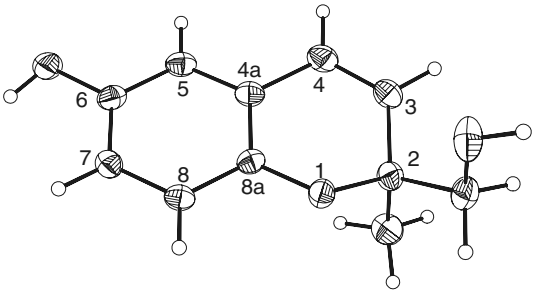
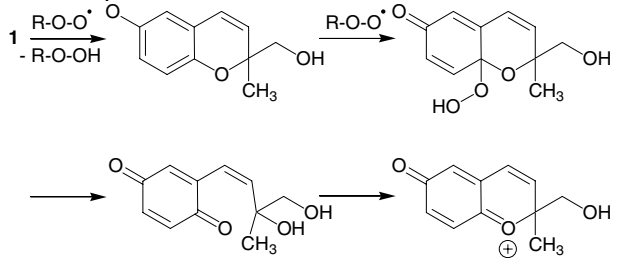


Figure 2. X-ray structure of quercinol (**1**).



Scheme 1. Model for ROS scavenging activity of **1**.

In summary we have isolated quercinol from a mycelial culture of the fungus *Quercina daedalea* and identified it as a new member of the chromene family of natural products. The structure and absolute configuration of **1** were established by NMR and X-ray studies. In various assays using enzymes that are commonly involved in inflammation processes and oxidative burst, **1** showed inhibitory activities at micromolar concentrations. It is remarkable that 1 showed a broad-band anti-inflammatory profile. In its natural context, **1** might play a role in fungal pathogenesis as it would efficiently neutralize the first line of plant defense upon infection. Thus, fungi growing on living trees should be investigated further as a promising source for anti-inflammatory natural products.

Acknowledgments

We gratefully acknowledge technical assistance by M.-G. Schwinger and C. Karkowski. This work was financially supported by the FCI (Frankfurt, Germany) and the Thuringian Ministry of Culture and Education.

Supplementary data

Supplemental material available: fermentation and isolation of **1**, spectroscopic and crystallographic data, biological assays. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.02.008.

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