Xyloketal H from the mangrove endophytic fungus *Xylaria* sp. 2508

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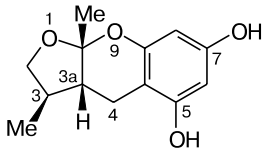
(3*R**,3a*R**,9a*R**)-3,9a-Dimethyl-2,3,3a,9a-tetrahydro-4*H*-furo[2,3-*b*]chromene-5,7-diol (xyloketal H), a representative of a new family of xyloketals, was isolated from the marine-derived mangrove fungus *Xylaria* sp. 2508. Its structure was elucidated by spectroscopic data and single-crystal X-ray diffraction analysis.

Key words: xyloketal, metabolites, marine fungi, mangrove, X-ray diffraction analysis.

Marine microorganisms live in special environments, such as high salinity, high pressure, poor nutrition, low temperature (especially in deep sea) or locally high temperature, and complex relations with different creatures. Thus, they have developed unique metabolic methods in these what is called extreme living environments, which ensure that they can live therein and provide the potential of producing metabolites never met in terrestrial ones. Among them, the marine mangrove fungus has gained more and more recognition from researchers for the special ecological status of mangrove. Metabolites with unique and novel structures have been isolated from mangrove fungi in recent years.[1—5] Xyloketals are a group of novel metabolites from the mangrove fungus *Xylaria* sp. 2508,[6—8] which have attracted some organic chemists to their synthesis.[9—13]

In the present report, we describe the isolation of a new optically active metabolite from the fungus *Xylaria* sp. 2508 with the structure of 3,9a-dimethyl-2,3,3a,9a-tetrahydro-4*H*-furo[2,3-*b*]chromene-5,7-diol (**1**), which we named xyloketal H. It should be mentioned that compound (±)-**1** has previously[11] been prepared as a mixture of epimeric intermediate products during non-enantioselective synthesis of xyloketals A and B.



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The structure of xyloketal **1** was determined by NMR spectroscopy and mass spectrometry. The X-ray diffraction data (Fig. 1) showed that its asymmetric centers have the mutual (3*R**,3a*R**,9a*R**)-configuration. Nevertheless, the absolute configuration of compound **1** remains unknown and has to be confirmed by other methods.

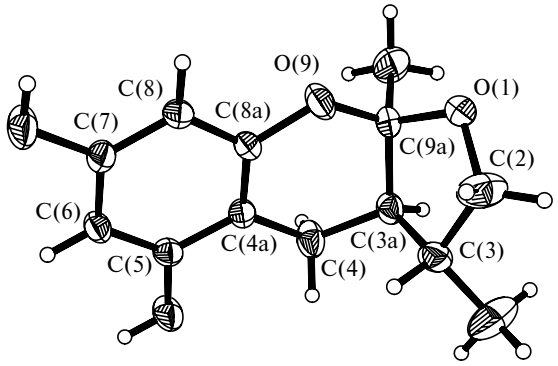

Fig. 1. Structure of xyloketal H (**1**).

In the preliminary bioassay, xyloketal H was not bioactive to Hep-2 cell line and against gram-positive bacteria *Staphylococcus aureus* in standard disk assays (200 μg disk^{-1}).

Experimental

A single crystal of compound **1** was prepared by crystallization from methanol. X-ray diffraction experiments were carried out for a faceted prismatic single crystal on a Bruker SMART 1000 CCD diffractometer (graphite monochromator, λ(Mo-Kα) = 0.71073 Å, temperature 293(2) K). The data were collected in groups of 600, 450, and 230 frames for ω = 0, 90, and 180°, respectively, in the ω-scan mode with a step of 0.3° and a 10-s exposure per frame. The crystallographic data were deposited with the Cambridge Crystallographic Center (CCDC 295186).

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^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometer (working frequencies 300.13 and 75.47 MHz, respectively) using Me_4Si as the internal standard. The mass spectrum was obtained on a VG-ZABHS instrument. The IR spectrum was measured on a Bruker VECTOR 22 spectrophotometer. The UV spectrum was obtained on a Shimadzu UV-2501PC spectrophotometer. The melting point was determined on an X-4 micromelting point apparatus and was uncorrected. Optical rotation was measured on a Schmidt+Hänsch Polartronic HH W5 polarimeter.

Cultivation of the fungus. A strain of the fungus *Xylaria* sp. 2508 was isolated from seeds of an angiosperm tree in Mai Po (Hong Kong) and stored at the Department of Applied Chemistry of the Zhongshan University (Guangzhou, China). The starting cultures (kindly presented by E. B. G. Jones and L. L. P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 500-mL Erlenmeyer flask containing 250 mL of liquid medium (glucose, 10 g L^{-1}; peptone, 2 g L^{-1}; yeast extract, 1 g L^{-1}; NaCl, 30 g L^{-1}). The flask was incubated at 30 °C on a rotary shaker for 5—7 days. The mycelium was aseptically transferred to a 300-L fermenter containing 170 L of GYT medium and incubated at 30 °C for 80 h.

Isolation of compound 1. The cultures (170 L) were filtered through cheesecloth. The filtrate was concentrated to 3.5 L below 60 °C and then extracted 5 times by shaking with an equal volume of AcOEt and 3 times with Bu^nOH. The butanolic fraction was condensed and then refluxed 3 times with 200 mL of AcOEt. The combined ethyl acetate extracts were chromatographed on silica gel using gradient elution from petroleum ether to AcOEt. Compound **1** was obtained in a yield of 213.6 mg from the fraction containing 30% AcOEt.

Compound 1. Colorless blocks, m.p. 210 °C (from MeOH, with decomp.), $[\alpha]_D^{25}$ −17±2 (*c* 0.24, $CHCl_3$). UV (MeOH), λ_{max}/nm (*c* 0.25, ε): 261 (1164), 244 (1132). IR (KBr), ν/cm^{-1}: 3368 br, 2960, 2908, 1616, 1514, 1472, 1383, 1275, 1237, 1148, 1103, 1061. Mass spectrum (FAB, 70 eV), *m/z*: 237 [M + 1], 219, 94, 65. ^{1}H NMR (acetone-d_6), δ: 1.04 (d, 3 H, C(3)Me, J = 6.3 Hz); 1.42 (s, 3 H, C(9a)Me); 1.97 (m, 1 H, C(3a)H); 2.06 (m, 1 H, C(3)H); 2.63 (d, 1 H, C(4)H, J = 17.1 Hz); 2.68 (dd, 1 H, C(4)H, J = 17.1 Hz, J = 6.0 Hz); 3.52, 4.17 (both dd, 1 H each, C(2)H, J = 8.4 Hz, J = 8.1 Hz); 5.79 (d, 1 H, C(8)H, J = 2.1 Hz); 5.99 (d, 1 H, C(6)H, J = 2.1 Hz); 7.95, 8.15 (both s, 1 H each, OH). ^{13}C NMR (acetone-d_6), δ: 16.2 (C(3)$\underline{C}H_3$), 19.2 (C(4)), 23.3 (C(9a)$\underline{C}H_3$), 36.2 (C(3)), 48.6 (C(3a)), 74.0 (C(2)), 95.8 (C(8)), 95.9 (C(6)), 98.2 (C(4a)), 107.8 (C(9a)), 155.4 (C(7)), 156.9 (C(5)), 157.5 (C(8a)) (signals were assigned using DEPT experiment).

The antibacterial activity of compound **1** was tested by a usual procedure.[14] The cytotoxicity against Hep-2 cells was estimated by the MTT colorimetric assay.[15]

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