Xyloketal F: A Strong L-Calcium Channel Blocker from the Mangrove Fungus *Xylaria* sp. (#2508) from the South China Sea Coast

Xiong Yu Wu,[a] Xiao Hong Liu,[a] Yong Cheng Lin,*[a] Jing Hui Luo,[a] Zhi Gang She,[a] Li Houjin,[a] Wing Lai Chan,*[b] Sándor Antus,[c] Tibor Kurtan,[c] Brigitta Elsässer,[d] and Karsten Krohn*[d]

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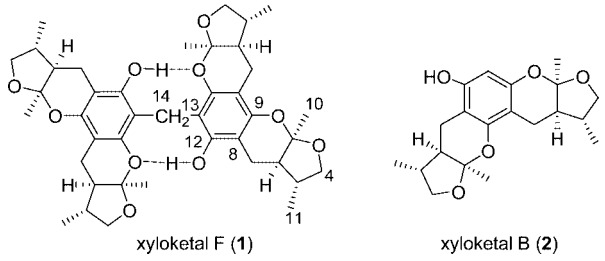
Xyloketal F (**1**), an unusual metabolite with strong L-calcium channel blocking activity, was isolated from the mangrove endophytic fungus *Xylaria* sp. (#2508) collected at the South China Sea coast. Its structure was elucidated by spectral and single-crystal X-ray diffraction analysis. The absolute configuration of **1** was determined by the method of quantum-mechanical calculation of CD spectra. Compound **1** was also synthesized by condensation of xyloketal B with formaldehyde. The L-calcium channel blocking activities of xyloketals B, A, and F were determined, and at the same concentration (0.03 μmol/L), the inhibiting rates were 12.05 %, 21.47 %, and 50.33 % respectively.

Introduction

A large variety of new bioactive compounds has recently been isolated from marine fungi.[1] It has been reported that most of the described marine fungi could be found associated with mangroves.[2] Mangroves are thus now considered the second most important hosts for marine fungi after driftwood. Recently, we embarked on a study of the metabolites of marine fungi including those from mangroves from the South China Sea. These studies have yielded a number of interesting compounds.[3–5] A series of new xyloketals was isolated from the mangrove fungus *Xylaria* sp. (#2508). In this paper, we report the isolation of xyloketal F (**1**), an unusual dimeric metabolite with strong L-calcium channel blocking activity, from the fungus *Xylaria* sp (#2508). Compounds that block the L-calcium channel are quite significant in the development of therapies for cardio- and cerebrovascular diseases and Alzheimer's dementia (Scheme 1).[6]



Scheme 1. Structures of xyloketal F (**1**) and xyloketal B (**2**).

The fungal strain #2508, isolated from seeds of the mangrove tree *Avicennia marina* in Hong Kong, was identified as an undescribed member of the genus *Xylaria.* The strain was cultured on 300-liter scale; the culture was concentrated, and extracted with ethyl acetate. Chromatography of the crude extract led to the isolation of xyloketal F (**1**) as a colorless crystalline solid (yield: 0.2 mg L^{-1}), $[\alpha]_D^{25} = -50.6$. The complete structural assignment of **1** was accomplished by spectral analysis and by a single-crystal X-ray diffraction study. High-resolution mass spectral analysis of **1** provided the molecular formula $C_{41}H_{52}O_{10}$. Similar to xyloketal B (**2**),[5] the ^1H NMR signals of **1** appeared in pairs, but there were two phenolic hydroxyl protons ($\delta_H = 6.14$), one CH_2 ($\delta_C = 17.2$ and $\delta_H = 3.72$), and no aromatic proton in the NMR spectra of **1**. The molecular weight of **1** (704 amu) was more than twice that of xyloketal B. That fact, together with a comprehensive analysis of 2D NMR spectroscopic data (COSY, HMQC, and HMBC experiments), enabled elucidation of the complete planar structure of xyloketal F as a dimeric form of xyloketal B, linked by a methylene

[a] Department of Applied Chemistry and Department of Pharmacy, Zhongshan University,
Guangzhou, P. R. China
E-mail: ceslyc@zsu.edu.cn
[b] Department of Applied Biology and Chemical Technology, Polytechnic University of Hong Kong,
Hong Kong
E-mail: bcwlchan@polyu.edu.hk
[c] Organic Chemistry Department, University of Debrecen,
4032 Debrecen, Hungary
[d] Department of Chemistry, University of Paderborn,
33098 Paderborn, Germany

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group. The correlation signals of the COSY and the HMBC spectrum of **1** were similar to those of Xyloketal B. In the HMBC spectrum, the correlations between CH_2-14 and C-9′, C-12 and C-13, respectively, located the position of CH_2-14. The relative stereochemistry and confirmation of the overall structure of xyloketal F were achieved by single-crystal X-ray diffraction analysis.[5] The four furan rings are in *cis* configurations. The two fragments attached to C-14 in **1** are in a *trans* array, and there are two hydrogen bonds, which are formed from the phenolic hydroxyl groups and oxygen atoms of the pyran rings. These hydrogen bonds appear to increase the stability of this conformation (Figure 1).

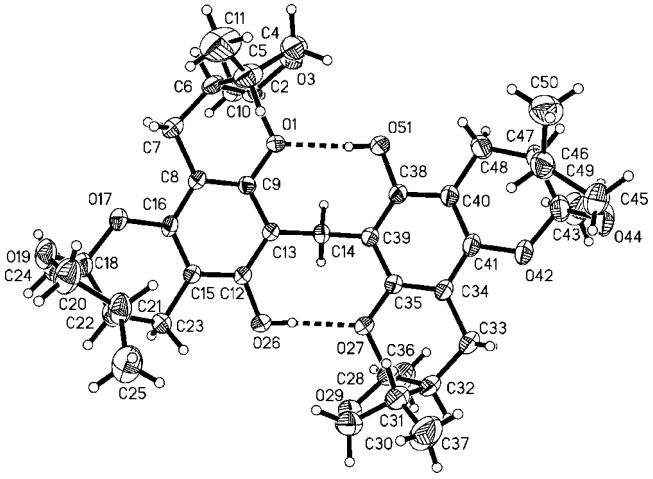

Figure 1. ORTEP plot of the X-ray structure of xyloketal F (**1**).

The absolute stereochemistry of **1** was not defined in the X-ray structure. However, in our previous communication[4] we solved the problem of absolute configuration of xyloketals A and D by quantum-mechanical calculation of the CD spectra and comparison with experimental values. The (*R*) configuration of xyloketal D was later confirmed by enantioselective synthesis.[7] Similar to xyloketals A and D,[5] the relative configuration of the new xyloketal F was established by X-ray analysis (see Figure 1). Calculation of the conformational minima showed the molecule to be quite rigid and conformationally fixed by strong hydrogen bonding. In addition, the calculated minimum conformation was virtually identical to that deduced from the X-ray analysis. Thus, the new xyloketal F was also a suitable compound for the method of quantum-mechanical calculation of CD spectra (for a review see ref.[8]).

Figure 2 shows the calculated spectrum taken from the X-ray data for the arbitrarily assumed (*R*) configuration (triangles) and the experimental spectrum (circles). The CD spectrum was similar to the previously reported spectra of xyloketals A and D.[5] In spite of less pronounced peaks in the 250 and 270 nm area, the good match of the strong and characteristic Cotton effect around 220 nm leaves no doubt that the new xyloketal F also has *all*-(*R*) configuration as shown in Figure 1 (X-ray) and the drawing in Scheme 1.

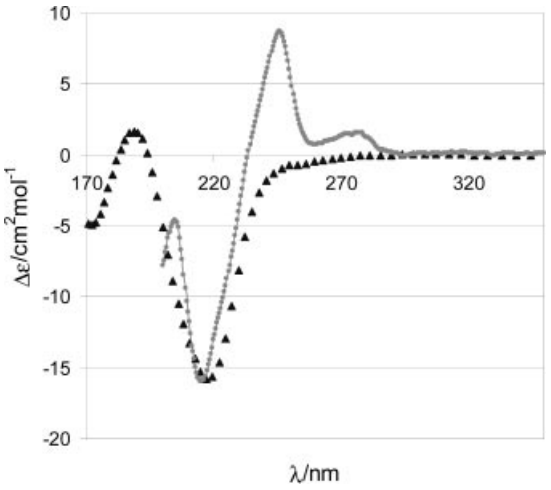

Figure 2. Experimental CD spectrum (circles) and calculated spectrum (triangles) of xyloketal F (**1**).

To demonstrate the relationship of xyloketal F (**1**) with xyloketal B (**2**), we successfully converted **2** to **1**, in 80% yield, in the presence of polyformaldehyde in analogy to the Lederer–Manasse reaction.[9] The optical rotation and NMR spectra of the synthetic product are identical with the data for xyloketal F (**1**). In the primary patch clamp biotest (using primary cultured hippocampal cells of new born rat and whole cell recording patch clamp technique), xyloketal F, xyloketal A, and xyloketal B showed the ability to block the L-calcium channel. Using the same concentration throughout (0.03 μmol/L), the inhibition rates were 50.33%, 21.47%, and 12.05%, respectively. Xyloketal F (**1**) was the most potent compound. Further studies on the bioactivity of xyloketal F (**1**) are in progress.

The isolation of xyloketal F strongly suggests that its biosynthesis in the fungus occurs by the reaction of two molecules of **2** and one molecule of formaldehyde (or a biological equivalent). It is becoming more and more evident that there is a formaldehyde biosynthetic system in nature. There is an endogenous formaldehyde level in humans and other animals, also in diverse plants (fruit),[10] but no evidence has been reported that fungi have the capacity to undertake reactions of this type. The effect of xyloketals inhibiting the L-calcium channel of hippocampal cells[6] suggests that these types of compounds, especially **1**, may be useful in restoring nerve damage and the treatment of Alzheimer's dementia.

Experimental Section

General Remarks: NMR spectroscopic data were recorded on a Varian Inova 500NB NMR spectrometer with tetramethyl silane as internal standard. Mass spectra were measured with a VG-ZAB-HS and a VG Autospec-500 mass spectrometer, IR spectra with a Bruker EQUINOX 55, UV spectra with a Shimadzu UV-2501PC spectrophotometer, and optical rotations with a Horiba High Sensitivity Polarimeter SEPA-300. The X-ray data were generated on a Bruker Smart 1000 CCD system diffractometer. CD spectra were recorded on a Jasco J-715 spectropolarimeter at room temperature with acetonitrile as the solvent.

Fungal Strain and Culture Conditions: A strain of the fungus *Xylaria* sp. (#2508) was isolated from seeds of the mangrove tree *Avicennia marina* in Hong Kong, and was stored at the Department of Applied Chemistry, Zhongshan University, Guangzhou, China. Starter cultures (from Professor E. B. G. Jones and Dr. L. L. P. Vrijmoed) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 250-mL Erlenmeyer flask containing 100 mL of liquid medium (glucose 10 g/L, peptone 2 g/L, yeast extract 1 g/L, NaCl 30 g/L). The flask was incubated at 30 °C on a rotary shaker for 5–7 days. The mycelium was aseptically transferred to a 300 liter fermenter containing 170 L of GYT medium, and cultivated at 30 °C for 80 h. The culture (170 L) was filtered and the filtrate was concentrated below 50 °C to 3.5 L and extracted five times by shaking with an equal volume of ethyl acetate.

Purification of Xyloketal F: The combined extracts were chromatographed on silica gel using a gradient elution from petroleum ether to ethyl acetate to obtain **1** from the 30% fraction and recrystallized to provide 30 mg of **1** as colorless needles (50% ethyl acetate/petroleum), m.p. 160–162 °C. $[\alpha]_D^{25}$ –50.6 (c = 0.2, CH_3OH). IR (KBr): $\tilde{\nu}$ = 3351, 2955, 2929, 1615, 1460, 1381, 1340, 1311, 1207, 1115, 1076, 1004, 869 cm^{-1}. UV/Vis (CH_3OH): λ_{max} (ε) = 226 (15411), 273 nm (2475). CD spectrum: see Figure 2. ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$, TMS): see Table 1. FABMS (70eV) 705 (M + 1). HRMS (EI) calcd. for [$C_{41}H_{52}O_{10}$ + Na]: m/z = 727.3458, found 727.3279.

Crystal Data for 1:[11] $C_{41}H_{52}O_{10}$, orthorhombic, $P2(1)2(1)2(1)$, a = 11.225(6) Å, α = 90°, b = 17.437(9) Å, β = 90°, c = 20.327(11) Å, c = 90°, Volume = 3979(4) $Å^3$, Z = 4, $\rho_{calcd.}$ = 1.177 Mg/m^3, μ = 0.083 mm^{-1}, reflections collected/unique 23646/8603 [R(int) = 0.0287], θ range for data collection from 1.54 to 27.05°. All single-crystal data were collected using the hemisphere technique on a Bruker SMART 1000 CCD system diffractometer with graphite-monochromated Mo-K_α, radiation λ = 0.7l073 Å at 293(2) K. The structures were solved by direct methods using SHELXTL V5.0 (Siemens Industrial Automation Inc., Madison, WI) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. Absorption corrections were applied with the Siemens Area Detector Absorption program (SADABS). The final value of R was 0.0332, wR_2 = 0.0903 [$I > 2\sigma(I)$].

Preparation of Xyloketal F from Xyloketal B: To a solution of xyloketal B (**2**) (4 mg) in THF (1 mL) was added paraformadehyde (3 mg) and 2 N HCl (0.05 mL). The mixture was stirred for 24 h at 20 °C, then neutralized by addition of K_2CO_3 solution, and extracted with ethyl acetate (2 mL). The solvent was removed in vacuo and the residue chromatographed on a column of silica gel (5 g, Ø 1 × 30 cm), eluted by 30% ethyl acetate/petroleum ether, to afford xyloketal F (**1**) (3 mg). The optical rotation and NMR spectra of the synthetic product were identical to those of xyloketal F (**1**).

L-Calcium Channel Inhibition Assay: Primary cultured hippocampal cells of newborn rats were used in the whole cell recording patch clamp technique, utilizing the CEZ-2300 patch clamp amplifier (Nihon Kohden). The data acquisition and analysis were carried out with the pClamp 6.0 software package (Axon Instruments, Foster City, CA). Patch pipettes were made from a borosilicate glass tube in a micropipette puller (MPT-1, Shimadzu, Kyoto, Japan), of which the inner diameter of the top was 1 μm. Pipette resistances ranged from 5 to 8 megaohms when filled with a solution (pH 7.35, 125 mM K-aspartate, 20 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 10 mM EGTA). Applying the holding potential of –40 mV, command potential of –70 to 10 mV, and the potential step of 10 mV, the effects of xyloketal F, A, and B on the L-calcium current of hippocampal cells were determined. In the same concentration (0.03 μmol/L), the inhibiting rate of xyloketal F, A, and B were 50.33%, 21.47%, and 12.05%, respectively.

Table 1. NMR spectroscopic data for xyloketal F (**1**).

C no.	δ_C	δ_H, mult., J [Hz]	HMBC correlations	H-HCOSY correlations
2	107.5 C			
4	74.0 CH_2	a. 4.14 (t, 8)	C-2, 6, 11	a. H-4b, 5
		b. 3.50 (t, 8)		b. H-4a, 5
5	35.2 CH	2.13 (m)	C-4, 7	H-4, 6, 11
6	47.5 CH	1.87 (dd, 7, 11)	C-2, 5, 8, 11	H-5, 7
7	19.2 CH_2	a. 2.86 (d,18)	C-2, 5, 6, 8, 9	a. H-6, 7b
		b. 2.72 (dd, 18, 7)		b. H-6, 7a
8	100.1 C			
9	150.2 C			
10	22.9 CH_3	1.48 (s)	C-2, 6	
11	15.9 CH_3	1.02 (d, 6.5)	C-4, 5, 6	H-5
12	152.5 C			
13	106.2 C			
14	17.2 CH_2	3.72 (s)	C-9′, 12, 13	
2′	109.3 C			
4′	74.5 CH_2	a. 4.30 (t, 6)	C-2′, 6′, 11′	a. H-4′b, 5′
		b. 3.61 (t, 8)		b. H-4′a, 5′
5′	35.3 CH	2.13 (m)	C-4′, 7′	H-4′, 6′, 11′
6′	47.5 CH	1.91(dd, 7, 11)	C-2′, 5′, 8′, 11′	H-5′, 7′
7′	19.2 CH_2	a. 2.84 (d,18)	C-2′, 5′, 6′, 8′, 9′	a. H-6′, 7′b
		b. 2.60 (dd, 7, 18)		b. H-6′, 7′a
8′	98.1 C			
9′	148.1 C			
10′	22.3 CH_3	1.58 (s)	C-2′, 6′	
11′	15.8 CH_3	1.04 (d, 6.5)	C-4′, 5′, 6′	H-5′
OH		8.40 (s)		

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[11] CCDC-270810 for xyloketal F contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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