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Yurinelide, a Novel 3-Benzylidene-1,4-benzodioxin-2(3*H*)-one Phytoalexin from *Lilium maximowiczii*[1]

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Key Words: yurinelide, phytoalexin, antifungal compound, *Lilium maximowiczii*.

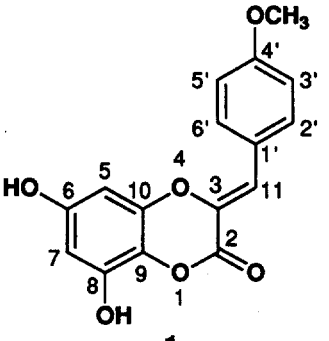
Abstract: *Infected lily bulbs afforded the first natural 3-benzylidene-1,4-benzodioxin-2(3H)-one derivative, yurinelide **1**. The structure was elucidated by interpretation of the spectral data and chemical reactions.*

Basal rot caused by *Fusarium oxysporum* has been a serious disease of *Lilium maximowiczii*.[2] In a continuation of our studies on vegetable phytoalexins[1] we examined *Lilium maximowiczii*, whose bulbs (Japanese name:Yurine) have been used in Japanese dishes. Diseased bulbs of cultivated *Lilium maximowiczii* cv. Hakugin (2.6 kg) were collected and homogenized in ethyl acetate. Fractionation of the ethyl acetate extract by column chromatography on silica gel (EtOAc), Sephadex LH-20 (MeOH),[3] and silica gel (benzene/EtOAc, 4:1) guided by TLC bioassay using *Bipolaris leersiae*,[4] afforded a main antifungal compound **1** (22 mg) which was named yurinelide. This was also induced in UV-irradiated or *Fusarium oxysporum*-inoculated bulb scales but not detected in untreated control tissue. Therefore, the compound **1** could be regarded as a phytoalexin of *Lilium maximowiczii*. We report herein the structural elucidation of the first lily phytoalexin, yurinelide **1**.

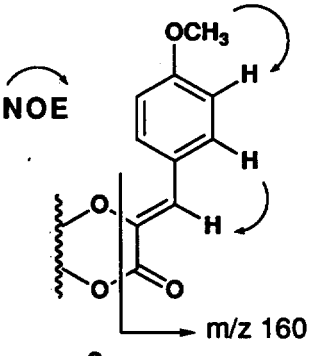
The high resolution mass spectrum of the compound **1**, mp 169-171 °C, gave a molecular ion at m/z 300.0630, consistent with a molecular formula $C_{16}H_{12}O_6$. The infrared spectrum (KBr) indicated the presence of hydroxyl (3284 cm^{-1}) and carbonyl (1732 cm^{-1}) groups. The ^1H NMR spectrum (acetone-d_6) was simple and showed the presence of one 1,4-disubstituted (δ 6.94, 2H, d, J = 8.3 Hz; 7.73, 2H, d, J = 8.3 Hz) and one 1, 2, 3, 5-tetrasubstituted (δ 6.10, 1H, d, J = 2.5 Hz; 6.21, 1H, d, J = 2.5 Hz) benzene rings, one methoxyl (δ 3.84) and two phenolic hydroxyl (δ 8.46 and 8.80, each s, D_2O exchangeable) groups, and a conjugated olefinic proton at δ 6.99 (s), accounting for all the 12 protons in **1**. The ^{13}C NMR spectrum (Table 1) showed 13 sp^2 (two are degenerated) and one methoxyl carbon signals, indicating that **1** is tricyclic. HMQC, HMBC, and NOE experiments allowed the construction of the following partial structure **2**.
Important long range correlations were as follows: the methoxyl exhibits coupling to C4' ; H2' correlates to C4', C6', and C11; the proton signal at δ 6.99 (H11) correlates to C2', C2 lactone carbonyl, and C3 oxygen-

bearing sp^2 carbon atoms. The mass fragment at m/z 160.0513 ($C_{10}H_8O_2$)[5], assignable to a retro Diels-Alder fission product of the lactone ring, also support the partial structure **2**.

The positions of the two *meta* hydroxyls (C5, C7 or C6, C8) and the stereochemistry at the C3-C11 double bond are ambiguous at this stage. The former was determined by examining the methanolysis product of **1**. While the compound **1** is stable in acetonitrile, the methanolic solution turned pink rapidly, indicating that the methanolysis product should be unstable. The expected methanolysis product was trapped by diazomethane as a stable methyl ether **3** in 80% yield. The ^1H NMR spectrum[6] of **3** showed five methoxyl and two *meta* coupled aromatic proton signals with independent chemical shifts, eliminating the other possible structure (two hydroxyls at C5 and C7). The instability of the methanolysis product can be attributed to the presence of labile hydroquinone structure.[7]



1



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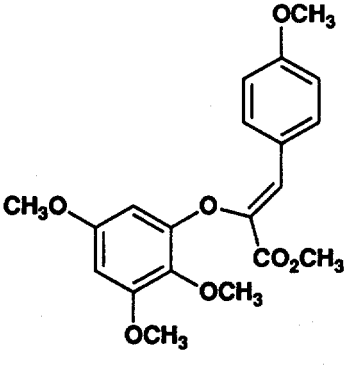

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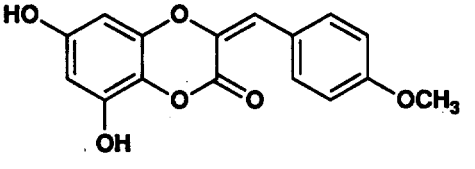

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The *Z* geometry of the C3-C11 double bond of **1** was deduced by comparing the spectral data of photoisomerization product **4** with those of **1**. Irradiation of **1** in acetone with a medium pressure Hg lamp gave an *E* isomer **4**. The UV spectrum (MeCN) of **4** showed hypsochromic shift of the absorption maximum with remarkable decrease in intensity (λ max 333 nm, ε 1710, cf. 337 nm, ε 10400 in **1**), indicating diminished coplanarity of the methoxyphenyl group in the conjugated system of **4**. The proton H2' in the ^1H NMR spectrum (acetone-d_6)[8] of **4** showed lower chemical shift (δ 7.92) as compared with that of **1** (δ 7.73). The whole structure of yurinelide is, therefore, represented by the formula **1**.

Structurally, yurinelide is rather simple. However, as far as we know, this is the first natural product of 3-benzylidene-1,4-benzodioxin-2(3*H*)-one structure. Biogenetically, **1** would be derived from chalcone or chalcone epoxide *via* a Baeyer-Villiger-type[9] reaction.

Yurinelide **1** inhibited completely the conidial germination of *Bipolaris leersiae* at a concentration of 100 ppm while the isomer **4** showed less antifungal activity even at 400 ppm. Hydrogenation product **5**[10] did not inhibit the germination at 400 ppm.

Table 1: NMR assignments for Yurinelide 1[a]

Atom no.	^{1}H NMR (mult., J (Hz))[b]	^{13}C[c]	LR ^{1}H to ^{13}C corr.
2		156.7 (s)	
3		135.6 (s)	
5	6.21[d] (d, 2.5)	99.8 (d)[d]	C6, C7, C9, C10
6		155.9 (s)	
7	6.10[e] (d, 2.5)	95.1 (d)[e]	C5, C6, C8, C9
8		144.1 (s)[d]	
9		123.8 (s)	
10		147.2 (s)[e]	
11	6.99 (s)	127.1 (d)	C2, C3, C2'
1'		125.8 (s)	
2'	7.73 (d, 8.3)	133.7 (d)	C11, C4', C6'
3'	6.94 (d, 8.3)	114.7 (d)	C1', C4', C5'
4'		161.8 (s)	
OCH_3	3.84 (s)	56.1 (q)	C4'

[a]All data were recorded in acetone-d_6; [b]Measured at 400 MHz;
[c]Measured at 100 MHz; [d,e]Indicated assignments may be reversed.

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5. Additional mass fragments of **1**: MS(m/z) 300 (M^+, 100), 257 (43), 202 (30), 160 (54), 148 (57), and 117 (27).

6. Additional data of **3**: MS(m/z) 374 (M^+, 93); UV (MeOH) 299 nm (ε 3880); IR ($CHCl_3$) 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.70, 3.73, 3.82, 3.86, and 3.87 (each 3H, s), 6.17 (1H, d, J = 2.9 Hz), 6.26 (1H, d, J = 2.9 Hz), 6.68 (1H, s), 6.87 (2H, d, J = 8.8 Hz), and 7.39 (2H, d, J = 8.8. Hz).
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8. Additional data of **4**: ^1H NMR (acetone-d_6) δ 3.82 (3H, s), 6.25 (1H, d, J = 3 Hz), 6.30 (1H, d, J = 3 Hz), 7.02 (1H, s), 7.04 (2H, d, J = 9 Hz), and 7.92 (2H, d, J = 9 Hz).
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10. The compound **5** was obtained by catalytic hydrogenation of **1** (10% Pd-C, acetone). Spectral data of **5**: MS(m/z) 302 (M^+, 11), 274 (9), 153 (100), 134 (25), and 121 (79); UV (MeCN) 223 (ε 16200), 277 (2660), and 281 (2490) nm; IR (KBr) 1760 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.13 (1H, dd, J = 14.7 and 8.3 Hz), 3.25 (1H, dd, J = 14.7 and 4.2 Hz), 3.78 (3H, s), 4.87 (1H, dd, J = 8.4 and 4.2 Hz), 6.00 (1H, d, J = 2.7 Hz), 6.18 (1H, d, J = 2.7 Hz), 6.87 (2H, d, J = 8.6 Hz), and 7.26 (2H, d, J = 8.6 Hz).

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