Derrisin, a New Rotenoid from *Derris malaccensis* Plain and Anti-*Helicobacter pylori* Activity of Its Related Constituents

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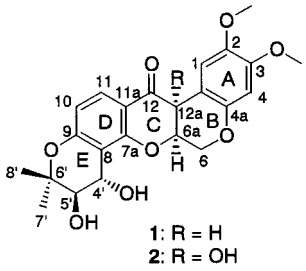
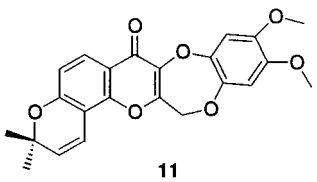
A new rotenoid, derrisin (**1**), together with 10 known rotenoids (**2**−**11**) were isolated from the roots of *Derris malaccensis* Plain. The structure of **1** was elucidated by spectroscopic analysis. Nine of the isolated rotenoids (**3**−**11**) showed antibacterial activity against *Helicobacter pylori*.

Infection with *Helicobacter pylori* is now recognized as the major cause of chronic active gastritis and peptic ulcer disease, and eradication of the infection will prevent recurrence of the majority of such ulcers.[1] Although some antibiotics have been effective in the clinic, their adverse effects and acquired resistance have been problematic; hence there is a need to develop more effective drugs.

A screening of Brazilian medicinal plants for anti-*H. pylori* actives resulted in the isolation of cabreuvin (**12**), an isoflavone from *Myroxylon peruiferum* (Leguminosae), which exhibited selective activity against *H. pylori* with a minimum inhibitory concentration (MIC) of 7.8 μg/mL.[2] To find more effective compounds, we screened several related isoflavonoids for activity. We found rotenone (**3**) is more active (MIC 1.3 μg/mL) against *H. pylori* but does not affect other microorganisms. Thus, we regarded rotenone as the second lead compound and focused our screening targets on related rotenoids. Rotenoids are known to occur in various plants belonging to the family Leguminosae. In this paper, we describe the isolation and structure elucidation of a new rotenoid, derrisin (**1**), from the roots of *Derris malaccensis* Plain (Leguminosae), as well as the anti-*Helicobacter pylori* activity of **1** and 10 known rotenoids (**2**−**11**) obtained from the same plant.





The dried powdered roots of *D. malaccensis* were extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble materials exhibited significant inhibitory activity (IC_{50} < 1 μg/mL) against *H. pylori*. Activity-guided fractionations of the EtOAc-soluble fraction resulted in isolation of a new rotenoid, derrisin (**1**), together with 10 known rotenoids, **2**,[3] rotenone (**3**),[4] rotenolone (**4**),[4] dehydrorotenone (**5**),[5] deguelin (**6**),[5] tephrosin (**7**),[5] toxicarol (**8**),[5] dehydrodeguelin (**9**),[5] elliptone (**10**),[5] and **11**.[6] The structures of **2**−**11** were established on the basis of spectral analyses and by comparison with reported data.

Table 1. ^1H and ^{13}C NMR Data (δ, ppm) for Derrisin (**1**) in Acetone-d_6

position	^1H	^{13}C
1	6.77 s (1H)	112.7
1a		106.3
2		144.9
3		151.1
4	6.45 s (1H)	112.7
4a		149.1
6	4.27 d (*J* = 11.9 Hz, 1H) 4.75 m (1H)	67.0
6a	5.20 m (1H)	73.8
7a		162.6
8		113.2
9		160.4
10	6.47 d (*J* = 8.8 Hz, 1H)	102.3
11	7.73 d (*J* = 8.8 Hz, 1H)	128.5
11a		112.5
12		189.6
12a	3.91 d (*J* = 4.4 Hz, 1H)	44.8
4′	4.75 d (*J* = 13.5 Hz,[a] 1H)	66.0
5′	3.73 d (*J* = 13.5 Hz,[a] 1H)	74.3
6′		79.5
7′	1.42 s (3H)	24.9
8′	1.33 s (3H)	23.3
2-OMe	3.65 s (3H)	56.9
3-OMe	3.75 s (3H)	56.0

[a] From $^1H-^1H$ COSY cross-peaks.

The molecular formula, $C_{23}H_{24}O_8$, of derrisin (**1**) was established by HRFABMS [*m*/*z* 428.1494 (M^+), $C_{23}H_{24}O_8$, Δ +2.3 mmu]. The structure of **1** was deduced from analysis of the ^1H and ^{13}C NMR data (Table 1) aided with 2D NMR measurements ($^1H-^1H$ COSY, HMQC, and HMBC). These data indicated the presence of one carbonyl, 12 aromatic carbons (of which five are linked to oxygen atoms and three are quaternary carbons), two methoxy groups, two methyl groups, one oxygenated methylene, four methines (three of which are oxygenated methines), and one oxygenated quaternary carbon. Since nine (two aromatic rings and one ketone) of 12 unsaturations were accounted for, it was concluded that **1** contained three more rings in the molecule. The $^1H-^1H$ COSY spectrum revealed connectivities of C-6a to C-12a and C-6, C-10 to C-11, and C-4′ to C-5′. The HMBC correlations of H-1 to C-12a, H-6 to C-4a, C-12a, and C-6a, H-6a to C-1a, H-12a to C-12 (δc 189.6), H-11 to C-12, H-4′ to C-9 and C-6′, and H-5′ to C-8 and

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Table 2. Anti-*Helicobacter pylori* Activity of Compounds **1**−**12**

compound	MIC (mg/mL)
derrisin (**1**)	85.0
2	>88.0
rotenone (**3**)	1.3
rotenolone (**4**)	1.3
dehydrorotenone (**5**)	9.8
deguelin (**6**)	0.6
tephlosin (**7**)	0.3
toxicarol (**8**)	0.3
dehydrodeguelin (**9**)	4.0
elliptone (**10**)	3.0
11	4.0
clarithromycin	0.05

C-4′ revealed the presence of a rotenoid skeleton. Furthermore, two methoxy groups were elucidated to be attached to C-2 and C-3 (ring A), respectively, and the two methyl groups were attached to C-6′, judging from the presence of cross-peaks from H-4′ to C-6′, H-7′ to C-6′ and C-8′, and H-8′ to C-6′ and C-7′ in HMBC spectrum (ring E). The related compound **2** was also isolated from the same plant and has been previously reported as a constituent of the roots of *Lonchocarpus utilis* and *L. urucu*.[3] In the previous report, all the carbon and proton signals of the ring E of **2** were assigned and the absolute stereochemistry of **2** was determined. All the carbon signals of **1** are similar to those of **2** except for the signal at C-12a: δ 68.3 (s) for **2** and δ 44.8 (d) for **1**. In place of the ^{1}H NMR signal at δ 5.52 (OH-12a) for **2**, a doublet signal at δ 3.91 (H-12a) appears for **1**. From these results, the gross structure of derrisin was elucidated to be **1**. NOESY correlations of H_3-8′ to H-4′, and H_3-7′ to H-5′, in ring E indicated a *trans* stereochemistry of 4′,5′-diol. The *trans* stereochemistry was also confirmed by a large diaxial-like coupling (13.5 Hz) to protons identified as H-4′ and H-5′. A *cis* relationship between rings B and C was elucidated by NOESY correlation of H-6a to H-12a. Thus the relative stereochemistry of derrisin was assigned as shown in **1**.

In 1998, Fang and Casida[7] reported the correlation of inhibition of NADH: ubiquinone oxidoreductase and induced ornithine decarboxylase activities in rotenoid constituents. The source of the rotenoids examined in this study was cube resin (*Lonchocarpus utilis* and *L. urucu*). It was reported that the methanolic extract of the cube resin was fractionated using the bioassays to guide fractionation and led to the isolation of 29 rotenoid compounds (12 of which were new). One of these reported compounds included the *trans*-4′,5′-dihydro-4′β,5′α-dihydroxy analogue of deguelin, which corresponds to the structure proposed for derrisin (**1**) reported herein. However, the structure of this compound was incorrectly reported in that paper.[8] The correct structure, reported in a subsequent paper,[3] is the *trans*-4′,5′-dihydro-12aβ-4′β,5′α-trihydroxydeguelin (compound **2** reported herein). Therefore, the data presented in the current manuscript represents the first report of derrisin (**1**) as a new rotenoid compound.

The antibacterial activities against *H. pylori* of compounds **1**−**11** are shown in Table 2. The rotenoids (**6**−**8**) with a 4′,5′-unsaturated six-membered ring E were 2−4 times more active than the corresponding rotenoids (**3** and **4**) with a five-membered ring E. The rotenoids (**5**, **9**, and **11**) with an olefin at 6a and 12a showed less activity than the rotenoids (**3**, **4**, and **6**−**8**) with sp^3 methines at the corresponding positions. The rotenoids (**1**, **2**, and **10**) with a modified ring E were also less active. In particular, the rotenoids with a 4′,5′-dihydoxy ring E (**1** and **2**) were inactive. These results indicate that the six-membered hydrophobic ring E together with the 6a and 12a saturated carbons may be important for activity.

Their antimicrobial activity was also tested against other microorganisms: Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 9341, and *Bacteriodes fragilis* ATCC 25285), Gram-negative bacteria (*Escherichia coli* ATCC 10536 and *Pseudomonas aeruginosa* ATCC 9027), and fungi (*Candida albicans* ATCC 10231). They were inactive against all of these organisms, even at a concentration of 625 μg/mL.

Rotenoids are known for their insecticide and piscicide activities. Their toxicity is caused by the inhibition of NADH oxidation in the respiratory chain.[9] The selective anti-*H. pylori* activity of the rotenoids examined might also relate to the inhibition of NADH oxidation. An investigation to examine the effects of rotenoids to the respiration of *H. pylori* is now being undertaken.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX500 spectrometer using tetramethylsilane as the internal standard. HRFABMS was measured on a JEOL MS-700 spectrometer. Optical rotation was recorded on a Jasco DIP-370 digital polarimeter. UV and IR spectra were measured on a Shimadzu UV-260 and JASCO FTIR-5300 spectrometer, respectively.

Plant Materials. The roots of *Derris malaccensis* were cultivated in Kwanxi Prefecture, China. The plant was identified by Dr. K. Yoneda (Osaka University), and the voucher specimen has been deposited at the Graduate School of Pharmaceutical Sciences, Osaka University.

Extraction and Isolation. The dried powdered roots of *D. malaccensis* (25.23 g) were extracted with MeOH. The MeOH extract (2.21 g) was partitioned with EtOAc and H_2O. The EtOAc solubles (0.86 g) were subjected to reversed-phase column chromatography (MCI Gel ODS IMY, Mitsubishi Chemical Corp., H_2O−CH_3CN) to give six fractions. Further purification of each fraction was achieved by C_{18} HPLC (Capcell pak C18 UG 80, Shiseido, H_2O−CH_3CN) or polymer C_{18} column (YMC-Pack polymer C18, YMC, H_2O−CH_3CN) to afford derrisin (**1**, 1.4 mg) and compounds **2**−**11**.

Derrisin (1): colorless amorphous solid; $[\alpha]^{23}_D$ −100° (*c* 0.01, $CHCl_3$); ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m*/*z* 428.1494 (calcd for $C_{23}H_{24}O_8$, 428.1471); IR (KBr) 2928, 1605, 1512, 1449, 1385, 1262, 1200, 1096, 1030 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 233 (15 100), 240 (sh, 11 100), 284 (11 500), 315 (sh, 6900) nm.

***H. pylori* and Culture Medium.** *H. pylori* 31 A strain derived from stomach ulcer was kindly provided by Dr. Takeshi Itoh (Tokyo Metropolitan Research Laboratory of Public Health). *H. pylori* was grown in test tubes in a liquid medium (5 mL) containing brain-heart infusion broth (Difco, Detroit, MI) with 10% fetal bovine serum (Upstate Biotechnology Inc., Lake Placid, NY) in an atmosphere of 10% CO_2, 5% O_2, and 85% N_2 at 37 °C for 48 h.

Determination of MIC against *H. pylori*. The assay for activity against *H. pylori* was performed using the method previously reported.[2] Serial doubling dilutions of each rotenoid and clarithromycin were made in 10% aqueous dimethyl sulfoxide. The culture medium containing 5% of the culture of *H. pylori* and 10% of the solution of each compound was incubated at 37 °C for 48 h. The MIC was defined as the lowest concentration of compounds at which visible growth was inhibited.

Determination of MIC against Other Microorganisms. MICs were determined by a conventional well agar method.[2] The plates were incubated at 37 °C for 24 h.

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Supporting Information Available: Structures of compounds **3**−**10** and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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