Cytotoxic and Novel Compounds from *Solanum indicum*

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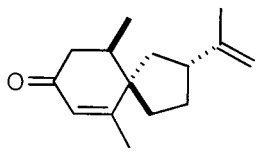
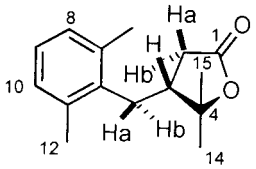
Solavetivone (**1**), cytotoxic to OVCAR-3 cells with an IC_{50} value of 0.1 mM, has been isolated from *Solanum indicum*. In addition, a novel solafuranone (**2**) and three known compounds, scopoletin, *N*-(*p-trans*-coumaroyl)tyramine, and *N-trans*-feruloyltyramine, were isolated for the first time from this plant. The structures of the above compounds were established by means of spectroscopic and X-ray analyses.

The Chinese folk medicine *Solanum indicum* (Solanaceae), an indigenous plant grown widely in Taiwan, has the property of jie du (toxin eliminating) and is used as a treatment for swelling.[1] It has been widely used in folk medicine as an analgesic for toothache, rhinitis, and breast cancer.[2] Previously several steroidal derivatives[3−10] have been isolated from this plant and some of these components have been studied for their antitumor activities.[8−10]

In our continuing search for antitumor constituents from plants,[11,12] the dried *S. indicum* was extracted with warm EtOH. Bioassay-directed fractionation led to isolation of a bioactive compound **1**, a novel solafuranone (**2**), and three additional known compounds. The known compounds **1** and **3**−**5** were identified as solavetivone,[13] scopoletine,[14,15] *N*-(*p-trans*-coumaroyl)tyramine,[16] and *N-trans*-feruloyltyramine,[17] respectively, by their spectral data. This is the first reported isolation of these compounds from this plant. Herein, we also report the cytotoxicity of **1** against OVCAR-3 cells and the structure determination of compound **2** using spectroscopic and X-ray methods.



1



2

Compound **2** was obtained as colorless plates from EtOAc/hexane, mp 132−133 °C. The HREIMS results corresponded to the molecular formula $C_{15}H_{20}O_2$. The EIMS of **2** gave a molecular ion peak at *m/z* 232 and the base peak at *m/z* 119, implying the loss of a lactone ring [M − $C_6H_9O_2$]. The ^{13}C NMR and DEPT spectra of **2** (Table 1) showed four quaternary (two aromatic carbons, one carbonyl carbon), three methine (two aromatic carbons), two methylene, and three methyl carbons (one aromatic carbon). The ^1H NMR data (Table 1) exhibited four methyl signals at δ 1.43, 1.55, 2.34 (two aromatic methyl groups), a methylene group at δ 2.21, 2.53, a benzylic methylene group at δ 2.78, 2.86, and three aromatic protons at δ 7.00. The stereochemistry of **2** was established by NOE difference experiments. When the protons at δ 1.55 (H-15) were irradiated, the protons at δ 2.86 (H-5a), 2.53 (H-3, H-2a), and 2.34 (H-12, H-13) showed NOE enhancements. In addition, when the protons at δ 1.43 (H-14) were irradiated, the signals at δ 2.86 (H-5a), 2.78 (H-5b), and 2.53 (H-3) showed NOE enhancements. Recrystallization of **2** gave crystals suitable for X-ray diffraction analysis, which established the molecular structure and relative stereochemistry as shown in Figure 1. Since compound **2** was optically active, $[\alpha]_D$ of +14.0°, the absolute configuration at C-4 was assigned as *R* by comparison with (+)-(*R*)-4-benzyl-2-furanone ($[\alpha]_D$ +8.79°).[18] Therefore, compound **2** was deduced to be (+)-(*R*)-5,5-dimethyl-4-(2,6-dimethylbenzyl)dihydrofuran-2-one, which has been given the trivial name solafuranone. Structure **2** contains a 2,6-dimethylbenzyl moiety, which occurs rarely in nature. Also, since **2** contains a carbon skeleton similar to solavetivone (**1**), it is possible that **1** may be biologically converted to **2** as shown in Scheme 1 in the Supporting Information.

Table 1. ^1H and ^{13}C NMR Assignments of Solafuranone (**2**) by DEPT and HMQC Experiments in Acetone-d_6[a]

position	δ_C	δ_H (*J*)[b]	DEPT
1	175.2		C
2	34.8	a 2.53 m	CH_2
		b 2.21 (13, 22)	
3	46.4	2.53 m	CH
4	86.7		C
5	29.1	a 2.86 (3, 14)	CH_2
		b 2.78 (11, 14)	
6	137.2		C
7	137.0		C
8	129.3	7.00 s	CH
9	127.0	7.00 s	CH
10	129.3	7.00 s	CH
11	137.0		C
12	20.4	2.34 s	CH_3
13	20.4	2.34 s	CH_3
14	21.7	1.43 s	CH_3
15	27.1	1.55 s	CH_3

[a] ^1H NMR (500 MHz), ^{13}C NMR (125 MHz), DEPT, and HMQC (J = 150 Hz) experiments were measured with a Varian Unity Inova 500 instrument. [b] J = coupling constants in Hz.

Compound **1** was optically active with a specific rotation $[\alpha]_D$ of −140°, and the stereochemistry of **1** was revealed as (−)-(2*R*,5*S*,10*R*)-2-isopropenyl-6,10-dimethylspiro[4.5]-dec-6-en-8-one by comparison with the previously reported synthetic solavetivone (lit.[13] $[\alpha]_D$ −135°). Solavetivone is a representative phytoalexin,[19] and its antibacterial activity has been studied.[20] Compound **1** was evaluated for its cytotoxicity on OVCAR-3, and it expressed IC_{50} at 0.1 mM.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-I3 micro-melting point ap-

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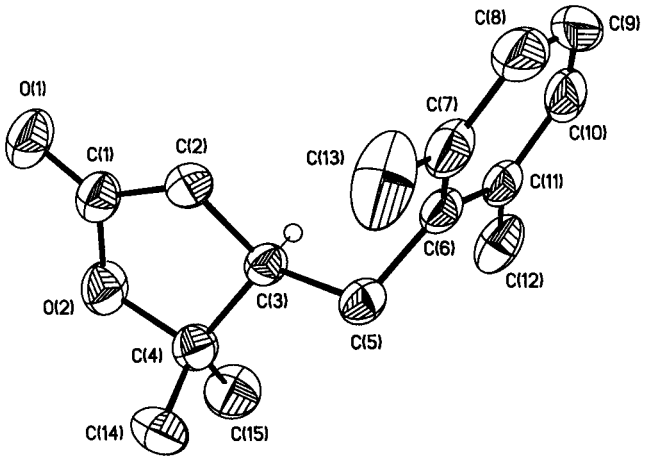

Figure 1. Perspective structure of **2** showing relative configuration. Hydrogen atoms have been omitted for clarity except at C(3).

paratus and are uncorrected. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a Nicolet AVATAR 320 FT-IR spectrometer. EIMS was measured with the direct insertion probe on a Finnigan GCQ GC/MS spectrometer at 70 eV. HREIMS data were taken on a JEOL JMS-HX 110 mass spectrometer. TLC analysis was carried out on precoated silica gel plates (Kieselgel 60 F_{254}, Merck Art. 5554). For column chromatography, silica gel (MN Kieselgel 60, 70−230 and 230−400 mesh) was employed.

Biological Assays. The procedures and conditions for the cytotoxicity assay were as previously described.[11]

Plant Material. The root of *S. indicum* was purchased from a Chinese herbal shop in Taipei in September, 1997. The material was identified by J.-C. Ou. A voucher specimen is retained in the National Research Institute of Chinese Medicine, Taipei.

Extraction and Isolation. Whole specimens of the air-dried plant (5 kg) were cut into small pieces and then extracted with EtOH (50 L) three times at 60 °C for 24 h. The ethanolic extracts were combined and concentrated in vacuo to 1 L. The concentrated extract was then suspended in water and then successively partitioned with hexane, $CHCl_3$, and *n*-butanol. The $CHCl_3$ layer was found to exhibit activity against the human ovarian cancer cell line OVCAR-3. The $CHCl_3$ fraction was then mixed with 200 g of silica gel (230−400 mesh). The air-dried mixture was subjected to a chromatographic column (4 × 100 cm, 70−230 mesh) and then eluted with hexane (3 L) followed by 10%, 20%, 40%, and 80% EtOAc/hexane and EtOAc (3 L each). Fractions (500 mL) were collected, and like fractions were combined to afford a total five pooled fractions.

The third fraction showed cytotoxicity to OVCAR-3 cells and was rechromatographed (2 × 100 cm, 70−230 mesh) using gradient elution. The solvent gradient used was 5%, 10%, 15%, and 20% EtOAc/hexane (1 L each). Fractions (200 mL per flask) were collected, and like fractions were combined and further chromatographed (1 × 100 cm, 230−400 mesh) using gradient solvents of 5%, 10%, 15%, and 20% EtOAc/hexane (20 mL each) to yield solavetivone **1** (22 mg) and a novel **2** (15 mg). Nonbioactive fractions were rechromatographed using gradient solvents of EtOAc and hexane to afford scopoletin (45 mg), *N*-(*p-trans*-coumaroyl)tyramine (27 mg), and *N-trans*-feruloyltyramine (33 mg).

Solafuranone (2): white solid (EtOAc/hexane); mp 132−133 °C; $[\alpha]^{25}_D$ +14.0° (*c* 1.0, CH_3CN); FT-IR (KBr) ν_{max} 3054, 3033, 2979, 2923, 1753, 1467, 1389, 1276, 1219, 1124, 936, 776 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (rel int) 232 (M^+, 12), 217 (3), 172 (31), 171 (13), 157 (16), 146 (8), 131 (30), 119 (100), 117 (33), 91 (18), 77 (7); HREIMS *m*/*z* 232.1461 (calcd for $C_{15}H_{20}O_2$, 232.1463).

X-ray Crystal Structure Analysis of Solafuranone (2).[21] A colorless crystal of **2** with dimensions 0.5 × 0.3 × 0.3 mm was selected for X-ray analysis. The crystallographic data were collected on a Siemens P4 diffractometer using graphite-monochromated Mo Kα radiation. Structure analysis was made by using the SHELXTL PLUS package. The compound crystallized in the space group $P2_12_12_1$, a = 7.443(10) Å, b = 11.455(10) Å, c = 16.021(2) Å, orthorhombic, V = 1365.9(3) Å^3, Z = 4, D_{calc} = 1.130 g/cm^3, λ = 0.71073 Å, μ(Mo Kα) = 0.073 mm^{-1}, $F(000)$ = 504, and T = 293 K. A total of 2470 reflections were collected in the range of 2.19° ≤ θ ≤ 24.00°, of which only 1826 unique reflections with $I > 2\sigma(I)$ were used for the analysis. The structure was solved using direct methods and refined by full-matrix least-squares on F^2 values. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were fixed at calculated positions and refined using a riding mode. The final indices were R_1 = 0.0525, wR_2 = 0.1616 with goodness-of-fit = 1.565. Scattering factors were taken from International Tables for X-ray Crystallography.[22]

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Supporting Information Available: Scheme 1 showing the possible biological conversion route to **2** from **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Crystallographic data for compound **2** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 160627). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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