Excelsione, a Depsidone from an Endophytic Fungus Isolated from the New Zealand Endemic Tree *Knightia excelsa*

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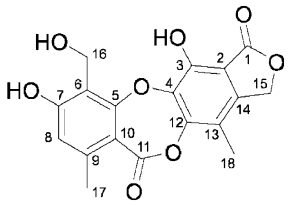
A new tetracyclic depsidone, excelsione (**1**), was isolated from the extract of an unidentified fungal endophyte obtained from the New Zealand endemic tree *Knightia excelsa*. The structure was elucidated by X-ray crystallography and NMR spectroscopy.

Endophytic fungi are a well-established source for structurally diverse and biologically active secondary metabolites.[1] In the course of our ongoing project targeted toward the isolation of novel natural products from New Zealand filamentous fungi,[2,3] we have investigated endophytes derived from plants endemic to New Zealand.[4]

A dark, pigmented fungus was isolated from the inner stem of *Knightia excelsa* R. Br. (Proteaceae), a tree also known as rewarewa or "New Zealand honeysuckle". The extract from a small-scale culture was cytotoxic against P388 cells (IC_{50} < 1 μg/mL). On the basis of this activity the fungus was grown on a larger scale for a more detailed chemical analysis. Unfortunately, the cytotoxic activity was not reproduced in the second culture, but one major metabolite, excelsione (**1**), crystallized from the extract. The structure was elucidated in part by NMR spectroscopy and confirmed by X-ray crystallography.



1

The ^{1}H NMR spectrum of **1** contained only five signals, all singlets: one aromatic proton (δ 6.79), two oxymethylene groups (δ 5.32 and 4.87), and two methyl groups (δ 2.46 and 2.22). The 18 resonances in the ^{13}C NMR spectrum together with the results from accurate mass measurement of the $[M + H]^+$ pseudomolecular ion in the positive mode HRESIMS suggested the molecular formula $C_{18}H_{14}O_8$. HMBC and ROESY data as well as the interpretation of ^{13}C chemical shifts (for NMR data see Table S1 in Supporting Information) made it possible to identify two substructures corresponding to the two substituted phenyl groups of **1**. Taking into account the molecular formula, these substructures had to be linked by either two ether or one ether and one ester bridge, leaving 12 potential structural possibilities, all with a central seven-membered ring. A single long-range correlation linking the substructures was observed but could not be used to unequivocally support one of the possible isomers. An alternative approach for the structural elucidation of **1** was thus sought.

By slow crystallization from THF, crystals suitable for X-ray diffraction analysis were obtained and the structure of excelsione was unequivocally established as **1**, with an ester linkage between C-11 and C-12 and a biaryl ether between C-5 and C-4 (Figure 1).

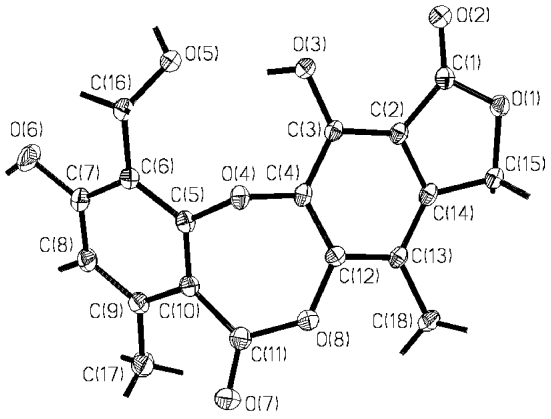


Figure 1. ORTEP diagram showing the structure of excelsione (**1**) in the crystal.

In the HMBC spectrum of excelsione (**1**) (see S5 in the Supporting Information) a remarkable number of $^nJ_{CH}$ couplings (n > 3) were observed and had been initially useful in defining the substructures. Normally H−H couplings cause significant attenuation on C−H correlation intensities, which is why $^nJ_{CH}$ correlations for n > 3 are very weak or nonobservable.[5] As excelsione (**1**) has no H−H couplings, the observation of longer range $^nJ_{CH}$ coupling becomes possible. The long-range coupling linking the substructures (H-15/C-5) was a $^7J_{CH}$ coupling, and a $^5J_{CH}$ coupling was also observed (H-15/C-4).

Structurally, excelsione (**1**) belongs to the depsidones, a group of secondary metabolites more commonly found in lichens than in fungi lacking an association with phytobionts. Related to **1** are the lichen compounds neotricone[6] and norstictic acid,[7] but the substitution pattern of the right-hand ring of **1** is unprecedented in natural products.

Excelsione (**1**) was inactive against P388 murine leukemia cells at 12.5 μg/mL and in disk agar diffusion assays against various bacterial and fungal strains using 40 μg of the compound per disk.

Experimental Section

General Experimental Procedures. UV spectra were measured on a GBC UV/vis 918 spectrophotometer. NMR spectra were recorded on a Varian INOVA AS-500 spectrometer (500 and 125 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 2.60 and δ_C 39.6 ppm for DMSO-d_6). For HRESIMS a Micromass LCT TOF mass spectrometer was used. Solvents for extraction and isolation were distilled prior to use. Antimicrobial and cytotoxic activities were assayed using standard protocols.[8]

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Fungus. The fungus was isolated from a surface-sterilized stem part of *Knightia excelsa* collected near Katikati in the Bay of Plenty, New Zealand. Cultures of the fungus were dematiaceous and sterile. Colonies superficially resembled *Phomopsis*, but the absence of sporulation prevented positive identification. A voucher culture of the fungus was deposited in the culture collection of the School of Biological Sciences, University of Canterbury (CANU E584). For chemical investigations, the fungus was cultivated for 21 days on 23 plates of malt yeast extract (MYE) agar at 26 °C.

Extraction and Isolation. The agar cultures were exhaustively extracted with EtOAc (3 × 800 mL). The solvent was then evaporated in vacuo and the residue partitioned between EtOAc (150 mL) and H_2O (50 mL). After concentrating the organic phase (∼10 mL) and leaving overnight at 4 °C, white needle-shaped crystals formed. These crystals were then separated by filtration and recrystallized from THF to give **1** (5.1 mg).

Excelsione (1): white needles; mp 195 °C (dec); IR (nujol) ν_{max} 3340, 3160, 1744, 1697, 1604, 1257, 1149, 1010, 779, 725 cm^{-1}; UV (acetone) λ_{max} (log ϵ) 213 (3.77), 283 (3.21), 326 (3.30); ^1H NMR (DMSO-d_6, 500 MHz) δ 6.79 (1H, s, 8-H), 5.32 (2H, s, 15-H), 4.87 (2H, s, 16-H), 2.46 (3H, s, 17-H), 2.22 (3H, s, 18-H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 168.2 (C, C-1), 162.1 (C, C-5), 161.2 (C, C-11), 159.9 (C, C-7), 148.2 (C, C-12), 147.2 (C, C-3), 144.9 (C, C-9), 144.8 (C, C-14), 138.8 (C, C-4), 115.9 (CH, C-8), 115.3 (C, C-6), 113.9 (C, C-13), 110.9 (C, C-10), 109.4 (C, C-2), 68.0 (CH_2, C-15), 52.3 (CH_2, C-16), 21.1 (CH_3, C-17), 11.0 (CH_3, C-18); for HMBC data, see Supporting Information; ESIMS (neg., %) *m*/*z* 715.3 (27) $[2M - H]^-$, 357.1 (100) $[M - H]^-$, 339.1 (57) $[M - H_2O - H]^-$; HRESIMS (pos.) *m*/*z* 359.0704 $[M + H]^+$ (calcd for $C_{18}H_{15}O_8$ 359.0767).

X-ray Structure Determination of 1. A plate crystal with dimensions 0.60 × 0.16 × 0.08 mm^3 was used for data collection. A Bruker-Nonius APEX II area detector system equipped with a nitrogen low-temperature gas-flow device was used to collect a full sphere of data with Mo Kα radiation. The data processing program SAINT[9] yielded 38 971 Bragg reflections, of which 14.8% were unique. The structure solution, refinement, and resulting table and diagrams were all produced using the SHELXTL suite of programs.[10,11] The final *R*-factor was 5.26%.

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Supporting Information Available: ^1H, ^{13}C, HSQC, HMBC, and ROESY NMR spectra as well as X-ray data tables for **1** are available free of charge via the Internet at http://pubs.acs.org. X-ray data are also available from the Cambridge Crystallographic Data Centre (http://www.ccdc.cam.ac.uk).

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