Cytotoxic and novel skeleton compounds from the heartwood of *Chamaecyparis obtusa* var. *formosana*

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Received 25 September 2006; revised 28 December 2006; accepted 4 January 2007

Available online 7 January 2007

Abstract—The novel skeleton compounds, chamaecypanone C (3) and obtunorlignan A (4) were isolated from the heartwood of *Chamaecyparis obtusa* var. *formosana*. The structure of 3 was elucidated as a dimer of monoterpene and norlignan with tricyclo[3.2.2.02.6]undecane and the structure of 4 was elucidated as a norlignan skeleton by spectroscopic methods. Compound 3 exhibits potent cytotoxic activity against several human cancer cells with IC50 values ranging from 0.19 to 0.52 μM, whereas 4 has no activity.

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The trunk of *Chamaecyparis obtusa* var. *formosana* Rehd. (Taiwan hinoki; Cupressaceae) is an important building material in Taiwan due to its decay-resistant characteristics. We have previously investigated the chemical components of the heartwood of this plant, and found various monoterpenes, sesquiterpenes, diterpenes and lignans.1–8 Two interesting compounds, bicyclo[2.2.2]octane skeleton diterpenes, obtunone (1)1 and 8,12-dihydroxydielmentha-5,9-diene-7,11-dione (2)1 were observed. The biosyntheses of 1 and 2 were proposed as the adducts from 1-hydroxymetha-3,5-dien-2-one with myrcene and itself, respectively, via bio-Diels–Alder reaction. Further detailed investigation of the same extraction from the heartwood has furnished two novel skeleton compounds, chamaecypanone C (3) and obtunorlignan A (4). The structural elucidation of these compounds are reported here.

The air-dried slices of heartwood of *C. obtusa* var. *formosana* were extracted with Me2CO at room temperature. After evaporation of Me2CO, the extract was partitioned with an EtOAc–water mixture to give an EtOAc-soluble fraction and an aqueous phase. The EtOAc-soluble fraction (680 g) was repeatedly chromatographed on SiO2 column and HPLC [Merck Lichrosorb Si 60, 250 × 10 mm i.d., EtOAc–CH2Cl2 (3:2)] to give chamaecypanone C (3) and obtunorlignan A (4).

Chamaecypanone C (3) was isolated as an amorphous solid with a positive optical rotation [α]23 D +175.7 (c 0.85, MeOH)] and UV λmax at 227, 282 and 302 nm. The positive-ion fast atom bombardment (FAB-MS) of 3 showed a quasi-molecular ion peak at m/z 431 (M+H)+, and the molecular formula C27H36O5 of 3 was resolved using high-resolution MS measurement.9

Keywords: *Chamaecyparis obtusa* var. *formosana*; Dimer of monoterpene and norlignan; Norlignan.
The IR (KBr) spectrum of 3 showed absorption bands at 3379, 1740, 1701, 1616 and 1517 cm\(^{-1}\) ascribable to hydroxyl, carbonyl and aromatic groups. In the UV spectrum of 3, absorption maxima were observed at 227, 282 and 302 nm revealing the presence of the conjugated system. The \(^1\)H–\(^1\)H COSY (acetone-\(d_6\)) spectrum\(^9,10\) of 3 showed an isopropyl group attached to a double bond [1380 and 1368 cm\(^{-1}\), \(\delta\) 0.91 and 0.94 (3H each, d, \(J = 6.8\) Hz), 2.29 (1H, sep, \(J = 6.8\) Hz)], one trisubstituted double bond [\(\delta\) 5.83 (1H, dd, \(J = 6.5, 1.2\) Hz, H-5)], a methyl group attached to a quaternary carbon bearing a hydroxyl group [\(\delta\) 1.26 (3H, s, H-15)], a methine proton located between the carbonyl and olefinic groups (\(\delta\) 3.71, d, \(J = 1.2\) Hz, H-1) and a methine proton considered to be linked between an olefinic and methine group (\(\delta\) 3.18, 1H, dd, \(J = 6.5, 3.5\) Hz, H-4). These data together with \(^13\)C NMR data [\(\delta_C\) 59.8 (CH, C-1), 47.8 (CH, C-4), 124.5 (CH, C-5), 147.9 (C, C-6), 209.8 (C, C-7), 71.0 (C, C-8), 33.9 (CH, C-12), 20.7 (CH\(_3\), C-13), 21.2 (CH\(_3\), C-14) and 26.7 (CH\(_3\), C-15)] are also similar to \(p\)-methenone moiety in compounds 1 and 2. The partial structure was further proved by \(^1\)H–\(^1\)H COSY, HMBC (see Fig. 1) and NOESY spectra (see Fig. 2). The \(^1\)H–\(^1\)H COSY experiment on 3 indicated the presence of partial structure in bold lines as in Figure 1. The pronounced NOESY correlation between H-5 and H-15 established two protons are in \(syn\) face. The differences between 3 and 2 are that H-2 is not observed, and H-1 only couples with H-5 via allylic coupling. Two \(p\)-hydroxyphenyl groups were obviously revealed from the \(^1\)H NMR data [\(\delta\) 6.73 and 7.35 (2H each, d, \(J = 8.7\) Hz, H-2\(_3\)-, \(5\)' and H-2\(_2\)-, \(6\)' respectively), 6.80 and 7.65 (2H each, d, \(J = 8.7\) Hz, H-2\(_3\)-, \(5\)'' and H-2\(_2\)-, \(6\)'' respectively) and 8.23, 8.50 (1H each, exchangeable)], H-1 exhibited NOESY correlations with H-12, 13, 14, and H-2\(_2\)-, \(6\)' as well as HMBC correlation with C-1\(_1\). This evidence suggests that the \(p\)-hydroxyphenyl group linked at C-2 with \(\beta\)-orientation. The remaining \(sp^3\) methine proton at \(\delta\) 3.58 (d, \(J = 3.5\) Hz) was assigned as H-3 due to coupling with H-4 (\(\delta\) 3.18, dd, \(J = 6.5, 3.5\) Hz) and HMBC correlation with C-8, C-9 (\(\delta_C\) 71.0, 160.1) and C-1\(_1\) (\(\delta_C\) 133.1). The pronounced NOESY correlation between H-3 and H-2\(_2\)-, \(6\)' confirmed the H-3 and one of \(p\)-hydroxyphenyl group are in \(syn\) face. The UV, IR (1740 cm\(^{-1}\)) and \(^1\)H and \(^13\)C NMR [\(\delta_C\) 209.0 (C-11), \(\delta_H\) 7.58 (H-9, \(\delta_C\) 160.1), \(\delta_C\) 142.8 (C-10)] signals indicated the presence of cyclopentenone with a \(p\)-hydroxyphenyl substituent at the \(\alpha\)-position. Fifteen indices of hydrogen deficiency (IHD) were determined from the \(^13\)C NMR, DEPT and HR-FAB-MS experiments. On the basis of the above evidence, the structure of 3 was elucidated as shown in the formula, a dimer of monoterpane and norlignan with tricyclo[5.2.2.0\(^2.6\)]undecane skeleton.

The absolute configuration of 2 was obtained from CD measurements and determined to be 8R. Compound 2, isolated from this plant, expressed the same specific rotation value as isolated from Callitric macleayana.\(^{11,12}\)

Based on the same biological pathway, C-8 in chamacepynane C was assigned as \(R\)-configuration. The biosynthesis of this novel skeleton may occur from 1-hydroxymenth-3,5-dien-2-one (5) and 1,3-bis(4-hydroxyphenyl)cyclopenta-1,3-diene (6, a norlignan) via endo addition of bio-Dieels–Alder reaction, and then was oxidized to produce compound 3.

Obtunorlignan A (4) was isolated as an amorphous solid with negative optical rotation [\(\left[\alpha\right]_{D}^{20} = -10.3\) (c 0.35, MeOH)] and UV\(_{\text{max}}\) at 225 and 255 nm. The IR spectrum showed aromatic (1616 and 1516 cm\(^{-1}\)) and hydroxyl (3387 cm\(^{-1}\)) groups. The \(^1\)H NMR spectrum\(^13,14\) revealed two \(p\)-hydroxyphenyls [\(\delta\) 6.75, 7.30 (2H each, d, \(J = 8.8\) Hz) and 6.77, 7.26 (2H each, d, \(J = 8.8\) Hz)]. Three lower shift oxygenated \(sp^3\) carbons at \(\delta_C\) 66.2 (CH\(_2\), C-9), 69.0 (CH, C-8') and 82.6 (CH, C-7') indicated the remaining 20-atoms which existed as one ether and one alcohol group. One trisubstitutes olefinic proton at the lower field \(\delta_H\) 6.04 (H-8, resonated at \(\delta_C\) 124.5) exhibited coupling with H-9 with dd, \(J = 2.4, 2.0\) Hz and conjugating with \(p\)-hydroxyphenyl (UV\(_{\text{max}}\) 255 nm). H-8' expressed signal at \(\delta_H\) 4.64 with diaxial coupling to H-7' (\(\delta_H\) 4.41, \(J = 6.8\) Hz) and homoallylic coupling to H-9\(_B\) (\(\delta_H\) 4.22, \(J = 3.6\) Hz) and to H-9\(_X\) (\(\delta_H\) 4.36, \(J = 2.4\) Hz). Except with geminal coupling (\(J = 16.8\) Hz), H-9\(_B\) and H-9\(_X\) exhibited vicinal coupling with H-8 with \(J = 2.0\) and 2.4 Hz, respectively. From the above evidence, the gross structure of 4 can be elucidated as 2,4-bis(4-hydroxyphenyl)-3-hydroxy-4,5-dehydrotetrahydropyranone. The further proof was confirmed from its HMBC correlation. The HMBC

![Figure 1](image1.png)

Figure 1. \(^1\)H–\(^1\)H COSY and key HMBC correlations of 3.

![Figure 2](image2.png)

Figure 2. Key NOESY correlations of 3.
spectrum (Fig. 3) indicated that the two p-hydroxyphenyl groups were located at C-7 and C-7’. The 1H–1H COSY experiment on 4 indicated the presence of partial structure in bold lines as in Figure 3. H-8’ and H-2’, 6’ have NOESY (Fig. 4) correlation to give the same side on the basis of the above evidence, the structure of (4) was elucidated.

Chamaecyparicin C (3) and obtunorignan A (4) were evaluated for their cytotoxicity against KB (human oral epidermoid carcinoma), HONE-1 (human nasopharyngeal carcinoma) and TSGH (human gastric carcinoma) cells. The cell viability were assessed through a methyl blue dye assay, and the results are shown in Table 1.

Table 1. Cytotoxicity of 3 and 4

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 (µM)</th>
<th>3</th>
<th>4</th>
<th>VP-16*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KB</td>
<td>0.19 ± 0.08</td>
<td>&gt;50</td>
<td>1.10 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>HONE-1</td>
<td>0.24 ± 0.09</td>
<td>&gt;50</td>
<td>0.51 ± 0.35</td>
<td></td>
</tr>
<tr>
<td>TSGH</td>
<td>0.52 ± 0.11</td>
<td>&gt;50</td>
<td>2.74 ± 0.94</td>
<td></td>
</tr>
</tbody>
</table>

*Positive control substance.

Compound 3 exhibited the higher susceptibility with IC50 ranges from 0.19 to 0.52 µM than that of the clinically used anticancer drug etoposide (VP-16). However, 4 displayed no cytotoxic activity. Further studies, aiming to investigate a possible mechanism responsible for 3-mediated cytotoxic effect among human cancer cells, are actually in progress.

Acknowledgments

The authors thank the National Science Council of the Republic of China, for financial support.

References and notes

9. Compound 3: amorphous solid; [α]23D +175.7 (c 0.85, MeOH); high-resolution positive-ion FAB-MS calcd for C17H16O4 (M+H)+ 431.935; found 431.930; UV (MeOH, logε 227 (4.32), 282 (3.93), 302 (3.86, sh) nm; IR (KBr) 3379, 1740, 1701, 1615, 1574, 1380, 1368, 1271, 1225, 1174, 837, 757 cm−1; positive ion FAB-MS m/z 431 (M+H)+; 1H NMR (acetone-d6) δ 0.89, 0.94 (3H each, d, J = 6.8 Hz, H-13, 14), 1.26 (3H, s, H-15), 2.29 (1H, sep, J = 6.8 Hz, H-12), 3.18 (1H, dd, J = 6.5, 3.5 Hz, H-4), 3.58 (1H, d, J = 3.5 Hz, H-3), 3.71 (1H, d, J = 1.2 Hz, H-1), 4.41, 8.23, 8.50 (1H each, br s, OH-8, 4’, 5’); 5.83 (1H, dd, J = 6.5, 1.2 Hz, H-5), 6.73 (2H, d, J = 8.7 Hz, H-3’, 5’), 6.80 (2H, d, J = 8.7 Hz, H-3, 5), 7.35 (2H, d, J = 8.7 Hz, H-2’, 6’), 7.58 (1H, s, H-9), 7.65 (2H, d, J = 8.7 Hz, H-2, 6’); 13C NMR (acetone-d6) δC 20.7 (C-13), 21.2 (C-14), 26.7 (C-15), 33.9 (C-12), 47.8 (C-4), 53.3 (C-2), 53.6 (C-3), 59.8 (C-1), 71.0 (C-8), 116.1 (C-3’, 5’, 5’), 123.7 (C-11’), 124.5 (C-5’), 129.4 (C-2’), 129.5 (C-2’, 6’), 133.1 (C-1’), 142.8 (C-10), 147.9 (C-6), 157.0 (C-4’), 158.8 (C-4), 160.1 (C-9), 209.0 (C-11), 209.8 (C-7’).
10. The 1H and 13C NMR spectra of 3 were assigned with the aid of NOESY, 1H–1H COSY, DEPT, HSOQC and HMBC experiments.
13. Compound 4: amorphous solid; [α]23D +10.3 (c 0.35, MeOH); high-resolution EI–MS calcd for C18H12O7 H2O (M+H)+ 366.0939, found 366.0941; UV (MeOH, logε 225 (4.02, sh), 250 (4.01) nm; IR (KBr) 3387, 1616, 1516, 1233, 833 cm−1; EI–MS (rel. int. %) 266 (17), 163 (100), 133 (68), 121 (17); 1H NMR (methanol-d4) δ 4.22 (1H, ddd, J = 16.8, 3.6, 2.0 Hz, H-9), 4.36 (1H, dt, J = 16.8, 2.4 Hz, H-9), 4.41 (1H, d, J = 6.8 Hz, H-7), 4.64 (1H, ddd, J = 6.8, 3.6, 2.4 Hz, H-8), 6.04 (1H, dd, J = 2.4, 2.0 Hz, H-8), 6.75 (2H, d, J = 8.8 Hz, H-3, 5), 6.75 (2H, d, J = 8.8 Hz, H-3’, 5’), 7.26 (2H, d, J = 8.8 Hz, H-2’, 6’), 7.30 (2H, d, J = 8.8 Hz, H-2, 6’); 13C NMR (methanol-d4) δC 66.2 (C-9), 69.0 (C-8), 82.6 (C-7), 116.1 (C-3, 5), 116.1 (C-3’, 5’), 124.5 (C-8), 128.7 (C-2, 6), 130.2 (C-2’, 6’), 131.5 (C-1), 131.3 (C-1’), 133.0 (C-7), 158.8 (C-4), 163.1 (C-9), 209.0 (C-11), 209.8 (C-7’).
14. The 1H and 13C NMR spectra of 4 were assigned with the aid of NOESY, 1H–1H COSY, DEPT, HSOQC and HMBC experiments.