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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 1567–1569

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

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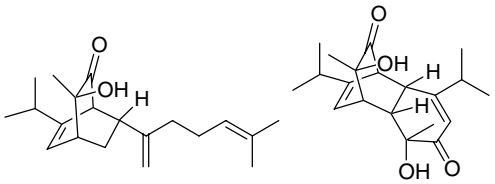
Received 25 September 2006; revised 28 December 2006; accepted 4 January 2007
Available online 7 January 2007

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 dimeric of monoterpene and norlignan with tricyclo-[5.2.2.$0^{2,6}$]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 IC_{50} values ranging from 0.19 to 0.52 μM, whereas **4** has no activity.

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 Me_2CO at room temperature. After evaporation of Me_2CO, 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 SiO_2 column and HPLC [Merck Lichrosorb Si 60, 250 × 10 mm i.d., EtOAc–CH_2Cl_2 (3:2)] to give chamaecypanone C (**3**) and obtunorlignan A (**4**).



1 **2**

Chamaecypanone C (**3**) was isolated as an amorphous solid with a positive optical rotation $[[\alpha]_D^{23}$ +175.7 (*c* 0.85, MeOH)] and UV λ_{max} 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 $C_{27}H_{26}O_5$ of **3** was resolved using high-resolution MS measurement.[9]

Keywords: *Chamaecyparis obtusa* var. *formosana*; Dimer of monoterpene and norlignan; Norlignan.
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0040-4039/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2007.01.011

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 ^1H NMR (acetone-d_6) spectrum[9,10] of **3** showed an isopropyl group attached to a double bond [1380 and 1368 cm^{-1}, δ 0.91 and 0.94 (3H each, d, $J = 6.8$ Hz), 2.29 (1H, sep, $J = 6.8$ Hz)], one trisubstituted double bond [δ 5.83 (1H, dd, $J = 6.5$, 1.2 Hz, H-5)], a methyl group attached to a quaternary carbon bearing a hydroxyl group [δ 1.26 (3H, s, H-15)], a methine proton located between the carbonyl and olefinic groups (δ 3.71, d, $J = 1.2$ Hz, H-1) and a methine proton considered to be linked between an olefinic and methine group (δ 3.18, 1H, dd, $J = 6.5$, 3.5 Hz, H-4). These data together with ^{13}C NMR data [δ_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 ^1H–^1H COSY, HMBC (see Fig. 1) and NOESY spectra (see Fig. 2). The ^1H–^1H 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_3-15 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 ^1H NMR data [δ 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′, 6′, as well as HMBC correlation with C-1′. This evidence suggests that the *p*-hydroxyphenyl group linked at C-2 with β-orientation. The remaining sp^3 methine proton at δ 3.58 (d, $J = 3.5$ Hz) was assigned as H-3 due to coupling with H-4 (δ 3.18, dd, $J = 6.5$, 3.5 Hz) and HMBC correlation with C-8, C-9 (δ_C 71.0, 160.1) and C-1′ (δ_C 133.1). The pronounced NOESY correlation between H-3 and H-2′, 6′ confirmed the H-3 and one of *p*-hydroxyphenyl group are in *syn* face. The UV, IR (1740 cm^{-1}) and ^1H and ^{13}C NMR [δ_C 209.0 (C-11), δ_H 7.58 (H-9, δ_C 160.1), δ_C 142.8 (C-10)] signals indicated the presence of cyclopentenone with a *p*-hydroxyphenyl substituent at the α-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 dimeric of monoterpene and norlignan with tricyclo[5.2.2.$0^{2,6}$]undecane skeleton.

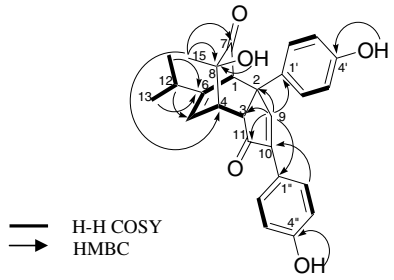


Figure 1. ^1H–^1H COSY and key HMBC correlations of **3**.

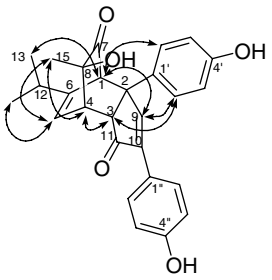
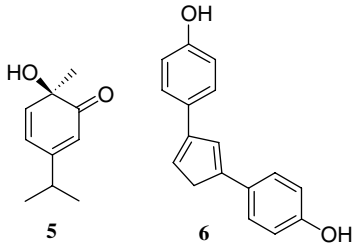


Figure 2. Key NOESY correlations of **3**.

The absolute configuration of **2** was obtained from CD measurements and determined to be 8*R*. 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 chamaecypanone C was assigned as *R*-configuration. The biosynthesis of this novel skeleton may occur from 1-hydroxymentha-3,5-dien-2-one (**5**) and 1,3-bis(4-hydroxyphenyl)cyclopenta-1,3-diene (**6**, a norlignan) via endo addition of bio-Diels–Alder reaction, and then was oxidized to produce compound **3**.



5 **6**

Obtunorlignan A (**4**) was isolated as an amorphous solid with negative optical rotation [$[\alpha]_D^{20}$ −10.3 (*c* 0.35, MeOH)] and UV_{max} at 225 and 255 nm. The IR spectrum showed aromatic (1616 and 1516 cm^{-1}) and hydroxy (3387 cm^{-1}) groups. The ^1H NMR spectrum[13,14] revealed two *p*-hydroxyphenyls [δ 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 δ_C 66.2 (CH_2, C-9), 69.0 (CH, C-8′) and 82.6 (CH, C-7′) indicated the remaining 2O-atoms which existed as one ether and one alcohol group. One trisubstitutes olefinic proton at the lower field δ_H 6.04 (H-8, resonanted at δ_C 124.5) exhibited coupling with H_2-9 with dd, $J = 2.4$, 2.0 Hz and conjugating with *p*-hydroxyphenyl (UV_{max} 255 nm). H-8′ expressed signal at δ_H 4.64 with diaxial coupling to H-7′ (δ_H 4.41, $J = 6.8$ Hz) and homoallylic compiling to H-9β (δ_H 4.22, $J = 3.6$ Hz) and to H-9α (δ_H 4.36, $J = 2.4$ Hz). Except with geminal coupling ($J = 16.8$ Hz), H-9β and H-9α 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-dehydrotetrahydropyranane. The further proof was confirmed from its HMBC correlation. The HMBC

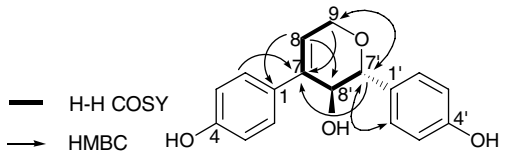


Figure 3. ^{1}H–^{1}H COSY and key HMBC correlations of **4**.

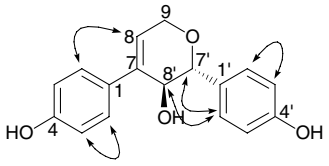


Figure 4. Key NOESY correlations of **4**.

Table 1. Cytotoxicity of **3** and **4**

Cell line	IC50 (μM)		
	3	**4**	VP-16[a]
KB	0.19 ± 0.08	>50	1.10 ± 0.12
HONE-1	0.24 ± 0.09	>50	0.51 ± 0.35
TSGH	0.52 ± 0.11	>50	2.74 ± 0.94

[a] Positive control substance.

spectrum (Fig. 3) indicated that the two *p*-hydroxyphenyl groups were located at C-7 and C-7′. The ^{1}H–^{1}H COSY experiment on **4** indicated the presence of partial structure in bold lines as in Figure 3. H-8′ and H-2′, H-6′ have NOESY (Fig. 4) correlation to give the same side evidence. The coupling constant ($J = 6.8$ Hz) between H-7′ and H-8′ determined the *quasi*-diaxial correlation. On the basis of the above evidence, the structure of (**4**) was elucidated.

Chamaecypanone C (**3**) and obtunorlignan 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 methylene blue dye assay,[15] and the results are shown in Table 1.

Compound **3** exhibited the higher susceptibility with IC_{50} 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

1. Kuo, Y. H.; Chen, C. H.; Huang, S. L. *Chem. Pharm. Bull.* **1998**, *46*, 181–183.
2. Kuo, Y. H.; Chen, C. H.; Huang, S. L. *J. Nat. Prod.* **1998**, *61*, 829–831.
3. Kuo, Y. H.; Chen, C. H. *Tetrahedron Lett.* **2001**, *42*, 2985–2986.
4. Kuo, Y. H.; Chen, C. H.; Chiang, Y. M. *Tetrahedron Lett.* **2001**, *42*, 6731–6735.
5. Kuo, Y. H.; Chen, C. H.; Chien, S. C.; Lin, Y. L. *J. Nat. Prod.* **2002**, *65*, 25–28.
6. Kuo, Y. H.; Chen, C. H.; Lin, Y. L. *Chem. Pharm. Bull.* **2002**, *50*, 978–980.
7. Kuo, Y. H.; Chen, C. H.; Wein, Y. S. *Helv. Chem. Acta* **2002**, *85*, 2657–2663.
8. Kuo, Y. H.; Chen, C. H.; Chien, S. C.; Lin, H. C. *Chem. Pharm. Bull.* **2004**, *52*, 764–766.
9. Compound **3**: amorphous solid; $[\alpha]_D^{23}$ +175.7 (*c* 0.85, MeOH); high-resolution positive-ion FAB-MS calcd for $C_{27}H_{27}O_5$ $(M+H)^+$ 431.1935, found 431.1930; UV (MeOH, log ε) 227 (4.32), 282 (3.93), 302 (3.86, sh) nm; IR (KBr) 3379, 1740, 1701, 1616, 1517, 1474, 1380, 1368, 1271, 1225, 1174, 837, 757 cm^{-1}; positive-ion FAB-MS *m/z* 431 $(M+H)^+$; ^{1}H NMR (acetone-d_6) δ 0.91, 0.94 (3H each, d, $J = 6.8$ Hz, H_3-13, 14), 1.26 (3H, s, H_3-15), 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′, 4″), 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″); ^{13}C NMR (acetone-d_6) δ_C 20.7 (C-13), 21.2 (C-14), 26.7 (C-15), 33.9 (C-12), 47.8 (C-4), 53.5 (C-2), 53.6 (C-3), 59.8 (C-1), 71.0 (C-8), 116.1 (C-3′, 5′, 3″, 5″), 123.7 (C-1″), 124.5 (C-5), 129.4 (C-2″, 6″), 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 ^{1}H and ^{13}C NMR spectra of **3** were assigned with the aid of NOESY, ^{1}H–^{1}H COSY, DEPT, HSQC and HMBC experiments.
11. Carmam, R. M.; Lambert, L. K.; Robinson, W. T.; Van Dongen, J. M. A. *Aust. J. Chem.* **1986**, *39*, 1843–1850.
12. Carmam, R. M.; Owsia, S.; Van Dongen, J. M. A. *Aust. J. Chem.* **1987**, *40*, 333–340.
13. Compound **4**: amorphous solid; $[\alpha]_D^{20}$ −10.3 (*c* 0.35, MeOH); high-resolution EI–MS calcd for $C_{17}H_{16}O_4$–H_2O $(M-H_2O)^+$ 266.0939, found 266.0941; UV (MeOH, log ε) 225 (4.02, sh), 255 (4.01) nm; IR (KBr) 3387, 1616, 1516, 1233, 833 cm^{-1}; EI–MS (rel. int. %) *m/z* 266 $(M-H_2O)^+$ (3), 250 (4), 163 (17), 162 (100), 133 (68), 121 (17); ^{1}H NMR (methanol-d_4) 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), 6.77 (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); ^{13}C NMR (methanol-d_4) δ_C 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), 132.1 (C-1′), 139.8 (C-7), 158.0 (C-4), 158.5 (C-4′).
14. The ^{1}H and ^{13}C NMR spectra of **4** were assigned with the aid of NOESY, ^{1}H–^{1}H COSY, DEPT, HSQC and HMBC experiments.
15. Finlay, G. J.; Baguley, B. C.; Wilson, W. R. *Anal. Biochem.* **1984**, *139*, 272–277.