Cytotoxic C-35 Terpenoid Cryptotrione from the Bark of Cryptomeria japonica

ARTICLE *in* ORGANIC LETTERS · JUNE 2010

Impact Factor: 6.36 · DOI: 10.1021/ol1009027 · Source: PubMed

ORGANIC LETTERS

2010
Vol. 12, No. 12
2786−2789

Cytotoxic C_{35} Terpenoid Cryptotrione from the Bark of *Cryptomeria japonica*

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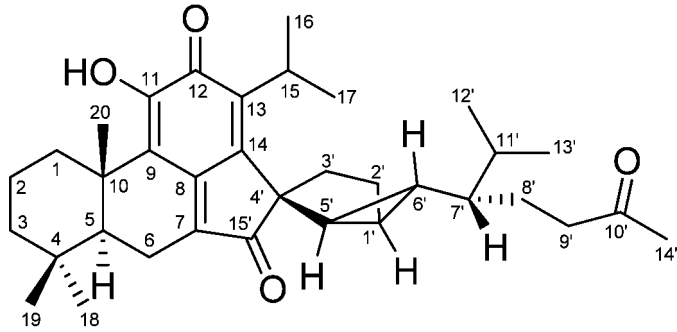
Received April 19, 2010

ABSTRACT



Cryptotrione (1)

A novel C_{35}-terpene, designated as cryptotrione (1), with an unprecedented skeleton possessing an abietane diterpene with a unique bicyclic sesquiterpene, is identified from the bark of *Cryptomeria japonica*. The carbon skeleton of 1 represents a new structural entity, and this is an intriguing addition to the structurally diverse diterpene-sesquiterpene class. A unique biosynthetic pathway is proposed to support the production of this phytocompound. Notably, 1 exhibits anticancer activity with an IC_{50} value of 6.44 ± 2.23 μM.

Cryptomeria is a genus of conifer in the cypress family Cupressaceae; it includes only one species, *Cryptomeria japonica D. DON. It is endemic to Japan, where it is known as Sugi (Japanese cedar),*[1] the national tree of Japan. The wood is scented, reddish-pink in color, lightweight but strong, waterproof, and resistant to natural decay. It is favored in Japan for a wide range of construction works as well as for interior paneling and other usage. Because of its industrial importance, the constituents of the leaves and heartwood of *C. japonica* including terpenoids have been actively investigated by many research groups.[2−20] In regard to biotech-

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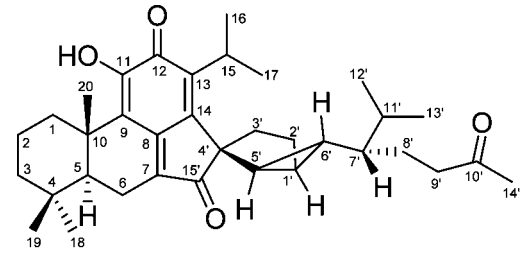
10.1021/ol1009027
Published on Web 05/18/2010

nological applications, the bark extracts have been found to exhibit antimicrobial activities against some plant pathogenic fungi.[21] The bark of *C. japonica* has also been employed as a substrate for horticultural crops in soil-less culture, and it has been reported that the replacement of rock wool with processed bark reduces losses caused by soil-borne plant pathogens.[22,23]

A previous study on the bark of *C. japonica* resulted in the isolation of an abietane-type diterpene quinone, and its antifungal and cytotoxic activities were reported.[24] Recently, an investigation of the acetone extract of its bark revealed eight new compounds.[25,26] It was contemplated that some of the newly reported abietane-type diterpenes might have unique skeleton structures that could incorporate an abietane diterpene and a cadinane sesquiterpene or a 1,10-secocadinane sesquiterpene. Therefore, we conducted a study on the chemical ingredients of the bark of *C. japonica* as an investigation on the bioactive constituents, and we report here a new compound with a unprecedented skeleton that can confer cytotoxic activity.

The bark of *C. japonica* D. Don was collected in Sitou, Taiwan in June, 2000. The air-dried bark (16.0 kg) was extracted with MeOH (3 × 100 L) at room temperature and concentrated to yield a crude extract (520 g), which was then partitioned between H_2O and EtOAc. The EtOAc fraction (430 g) was subsequently chromatographed repeatedly on silica gel and by HPLC (LDC Analytical-III; Purospher STAR, Merck, 250 mm ×10 mm, EtOAc/hexane 3:17) to give **1**.



1

Crytotrione (**1**)[27] was considered to have the molecular formula of $C_{35}H_{48}O_4$ based on the HREIMS of the molecular ion peak at *m/z* 532.3548 $[M]^+$ (calcd 532.3554), suggesting the presence of 12 degrees of unsaturation. The IR spectrum of **1** showed absorption bands at 3310 cm^{-1}, indicating the presence of hydroxyl, and 1699, 1613 cm^{-1} for the carbonyl groups (including the isolated and conjugated ones) and conjugated double bond. The presence of conjugated carbonyls was also supported by UV absorptions (λ_{max} 335, 348 nm). There were 35 signals observed in the ^{13}C NMR spectrum.[27] Analysis of the ^{13}C NMR, DEPT, and HMQC spectra revealed that **1** contained eight sp^3 methyls, eight sp^3 methylenes (δ_C 42.0, 41.6, 36.6, 31.3, 26.9, 23.9, 19.5 and 18.6), seven sp^3 methines (δ_C 50.7, 46.6, 31.3, 32.0, 29.0, 25.5 and 24.3), 12 quaternary carbons {three sp^3 (δ_C 58.3, 38.7 and 33.8), nine sp^2 including six olefinic carbons (δ_C 152.0, 149.0, 144.8, 144.5, 136.2 and 123.6), and three carbonyls [δ_C 209.0 (C-10′, acetyl), δ_C 205.1 (C-15′, cyclopentenone), and δ_C 182.5 (C-12, cyclohexadienone chelating with enol)]}. Careful analysis of the ^1H NMR spectrum indicated the presence of three tertiary methyl signals at δ_H 1.17 (s), 0.98 (s), and 0.94 (s), an isopropyl group signal at δ_H 3.00 (sep, J = 7.0 Hz, 1H), 1.35 and 1.24 (d, J = 7.0 Hz, 3H each), an allylic methylene at δ_H 2.66 (dd, J = 15.5, 3.5 Hz, H-6e) and 2.20 (dd, J = 15.5, 12.5 Hz, H-6a), a 11-hydroxy-12-oxoabietatriene H_β-1 type signal at δ_H 2.79 (br d, J = 12.5 Hz),[28] and an intramolecular five-membered ring hydrogen bonding at at δ_H 7.70 (s), together indicating

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(27) Crytotrione (**1**): yellow needles, mp 185−187°; $[\alpha]^{23}_D$ 37.1 (*c* 0.5, $CHCl_3$). UV (MeOH) λ_{max} (log ε) nm: 266 (4.21), 335 (4.49), 348 (4.56). IR ν_{max} cm^{-1}: 3310, 1699, 1646, 1613, 1580, 1427, 1374, 1295, 1208, 1122, 731. EIMS *m/z* (%): 532 (M^+, 100), 514 (9), 489 (23), 406 (13), 353 (41), 109 (6), 69 (8), 55 (12). HREIMS *m/z* 532.3548 $[M]^+$ (calcd for $C_{35}H_{48}O_4$ 532.3554). ^1H NMR ($CDCl_3$): δ 0.73 (1H, m, H-7′), 0.85 (3H, d, J = 7.0 Hz, H-12′), 0.90 (d, J = 7.0 Hz, H-13′), 0.93 (1H, overlap, H-6′), 0.94 (3H, s, H-18), 0.98 (3H, s, H-19), 1.07 (1H, dd, J = 6.5, 3.5 Hz, H-5′), 1.17 (3H, s, H-20), 1.23 (1H, overlap, H-3a), 1.24 (3H, d, J = 7.0 Hz, H-17), 1.35 (3H, d, J = 7.0 Hz, H-16), 1.47 (1H, overlap, H-3b), 1.48 (1H, overlap, H-1′), 1.49 (1H, overlap, H-5), 1.52 (1H, overlap, H-3′), 1.59 (1H, m, H-1a), 1.60 (3H, m, H-8′, -2a), 1.67 (1H, overlap, H-2b), 1.71 (1H, overlap, H-11′), 1.76 (3H, m, H-3′, -2′), 2.12 (3H, s, H-14′), 2.20 (1H, dd, J = 15.5, 12.5 Hz, H-6b), 2.29 (1H, m, H-2′), 2.46 (2H, m, H-9′), 2.66 (1H, dd, J = 15.5, 3.5 Hz, H-6a), 2.79 (1H, br d, J = 12.5 Hz, H-1b), 3.00 (3H, sep, J = 7.0 Hz, H-15). ^{13}C NMR ($CDCl_3$) δ 18.6 (C-2), 19.3 (C-20), 19.5 (C-6), 20.0 (C-13′), 20.4 (C-12′), 20.5 (C-16), 20.8 (C-17), 22.0 (C-19), 23.9 (C-8′), 24.3 (C-1′), 25.5 (C-6′), 29.0 (C-15), 26.9 (C-2′), 29.9 (C-14′), 31.3 (C-11′, -3′), 32.0 (C-5′), 33.3 (C-4), 36.6 (C-1), 33.8 (C-18), 38.7 (C-10), 41.6 (C-3), 42.1 (C-9′), 46.6 (C-7′), 50.7 (C-5), 58.3 (C-4′), 123.6 (C-9), 136.2 (C-13), 144.5 (C-8), 144.8 (C-11), 149.0 (C-14), 152.0 (C-7), 182.5 (C-12), 205.1 (C-15′), 209.0 (C-10′).

that **1** possessed a 11-hydroxy-12-oxoabietatriene type moiety. The gross structure of **1** was deduced from extensive analyses of the 2D NMR data, including the $^1H-^1H$ COSY, HMQC, and HMBC spectra in $CDCl_3$ (Figure 1). The $^1H-^1H$ COSY spectrum revealed coupling spin systems of the sequences of H-1 to H-3 and H-5 to H-6. Long range HMBC correlations from CH_3-19 to C-3, C-5, and C-18; CH_3-20 to C-1, C-5, and C-9; CH_2-6 to C-8, and C-10; 11-OH to C-9, C-11, and C-12; and H-15 to C-12, and C-14, constructed the ABC rings of the abietane skeleton.

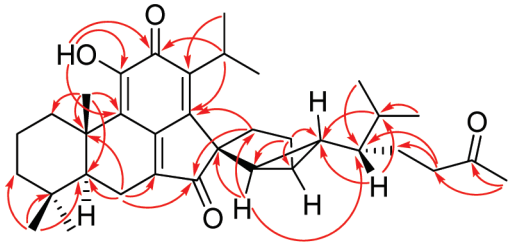


Figure 1. Key HMBC correlations of compound **1**.

These NMR spectral data resembled those of 11-hydroxy-12-oxoabieta-7,9(11),13-triene (**2**) except for the absence of two olefinic signals (H-7 and H-14) of **1**.[28] It is suggested that H-7 and H-14 in **2** were substituted as in the case of **1**. Two sets of signals [δ_H 2.12 (s), δ_C 209.0 and 29.9; δ_H 0.90, 0.85 (3H each, d, $J = 7.0$ Hz), having COSY correlation to δ_H 1.71 (1H, m, H-11′)] indicated the presence of an acetyl and an isopropyl groups. The consecutive protons H-6′ (δ_H 0.93, m) → H-7′ (δ_H 0.73) → H-11′ → H-12′ (H-13′); H-7′ → H-8′ → H-9′ revealed from the COSY correlation together with the correlations H-14′/C-9′, -10′; H-8′/C-9′, -10′ suggested that the side chain is a 1-isopropyl-4-pentanone. HMBC and COSY cross-peaks indicated the connectivities of H-3′ to C-1′-C-5′; H-6′ to C-1′, C-5′, C-7′, C-8′; and H-7′ to C-6′, H-11′, H-8′, H-9′ The result disclosed the presence of a moiety of bicyclo[3,1,0]hexane, and the side chain linked on C-6′ of cyclopropane. Three methine protons presented higher field at δ_H 1.07 (H-5′), 0.93 (H-6′) and 0.73 (H-7′), and this is an additional proof of the existence of cyclopropane. The HMBC correlations from CH_2-6 to C-15′ showed that C-15′ must be connected to C-7. HMBC correlations, H-1′, H_2-2′, H_2-3′, H-5′/C-4′ (δ_C 58.3); H_2-3′/C-14, C-15′ revealed that C-4′ is a spiro carbon which bonded four carbons, C-3′, C-5′, C-14 and C-15′. On the basis of the above evidence, the gross structure of **1** was elucidated as the structure shown in Figure 1.

For the stereochemistry of **1**, NOESY cross-peaks between CH_3-19, CH_3-20 and H_β-6 showed that **1** has identical relative configuration of trans-fused AB rings to those of common diterpenes reported from *C. japonica*. NOESY correlations of H-15/H-6′ and H-1′/H-5′, H-7′ established the relative configurations at C-1′, C-5′, and C-6′. X-ray crystallographic

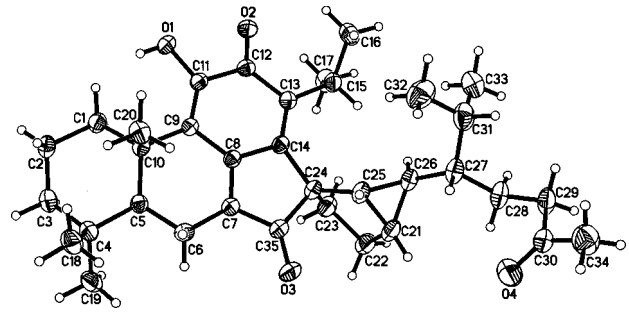
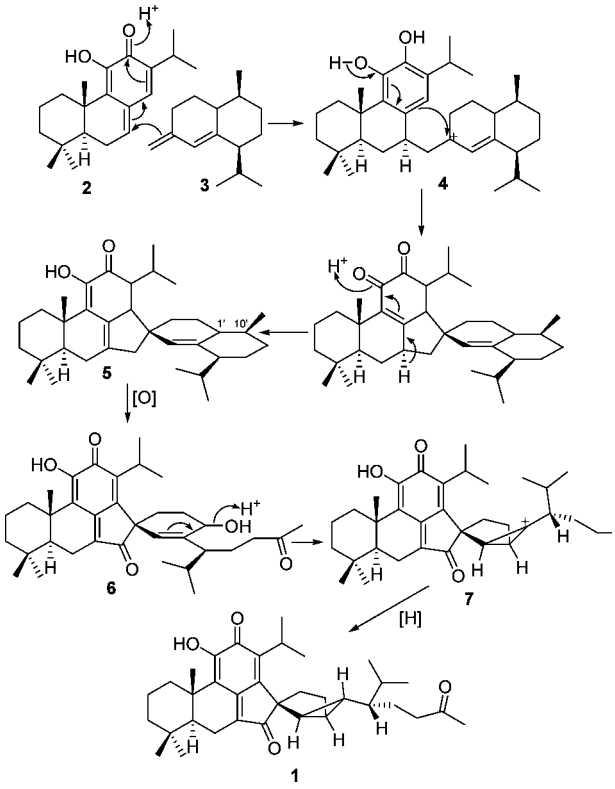


Figure 2. ORTEP drawing of **1**.

Scheme 1. Plausible Biosynthetic Pathway for **1**



analysis (Figure 2), gave the relative configuration of **1**.[30] Su et al. has published many new abietane type derivatives from the same plant and elucidated their absolute configuration as 5*S* and 10*S*.[11−16] The compounds from the same plant would give the same absolute configuration. Consequently, the structure and absolute configuration of **1** was unambiguously established.

A plausible biosynthetic pathway for cryptotrione (**1**) was proposed as shown in Scheme 1. Cryptotrione (**1**) might be derived from a combination of **2** and **3** to form intermediate cation **4** by acid catalytic bond formation, which was

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(30) X-ray data have been included in Supporting Information.

followed via cyclization and rearrangement to yield **5**. After dehydrogenation, oxidation at C-15′ and oxidative cleavage of C1′-C10′ bond of the sesquiterpene moiety produced trione **6**. Finally, **6** was cyclized by acidic catalysis to generate the compound **1**.

Ctyptotrione (**1**) was evaluated for potential antitumoral cytotoxicity against human oral epidermoid carcinoma KB cells. After treating KB cells with various concentrations of test compounds for population doubling times (each time about 72 h), the cell viability was assessed using a methylene blue dye assay.[29] The result demonstrated that **1** exhibited an IC_{50} value of 6.44 ± 2.23 μM. This compound thus possesses a medium cytotoxic property that is slightly weaker than that of the clinically used anticancer drug, etoposide (VP-16, IC_{50} value 2.0 μM).

Acknowledgment. The authors thank China Medical University (CMU96-104), Taiwan Department of Health Clinical Trial Research Center of Excellence (DOH99-TD-B-111-004) and of Health Cancer Research Center of Excellence for financial support (DOH99-TD-C-111-005).

Supporting Information Available: ^{1}H and ^{13}C NMR, 2D spectra, and X-ray data of cryptotrione (**1**) and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

OL1009027