Bioactive Compounds from *Taiwania cryptomerioides*

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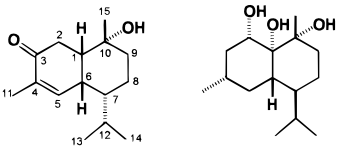
Two new sesquiterpenes, 10α-hydroxyamorphan-4-en-3-one (**1**) and 4α-methylcadinane-4α-methyl-1α,2α,10α-triol (**2**), together with four known compounds, sesquiterpenes 10α-hydroxycadinan-4-en-3-one (**3**) and α-cadinol (**4**), diterpene ferruginol, and lignan helioxanthin, were isolated from the whole plant of *Taiwania cryptomerioides* under bioassay-guided fractionations. The structures of **1** and **2** were elucidated mainly by the NMR spectroscopic analyses. Bioactivities of the isolated compounds against brine shrimp, yellow fever mosquito larvae, and human tumor cells are reported; compound **4** was the most bioactive, showing selectivity for the human colon tumor cell line (HT-29).

A number of sesquiterpenes,[1−5] diterpenes,[6] biflavones,[7] and lignans[8] were previously isolated from the heartwood of *Taiwania cryptomerioides* Hayata (Taxodiaceae). Our work on this plant began as a part of a program to isolate biologically active products and was directed by the brine shrimp lethality test (BST) for antitumor compounds and the yellow fever mosquito microtiter plate (YFM) assay for pesticides.[9] The bioactive compounds were then evaluated for cytotoxicities in a panel of human solid tumor cell lines. Under these bioassay- guided fractionations, the active crude extract of the whole plant of *T. cryptomerioides* [CH_2Cl_2−MeOH (1:1)] was subjected to repeated chromatography over Si gel and preparative HPLC to yield several sesquiterpenes, diterpenes, and lignans. We report here two new sesquiterpenes (**1**, **2**) together with four known compounds—(10α-hydroxycadinane-4-en-3-one (**3**), α-cadinol (**4**), ferruginol, and helioxanthin—all of which showed interesting bioactivities.



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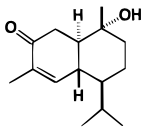

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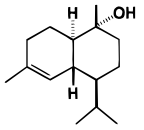

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Compound **1** was isolated as an oil, and its molecular formula, $C_{15}H_{24}O_2$, was determined by analyzing its HREIMS molecular ion at *m/z* 236.1776 (calcd 236.1776). The ^{13}C-NMR spectrum of **1** showed 15 carbon signals, including one carbonyl, one double bond, one oxygenated carbon, three methylenes, four methines, and four methyl groups. A downfield broad singlet at δ 6.95 (H-5) in its ^{1}H-NMR spectrum indicated the presence of a double bond conjugated with a carbonyl group. H-5 had a long-range coupling with a methyl group at δ 1.79, indicating that this methyl was attached to the other end of the double bond (CH_3-11). Two protons adjacent to the carbonyl group at δ 2.39 showed the same chemical shift value, which was confirmed by a spin decoupling experiment. An irradiation of the bridgehead proton at δ 2.15 (H-1) converted this methylene signal from a doublet to a singlet. The doublets of two methyl groups and the septet of one methine were assigned to an isopropyl unit. An additional methyl group was shifted relatively downfield to δ 1.18 and appeared as a singlet, indicating it was bonded to C-10 where an hydroxyl group was attached.

Correlations present in the ^{1}H−^{1}H COSY spectrum of **1** showed connectivities for H_2-2 and bridgehead proton H-1, which in turn was connected to the other bridgehead proton (H-6). The latter proton coupled to the methine proton at δ 1.54 (H-7) and the coupling chain continued from H-7 to H-8. The location of the isopropyl at C-7 was confirmed by correlations observed between H-5 and the two methyls of the isopropyl in the NOESY spectrum, suggesting that **1** had the skeleton of the cadinane type of sesquiterpene.[10] Protons bonded to C-8 and C-9 overlapped at δ 1.47−1.61 and could not be unambiguously assigned by homonuclear correlations.

The relative stereochemistry of **1** was deduced from a combination of coupling constant-analyses and the NOESY spectrum. H-1 of **1** in the ^{1}H-NMR displayed a doublet of triplets at δ 2.15, and this triplet was confirmed to be derived from its coupling with H_2-2 by a decoupling experiment. On the other hand, when irradiating H-6 at δ 2.67, H-1 was changed from a doublet of triplets into a triplet. Based on the analysis of the molecular model, the two six-membered rings were determined to be fused in a *cis* fashion because only in this form could H_2-2 exhibit the same dihedral angle with H-1 and afford a triplet coupling pattern. The *cis*-fused **1** showed a significant coupling (6.5 Hz) between the olefinic proton (H-5)[3] and the bridge proton (H-6); whereas, in the *trans*-fused isomer **3**,[2] the olefinic proton appeared as a broaden singlet peak in the ^{1}H-NMR. The phase-sensitive NOESY spectrum of **1** also gave proof for the proposed *cis*-fused ring system as the NOE correlation between H-1 and H-6 was observed. The equatorial orientation of the isopropyl group at C-7

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Table 1. ^{1}H-NMR δ Values for **1-3** ($CDCl_3$)

no.	1	2	2 (C_6D_6)	3
1	2.15 td (9.5, 4.5)			1.83ddd(13.8,8.4,3.0)
2α	2.39 d (9.5)			2.75 dd (15.0, 3.0)
2β	2.39 d (9.5)	3.44 br d (10.0)	3.47 br s	2.10 dd (15.0, 13.8)
3α		1.07 br t (12.5)	1.29 m	
3β		1.66 m	1.60 br d (12.0)	
4		1.32 m	1.03 m	
5α	6.95 d (6.5)	1.57 td (13.9, 4.4)	1.16 td (14.8, 3.3)	6.80 br s
5β		1.39 ddd (13.9, 4.4, 2.6)	1.44 m	
6	2.67 br s	1.92 td (13.9, 4.4)	1.84 td (14.8, 6.3)	2.07 br t (8.4)
7	1.54 m	1.75 tdd (13.9, 4.5, 2.5)	1.87 m	1.22 m
8α	1.47−1.61 m	1.68 m	1.64 m	1.84 m
8β	1.47−1.61 m	1.05 qd like	0.92 qd (13.3, 3.4)	1.44 qd (12.1 4.0)
9α	1.47−1.61 m	1.31 td (12.7, 4.1)	1.45 m	1.69 dt (12.1, 3.2)
9β	1.47−1.61 m	1.49 m	1.30 dt (16.6, 3.4)	1.20 m
11	1.79 d (1.0)	0.81 d (6.6)	0.62 d (7.0)	1.79 br s
12	1.89 br sept (7.0)	2.05 septd (7.1, 2.9)	2.19 septd (7.0, 3.0)	2.20 septd (6.9, 2.5)
13	0.92 d (7.0)	0.92* d (7.1)	0.96* d (7.0)	0.83* d (6.9)
14	0.92 d (7.0)	0.72* d (7.1)	0.79* d (7.0)	0.99* d (6.9)
15	1.18 s	1.32 s	1.25 s	1.16 s
2-OH		3.69 d (10.0)		

[a] Items marked with asterisks within the same column are interchangeable.

was confirmed by observing the NOE correlations between H-7 and H-6, as well as between CH_3-13 (14) and H-5. The strong NOE correlation between CH_3-15 and H-1β indicated an equatorial CH_3-15 at C-10. The molecular model for **1** was consistent with the NOESY result. The same coupling constants between H_2-2 and H-1, together with an NOE correlation between H-2 and CH_3-15, indicated the ring conformation as shown. Compound **1** was identified as 10α-hydroxyamorphane-4-en-3-one, which is a new sesquiterpene. The complete assignments of carbon signals were achieved by HMQC and HMBC experiments (Table 2).

Compound **2** was also isolated as an oil and showed a molecular ion at *m/z* 256.2043 (calcd 256.2038) in the HREIMS, analyzing for $C_{15}H_{28}O_3$. The ^{1}H-NMR spectrum showed a similar pattern with **1** including an isopropyl and a methyl attached to a quaternary carbon bearing a hydroxyl group. The ^{13}C-NMR spectrum indicated three oxygenated carbons in **2**, and two of them were quaternary as determined by a DEPT experiment. The COSY and DQF−COSY spectra of **2** enabled extensive chains of coupling to be delineated, and the cadinane-type skeleton[10] was established for **2**. As in **1** and later in **3,** COSY correlations were blocked at C-10, suggesting, therefore, that one hydroxyl group was located at C-10. However, the downfield shift of CH_3-15 of **2**, compared with **1** and **3**, indicated that another hydroxyl group was attached at C-1. The COSY correlation between H-2 at δ 3.44 and H-3 at δ 1.66 helped to place the third hydroxyl group at the C-2 position; this placement was supported by the single-relayed COSY spectrum which exhibited the long-range proton coupling between H-4 and H-2. The correlation between H-11 at δ 0.81 and C-2 at δ 74.0 in HMBC spectrum of **2** further confirmed the above conclusion.

The large vicinal coupling constant of 13.9 Hz for H-6 in **2** was justified by assuming that diaxial coupling occurred between H-6 and H-5β (δ 1.39) as well as H-7 (δ 1.75), suggesting a *trans*-junction between the two six-membered rings. Correspondingly, the axial H-7 indicated that the isopropyl group at C-7 was equatorial. The equatorial CH_3-11 and axial CH_3-15 were verified by a strong NOE correlation between H-4 and H-6, as well as a correlation between CH_3-15 and H-6 in the NOESY spectrum (C_6D_6). H-2 showed a NOE correlation with CH_3-15, indicating that H-2 was in the axial

Table 2. ^{13}C-NMR Data for **1-3** ($CDCl_3$)[a]

no.	1	2	3
1	45.8 d	72.1 s	51.1 d
2	37.1 t	74.0 d	38.3 t
3	199.2 s	28.7 t	200.1 s
4	134.9 s	41.0 d	135.4 s
5	150.5 d	30.3 t	146.0 d
6	35.6 d	42.7 d	40.8 d
7	43.1 d	37.3 d	45.0 d
8	19.4* t	23.7 t	21.5* t
9	34.1* t	32.1 t	41.6* t
10	71.3 s	74.7 s	71.2 s
11	16.0 q	14.1 q	15.1 q
12	27.8 d	25.5 d	26.2 d
13	15.7* q	15.0*	15.9* q
14	21.3* q	21.5* q	21.4* q
15	28.7 q	28.2 q	26.2 q

[a] Items marked with asterisks within the same column are interchangeable.

podition. Consequently, an equatorial orientation of the hydroxyl at C-2 was assigned. The relative configuration of **2** is as illustrated.

In the ^{1}H-NMR spectrum of **2**, the peak of H-2 exhibited a broad doublet (J = 10 Hz), while a doublet of doublets was expected. The doublet was apparently from the coupling between H-2 and the hydroxylic proton of OH-2 (δ 3.69), because the latter also appeared as a 10-Hz doublet and was lost to D_2O exchange. Also, a likely hydrogen bond bridging the C-2 and C-1 hydroxyls could have twisted the left six-membered ring into a less than ideal chair conformation, resulting in very small coupling constants of H-2 with both H-3α and H-3β. Compound **2** was identified as a new sesquitepene and was named 4α-methylcadinane-1α, 2α,-10α-triol. The assignments of carbon signals were achieved by using HMQC and HMBC results.

Isolate **3** was identified as 10α-hydroxycadinan-4-en-3-one, which was originally isolated from this plant but not reported with detailed ^{1}H- and ^{13}C-NMR data.[2] The ^{1}H-NMR assignments for **3** are presented in Table 1 and are based on the COSY spectrum. ^{13}C-NMR data for **3** are shown in Table 2. The relative stereochemistry of **3** was confirmed by the correlations observed in its NOESY spectrum.

Three additional known compounds, α-cadinol[11] (**4**), ferruginol,[12] and helioxanthin,[13] were also isolated and identified based on the complete agreement of their spectral data with those reported in the literature.

Table 3. Lethalities and Cytotoxicities of Compounds **1-4**, Ferruginol and Helioxanthin in the BST (LC_{50} μg/mL), Human Solid Tumor Cell Line (ED_{50} μg/mL) and YFM (LC_{50} μg/mL) Assays

compounds	BST[a]	A-549[b]	MCF-7[c]	HT-29[d]	YFM[e]
1	54.4	8.78	12.89	7.85	200.0
2	84.8	13.34	41.05	12.01	> 250.0
3	199.9	32.21	30.08	32.50	50.0
4	9.4	11.09	13.05	7.78×10^{-1}	2.0
ferruginol	42.3	6.47	23.42	6.43	3.0
helioxanthin	> 500				3.0
adriamycin[f]		4.99×10^{-3}	1.89×10^{-1}	3.57×10^{-2}	

[a] Brine shrimp lethality test. [b] Human lung carcinoma. [c] Human breast carcinoma. [d] Human colon adenocarcinoma. [e] Yellow fever mosquito larvae test. [f] The standard positive control.

Results of the BST and YFM assays for the above six compounds, as well as their in vitro cytotoxicities against three human solid tumor cell lines, are summarized in Table 3. Compounds **1**−**4** and ferruginol showed moderate activities in the BST. Compound **4** showed the best activities in all of the bioassays and was selectively cytotoxic against the human colon adenocarcinoma (HT-29) cell line with an ED_{50} value of 7.78×10^{-1} μg/mL. ED_{50} values of less than 4 μg/mL for pure compounds are considered significant in the search of antitumor compounds. However, the slight cytotoxicities exhibited by the other compounds might imply a potential for bioactivities in other uses. In the YFM assay, α-cadinol (**4**), ferruginol, and helioxanthin displayed LC_{50} values of around 2−3 μg/mL; compounds showing LC_{50} values below 1.0 μg/mL are considered as significant new leads for insecticide development.

Experimental Section

Instruments. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. The IR spectrum was recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. The LREIMS and HREIMS were obtained on Finnigan 4000 and on Kratos 50 spectrometers, respectively. The NMR spectra were recorded on a Varian VXR-500 (^{1}H at 500 MHz and ^{13}C at 125 MHz) or a Bruker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometer with $CDCl_3$ as solvent and TMS as internal reference. The mixing time used in the NOESY spectra was 0.3 s. A Rainin HPLC system with Dynamax software and a Dynamax UV-1 variable wavelength detector were used for preparative separations.

Plant Material. The whole plant of *T. cryptomeriodes* Hayata was collected in Taiwan by the Medicinal Plant Laboratory of the USDA, Beltsville, MD, where the voucher specimen is deposited. Its identification numbers are B641393 and PU000509.

Bioassays. The brine shrimp (*Artemia salina* Leach) test (BST)[14,15] and the yellow fever mosquito (YFM) assay[9] were routinely employed for evaluating the extracts, fractions, and isolated compounds from the title plant. Seven-day MTT in vitro cytotoxicities against A-549 (human lung carcinoma),[16] MCF-7 (human breast carcinoma),[17] and HT-29 (human colon adenocarcinoma)[18] cell lines were performed at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive control. The YFM (*Aedes aegypti*) microtiter plate assay was performed both at Purdue University and FMC Corporation where standard protocols are provided.[9]

Extraction and Isolation. The pulverized material (700 g) was extracted with CH_2Cl_2−MeOH (1:1) at room temperature and condensed *in vacuo* to yield a crude extract (50 g). This extract was repeatedly chromatographed over Si gel columns using gradients of hexane−EtOAc and then further separated by HPLC (Dynamax-60 A 8-μm Si gel column, 250 × 21 mm) with gradients of hexane−MeOH−THF or CH_2Cl_2−MeOH to give compounds **1**−**6**.

10α-Hydroxyamorphane-4-en-3-one (1): oil, $[\alpha]^{25}_D$ −63° (*c* 0.32, $CHCl_3$). IR (dry film) ν max 3325 (OH), 2956, 1712 (C═C, ketone), 1667 (C═C), 1464, 1369, 1255, 1192, 1147, 1045, 907 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); HREIMS (70 eV) *m*/*z* $[M]^+$ 236.1776 for $C_{15}H_{24}O_2$, calcd 236.1776; LREIMS (70 eV) *m*/*z* of $[M + 1]^+$ 237 (43), 236 (11), 219 (18), 201 (3), 193 (24), 175 (37), 165 (14), 147 (15), 135 (31), 121 (18), 109 (100), 95 (28), 69 (48).

4α-Methylcadinane-1α,2α,10α-triol (2): oil, $[\alpha]^{25}_D$ −1.3° (*c* 0.15, $CHCl_3$); IR ν max 3374 (OH), 2926, 1547, 1462, 1401, 1217, 1078, 1057, 1010, 941, 854 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); HREIMS (70 eV) *m*/*z* of $[M]^+$ 256.2043 for $C_{15}H_{28}O_3$, calcd 256.2038; LREIMS (70 eV) *m*/*z* $[M]^+$ 256 (0.5), 239 (5), 221 (14), 203 (3), 195 (9), 185 (100), 149 (27), 137 (21), 121 (24), 119 (17), 111 (40), 97 (25), 69 (51).

10α-Hydroxycandinan-4-en-3-one (3): ^{1}H-NMR, see Table 1; for ^{13}C NMR; see Table 2.

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