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New Terpenoids from *Amentotaxus formosana*

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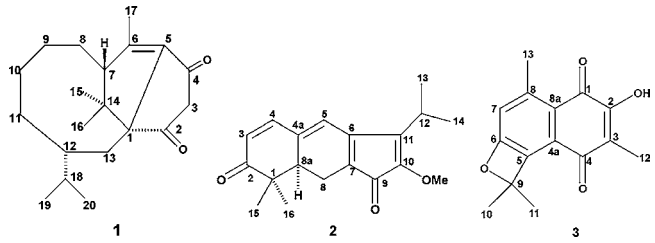
ABSTRACT



1 **2** **3**

Amentoditaxone (1), possessing an unprecedented diterpenoid skeleton, along with two new terpenoids, amentotaxin WC (2) and amentotaxone (3), were established by extensive analysis of spectroscopic data.

In previous papers, we have reported the isolation and biological activity of constituents from *Amentotaxus formosana*.[1−5] As part of a continued investigation on the bioactive constituents of Formosan plants, a novel diterpenoid, with a new skeleton, amentoditaxone (**1**), and two new terpenoids, amentotaxin WC (**2**) and amentotaxone (**3**), were isolated from the leaf and heart wood of this plant, respectively. In the present paper, the structure elucidation of these new compounds and the hypothetical biogenetic route for the formation of **1**, **2**, and **3** are reported.

The whole plants of *A. formosana* were collected at Kaohsiung Hsien, Taiwan, during July 2001. The $CHCl_3$ extracts (80 g) of the leaf (3.1 kg) of this plant were chromatographed over silica gel. Elution with *n*-hexane/acetone (8.5:2.5) yielded **1** (4 mg). The $CHCl_3$ extracts (6.5 g) of the heart wood (1.6 kg) of this plant also were chromatographed over silica gel. Elution with CH_2Cl_2/acetone (1:1) and CH_2Cl_2/MeOH (9:1) yielded **2** (5 mg) and **3** (10 mg), respectively.

Compound **1** was isolated as colorless powder, $[\alpha]_D^{25}$ −32° (*c* 0.075, $CHCl_3$). The IR spectrum of **1** exhibited an absorption band for the saturated carbonyl moiety (1706 cm^{-1}).[6] The presence of an unsaturated carbonyl moiety was confirmed by the UV spectrum [λ_{max} 265 nm (4.381)].[7] HREIMS suggested a molecular formula of $C_{20}H_{30}O_2$ ([M]$^+$, 302.2244, Δ −0.0002 mmu).

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Table 1. ^{1}H and^{13}C NMR Spectroscopic Data (δ in ppm, J in Hz) of **1**, **2** (in $CDCl_3$), and **3** (in CD_3OD)

1			**2**			**3**		
position	δ_H	δ_C	position	δ_H	δ_C	position	δ_H	δ_C
		67.8	1		45.6	1		178.8
		207.3	2		203.0	2		170.8
3	α 2.15 d (18.8)	38.0	3	5.96 d (9.6)	124.5	3		109.0
	β 3.23 d (18.8)		4	7.17 d (9.6)	147.8	4		182.7
4		208.8	4a		137.7	4a		137.6
5		152.3	5	7.30s	118.7	5		132.9
6		153.3	6		147.9	6		160.1
7	1.87 dd (13.2, 2.8)	47.1	7		135.0	7	6.67	121.5
8	α 1.77 ddd (21.2, 13.2, 5.2)	23.4	8	α 2.93 dd (14.0, 11.6)	43.2	8		148.7
	β 2.13 m			β 3.11 t (14.0)				
9	α 2.52 ddd (9.6, 5.2, 2.0)	39.0	8a	α 2.93 t (11.6)	43.8	8a		117.2
	β 2.60 m		9		201.1	9		98.0
10	α 1.15 m	33.6	10		147.4	10	1.76 s	25.9
	β 1.33 m		11		142.4	11	1.76 s	25.9
11	α 1.24 m	41.7	12	3.26 m	27.0	12	1.89 s	7.7
	β 1.48 m		13	1.27 d (6.8)	23.5	13	2.56 s	20.6
12	1.15 m	33.7	14	1.27 d (6.8)	23.3			
13	1.56 m	18.3	15	1.05 s	21.2			
14		44.0	16	1.31 s	23.5			
15	0.91 s	17.6	OMe	3.85 s	62.0			
16	0.89 s	22.0						
17	1.04 s	33.8						
18	2.60 m	24.9						
19	1.07 d (6.8)	21.4	21.4					
20	1.11 d (6.8)	21.1	21.1					

The structure of **1** was completely assigned by a combination of one- and two-dimensional NMR methods. Its ^{1}H NMR spectrum (Table 1) contained the signals of two secondary methyls, three tertiary methyls, six methylene groups, and three methine groups. Its ^{13}C NMR spectrum (Table 1) further indicated the presence of five methyl, six methylene, three methine, and six quaternary carbons.

Analysis of the ^{1}H−^{1}H COSY and HMQC experiment for **1** established the connectivities of eight ^{1}H−^{1}H and ^{1}H−^{13}C spin systems represented as bold lines (Figure 1). The HMBC correlation of H-12/C-18 and Me-19 and Me-20/C-12 confirmed connectivity between C-12 and C-18. The HMBC correlations of H-7/C-8, C-15, C-16 and C-14, Me-15/C-1, C-7, and C-14, Me-16/C-1, C-7, and C-14, Me-15/C-16, and Me-16/C-15 established the connectivities between C-7/C-14 and C-14/C-1. The HMBC correlations of Me-17/C-5, C-6, and C-7 and H-13/C-5 and C-2 established the connectivities between C-7/C-6 and C-5/C-6, and C-13 was linked through C-1 to C-5. The HMBC correlations of H-3/C-1, C-2, and C-4, and C-5, along with the above evidence of IR and UV spectra, confirmed that the β-diketone group was linked between C-4/C-5 and C-1/C-2. Thus, the structure of amentoditaxone was established as **1**.

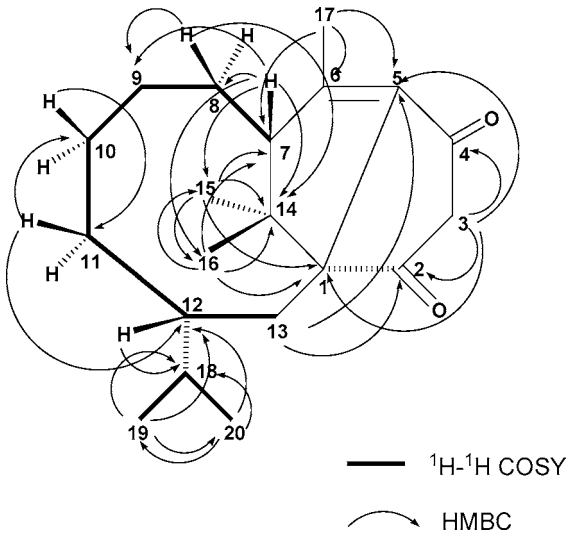


Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of **1**.

The relative configurations at C-1, C-7, and C-12 were determined as shown in **1** from the results of a NOESY experiment (Figure 2). A NOESY experiment on **1** showed cross-peaks between H_β-8/Me-17, Me-17/H-7, H_β-11/H-12, and Me-16/H_β-11. The above result suggested that the proton groups at C-7 and C-12 are on the β-side of **1** and the chemical bond between C-1 and C-2 is on the α-side of **1**. From the ^{1}H NMR, COSY, and NOESY spectra, a computer-generated 3D structure of **1** was obtained by using the molecular modeling program, CS CHEM 3D V 3.5.1, with MM2 force-field calculations for energy minimization (Figure 2). The calculated distances between H_β-8/Me-17 (2.797 Å), Me-17/H-7 (2.679 Å), H_β-11/ Me-16 (1.881 Å), and H_β-11/H-12 (3.024 Å) are all less than 4.00 Å. This is consistent with the well-defined NOESY interactions observed for each of these proton pairs.

Compound **2** was isolated as yellowish powder, $[\alpha]_D^{25}$ + 30° (*c* 0.20, $CHCl_3$). The HREIMS of **2** indicated a $[M]^+$ peak at *m*/*z* 298.1550, Δ −0.0019 mmu, which corresponded to a molecular formula of $C_{19}H_{22}O_3$. The IR absorption of **2** implied the presence of a conjugated ketone in a five-membered ring (1718 cm^{-1}), an α,β-unsaturated carbonyl

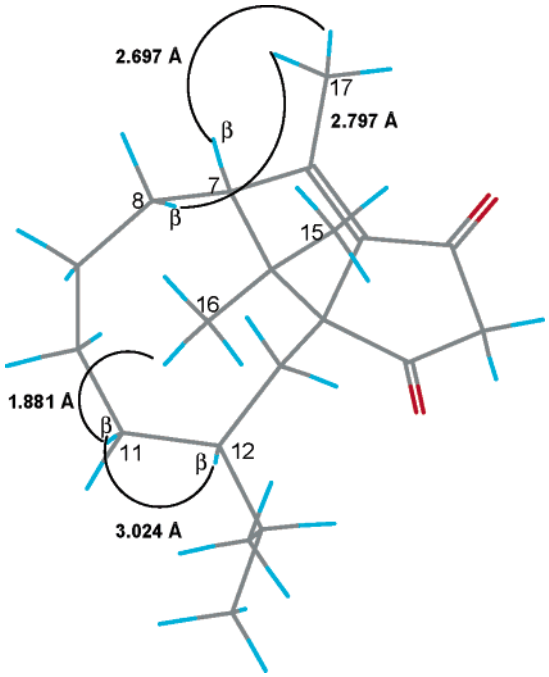


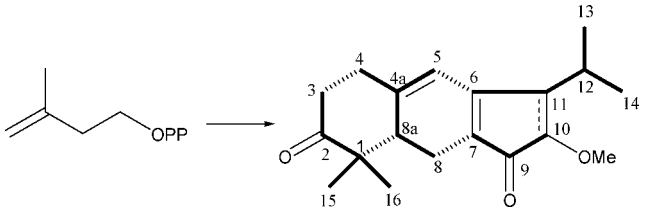
Figure 2. Selective NOESY correlations and relative stereochemistry for **1**.

group (1647 cm^{-1}),[8] and a conjugated C═C moiety (1594 cm^{-1}). The UV spectrum of **2** exhibited absorption maxima at 260 (4.23), 320 (3.55), and 375 (4.06) nm. The ^1H NMR spectrum of **2** (Table 1) showed proton signals for two tertiary methyl groups at δ 1.05 and 1.31, two secondary methyl groups at δ 1.27 (6H, d, J = 6.8 Hz), a methylene group at δ 2.93 (1H, dd, J = 14.0, 11.6 Hz) and 3.11 (1H, t, J = 14.0 Hz), two methine groups at δ 2.93 (1H, t, J = 11.6 Hz) and 3.26 (1H, m), a methoxy group at δ 3.85 (s), a *cis*-disubstituted olefin moiety at δ 5.96 (1H, d, J = 9.6 Hz) and 7.17 (1H, d, J = 9.6 Hz), and an olefinic proton at δ 7.30 (s). The ^{13}C NMR spectrum (Table 1) showed signals for four methyl, a methoxyl, a methylene, two methine, three olefinic carbons, and eight quaternary carbons. The above evidence suggested that **2** contained an α,β-unsaturated carbonyl moiety. The HMBC correlations of H-3/C-4a, H-4/C-2 and C-8a, H-5/C-6, C-7 and C-8a, Me-15/C-2, C-1, C-8a and C-16, Me-16/C-1, C-2, C-8a and C-15, H_β-8/ C-8a, H_α-8/C-4a and H-8a/C-4a, the $^1H-^1H$ COSY correlation between H_2-8 and H-8a and the NOESY correlations of Me-16/H_α-8, H-8a and Me-15 and Me-15/Me-16 suggested that **2** possessed a disubstituted 1,1-dimethyl-8,8a-dihydro-2*H*,8*H*-naphthalene-2-one moiety. The HMBC correlations of H-5/C-6 and C-7, H_2-8/C-9, MeO-10/C-10, Me-13/C-11, Me-14/C-11, the $^1H-^1H$ COSY correlations of H-12/Me-13 and Me-14, and the NOESY correlations of H-5/Me-14 and OMe-10/Me-13, with C-9 and C-10 presented as quaternary carbon, confirmed the structure of amentotaxin WC as **2**. The NOESY experiment of **2** showed a cross-peak between H_α-8/Me-16 and Me-16/H-8a and suggested that H-8a and Me-16 are on the α-side of **2**. The presence of a fragment peak at m/z 283 $[M - Me]^+$, 269 $[M - 2 \times Me + H]^+$ and 255 $[M - CH(CH_3)_2]^+$ in the EIMS also supported the characterization of **2**.

The HREIMS of **3** gave a molecular peak at m/z 258.0899, Δ −0.0007 mmu, which corresponded to a molecular formula $C_{15}H_{14}O_4$. Its IR spectrum showed absorptions due to hydroxyl (3240 cm^{-1}), carbonyl (1684 and 1635 cm^{-1}), and aromatic (1598 cm^{-1}) functionalities. The UV spectrum of **3** exhibited absorption maxima similar to that of hydroxynaphthaquinones.[9] The ^1H NMR spectrum of **3** (Table 1) showed proton signals for four tertiary methyl groups at δ 1.76 (6H, s), 1.89 (3H, s) and 2.56 (3H, s), and an aromatic proton at δ 6.67. The ^{13}C NMR spectrum (Table 1) showed signals for four methyl, a tertiary aromatic carbon, and 10 quaternary carbons, including two carbonyl carbons. The two tertiary methyl proton signals (Me − 3 and −8) in **3** were identical to those of aristolindiquinone, a 3,8-dimethylnaphthaquinone derivative,[10] and the HMBC correlations of Me-12/C-2, C-3 and C-4, Me-10 or Me-11/C-9, Me-10 or Me-11/C-5, H-7/C-5 and C-8a, Me-13/C-7, C-8 and C-8a, and NOESY correlation of H-7/Me-13 suggested that **3** was characterized as **3** or **3a**. On the basis of the above result, and the comparison of NMR spectra with those of aristolindiquinone[10] and reported data in the literature,[11] it was confirmed that the structure of amentotaxone as **3**. The presence of fragment peaks at m/z 243 $[M - Me]^+$, 215 $[M - C(CH_3)_2 - H]^+$, 187 $[215 - CO]^+$, and 115 [M − b − Me × 2 − H] in the EIMS also supported the characterization of **3**.

Compounds **1**, **2**, and **3** showed no cytotoxic activity against several human cancer cell lines. Biogenetically, the C-1, C-2, C3, C-15, C-16, C-4, C-4a, C-5, C-8a, C-8, C-6, C-11, C-12, C-13 and C-14 in the skeleton of **2** seem to be derived from isopentenyl diphosphate (Scheme). The C-7, C-9, and C-10 may derive from pyruvate (Scheme 1).[11]

Scheme 1. Hypothetical Biogenetic Route for the Formation of **2**



Therefore, this hypothetical result indicates that *Amentotaxus*

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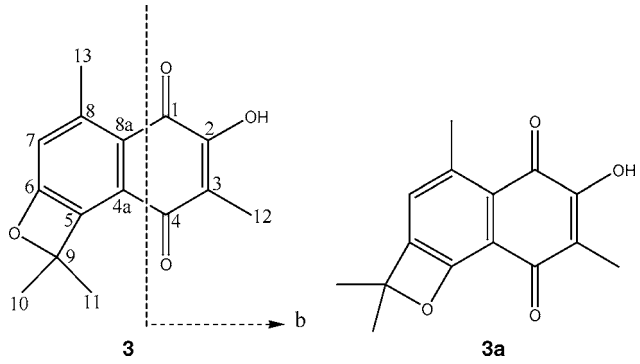

Figure 3. Chemical structure of compounds **3**, **3a**, and EIMS fragmentation pattern of **3**.

formosana uses this hypothetical pathway in the synthesis of new compound **2.**[12] Previously reported terpenoids, amentotaxins WA and WB,[13] may also use the same biosynthetic pathway. The biosynthesis of **3** agrees with three head-to-tail isoprenoid units, whereas structure **3a** does not. It further supported the characterization of **3**. It is valuable to study the biosynthetic pathway of the unprecedented diterpenoid **1**.

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Note Added after ASAP Publication. The version of SI posted January 17, 2006 contained errors. The correct version posted January 26, 2006.

Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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