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Obtusinones D and E, linear and angular fused dimeric icetexane diterpenoids from *Premna obtusifolia* roots

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ABSTRACT

Two new dimeric icetexane diterpenoids, obtusinones D and E (**1** and **2**), were isolated from the root extracts of *Premna obtusifolia*. Obtusinone D (**1**) represents the first example of a novel linear fused dimeric icetexane, whereas obtusinone E (**2**) is an angular fused icetexane. The structures of **1** and **2** were elucidated on the basis of 1D and 2D NMR spectral data analysis.

Dimeric icetexane diterpenes are rarely found in Nature. Grandione was the first example of such a dimeric icetexane diterpene, which was isolated from the wood of *Torreya grandis* Fort by Riccio and co-workers.[1] In 2005, Takeya and co-workers attempted to synthesize grandione from demethylsalvicanol via a solid-state hetero-Diels–Alder type dimerization reaction.[2] These results

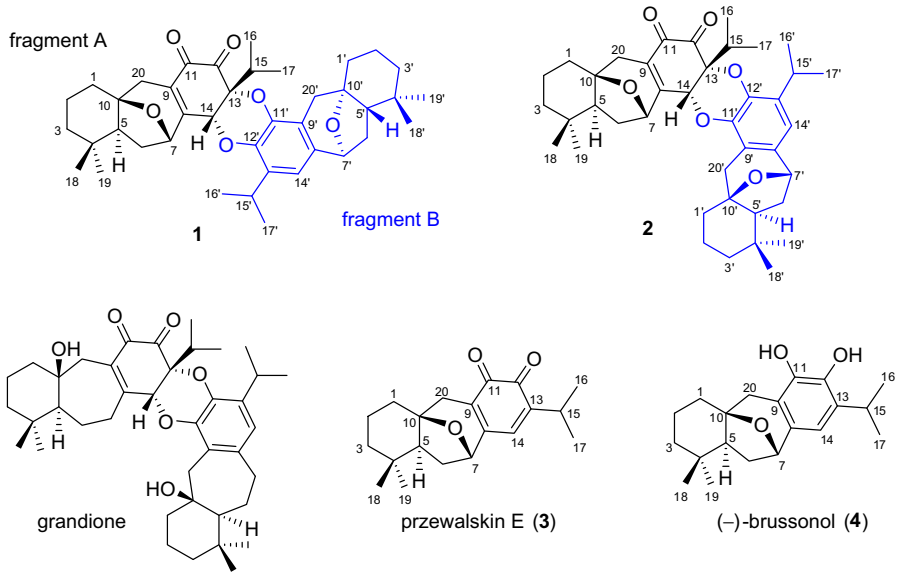

Figure 1. Structures of dimeric icetexanes **1** and **2** and other related structures.

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Table 1
^{13}C NMR spectroscopic data of compounds **1**–**4** in $CDCl_3$

Position	**1**[a]	**2**[b]	**3**[c]	**4**[d]
1	29.5	29.7	29.8	30.7
2	15.7	15.8	15.8	16.1
3	31.2	31.3	31.6	32.2
4	31.9	31.8	32.0	31.8
5	51.6	51.7	51.5	51.0
6	37.7	37.7	37.9	39.6
7	73.9	73.9	74.9	76.1
8	158.2	158.1	153.0	134.2
9	135.6	135.8	129.5	116.4
10	80.1[e]	80.2	80.4	80.0
11	185.7	185.5	180.3	141.5
12	191.7	191.2	179.7	139.4
13	86.3	86.0	147.9	131.9
14	72.9	72.4	131.9	112.7
15	31.0[e]	31.0	27.2	27.1
16	16.1	16.1	21.6	22.5
17	16.9	16.9	21.6	22.8
18	30.2	30.3	30.3	30.6
19	26.9	26.9	26.7	26.6
20	38.4	38.9	38.0	38.7
1′	31.0[e]	30.0		
2′	16.4	15.9		
3′	32.6	32.1		
4′	32.0	31.4		
5′	51.1	50.7		
6′	39.5	40.1		
7′	76.0	75.8		
8′	136.5	134.3		
9′	118.6	118.7		
10′	80.1[e]	79.9		
11′	139.7	139.9		
12′	138.3	137.6		
13′	134.2	134.0		
14′	113.1	114.8		
15′	26.4	27.5		
16′	23.2	22.4		
17′	22.3	22.1		
18′	30.6	30.4		
19′	26.5	27.1		
20′	38.7	38.5		

[a] Spectra were recorded at 300 MHz.
[b] Spectra were recorded at 400 MHz.
[c] Spectra were recorded at 400 MHz and previously reported by Xu et al.[17]
[d] Spectra were recorded at 500 MHz and previously reported by Fraga et al.[18]
[e] Duplicated signals.

showed that the correct structure of grandione was that of an angular fused dimeric icetexane. The complex architecture and the fact that these compounds are rarely found in Nature, prompted us to search for dimeric icetexane diterpenoids from Thai plants. The previous phytochemical investigation of plants in the *Premna* genus[3–13] revealed that *Premna obtusifolia* produced many types of diterpenoids including the icetexane diterpene.[14,15] *P. obtusifolia* is commonly found in the southern part of Thailand and is known locally in Thai as 'Akkhi Thawan Thale'.[16] It has been used as a folk medicine for the treatment of fever, coughs, skin rash, and diarrhea. Some of these isolated compounds exhibited significant antibacterial[6,7,10,11] and cytotoxic[12,13] activities. The crude hexane extract of the roots of *P. obtusifolia* was subjected to chemical investigation leading to the isolation of two new examples of rare dimeric icetexane diterpenes, **1** and **2** (Fig. 1).

Compound **1** was obtained as a yellow amorphous powder with a negative optical rotation ($[\alpha]_D^{24}$ −359.0 in $CHCl_3$). The high resolution EIMS spectrum of **1** showed a molecular ion peak at *m*/*z* 628.2798 $[M]^+$, consistent with the molecular formula $C_{40}H_{52}O_6$, indicating 15 degree of unsaturation. The ^{13}C NMR spectrum of **1** (Table 1) exhibited a total of 40 carbon signals (including two duplicated carbon signals), which suggested that compound **1** was a dimer of a diterpene. Moreover, the EIMS spectrum contained molecular ion

Table 2
^{1}H NMR spectroscopic data of compounds **1**–**4** in $CDCl_3$ (*J* values in Hz). Assignments based on HMQC, HMBC, and COSY experiments

Position	**1**[a]	**2**[b]	**3**[c]	**4**[d]
1	1.83 m, 2.05 m	1.90 m	1.45 m, 1.93 m	1.77 m, 1.98 m
2	1.62 m, 1.82 m	1.87 m	1.44 m, 1.75 m	1.58 m, 1.77 m
3	1.17 m, 1.54 m	1.51 m	1.18 m, 1.60 m	1.14 m, 1.50 m
5	1.79 m	1.77 m	1.23 m	1.79 m
6	2.05 m, 2.22 m	2.07 m	1.63 m, 2.10 m	1.87 dd, 12.0, 7.0
				2.10 dd, 12.0, 7.0
7	4.76 d, 6.6	4.80 d, 6.8	4.40 d, 6.8	4.83 d, 6.7
14	4.49 s	4.62 s	1.23 m	6.42 s
15	2.27 m	2.30 m	1.63 m, 2.10 m	3.09 *sept*, 7.0
16	1.08 d, 6.9	1.12 d, 6.8	4.40 d, 6.8	1.21 *d*, 7.0
17	1.02 m	1.01 d, 6.8	1.23 m	1.20 d, 7.0
18	1.02 s	1.02 s	1.63 m, 2.10 m	0.93 s
19	0.88 s	0.89 s	4.40 d, 6.8	0.82 s
20	2.59 d, 18.3	2.59 d, 18.4	2.46 d, 18.3	2.71 d, 16.0
	2.24 d, 18.3	2.24 d, 18.4	2.15 d, 18.3	2.37 d, 16.0
1′	1.59 m, 1.98, m	2.10 m		
2′	1.55 m, 1.84 m	1.55 m, 1.78 m		
3′	1.21 m, 1.53 m	1.52 m		
5′	1.90 m	1.81 m		
6′	1.93 m, 2.15 m	1.98 m, 2.01 m		
7′	4.83 d, 6.6	4.83 d, 6.4		
14′	6.45 s	6.57 s		
15′	3.13 *sept*, 6.9	3.29 *sept*, 6.8		
16′	1.13 d, 6.9	1.31 d, 6.9		
17′	1.12 d, 6.9	1.28 d, 6.9		
18′	0.99 s	0.96 s		
19′	0.86 s	0.81 s		
20′	2.76 d, 17.1	2.62 d, 17.2		
	2.57 d, 17.1	2.31 d, 17.2		

[a] Spectra were recorded at 300 MHz.
[b] Spectra were recorded at 400 MHz.
[c] Spectra were recorded at 400 MHz and previously reported by Xu et al.[17]
[d] Spectra were recorded at 500 MHz and previously reported by Fraga et al.[18]

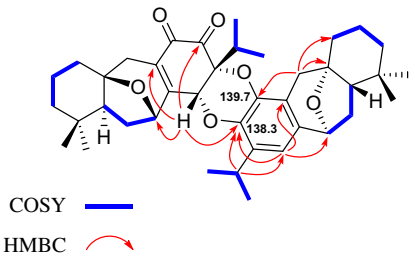


Figure 2. Key HMBC correlations of **1**.

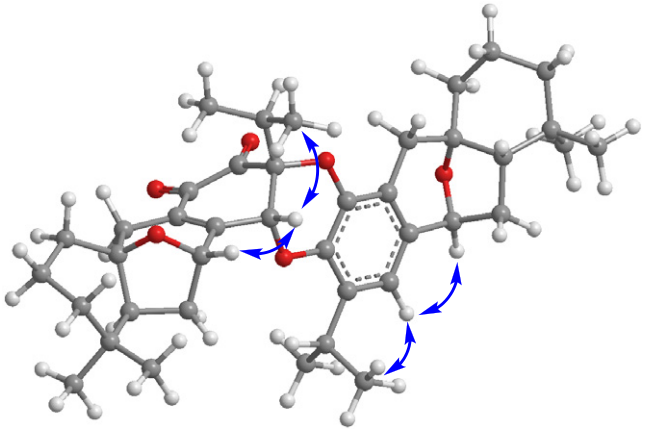


Figure 3. Key NOESY correlations of **1**.

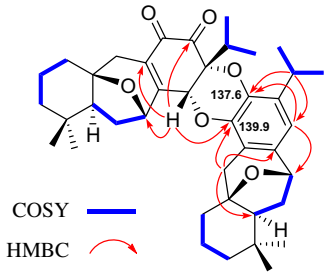


Figure 4. Key HMBC correlations of **2**.

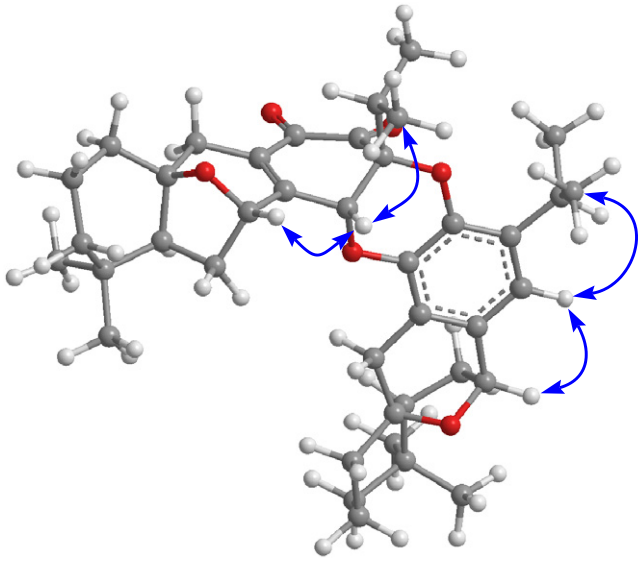
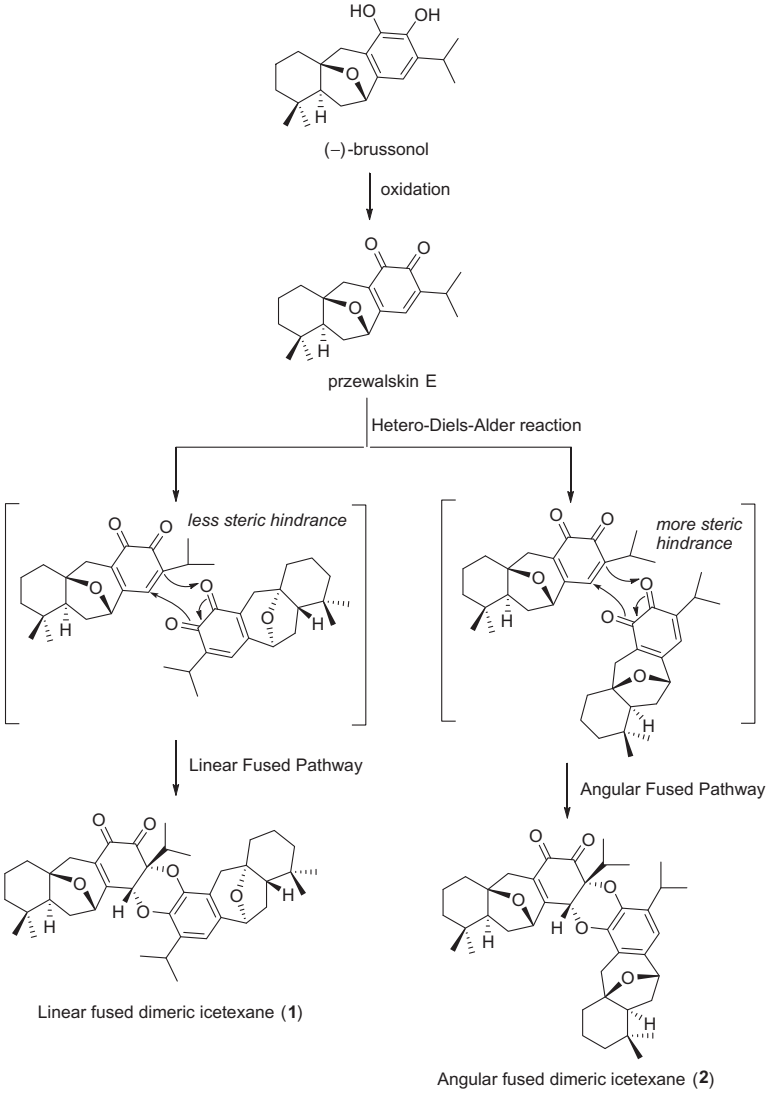


Figure 5. Key NOESY correlations of **2**.

peaks at m/z 316 $[M-314]^+$ and 314 $[M-316]^+$ corresponding to the fragment ions $C_{20}H_{28}O_3$ and $C_{20}H_{26}O_3$, respectively, which corresponded with the half structures of dimeric diterpene **1**. The UV spectrum (MeOH) showed absorption bands at 211 and 269 nm. The IR absorption bands at 1684, 1710, and 1750 cm^{-1} indicated the presence of an aromatic unit and two carbonyl moieties of a conjugated α-diketone group.

The ^{13}C NMR (Table 1) data, with the aid of DEPT analysis, revealed the presence of eight methyls, 10 methylenes, eight



Scheme 1. Proposed biosynthesis of compounds **1** and **2**.

methines (including three oxygenated and an aromatic), and 14 quaternary carbons [including two carbonyls (δ_C 185.7 and δ_C 191.7) and five oxygenated], respectively. The ^{13}C and ^{1}H NMR spectral data of a fragment A of compound **1** (Tables 1 and 2), were similar to those of przewalskin E (**3**), an *ortho*-quinone-type icetexane diterpenoid isolated from *Salvia prezwalskii* MAXIM,[17] except for the presence of a singlet oxymethine proton signal at δ_H 4.49 (1H, *s*, H-14; δ_C 72.9) instead of a singlet olefinic proton signal at δ 6.45 (1H, *s*, H-14; δ_C 131.9). For the fragment B of **1**, the ^{13}C and ^{1}H NMR (Tables 1 and 2) spectral data were almost identical to those of (−)-brussonol (**4**), an icetexane diterpenoid isolated from *Salvia broussonetii*.[18]

From the selected HMBC spectral data (Fig. 2), the oxymethine proton H-14 of fragment A at δ_H 4.49 showed correlations with C-12 (δ_C 191.7), C-9 (δ_C 135.6), C-7 (δ_C 73.9), and C-12′ (δ_C 138.3), while the methine proton H-15′ of the isopropyl side chain on fragment B at δ_H 3.13 showed correlations with C-12′ (δ_C 138.9) and C-14′ (δ_C 113.1), respectively, which proved that fragment A was connected to fragment B through a dioxane ring in a linear fashion.

In the NOESY spectrum of **1** (Fig. 3), the correlations between H-7 (δ_H 4.76) and H-14 (δ_H 4.49), and H-14 (δ_H 4.49) and Me-16 (δ_H 1.08), suggested that they were of co-facial orientation. Compound **1** was named obtusidione D, a linear fused dimeric icetexane diterpenoid.

Compound **2** was obtained as a yellow oil with negative optical rotation ($[\alpha]_D^{24}$ −195.5 in $CHCl_3$). The HREIMS spectrum of **2** showed a molecular ion peak at *m*/*z* 628.3684 $[M]^+$ (Calcd 628.8186), corresponding to a molecular formula of $C_{40}H_{52}O_6$. The UV (MeOH) and IR spectra were quite similar to those of **1**. The ^{1}H (Table 2 and Fig. S1) and ^{13}C NMR (Table 1) spectral data of **2** were also very similar to those of **1**. From selected HMBC spectral data (Fig. 4) of **2**, the oxymethine proton H-14 of fragment A at δ_H 4.62 showed correlations with C-12 (δ_C 191.2), C-9 (δ_C 135.8), C-7 (δ_C 73.9), and C-11′ (δ_C 139.9). The methine proton H-15′ of the isopropyl side chain on fragment B at δ_H 3.29 showed correlations with C-12′ (δ_C 137.6) and C-14′ (δ_C 114.8), respectively, which implied that fragment A was connected to fragment B via a dioxane ring in an angular fashion.

In the NOESY spectrum (Fig. 5) of **2**, the correlations between H-7 (δ_H 4.80) and H-14 (δ_H 4.62), and H-14 (δ_H 4.62) and Me-16 (δ_H 1.12), suggested that they were of co-facial orientation. Therefore, compound **2** was a structural isomer of **1** and was named obtusidione E.

Linear fused dimeric icetexane **1** was isolated in a higher yield than angular fused dimeric icetexane **2** (4:1 ratio). From the proposed biosynthetic pathway (Scheme 1), it is thought that compounds **1** and **2** were formed through hetero-Diels–Alder reactions of przewalskin E, involving the *ortho*-quinone units. To understand the origin of the regioselectivity of the hetero-Diels–Alder products **1** and **2**, a simple charge distribution was calculated and is summarized in Table S1. This study revealed that there was no significant difference between the electron density of the carbonyl oxygens at C-11 and C-12. Therefore, it is possible that a steric effect of bimolecular przewalskin E in the transition state could be a main factor in controlling the regioselectivity of the dimerization, giving compound **1** as the major product, that is, compound **1** was formed more favorably than **2** via a less sterically demanding route.

In conclusion, two new dimeric icetexane diterpenoids, obtusinones D and E (**1** and **2**), were isolated from the root extracts of *Premna obtusifolia*. Obtusinone D (**1**) represents the first example of a linear fused dimeric icetexane. The structures were fully characterized through spectroscopic methods. Our proposed biosynthesis indicates that **1** and **2** were formed through a hetero-Diels–Alder reaction of przewalskin E (**3**).

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Supplementary data

Supplementary data (experimental section, 1D and 2D NMR and mass spectra of compounds **1** and **2** and charge distribution calculations on przewalskin E) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.12.096.

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