ent-Halimane Diterpenes and a Guaiane Sesquiterpene from *Cladogynos orientalis*

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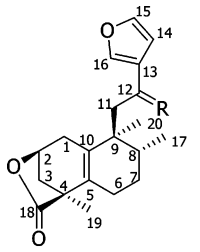
Four new *ent*-halimane diterpenes (**1**−**4**) and one new guaiane sesquiterpene (**5**) were isolated from the $CHCl_3$ extract of the roots of *Cladogynos orientalis*, together with six known compounds. The structures of compounds **1**−**5** were established using spectroscopic methods, and the stereochemistry of chettaphanin I (**6**) was confirmed by X-ray crystallography.

Cladogynos orientalis Zipp. ex Span. (syn. *Adenochlaena siamensis* Ridl.) (Euphorbiaceae), commonly known as "Chettaphangki" in Thailand, is the only member of the genus *Cladogynos*.[1] It is distributed sporadically in central and northeast Thailand, and the roots are used as a carminative in Thai folk medicine.[2] However, very little is known about the chemical constituents of *C. orientalis* and, so far, there have been only two previous phytochemical investigations, which reported the isolation and structure elucidation of two *ent*-halimane diterpenes, chettaphanins I and II,[3,4] from the roots of this plant. As a part of our chemical studies on Thai medicinal plants, we describe herein the isolation of four new *ent*-halimane diterpenes (**1**−**4**) and one new guaiane sesquiterpene (**5**), along with six known compounds, chettaphanin I (**6**),[3,5] chettaphanin II,[4,5] spathulenol,[6] cyperenoic acid,[7,8] taraxerol,[9,10] and acetoxyaleuritolate,[11−13] from the $CHCl_3$ extract of the roots of *C. orientalis*. The absolute stereochemistry of **6** was established by X-ray crystallographic analysis.



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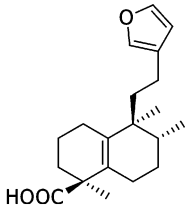
2 R = H_2



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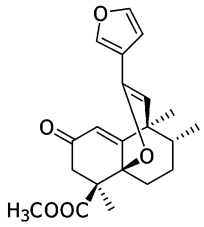

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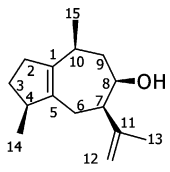

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Results and Discussion

Among four new diterpenes, the molecular formula of compound **1** was established as $C_{20}H_{24}O_4$ from its HR-FABMS and NMR data. The IR spectrum showed absorption bands due to a conjugated keto carbonyl (1671 cm^{-1}), a lactone ring (1757, 1276 cm^{-1}), and a furan ring (3122, 1509, 872 cm^{-1}). In the ^1H NMR spectrum, one secondary methyl group at δ_H 0.86 (d, J = 7.0 Hz, H_3-17) and two tertiary methyl groups at δ_H 1.07 and 1.32 (each s, H_3-20 and H_3-19) were observed. The signals at δ_H 6.73, 7.41, and 7.95 (1H each, H-14, H-15, and H-16) were characteristic of a β-substituted furan ring. In the ^{13}C NMR spectrum, 20 carbon signals appeared including three methyl carbons at δ_C 15.2 (C-17), 16.5 (C-19), and 21.9 (C-20), four furan ring carbons at δ_C 108.7 (C-14), 129.3 (C-13), 144.2 (C-15), and 147.6 (C-16), a lactone carbonyl carbon at δ_C 178.3 (C-18), and a ketone carbon at δ_C 193.6 (C-12). The signal patterns of the ^{13}C NMR spectrum were similar to those of methyl 15,16-epoxy-2-ethylenedioxy-12-oxo-5(10),13(16),-14-*ent*-halimatrien-18-oate.[5] This compound has been obtained as a semisynthetic product from *ent*-halimic acid (a bicyclic diterpene with known absolute configuration). In the case of **1**, there was evidence that a lactone ring was formed between C-2 and C-4, based on HMBC correlations from H_3-19 and H_a-3 to C-18, from H_a-1 and H_b-1 to C-2, and from H-2 to C-4 and C-10. The assignments of each signal were completed by analysis of HMQC, HMBC, and $^1H-^1H$ COSY NMR correlations (Figure 1). Thus, **1** was assigned as 6-[2-(furan-3-yl)-2-oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one.

Compound **2** was assigned to have the molecular formula $C_{20}H_{26}O_3$ from its HRFABMS and NMR data. The IR absorption bands indicated the presence of a lactone ring (1773, 1290 cm^{-1}) and a furan ring (3120, 1459, 873 cm^{-1}). Comparison of the NMR spectra of **1** and **2** (Tables 1 and 2) showed similarities except for the substitution of the keto carbonyl group at C-12 in **1** with a methylene group (δ_C 19.1) in **2**. The examination of 2D NMR data allowed us to deduce that **2** is 6-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.$0^{2,7}$]dodec-2(7)-en-11-one.

Compound **3** was assigned as a reductive cleaved product between the alkoxy bond of the lactone ring in **2** from its HRFABMS and NMR data. The ^1H (Table 1) and ^{13}C NMR (Table 2) spectra of **3** were similar to those of **2** except that the C-2 signal was assignable as a methylene group. The IR spectrum showed absorption bands due to a carboxylic acid (3600−2400, 1699 cm^{-1}), and the ^{13}C NMR spectrum also supported the presence of a carboxylic acid group (δ_C

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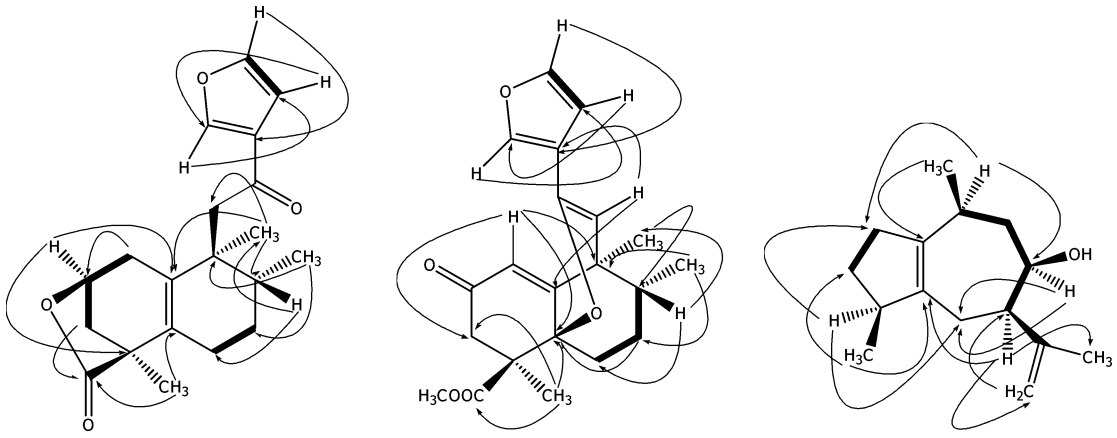


1 **4** **5**

Figure 1. Selected HMBC (curved arrows) and ^{1}H−^{1}H COSY (solid bold lines) correlations for **1**, **4**, and **5**.

Table 1. ^{1}H NMR Data of Compounds **1**−**5**[a]

position	1	2	3	4	5
1	2.33 (dd, 17.9, 2.7, H_a-1)[b]	2.39−2.45 (m)	1.89−2.02 (m, H_a-1)	5.90 (s)	
	2.40 (dddd, 17.9, 2.8, 2.8, 2.5, H_b-1)[b]		2.07−2.17 (m, H_b-1)		
2	4.76 (ddd, 6.0, 2.8, 2.7)[b]	4.81 (ddd, 5.5, 2.8, 2.5)[c]	1.74−1.81 (m)		2.10−2.17 (m, H_a-2)
					2.43−2.56 (m, H_b-2)
3	1.93 (d, 11.0, H_a-3)[b]	1.96 (d, 11.0, H_a-3)[c]	1.64−1.69 (m, H_a-3)	2.38 (d, 16.3, H_a-3)	1.26−1.32 (m, H_a-3)
	2.13 (dd, 11.0, 6.0, H_b-3)[b]	2.13 (dd, 11.0, 5.5, H_b-3)[c]	1.89−2.02 (m, H_b-3)	2.39 (d, 16.3, H_b-3)	1.93−2.01 (m, H_b-3)
4					2.43−2.56 (m)
6	2.10−2.19 (m)	1.99−2.21 (m)	1.34−1.44 (m, H_a-6)	1.89 (dd, 13.3, 4.8, H_a-6)	1.67−1.77 (m, H_a-6)
			1.89−2.02 (m, H_b-6)	2.34 (dd, 13.3, 4.8, H_b-6)	2.43−2.56 (m, H_b-6)
7	1.42−1.49 (m, H_a-7)	1.38−1.46 (m, H_a-7)	1.50−1.56 (m)	1.37−1.41 (m, H_a-7)	2.43−2.56 (m)
	1.74−1.81 (m, H_b-7)	1.61−1.65 (m, H_b-7)		2.12−2.20 (m, H_b-7)	
8	2.01−2.08 (m)	1.67−1.76 (m)	1.74−1.81 (m)	1.92−1.97 (m)	3.97−4.01 (m)
9					1.67−1.77 (m, H_a-9)
					1.93−2.01 (m, H_b-9)
10					2.32 (br s)
11	2.74 (d, 15.5, H_a-11)	1.58−1.59 (m, H_a-11)	1.64−1.69 (m)	4.80 (s)	
	2.85 (d, 15.5, H_b-11)	1.67−1.76 (m, H_b-11)			
12		1.99−2.21 (m, H_a-12)	2.07−2.17 (m, H_a-12)		4.78 (s, H_a-12)
		2.27−2.35 (m, H_b-12)	2.33−2.40 (m, H_b-12)		5.02 (s, H_b-12)
13					1.80 (s)
14	6.73 (dd, 2.0, 1.0)	6.24 (s)	6.26 (dd, 0.8, 0.8)	6.40 (dd, 0.8, 0.8)	0.98 (d, 7.0)
15	7.41 (dd, 2.0, 1.5)	7.33 (dd, 1.5, 1.5)	7.34 (dd, 1.5, 1.5)	7.33 (dd, 1.8, 1.8)	1.06 (d, 7.5)
16	7.95 (dd, 1.5, 0.5)	7.19 (d, 1.0)	7.20 (s)	7.47 (d, 1.0)	
17	0.86 (d, 7.0)	0.88 (d, 7.0)	0.87 (d, 7.0)	0.88 (d, 7.0)	
18					
19	1.32 (s)	1.31 (s)	1.30 (s)	1.42 (s)	
20	1.07 (s)	0.90 (s)	0.86 (s)	1.17 (s)	
OCH_3				3.54 (s)	
OH					1.57 (s)

[a] Chemical shift values are in ppm from $CDCl_3$ and J values (in Hz) are presented in parentheses. The assignments are based on decoupling, HMQC, HMBC, and ^{1}H−^{1}H COSY experiments. [b] Assignment of coupling constants: δ_H 1.93 (d, $J_{Ha-3,Hb-3}$ = 11.0 Hz), 2.13 (dd, $J_{Hb-3,Ha-3}$ = 11.0 Hz, $J_{Hb-3,H-2}$ = 6.0 Hz), 2.33 (dd, $J_{Ha-1,Hb-1}$ = 17.9 Hz, $J_{Ha-1,H-2}$ = 2.7 Hz), 2.40 (dddd, $J_{Hb-1,Ha-1}$ = 17.9 Hz, $J_{Hb-1,H-2}$ = 2.8 Hz, $J_{Hb-1,Hb-3}$ = 2.8 Hz, $J_{Hb-1,H-6}$ = 2.5 Hz), 4.76 (ddd, $J_{H-2,Hb-3}$ = 6.0 Hz, $J_{H-2,Ha-1}$ = 2.7 Hz, $J_{H-2,Hb-1}$ = 2.8 Hz). [c] Assignment of coupling constants: δ_H 1.96 (d, $J_{Ha-3,Hb-3}$ = 11.0 Hz), 2.13 (dd, $J_{Hb-3,Ha-3}$ = 11.0 Hz, $J_{Hb-3,H-2}$ = 5.5 Hz), 4.81 (ddd, $J_{H-2,Hb-3}$ = 5.5 Hz, $J_{H-2,Ha-1\ and\ Hb-1}$ = 2.5 and 2.8 Hz).

183.1). Thus, **3** was deduced to be 5-[2-(furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid.

The relative stereochemistry of compounds **1**−**3** could not be completely established by application of NOE experiments. However, it would be reasonable to deduce that the three methyl groups at C-4, C-8, and C-9 in these diterpenes are in a *cis* orientation to one another because of the co-isolation of the structurally related **6** in this study, the absolute stereochemistry of which was determined by X-ray crystallography, as described below.

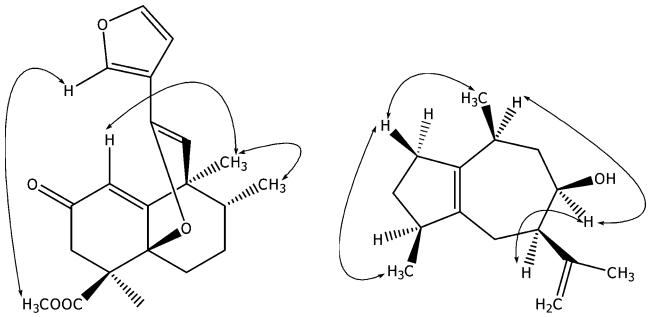
The molecular formula of compound **4** was analyzed as $C_{21}H_{24}O_5$ from its HRFABMS and NMR data. The IR spectrum showed absorption bands due to an ester carbonyl group (1763, 1276 cm^{-1}), a conjugated keto carbonyl group (1676 cm^{-1}), and a furan ring (3150, 1458, 920 cm^{-1}). The ^{13}C NMR spectrum was similar to that of chettaphanin I (**6**),[6] except for the lack of a C-12 keto carbonyl group, instead of the presence of olefinic carbons at δ_C 103.2 (C-11) and 146.2 (C-12). Detailed assignments of each signal using 2D NMR data (Figure 1) including NOE correlations (Figure 2) led to a tricyclic system through an enol ether linkage to the structure of **4**. The close proximity of the methyl ester group at C-4 to the H-16 furan ring proton was also indicated by a NOE experiment.

It is reasonable to suppose that the ether bridge between C-5 and C-12 in **4** could be built up by intramolecular hemiacetal formation of the 12-keto group with a *cis*-oriented OH-5 group, as in compound **7**, the C-5 epimer of **6**, followed by dehydration, as shown in Scheme 1. However, the epimer is unknown. The absolute stereochemistry of **6** has been determined by chemical correlation to natural

Table 2. ^{13}C NMR Data of Compounds **1**−**5**[a]

carbon	1	2	3	4	5
1	31.6	31.2	25.1	121.8	139.6
2	74.0	74.4	19.5	196.4	33.8
3	41.1	41.2	35.4	45.5	30.8
4	43.6	43.5	47.4	51.4	46.1
5	132.1	133.5	131.0	79.5	140.9
6	22.2	24.5	25.9	31.7	26.0
7	25.5	26.2	26.8	26.5	49.7
8	33.2	32.4	33.3	42.5	68.3
9	40.3	39.9	40.9	41.4	42.0
10	132.4	133.9	136.0	157.7	29.2
11	47.7	37.9	36.5	103.2	148.0
12	193.6	19.1	19.5	146.2	112.3
13	129.3	125.3	125.8	121.3	23.0
14	108.7	110.9	111.0	107.2	20.0
15	144.2	142.7	142.6	143.2	21.6
16	147.6	138.5	138.4	139.5	
17	15.2	15.7	16.0	14.3	
18	178.3	178.8	183.1	173.9	
19	16.5	17.0	22.9	20.1	
20	21.9	21.4	20.8	22.3	
OCH_3				52.1	

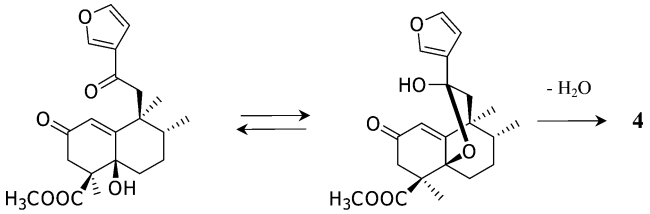
[a] Chemical shift values are in ppm from $CDCl_3$. The assignments are based on decoupling, HMQC, HMBC, and $^1H-^1H$ COSY experiments.



4 **5**

Figure 2. Selected NOE correlations for **4** and **5**.

Scheme 1. Possible Formation of **4** from **7**, the C-5 Epimer of **6**



ent-halimic acid and by NOE experiments,[5] in which these functionalities are situated in a *trans* arrangement. Accordingly, the stereochemistry of **6** isolated in this study was re-examined. A single crystal of **6** was prepared carrying $CHCl_3$ in its molecule by recrystallization from hexane−$CHCl_3$. The X-ray crystallographic analysis of the $CHCl_3$-containing crystal (Figure 3) indicated that the reported stereochemistry of **6** including the absolute configuration was correct, in which C-5 is in the *S* configuration.

Compound **5** was analyzed as a sesquiterpene with the molecular formula $C_{15}H_{24}O$ from its HRFABMS and NMR data. The IR spectrum showed an absorption band due to a hydroxyl group (3448 cm^{-1}). The ^1H and ^{13}C NMR data of **5** were almost identical to those of the known α-guaiene,[14] except for an additional hydroxyl signal. The downfield shift of the ^1H NMR signal due to H-8 (δ_H 3.39) implied **5** to be 8-hydroxy-α-guaiene. The protons and carbons in **5** were completely assigned by analysis of its 2D NMR spectra (Figure 1). The NOE correlation (Figure 2) between H-8 and H-7 and H-10 indicated that substitutions at those positions were situated in a *cis* orientation to each other. Thus, **5** was identified as (4*S**,7*R**,8*R**,10*S**)-8-hydroxy-α-guaiene.

All isolated products were tested for cytotoxic activity toward a human small cell lung cancer cell line (NCI-H187) and for antituberculosis activity using *M. tuberculosis* $H_{37}Ra$. However, none of these compounds exhibited significant cytotoxicity (IC_{50} < 5 μg/mL) or antituberculosis activity (MIC < 12.5 μg/mL).

Experimental Section

General Experimental Procedures. Melting points were determined using a MP-S3 micromelting point hot stage (Yanagimoto). Optical rotations were measured on a JASCO P-1020 polarimeter. UV absorption spectra were measured on a JASCO V-560 UV spectrophotometer. IR spectra were recorded on a JASCO FT/IR-300E spectrometer. ^1H NMR (400, 500 MHz) and ^{13}C NMR (100, 125 MHz) spectra were measured on JEOL JNM-ECP400 and JNM-GSX500A NMR spectrometers. Mass spectra were obtained by a JEOL JMS-HX110 (FABMS) spectrometer. TLC was performed using Merck precoated plates (Silica gel 60 F_{254}) of 0.25 mm thickness. Silica gel FL100D (Fuji Silysia Chemical Ltd.) was used for column chromatography.

Plant Material. The plant material was collected from the World Biosphere Reserve, Sakaeraj Environmental Research Station, Nakorn-Rachasima Province, Thailand, in October 2002. Authentication was achieved by comparison with a herbarium specimen (BKF No. 28024) at the National Park, Wildlife and Plant Conservation Department, Ministry of Natural Resources and Environment, Bangkok, Thailand. A voucher specimen (NSR 090251) has been deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

Extraction and Isolation. The dried, powdered roots of *C. orientalis* (4.5 kg) were extracted successively with $CHCl_3$ (5 × 20 L) and then with MeOH (3 × 20 L). Removal of the solvent from the extract under reduced pressure gave 208.6 g ($CHCl_3$) and 227.3 g (MeOH) of dark brown oily residues. The $CHCl_3$-soluble part (208.6 g) was chromatographed on a silica gel column with hexane−$CHCl_3$−MeOH (1:0:0 → 0:1:0 → 0:0:1). The eluted fractions were evaluated by TLC to give eight main fractions. Fraction 2 (18.2 g), eluted with a gradient of hexane−EtOAc (50:1 → 0:1), was purified by column chromatography (hexane−EtOAc, 20:1) to furnish compound **5** (50.7 mg). Fraction 3 (6.8 g), eluted with hexane−EtOAc (20:1 → 0:1), was rechromatographed on a silica gel column (hexane−CH_2Cl_2, 2:1) to give the following compounds, in order of increasing polarity: spathulenol (62.5 mg), compound **4** (32.7 mg), and compound **3** (3.2 mg). Fraction 4 (16.6 g) was subjected to a silica gel column (hexane−ether, 5:1 → 0:1) to give fractions 4-1 to 4-9. Fraction 4-2 (2.1 g) was crystallized from a hexane−$CHCl_3$ mixture to afford acetoxyaleuritolate (162.5 mg). Fraction 4-7 (1.4 g) was crystallized from hexane−$CHCl_3$ to afford taraxerol (79.0 mg), and the mother liquid was further purified with silica gel column chromatography (hexane−CH_2Cl_2, 1:1) to give chettaphanin II (25.2 mg). Fraction 5 (20.2 g), eluted with hexane−$CHCl_3$ (1:2 → 0:1) and then $CHCl_3$− EtOAc (1:0 → 0:1), was chromatographed over silica gel (CH_2Cl_2−EtOAc, 1:0 → 0:1) to give compound **2** (4.1 mg) and compound **1** (33.4 mg). Repeated chromatography of fraction 6 (9.8 g) with hexane−EtOAc (3:1) yielded chettaphanin I (**6**) (258.0 mg). Fraction 7 (113.9 g) was crystallized from hexane−$CHCl_3$ to yield cyperenoic acid (295.9 mg).

6-[2-(Furan-3-yl)-2-oxoethyl]-1,5,6-trimethyl-10-oxatricyclo[7.2.1.0^{2,7}]dodec-2(7)-en-11-one (1): pale yellow amorphous solid; mp 103−105 °C; $[\alpha]^{23}_D$ −151.5° (*c* 0.017, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 230 (4.13) nm; IR (KBr) ν_{max} 3122, 1757, 1671, 1509, 1276, 872 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 329.1727 (calcd for $C_{20}H_{25}O_4$, $[M + H]^+$ 329.1753).

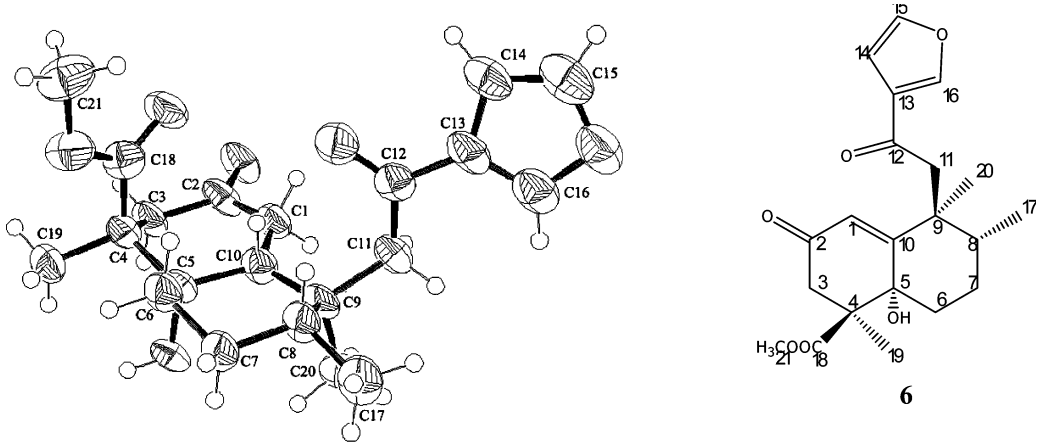


Figure 3. ORTEP drawing of **6**. The chloroform molecule is omitted for clarity.

6-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-10-oxatricyclo-[7.2.1.0^{2,7}]dodec-2(7)-en-11-one (2): pale yellow oil; $[\alpha]^{23}_{D}$ −88.6° (*c* 0.0017, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.19) nm; IR (film) ν_{max} 3120, 1773, 1459, 1290, 1024, 873 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 315.1990 (calcd for $C_{20}H_{27}O_3$, $[M + H]^+$ 315.1960).

5-[2-(Furan-3-yl)ethyl]-1,5,6-trimethyl-1,2,3,4,5,6,7,8-octahydronaphthalene-1-carboxylic acid (3): pale yellow oil; $[\alpha]^{23}_{D}$ −23.2° (*c* 0.0013, MeOH); UV (MeOH) λ_{max} (log ϵ) 299 (3.23) nm; IR (film) ν_{max} 3600−2400 (br), 2929, 1699, 1458, 1190, 938 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 317.2108 (calcd for $C_{20}H_{29}O_3$, $[M + H]^+$ 317.2117).

Methyl 9-(furan-3-yl)-2,7,13-trimethyl-4-oxo-10-oxatricyclo[5.3.3.0^{1,6}]trideca-5,8-diene-2-carboxylate (4): pale yellow oil; $[\alpha]^{23}_{D}$ +56.1° (*c* 0.015, MeOH); UV (MeOH) λ_{max} (log ϵ) 239 (4.19) nm; IR (film) ν_{max} 3150, 1736, 1676, 1458, 1276, 920 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 357.1685 (calcd for $C_{21}H_{25}O_5$, $[M + H]^+$ 357.1702).

8-Hydroxy-α-guaiene (5): pale yellow oil; $[\alpha]^{23}_{D}$ −65.1° (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ϵ) 264 (2.99) nm; IR (film) ν_{max} 3448, 3100, 2926, 1457, 1023 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRFABMS *m/z* 259.1487 (calcd for $C_{15}H_{24}OK$, $[M + K]^+$ 259.1464).

X-ray data for Chettaphanin I (6)·CHCl$_3$:[15] colorless prisms, mp 157−158 °C by crystallization of **6** from hexane−$CHCl_3$ and selected for data collection. Crystal data: $C_{22}H_{29}Cl_3O_6$, M = 495.80 g mol^{-1}, orthorhombic, $P2_12_12_1$, a = 7.338(3) Å, b = 11.777(5) Å, c = 26.354(12) Å, U = 2277.5(18) Å^3, T = 173 K, Z = 4, μ(Mo Kα) = 0.439 mm^{-1}, 5438 reflections measured, 2094 were unique (R_{int} = 0.1894) and used in all calculations. The final $wR(F^2)$ was 0.3330 (all data). The data were measured using a Bruker SMART CCD diffractometer, using Mo Kα graphite-monochromated radiation (λ = 0.71073 Å). The structures were solved by direct methods using the program SHELXS-97.[16] The refinement and all further calculations were carried out using SHELXL-97. The H atoms were included in calculated positions and treated as riding atoms using the SHELXL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Figure 3 is drawn with ORTEP.[17]

Cytotoxicity Assays.[18] Cytotoxicity was assessed using the sulforhodamine B (SRB) assay using human tumor cell lines of NCI-H187 (small cell lung cancer). The cells were incubated at 37 °C for 72 h, at which time the SRB was added. The results are expressed as an IC_{50} (μg/mL), and the reference substance was ellipticine.

Antituberculosis Assays.[19] Antituberculosis activity was performed by a microplate alamar blue assay. *M. tuberculosis* H_{37}Ra was used as a tested microorganism. The MICs of the tested compounds were measured in μg/mL, and the reference substances were isoniazid and kanamycin sulfate.

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Supporting Information Available: X-ray data for chettaphanin I. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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