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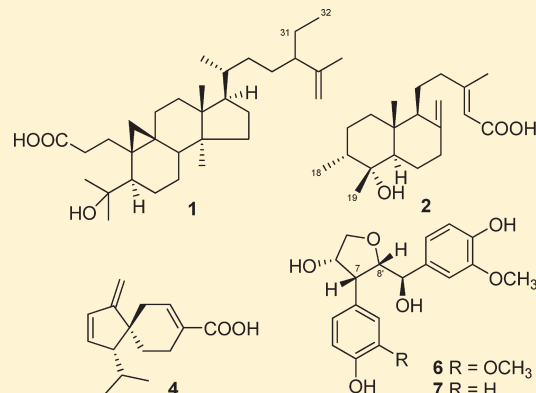
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Terpenoids and Norlignans from *Metasequoia glyptostroboides*Liao-Bin Dong,^{†,‡} Juan He,[†] Yuan-Yuan Wang,[†] Xing-De Wu,^{†,‡} Xu Deng,^{†,‡} Zheng-Hong Pan,[§] Gang Xu,[†] Li-Yan Peng,[†] Yu Zhao,[†] Yan Li,[†] Xun Gong,[†] and Qin-Shi Zhao^{*,†}[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming 650204, People's Republic of China[‡]Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China[§]Guangxi Institute of Botany, Chinese Academy of Science, Guilin 541006, People's Republic of China

Supporting Information

ABSTRACT: Four new terpenoids, metaseglyptorin A (1), metasequoic acid C (2), 12 α -hydroxy-8,15-isopimaradien-18-oic acid (3), and (–)-acora-2,4(14),8-trien-15-oic acid (4), and three new norlignans, metasequirins D–F (5–7), were isolated from *Metasequoia glyptostroboides*, together with 15 known compounds. Structures of the new compounds were determined by analysis of their spectroscopic data, and the absolute configuration of 7 was established by the modified Mosher method. All of the compounds were evaluated for cytotoxicity against five human tumor cell lines.

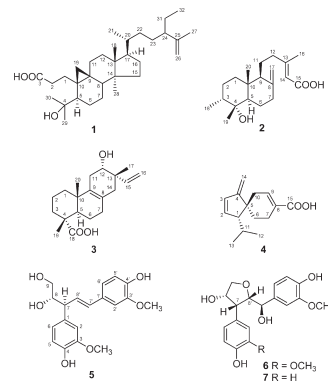


Metasequoia glyptostroboides Hu et Cheng (Taxodiaceae) is a monotypic genus, endemic in China, which is well known as a “living fossil” species. Since the first living *M. glyptostroboides* was discovered in the south of China in the early 1940s, many chemical investigations were carried out to get a better understanding of this genus. The earlier studies on this plant reported flavonoids,¹ norlignans,² sesquiterpenoids,³ labdane-type diterpenoids,^{4,5} abietane-type diterpenoids,⁶ and sterols.⁷ Our current investigation of the stems and leaves of *M. glyptostroboides* led to the isolation of four new terpenoids (1–4), three new norlignans (5–7), and 15 known compounds. All compounds were evaluated for cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480). This paper describes the isolation, structural characterization, and cytotoxic activities of the compounds from *M. glyptostroboides*.

RESULTS AND DISCUSSION

An EtOH extract of the stems and leaves of *M. glyptostroboides* was partitioned between H₂O and EtOAc. The EtOAc portion was subjected to MCI, silica gel, Sephadex LH-20, and semipreparative HPLC to afford the new compounds metaseglyptorin A (1), metasequoic acid C (2), 12 α -hydroxy-8,15-isopimaradien-18-oic acid (3), (–)-acora-2,4(14),8-trien-15-oic acid (4), metasequirins D–F (5–7), and 15 known compounds. The known compounds (see Supporting Information), compared with literature data, were identified as 3-hydroxylabda-8(20),13-dien-15-oic

acid, 3-acetoxylabda-8(20),13-dien-15-oic acid,⁴ 8 α -hydroxylabda-13(16),14-dien-19-yl-*cis*-4-hydroxycinnamate,⁸ 12S,13R-dihydroxylabda-8(17),14-dien-19-oic acid, 12S,13S-dihydroxylabda-8(17),14-dien-19-oic acid,^{9,10} 15-norlabda-8(20),12E-diene-14-carboxaldehyde-19-oic acid,¹¹ 15,16-bisnor-13-oxo-8(17),11E-labdadien-19-oic acid,¹² 8 β -hydroxy-isopimar-15-en-19-oic acid,¹³ sequoempervirin B, sequoempervirin D, sequoempervirin F,¹⁴ agatharesinol,¹⁵ agatharesinol acetonide,¹⁶ sequirin C,¹⁷ and hinokiresinol.¹⁸



Compound 1 had the molecular formula C₃₂H₅₄O₃, as evidenced by the positive HRFABMS at *m/z* 487.4163 [M + H]⁺,

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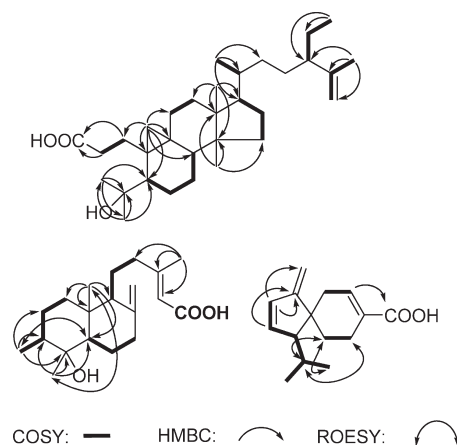
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Table 1. ^{13}C NMR and ^1H NMR Spectroscopic Data for Compound **1**^a in CDCl_3 (125 and 500 MHz)

no.	δ_{C} , mult	δ_{H} (J in Hz)	no.	δ_{C} , mult	δ_{H} (J in Hz)
1a	30.2, CH_2	2.65, m	17	52.4, CH	1.53, m
1b		1.37, m			
2a	32.0, CH_2	2.65, m	18	18.4, CH_3	0.94, s
2b		2.30, m			
3	179.5, qC		19a	31.1, CH_2	0.66, d (6.0)
			19b		0.55, d (6.0)
4	76.7, qC		20	35.8, CH	1.35, m
5	45.1, CH	1.88, m	21	18.1, CH_3	0.84, d (6.0)
6a	25.4, CH_2	1.71, m	22a	33.9, CH_2	1.35, m
6b		0.66, m	22b		1.27, m
7a	25.7, CH_2	1.27, m	23	29.7, CH_2	1.20, m
7b		0.98, d (11.5)			
8	48.6, CH	1.27, m	24	49.5, CH	1.85, m
9	22.7, qC		25	147.6, qC	
10	26.6, qC		26a	111.4, CH_2	4.73, br s
			26b		4.64, br s
11a	26.6, CH_2	2.11, m	27	17.7, CH_3	1.56, s
11b		1.15, m			
12	33.1, CH_2	1.65, d (8.8)	28	19.5, CH_3	0.90, s
13	44.8, qC		29	26.0, CH_3	1.22, s
14	48.8, qC		30	31.7, CH_3	1.24, s
15a	36.0, CH_2	1.35, m	31a	26.6, CH_2	1.31, m
15b		1.26, m	31b		1.12, m
16a	28.1, CH_2	1.87, m	32	12.1, CH_3	0.80, t (7.4)
16b		1.28, m			

^a Assignments are based on 1D and 2D NMR experiments.

indicating six degrees of unsaturation. IR absorption bands at 3441, 1709, and 2959–2871 cm^{-1} indicated the presence of OH and carbonyl functionalities. In the ^1H NMR spectrum (Table 1), seven methyls [δ_{H} 0.80 (3H, t, $J = 7.4$ Hz, Me-32), 0.84 (3H, d, $J = 6.0$ Hz, Me-21), 0.90 (3H, s), 0.94 (3H, s), 1.22 (3H, s), 1.24 (3H, s), and 1.56 (3H, s)], a pair of upfield doublets [δ_{H} 0.66 (1H, d, $J = 6.0$ Hz, Ha-19) and 0.55 (1H, d, $J = 6.0$ Hz, Hb-19)], and terminal olefinic protons at δ_{H} 4.73 (1H, br s, Ha-26) and 4.64 (1H, br s, Hb-26) suggested that **1** was a typical cycloartane triterpenoid with a 24-ethyl side-chain. This was supported by the ^{13}C and DEPT NMR spectra, which exhibited 32 carbon signals ascribable to seven methyls, 12 methylenes, five methines, five quaternary carbons [including one oxygenated carbon (δ_{C} 76.7, s, C-4)], a terminal double bond (δ_{C} 147.6, s, C-25; 111.4, t, C-26), and a carbonyl group (δ_{C} 179.5, s, C-3). In the HMBC spectrum, correlations of δ_{H} 1.24 (3H, s, Me-30) with δ_{C} 76.7 (s, C-4), 45.1 (d, C-5), and 26.0 (q, C-29) and of δ_{H} 2.65 (1H, m, Ha-2) and 2.30 (1H, m, Hb-2) with δ_{C} 179.5 (s, C-3) suggested that the usual A ring of **1** was open between C-3 and C-4. Detailed 2D NMR (HSQC, ^1H – ^1H COSY, HMBC, ROESY) data analysis (Figure 1) indicated that the other parts of **1** were the same as those of 24S-ethyl-4,4-dimethylphytosterol.^{19,20} However, since the crystals obtained could not meet the required standard for a single-crystal X-ray experiment after many attempts, the configuration at C-24 was left undetermined. Thus, the structure of **1** was determined as 3,4-secocycloarta-4-hydroxy-24-ethyl-25-en-3-oic acid, and it was named metaseglyptorin A.

**Figure 1.** Key 2D NMR correlations of **1**, **2**, and **4**.

Compound **2** had the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}_3$ (by HRE-IMS), and the IR spectrum exhibited bands for OH (3440 cm^{-1}) and α,β -unsaturated carbonyl groups (1692, 1641 cm^{-1}). The ^{13}C and DEPT NMR data (Table 2) showed the existence of four methyls (δ_{C} 13.0, 13.5, 18.2, 24.1), six methylenes, three methines, one quaternary carbon, a cyclohexane methyldene (δ_{C} 147.3, 106.3), and an α,β -unsaturated carbonyl group (δ_{C} 168.9, 160.8, 115.0, 73.4). The spectroscopic data of **2** were very similar to those of 3-hydroxylabda-8(20),13-dien-15-oic acid.⁴ Analysis of the 1D NMR spectra of the two compounds revealed marked differences: signals for a singlet methyl and an oxymethine in 3-hydroxylabda-8(20),13-dien-15-oic acid were replaced by a doublet methyl (δ_{H} 0.84, d, $J = 7.3$ Hz, Me-18) and an oxygenated quaternary carbon (δ_{C} 73.4, s, C-4) in **2**. This information suggested that one of the *gem*-dimethyl groups at C-4 in 3-hydroxylabda-8(20),13-dien-15-oic acid was at C-3 in **2**. This was confirmed by the HMBC correlations of δ_{H} 0.84 (d, $J = 7.3$ Hz, Me-18) with δ_{C} 26.4 (t, C-2), 39.6 (d, C-3), and 73.4 (s, C-4) and of δ_{H} 0.96 (s, Me-19) with δ_{C} 39.6 (d, C-3), 49.1 (d, C-5), and 73.4 (s, C-4) (Figure 1).

The relative configuration of compound **2** was determined by a ROESY experiment (Figure 1). Biogenetically, Me-20 was β -oriented and H-5 was α -oriented in labdane-type diterpenoids.^{4,5} The ROESY correlations of Me-20/Me-19 and H-5/Me-18 demonstrated that Me-19 was β -oriented and Me-18 was α -oriented. Thus, compound **2** was established as 4 α -hydroxy-methyl-18-(4 \rightarrow 3 α)-abeolabda-8(17),*E*-13-dien-15-oic acid, designated as metasequoic acid C.

Compound **3** had the molecular formula $\text{C}_{20}\text{H}_{30}\text{O}_3$, and the IR spectrum indicated OH (3432 cm^{-1}), carbonyl (1696 cm^{-1}), and olefinic (1640 cm^{-1}) functionalities. 1D NMR experiments showed that **3** had a carbonyl group (δ_{C} 182.4, qC, C-18), a tetrasubstituted olefin (δ_{C} 134.1, s, C-9; 125.1, s, C-8), a terminal double bond [δ_{C} 140.8, d, C-15; δ_{H} 5.88 (1H, dd, $J = 17.4$, 11.0 Hz, H-15); δ_{C} 114.7, t, C-16; δ_{H} 5.13 (1H, dd, $J = 10.7$, 1.2 Hz, Ha-16); 5.08 (1H, dd, $J = 17.9$, 1.4 Hz, Hb-16)], one oxymethine group [δ_{C} 74.6, d, C-12; δ_{H} 3.53 (1H, dd, $J = 9.2$, 5.5 Hz, H-12)], three quaternary carbons, one tertiary carbon, seven secondary carbons, and three methyl groups. The data were similar to those of 8,15-isopimaradiene-18-oic acid,²¹ except that compound **3** had one more OH group (at C-12), as evidenced by the ^1H – ^1H COSY correlations of δ_{H} 3.53 (1H, dd, $J = 9.2$, 5.5 Hz, H-12) with δ_{H} 1.90 (1H, m, Ha-11) and 1.81 (1H, m, Hb-11) and the HMBC correlation of δ_{H} 3.53 (1H, dd,

Table 2. ^{13}C NMR and ^1H NMR Spectroscopic Data for Compounds 2–4^a in CDCl_3 (100 and 400 MHz)

no.	2 ^b		3		4	
	δ_{C} , mult	δ_{H} (J in Hz)	δ_{C} , mult	δ_{H} (J in Hz)	δ_{C} , mult	δ_{H} ^c (J in Hz)
1a	31.3, CH_2	1.24, m	36.6, CH_2	1.75, m	60.6, CH	2.31, m
1b		1.17, m		1.03, m		
2a	26.4, CH_2	1.59, m	19.3, CH_2	1.83, m	137.2, CH	6.03, br d (7.0)
2b		1.27, m		1.49, br d (14.1)		
3a	39.6, CH	1.60, m	37.4, CH_2	2.16, m	133.5, CH	6.20, dd (7.0, 2.0)
3b				1.00, m		
4	73.4, qC		43.6, qC		162.0, qC	
5	49.1, CH	1.41, dd (10.9, 2.1)	53.1, CH	1.35, br d (11.9)	45.6, qC	
6a	22.7, CH_2	1.68, br d (10.4)	20.4, CH_2	1.90, m	25.1, CH_2	1.72, m
6b		1.18, m		1.81, m		
7a	37.4, CH_2	2.23, br d (13.0)	32.3, CH_2	1.91, m	21.6, CH_2	2.45, m
7b		1.80, m				
8	147.3, qC		125.1, qC		129.0, qC	
9	55.7, CH	1.50, br d (10.9)	134.1, qC		142.1, CH	7.13, m
10a	39.9, qC		38.1, qC		40.2, CH_2	2.42, br d (19.6)
10b						2.19, ddd (19.6, 6.0, 2.9)
11a	21.4, CH_2	1.47, m	30.9, CH_2	1.90, m	27.7, CH	1.92, m
11b		1.32, m		1.81, m		
12a	39.4, CH_2	2.12, m	74.6, CH	3.53, dd (9.2, 5.5)	19.7, CH	0.80, d (6.7)
12b		1.79, m				
13	160.8, qC		40.3, qC		23.1, CH_3	0.96, d (6.7)
14a	115.0, CH	5.45, br s	42.8, CH_2	1.96, m	101.8, CH_2	4.82, br s
14b				1.90, m		4.59, br s
15	168.9, qC		140.8, CH	5.88, dd (17.4, 11.0)	172.4, qC	
16a	18.2, CH_3	1.94, br s	114.7, CH_2	5.13, dd (10.7, 1.2)		
16b				5.06, dd (17.9, 1.4)		
17a	106.3, CH_2	4.68, s	23.8, CH_3	1.09, s		
17b		4.33, s				
18	13.0, CH_3	0.84, d (7.3)	182.4, qC			
19	24.1, CH_3	0.96, s	28.5, CH_3	1.26, s		
20	13.5, CH_3	0.48, s	17.5, CH_3	0.89, s		

^a Assignments are based on 1D and 2D NMR experiments. ^b In CDCl_3 – CD_3OD . ^c 500 MHz.

$J = 9.2, 5.5$ Hz, H-12) with δ_{C} 40.3 (s, C-13). The ROESY correlation of H-12 with Me-17 revealed β -orientation of H-12. Thus, compound 3 was identified as 12 α -hydroxy-8,15-isopimaradiene-18-oic acid.

The HRESIMS of compound 4 showed a protonated molecular ion at m/z 233.1538 $[\text{M} + \text{H}]^+$, corresponding to the molecular formula $\text{C}_{15}\text{H}_{20}\text{O}_2$ with six degrees of unsaturation. The ^{13}C and DEPT NMR spectra showed that 4 had an α,β -unsaturated carbonyl group (δ_{C} 172.4, 129.0, and 142.1), a cyclopentane methylenes (δ_{C} 162.0 and 101.8), a disubstituted olefin (δ_{C} 137.2 and 133.5), one quaternary carbon, two tertiary carbons, three secondary carbons, and two methyls. In the ^1H NMR spectrum, the two methyls at δ_{H} 0.80 and 0.96 (each 3H, d, $J = 6.7$ Hz, Me-12, 13) and a methine at δ_{H} 1.92 (1H, m, H-11) suggested the presence of an isopropyl group. These data suggested that 4 possessed a structure similar to that of 15-hydroxyacora-4(14),8-diene,²² except that C-15 was oxidized to a carboxyl group and the presence of one more double bond was located at C-2/C-3 in 4. These structural differences were confirmed by HMBC correlation of δ_{H} 7.13 (1H, m, H-9) with δ_{C} 172.4 (s, C-15) and the ^1H – ^1H COSY correlation of δ_{H} 2.31

(1H, m, H-1) with δ_{H} 6.03 (1H, br d, $J = 7.0$ Hz, H-2). The relative configuration of 4 was elucidated by the ROESY experiment (Figure 1), and the correlations of H-11/H-6, H-11/H-7, and H-12/H-6 placed these groups in close proximity. Thus, compound 4 was determined to be (–)-acora-2,4(14),8-triene-15-oic acid.

Compound 5 had the molecular formula $\text{C}_{19}\text{H}_{22}\text{O}_6$. The IR spectrum showed the presence of OH (3433 cm^{-1}) and aromatic (1629 and 1514 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 3) revealed signals of two ABX systems of two benzene rings [δ_{H} 6.99 (1H, d, $J = 1.8$ Hz, H-2), 6.75 (1H, d, $J = 8.0$ Hz, H-5), 6.81 (1H, dd, $J = 8.0, 2.0$ Hz, H-6) and δ_{H} 7.03 (1H, d, $J = 2.0$ Hz, H-2'), 6.73 (1H, d, $J = 8.2$ Hz, H-5'), 6.83 (1H, d, $J = 8.2$ Hz, H-6')] together with signals of two aromatic O-methyl groups at δ_{H} 3.83 (6H, s), indicating the presence of two 1,3,4-trisubstituted aromatic rings. Correlations in the ^1H – ^1H COSY and HSQC spectra revealed the presence of a $\text{CH}_2(9)$ – $\text{CH}(8)$ – $\text{CH}(7)$ – $\text{CH}(8')=\text{CH}(7')$ unit. The HMBC correlations from H-7 at δ_{H} 3.49 (1H, t, $J = 7.5$ Hz) to C-1 (δ_{C} 134.2, s) and H-7' at δ_{H} 6.35 (1H, d, $J = 15.8$ Hz) to C-1' (δ_{C} 130.7, s) suggested that C-7 was connected to C-1 and C-7' was linked

Table 3. ^{13}C NMR and ^1H NMR Spectroscopic Data for Compounds 5–7^a in CD_3COCD_3 (100 and 400 MHz)

no.	5		6		7	
	δ_{C}^b , mult	δ_{H} (J in Hz)	δ_{C} , mult	δ_{H} (J in Hz)	δ_{C} , mult	δ_{H} (J in Hz)
1	134.2, qC		134.7, qC		134.1, qC	
2	113.2, CH	6.99, d (1.8)	112.0, CH	6.36, d (2.0)	129.2, CH	6.86, d (8.6)
3	148.0, qC		148.0, qC		115.9, CH	6.67, d (8.6)
4	145.8, qC		145.6, qC		156.5, qC	
5	115.5, CH	6.75, d (8.0)	115.5, CH	6.65, d (8.0)	115.9, CH	6.67, d (8.6)
6	122.1, CH	6.81, dd (8.0, 2.0)	120.0, CH	6.51, dd (8.0, 2.0)	129.2, CH	6.86, d (8.6)
7	53.4, CH	3.49, t (7.5)	54.0, CH	3.25, dd (4.8, 2.8)	54.1, CH	3.25, dd (4.8, 3.0)
8	75.7, CH	3.97, m	79.2, CH	4.23, br s	79.7, CH	4.15, br s
9a	65.5, CH_2	3.61, dd (11.1, 3.6)	75.9, CH_2	4.04, dd (9.2, 4.4)	75.6, CH_2	4.04, dd (9.2, 4.6)
9b		3.45, dd (10.8, 7.2)		3.84, dd (9.2, 2.7)		3.81, dd (8.8, 3.2)
1'	130.7, qC		134.1, qC		134.0, qC	
2'	109.9, CH	7.03, d (2.0)	110.8, CH	7.02, d (1.6)	110.9, CH	6.99, d (1.8)
3'	148.3, qC		148.0, qC		147.9, qC	
4'	146.9, qC		146.4, qC		146.3, qC	
5'	115.7, CH	6.73, d (8.2)	115.2, CH	6.75, d (8.0)	115.1, CH	6.73, d (8.0)
6'	120.3, CH	6.83, d (8.2)	119.9, CH	6.86, dd (8.2, 1.8)	120.0, CH	6.82, dd (8.0, 2.0)
7'	131.0, CH	6.35, d (15.8)	74.8, CH	4.88, br s	75.0, CH	4.85, br s
8'	129.6, CH	6.40, dd (15.8, 8.0)	91.2, CH	4.18, dd (4.8, 3.5)	90.8, CH	4.19, dd (4.8, 3.6)
3-OCH ₃	56.1, CH ₃	3.83, s	56.0, CH ₃	3.68, s		
3'-OCH ₃	56.1, CH ₃	3.83, s	56.2, CH ₃	3.78, s	56.1, CH ₃	3.77, s

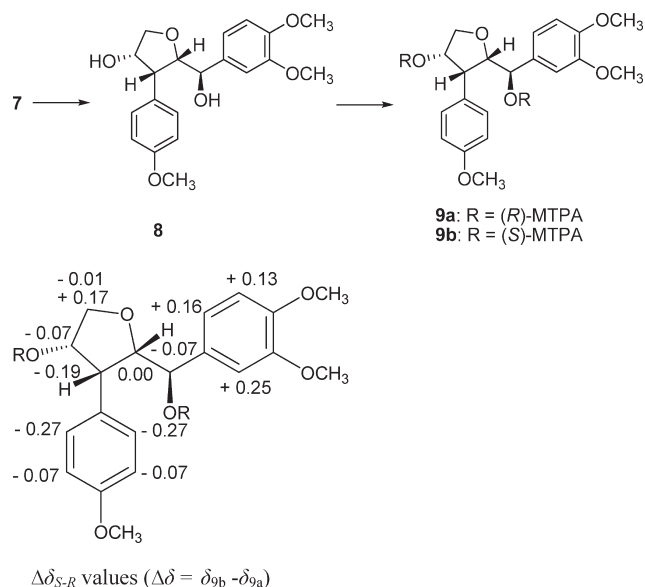
^a Assignments are based on 1D and 2D NMR experiments. ^b 500 MHz.

to C-1'. The assignments of 7S, 8S of **5** were confirmed by comparing its 1D NMR and specific rotation ($[\alpha]_{\text{D}}^{25} -40.5$) data with those of sequeempervirin B, whose structure was unambiguously established by a single-crystal X-ray experiment.¹⁴ Thus, compound **5** was named as metasequirin D.

Compound **6** gave a peak at 385.1272 $[\text{M} + \text{Na}]^+$ in the HRESIMS, providing a molecular formula of $\text{C}_{19}\text{H}_{22}\text{O}_7$, 16 mass units higher than that of **5**. The ^1H NMR spectrum (Table 3) also displayed signals of two 1,3,4-trisubstituted aromatic rings. Compared to **5**, signals for the C-7'–C-8' double bond were absent in **6** and were replaced by two oxymethine carbons at δ_{C} 74.8 (d, C-7') and 91.2 (d, C-8'). On the basis of the molecular formula, one ring was needed to meet the required degrees of unsaturation. HMBC correlations were observed from H-9a (δ_{H} 4.04, dd, $J = 9.2, 4.4$ Hz) and H-9b (δ_{H} 3.84, dd, $J = 9.2, 2.7$ Hz) to C-8' (δ_{C} 91.2, d), supporting the structure of metasequirin E as **6**.

Compound **7** was isolated as a colorless gum. The HRESIMS displayed an $[\text{M} + \text{Na}]^+$ peak at m/z 355.1160 ($\text{C}_{18}\text{H}_{20}\text{O}_6$), 30 mass units less than that of **6**. According to the 1D NMR data (Table 3), compound **7** was readily identified as an analogue of **6** in which the OMe at C-3 was replaced by H. Analysis of 2D NMR data confirmed that the other parts of **7** were the same as those of **6**. Thus, compound **7** was named metasequirin F.

The absolute configurations of **6** and **7** were deduced from interpretation of ROESY experiments and utilization of the modified Mosher method. The modified Mosher method²³ was applied to determine the absolute configurations of the secondary alcohols in **7**. From the values of $\Delta\delta$ ($\delta_{\text{S}} - \delta_{\text{R}}$) (Figure 2), the absolute configurations at C-8 and C-7' were assigned as 8S and 7'R, respectively. In **6** and **7**, the ROESY correlations of H-7/H-8', H-7'/H-8, H-8/H-9a, and H-6/H-9b as well as the small coupling constants of H-7 ($J = 4.8, 3.0$ Hz)²⁴ indicated H-7, H-8, and H-8'

Figure 2. Determination of the absolute configuration of **7**.

were on one side. Accordingly, the absolute configurations of **6** and **7** were established as 7R, 8S, 7'R, 8'S.

Structurally, compound **1** is a 24-ethyl cycloartane triterpenoid with an opened A ring, which is also the first triterpenoid reported from the genus *Metasequoia*. Compound **4** represents the first example of an acorane-type sesquiterpenoid from the family Taxodiaceae.

All of the compounds were evaluated for cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) using the MTT method as reported previously.²⁵

Cisplatin was used as the positive control. 8 α -Hydroxyabda-13(16),14-dien-19-yl-*cis*-4-hydroxycinnamate and sequirin C showed moderate cytotoxicity against HL-60, with IC₅₀ values of 14.3 and 5.5 μ M, respectively, while cisplatin gave IC₅₀ values of 2.0 μ M. The other compounds were inactive (IC₅₀ values >40 μ M).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 micro melting point apparatus. Optical rotations were measured on a JASCO-20C digital polarimeter. IR spectra were obtained on a Tensor 27 spectrometer with KBr pellets. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer. 1D and 2D NMR spectra were performed on Bruker AM-400, DRX-500, or AVANCE III-600 spectrometers with TMS as an internal standard. Mass spectra were taken on VG Auto Spec-3000 or API-Qstar-Pulsar instruments. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm \times 25 cm) column. Column chromatography (CC) was performed using silica gel (100–200 and 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, People's Republic of China), MCI gel (75–150 μ m; Mitsubishi Chemical Corporation, Japan), and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden).

Plant Material. Stems and leaves of *M. glyptostroboides* were collected in the Kunming Botany Garden, Kunming, Yunnan Province, People's Republic of China, in May 2009, and were identified by one of the authors (X.G.). A voucher specimen (200905M) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The air-dried and powdered stems and leaves of *M. glyptostroboides* (19 kg) were extracted with 95% EtOH (3 \times 70 L), each for 48 h, at room temperature, and concentrated in vacuo. The crude extract was partitioned between H₂O and EtOAc. The EtOAc portion (436 g) was decolorized on MCI gel (eluted with 90% MeOH) and then was chromatographed on a silica gel column (100–200 mesh) eluting with a gradient of petroleum ether–acetone (1:0, 9:1, 8:2, 7:3, 3:2, and 0:1) to afford six fractions (A–F). Fraction C (21 g) was fractionated by MPLC (MCI) eluting with MeOH–H₂O (from 30% to 100%) to provide subfractions (C₁–C₃). Subfraction C₂ was recrystallized to afford 3-acetoxyabda-8(20),13-dien-15-oic acid (5 g). Subfraction C₃ was chromatographed over Sephadex LH-20 eluted with MeOH and then chromatographed repeatedly over silica gel, then by semipreparative HPLC (83% MeOH–H₂O), to give 4 (6 mg), 8 α -hydroxyabda-13(16),14-dien-19-yl *cis*-4-hydroxycinnamate (30 mg), 15,16-bisnor-13-oxo-8(17),11E-labdadien-19-oic acid (7 mg), and 8 β -hydroxy-isopimar-15-en-19-oic acid (20 mg). Fraction D (40.5 g) was chromatographed on MPLC (MCI gel) (0:1 \rightarrow 1:0, MeOH–H₂O) to give subfractions D₁–D₃. Compounds 2 (5 mg), 3 (1.8 mg), 12S,13R-dihydroxyabda-8(17),14-dien-19-oic acid (3 mg), 12S,13S-dihydroxyabda-8(17),14-dien-19-oic acid (4 mg), and 15-norabda-8(20),12E-diene-14-carboxaldehyd-19-oic acid (6 mg) were isolated from subfraction D₁ by repeated chromatography including silica gel, MCI, and Sephadex LH-20. 3-Hydroxyabda-8(20),13-dien-15-oic acid (2 g) was crystallized from subfraction D₂ directly. Subfraction D₃ was subjected to repeated silica gel CC eluted with petroleum ether–acetone (9:1 \rightarrow 0:1) and then by semipreparative HPLC (69% MeOH–H₂O) to obtain sequeempervirin D (20 mg), agatharesinol acetonide (30 mg), and hinokiresinol (15 mg). The acetone-insoluble part of fraction D (2 g) was chromatographed over Sephadex LH-20 eluting with CHCl₃–MeOH (1:1) and then by semipreparative HPLC (94% MeOH–H₂O) to afford 1 (20 mg). Fraction E (36.6 g) was submitted to repeated chromatography and purified by Sephadex LH-20 and semipreparative HPLC to afford 5 (35 mg), 6 (12 mg), 7 (50 mg), sequeempervirin B (60 mg), sequeempervirin F (45 mg), agatharesinol (20 mg), and sequirin C (50 mg).

Metaseglyptorin A (1): colorless plates (MeOH); mp 140–142 $^{\circ}$ C; [α]_D²⁵ +29.6 (c 0.41, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 239 (2.49), 235 (2.35), 229 (2.44), 217 (2.64), 198 (2.84) nm; IR (KBr) ν_{\max} 3441, 2959, 2933, 2871, 1709, 1642, 1460, 1375, 1192, 1034 cm^{−1}; ¹H and ¹³C NMR data, see Table 1; positive FABMS m/z 487 [M + H]⁺; positive HRFABMS m/z 487.4163 [M + H]⁺ (calcd for C₃₂H₅₅O₃, 487.4151).

Metasequoic Acid C (2): colorless gum; [α]_D²⁵ +20.5 (c 0.30, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 240 (3.35), 222 (2.71), 217 (2.73) nm; IR (KBr) ν_{\max} 3440, 2930, 1692, 1641, 1463, 1384, 1247, 1157 cm^{−1}; ¹H and ¹³C NMR data, see Table 2; positive EIMS m/z 320 [M]⁺; positive HREIMS m/z 320.2356 [M]⁺ (calcd for C₂₀H₃₂O₃, 320.2351).

12 α -Hydroxy-8,15-isopimaradien-18-oic acid (3): colorless gum; [α]_D²⁵ +135.6 (c 0.09, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 240 (3.01), 227 (2.78), 204 (2.88), 198 (2.90) nm; IR (KBr) ν_{\max} 3432, 2957, 2927, 1696, 1640, 1466, 1377, 1252 cm^{−1}; ¹H and ¹³C NMR data, see Table 2; positive ESIMS m/z 341 [M + Na]⁺; positive HRESIMS m/z 341.2091 [M + Na]⁺ (calcd for C₂₀H₃₀O₃Na, 341.2092).

(−)-*Acora-2,4(14),8-trien-15-oic acid (4)*: colorless gum; [α]_D²⁵ −59.2 (c 0.32, CHCl₃); UV (CHCl₃) λ_{\max} (log ϵ) 241 (3.60), 231 (3.11), 222 (3.14), 212 (3.10), 203 (3.10) nm; IR (KBr) ν_{\max} 3440, 2960, 1689, 1640, 1423, 1273 cm^{−1}; ¹H and ¹³C NMR data, see Table 2; positive ESIMS m/z 233 [M + H]⁺; positive HRESIMS m/z 233.1538 [M + H]⁺ (calcd for C₁₅H₂₁O₂, 233.1541).

Metasequirin D (5): colorless gum; [α]_D²⁵ −40.5 (c 0.17, MeOH); UV (MeOH) λ_{\max} (log ϵ) 269 (4.03), 204 (4.41), 194 (4.11) nm; IR (KBr) ν_{\max} 3433, 1629, 1514, 1272, 1032 cm^{−1}; ¹H and ¹³C NMR data, see Table 3; positive ESIMS m/z 369 [M + Na]⁺; positive HRESIMS m/z 369.1317 [M + Na]⁺ (calcd for C₁₉H₂₂O₆Na, 369.1314).

Metasequirin E (6): colorless gum; [α]_D²⁵ +22.2 (c 0.25, MeOH); UV (MeOH) λ_{\max} (log ϵ) 281(3.49), 229 (3.84), 204 (4.41) nm; IR (KBr) ν_{\max} 3430, 1612, 1517, 1273, 1033 cm^{−1}; ¹H and ¹³C NMR data, see Table 3; positive ESIMS m/z 385 [M + Na]⁺; positive HRESIMS m/z 385.1272 [M + Na]⁺ (calcd for C₁₉H₂₂O₇Na, 385.1263).

Metasequirin F (7): colorless gum; [α]_D²⁵ +8.7 (c 0.32, MeOH); UV (MeOH) λ_{\max} (log ϵ) 279 (3.18), 226 (3.71), 204 (3.91), 193 (3.58) nm; IR (KBr) ν_{\max} 3441, 1620, 1516, 1271, 1033 cm^{−1}; ¹H and ¹³C NMR data, see Table 3; positive ESIMS m/z 355 [M + Na]⁺; positive HRESIMS m/z 355.1160 [M + Na]⁺ (calcd for C₁₈H₂₀O₆Na, 355.1157).

Methylation of the Phenolic OH Groups of 7. Diazomethane in ether was added to a solution of 7 (8 mg, 24 μ mol) in anhydrous tetrahydrofuran (1 mL), which was stirred under N₂ at room temperature overnight. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel CC (petroleum ether–acetone, 7:3) to afford 8 (8 mg, 100%): ¹H NMR (400 MHz, CD₃COCD₃) δ 3.27 (1H, m, H-7), 3.71, 3.73, and 3.74 (each 3H, s, −OCH₃), 3.80 (1H, dd, J = 9.1, 3.0 Hz, H-9b), 4.04 (1H, dd, J = 9.1, 4.8 Hz, H-9a), 4.16 (1H, m, H-8), 4.20 (1H, t, J = 4.8 Hz, H-8'), 4.86 (1H, br s, H-7'), 6.74 (2H, d, J = 8.5 Hz, H-3/5), 6.81 (1H, d, J = 8.5 Hz, H-6'), 6.89 (1H, dd, J = 8.5, 1.8 Hz, H-5'), 6.94 (2H, d, J = 8.5 Hz, H-2/6), 6.98 (1H, d, J = 1.8 Hz, H-2').

Preparation of the (S)- and (R)-MTPA Esters of 8. A mixture of 8 (2 mg, 5.5 μ mol), (R)-MTPA (5 mg, 21.4 μ mol, 3.9 equiv), DCC (4 mg, 19.4 μ mol, 3.5 equiv), and DMAP (2 mg, 16.4 μ mol, 3.0 equiv) was dissolved in anhydrous CH₂Cl₂ (0.5 mL), which was stirred under N₂ at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by silica gel CC (petroleum ether–acetone, 9:1) to yield bis-(R)-MTPA ester 9a (1.5 mg). Bis-(S)-MTPA ester 9b (1.2 mg) was prepared in the same manner.

Bis-(R)-MTPA Ester of 8 (9a): ¹H NMR (600 MHz, CDCl₃) δ 3.33 (1H, dd, J = 6.2, 4.8 Hz, H-7), 3.79 (1H, dd, J = 10.2, 3.0 Hz, H-9b), 4.26 (1H, dd, J = 10.2, 5.4 Hz, H-9a), 4.42 (1H, t, J = 6.2 Hz, H-8'), 5.34 (1H, m, H-8), 5.84 (1H, d, J = 6.2 Hz, H-7'), 6.55 (1H, d, J = 1.8 Hz,

H-2'), 6.68 (1H, dd, $J = 8.2, 1.8$ Hz, H-5'), 6.71 (1H, d, $J = 8.2$ Hz, H-6'), 6.81 (2H, d, $J = 8.4$ Hz, H-3/5), 7.01 (2H, d, $J = 8.4$ Hz, H-2/6).

Bis-(S)-MTPA Ester of **8 (**9b**):** ^1H NMR (600 MHz, CDCl_3) δ 3.14 (1H, dd, $J = 4.8, 3.0$ Hz, H-7), 3.96 (1H, dd, $J = 11.4, 3.0$ Hz, H-9b), 4.25 (1H, dd, $J = 11.4, 4.8$ Hz, H-9a), 4.42 (1H, dd, $J = 7.9, 4.8$ Hz, H-8'), 5.27 (1H, m, H-8), 5.77 (1H, d, $J = 7.9$ Hz, H-7'), 6.74 (4H, br s, H-2/3/5/6), 6.80 (1H, s, H-2'), 6.81 (1H, d, $J = 8.2$ Hz, H-5'), 6.87 (1H, dd, $J = 8.2, 1.8$ Hz, H-6').

Cytotoxicity Assay. Cytotoxicity of all compounds against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines was assessed using the MTT method.²⁵ Cells were plated in 96-well plates 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well, which were incubated for another 4 h. Then 20% SDS (100 μL) was added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The IC_{50} value of each compound was calculated by the Reed and Muench method.²⁶

■ ASSOCIATED CONTENT

S Supporting Information. 1D and 2D NMR spectra of **1**, **2**, **4**, and **6**, 1D NMR spectra of **3**, **5**, and **7**, MS spectra of **1**–**7**, ^1H NMR spectra of **8**, **9a**, and **9b**, and COSY NMR spectra of the MTPA esters **9a** and **9b** are available free of charge via the Internet at <http://pubs.acs.org>.

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