ent-Rosane and Labdane Diterpenoids from *Sagittaria sagittifolia* and Their Antibacterial Activity against Three Oral Pathogens

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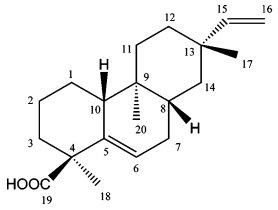
Seven new *ent*-rosane diterpenoids, sagittines A−G (**1**−**7**), together with one new labdane diterpene, 13-epi-manoyl oxide-19-*O*-α-L-2′,5′-diacetoxyarabinofuranoside (**8**), were isolated from the whole plant of *Sagittaria sagittifolia*. The structures and relative configurations of **1**−**8** were characterized using spectroscopic means, chemical methods, and X-ray crystallography. Compounds **1**−**4** exhibited antibacterial activity against the oral pathogens *Streptococcus mutans* ATCC 25175 and *Actinomyces naeslundiis* ATCC 12104, with MIC values between 62.5 and 125 μg/mL. Compound **5** was active against only *A. naeslundiis* ATCC 12104, with an MIC value of 62.5 μg/mL.

Sagittaria sagittifolia L. (Alismaceae) is an aquatic herb found locally in the ponds and banks of rivers of the People's Republic of China. The tubers of *S. trifolia* have been used for treatment of skin diseases in traditional Chinese medicine. In early work, sagittariol and 18-deoxysagittariol, isolated from the whole plant of *S. trifolia* collected in India, were shown to be clerodane diterpenes.[1−3] A series of pimarane- and labdane-type diterpenoids were obtained from fresh tubers of *S. sagittifolia* collected in Japan, among which trifoliones A−D exhibited inhibitory effects on histamine release from rat mast cells induced by compound 48/80 and calcium ionophore A-23187.[4,5]

In the present work, seven new *ent*-rosane diterpenoids, named sagittines A−G (**1**−**7**), together with one new labdane diterpene, 13-epi-manoyl oxide-19-*O*-α-L-2′,5′-diacetoxyarabinofuranoside (**8**), were isolated. In this paper, we report the isolation and structure elucidation of these new diterpenoids from the whole plant of *S. sagittifolia*. The antibacterial activity of the isolated compounds against three oral pathogens (*Streptococcus mutans* ATCC 25175, *Actinomyces naeslundiis* ATCC 12104, and *Actinobacillus actinomycetemcomitans* ATCC 43717) has been determined.



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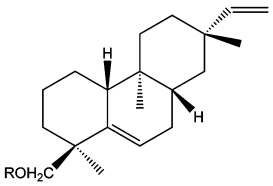
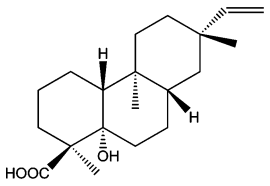


2 R=arabinofuranosyl
3 R=5'-acetoxyarabinofuranosyl
4 R=2'-acetoxyarabinofuranosyl
5 R=2',5'-diacetoxyarabinofuranosyl
6 R=3',5'-diacetoxyarabinofuranosyl



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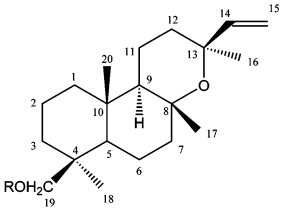

8 R=2',5'-diacetoxyarabinofuranosyl

Results and Discussion

The dried herb of *S. sagittifolia* was extracted with 95% EtOH. The concentrated extract was partitioned between petroleum ether and water, with the petroleum ether fraction further fractionated by silica gel column chromatography followed by passage over Sephadex LH-20 to afford eight new diterpenoids (**1**−**8**).

Compound **1** was obtained as colorless needles, and its molecular formula $C_{20}H_{30}O_2$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 325.2247, calcd 325.2245). The IR spectrum showed absorption peaks at 3470 cm^{-1} (hydroxyl group) and 1725 cm^{-1} (carboxyl group). The ^1H and ^{13}C NMR signals (Tables 1 and 2) of **1** were assigned by different 2D NMR experiments. The combined analysis of its ^1H and ^{13}C NMR spectra revealed the presence of 20 carbons assigned to three methyl groups [(δ_H 0.67 (δ_C 12.6), 1.01 (22.4), and 1.36 (25.2)], a vinyl group on a quaternary carbon [δ 4.83 (d, J = 10.8 Hz, H-16b), 4.90 (d, J = 17.6 Hz, H-16a), 5.80 (dd, J = 10.8, 17.6 Hz, H-15)], a trisubstituted olefin [δ_H 5.70 (d, J = 5.6 Hz, H-6), δ_C 121.9 (C-6), 139.3 (C-5)], two methines, seven methylenes, three quaternary carbons, and a carboxyl group (δ 183.6). From the above data, accordingly, a pimarane or a rosane derivative with a double bond was proposed. The HMBC spectrum (Figure 1) of compound **1** showed the $^2J_{H,C}$-connectivities between CH_3-20 and C-9, as well as the $^3J_{H,C}$-connectivities of CH_3-20/C-8 and CH_3-20/C-10 indicating that CH_3-20 should be placed at C-9. Further comparison of the chemical shifts with those of related compounds indicated that an *ent*-rosane with a 5(6)-double bond was present.[6−13] In the HMBC spectrum (Figure 1), the correlations of H-6/C-4, H-6/C-10, and H-18/C-5 also indicated the presence of a 5(6)-double bond group, and the correlations of H-3b/C-19 and H-18/C-19 showed that the carboxyl group was at the C-19 position. The stereochemistry of **1** was determined by a NOESY experiment (Figure 1). The NOE correlations of CH_3-20/H-1b, H-2b/H-10, and H-8/H-10 suggested the α-axial orientation of CH_3-20 and thus the β-axial orientations of H-8 and H-10. The absence of correlations between CH_3-18 and both H-2b and H-10 revealed that CH_3-18 was at the α-equatorial position. Observation of NOE effects at CH_3-17/H-8 and CH_3-17/H-11b indicated the β-axial

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Table 1. ^{1}H NMR (400 MHz) Data of Compounds **1**−**8** in $CDCl_3$ (δ values; *J* values in parentheses)

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1a	1.78 dd (3.2, 12.8)	1.76 d (12.4)	1.78 d (13.2)	1.77 d (12.9)	1.79 d (13.0)	1.75 d (13.2)	1.62 m	1.59 d (11.6)
1b	1.07 d (9.6)	1.01 m	1.06 m	1.02 m	1.03 m	1.03 m	1.56 m	0.83 m
2a	1.68 d (10.4)	1.53 m	1.57 m	1.55 m	1.55 m	1.51 m	1.62 m	1.60 m
2b	1.45 d (11.2)	1.39 m	1.41 m	1.41 m	1.44 m	1.44 m	1.35 d (10.4)	1.47 m
3a	2.18 d (13.2)	1.62 d (12.5)	1.61 d (11.6)	1.70 d (12.0)	1.69 d (11.5)	1.77 d (13.2)	1.81 d (15.2)	1.36 m
3b	1.18 d (13.2)	1.06 d (12.0)	1.10 d (13.2)	1.07 m	1.10 d (11.9)	1.01 m	1.66 d (10.4)	1.27 m
4								
5								1.23 m
6a	5.70 d (5.6)	5.52 t (2.4)	5.54 t (2.8)	5.54 t (2.4)	5.54 t (2.4)	5.54 t (2.4)	2.09 dt (4.4, 13.2)	1.46 m
6b							1.72 d (14.0)	1.23 m
7a	1.80 d (17.6)	1.67 m	1.71 m	1.68 m	1.68 m	1.69 m	1.46 d (20)	1.72 d (10.4)
7b	1.71 d (12.0)	1.65 m	1.65 m	1.66 m	1.66 m	1.64 m	1.13 d (14.8)	1.32 m
8	1.56 m	1.42 m	1.43 m	1.43 m	1.42 s	1.42 m	1.42 m	
9								1.20 m
10	1.95 d (12.8)	1.83 d (12.8)	1.86 d (11.8)	1.84 d (12.5)	1.83 d (12.0)	1.86 d (12.8)	1.77 d (14.0)	
11a	1.62 d (10.8)	1.61 d (13.0)	1.63 d (13.2)	1.63 d (12.6)	1.64 d (12.8)	1.61 d (11.7)	1.53 d (8.0)	1.48 m
11b	1.29 d (9.6)	1.20 d (12.6)	1.23 d (12.0)	1.23 d (13.0)	1.23 d (11.5)	1.23 d (12.0)	1.14 t (13.8)	1.43 m
12a	1.47 t (12.8)	1.43 t (16.0)	1.45 t (18.0)	1.45 t (15.6)	1.45 t (15.6)	1.44 t (13.9)	1.53 t (15.2)	2.18 m
12b	1.22 d (13.6)	1.21 d (11.2)	1.22 d (10.4)	1.23 d (11.0)	1.21 d (11.5)	1.21 d (12.2)	1.16 d (12.8)	1.44 m
13								
14a	1.26 dd (6.4, 12.0)	1.22 dd (6.0, 14.0)	1.24 dd (6.0, 13.2)	1.23 dd (5.6, 13.2)	1.24 dd (6.0, 13.8)	1.23 m	1.38 m	5.99 dd (10.8, 18.0)
14b	1.12 d (10.0)	1.08 d (13.2)	1.09d (13.6)	1.08 dd (4.0, 13.6)	1.07 d (12.9)	1.07 t (3.2)	0.98 d (11.6)	
15a	5.80 d (10.8, 17.6)	5.77 dd (10.8, 17.6)	5.79 dd (10.8, 17.6)	5.78 dd (10.4, 17.2)	5.78 dd (10.8, 17.6)	5.78 dd (10.8, 17.6)	5.79 dd (10.8, 17.6)	4.95 d (18.0)
15b								4.89 d (10.8)
16a	4.90 d (17.6)	4.87 dd (1.2, 17.2)	4.89 dd (1.2, 17.6)	4.89 dd (1.2, 17.6)	4.89 dd (1.2, 17.6)	4.89 dd (1.2, 17.6)	4.89 dd (0.8, 17.2)	1.11 s
16b	4.83 d (10.8)	4.80 dd (1.2, 10.8)	4.82 dd (1.2, 10.8)	4.82 dd (1.2, 10.8)	4.82 dd (1.2, 10.2)	4.81 dd (1.2, 10.4)	4.82 dd (0.8, 10.8)	
17	1.01 s	0.96 s	0.98 s	0.97 s	0.98 s	0.98 s	1.02 s	1.19 s
18	1.36 s	1.02 s	1.04 s	1.04 s	1.04 s	1.03 s	1.20 s	1.23 s
19a		3.74 d (9.2)	3.75 d (9.2)	3.73 d (9.2)	3.73 d (9.2)	3.77 d (9.2)		3.27 d (9.2)
19b		2.99 d (9.2)	3.07 d (9.2)	3.04 d (9.2)	3.07 d (9.2)	2.93 d (9.2)		3.13 d (9.2)
20	0.67 s	0.61 s	0.64 s	0.63 s	0.63 s	0.63 s	0.88 s	0.73 s
1′		4.90 br s	4.94 br s	4.99 br s	4.99 br s	4.93 br s		4.99 br s
2′		3.98 s	4.06 d (1.2)	4.81 d (2.4)	4.82 dd (0.8, 2.8)	4.21 d (5.6)		4.82 d (2.8)
3′		3.95 s	3.86 br s	3.97 br s	3.87 dd (2.1, 5.6)	4.62 dd (2.0, 5.6)		3.89 dd (2.8, 5.6)
4′		4.06 d (2.8)	4.19 dd (2.4, 4.0)	4.07 m	4.17 d (2.0)	4.10 d (2.0)		4.18 m
5′**a**		3.82 d (2.8)	4.27 d (4.4)	3.86 dd (2.8, 12.0)	4.30 dd (3.0, 4.5)	4.38 dd (3.0, 4.8)		4.30 d (7.2)
5′**b**		3.79 d (2.4)	4.27 d (4.4)	3.70 d (9.2)	4.16 d (2.2)	4.23 d (2.0)		4.16 d (7.2)
2′-OAc COC*H*$_3$				2.08 s	2.08 s			2.11 s
3′-OAc COC*H*$_3$						2.08 s		
5′-OAc COC*H*$_3$			2.08 s		2.07 s	2.09 s		2.08 s

orientation of CH_3-17. This was confirmed by the relatively upfield signal of CH_3-17 (δ 22.4), showing that this group was axial, i.e., with β orientation.[11] The relative configuration of **1** was further confirmed by X-ray crystallographic analysis (Figure 2). In conclusion, the structure of compound **1** (sagittine A) was established as *ent*-rosa-5,15-dien-19-oic acid.

Compound **2** was obtained as a colorless oil, and its molecular formula $C_{25}H_{40}O_5$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 443.2872, calcd 443.2875). The IR spectrum showed 1640 cm^{-1} (olefinic bond) and 3505 cm^{-1} (hydroxyl group) absorption peaks. The molecule appeared to be a glycoside, and the ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed the presence of 25 carbons assigned to three methyl groups, a vinyl moiety on a quaternary carbon, a trisubstituted olefin, two methines, seven methylenes, three quaternary carbons, a hydroxymethyl group from the aglycon, and a pentosyl moiety. The aglycon of compound **2** was the same as that of compound **1**, except that the carboxylic acid in **1** was replaced with a hydroxymethyl group (δ 3.74, d, J = 9.2 Hz, H-19a; and 2.99, d, J = 9.2 Hz, H-19b) in **2**. The position of the hydroxymethyl group was determined at C-19 by the HMBC correlations between H-19a (and H-19b) and C-4, C-5, and C-18 (Figure 1). The ^{13}C NMR signals measured in $CDCl_3$ of the pentosyl moiety at δ 108.0, 79.2, 77.5, 86.3, and 61.4 (Table 2) and the anomeric proton signal at δ 4.90 (br s) (Table 1), in addition to the NOESY correlations of H-1′/H-3′and H-2′/H-4′, suggested the presence of an arabinofuranosyl group in the α-form.[14−16] The identification of the sugar moiety was confirmed by acid hydrolysis of **1** and GC-MS analysis with an authentic sample. The absolute configuration of the pentose was determined to be L-arabinose by GC-MS analysis of its 1-[(*S*)-*N*-acetyl-α-methylbenzylamine)]-1-deoxyalditol acetate prepared with chiral α-methylbenzylamine (MBA) in the presence of sodium cyanoborohydride followed by peracetylation, as previously reported.[18,19] The HMBC correlations between the anomeric proton at δ 4.90 (H-1′) and C-19 indicated that the sugar residue was linked to the C-19 position. The NOESY experiment (Figure 1) showed that the stereochemistry of compound **2** was consistent with

Table 2. ^{13}C NMR (100 MHz) Data (δ) of Compounds **1**−**8** in $CDCl_3$

position	**1**[a]	**2**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]
1	25.6	25.8	26.4	25.8	25.9	25.9	26.0	23.0	38.8
2	22.9	21.1	21.8	21.2	21.2	21.2	21.3	20.4	17.8
3	37.6	35.0	35.2	35.2	35.0	35.0	34.8	33.7	36.0
4	48.2	39.7	40.5	39.8	39.9	39.8	39.9	51.8	36.9
5	139.3	141.2	142.3	141.1	141.2	141.1	141.4	75.0	49.9
6	121.9	120.7	120.4	120.8	120.7	120.8	120.5	33.6	19.8
7	30.1	30.2	30.7	30.3	30.3	30.2	30.3	25.0	42.7
8	35.7	35.9	36.4	36.0	36.0	36.0	36.0	40.7	75.8
9	34.9	34.7	35.1	34.8	34.8	34.8	34.7	36.3	58.4
10	48.8	47.6	48.1	47.7	47.7	47.7	47.8	49.3	36.7
11	34.0	34.0	34.2	34.1	34.0	34.0	34.1	34.8	15.8
12	32.3	32.3	32.8	32.3	32.3	32.3	32.4	31.9	34.8
13	36.3	36.1	36.6	36.2	36.2	36.2	36.2	36.6	73.3
14	38.9	38.9	39.4	39.0	39.0	39.0	39.0	39.1	147.6
15	151.3	151.2	151.5	151.3	151.3	151.3	151.3	151.3	109.5
16	108.6	108.6	109.6	108.6	108.6	108.6	108.6	108.6	32.6
17	22.4	22.2	22.6	22.3	22.2	22.2	22.2	23.1	23.8
18	25.2	24.9	25.5	24.9	24.8	24.8	24.8	20.1	29.6
19	183.6	73.7	74.1	74.0	73.8	74.0	73.6	182.3	76.8
20	12.6	12.1	12.4	12.1	12.1	12.1	12.1	12.2	17.3
1′		108.0	109.0	108.0	106.0	106.0	107.6		105.4
2′		79.2	83.9	79.5	85.8	84.5	79.0		85.1
3′		77.5	79.0	78.0	76.3	76.6	81.1		77.1
4′		86.3	85.6	84.2	84.0	81.8	80.7		81.3
5′		61.4	62.9	64.1	61.8	63.6	63.4		63.5
2′-OAc									
*C*OCH$_3$					171.1	170.9			171.2
COC*H*$_3$					20.7	20.7			20.8
3′-OAc									
*C*OCH$_3$							171.6		
COC*H*$_3$							20.6		
5′-OAc									
*C*OCH$_3$				170.4		170.6	170.5		170.7
COC*H*$_3$				20.8		20.7	20.7		20.8

[a] ^{13}C NMR data were measured in $CDCl_3$. [b] ^{13}C NMR data were measured in C_5D_5N.

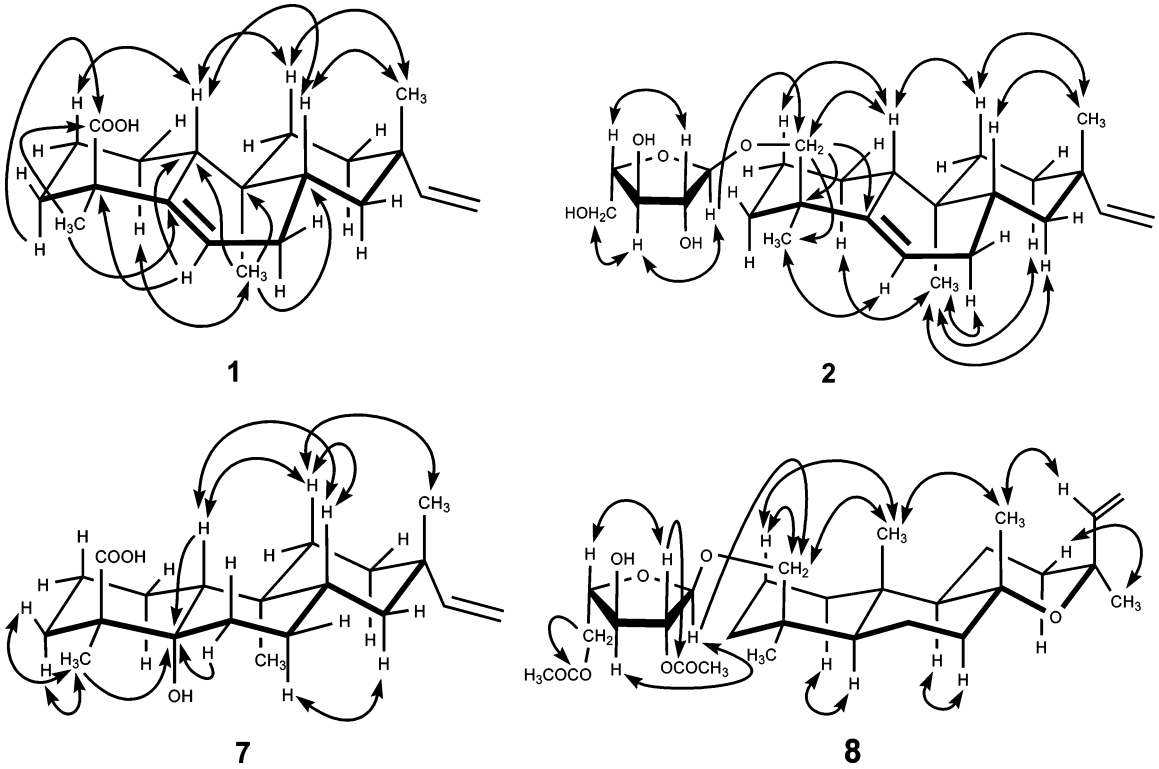

Figure 1. Key HMBC correlations (arrow) and key NOE correlations (arrows) of compounds **1**, **2**, **7**, and **8**.

compound **1**. Therefore, the structure of compound **2** (sagittine B) was characterized as 19-*O*-α-L-arabinofuranosyl-*ent*-rosa-5,15-diene.

Compounds **3**−**6** showed a positive reaction with Molisch reagent. The structures of these compounds were determined from their molecular mass, sugar analysis, 2D NMR experiments applied, and similarities of the ^{1}H and ^{13}C NMR signals with those of compound **2**. They all were assigned the same aglycon, characterized as 19-hydroxy-*ent*-rosa-5,15-diene and one L-arabinofuranosyl moiety with different acetyl substituents. All the ^{1}H and ^{13}C NMR

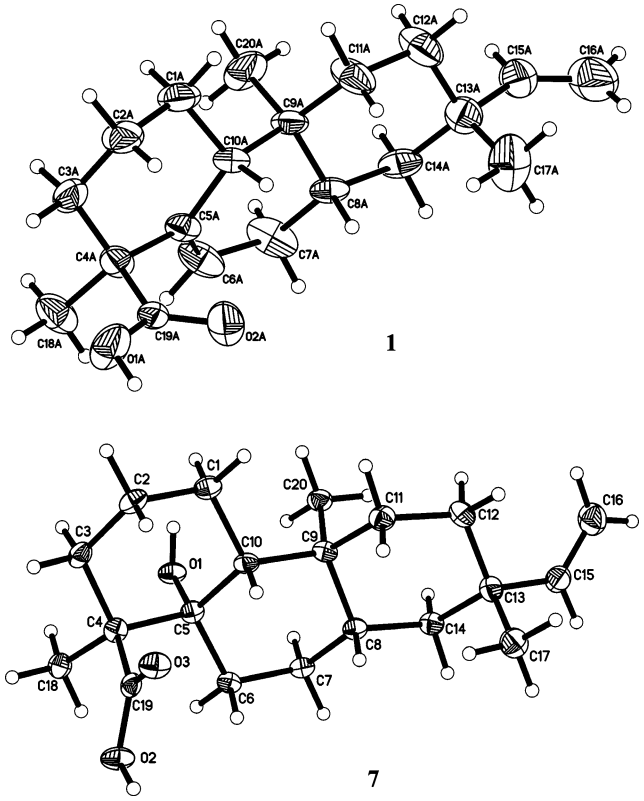

Figure 2. X-ray crystal structures of compounds **1** and **7** (thermal ellipsoids at the 20% and 50% probability level for **1** and **7**, respectively).

signals of compounds **3**−**6** were assigned unambiguously (Tables 1 and 2), and their relative configurations were established by NOESY experiments.

Compound **3** was obtained as a colorless oil, and its molecular formula of $C_{27}H_{42}O_6$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 485.2985, calcd 485.2981). The ^1H and ^{13}C NMR data of **3** (Tables 1 and 2) together with sugar analysis demonstrated that compound **3** consisted of an aglycon characterized as 19-hydroxy-*ent*-rosa-5,15-diene, an α-L-arabinofuranosyl moiety, and an acetyl group (δ_C 170.4, 20.8). The HMBC correlations between the anomeric proton at δ 4.94 and the C-19 signal indicated that the sugar residue was linked to C-19. The presence of the acetyl group at the C-5′ position was confirmed by the observation of the $^3J_{H,C}$-connectivities between H-5′ and the carbonyl carbon of the acetyl group (δ 4.27/170.4). Thus, the structure of compound **3** (sagittine C) was established as 19-*O*-α-L-5′-acetoxyarabinofuranosyl-*ent*-rosa-5,15-diene.

Compound **4** was obtained as a colorless oil, and its molecular formula of $C_{27}H_{42}O_6$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 485.2978, calcd 485.2981). According to the NMR data (Tables 1 and 2) and sugar analysis, the structure of **4** was found to consist of an aglycon characterized as 19-hydroxy-*ent*-rosa-5,15-diene, an α-L-arabinofuranosyl unit, and an acetyl group (δ_C 171.1, 20.7). The HMBC correlations between the anomeric proton at δ 4.99 and the C-19 signal indicated that the sugar residue was linked to C-19, and the acetyl group was substituted at the C-2′ position by the observation of the HMBC correlation between H-2′ and the carbonyl carbon of the acetyl group (δ 4.81/171.1). Thus, the structure of compound **4** (sagittine D) was established as 19-*O*-α-L-2′-acetoxyarabinofuranosyl-*ent*-rosa-5,15-diene.

Compound **5** was obtained as a colorless oil, and its molecular formula of $C_{29}H_{44}O_7$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 527.3085, calcd 527.3087). The ^1H and ^{13}C NMR spectra of compound **5** (Tables 1 and 2) were similar to those of compounds **3** and **4**, except that compound **5** had two acetyl substituents on the sugar moiety. In the HMBC spectrum, the correlations between the anomeric proton at δ 4.99 and C-19 indicated that the sugar residue was linked to C-19, and the correlations of H-2′ (δ 4.82)/CO-2′ (δ 170.9) and H-5′a (δ 4.30)/CO-5′ (δ 170.6) demonstrated that the acetyl groups were substituted at C-2′ and C-5′, respectively. Therefore, the structure of compound **5** (sagittine E) was established as 19-*O*-α-L-2′,5′-diacetoxyarabinofuranosyl-*ent*-rosa-5,15-diene.

Compound **6** was obtained as a colorless oil, and its molecular formula of $C_{29}H_{44}O_7$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 527.3089, calcd 527.3087). The ^1H and ^{13}C NMR data of compound **6** (Tables 1 and 2) were similar to those of compound **5** and again included two acetyl groups. The HMBC correlations between the anomeric proton at δ 4.93 and C-19 indicated the sugar residue was linked to C-19, and the $^3J_{H,C}$-connectivities of H-3′ (δ 4.62)/CO-3′ (δ 171.6) and H-5′a (δ 4.38)/CO-5′ (δ 170.5) indicated that the acetyl groups were substituted at C-3′ and C-5′, respectively. Therefore, the structure of **6** (sagittine F) was characterized as 19-*O*-α-L-3′,5′-diacetoxyarabinofuranosyl-*ent*-rosa-5,15-diene.

Compound **7** was obtained as colorless needles, and its molecular formula of $C_{20}H_{32}O_3$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 343.2349, calcd 343.2351). The ^1H and ^{13}C NMR spectra of compound **7** (Tables 1 and 2) were almost identical with those of compound **1**, except the olefinic bond in compound **1** was replaced with a hydroxyl group in compound **7** at C-5. The tertiary alcohol was placed at the C-5 position because of the correlations between C-5 (δ 75.0) and CH_3-18 (δ 1.20), H-6 (δ 1.72), and H-10 (δ 1.77) in the HMBC spectrum (Figure 1). The relative configuration of **7** was determined to be consistent with that of compound **2** by a NOESY experiment (Figure 1). The relative orientation of the hydroxyl group was determined by a NOESY experiement revealing correlations from H-10 to both H-8 and H-11b and from H-7b to H-14b. These results established the hydroxyl group at C-5 as having an α-axial orientation. The relative configuration of **7** was further confirmed by X-ray crystallographic analysis (Figure 2). Therefore, the structure of compound **7** (sagittine G) was proposed as 5α-hydroxy-*ent*-rosa-15-en-19-oic acid.

Compound **8** was obtained as a colorless oil, and its molecular formula of $C_{29}H_{46}O_8$ was established by HRESIMS ($[M + Na]^+$, *m*/*z* 545.3195, calcd 545.3192). This compound showed a positive reaction with Molisch reagent. The ^1H and ^{13}C NMR data (Tables 1 and 2) revealed the presence of 29 carbons assigned to four methyls, seven methylenes, three methines, a vinyl moiety, three quaternary carbons, a hydroxymethyl group, two acetyl groups, and a pentosyl moiety. According to the ^1H and ^{13}C NMR spectra, the agylcon of compound **8** was assigned as the same as those of the known labdane diterpenoid 13-epi-manoyl oxides.[4,17] This was further supported by the results of NOE experiments (Figure 1) revealing the NOE correlations of CH_3-17/H-14 and CH_3-16/H-12a. The assignment of the methyl signals and the position of the hydroxymethyl group followed from the NOE correlations of CH_3-17/CH_3-20, H-19a (and H-19b)/CH_3-20, H-2b /CH_3-20, and H-19a (H-19b)/H-2b. The NMR data of the sugar moiety in **8** were similar to those of the sugar moiety in compound **5**, and the fragment was characterized as an α-L-arabinofuranosyl unit by the combined analysis of NMR data and sugar analysis. The HMBC correlations between the anomeric proton at δ 4.99 (H-1′) and C-19 indicated that the sugar residue was linked to the C-19 position. Additional $^3J_{H,C}$-connectivities were observed between H-2′ and the carbonyl carbon of the acetyl group (δ 4.82/171.2) and between H-5′a and the carbonyl carbon of the second acetyl group (δ 4.30/170.7), demonstrating that the acetyl groups were substituted at C-2′ and C-5′, respectively (Figure 1). The stereochemistry of **8** was established by NOESY experiment (Figure 1). Thus, compound **8** was characterized as 13-epi-manoyl oxide-19-*O*-α-L-2′,5′-di-acetoxyarabinofuranoside.

Compounds **1**−**8** were evaluated for their antibacterial activity against oral pathogens *Streptococcus* mutans ATCC 25175, *Acti-*

Table 3. MIC Values of Compounds **1**−**8** against Three Oral Pathogens

	MIC (μg/mL)		
compound	*Actinobacillus actinomycetemcomitans* ATCC 43717	*Actinomyces naeslundiis* ATCC 12104	*Streptococcus mutans* ATCC 25175
1	>250	62.5	62.5
2	250	125	62.5
3	250	62.5	62.5
4	250	125	125
5	>250	62.5	>250

nomyces naeslundiis ATCC 12104, and *Actinobacillus actinomycetemcomitans* ATCC 43717. Compounds **1**−**4** exhibited strong inhibitory activity against the oral pathogens *S. mutans* ATCC 25175 and *A. naeslundiis* ATCC 12104, with MICs values between 62.5 and 125 μg/mL. Compound **5** was active against only *A. naeslundiis* ATCC 12104 (MIC value 62.5 μg/mL). All compounds showed weak or no activity against *A. actinomycetemcomitans* ATCC 43717 (Table 3).

Experimental Section

General Experimental Procedures. Melting points were determined on a Leica Galen III apparatus and were uncorrected. Optical rotations were taken in $CHCl_3$ on a Perkin-Elmer PE 241 polarimeter. The IR spectra were obtained on a Perkin-Elmer 16 PC FT-IR spectrophotometer. The 1D and 2D NMR spectra were run on a Bruker AV400 spectrometer, with TMS as an internal standard. The HRESIMS were obtained on a PE Biosystems Mariner System 5140 LC/MS spectrometer. Column chromatography was carried out using silica gel (Qingdao) and Sephadex LH-20 (Pharmacia). TLC was performed on HPTLC plates (Merck), with compounds visualized by spraying with 10% sulfuric acid following by heating.

Plant Material. The dried herb of *Sagittaria sagittifolia* L. was collected from Nanning, Guangxi Province, People's Republic of China, in September 2003, and was identified by Prof. Lai Maoxiang (Nanning Institute of Traditional Chinese Medicine, Nanning, People's Republic of China). A voucher specimen (no. 03-110045) was deposited in the herbarium of China Pharmceutical University.

Extraction and Isolation. The dried herb of *S. sagittifolia* (10 kg) was crushed, extracted with 95% EtOH, and concentrated in vacuo to leave a black liquid. The EtOH extract was partitioned between petroleum ether and H_2O (1:1) to afford a petroleum ether fraction (fraction A, 300 g). Half of fraction A was subjected to silica gel column chromatography with a gradient of petroleum ether−EtOAc (95:5, 90:10, 85:15, 80:20, 70:30, 60:40) to afford fractions 1−20. Fraction 4 (8.0 g) was chromatographed on silica gel, eluting with petroleum ether−EtOAc (97:3) to give **1** (50 mg). Fraction 7 (6.5 g) was chromatographed on silica gel, eluting with petroleum ether−acetone (95:5) to give **7** (35 mg). Fraction 9 (3.5 g) was chromatographed on silica gel, eluting with petroleum ether−acetone (90:10), which was purified further on silica gel column chromatography eluting with $CHCl_3$−acetone (100:2) to give **5** (30 mg) and **6** (45 mg). Fractions 12−14 (5.0 g) were subjected to silica gel column chromatography with petroleum ether−EtOAc (8:2), followed by purification on Sephadex LH-20 [$CHCl_3$−MeOH (1:1)] to give **8** (25 mg). Fractions 15 and 16 (9.0 g) were subjected to silica gel column chromatography eluting with a gradient of petroleum ether−EtOAc (95:5, 90:10, 85:15, 80:20) to afford fractions 15-16-1, 15-16-2, 15-16-3, and 15-16-4. Fraction 15-16-3 (1.2 g) was chromatographed on silica gel, eluting with $CHCl_3$−MeOH (100:1), to give **3** (20 mg) and **4** (25 mg). Fraction 19 (4.0 g) was subjected to silica gel column chromatography eluting with petroleum ether−EtOAc (7:3) to afford fractions 19-1, 19-2, and 19-3. Fraction 19-2 (1.1 g) was further chromatographed on silica gel, eluting with $CHCl_3$−MeOH (100:3), followed by purification on Sephadex LH-20 [$CHCl_3$−MeOH (1:1)] to give **2** (60 mg).

Sagittine A (1): colorless needles (petroleum ether−acetone); mp 162−163 °C; $[\alpha]^{25}_D$ −13.0 (*c* 1.15, $CHCl_3$); IR (KBr) ν_{max} 3470, 3080, 1725, 1635, 1080, 915, 790 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 325.2247 $[M + Na]^+$ (calcd for $C_{20}H_{30}O_2Na$, 325.2245).

Sagittine B (2): colorless oil; $[\alpha]^{25}_D$ −29.6 (*c* 1.25, $CHCl_3$); IR (KBr) ν_{max} 3505, 1640, 1100, 918, 801 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 443.2872 $[M + Na]^+$ (calcd for $C_{25}H_{40}O_5Na$, 443.2875).

Sagittine C (3): colorless oil; $[\alpha]^{25}_D$ −36.9 (*c* 1.30, $CHCl_3$); IR (KBr) ν_{max} 3480, 1738, 1636, 1240, 1105, 925, 808 cm^{-1}; ^{1}H NMR ($CHCl_3$,-400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 485.2985 $[M + Na]^+$ (calcd for $C_{27}H_{42}O_6Na$, 485.2981).

Sagittine D (4): colorless oil; $[\alpha]^{25}_D$ −42.6 (*c* 1.15, $CHCl_3$); IR (KBr) ν_{max} 3506, 1740, 1650, 1220, 1085, 910, 802 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 485.2978 $[M + Na]^+$ (calcd for $C_{27}H_{42}O_6Na$, 485.2981).

Sagittine E (5): colorless oil; $[\alpha]^{25}_D$ −57.8 (*c* 0.90, $CHCl_3$); IR (KBr) ν_{max} 3450, 1732, 1647, 1245, 1031, 970, 916 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 527.3085 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_7Na$, 527.3087).

Sagittine F (6): colorless oil; $[\alpha]^{25}_D$ −62.8 (*c* 1.70, $CHCl_3$); IR (KBr) ν_{max} 3400, 1750, 1645, 1229, 1070, 935, 800 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 527.3089 $[M + Na]^+$ (calcd for $C_{29}H_{44}O_7Na$, 527.3087).

Sagittine G (7): colorless needles (petroleum ether−acetone); mp 162−163 °C; $[\alpha]^{25}_D$ −48.4 (*c* 1.55, $CHCl_3$); IR (KBr) ν_{max} 3500, 1695, 1260, 1080, 920, 860 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 343.2349 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_3Na$, 343.2351).

13-Epi-manoyl oxide-19-*O*-α-L-2′,5′-diacetoxyarabinofuranoside (8): colorless oil; $[\alpha]^{25}_D$ −97.4 (*c* 1.15, $CHCl_3$); IR (KBr) ν_{max} 3386, 2870, 1665, 1420, 1075, 910, 815 cm^{-1}; ^{1}H NMR ($CHCl_3$, 400 MHz), see Table 1; ^{13}C NMR ($CHCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z* 545.3195 $[M + Na]^+$ (calcd for $C_{29}H_{46}O_8Na$, 545.3192).

Acid Hydrolysis of Compounds 2−6 and 8 and Sugar Analysis. Compounds **2**−**6** and **8** (each 3 mg) were hydrolyzed with 4 M TFA (0.5 mL) at 120 °C for 1 h, after which the solvent was evaporated with a stream of N_2. The products were dissolved in water (0.5 mL), which were then neutralized with NH_4OH. The (*S*)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (6 mg) in EtOH (0.5 mL) were added to the solution and heated at 40 °C for 5 h. Excess $Na[BH_3CN]$ was quenched with a few drops of HOAc and the formed boric acid removed by co-distillation with 10% HOAc in MeOH (3 × 0.5 mL) and MeOH (3 × 0.5 mL). The reaction mixtures were acetylated with Ac_2O−pyridine (1:1, 1.0 mL) at 120 °C for 30 min and analyzed by GC-MS using an Agilent 6890 Plus GC with 7683 autoinjector and Agilent 5973 Network MSD detector apparatus, employing an HP-5MS capillary column of 30 m × 0.25 mm, 0.25 μm film thickness, He gas as carrier gas at 1.0 mL/min, inlet pressure of 12.8 psi, using a temperature program, from 140 °C (1 min) to 230 °C at 3 °C min^{-1}. The diasteromeric alditol derivative of the standard sugar arabinose was used as reference. The derivative of L-arabinose was detected at t_R 15.64 min.[18,19]

X-ray Structure Determinations of 1 and 7. Selected crystals were mounted on glass fibers, and the diffraction intensity data were collected by a Bruker CCD diffractometer with graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å). Lattice determination and data collection were carried out using SMART v. 5.625 software. Data reduction and absorption correction were performed using SAINT v. 6.26 and SADABS v. 2.03. Structure solution and refinement were performed using the SHELXTL v. 6.10 software package. Both structures were solved using direct methods. All non-hydrogen atoms were refined anisotropically by full-matrix least squares with a riding model for the hydrogen atoms.

X-ray Crystal Structure of Compound 1. Diffraction intensity data were acquired with a Bruker APEX CCD single-crystal X-ray diffractometer with Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator. Crystal data: $C_{20}H_{30}O_2$ (302.44 g/mol), colorless block-shaped crystal with size 0.40 × 0.35 × 0.30 mm^3, monoclinic, space group $P2_1$, T = 253(2) K, a = 12.6906(15) Å, b = 10.2956(13) Å, c = 14.9114(18) Å, β = 112.175(2)°, V = 1804.2(4) Å^3, D_c = 1.113 Mg/m^3, Z = 4, $F_{(000)}$ = 664, $\mu_{(Mo\ K\alpha)}$ = 0.070 mm^{-1}. A total of 11 440 reflections were collected in the range 1.47° < θ < 28.33°, with 4082 independent reflections [R(int) = 0.0252], completeness to $\theta_{max.}$ was 89.2%; absorption correction was by SADABS with max. and min. transmission 1.00 and 0.97; refinement method (Bruker AXS SHELXTL 6.10), full-matrix least-squares on F^2, the number of data/restraints/parameters were 4082/1/397; goodness-of-fit on F^2 = 1.009; final R indices [$I > 2\sigma(I)$], R_1 = 0.0526, wR_2 = 0.1199; R indices (all data),

$R_1 = 0.1094$, $wR_2 = 0.1511$, largest difference peak and hole, 0.21 and −0.12 e/Å^3.

X-ray Crystal Structure of Compound 7. Experimental protocols were as for **1**, crystal data for **7**: $C_{20}H_{32}O_3$ (320.46 g/mol), colorless block-shaped crystal with size 0.35 × 0.30 × 0.25 mm^3, monoclinic, space group $P2_1$, $T = 100(2)$ K, $a = 11.4901(13)$ Å, $b = 6.5871(8)$ Å, $c = 12.0565(14)$ Å, $\beta = 107.110(2)°$, $V = 872.13(18)$ Å^3, $D_c = 1.220$ Mg/m^3, $Z = 2$, $F_{(000)} = 352$, $\mu_{(Mo\ K\alpha)} = 0.080$ mm^{-1}. A total of 5552 reflections were collected in the range $1.77° < \theta < 28.25°$, with 2140 independent reflections [R(int) = 0.0252], completeness to $\theta_{max.}$ was 99.4%; absorption correction was by SADABS with max. and min. transmission 1.00 and 0.89; refinement method (Bruker AXS SHELXTL 6.10), full-matrix least-squares on F^2, the number of data/restraints/parameters were 2140/1/208; goodness-of-fit on $F^2 = 1.039$; final R indices [$I > 2\sigma(I)$], $R_1 = 0.0390$, $wR_2 = 0.0870$; R indices (all data), $R_1 = 0.0459$, $wR_2 = 0.0903$, largest difference peak and hole, 0.28 and −0.23 e/Å^3.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (Accession No. CCDC 289377 for **1** and 289378 for **7**). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

MIC Determinations. The MIC values of isolates **1**−**8** against selected oral bacteria were determined using liquid cultures in 96-well culture plates according to a modification of the method described by Shapiro et al.[20] Trypticase soy broth (TSA, Becton-Dickinson Microbiology Systems, Cockeysville, MD) was used for *Streptococcus mutans*, *Actinomyces naeslundiis*, and *Actinobacillus actinomycetemcomitans*. Todd Hewitt broth supplemented with 1% yeast extract (Difco Laboratories, Detroit, MI) was used. Serial dilutions (1.0−0.002%) of each extract and compound were prepared in each culture medium. Aliquots (200 μL) of each dilution were dispensed in 96-well cell culture plates (Becton-Dickinson Microbiology Systems). Subsequently, 10^5−10^6 test bacteria that had been cultured overnight in each culture medium were inoculated into each well and cultured for 1−2 days under anaerobic conditions. Then the absorbance was measured at 630 nm (Bio-tek, ELX808). The highest dilution at which no growth ($OD_{630} \leq 0.05$) was observed was defined as the minimum inhibitory concentration (MIC).

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Supporting Information Available: ^{1}H and ^{13}C NMR and 2D NMR data of compounds **1**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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