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Caesanines A–D, New Cassane Diterpenes with Unprecedented N Bridge from *Caesalpinia sappan*

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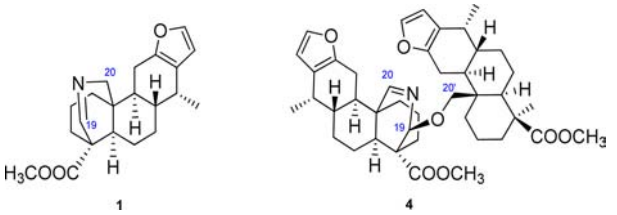
ABSTRACT



Serial antibacterial furanoditerpenes caesanines A–D (1–4), possessing a cassane-type diterpenoid skeleton with an unusual N bridge between C-19/C-20, were identified from a Chinese herb *Caesalpinia sappan* Linn. In addition, caesanine D (4) showed the first class of dicassane diterpenoid ethers. Their structures were determined by different spectroscopic methods and ECD calculation. Caesanines A and B exhibited strong activities against MRSA suggesting a promising entry point for the development of anti-infective drugs.

Multidrug resistance of pathogenic microorganisms is a serious threat to human health. Clinically important examples include methicillin-resistant *Staphylococus aureus* (MRSA), highly resistant Gram-negative pathogens, and *Mycobacterium tuberculosis*.[1] New antibiotics and new therapeutic strategies are needed to address this challenge. From our previous screening efforts on the constructed Natural Product Library (NPL), including samples from traditional Chinese Medicines (TCMs) and microorganisms, a series of active compounds have been described.[2] During the screening work, the TCM *Caesalpinia sappan* was highlighted for having good activity against MRSA with an MIC value of 12.5 μg/mL.

Caesalpinia sappan Linn. (Leguminosae) is a shrubby tree distributed in southeast Asia. The heartwood of

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C. sappan contains diverse structural types of phenolic components,[3] with various biological activities including antimicrobial activity, anti-inflammatory activity, aldose reductase inhibition, hypoglycemic activity, antihepatotoxicity, inhibition of protein kinase C, anit-platelet aggregation, and vasorelaxation effects.[4] Previous chemical investigations revealed several cassane-type diterpenoids, exhibiting a cytotoxic effect against the KB cell line, from the crude extract of seeds of *C. sappan*.[5] We report herein the isolation and structure elucidation of five new cassane diterpenes (**1**–**5**), together with three known furanoditerpenoids (**6**–**8**)[5a] from the seeds of *C. sappan* (Figure 1). The new structures were determined by HRESIMS, 1D and 2D NMR techniques, and CD experiments. The skeleton of compounds **1**–**4** is rather unusual, consisting of a cassane-type diterpene with an N bridge between C-19 and C-20. In addition, compound **4** possesses a cassane diterpenoid alkaloid–cassane diterpene skeleton, which is the first class of dicassane diterpenoid ethers. The antibacterial activities of these compounds were evaluated as well.

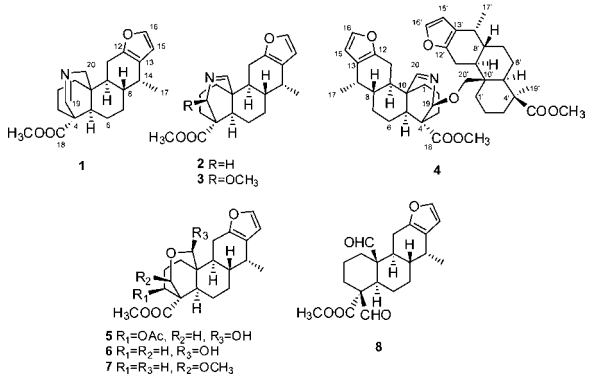

Figure 1. Structures of compounds **1**–**8**.

Compound **1** was isolated as a white amorphous powder, and its molecular formula was determined to be $C_{21}H_{27}NO_3$, which possessed 9 degrees of unsaturation, by HRESIMS ($[M + H]^+$ m/z 342.2063, calcd for $C_{21}H_{28}NO_3$ 342.2064). IR absorption at 1735 cm^{-1} indicated the presence of carbonyl. Combined analysis of ^1H NMR, ^{13}C NMR, HSQC, and HMBC data of **1** (Table 1 and Figures S1–S4) revealed there were 21 carbons, which included an ester carbonyl carbon (δ_C 175.9), an olefinic carbon in an imine system [δ_C/δ_H 165.2/8.03 (s)], a 1,2-disubstituted furan ring moiety [δ_C/δ_H 150.4, 142.3/7.21 (d, J = 1.8 Hz), 124.1, and 110.9/6.16 (d, J = 1.8 Hz)], one methoxy group [δ_C/δ_H 53.1/3.76 (s)], one secondary methyl group [δ_C/δ_H 18.2/0.97 (d, J = 6.9 Hz)], four methines, seven methylenes, and two quaternary carbons. These data revealed that **1** had a cassane-type furanoditerpene framework, by comparison to those of previously reported compounds.[5a]

The imine proton at δ_H 8.03 (s) was deduced to be proton H-19 from the HMBC correlations of H-3b/C-19 and H-5/C-19 (Figure 2). Furthermore, the HMBC cross peaks of H_2-20/C-19, H_2-20/C-10, H-9/C-20, and H-5/C-20, as well as the ^1H–^{15}N HMBC (Figure S6) correlation of

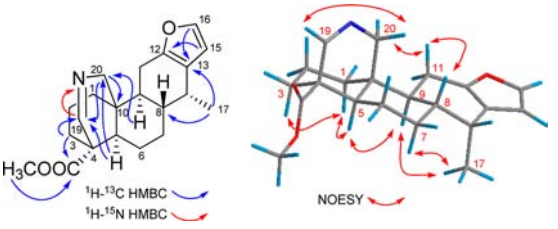

Figure 2. Key HMBC and NOESY correlations of **1**.

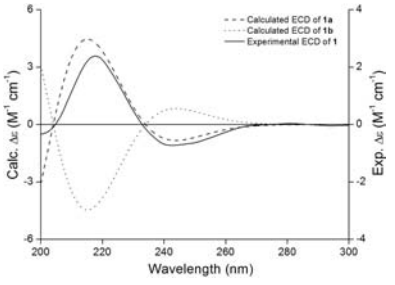

Figure 3. Computed and observed ECD of **1a**, **1b**, and **1**.

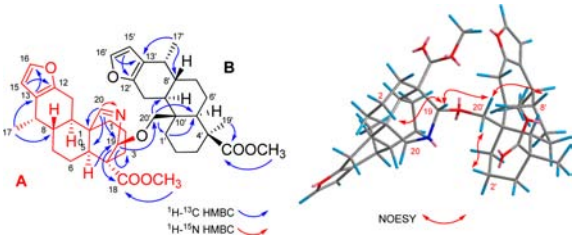

Figure 4. Key HMBC and NOESY correlations of **4**.

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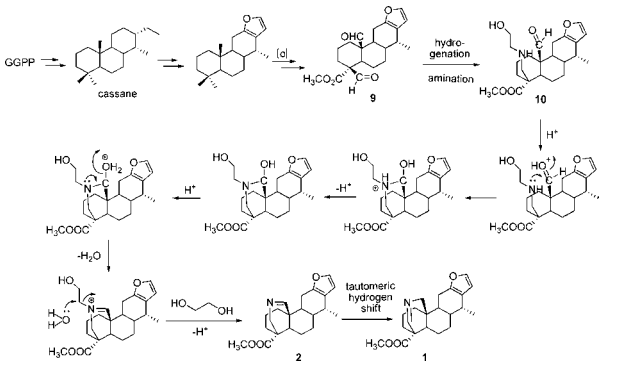
Scheme 1. A Proposed Biogenetic Relationship between **1** and **2**



N/H-19 (δ_N/δ_H 311.8/8.03 (s)) (Figure 2), revealed the presense of a N=C functionality between C-4 and C-20. The loaction of the carbonyl group at C-18 was deduced from the HMBC correlation of H-3b/C-18. The location of the methoxy group could be determined by the HMBC data as well.

Thus, the planar structure of **1** was established (Figure 2), and all of the signals in the 2D NMR spectra were assigned unambiguously. **1** contains an unusual cassane diterpene skeleton with an N bridge between C-19 and C-20 representing a new class of diterpenoid alkaloids.

The relative configurations of **1** could be determined according to the NOESY (Figure 2) correlations of H-1a/H-3a, H-5/H-1a, H-5/H-9, H-7a/H_3-17, H-9/H_3-17, H-20a/H-19, H-11a/H-8, and H-11a/H-20a.

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1** and **4** (*J* in Hz)

	1[a]		**4**[b]	
Pos.	δ_H (mult, *J*)	δ_C	δ_H (mult, *J*)	δ_C
1a, 1′a	1.24 m	40.5	1.28 m	32.4, 34.1
			0.85 td (12.5, 3.3)	
1b, 1′b	2.03 m		1.78 m	
			2.19 d (12.5)	
2a, 2′a	1.72 m	21.1	1.06 m, 1.50 m	23.7, 19.1
2b, 2′b			1.33 m, 1.61 m	
3a, 3′a	1.78 m	33.7	1.60 m, 1.52 m	31.3, 36.7
3b, 3′b	1.92 m		1.67 m, 1.73 m	
4, 4′	–	43.3	–	49.6, 47.0
5, 5′	1.71 m	45.7	1.66 m, 1.73 m	47.0, 49.7
6a, 6′a	1.03 m	26.6	1.06 m, 1.23 m	25.4, 29.7
6b, 6′b	1.36 m		1.26 m, 1.57 m	
7a, 7′a	1.39 m	31.0	1.62 m, 1.32 m	30.7
7b, 7′b	1.68 m		2.00 d (11.0) 1.62 m	
8, 8′	1.80 m	38.3	1.52 m 1.88 m	37.7, 36.7
9, 9′	1.61 m	43.3	1.79 m, 1.52 m	40.1, 45.3
10, 10′	–	37.5	–	44.2, 40.6
11a, 11′a	2.30 dd (16.5, 10.8)	23.2	2.71 dd (16.8, 10.5) 2.49 d (6.24)	21.6, 22.6
11b, 11′b	2.72 dd (16.5, 6.0)		2.91 dd (16.8, 11.5)	
			2.67 d (6.24)	
12, 12′	–	150.4	–	148.7, 150.0
13, 13′	–	124.1	–	122.4, 122.1
14, 14′	2.66 m	33.3	2.61 m, 2.57 m	31.4
15, 15′	6.16 d (1.8)	110.9	6.28 d (1.8)	110.1
			6.25 d (1.8)	
16, 16′	7.21 d (1.8)	142.3	7.41 d (1.8)	141.3, 140.9
			7.35 d (1.8)	
17, 17′	0.97 d (6.9)	18.2	0.94 d (7.0)	19.0, 18.0
			0.91 d (7.0)	
18, 18′	–	175.9	–	175.2, 178.7
19	8.03 s	165.2	5.00 d (1.8)	89.5
19′-Me			1.28 s	17.5
20a	3.48 d (18.9, 2.7)	55.6	7.75 s	163.7
20b	3.76 d (18.9)			
20′a			3.61 d (10.4)	66.6
20′b			4.47 d (10.4)	
18-OMe, 18′-OMe	3.76 s	53.1	3.48 s	52.3
			3.60 s	52.1

[a] Was measured in CD_3OD, ^{1}H 300 MHz; ^{13}C 75 MHz, [b] Was measured in DMSO-d_6, ^{1}H 600 MHz; ^{13}C 150 MHz.

In order to determine the absolute configurations of **1**, quantum chemical CD calculations were carried out. After a conformational search, two stereoisomers with the lowest energy, (4*S*,5*R*,8*S*,9*S*,10*S*,14*R*)-**1a** and (4*R*,5*S*,8*R*,9*R*,-10*R*,14*S*)-**1b**, were found (Figure S10). A conformational analysis of **1a** and **1b** was carried out by the Molecular Operating Environment (MOE) software package using the MMFF94 molecular mechanics force field calculation. The calculated ECD spectra of **1a** and **1b** were obtained by Boltzman statistics at the B3LYP/6−31+G(d) level. The calculated ECD of **1a** was consistent with experimental data. The recorded and the computed ECD curves are illustrated in Figure 3. Therefore, the absolute configurations of the chiral carbons were determined to be 4*S*,5*R*,8*S*,9*S*,10*S*,14*R* in **1**, which was named as caesanine A.

Compound **4** was isolated as a white powder. HRESIMS measurements on **4** revealed an adduct of $[M + H]^+$ (*m*/*z* 686.4043, calcd for $C_{42}H_{56}NO_7$ 686.4081) consistent with a molecular formula of $C_{42}H_{55}NO_7$. Combined analysis of the ^1H and ^{13}C NMR (Table 1) data supported a pair of cassane-type furanoditerpenoid frameworks. Examination on the HSQC of **4** allowed the following assigments. Unit A exhibited similar NMR spectra to **3**. In the $^1H-^{15}N$ HMBC (Figure S34 in the Supporting Information and Figure 4) spectra, the resonance at δ_N 331.9 showed correlation with the olefinic proton at δ_H 7.75 (H-20). In unit B, the ^1H and ^{13}C NMR data were similar to those of phanginin I,[5a] except that the aldehyde group was replaced by an oxymethene group. The presence of the oxymethene group [δ_C/δ_H 66.6/4.47 (d, J = 10.4 Hz), 3.61 (d, J = 10.4 Hz)] at C-20′ could be determined by the HMBC (Figure 4) correlations of the lower field signals at δ_H 4.47 (d, J = 10.4 Hz) and 3.61 (d, J = 10.4 Hz) with C-1′ (δ_C 34.1), C-5′ (δ_C 49.7), and C-9′ (δ_C 45.3), respectively.

The connectivity of both fragments was confirmed by the HMBC correlations. Resonances at δ_H 5.00 (s) showed correlations with carbons at δ_C 31.3 (C-3), 66.9 (C-20′), and 175.2 (C-18), and oxymethine protons at δ_H 4.47 and 3.61 showed correlations with carbons at δ_C 89.5 (C-19), revealing that subunits A and B were connected at C-19 and C-20′ via an oxygen bridge. The relative configurations of **4** could be determined by the NOESY (Figure 4) correlations of H-19/H-2a, H-19/H-20′a, H-20′a/H-8′, and H-20′b/H-2′b. Accordingly, the structure of **4** was determined and named as caesanine D, which was the first reported compound composed of a cassane-type diterpene alkaloid and cassane diterpenoid. The structure elucidations of **2**, **3**, and **5** were included in the Supporting Information.

The manner of the CD split of compounds **3** and **4** (Figures S28 and S37) resembled that of **1**, indicating that the chiral centers of compounds **3** and **4** at C-19 were both *R*.

The key intermediate product **9** was produced from the precursor of geranylgeranyl pyrophosphate (GGPP) by a intramolecular cyclization, followed by oxidation, dehydration, and dehydrogenation reaction. Intermediate product **10** was proposed to be generated from **9** through the amination and hydrogenation reactions with the participation of β-aminoethanol.[6] A plausible biosynthetic pathway to caesanines A and B (**1** and **2**) was proposed in detail (Scheme 1).

All these compounds were tested for their activities against *Bacillus subtilis*, *S. aureus*, MRSA, and bacillus Calmette-Guérin (BCG) (Table 2). Both caesanines A (**1**) and B (**2**) showed good activities against MRSA and SA with a range of MIC values between 3.125 and 12.5 μg/mL. A comparison of the activity of caesanine serial compounds (**1**−**4**) and phangnins (**5**−**8**) revealed that the structural formation of the unusual N bridge between C-19 and C-20 offered a potential enhancement of efficacy. Furthermore, the oxidation on the six-membered imine ring of the structure of **3** and **4** reduced the activity compared to that of **1** and **2**. Our study on caesanines A−D suggested a promising entry point for the development of anti-infective drugs of novel structures.

Table 2. Antibacterial Activities of Compounds **1**−**8**

	minimum inhibitory concentration (μg/mL)			
compd	BCG[a]	SA[a]	MRSA[a]	BS[a]
1	50	12.5	12.5	50
2	100	3.125	6.25	>100
3	>100	>100	>100	>100
4	>100	>100	>100	>100
5	50	>100	>100	>100
6	>100	12.5	25	12.5
7	50	>100	>100	>100
8	>100	100	100	50
control	0.05[b]	1[c]	1[c]	0.5[c]

[a] BCG: Bacillus Calmette-Guérin (Pasteur 1173P2). SA: *Staphylococcus aureus* (ATCC 6538). MRSA: methicillin-resistant *Staphylococcus aureus*. BS: *Bacillus subtilis* (ATCC 6633). [b] Isoniazid. [c] Vancomycin.

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Supporting Information Available. Experimental section; UV, IR, HRESIMS, CD, 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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