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Structure and antiviral properties of macrocaesalmin, a novel cassane furanoditerpenoid lactone from the seeds of *Caesalpinia minax* Hance†

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Abstract—A novel cassane-type furanoditerpenoid lactone, named macrocaesalmin, possessing a ten-membered macrocyclic 1,5-diketone ring and an unprecedented *cis* B/D ring fusion mode, has been isolated from the seeds of *Caesalpinia minax* Hance. Its structure was elucidated from detailed 1D and 2D NMR spectral analyses and X-ray crystallography, and density-functional optimization showed that *cis* fusion of the B/D ring is more stable than the *trans* mode. Macrocaesalmin was found to exhibit inhibitory activity against RSV, but not for Flu-A and Para-3 viruses.

Cassane-type diterpenoids are characteristic components of the genera *Caesalpinia* and *Pterodon* of the Leguminosae family, and they differ by the presence of an oxygenated group at C-5 in the former genus. The skeletons of these diterpenoids can be classified into five categories: (1) tricarbocyclic derivatives fused with a furan ring,[1,2] e.g. ε-caesalpin (**2**);[1b] (2) tricarbocyclic derivatives fused with an α,β-butenolide,[3] e.g. neocaesalpin C (**3**);[3b] (3) tricarbocyclic derivatives with cleavage of the furan ring,[4] e.g. caesaldekarin G (**4**).[4b] (4) rearranged furanoditerpenoids with migration of the C-4 methyl group to C-3, e.g. caesalpinin (**5**);[5] and (5) furanoditerpenoid lactones constructed from ring closure involving the oxygen atoms bridged to C-7 and C-17,[1h,6] e.g. caesalmin A(**6**)[6] (Fig. 1). Some of these furanoditerpenoids have been found to manifest anti-inflammatory, anti-analgesic,[7] radical growth regulation[2c] and anticancer activities.[1i] Cassane diterpenoids are therefore of interest in the context of structural diversity, and also in regard to their broad spectrum of biological activities.

The seeds of *Caesalpinia minax* Hance ('ku-shi-lian' in Chinese) have been used as a folk remedy for some diseases including fever, the common cold and dysentery.[8] Taking a lead from these ethnomedical uses, we investigated the chemical components of the seeds and found that some cassane-type furanoditerpenoids possess potent anti-Para3 virus (parainfluenza virus type 3) activity.[9] Following this discovery, we became engaged in further studies that led to the isolation of a novel macrocyclic furanoditerpenoid lactone, namely macrocaesalmin (**1**), which features the presence of a ten-membered, macrocyclic, 1,5-diketone ring and a *cis*-fusion mode of the B/D ring. The present report is concerned with its structural elucidation and inhibitory effects on Para-3, RSV (respiratory syncytial virus) and Flu-A (influenza type A virus), all of which are major pathogens of some respiratory infections.[10]

Macrocaesalmin (**1**) was isolated from the chloroform fraction of a 95% ethanol extract of the seeds followed by column chromatography, preparative TLC (hexane:acetone = 2:1, $R_f = 0.42$) and recrystallization from methanol solution (12 mg, yield 0.00022%). The HRLSI mass spectrum of **1** indicated a quasimolecular ion $[MH]^+$ at m/z 403.1747, corresponding to $C_{22}H_{26}O_7$ with ten units of unsaturation.

Keywords: *Caesalpinia minax*; antiviral activity; diterpene; cassane; furanoditerpenoid; macrocaesalmin.

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† Dedicated to Professor Wei-Yuan on the occasion of his 80th birthday.

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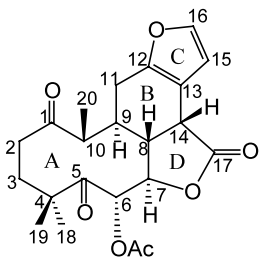
The ^{1}H NMR spectrum[11] showed the presence of two tertiary methyl groups at δ 1.02 (3H, s, H-18), δ 1.25 (3H, s, H-19), one secondary methyl group at δ 1.29 (3H, d, J=8.1 Hz, H-20), one acetoxyl group at δ 2.00 (3H, s, H-22), two oxymethine resonances at δ 5.05 (1H, d, J=9.2 Hz, H-6) and δ 5.00 (1H, dd, J=9.2, 11.2 Hz, H-7), and a 1,2-disubstituted furan ring which was evident from a pair of doublets at δ 6.10 (1H, d, J=2.0 Hz, H-15) and δ 7.22 (1H, d, J=2.0 Hz, H-16). The identities of all the 22 carbon atoms were revealed from ^{13}C NMR and DEPT measurements, which showed that **1** has four carbonyls, four methyls, three methylenes, six sp^3 methines and two sp^2 methines, one sp^3 quaternary carbon and two sp^2 quaternary carbons. The low-field region of the ^{13}C NMR spectrum contained signals for four carbonyls including two ketone carbonyls at δ 212.98 (s, C-1 and C-5, overlapping), one lactonic carbonyl at δ 174.72 (s, C-17) and one acetate carbonyl at δ_C 170.46 (s, C-21), four olefinic carbon atoms of the furan ring at δ 151.12 (s, C-12), 112.16, (s, C-13), 108.10, (d, C-15) and 141.02 (d, C-16), and two carbon atoms bearing oxygen functions at δ 75.71 (d, C-6) and 82.82 (d, C-7). The full assignments and connectivities were determined by ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC spectra. The ^{1}H–^{1}H COSY spectrum established spin systems involving H-6, H-7, H-8, H-9, H-10 and H_2-11, and H-15 and H-16. The HMQC spectrum revealed that the signal at δ 5.00 (H-7) corresponds to the proton attached to the carbon at δ 82.82 (C-7) and the HMBC spectrum showed that H-7 is correlated to C-5, C-6, C-8, C-9, C-14 and C-17, which established that the γ-lactone is formed by ring closure involving the oxygen atom bridged to C-7 and C-17. Since eight (two ketone carbonyls, an acetoxyl group, a lactonic ring and a furan ring) out of ten units of unsaturation are accounted for, **1** is inferred to possess two additional rings. As cassane furanoditerpenoids are tricarbocyclic derivatives, two of the cyclohexane rings might be merged into a macro-ring by cleavage of C-5–C-10, which is supported by the absence of the HMBC correlation between the ketone at C-5 and protons at C-9 and C-10.



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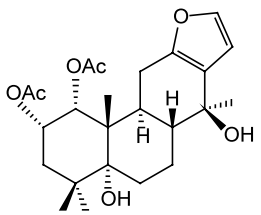

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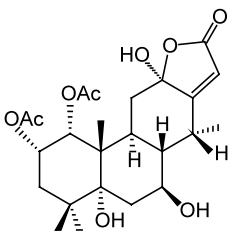

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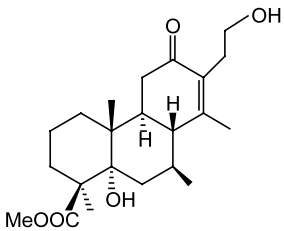

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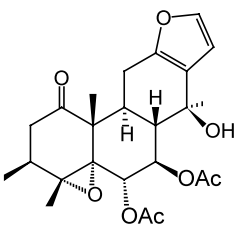

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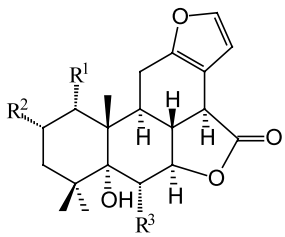

6 R^1 = OH, R^2 =OH, R^3 = OAc
7 R^1 = OAc, R^2 =H_2, R^3 = H_2
8 R^1 = OH, R^2 =H_2, R^3 = H_2
9 R^1 = OH, R^2 =H_2, R^3 = OAc

Figure 1. Chemical formulae of macrocaesalmin and some representative cassane-type diterpenoids.

The complete molecular structure and relative stereochemistry of **1** were established by X-ray crystallographic analysis[12] (Fig. 2a). In the solid state, compound **1** exists as a dimer through weak intermolecular C–H···O interactions involving pairing of the C-1 carbonyl with methine groups[13] (Fig. 2b). The two independent molecules **I** and **II** have nearly the same conformation; however, differences between them were observed in some bond distances, and several atoms of **II** exhibit significantly more pronounced thermal motions. The ten-membered ring A takes a chair–boat–chair conformation. In contrast, a synthetic ten-membered 1,6-diketone ring reported by Gudmundsdottir et al. has a boat–chair–boat conformation.[14] The unsaturated ring B exists in a twisted half-chair conformation due to fusion with the planar furan ring C. The five-membered lactone ring D adopts an envelope conformation.

In the crystal structure, the distances between H-6 and H-8, H-8 and H-14, and H-7 and H-9 are 2.349, 2.230 and 2.490 Å, respectively, and they show the same orientations as disclosed from the NOESY spectrum. The coupling constant for H-8 and H-14 (6.8 Hz) is also consistent with their small dihedral angle of 28.5° in the crystal structure. Thus, the X-ray structure would be expected to closely resemble the solution conformation in chloroform. In contrast, the conformation of spiro-prorocentrimine in the solid state is different from that in DMSO solution.[15] A plausible reason for this difference is that compound **1** bears a rigid ring system, whereas a soft macrolide moiety is present in spiro-prorocentrimine.

It is noteworthy that lactone ring D forms a dihedral angle of 66.6° (66.9° for comformer **II**) with the plane of the B/C rings (C-11 to C-16), due to its *cis*-fusion with ring B. Compound **1** is the first example possessing a *cis*-fusion mode of the B/D rings in contrast to other furanoditerpenoid lactones, e.g. caesalmin A (**6**), cae-

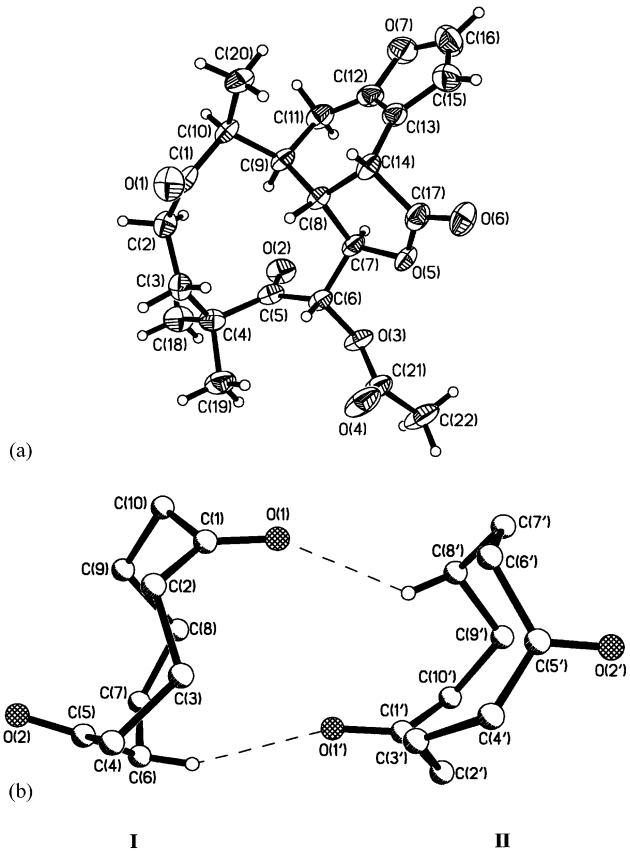

Figure 2. (a) Molecular structure of macrocaesalmin (**1**). (b) Two independent molecules of **1** (from left to right: **I** and **II**) forming a weakly-bound dimer. Only selected atoms highlighting the C–H···O interactions are shown. Relevant distances and angles: C(6)–O(1′) 3.456 Å, O(1′)–H(6A) 2.548 Å, C(8′)–O(1) 3.263 Å, O(1)–H(8′A) 2.512 Å, C(6)–H(6A)–O(1′) 154.0° and C(8′)–H(8′A)–O(1) 133.3°.

salmin B (**7**),[6] caesalmin G (**8**)[9] and bonducellpin (**9**)[1h] (Fig. 1). In order to investigate if the *cis*-fusion mode is indeed stable, structural optimization using density functional theory (DFT)[16] was used to compare the energies of the *cis*-isomer (**1**, macrocaesalmin) and the *trans*-isomer (**1a**, 14-epimacrocaesalmin, which was modeled by inverting the configuration at C-14). The calculations starting with X-ray coordinates were performed using the Gaussian 98 software package.[17] The result showed that **1** is more stable with a ground state energy 7.51 kcal/mol lower than that of **1a** (Fig. 3). In contrast, the same computation on the *trans*-isomers (original) and *cis*-isomers of compounds **6**, **7** and **8** showed that the *trans*-isomers are more stable by 2.40, 2.49 and 2.21 kcal/mol, respectively, versus the *cis* isomers. It can be inferred that the cleavage of the C-5–C-10 bond is responsible for the *cis*-fusion of the B/D rings in **1**. To our knowledge, such an inference regarding the ring fusion mechanism of natural products based on X-ray crystallography in combination with DFT optimization is made for the first time.

Compound **1** was evaluated for antiviral activities against RSV, Para-3 and Flu-A viruses according to an established protocol.[18] **1** showed inhibitory activity against the RSV with $IC_{50}=24.2$ μg/mL, $TC_{50}=138.3$ μg/mL and $SI=5.7$ in cell culture, and the corresponding values for positive control (ribavirin) are 3.4, 60.6 and 17.8 μg/mL, respectively. The antiviral activity of **1** was not better than the positive control; however, the selectivity index SI>4 for natural products was considered significant.[19] However, unlike other furanoditerpenoids,[9] **1** was inactive against the para-3 virus with $IC_{50}=51.9$ μg/mL, $TC_{50}=137.5$ μg/mL and $SI=2.6$, and the corresponding values for the positive control are 2.7 μg/mL, 62.5 μg/mL and 23.1 μg/mL, respectively. Similarly, **1** was also inactive against the Flu-A virus. Respiratory viral infections have long been recognized as important contributors to morbidity and mortality in young children and older adults,[20] and the search for natural products as antiviral agents against respiratory viruses has attracted considerable attention in recent years.[21] Based on an ethnomedical lead, our isolation of macrocaesalmin and the first simultaneous evaluation of its efficacy on three major respiratory pathogens thus provide a useful clue to the search for antiviral drugs against RSV infection.

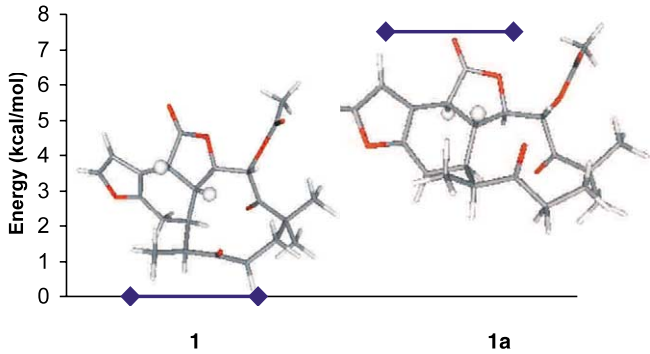

Figure 3. DFT-based structural optimization showing that macrocaesalmin (**1**) with *cis* B/D ring fusion is 7.51 kcal/mol more stable than 14-epimacrocaesalmin (**1a**) with *trans* B/D ring fusion.

Acknowledgements

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11. IR (KBr): 1747 cm^{-1} (acetate carbonyl) and 1792 cm^{-1} (saturated γ-lactone); ESI-MS *m/z* (%): $[M+Na]^+$ 425(100); ^1H NMR (400 MHz, $CDCl_3$): 1.68 (1H, m, H-2α), 2.10 (1H, m, H-2β), 1.37 (1H, m, H-3α), 1.87 (1H, m, H-3β), 5.05 (1H, d, $J=9.2$ Hz, H-6), 5.00 (1H, dd, $J=9.2$, 11.2 Hz, H-7), 2.23 (1H, ddd, $J=4.8$, 6.8, 11.2 Hz, H-8), 3.27 (1H, m, H-9), 2.40 (1H, dq, $J=4.2$, 8.1 Hz, H-10), 2.79 (1H, dd, $J=4.6$, 12.5 Hz, H-11α), 2.89 (1H, dd, $J=6.0$. 12.5 Hz, H-11β), 3.40 (1H, br d, $J=6.8$, H-14), 6.10 (1H, $J=2.0$ Hz, H-15), 7.22 (1H, $J=2.0$ Hz, H-16), 1.02 (3H, s, H-18), 1.25 (3H, s, H-19), 1.29 (3H, d, $J=8.1$ Hz, H-20), 2.00 (3H, s, 6-OAc); ^{13}C NMR (100 MHz, $CDCl_3$): 212.98 (s, C-1), 35.50 (t, C-2), 32.68 (t, C-3), 38.88 (s, C-4), 212.98 (s, C-5), 75.71 (d, C-6), 82.82 (d, C-7), 37.68 (d, C-8), 29.77 (d, C-9), 46.12 (d, C-10), 23.82 (t, C-11), 151.12 (s, C-12), 112.16 (s, C-13), 51.96 (d, C-14), 108.10 (d, C-15), 141.02 (d, C-16), 174.72 (s, C-17), 27.22 (q, C-18), 25.74 (q, C-19), 16.43 (q, C-20), 170.46 (s, C-21), 21.21 (q, C-22).
12. Crystal data: $C_{22}H_{26}O_7$, $M=402.43$, monoclinic, space group $C2$ (No. 5), $a=21.400(3)$, $b=8.560(1)$, $c=23.813(3)$ Å, $\beta=106.599(3)°$, $V=4180.3(8)$ $Å^3$, $Z=8$, $D_{calcd}=1.279$ g/cm^3, $F(000)=1712$, μ(Mo-Kα)$=0.095$ mm^{-1}. $R=0.0495$, $Rw=0.0919$ and $S=0.943$. CCDC Ref. No. 170840.
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