ORGANIC LETTERS

2011
Vol. 13, No. 19
5032–5035

Paracaseolide A, First α-Alkylbutenolide Dimer with an Unusual Tetraquinane Oxa-Cage Bislactone Skeleton from Chinese Mangrove *Sonneratia paracaseolaris*

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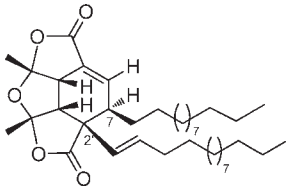
Received July 6, 2011

ABSTRACT



A novel α-alkylbutenolide dimer, paracaseolide A (2), characterized by an unusual tetraquinane oxa-cage bislactone skeleton bearing two linear alkyl chains, was isolated from the mangrove plant *Sonneratia paracaseolaris*. The structure of 2 was elucidated by extensive spectroscopic analysis. A plausible retrosynthetic pathway for paracaseolide A (2) was proposed. Compound 2 exhibited significant inhibitory activity against dual specificity phosphatase CDC25B, a key enzyme for cell cycle progression, with an IC_{50} value of 6.44 μM.

The α-alkylbutenolide substructure is frequently encountered in a variety of pharmacologically active natural products, as represented by the antileukemic/neuroprotective labdane pinusolide,[1] the novel antimalarial clerodane gomphostinin,[2] a potent antitumor acetogenin (annomolon A),[3] and the anti-inflammatory gorgonian lipid (**1**)[4] (Figure 1). In addition, α-substituted butenolides are also valuable intermediates in the synthesis of other important targets,[5] including a host of antimicrobial[6] and herbicidal lactones.[7] Although natural products containing such a scaffold are common, to our knowledge, the dimeric α-alkylbutenolide has not been reported to date.

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10.1021/ol201809q
Published on Web 08/29/2011

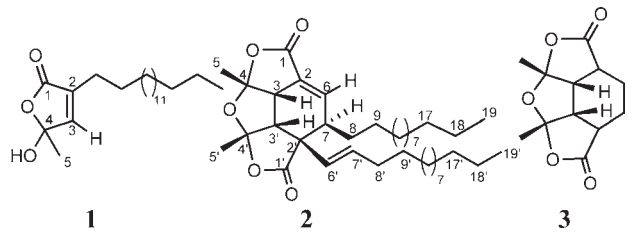

Figure 1. Structures of compounds **1**–**3**.

The mangrove plants belonging to the genus *Sonneratia* (family Sonneratiaceae) are widely distributed on seashores and their edges from eastern Africa to Indo-Malaysia, Australia, New Guinea, and the western Pacific islands. There are six *Sonneratia* species distributed in China.[8] Among them, *Sonneratia paracaseolaris* is an endemic mangrove species found in China.[9] A review of the literature revealed that no phytochemical studies have been carried out on this species. In the course of our research on biologically active substances from Chinese mangrove plants,[10] the title plant was recently collected from Zhanjiang, Guangdong Province, China. A chemical investigation of the stem bark of the plant has resulted in the isolation of a novel α-alkylbutenolide dimer, named paracaseolide A (**2**), characterized by an unusual tetraquinane oxa-cage bislactone system bearing two linear alkyl chains. In this paper, we report the isolation, structure elucidation and postulated biogenetic origin of this novel compound.

The dried stem bark (1.5 kg) of *S. paracaseolaris* was extracted exhaustively with methanol at room temperature. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc-soluble portion was repeatedly chromatographed over silica gel and Sephadex LH-20 to afford paracaseolide A (**2**, 12.0 mg).

Table 1. ^{1}H and ^{13}C NMR Data[a] of Compound **2** in $CDCl_3$

no.	δ_H[b] (J in Hz)	δ_C[c]	no.	δ_H[b] (J in Hz)	δ_C[c]
1		166.2, s	1′		175.1, s
2		129.3, s	2′		58.3, s
3	3.36 (dd, 9.6, 3.0)	46.7, d	3′	3.30 (d, 9.6)	50.2, d
4		113.8, s	4′		115.4, s
5	1.75 (s)	25.7, q	5′	1.61 (s)	26.5, q
6	7.24 (dd, 7.8, 3.0)	144.7, d	6′	5.48 (d, 15.6)	126.2, d
7	3.03 (m)	45.0, d	7′	5.82 (dt, 15.6, 6.6)	135.1, d
8a	1.16 (m)	27.9, t	8′	2.10 (m)	32.7, t
8b	1.67 (m)				
9	1.28 (m)	[c]	9′	1.36 (m)	29.1, t
17	1.24 (m)	31.9, t	17′	1.24 (m)	31.9, t
18	1.26 (m)	22.7, t	18′	1.26 (m)	22.7, t
19	0.87 (t, 7.2)	14.1, t	19′	0.87 (t, 7.2)	14.1, t

[a] Varian Mercury. Recorded at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. Assignments were deduced by analysis of 1D and 2D NMR spectra. [b] Methylene protons not reported appeared as a large signal at δ 1.24. [c] Methylene carbons not reported resonated between δ 28.2 and 29.6.

Paracaseolide A (**2**)[11] was isolated as an UV absorbing [λ_{max} = 213 nm (ε 3800)] amorphous white powder. The molecular formula, $C_{38}H_{62}O_5$, was determined by the HREIMS ion at m/z 598.4589 $[M]^+$ (calcd 598.4597), suggesting the presence of eight degrees of unsaturation. The IR spectrum showed the absorptions indicative of ester carbonyls (1757, 1772 cm^{-1}). The ^{13}C NMR spectrum of **2** (Table 1) disclosed 4 sp^3 methyls, 3 sp^2 methines, 3 sp^3 methines, 6 quaternary carbons, and 22 sp^3 methylenes. All of the protons were connected to carbons by HSQC experiments. The presence of one trisubstituted and one disubstituted double bonds was easily recognized by the ^{1}H and ^{13}C NMR resonances {[δ_H 7.24, (1H, dd, J = 7.8, 3.0 Hz, H-6); δ_C129.3 (C-2) and 144.7 (C-6)]; [δ_H 5.48 (1H, d, J = 15.6 Hz, H-6′) and 5.82 (1H, dt, J = 15.6, 6.6 Hz, H-7′); δ_C 126.2 (C-6′) and 135.1 (C-7′)]}. These data account for two of the required eight sites of unsaturation. Consequently, bearing in mind the presence of two ester carbonyls, paracaseolide A must possess four rings containing three oxygen atoms. In the ^{1}H NMR spectrum, the signal at δ_H 7.24 (H-6) was assigned to a vinylic proton conjugated to the ester carbonyl resonated at δ_C 166.2 (C-1) by HMBC cross peak between H-6 to C-1. This experiment provided an important anchor point to begin the substructure analysis on the basis of ^{1}H–^{1}H COSY, HMBC, HSQC, and ROESY spectral data.

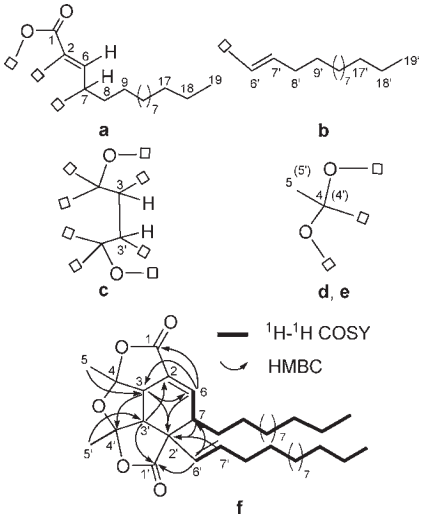

Figure 2. Partial structures **a**–**e** and selected key HMBC and ^{1}H–^{1}H COSY correlations of compound **2**.

Analysis of the ^{1}H NMR spectrum of **2**, aided by ^{1}H–^{1}H COSY, HSQC, and HMBC experiments, revealed the

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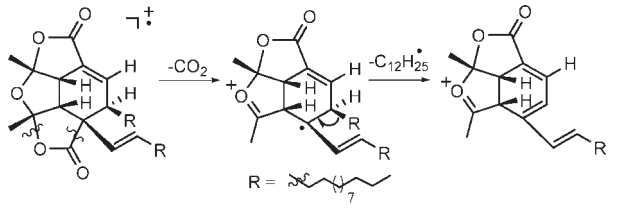
proton connectivities for the partial structures **a**–**c** and also allowed recognization of two methyls linked to oxygen-bearing carbons (partial structures **d**, **e**), respectively. For the partial structure **a**, the olefinic methine (δ_H 7.24, H-6) exhibited a clear connection with the adjacent methine proton (δ_H 3.03, H-7), which in turn, was further correlated with the methylene at δ_H 1.16 (Ha-8) and 1.67 (Hb-8). On the other hand, the terminal methyl (δ_H 0.87, H_3-19) showed correlation with H_2-18 (δ 1.26) that was further coupled with the signals centered at δ_H 1.24 assignable to methylenes (H_2-17 to H_2-9). Analogously, starting from H-6′ (δ 5.48), a series of distinct correlations between H-6′ to H-7′ (δ 5.82), H_2-8′ (δ 2.10), up to H_3-19′ (δ 0.87) were observed. Finally, the $^1H-^1H$ COSY spectrum of **2** showed another well-resolved cross-peak between methine protons resonated at δ_H 3.36 (H-3) and δ_H 3.30 (H-3′) according to the partial structure **c**, being linked to two oxygen-bearing carbons and two quaternary carbons, respectively. All of the elaborated subunits, including two ester carbonyls and two unassigned quaternary carbons (δ_C 129.3, C-2; δ_C 58.3, C-2′), were connected by extensive interpretation of well-resolved HMBC spectrum. Significant $^1H-^{13}C$ long-range correlations as shown in Figure 2 connected H-3 to C-2, C-4 (δ 113.8) and H-3′ to C-2′, C-4′ (δ 115.4). Thus, all of the NMR data of **2** were completely assigned according to the planar structure **f** for **2**. It is necessary to point out that determining the length of the alkyl chains at C-7 and C-2′ was not easy. They were tentatively assigned on the basis of the analysis of the HREIMS fragmentation patterns of **2** (Scheme 1). The characteristic fragment ion peak at *m*/*z* 554 [*m*/*z* 554.4715, $(C_{37}H_{62}O_3)^+$, Δ −1.6 mmu] could be ascribed to the loss of CO_2 from the molecular formula of **2**. Furthermore, an intense fragment ion peak at *m*/*z* 385 [*m*/*z* 385.2756, $(C_{25}H_{37}O_3)^+$, −1.4 mmu] could be rationalized by the successive loss of a *n*-C_{12} alkyl chain to form a more stable conjugated structure **6**. As a consequence, the other alkyl chain at C-2′ should contain 14 carbons including an olefin.

Scheme 1. Key EIMS Fragmentations of Paracaseolide A (**2**) (*m*/*z* Values; % Relative Abundance)



(11) Paracaseolide A: amorphous white powder; $[\alpha]^{24}_D$ +0.9 (*c* 0.33, $CHCl_3$); UV(MeOH) λ_{max} = 213 nm (ε 3800); IR (KBr) ν_{max} cm^{-1} 2921, 2850, 1772, 1757, 1458, 1389, 1313, 1284, 1057, 912; ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 598 $[M]^+$; HREIMS *m*/*z* 598.4589 $[M]^+$ (calcd for $C_{38}H_{62}O_5$ 598.4597).

The relative configurations for the chiral carbons around the tetracyclic core was established by detailed analysis of ROESY spectrum of **2**. As shown in the ROESY data (Figure 3), the correlations of H-3/CH_3-5, H-3/H_2-8, H-3′/CH_3-5′, H-3′/CH_3-5, H-3′/H-6′, and H-3′/H-7′ indicated that H-3, H-3′, H_3-5, H_3-5′, the *n*-C_{12} alkyl side chain at C-7, and the $\Delta^{6'(7')}$ tetradecenyl side chain at C-2′ were all cofacial, arbitrarily assigned to be β-oriented. The *trans* nature of the olefin at $\Delta^{6'(7')}$ was deduced by large coupling constants (J = 15.6 Hz) between H-6′ and H-7′. Thus, the structure of compound **2** was demonstrated as depicted.

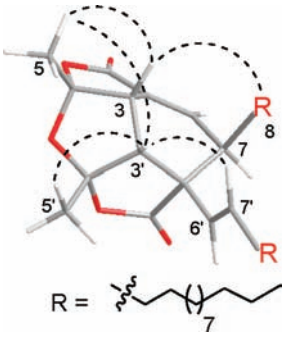
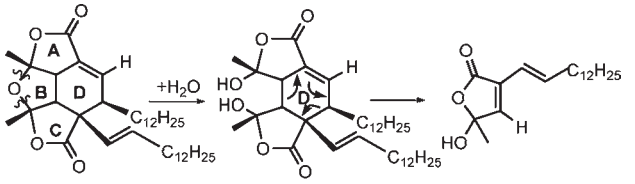


Figure 3. Key ROESY correlations for paracaseolide A (**2**).

Now, we can conclude that the structure of **2** is characterized by a tetracyclic nucleus (tetraquinane oxa-cage bislactones system) bearing two linear alkyl chains at C-2′ and C-7. Paracaseolide A (**2**) has an unusual bisacetal oxa-cage core related to the model compound **3** that was synthesized previously by Lin et al.[12] A plausible retrosynthetic pathway for paracaseolide A is proposed as shown in Scheme 2. The cleavage of the ether bridge of the THF ring (ring B) generated the tricyclic bislactones **7**, which after cleavage opening of the ring D yields butenolide lipid **8**. It is clear now that paracaseolide A (**2**) should be formally biosynthesized through a Diels–Alder [4 + 2] cycloaddition starting from two molecules of **8**. Paracaseolide A represents the first example of an α-alkylbutenolide dimer from a natural source.

Scheme 2. Plausible Retrosynthetic Pathway for Paracaseolide A (**2**)



As mentioned above, the monomeric α-alkylbutenolides or natural products containing butenolide moiety often showed a variety of promising bioactivities,[1−4] which inspired us to test other bioactivities of the dimeric compound **2**. In the bioassay of inhibitory activity against dual specificity phosphatase CDC25B, which is a key enzyme for cell cycle progression and was observed in a variety of cancers with a striking association with tumor aggressiveness and poor prognosis,[13] paracaseolide A (**2**) showed significant bioactivity with an IC_{50} value of 6.44 μM (4.92−7.96 μM, 91.3% CI).

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Acknowledgment. This research work was financially supported by the National S & T Major Project (No. 2009ZX09301-001), the National Marine "863" Project (No. 2011AA090071-2), the Natural Science Foundation of China (Nos. 40976048, 31070310, and 21021063), the EU seventh Framework Programme-IRSES Project (2010-2014), and the NSFC-TRF International Cooperation Project (No. 20911140471) and was partially funded by a grant from CAS (KSCX2-YW-R-18) and the STCSM Project (No. 10540702900).

Supporting Information Available. Experimental procedures and full NMR data of paracaseolide A. This material is available free of charge via the Internet at http://pubs.acs.org.