Tetrahedron Letters 48 (2007) 7194-7198

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New cassane butenolide hemiketal diterpenes from the marine creeper *Caesalpinia honduc* and their antiproliferative activity

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Abstract-Two new cassane butenolides, caesalpinolide A (1) and B (2), epimeric at the hemiketal position were isolated from the marine creeper *Caesalpinia bonduc.* The structures were elucidated by the analysis of spectroscopic data and relative stereochemistry was assigned on the basis of ROESY correlations. Compounds 1 and 2 were found to inhibit MCF-7 breast cancer cell lines with ICso values of (IlM) 12.8 and 6.1, respectively, along with the inhibition of endometrial and cervical cancer cell lines.

In the process of drug discovery, Nature has always been an important source with unmatched biological and chemical diversities along with a plethora of unexplored,,"~abitats. *Caesalpinia bonduc* L. Roxb. (Fabaceae), is a medicinal plant widely distributed throughout the tropics and subtropics. I C. *bonduc* in the present study was a marine creeper of mangrove plants found in the intertidal zone of the south Andaman Islands, India. In traditional medicine, different parts of the plant are used to treat asthma, chronic fever, coughs, headache and stomach upset. 2 Different parts of the plant have shown a variety of pharmacological activities such as antimicrobial, adaptogenic, contractile activity in smooth muscles and skeletal muscles and antifilaria1.3 A wide variety of cassane furanoditerpenes have been isolated from the genus *Caesalpinia.4* Among this class of compounds, cas sane butenolides and butenolide hemiketals, are rare photo oxygenation products of the furan ring.4d These butenolide hemiketals of cassane type have shown very limited distribution among a few genera of Fabaceae including *Caesalpinia,* which reinforces their importance as biogenetic and chemotaxonomic markers.

As a part of an ongoing program designated to drug discovery from natural products, a cell based assay was used to screen the crude extract of the dried whole plant of C. *bonduc* for the ability to arrest human breast cancer MCF-7 ceUs. The ethanolic extract showed promising activity in the assay.

Further fractionation of the EtOH extract and column chromatographic purification of the hexane fraction

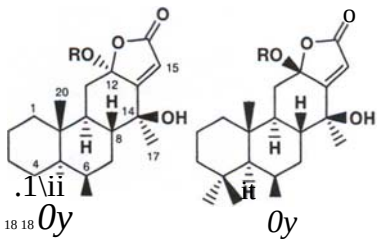

Figure I. Structure of compounds.

"CDRI Communication No. 7219.

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Table 1. ^{1}H and ^{13}C NMR data for compounds **1**, **1a**, **2** and **2a**

	1 (Pyr-d_5)[a] δ_H [mult., J (Hz)]	**1** (Pyr-d_5)[b] δ_C	**1a** ($CDCl_3$)[a] δ_H [mult., J (Hz)]	**1a** ($CDCl_3$)[a] δ_C	**2** (Pyr-d_5)[a] δ_H [mult., J (Hz)]	**2** (Pyr-d_5)[b] δ_C	**2a** ($CDCl_3$)[a] δ_H [mult., J (Hz)]	**2a** ($CDCl_3$)[a] δ_C[a]
1	1.66 [m]*	42.5	1.75 [br d, 13.2]	42.1	1.63 [br d, 12.6]	42.4	1.75 [br d, 13.2]	41.8
	1.03 [s]*		1.03 [dt, 13.2, 3.6]		0.95 [m]*		1.08 [dt, 13.2, 3.6]	
2	1.53 [m]	19.6	1.60 [m]	18.7	1.51 [m]*	19.6	1.61 [m]	18.8
	1.35 [m]		1.49 [td, 13.8, 3.0]		1.31 [m]		1.51 [td, 13.8, 3.0]	
3	1.32 [m]	44.2	1.41 [m]	43.5	1.28 [m]	44.3	1.40 [m]	43.6
	1.14 [m]*		1.19 [dt, 13.8, 3.6]		1.05 [m]		1.20 [dt, 13.8, 3.6]	
4	—	34.4	—	33.9	—	34.3	—	33.8
5	1.15 [br s]*	55.7	1.07 [br s]	55.3	1.03 [br s]	55.3	1.12 [br s]	54.7
6	5.77 [br s]	69.8	5.56 [br s]	68.5	5.81 [br s]	70.0	5.57 [br s]	69.2
7	2.65 [td, 14.4, 3.0]	32.7	2.15 [br td, 13.8] 1.42 [m]	31.4	2.25 [td, 14.4, 3.0] 2.09 [dt, 14.4, 3.0]	32.0	1.97 [br td, 13.8] 1.77 [m]	30.7
	1.66 [m]*							
8	2.25 [br t, 11.4]	45.3	1.80 [dt, 12.0, 3.6]	44.2	1.72 [br dt, 11.4, 3.0]	44.2	1.56 [dt, 12.0, 3.6]	43.2
9	1.73 [m]*	48.2	1.26 [br dt]*	46.7	2.01 [dt, 12.6, 1.8]	46.9	1.66 [br d]	45.4
10	—	38.7	—	37.9	—	38.2	—	37.5
11	2.80 [dd, 20.1, 11.5]	39.6	2.70 [dd, 13.2, 2.4] 1.35 [br t, 13.2]	37.4	2.80 [dd, 13.2, 2.4]	39.6	2.69 [dd, 13.2, 2.4]	38.5
	1.73 [m]*				1.58 [t, 12.8]		1.28 [br t, 13.2]	
12	—	107.0	—	104.7	—	107.2	—	104.6
13	—	179.1	—	171.8	—	172.1	—	169.1
14	—	74.0	—	73.5	—	71.5	—	70.9
15	6.48 [s]	113.3	6.08 [s]	114.8	6.09 [s]	114.0	5.91 [s]	115.1
16	—	171.3	—	168.7	—	171.0	—	168.5
17	1.78 [s]	22.2	1.26 [s]*	20.1	1.50 [s]*	24.0	1.49 [s]	24.3
18	1.00 [s]	34.1	0.98 [s]	33.6	0.95 [s]*	33.9	0.97 [s]	33.4
19	1.02 [s]*	24.0	0.99 [s]	23.2	1.02 [s]	23.9	0.98 [s]	23.3
20	1.12 [s]	17.8	1.11 [s]	17.0	1.12 [s]	17.8	1.08 [s]	16.9
$COCH_3$-6	1.91 [s]	21.8	2.03 [s]	21.7	2.14 [s]	22.1	2.03 [s]	21.7
		170.6		170.5		170.8		170.4
$COCH_3$-12	—	—	2.04 [s]	21.6	—	—	2.04 [s]	21.5
				168.5				170.0

* Overlapped signals.

[a] Data recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C.

[b] Data recorded at 75 MHz for ^{13}C.

resulted in the isolation of two new cassane butenolide hemiketals **1** and **2**,[5] which were epimeric at the hemiketal carbon (Fig. 1). The β-orientation of the hemiketal hydroxyl in **2** is an unusual observation as all the cassane butenolide hemiketals isolated thus far possess an α-OH at the hemiketal C-12.[6]

Compound **1** was isolated as white crystals, mp 218–219 °C, $[\alpha]_D^{25}$ −143.52 (*c* 0.085, MeOH), and gave a molecular ion peak at *m/z* 392.22690 in the DART-HRMS spectrum consistent with the molecular formula $C_{22}H_{32}O_6$. It exhibited IR maxima at 3420 and 1731 cm^{-1}, indicative of hydroxyl and α,β-unsaturated γ-lactone functionalities, respectively. Unambiguous NMR asssignments (Table 1) were made by 2D-homo- and heteronuclear NMR experiments. The presence of the α,β-unsaturated γ-lactone moiety was further substantiated by the signals for the butenolide olefin H/C at δ 6.48/113.3, the downfield quaternary olefin carbon at δ 179.1, the lactone carbonyl at δ 171.3 and the hemiketal carbon at δ 107.0. The DEPT edited ^{13}C NMR spectrum indicated the presence of 22 carbons accounting for five methyl, one olefin methine, four sp^3 methine, five methylene, four sp^3 quaternary, one olefinic quaternary and two carbonyl carbons. The ^1H NMR spectrum showed singlets for five methyl groups at δ 1.00, 1.02, 1.12, 1.78 and 1.91. The signal at δ 1.91 corresponds to an acetate methyl from its HMBC correlation to the carbonyl at δ 170.6 and the signal at δ 1.78 corresponds to CH_3-17 as indicated by the HMBC correlation with the quaternary olefinic carbon at δ 179.1. The ^3J and/or ^2J HMBC correlations of CH_3-17 and butenolide olefin at δ 6.48 with the quaternary hydroxylated carbon at δ 74.0 indicated that C-14 of the cassane skeleton was hydroxylated. The other two methyl signals at δ 1.00 and 1.02 showed common HMBC correlations, which indicated that they were the CH_3-18 and CH_3-19 methyls. The remaining methyl at δ 1.12 (CH_3-20) along with CH_3-18 and CH_3-19 showed HMBC correlations with the deshielded angular methine at δ 55.7 indicating an unsubstituted C-5 position. Among other distinct proton signals were resonances for H-5 at δ 1.15 which showed an HSQC with C-5 at δ 55.7. The ^1H spectrum also revealed the presence of an oxymethine at δ 5.77/69.8. The downfield appearance of the oxymethine along with its HMBC correlation with the acetate carbonyl at δ 170.6 indicated it to be an acetylated oxymethine. Further, the position of the oxymethine at C-6 was confirmed by its COSY correlation with the characteristic signal for H-5 at δ 1.15. Among other resolved signals were those for H-8 at δ 2.25 (br t, $J = 11.4$ Hz), H-7β at δ 2.65 (td, $J = 14.4$, 3.0 Hz) and H-11α at δ 2.80 (dd, $J = 20.1$, 11.5 Hz). Most of the methylene signals appeared as complex multiplets overlapping with other signals and were assigned based on their correlations in the COSY, HSQC and HMBC spectra. To further simplify the spectrum and assign the relative stereochemistry at the hemiketal C-12 position, **1** was acetylated with acetic anhydride and pyridine at room temperature to afford the monoacylated product **1a**. Monoacetylation at the hemiketal hydroxyl was confirmed by the appearance of a C-12 ketal at δ 104.7, upfield relatively to δ 107.0 in **1**. The ^1H NMR spectrum of the acetate showed resolved signals for H-8 at δ 1.80 [dt, $J = 12.0$, 3.6 Hz], H-9 at δ 1.26 [br dt, overlapped with the singlet for CH_3-17], H-11β at δ 1.35 [br t, $J = 13.2$ Hz], H-11α at δ 2.70 [dd, $J = 13.2$, 2.4 Hz], H-7β at δ 2.15 [br td, $J = 13.8$ Hz] and H-7α at δ 1.42 [m], along with the appearance of one singlet for the C-12-acetate at δ 2.04. The relative stereochemistries were assigned based on ROESY correlations observed among signals for CH_3-20 with CH_3-19, H-8, H-11β, $COCH_3$-6, from CH_3-18 to signals for H-5, H-6 and from the overlapped signal for CH_3-17 and H-9 with signals for H-11α, H-7α, H-1α and $COCH_3$-12. ROESY correlation of the singlet for CH_3-17 at δ 1.26 with that of the acetate methyl at δ 2.04 clearly revealed the ketal acetyl to be α-oriented (Fig. 2). Based on the above experimental observations, the structure of **1** was assigned as 6β-acetoxy-12β,14β-dihydroxycassa-(13)15-en-16,12-olide.

Compound **2** was isolated as white fluffy crystals, mp 212–213 °C, $[\alpha]_D^{25}$ −123.07 (*c* 0.078, MeOH), which gave a $[M+H]^+$ molecular ion peak at *m/z* 393.22793 in the DART-HRMS spectrum consistent with the molecular formula $C_{22}H_{32}O_6$. It exhibited IR maxima at 3403 and 1742 cm^{-1}, indicative of hydroxyl and α,β-unsatu-

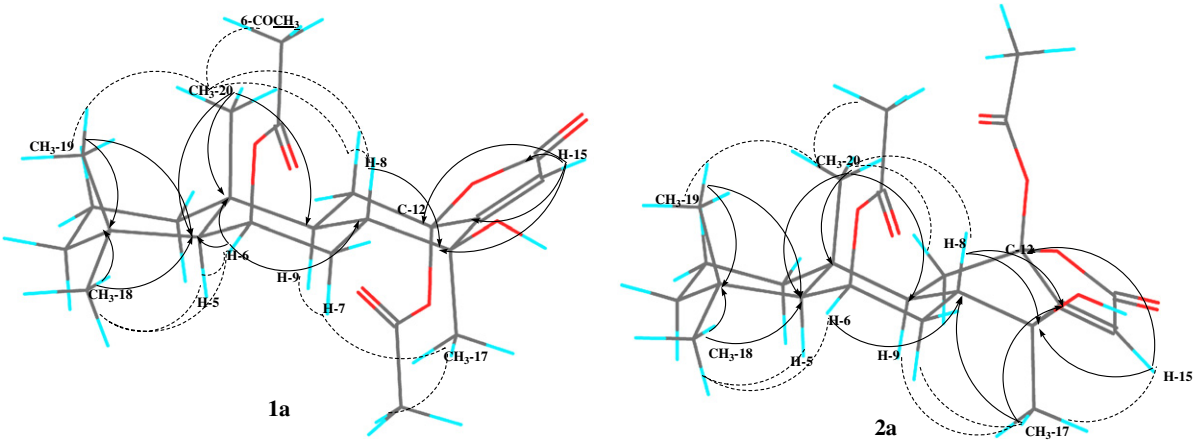

Figure 2. Energy minimized conformations of **1a** and **2a** and their HMBC (→) and ROESY (···) correlations.

rated γ-lactone functionalities, respectively. The presence of the α,β-butenolide hemiketal ring was characterized by signals at δ 6.09 (H-15) in the ^{1}H NMR spectrum and a hemiketal carbon at δ 107.2 along with lactone carbonyl at δ 171.0 and olefinic carbons of the butenolide at δ 114.0 (C-15) and 172.1 (C-13). Resonances for the four methyl groups appeared at δ 0.95, 1.02, 1.12 and 1.50, and the acetyl group appeared at δ 2.14. The spectrum was more or less similar to that of compound **1** with differences in the ^{1}H NMR shifts for H-8, δ 1.72 (br dt, $J = 11.4$, 3.0 Hz), H-9, δ 2.01 (dt, $J = 12.6$, 1.8 Hz), CH_3-17, δ 1.50 (s) and H-15, δ 6.09 (s). The ^{13}C NMR chemical shift values were also similar to those of **1** with differences in the resonances of the quaternary olefinic butenolide C-13 (δ 172.1) and C-17 (δ 24.0). Both these compounds have an R_f difference of 0.1 on TLC when run in $Me_2CO/CHCl_3$ (4:6). Acetate (**2a**) was also prepared in similar fashion and showed very similar resonance patterns to **1a** with differences in the ^{1}H NMR spectrum for H-8 [δ 1.56 (dt, $J = 12.0$, 3.6 Hz)] and H-9 [δ 1.66, br d]. Further study of the NMR data for the two compounds revealed that the A/B rings were very similar in both compounds and the suspected orientation of the acetoxy function at C-6 was also found to be the same based on a ROESY correlation between H-6 and CH_3-18 and H-5 (Fig. 2). The appearance of significant ROESY correlation between CH_3-17 and the butenolide olefin H-15 and the absence of a ROESY correlation between –OCO*CH*$_3$ at C-12 and CH_3-17 clearly accounted for the β-orientation of the C-12 hemiketal hydroxyl. The relative abundance of the product ions at *m*/*z* 375 corresponding to the elimination of water in tandem mass spectrometry was found to be prominent in **1** but not in compound **2** (see Supplementary data). The significant difference in the stability of the product ion at *m*/*z* 375 also revealed the difference in the conformation of the fused butenolide hemiketal ring system.

It can be visualized from the energy minimized conformation (MOPAC AM-1, 0.01 RMS gradient) of both compounds **1** and **2** that the chair conformation of ring-C in compound **1** would have prevented the spacial interaction of CH_3-17 and H-15, whereas the β-oriented hemiketal hydroxyl forced ring-C to adopt a twisted chair conformation in **2**, which forced CH_3-17 and H-15 into a *syn*-orientation and thus ROESY correlations were observed among these signals (Figs. 2 and 3). All the experimental results and the key ROESY correlations led to the characterization of compound **2** as 6β-acetoxy-12β,14β-dihydroxycassa-(13)15-en-16,12-olide.

Antiproliferative activity of compounds **1** and **2** against breast carcinoma cell lines (MCF-7 and MDA-MB-231), endometrial carcinoma cell line (Ishikawa), and cervical carcinoma cell line (Hela) was evaluated (Table 2). Compounds **1** and **2** showed significant inhibition of MCF-7 cell lines with IC_{50} (μM) values 12.8 and 6.1, respectively.

In conclusion, cassane butenolide hemiketals **1** and **2** were isolated with the first example of a β-oriented hemiketal hydroxyl. Together with the observed stereochemical differences and biological activity profile, our results might be a promising basis for the development of a new class of antiproliferative agents.

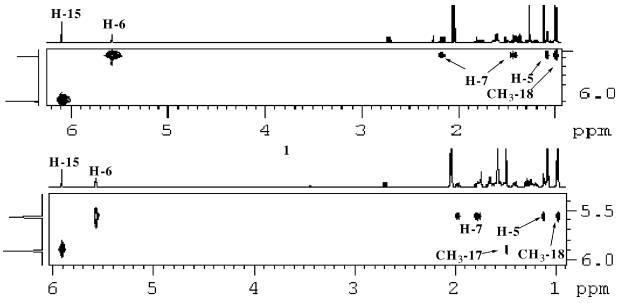

Figure 3. ROESY correlations (part of the spectrum) observed for H-15 and H-6 of **1** and **2**, respectively.

Table 2. In vitro antiproliferative activity of compounds **1** and **2**

Compound	IC_{50} (μM)			
	MCF-7	MDA-MB-231	Ishikawa	Hela
1	12.8	17.0	12.7	18.5
2	6.1	11.2	11.3	18.8

Acknowledgements

Thanks are due to DOD for financial assistance, Dr. M. N. Srivastava for the collection of the plant, Dr. C. Singh, Dr. R. Maurya, Dr. R. Raghubir, and Dr. N. Chattopadhyay for their constant encouragement and critical suggestions.

Supplementary data

Supplementary data (Experimental details on the isolation of compounds **1** and **2** and their spectral data (^{1}H, ^{13}C, HSQC, HMBC, ROESY and tandem mass spectrum)) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.07.206.

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5. Air dried and powdered plant material (4 kg) was extracted at room temperature with EtOH. The EtOH extract (230 g) was then fractionated successively into four fractions: *n*-hexane (38 g), $CHCl_3$ (20 g), *n*-BuOH (58 g), and aqueous (80 g). The *n*-hexane (32 g) fraction was subjected to column chromatography over silica gel, eluting with EtOAc:hexane gradient (0–100%) to afford 18 fractions (F1–F18). Fraction F-14 was subjected to chromatographic purification over silica gel eluting with a gradient of MeOH:$CHCl_3$ (0.2–6%) to give impure sub-fractions containing **1** and **2**. The impure fractions when subjected to flash silica column chromatography using a gradient of Me_2CO:$CHCl_3$ (1–10%) eluted pure compound **1** (15 mg) and **2** (10 mg).
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