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# Cassane Diterpenes from *Caesalpinia bonduc*

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ABSTRACT

Three cassane diterpene hemiketals, caesalpinolide-C, caesalpinolide-D, caesalpinolide-E and one cassane furanoditerpene were isolated from *Caesalpinia bonduc*. The molecular structures were elucidated using NMR spectroscopy in combination with IR, UV and mass spectral data and relative stereochemistries were determined through ROESY correlation. The isolated compounds were tested for their antiproliferative activity against MCF-7 (breast adenocarcinoma), DU145 (prostate carcinoma), C33A (Cervical carcinoma) and Vero (African green monkey kidney fibroblast) cells.



## 1. Introduction

*Caesalpinia bonduc* L. Roxb., (Fabaceae), was collected from the intertidal zone of south Andaman Islands, India as a marine creeper of the mangrove plants (Bennet, 1987). In traditional medicine different parts of the plant are used to treat asthma, chronic fever, cough, headache and stomach upset (Nandkarni, 1976; Satyavati et al., 1956; Chopra et al., 1956). 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 antifilarial activity (Simin et al., 2001; Kannur et al., 2006; Datté et al., 2004; Rastogi et al., 1996).

We previously described the isolation of two epimeric cassane butenolide hemiketals and their antiproliferative activity against different cancer cell lines (Yadav et al., 2007; Kanojiya and Yadav, 2008). Continuing with our earlier studies on *C. bonduc*, here we report the isolation and structure elucidation of four new cassane diterpenes caesalpinolide-C (**1**), caesalpinolide-D (**2**), caesalpinolide-E (**3**) and cassane furanoditerpene (**4**) (Fig. 1) along with other known compounds. *Caesalpinia* sp. has a propensity for producing cassane furanoditerpenes (Kinoshita et al., 2005; Kaluni et al., 2005; Lyder et al., 1998a; Jiang et al., 2001; Linn et al., 2005; Ragasa et al., 2002; Jiang et al., 2002; Roengsumran et al., 1998). Among cassane diterpenes production of the cassane butenolides or butenolide hemiketals is possibly via photooxygenation of the furan ring and these compounds have very limited distribution (Lyder et al., 1998b; Torres-Mendoza et al., 2004; Roach et al., 2003; Kinoshita et al., 2005; Jiang et al., 2002).

These compounds were isolated following fractionation of the ethanolic extract from which a hexane fraction was found to be active against MCF-7 cell lines (Yadav et al., 2007). The isolated compounds were tested for their antiprolifertative activity against MCF-7 (breast adenocarcinoma), DU145 (prostate carcinoma), C33A (Cervical carcinoma) and Vero (African green monkey kidney fibroblast) cell lines and were found to show a low to moderate activity profile. The structure elucidation of new compounds and their activity profile are discussed in the following sections.

## 2. Results and discussion

The ethanol extract of the plant showed inhibition of breast cancer cell lines and was fractionated in to n-hexane, chloroform, n-butanol and water soluble fractions. The active n-hexane fraction was further subjected to repeated chromatographic purifications to yield new cassane butenolide hemiketals named as caesalpinolide-C-E (**1**–**3**) and one cassane furanoditerpene (**4**).

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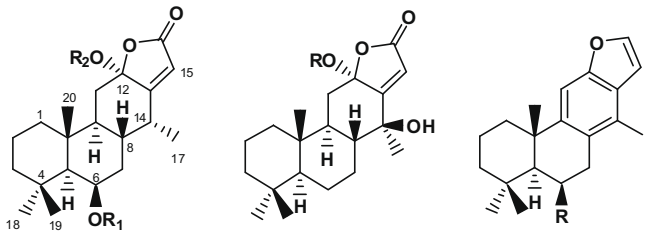

**1** $R_1=R_2=H$
**1a** $R_1=H$, $R_2=COCH_3$
**1b** $R_1=R_2=COCH_3$
**3** $R_1=COCH_3$, $R_2=H$

**2** R = H
**2a** R = $COCH_3$

**4** R = $OCOCH_3$
**5** R = H

**Fig. 1.** Structrure of compounds.

Compound **1** was isolated as white crystals, mp. 216–218 °C, $[a]_D^{25}$ −95.0 (*c* 0.12, MeOH), gave a pseudomolecular ion $[M+H]^+$ peak at *m/z* 335.2097 in the DART-HRMS spectrum consistent with the molecular formula of $C_{20}H_{30}O_4$ (calc. for $C_{20}H_{31}O_4 \cdot$ 335.2222). It exhibited IR maxima at 3420 and 1725 $cm^{-1}$, indicative of hydroxyl and α,β-unsaturated γ-lactone functionalities, respectively. The $^1H$ NMR spectrum (Table 1) together with the COSY, *g*HSQC, and *g*HMBC data revealed the presence of a cassane butenolide skeleton with an α/β-unsaturated γ-lactone moiety. The presence of an α-β-unsaturated γ-lactone moiety was substantiated by the signals for butenolide olefin H/C at δ 5.87/ 113.4, downfield quaternary olefin carbon at δ 175.1, lactone carbonyl at δ 171.8 and hemiketal carbon at δ 107.5. $^{13}C$ NMR spectrum showed presence of twenty carbons accounting for four methyl, one olefin methine, five $sp^3$ methine, five methylene, three $sp^3$ quaternary, one olefinic quaternary and one carbonyl carbon. $^1H$ NMR showed signals for four methyl groups at δ 1.08(*s*), 1.37(*s*), 1.51(*s*) and 1.35(*d*, 7.2). Signal at δ 1.35 corresponds to $CH_3$-17 of cassane skeleton as indicated by its HMBC correlation with the quaternary olefinic carbon at δ 175.1. Appearance of the $CH_3$-17 as doublet indicated C-14 of cassane skeleton to be a methine appearing at δ 2.87 (*m*) which in turn showed correlations in HMBC spectrum with signals at 175.1(C-13), 113.4(C-15), 107.5(C-12) of the butenolide framework. The other two methyl signals at δ 1.08 and 1.51 showed common HMBC correlations which indicated them to be the $CH_3$-18 and $CH_3$-19 methyl of cassane skeleton. The remaining methyl at δ 1.37($CH_3$-20) along with $CH_3$-18 and $CH_3$-19 showed HMBC correlations with the deshielded angular methine at δ 56.8 indicating an unsubstituted C-5 position.

Among other distinct proton signals are signals for H-5 at δ 0.97 which showed HSQC with the C-5 at δ 56.8. The spectrum also revealed the presence of an oxymethine at δ 4.68/ 66.3 which was assigned to C-6 based on its COSY correlation with characteristic signal for H-5 at δ 0.97. Other resolved signals were those for H-8 at δ 2.36 (*tt*, *J* = 12.2, 4.2 Hz), H-7β and H-9 at δ 1.80 (*m*) and H-11α at δ 2.76 (*dd*, *J* = 12.6, 3.0 Hz). Unambiguous assignments were made by analysing 2D NMR correlations (DQF-COSY, HSQC and HMBC). Most of the methylene signals appeared as complex multiplets overlapped with other signals in $^1H$ NMR spectrum

**Table 1**
NMR data for compounds **1**, **1a**, **2**, **2a** and **2b**.

| Position | **1** (Pyr–$d_5$)[a] δH [mult., *J*(Hz)] | **1** (Pyr–$d_5$)[a] δC | **1a** ($CDCl_3$)[a] δH [mult., *J*(Hz)] | **1a** ($CDCl_3$)[a] δC | **1b** ($CDCl_3$)[a] δH [mult., *J*(Hz)] | **1b** ($CDCl_3$)[a] δC | **2** (Pyr–$d_5$)[a] δH [mult., *J*(Hz)] | **2** (Pyr–$d_5$)[a] δC | **2a** ($CDCl_3$)[a] δH [mult., *J*(Hz)] | **2a** ($CDCl_3$)[a] δC |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.66 [*m*]* 1.03 [*brt*, 13.2 Hz] | 42.4 | 1.73 [*brd*, 12.6 Hz] 1.03 [*m*]* | 42.1 | 1.76 [*m*]* 1.06 [*m*]* | 42.2 | 1.59 [*m*]* 0.96 [*dd*, 13.2, 3.0 Hz] | 40.1 | 1.72 [*m*]* 1.00 [*dt*, 12.9, 3.6 Hz] | 39.8 |
| 2 | 1.66 [*m*]* 1.37 [*m*]* | 19.7 | 1.65[*m*]* 1.42 [*m*]* | 18.8 | 1.62 [*m*] 1.49 [*m*] | 18.9 | 1.45 [*m*]* 1.32 [*m*]* | 19.5 | 1.55 [*m*]* 1.48 [*m*]* | 18.9 |
| 3 | 1.37 [*m*]* 1.20 [*brt*, 12.6 Hz] | 44.3 | 1.42 [*m*]* 1.19 [*m*]* | 43.7 | 1.42 [*m*]* 1.21 [*dt*, 13.8, 3.6 Hz] | 43.8 | 1.32 [*m*]* 1.12 [*dt*, 13.2, 3.6 Hz] | 42.4 | 1.43 [*m*]* 1.16 [*brd*t, 13.3, 3.9 Hz] | 41.9 |
| 4 | – | 34.6 | – | 34.1 | – | 34.1 | – | 33.7 | – | 33.4 |
| 5 | 0.97 [*brs*]* | 56.8 | 0.96 [*brs*] | 56.1 | 1.06 [*brd*, 1.02 Hz] | 55.4 | 0.92 [*brd*, 10.8 Hz] | 55.6 | 0.91 [*m*]* | 55.3 |
| 6 | 4.68[*brs*] | 66.3 | 4.51 [*brs*] | 67.1 | 5.51 [*brs*] | 69.3 | 1.66 [*m*]* 1.23 [*dq*, 12.6, 3.0 Hz] | 22.1 | 1.72 [*m*]* 1.27 [*m*]* | 21.2 |
| 7 | 1.80[*m*]* 1.66 [*m*]* | 41.0 | 1.65 [*m*]* 1.57 [*m*]* | 39.2 | 1.76 [*m*]* 1.53 [*m*] | 35.5 | 2.44 [*dd*, 12.2, 3.6 Hz] 1.45 [*m*]* | 27.4 | 2.04 [*m*] 1.27 [*m*]* | 26.4 |
| 8 | 2.36 [*tt*,12.2, 4.2 Hz] | 36.5 | 2.08 [*tt*, 12.0, 4.8 Hz] | 35.5 | 1.89 [*m*] | 36.2 | 1.91 [*dt*,11.4, 3.6 Hz] | 49.8 | 1.55 [*m*]* | 49.0 |
| 9 | 1.80 [*m*]* | 46.5 | 1.42 [*m*]* | 44.8 | 1.46 [*m*]* | 44.7 | 1.63 [*m*]* | 48.2 | 1.21 [*m*]* | 47.0 |
| 10 | – | 38.2 | – | 37.4 | – | 37.95 | – | 37.9 | – | 37.5 |
| 11 | 2.76 [*dd*, 12.6, 3.0 Hz] 1.66 [*m*]* | 39.5 | 2.67 [*dd*, 13.8, 3.0 Hz] 1.30 [*brt*, 13.2 Hz] | 37.8 | 2.69 [*dd*, 13.2, 3.0 Hz] 1.31 [*t*, 13.2 Hz] | 37.93 | 2.74 [*d*, 10.2 Hz] 1.59 [*m*]* | 39.6 | 2.66 [*dd*, 13.2, 2.4 Hz] 1.27 [*m*]* | 37.8 |
| 12 | – | 107.5 | – | 105.1 | – | 105.2 | – | 107.1 | – | 105.1 |
| 13 | – | 175.1 | – | 169.9 | – | 169.9 | – | 179.4 | – | 172.5 |
| 14 | 2.87 [*m*] | 37.3 | 3.00 [*m*] | 36.0 | 2.98 [*m*] | 36.0 | – | 74.5 | – | 74.4 |
| 15 | 5.87 [*s*] | 113.4 | 5.86 [*s*] | 115.0 | 5.87 [*s*] | 115.5 | 6.48 [*s*] | 113.2 | 6.06 [*s*] | 114.8 |
| 16 | – | 171.8 | – | 169.7 | – | 169.7 | – | 171.4 | – | 169.1 |
| 17 | 1.35 [*d*, 7.2 Hz] | 13.8 | 1.03 [*d*, 7.2 Hz] | 11.6 | 1.03 [*d*, 7.2 Hz] | 11.8 | 1.81 [*s*] | 22.1 | 1.28 [*s*] | 20.2 |
| 18 | 1.51 [*s*] | 34.5 | 1.01 [*s*] | 33.8 | 0.98 [*s*] | 33.9 | 0.88 [*s*] | 34.2 | 0.89 [*s*] | 33.8 |
| 19 | 1.08 [*s*]* | 24.5 | 1.24 [*s*] | 24.3 | 1.00 [*s*] | 23.5 | 0.79 [*s*] | 22.5 | 0.84 [*s*] | 22.2 |
| 20 | 1.37 [*s*] | 18.1 | 1.13 [*s*] | 17.5 | 1.10 [*s*] | 17.3 | 0.75 [*s*] | 14.8 | 0.82 [*s*] | 14.4 |
| $COCH_3$ –6 | – | – | – | – | 2.04 [*s*] | 21.9 170.6 | – | – | – | – |
| $COCH_3$–12 | – | – | 2.04 [*s*] | 21.7 168.6 | 2.04 [*s*] | 22.0 168.8 | – | – | 2.05 [*s*] | – |

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

and were assigned based on their correlations in the COSY, HSQC and HMBC spectrum. 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** and diacetylated product **1b** (major), whereas at elevated temperatures afforded diacetylated product **1b** exclusively. Monoacetylation at the hemiketal hydroxyl in **1a** was confirmed by the appearance of C-12 ketal carbon at $\delta$ 105.1, relatively upfield to $\delta$ 107.5 in **1** whereas the diacetylated product **1b** has a similar shift in the signals for C-12 in addition to a downfield shift in the signal for H-6 which appeared at $\delta$ 5.51 as compared to $\delta$ 4.68 in the parent compound. The $^1$H NMR spectrum of diacetate **1b** showed resolved signals for the H-8 at $\delta$ 1.89 (*m*), H-11β at $\delta$ 2.69 (*dd*, *J* = 13.2, 3.0 Hz), H-11α at $\delta$ 1.31 (*t*, *J* = 13.2 Hz), H-7α at $\delta$ 1.53 (*m*) and H-9 at $\delta$ 1.46 (*m*), partially overlapped with signals for H-3, along with appearance of singlets for two acetate methyls at $\delta$ 2.04. Relative stereochemistries were assigned based on ROESY correlations for **1b** (Fig. 2). ROESY correlations among signals for $CH_3$-20 with $CH_3$-19, H-8 and $COCH_3$-6 and from $CH_3$-18 with H-5, H-6 indicated for the relative stereochemistry of the ring A and B and the α-orientation of H-5/6. ROESY correlations from H-14 with the signals for H-15 and from $CH_3$-17 with the signals for $COCH_3$-12 clearly revealed the orientation of the ketal hydroxyl to be α (Fig. 2). Based on the above experimental observations the structure of **1** was assigned as 6β-hydroxy-12α-hydroxycassa-(13)15-en-16,12-olide.

Compound **2** was isolated as white fluffy crystals, mp. 220–222 °C, $[a]_D^{25}$ −44.0 (*c* 0.05, MeOH), showed a pseudomolecular ion $[M+H]^+$ peak at *m/z* 335.2200 in the DART-HRMS spectrum consistent with the molecular formula of $C_{20}H_{30}O_4$ (calc. for $C_{20}H_{31}O_4$ 335.22223). It exhibited IR maxima at 3382 and 1728 cm$^{-1}$, indicative of hydroxyl and α,β-unsaturated γ-lactone functionalities, respectively. The presence of an α,β-butenolide hemiketal ring was characterized by signals at $\delta$ 6.48 (H-15) in the $^1$H NMR spectrum and a hemiketal carbon at $\delta$ 107.1 along with a lactone carbonyl at $\delta$ 171.4 and olefinic carbons of the butenolide at $\delta$ 113.2(C-15), 179.4(C-13). Resonances for four tertiary methyl groups appeared at $\delta$ 0.75, 0.79, 0.88 and 1.81. A singlet at $\delta$ 1.81 corresponds to $CH_3$-17 of the cassane skeleton as indicated by its $^2J/^3J$ HMBC correlations with the quarternary oxygenated carbon C-14 ($\delta$ 74.5), C-13 ($\delta$ 179.4) and C-8 ($\delta$ 49.8). The other two methyl signals at $\delta$ 0.88 and 0.79 showed common HMBC correlations which indicated them to be the $CH_3$-18 and $CH_3$-19 methyl of cassane skeleton. The remaining methyl at $\delta$ 0.75($CH_3$-20) along with $CH_3$-18 and $CH_3$-19 showed HMBC correlations with the deshielded angular methine at $\delta$ 55.6 indicating an unsubstituted C-5 position. A signal for H-5 at $\delta$ 0.92 (*brd*, 10.8 Hz) showed HSQC with the C-5 at $\delta$ 55.6 and indicated for an unsubstituted C-6 position. Other resolved signals were those for H-8 at $\delta$ 1.91 (*dt*, *J* = 11.4, 3.6 Hz), H-9 at $\delta$ 1.63 (*m*), H-7β $\delta$ 2.44 (*dd*, *J* = 12.2, 3.6 Hz) and H-11α at $\delta$ 2.74 (*d*, 10.2 Hz). Unambiguous assignments were made by analysing DQF-COSY, HSQC and HMBC experiments. Most of the methylene signals appeared as complex multiplets overlapped with other signals in the $^1$H NMR spectrum 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, **2** was acetylated with acetic anhydride and pyridine at room temperature to afford the monoacylated product **2a**. Monoacetylation at hemiketal hydroxyl in **2a** was confirmed by the appearance of C-12 ketal at $\delta$ 105.1, relatively upfield to $\delta$ 107.1 in 2. The $^1$H NMR spectrum of **2a** showed signals for the H-8 at 1.55 (*m*, partially overlapped with signals for H-2), H-9 at $\delta$ 1.21 (*m*, partially overlapped with signals for H-3), H-11β at $\delta$ 2.66 (*dd*, *J*=13.2, 2.4 Hz), and H-7α at $\delta$ 2.04 (*m*) along with appearance of a singlet for acetate methyl at $\delta$ 2.05. Relative stereochemistries were assigned based on ROESY correlations (Fig. 3) observed among signals for $CH_3$-20 with $CH_3$-19, H-8, H-11β, $COCH_3$-12, and from $CH_3$-18 to signals for H-5, H-6 which characterize the relative stereochemistry of the A and B rings. ROESY correlation of the acetate methyl singlet at $\delta$ 2.05 with signals for $CH_3$-17 at $\delta$ 1.28 clearly revealed that the ketal hydroxyl was α-oriented (Fig. 2). Based on the above experimental observations the structure of **2** was assigned as 12α,14β-dihydroxycassa-(13)15-en-16,12-olide.

Compound **3** gave a $[M+H]^+$ molecular ion peak at m/z 377.2292, and an $[M+H_2O]^+$ peak at m/z 394.25667 in the DART-HRMS spectrum consistent with the molecular formula $C_{22}H_{32}O_5$. It exhibited IR maxima at 3403 and 1742 cm$^{-1}$, indicative of hydroxyl and α,β-unsaturated γ-lactone functionalities, respectively. The presence of an α,β-butenolide hemiketal ring was characterized by signals at $\delta$ 5.72 (H-15) in the $^1$H NMR spectrum and a hemiketal carbon at $\delta$ 105.7 along with a lactone carbonyl at $\delta$ 170.5 and olefinic carbons of butenolide at $\delta$ 113.8(C-15), 172.7(C-13). $^{13}$C NMR and phase sensitive gHSQC spectrum showed the presence of twenty carbons accounting for four methyl, one olefin methine, five $sp^3$ methine, five methylene, three $sp^3$ quaternary, one olefinic quaternary and one carbonyl carbon. $^1$H NMR showed signals for four methyl groups at $\delta$ 0.98(*s*), 1.00(*s*), 1.10(*s*) and 1.16(*d*, 7.2) and one acetate methyl at $\delta$ 2.04(*s*). A signal at $\delta$ 1.16 corresponds to $CH_3$-17 of the cassane skeleton as indicated by its HMBC correlation with the quaternary olefinic carbon at $\delta$ 172.7. The appearance of the $CH_3$-17 as a doublet indicated C-14 of cassane skeleton to be a methine appearing at $\delta$ 2.92 (*m*) which in turn showed correlations in the HMBC spectrum with signals at 172.7(C-13), 113.8(C-15), 105.7(C-12) of the butenolide framework. The other two methyl signals at $\delta$ 1.08 and 1.51 showed common HMBC correlations which indicated

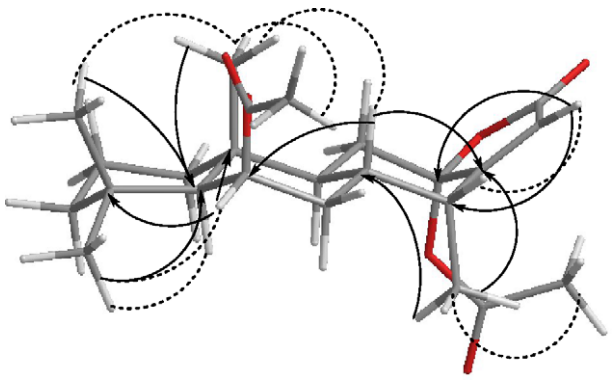

**Fig. 2.** Selected ROESY (. . . . . .) and HMBC (→) correlations for 1b.

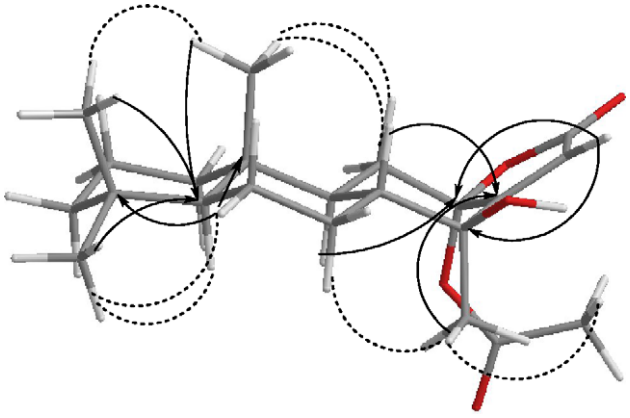

**Fig. 3.** Selected ROESY (. . . . . .) and HMBC (→) correlations for 2a.

**Table 2**
NMR Data for Compounds **3** and **4**.

| Position | **3** (Pyr-$d_5$)[a] $\delta$H [mult., $J$ (Hz)] | **3** (Pyr-$d_5$)[b] $\delta$C | **4** ($CDCl_3$)[a] $\delta$H [mult., $J$ (Hz)] | **4** ($CDCl_3$)[a] $\delta$C |
|---|---|---|---|---|
| 1 | 1.76 [*m*]* 1.07 [*m*]* | 42.2 | 2.31 [*brd*, 12.6] 1.49 [*brdd*, 13.5, 1.4 Hz] | 43.2 |
| 2 | 1.61 [*m*] 1.49 [*m*] | 19.0 | 1.88 [*tt*, 13.8, 3.3] 1.65 [*td*, 13.9, 3.4 Hz] | 19.9 |
| 3 | 1.41 [*brd*, 12.6 Hz] 1.20 [*dt*, 13.2, 3.6 Hz] | 43.9 | 1.45 [*dd*, 13.0, 3.1] 1.25 [*m*]* | 43.3 |
| 4 | – | 34.1 | – | 34.3 |
| 5 | 1.07 [*brs*]* | 55.4 | 1.55 [*brs*] | 51.8 |
| 6 | 5.51 [*brs*] | 69.5 | 5.84 [*brd*, 5.5] | 67.9 |
| 7 | 1.76[*m*]*1.56 [*m*]* | 35.7 | 3.06 [*dd*, 18.1, 5.4] 2.98 [*d*, 18.0] | 35.3 |
| 8 | 1.87 [*m*] | 36.1 | – | 123.9 |
| 9 | 1.56 [*m*] | 45.3 | – | 146.3 |
| 10 | – | 37.9 | – | 38.7 |
| 11 | 2.43 [*dd*, 13.2, 3.6 Hz] 1.44 [*t*, 13.2 Hz] | 38.0 | 7.39 [*brs*] | 105.4 |
| 12 | – | 105.7 | – | 154.0 |
| 13 | – | 172.7 | – | 125.8 |
| 14 | 2.92 [*m*] | 36.2 | – | 128.6 |
| 15 | 5.72 [*s*] | 113.8 | 6.72 [*dd*, 2.1, 0.7] | 105.1 |
| 16 | – | 170.5 | 7.53 [*d*, 2.2] | 144.6 |
| 17 | 1.16 [*d*, 7.2 Hz] | 13.1 | 2.33 [*s*] | 16.2 |
| 18 | 0.98 [*s*] | 33.9 | 1.07 [*s*] | 33.7 |
| 19 | 1.00 [*s*] | 23.6 | 1.10 [*s*] | 23.3 |
| 20 | 1.10 [*s*] | 17.4 | 1.61 [*s*] | 27.7 |
| $COCH_3$–6 | 2.04 [*s*] | 22.0 170.7 | 2.01 [*s*] | 22.0 171.2 |

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

them to be the $CH_3$-18 and $CH_3$-19 of the cassane skeleton. The remaining methyl at $\delta$ 1.37($CH_3$-20) along with $CH_3$-18 and $CH_3$-19 showed HMBC correlations with the deshielded angular methine at $\delta$ 55.4 indicating an unsubstituted C-5 position. Comparison of the NMR data of **3** with **1a** suggested them to be isomeric with the only difference being the position of the acetate substitution. In **1a** acetate was at the C-12 position whereas in **3** it was found to be at C-6 as indicated by HMBC correlation of the H-6 with the acetate carbonyl at $\delta$ 170.5. Acetylation of **3** with acetic anhydride and pyridine at room temperature afforded the same compound as **1b** as characterized by its TLC profile and $^1$H and $^{13}$CNMR data. Recently isolation of the 6α-acetoxy epimer, named as sucutinarane A, of compound **3** has been reported from *Bowdichia nitida* (Matsuno et al., 2008). The report showed an antiperiplaner orientation for H-5 ($\delta$ 1.31, *d*, 11.0 Hz) and H-6 ($\delta$ 5.12, *ddd*, 11.0, 11.0, 4.1 Hz) whereas in our case H-5 and H-6 have α-orientation with very low order of coupling observed in the COSY spectrum. Based on the above experimental observations the structure of **3** was assigned as 6β-acetoxy-12α-hydroxycassa-(13)15-en-16,12-olide.

Compound **4** gave an $[M + H_2O]^+$ molecular ion peak at m/z 358.2288 in the DART-HRMS spectrum consistent with the molecular formula of $C_{22}H_{28}O_3$. It exhibited IR maxima at 3403 and 1742 $cm^{-1}$, indicative of acetate and furan ring systems. The $^{13}$C NMR spectrum showed the presence of twenty two carbons accounting for three aromatic methine, five aromatic quaternary, five methyl, one oxymethine, four methylene, two $sp^3$ quaternary and one carbonyl carbon. The $^1$H NMR spectrum showed signals for one acetate methyl at $\delta$ 2.01 (*s*), one aromatic methyl at $\delta$ 2.33 (*s*), and other three methyls at $\delta$ 1.61(*s*), 1.10(*s*), and 1.07(*s*). The presence of a trisubstituted benzofuran moiety was characterized by doublets for 1,2 substituted furan at $\delta$ 7.53 (*d*, $J$ = 2.2 Hz) and 6.72 (*dd*, $J$ = 2.1, 0.7 Hz), one proton singlet at $\delta$ 7.39 and an aromatic methyl at $\delta$ 2.33. From the HSQC and HMBC correlations it was evident that the aromatic methyl was present at the C-14 position and a signal for H-11 appeared as an isolated broad singlet. Two methyl signals at $\delta$ 1.07 and 1.10 showed common HMBC correlations which indicated them to be the $CH_3$-18 and $CH_3$-19 and the remaining methyl at $\delta$ 1.61was assigned as $CH_3$-20 of the cassane skeleton. $CH_3$-18 and $CH_3$-19 showed HMBC correlations with the deshielded angular methine at $\delta$ 55.4 indicating an unsubstituted C-5 position which was further substantiated by its HSQC correlation with the proton at $\delta$ 1.55. That the acetoxymethine $\delta$ 5.84 (*brd*, 5.5) was present at the C-6 position was confirmed by its HMBC correlations with the C-5, C-10 and C-8. The relative stereochemistry of the acetoxymethyl function was established from ROESY correlations observed for H-6 with signals for $CH_3$-18, and for acetate methyl with the signals for $CH_3$-19 and $CH_3$-20. Based on the above experimental results the structure of **4** was characterized as 6β-acetoxy-17-methylvoucapane-8(14),-9(11)-diene.

Other known compounds isolated form the plant are furanoditerpene (**5**) (Jadhav et al., 2003), friedelin (Ageta et al., 1995), lupeol (Burns et al., 2000), mixture of α and β-amyrin (Mahato and Kundu, 1994) and mixture of β-sitosterol and stigmasterol (Rubinstein et al., 1976). The structures of known compounds were elucidated based on their spectroscopic comparison to the data reported in the literature.

Isolated compounds were tested for their antiproliferative activity against MCF-7 (breast adenocarcinoma), DU145 (prostate carcinoma), C33A (Cervical carcinoma) and Vero (African green monkey kidney fibroblast) cancer cell lines and were found to show moderate to low order $IC_{50}$ (μM) values (Table 3). Among the tested compounds, **2** was found to show a better activity profile in cervical and prostate and breast carcinoma cell lines whereas compound **5** was found to be most active in breast and cervical carcinoma cell lines.

As pointed out earlier (Kinoshita et al., 2005) there has been enormous confusion of botanical names because of enormous morphologic variations (and possibly chemical variations as well) at

**Table 3**
In-vitro antiproliferative activity of compounds.

| Compounds | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | MCF-7 | DU145 | C33A | Vero |
| 1 | 89.82 | 89.21 | 77 | >150 |
| 2 | 75.23 | 68.52 | 58.2 | >150 |
| 3 | 97.90 | 113.50 | 101.03 | >133 |
| 5 | 64.03 | 119.72 | 75.83 | 166.44 |
| Staurosporine* | 9.4 | 5.4 | 1.5 | 9.0 |

* Staurosporine was used as positive control in antiproliferative assay.

the infra-specific level in some *Caesalpinia* species. The isolation of furanoditerpene **5** from species of *Caesalpinia* i.e. *C. crista* has been reported as a chemotaxonomic marker for that species to differentiate it from the synonymous *C. bonduc* (Jadhav et al., 2003). Whereas, isolation of the **5** from *C. bonduc* L. Roxb. in our report revealed that the *C. crista* in the earlier report was actually *C. bonduc* L. Roxb. Further isolation of the cassane butenolide hemiketals in present study may serve as distinguishing feature for *C. bonduc* from other related species of genus.

## 3. Experimental

### 3.1. General experimental procedure

Melting points were recorded on a Complab melting point apparatus and are uncorrected. IR spectra (KBr) were recorded on a Perkin–Elmer RX-1 spectrophotometer. UV spectra were obtained on a Perkin–Elmer $\lambda$-15 UV spectrophotometer. Optical rotation was measured on Rudolf Autopol III polarimeter, NMR spectra were run on 600 MHz Varian Inova Spectrometer, Electrospray mass spectra were recorded on MIMASS QUATTRO II triple quadrupole mass spectrometer. DART-HRMS were recorded on JEOL AccuTOF-DART instrument. Column chromatography was performed using silica gel (60–120 mesh) and flash silica gel (230–400 mesh).

### 3.2. Plant material

The whole plant of *C. bonduc* were collected from intertidal zone of south Andaman Islands, India, and identified by Srivastava, Botany Division, Central Drug Research Institute. A voucher specimen (No. 464) is kept in the herbarium of the Institute.

### 3.3. Extraction and isolation

Air dried and powdered plant material (4 kg) were extracted at room temperature with EtOH. The concentrated 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 fraction (32 g) was subjected to column chromatography over silica gel, eluted with EtOAc:hexane gradient (0–100%) to afford eighteen fractions (F-1–F-17). Fraction F-4 was subjected to repeated chromatographic purification over silica gel eluting with gradient of EtOAc:hexane (0–2%) to give pure compounds **5** (25 mg) and friedelin (15 mg). Fraction F-5 was subjected to repeated chromatographic purification over silica gel using isocratic elution with $Me_2CO$:$CHCl_3$ (2%). A slightly impure compound was obtained which was further purified by preparative TLC (MERCK PTLC plates concentration zone silica gel 0.5 mm) using $Me_2CO$:$CHCl_3$ (15%) as eluent to afford compound **4** (4 mg). F-7 was chromatographed over flash silica gel eluting with gradient of EtOAc:hexane (20–30%) to give **3**(6 mg). Fraction F-8 upon chromatography over silica gel eluting with gradient of $Me_2CO$:$CHCl_3$ (0.2%) gave a fraction which was found to be a mixture of at least two compounds from its $^{13}C$ NMR spectrum. TLC was run over silver nitrate (10%) impregenated silica gel which showed two distinct spots. Further chromatographic purification over $AgNO_3$ impregenated (10%) silica gel using $Me_2CO$:$CHCl_3$ (0.5%) afforded pure lupeol (20 mg) and mixture of $\alpha$ and $\beta$-amyrin (25 mg). Fraction F-9 after chromatograhic purification over silica gel eluting with EtOAc:hexane (15%) afforded a mixture of $\beta$-sitosterol and stigmasterol (40 mg). Fraction F-13 was subjected to repeated chromatographic purification over silica gel eluting with a gradient of MeOH:$CHCl_3$ (0.5–6%) to give pure compounds **1**(12 mg) and **2**(10 mg).

**1**: M.p. 216–218 °C; $[a]_D^{25}$ −95.0 (*c* 0.12, MeOH); UV $\lambda_{max}^{MeOH}$ nm: 215; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3493, 2929, 1731, 1650, 1228,1029, 934; for $^1H$ NMR (600 MHz, Pyr-$d_5$) and $^{13}C$ NMR (150 MHz, Pyr-$d_5$) spectroscopic data see Table 1; ESIMS (pos.): *m/z* 357 $[M + Na]^+$, 335 $[M + H]^+$, 316 $[M–H_2O]^+$; DART-HRMS: $[M + H]^+$ 335.2097; calc. for $C_{20}H_{31}O_4$;335.2222.

**1** (3 mg) was acetylated with acetic anhydride (1 ml) and pyridine (0.2 ml) at room temperature, kept overnight and was concentrated under reduced pressure and purified by column chromatography using gradient of $Me_2CO$:$CHCl_3$ (0.5–4%) to afford pure monoacylated product **1a**(2.0 mg) and and very minute amount of diacetylated produce **1b**; Again 3 mg of **1** was acetylated by acetic anhydride (1 ml) and pyridine (0.4 ml) at elevated temperature of 70 °C to afford exclusively **1b**(1.8 mg). **1a**; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3447, 2930, 1752, 1643, 1461, 1378, 1160, 1030. $^1H$ NMR ($CDCl_3$, 600 MHz) and $^{13}C$ NMR ($CDCl_3$, 150 MHz); see Table 1. ESIMS (pos.): *m/z* 399 $[M + Na]^+$, 394 $[M + H_2O]^+$, 377 $[M + H]^+$, 316 $[M–CH_3COOH]^+$. **1b**; IR $\nu_{max}^{KBr}$ $cm^{-1}$:2928, 1745, 1643, 1459, 1370, 1204, 1157, 1024, 966. $^1H$ NMR ($CDCl_3$, 600 MHz) and $^{13}C$ NMR ($CDCl_3$, 150 MHz); see Table 1. ESIMS (pos.): *m/z* 441 $[M +Na]^+$, 359 $[M–CH_3COOH]^+$.

**2**: M.p.: 220–222 °C; $[a]_D^{25}$ −44.0 (*c* 0.05, MeOH); UV $\lambda_{max}^{MeOH}$ nm: 216; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3401, 2927, 1729, 1659, 1593,1216, 931; for $^1H$ NMR (600 MHz, Pyr-$d_5$) and $^{13}C$ NMR (150 MHz, Pyr-$d_5$) spectroscopic data see Table 1; ESIMS (pos.): *m/z* 357 $[M + Na]^+$, 335 $[M + H]^+$, 316 $[M-H_2O]^+$; DART-HRMS: $[M + H]^+$ 335.2200; calc. for $C_{20}H_{31}O_4$;335.2222.

**2** (3 mg) was acetylated with acetic anhydride (1 ml) and pyridine (0.2 ml) at room temperature, kept overnight. The reaction mixture was concentrated under reduced pressure and purified by column chromatography using gradient of $Me_2CO$:$CHCl_3$ (0.5–4%) to afford pure monoacylated product **2a**(2.3 mg); **2a**; white solids; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3447, 2930, 1770, 1745, 1651, 1461, 1430, 1378, 1245, 1165, 1030. $^1H$ NMR ($CDCl_3$, 600 MHz) and $^{13}C$ NMR ($CDCl_3$, 150 MHz); see Table 1. ESIMS (pos.): *m/z* 399 $[M + Na]^+$, 377 $[M + H]^+$, 316 $[M–CH_3COOH]^+$.

**3**: $[a]_D^{25}$ -44.82 (*c* 0.029, MeOH); UV $\lambda_{max}^{MeOH}$ nm: 214; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3490, 2930, 1730, 1656, 1590, 1216, 929; for $^1H$ NMR (600 MHz, Pyr-$d_5$) and $^{13}C$ NMR (150 MHz, Pyr-$d_5$) spectroscopic data see Table 2; DART-HRMS: $[M + H]^+$ 377.2292; calc. for $C_{22}H_{33}O_5$; 377.2328.

**3** (2 mg) was acetylated with acetic anhydride (1 ml) and pyridine (0.2 ml) at room temperature, kept overnight. The reaction mixture was concentrated under reduced pressure and purified by column chromatography using gradient of $Me_2CO$:$CHCl_3$ (0.5–4%) to afford pure compound 3**a** (1 mg); It was found to be similar to **1b** from its TLC mobility and NMR data.

**4**: oily, $[a]_D^{25}$ -45.0 (*c* 0.02, MeOH); UV $\lambda_{max}^{MeOH}$ nm: 212, 266, 282, 294; IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3436, 2929, 1729, 1624, 1458, 1134, 1033, 7651386, 1696, 756 for $^1H$ NMR (600 MHz, $CDCl_3$) and $^{13}C$ NMR (150 MHz, $CDCl_3$) spectroscopic data see Table 2; ESIMS (pos.): *m/z* 363 $[M + Na]^+$, 280 $[M–CH_3COOH]^+$; DART-HRMS: $[M + H_2O]^+$ 358.2288; calc. for $C_{22}H_{30}O_4$; 358.2144.

### 3.4. Antiproliferative assays

Antiproliferative activity of these compounds was measured by using the MTT-micro culture tetrazolium assay described by Mosmann (1983) using staurosporine (a broad spectrum inhibitor of protein kinases) as a positive control. Briefly, cells at the exponential growth phase were harvested and seeded into a flat bottom 96 well plate. A total of 180 µl volume containing $10^4$ cells were added to each well of the plate. After 24 h incubation in a 5% humidified $CO_2$ incubator at 37 °C, 20 µl of the test agent was added in triplicates to give final concentration of 150, 75, 37.5, 18.75 and 9.375 µM. After 48 h of incubation at 37 °C, 25 µl/well MTT (stock solution 5 mg/ml PBS) was added and the plate was again incubated at 37 °C for 4 h. Supernatant was removed care-

fully by aspiration and DMSO (150 μl) was added to each well to dissolve the formazan dye by gentle shaking for 15 min. The plates were read immediately in a plate reader operating at 540 nm. Mean values were calculated and the $IC_{50}$ values for each cell line were interpolated from the dose response curve.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2008.12.008.

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