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## CASSANE AND NORCASSANE DITERPENOIDS OF *CAESALPINIA BONDUC*

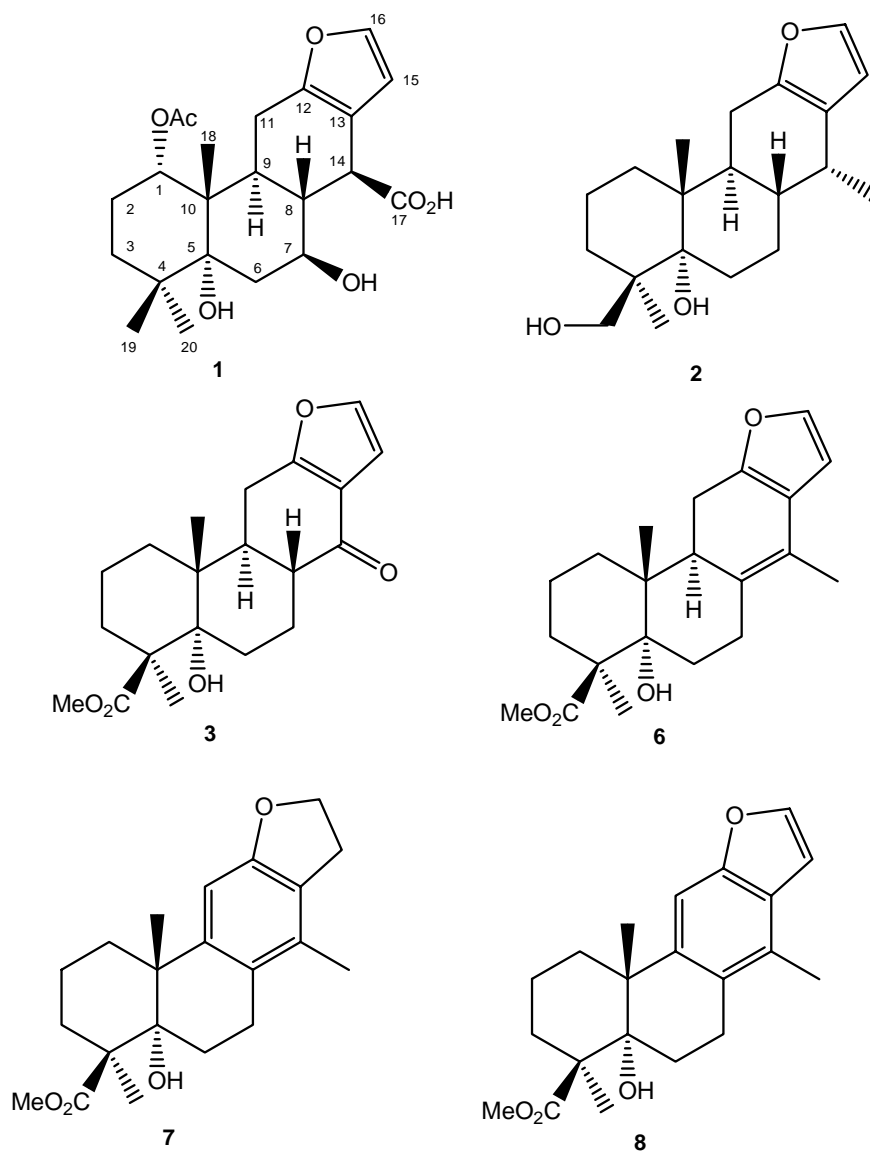
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**Abstract** – Two new cassane and one norcassane diterpenoid (**1-3**) were isolated from *Caesalpinia bonduc* along with caesaldekarins C (**4**) and F (**5**). During acquisition of NMR spectral data on compound **5**, it rearranged to produce compounds **7** and **8**, via compound **6**. The <sup>1</sup>H and <sup>13</sup>C NMR spectra of all compounds were completely assigned using a combination of 2D NMR experiments, including <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, HMBC, and T-ROESY sequences. Compounds **4** and **5** showed modest cytotoxic activity.

## INTRODUCTION

*Caesalpinia bonduc* Roxb. (Fabaceae) is a sprawling evergreen shrub found in the tropics and sub-tropics. The roots, bark, leaves, and mostly the seeds have been used in folk medicine in various parts of the world to treat a myriad of disorders.<sup>1,2</sup> Roasted seeds are given internally in leprosy and have been found useful in some cases of asthma and to treat malarial fevers. The seeds are also known for their anti-periodic, anti-pyretic, and tonic properties.<sup>3</sup> *C. bonduc* seeds have been extensively studied since the late 19<sup>th</sup> century.<sup>4</sup> More recently, *C. bonduc* roots have been investigated and have proven to be a rich source of cassane diterpenoids.<sup>5-9</sup> We report here the isolation and structure elucidation of two cassane (**1** and **2**) and one norcassane diterpenoid (**3**), along with the known caesaldekarins C (**4**), F (**5**), and J (**8**), and the artifacts (**6**) and (**7**).



## RESULTS AND DISCUSSION

The dichloromethane extract of the roots of *C. bonduc* was fractionated by silica gel chromatography and subsequent preparative HPLC to give compounds (**1-5**). A sample of compound **5** rearranged to compound **6**, during spectral data acquisition. Subsequently compound **6** rearranged to compounds **7** and **8**. The structures for compounds **1-8** were elucidated using a combination of 1D and 2D NMR techniques and HREIMS as described in the following.

The molecular formula for **1**,  $C_{22}H_{30}O_7$ , was established by HREIMS. The  $^1H$  NMR spectrum showed the presence of three tertiary methyl groups at  $\delta$  1.09, 1.12, and 1.14, and an acetoxy methyl group at  $\delta$  2.14. Two oxymethine protons were observed at  $\delta$  4.70 (td,  $J = 10.9, 5.0$ , H-7) and 4.95 (t,  $J = 2.9$  Hz, H-1). Signals for a 2,3-disubstituted furan ring were seen at  $\delta$  6.61 (d,  $J = 1.9$  Hz, H-15) and 7.30 (d,  $J = 1.9$  Hz,

TABLE 1. NMR SPECTRAL DATA FOR COMPOUNDS (1-3)

Position	1		2		3	
	$\delta_C$	$\delta_H$ (mult. J/Hz)	$\delta_C$	$\delta_H$ (mult. J/Hz)	$\delta_C$	$\delta_H$ (mult. J/Hz)
1	74.7	4.95 (t, 2.9)	32.5	1.48 (m) 1.51 (m)	31.6	1.40 (m) 1.58 (m)
2	22.5	1.72 (m) 2.05 (m)	18.0	1.50 (m) 1.58 (m)	18.7	1.53 (m) 1.86 (m)
3	30.0	1.20 (m) 1.81 (m)	30.4	1.40 (m) 1.55 (m)	31.7	1.64 (m) 1.94 (m)
4	39.2		44.2		49.0	
5	82.1		76.6		76.4	
6	30.2	1.75 (m) 2.39 (dd, 12.2, 5.0)	26.5	1.64 (m) 1.82 (m)	27.9	1.88 (m) 2.40 (m)
7	80.6	4.70 (dt, 10.9, 5.0)	24.4	1.44 (m) 1.84 (m)	21.5	1.60 (m) 2.33 (m)
8	46.4	1.93 (ddd, 10.9, 13.3, 13.3)	34.4	1.76 (m)	44.2	2.33 (m)
9	33.3	2.81 (td, 13.3, 8.6)	38.0	2.39 (m)	44.5	2.62 (m)
10	44.8		41.3		41.7	
11	21.3	2.49 (m) 2.58 (m)	22.4	2.36 (m) 2.47 (m)	23.2	2.81 (m)
12	151.5		149.6		166.8	
13	114.0		122.6		119.8	
14	41.3	3.24 (d, 13.3)	31.5	2.59 (dq, 7.0, 4.8)	196.1	
15	107.9	6.61 (d, 1.9)	140.4	6.18 (d, 1.8)	106.5	6.64 (d, 2.0)
16	141.7	7.30 (d, 1.9)	108.4	7.22 (d, 1.8)	142.2	7.30 (d, 2.0)
17	174.5		17.5	1.01 (d, 7.0)		
18	17.4	1.14 (s)	17.0	1.01 (s)	15.1	0.89 (s)
19	24.6	1.12 (s)	66.1	3.65 (d, 11.0) 3.92 (d, 11.0)	177.2	
20	28.1	1.09 (s)	20.6	1.03 (s)	23.7	1.19 (s)
OAc	168.7					
OMe	21.3	2.14 (s)			51.7	3.69 (s)

H-16). In the  $^{13}\text{C}$  NMR spectrum the furan carbons resonated at  $\delta$  151.5, 141.7, 114.0, and 107.9. A quaternary oxygenated carbon was observed at  $\delta$  82.1 and two secondary oxygenated carbons were seen at  $\delta$  80.6, and 74.7 representing C-5, C-7 and C-1, respectively. An acetate carbonyl appeared at  $\delta$  168.7, while a carboxylic acid moiety resonated at  $\delta$  174.5. The full  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments were made on the basis of interpretation of HSQC, HMBC, and  $^1\text{H}$ - $^1\text{H}$  COSY spectral data. The HSQC spectrum showed that the oxymethine proton at  $\delta$  4.95 (t,  $J = 2.9$  Hz, H-1) was directly attached to the carbon at  $\delta$  74.7 (C-1). The HMBC cross-section for H-1 showed correlations to C-1', C-3, and C-5, which placed the acetate at C-1). Similarly, the HMBC data supported placement of the hydroxy group at C-7 and the carboxylic acid moiety at C-14. An analysis of coupling constants indicated that the substituent at C-1 was  $\alpha$ -oriented while the C-7 hydroxy group was  $\beta$ -oriented. The  $^3J_{\text{HH}}$  for H-8 and H-14 was 13.3 Hz, indicating that H-14 was  $\alpha$ -oriented. Strong T-ROESY cross-peaks from H-14 to H-7 and H-9, confirmed this stereochemistry. Accordingly, compound **1** was assigned as

1 $\alpha$ -acetoxy-5 $\alpha$ ,7 $\beta$ -dihydroxy-12,16-epoxycassa-12,15-dien-17 $\beta$ -oic acid. The trivial name proposed for compound **1** is 17-*O*-demethylbonducellpin C.<sup>5</sup>

**TABLE 2. NMR SPECTRAL DATA FOR COMPOUNDS (6-7)**

Position	<b>6</b>		<b>7</b>	
	$\delta_C$	$\delta_H$ (mult. J/Hz)	$\delta_C$	$\delta_H$ (mult. J/Hz)
1	30.2	<1.50> <sup>a</sup> (m)	32.4	1.86 (m) 1.95 (m)
2	18.9	1.52 (m) 1.77 (m)	19.4	1.64 (m) 2.04 (m)
3	31.9	1.59 (m) 1.93 (m)	31.9	1.75 (m) 2.00 (m)
4	49.1		48.6	
5	77.6		75.5	
6	30.3	1.89 (m) 2.37 (m)	25.4	2.21 (m) 2.54 (m)
7	25.0	2.27 (m) 2.67 (m)	23.5	<2.72> <sup>a</sup> (m)
8	118.9		124.4 <sup>b</sup>	
9	44.6	3.15 (m)	145.6	
10	45.6		43.7	
11	20.8	<2.80> <sup>a</sup> (m)	103.7	6.64 (s)
12	149.2		158.3	
13	118.5		124.3 <sup>b</sup>	
14	126.9		132.7	
15	106.8	6.25 (d, 1.9)	29.2	<3.12> <sup>a</sup> (m)
16	140.5	7.19 (d, 1.9)	70.9	<4.53> <sup>a</sup> (m)
17	14.4	1.90 (m)	16.3	2.14 (s)
18	15.1	0.70 (s)	27.0	1.10 (s)
19	177.3		177.3	
20	23.9	1.19 (s)	23.8	1.30 (s)
OMe	51.6	3.64 (s)	51.6	3.68 (s)

<sup>a</sup> Value for an incompletely resolved methylene group.

<sup>b</sup> Assignments may be reversed.

The <sup>1</sup>H NMR data for compound **2**, C<sub>20</sub>H<sub>30</sub>O<sub>3</sub>, showed two tertiary methyl groups at  $\delta$  1.01 and 1.03 and a secondary methyl group at  $\delta$  1.01 (d,  $J$  = 7.0 Hz, H-17). Signals for a 2,3-disubstituted furan ring resonated at  $\delta$  6.18 (d,  $J$  = 1.8 Hz, H-15) and 7.22 (d,  $J$  = 1.8 Hz, H-16), while a pair of oxymethylene protons occurred at  $\delta$  3.65 (d,  $J$  = 11.0 Hz, H-19) and 3.92 (d,  $J$  = 11.0 Hz, H-19). HMBC correlations from these oxymethylene protons to C-3, C-4, C-5 and C-20 and conversely from the C-20 methyl protons to C-3, C-4, C-5, and C-19 indicated that the oxymethylene group was geminal to the C-20 methyl group and attached to C-4. Compound **2** was thus assigned as 12,16-epoxy-5 $\alpha$ -hydroxycassa-12,15-dien-19-ol and the trivial name proposed for compound **2** is 7-dehydroxycaesaldekarin I.<sup>8</sup>

Compound **3** has the molecular formula  $C_{20}H_{26}O_5$  as established by HREIMS. The  $^1H$  NMR data showed two tertiary methyl groups at  $\delta$  0.89 and 1.19, and a methoxy group resonated at  $\delta$  3.69. A signal for a C-17 secondary methyl group was notably absent. The  $^{13}C$  NMR spectrum showed signals for twenty carbons including signals for two carbonyl carbons at  $\delta$  196.1 and 177.2 corresponding to a C-14 ketone functionality and the C-19 methyl ester, respectively. HMBC correlations from a methine proton at  $\delta$  2.33 (m, H-8) to the C-14 carbonyl carbon supported its placement. Compound **3** was assigned as methyl-12,16-epoxy-5 $\alpha$ -hydroxynorcassa-12,15-dien-14-one-19-carboxylate. This is the first example of a norcassane diterpene being isolated from *C. bonduc*. Norcaesalpins A and B possessing 17-norcassane skeletons and norcaesalpin C possessing a 16-norcassane skeleton were isolated from *C. crista* seed kernels.<sup>10</sup> Accordingly, the trivial name proposed for compound **3** is norcaesalpin D.

The known cassane diterpenoids caesaldekarin C (**4**), F (**5**), and J (**8**) were identified by comparison to literature data,<sup>7-8,11</sup> During NMR data acquisition of compound **5**, the sample underwent acid-catalyzed rearrangement to compound **6**, and subsequently to compounds **7** and **8**. This, no doubt, was due to the traces of acid that are usually present in deuterated chloroform. The structures for compounds **6** and **7** were in complete accord with the 2D NMR and mass spectral data. The  $^1H$  and  $^{13}C$  assignments are shown in Table 2. Compounds **4**, and **5** showed modest cytotoxic activity against a number of cancer cell lines (Table 3), with compound **5** showing the best activity with an  $IC_{50}$  value of 5.2  $\mu g/mL$  against the breast cancer cell line MCF7.

**TABLE 3. CYTOTOXIC ACITVITY FOR COMPOUNDS 4-5**

Panel	Cell	Compound $IC_{50}$ $\mu g/mL$	
		4	5
Leukemia	SR	7.7	9.0
Non-small cell lung cancer	NCI-H460	9.3	10.4
Colon cancer	HCT-116	9.7	9.4
Colon cancer	HT-29	8.3	7.2
Breast cancer	MCF7	8.7	5.2

## EXPERIMENTAL

**General Experimental Procedures.** Melting points were determined using a Fisher/Johns melting point apparatus. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with Na 589 nm at 20 °C. UV spectra were recorded on a HP8452A diode array spectrophotometer and IR spectra were recorded on a Nexus 670 FT-IR spectrophotometer. NMR spectra were recorded on a Varian UNITY 500 MHz spectrometer in CDCl<sub>3</sub> with TMS as internal standard. The High- and Low-resolution EIMS were recorded on a Micromass 70-250S (double focusing) mass spectrometer at an ionizing voltage of 70 eV. Flash column chromatography was performed using Merck Grade 9385 silica gel 60 (230-400 mesh). TLC was performed using precoated silica gel plates of 0.2 mm thickness; the plates were visualized by spraying with Ehrlich's reagent and warming.

**Plant Material.** *C. bonduc* was collected from the East Coast Road, St. Andrew in February, 2004. Professor Sean Carrington identified the plant as a genuine sample of *Caesalpinia bonduc* and a voucher specimen, JSR2, is maintained in the National Herbarium (BAR) located on the Campus.

**Extraction and Isolation.** The air-dried roots (150 g) were ground in MeOH (3 L) and left to soak for one week. The roots were filtered and the filtrate was concentrated *in vacuo* to yield a crude extract (10 g). The crude extract was suspended in MeOH/H<sub>2</sub>O, 9:1 (200 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x 150 mL). The crude CH<sub>2</sub>Cl<sub>2</sub> extract was reduced and suspended in MeOH/H<sub>2</sub>O, 9:1 (200 mL) and defatted with hexane (3 x 150 mL). Water (100 mL) was added and the aqueous layer was re-extracted with CH<sub>2</sub>Cl<sub>2</sub> to give a final CH<sub>2</sub>Cl<sub>2</sub> extract (5.50 g). The CH<sub>2</sub>Cl<sub>2</sub> extract (5.50 g) was chromatographed on silica gel using increasing proportions of acetone-hexane solvent systems, starting with 10% acetone. Forty-two fractions (75 mL) were collected. The 2.5 % acetone fractions afforded compound **4** (56 mg) as colorless rectangular plates while the 5 and 15% acetone fractions afforded compounds **5** (114 mg) and **1** (15 mg), respectively, also in crystalline form. Subsequent concentration of the 15% acetone fractions *in vacuo* followed by preparative reversed-phase recycling HPLC in chloroform gave additional compound **1** (20 mg) and compound **2** (7 mg). Preparative reversed-phase HPLC on the 10 % acetone fractions gave compound **3** (10 mg). A sample of compound **5** rearranged during spectral data acquisition to compound **6**, which subsequently rearranged to compounds **7** and **8**.

**17-O-Demethylbonducellpin C (1).** Obtained as colorless needles (acetone/n-hexane): mp 145-147 °C;  $[\alpha]_D^{20} +52.3^\circ$  (*c* 1.28, MeOH); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 236 (2.39); IR  $\nu_{\max}$  (KBr): 3449, 1774, 1736, 1236, 934, 732 cm<sup>-1</sup>; <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data, Table 1; EIMS *m/z* (rel. int.): 405 [M-H]<sup>+</sup> (10), 328

(100), 174 (54), 146 (64), 131 (48), 109 (50), 91 (49); HREIMS  $m/z$  405.1914 (calcd. for  $C_{22}H_{29}O_7$ , 405.1913).

**7-Dehydroxycaesaldekarin I (2).** White amorphous solid;  $[\alpha]_D^{20}$  +24.0° ( $c$  0.20, MeOH); UV  $\lambda_{max}$  (MeOH) nm (log  $\epsilon$ ): 204 (2.42); IR  $\nu_{max}$  (film)  $cm^{-1}$ : 3454;  $^1H$ -NMR and  $^{13}C$ -NMR data, Table 1; EIMS  $m/z$  (rel. int.): 318  $[M]^+$  (2), 119 (100), 91 (33); HREIMS  $m/z$  318.2192 (calcd. for  $C_{20}H_{30}O_3$ , 318.2195).

**Norcaesalpin D (3).** White amorphous solid;  $[\alpha]_D^{20}$  +41.9° ( $c$  0.21, MeOH); UV  $\lambda_{max}$  (MeOH) nm (log  $\epsilon$ ): 206 (3.41), 260 (3.07); IR  $\nu_{max}$  (film)  $cm^{-1}$ : 3423, 1647;  $^1H$ -NMR and  $^{13}C$ -NMR data, Table 1; EIMS  $m/z$  (rel. int.): 346  $[M]^+$  (55), 314 (100), 147 (64), 135 (58), 64 (45); HREIMS  $m/z$  346.1788 (calcd. for  $C_{20}H_{26}O_5$ , 346.1780).

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## REFERENCES

1. S. Carrington, 'Wild Plants of Barbados', Macmillan Press, London, 1993; p. 37.
2. D. W. Nellis, 'Seashore Plants of South Florida and the Caribbean', Pineapple Press, Sarasota, 1994; pp. 62-63.
3. N. Ghatak, *Proc. Acad. Sci. Agra and Oudh.*, 1934, **4**, 141.
4. E. Heckel and F. Schlagdenhauffen, *Compt. Rend. Acad. Sci.*, 1886, **103**, 89.
5. S. Peter, W. Tinto, S. McLean, W. F. Reynolds, and M. Yu, *J. Nat. Prod.*, 1997, **60**, 1219.
6. S. Peter, W. Tinto, S. McLean, W. F. Reynolds, and L. Tay, *Tetrahedron Lett.*, 1997, **38**, 5767.
7. S. Peter, W. Tinto, S. McLean, W. F. Reynolds, and M. Yu, *Phytochemistry*, 1998, **47**, 1153.
8. D. L. Lyder, S. Peter, W. Tinto, S. M. Bissada, S. McLean, and W. F. Reynolds, *J. Nat. Prod.*, 1998, **61**, 1462.
9. D. L. Lyder, S. Peter, W. Tinto, S. M. Bissada, S. McLean, and W. F. Reynolds, *Heterocycles*, 1998, **48**, 1465.
10. A. H. Banskota, F. Attamimi, T. Usia, T. Z. Linn, Y. Tezuka, S. K. Kalami, and S. Kadota, *Tetrahedron Lett.*, 2003, **44**, 6879.
11. I. Kitagawa, P. Simanjuntak, T. Mahnmud, M. Kobayashi, S. Fujii, T. Uji, and H. Shibuya, *Chem. Pharm. Bull.*, 1996, **44**, 1157.