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CASSANE AND NORCASSANE DITERPENOIDS OF *CAESALPINIA BONDUC*

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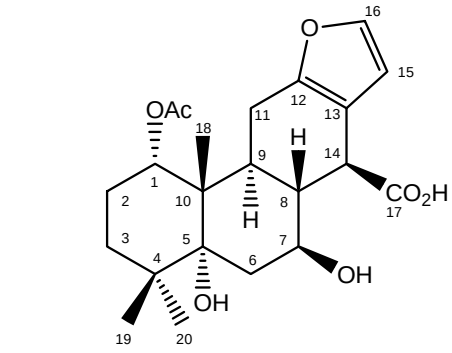
Abstract – Two new cassane and one norcassane diterpenoid (**1**-**3**) were isolated from *Caesalpinia bonduc* along with caesaldekarins C (**4**) and F (**5**). During acquisition of NMR spectral data on compound **5**, it rearranged to produce compounds **7** and **8**, via compound **6**. The ^{1}H and ^{13}C NMR spectra of all compounds were completely assigned using a combination of 2D NMR experiments, including ^{1}H-^{1}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.[1,2] 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.[3] *C. bonduc* seeds have been extensively studied since the late 19th century.[4] More recently, *C. bonduc* roots have been investigated and have proven to be a rich source of cassane diterpenoids.[5-9] 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**).



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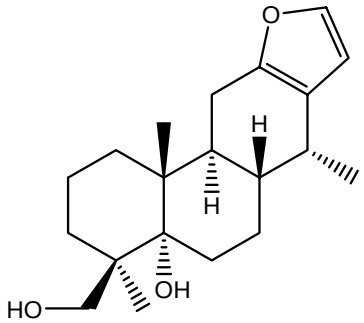

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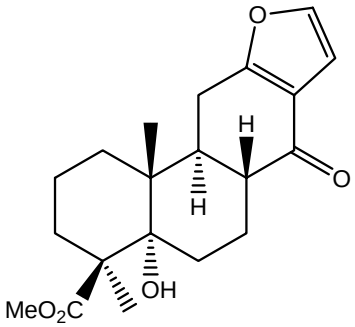

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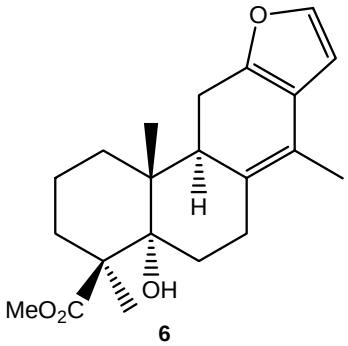

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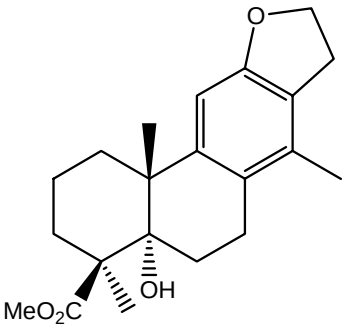

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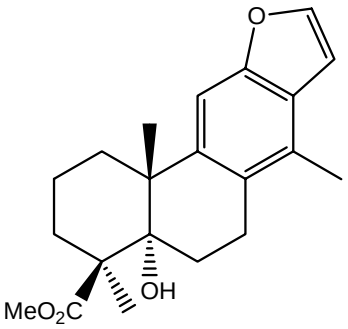

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RESULTS AND DISCUSSION

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 ^{1}H NMR spectrum showed the presence of three tertiary methyl groups at δ 1.09, 1.12, and 1.14, and an acetoxy methyl group at δ 2.14. Two oxymethine protons were observed at δ 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 δ 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)

	1		2		3	
Position	δ_C	δ_H (mult. *J* Hz)	δ_C	δ_H (mult. *J* Hz)	δ_C	δ_H (mult. *J* Hz)
1	74.7	4.95 (t, 2.9)	32.5	1.48 (m)	31.6	1.40 (m)
				1.51 (m)		1.58 (m)
2	22.5	1.72 (m)	18.0	1.50 (m)	18.7	1.53 (m)
		2.05 (m)		1.58 (m)		1.86 (m)
3	30.0	1.20 (m)	30.4	1.40 (m)	31.7	1.64 (m)
		1.81 (m)		1.55 (m)		1.94 (m)
4	39.2		44.2		49.0	
5	82.1		76.6		76.4	
6	30.2	1.75 (m)	26.5	1.64 (m)	27.9	1.88 (m)
		2.39 (dd, 12.2, 5.0)		1.82 (m)		2.40 (m)
7	80.6	4.70 (dt, 10.9, 5.0)	24.4	1.44 (m)	21.5	1.60 (m)
				1.84 (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)	22.4	2.36 (m)	23.2	2.81 (m)
		2.58 (m)		2.47 (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)	177.2	
				3.92 (d, 11.0)		
20	28.1	1.09 (s)	20.6	1.03 (s)	23.7	1.19 (s)
OAc	168.7					
	21.3	2.14 (s)				
OMe					51.7	3.69 (s)

H-16). In the ^{13}C NMR spectrum the furan carbons resonated at δ 151.5, 141.7, 114.0, and 107.9. A quaternary oxygenated carbon was observed at δ 82.1 and two secondary oxygenated carbons were seen at δ 80.6, and 74.7 representing C-5, C-7 and C-1, respectively. An acetate carbonyl appeared at δ 168.7, while a carboxylic acid moiety resonated at δ 174.5. The full ^{1}H and ^{13}C NMR assignments were made on the basis of interpretation of HSQC, HMBC, and ^{1}H-^{1}H COSY spectral data. The HSQC spectrum showed that the oxymethine proton at δ 4.95 (t, J = 2.9 Hz, H-1) was directly attached to the carbon at δ 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 α-oriented while the C-7 hydroxy group was β-oriented. The $^{3}J_{HH}$ for H-8 and H-14 was 13.3 Hz, indicating that H-14 was α-oriented. Strong T-ROESY cross-peaks from H-14 to H-7 and H-9, confirmed this stereochemistry. Accordingly, compound **1** was assigned as

1α-acetoxy-5α,7β-dihydroxy-12,16-epoxycassa-12,15-dien-17β-oic acid. The trivial name proposed for compound **1** is 17-*O*-demethylbonducellpin C.[5]

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

	6		7	
Position	δ_C	δ_H (mult. *J* Hz)	δ_C	δ_H (mult. *J* Hz)
1	30.2	<1.50>[a] (m)	32.4	1.86 (m)
				1.95 (m)
2	18.9	1.52 (m)	19.4	1.64 (m)
		1.77 (m)		2.04 (m)
3	31.9	1.59 (m)	31.9	1.75 (m)
		1.93 (m)		2.00 (m)
4	49.1		48.6	
5	77.6		75.5	
6	30.3	1.89 (m)	25.4	2.21 (m)
		2.37 (m)		2.54 (m)
7	25.0	2.27 (m)	23.5	<2.72>[a] (m)
		2.67 (m)		
8	118.9		124.4[b]	
9	44.6	3.15 (m)	145.6	
10	45.6		43.7	
11	20.8	<2.80>[a] (m)	103.7	6.64 (s)
12	149.2		158.3	
13	118.5		124.3[b]	
14	126.9		132.7	
15	106.8	6.25 (d, 1.9)	29.2	<3.12>[a] (m)
16	140.5	7.19 (d, 1.9)	70.9	<4.53>[a] (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)

[a] Value for an incompletely resolved methylene group.

[b] Assignments may be reversed.

The ^{1}H NMR data for compound **2**, $C_{20}H_{30}O_3$, showed two tertiary methyl groups at δ 1.01 and 1.03 and a secondary methyl group at δ 1.01 (d, *J* = 7.0 Hz, H-17). Signals for a 2,3-disubstituted furan ring resonated at δ 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 δ 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-5α-hydroxycassa-12,15-dien-19-ol and the trivial name proposed for compound **2** is 7-dehydroxycaesaldekarin I.[8]

Compound **3** has the molecular formula $C_{20}H_{26}O_5$ as established by HREIMS. The ^{1}H NMR data showed two tertiary methyl groups at δ 0.89 and 1.19, and a methoxy group resonated at δ 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 δ 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 δ 2.33 (m, H-8) to the C-14 carbonyl carbon supported its placement. Compound **3** was assigned as methyl-12,16-epoxy-5α-hydroxynorcasa-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.[10] 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,[7-8,11] 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 ^{1}H 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 μg/mL against the breast cancer cell line MCF7.

TABLE 3. CYTOTOXIC ACITVITY FOR COMPOUNDS 4-5

Panel	Cell	Compound IC_{50} μg/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 $CDCl_3$ 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* a yield a crude extract (10 g). The crude extract was suspended in MeOH/H_2O, 9:1 (200 mL) and extracted with CH_2Cl_2 (3 x 150 mL). The crude CH_2Cl_2 extract was reduced and suspended in MeOH/H_2O, 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_2Cl_2 to give a final CH_2Cl_2 extract (5.50 g). The CH_2Cl_2 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° (*c* 1.28, MeOH); UV (MeOH) λ_{max} nm (log ε): 236 (2.39); IR ν_{max} (KBr: 3449, 1774, 1736, 1236, 934, 732) cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data, Table 1; EIMS *m/z* (rel. int.): 405 $[M-H]^+$ (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 λ_{max} (MeOH) nm (log ε): 204 (2.42); IR ν_{max} (film) cm^{-1}: 3454; ^{1}H-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 λ_{max} (MeOH) nm (log ε): 206 (3.41), 260 (3.07); IR ν_{max} (film) cm^{-1}: 3423, 1647; ^{1}H-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.