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New diterpenoids from *Caesalpinia* species and their cytotoxic activity ☆

Biswanath Das [a,*], Yallamalla Srinivas [a], Chithaluri Sudhakar [a], Ibram Mahender [a], Keetha Laxminarayana [a], Parigi Raghavendar Reddy [a], Tuniki Venugopal Raju [b], Naga Mahesh Jakka [c], Janapala Venkateswara Rao [c]

[a] *Organic Chemistry Division-I, Indian Institute of Chemical Technology, Hyderabad 500 007, India*
[b] *NMR Division, Indian Institute of Chemical Technology, Hyderabad 500 007, India*
[c] *Biology Division, Indian Institute of Chemical Technology, Hyderabad 500 007, India*

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ABSTRACT

Chemical investigation on *Caesalpinia crista* afforded two new diterpenoids, 6β-cinnamoyloxy-7β-acetoxyvouacapen-5α-ol and 6β,7β-dibenzoyloxyvouacapen-5α-ol and on *Caesalpinia pulcherrima* another new diterpenoid, 12-demethyl neocaesalpin F along with several known constituents. The structures of the new compounds were settled from their 1D and 2D NMR spectral data. The cytotoxicity of these compounds was measured on two different cancer cell lines.

The species belong to *Caesalpinia* (Leguminosae) are evergreen and deciduous trees and shrubs grown as ornamental plants in tropical and subtropical countries. These plants are known for their medicinal properties, such as *Caesalpinia crista* is used[1] as tonic for the treatment of rheumatism and backache while *Caesalpinia pulcherrima* is applied[2] as abortifacient and emmenagogue. The metabolites of these two species are mainly diterpenoids[3–7] flavonoids[8,9] and peltogynoids.[8] Some of the constituents are known to possess antitumor, antimicrobial and antimaterial properties.[4–6] Here, we report the isolation, structure elucidation and cytotoxicity of two new diterpenoids, **1** and **2** from *C. crista* and of another new diterpenoid, **3** from *C. pulcherrima.*

Compound **1** was isolated as a white solid.[10] Its molecular formula was decided to be $C_{31}H_{38}O_6$ from its HREIMS (*m/z* 529.2578 for $C_{31}H_{38}O_6Na$). The IR spectrum showed the presence of carbonyl and hydroxyl groups as well as unsaturation in the molecule. The structure of the compound was established from its ^1H and ^{13}C NMR spectral data (Table 1) which indicated it to be a cassane diterpenoid.[7,8] The proton and carbon signals were clearly assigned with the help of 2D NMR (^1H–^1H COSY, NOESY, HSQC and HMBC) and DEPT experiments. The spectral data suggested that the structure of **1** was related to 6β-cinnamoyloxy-7β-hydroxy-vouacapen-5α-ol (**4**)[3] (Fig. 1), previously reported from *C. pulcherrima.* However, the former contained an acetoxy group instead of the hydroxyl group present at C-7 in **4**. The acetoxy group was reasonably placed at C-7 in **1** as H-7 appeared downfield (δ 5.52, dd, *J* = 10.0, 4.0 Hz) and was related to H-6 (δ 5.72, d, *J* = 4.0 Hz) as indicated by coupling constant. Both the compounds **1** and **4** contained a *trans*-cinnamoyloxy moiety at C-6. The HMBC experiment (Fig. 2) showed that H-6 was correlated to C-1′ (δ 166.1) which in turn was related to C-3′ (δ 145.4). Similarly, H-7 was related to C-1″ (δ 170.5). The coupling constant (4.0 Hz) between H-6 and H-7 and the NOESY correlation clearly suggested their β-configuration. The ester groups of **1** were hydrolyzed with methanolic KOH to afford a known compound, 6β-hydroxyisovouacapenol C (**5**)[11] which was characterized by comparison of its physical (mp and optical rotation) and spectral (IR, ^1H NMR and MS) data with those reported earlier. Thus the compound **1** was characterized as 6β-cinnamoyloxy-7β-acetoxyvouacapen-5α-ol.

Compound **2** was obtained as a white solid.[10] Its molecular formula was decided to be $C_{34}H_{38}O_6$ from its HREIMS (*m/z* 565.2555 for $C_{34}H_{38}O_6Na$). Its IR absorption peaks as well as ^1H and ^{13}C NMR data (Table 1) suggested that its structure was related to that of **1** (Fig. 1). However, the ester moieties at C-6 and C-7 were different. In **2** two dibenzoyloxy groups were present at these two positions. The HMBC spectrum revealed that H-6 (δ 6.03, d, *J* = 4.0 Hz) was related to the carbonyl group (δ 166.1) of a benzoyloxy moiety while H-7 (δ 5.85, dd, *J* = 10.0, 4.0 Hz) to another

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* Corresponding author. Tel./fax: +91 40 7160512.
E-mail address: biswanathdas@yahoo.com (B. Das).

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Table 1
NMR spectral data of compound **1**, **2** and **3**[a]

Position	Compound **1**			Compound **2**			Compound **3**		
	^{1}H NMR	Multiplicity (*J* in Hz)	^{13}C NMR	^{1}H NMR	Multiplicity (*J* in Hz)	^{13}C NMR	^{1}H NMR	Multiplicity (*J* in Hz)	^{13}C NMR
1	1.60(a)1.44(b)	M	35.0	1.70(a) 1.52(b)	m	35.0	1.60(a) 1.53(b)	m	34.7
2	1.70(a) 1.52(b)	M	18.1	1.74(a) 1.55(b)	m	18.2	1.70(a) 1.54(b)	m	18.1
3	1.69(a) 1.16(b)	M	37.7	1.76(a) 1.16(b)	m	37.6	1.67(a) 1.15(b)	m	37.6
4	—	—	41.2	—	—	39.3	—	—	39.2
5	—	—	77.3	—	—	77.3	—	—	77.97
6	5.72	d (4.0)	70.2	6.03	d (4.0)	70.9	5.78	d (4.0)	73.9
7	5.52	dd (10.0, 4.0)	71.6	5.85	dd (10.0, 4.0)	72.3	4.48	dd (10.0, 4.0)	68.0
8	2.26	M	35.4	2.66	m	37.2	1.89	m	41.9
9	2.54	M	37.1	2.46	m	35.8	2.49	m	36.9
10	—	—	39.2	—	—	41.2	—	—	40.8
11	2.57	M	21.7	2.63	m	21.8	2.26(a) 1.44(b)	m	37.7
12	—	—	149.2	—	—	149.8	—	—	106.1
13	—	—	121.6	—	—	121.9	—	—	171.5
14	2.83	M	27.4	2.87	m	27.5	3.37	m	32.1
15	6.19	d (2.0)	109.5	6.15	d (2.0)	109.5	5.70	s	113.6
16	7.21	d (2.0)	140.5	7.24	d (2.0)	140.5	—	—	173.3
17	0.98	d (7.0)	17.1	1.02	d (7.0)	17.1	1.26	d (7.0)	12.2
18	1.47	S	27.7	1.61	s	17.6	1.42	s	17.5
19	1.18	S	25.4	1.18	s	25.4	1.07	s	27.8
20	1.02	S	17.6	1.10	s	27.7	1.13	s	25.4
1′	—	—	166.1	—	—	166.1	—	—	167.5
2′	6.43	d (16.0)	117.9	—	—	129.7	—	—	129.9
3′	7.70	d (16.0)	145.4	7.80	dd (8.0, 2.0)	129.2	8.02	dd (8.0, 1.5)	129.6
4′	—	—	134.1	7.42	t (8.0)	128.1	7.45	t (8.0)	128.6
5′	7.58–7.50	M	128.9	7.46	br t (8.0)	132.8	7.59	t (8.0)	133.4
6′	7.43–7.37	M	128.2	7.42	t (8.0)	128.1	7.45	t (8.0)	128.6
7′	7.43–7.37	M	130.5	7.80	dd (8.0, 2.0)	129.2	8.02	dd (8.0, 1.5)	129.6
8′	7.43–7.37	M	128.2	—	—	—	—	—	—
9′	7.58–7.50	M	128.9	—	—	—	—	—	—
1″	—	—	170.5	—	—	171.2	—	—	—
2″	1.98	S	20.9	—	—	130.2	—	—	—
3″	—	—	—	7.89	dd (8.0, 2.0)	129.6	—	—	—
4″	—	—	—	7.49	t (8.0)	128.5	—	—	—
5″	—	—	—	7.57	brt (8.0)	133.8	—	—	—
6″	—	—	—	7.49	t (8.0)	128.5	—	—	—
7″	—	—	—	7.89	dd (8.0, 2.0)	129.6	—	—	—
OH-2	1.82	br s	—	1.98	br s	—	2.16	br s	—
OH-12	—	—	—	—	—	—	2.16	br s	—

[a] The spectra were run in $CDCl_3$ at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR).

carbonyl group of the second benzoyloxy moiety (δ 171.2). The NOESY experiment decided the β-configuration of both H-6 and H-7. The hydrolysis of the ester groups of **2** with methanolic KOH afforded the same known compound, 6β-hydroxyisovouacapenol C (**5**)[11] as was obtained by hydrolysis of **1**. Thus the structure of **2** was settled as 6β,7β-dibenzoyloxyvouacapen-5α-ol.

Compound **3** was isolated as a white solid.[10] Its molecular formula was established as $C_{27}H_{34}O_7$ from its HREIMS (*m*/*z* 471.2395 for $C_{27}H_{35}O_7$). The IR spectrum showed the presence of hydroxyl and carbonyl groups and aromatic residue. The ^{1}H and ^{13}C NMR spectral data (Table 1) suggested the compound to be an α,β-butenolide cassane diterpenoid while the earlier two compounds **1** and **2** were furano cassane diterpenoid. The spectral data revealed that the compound **3** was structurally related to neocaesalpin F (**6**) (Fig. 3) previously reported[11] from the same species. However, the former contained no methoxy group present at C-12 in the latter. Instead, a hydroxyl group was present in **3** at the same position. In the ^{13}C NMR spectrum C-12 appeared at δ 106.1 and in the HMBC spectrum H-15 (δ 5.70, s) showed correlation with this carbon. In the NOESY experiment (Fig. 4) Me-17 (δ 1.26, d, J = 7.0 Hz) was correlated to H-7 (δ 4.48, dd, J = 10.0, 4.0 Hz) and H-9 (δ 2.49, td, J = 10.0, 2.0 Hz) which indicated that the C-ring is in the chair form and consequently HO-12 is α-oriented.[12] Me-17 also showed NOESY correlation with HO-12 (δ 2.16, br s). Thus the structure of **3** was deduced to be 12-demethyl neocaesalpin F.

The known homoisoflavanoids, 7-*O*-methyl bonducellin[13] sappanone A[14] and 4′-*O*-methyl sappanone A[15] were also isolated from *C. crista* and bonducellin[9] and isobonducellin[9] from *C. pulcherrima*. In addition to these compounds the latter species afforded the simple flavanoids, 5,7-dimethoxy flavanone 5,7-dimethoxy-3′,4′-methylenedioxy flavanone and 2′-hydroxy-2,3,4′,6′-tetramethoxy chalcone.[9]

Compounds **1**–**3** were tested for in vitro cytotoxic activity against two cancer lines, HL-60 (Human promyelocytic leukemia) and HeLa (Human cervical carcinoma) using the MTT assay according to the protocol of Mossmann.[16] Camptothecin and etoposide were considered as the positive control. All the three compounds (**1**–**3**) showed significant decrease in cell viability in the test cell lines in a concentration dependent manner. IC_{50} value was measured with each cell line after four individual observations (Table 2). The cytotoxic activity of the test compounds was found to be

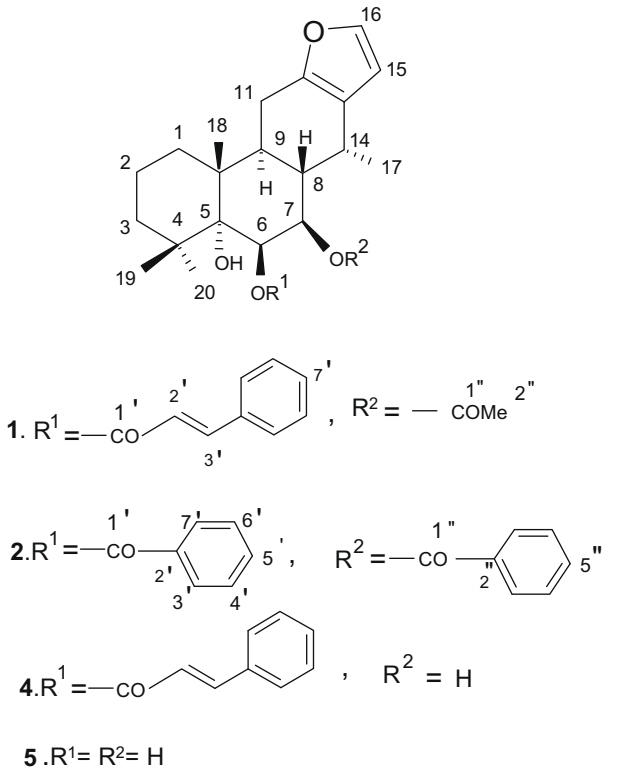

Figure 1.

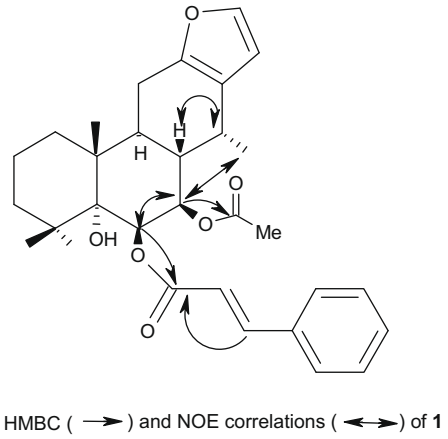

Figure 2.

Table 2
Cytotoxic activity of compounds **1**–**3** on two different cancer cell lines

Sample	IC_{50}[a] (μM)	
	HL-60	HeLa
Compound **1**	17.37 ± 0.88	33.41 ± 0.75
Compound **2**	19.77 ± 1.54	33.90 ± 0.66
Compound **3**	44.89 ± 2.58	52.69 ± 2.48
Camptothecin	0.59 ± 0.04	0.94 ± 0.07
Etoposide	1.81 ± 0.20	8.93 ± 0.69

[a] The results represent the mean ± standard error of four individual observations.

in the order of camptothecin > etoposide > **1** > **2** > **3** on both HL-60 and HeLa cells.

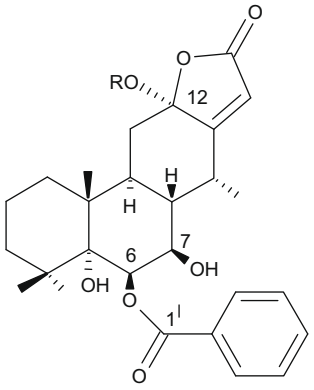

Figure 3.

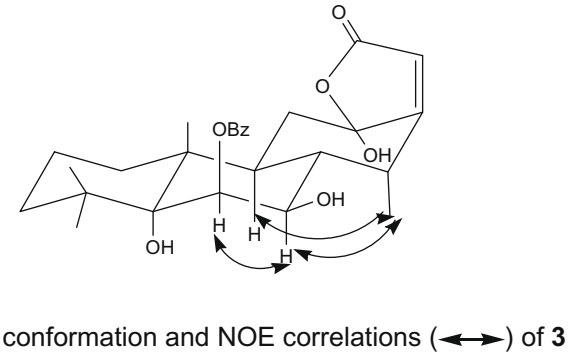

Figure 4.

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10. Isolation of constituents of *C. crista* and *C. pulcherrima*: The aerial parts of *C. crista* and *C. pulicherrima* were collected for Osmania University campus, Hyderabad in May, 2007 and were botanically identified. Voucher specimen (Nos. CC-150509 for *C. crista* and C.P-150509 for *C. pulicherrima*) are preserved in IICT herbarium.
The air dried plant material (2 kg) of *C. crista* was powdered and extracted thrice with $CHCl_3$/MeOH (1:1) at room temperature. Each extraction was continued for 120 h using 5 L of solvent. The combined solvent was concentrated under reduced pressure to afford a thick brown gummy material (7 g). The residue was subjected to column chromatography over silica gel. The column was eluted with solvents of increasing polarity using

hexane and mixtures of hexane and EtOAc. The following compounds (amounts and eluting solvents are given) were obtained according to the increasing order of polarity: compound **2** (18 mg, hexane/EtOAc, 80:20), compound **1** (22 mg, hexane/EtOAc, 70:30), 7-methoxy bonducellin (32 mg, hexane/EtOAc, 60:40), 7-*O*-methyl sappanone (27 mg, hexane/EtOAc, 30:70) and sappanone (35 mg, hexane/EtOAc, 10:90).

The air dried and powdered plant material (2 kg) of *C. pulcherrima* was extracted thrice with $CHCl_3$/MeOH (1:1) at room temperature following the above method as described for extraction of *C. crista*. The concentrated extract (a thick brown gummy residue, 8 g) was subjected to column chromatography over silica gel. The column was eluted with solvents of increasing polarity using hexane and mixtures of hexane and EtOAc. The following compounds (amounts and eluting solvents are given) were isolated according to the increasing order of polarity: 2′-hydroxy-2,3,4′,6′-tetramethoxy chalcone (9 mg, hexane/EtOAc, 80:20), isobonducellin (7 mg, hexane/EtOAc, 70:30), bonducellin (8 mg, hexane/EtOAc, 60:40), 5,7-dimethoxyflavone (10 mg, hexane/EtOAc, 50:50), 5,7-dimethoxy-3′,4′-methylenedioxy flavone (12 mg, hexane/EtOAc, 40:60), and compound **3** (12 mg, EtOAc).

Compound **1** (6β-cinnamoyloxy-7β-acetoxyvouacapen-5α-ol): White solid, mp 127–128 °C, $[\alpha]_D^{25}$ +63.8 (*c* 0.3, $CHCl_3$), IR: 3448, 1721, 1636, 1456, 1238 cm^{-1}; ^1H and ^{13}C NMR: Table 1; HREIMS: *m*/*z* 529.2578 $[M+Na]^+$ (Calcd for $C_{31}H_{38}O_6Na$: *m*/*z* 529.2566).

Compound **2** (6β,7β-dibenzoyloxyyvouacapen-5α-ol): White solid, mp 69–70 °C, $[\alpha]_D^{25}$ +13.7 (*c* 0.3, $CHCl_3$); IR: 3446, 1724, 1459, 1280 cm^{-1}; ^1H and ^{13}C NMR: Table 1; HREIMS: *m*/*z* 565.2555 $[M+Na]^+$ (Calcd for $C_{34}H_{38}O_6Na$: *m*/*z* 565.2566).

Compound **3** (12-demethyl neocaesalpin F): White solid, mp 159–161 °C, $[\alpha]_D^{25}$ −56.6 (*c* 1, $CHCl_3$); IR: 3425, 1722, 1449, 1276 cm^{-1}; ^1H and ^{13}C NMR: Table 1; HREIMS: *m*/*z* 471.2395 $[M+H]^+$ (Calcd for $C_{27}H_{35}O_7$: *m*/*z* 471.2382).

Hydrolysis of compounds **1** and **2**: Compounds **1** and **2** (10 mg each) were separately heated under reflux with 5% KOH in MeOH (2 mL) for 30 min. After usual work-up followed by purification of the reaction mixture our column chromatography both the compounds afforded **5** (5 mg from **1** and 6 mg from **2**) mp 106–107 °C, $[\alpha]_D^{25}$ +25.4 (*c* 0.05, $CHCl_3$). The physical and spectral (IR, ^1H NMR and MS) properties of the compound were found to be identical to those reported in the literature.[11]

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