A New Furanoid Diterpene from *Caesalpinia pulcherrima*

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A new cassane-type diterpene isovouacapenol E (1) was isolated from the leaves of *Caesalpinia pulcherrima*, together with the known compounds caesaldekarin A (3), spathulenol (4), caryophyllene oxide (5), phytol, and sitosterol. The structure of 1 was elucidated by spectral data interpretation.

Key words *Caesalpinia pulcherrima*; Leguminosae; isovouacapenol E; caesaldekarin A

Caesalpinia pulcherrima, locally known as *caballero*, is an ornamental medicinal plant cultivated throughout the Philippines. A decoction of the leaves, bark, and roots is used to alleviate fungal infections, reduce fever, and cause abortion. It is also used to treat liver disorders and ulcers of the mouth and throat.[1)] A number of studies have been reported on the chemical constituents of *C. pulcherrima*.[2—11)] We have previously reported the isolation of four new furanoid diterpenes from the leaves of the plant.[12)]

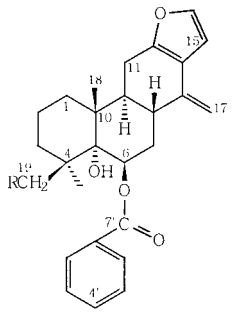
We report here the isolation and structure elucidation of another new furanoid diterpene dibenzoate (**1**) from the leaves of the plant and the known compounds caesaldekarin A (**3**), spathulenol (**4**), caryophyllene oxide (**5**), phytol, and sitosterol. To the best of our knowledge, this is the first report on the isolation of **3**, **4**, and **5** from *C. pulcherrima*.

Results and Discussion

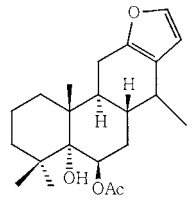
The dichloromethane extract of the air-dried leaves of *C. pulcherrima* afforded the new furanoid diterpene dibenzoate isovouacapenol E (**1**) and the known compounds caesaldekarin A,[13)] spathulenol,[14)] caryophyllene oxide,[15)] phytol,[16)] and sitosterol,[17)] by silica gel chromatography. The structures of the known compounds were identified by comparison of their ^{1}H and ^{13}C with those found in the literature.[13—17)] The structure of **1** was elucidated by NMR and mass spectrometry as follows. The ^{1}H-NMR spectral data of **1** (Table 1) indicated resonances for two benzoates [δ 8.05 (2H), 7.47 (2H), 7.56 (1H), and δ 7.92 (2H), 7.37 (2H), 7.51 (1H)]; two methyl singlets (δ 1.24, 1.65); an exocyclic methylene (δ 4.85, 5.10); 2,3-disubstituted furan (δ 6.44, 7.24); a methine carbinyl (δ 5.63), and a methylene carbinyl (δ 4.23, 5.26). These were similar to the resonances of the furanoid diterpenes, particularly isovouacapenol A (**2**), which we earlier reported.[12)] The major difference was the presence of a second benzoate and a methylene carbinyl in **1** in place of a methyl group in **2**. ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectral data (Table 1) confirmed the presence of 34 carbons in **1** with the following functionalities: dibenzoates (δ 128.3—133.4, 165.7, 166.2); an exocyclic methylene (δ 104.4, 141.7); a furan (δ 106.3, 141.5—151.9); oxygenated methylene (δ 66.9), methine (δ 72.2), and quaternary (δ 76.2) carbons; and two methyls, five methylenes, two methines, and two quaternary carbons. Assuming a furanoditerpene dibenzoate with an alcohol functionality, the proposed molecular formula for **1** was $C_{34}H_{36}O_6$, and this was confirmed by high resolution electron impact-mass spectra (HR-EI-MS).

The correlation spectroscopy (COSY) 2D NMR spectrum (Fig. 1) showed correlations for seven spin systems as follows: H_2-1/H_2-2/H_2-3; H-6/H_2-7/H-8/H-9/H_2-11; H-15/H-16; H-17a/H-17b; H-19a/H-19b; H-4′/H-3′(5′)/H-2′(6′); and H-4″/H-3″(5″)/H-2″(6″) (Fig. 1).

The heteronuclear single quantum coherence (HSQC) 2D NMR data enabled assignments of protons attached to carbons, and connectivities were deduced from heteronuclear multiple bond coherence (HMBC) 2D NMR data (Table 1). One of the benzoates was attached to C-19 due to long-range correlations between the oxygenated methylene carbon (δ 66.9, C-19) and the methyl singlet (δ 1.24, H-20). The other benzoate was attached to C-6 due to the deshielded nature of this carbon (δ 72.2) and the proton (δ 5.63) attached to it. The quaternary oxygenated carbon at δ 76.2 was assigned to C-5 because of its long-range correlation with the methyl singlets at δ 1.65 (H-18) and 1.24 (H-20). The exocyclic double bond was assigned to C-14 due to long-range correlations of the exocyclic methylene (δ 4.85, 5.09) to the methine carbon at δ 31.37 (C-8) and the quaternary furan carbon at δ 118.8 (C-13). The oxygen of the furan ring was as-



1 R = OCOPh
2 R = H



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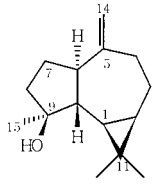

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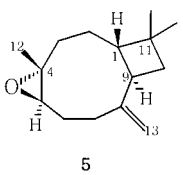

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signed to C-12 since this carbon (δ 151.9) was long-range correlated to the methylene protons at δ 2.68 (H-11) and the furan protons at δ 6.44 (H-15) and 7.24 (H-16). All other long-range correlations observed were consistent with the structure of **1**. Key HMBC correlations for **1** are shown in Fig. 1.

The relative stereochemistry of **1** was determined by a combination of analysis of coupling constants and nuclear Overhauser effect spectroscopy (NOESY) 2D NMR data (Table 1, Fig. 2). The carbinyl proton at δ 5.63 (H-6) is in the equatorial position due to small coupling constants (2.8, 3.6 Hz). An NOE was observed between this carbinyl proton and the methyl at δ 1.24 (H-20), indicating that this methyl is in the equatorial position. The methyl singlet at δ 1.65 (H-18) is close to the methine proton at δ 2.57 (H-8), and methylene proton at δ 5.26 (H-19b), indicating that they are on the same side of the molecule. No NOE correlation was observed between the methine proton at δ 2.46 (H-9) and the C-18 methyl and H-8, and hence H-9 should be on the opposite side of the molecule. The trivial name isovouacapenol E

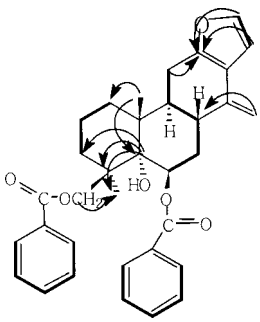

Fig. 1. Key COSY (—) and HMBC (→) Correlations for **1**

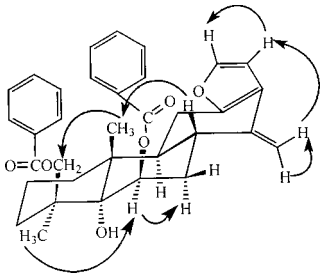

Fig. 2. Key NOESY Correlations of **1**

Table 1. 400-MHz ^{1}H-NMR and 100-MHz ^{13}C-NMR, HMBC, and NOESY Correlations of **1**

Position	δ_C	δ_H mult. (*J* Hz)	HMBC correlations	NOESY correlations	δ_C of **2**[12]
1	34.2	1.6	H-18 (Me)	H-1b	34.5
		1.7		H-1a	
2	18.0	1.55		H-1a, H-3a	18.2
3	31.0	1.65	H-20 (Me)	H-3b	38.0
		1.75	H-19	H-3a	
4	44.2		H-18 (Me)		38.9
			H-20 (Me)		
5	76.2		H-18 (Me)		76.1
			H-20 (Me)		
6	72.2	5.63 dd (2.8, 3.6)	H-7	H-20, H-2′/6′, H-2a, H-7a, H-7b	72.3
7	31.44	2.25		H-7b	31.6
		2.35		H-7a	
8	31.37	2.57	H_2-17	H-18	31.6
9	44.3	2.46			44.1
10	41.4		H-18 (Me)		41.3
11	22.3	2.68		H-18	22.3
12	151.9		H_2-11		152.2
			H-15		
			H-16		
13	118.8		H_2-17		118.7[a]
14	141.7				142.0[a]
15	106.3	6.44 d (2.0)		H-16, H-17b	106.3
16	141.5	7.24 d (2.0)			141.3
17	104.4	4.85 d (2.0)		H-17b, H-8	104.2
		5.10 d (2.0)		H-15, H-17a	
18	21.2	1.65 s (Me)		H-11, H-8, H-19b	17.1
19	66.9	4.23 d (11.2)	H-20 (Me)	H-19b, H-2′/6′, H-20	27.6
		5.26 d (11.2)		H-19a, H-2′/6′	
20	16.8	1.24 s (Me)		H-6, H-2″/6″	25.9
1′	130.3				130.4
2′/6′	129.7	8.05		H-18, H-19a, H-3′/H-5′	129.7
3′/5′	128.7	7.47		H-2′/6′, H-4′	128.6
4′	133.4	7.56		H-3′/5′	133.1
7′	165.7				165.7
1″	129.9				
2″/6″	129.5	7.92		H-20, H-3″/5″	
3″/5″	128.3	7.37		H-2″/6″, H-4″	
4″	132.8	7.51		H-3″/5″	
7″	166.2				

a) These two carbons were accidentally interchanged in our earlier paper.[12]

is proposed.

Compounds **1** and **5** were obtained from specimen #067, while 2—4, phytol and sitosterol were isolated from specimen #052. The difference in the constituents of the two batches of samples may be explained by the fact that voucher specimen #052 was collected in July 2001, which is the rainy season in the Philippines, while specimen #067 was collected in January 2002, which is the dry season. The change in climate may have resulted in different isolates. Furthermore, in specimen #067 compounds with *Rf* values similar those of the diterpenes[12] and other compounds obtained in specimen #052 were no longer purified.

Experimental

General Experimental Procedures Optical rotation was measured on a Perkin-Elmer 341 polarimeter, IR spectra on a Perkin Elmer Spectrum One FTIR, and ultraviolet absorption data on a Varian Cary 4E spectrophotometer. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer in $CDCl_3$ (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). The high- and low-resolution EI-MS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70—230 mesh). TLC was performed with plastic-backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin-H_2SO_4 and warming.

Sample Collection The samples were obtained from Metro Manila in January 2002 and July 2001. They were identified as *C. pulcherrima* (L.) SWARTZ at the Philippine National Museum and voucher specimens #052 and #067 were deposited in the Chemistry Department of De La Salle University.

Extraction and Isolation Air-dried leaves (2 kg) of *C. pulcherrima* were extracted with dichloromethane to afford a crude extract (125 g). The crude extract was treated with 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components.[12] The treated extract was fractionated by gravity column chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. The dichloromethane and 10% acetone in dichloromethane fractions were rechromatographed on silica gel with 10% ethyl acetate in petroleum ether as eluent to afford **5** (5 mg). The more polar fractions were rechromatographed (3 times) in 10% ethyl acetate in petroleum ether to afford **1** (3 mg).

From another batch of samples collected in July 2001,[12] **3** and **4**, phytol, and sitosterol were obtained. Dried leaves (2.3 kg) were extracted with dichloromethane to afford a crude extract (150.5 g) which was treated with 4% aqueous $Pb(OAc)_2$ to precipitate the more polar components.[12] The extract was fractionated by gravity column chromatography using increasing proportions of acetone in dichloromethane (10% increment) as eluents. The 10% acetone in dichloromethane fraction was rechromatographed (3 times) in 10% ethyl acetate in petroleum ether to afford **4** (6 mg). The 20% acetone in dichloromethane fraction was rechromatographed in dichloromethane, followed by acetonitrile : diethyl ether : dichloromethane (0.5 : 0.5 : 9), then acetonitrile : diethyl ether : dichloromethane (1 : 1 : 8). The dichloromethane fraction was rechromatographed in 10% ethyl acetate in petroleum ether to afford **3** (4 mg) and phytol (10 mg). The acetonitrile : diethyl ether : dichloromethane (0.5 : 0.5 : 9) fraction was rechromatographed in 10% ethyl acetate in petroleum ether to afford sitosterol (20 mg).

Isovouacapenol E (**1**): Colorless gum, $[\alpha]_D^{20}$ −7° (c=0.001, $CHCl_3$); ^{1}H- and ^{13}C-NMR data, Table 1; IR ($CHCl_3$) 2933, 1718, 1277, 1094 cm^{-1}; UV (MeOH) (ε) 230 (26000), 279 nm (sh) (2000); EI-MS m/z 540 [M^+] (92), 418 (61), 400 (35), 385 (49), 338 (22), 278 (36), 265 (43), 263 (100); HR-EI-MS m/z 540.2508 [M^+] ($C_{34}H_{36}O_2$ requires 540.2512).

Caesaldekarin A (**3**): Colorless gum; ^{1}H-NMR: 0.99 (3H, d, J=7.2 Hz, H-17), 0.99 (3H, s, H-18), 1.25 (3H, s, H-19), 1.34 (3H, s, H-20), 2.06 (3H, s, OAc), 5.23 (1H, t, J=3.0 Hz, H-6), 6.18 (1H, d, J=1.8 Hz, H-15), 7.23 (1H, d, J=1.8 Hz, H-16); ^{13}C-NMR: 34.8 (C-1), 18.1 (C-2), 38.1 (C-3), 38.9 (C-4), 76.1 (C-5), 72.2 (C-6), 31.3 (C-7), 30.5 (C-8), 38.0 (C-9), 41.4 (C-10), 21.8 (C-11, $OCO\underline{C}H_3$), 149.5 (C-12), 122.3 (C-13), 31.1 (C-14), 109.5 (C-15), 140.4 (C-16), 17.5 (C-17), 27.6 (C-18), 25.7 (C-19), 16.5 (C-20), 169.8 ($O\underline{C}OCH_3$).

Spathulenol (**4**): Colorless oil, ^{1}H-NMR: 0.47 (H-1, dd, J=9.6, 11.6 Hz), 0.71 (H-2), 1.01 (H-3a), 1.96 (H-3b), 2.05 (H-4a), 2.42 (H-4b, dd, J=5.2, 13.6 Hz), 2.20 (H-6), 1.64 (H-7a), 1.91 (H-7b), 1.54 (H-8a), 1.77 (H-8b), 1.31 (H-10), 1.05 (H-12), 1.04 (H-13), 4.66 (H-14a), 4.68 (H-14b), 1.28 (H-15); ^{13}C-NMR: 29.9 (C-1), 27.5 (C-2), 24.8 (C-3), 38.9 (C-4), 153.5 (C-5), 53.4 (C-6), 26.7 (C-7), 41.8 (C-8), 81.0 (C-9), 54.4 (C-10), 20.3 (C-11), 28.7 (C-12), 16.3 (C-13), 106.3 (C-14), 26.1 (C-15).

Caryophyllene Oxide (**5**): Colorless oil; ^{1}H-NMR: 1.75 (H-1), 1.45, 1.64 (H_2-2), 2.10, 0.95 (H_2-3), 2.85 (H-5), 1.35, 2.25 (H_2-6), 2.10, 2.35 (H_2-7), 2.60 (H-9), 1.60, 1.69 (H_2-10), 1.20 (s, H-12, Me), 4.86, 4.97 (H_2-13), 1.01 (s, H-14, Me), 0.99 (s, H-15, Me); ^{13}C-NMR: 50.8 (C-1), 27.2 (C-2), 39.2 (C-3), 59.8 (C-4), 63.7 (C-5), 29.94 (C-6), 29.88 (C-7), 151.9 (C-8), 48.7 (C-9), 39.8 (C-10), 34.5 (C-11), 17.0 (C-12), 112.7 (C-13), 21.6 (C-14), 30.2 (C-15).

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References

1) Quisumbing E., "Medicinal Plants of the Philippines," Bureau of Printing, Manila, 1978, pp. 372—377.
2) Padma P. R., Samathi S., Aparna S., *J. Med. Aromat. Plant Sci.*, **22/4A—23/1A**, 78—83 (2001).
3) Patel A. D., Freyer A. J., Webb R. L., Zuber G., Reichwein R., Bean M. F., Faucette L., Johnson R. K., *Tetrahedron*, **53**, 1593—1597 (1997).
4) Che C. T., McPherson D. D., Cordell G. A., Fong H. H. S., *J. Nat. Prod.*, **49**, 561—569 (1986).
5) McPherson D. D., Che C. T., Cordell G. A., Soejarto D. D., Pezzuto J. M., Fong H. H. S., *Phytochemistry*, **25**, 167—170 (1986).
6) McPherson D. D., Cordell G. A., Pezzuto J. M., Fong H. H. S., *Phytochemistry*, **22**, 2835—2838 (1983).
7) Unrau A. M., Choi Y. M., *Carbohydr. Res.*, **14**, 157—158 (1970).
8) Paris R., Delaveau P. Q., *J. Crude Drug Res.*, **7**, 964—966 (1967).
9) Rao R. V. K., Prasad G. R., *Indian J. Pharm. Sci.*, **40**, 103—104 (1978).
10) Awasthi K. K., Kumar A., Misra K., *Phytochemistry*, **19**, 1995—1997 (1980).
11) Awasthi K. K., Misra K. J., *J. Indian Chem. Soc.*, **54**, 646—647 (1977).
12) Ragasa C. Y., Hofileña J., Rideout J. A., *J. Nat. Prod.*, **65**, 1107—1110 (2002).
13) Kitagawa I., Simanjuntak P., Watano T., Shibuya H., Fujii S., Yamagata Y., Kobayashi M., *Chem. Pharm. Bull.*, **42**, 1798—1800 (1994).
14) Inagaki F., Abe A., *J. Chem. Soc. Perkin Trans II*, **1985**, 1773—1778 (1985).
15) Silverstein R. M., Webster F. X., "Spectrometric Identification of Organic Compounds," 6th ed., John Wiley and Sons, New York, 1998, pp. 258—268.
16) Goodman R. A., Oldfield E., Allerhand A., *J. Am. Chem. Soc.*, **95**, 7553—7558 (1973).
17) Rubinstein I., Goad J., Clague A. D. A., Mulheim L. J., *Phytochemistry*, **15**, 195—200 (1976).