Pergamon

Tetrahedron, Vol. 53, No. 5, pp. 1583-1592, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01135-0

Pulcherrimins A - D, Novel Diterpene Dibenzoates from *Caesalpinia pulcherrima* with Selective Activity against DNA Repair-Deficient Yeast Mutants

Ashok D. Patil*, Alan J. Freyer, R. Lee Webb, Gary Zuber, Rex Reichwein, Mark F. Bean, Leo Faucette and Randall K. Johnson

Departments of Biomolecular Discovery, Analytical Sciences and Physical & Structural Chemistry
SmithKline Beecham Pharmaceuticals, R & D, 709 Swedeland Road
King of Prussia, Pennsylvania 19406-0939

Abstract: Bioassay-guided fractionation of the MeOH/CH_2Cl_2 extract of the roots of *Caesalpinia pulcherrima* yielded four novel dibenzoate diterpenes, pulcherrimins A, B, C and D (**1-4**). The structures of these compounds including their absolute configuration were established by interpretation of spectral data and CD measurements. Pulcherrimins A and B (**1** and **2**) were found to be active in DNA repair-deficient yeast mutant.

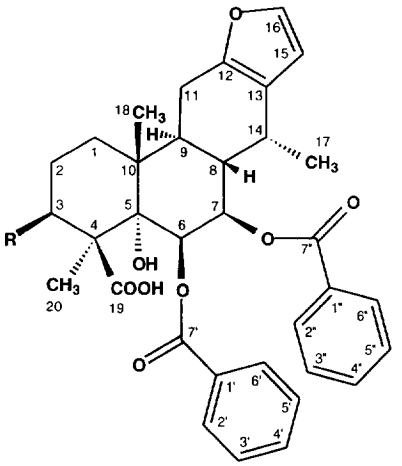
Introduction: *Caesalpinia pulcherrima* (L) Swartz is a large perennial shrub or small tree of the cassane family found throughout the tropics. It has been used ornamentally and is commonly known as 'Pride of Barbados', 'Peacock Flower' and 'Paradise Flower'.[1] According to folklore, the stem has been used as an abortifacient and an emmenagogue.[1] Earlier workers have reported the isolation of peltogynoids,[2] homoisoflavonoids,[2] caesalpins,[3] diterpenoids, 6β-cinnamoyl-7β-hydroxy-vouacapen-5α-ol, 6-methoxypulcherrimin,[2] pulcherrimin,[2] bonducellin and 8-methoxybonducellin, 2,6-dimethoxybenzoquinones and 4'-methylisoliquiritigenin[4] from various parts of the plant.

Results and Discussion

In our search for potential anticancer agents employing a mechanism-based yeast bioassay for DNA-damaging agents,[5,6] we screened several thousand natural product extracts. One of the extracts which demonstrated selective activity in a DNA repair-deficient yeast mutant was from a Mexican plant, *C. pulcherrima*, and therefore was selected for fractionation. We now report the isolation and structure determination of four novel diterpene dibenzoates, pulcherrimins A-D (**1-4**).

Powdered roots of *C. pulcherrima* were extracted sequentially with ethyl acetate and methanol. The methanol extract, which showed activity suggestive of induction of DNA lesions, was

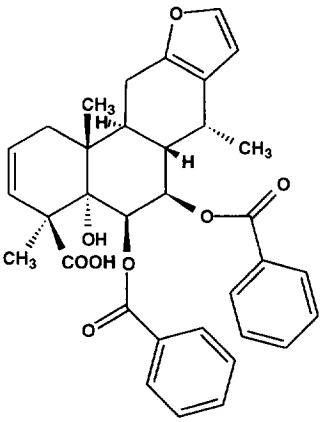
chromatographed over an RP-18 column to yield several active fractions. Further purification of these active fractions by preparative TLC and HPLC led to the isolation of pulcherrimins A-D (**1-4**).



1 R = OH

3 R = H

4 R = CH_3CO_2



2

Pulcherrimin A (**1**) was isolated as a white powder, $[\alpha]_D$ = +89.1°. The positive ESIMS of **1** had a molecular ion $(M+H)^+$ at *m/z* 589 (three exchangeables) and exhibited fragment ions at *m/z* 571 $(M+H-H_2O)$ and *m/z* 553 $(M+H-2H_2O)$. Intense fragment ions at *mz* 467 and *m/z* 345 corresponded to the loss of two benzoic acid molecules from the molecular ion. The molecular formula of pulcherrimin A (**1**) was deduced as $C_{34}H_{36}O_9$ from high resolution FABMS [*m/z* 589.2449 for $(M+H)^+$] which required seventeen degrees of unsaturation. The IR spectrum showed carbonyl absorptions at 1730 (ester) and 1695 cm^{-1} (carboxyl). The UV absorptions at 221 nm along with IR absorption at 1451 cm^{-1} attested to the presence of a 2,3-disubstituted furan ring, and this was further supported by a pair of doublets (J = 1.8 Hz) at δ7.23 and 6.13 in its ^1H NMR spectrum. In addition, the ^1H NMR spectrum which is summarized in Table 1 revealed the presence of ten aromatic multiplets resonating between δ7.85 and 7.31, a methine doublet at δ6.12 and a doublet of doublets at δ5.72, as well as a doublet of doublet at δ3.42, nine proton multiplets between δ2.86 and 1.52 and three methyl signals at δ1.44, 1.34 and 1.00 (d, J = 7.0 Hz) in the aliphatic region.

The COSY data provided evidence that ten of the downfield protons were attached to two mono-substituted aromatic rings and that the narrow furan doublets were coupled to each other. The remaining aliphatic protons were divided into two extended spin systems. The first of these systems included four consecutive methine signals, H-6 through H-9 at δ6.12, 5.72, 2.42 and 2.57 respectively, attached to an H-11 methylene group at δ2.63 and δ2.57 with an additional H-8 branch consisting of methine H-14 at

TABLE 1. ^{1}H NMR Assignment for **1** - **4** in $CDCl_3$

H	1	2	3	4
1	1.80 (1H,ddd, *J*=3.3,11.4,12.6)	2.27 (1H,dm, *J*=17.2)	1.70 (1H,m)	2.00 (1H,m)
	1.52 (1H,ddd, *J*=3.3,3.3,12.6)	2.07 (1H,dd, *J*=5.8,17.2)	1.53 (1H,m)	1.70 (1H,m)
2	2.14 (1H,ddd, *J*=3.3,11.4,12.2)	5.78 (1H,dm, *J*=10.7)	1.93 (1H,m)	2.65 (1H,m)
	1.58 (1H,m)		1.44 (1H,m)	1.97 (1H,m)
3	3.42 (1H,dd, *J*=4.7,12.2)	5.22 (1H,dm, *J*=10.7)	1.76 (1H,m)	5.33 (1H,dd, *J*=4.8,12.0)
			1.55 (1H,m)	
6	6.12 (1H,d, *J*=3.8)	5.97 (1H,d, *J*=3.4)	6.05 (1H,d, *J*=3.7)	5.98 (1H,d, *J*=3.8)
7	5.72 (1H,dd, *J*=3.8,11.4)	5.75 (1H,dd, *J*=3.4,11.3)	5.76 (1H,dd, *J*=3.7,11.1)	5.52 (1H,dd, *J*=3.8,11.7)
8	2.42 (1H,ddd, *J*=5.0,11.4,11.8)	2.41 (1H,ddd, *J*=5.0,11.2,11.3)	2.44 (1H,ddd, *J*=5.0,11.1,12.0)	2.31 (1H,m)
9	2.57 (1H,m)	2.53 (1H,ddd, *J*=6.5,11.2,11.4)	2.52 (1H,m)	2.63 (1H,m)
11	2.63 (1H,m)	2.67 (2H,m)	2.67 (1H,m)	2.66 (1H,m)
	2.57 (1H,m)		2.63 (1H,m)	2.59 (1H,m)
14	2.86 (1H,dq, *J*=5.0,7.0)	2.88 (1H,dq, *J*=5.0,7.0)	2.86 (1H,dq, *J*=5.0,7.0)	2.85 (1H,dq, *J*=5.0,7.0)
15	6.13 (1H,d, *J*=1.8)	6.12 (1H,d, *J*=1.8)	6.14 (1H,d, *J*=1.8)	6.19 (1H,d, *J*=1.9)
16	7.23 (1H,d, *J*=1.8)	7.22 (1H,d, *J*=1.8)	7.24 (1H,d, *J*=1.8)	7.26 (1H,d, *J*=1.9)
17	1.00 (3H,d, *J*=7.0)	0.98 (3H,d, *J*=7.0)	1.00 (3H,d, *J*=7.0)	1.00 (3H,d, *J*=7.0)
18	1.44 (3H,s)	1.56 (3H,s)	1.35 (3H,s)	1.42 (3H,s)
20	1.34 (3H,s)	1.26 (3H,s)	1.12 (3H,s)	1.24 (3H,s)
2'6'	7.70 (2H,dd, *J*=1.3,8.4)	7.72 (2H,dd, *J*=1.3,8.4)	7.76 (2H,dd, *J*=1.3,8.4)	7.95 (2H,dd, *J*=1.3,8.4)
3'5'	7.31 (2H,dd, *J*=8.4,8.4)	7.35 (2H,dd, *J*=8.4,8.4)	7.36 (2H,dd, *J*=8.4,8.4)	7.39 (2H,m)
4'	7.49 (1H,tm, *J*=8.4)	7.55 (1H,tm, *J*=8.4)	7.50 (1H,tm, *J*=8.4)	7.54 (1H,tm, *J*=8.4)
2"6"	7.85 (2H,dd, *J*=1.3,8.4)	7.85 (2H,dd, *J*=1.3,8.4)	7.78 (2H,dd, *J*=1.3,8.4)	7.98 (2H,dd, *J*=1.3,8.4)
3"5"	7.35 (2H,dd, *J*=8.4,8.4)	7.35 (2H,dd, *J*=8.4,8.4)	7.28 (2H,dd, *J*=8.4,8.4)	7.42 (2H,m)
4"	7.53 (1H,tm, *J*=8.4)	7.55 (1H,tm, *J*=8.4)	7.48 (1H,tm, *J*=8.4)	7.54 (1H,tm, *J*=8.4)

δ2.86 and the CH_3-17 doublet at δ1.00. The second spin system consisted of two adjacent methylene groups, H-1 at δ1.80 and 1.52 and H-2 at δ2.14 and 1.58, attached to the oxygen-bearing H-3 methine doublet of doublets at δ3.42. Treatment of pulcherrimin A (**1**) with acetic anhydride/pyridine yielded a monoacetate (**4**) whose ^{1}H NMR displayed a singlet (3H) at δ2.05 instead of an expected diacetate, suggesting the presence of a tertiary hydroxyl at C-5 as in all caesalpins that had previously been isolated from *Caesalpinia* sp.[3,4] Based on the mass spectral fragmentation, proton and COSY data, and formation of a monoacetate, compound **1** was considered to be a tricarbocyclic furanoditerpenoid with two pendant benzoate substituents.

The ^{13}C GASPE NMR spectrum of pulcherrimin A (**1**) which is summarized in Table 2 displayed three carbonyl signals, eight protonated aromatic/olefinic carbons (four of which represented two symmetrical carbons each) and four quaternary carbons in the downfield region. Of the four oxygenated carbons near δ75, one was quaternary (δ79.2) and three were methines. The remainder of the ^{13}C spectrum included two quaternary and three methylene aliphatic signals along with three methines and three methyl groups.

Differentiation between the two benzoate carbonyls was based on their HMBC correlations with the respective ortho ring protons. HMBC correlations between the H-6 methine at δ6.12 and carbonyl C-7' at δ166.3 and between H-7 at δ5.72 and carbonyl C-7" at δ166.5 established that these two benzoate groups were attached to the diterpene moiety at C-6 and C-7. The angular CH_3-18 at δ1.44 shared long-range correlations with methylene carbon C-1 (δ33.4), with quaternary carbons C-5 (δ79.2) and C-10 (δ41.3) and with angular methine C-9 (δ36.9). The only oxygenated quaternary carbon at δ79.2 was assigned to C-5. Similarly, CH_3-20 at δ1.34 correlated with the oxygenated C-3 (δ74.6) methine carbon, with quaternary carbons C-4 (δ54.6) and C-5 and with the C-19 carboxylic acid carbonyl at δ177.9. The CH_3-17 doublet at δ1.00 shared long-range correlations with methines C-8 (δ35.4) and C-14 (δ27.3) as well as with the quaternary C-13 at δ121.3. The H-11 methylene protons also correlated to C-13 as well as to C-12 at δ149.0. Finally, HMBC correlations were observed between H-16 at δ7.23 and C-12, C-13 and C-15 and between H-15 at δ6.13 and C-12, C-13 and C-16.

The relative stereochemistry of **1** was determined by coupling constants and NOESY data. Axial methine protons H-7 and H-9 are both depicted transperiplanar to H-8 in Fig.1 based on the large coupling constants which H-8 shared with H-7 and H-9. H-6 was equatorial as reflected by the small coupling constant which it shared with H-7. Likewise, H-3 occupied an axial position because it shared a large coupling constant with H-2_{ax} which in turn shared a large coupling constant with H-1_{ax}.

The relative stereochemistry of **1** suggested by coupling constants was born out by NOESY results which are summarized in Fig. 1 by arrows about an energy-minimized structure. The benzoate phenyl groups are represented by "Ph" for clarity. NOESY correlations from H-3 to H-2_{eq} at δ1.58 and to H-1_{ax} at δ1.80 confirmed that H-3 was axial and that the C-3 hydroxyl group was equatorial. H-1_{ax} correlated

TABLE 2. ^{13}C NMR Assignment for **1** - **3** in $CDCl_3$

C	1	2	3
1	33.4	36.8	34.6
2	27.5	123.5	18.7
3	74.6	129.3	33.4
4	54.6	50.5	49.0
5	79.2	77.6	77.8
6	69.3	69.5	68.9
7	72.5	72.0	72.4
8	35.4	35.4	35.6
9	36.9	37.3	37.3
10	41.3	40.8	41.5
11	22.3	22.2	22.2
12	149.0	148.7	149.1
13	121.3	121.3	121.4
14	27.3	27.4	27.4
15	109.4	109.4	109.5
16	140.7	140.8	140.8
17	17.0	17.1	17.1
18	17.7	16.9	17.8
19	177.9	177.8	181.6
20	20.2	22.7	24.2
1'	129.8	130.2	130.5
2',6'	129.5 (2C)	129.6 (2C)	129.5 (2C)
3',5'	128.4 (2C)	128.5 (2C)	128.3 (2C)
4'	132.9	132.8	132.6
7'	166.3	165.0	165.6
1"	130.1	130.1	129.9
2",6"	129.7 (2C)	129.7 (2C)	129.6 (2C)
3",5"	128.2 (2C)	128.1 (2C)	128.1 (2C)
4"	133.1	132.9	132.9
7"	166.5	165.7	166.2

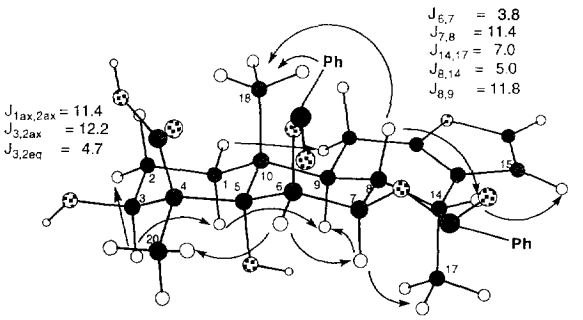


Figure 1. Molecular model of pulcherrimin A (**1**) with arrows representing NOESY correlations.

to H-9, and H-1_{eq} at δ1.52 correlated to H-11 at δ2.57. H-6 correlated to methyl CH_3-20 and H-7. H-7 had additional correlations to methyl CH_3-17 and H-9. Therefore H-3, H-1_{ax}, H-9, H-7 and CH_3-17 were all axial and appeared on the same face of **1**. H-8 on the opposite face of the molecule correlated to methine H-14 and methyl CH_3-18. The 2' and 6' hydrogens of the benzoate substituent attached at C-6 shared a correlation with methyl CH_3-18 which confirmed that this phenyl group was situated axially on the same face of **1** as CH_3-18, making H-6 equatorial.

The CD spectrum of **1** (Figure 2) exhibited a positive screw sense.[7] Based on the relative stereochemistry determined by NMR, the CD data established that the absolute stereochemistry about the vicinal benzyloxy groups was 6R, 7R.

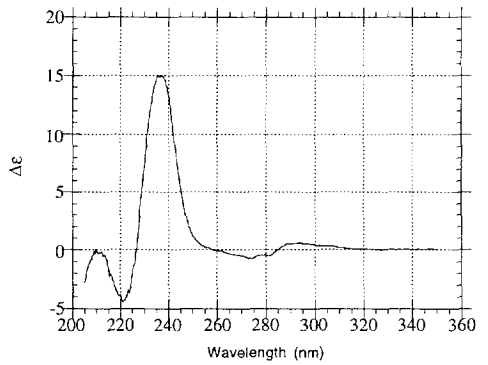


Figure 2. CD spectrum of pulcherrimin A (**1**)

With the structure of pulcherrimin A (**1**) in hand, we readily assigned structures of the closely related pulcherrimins B-D (**2-4**). Pulcherrimin B (**2**) was isolated as a colorless powder and displayed a

molecular ion at *mz* 571 $(M+H)^+$, 18 mass units less than **1** which implied that **2** was a dehydrated analog of **1**. The UV and IR spectra were similar to those of **1**, and the ^1H NMR spectrum which is summarized in Table 1 was almost identical to that of **1**, except that it had two additional olefinic doublets at δ 5.78 and 5.22 which shared a 10.7 Hz coupling. Absence of the H-3 methine and the H-2 methylene protons in **2**, which were present in **1**, and emergence of two olefinic methine carbon signals at δ 123.5 and 129.3 in the ^{13}C GASPE NMR spectrum of **2** which is summarized in Table 2, confirmed that there was an additional C2/3 double bond in pulcherrimin B (**2**) with the remainder of the molecule remaining the same as **1**. This was further supported by homo- and heteronuclear correlation data. The ^1H coupling constants and NOESY correlations for all the protons other than ring A in pulcherrimin B (**2**) were identical to their counterparts in **1** establishing that the relative stereochemistry was preserved. Optical rotation indicated that the absolute stereochemistry was also retained.

Pulcherrimin C (**3**), also obtained as white powder, showed a molecular ion at *m/z* 573 $(M+H)^+$, two mass units higher than that of **2**. The UV and IR absorptions were nearly identical to those of **1** and **2**, and the ^1H NMR spectrum (see Table 1) was similar to that of **2** except that it lacked the two olefinic doublets. Instead there were two additional methylenes present indicating that **3** was the dihydro analog of **2**. Homo- and heteronuclear data confirmed that the C-2/3 double bond in **2** had been reduced in **3**. The coupling constants and NOESY correlations of all the protons in **3** were identical to those in **1** and **2** suggesting that the relative stereochemistry was same as in **1** and **2**, and the optical rotation confirmed that the absolute stereochemistry was also retained.

Pulcherrimin D (**4**) was isolated as a white amorphous powder with an $[\alpha]_D = +55.4°$ and had a molecular weight of 631 Daltons $(M+H)^+$ by positive ESIMS, 42 Daltons more than pulcherrimin A (**1**). The molecular formula of $C_{36}H_{38}H_{10}$ was deduced from the HRFABMS. The UV, IR, NMR and mass spectra of **4** were identical to those of the acetyl analog of **1**. A three proton acetyl singlet observed at δ1.98 in the ^1H NMR spectrum helped confirm this conclusion, as did the nearly 2 ppm downfield shift of H-3 from δ3.42 in **1** to δ5.33 in **4**. The coupling constants and NOESY correlations of all the protons in pulcherrimin D (**4**) were identical to those in **1** implying identical relative stereochemistry. The H-3 doublet of doublets in **4** shared 4.8 and 12.0 Hz coupling constants with the H-2 methylene protons indicating that H-3 occupied an axial position as in **1**. A positive optical rotation also confirmed that the absolute stereochemistry was the same as for **1-3.** Paucity of sample prevented ^{13}C analysis.

Pulcherrimins A, B and C (**1-3**) were selectively active in the yeast strains in which both the RAD 52 and TOP1 genes had been deleted. The greatest potency was evident for pulcherrimin B (Table 3). Acetylation of the 3-hydroxyl group resulted in loss of activity. This pattern of activity in the yeast strain

Table 3 Bioactivity of pulcherrimins A-D (1-4)

IC_{12} (μg/well) in yeast strain

Compound	Wild type	Δ RAD 52	Δ RAD 52 Δ TOP1	IC_{50} in Vero cells (μg/ml)
1	88	> 100	16	66
2	> 100	66	0.64	not tested
3	> 100	> 100	4.4	59
4	> 100	> 100	> 100	not tested

is similar to what is seen with inhibitors of topoisomerase II such as amsacrine, ellipticine or etoposide. Hypersensitivity to topoisomerase II inhibitors likely occurs due to increase in topoisomerase II expression and/or activity to compensate for deletion of topoisomerase I. Pulcherrimins A (**1**) and C (**3**) were tested for cytotoxicity in Vero cells in culture and were found to have weak activity (IC_{50} of about 60 μg/ml).

Experimental Section

General: The IR spectra were recorded on a Nicolet Model 20 DXB FTIR spectrometer. All homonuclear and heteronuclear one and two-dimensional NMR data were recorded on a Bruker AMX-400 spectrometer in $CDCl_3$ and are presented in Tables 1 and 2. The ESIMS were obtained on a Perkin-Elmer Sciex API-III triple quadruple mass spectrometer. HRFAB mass spectra were performed on a VG ZAB SE4F tandem sector mass spectrometer. Analytical and preparative TLC were carried out on precoated Si gel G (Kiesel gel G254) and reversed-phase (Whatman KC18F) plates. The UV spectra were recorded on a Beckman DV-7 spectrophotometer. Optical rotations were recorded on Perkin-Elmer 241 MC polarimeter. The CD spectrum was obtained on a JASCO J 500-C spectropolarimeter from 350 to 205 nm in a 1 mm cell (6 computer averaged scans). Reagent grade chemicals (Fisher and Baker) were used throughout.

Plant Material: *C. pulcherrima* (Fabaceae) was collected near Sonoro in Mexico for phytochemical investigators by the USDA under a cooperative agreement with the National Cancer Institute in January 1963. A voucher sample is preserved at the National Herbarium, Washington D. C.

Bioassays: Activity of crude extracts, fractions and purified components was monitored by the use of specific deletion mutants of the yeast *Saccharomyces cerevisiae*. Three strains were used, all of which were selected for resistance to nystatin and were correspondingly hypersensitive to a broad spectrum of unrelated compounds by virtue of altered permeability. The induction of DNA damage was signified by differential mutation of strains in which the RAD 52 gene was knocked out by homologous recombination. Such strains are incapable of repairing DNA double strand breaks and are specifically hypersensitive to compounds which produce DNA lesions by a variety of mechanisms such as topoisomerase inhibitors, DNA intercalations and minor groove binders and DNA alkylators and cleavers. Additionally, one of the test strains also had the gene for topoisomerase I deleted; this strain is useful for identifying inhibitors of the topoisomerases in that it is hypersensitive to diverse inhibitors of topoisomerase II like amsacrine, ellipticine and etoposide and is specifically resistant to the camptothecins that inhibit topoisomerase I.[8] Activity in these strains was determined by a standard agar diffusion technique in which 0.1 ml aliquot of sample solution was placed in a well punched in an agar plate (YEPD complete yeast medium) on which a test strain was seeded. Zones of inhibition were measured after 48 hours at 30° and the concentration (in μg/well) which produced a 12 mm zone (IC_{12}) was determined by linear regression. Cytotoxicity was determined by an XTT assay using Vero monkey kidney cells exposed to graded concentrations of test agents for 72 hours.

Extraction and Isolation: Air dried roots (57 g) were powdered and extracted with methanol/methylene chloride (1:1) by the cold percolation procedure to yield a pale brown residue (1.97 g). This residue was chromatographed on a column of RP-18 SI gel eluting with H_2O:MeOH (15:85) first, and finally with methanol to afford several fractions that were tested against a wild type yeast strain and a strain deficient in double-stranded DNA repair (RAD-52) and a strain deficient in both double-stranded DNA repair and topoisomerase I. Further purification of the residue (0.29 g) obtained from the active fractions using a Si gel column (EtOAc:Hex,1:1) yielded fractions which were followed by bioactivity. The RP-18 HPLC (Whatman, ODS-3, Magnum-9 column, H_2O:CH_3CN:TFA,25:75:0.2) of the active fraction (79 mg) using a refractive index detector yielded pulcherrimin A (**1**, 11.2 mg), pulcherrimin B (**2**, 3.1 mg), pulcherrimin C (**3**, 56 mg) and pulcherrimin D (**4**, 1.8 mg).

Pulcherrimin A (**1**): White amorphous solid, $[\alpha]_D$ + 89.1° (*c* = 0.46, MeOH); UV (MeOH) λ_{max} 221, 238, 273 nm; ν_{max} (KBr) 3400, 3000-3100, 2800-3000, 1730, 1695, 1451, 1286, 1072-1122, 709 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m/z* 589 $(M+H)^+$, HRFABMS, calcd for $(M+H)^+$, $C_{34}H_{37}O_9$ *m/z* 589.2438, $(M+H)^+$, found *m/z* 589.2449.

Pulcherrimin B (**2**): White amorphous powder, $[\alpha]_D$ +155.4° (*c* = 0.12, MeOH); UV (MeOH) λ_{max} 220, 230, 273 nm; ν_{max} (KBr) 3440, 3000-3100, 2800-3000, 1733, 1696, 1451, 1384, 1283, 1117, 714 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m/z* 571 $(M+H)^+$, HRFABMS, calcd for $(M+H)^+$, $C_{34}H_{35}O_8$ *m/z* 571.2332, $(M+H)^+$, found *m/z* 571.2353.

Pulcherrimin C (**3**): White amorphous powder, $[\alpha]_D$ +78.4° (*c* = 0.14, MeOH); UV (MeOH) λ_{max} 222, 237, 278 nm; ν_{max} (KBr) 3487, 3000-3100, 2800-3000, 1736, 1692, 1651, 1602, 1585, 1316, 1108 cm^{-1}; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; ESIMS, *m/z* 573 $(M+H)^+$, HRFABMS, calcd for $(M+H)^+$, $C_{34}H_{37}O_8$ *m/z* 573.2488, $(M+H)^+$, found *m/z* 573.2499.

Pulcherrimin D (**4**): White amorphous powder, $[\alpha]_D$ +55.4° (*c* = 0.1, MeOH); UV (MeOH) λ_{max} 220, 237, 274 nm; ν_{max} (KBr) 3440, 3000-3100, 2800-3000, 1725, 1699, 1632, 1602, 1452, 1384, 1273, 1111, 710 cm^{-1}; ^1H NMR, see Table 1; ESIMS, *m/z* 631 $(M+H)^+$, HRFABMS, calcd for $(M+H)^+$, $C_{36}H_{39}O_{10}$ *m/z* 631.2543, $(M+H)^+$, found *m/z* 631.2559.

Acknowledgment

This work was supported in part by a grant (CA-50771) from the National Institutes of Health.

References

1. Quisimbing, E. "Medicinal Plants of the Philippines" Manila Bureau of Printing, **1951**, pp 372-374.

2. McPherson, D. D.; Cordell, G. A.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. *Phytochem.* **1983**, *22*, 2835-2838.

3. Sengupta, P., Roy, S.; Das, K. G. *Chem. Ind.* **1970**, 534-535.

4. McPherson, D. D.; Che, T-C.; Cordell, G. A.; Soejarto, D. D.; Pezzuto, J. M.; Fong, H. H. *Phytochem.* **1986**, *25*, 167-170.

5. Guntilaka, A. A. L.; Samarnayke, G.; Kingston, D. G. I.; Hoffman, G.; Johnson, R. K. *J. Nat. Prod.* **1992**, *55*, 1648-1654.

6. Gunatilaka, A. A. L.; Kingston, D. G. I.; Johnson, R. K. *Pure & Appl. Chem.* **1994**, *66*, 2219-2222.

7. Harada, N.; Nakanishi, K. "Circular Dichroic Spectroscopy - Exiton Coupling in Organic Stereochemistry " **1983**, University Science Books, p 76.

8. Eng, W. K.; Faucette, L.; Johnson, R. K.; Sterngalnz, R. *Mol. Pharm.*, **1988**, *34*, 755-760.

(*Received in USA* 3 *October* 1996; *revised* 8 *November* 1996; *accepted* 3 *December* 1996)