JOURNAL OF NATURAL PRODUCTS

Volume 60, Number 6 June 1997

Full Papers

Phospholipase A_2 Inhibitors from an *Erythrina* Species from Samoa

V. R. Hegde,* P. Dai, M. G. Patel, M. S. Puar, P. Das, J. Pai, R. Bryant, and P. A. Cox†

Microbial Products, Schering−Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033

Received June 25, 1996⊗

Three flavonoid phospholipase A_2 (PLA_2) inhibitors were isolated from a MeOH extract of the bark of the Samoan medicinal plant *Erythrina variegata*. Two of these compounds, 4′-hydroxy-3′,5′-diprenylisoflavonone (abyssinone V) (**1**) and 3,9-dihydroxy-2,10-diprenylpterocarp-6a-ene (erycrystagallin) (**3**), have been previously reported as antimicrobial agents from plants belonging to other *Erythrina* species. The isoflavonone 4′-hydroxy-6,3′,5′-triprenylisoflavonone (**2**) is a new compound, and its structure was determined by spectroscopic techniques. The IC_{50} values for the PLA_2 enzyme were 6, 10, and 3 μM for **1**−**3**, respectively.

Phospholipase A_2 (PLA_2) is a lipolytic enzyme that specifically hydrolyzes the SN-2 ester bond of phospholipids. In addition to a role in phospholipid catabolism, this enzyme is believed to be involved in a series of vital regulatory processes through its ability to release arachadonic acid (AA) for the subsequent biosynthesis of eicosanoids. Eicosanoids are implicated in the pathobiology of many diseases, especially those involving inflammation and allergy. Due to the biological importance of PLA_2 in inflammatory processes, the inhibition of PLA_2 activity offers an attractive therapeutic target for the design of novel antiinflammatory agents.

As part of a screen for PLA_2 inhibition from natural sources, we have isolated three flavonoids (**1**−**3**) from the bark of the Samoan plant *Erythrina variegata*, which demonstrated strong PLA_2 activity. *E. variegata* (Leguminosae) is a Samoan tree with brilliant red flowers. Locally it is known as "gatae". Samoan healers prepare extracts of the bark in coconut oil and apply the extracts externally for swellings and inflammation, called in Samoa "fula" and "fula la'au". It is also used to treat "Ati loto", which are skin ulcers that appear as boils or burns and which burst and suppurate. Given these ethnomedical data, it appears that *E. variegata* is effective in Samoa in the treatment of inflammation or inflammation-like processes. It is also used to treat inflammation in the Philippines[1] and inflammation from insect bites in Tahiti.[2]

The MeOH extract of the bark of *E. variegata*, on chromatographic purification on Si gel and HPLC, afforded abyssinone V (**1**), a new isoflavonone (**2**), and erycristagallin (**3**).

Results and Discussion

Compound **1**, obtained as a white amorphous powder, showed a $[M + H]^+$ peak at *m*/*z* 409 by FABMS revealing its molecular weight to be 408 Da. The spectral values obtained for **1** when compared those with those of known isoflavonones from the literature revealed this compound to be identical to abyssinone V, isolated from an East African medicinal plant *Erythrina abyssinica*.[3,4]

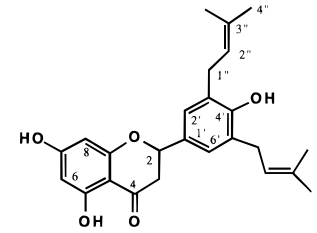
Compound **2** was obtained as a white solid. It showed a protonated molecular ion peak at *m*/*z* 477 in the FABMS, revealing a molecular weight of 476 Da. HRMS measurements established the molecular formula of **2** as $C_{30}H_{36}O_5$ (calcd for $C_{30}H_{37}O_5$ 477.2641, found 477.2638). It differs from **1** by having an ad-

* To whom correspondence should be addressed. Phone: (908) 298-7296. FAX: (908) 298-7115.

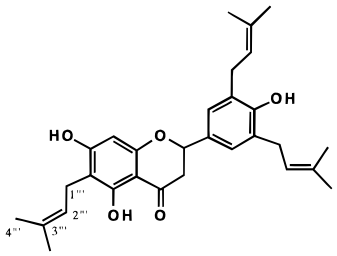
† Department of Botany and Range Science, Brigham Young University, Provo, UT 84602.

⊗ Abstract published in *Advance ACS Abstracts*, March 15, 1997.

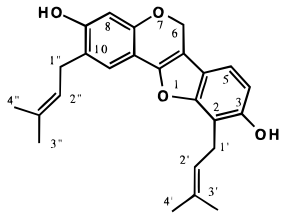
S0163-3864(96)00533-2 CCC: $14.00



1



2



3

ditional 68 mass units corresponding to a C_5H_8 group. The IR spectrum showed absorption bands at 3450, 3435 (free OH), 1635 (>C=O), 1605, 1435 (aromatic), 1270, and 1170 (C−O) cm^{-1}, and the UV spectrum showed maxima at 232 and 296 nm, characteristic for an isoflavonone. This inference was also strengthened by the presence of characteristic signals (H-2 at δ 5.32, H_2-3 at δ 2.80 and δ 3.06) in the ^{1}H-NMR spectrum. It also showed additional proton signals for three phenolic hydroxyl groups (δ 5.50, OH-4′; δ 6.05, OH-7; and δ 12.02, OH-5), three isolated aromatic protons (δ 6.02, H-8; and two proton signals at δ 7.05, H- 2′ and H-6′), and three isoprenyl groups (two methyls at δ 1.74; four methyls at δ 1.80; two allylic protons at δ 3.30; four allylic protons at δ 3.38; three vinylic proton signals, with one at δ 5.20 and two at δ 5.32). The two methyls and another double bond revealed the presence of an additional isoprenyl group compared with **1**. Analysis of the ^{1}H-NMR spectrum of **2** indicated the position of the additional isoprenyl group to be at either C-6 or C-8. The position of the isoprenyl group was suggested to be at C-6 based on the ^{13}C-NMR chemical shift assignments. This structure was further supported by the behavior of the isoflavonone in the Gibb's test,[5,6] which is diagnostic for phenols with a free *para* position. The characteristic absorption at 615 nm for the indophenol formed established the position of the isoprenyl group at C-6 as shown in structure **2**. This compound is therefore now established as a novel isoflavonone with three isoprenyl groups at the 6, 3′, and 5′ positions.

Compound **3** showed UV maxima at 214, 245, 252, 293, 339, and 356 nm, revealing it to be structurally distinct from compounds **1** and **2**. Comparison of the UV spectrum of **3** with those of various classes of compounds originating from plants indicated its similarity with anhydroglucinol (pterocarpan), a fused benzopyran, and benzfuran type of compound isolated from *Tetragonolobus maritimus*.[7] Analysis of the ^{1}H- and ^{13}C-NMR spectral data revealed the structure of **3** to be erycristagallin,[8] a pterocarpan isolated from the Bolivian coral tree, *Erythrina crysta-galli*.

In addition to compounds **1**−**3** reported here, similar pterocarpans[8−10] and isoflavones[11−14] have been isolated from other *Erythrina* species. Compounds **1**−**3** showed IC_{50} values of 6, 10, and 3 μM in the PLA_2 assay, respectively. Trifluropirazine at 20 μM showed >80% inhibition under these assay conditions.

The Samoans recognize two varieties of *E. variegata*, which they call "gatae samoa" and "gatae palagi", respectively. Healers claim that only the "gatae samoa" variety is active against inflammation. This observation was confirmed by us by testing both varieties against our PLA_2 assay; only the "gatae samoa" variety showed activity.

Experimental Section

General Experimental Procedures. IR spectra were determined on a Nicolet model 10-MX FTIR instrument. UV spectra were obtained using a Hewlett-Packard 8450 A UV-vis spectrophotometer equipped with a HP-9872B plotter. NMR spectra were measured on a Varian XL-300 instrument operating at 300 and 75 MHz for ^{1}H- and ^{13}C-NMR, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded relative to TMS as internal standard. FABMS and HRFABMS were obtained using a Finnigan MAT-312 mass spectrometer in a glycerol−thioglycerol matrix.

Plant Material. A bark sample of *E. variegata* C. A. Rich Allen was collected in August 1986, at Potogoa, Upolu Island, Samoa. Voucher specimens (COX 1062) are on deposit at the Brigham Young University Herbarium.

Extraction and Isolation. The outer bark (500 g) of the plant material was chopped into small pieces and blended in MeOH. The mixture was soaked for a day in a refrigerator, stirred, and filtered. The dried extract (gummy solid, 6.0 g) was partitioned between H_2O and EtOAc. The organic layer was separated, dried over anhydrous Na_2SO_4, and evaporated to obtain 2.2 g of solids containing a mixture of isoflavonones. The individual compounds were separated with a Si gel column eluting with a gradient mixture of hexane and EtOAc. Further purification by HPLC using a μBondapak C_{18} Si column and elution with MeOH−H_2O (80:20), yielded 20, 50, and 35 mg of **1**, **2**, and **3**, respectively.

Compound 1: white powder; UV λ_{max} (MeOH) 227, 291 nm (+ NaOH) 229, 248, 333 nm; IR ν_{max} 3430, 2915, 1640, 1605, 1475, 1375, 1340, 1270, 1165, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.60 (3H, s, CH_3), 1.75 (9H, s, 3 × CH_3), 2.75 (1H, dd, J = 14.5, 2.5 Hz, H-3), 3.10 (1H, dd, J = 14.5, 12.5 Hz, H-3), 3.35 (4H, d, J = 6 Hz, 2 × H_2-1″), 5.30 (3H, m, 2 × H-2″ and OH-7), 6.00 (2H, s, H-6 and H-8), 7.05 (2H, s, H-2′ and H-6′), 12.06 (1H, s, OH-5); ^{13}C NMR ($CDCl_3$) δ 17.9 (C-4″), 25.5 (C-5″), 29.7 (C-1″), 43.1 (C-3), 79.5 (C-2), 95.5 (C-8), 96.6 (C-6), 103.2 (C-4a), 121.6 (C-2″), 126.1 (C-2′ and C-6′), 127.6 (C-3′ and C-5′), 129.7 (C-1′), 134.9 (C-3″), 153.4 (C-4′), 163.4 (C-8a), 164.3 (C-5), 164.5 (C-7), 196.4 (C-4); FABMS *m*/*z* $[M + H]^+$ 409.

Compound 2: white powder; UV(MeOH) λ_{max} 232, 296 nm (+ NaOH) 249, 348 nm; IR (KBr) ν_{max} 3450, 3435, 3151, 2915, 1635, 1605, 1435, 1405, 1345, 1270, 1170, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.74 (6H, s, CH_3-4‴), 1.80 (12H, s, CH_3-4″), 2.80 (1H, dd, J = 14.5, 2.5 Hz, H-3), 3.06 (1H, dd, J = 14.5, 2.5 Hz, H-3), 3.30 (2H, d, J = 6 Hz, H-1‴), 3.38 (4H, d, J = 6.5 Hz, H-1″), 5.20 (1H, t, J = 6.5 Hz, H-2‴), 5.32 (3H, m, H-2, H-2″), 5.50 (1H, s, OH-4), 6.02 (1H, s, H-8), 6.05 (1H, br s, OH-7), 7.05 (2H, s, H-2′ and H-6′), 12.02 (1H, s, OH-5); ^{13}C NMR ($CDCl_3$) δ 17.9 (C-4″ and C-4‴), 21.8 (C-1‴), 25.8 (C-5″ and C-5‴), 29.7 (C-1″), 43.2 (C-3), 79.1 (C-2), 96.7 (C-8), 103.2 (C-8a), 106.0 (C-6), 121.5 (C-2″ and C-2‴), 125.7 (C-2′ and C-6′), 127.4 (C-3′ and C-5′), 130.2 (C-1′), 134.8 (C-3″), 135.0 (C-3‴), 153.1 (C-4′), 159.9 (C-5), 162.3 (C-4a), 163.6 (C-7), 196.4 (C-4); positive FABMS m/z $[M + H]^+$ 477; HRFABMS m/z $[M + H]^+$ $C_{30}H_{36}O_5$ (calcd for $C_{30}H_{37}O_5$ 477.2641, found 477.2638).

Compound 3: white powder; UV (MeOH) λ_{max} 214, 245, 252, 293, 339, 356 nm (+ NaOH) 212, 250, 287, 355, 367 nm; IR (KBr) ν_{max} 3430, 2970, 2915, 1650, 1615, 1505, 1445, 1375, 1250, 1165, 1035 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.85 (9H, s, 3 × CH_3), 1.92 (3H, s, CH_3), 3.32 (2H, d, J = 8 Hz, H-1″), 3.70 (2H, d, J = 8 Hz, H-1′), 5.18 (2H, s, H-6), 5.32 (1H, t, J = 8 Hz, H-2″), 5.39 (1H, t, J = 8 Hz, H-2′), 5.50 (2H, s, OH-3 and OH-9), 6.41 (1H, s, H-8), 6.78 (1H, d, J = 10 Hz, H-4), 7.05 (1H, d, J = 10 Hz, H-5), 7.21 (1H, s, H-11); ^{13}C NMR ($CDCl_3$) δ 17.9 (C-4′), 18.0 (C-4″), 23.2 (C-1″), 25.8 (C-4′), 25.8 (C-4″), 29.3 (C-1′), 65.5 (C-6), 104.3 (C-8), 106.3 (C-2), 109.9 (C-10), 111.1 (C-11a), 112.5 (C-4), 116.0 (C-5), 119.2 (C-5a), 119.7 (C-5b), 121.0 (C-2′), 121.4 (C-2″), 121.9 (C-11), 135.1 (C-3″), 135.4 (C-3′), 147.1 (C-1a), 151.9 (C-11b), 153.5 (C-7a), 154.4 (C-9), 155.2 (C-3); positive FABMS m/z $[M + H]^+$ 391.

Phospholipase A_2 (PLA_2) Inhibition. [^3H] Arachidonic acid 50 μCi was incubated overnight (18−20 h) with 250 mL of differentiated HL60 cells (1−1.5 × 10^6 cells/mL). After the overnight labeling, the cells were washed twice with Hepes−EGTA buffer and finally resuspended in Hepes−saline−BSA buffer at a concentration of 1.5 × 10^8 cells/mL.

A23187 (ionophore) and fMLP (formylated chemotactic peptide) were initially dissolved in DMSO and then diluted with buffer so that the concentration of DMSO in the assays did not exceed 0.1%. This concentration of DMSO had no discernible effects on the parameters being measured.

The assay mixtures contained 7−8 × 10^6 prelabeled cells, 1.5 mM of $CaCl_2$, 30 μg/mL of nordihydroguaiaretic acid (NDGA), and various concentrations of the compounds (**1**−**3**) in a total volume of 180 μL Hepes−saline−BSA buffer. These assay mixtures were incubated at 37 °C for 5 min before initiating the reaction by adding buffer of fMLP (100 nM) in a volume of 20 μL. The 5-min preincubation period was omitted in the assay mixtures where A23187 (1 μM) initiated the reaction.

After the incubation times of 2 min for fMLP and 5 min for A23187, the reaction was stopped by adding 40 μL of glutaraldehyde. After spinning the mixture for 10 min at 2000 rpm, 160 μL of supernatant was added to the scintillation cocktail and counted for 1 min to determine the amount of [^3H]arachidonic acid release.

Human promyelocitic leukemic (HL60) cells were grown in suspension culture and differentiated by culturing for 6 days in the presence of 1.3% dimethyl sulfoxide (DMSO) as described previously.[15,16]

Acknowledgment. The authors wish to thank Miss Emily Dobek and Miss Andrea Pascale for the assay support.

References and Notes

(1) Roi, J. *Atlas de Plantes Medicinales Chinoises*; Universite L'Aurore: Chang-Hai, 1946; p 125. (Shanghai Université L'Aurore. Musce Heude. Notes de Botanique Chinoise, no. 8).
(2) Florence, J.; Guerin, M.; Margueron, F.; Margueron, D.; Koenig D.; Koenig R. Ed.; *Archipel de Tahiti Recherches sur les Principales Productions Vegetales*; Editions Haere Po No: Tahiti, 1983; p 139.
(3) Kokwaro, J. O. *Medicinal Plants of East Africa*; East African Literature Bureau: Nairobi, 1976.
(4) Kamat, V. S.; Chuo, F. Y.; Kubo, I.; Nakanishi, K. *Heterocycles* **1981**, *15*, 1163−1170.
(5) Gibbs, H. D. *J. Biol. Chem.* **1927**, *72*, 649−664.
(6) King, F. E.; King, T. J.; Manning, L. C. *J. Chem Soc.* **1957**, 563−566.
(7) Ingham, J. L.; Dewick, P. M. *Phytochemistry* **1978**, *17*, 535−538.
(8) Mitscher, L. A.; Ward, J. A.; Drake, S.; Rao, G. S. *Heterocycles* **1984**, *22*, 1673−1675.
(9) Ingham, J. L.; *Z. Naturforsch.* **1980**, *35C*, 384−386.
(10) Ingham, J. L.; Markham, K. R. *Phytochemistry* **1980**, *19*, 1203−1207.
(11) Deshpande, V. H.; Pendse, A. D.; Pendse, R. *Ind. J. Chem.* **1977**, *15B*, 205−207.
(12) Wandji, J.; Nkengfack, A. E.; Fomum, Z. T.; Ubillas, R.; Killday, K. B.; Tempesta, M. J. *J. Nat. Prod.* **1990**, *53*, 1425−1429.
(13) Nkengfack, A.; Fomum, Z. T.; Ubillas, U.; Tempesta, M. S. *J. Nat. Prod.* **1990**, *53*, 1552−1556.
(14) Fomum, Z. T.; Ayafor, J. F.; Mbafor, J. T. *Tetrahedron Lett.* **1983**, *24*, 4127−4130.
(15) Billah, M. M.; Eckel, S.; Myers, R. F.; Siegel, M. T. *J. Biol. Chem.* **1986**, *261*, 5824−5831.
(16) Collins, S. J.; Ruscelli, F. W.; Gallagher, R. E.; Gallo, R. C. *J. Exp. Med.* **1979**, *149*, 969−974.

NP960533E