A New Anti-HIV Alkaloid, Drymaritin, and a New C-Glycoside Flavonoid, Diandraflavone, from *Drymaria diandra*

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A novel anti-HIV alkaloid, drymaritin (**1**), and a new C-glycoside flavonoid, diandraflavone (**2**), along with eight known compounds, torosaflavone A, isovitexin, spinasterol β-D-glycoside, *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, *cis*-*p*-coumarate, methyl 5-hydroxy-4-oxopentanoate, and glycerol-α-lignocerate, were isolated from *Drymaria diandra*. Drymaritin (**1**) exhibited anti-HIV effects in H9 lymphocytes with an EC_{50} value of 0.699 μg/mL and a TI of 20.6. Compound **2** showed significantly selective inhibition on superoxide anion generation from human neutrophils stimulated by fMLP/CB with an IC_{50} value of 10.0 μg/mL.

The genus *Drymaria* Willd. (Caryophyllaceae) includes 40 species, which are mainly distributed in tropical America. There is one species, however, *Drymaria diandra* Bl. [*Drymaria cordata* (L.) Willd. subsp. *diandra* (Bl.) I. Duke ex Hatusima], that grows in Taiwan.[1] This plant is a folk medicine used in Taiwan for treating fever, rheumatism, hepatoma, malaria, and cancer. Recently, several cyclopeptides,[2,3] flavonoids,[4] and norditerpenes[5] were isolated from this genus. In our previous studies, four cyclicpeptides[6] were isolated from *D. diandra* methanolic extracts. After further separation, a new alkaloid, drymaritin (**1**), and a C-glycoside flavonoid, diandraflavone (**2**), along with eight known compounds, torosaflavone A,[7] isovitexin,[8,9] spinasterol β-D-glycoside,[10] *p*-hydroxybenzoic acid,[11] *p*-hydroxybenzaldehyde,[12] methyl 5-hydroxy-4-oxopentanoate,[13] and glycerol-α-lignocerate,[14] were isolated. Screen tests of inhibition of superoxide anion generation from human neutrophils were processed on all 10 of these isolates. Compound **2** showed significant selective inhibition of superoxide anion generation from human neutrophils that were stimulated by fMLP/CB with an IC_{50} value of 10 μg/mL. Furthermore, compound **1** exhibited an anti-HIV effect in H9 lymphocytes (EC_{50} 0.699 μg/mL; TI 20.6).

Compound **1** was obtained as a pale yellow amorphous powder. IR (KBr) absorptions appeared at 1673, 1562, and 1474 cm^{-1}, and the UV absorptions maximized at 366, 349, 298, 288, and 264 nm, which suggested that **1** was a β-carboline analogue.[15,16] The molecular formula, $C_{15}H_{10}N_2O_2$, was obtained by HREIMS $[M]^+$ at *m*/*z* 250.0748 (calcd 250.0742). In the ^1H NMR spectrum of **1**, two pairs of doublets at δ 8.75 (1H, d, J = 4.8 Hz) and 7.90 (1H, d, J = 4.8 Hz), along with four *ortho*-coupled aromatic signals at δ 8.53 (1H, d, J = 8.4 Hz), 7.99 (1H, d, J = 7.2 Hz), 7.63 (1H, dd, J = 7.2, 8.4 Hz), and 7.43 (1H, dd, J = 7.2, 8.4 Hz), also strongly indicated its β-carboline nature.[15,16] In addition, an isolated aromatic proton signal at δ 6.11 (1H, s) and a methoxyl group at δ 4.08 (3H, s) were observed. On the basis of the analyses of NOESY, HMQC, $^1H-^{13}C$ HMBC, and $^1H-^{15}N$ HMBC spectra (Table 1), the proton at δ 6.11 (1H, s) and methoxyl were linked to C-6 and C-5, respectively, and the carbonyl was assigned at C-4. Besides the ^1H NMR evidence, the key EIMS fragment showed at *m*/*z* 192 ($C_{13}H_8N_2$), produced by the sequential loss of carbonyl and methoxyl groups from the molecular ion. In comparison with the spectroscopic data of toindolo[3,2,1-*de*][1,5]naphthyridin-4-ones,[15,16] the skeleton of **1** was confirmed. The structure was established as shown in Figure 1 and named drymaritin.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data[a] of **1** in $CDCl_3$

	δ_H (mult., J in Hz)	δ_C (mult.)	HMBC (δ_H)	$^1H-^{15}N$ HMBC (δ_H)[b]
1	8.75 (d, J = 4.8 Hz)	144.9 (d)	7.90	
2	7.90 (d, J = 4.8 Hz)	116.8 (d)	8.75	
3				7.90, 8.75
4		160.8 (s)		
5		163.9 (s)	6.11	
6	6.11 (s)	101.8 (d)		
7				6.11, 8.53, 7.63
8	8.53 (d, J = 8.4 Hz)	116.9 (d)	7.43, 7.63	
9	7.63 (dd, J = 8.4, 2.0 Hz)	130.9 (d)	7.43, 7.99	
10	7.43 (dd, J = 7.2, 2.0 Hz)	125.0 (d)	8.53	
11	7.99 (d, J = 7.2 Hz)	122.5 (d)	7.63	
12		124.2 (s)	7.43, 7.90, 8.53	
13		139.2 (s)	6.11, 7.63, 7.99, 8.53	
14		130.5 (s)	8.75	
15		131.8 (s)	6.11, 7.90	
16		131.9 (s)		
OCH_3	4.08 (s)	56.8 (q)		

[a] All assignments were confirmed by DEPT, HMQC, and HMBC. [b] The $^1H-^{15}N$ NMR were measured on a Bruker AMX-600 NMR.

In previous studies, the canthin-6-one alkaloids exhibited diverse, for example, anti-HIV,[17] cytotoxic,[17] and antifungal[18] bioactivities. For instance, 1-methoxycanthinone (**3**), a canthin-6-one, exhibited excellent activity with an im-

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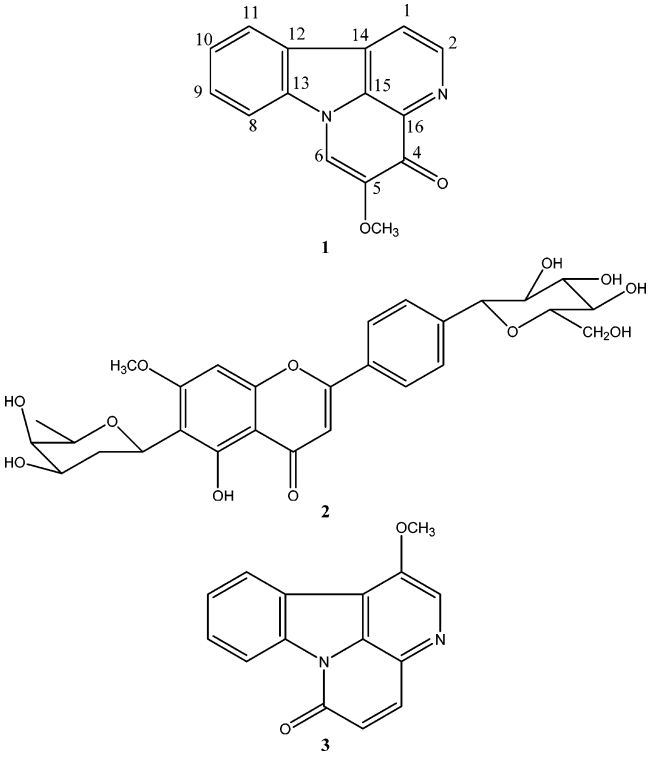

Figure 1. Structures of compounds **1**−**3**.

pressive TI value of >391 in an anti-HIV assay.[17] However, the canthin-4-one alkaloids, isomers of canthin-6-ones, had been isolated from *Pleiocarpa tubicina*[15] without bioactivity reported and synthesized from β-carboline-1-carboxylate.[16] Compound **1**, a canthin-4-one and isomer of **3**, showed anti-HIV activity with EC_{50} and TI values of 0.699 μg/mL and 20.6, respectively. This is the first report of bioactivity among the canthin-4-one series of compounds. The positive results of **1** and **3** indicate the potential for further studies on these two series of compounds.

Compound **2** was obtained in the form of a yellow amorphous solid. The magnesium ribbon test was positive. The molecular formula, $C_{28}H_{32}O_{13}$, was obtained on the basis of the $[M + H]^+$ ion at *m*/*z* 577.1918 (calcd 577.1843) in HRFABMS. The IR (KBr) absorptions displayed a hydroxyl at 3373 cm^{-1}, and UV absorption appeared at 273 and 326 nm, which suggested that **2** was a flavone. The ^{1}H NMR spectrum showed an A_2B_2 spin system at δ 7.86 (2H, d, J = 8.4 Hz) and 7.45 (2H, d, J = 8.4 Hz), together with two aromatic signals at δ 6.91 (1H, s) and 6.74 (1H, s). Furthermore, the ^{1}H NMR signal at δ 14.10 (1H, s), along with the UV spectroscopic data [bathochromic effect when adding $AlCl_3$ (λ_1 = +16 nm) and no bathochromic effect when adding NaOAc], indicated the presence of 5-hydroxyl and the absence of hydroxyl substitution at C-7. ^{13}C NMR signals of a flavone skeleton, one methoxy and two sugar moieties, one β-D-glucosyl, and one 2,6-dideoxy-hexosyl were observed (Table 2). In combining analyses by HMBC, HMQC, and NOESY experiments, the β-D-glucosyl, β-D-oliopyranosyl, and methoxyl groups were attached to C-4′, C-6, and C-7, respectively. Thus, the structure was established as **2** and named diandraflavone.

The known compounds were identified by comparison of their physical and spectroscopic data with those in the relevant literature.[7−14] Additionally, these compounds were isolated for the first time from this plant, and all of them were screened for inhibition of superoxide anion generation from human neutrophils.

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of **2** in C_5D_5N[a]

	δ_H (mult., J in Hz)	δ_C (mult.)	HMBC (δ_H)
2		163.8 (s)	6.91
3	6.91 (s)	104.9 (d)	
4		182.9 (s)	6.91
4a		104.9 (s)	6.74, 6.91
5		161.3 (s)	5.50
6		112.6 (s)	5.50, 3.39, 6.74
7		164.2 (s)	3.83, 5.50, 6.74
8	6.74 (s)	91.0 (d)	
8a		157.4 (s)	6.74
1′		124.7 (s)	6.91, 7.45
2′, 6′	7.86 (d, 8.8)	128.5 (d)	7.45
3′, 5′	7.45 (d, 8.8)	117.2 (d)	7.86
4′		161.3 (s)	7.45, 7.86, 5.77
OH	14.1 (br s)		
OCH_3	3.83 (s)	56.3 (q)	
G-1	5.50 (dd, 10.8, 2.4)	69.8 (d)	3.39
G-2	2.13 (ddd, 12.0, 3.2, 2.4)	32.5 (t)	
	3.39 (ddd, 12.0, 10.8, 3.2)		
G-3	4.21 (m)	71.1 (d)	2.13, 3.39
G-4	4.31 (m)	71.3 (d)	2.13, 3.39, 4.21
G-5	3.78 (q, 6.0)	75.5 (d)	1.50, 4.31
G-6	1.50 (d, 6.0)	18.1 (q)	3.78
G′-1	5.77 (d, 6.8)	101.6 (d)	
G′-2	4.36 (m)	74.8 (d)	
G′-3	4.33 (m)	78.4 (d)	
G′-4	3.98 (br s)	72.1 (d)	
G′-5	4.23 (m)	79.2 (d)	
G′-6	4.33 (m)	62.4 (d)	
	4.63 (d, 11.2)		

[a] All assignments were confirmed by DEPT, HMQC, and HMBC.

Neutrophils are important in a host's defenses against invasion by microorganisms and are extensively involved in inflammatory processes. In response to diverse stimuli, activated neutrophils exhibit adhesion chemotaxis, degranulation, and superoxide anion production.[19] In a screening test for superoxide anion generation from human neutrophils, compound **2** significantly inhibited the fMLP-induced superoxide anion generation from human neutrophils, but not the PMA-activated ones. The results suggested that **2** may inhibit signaling upstream of protein kinase C.[20]

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were obtained on a Hitachi 200-20 spectrophotometer. IR spectra were measured on a Mattson Genesis II spectrophotometer. ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMBC, HMQC, and NOESY spectra were obtained on Varian NMR (Unity Plus 400) and Bruker AMX-600 NMR spectrometer. Low-resolution EIMS were collected on a Bruker APEX II mass spectrometer or Quattro GC/MS spectrometer having a direct inlet system. High-resolution EIMS and FABMS were collected on a JEOL JMS SX/SX 102A mass spectrometer or Quattro GC/MS spectrometer. Silica gel 60 (Merck, 230−400 mesh) was used for column chromatography. Shimadzu LC-10AT pumps, a SPD-10A UV−vis detector, Hypersil ODS 5 μm (250 × 4.6 mm i.d.), and preparative ODS 5 μm (250 × 21.2 mm i.d.) columns were employed for the HPLC. The TLC spots were detected by spraying with 50% H_2SO_4 and then heating TLC on the hot plate.

Plant Material. Whole plants of *D. diandra* were collected from Ping-Tung Hsien, Taiwan, in June 2000 and identified by botanist Dr. Hsin-Fu Yen. A voucher specimen (TNM-S0773305) was deposited at the National Museum of Natural Science, Taichung, Taiwan.

Extraction and Isolation. The air-dried whole plants (20 kg) of *D. diandra* were extracted and partitioned as previously described.[6] The residue of aqueous extracts (300 g) was separated on a Diaion HP-20 (1.2 kg) column with gradient systems of MeOH/H_2O (0%, 25%, 50%, 75%, and 100%, each 2000 mL) to obtain five fractions (WA1−5). Fraction WA3 (50% MeOH, 3 g) was processed using HPLC eluting with 45% MeOH/H_2O to give diandraflavone (**2**) (5.3 mg). Fraction WA2 (70% MeOH, 2 g) was further separated using preparative reverse-phase HPLC eluting with 50% MeOH/H_2O to give torosaflavone A (32.5 mg) and isovitexin (3.6 mg).

The residue of $CHCl_3$ extracts (125 g) was separated by column chromatography on silica gel with gradient systems of $CHCl_3$/MeOH to give 10 fractions (A−J). Fraction F (12 g) was processed using silica gel column chromatography eluting with a gradient of $CHCl_3$/acetone/MeOH to yield 10 subfractions. The subfraction F-5 was further separated on a silica gel column (eluted with $CHCl_3$/acetone) and purified by an RP-18 (25−40 μm, LiChroprep, Merck) column (eluting with H_2O/MeOH) to give methyl 5-hydroxy-4-oxopentanoate (41.0 mg). The subfraction F-7 was further partitioned with *n*-hexane/MeOH and purified by a silica gel column (eluting with *n*-hexane/EtOAc) to afford glycerol-α-lignocerate (11.2 mg). Drymaritin (**1**, 214 mg), *p*-hydroxybenzoic aicd (8.3 mg), and *p*-hydroxybenzaldehyde (5.6 mg) were obtained and purified from subfraction F-8 over Sephadex LH-20 (eluted with $CHCl_3$/MeOH, 2:1) and silica gel (eluted with *n*-hexane/EtOAc, 3:1) chromatography. Fraction J (15 g) was further separated using a silica gel column eluting with a gradient of EtOAc/MeOH, to afford 10 subfractions. Spinasterol β-D-glycoside (210 mg) was obtained from subfraction J-6 by recrystallization from MeOH. Fraction I (3.3 g) was separated on Sephadex LH-20 eluting with a gradient of $CHCl_3$/MeOH (1:2) to yield 10 subfractions. Subfraction I-6 was further separated by a silica gel column (eluting with EtOAc/*n*-hexane, 2:1), which afforded compound **7** (11.2 mg).

Drymaritin (1): pale yellow amorphous solid; mp 181−183 °C; $[\alpha]_D^{25}$ 0° (*c* 0.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 366 (3.41), 349 (3.43), 333 (sh, 3.18), 297 (3.41), 288 (3.41), 264 (3.60), 237 (sh, 3.64), 222 (3.75) nm; IR ν_{max} 1673, 1562, 1474, 1441, 1397, 1331, 1252, 1212, 1093, 864, 753 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, Table 1; EIMS *m*/*z* (rel int) 250 ($[M]^+$, 41), 249 (61), 222 (50), 192 (100), 164 (14), 152 (11), 139 (18), 125 (13), 113 (15), 96 (24), 89 (12), 83 (36), 69 (48), 55 (30); HREIMS *m*/*z* 250.0748 $[M]^+$ (calcd for $C_{15}H_{10}N_2O_2$, 250.0742).

Diandraflavone A (2): yellow amorphous solid; $[\alpha]_D^{25}$ +38.5° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.41), 215 (*sh*, 4.32), 273 (4.27), 326 (4.21) nm; IR ν_{max} 3373, 2924, 2854, 1654, 1605, 1508, 1451, 1355, 1299, 1245, 1204, 1186, 1073, 838, 515 cm^{-1}; FABMS *m*/*z* (rel int) 599 ($[M + Na]^+$, 1), 577 ($[M + H]^+$, 1), 413 (1), 391 (2), 338 (2), 329 (3), 307 (5), 289 (5), 176 (41), 154 (85), 136 (83), 107 (55), 89 (69), 77 (95), 69 (86), 56 (100); HRFABMS *m*/*z* 577.1918 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{13}$, 577.1843).

HIV Inhibition Assay. This assay was performed using methods described previously.[21]

Neutrophil Superoxide Anion Formation.[20] Human neutrophils from the venous blood of healthy, adult volunteers (18−32 years old) were isolated with a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes. Neutrophil superoxide anion generation was determined using superoxide dismutase (SOD)-inhibitable cytochrome *c* reduction. In brief, after supplementing with ferricytochrome *c* (0.5 mg/mL), neutrophils (10^6/mL) were equilibrated at 37 °C for 2 min and incubated with either control or different concentrations of tested compounds for 5 min. Cells were activated by formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) for 10 min. When fMLP was used as a stimulant, cytochalasin B (1 μg/mL) (CB) was incubated for 3 min before peptide activation.

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