Three Novel Triterpenoid Dienolides from *Phyllanthus myrtifolius*

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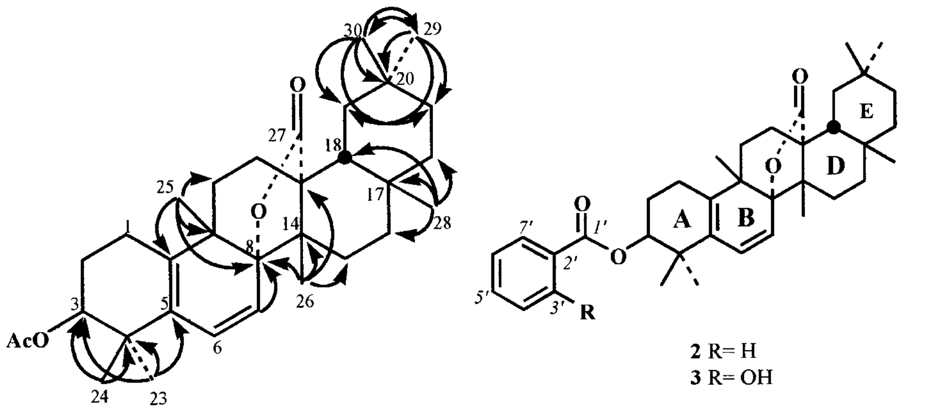
Three novel pentacyclic triterpenoid dienolides, phyllenolide A (= 3β-acetoxyglutina-5(10), 6-dien-27,8α-olide; **1**), phyllenolide B (= 3β-(benzoyloxy)glutina-5(10),6-dien-27,8α-olide; **2**), and phyllenolide C (= 3β-(2-hydroxybenzoyloxy)glutina-5(10),6-dien-27,8α-olide; **3**), were isolated from the aerial parts of *Phyllanthus myrtifolius* Moon. (Euphorbiaceae). These three compounds possess an endocyclic γ-lactone moiety across ring C and a homo-annular diene system in ring B. Their structures were established by analyses of CD, NOED, and 2D-NMR spectra.

1. Introduction. – The plants of the genus *Phyllanthus* (Euphorbiaceae) have been popularly employed for the treatment of kidney and bladder calculi, diabetes, hepatitis, and dysentery, and have also been used against affections of the intestines [1][2]. *Phyllanthus myrtifolius* Moon. (Euphorbiaceae) is a small garden shrub indigenous to India and Srilanka [3]. Our earlier chemical investigation of this plant was focused on the lignans and has led to the isolation of ten new compounds of such a skeleton, *i.e.*, phyllamyricins A–F, retrojusticidin B and phyllamyricosides A–C [4][5]. Of these, retrojusticidin B and phyllamyricin B were found to possess high selectivity toward the HIV-1 reverse transcriptase [6]. Continuing our efforts in systematic phytochemical investigation of this plant, three novel pentacyclic triterpenes **1**–**3** of glutinane type were isolated from its aerial parts. In this paper, we describe the isolation and structure elucidation of these three novel compounds.

2. Results and Discussion. – Compound **1** has a molecular formula $C_{32}H_{46}O_4$, as deduced from its HR-FAB-MS. It contains an AcO group, as exemplified by the NMR data, δ(H) 2.04 (*Me*CO) and δ(C) at 170.8 (Me*C*O) and 21.2 (*Me*CO). Its ^{1}H-NMR spectrum exhibited seven additional Me *singlets*, an *AX* system for two *cis*-coupled olefinic H-atoms (6.35 (*d*) and 5.84 (*d*, *J* = 9.9), and an AcO-attached carbinoyl H-atom (4.74 (*dd*, *J* = 3.3, 11.3, H_α–C(3)). Its ^{13}C-NMR and DEPT spectra indicated the presence of a lactone C-atom (180.8 (*s*)), two quaternary C-atoms (139.5 and 131.1), two tertiary olefinic C-atoms (131.9 and 119.0), and an oxygenated quaternary C-atom (90.2). These data suggest that **1** contains a γ-lactone moiety and a homodiene chromophore, the latter being supported by its UV absorption maximum at 269 nm (calc. 273 nm) [7]. An HMBC spectrum revealed the correlations of two Me *singlets* at 1.02 (Me(23)) and 1.03 (Me(24)) to a quaternary olefinic C-atom at 131.9 (C(5)), the carbinoyl C-atom at 79.0 (C(3)), and a quaternary aliphatic C-atom at 36.48 (C(4)), indicating that the homodiene moiety to be located at ring B. This spectrum also revealed the correlations of an olefinic H-atom at 5.84, and two Me *singlets* at 0.99 and 1.27 to the oxygenated quaternary C-atom at 90.2 (C(8)), suggesting that C(8) to be oxygenated, and both C(9) and C(14) to be methylated. Thus, the γ-lactone is located between C(13) and C(8). The presence of a Me group on C(9) is also supported by the observation of a correlation between C(10) at 139.4 (*s*) to the Me *singlet* at 0.99 (Me–C(9)). These data and other correlations in the HMBC spectrum established the structure of **1** as shown in *Fig. 1*, leaving the configuration to be determined.



1

2 R= H
3 R= OH

Fig. 1. *Key HMBC for* **1**

The relative configuration of **1** was determined by a series of NOED experiments. The key NOEDs include Me–C(9) (H–C(25)) ↔ Me–C(14) (H–C(26)), H–C(18) (1.37) ↔ Me–C(17) (H–C(28)), Me_{β}–C(20) (H–C(30)) ↔ H–C(18), H–C(3) ↔ Me_{α}–C(4) (H–C(23)) ↔ H–C(6), Me_{β}–C(4) (H–C(24)) ↔ H–C(6), Me_{α}–C(20) (H–C(29)) ↔ H_{α}–C(19) (2.47). These data established the *cis*-relationship between Me–C(9) and Me–C(14), and also H–C(18) and Me–C(17). Hence, compound **1** possesses a glutinane skeleton having all these substitutents (Me–C(9), Me–C(14), H–C(18), and Me–C(17)) in β-orientation. Consequently, the γ-lactone should be α-oriented from a chemical model and biogenetics point of view. Pooling these data together established the structure of **1** as 3β-acetoxyglutina-5(10),6-dien-27,8α-olide. Complete ^{1}H- and ^{13}C-NMR assignments for **1** (*Table*) were based on the analysis of NOED and 2D NMR spectra (COSY-45, HMQC, and HMBC (see *Fig. 1*)). For example, the signals of C(16) at δ 35.6 (*t*) and C(22) at 36.5 (*t*), both being three-bond coupled to Me(28) singlet at 1.05, were distinguished by the observation of ^{1}J correlation of δ(C) 36.5 (*t*) to δ(H) 1.56 and 0.89, the latter being, in turn, coupled to the signals of H–C(21) at 1.37 by COSY spectral analysis.

Compound **2** has a molecular formula $C_{37}H_{48}O_4$, as deduced from its HR-FAB-MS, which was consistent with the analysis of ^{1}H- and ^{13}C-NMR spectra (*Table*). In comparison with **1**, the ^{1}H-NMR spectrum of **2** exhibited five additional arom. H-atom signals at 8.02 (br. *d*, 2 H), 7.53 (br. *t*, 1 H), and 7.42 (*dd*, 2 H) with the absence of *Me*CO *singlet* signal, indicating a 3-benzoyloxy group in place of the 3-AcO group in **1**.

Table. *¹H-* (δ/ppm, multiplicity (*J*/Hz)) *and ¹³C-NMR* (δ/ppm (multiplicity[a])) *Data for* **1**–**3**[b]) *in* $CDCl_3$

Position	**1**		**2**	**3**
	¹H	¹³C	¹³C	¹³C
1	2.27, *m* (α); 1.95 *m* (β)	22.5 (*t*)	22.3 (*t*)	22.3 (*t*)
2	1.83, *m* (α); 1.94 *m* (β)	24.2 (*t*)	24.2 (*t*)	24.2 (*t*)
3	4.74, *dd* (11.3, 3.3)	77.3 (*d*)	77.9 (*d*)	77.1 (*d*)
4		36.48 (*s*)	36.9 (*s*)	36.8 (*s*)
5		131.1 (*s*)	131.0 (*s*)	131.0 (*s*)
6	6.35 *d* (9.9)	131.9 (*d*)	131.9 (*d*)	131.9 (*d*)
7	5.84 *d* (9.9)	119.0 (*d*)	119.1 (*d*)	119.0 (*d*)
8		90.2 (*s*)	90.2 (*s*)	90.3 (*s*)
9		42.5 (*s*)	42.5 (*s*)	42.5 (*s*)
10		139.5 (*s*)	139.7 (*s*)	139.7 (*s*)
11	1.63, *m*	28.1 (*t*)	28.3 (*t*)	28.2 (*t*)
12	1.58, *m*	23.7 (*t*)	23.8 (*t*)	23.7 (*t*)
13		51.1 (*s*)	51.2 (*s*)	51.2 (*s*)
14		45.8 (*s*)	45.9 (*s*)	45.8 (*s*)
15	2.19, *m* (α); 1.53, *m* (β)	31.0 (*t*)	31.0 (*t*)	31.0 (*t*)
16	1.14, *dd* (13.4, 4.5) (α); 1.52 *m* (β)	35.6 (*t*)	35.6 (*t*)	45.6 (*t*)
17		30.8 (*s*)	30.8 (s)	30.8 (*s*)
18	1.39 *dd* (13.5, 4.4)	39.7 (*d*)	39.7 (*d*)	39.6 (*d*)
19	2.47 *t* (13.5) (α); 1.14 *dd* (13.5, 4.4) (β)	34.2 (*t*)	34.2 (*t*)	34.2 (*t*)
20		28.8 (*s*)	28.8 (*s*)	28.7 (*s*)
21	1.37, *m*	32.2 (*t*)	32.2 (*t*)	32.2 (*t*)
22	0.89 *m*, 1.56 *m*	36.5 (*t*)	36.5 (*t*)	36.5 (*t*)
23	1.02 *s*	25.3 (*q*)	25.7 (*q*)	25.7 (*q*)
24	1.03 *s*	21.7 (*q*)	22.1 (*q*)	22.2 (*q*)
25	0.99 *s*	19.1 (*q*)	19.2 (*q*)	19.2 (*q*)
26	1.27 *s*	20.3 (*q*)	20.3 (*q*)	20.3 (*q*)
27		180.8 (*s*)	180.8 (*s*)	180.8 (*s*)
28	1.05 *s*	27.9 (*q*)	27.9 (*q*)	27.9 (*q*)
29	0.98 *s*	34.8 (*q*)	34.8 (*q*)	34.8 (*q*)
30	0.96 *s*	30.2 (*q*)	30.2 (*q*)	30.1 (*q*)
Me*C*O		170.8 (*s*)		
*Me*CO	2.04 *s*	21.2 (*q*)		

[a]) Multiplicity was obtained from DEPT experiments. [b]) The signals for aryl groups in **2** and **3**, and those distinct signals from **1**: **2**: ¹H-NMR: 8.01 (*dd*, *J* = 7.8, 1.2, H–C(3′), H–C(7′)); 7.42 (*dd*, *J* = 7.8, 7.5, H–(4′), H–C(6′)); 7.54 (br. *t*, *J* = 7.5, H–C(5′)); 5.00 (*dd*, *J* = 10.9, 3.6, H–C(3)); 1.10 (*s*, Me(23)); 1.18 (*s*, Me(24)). ¹³C-NMR: δ 166.1 (*s*, C(1′)); 110.6 (*s*, C(2′)); 129.5 (*d*, C(3′), C(7′)); 128.3 (*d*, C(4′), C(6′)); 132.8 (*d*, C(5′)); 77.9 (*d*, C(3)); 36.9 (*s*, C(4)); 25.7 (*q*, C(23)); 22.1 (*q*, C(24)). **3**: ¹H-NMR: 7.81 (br. *d*, *J* = 8.0, H–C(7′)); 7.24 (*dt*, *J* = 1.6, 8.0, H–C(5′)); 6.63 (br. *d*, *J* = 8.0, H–C(4′)); 6.60 (br. *t*, *J* = 8.0, H–C(6′)); 5.73 (*s*, OH); 4.95 (*dd*, *J* = 10.7, 3.1, H–C(3)); 1.09 (*s*, Me(23)); 1.17 (*s*, Me(24)). ¹³C-NMR: 167.6 (*s*, C(1′)); 111.2 (*s*, C(2′)); 150.6 (*s*, C(3′)); 116.1 (*d*, C(4′)); 133.9 (*d*, C(5′)); 116.7 (*d*, C(6′)); 130.9 (*d*, C(7′)); 77.1 (*d*, C(3)); 36.8 (*s*, C(4)); 25.7 (*q*, C(23)); 22.2 (*q*, C(24)).

This finding was also supported by its ¹³C-NMR spectrum (*Table*), which showed the benzoyl signals at δ(C) 166.1 (*s*), 132.8 (*d*), 129.5 (*d*, 2 C), 128.3 (*d*, 2 C), and 110.6 (*s*). Other than these differences, the remaining signals in its ¹³C-NMR spectrum are almost superimposable to the corresponding signals in **1**. Thus, **2** is established as 3-benzoyl derivative of **1** and has structure as shown in the *Fig. 1*. Since the ring-current effect has great influence on the chemical shifts of the adjacent H-atoms, the signals for H–C(1), H–C(23), and H–C(24) were assigned based on the HMQC spectral analysis.

Compound **3** has a molecular formula $C_{37}H_{48}O_5$, as deduced from HR-FAB-MS. It contains a phenolic function, as evidenced by the observation of a bathochromic shift under alkaline conditions during UV measurement. Instead of an AcO group as in **1**, **3** has a 2-hydroxybenzoyloxy group, as evidenced by the characteristic ^{1}H-NMR signals for this moiety, two broad *doublets* for H–C(4′) (6.63) and H–C(7′) (7.81), one broad *triplet* for H–C(6′) (6.60), and one double *triplet* for H–C(5′) (7.24). The ^{13}C-NMR data (*Table*) also confirmed the existence of this moiety, the chemical-shift assignment of which was achieved by correlation with the reported data [8]. Other than these differences, the remaining data are almost superimposable on those of **1**. Thus, **3** is established as 3-(2-hydroxybenzoyl) derivative of **1** and has a structure as shown in *Fig. 1*.

The chemical models of **1**–**3** conformed to the structure of the glutinane skeleton that accommodates a homo-annular diene in ring B as depicted in *Fig. 2*. The chirality as shown will give rise to a negative *Cotton* effect [9], consistent with that observed in their CD curves, all exhibiting large negative *Cotton* effects having maximum at the same wavelength as UV absorption (*ca.* 268.5 nm). Thus, the absolute configuration of **1**–**3** was determined as depicted in the structures. The exciton coupling centered around 229 nm in **2** is not prominent, indicating the near co-planar relationship between benzyoyloxy and diene chromophores. The conformations of these com-

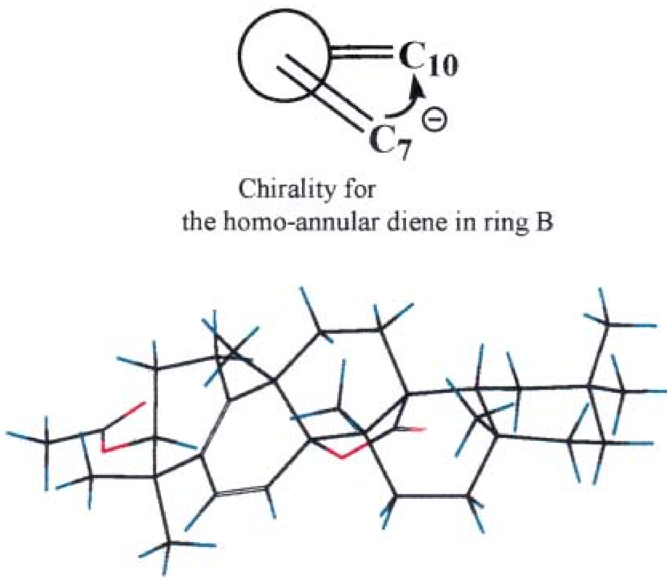


Fig. 2. *Molecular modeling for* **1**–**3** *performed by the simulated annealing module of Sybyl with generic Tripos force fields* (*TRIPOS, Inc.*). The energy-minimized conformation was obtained after adjusting for the NOED data. The distances [Å] for the spatially adjacent H-atom pairs are as follows: H(3)–H(23): 2.43, H(6)–H(23): 2.35, H(6)–H(24): 2.38, H(25)–H(26): 2.31–2.42, H(26)–H(28): 2.28–2.37, H(18)–H(28): 2.35, and H(18)–H(30): 2.33.

pounds, *i.e.*, twisted-chair forms for rings A and C, twisted-boat form for ring D, and chair form for ring E, were determined by the NOED experiments as described earlier. Based on these data, a computer-assisted molecular modeling was performed to obtain an energy-minimized conformer as shown in *Fig. 2*. The torsion angle, $\measuredangle$ C(7)−C(6)/C(5)−C(10), determined from this conformer is −20.3°, confirming the chirality indicated above.

To the best of our knowledge, compounds **1**–**3** are the first triterpenes of the glutinane type containing a 27(8)-γ-lactone moiety, as well as a homo-annular diene chromophore. The other related glutinanes contain either a monoene (Δ^5) or a 5,6-epoxy at ring B, such as 5,6-epoxy-3-hydroxy-29-glutinanoic acid isolated from *Tripterygium wilfordii* [10]. These three compounds were named as phyllenolides A–C (**1**–**3**, resp.), after their plant origin.

Experimental Part

General. M.p.: *Fischer-Johns* melting-point apparatus (uncorrected). Optical rotations: *JASCO DIP-370* digital polarimeter. UV Spectra: *Hitachi U-2000* UV spectrophotometer. CD Spectra: *JASCO J-710* spectropolarimeter. IR Spectra: *JASCO FT/IR-410* spectrophotometer. NMR Spectra: *Bruker AMX-400* and *DPX 200* spectrometer; Me_4Si as reference standard; 2D-NMR spectra were recorded by using *Bruker*'s standard pulse program. MS: *JEOL JMX-HX 100* mass spectrometer.

Plant Material. The aerial parts of *P. myrtifolius* were harvested in June 2000, from the hedge of College of Medicine, National Taiwan University. A voucher specimen was deposited in the herbarium of School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The dried twigs and stems (64 kg) were powdered and macerated with MeOH (200 l × 5) at r.t. The MeOH extract (1.64 kg) was then triturated with $CHCl_3$ (2 l × 3). The residue was partitioned between $CHCl_3$ and H_2O (each 1 l). The $CHCl_3$-soluble and $CHCl_3$ layer were combined and condensed to give 169 g of $CHCl_3$ extract. The aq. layer was further partitioned against BuOH saturated with H_2O, to give fractions soluble in BuOH (778 g) and H_2O (560 g). The $CHCl_3$ extract was further fractionated into hexane- (52 g) and MeCN- (49 g) soluble parts. The MeCN-soluble part (44 g) was chromatographed on a silica gel (70–230 mesh, 450 g) column (hexane/AcOEt 9:1, 8:2, 1:1) to give 42 fractions. *Fr. 11–14* (98 mg) from 10% AcOEt elution were purified on a silica gel (230–400 mesh, 1 g) column (10% AcOEtc in hexane) to give compound **2** (6.8 mg). *Fr. 17–18* (1.2 g) from 20% AcOEt elution were rechromatographed on a *Sephadex LH-20* (120 g) column ($CHCl_3$/MeOH 1:19) to give 11 fractions. *Fr. 4–7* (43.2 mg) were further purified on a silica gel (230–400 mesh, 500 mg) column (20% AcOEt in hexane) to afford compounds **1** (11.9 mg) and **3** (8.4 mg).

3β-Acetoxyglutina-5(10),6-dien-27,8α-olide (= *Phyllenolide A*; **1**). Colorless solid. M.p. 275–276° (dec.). $[\alpha]^{23}_D = -110.0$ ($CHCl_3$, $c = 0.1$). UV (MeOH): 269 (3.87). CD (MeOH): 306 (0), 268.5 (−14.14), 234.2 (0), 228.6 (+0.83), 215.5 (−0.89). IR (KBr): 2948, 1753, 1728, 1252, 1178, 1026, 989, 903. FAB-MS: 495 (41.5, $[M+H]^+$), 435 (21.8), 434 (13.8), 375 (3.8), 307 (20.7), 289 (18.5), 243 (9.9), 154 (100.0), 136 (86.5), 77 (48.5), 43 (30.4), 39 (29.6). HR-FAB-MS: 495.3468 (100, $[M+H]^+$, $C_{32}H_{47}O_4^+$; calc. 495.3474), 435.3222 (67.9, $[M - MeCOOH + H]^+$, $C_{30}H_{43}O_2^+$; calc. 435.3263), 434.3167 (1.4, $[M - MeCOOH]^+$, $C_{30}H_{42}O_2^+$; calc. 434.3184), 419.2894 (29.3, $[M - MeCOOH - Me]^+$, $C_{29}H_{39}O_2^+$; calc. 419.2950).

3β-(Benzoyloxy)glutina-5(10),6-dien-27,8α-olide (= *Phyllenolide B*; **2**). Colorless solid. M.p. 284–285° (dec.). $[\alpha]^{23}_D = -40.0$ ($CHCl_3$, $c = 0.2$). UV (MeOH): 228.5 (4.1), 268.5 (3.8). CD (MeOH): 366 (0), 359.3 (+0.25), 310 (0), 269.6 (−5.31), 228.4 (−0.15), 222.4 (−0.51). FAB-MS: (100, $[M+H]^+$), 511 (13.5), 435 (56.9), 434 (32.5), 389 (16.5), 333 (30.3), 281 (9.5), 221 (13.0), 207 (7.3), 128 (7.0), 105 (47.9), 41 (32.6), 39 (12.3). IR (KBr): 2949, 2921, 2866, 1747, 1716, 1588, 1467, 1276, 1178, 1026, 989, 903. HR-FAB-MS: 557.3629 (100, $[M+H]^+$, $C_{37}H_{49}O_4^+$; calc. 557.3631), 511.3484 (14.6, $[M - COO - H]^+$, $C_{36}H_{47}O_2^+$; calc. 511.3586), 435.3291 (97.8, $[M - C_7H_6O_2 + H^+]$, $C_{30}H_{43}O_2^+$; calc. 435.3263), 434.3213 (83.6, $[M - C_7H_6O_2]^+$, $C_{30}H_{42}O_2^+$; calc. 434.3184), 419.2997 (49.2, $[M - C_7H_6O_2 - Me]^+$, $C_{29}H_{39}O_2^+$; calc. 419.2950).

3β-[(2-Hydroxybenzoyl)oxy]glutina-5(10),6-dien-27,8α-olide (= *Phyllenolide C*; **3**). Colorless solid. M.p. 282–283° (dec.). $[\alpha]^{23}_D = -25.0$ ($CHCl_3$, $c = 0.1$). UV (MeOH): 218.5 (4.49), 253.5 (4.22), 271.5 (4.17), 338.5 (3.76); UV (MeOH + KOH): 226 (5.14), 333.5 (3.55). CD (MeOH): 368 (−0.13), 361.3 (+0.10), 356 (0), 351

(+0.15), 345 (0), 335 (−0.16), 330.3 (−0.05), 325.1 (−0.15), 318.3 (−0.05), 311.1 (−0.24), 268.3 (−5.84), 237.1 (0), 233.1 (+0.15), 229.6 (0), 219.7 (−1.93). IR (KBr): 3502, 3378, 1747, 1686, 1617, 1561, 1459, 1294, 1245, 1107, 902. FAB-MS: 572 (37.7, M^+), 435 (12.1), 375 (3.5), 211 (7.5), 154 (11.7), 120 (100.0), 41 (35.4), 39 (17.4). HR-FAB-MS: 573.3551 (18.9, $[M+H]^+$, $C_{37}H_{49}O_5^+$; calc. 573.3580), 572.3519 (45.6, M^+, $C_{37}H_{48}O_5^+$; calc. 572.3501), 435.3172 (100, $[M-C_7H_6O_3+H]^+$, $C_{30}H_{43}O_2^+$; calc. 435.3263), 434.3133 (83.6, $[M-C_7H_6O_3]^+$, $C_{30}H_{42}O_2^+$; calc. 434.3184), 419.2883 (56.1, $[M-C_7H_6O_3-Me]^+$, $C_{29}H_{39}O_2^+$; calc. 419.2950), 389.3140 (24.6, $[M-C_7H_6O_3-COO-H]^+$, $C_{29}H_{41}^+$; calc. 389.3208).

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