Antimalarial and Antituberculous Poly-*O*-acylated Jatrophane Diterpenoids from *Pedilanthus tithymaloides*†

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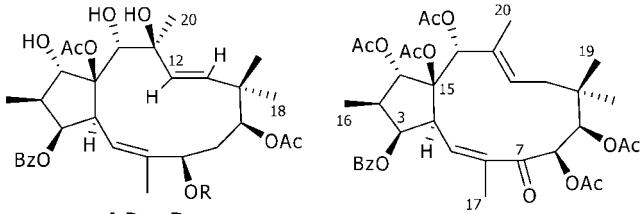
Received April 16, 2007

Six new poly-*O*-acylated jatrophane diterpenes (**1**–**6**) have been isolated along with five known compounds from the white latex of *Pedilanthus tithymaloides*. The structural identification was accomplished on the basis of 2D NMR and MS investigations. Some of these highly oxygenated jatrophane diterpenes possess a rare *O*-acetyl enol moiety. Compounds **1** and **3**–**5** showed antiplasmodial activity with IC_{50} values of 3.4–4.4 μg/mL and antimycobacterial activity against *Mycobacterium tuberculosis* with minimum inhibition concentration (MIC) values ranging from 12.5 to 100 μg/mL.

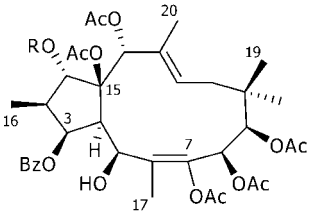
In our ongoing search for biologically active compounds from plants in the family Euphorbiaceae,[1] we have studied *Pedilanthus tithymaloides*, which is an ornamental plant, known in Thailand as "sa yaek" or "sa yaek sam si".[2] The plant has been reported to possess febrifuge, anticancer, and anti-inflammatory properties.[3] The stem and stem latex were used to treat warts, chloasma, scorpion stings and centipede bites,[4] but may cause stomach upset if accidentally chewed.[4]

Previous investigations on the chemical constituents of *P. tithymaloides* have led to the isolation of long-chain alcohol,[5] sterol,[5] triterpenes,[5] a carotene derivative, azafrin,[6] and a cancer growth inhibitor phorbol ester derivative designated pedilstatin.[7] The present work reports the isolation and structural elucidation of the isolates including **1**–**6** and their biological activities.

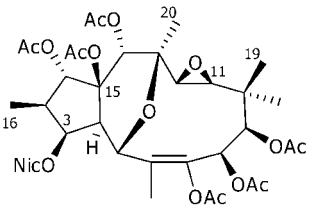


1 R = Bz
2 R = H

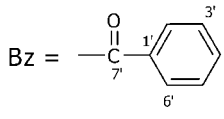
3

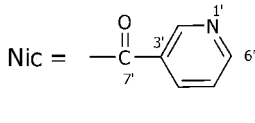


4 R = H
5 R = Ac



6





Results and Discussion

Column chromatographic separation of the CH_2Cl_2 extract of the latex of *Pedilanthus tithymaloides*, which exhibited inhibitory activity against *Mycobacterium tuberculosis* H37 Ra, with a minimum inhibition concentration (MIC) value of 50 μg/mL, resulted in the isolation of six new highly oxygenated diterpenes (**1**–**6**), in addition to five known compounds, which were identified as β-amyrin,[8] cycloartenol,[9] lupeol,[10] β-sitosterol,[11] and tirucalla-7,24-dien-3β-ol,[12] through comparison with reported physical and spectroscopic data.

Compound **1** was obtained as a colorless solid, mp 82–84 °C. The HRESIMS gave $[M-H]^-$ ion at *m/z* 677.2948, corresponding to a molecular formula of $C_{38}H_{46}O_{11}$. The FTIR absorption bands at ν_{max} 3503 and 1718 cm^{-1} indicated hydroxyl and ester functions, respectively. The presence of four carbonyl carbon signals at δ_C 173.3, 170.1, 165.1, and 164.9 indicated four ester groups, among which two acetyl groups [δ_H 1.66 (s), δ_C 20.8 (q), 170.1 (s) and δ_H 2.404 (s), δ_C 22.1 (q), 173.5 (s)] and two benzoyl groups (δ_H 7.64, 7.24, 7.06, and 7.53, 7.24, 6.97) could be assigned. The jatrophane diterpene nucleus was established from the presence of five methyl groups, among which one was a secondary methyl and two were tertiary methyls bonding to the same carbon (CH_3–C–CH_3), as detected from the mutual $^3J_{H,C}$ correlations of H_3-18/C-19 and H_3-19/C-18.[13] In addition to the ^1H–^1H COSY correlations of H-1/H-2, H-2/H-3 and H_3-16, H-3/H-4, the key long-range ^1H–^{13}C correlations between H-1/oxygenated C-15 (s), C-16 and H-4/C-15 helped disclose a methyl-substituted cyclopentyl moiety of the jatrophane nucleus.[13] The placement of double bonds at C-5(6) and C-11(12) in **1** was based on the long-range $^3J_{H,C}$ correlations of H-5/C-6, C-15, C-17 and H-12/C-10, C-20, respectively. The *E* C-11/C-12 double bond was deduced from the large vicinal coupling constants of signals at δ_H 5.49 (H-11, d, J = 15.5 Hz) and 5.28 (H-12, d, J = 15.5 Hz). Attachments of benzoyloxy groups at C-3 and C-7, and of an acetoxy group at C-9, were deduced from the HMBC correlations of H-3, H-2′ and H-6′/O*CO*-3, of H-7, H-2″, and H-6″/O*CO*-7, and of *CH*$_3$COO-9, H-9/O*CO*-9, respectively. The *O*-acetylated C-15 carbon signal was detected at a less shielded position (δ_C 91.3) than that found for a hydroxylated C-13 (δ_C 74.6).[13] The shielded acetyl methyl signal at δ_H 1.66, assigned for *CH*$_3$CO-9, was proposed to be due to a shielding effect caused by the *O*-benzoyloxy group at C-7. Two doublet signals at δ_H 4.34 (J = 3.2 Hz) and 4.42 (J = 4.2 Hz), as well as a singlet signal at δ_H 2.71 that showed no HMQC correlation with any carbon and also disappeared after D_2O exchange, indicated three hydroxyl groups. The connectivities of three hydroxyl groups at C-1, C-13, and C-14 were also detected from 2D NMR experiments. The ^1H and ^{13}C NMR chemical shifts were assigned for **1** as shown in Table 1. The relative stereochemistry of **1** was obtained from the NOESY spectrum indicating NOE effects as

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† This work is dedicated to Professor Apichart Suksamrarn in honor of his 60th birthday.

10.1021/np070174v CCC: $37.00
Published on Web 09/11/2007

Table 1. ^{1}H NMR Spectroscopic Data of **1–6** [400 MHz, $CDCl_3$, δ_H (ppm), and mult (*J* in Hz)[a]]

H	**1**[b]	**2**	**3**	**4**	**5**	**6**
1	4.19 dd (11.8, 3.2)[c]	4.19 dd (11.9, 3.2)	5.41 d (11.2)	4.09 d (11.6)	5.43 d (11.6)[k]	5.63 d (11.0)[l]
2	2.26 m	2.29 m	2.23 m	2.41 m	2.44 m	2.57 obs dq (11.0, 7.2)
3	5.42 dd (4.7, 4.7)	5.39 dd (4.8, 4.8)	5.68 dd (3.6, 3.6)	5.44 dd (4.5, 4.5)	5.45 dd (4.4, 4.4)[k]	5.63 obs d (6.8)
4	4.08 dd (9.7, 4.7)	4.08 dd (9.8, 4.8)	3.58 dd (5.9, 3.6)	4.25 dd (11.1, 4.5)	4.30 dd (11.2, 4.4)	3.79 dd (12.4, 6.8)
5	5.76 d (9.7)	5.63 d (9.8)	6.75 dd (5.9, 1.1)	4.49 d (11.1)	4.54 d (11.2)	4.75 d (12.4)
6						
7	5.25 m[f–m]	4.05 obs d (5.8)				
8	2.03 m	1.95 m, 1.77 m	5.39 obs d (1.6)	6.53 s	6.52 s	6.26 d (4.4)
9	5.13 dd (3.2, 3.2)	4.71 dd (3.3, 3.3)	5.48 d (1.6)	5.23 s	5.22 s	5.16 d (4.4)
10						
11	5.49 d (15.5)	5.40 d (15.4)	2.37 dd (15.4, 11.1) 1.99	2.35 dd (16.4, 9.2) 1.98 m	2.36 dd (16.2, 9.2), 1.94 m	2.88 br s
12	5.28 d (15.5)[f–m]	5.17 d (15.4)	5.39 d (2.4)	5.93 br d (9.2)	5.94 d (9.2)	3.56 d (2.2)
13						
14	4.30 d (4.2)[d]	4.25 d (4.0)	6.36 br s	5.84 s	6.06 s	5.64 s[l]
15						
16	0.93 obs d (7.0)[g]	1.01 d (6.6)	1.02 d (6.7)	0.97 d (5.7)	0.88 d (6.8)	0.99 d (7.2)
17	1.81 s	1.66 s	1.73 s	1.29 s	1.29 s	1.42 s
18	0.93 s[g]	0.94 s[h]	1.02 s[i]	0.87 s	0.97 s	1.02 s
19	0.95 s	0.94 s[f]	0.97 s[i]	0.97 s	0.86 s	0.77 s
20	1.31 s	1.28 s	1.68 s	1.63 s	1.62 br s	1.04 s
OAc-1			2.05 s		2.08 s	2.11 s[m]
OAc-7				2.21 s	2.17 s	2.11 s[m]
OAc-8			2.00 s[j]	2.00 s	2.00 s	2.08 s
OAc-9	1.66 s	2.03 s	2.02 s[j]	2.11 s	2.14 s	2.22 s
OAc-14			2.18 s	2.15 s	2.11 s	2.15 s
OAc-15	2.40 s	2.27 s	2.27 s	2.07 s	2.07 s	2.11 s[m]
OBz-3						ONic-3 2′, 9.16 br s
2′, 6′	7.64 dd (7.5, 1.0)	7.89 dd (7.1, 1.3)	8.01 dd (7.0, 1.4)	7.89 dd (7.6, 1.0)	7.94 dd (7.1, 1.2)	4′, 8.33 br d (7.6)
3′, 5′	7.06 dd (7.5, 7.3)	7.41 dd (7.8, 7.1)	7.42 dd d (7.5, 7.0, 1.4)	7.41 dd (7.6, 7.6)	7.42 dd (7.6, 7.1)	5′, 7.49 dd (7.6, 4.1)
4′	7.24 m	7.52 dd (7.8, 7.6)	7.54 ddd (7.5, 7.4, 1.4)	7.55 dd (7.6, 7.6)	7.56 ddd (7.6, 7.1, 1.2)	6′, 8.79 d (4.1)
OH-1	4.34 d (3.2)[e]	4.35 d (3.2)				
OH-13	2.71 s[e]	2.69 s				
OH-14	4.42 d (4.2)[e]	4.41 d (4.0)				

[a] Coupling constants were averaged and were obtained from an expanded ^{1}H NMR spectra. [b] OBz-7 of **1**, δ_H 7.53 (dd, J = 7.1, 1.0 Hz, H-2″, 6″), 6.97 (dd, J = 7.7, 7.6 Hz, H-3″, 5″), 7.24 (m, H-4″). [c] Signal became doublet with J = 11.8 Hz after D_2O exchange. [d] Signal became singlet after D_2O exchange. [e] Signal disappeared after D_2O exchange. [f–m] Overlapping signals.

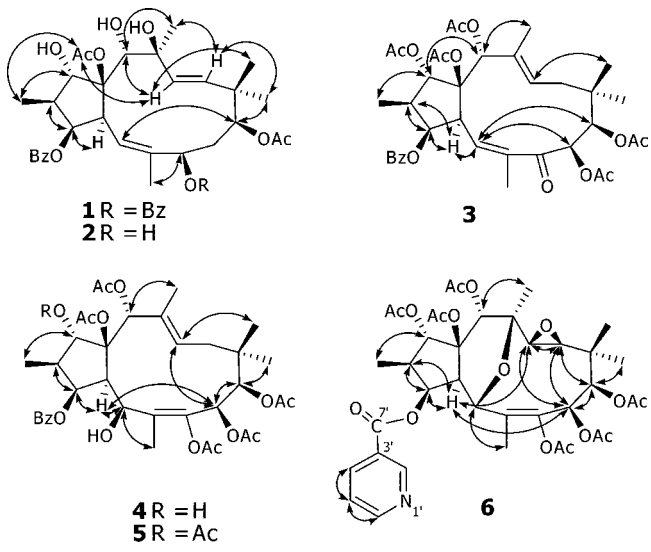

Figure 1. Selected NOE effects in **1–6**.

given in Figure 1. Compound **1** was concluded to be 1α,13*β*,14α-trihydroxy-3*β*,7*β*-dibenzoyloxy-9*β*,15*β*-diacetoxyjatropha-5,11 *E*-diene.

Compound **2** was isolated as a colorless solid, mp 98–100 °C, with the molecular formula of $C_{31}H_{42}O_{10}$ being obtained from HRESIMS. The ^{1}H and ^{13}C NMR spectra resembled those of **1**, except for the presence of only one *O*-benzoyloxy group as observed from the ^{1}H NMR signals at δ_H 7.89, 7.52, and 7.41. The ^{1}H–^{1}H COSY spectrum revealed a correlation between H-2 (δ_H 2.29)/H-3 (δ_H 5.39, dd, 4.8, 4.8 Hz), as well as the $^3J_{H,C}$ correlations of H-3, H-2′ and H-6′/3-O*CO* (δ_C 166.1), and supported the placement of a OBz group to C-3 as deduced also for **1**. A carbinolic proton signal at δ_H 4.05 could thus be assigned for H-7. The absence of an *O*-benzoyloxy at C-7 also caused, as a consequence, a downfield shift of the *CH*$_3$CO-9 proton signal relative to that resonating for **1** at δ_H 2.03. The use of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra led to the full assignment of the ^{1}H and ^{13}C NMR chemical shifts as shown in Table 1. Compound **2** was proposed as 1α,7*β*,13*β*,14α-tetrahydroxy-3*β*-benzoyloxy-9*β*,15*β*-diacetoxyjatropha-5,11 *E*-diene. The key NOE effects from the NOESY spectrum (Figure 1) showed the relative configuration of **2** to be the same as that of **1**.

Compound **3**, a colorless solid, mp 106–108 °C, was determined to have the following molecular formula, $C_{37}H_{46}O_{13}$, from HRESIMS. The ^{13}C NMR signals revealed the presence of two trisubstituted double bonds (δ_C132.1 d, 136.9 s, 124.1 d, 133.9 s), a keto group (δ_C 197.5), and six ester groups (δ_C 166.2, 169.0, 169.7, 170.0, 170.2, 170.5), of which one is a benzoyl [δ_H 8.01(dd), 7.54 (ddd), 7.42 (ddd) and δ_C 166.2 (s), 133.1 (d), 130.0 (d and s), 128.5 (d)]. The ^{1}H–^{1}H COSY spectrum revealed connectivities from C-1 to C-5, a long-range ^{1}H–^{1}H cross-peak between H-5/H$_3$-17, as well as cross-peaks of H-8/H-9 and H$_2$-11/H-12. An α-substituted-α,*β*-unsaturated carbonyl function was established from the presence of a less shielded doublet signal at δ_H 6.75 (H-5) and a keto function at C-7 was elucidated from the $^3J_{H,C}$ correlations of H-5, H-9, and H-17 with C-7. Assignment of a broad singlet at δ_H 6.36 for H-14 was based on the long-range HMBC correlations of H-14/C-4, C-12, C-20, and O*CO*-14. The placement of double bonds at C-5(6) and C-12(13) was elucidated from $^3J_{H,C}$ correlations of H-5/C-3, C-15, and C-17 and H-12/C-10 and C-20, respectively. The NOESY spectrum revealed the relative configuration of **3** as illustrated in Figure 1. Compound **3** was thus proposed as 1α,8*β*,9*β*,14α,15*β*-pentaacetoxy-3*β*-benzoyloxy-7-oxojatropha-5,12-diene.

Table 2. ^{13}C NMR Spectroscopic Data of **1–6** [100 MHz, $CDCl_3$, δ_C (ppm), and mult]

C	**1**[a]	**2**	**3**	**4**	**5**	**6**
1	87.1 d	86.9 d	84.6 d	85.5 d	84.5 d	73.5 d
2	43.3 d	43.2 d	45.9 d	43.5 d	44.2 d	46.8 d
3	77.6 d	78.4 d	76.5 d	76.3 d	76.2 d	80.4 d
4	41.3 d	41.3 d	46.8 d	47.0 d	47.5 d	47.0 d
5	119.2 d	117.9 d	132.1 d	71.5 d[c]	71.3 d	71.2 d
6	134.3 s	138.6 s	136.9 s	129.4 s	129.4 s	128.4 s
7	74.3 d	72.1 d	197.5 s	143.9 s	143.9 s	141.6 s
8	32.3 t	34.6 t	74.3 d	69.6 d	69.7 d	65.9 d
9	74.1 d	74.2 d	74.1 d	78.6 d	78.6 d	74.4 d
10	39.7 s	39.1 s	39.2 s	38.8 s	38.8 s	39.5 s
11	132.1 d	131.8 d	39.9 t	41.3 t	41.2 t	59.0 d
12	130.1 d	129.6 d	124.1 d	125.1 d	124.9 d	57.7 d
13	74.6 s	74.6 s	133.9 s	131.4 s	131.5 s	78.4 s
14	72.6 d	72.6 d	71.2 d	71.5 d[c]	72.0 d	75.8 d
15	91.3 s	91.4 s	89.4 s	93.8 s	91.8 s	86.1 s
16	11.7 q	11.7 q	12.1 q	12.3 q	11.8 q	12.8 q
17	16.3 q	16.3 q	13.7 q	17.7 q	17.7 q	18.9 q
18	23.0 q	23.2 q	24.1 q	29.4 q	29.3 q	24.1 q
19	20.6 q	20.8 q	25.5 q	20.5 q	20.5 q	13.5 q
20	31.3 q	31.3 q	15.2 q	17.4 q	17.2 q	22.3 q
OAc-1			20.6 q 169.7 s		20.7 q 170.1 s	20.3 q 168.8 s
OAc-7				22.0 q 171.7 s	20.9 q, 168.9 s	20.6 q, 169.8 s
OAc-8			20.5 q[b] 170.2 s	21.0 q[d] 170.0 s	21.0 q[e] 170.1 s	20.6 q 170.1 s
OAc-9	20.8 q, 170.1 s	21.2 q, 171.0 s	20.5 q[b] 170.0 s	20.9 q 171.3 s	21.8 q 170.1 s	22.1 q 169.4 s
OAc-14			21.0 q 169.0 s	21.0 q[d] 169.1 s	21.0 q[e] 169.1 s	20.5 q 169.0 s
OAc-15	22.1 q 173.3 s	22.2 q 173.3 s	22.1 q 170.5 s	20.1 q 167.7 s	20.1 q 167.7 s	20.9 q 169.4 s
OBz-3	165.1 s	166.1 s	166.2 s	165.2 s	165.4 s	ONic-3, 163.6 s
1′	129.7 s	130.2 s	130.0 s	130.3 s	130.1 s	2′, 149.3 d
2′, 6′	128.9 d	129.4 d	130.0 d	129.3 d	129.5 d	3′, 126.3 s
3′, 5′	128.0 d	128.5 d	128.5 d	128.6 d	128.6 d	4′, 138.1 d
4′	132.5 d	133.0 d	133.1 d	133.1 d	133.2 d	5′, 124.0 d
OBz-7[a]						6′, 152.5 d

[a] OBz-7 of **1**, δ_C 164.9 (s) C-7″, 129.7 (s) C-1″; 129.3 (d) C-2″ and C-6″, 127.8 (d) C-3″ and C-5″, 132.1 (d) C-4. [b–e] Overlapping signals.

Compound **4** was obtained as a solid, mp 178–180 °C, and HRMS indicated the molecular formula to be $C_{37}H_{48}O_{14}$. The ^1H NMR spectrum showed five sharp singlet signals of acetyl groups at δ_H 2.00, 2.07, 2.11, 2.15 and 2.21, and the ^{13}C NMR spectrum exhibited six oxymethine carbons at δ_C 69.6, 71.5 (2×), 76.3, 78.6, 85.5 and one quaternary oxygenated carbon (δ_C 93.8). The ^1H–^1H COSY spectrum showed connnectivities from H-1 to H-5 as well as from H_3-17 to H-9 and H_2-11 to H-20. A broad singlet signal at δ_H 5.84 was elucidated for H-14 as indicated from the $^3J_{H,C}$ correlations between H-14/C-4, C-12, C-13, and O*CO*-14 (δ_C 169.1). A benzoyloxy group was apparent from ^1H and ^{13}C NMR signals at δ_H 7.89, 7.55, 7.41 and δ_C 165.2 (s), 130.3 (s), 129.3 (d), 128.6 (d), 133.1 (d), and could be deduced to be bonded to C-3, as indicated from the HMBC correlations of H-3, H-2′, and H-6′/O*CO*-3 (δ_C 165.2). The FTIR spectrum showed absorption bands of an enol acetate group at ν_{max} 1743 (br) and 1231 cm^{-1}.[14] The locations of a tetrasubstituted double bond of an enol acetate at C-6(7) and a trisubstituted double bond at C-12(13) were established from the $^3J_{H,C}$ correlations of H-5/C-4, C-6, C-7, C-17 and H-12/C-14, C-20, respectively. The interactions obtained from a NOESY experiment are shown in Figure 1. Compound **4** therefore could be proposed as 7,8β,9β,14α,15β-pentaacetoxy-3β-benzoyloxy-1α,5β-dihydroxyjatropha-6(7),12-diene.

Compound **5** was isolated as a colorless solid, mp 232–234 °C, and its molecular formula of $C_{39}H_{50}O_{15}$ was determined by HRMS. The IR and ^1H and ^{13}C NMR spectra of **5** resembled those of **4**, except for the presence of six instead of five acetate groups as found in **4**. The doublet signal at δ_H 5.43, assignable to H-1, was found to be at a less shielded position than that encountered in **4**, indicating the attachment of an OAc group to C-1. A deshielded singlet signal of H-14 at δ_H 6.06 due to an anisotropic effect by the C-1 acetoxy group gave further support to this assignment. The NOESY spectrum (Figure 1) also gave evidence of the same relative configuration as found for **4**. Compound **5** could be proposed as 1α,7,8β,9β,14α,15β-hexaacetoxy-3β-benzoyloxy-5β-hydroxyjatropha-6(7),12-diene.

Compound **6** was obtained as colorless needles with mp 238–240 °C and the HRESIMS indicated a molecular formula of $C_{38}H_{47}NO_{16}$. Connectivities from C-1 to C-5 were recognized from the ^1H–^1H COSY spectrum as in compounds **1–5**. The FTIR spectrum indicated absorption bands of an enol acetate group at ν_{max} 1755 (br), 1222 cm^{-1},[14] in addition to an epoxide C–O stretching at ν_{max} 1242 cm^{-1}. The presence of an epoxide ring was also supported by the shielded oxymethine ^{13}C NMR signals at δ_C 59.0 and 57.7. The long-range ^1H–^{13}C correlations between both H_3-18 and H_3-19 to C-9, C-10, and C-11 was used to locate a strained epoxide ring at C-11 and C-12, and a small $^3J_{11,12}$ value of around 2.0 Hz indicated a cis conformation. Diagnostic long-range ^1H–^{13}C HMBC correlations between H-5/C-4, C-6, C-7, C-13, C-14, and C-17 allowed the detection of the second epoxide ring between C-5/C-13 and a tetrasubstituted double bond at C-6(7). A nicotinoyloxy group,[13] evident from characteristic signals at δ_H 9.16 (s), 8.79 (d), 8.33 (br d), 7.49 (dd) and δ_C 163.6 (s, O*CO*-3), 152.5 (d, C-6′), 149.3 (d, C-2′), 138.1 (d, C-4′), 126.3 (s, C-3′), 124.0 (d, C-5′) was implied to be positioned at C-3 from key $^3J_{H,C}$ correlations between H-3 (δ_H 5.63) and H-4′ (δ_H 8.33)/ O*CO*-3. Six acetoxy groups and their positions of substitution were revealed from acetate ^1H and ^{13}C NMR signals and from the analysis of the 2D NMR data. Full assignments of the ^1H and ^{13}C NMR chemical shifts are shown in Tables 1 and 2. The relative configuration in **6** was investigated thoroughly using NOESY and NOEDIFF techniques, and important NOE effects are indicated in Figure 1. Compound **6** was thus proposed as 1α,7,8β,9β,14α,15β-hexaacetoxy-3β-nicotinoyloxy-5,13β,11,12β-diepoxyjatropha-6(7)-ene.

This work has indicated the genus *Pedilanthus* as a new source of highly oxygenated jatrophane diterpenoids, including some with an *O*-acetyl enol moiety (**4–6**). Compound **6** also possesses two extra epoxide rings that make the structure more rigid than other compounds in this compound class.

The pure compounds **1–5** were investigated for their biological activities including antimalarial effects against *Plasmodium falciparum* K1 strain and antitubercular effects against *Mycobacterium*

Table 3. Biological Activities of Compounds **1**–**5**

compound	antimalarial[a]	anti-TB[b]
1	4.0 (5.9)	12.5
2	inactive[c]	100
3	3.4 (4.0)	50
4	4.3 (6.0)	100
5	4.4 (5.8)	50
dihydroartemisinin	(0.0036)	
isoniazide		0.1
kanamycin		2.5

[a] IC_{50} values are reported in micrograms per milliliter, with values in parentheses in micromolar. [b] Minimum inhibitory concentration (MIC) in micrograms per milliliter. [c] Inactive at 10 μg/mL.

tuberculosis H37 Ra. Compounds **1** and **3**–**5** were found to be active against *P. falciparum* with LC_{50} values of 3.4–4.4 μg/mL, whereas **2** was not active at 10 μg/mL. Compounds **1**–**5** also showed moderate to mild antimycobacterial activity, with **1** being the most active (Table 3). In an additional antifungal assay of compounds **1**, **2**, and **5** against *Candida albicans* at 50 μg/mL, none was active.

Experimental Section

General Experimental Procedures. Melting points were measured on a electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signals ($CDCl_3$: δ_H 7.24 and δ_C 77.0 ppm). HRESIMS and HRAPCIMS were recorded on a Bruker Daltonics micro-TOF instrument.

Plant Material. The milky juice or latex of *Pedilanthus tithymaloides* was collected from plants growing within the Ramkhamhaeng University area during March–July, 2004. Botanical identification of the plant was carried out through comparison with a voucher specimen No. SN240277 kept in the herbarium collection of the Sirindhorn Museum (Bangkok Herbarium), Botany and Weed Section, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok. A voucher specimen (SSPT/2004) was deposited at the Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok.

Extraction and Isolation. The crude latex of *P. tithymaloides* was collected by drawing liquid using a pipette from newly cut leaf stalks, and also from 1–2 mm depth incisions made by a sharp clean knife and kept in MeOH–water. After concentration, a mixture of water and MeOH was added to the crude MeOH extract (6.48 g), and the resulting solution was then partitioned with CH_2Cl_2 to afford a CH_2Cl_2 extract (3.76 g) after solvent evaporation.

The CH_2Cl_2 extract of the latex was subjected to silica gel column chromatography (CC) with a gradient of hexane–CH_2Cl_2 (100:0) to CH_2Cl_2–MeOH (90:10) to afford 12 major fractions. Fraction 2 was chromatographed (silica gel, hexane–EtOAc, 97:3 to 70:30) to yield four subfractions (2.1–2.4). Subfraction 2.2 after reversed-phase CC (C_{18}, MeOH–H_2O, 80:20 to 100:0) yielded four subfractions (2.2.1–2.2.4). Tirucalla-7,24-dien-3β-ol (6.5 mg) was obtained from subfraction 2.2.2. Subfraction 2.2.3 was further purified by RP HPLC (Lichrospher 100 C_{18}, 4 × 250 mm, 1.0 mL/min) to yield cycloartenol (2.1 mg; t_R = 17.3 min). Fraction 3 was rechromatographed (silica gel, hexane–CH_2Cl_2, 75:25 to CH_2Cl_2–MeOH, 90:10) to give six subfractions (3.1–3.6). Subfraction 3.2 after CC (silica gel, hexane–EtOAc, 97:3 to 70:30) gave five subfractions (3.2.1–3.2.5). Subfraction 3.2.2 was purified (C_{18}, MeOH–H_2O, 90:10 to 100:0) and gave three subfraction (3.2.2.1–3.2.2.3). Subfraction 3.2.2.2 yielded lupeol (3.4 mg; t_R = 18.6 min), an additional quantity of tirucalla-7,24-dien-3β-ol (17.8 mg; t_R = 20.1 min), and β-amyrin (2.9 mg; t_R = 22.6 min) after purification using HPLC (C_{18}, MeOH). Fraction 4 after CC (silica gel, hexane–EtOAc, 95:5 to 100% MeOH) yielded three subfractions (4.1–4.3). Subfraction 4.2 yielded β-sitosterol (32.4 mg). Fraction 7 (162.3 mg) was purified using reversed-phase CC (C_{18}, MeOH–H_2O, 50:50 to 100:0) to give seven subfractions (7.1–7.7). Subfraction 7.2 was column chromatographed (silica gel, hexane–EtOAc, 88:22 to 65:35) to yield compound **2** (8.1 mg). Subfraction 7.3, after purification using a gradient of hexane–EtOAc, 88:12 to 85:15, yielded four subfractions (7.3.1–7.3.4). Subfraction 7.3.2 gave an additional quantity of **2** (12.2 mg), whereas subfraction 7.3.3 after further reversed-phase CC (C_{18}, MeOH–H_2O, 45:55 to 100:0) yielded compound **3** (5.4 mg). Subfraction 7.5 afforded compound **1** (31.1 mg). Fraction 9 was fractionated using silica gel CC (hexane–EtOAc, 88:12 to 80:20) to afford six subfractions (9.1–9.6). Subfraction 9.2 after further purification afforded compound **4** (5.3 mg). Subfraction 9.4 gave compound **5** (11.5 mg) after reversed-phase CC (C_{18}, MeOH–H_2O, 65:35 to 100:0). Fraction 12 after fractionation (silica gel, hexane–EtOAc, 70:30 to 50:50) and further reversed-phase CC (C_{18}, MeOH–H_2O, 20:80 to 100:0) gave compound **6** (4.0 mg).

1α,13*β*,14α-Trihydroxy-3*β*,7*β*-dibenzoyloxy-9*β*,15*β*-diacetoxyjatropha-5,11 *E*-diene (1). Colorless solid. mp 82–84 °C. $[\alpha]_D^{27}$ −8.1 (*c* 0.45, $CHCl_3$). IR (KBr) ν_{max} 3503, 2971, 2931, 1718, 1542, 1457, 1373, 1280, 1114, 1070, 1027, 709, 527 cm^{-1}. ^{1}H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-15, C-16; H-2/C-1, C-16; H-3/C-1, C-15, O*CO*-3; H-4/ C-5, C-6, C-14, C-15; H-5/C-6, C-7, C-15, C-17; H-7/C-5, C-6, C-8, C-9, C-17, O*CO*-7; H-8/C-6, C-9,C-10; H-9/C-7, C-11, C-18, C-19, O *CO*-9; H-11/C-9, C-10, C-12, C-13, C-14, C-18, C-19, C-20; H-12/C-10, C-11, C-13,C-20; H-14/C-4, C-15, C-20; H-16/C-1, C-2, C-3, C-4, C-14; H-17/C-4, C-5, C-6, C-7, C-8; H-18/C-9, C-10, C-11, C-12, 19; H-19/C-9, C-10, C-11, C-12, C-18; H-20/C-12, C-13, C-14; OCOC *H*$_3$-15/C-15, O*CO*-15; H-2′,6′/C-2′, C-6′, O *CO*-3; H-3′,5′/C-1′, C-3′, C-5′; H-4′/C-2′, C-6′; H-2″,6″/C-2″, C-6″, O *CO*-7; H-3″,5″/C-1″, C-3″, C-4″, C-5″; H-4″/C-2″, C-6″; OH-1/C-1, C-2; OH-13/C-12, C-14, C-20; OH-14/C-13, C-14. HRESIMS [M–H]$^-$ *m/z* 677.2948 (calcd for $C_{38}H_{45}O_{11}$, 677.2962). R_f = 0.63 (silica gel, hexane–EtOAc, 6:4). Purple color after staining with anisaldehyde–sulfuric acid reagent.

1α,7*β*,13*β*,14α-Tetrahydroxy-3*β*-benzoyloxy-9*β*,15*β*-diacetoxyjatropha-5,11 *E*-diene (2): colorless solid. mp 98–100 °C. $[\alpha]_D^{27}$ +9.6 (*c* 0.42, $CHCl_3$). IR (KBr) ν_{max} 3446, 2970, 2929, 1717, 1373, 1273, 1168, 1114, 1070, 1027, 983, 936, 899, 762, 712, 670, 519, 473 cm^{-1}. ^{1}H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-15, C-16; H-2/C-1, C-16; H-3/C-1, C-15, O*CO*-3; H-4/ C-5, C-6, C-14, C-15; H-5/C-6, C-7, C-15, C-17; H-7/C-5, C-6, C-8, C-9, C-17; H-8/C-6, C-7, C-9, C-10; H-9/C-7, C-10, C-18, C-19, O *CO*-9; H-11/C-9, C-10, C-12, C-13, C-18, C-19; H-12/C-10, C-11, C-13, C-14, C-20; H-14/C-1, C-4, C-15, C-20; H-16/C-1, C-2, C-3; H-17/C-5, C-6, C-7, C-8; H-18/C-9, C-10, C-11, C-19; H-19/C-9, C-10, C-12, C-13, C-14; OCO *CH*$_3$-9/C-9, O *CO*-9; COC *H*$_3$-15/O*CO*-15; H-2′, 6′/C-2′, C-4′, C-6′, O*CO*-3; H-3′, 5′/C-1′, C-3′, C-5′, O*CO*-3; H-4′/ C-2′, C-6′; OH-1/C-1, C-2; OH-13/C-12, C-14, C-20; OH-14/C-13, C-14. HRESIMS [M–H]$^-$ *m/z* 573.2711 (calcd for $C_{31}H_{41}O_{10}$, 573.2700). R_f = 0.50 (silica gel, hexane–EtOAc, 6:4). Purple color after staining with anisaldehyde–sulfuric acid reagent.

1α,8*β*,9*β*,14α,15*β*-Pentaacetoxy-3*β*-benzoyloxy-7-oxojatropha-5,12-diene (3). Colorless solid. mp 106–108 °C. $[\alpha]_D^{27}$ −5.7 (*c* 0.54, $CHCl_3$). IR (KBr) ν_{max} 2975, 1749, 1508, 1456, 1374, 1245, 1102, 1039, 949, 756, 715, 608 cm^{-1}. ^{1}H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-14, C-15, C-16, O*CO*-1; H-3/C-1, C-15; H-4/ C-5, C-6, C-14, C-15; H-5/C-3, C-7, C-15, H-8/C-7, C-9, O*CO*-8; H-9/C-7, C-10, C-18, C-19, O*CO*-9; H-11/C-9, C-10, C-12, C-13, C-19; H-12/C-10, C-20; H-14/C-4, C-12, C-13, C-15, C-20, O*CO*-14; H-16/C-1, C-2, C-3; H-17/C-5, C-6, C-7; H-18/C-9, C-10, C-11, C-19; H-19/C-9, C-10, C-11, C-18; H-20/C-12, C-13, C-14; OCO *CH*$_3$-1/C-1, O*CO*-1; COC *H*$_3$-8/O*CO*-8; OCO *CH*$_3$-9/O*CO*-9; OCOC *H*$_3$-14/O*CO*-14; O*COC H*$_3$-15/O*CO*-15; H-2′, 6′/C-2′, C-4′, C-6′, O*CO*-3; H-3′, 5′/C-2′, C-3′, C-5′, C-6′; H-4′/C-1′, C-2′, C-6′. HRESIMS [M+Na]$^+$ *m/z* 721.2826 (calcd for $C_{37}H_{46}NaO_{13}$, 721.2836). R_f = 0.38 (silica gel, hexane–EtOAc, 6:4). Blue color after staining with anisaldehyde–sulfuric acid reagent.

7,8*β*,9*β*,14α,15*β*-Pentaacetoxy-3*β*-benzoyloxy-1α,5*β*-dihydroxyjatropha-6(7),12-diene (4). Colorless solid. mp 178–180 °C. $[\alpha]_D^{27}$ −27.6 (*c* 0.48, $CHCl_3$). IR (KBr) ν_{max} 3524, 2969, 2930, 1743, 1456, 1371, 1274, 1231, 1201, 1110, 1027, 957, 714 and 614 cm^{-1}. ^{1}H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-15, C-16; H-4/ C-5, C-6, C-14, C-15; H-5/C-4, C-6, C-7, C-17; H-8/C-6, C-10, O*CO*-8; H-9/C-6, C-7, C-10, C-11, C-18, C-19, O*CO*-9; H-11/C-9, C-10, C-12, C-13, C-18; H-12/C-14, C-20; H-14/C-4, C-12, C-13, C-15, O*CO*-14; H-16/C-1, C-2, C-3; H-17/C-5, C-6, C-7, C-8, C-9; H-18/C-10, C-11, C-19; H-19/C-10, C-11, C-12, C-18; H-20/C-12, C-13, C-14; OCO *CH*$_3$-7/O*CO*-7; OCO *CH*$_3$-8/O*CO*-8; OCO *CH*$_3$-9/O*CO*-9; OCO *CH*$_3$-14/O*CO*-14; OCOC *H*$_3$-15/

O*CO*-15; H-2′, 6′/C-2′, C-4′, C-6′, O*CO*-3; H-3′, 5′/C-1′, C-3′, C-5′; H-4′/ C-3′, C-5′. HRESIMS $[M+Na]^+$ *m/z* 739.2941 (calcd for $C_{37}H_{48}NaO_{14}$, 739.2942). $R_f = 0.30$ (silica gel, hexane–EtOAc, 6:4). Blue color after staining with anisaldehyde–sulfuric acid reagent.

1α,7,8*β*,9*β*,14α,15*β*-Hexaacetoxy-3*β*-benzoyloxy-5*β*-hydroxyjatropha-6(7),12-diene (5). Colorless needles. mp 232–234 °C. $[\alpha]_D^{27}$ −46.0 (*c* 0.63, $CHCl_3$). IR (KBr) ν_{max} 2976, 2936, 2878, 1748, 1508, 1457, 1373, 1229, 1162, 1111, 1085, 1027, 956, 930, 839, 715, 607 cm^{-1}. ^1H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-15, C-16, O*CO*-1; H-3/ C-2, C-4, C-16, O*CO*-3; H-4/ C-5, C-15; H-5/C-3, C-4, C-6, C-7, C-17; H-8/O*CO*-8; H-9/C-7, C-10, C-11, C-18, C-19, O*CO*-9; H-11/C-10, C-12, C-13; H-12/C-14, C-20; H-14/C-12, C-13, C-15, O*CO*-14; H-16/ C-1, C-2, C-3; H-17/C-5, C-6, C-7; H-18/C-9, C-10, C-11, C-19; H-19/ C-8, C-9, C-10, C-11, C-18; H-20/C-12, C-13, C-14; OCO*CH*$_3$-1/O*CO*-1;OCO*CH*$_3$-7/O*CO*-7;OCO*CH*$_3$-8/O*CO*-8;OCO*CH*$_3$-9/O*CO*-9;OCO*CH*$_3$-14/O*CO*-14; OCOC *H*$_3$-15/O*CO*-15; H-2′, 6′/C-2′, C-4′, C-6′, O*CO*-3; H-3′, 5′/C-1′, C-3′, C-5′; H-4′/C-2′, C-6′. HRESIMS $[M+Na]^+$ *m/z* 781.3035 (calcd for $C_{39}H_{50}NaO_{15}$, 781.3047). $R_f = 0.30$ (silica gel, hexane–EtOAc, 6:4). Blue color after staining with anisaldehyde–sulfuric acid reagent.

1α,7,8*β*,9*β*,14α,15*β*-Hexaacetoxy-3*β*-nicotinoyloxy-5,13*β*,11,12*β*-diepoxyjatropha-6(7)-ene (6). Colorless needles. mp 238–240 °C. $[\alpha]_D^{27}$ −54.7 (*c* 0.12, $CHCl_3$). IR (KBr) ν_{max} 3448, 2979, 2937, 1755 (d), 1591, 1423,1372, 1283, 1242, 1222, 1113, 1068, 1038, 946, 922, 889, 741, 702 cm^{-1}. ^1H NMR ($CDCl_3$) data, see Table 1. ^{13}C NMR ($CDCl_3$) data, see Table 2. HMBC correlations: H-1/C-2, C-3, C-4, C-14, C-15, C-16, O*CO*-1; H-3/C-2, C-4, C-15, C-16, O*CO*-3; H-4/ C-5, C-6; H-5/C-4, C-6, C-7, C-13, C-15, C-17; H-8/C-10, O*CO*-8; H-9/C-7, C-9, C-10, C-18, C-19, O*CO*-9; H-11/C-10; H-12/C-11, C-13; H-14/C-13, C-15, C-20; H-16/C-1, C-2, C-3; H-17/C-5, C-6, C-7; H-18/ C-9, C-10, C-11, C-19; H-19/C-9, C-10, C-11, C-18; H-20/C-13, C-14; OCO*CH*$_3$-1/C-1, O*CO*-1; OCO*CH*$_3$-7/O*CO*-7; OCO*CH*$_3$-8/C-8, O*CO*-8; OCO*CH*$_3$-9/OCO-9; OCO*CH*$_3$-14/O*CO*-14; OCO*CH*$_3$-15/O*CO*-15; H-2′/C-3′, C-4′, C-6′; H-4′/C-2′, C-6′, O*CO*-3; H-6′/C-5′. HRAPCIMS $[M+ H]^+$ *m/z* 774.2952 (calcd for $C_{38}H_{48}NO_{16}$, 774.2973). $R_f = 0.33$ (silica gel, hexane–EtOAc, 3:7). Blue color after staining with anisaldehyde–sulfuric acid reagent.

Bioassays. Antimalarial activity was evaluated against *Plasmodium falciparum* (K1 multidrug-resistant strain) cultured continuously according to the method of Trager and Jensen.[15] A quantitative determination of antimalarial activity in vitro was conducted by means of the microculture radioisotope technique based on the method of Desjardins et al.[15] The antimycobacterial activity (anti-TB) assay was performed against *Mycobacterium tuberculosis* H37Ra using an Alamar Blue microplate assay.[16] An antifungal test was undertaken against *Candida albicans* (ATCC 90028) using a tetrazolium/formazan assay method.[17] Amphotericin B and DMSO were used as a positive control (IC_{50} value of 0.068–0.092 μg/mL) and a negative control, respectively.

Acknowledgment. We are grateful to the Thailand Research Fund and Ramkhamhaeng University for financial support. W.M. acknowledges the Royal Golden Jubilee Ph.D. Program, Thailand Research Fund, for a scholarship. We acknowledge Mr. N. Chimnoi, Chulabhorn Research Institute, for HRMS measurements, the Bioassay Laboratory of the National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand, for biological activity assays, and the Center for Innovation in Chemistry, Postgraduate Education and Research Program in Chemistry (PERCH-CIC), for partial support.

Supporting Information Available: ^1H and ^{13}C NMR spectra of compounds **1–6** (Figures S1–S12). This material is available free of charge via the Internet at http://pubs.acs.org.

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NP070174V