New Jatrophane Diterpenoid Esters from *Euphorbia turczaninowii*

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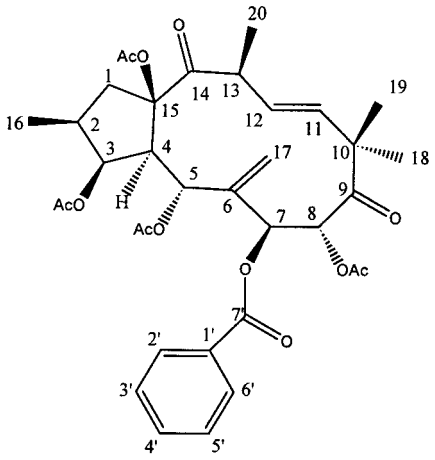
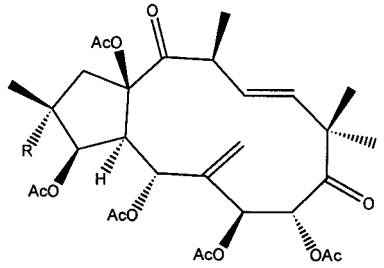
Five new (**1**−**5**) and one known (**6**) jatrophane diterpenoid esters were isolated from the ethanol extract of the whole herb of *Euphorbia turczaninowii*. Their structures were established by extensive spectroscopic methods. The absolute stereochemistry of 3*β*,5α,8α,15*β*-tetraacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-9,14-dione (**1**) was confirmed by a single-crystal X-ray analysis coupled with the exciton chirality circular dichroism method. Compounds **1**−**6** were inactive when evaluated both in a mouse ear inflammation assay and for cytotoxicity against the B16 mouse melanoma cell line.

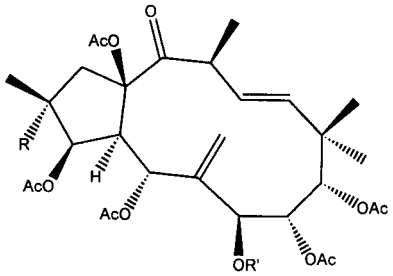
Euphorbia species (Euphorbiaceae) are well-known for the production of skin irritant, proinflammatory, and tumor-promoting tigliane,[1−3] ingenane,[4−6] and daphnane diterpene esters.[7] Previous phytochemical investigation has revealed that members of the genus also produce jatrophane diterpenoids,[8−18] which have attracted attention because of the high conformational flexibility of the 12-membered ring present and their antiwrithing,[14] analgesic,[14] and phytotoxic activities.[15] *Euphorbia turczaninowii* Kar. et Kir., distributed mainly in the northern part of mainland China, is occasionally used locally in the treatment of swellings. However, no previous studies have been published on its chemical composition. Thus, we have examined the title plant phytochemically to characterize five new (**1**−**5**) and one known jatrophane diterpenoids (**6**). The absolute stereochemistry of **1** was established by X-ray crystallographic analysis coupled with the exciton chirality circular dichroism method. The biological activities of **1**−**6** were evaluated in a mouse ear inflammation assay and for cytotoxicity against the B16 mouse melanoma cell line.



1



2 R = H
6 R = OBz



3 R = OAc R' = Bz
4 R = H R' = Bz
5 R = OBz R' = OAc

Results and Discussion

Repeated chromatographic fractionation of the ethanol extract of *E. turczaninowii* afforded five new compounds (**1**−**5**) and one known diterpene (**6**), which was identified as 3*β*,5α,7*β*,8α,15*β*-pentaacetoxy-2α-benzoyloxyjatropha-6(17),11*E*-dien-9,14-dione, previously characterized as a gum from *Euporbia semiperfoliata*, by comparison of its ^{1}H NMR spectrum with values reported in the literature.[18]

Compound **1** ($C_{35}H_{42}O_{12}$) was crystallized from MeOH. The EIMS revealed fragment ions (*m*/*z* 594, 552, 534, 492, and 396) from the molecular ion (*m*/*z* 654) produced by the sequential elimination of four acetic acid units and ketenes of acetoxy groups in the compound. A base peak at *m*/*z* 105 suggested that the molecule carried a benzoyl group. Fragment peaks at *m*/*z* 123 and 96 corresponded to the ions $[(CH_3)_2C{=}CH{-}CH{=}CCH_3C{=}O]^+$ and $[(CH_3)_2C{=}CH{-}CH{=}CHCH_3]^+$, characteristic of a jatroph-11-ene-9,14-dione derivative.[17] All these mass spectral features suggested that compound **1** was a highly acylated jatrophane diterpenoid. This was reinforced by the ^{13}C NMR spectral data for the jatrophane core polyol moiety of **1** ($C_{20}H_{30}O_7$), containing 20 resonance signals corresponding to two tertiary and two secondary methyls, a *trans*-disubstituted double bond, one methylene, an exomethylene, seven methines, and two carbonyl and three quaternary carbons. Four methyl groups were apparent from the signals at *δ*

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Table 1. NMR Data of Compound **1** (*J* in Hz in parentheses)

position	δ_H	δ_C	COLOC (C→H)	NOESY
1α	2.93 dd (16.0, 8.0)	44.8 t		1*β*, 2, 16, 20
1*β*	2.11 dd (16.0, 12.5)			1α, 16
2	2.37 m	38.5 d	1*β*, 16	1α, 3, 4, 13, 16
3	5.59 t (3.6)	76.7 d	1α, 16	2, 4, 16
4	2.93 dd (10.0, 3.6)	49.5 d		2, 3, 7, 13
5	5.74 d (10.0)	72.3 d		17a
6		136.7 s	7	
7	6.11 brs	63.9 d		4, 8
8	5.44 brs	73.9 d		7, 12, 17b, 19
9		204.6 s	18, 19	
10		49.2 s	11, 18, 19	
11	6.08 d (16.0)	135.2 d	12, 18, 19	13, 18
12	5.95 dd (16.0, 9.0)	133.4 d	20	8, 19, 20
13	3.47 dq (9.0, 6.5)	43.3 d	11, 20	2, 4, 11, 20
14		204.0 s	20	
15		90.3 s	1*β*, 3	
16	1.01 d (6.5)	13.6 q	1*β*	1α, 1*β*, 2, 3, AcO-3
17a	5.62 brs	123.7 t		5, 17b, AcO-15
17b	5.55 brs			8, 17a
18	1.26 s	23.8 q		11
19	1.34 s	25.8 q		8, 12
20	1.44 d (6.5)	20.3 q		1α, 12, 13
AcO-3	2.01 s	20.6 q	3	16
		170.1 s		
AcO-5	1.34 s	20.4 q	5	
		169.3 s		
AcO-8,15	2.18 × 2 s	21.2 q		17a
		20.7 q		
		169.0 s		
		169.8 s		
BzO-7				
1′	8.00 br d (8.0)	129.2 s		
2′,6′	7.43 br t (8.0)	129.9 d		
3′,5′	7.56 br t (8.0)	128.5 d		
4′		133.5 d	7	
7′		165.8 s		

1.01 (d, H-16), 1.26 (s, H-18), 1.34 (s, H-19), and 1.44 (d, H-20) in the ^{1}H NMR spectrum of **1**. Additionally, a *trans*-disubstituted double bond was indicated by a pair of mutually coupled proton signals at δ 6.08 (d, J = 16 Hz, H-11) and 5.95 (dd, J = 16, 9 Hz, H-12). Moreover, the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) confirmed the presence of four acetoxy groups [δ_H 2.18 × 2, 2.01, 1.34; δ_C 170.1, 169.8, 169.3, 169.0 (CO), 21.2, 20.7, 20.6, 20.4 (CH_3)] and a benzoate group [δ_H 7.43, 7.56, 8.00; δ_C 128.5 × 2, 129.2, 129.9 × 2, 133.5, 165.8 (CO)]. All proton and carbon signals of **1** were assigned by extensive 2D NMR experiments, including the ^{1}H−^{1}H COSY, TOCSY, HMQC, COLOC, and NOESY techniques. In the COLOC spectrum of **1**, the correlations of the benzoate carbonyl C-7′ (δ_C 165.8) with H-7 (δ_H 6.11) demonstrated that the benzoyloxy group was situated at C-7. Furthermore, the attachment of a 3,5-diacetyl functionality was ascertained in the same manner. It was noteworthy that the C-5 acetyl group methyl signal was moved upfield substantially (δ_H 1.34) owing to the deshielding effect of the benzoyloxy group at C-7. The remaining two acetyl groups, showing indiscernible correlations in the COLOC spectrum, were placed at C-15 (quaternary) and C-8, as evidenced by the chemical shift of H-8 (δ_H 5.44).

The relative stereochemistry of **1** was assigned on the basis of a NOESY experiment. Relative to the angular proton H-4, which was assumed to be α-oriented on a biogenetic basis,[13] the C-2 methyl and C-3 acetoxy groups were proposed in the *β*-configuration on the basis of the NOE effect of H-3 with H-2 and H-4. The *β*-orientation of H-5 was established by the magnitude of $J_{4,5}$ = 10 Hz, as in the case of enukokurin.[13] The NOE effect between H-4 and H-7 and the magnitude of $J_{7,8}$ = 0 Hz could be explained by assuming the presence of H-7α and H-8*β*, which, as shown by inspection of a model of **1**, were mutually at right angles in this case. Furthermore, the NOE correlations of H-12 with H-8 and H-19, and H-11 with H-13 and H-18 in the NOESY spectrum, indicated that H-19 was *β*-oriented, with H-13 and H-18 α-oriented.

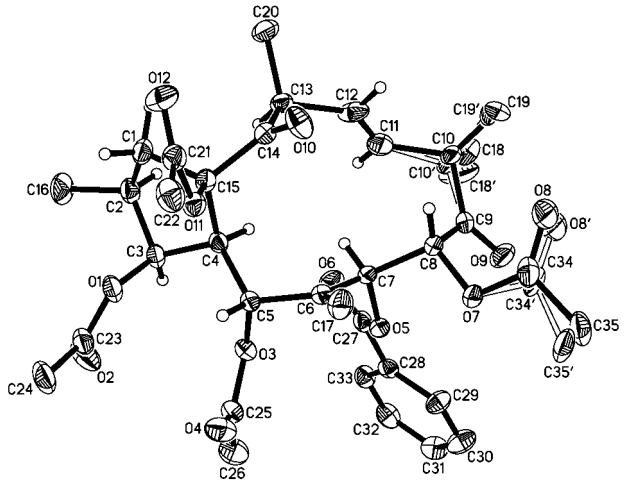

Figure 1. Perspective view of **1** with crystallographic numbering scheme.

The absolute stereochemistry of **1** was determined by X-ray crystallographic analysis (Figure 1) coupled with the circular dichroism exciton chirality method.[15] In the case of compound **1**, the interaction between the C-6, C-17 double bond and the C-7 benzoate chromophore produced a positive first Cotton effect (longer wavelength) ($\Delta\epsilon_{232}$ +15.5) that clearly specified the 7*S*-configuration of the diterpene.[19] The absolute configuration at other chiral centers could thus be settled readily on the basis of the X-ray crystallographic data by correlation with the C-7 stereochemistry. In conclusion, the structure of compound **1** was established as 3*β*,5α,8α,15*β*-tetraacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-9,14-dione.

Compound **2** ($C_{30}H_{40}O_{12}$) was obtained as colorless crystals. The ^{1}H and ^{13}C NMR spectra of **2** were quite similar to those of **1**. Comparison between the ^{1}H NMR spectra of **1** and **2** demonstrated that the C-7 benzoyloxy moiety in the former was replaced by an acetyl group in the latter as the H-7 signal of **2** resonated at δ_H 5.93, moving upfield by 0.18 ppm compared with **1**. Furthermore, the stereochemistry of **2**, identical to that of **1**, was established by the close resemblances between the ^{13}C NMR spectra of the two diterpenes ($\Delta\delta_C$ < 0.7 ppm) and the coupling constant of the ^{1}H NMR signals in both compounds. These observations, along with the optical rotations of both compounds, permitted the assignment of compound **2** as 3*β*,5α,7*β*,8α,15*β*-pentaacetoxyjatropha-6(17),11*E*-dien-9,14-dione.

Compound **3** ($C_{39}H_{48}O_{15}$) was obtained as colorless crystals. The ^{1}H and ^{13}C NMR spectra of **3**, which were similar to those of **1**, indicated that **3** shared the same carbon framework as that of **1**. The presence of acetyl groups at C-3, C-5, and C-9 was suggested by long-range correlations with carbonyl ^{13}C NMR signals at δ 169.0 (×2) and δ 169.6, respectively. The carbonyl signals of the remaining acetoxy groups (δ 170.0 and 170.2) exhibited no HMBC correlations with any protons, thereby placing the acetoxy groups at quaternary carbons (C-2 and C-15). As in the case of **1**, the stereochemistry of **3** was determined on the basis of the NOESY experiment together with ^{1}H NMR coupling patterns. A strong cross-peak between H-1*β*

and H-16 suggested that the AcO-2 group was α-oriented. Furthermore, inspection of a model of **3** revealed that the zero coupling of $J_{7,8}$ and $J_{8,9}$ was consistent with the α-orientation of C-8 and C-9 acetoxy groups, respectively.[8,10,11,20] Thus, the structure of **3** was established as 2α,3*β*,5α,8α,9α,15*β*-hexaacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-14-one.

Compound **4** ($C_{37}H_{46}O_{13}$) was obtained as colorless crystals. The ^{1}H and ^{13}C NMR spectra of compound **4** were very similar to those of **3**. All proton and carbon signals of **4** were assigned by a HMBC experiment. The chemical shift of C-2 (*δ* 38.0) and the doublet of H-16 clearly indicated that diterpene **4** was a 2-deacetoxy derivative of **3**. Thus, the structure of diterpene **4** was determined as 3*β*,5α,8α,9α,-15*β*-pentaacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-14-one.

Compound **5** ($C_{39}H_{48}O_{15}$) was obtained as colorless crystals and possessed the same molecular formula as **3**. Comparison of the ^{1}H and ^{13}C NMR spectral data of **5** with those of **3** suggested that **5** is an isomer of **3** having the same parent jatrophane core polyol moiety. In the ^{1}H NMR spectrum of **5**, the H-16 resonance appeared at lower field (*δ* 1.71) than that (*δ* 1.58) in diterpene **3**, suggesting the presence of a C-2 benzoyloxy group, which exerted a substantial paramagnetic effect on the C-2 methyl group. Furthermore, the 3,5,7,8,9-pentaacetoxy groups were readily assigned on the basis of a HMBC experiment. The final acetoxy group was placed at C-15 by a process of elimination. The stereochemistry of **5** was shown to be identical to that of **3** as demonstrated by the similarity of the splitting patterns of proton signals and the NOE correlations for **3** and **5**. Compound **5** was therefore determined to be 3*β*,5α,7*β*,8α,9α,15*β*-hexaacetoxy-2α-benzoyloxyjatropha-6(17),11*E*-dien-14-one.

When evaluated biologically, all six compounds exhibited no irritant activity (ID^{24}_{50} > 100 *μ*g/ear) in a mouse ear inflammation model and no significant cytotoxicity when evaluated against the B16 melanoma cell line (IC_{50} > 5 *μ*g/mL).

Experimental Section

General Experimental Procedures. Melting points were measured on a Kofler hot stage microscope and were uncorrected. Optical rotations were measured on a P-E-241MC polarimeter. UV spectra were recorded on a UV-2100 spectrophometer. The CD spectrum was recorded on a JASCO-600 CD spectrometer. IR spectra were recorded on a Nicolet Impact 410 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM 400 spectrometer with standard pulse sequences operating at 400 and 100 MHz, respectively. $CDCl_3$ was used as the solvent and TMS as internal standard. EIMS were recorded on a ZAB-HS spectrometer. HRESIMS were recorded on a Bruker FTMS 4.7T spectrometer. The X-ray crystallographic data were collected on a Siemens P4 diffractometer using graphic-monochromated Mo Kα radiation. Column chromatography was performed over Si gel (200−300 mesh). Chromatographic fractions were monitored by TLC (Merck) visualized by spraying with 5% vanillin-sulfuric acid followed by heating. All other chemicals used in this study were of analytical grade.

Plant Material. The whole herb of *E. turczaninowii* was collected in June 1998 at Fukang, Xingjiang, People's Republic of China. A voucher specimen (9806), identified by Chang You Yang (Xingjiang Agricultural University), has been deposited in the Herbarium of Nanjing University, Nanjing, People's Republic of China.

Extraction and Isolation. The air-dried plant material (6 kg) was pulverized and then extracted exhaustively with EtOH (90 L) at room temperature. Removal of the solvent from the crude extract under reduced pressure gave a dark residue (633 g), which was suspended in H_2O and then partitioned with $CHCl_3$ (12 L). The $CHCl_3$ layer was concentrated in vacuo to afford a brown gum (367 g), which, after being dissolved in 90% aqueous MeOH, was further partitioned with petroleum ether. Evaporation of the solvent gave a gum (155 g). The 90% aqueous MeOH layer was diluted with H_2O to MeOH−H_2O (ca. 1:1) followed by repeated extraction with $CHCl_3$. The $CHCl_3$ layer was dried with Na_2SO_4 and then filtered. The filtrate was concentrated in vacuo to give a residue (89.5 g) that was chromatographed over a Si gel column (1 kg) with petroleum ether−Me_2CO mixtures (10:1 → 1:99). The column chromatographic fractions (200 mL each) obtained were combined according to TLC monitoring into five portions (F-1, 25.3 g; F-2, 13.8 g; F-3, 22.4 g; F-4, 11.5 g; F-5, 13.1 g). F-1 and F-5 contained nothing of interest. Detailed Si gel chromatography on F-2 afforded compounds **1** (373 mg) and **2** (15 mg), respectively. F-3 was chromatograped on a Si gel column eluting with petroleum ether−EtOAc (2:1) to give compounds **3** (9 mg) and **4** (12 mg). Repeated chromatography on a Si gel column and subsequently gel filtration over Sephadex LH-20 of F-4 afforded compounds **5** (8 mg) and **6** (135 mg), respectively.

3*β*,5α,8α,15*β*-Tetraacetoxy-7*β*-benzoyloxyjatropha-6(17),-11*E*-dien-9,14-dione (1): colorless crystals; mp 230−231 °C; $[\alpha]^{25}_D$ +88.3° (*c* 0.49, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 203 (4.18), 230 (4.13) nm; CD (MeOH) (nm) $\Delta\epsilon_{232}$ +15.5; IR (KBr) ν_{max} 2977, 2938, 1747 (ester), 1721 (ketone), 1600 (phenyl), 1580 (phenyl), 1493, 1455, 1436, 1372, 1321, 1248, 1228, 1178, 1163, 1138, 1094, 1071, 1052, 1024, 722 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 654 [M$^+$] (2), 594 (1), 552 (1), 534 (2), 492 (1), 396 (1), 123 (27), 105 (100), 96 (43), 77 (19); HRFABMS *m*/*z* 661.2836 (calcd for $C_{35}H_{42}O_{12}Li$, 661.2836).

3*β*,5α,7*β*,8α,15*β*-Pentaacetoxyjatropha-6(17),11*E*-dien-9,14-dione (2): colorless crystals; mp 287−289 °C; $[\alpha]^{25}_D$ +41.9° (*c* 0.48, $CHCl_3$); IR (KBr) ν_{max} 3028, 2983, 2936, 2901, 2877, 1743 (ester), 1724 (ketone), 1471, 1456, 1436, 1373, 1348, 1311, 1254, 1236, 1159, 1145, 1134, 1105, 1070, 1056, 1029, 1013 cm^{-1}; ^{1}H NMR *δ* 5.96 (1H, d, *J* = 16.0 Hz, H-11), 5.93 (1H, brs, H-7), 5.88 (1H, dd, *J* = 16.0, 9.0 Hz, H-12), 5.72 (1H, d, *J* = 9.4 Hz, H-5), 5.57 (1H, dd, *J* = 3.3, 3.5 Hz, H-3), 5.54 (1H, s, H-17a), 5.47 (1H, s, H-17b), 5.33 (1H, brs, H-8), 3.43 (1H, dq, *J* = 9.0, 6.6 Hz, H-13), 2.91 (1H, dd, *J* = 15.6, 8.1 Hz, H-1α), 2.77 (1H, dd, *J* = 9.4, 3.5 Hz, H-4), 2.33 (1H, m, H-2), 2.14, 2.13, 2.06, 2.03, 1.99 (each 3H, s, acetate methyl), 2.09 (1H, dd, *J* = 15.6, 12.0 Hz, H-1*β*), 1.41 (3H, d, *J* = 6.5 Hz, H-20), 1.32 (3H, s, H-19), 1.23 (3H, s, H-18), 1.01 (3H, d, *J* = 6.5 Hz, H-16); ^{13}C NMR *δ* 205.1 (s, C-9), 203.9 (s, C-14), 170.2 × 2, 169.8, 169.0, 168.8 (s, acetate carbonyl), 136.9 (s, C-6), 135.0 (d, C-11), 133.5 (d, C-12), 123.0 (t, C-17), 90.3 (s, C-15), 76.5 (d, C-3), 73.6 (d, C-8), 71.8 (d, C-5), 63.7 (d, C-7), 49.5 (d, C-4), 49.2 (s, C-10), 44.7 (t, C-1), 43.3 (d, C-13), 38.5 (d, C-2), 25.7 (q, C-19), 23.7 (q, C-18), 20.7 (q, C-20), 13.6 (s, C-16), 21.2, 20.9, 20.7, 20.5, 20.1 (each s, acetate methyl); EIMS *m*/*z* 592 [M$^+$] (0.5), 532 (0.7), 123 (40), 96 (94), 42 (100); HRESIMS *m*/*z* 615.2422 (calcd for $C_{30}H_{40}O_{12}Na$, 615.2417).

2α,3*β*,5α,8α,9α,15*β*-Hexaacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-14-one (3): colorless crystals; mp 166−168 °C; $[\alpha]^{25}_D$ +12.9° (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.39), 231 (4.22), 274 (3.05), 281 (2.97) nm; IR (KBr) ν_{max} 2981, 2936, 2875, 1747 (ester), 1723 (ketone), 1650, 1601 (phenyl), 1583 (phenyl), 1453, 1433, 1373, 1273, 1236, 1179, 1156, 1114, 1099, 1071, 716 cm^{-1}; ^{1}H NMR *δ* 8.07 (2H, brd, *J* = 8.0 Hz, H-2′, 6′), 7.58 (1H, brt, *J* = 8.0 Hz, H-4′), 7.46 (2H, brt, *J* = 8.0 Hz, H-3′, 5′), 6.25 (1H, brs, H-7), 5.82 (1H, d, *J* = 16.0 Hz, H-11), 5.72 (1H, d, *J* = 8.0 Hz, H-5), 5.65 (1H, brs, H-3), 5.64 (1H, dd, *J* = 16.0, 9.4 Hz, H-12), 5.30 (1H, s, H-17a), 5.20 (1H, brs, H-8), 5.17 (1H, s, H-17b), 4.95 (1H, brs, H-9), 3.73 (1H, m, H-4), 3.72 (1H, m, H-13), 3.25 (1H, d, *J* = 17.0 Hz, H-1α), 2.60 (1H, d, *J* = 17.0 Hz, H-1*β*), 2.34, 2.14 × 2, 1.98, 1.49, 1.37 (each 3H, s, acetate methyl), 1.58 (3H, s, H-16), 1.32 (3H, d, *J* = 6.6 Hz, H-20), 1.29 (3H, s, H-19), 0.92 (3H, s, H-18); ^{13}C NMR *δ* 204.2 (s, C-14), 170.2, 170.0, 169.6, 169.5, 169.0 × 2 (each s, acetate carbonyl), 165.2 (s, C-7′), 138.8 (s, C-6), 133.3 (d, C-11), 130.5 (s, C-1′), 130.5 (d, C-12), 129.7 × 2 (d, C-2′,

6′), 128.7 × 2 (d, C-3′, 5′), 121.0 (t, C-17), 90.6 (s, C-15), 87.3 (s, C-2), 81.1 (d, C-9), 77.3 (d, C-3), 71.1 (d, C-5), 70.0 (d, C-8), 66.8 (d, C-7), 48.7 (t, C-1), 46.4 (d, C-4), 43.7 (d, C-13), 40.1 (s, C-10), 25.4 (q, C-18), 23.5 (q, C-19), 20.1 (q, C-20), 18.8 (q, C-16), 22.3, 21.3, 20.9, 20.7, 20.4, 20.2 (each q, acetate methyl); EIMS *m*/*z* 756 [M^+] (0.1), 123 (13), 105 (100), 96 (37), 77 (10), 42 (89); HRESIMS *m*/*z* 779.2907 (calcd for $C_{39}H_{48}O_{15}Na$, 779.2891).

3*β*,5α,8α,9α,15*β*-Pentaacetoxy-7*β*-benzoyloxyjatropha-6(17),11*E*-dien-14-one (4): colorless crystals; mp 160−161 °C; $[\alpha]^{25}_D$ + 45.8° (*c* 0.46, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.45), 231 (4.25), 274 (3.08), 281 (3.00) nm; IR (KBr) ν_{max} 2972, 2934, 1745 (ester), 1722 (ketone), 1650, 1600 (phenyl), 1473, 1454, 1433, 1373, 1318, 1272, 1253, 1237, 1177, 1157, 1147, 1113, 1085, 1062, 1026, 716 cm^{-1}; ^1H NMR δ 8.08 (2H, brd, *J* = 8.0 Hz, H-2′, 6′), 7.59 (1H, brt, *J* = 8.0 Hz, H-4′), 7.47 (2H, brt, *J* = 8.0 Hz, H-3′, 5′), 6.13 (1H, brs, H-7), 5.85 (1H, d, *J* = 16.0 Hz, H-11), 5.71 (1H, d, *J* = 7.6 Hz, H-5), 5.62 (1H, dd, *J* = 16.0, 9.5 Hz, H-12), 5.59 (1H, d, *J* = 3.3 Hz, H-3), 5.30 (1H, s, H-17a), 5.19 (1H, s, H-17b), 5.18 (1H, brs, H-8), 4.94 (1H, brs, H-9), 3.53 (1H, dq, *J* = 9.5, 6.6 Hz, H-13), 3.25 (1H, dd, *J* = 7.6, 3.3 Hz, H-4), 2.85 (1H, dd, *J* = 15.6, 8.9 Hz, H-1α), 2.50 (1H, m, H-2), 2.15 (1H, d, *J* = 15.6 Hz, H-1*β*), 2.14 × 2, 1.98, 1.51, 1.44 (each 3H, s, acetate methyl), 1.34 (3H, d, *J* = 6.6 Hz, H-20), 1.28 (3H, s, H-19), 1.02 (3H, d, *J* = 6.6 Hz, H-16), 0.92 (3H, s, H-18); ^{13}C NMR δ 204.4 (s, C-14), 170.2, 170.0, 169.6, 168.9, 166.4 (each s, acetate carbonyl), 165.2 (s, C-7′), 139.2 (s, C-6), 135.3 (d, C-4′), 133.5 (d, C-11), 130.7 (d, C-12), 130.5 (s, C-1′), 129.7 × 2 (d, C-2′, 6′), 128.7 × 2 (d, C-3′, 5′), 120.6 (t, C-17), 91.3 (s, C-15), 81.2 (d, C-9), 77.0 (d, C-3), 71.1 (d, C-5), 70.2 (d, C-8), 67.0 (d, C-7), 49.6 (d, C-4), 44.4 (d, C-13), 43.8 (t, C-1), 40.1 (s, C-10), 38.0 (d, C-2), 14.1 (q, C-16), 25.4 (q, C-18), 23.8 (q, C-19), 20.4 (q, C-20), 21.4, 20.9, 20.7, 20.5, 20.2 (each s, acetate methyl); EIMS *m*/*z* 698 [M^+] (0.3), 123 (12), 121 (15), 105 (100), 96 (44), 77 (16), 42 (100); HRESIMS *m*/*z* 721.2837 (calcd for $C_{37}H_{46}O_{13}Na$, 721.2836).

3*β*,5α,7*β*,8α,9α,15*β*-Hexaacetoxy-2α-benzoyloxyjatropha-6(17),11*E*-dien-14-one (5): colorless crystals; mp 139−141 °C; $[\alpha]^{25}_D$ −59.1° (*c* 0.62, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.39), 230 (4.16), 274 (3.10), 281 (3.02) nm; IR (KBr) ν_{max} 3024, 2980, 2940, 2906, 1751 (ester), 1721 (ketone), 1602 (phenyl), 1452, 1434, 1374, 1317, 1280, 1248, 1228, 1178, 1135, 1108, 1075, 1035, 721 cm^{-1}; ^1H NMR δ 8.35 (2H, brd, *J* = 8.0 Hz, H-2′, 6′), 7.60 (1H, brt, *J* = 8.0 Hz, H-4′), 7.47 (2H, brt, *J* = 8.0 Hz, H-3′, 5′), 5.84 (1H, d, *J* = 3.7 Hz, H-3), 5.82 (1H, brs, H-7), 5.79 (1H, d, *J* = 7.4 Hz, H-5), 5.41 (1H, dd, *J* = 15.8, 9.5 Hz, H-12), 5.25 (1H, brs, H-17a), 5.20 (1H, d, *J* = 15.8 Hz, H-11), 5.10 (1H, brs, H-17b), 5.02 (1H, brs, H-8), 4.82 (1H, s, H-9), 3.69 (1H, d, *J* = 17.7 Hz, H-1α), 3.61 (1H, dd, *J* = 7.4, 3.7 Hz, H-4), 3.29 (1H, dq, *J* = 9.5, 7.1 Hz, H-13), 2.67 (1H, d, *J* = 17.7 Hz, H-1*β*), 2.16, 2.14, 2.10, 2.08, 2.05, 2.00 (each 3H, s, acetate methyl), 1.71 (3H, s, H-16), 1.12 (3H, s, H-19), 1.10 (3H, d, *J* = 7.1 Hz, H-20), 0.68 (3H, s, H-18); ^{13}C NMR δ 203.7 (s, C-14), 169.9, 169.5, 169.4 × 2, 169.3, 168.5 (each s, acetate carbonyl), 165.3 (s, C-7′), 139.1 (s, C-6), 134.8 (d, C-11), 133.2 (d, C-4′), 130.7 (s, C-1′), 130.6 (d, C-12), 130.1 × 2 (d, C-2′, 6′), 128.4 × 2 (d, C-3′, 5′), 120.4 (t, C-17), 91.1 (s, C-15), 88.6 (s, C-2), 81.3 (d, C-9), 78.6 (d, C-3), 70.5 (d, C-5), 69.6 (d, C-8), 66.8 (d, C-7), 46.6 (t, C-1), 46.4 (d, C-4), 43.5 (d, C-13), 40.0 (s, C-10), 25.0 (q, C-18), 23.4 (q, C-19), 19.5 (q, C-20), 18.9 (q, C-16), 21.5, 21.3 × 2, 20.9 × 2, 20.6 (each q, acetate methyl); EIMS *m*/*z* 756 [M^+] (0.2), 123 (30), 121 (27), 105 (100), 96 (90), 77 (24), 42 (100); HRESIMS *m*/*z* 779.2891 (calcd for $C_{39}H_{48}O_{15}$-Na, 779.2891).

3*β*,5α,7*β*,8α,15*β*-Pentaacetoxy-2α-benzoyloxyjatropha-6(17),11*E*-dien-9,14-dione (6): white crystals; mp 228−229 °C; $[\alpha]^{25}_D$ −215° (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 201 (4.38), 228 (4.17), 274 (3.08), 281 (3.02) nm; ^{13}C NMR δ 209.5 (s, C-14), 205.6 (s, C-9), 170.1, 169.8, 169.3, 169.1 (each s, acetate carbonyl), 165.5 (s, C-7′), 142.9 (s, C-6), 136.4 (d, C-11), 133.2 (d, C-12), 132.5 (d, C-4′), 132.2 (s, C-1′), 130.0 × 2 (d, C-2′, 6′), 128.1 × 2 (d, C-3′, 5′), 113.3 (t, C-17), 91.7 (s, C-15), 87.2 (s, C-2), 78.2 (d, C-3), 74.3 (d, C-8), 67.9 (d, C-5), 67.8 (d, C-7), 49.6 (d, C-4), 48.4 (s, C-10), 46.4 (t, C-1), 44.7 (d, C-13), 27.7 (q, C-18), 22.5 (q, C-19), 20.2 (q, C-20), 17.9 (q, C-16), 21.3, 21.1, 20.8, 20.5, 20.2 (each q, acetate methyl); EIMS *m*/*z* 712 [M^+] (0.2), 123 (27), 105 (76), 96 (74), 77 (14), 42 (100); HRESIMS *m*/*z* 735.2611 (cacld for $C_{37}H_{44}O_{14}Na$, 735.2629).

Single-Crystal X-ray Crystallography of 1. Suitable colorless prisms of **1** were obtained from a solution in MeOH. The crystal (0.48 × 0.48 × 0.48 mm) belongs to the trigonal system, space group *P*3(2)21 with a = 11.9291(14) Å, b = 11.921(14) Å, c = 43.899(8) Å, α = 90°, β = 90°, γ = 90°, V = 5410.1 (14) $Å^3$, Z = 6, D_{calcd} = 1.206 g/cm^3, λ(Mo Kα) = 0.71073 Å, F(000) = 2088, and T = 295(2) K. Intensity data were measured on a Siemens P4 diffractometer in the range 1.97° ≤ θ ≤ 25.00°. All 7021 reflections were collected. The structure was solved by direct methods and refined by full matrix least-squares on F^2 values for 6338 reflections with $I > 2\sigma(I)$. The final indices were R = 0.0471, R_w = 0.1016. The goodness-of-fit = 0.839. The crystal structure is shown in Figure 1.[21]

Biological Assays. Irritant doses 50% (ID_{50}) were determined on the mouse ear as described previously.[22] The redness of the ear was estimated 4 and 24 h after the application of solution in Me_2CO. TPA (12-*O*-tetradecanoylphorbol 13-acetate) was used as a reference: ID^4_{50} 0.012 μg/ear, ID^{24}_{50} 0.0085 μg/ear.

The B16 mouse melanoma cell line was kindly provided by Beijing University, People's Republic of China. The cytotoxicity against human tumor cells was measured with a MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test[23] against the B16 mouse melanoma cell line. VLB (Vinblastine) was used as a reference (IC_{50} = 3.6 μg/mL).

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Supporting Information Available: Selected tables of 2D NMR for compounds **3**−**5** are available free of charge via the Internet at http://pubs.acs.org.

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(21) Tables of X-ray crystallographic data of **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of these data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposited@ccdc.cam.ac.uk).

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