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New Bioactive Macrocyclic Diterpenoids from *Euphorbia helioscopia*

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Three new macrocyclic diterpenoids, euphoscopoids A – C (**1** – **3**), including two new jatrophanes and a new lathyrane, were isolated from the whole plant of *Euphorbia helioscopia*. Their structures were elucidated by spectroscopic methods. Antifeedant and cytotoxic activities of these isolates were evaluated. All compounds showed significant antifeedant activity against a generalist plant-feeding insect, *Helicoverpa armigera*, with EC_{50} values ranging from 2.05 to 4.34 μg/cm^2. In addition, compound **2** showed moderate cytotoxicity against tumor cell lines NCI-H1975, HepG2, and MCF-2, while compounds **1** and **3** were not active at 80 μM. The results suggested not only the defensive function of macrocyclic diterpenoids in *E. helioscopia* against insect herbivores, but also their potential applications as new natural insect antifeedants.

Keywords: *Euphorbia helioscopia*, Euphoscopoids A – C, Diterpenoids, Antifeedant activities, Cytotoxic activities.

Introduction

Euphorbia is the largest genus in the family Euphorbiaceae, which is well-known for the chemical diversity of terpenoids.[1] Diterpenoids are particularly rich and diversified in *Euphorbia* species, from which more than 650 diterpenoids have been isolated and identified, including jatrophanes, lathyranes, ingenanes, toglianes, abietanes, kauranes, pimaranes, daphnanes, casbbanes, myrsinanes, and so on.[1][2] Many of them possessed significant biological activities, including cytotoxic, antimicrobial, anti-inflammatory, and pesticidal activities.[3 – 6] In particular, some ingenol and phorbol esters are potent and promising diterpenoids that are under clinical investigation.[2] For example, prostratin, a non-tumor-promoter phorbol ester, has been known as a stimulator of protein kinase C, and might be a potential inductive adjuvant therapy for highly active antiretroviral therapy (HAART) through activation of latent HIV-1 expression.[7][8] Ingenol 3-angelate (PEP-005), a diterpenoid isolated from *Euphorbia peplus* with antitumor and antileukemic activities, has been approved by the FDA in January 2012 for treatment of actinic keratosis in a pre-cancerous skin condition.[9][10] Therefore, the diterpenoids in *Euphorbia* spp. have attracted extensive research interest in recent years.

Euphorbia helioscopia L., a traditional Chinese medicine with milky latex, is widely distributed in China and has been often used to treat malaria, osteomyelitis, edema, and ascites.[5][11] The methanolic and aqueous extracts of *E. helioscopia* exhibited significant anthelmintic effect on *Haemonchus contortus*,[12] but the responsible compounds still remain unknown. Phytochemical investigation on this plant has uncovered over 80 diterpenoids, especially macrocyclic jatrophane diterpenoids, most of which exhibited significant pharmacological activities.[5][13 – 16] Our previous study showed that the latex of *E. helioscopia* mainly contains five macrocyclic (jatrophane and lathyrane) diterpenoids with anti-inflammatory activity.[17] A continuing phytochemical investigation on the whole plant of *E. helioscopia* led to the isolation of three additional new diterpenoids (*Fig. 1*), and their antifeedant and cytotoxic activities were observed.

Results and Discussion

Compound **1** was obtained as colorless oil. Its molecular formula was deduced as $C_{31}H_{40}O_8$ according to the ^{13}C-NMR (*Fig. S2*) and HR-EI-MS data at *m/z* 540.2725 (M^+, $C_{31}H_{40}O_8^+$; calc. 540.2723). The ^{1}H- and ^{13}C-NMR spectra (*Table 1* and *Figs. S1* and *S2*) revealed the

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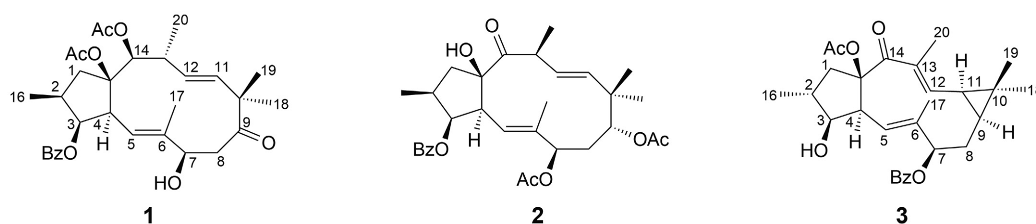

Figure 1. Chemical structures of compounds **1** – **3** from *Euphorbia helioscopia*.

presence of a typical benzoyloxy group (δ(H) 8.01 (*d*, *J* = 7.4 Hz, H–C(2′) and H–C(6′)), 7.61 (*t*, *J* = 7.4 Hz, H–C(4′)), and 7.50 (*t*, *J* = 7.4 Hz, H–C(3′) and H–C(5′)); δ(C) 166.1, 133.6, 131.5, 130.2, and 129.3), and two acetoxy groups (δ(H) 2.15 (*s*, AcO–C(15)) and 2.13 (*s*, AcO–C(14)); δ(C) 170.5, 169.9, 22.2, and 20.9). Other signals indicated the existence of a trisubstituted double bond (δ(H) 5.62 (*d*, *J* = 10.4 Hz, H–C(5)); δ(C) 120.6 and 137.5), a *trans*-disubstituted double bond (δ(H) 5.32 (*dd*, *J* = 7.2, 15.4 Hz, H–C(12)) and 5.35 (*d*,

Table 1. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectral data of compounds **1** – **3** in (D_6)acetone

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	2.47 – 2.51 (*m*, H_α), 1.90 – 1.96 (*m*, H_β)	47.4 (*t*)	2.55 – 2.63 (*m*, H_α), 1.80 – 1.84 (*m*, H_β)	44.4 (*t*)	2.53 – 2.55 (*m*, H_α), 2.18 – 2.21 (*m*, H_β)	41.4 (*t*)
2	2.18 – 2.23 (*m*)	39.2 (*d*)	2.66 – 2.73 (*m*)	37.3 (*d*)	2.13 – 2.17 (*m*)	41.7 (*d*)
3	5.41 (*t*, *J* = 3.8)	81.8 (*d*)	5.49 (*t*, *J* = 4.9)	80.4 (*d*)	3.73 – 3.77 (*m*)	81.5 (*d*)
4	3.42 (*dd*, *J* = 4.5, 10.4)	46.7 (*d*)	3.42 (*dd*, *J* = 5.6, 10.5)	56.0 (*d*)	2.61 (*dd*, *J* = 6.8, 10.9)	49.8 (*d*)
5	5.62 (*d*, *J* = 10.4)	120.6 (*d*)	5.09 (*d*, *J* = 10.5)	119.5 (*d*)	6.32 (*d*, *J* = 10.9)	124.2 (*d*)
6	–	137.5 (*s*)	–	137.7 (*s*)	–	142.0 (*s*)
7	4.09 (*t*, *J* = 7.6)	73.8 (*d*)	4.85 (*d*, *J* = 9.2)	73.2 (*d*)	5.08 (*dd*, *J* = 2.9, 11.6)	78.2 (*d*)
8	3.18 (*dd*, *J* = 1.5, 14.6, H_α), 2.42 (*dd*, *J* = 7.6, 14.6, H_β)	39.9 (*d*)	2.07 – 2.11 (*m*, H_α), 1.86 – 1.90 (*m*, H_β)	33.5 (*d*)	2.50 – 2.53 (*m*, H_α), 2.04 – 2.07 (*m*, H_β)	33.9 (*d*)
9	–	212.9 (*s*)	4.73 (*dd*, *J* = 2.9, 5.8)	75.8 (*d*)	1.31 – 1.35 (*m*)	30.6 (*d*)
10	–	51.4 (*s*)	–	39.9 (*s*)	–	24.8 (*s*)
11	5.35 (*d*, *J* = 15.4)	131.5 (*d*)	5.26 (*d*, *J* = 15.8)	136.8 (*d*)	1.36 (*dd*, *J* = 8.2, 11.6)	30.9 (*d*)
12	5.32 (*dd*, *J* = 7.2, 15.4)	132.9 (*d*)	5.59 (*dd*, *J* = 9.6, 15.8)	132.4 (*d*)	6.74 (*d*, *J* = 11.6)	145.7 (*d*)
13	2.43 – 2.46 (*m*)	41.7 (*d*)	3.95 – 4.00 (*m*)	44.9 (*d*)	–	133.4 (*s*)
14	5.91 (*d*, *J* = 8.5)	76.2 (*s*)	–	214.6 (*s*)	–	195.1 (*s*)
15	–	90.8 (*s*)	–	90.6 (*s*)	–	96.7 (*s*)
16	0.88 (*d*, *J* = 6.7)	14.0 (*q*)	1.00 (*d*, *J* = 6.6)	14.0 (*q*)	1.01 (*d*, *J* = 6.8)	18.5 (*q*)
17	1.75 (*s*)	16.1 (*q*)	1.75 (*s*)	16.2 (*q*)	1.61 (*s*)	19.0 (*q*)
18	1.22 (*s*)	24.5 (*q*)	0.96 (*s*)	21.7 (*q*)	1.24 (*s*)	29.0 (*q*)
19	1.11 (*s*)	20.0 (*q*)	0.94 (*s*)	24.6 (*q*)	1.19 (*s*)	16.6 (*q*)
20	0.92 (*d*, *J* = 6.7)	21.3 (*q*)	1.15 (*d*, *J* = 6.9)	19.9 (*q*)	1.81 (*s*)	12.4 (*q*)
PhCOO	–	166.1 (*s*)	–	165.9 (*s*)	–	166.1 (*s*)
1′	–	131.5 (*s*)	–	131.3 (*s*)	–	131.7 (*s*)
2′/6′	8.01 (*d*, *J* = 7.4)	130.2 (*d*)	8.11 (*d*, *J* = 7.4)	130.3 (*d*)	8.01 (*d*, *J* = 7.4)	130.2 (*d*)
3′/5′	7.50 (*t*, *J* = 7.4)	129.3 (*d*)	7.55 (*t*, *J* = 7.4)	129.5 (*d*)	7.50 (*t*, *J* = 7.4)	129.3 (*d*)
4′	7.61 (*t*, *J* = 7.4)	133.6 (*d*)	7.61 (*t*, *J* = 7.4)	133.7 (*d*)	7.61 (*t*, *J* = 7.4)	133.7 (*d*)
7-AcO	–	–	1.35 (*s*)	20.3 (*q*), 169.5 (*s*)	–	–
9-AcO	–	–	1.94 (*s*)	21.0 (*q*), 169.9 (*s*)	–	–
14-AcO	2.13 (*s*)	20.9 (*q*), 170.5 (*s*)	–	–	–	–
15-AcO	2.15 (*s*)	22.2 (*q*), 169.9 (*s*)	–	–	2.03 (*s*)	21.7 (*q*), 170.3 (*s*)
7-OH	4.55 (*d*, *J* = 7.6)	–	–	–	–	–

δ in ppm, *J* in Hz.

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$J = 15.4$ Hz, H–C(11)); δ(C) 132.9 and 131.5), three oxymethines (δ(H) 5.91 (*d*, $J = 8.5$ Hz, H–C(14)), 5.41 (*t*, $J = 3.8$ Hz, H–C(3)), and 4.09 (*t*, $J = 7.6$ Hz, H–C(7)); δ(C) 81.8, 76.2, and 73.8), three tertiary methyls (δ(H) 1.75 (*s*, Me(17)), 1.22 (*s*, Me(18)), and 1.11 (*s*, Me(19)), δ(C) 24.5, 20.0, and 16.1), and two secondary methyls (δ(H) 0.92 (*d*, $J = 6.7$ Hz, Me(20)) and 0.88 (*d*, $J = 6.7$ Hz, Me(16)), δ(C) 21.3 and 14.0). From the above evidence, compound **1** was deduced to be a jatrophane diterpenoid.[5][18][19] In the HMBC spectrum of **1** (*Fig. 2* and *Fig. S4*), the ^{1}H-^{13}C long-range correlation of the H-atom at δ(H) 5.41 (H–C(3)) with the carbon at δ(C) 166.1 disclosed that the benzoyloxy group was connected to C(3). The HMBC correlations of CH_2(8), H–C(11), Me(18), and Me(19) with a keto carbon at δ(C) 212.9 indicated that the keto group was assignable to C(9). On the basis of the HMBC correlation of HO–C(7) (δ(H) 4.55) with C(7) (δ(C) 73.8) and C(8) (δ(C) 39.9), the only OH group was placed at C(7). Similarly, the HMBC correlation between the H-atom at δ(H) 5.91 (H–C(14)) and the carbonyl carbon at δ(C) 170.5 indicated the presence of one acetoxy group at C(14). The other acetoxy group could only be assigned to C(15) because no HMBC correlation was observed between the carbonyl carbon (δ(C) 169.9) and any H-atoms from the jatrophane skeleton, which was further supported by the dramatically downfield shifted C(15) (δ(C) 90.8) comparing with the chemical shift (δ(C) 83.6) of euphornin G, which is a similar jatrophane diterpenoid with a OH group at C(15).[5]

The relative configuration of **1** was deduced from the coupling constant pattern and ROESY spectrum (*Fig. S6*). Since the angular H-atom H–C(4) in jatrophane diterpenoids is exclusively α-oriented,[14][19] the ROESY correlations of H–C(4)/$H_α$–C(1), H–C(4)/H–C(2), H–C(4)/H–C(3), $H_α$–C(1)/H–C(14), H–C(14)/Me(20), Me(18)/$H_α$–C(8), and $H_α$–C(8)/H–C(7) indicated an α-orientation of all these H-atoms. Therefore, the structure of **1** was elucidated as 14β,15β-diacetoxy-3β-(benzoyloxy)-7β-hydroxy-9-oxojatropha-5*E*,11*E*-diene and was named euphoscopoid A.

Compound **2**, colorless oil, was assigned a molecular formula of $C_{31}H_{40}O_8$ from its ^{13}C-NMR (*Fig. S8*) and HR-EI-MS data at *m/z* 540.2734 (M^+, $C_{31}H_{40}O_8^+$; calc. 540.2723). The 1D-NMR spectra of **2** (*Table 1* and *Figs. S7* and *S8*) showed close similarity with those of **1**, including the jatrophane skeleton and functionalities (two double bonds, a benzoyloxy, a hydroxy, a keto, and two acetoxy groups). By inspection of the HSQC (*Fig. S9*) and HMBC spectra (*Fig. 2* and *Fig. S10*), the four oxygenated carbons at δ(C) 80.4, 73.2, 75.8, and 90.6 were assignable to C(3), C(7), C(9), and C(15), respectively, and the carbonyl carbon at δ(C) 214.6 was ascribable to C(14). In the HMBC spectrum, correlations of H–C(7) (δ(H) 4.85) and H–C(9) (δ(H) 4.73) with the ester carbonyl carbons at δ(C) 169.5 and 169.9, respectively, indicated that the two acetoxy groups were located at C(7) and C(9). Similarly, the simultaneous HMBC correlations of the H-atom signals at δ(H) 5.49 (H–C(3)) and 8.11 (H–C(2′)/H–C(6′)) with the ester carbonyl carbon at δ(C) 165.9 indicated that the benzoyloxy group was attached to C(3). Consequently, the OH group could be assigned to C(15). In the ROESY spectrum, the correlations of H–C(4)/$H_α$–C(1), H–C(4)/H–C(2), H–C(4)/H–C(3), H–C(13)/H–C(4), Me(18)/H–C(11), Me(18)/$H_α$–C(8), and $H_α$–C(8)/H–C(7) indicated that H–C(2), H–C(3), H–C(7), and H–C(13) were all α-oriented. The ROESY correlations (*Fig. S12*) of H–C(9)/Me(19) and Me(19)/H–C(12) indicated that H–C(9) was β-oriented. Therefore, the structure of **2** was determined as 7β,9α-diacetoxy-3β-(benzoyloxy)-15β-hydroxy-14-oxojatropha-5*E*,11*E*-diene and was named euphoscopoid B.

Compound **3**, colorless oil, gave a molecular formula of $C_{29}H_{36}O_6$, as established by its ^{13}C-NMR (*Fig. S14*) and HR-EI-MS data at *m/z* 480.2501(M^+, $C_{29}H_{36}O_6^+$; calc. 480.2512). The ^{1}H-NMR spectrum of **3** (*Table 1*

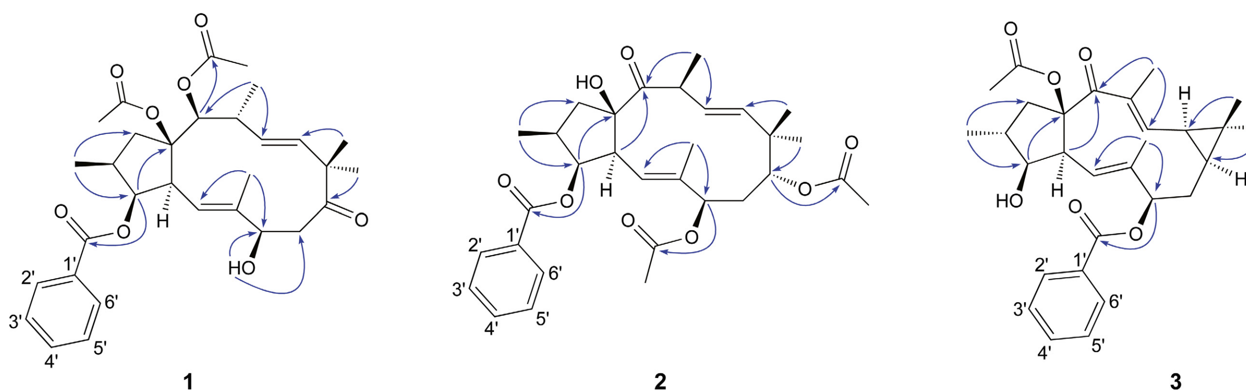

Figure 2. Significant HMBC correlations (H → C) of compounds **1** – **3**.

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and *Fig. S13*) revealed a benzoyl group (δ(H) 8.01 (*d*, *J* = 7.4 Hz, H–C(2′) and H–C(6′)), 7.61 (*t*, *J* = 7.4 Hz, H–C(4′)), and 7.50 (*t*, *J* = 7.4 Hz, H–C(3′) and H–C(5′))), an acetyl group (δ(H) 2.03 (*s*, AcO–C(15))), two olefinic methines (δ(H) 6.74 (*d*, *J* = 11.6 Hz, H–C(12)) and 6.32 (*d*, *J* = 10.9 Hz, H–C(5))), four tertiary methyls (δ(H) 1.81 (*s*, Me(20)), 1.61 (*s*, Me(17)), 1.24 (*s*, Me(18)), and 1.19 (*s*, Me(19))), and one secondary methyl (δ(H) 1.01 (*d*, *J* = 6.8 Hz, Me(16))). The ^{13}C-NMR and DEPT spectra (*Table 1* and *Fig. S14*) displayed 29 carbon resonances. Besides the resonances for the acetoxy and benzyloxy groups, the remaining ^{13}C-NMR data demonstrated five methyls, two methylenes, eight methines, and five quaternary carbons, suggesting that **3** might be a diterpenoid. The characteristic signals at δ(H) 1.31 – 1.35 (*m*, H–C(9)), 1.36 (*dd*, *J* = 8.2 and 11.6 Hz, H–C(11)), 1.24 (*s*, Me(18)), 1.19 (*s*, Me(19)) and δ(C) 30.9, 30.6, 29.0, 24.8, and 16.6 suggested the existence of a *gem*-dimethyl-substituted cyclopropane ring,[11] indicative of a lathyrane-type diterpenoid similar to altotibetol.[20] The only difference between **3** and altotibetol was that the OH group at C(7) in altotibetol was replaced by a benzyloxy group in **3**. In the HMBC spectrum of **3** (*Fig. 2* and *Fig. S16*), the ^{1}H-^{13}C correlation of the H-atom signal at δ(H) 5.08 (H–C(7)) with the carbonyl carbon at δ(C) 166.1 supported that the benzoyloxy group was connected to C(7). The ROESY spectrum (*Fig. S18*), coupling constant pattern, and chemical shifts of chrial carbons of **3** indicated that the relative configurations of all stereogenic centers in **3** were the same as those in altotibetol. Consequently, compound **3** was identified as 15β-acetoxy-7β-(benzoyloxy)-3β-hydroxy-14-oxolathyra-5*E*,12*E*-diene and was named euphoscopoid C.

Since the macrocyclic diterpenoids showed potent antifeedant activity against the generalist plant-feeding insect *Helicoverpa armigera* and functioned as constitutive defense metabolites for *E. peplus*,[21] it was interesting to know whether these compounds with similar structures also play the same function in *E. helioscopia*. Compounds **1** – **3** were therefore tested for their antifeedant activity against *H. armigera*. As shown in *Table 2*, all three compounds showed significant antifeedant activity, with EC_{50} values of 2.97 ± 0.30, 2.05 ± 0.11, and 4.34 ± 0.16 μg/cm^2 for **1** – **3**, respectively (neem oil: EC_{50} = 5.62 ± 0.29 μg/cm^2). In addition, considering that a number of *Euphorbia* diterpenoids including jatrophanes and lathyranes were reported to be cytotoxic,[1][2] the cytotoxic activity of compounds **1** – **3** were also evaluated against three human cancer cell lines, human lung adenocarcinoma cells (NCI-H1975), human hepatocellular carcinoma cells (HepG2), and human breast adenocarcinoma cells (MCF-7). Compound **2** exhibited moderate cytotoxicity, with IC_{50} of 34.12 ± 0.57, 31.38 ± 1.29 and 30.23 ± 4.18 μM, respectively (*Table 3*). In contrast, compounds **1** and **3** were inactive at 80 μM.

Table 2. Antifeedant activity of compounds **1** – **3** against *Helicoverpa armigera*

Compound	EC_{50} (μg/cm^2 ± SD) (*n* = 5)
1	2.97 ± 0.30
2	2.05 ± 0.11
3	4.34 ± 0.16
Neem oil	5.62 ± 0.29

Conclusions

Previous phytochemical investigation has revealed that diterpenoids, including jatrophane, ingenane, lathyrane types, were the major constituents of genus *Euphorbia*.[1][2] Biological study on these macrocyclic diterpenoids has revealed their broad pharmacological especially cytotoxic, antimicrobial, and anti-inflammatory activities, as well as defensive roles.[1 – 3][21] In this work, three new compounds, belonging to jatrophane (**1** and **2**) and lathyrane (**3**) diterpenoids, were isolated from the whole plant of *E. helioscopia*. Although cytotxoic activity of jatrophane and lathyrane diterpenoids from *Euphorbia* has been reported,[14][19][22] only compound **2** was moderately active while the other two compounds were inactive, therefore the apoptosis and other related mechanisms of these diterpenoids were not investigated. Interestingly, all three compounds exhibited significant antifeedant activity against the generalist insect herbivore *H. armigera*, suggesting that they should also be constitutive defense chemicals in *E. helioscopia* against insect herbivores. The results implied not only the natural function of the macrocyclic diterpenoids in *E. helioscopia*, but also their potential application as new natural insect antifeedants.

Experimental Section

General

TLC: Silica gel *GF254* (SiO_2; *Qingdao Dingkang Inc.*, P. R. China); spots were visualized under UV light and by spraying with 5% H_2SO_4 in EtOH followed by heating. Column chromatography (CC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Marine Chemical Factory*, P. R. China), or *Sephadex LH-20* (*Amersham Phamacia*

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Table 3. Cytotoxicity of compounds **1** – **3**

Compound	IC_{50} (µM ± SD) (n = 3)		
	NCI-H1975	HepG2	MCF-7
1	> 80	> 80	> 80
2	34.12 ± 0.57	31.38 ± 1.29	30.23 ± 4.18
3	> 80	> 80	> 80
Taxol	(4.79 ± 0.18) × 10^{-3}	(25.60 ± 1.70) × 10^{-3}	(41.15 ± 6.73) × 10^{-3}

Biotech, Sweden), or *MCI* gel *CHP-20P* (75 – 150 µm; *Mitsubishi Chemical Corp.*, Tokyo, Japan). Semi-preparative HPLC was performed on an *Agilent 1200* series instrument (*Agilent*, Santa Clara, CA, USA) equipped with a quaternary pump, an autosampler, a vacuum degasser, a thermostatted column compartment, a diode array detector and a *Zorbax SB-C$_{18}$* column (5 µm, 9.4 × 250 mm). Optical rotations were obtained on a *Jasco P-1020* spectropolarimeter (*Jasco*, Tokyo, Japan). UV Spectroscopic data were measured on a *Shimadzu-210A* double-beam spectrophotometer (*Shimadzu*, Tokyo, Japan); λ_{max} (log ε) in nm. IR Spectra of samples in KBr discs were recorded on a *Bruker-Tensor-27* spectrometer (*Bruker*, Karlsruhe, Germany) with KBr pellets; v in cm^{-1}. NMR Spectra were measured on a *Bruker AM-400* spectrometer (*Bruker*) in (D$_6$)acetone; δ in ppm rel. to Me_4Si as internal standard, J in Hz. Mass spectra were obtained on a *Waters AutoSpec Premier P776* spectrometer (*Waters Corp.*, Massachusetts, USA); in *m/z*.

Plant Material

The whole plant of *E. helioscopia* was purchased from local herbal medicine of Kunming, in August 2014. An authentic sample (KB-2014-0819) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation

Dried and powered whole plant of *E. helioscopia* (35.0 kg) was extracted with EtOH at r.t. The crude extract was concentrated *in vacuo*, and then partitioned between AcOEt and H_2O (3 × 5 l). The AcOEt fraction (1.7 kg) was chromatographed on a silica gel column, eluting successively with a solvent gradient system ($CHCl_3$/acetone, 100:0 – 0:100) to give ten fractions (*Frs. 1* – *10*). *Fr. 3* (90 g) was further subjected to column chromatography on silica gel, eluting with petroleum ether/acetone (90:10 – 0:100) to afford seven subfractions (*Frs. 3.1* – *3.7*). *Fr. 3.4* (18 g) was subjected to *MCI* gel column chromatography (MeOH/H_2O, 60:40 – 100:0) to obtain five subfractions (*Frs. 3.4.1* – *3.4.5*). *Fr. 3.4.2* (MeOH/H_2O 70:30) (2 g) was chromatographed on *Sephadex LH-20* column (acetone as eluent) and then purified by semi-preparative HPLC (MeOH/H_2O 60:40, 3 ml/min), to give **1** (12 mg, t_R 15.3 min) and **2** (9 mg, t_R 16.1 min). *Fr. 3.4.3* (MeOH/H_2O, 75:25) was subjected to column chromatography on silica gel (petroleum ether/AcOEt 2:1) and purified by semi-preparative HPLC (MeOH/H_2O 60:40, 3 ml/min), to give **3** (7 mg, t_R 13.9 min).

Euphoscopoid A (= **14*β*,15*β*-Diacetoxy-3*β*-(benzoyloxy)-7*β*-hydroxy-9-oxojatropha-5*E*,11*E*-diene 1**). Colorless oil. $[\alpha]_D^{25}$ = −41.3 (c = 0.15, MeOH). UV (MeOH): 227 (4.20), 273 (3.04). IR: 3452, 2973, 1746, 1635, 1605, 1451, 1244, 1114, 1026, 713. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 540.2725 (M^+, $C_{31}H_{40}O_8^+$; calc. 540.2723).

Euphoscopoid B (= **7*β*,9*α*-Diacetoxy-3*β*-(benzoyloxy)-15*β*-hydroxy-14-oxojatropha-5*E*,11*E*-diene 2**). Colorless oil. $[\alpha]_D^{25}$ = +33.1 (c = 0.15, MeOH). UV (MeOH): 201 (4.18), 272 (3.13). IR: 3446, 2971, 1717, 1633, 1452, 1277, 1117, 1023, 714. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 540.2734 (M^+, $C_{31}H_{40}O_8^+$; calc. 540.2723).

Euphoscopoid C (= **15*β*-Acetoxy-7*β*-(benzoyloxy)-3*β*-hydroxy-14-oxolathyra-5*E*,12*E*-diene 3**). Colorless oil. $[\alpha]_D^{25}$ = +30.7 (c = 0.19, MeOH). UV: 201 (4.02), 228 (3.99), 274 (2.88). IR: 3442, 2928, 1717, 1646, 1619, 1452, 1273, 1112, 1068, 716. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-EI-MS: 480.2501 (M^+, $C_{29}H_{36}O_6^+$; calc. 480.2512).

Cytotoxic Assay

The *in vitro* cytotoxicities of compounds **1** – **3** were determined by MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2*H*-tetrazolium) assay.[23][24] Three human cancer cell lines (NCI-H1975, HepG2, and MCF-7) were selected for the assay. The tested cell lines were seeded in 96-well plates, and then the plates were incubated for 24 h at 37 °C in 5% CO_2 incubator. Subsequently, the compounds

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were added at a dosage of 0.128 – 80 μM. After 72 h, MTS was added to the culture medium and the absorbance at 490 nm was measured with a microplate reader (*SpectraMax plus 384*, MD, USA). Each experiment was carried out in triplicate. The IC_{50} values were calculated with the Graphpad Prism 5.01 software. Taxol was used a positive control.

Antifeedant Activity

The insect cotton bollworm (*H. armigera*) was purchased from the Pilot-Scale Base of Bio-Pesticides, Institute of Zoology, Chinese Academy of Sciences. A dual-choice bioassay as previously described was performed for antifeedant test.[25] In brief, fresh leaf discs were cut from *Brassica chinensis* by a borer (0.9 mm in diameter). Treated leaf discs were painted with 10 μl of acetone solution containing the test compound, and control leaf discs with the same amount of acetone. After air drying, two tested leaf discs and two control ones were set in alternating position in the same *Petri* dish (90 mm in diameter) with moistened filter paper at the bottom. Two 3rd instar larvae which were starved 4 – 5 h were placed at the center of the *Petri* dish. Compound was tested in five different concentrations, started from 62.5 to 1000 μg/ml. Five replicates were run for each treatment. After feeding for 24 h, areas of leaf discs consumed were measured. The insect antifeedant potency of the tested compound was evaluated in terms of the EC_{50} value which was determined by Probit analysis for each insect species. Commercial neem oil was used as positive control.

Supplementary Material

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.201700327.

Acknowledgements

We thank the members of the Analytical Center of Kunming Institute of Botany, Chinese Academy of Sciences, for spectral measurements. This research was financially supported by *973 Program on Biological Control of Key Crop Pathogenic Nematodes* (2013CB127505), the *NSFC-Yunnan Joint Fund* (U1502262), the *National Natural Science Foundation of China* (31200263 and 31470169), the *National Science Fund for Distinguished Young Scholars* (31525005), the *Youth Innovation Promotion association of Chinese Academy of Sciences* (awarded to *Y. Liu* and *S.-H. Luo*), the *'Western Light' Program of Chinese Academy of Sciences* (awarded to *S.-H. Luo*), and *Yunnan University Program for Excellent Young Talents* awarded to *X.-M. Niu* (XT412003).

Author Contribution Statement

Juan Hua and *Shi-Hong Luo* isolated and identified the compounds, *Yan-Chun Liu* conducted cytotoxic assay, *Juan Hua*, *Yan-Yun Tan*, *Ling Feng*, and *Chao-Jiang Xiao* tested antifeedant activity. *Shi-Hong Luo*, *Yan Liu*, *Ke-Qin Zhang*, *Sheng-Hong Li,* and *Xue-Mei Niu* designed the study and wrote the manuscript.

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Received July 8, 2017
Accepted July 31, 2017

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