Supporting Information

Jatrophalactam, A Novel Diterpenoid Lactam Isolated from *Jatropha curcas*

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S1. Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. IR Spectra were recorded on a Nicolet FTIR 750 spectrophotometer; in cm^{-1} . Melting point was measured on a BüCHI Melting Point B-540 apparatus. ^1H - and ^{13}C -NMR spectra were determined on a Bruker AMX-300/400 NMR spectrometer with TMS as an internal standard. HREIMS were measured on a Finnigan MAT 95 mass spectrometer. ESIMS were measured on a Bruker Esquire 3000 plus spectrometer. Single-crystal X-ray diffraction was measured on a Bruker APEX SMART-CCD diffractometer. All solvents used were of anal. grade (Shanghai Chemical Plant, Shanghai, P. R. China). Column chromatography (CC): silica gel H (200–300 mesh) was purchased from Qingdao Marine Chemical Ltd., Qingdao, P. R. China, MCI gel CHP 20P (75–150 μm) was purchased from Mitsubishi Chemical Ind., Tokyo, Japan), RP-18 (20-45 μm) was purchased from Fuji Silysia Chemical Ltd., silica gel GF₂₅₄ for TLC was purchased from Yantai Huiyou Inc., Yantai, P. R. China. 96-well plates was purchased from Costar. RMPI 1640 was purchased from Gibco. McCoy's 5A was purchased from SIGMA. Newborn Calf Serum was purchased from Hanzhou Sijiqing Biological Engineering Material Co., Ltd. MTT and DMSO were purchased from Amresco.

Plant Material. The roots of *J. curcas* were collected from Panzhihua City, Sichuan Province, China, in Dec 2006 and identified by Xian-Feng He (Panzhihua Institute of Forest Research, Sichuan, 617027, P. R. China). A voucher sample (20061214) was deposited at the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai, China.

Extraction and Isolation. The dried and powdered roots of *J. curcas* (3 kg) were extracted with MeOH at room temperature three times, which afforded a dark residue (245 g) after evaporation under reduced pressure. The residue was suspended in H_2O and then partitioned with petroleum ether and EtOAc successively. The EtOAc extract (35 g) was subjected to a MCI gel column chromatography eluted with MeOH– H_2O (20:80 to 100:0) to give six fractions A–F. Fraction D (10.2 g) was chromatographed over a silica gel column and then an RP-18 column to yield **jatropha lactam (1, 35mg)**.

Jatrophalactam (I): C₂₀H₂₉NO₃, colorless crystal (EtOAc), mp 185–186 °C; [α]_D²⁰ + 148.7 (*c* 0.3, MeOH); IR (KBr) ν_{max} 3392, 3266, 2933, 2969, 1704, 1664, 1457, 1384, 1093, 985 cm⁻¹; ¹H and ¹³C NMR see Table 1; ESI-MS *m/z* 332.2 [M + H]⁺, 685.4 [2M + Na]⁺, 330.1 [M – H]⁻; HREIMS *m/z* 331.2153 [M]⁺ (calcd for C₂₀H₂₉NO₃, 331.2147).

Cytotoxicity assay. In 96-well plates, each well was plated with 5×10⁵ cells (human lung cancer cell line A549, human colon cancer cell line HT-29 and human epidermal squamous cell line A431, respectively). After cell attachment overnight, the medium was removed, and each well was treated with 100 μ L of medium containing 0.2% DMSO or appropriate concentration of test compounds (10 μ M as stock solution of a compound in DMSO and serial dilutions). Cells were treated at 37 °C for 4 h in a humidified incubator at 5% CO₂ first, and then were allowed to grow for another 72 h after the medium was changed to fresh Dulbecco's Modified Eagle Medium (DMEM). MTT (Sigma) was dissolved in serum free-medium or PBS at 0.5 mg/mL and sonicated briefly. In the dark, 20 μ L of MTT/medium was added into each well after the medium was removed from wells, and incubated at 37 °C for 4 h. Upon removal of MTT/medium, 150 μ L of DMSO was added to each well, and shaken at 60 rpm for 10 min to dissolve the precipitate. The assay plate was read at 490 nm using a microplate reader. 5-fluorouracil was used as the positive control.

S2. ^1H NMR spectrum (300 MHz) of jatropha lactam (1) in CDCl_3 .

S3. ^{13}C NMR spectrum (75 MHz) of jatrophenolactam (1) in CDCl_3 .

S4. HSQC spectrum (400 MHz) of jatropha lactam (1) in CDCl₃.

S5. HMBC spectrum (400 MHz) of jatrophalactam (1) in CDCl₃.

JCE-18 CDCl₃ HMBC

S6. ^1H - ^1H COSY spectrum (300 MHz) of jatropha lactam (1) in CDCl_3 .

S7. ROESY spectrum (600 MHz) of jatropha lactam (1) in CDCl₃.

S8. ESIMS spectrum (positive mode) of jatrophalactam (1).

Display Report

Analysis Info

Analysis Name 011-1901.D
 Method Copy of SOPMSMSP.M
 Sample Name hmh-JCE-18
 Comment W

Acquisition Date 07/02/07 15:43:36
 Operator Administrator
 Instrument esquire3000plus

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	100 m/z	Scan End	1750 m/z
Capillary Exit	158.5 Volt	Skim 1	40.0 Volt	Trap Drive	85.4
Accumulation Time	9656 經	Averages	3 Spectra	Auto MS/MS	on

S9. ESIMS spectrum (negative mode) of jatrophalactam (1).

Display Report

Analysis Info

Analysis Name 011-2901.D
Method Copy of SOPMSMSN.M
Sample Name hmh-JCE-18
Comment W

Acquisition Date 07/02/07 18:32:08
Operator Administrator
Instrument esquire3000plus

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Negative	Alternating Ion Polarity	off
Mass Range Mode	Std/Normal	Scan Begin	100 m/z	Scan End	1750 m/z
Capillary Exit	-158.5 Volt	Skim 1	-40.0 Volt	Trap Drive	92.9
Accumulation Time	7528 罫	Averages	3 Spectra	Auto MS/MS	on

S10. IR spectrum (KBr) of jatrophalactam (1).

S11. X-ray Crystallographic Analysis of jatrophalactam (1).

Crystallographic data of compound **1**: colorless crystal (EtOAc), C₂₀H₂₉NO₃, MW = 331.44, monoclinic, space group *P*2(1); *a* = 6.872 (4), *b* = 9.187 (5), *c* = 15.595 (9) Å, α = 90.00, β = 97.384 (9), γ = 90.00, *V* = 976.4 (10) Å³, *Z* = 2, ρ_{calc} = 1.127 g/cm³, crystal dimensions 0.02×0.07×0.10 mm was used for measurement on a Bruker APEX SMART-CCD diffractometer with a graphite monochromator. The total number of reflections measured was 4813, of which 3157, were observed, $I > 2\sigma(I)$. Final indices: *R* = 0.0702, *wR*2 = 0.1990. The crystal structure of **1** was solved by direct method SHLXS-97 (Sheldrick, 1990) and expanded using difference Fourier technique, refined by the program SHLXL-97 (Sheldrick, 1997) and the full-matrix least-squares calculations. Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 743516). Copies of the data can be obtained, free of charge, on application to the director, CCDC 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or email: deposit@ccdc.cam.ac.uk).

Table 1. The atom site of jatrophalactam (**1**)

	X	Y	Z	Uiso
N1	0.6771(6)	0.3385(5)	0.0059(3)	0.0507(11)
H1	0.633(7)	0.405(6)	-0.037(3)	0.045(15)
O1	0.3709(5)	0.2560(4)	0.0241(2)	0.0611(10)
O2	0.8519(8)	0.8094(4)	0.1425(3)	0.0806(13)
H2	0.8148	0.7946	0.0912	0.121
O3	0.4545(5)	-0.0058(4)	0.1528(2)	0.0561(10)
C1	0.5502(8)	0.2573(5)	0.0414(3)	0.0451(12)
C2	0.8599(6)	0.2003(5)	0.1073(3)	0.0405(11)
C3	0.8796(7)	0.3193(6)	0.0422(3)	0.0504(13)
H3	0.9610	0.2884	-0.0017	0.061
C4	0.9652(7)	0.4505(5)	0.0918(3)	0.0486(13)
H4	1.1011	0.4597	0.1001	0.058
C5	0.8630(8)	0.5520(6)	0.1237(3)	0.0500(13)
H5	0.7282	0.5455	0.1075	0.060
C6	0.9266(8)	0.6776(6)	0.1823(3)	0.0567(14)
C7	0.8347(9)	0.6581(6)	0.2662(3)	0.0677(16)

H7A	0.6954	0.6400	0.2512	0.081
H7B	0.8486	0.7487	0.2983	0.081
C8	0.9219(9)	0.5349(6)	0.3258(3)	0.0677(17)
H8A	0.9537	0.4535	0.2905	0.081
H8B	1.0430	0.5687	0.3585	0.081
C9	0.7875(10)	0.4834(6)	0.3872(3)	0.0689(17)
H9	0.7385	0.5607	0.4221	0.083
C10	0.8216(11)	0.3416(7)	0.4330(3)	0.0717(18)
C11	0.6452(9)	0.3609(6)	0.3648(3)	0.0643(16)
H11	0.5216	0.3730	0.3891	0.077
C12	0.6188(9)	0.2960(6)	0.2757(3)	0.0614(16)
H12A	0.7188	0.3368	0.2442	0.074
H12B	0.4927	0.3279	0.2467	0.074
C13	0.6279(8)	0.1274(5)	0.2679(3)	0.0520(14)
H13	0.7649	0.0994	0.2851	0.062
C14	0.5779(7)	0.0844(5)	0.1740(3)	0.0426(12)
C15	0.6711(7)	0.1702(5)	0.1106(3)	0.0391(11)
C16	1.0391(8)	0.1419(6)	0.1602(4)	0.0617(15)
H16A	1.0653	0.1970	0.2127	0.093
H16B	1.1488	0.1496	0.1280	0.093
H16C	1.0187	0.0416	0.1738	0.093
C17	1.1497(9)	0.6960(7)	0.2006(4)	0.0804(19)
H17A	1.2017	0.7150	0.1474	0.121
H17B	1.2071	0.6085	0.2263	0.121
H17C	1.1800	0.7761	0.2396	0.121
C18	0.7667(15)	0.3342(10)	0.5258(4)	0.116(3)
H18A	0.7328	0.2359	0.5387	0.175
H18B	0.6568	0.3969	0.5304	0.175
H18C	0.8765	0.3652	0.5660	0.175
C19	0.9926(11)	0.2508(9)	0.4180(4)	0.093(2)
H19A	1.0190	0.2632	0.3595	0.139
H19B	0.9642	0.1503	0.4278	0.139
H19C	1.1053	0.2802	0.4570	0.139
C20	0.5087(11)	0.0482(8)	0.3271(4)	0.083(2)
H20A	0.3723	0.0706	0.3115	0.124

H20B	0.5490	0.0782	0.3857	0.124
H20C	0.5284	-0.0547	0.3220	0.124

Table 2. The geometric parameters of jatrophalactam (**1**) (Å, °)

N1-C1	1.322(6)	C10-C19	1.484(10)
N1-C3	1.445(7)	C10-C11	1.517(8)
N1-H1	0.92(5)	C10-C18	1.543(8)
O1-C1	1.227(5)	C11-C12	1.501(7)
O2-C6	1.425(6)	C11-H11	0.9800
O2-H2	0.8200	C12-C13	1.556(7)
O3-C14	1.202(6)	C12-H12A	0.9700
C1-C15	1.505(7)	C12-H12B	0.9700
C2-C15	1.334(6)	C13-C20	1.499(7)
C2-C16	1.491(7)	C13-C14	1.513(7)
C2-C3	1.511(7)	C13-H13	0.9800
C3-C4	1.510(7)	C14-C15	1.474(7)
C3-H3	0.9800	C16-H16A	0.9600
C4-C5	1.304(7)	C16-H16B	0.9600
C4-H4	0.9300	C16-H16C	0.9600
C5-C6	1.501(7)	C17-H17A	0.9600
C5-H5	0.9300	C17-H17B	0.9600
C6-C17	1.533(8)	C17-H17C	0.9600
C6-C7	1.535(8)	C18-H18A	0.9600
C7-C8	1.536(8)	C18-H18B	0.9600
C7-H7A	0.9700	C18-H18C	0.9600
C7-H7B	0.9700	C19-H19A	0.9600
C8-C9	1.491(8)	C19-H19B	0.9600
C8-H8A	0.9700	C19-H19C	0.9600
C8-H8B	0.9700	C20-H20A	0.9600
C9-C10	1.490(8)	C20-H20B	0.9600
C9-C11	1.502(8)	C20-H20C	0.9600
C9-H9	0.9800		

Table 3. The atom angle of jatropha lactam (**1**)

C1-N1-C3	114.9(5)	C12-C11-C10	126.3(5)
C1-N1-H1	120(3)	C9-C11-C10	59.1(4)
C3-N1-H1	125(3)	C12-C11-H11	113.3
C6-O2-H2	109.5	C9-C11-H11	113.3
O1-C1-N1	127.7(5)	C10-C11-H11	113.3
O1-C1-C15	126.7(4)	C11-C12-C13	117.8(4)
N1-C1-C15	105.5(4)	C11-C12-H12A	107.8
C15-C2-C16	130.0(4)	C13-C12-H12A	107.8
C15-C2-C3	110.3(4)	C11-C12-H12B	107.8
C16-C2-C3	119.5(4)	C13-C12-H12B	107.8
N1-C3-C4	113.3(5)	H12A-C12-H12B	107.2
N1-C3-C2	100.8(4)	C20-C13-C14	113.3(4)
C4-C3-C2	107.2(4)	C20-C13-C12	113.8(5)
N1-C3-H3	111.7	C14-C13-C12	109.2(4)
C4-C3-H3	111.7	C20-C13-H13	106.7
C2-C3-H3	111.7	C14-C13-H13	106.7
C5-C4-C3	125.0(5)	C12-C13-H13	106.7
C5-C4-H4	117.5	O3-C14-C15	122.5(4)
C3-C4-H4	117.5	O3-C14-C13	120.7(4)
C4-C5-C6	130.8(5)	C15-C14-C13	116.5(4)
C4-C5-H5	114.6	C2-C15-C14	130.1(4)
C6-C5-H5	114.6	C2-C15-C1	108.2(4)
O2-C6-C5	109.2(4)	C14-C15-C1	121.2(4)
O2-C6-C17	106.4(5)	C2-C16-H16A	109.5
C5-C6-C17	113.9(5)	C2-C16-H16B	109.5
O2-C6-C7	107.8(5)	H16A-C16-H16B	109.5
C5-C6-C7	108.0(4)	C2-C16-H16C	109.5
C17-C6-C7	111.4(5)	H16A-C16-H16C	109.5
C6-C7-C8	115.5(5)	H16B-C16-H16C	109.5
C6-C7-H7A	108.4	C6-C17-H17A	109.5
C8-C7-H7A	108.4	C6-C17-H17B	109.5
C6-C7-H7B	108.4	H17A-C17-H17B	109.5
C8-C7-H7B	108.4	C6-C17-H17C	109.5
H7A-C7-H7B	107.5	H17A-C17-H17C	109.5

C9-C8-C7	113.4(5)	H17B-C17-H17C	109.5
C9-C8-H8A	108.9	C10-C18-H18A	109.5
C7-C8-H8A	108.9	C10-C18-H18B	109.5
C9-C8-H8B	108.9	H18A-C18-H18B	109.5
C7-C8-H8B	108.9	C10-C18-H18C	109.5
H8A-C8-H8B	107.7	H18A-C18-H18C	109.5
C10-C9-C8	120.9(5)	H18B-C18-H18C	109.5
C10-C9-C11	60.9(4)	C10-C19-H19A	109.5
C8-C9-C11	121.8(5)	C10-C19-H19B	109.5
C10-C9-H9	114.3	H19A-C19-H19B	109.5
C8-C9-H9	114.3	C10-C19-H19C	109.5
C11-C9-H9	114.3	H19A-C19-H19C	109.5
C19-C10-C9	119.7(6)	H19B-C19-H19C	109.5
C19-C10-C11	122.2(5)	C13-C20-H20A	109.5
C9-C10-C11	60.0(4)	C13-C20-H20B	109.5
C19-C10-C18	114.6(6)	H20A-C20-H20B	109.5
C9-C10-C18	116.4(6)	C13-C20-H20C	109.5
C11-C10-C18	113.1(6)	H20A-C20-H20C	109.5
C12-C11-C9	120.7(5)	H20B-C20-H20C	109.5

Figure 1. X-ray Crystal-Structure of jatropha lactam (**1**)

Figure 2. The crystal packing of jatropha lactam (**1**)

S12. A plausible biogenetic pathway of jatrophalactam (1)

Jatrophalactam (**1**) was the first novel diterpenoid lactam possessing an unprecedented 5/13/3 tricyclic framework. It is presumably biosynthesized from the biogenetically acceptable casbene. Casbene, which distributed widely on the genus *Jatropha*, was firstly via an oxidation to give an intermediate (**2**) with a broken C1-C2 chain. Followed by an Aldol condensation, a double bond between C-2 and C-15 was built. Subsequently, a series of oxidation, hydroxylation and rearrangement afford a key intermediate (**4**). After transamination of 3-OH and then dehydrated cyclization, jatrophalactam (**1**) was finally produced (Scheme 1).

Scheme 1. A plausible biogenetic pathway of jatrophalactam (**1**)