A New Tetracyclic Diterpene from *Jatropha curcas*

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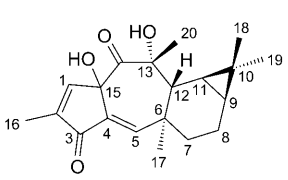
Jatrophodione A (**1**), a new diterpene with four rings, together with nine known compounds, caniojane (**2**), jatropholone A (**3**), jatropholone B (**4**), jatrogrossidione (**5**), 2-epijatrogrossidione (**6**), heudelotinone (**7**), gossweilone (**8**), (3α)-3-hydroxy-*ent*-pimara-8(14),15-dien-12-one (**9**), and 12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one (**10**), was isolated from the aerial parts of *Jatropha curcas*. Compounds **5**, **6**, **9**, and **10** were found for the first time in this plant. Their structures were established by spectroscopic analysis, including 2D-NMR spectroscopic techniques. Cytotoxicities of compounds **1**, **2**, **7**, **8**, and **9** were tested on the three cancer cell lines A549, Hela, and SMMC-7721. Results showed that **7** exhibited cytotoxicity against SMMC-7721 with an IC_{50} value of 21.68 μM, whereas **7** and **8** were active against A549 with the IC_{50} values of 16.04 and 20.47 μM, and against Hela with the IC_{50} values of 10.67 and 22.83 μM, respectively.

Introduction. – *Jatropha curcas* LINN. (Euphorbiaceae) is distributed in tropical and subtropical areas in many countries, including southern China, and is used as a medicinal plant to treat, for example, malarial fever, arthritis, gout, jaundice, wounds, and ulcers [1–4]. *J. curcas* mainly contains diterpenes, phorbol esters, and cyclopeptides [5–12]. The further investigation on this plant led to the isolation of a novel tetracyclic diterpene, jatrophodione A (**1**), as well as of nine known compounds, caniojane (**2**) [7][13], jatropholone A (**3**) [5][7][14], jatropholone B (**4**) [5][7][14], jatrogrossidione (**5**) [13][15], 2-epijatrogrossidione (**6**) [13][15], heudelotinone (**7**) [16], gossweilone (**8**) [17], (3α)-3-hydroxy-*ent*-pimara-8(14),15-dien-12-one (**9**) [18][19], and 12-hydroxy-13-methylpodocarpa-8,11,13-trien-3-one (**10**) [20]. Compounds **5**, **6**, **9**, and **10** were found for the first time in this plant. This article mainly deals with the isolation and structure determination of compound **1** and with the cytotoxicities of compounds **1**, **2**, **7**, **8**, and **9**.

Results and Discussion. – Jatrophodione A (**1**) was obtained as a colorless oil and exhibited the molecular formula $C_{20}H_{26}O_4$ with eight degrees of unsaturation, as shown by HR-ESI-MS (*m*/*z* 353.1746 ([M + Na]$^+$)). The NMR data (*Table*) showed five CH signals including two olefinic H-atom signals at δ(H) 7.20 (br. *s*, H–C(1)) and 6.52 (*s*, H–C(5)), two CH_2 signals at δ(C) 37.89 (*t*, C(7)) and 15.98 (*t*, C(8)), five Me signals, and eight quaternary C-atom signals including a characteristic signal due to the three-membered ring resonating at δ(C) 17.42 (*s*, C(10)) and two ketone C-atom signals (δ(C) 195.19 (*s*, C(3)), 208.33 (*s*, C(14)). Among the eight degrees of unsaturation, two of



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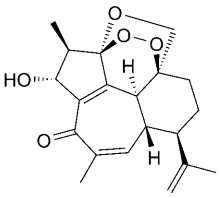

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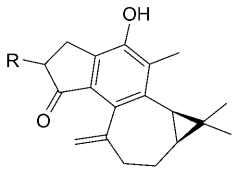

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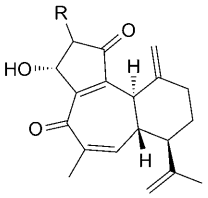
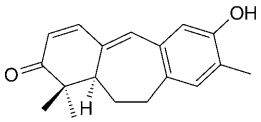
4 R = α-Me



5 R = α-Me
6 R = β-Me



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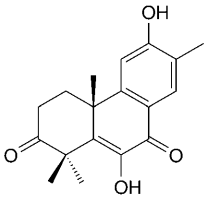

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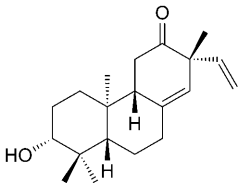

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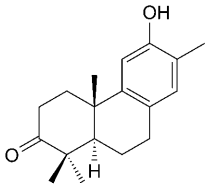

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them were assigned to the C=O groups, and two to the C=C bonds (δ(C) 150.89 (*d*, C(1)), 144.60 (*s*, C(2)), 129.83 (*s*, C(4)), and 149.32 (*d*, C(5)), thus **1** was inferred to possess four rings. The constitution of **1** was determined by HSQC, ^{1}H,^{1}H-COSY, and HMBC data (*Fig. 1*). Analysis of the ^{1}H,^{1}H-COSY plot revealed a CH_2–CH_2–CH–CH–CH moiety by the correlations CH_2(7)/CH_2(8), CH_2(8)/H–C(9), H–C(9)/H–C(11), and H–C(11)/H–C(12). In addition, the HMBC experiment showed the key correlations H–C(1)/C(2), C(4), and C(15), H–C(5)/C(3), C(4), C(12), and C(15), H–C(12)/C(10), Me(16)/C(2) and C(3), Me(17)/C(6), C(7), and C(12), Me(18) and Me(19)/C(10), Me(20)/C(13) and C(14), and OH–C(13)/C(13). Particularly, the unusual linkage between C(6) and C(12) was confirmed by the correlations of Me(17) and H–C(5) with C(12). The above information suggested that compound **1** was a myrsinane-type diterpene [21–24]. From the key NOESY correlations (*Fig. 2*) H–C(12)/H_β-C(7), Me(18), and Me(20), and Me(17)/H_α–C(7), the β-configurations of Me(20) and H–C(12) were deduced. The correlations H–C(9)/H–C(11)/Me(17) indicated their α-orientation. Thus, the relative configuration of **1** was established.

The cytotoxicities against the three cancer cell lines A549, Hela, and SMMC-7721 of compounds **1**, **2**, **7**, **8**, and **9** were tested *in vitro*. The results showed that **7** exhibited cytotoxicity against SMMC-7721 with an IC_{50} value of 21.68 μM, while **7** and **8** were

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) *of* **1**. δ in ppm, J in Hz.

	δ(H)	δ(C)
H–C(1)	7.20 (br. *s*)	150.89 (*d*)
C(2)		144.60 (*s*)
C(3)		195.19 (*s*)
C(4)		129.83 (*s*)
H–C(5)	6.52 (*s*)	149.32 (*d*)
C(6)		39.42 (*s*)
CH_2(7)	1.35–1.45 (*m*, H_α), 0.98–1.08 (*m*, H_β)	37.89 (*t*)
CH_2(8)	1.94–2.05 (*m*, H_α), 1.62–1.70 (*m*, H_β)	15.98 (*t*)
H–C(9)	0.74–0.78 (*m*, H_α)	19.24 (*d*)
C(10)		17.42 (*s*)
H–C(11)	1.13–1.17 (*m,* H_α)	20.93 (*d*)
H–C(12)	2.61 (*d*, $J = 6.4$)	40.90 (*d*)
C(13)		81.70 (*s*)
C(14)		208.33 (*s*)
C(15)		81.29 (*s*)
Me(16)	1.92 (*s*)	10.79 (*q*)
Me(17)	1.11 (*s*)	19.42 (*q*)
Me(18)	0.95 (*s*)	15.66 (*q*)
Me(19)	1.11 (*s*)	28.45 (*q*)
Me(20)	1.58 (*s*)	27.68 (*q*)
OH–C(13)	3.43 (*s*)	

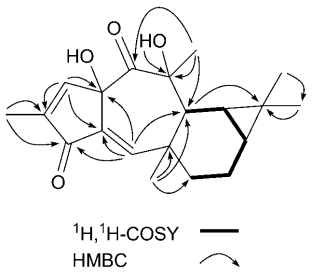

Fig. 1. *^{1}H,^{1}H-COSY and key HMBC features of compound* **1**

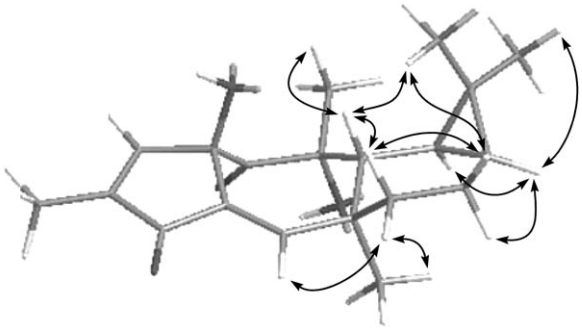

Fig. 2. *Key NOESY correlations of compound* **1**

active against A549 with the IC_{50} values of 16.04 and 20.47 μM, respectively, and against Hela with the IC_{50} values of 10.67 and 22.83 μM, respectively.

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 100–200 or 200–300 mesh; *Qingdao Marine Chemical Ltd. Co.*, P. R. China), silica gel *H* (60 μm; *Qingdao Marine Chemical Ltd. Co.*, P. R. China), *Lichroprep RP-18* gel (40–63 μM; *Merck*, Darmstadt, Germany), and *MCI* gel *CHP-20P* (75–150 μm; *Mitsubishi Chemical Co.*). TLC: silica gel *GF254* (*Qingdao Marine Chemical Ltd. Co.*, P. R. China). Semiprep. HPLC: *Agilent-1100* liquid chromatograph; reversed-phase *Zorbax SB-C_{18}* column. Optical rotations: *Jasco-DIP-370* digital polarimeter. UV Spectra: *Shimadzu-210A* double-beam spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad-FTS-135* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-DRX-500* and *Bruker-AM-400* instruments; $SiMe_4$ as internal standard; δ in ppm, *J* in Hz. ESI and HR-ESI-MS: *API-Qstar-Pulsar* instrument; in *m*/*z*.

Plant Material. The aerial parts of *Jatropha curcas* were collected from Luquan County of Kunming, Yunnan Province, P. R. China, in November, 2008, identified by Prof. *Chun-Lin Long* of the Kunming Institute of Botany, Chinese Academy of Sciences, and deposited with the KUN Herbarium (voucher number: 593204).

Extraction and Isolation. The dried and powdered plant material (35 kg) was extracted with MeOH under reflux for 8 h (3 × 30 l). The resulting residue was partitioned between AcOEt and H_2O, and then BuOH and H_2O. The AcOEt extract (220 g) was subjected to CC (SiO_2, $CHCl_3$/Me_2CO 9:1 → 1:1) and then to CC (*MCI*, MeOH/H_2O 85:15): *Fractions 1–7. Fr. 3* (15 g) was subjected to CC (*RP-18*, MeOH/H_2O 15:85 → 1:0): *Frs. 3.1–3.4. Fr. 3.3* was further purified by CC (SiO_2, petroleum ether/acetone 2:1) and HPLC (MeOH/H_2O 6:4): **1** (4 mg), **5** (2 mg), and **6** (3 mg). *Fr. 4* (23 g) was subjected to CC (*RP-18*, MeOH/H_2O 2:8 → 1:0): *Frs. 4.1–4.6. Fr. 4.1* was further purified by CC (SiO_2, petroleum ether/AcOEt 1:1) and HPLC (MeCN/H_2O 3:7): **2** (4 mg) and **10** (14 mg). *Fr. 2* (10 g) was subjected on CC (*RP-18*, MeOH/H_2O 2:8 → 1:0): *Frs. 2.1–2.4. Fr. 2.1* was further purified by CC (SiO_2, $CHCl_3$/Me_2CO 30:1) and HPLC (MeCN/H_2O 7:3): **3** (6 mg) and **4** (7 mg). *Fr. 2.2* was further purified by HPLC (MeCN/H_2O 45:55): **7** (9 mg). *Fr. 5* (9 g) was subjected on CC (*RP-18*, MeOH/H_2O 2:8 → 1:0): *Frs. 5.1–5.5. Fr. 5.4* was further purified by CC (SiO_2, petroleum ether/acetone 1:1) and HPLC (MeCN/H_2O 2:8): **9** (11 mg). *Fr. 7* (11 g) was subjected on CC (*RP-18*, MeOH/H_2O 1:9 → 1:0): *Frs. 7.1–7.6. Fr. 7.4* was further purified by CC (SiO_2, petroleum ether/acetone 1:1) and HPLC (MeCN/H_2O 3:7): **8** (7 mg).

Jatrophodione A (= rel-*(1aR,3aR,7aζ,9S,9aS,9bR)-1a,2,3,3a,7a,9,9a,9b-Octahydro-9-hydroxy-1,1,3a,6,9-pentamethyl-1*H*-cyclopropa[3,4]benz[1,2-f]azulene-5,8-dione*; **1**): Colorless oil. $[\alpha]_D^{24.6} = -61.44$ ($c = 0.38$, MeOH). UV (MeOH): 202 (4.10), 217 (4.04), 260 (3.79). IR (KBr): 3434, 2932, 1705, 1659, 1164, 1074, 886. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 353.17 ($[M+Na]^+$, $C_{20}H_{26}NaO_4^+$; calc. 353.1728).

Assessment of Cytotoxicity (SRB method). Cytotoxicities of compounds **1**, **2**, **7**, **8**, and **9** against three cancer cell lines, A549, Hela, and SMMC-7721, were measured by the SRB (sulforhodamine B) method. Briefly, cells were plated in 96-well culture plates. After 24 h, the cells were treated with serial dilutions of compounds **1**, **2**, **7**, **8**, and **9** with the maximum concentration of 50 μg/ml. Each compound was initially dissolved in DMSO and further diluted in medium to produce different concentrations. After 48 h, cells were fixed by the addition of 25 μl of ice-cold 50% CF_3COOH soln. and incubated at 4° for 1 h. After being washed with distilled H_2O and air-dried, the plate was stained for 15 min with 100 μl of 0.4% SRB (*Sigma*) in 1% glacial AcOH. The plates were washed with 1% AcOH and air-dried overnight. For

reading the plate, the bound dye (SRB) was solubilized with 100 µl of 10 mM *Tris* base soln., and the absorbance was measured at 560 nm with a *Molecular-Devices-SpectraMax-340* microplate spectrophotometer (*MWG-Biotech, Inc.*, Sunnyvale, USA). Cell survival was measured as the percentage absorbance compared to the untreated control. Taxol was used as a positive control.

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