Chemical Studies on the Constituents of the Chinese Medicinal Herb *Euphorbia helioscopia* L.

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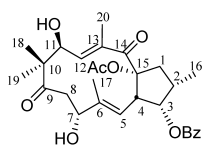
Euphoheliosnoid D (1), a new jatrophone-type diterpenoid, was isolated together with 22 known metabolites from the Chinese medicinal herb *Euphorbia helioscopia* L. The structure and relative stereochemistry of 1 was elucidated on the basis of spectroscopic methods. Compounds 14—23 were obtained from the species for the first time while compound 14, 2α-hydroxy helioscopinolide B, was isolated as a new natural product.

Key words *Euphorbia helioscopia*; jatrophone-type; euphoheliosnoid; diterpenoid; sesquiterpenoid; flavonoid

Euphorbia helioscopia L. (Family Euphorbiaceae), a Chinese medicinal herb, is widely distributed in most parts of China.[1] A literature survey revealed that the chemical constituents of *E. helioscopia* L. have been intensively investigated. Different kinds of secondary metabolites, such as triterpenoids,[2] diterpenoids,[3—9] flavonoids,[10,11] tannins,[12,13] steroids,[3] and lipids,[3] have been isolated from the titled plant by several groups during the past four decades. Some of these compounds were responsible for the antitussis,[12] antitumor,[6,14] anti-allergy, anti-asthma,[15] and inhibitory effect on mushroom tyrosinase *in vitro*.[16] In the course of our research directed to investigate Chinese medicinal plants,[17,18] we chemically examined the plant *E. helioscopia* L. collected from Zhejiang Province of China, resulting in the isolation of three new jatrophone-type diterpenoids, namely euphoheliosnoids A—C.[19] Interestingly, two of the new diterpenoids, euphoheliosnoids A and B, were characterized by the presence of an uncommon picolinic ester moiety that had never been found before in the species. Our continuous investigation of this plant has now led to the isolation of an additional diterpene with a jatrophone-type skeleton, named euphoheliosnoid D (**1**), together with 22 known metabolites (**2**—**23**). Careful analysis of NMR spectra of these isolates and in comparison with the NMR data reported in the literature, the 22 known compounds were readily identified as thirteen diterpenoids, namely euphornin (**2**),[4,5,20] euphornins B (**3**) and C (**4**), euphoscopins A—C (**5**—**7**), F (**8**) and J (**9**),[5,6] epieuphorscopins A (**10**)[4,5,9,20] and B (**11**),[5] euphoscopin A (**12**),[5,9] helioscopinolide B (**13**), and 2α-hydroxy helioscopinolide B (**14**)[7]; a guaiane lactone, hemistepsin (**15**)[21]; two nor-sesquiterpenoids, namely 4,5-dihydroblumenol A (**16**)[22] and aglycone of icariside B2 (**17**)[23]; and six flavonoids, namely licochalcone A (**18**),[24] 2′,4,4′-trihydroxychalcone (**19**),[25] echinatia (**20**),[26] licochalcone B (**21**),[24,27] glabrone (**22**),[28,29] and 4′,5,7-trihydroxyflavanone (**23**),[30] respectively. Among these metabolites, diterpenoids **2**—**13** were obtained previously from the same species of Japanese origin,[4,5,9] while compounds **14**—**23** were isolated from the species for the first time. In addition, 2α-hydroxy helioscopinolide B (**14**), which was reported as an intermediate in the course of structure elucidation of helioscopinolide C (**24**),[7] was isolated from natural sources for the first time.

Euphoheliosnoid D (**1**) was obtained as an optically active oil ($[\alpha]_D^{20} = -15°$). The molecular formula $C_{29}H_{36}O_8$ was established by HR-EI-MS molecular ions at *m/z* 512.2435 (M^+), indicating twelve degrees of unsaturation. IR absorption demonstrated the presence of hydroxyl groups (3457 cm^{-1}), ester groups, carbonyl functionalities (1752, 1717 cm^{-1}), and an aromatic system in the structure (1617, 1541, 791 cm^{-1}), in agreement with the presence of two secondary alcohol carbons (δ 73.1, 73.8), two ester carbons (δ 165.8, 170.6), two ketone signals (δ 201.8, 219.5) and one phenyl group (δ 128.5×2, 129.6×2, 130.4, 133.0) in the ^{13}C-NMR spectrum (Table 1), taking account of eight degrees of unsaturation. The remaining four degrees of unsaturation were due to two double bonds (δ 135.5, 138.9, 117.7, 142.5) and two cycles in the molecule. Also, the ^{13}C-NMR and DEPT spectra revealed additional 13 sp^3 carbon signals (2×C, 3×CH, 2×CH_2, 6×CH_3), which were completely assigned to their correspondent proton signals by HMQC experiments



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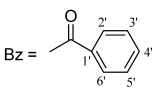

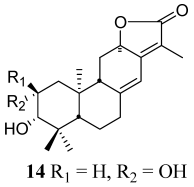

14 R_1 = H, R_2 = OH
24 R_1,R_2 = O

Table 1. NMR Data[a,b] for Euphoheliosnoid D (**1**)

No.	δ_H m, *J* in Hz	δ_C[c] m	No.	δ_H m, *J* in Hz	δ_C[c] m
1α	1.70, dd, 13.7, 8.2	47.1, t	13		138.9, s
1β	3.27, dd, 13.7, 7.3		14		201.8, s
2	2.38, m	39.4, d	15		92.7, s
3	5.52, dd, 4.1, 3.3	81.4, d	16	1.00, d, 6.7	13.8, q
4	2.96, dd, 10.6, 4.1	51.3, d	17	1.51, s	16.0, q
5	5.86, d, 10.6	117.7, d	18	1.28, s	20.2, q
6		142.5, s	19	0.87, s	22.4, q
7	4.17, m	73.8, d	20	1.83, s	11.9, q
8α	2.49, dd, 16.0, 5.6	40.3, t	15-OAc	2.20, s	21.7, q
					170.6, s
8β	3.67, d, 16.0		3-OBz		165.8, s
9		219.5, s	Bz (1′)		130.4, s
10		52.7, s	Bz (2′, 6′)	8.07, d, 7.4	129.6, d
11	4.19, d, 9.4	73.1, d	Bz (3′, 5′)	7.48, t, 7.4	128.5, d
12	6.38, d, 9.4	135.5, d	Bz (4′)	7.60, t, 7.5	133.0, d

a) Bruker DRX-400 NMR spectrometer, $CDCl_3$, chemical shifts (ppm) referred to $CHCl_3$ (δ 7.26) and $CDCl_3$ (δ 77.0), respectively. *b*) Assignments made by ^{1}H–^{1}H COSY, HMQC, HMBC. *c*) By DEPT sequence.

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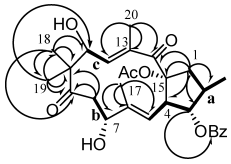

Fig. 1. ^{1}H–^{1}H COSY (——) and Selected HMBC ( ) Correlations of **1**

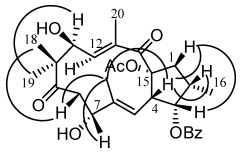

Fig. 2. Key NOESY Correlations ( ) of **1**

(Table 1).

A detailed analysis of the ^{1}H–^{1}H COSY spectrum of **1** revealed separated proton spin systems of three structural fragments **a** (H_2-1/H-2/(H_3-16)/H-3/H-4/H-5), **b** (H-7/H_2-8), and **c** (H-11/H-12) as illustrated in Fig. 1. For partial structure **a**, connectivity from H_2-1 to H-5 were evident from the DQF-COSY spectrum, which was further connected to Me-16 by the cross peak for H-2 (δ 2.38, m) and H_3-16 (δ 1.00, d, J=6.7 Hz). The proton connectivities of **b** and **c** were deduced from the clear cross peaks for H-7 (δ 4.17, m) and H_2-8 (δ 2.49, dd, J=16.0, 5.6 Hz; 3.67, d, J=16.0 Hz), and H-11 (δ 4.19, d, J=9.4 Hz) and H-12 (δ 6.38, d, J=9.4 Hz) in the DQF-COSY spectrum of **1**, respectively (Fig. 1). Partial fragments of **a** and **b** were linked together by the long-range correlation between H_3-17 and C-5, C-6 and C-7 in the HMBC spectrum. The clear HMBC correlations of H_3-18/C-9, C-10, C-11, C-19, H_3-19/C-9, C-10, C-11, C-18, and H_2-8/C-9, C-10 gave rise to the connectivity of partial structures **b** and **c** through the quaternary carbon linkage C-9/C-10. The significant HMBC correlations of H_3-20/C-12, C-13, C-14 and H_2-1/C-4, C-14, C-15 led to the connection of partial **a** and **c** and, consequently, allowed us to draw the planar structure of **1** (Fig. 1). In addition, the diagnostic long-range correlation between H-3 and one of the ester carbonyl signals (δ 165.8) implied that the ester of benzoic acid should be attached to C-3. The downfield shifted ^{13}C-NMR chemical shift value of C-15 (from δ 84.4 in euphornin C[5] to δ 92.7 in **1**) suggested that the 15-OH was acetylated. As a consequence, the two hydroxyl groups should be linked to both C-7 and C-11, respectively.

The relative stereochemistry of **1** was mainly deduced by the NOESY experiment (Fig. 2). Since the angular proton H-4 was assumed to be β-oriented on a biogenetic basis,[4—6] the diagnostic NOE cross peaks of H-4/H-1β (δ 3.27, dd, J=13.7, 7.3 Hz), H-2, and H-1β/H-3, H-4, were consistent with the α-orientations for both Me-16 at C-2 and the benzoic ester moiety at C-3. Meanwhile, the strong NOE effect between H-4 and H_3-17 indicated the relative *cis*-orientation of H-4 and the vinylic methyl group. The β-oriented H-7, H-8β and H_3-18 were consequently deduced from the distinct NOE interactions of H_3-17/H-7, H-8β, and H-8β/H_3-18. The absence of a cross peak between the H-4 and Me-OAc (δ 2.20) at C-15 supported a *trans*-fused cyclopentane ring which is usual in other jatrophone derivatives.[4—6,9,19] The absence of an NOE effect between H-12 and H_3-20 indicated an *E*-double bond of Δ^{12}. Finally, the NOE enhancement between H-11 and H_3-19 suggested an α-configuration of H-11 according to the structure **1**. It should be pointed out that the conformational mobility/flexibility of macrocyclic compounds might cause ambiguous judgment of the NOEs between the relevant chiral centers, making the stereochemistry determination based only on NOE cross-peaks somewhat risky. Therefore, to obtain correct stereochemical assignments, more advanced techniques, for example, X-ray diffraction analysis, are needed.

The crude EtOAc extract of the titled plant exhibited cytotoxic activity against murine leukemia P-388 cells, but euphoheliosnoid D proved to be inactive. Further biological assays for compound **1** as well as other known compounds are currently under investigation.

Experimental

General Experimental Procedures Optical rotations were measured on a PERKIN-ELMER polarimeter 341. IR spectra were determined on a Nicolet Magna FT-IR 750 spectrometer (ν_{max} in cm^{-1}). The NMR spectra were recorded on a Bruker DRX-400 NMR spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts (δ) are reported in parts per million, using the residual $CHCl_3$ (δ_H 7.26 ppm) as an internal standard for ^{1}H-NMR, and $CDCl_3$ (δ_C 77.0 ppm) as an internal standard for ^{13}C-NMR; coupling constant (J) in Hz. ^{1}H- and ^{13}C-NMR assignments were supported by ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY experiments. The ESI-MS spectra were recorded on a Q-TOF Micro LC-MS-MS mass spectrometer; EI-MS and HREIMS data were obtained on a Finnigan-MAT-95 mass spectrometer. Commercial silica gel (Qing Dao Hai Yang Chemical Group Co., 200—300 and 400—600 mesh) was used for column chromatography. Precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC.

Plant Material The plant of *Euphorbia heliscopia* cultivated at Shujiao, Zhejiang Province, China, was obtained in July 2003. A voucher specimen (Reg. No.: P-18) is available for inspection at the Herbarium of the Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences.

Extraction and Isolation The air-dried whole powdered plant (10 kg) was immersed in 95% EtOH at room temperature for 21 d. Evaporation of the EtOH solvent (under vacuum, 40 °C) from the crude extract gave a residue (623 g), which was partitioned between H_2O and EtOAc. The EtOAc solution was concentrated and subsequently partitioned between 90% aq MeOH and light petroleum ether. The 90% aq MeOH extract (203 g) was chromatographed on silica gel (200—300 mesh, 2.0 kg), using a gradient of petroleum ether–EtOAc (9 : 1—0 : 100) as eluent, to yield 16 fractions. Fraction 4 was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1), followed by a purification over CC on silica gel (400—600 mesh; petroleum ether/EtOAc, 9 : 1) to afford **8** (3.4 mg). Fraction 5 was purified over a CC on Sephadex LH-20, eluted with petroleum ether/$CHCl_3$/MeOH (2 : 1 : 1), to give four subfractions (A—D). The four subfractions were subjected to CC on silica gel (400—600 mesh; petroleum ether/EtOAc, 9 : 1), separately, yielding **7** (16.1 mg) from fraction A, **6** (12.3 mg), **10** (9.8 mg) and **11** (15.9 mg) from fraction B, **12** (21.0 mg) from fraction C, and **2** (27.4 mg) from fraction D, respectively. Fraction 6 was chromatographed over Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1), and then subjected to CC on silica gel (400—600 mesh; petroleum ether/EtOAc, 3 : 1), to afford **5** (54.8 mg). Fraction 7 was split into two subfractions (E, F) by a CC on silica gel (200—300 mesh; $CHCl_3$/Me_2CO, 24 : 1). Both subfractions (E, F) were purified by CC on Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1) to yield **18** (187.0 mg) from fraction E, and **19** (35.5 mg) and **23** (53.5 mg) from fraction F, respectively. Fraction 8 was chromatographed over Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1) to afford two subfractions (G, H) which were subsequently purified by CC on silica gel (400—600 mesh). Fraction G was eluted with petroleum ether/EtOAc (8 : 1) to give **4** (6.8 mg) and **9** (14.3 mg), while fraction H was eluted with petroleum ether/EtOAc (6 : 1) to afford **22** (14.0 mg). Fraction 9 was purified by CC on Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1), followed by CC on silica gel (400—600 mesh; petroleum ether/EtOAc, 3 : 2), yielding **13** (3.5 mg) and **17** (37.0 mg). Fraction 10 afforded **3** (18.6 mg) after CC on Sephadex LH-20 (petroleum ether/$CHCl_3$/MeOH, 2 : 1 : 1) and silica gel (petroleum ether/Me_2CO, 7 : 3). Fraction 11 was subjected to Sephadex LH-20

(petroleum ether/$CHCl_3$/MeOH; 2 : 1 : 1), followed by purification of CC on silica gel (400—600 mesh; $CHCl_3$/MeCO, 96 : 4) to afford **1** (2.6 mg). Fraction 13 was chromatographed over Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1), and then subjected to CC on silica gel ($CHCl_3$/MeCO, 4 : 1) to afford **14** (2.3 mg) and **16** (78.0 mg). Fraction 14 was subjected to CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) silica gel ($CHCl_3$/MeOH, 13 : 1) to yield **15** (74.9 mg) and **20** (83.5 mg). Finally, compound **21** (135.0 mg) was obtained from fraction 15 after purifications with CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1 : 1) and silica gel (petroleum ether/EtOAc, 3 : 7).

Euphoheliosnoid D (**1**): colorless oil. ^{1}H- and ^{13}C-NMR see Table 1. IR (KBr) cm^{-1}: 3457, 2921, 2856, 1752, 1717, 1617, 1541, 791. ESI-MS *m*/*z*: 535.019 ($[M+Na]^+$). EI-MS *m*/*z*: 512 (M^+), 494, 470, 452, 434, 400, 165, 123, 105, 77. HR-EI-MS *m*/*z*: 512.2435 (M^+, Calcd for $C_{29}H_{36}O_8$: 512.2410). $[\alpha]_D^{20}$ −15° (*c*=0.21, $CHCl_3$).

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