Eupatoric Acid: A Novel Triterpene from *Eupatorium odoratum* L. (Asteraceae)

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Phytochemical studies on the petroleum ether extract of the roots of *Eupatorium odoratum* have resulted in the isolation of a novel triterpene, 3β-hydroxy-28-carboxyolean-12-ene (**1**) along with seven known compounds – poriferasterol (**2**), octadecane (**3**), butyrospermol acetate (**4**), bis(2-ethylhexyl)phthalate (**5**), chrysophanol (**6**), physcion (**7**) and palmitic acid (**8**). Novel compound **1** is designated as eupatoric acid. Compounds **2** – **7** were reported here for the first time from this plant. Palmitic acid (**8**) was also isolated for the first time from this root. The structure of the novel compound was established on the basis of spectroscopic studies. The cytotoxicity of the compounds **1** – **7** was studied using a lethality test against *Artemia salina* (brine shrimp).

Key words: 3β-Hydroxy-28-carboxyolean-12-ene, Eupatoric Acid, *Eupatorium odoratum*, *Artemia salina*

Introduction

Eupatorium odoratum L. (Asteraceae: Eupatoriae), a perennial shrub, grows abundantly in the Central and Eastern regions of Nepal from 400-1500m altitude, being known as "Banmara". The juice of the aerial parts of this plant is used for cuts and wounds to arrest bleeding and promote healing [1]. The plant is suitable for treating fungal and protozoa diseases [2]. The ethanolic extract of the leaf is reported to possess antioxidant activity to protect cultured skin cells [3]. 4',5,6,7-Tetramethoxyflavone isolated from this plant, is found as a blood clotting enhancer factor when studied *in vitro* [4]. Extensive literature searches revealed that very few phytochemical analyses were done on that root [5]. Our preliminary test of the petroleum ether extract showed some cytotoxicity ($LC_{50} < 1000$ μg/ml) against brine shrimp. This encouraged us to perform a phytochemical analysis of the root of *E. odoratum*. We report a new triterpene which has been characterized as 3β-hydroxy-28-carboxyolean-12-ene (**1**) on the basis of spectral analyses and has been designated as eupatoric acid. In addition, we have isolated seven compounds – poriferasterol (**2**), octadecane (**3**), butyrospermol acetate (**4**), bis(2-ethylhexyl)phthalate (**5**), chrysophanol (**6**), physcion (**7**) and palmitic acid (**8**). Compounds **2** – **7** are reported here for the first time from this plant. Palmitic acid (**8**) is isolated for the first time from this root.

Results and Discussion

Compound **1**, eupatoric acid, showed positive on the Liebermann-Burchard test, Ferric chloride test, as well as the false Dragendorff test. Its IR spectrum exhibited bands for hydroxyl (3450 cm^{-1}) and carboxylic (1685 cm^{-1}) groups. The ^{1}H NMR spectrum of **1** showed seven methyls, one olefinic proton and one methine proton indicating an olean-12-ene skeleton. The methyls 23-H_3, 24-H_3, 25-H_3, 26-H_3, 27-H_3, 30-H_3 and 31-H_3 resonated as singlets at $\delta = 0.98$, 0.77, 0.90, 0.77, 1.13, 0.91 and 0.93, respectively. A one-proton broad triplet at $\delta = 5.28$ ($J = 3.3$ Hz) was assigned to the olefinic 12-H. Signals of double doublets at $\delta = 2.84$ (dd, $J = 14.9$, 4.9 Hz) were assigned to the methine proton of 18-H due to 19-H_2. In addition, it was possible to observe a signal typical of 3ax-H at $\delta = 3.22$, (dd, $J = 9.9$, 4.9 Hz) due to the presence of β-OH group at C-3 position. In ^{1}H-^{1}H COSY spectrum of **1**, the olefinic proton 12-H ($\delta = 5.28$) showed connectivity with the methylene group, 11-H_2 at $\delta = 1.85 - 1.90$ and the proton 3-H ($\delta = 3.22$) with the methylene group 2-H_2 at $\delta = 1.54 - 1.62$. The ^{1}H NMR spectrum (Table 1) bore a close resem-

C/H	δ_C [a]	DEPT[b]	δ_H [c] (*J*)	^{1}H-^{1}H COSY	HMQC	Cross peaks in HMBC ^2J	^3J	^4J
1	38.3	CH_2						
2	26.9	CH_2	1.54 – 1.62 *m*	H-3				
3	78.8	CH	3.22 *dd* (9.9, 4.9)	H-2	78.8			
4	38.6	C						
5	55.1	CH						
6	18.2	CH_2						
7	32.6	CH_2						
8	39.2	C						
9	47.5	CH						
10	36.9	C						
11	23.3	CH_2	1.85 – 1.9 *m*	H-12				
12	122.2	CH	5.28 *br t* (3.3)	H-11	122.2			
13	143.7	C						
14	41.6	C						
15	27.6	CH_2						
16	22.9	CH_2						
17	46.3	C						
18	41.1	CH	2.84 *dd* (14.9, 4.9)		41.1			
19	45.9	CH_2						
20	30.6	C						
21	33.8	CH_2						
22	32.4	CH_2						
23	27.9	CH_3	0.98 *s*		27.9	C-4	C-3, C-5	
24	15.2	CH_3	0.77 *s*		15.2	C-4	C-3, C-5, C-23	
25	15.4	CH_3	0.90 *s*		15.4	C-10	C-9, C-1,C-5	C-4
26	16.8	CH_3	0.77 *s*		16.8	C-8	C-7, C-9, C-14	
27	25.8	CH_3	1.13 *s*		25.8	C-14	C-8, C-15, C-13	
28	29.6	CH_2	1.25 *s*		29.6			
29	181.0	–						
30	33.0	CH_3	0.91 *s*		33.0	C-20	C-19, C-31	
31	23.4	CH_3	0.93 *s*		23.4	C-20	C-30	

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra of Eupatoric acid **1**.

J (in parenthesis) in Hertz; *s*: singlet, *dd*: double doublet, *m*: multiplet, *br t*: broad triplet; [a,c] ^{1}H NMR and ^{13}C NMR were measured in $CDCl_3$ and one drop CD_3OD, and δ is in ppm; [b] DEPT was measured in $CDCl_3$.

blance to the oleanolic acid [6], with the exception of the one methylene group which appeared at $\delta = 1.25$ as a singlet.

The ^{13}C NMR and DEPT spectra of **1** showed thirty one carbon resonances revealing the presence of seven methyl, eleven methylene, three methine carbons, one carbinol carbon, six quaternary carbons, two olefinic carbons and of one very weak signal for carbonyl carbon of carboxylic group ($\delta = 181.0$) (Table 1). Two olefinic carbons resonated at $\delta = 122.2$ and 143.7 were assigned for C-12 and C-13 respectively [7]. The remaining carbon atoms were assigned by HMQC and a comparison made with reported ^{13}C NMR data [8]. The observed HMQC and HMBC of **1** are presented in Table 1. Its ^{13}C NMR spectrum was in close agreement with that of oleanolic acid [8], except for one methylene group at $\delta = 29.6$, which showed one-bond connectivity with a singlet proton of methylene at $\delta = 1.25$ in the HMQC spectrum. Therefore, this methylene group ($\delta_C = 29.6$, $\delta_H = 1.25$) was seen to connect with quaternary C-17 and C-29. Its number was assigned as C-28 (Fig. 1). The HRMS spectrum of compound **1** showed the molecular ion peak at *m/z* 470.3387, accounting for the molecular composition $C_{31}H_{50}O_3$ (calcd. 470.3760). Furthermore, a small fragment ion in EIMS at *m/z* 425 $[M\text{-}COOH]^+$ and 411 $[M\text{-}CH_2COOH]^+$ confirmed the above results. These spectroscopic studies led to structure **1** for this new triterpene, 3β-hydroxy-28-carboxyolean-12-ene, designated as eupatoric acid.

Compound **2**, previously isolated from various natural sources [9 – 12], was confirmed as poriferasterol by comparison with the reported melting point [13] and ^{13}C NMR [14]. Compound **3** was confirmed as octadecane by ^{1}H NMR, ^{13}C NMR and GC/MS. In compound **4**, methyl protons and carbon signals were assigned by comparison with those of butyrospermol [15]. Its melting point and mass spectrum were

Table 2. LC_{50} and 95% Confidence interval of isolated compounds (**1** – **7**) from the roots of *E. odoratum* tested at 1000, 100 and 10 μg/ml in the Brine shrimp lethality test.

Compounds	LC_{50} (μg/ml)	95% Confidence intervals
Eupatoric acid (**1**)	> 1000	–
Poriferasterol (**2**)	> 1000	–
Octadecane (**3**)	> 1000	–
Butyrospermol acetate (**4**)	> 1000	–
Bis(2-ethylhexyl)phthalate (**5**)	538.15	642.21 – 434.09
Chrysophanol (**6**)	289.00	344.87 – 233.13
Physcion (**7**)	158.14	209.56 – 106.72
Berberine*	89.12	
Berberine chloride	22.50[e]	

[e] Meyer *et al.* (1982); * positive control.

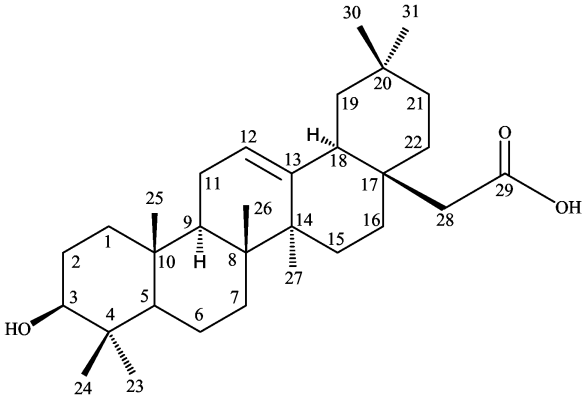

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Fig. 1. Eupatoric acid.

in close agreement with those reported in the literature [16]. Compound **5**, previously isolated from plants and microorganism [17 – 19], was confirmed as bis(2-ethylhexyl)phthalate by comparison with reported ^{1}H NMR, ^{13}C NMR and MS data [19]. Compound **6** and **7** were identified as chrysophanol and physcion, respectively, by comparison with reported ^{1}H NMR, ^{13}C NMR and MS data [20]. However, signals due to quaternary carbons C-11, C-12, C-13 and C-14 in **7** were difficult to observe due to their very long relaxation times [21]. Compound **8** was identified as palmitic acid by co-TLC with an authentic sample and further confirmed from spectroscopic data (GC/MS, ^{13}C NMR, ^{1}H NMR and IR).

Compounds **1** – **7** were tested for cytotoxicity using a brine shrimp lethality test [22]. From Table 2, it was revealed that compounds **1**, **2**, **3** and **4** exhibited non-significant cytotoxicity ($LC_{50} > 1000$ μg/ml), while **5**, **6** and **7** exhibited significant cytotoxicity ($LC_{50} < 1000$ μg/ml). LC_{50} values of **5**, **6** and **7** showed 538.15, 289.00 and 158.14 μg/ml, respectively, which were relatively, low as compared to the known cytotoxic compound, berberine chloride (22.5 μg/ml) [22].

Experimental Section

General

The Melting point was determined on Mettler FP61 and was uncorrected. IR spectra were recorded in KBr on Shimadzu spectrophotometer. The UV/vis spectra were measured on a Chemito UV-VIS 2500 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz), DEPT were recorded on a JEOL GX-400 with TMS as internal standard. FAB mass spectrum was recorded on a JEOL LMS-700T Spectrometer with glycerol as matrix. However, ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz), APT as well as EIMS were recorded at the University of Gö ttingen, Germany. ^{1}H NMR (100 MHz) and ^{13}C NMR (25 MHz) were recorded at the University of Wisconsin-La Crosse, USA. HRMS and EIMS for compound **1** were recorded on Finnigan Mat SSQ 710 (70 eV) and VG7035 instruments respectively at Institute of Chemical and Engineering Sciences (ICES), Singapore. GC/MS were recorded on Agilent 5973N plus 6890N at Tohoku University, Aoba-yama Campus, Japan. Silica gels (60 – 200 and 60 – 120 mesh) were used for column chromatography. TLC analyses were carried out on microscopic slide coated with silica gel GF_{254}. For PTLC, glass plates (20 × 20 cm) were coated with silica gel GF_{254} with thickness 0.25 mm. Petroleum ether of boiling point (40 – 60 °C) was used throughout the experiment, unless otherwise stated.

Plant materials

E. odoratum was collected from Tinpipalay (Kabhrepalanchok district), Nepal, in November 2001. A voucher specimen (TUCH 19) was deposited in the Tribhuvan University Central Herbarium, Central Department of Botany, TU, Nepal.

Brine shrimp lethality test

This test was performed as described by Meyer *et al*. [22]. LC_{50} values were calculated by Probit analysis [23]. LC_{50} value was expressed as the mean of three independently performed experiments in μg/ml with 95% confidence interval. Berberine was used as a positive control.

Extraction and isolation

Sun-dried root powder (1.99 kg) was extracted successively with petroleum ether (6.5 l, 5.5 l and 3.5 l) overnights by cold percolation and then filtered. The filtrate was concentrated to about 200 ml and refrigerated for two nights. The solid obtained was centrifuged and washed with cold

petroleum ether (10 ml × 5) to obtain a pale yellow solid and supernatant liquid (L1). The pale yellow solid was further washed, followed by a centrifuge with EtOAc-cold petroleum ether (3:97, 1 ml × 3), and recrystallized successively from EtOAc-hexane and MeOH, to yield the compound **1** [0.0143 g, $R_f = 0.34$ (MeOH-$CHCl_3$ 5:95)]. Supernatant liquid (L1) was concentrated to about 200 ml and left two overnights at room temperature, centrifuged and washed with cold petroleum ether (5 ml × 3) to obtain white mass and supernatant liquid (L2). White mass (0.0466 g) was chromatographed on silica gel column (1.6 × 37 cm, 15 g) by eluting with CH_2Cl_2 and CH_2Cl_2-EtOAc of increasing polarity: [CH_2Cl_2, 80 ml], [CH_2Cl_2-EtOAc (99:1, 150 ml)], [CH_2Cl_2-EtOAc (95:5, 100 ml)]. Fractions were collected at the rate of 25 ml/min in test tubes. They were pooled together into three fractions based on TLC characteristics. Fraction obtained from CH_2Cl_2-EtOAc (99:1), was evaporated to dryness and then recrystallized in methanol to yield white crystals of compound **2** [0.033 g, $R_f = 0.48$ (EtOAc-hexane 3:7), 0.51 (EtOAc-CH_2Cl_2 3:7)]. Yellow syrupy oil (4.2 g) obtained after evaporating supernatant liquid (L2) in Rotavapor, was chromatographed on a silica gel column (3 × 58 cm, 145 g). The column was eluted with hexane-CH_2Cl_2 (0 to 100%), CH_2Cl_2-EtOAc (0 to 100%) and EtOAc-MeOH (0 to 100%) in succession. Fractions were collected at the rate of 20 ml/15 min in test tubes. They were pooled together into fourteen fractions based on TLC characteristics.

Fraction 1, eluted with hexane, yielded compound **3** (0.681 g). Fraction 7 (oil, 0.078 g), eluted with hexane-CH_2Cl_2 (70:30), was dissolved in boiling MeOH, filtered off, allowed to cool over ice water, centrifuged and washed by cold MeOH to yield white amorphous powder **4** [0.019 g, $R_f = 0.48$ (CH_2Cl_2-hexane 50:50), 0.28 (hexane-CH_2Cl_2 70:30)].

Fraction 8 (0.260 g), eluted with hexane-CH_2Cl_2 (70:30 – 60:40) was rechromatographed on silica gel column (1.75 × 40 cm, 60 – 120 mesh, 20 g) by eluting with hexane-EtOAc gradients [hexane (40 ml), hexane-EtOAc (97:3, 270 ml), (90:10, 50 ml), (80:20, 50 ml), EtOAc (25 ml)]. Fractions were collected at the rate of 20 ml/30 min in test tubes. The collected fractions were monitored by TLC and pooled together to seven subfractions (S1 to S7). Subfraction S2 and S4 eluted with hexane-EtOAc (97:3) gave an oily compound **5** (0.166 g) and a sticky yellow mass (0.073 g), respectively after evaporation of solvent completely. Thus sticky yellow mass (0.065 g) was rechromatographed on a silica gel column (1 × 48 cm, 60 – 120 mesh, 7 g) by eluting with hexane/benzene 80:20. Fractions with $R_f = 0.58$ (benzene) were combined to yield a yellow mass (0.020 g), which, on recrystallization with chloroform produced compound **6** [0.0055 g, $R_f = 0.48$ (hexane-EtOAc 7:3)], 0.58 (benzene). Subfraction S6 (0.028 g), eluted with hexane-EtOAc (90:10 – 80:20), was chromatographed by preparative TLC in hexane-EtOAc (90:10) solvent to produce the compound **7** [0.019 g, $R_f = 0.12$ (EtOAc-hexane 1:9), 0.54 (benzene)].

Fraction 11 (greenish syrupy residue, 0.198 g), eluted from CH_2Cl_2-EtOAc (95:5 – 75:25), was dissolved in boiling hexane, cooled over ice water, filtered off and washed successively with cold hexane (10 ml × 3) and cold hexane-EtOAc 97:3 (10 ml) to obtain a white substance (0.084 g). It was confirmed as compound **2** by co-TLC, its melting point as well as the Liebermann-Burchard test.

Isolation of palmitic acid by PTLC from petroleum ether extract

Petroleum ether extract (2.6 g) obtained from root powder of *E. odoratum* (650 g) by cold percolation was dissolved in chloroform and chrmomatographed on PTLC plates with EtOAc solvent. The band with R_f value 0.41 [Hexane: Et_2O (1:1), fluorescein spray (0.01% in ethanol)] on usual work up with EtOAc yielded compound **8** (0.080 g).

Eupatoric acid (**1**): Pale yellow amorphous powder. – UV/vis ($CHCl_3$): $\lambda_{max} = 243.8$ nm. – IR (KBr): $\nu_{max} = 3450$ (OH), 1685 (COOH) cm^{-1}. – ^1H NMR and ^{13}C NMR (Table 1). – EIMS: $m/z = 470$ $[M]^+$. HRMS *m/z*: 470.3387 [$C_{31}H_{50}O_3$, calcd. 470.3760].

Poriferasterol (**2**): White soft powder. – M. p. 154 °C. – UV/vis ($CHCl_3$): $\lambda_{max} = 242$ nm. – IR ($CHCl_3$): $\nu_{max} = 3600$ (OH), 1040 (C-O stretch), 930 (-CH=CH-, *trans*) cm^{-1}. – ^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.69$ (s, 3H, 18-H), 0.79 (d, 3H, $J = 6.9$ Hz, 27-H), 0.80 (t, 3H, $J = 6.9$ Hz, 29-H), 0.84 (d, 3H, $J = 5.9$ Hz, 26-H), 1.01 (s, 3H, 19-H), 1.03 (d, 3H, $J = 5.9$ Hz, 21-H), 3.52 (m, 1H, 3α-H), 5.04 (dd, 1H, $J = 14.9$, 8.5 Hz, 23-H), 5.14 (dd, 1H, $J = 14.9$, 8.5 Hz, 22-H), 5.35 (br d, 1H, $J = 4.2$ Hz, 6-H). – ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.04$ (C-18), 12.22 (C-29), 18.98 (C-26), 19.39 (C-19), 21.07 (C-11), 21.07 (C-21), 21.21 (C-27), 24.36 (C-15), 25.39 (C-28), 28.90 (C-16), 31.67 (C-2), 31.91 (C-7), 31.91 (C-8), 31.91(C-25), 36.52 (C-10), 37.27 (C-1), 39.69 (C-12), 40.46 (C-20), 42.23 (C-4), 42.32 (C-13), 50.18 (C-9), 51.24 (C-24), 55.98 (C-17), 56.87 (C-14), 71.80 (C-3), 121.69 (C-6), 129.30 (C-23), 138.30 (C-22), 140.76 (C-5). – FABMS (positive ion, glycerol matrix): $m/z = 413$ $[M+1]^+$.

Octadecane (**3**): White translucent solid. – ^1H NMR (100 MHz, $CDCl_3$): $\delta = 0.8 - 0.9$ (t, 3H × 2), 1.2 – 1.3 (m, CH_2). – ^{13}C NMR (25 MHz, $CDCl_3$): $\delta = 14.1$ (C-1, C-18), 22.7 (C-2, C-17), 31.95 (C-3, C-16), 29.39 (C-4, C-15), 29.70 (C-5 to C-14). – GC/MS: $m/z = 254$ $[M]^+$.

Butyrospermol acetate (**4**): White amorphous powder. – M.p. 144 – 145 °C. – UV/vis (hexane): $\lambda_{max} = 218$, 263 nm. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.74$ (s, 3H, 25-H), 0.78 (s, 3H, 27-H), 0.85 (d like, 3H, 28-H), 0.86 (s, 3H, 24-H), 0.92 (s, 3H, 23-H), 0.96 (s, 3H, 26-H), 1.60 (s, 3H, 30-H), 1.63 (s, 3H, 29-H), 2.03 (CH_3COO), 4.50 (dd, 1H, $J = 10.9$,

5.4 Hz, 3-H), 5.07 (t, 1H, $J = 6$ Hz, 21-H), 5.22 (dd, 1H, $J = 5.4$, 2.7 Hz, 7-H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 13.1$ (C-25); 15.8 (C-24), 17.6 (C-30), 18.5 (C-28), 21.3 (OAc), 22.0 (C-27), 23.7 (C-6), 24.1 (C-2), 25.3 (C-20), 25.7 (C-29), 27.3 (C-26), 27.5 (C-23), 28.4 (C-16), 33.7 (C-15), 33.9 (C-12), 34.7 (C-10), 35.1 (C-19), 35.7 (C-18), 36.7 (C-1), 37.8 (C-4), 43.4 (C-13), 48.7 (C-9), 50.7 (C-5), 51.2 (C-14), 53.2 (C-17), 81.1 (C-3), 117.5 (C-7), 125.0 (C-21), 130.9 (C-22), 145.9 (C-8), 171.0 (CH_3COO). – EIMS: *m/z* (%) = 468 $[M]^+$ (43.2), 453 $[M\text{-}CH3]^+$ (100), 451 (5.6), 393 (44), 355 [M-side chain-2H]$^+$ (10.4), 315 (3.2), 301 (4), 271 (3.2), 255 (4), 241 (4), 229 (4), 227 (3.2), 187 (5.6), 121 (9.6), 109 (17.6), 95 (14.4), 69 (40), 55 (14.4), 43 $[CH_3CO]^+$ (21.6).

Bis(2-ethylhexyl)phthalate (**5**): Light yellow oil. – UV/vis (Et_2O): $\lambda_{max} = 246.2$, 273.4 nm. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.91$ (t, 6H, $J = 6.6$ Hz, 6-H, 2"-H); 1.20 – 1.50 (m, 2-H, 3-H, 4-H and 5-H, merged), 1.60 – 1.70 (q, 2H, 2'-H), 4.20 (dd like, 2H, 1-H), 7.51 (dd, 1H, $J = 6.6$, 3.3 Hz, 10-H), 7.68 (dd, 1H, $J = 6.6$, 3.3 Hz, 9-H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 10.8$ (C-6), 14.0 (C-2"), 23.6 (C-4), 22.9 (C-5), 28.8 (C-3), 30.2 (C-2'), 38.6 (C-2), 68.0 (C-1), 128.7 (C-10), 130.8 (C-9), 132.3 (C-8), 167.6 (C-7). – EIMS: m/z (%) = 390 $[M]^+$ (0.8), 279 (28.8), 167 (43.2), 149 (100), 132 (2.4), 113 (11.2), 83 (6.4), 71 (18.4).

Chrysophanol (**6**): Yellow powder. – UV/vis (MeOH): $\lambda_{max} = 225$, 253, 287, 429 nm; – UV/vis (MeOH + KOH): $\lambda_{max} = 214$, 233.5, 285.5, 507 nm. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.43$ (s, 3H, 3-Me); 7.06, (s, 1H, 2-H), 7.25 (d, 1H, $J = 6.6$ Hz, 7-H), 7.60 (s, 1H, 4-H), 7.63 (d, 1H, $J = 6.6$ Hz, 6-H), 7.79 (d, 1H, $J = 6.6$ Hz, 5-H), 11.98 (s, 1-OH), 12.09 (s, 8-OH). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 22.1$ (CH_3), 113.6 (C-12), 115.8 (C-13), 119.8 (C-7), 121.3 (C-4), 124.3 (C-2), 124.5 (C-5), 133.2 (C-14), 133.5 (C-11), 136.9 (C-6), 149.3 (C-3), 162.3 (C-1), 162.6 (C-8), 181.9 (C-10), 192.4 (C-9). – EIMS: m/z (%) = 254 $[M]^+$ (100), 239 $[M\text{-}CH_3]^+$ (1.6), 237 (2.4), 226 (8), 198 (4), 197 (6.4), 152 (6.4), 115 (4), 57 (3.2), 43 (3.2).

Physcion (**7**): Yellow powder. – UV/vis (MeOH): $\lambda_{max} = 223$, 282, 434 nm. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.42$ (s, 3-Me); 3.91 (s, 6-OMe), 6.66 (d, 1H, $J = 2.6$ Hz, 7-H), 7.06 (*d*-like, 1H, 2-H), 7.34 (d, 1H, $J = 2.6$ Hz, 5-H), 7.62 (*d*-like, 1H, 4-H), 12.10 (s, 1-OH), 12.30 (s, 8-OH). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 22.1$ (Me), 56.0 (OMe), 106.7 (C-7), 108.2 (C-5), 121.3 (C-4), 124.5 (C-2), 149.0 (C-3), 162.2 (C-8), 165.8 (C-1), 166.2 (C-6), 182.5 (C-10), 193.0 (C-9). – EIMS: m/z (%) = 284 $[M]^+$ (100), 255 (5.6), 241 (5.6), 227 (2.4), 213 (3.2), 198 (2.4), 185 (2.4), 128 (4.8), 43 (8.8).

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