Chemical Constituents of the Leaves of *Desmos cochinchinensis* var. *fulvescens* BAN

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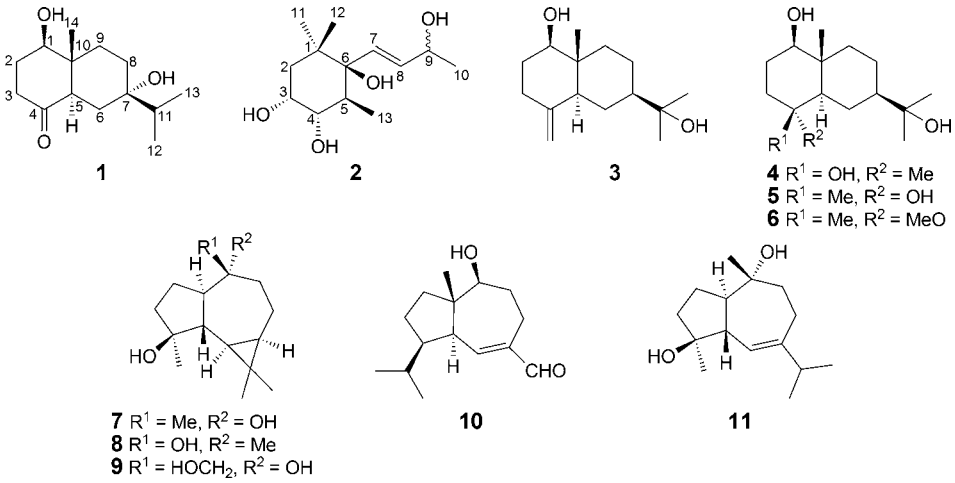
A phytochemical investigation of MeOH extract of *Desmos cochinchinensis* var. *fulvescens* BAN afforded two new compounds, 1β,7α-dihydroxyeudesman-4-one (**1**) and 5αH-megastigm-7-ene-3α,4α,6β,9-tetrol (**2**), together with nine known terpenoids. The structures of the new compounds were elucidated by 1D- and 2D-NMR spectroscopic analysis. Their relative configurations were assigned by NOESY experiments.

Introduction. – The genus of *Desmos*, belonging to the family Annonaceae, is a climbing or upright shrub. About 42 species have been found in Oceania and in tropic and subtropic Asia. The phytochemical studies of genus *Desmos* resulted in the isolation of triterpenoids [1], alkaloids [2–3], chalcones [4–6], flavonoids, and a cyclopeptide [7]. Some of these components showed cytotoxic [7], antiplasmodial [2], and anti-HIV [8] activities. In some folk-medicine records, *D. cochinchinensis* was used to treat malaria in the south part of China. *D. cochinchinensis* var. *fulvescens* BAN is a rare species growing in the coastal forest of Vietnam and has never been phytochemically studied. In our preliminary bioactivity test, the MeOH extract of *D. cochinchinensis* var. *fulvescens* not only showed cytotoxic activities against Hep G2, Hep 3B, and MCF-7 cell lines (all IC_{50} values < 20 μg/ml) but also exhibited anti-inflammatory effects in fMLP-induced superoxide and elastase-release assays. Herein, we report the isolation and structure elucidation of two new natural products, 1β,7α-dihydroxyeudesman-4-one (**1**) and 5αH-megastigm-7-ene-3α,4α,6β,9-tetrol (**2**), along with nine known sesquiterpenes, *i.e.*, selin-4(15)-ene-1β,11-diol (**3**) [9][10], 4-epicryptomeridiol (**4**), cryptomeridiol (**5**) [11], 11-hydroxy-4α-methoxyselinane (**6**) [12], 4β,10α-dihydroxyaromadendrane (**7**), 4β,10β-dihydroxyaromadendrane (**8**) [13], pipelol A (**9**) [14], 10β-hydroxyisodauc-6-en-14-al (**10**) [15], and alismoxide (**11**) [16] from an MeOH extract of the leaves of *D. cochinchinensis* var. *fulvescens* BAN. The structures of all compounds were established by interpretation of their spectroscopic data, especially 2D-NMR. The relative configurations of new structures were assigned by NOESY experiments.



Results and Discussion. – A combination of silica-gel and reversed-phase *RP-18* column chromatography of the MeOH extract of *D. cochinchinensis* var. *fulvescens* BAN gave two new and nine known compounds.

Structure Elucidation. Compound **1** was assigned the molecular formula $C_{14}H_{24}O_3$ as determined by HR-ESI-MS (*m/z* 263.1625 ($[M+Na]^+$)), corresponding to three degrees of unsaturation. The IR spectrum indicated the presence of OH (3434 cm^{-1}), and C=O (1705 cm^{-1}) groups. The ^{1}H-NMR data of **1** (*Table 1*) exhibited one Me *singlet* (δ(H) 0.74), two Me *doublet*s (δ(H) 0.94, $J = 8.8$), and the signal of a CH–O group (δ(H) 3.91 (*dd*, $J = 12.0, 4.0$)). The ^{13}C-NMR (*Table 2*) and DEPT spectra of **1** showed 14 C-atom signals, consisting those of a C=O group (δ(C) 211.5), an O-bearing quaternary C-atom (δ(C) 73.1), a CH–O group (δ(C) 77.1); two aliphatic CH (δ(C) 49.4 and 38.9), five aliphatic CH_2 (δ(C) 28.3, 28.7, 30.2, 32.1, and 39.0), and three Me groups (δ(C) 10.1, 16.7, and 16.7), and a aliphatic C-atom (δ(C) 42.0). The above findings accounted for one degree of unsaturation, indicating that **1** had two rings. In the COSY spectrum (*Fig. 1*) of **1**, correlations from three H-atom sequences, δ(H) 3.91 (H–C(1))/1.84–1.96, 2.10–2.18 (CH_2(2))/2.25–2.46 (CH_2(3)); δ(H) 2.67 (H–C(5))/1.49–1.56 (CH_2(6)); and δ(H) 1.50–1.70 (CH_2(8))/1.65–1.72 (CH_2(9)), as well as an ^{i}Pr moiety, δ(H) 0.94 (Me(12) and Me(13))/1.53–1.73 (H–C(11)), were observed. The ^{i}Pr group was attached to C(7) as evidenced by the HMBCs (*Fig. 1*) from Me(12) and Me(13) to C(7) (δ(C) 73.1). In addition, HMBCs from both CH_2(6) and CH_2(8) to C(7) indicated these CH_2 groups were connected by C(7). The HMBCs from CH_2(3) and H–C(5) to C(4) (δ(C) 211.5) revealed the presence of a C=O group between them. Moreover, the HMBC from Me(14) to C(1) (δ(C) 77.1), C(10) (42.0), C(5) (49.4), and C(9) (32.1) constructed the bicyclic system of **1**. Thus, the above 2D-NMR revealed that **1** was an eudesmane-type sesquiterpene.

The relative configuration of **1** was determined on the basis of NOESY correlations (*Fig. 2*) and comparison with previous literature. Since the ^{1}H- and ^{13}C-NMR data were similar to those eudesmane derivatives [17][18], the OH group at C(1) was considered to be β-oriented. The present NOESY correlation between H–C(1) and H–C(5), and

Table 1. *^{1}H-NMR Data*[a]) (400 MHz) *of Compounds* **1** (in $CDCl_3$) *and* **2** (in C_5D_5N). δ in ppm, J in Hz.

Position	**1**	**2**
1	3.91 (*dd*, *J* = 4.0, 12.0)	
2	2.10–2.18 (*m*), 1.84–1.96 (*m*)	1.92 (*dd*, *J* = 2.4, 13.8), 2.38 (*dd*, *J* = 3.6, 13.8)
3	2.25–2.46 (*m*)	4.49 (br. *s*)
4		4.21–4.27 (*m*)
5	2.67 (*dd*, *J* = 4.0, 12.0)	2.61–2.69 (*m*)
6	1.49–1.56 (*m*)	
7		6.07 (*dd*, *J* = 1.2, 15.6)
8	1.50–1.70 (*m*)	6.35 (*dd*, *J* = 6.0, 15.6)
9	1.65–1.72 (*m*)	4.70–4.78 (*m*)
10		1.49 (*d*, *J* = 6.0)
11	1.53–1.73 (*m*)	1.17 (*s*)
12	0.94 (*d*, *J* = 8.8)	1.55 (*s*)
13	0.94 (*d*, *J* = 8.8)	1.50 (*d*, *J* = 6.6)
14	0.74 (*s*)	
HO–C(6)		5.06 (*s*)

[a]) Assignments accomplished by COSY and HMBC techniques.

Table 2. *^{13}C-NMR Data*[a]) (100 MHz) *of compounds* **1** in ($CDCl_3$) *and* **2** (in C_5D_5N)

Position	**1**	**2**	Position	**1**	**2**
1	77.1	38.2	8	28.3	135.8
2	30.2	42.0	9	32.1	68.1
3	39.0	70.8	10	42.0	12.6
4	211.5	73.5	11	38.9	26.5
5	49.4	37.5	12	16.7	27.8
6	28.7	80.0	13	16.7	25.0
7	73.1	133.4	14	10.1	

[a]) Assignments accomplished by HMQC and HMBC techniques.

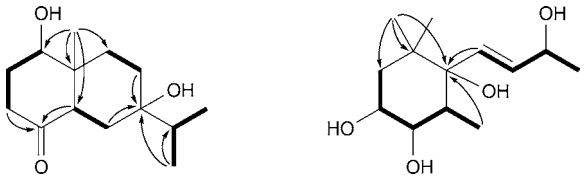


Fig. 1. *^{1}H,^{1}H-COSY* (—) *correlations and key HMBCs* (H → C) *of* **1** *and* **2**

the absence of NOESY correlation between H–C(5) and Me(14) indicated that the bicycle was *trans*-fused, and Me(14) and the OH group at C(1) were β-oriented. Moreover, the absence of NOESY cross-peaks H–C(5)/Me(12) and Me(13) suggested that the OH group at C(7) was located on the α-face of the molecule. These findings established the structure of 1β,7α-dihydroxyeudesman-4-one (**1**), unambiguously.

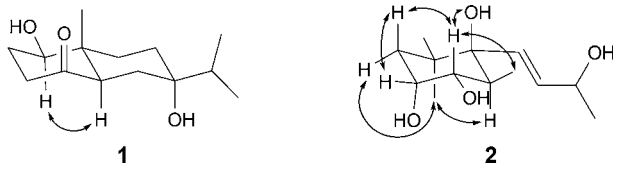


Fig. 2. *Key NOESY* (H ↔ H) *correlations and relative configurations of* **1** *and* **2**

Compound **2** was obtained as pale-yellow oil and assigned the molecular formula, $C_{13}H_{24}O_4$ ($\Delta = 2$), as deduced from HR-ESI-MS (*m/z* 267.1573 ($[M + Na]^+$)). The IR spectrum evidenced the presence of OH (3472 cm^{-1}) and C–O (1093 cm^{-1}) functionalities. The ^{1}H-NMR spectroscopic data (*Table 1*) disclosed two Me *singlet*s (δ(H) 1.17 and 1.55), two Me *doublet*s (δ(H) 1.49 ($J = 6.0$) and 1.50 ($J = 6.6$)), and signals of three CH–O groups (δ(H) 4.21 – 4.27 (*m*), 4.49 (br. *s*), 4.70 – 4.78 (*m*)) and two (*E*)-olefinic CH groups (δ(H) 6.07 (*dd*, $J = 1.2$, 15.6), 6.35 (*dd*, $J = 6.0$, 15.6)). The ^{13}C-NMR and DEPT spectra (*Table 2*) revealed the presence of two olefinic CH groups (δ(C) 133.4 and 135.8), an O-bearing quaternary C-atom (δ(C) 80.0), three CH–O groups (δ(C) 68.1, 70.8, and 73.5), a CH_2 group (δ(C) 42.0), an aliphatic C-atom (δ(C) 38.2), and four Me groups (δ(C) 27.8, 26.5, 25.0, and 12.6). In the COSY spectrum of **2** (*Fig. 1*), two spin systems, δ(H) 1.92 and 2.38 (CH_2(2))/4.49 (H–C(3))/4.21 – 4.27 (H–C(4))/2.61 – 2.69 (H–C(5))/1.50 (Me(13)) and δ(H) 6.07 (H–C(7))/6.35 (H–C(8))/4.70 – 4.78 (H–C(9))/1.49 (Me(10)) were observed. The HMBCs from H–C(7) (δ(H) 6.07) and Me(13) (δ(H) 1.50) to C(6) (δ(C) 80.0) revealed that these two spin systems were attached to C(6). In addition, HMBCs from both δ(H) 1.17 and 1.55 to δ(C) 38.2, 42.0, and 80.0 indicated two germinal Me groups at C(1), and the connection of adjacent C-atoms C(2) and C(6). On the basis of above 2D-NMR analysis, the constitution of **2** was established.

The configuration of **2** was determined by a NOESY experiment and coupling-constant analysis. The coupling constants between H_α–C(2) and H_β–C(2), and H–C(3) are 2.4 and 3.6 Hz, respectively. This finding suggested that the OH group at C(3) was axial and located on α-face of the molecule. In the NOESY spectrum, the correlations H_β–C(2)/H–C(4), H–C(4)/HO–C(6), and H–C(4)/Me(13) suggested the β-configuration of these substituents. On the other hand, NOESY cross-peaks H_α–C(2)/Me(12) and Me(12)/H–C(5) indicated the α-configuration of these groups. Therefore, **2** was assigned the structure 5α*H*-megastigm-7-ene-3α,4α,6β,9-tetrol.

Experimental Part

General. Prep. TLC: Precoated silica-gel plates (*60 F-254*, 1 mm; *Merck*). Column chromatography (CC): silica gel 60 (SiO_2; *Merck*) and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, SE-Uppsala). FC, Flash chromatography. HPLC: *Lichrosorb Si-60* (7 μm, 250 mm × 10 mm) and *Lichrosorb Rp-18* (7 μm, 250 mm × 10 mm) columns; *Hitachi L-6250 Intelligent* pump, *Hitachi L-4000 H* UV detector. Optical rotations: *Jasco DIP-1000* polarimeter. UV Spectra: *Hitachi U-3210* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi T-2001* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H-, ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY spectra: *Varian Unity-Inova-400* FT-NMR spectrometers at 400 (^{1}H) and 100 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Finingan Mat 95S* Mass spectrometer; in *m/z*.

Plant Material. D. cochinchinensis var. *fulvescens* BAN was collected from Ha Tinh province, Vietnam, in April, 2004, and was identified by one of the authors (*T. D. T.*). A voucher specimen (KMU-

Desmos-1) was deposited with Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Taiwan.

Extraction and Isolation. The dried aerial parts of *D. cochinchinensis* var. *fulvescens* BAN (10.0 kg) were air dried and extracted with MeOH at r.t. (5×3 d). After evaporation of the solvent, the crude extract (960.0 g) was partitioned with AcOEt/H_2O 1:1. The org. layer was concentrated under reduced pressure to afford an org. portion (178.3 g). This portion was repartitioned between hexane/MeOH/H_2O 4:3:1 to give a MeOH-soluble residue (80.3 g). This residue was subjected CC (silica gel; step gradient of hexane/AcOEt/MeOH) to give 19 fractions. *Fr. 11* was submitted to CC (*Sephadex LH-20*; MeOH) to afford seven fractions. *Subfr. 11-4* was separated by RP-HPLC (MeOH/H_2O 35:65) to yield **1** (1.9 mg), **2** (1.2 mg), and **9** (3.2 mg). *Fr. 4* was subjected to CC (silica gel; CH_2Cl_2 and MeOH) to give five fractions. *Subfr. 4-3* was further purified by prep. TLC (CH_2Cl_2/MeOH 50:1) to afford **6** (3.7 mg) and **10** (1.1 mg). *Fr. 7* was also separated by CC (*Sephadex LH-20*; MeOH) to afford nine fractions. *Subfr. 7-3* was purified by a RP-HPLC (MeOH/H_2O 73:27) to give **3** (5.4 mg), **4** (56.8 mg), **7** (6.0 mg), **8** (5.5 mg), and **11** (11.9 mg). *Fr. 8* was subjected to CC (*Sephadex LH-20*; MeOH) and then to RP-HPLC (MeOH/H_2O 73:27) to afford **5** (37.2 mg).

1β,7α-Dihydroxyleudesman-4-one (=*(4*R*,4a*R*,7*S*,8a*R*)-4,7-Dihydroxy-4a-methyl-7-(propan-2-yl)octahydronaphthalen-1(2*H*)-one*; **1**). Colorless oil. $[\alpha]_D^{25} = -12.7$ ($c = 0.48$, CH_2Cl_2). UV (MeOH): 207 (2.73). IR (neat): 3434, 2955, 2876, 1705, 1462, 1384, 1272, 1027, 906, 736. ^{1}H- and ^{13}C-NMR ($CDCl_3$): see *Tables 1* and *2*, resp. HR-ESI-MS: 263.1625 ($C_{14}H_{24}NaO_3^+$; calc. 263.1623).

*5α*H*-Megastigm-7-ene-3α,4α,6β,9-tetrol* (=*(1*R*,2*S*,3*S*,4*S*)-4-[(1*E*)-3-Hydroxybut-1-en-1-yl]-3,5,5-trimethylcyclohexane-1,2,4-triol*; **2**). Pale-yellow oil. $[\alpha]_D^{25} = -54.9$ ($c = 0.27$, CH_2Cl_2). UV (MeOH): 208 (3.42). IR (neat): 3472, 2924, 2855, 1460, 1376, 1261, 1093, 1028, 802. ^{1}H- and ^{13}C-NMR (C_5D_5N): see *Tables 1* and *2*, resp. HR-ESI-MS: 267.1573 ($C_{13}H_{24}NaO_4^+$; calc. 267.1572).

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REFERENCES

[1] J. D. Connolly, M. D. E. Haque, C. M. Hasan, S. Hossain, *Phytochemistry* **1994**, *36*, 1337.
[2] N. T. Nguyen, V. C. P. Litaudon, F. O. Gueritte, P. Grellier, V. T. Nguyen, V. H. Nguyen, *J. Nat. Prod.* **2008**, *71*, 2057.
[3] K. C. Chan, H. T. Toh, *Phytochemistry* **1986**, *25*, 1999.
[4] N. T. Nguyen, V. C. Pham, M. Litaudon, F. Gueritte, B. Bodo, V. T. Nguyen, V. H. Nguyen, *Tetrahedron* **2009**, *65*, 7171.
[5] M. M. Rahman, N. Qais, M. A. Rashid, *Fitoperapia* **2003**, *74*, 511.
[6] K. Awang, Z. Abdullah, M. R. Mukhtar, M. Litaudon, F. M. Jaafar, A. H. A. Hadi, N. F. Thomas, *Nat. Prod. Res.* **2009**, *23*, 652.
[7] M. Sulaiman, M.-T. Martin, M. Pas, A. H. A. Hadi, K. Awang, *Phytochemistry* **1998**, *49*, 2191.
[8] J.-H. Wu, X.-H. Wang, Y.-H. Yi, K.-H. Lee, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1813.
[9] D. Adinarayana, K. V. Syamasundar, *Phytochemistry* **1982**, *21*, 1083.
[10] M. Isaka, S. Palasarn, S. Lapanun, R. Chanthaket, N. Boonyuen, S. Lumyong, *J. Nat. Prod.* **2009**, *72*, 1720.
[11] W.-C. Su, J.-M. Fang, Y.-S. Cheng, *Phytochemistry* **1995**, *39*, 603.
[12] N. Kaneko, S. Hasegawa, Y. Hirose, *Phytochemistry* **1985**, *24*, 185.
[13] G. Goldsby, B. A. Bruke, *Phytochemistry* **1987**, *26*, 1059.
[14] C. Masuoka, M. Ono, Y. Ito, M. Okawa, T. Nohara, *Chem. Pharm. Bull.* **2002**, *50*, 1413.
[15] W. D. Xie, Y. F. Niu, P. X. Lai, K. H. Row, *Chem. Pharm. Bull.* **2010**, *58*, 991.
[16] G.-P. Peng, G. Tian, X.-F. Huang, F.-C. Lou, *Phytochemistry* **2003**, *63*, 877.
[17] X. Li, M. Yang, Y. F. Han, K. Gao, *Planta Med.* **2005**, *71*, 268.
[18] T. V. Sung, B. Steffan, W. Steglich, G. Klebe, G. Adam, *Phytochemistry* **1992**, *31*, 3515.

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