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Dioscorealides and Dioscoreanone, Novel Cytotoxic Naphthofuranoxepins, and 1,4-Phenanthraquinone from *Dioscorea membranacea* Pierre

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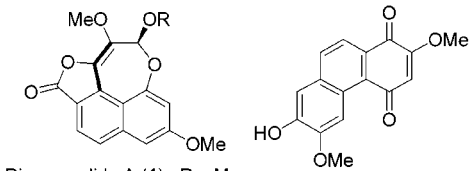
ABSTRACT



Dioscorealide A (**1**) ; R = Me
Dioscorealide B (**2**) ; R = H

Dioscoreanone (**3**)

Commonly used among ingredients in Thai traditional anticancer preparations, the rhizome of *Dioscorea membranacea* Pierre was found potently cytotoxic and possibly contributed to such a therapeutic effect. Bioassay-guided isolation resulted in two novel cytotoxic naphthofuranoxepins, dioscorealides A (1) and B (2), and a new 1,4-phenanthraquinone, dioscoreanone (3). The structure determination, achieved mainly by means of NMR and CD spectral and X-ray crystallographic analyses, and cytotoxicity are discussed here.

In Thai traditional medicines, herbal drugs named "Hua-Khao-Yen" have been long used as common ingredients in several preparations, including those used in the treatments of lymphopathy, dermopathy, venereal diseases, leprosy, and cancers. Interestingly, despite their close resemblance, the drugs available in traditional drug stores throughout the country are in fact rhizomes from different plant species from at least three genera, *Dioscorea* of the Dioscoreaceae, *Smilax* of the Smilacaceae, and *Pygmaeopremna* of the Verbenaceae.[1] However, whereas the plants in *Smilax* and *Pygmaeopremna* were practically inactive, the EtOH extract from *D. membranacea* Pierre rhizome was potently cytotoxic against various cancer cell lines, including COR-L23, LS-174T, MCF-7, and SVK-14 (cell mortality >90% at 50 μg/mL). Here, we report the isolation of three novel cytotoxic agents, dioscorealides A (**1**) and B (**2**) and dioscoreanone (**3**).

Mimicking the procedure practiced in Thai traditional remedies, the ground dried rhizomes of *D. membranacea*[2] were exhaustively percolated with EtOH. An aliquot of the dried percolate was chromatographed repeatedly over SiO_2

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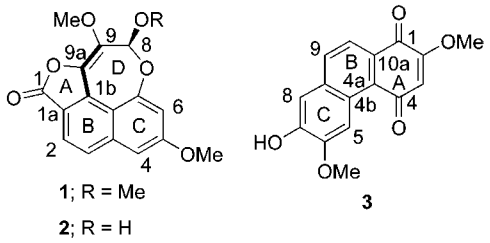
Table 1. NMR Spectral Data for **1** and **2** (500 MHz for ^{1}H)

	1 (in $CDCl_3$)			**2** (in DMSO-d_6)		
positions	^{1}H (mult, *J* in Hz)[a]	^{13}C	HMBC (C → H)	^{1}H (mult, *J* in Hz)[a]	^{13}C	HMBC (C → H)
1		166.8	H-2, H-3		168.9	
1a		115.6	H-3		114.9	H-3
1b		136.7	H-2, H-3		136.8	H-2
2	7.72 (d, 8.5)	121.4		7.76 (d, 8.5)	121.2	
3	7.66 (d, 8.5)	127.9	H-2, H-4	7.88 (d, 8.5)	128.2	H-4
3a		138.6	H-2, H-3		138.5	H-2
3b		116.2	H-2, H-3, H-4, H-6		116.3	H-3, H-4
4	7.03 (d, 2.0)	103.6	H-3, H-6	7.36 (d, 2.5)	103.9	
5		161.0	H-4, H-5, 5-OCH_3		162.2	5-OCH_3
6	6.94 (d, 2.0)	110.5	H-4	6.88 (d, 2.5)	110.7	H-4
6a		151.0	H-3, H-4, H-6, H-8		152.1	
8	5.57 (s)	102.7	8-OCH_3	6.03 (s)	94.1	
9		140.3	H-8, 9-OCH_3		141.9	9-OCH_3
9a		130.4	H-8		130.7	
5-OCH_3	3.96 (3H, s)	55.7		3.96 (3H, s)	56.7	
8-OCH_3	3.56 (3H, s)	56.8	H-8			
9-OCH_3	4.24 (3H, s)	60.9		4.13 (3H, s)	60.0	

[a] Unless stated otherwise, each proton signal was integrated as one proton.

(first column, gradient $CHCl_3$ to MeOH; second column, 20% $CHCl_3$ in *n*-hexane) to afford dioscorealides A (**1**) and B (**2**), and dioscoreanone (**3**)[3] (11, 31, and 8 mg, respectively).



The molecular formula of dioscorealide A (**1**)[3a] was proposed to be $C_{17}H_{14}O_6$, as rationalized from its ^{13}C NMR spectrum and its molecular mass from HR-EIMS. This leads to the unsaturation degree of 11 (one carbonyl, six olefins, and four rings), suggesting a condensed aromatic skeleton.

In its ^{1}H NMR spectrum (Table 1), two aromatic spin systems were observed. One is two *ortho* protons of a tetrasubstituted benzene ring (δ 7.72, d, J = 8.5 Hz, H-2; and 7.66, d, J = 8.5 Hz, H-3), and the other is two *meta* protons of another tetrasubstituted benzene (δ 7.03, d, J = 2.0 Hz, H-4; and 6.94, d, J = 2.0 Hz, H-6). Both systems were connected through HMBC correlations from C-3 (δ 127.9) to H-4 and from C-4 (δ 103.6) to H-3, to compose rings B and C as a naphthalene moiety. The HMBC correlations also help in connecting a methoxyl group (δ 3.96, s, 5-OCH_3) onto C-5 (δ 161.0), a methoxy-methine-oxy moiety (δ 5.57, s, H-8) onto C-6a (δ 151.0), and an ester carbonyl carbon (δ 166.8, C-1) onto C-1a (δ 115.6).

The HMBC correlations from two remaining olefinic carbons (δ 140.3, C-9; and 130.4, C-9a) that were observed only to H-8, however, led to an ambivalent structure determination. Without additional observable correlations from C-9 and C-9a to other protons, either may therefore be directly attached to C-8; two possible structures, **A** and **B**, thus arose (Figure 1). Structure **A** was proposed as a naphthofuranoxepin, of which C-8 connected directly to C-9, and **B** was as a naphthopyranopyran, where C-8 instead connected to C-9a.

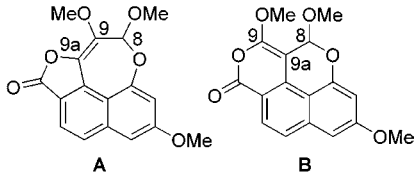


Figure 1. Possible structures of compound **1**, based on the HMBC correlation from C-9 and C-9a to H-8.

(2) *Dioscorea membranacea* Pierre was kindly identified by Dr. Tippan Sdakorn of the Department of Forestry, Ministry of Agriculture and Cooperation, and by Mr. Cherdsak Thapyai of the Faculty of Pharmaceutical Sciences, Naresuan University. The authentic specimen is deposited at the herbarium of the Department of Forestry, Bangkok, Thailand.

(3) (a) Dioscorealide A (**1**): white crystal; mp 155−156 °C.; $[\alpha]_D$ +73.1 (*c* 0.1, $CHCl_3$); CD (*c* 1.6 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) 0 (412), −0.8 (360), 0 (327), 1.5 (310), 0.7 (275), 1.3 (250), 0 (220) M^{-1} cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 216 (4.33), 270 (4.43), 308 (4.26) nm; IR (KBr) ν_{max} 2940, 1750, 1680, 1620 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HR-EIMS *m/z* M^+ 314.0790 (calcd for $C_{17}H_{14}O_6$, 314.0790); EIMS *m/z* (relative intensity) 314 (M^+, 81), 283 (100), 227 (18), 83 (83). b) Diocorealide B (**2**): white crystal; mp 282−283 °C (dec); $[\alpha]_D$ +57.4 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (4.29), 266 (4.39), 310 (4.22) nm; IR (KBr) ν_{max} 3400, 2950, 1740, 1680, 1630 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HR-EIMS *m/z* M^+ 300.0634 (calcd for $C_{16}H_{12}O_6$, 300.0634); EIMS *m/z* (relative intensity) 300 (M^+, 100), 277 (45), 257 (27), 229 (77). (c) Dioscoreanone (**3**): yellow crystal; mp 266−267 °C.; UV (MeOH) λ_{max} (log ϵ) 246 (4.44), 280 (4.04), 302 (4.01) nm; IR (KBr) ν_{max} 3450, 3140, 1665, 1630, 1605, 1580 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HR-FABMS *m/z* MH^+ 285.0763 (calcd for $C_{16}H_{13}O_5$, 285.0763); EIMS *m/z* (relative intensity) 284 (M^+, 100), 267 (9), 254 (22), 231 (19).

To resolve such ambiguity, an X-ray crystallographic analysis was employed. The perspective drawing (Figure 2)

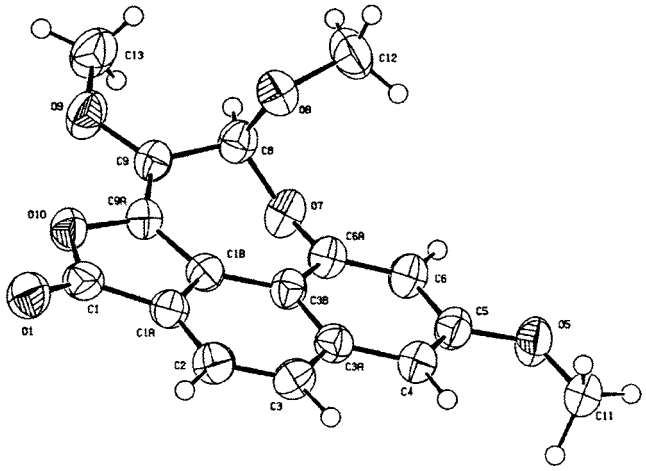


Figure 2. Computer-generated perspective drawing of **1**.

clearly demonstrated the unusual yet novel naphthofuran-oxepin skeleton of compound **1** as **A**. This structure was confirmed by a cascade of dipolar coupling along the circumference of the molecule; i.e., H-3 ↔ H-4 ↔ 5-OCH_3 ↔ H-6 ↔ 8-OCH_3 and H-8 ↔ 9-OCH_3, as observed in the NOE−ds experiments.

Besides the unambiguous observation of the preferred pseudoaxial orientation of the 8-OCH_3 group as contributed by the anomeric effect, the drawing also shows that the core skeleton of **1** is in fact not completely flat but twisted in the oxepin ring D. Such twist leads to the skew position between ring C of the naphthyl moiety and the olefinic bond of C-9−C-9a, with an observed torsion angle, i.e., between the plane of C-3b−C-6a and that of C-9−C-9a, of 12.86°. Counterclockwise helicity of the two planes, suggested by the first negative Cotton effect at 356 nm in the CD spectrum, indicates that the oxepin ring actually adopts the *M* conformation (Figure 3). Here, the proposed *S* configuration at C-8 is deduced indirectly from the 8-OCH_3 pseudoaxial orientation on the nonflipping *M*-oxepin.

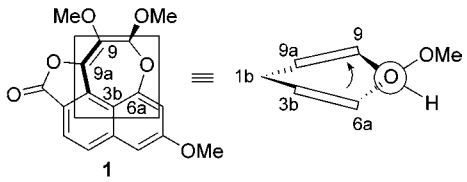


Figure 3. *M*-oxepin moiety of **1** showing the C-3b−C-6a ↔ C-9−C-9a skew conformation, with O-7 projected on C-8.

The molecular formula of dioscorealide B (**2**),[3b] as suggested by its ^{13}C NMR and HR-EI mass spectra, was proposed to be $C_{16}H_{12}O_6$. Despite different operating solvents, the ^1H and ^{13}C NMR spectra of **2** (Table 1) are almost identical to that of **1**, clearly indicating that the two share a similar skeleton. The major differences observed between the compounds are the absence of the methoxy signals previously assigned for 8-OCH_3 and the significant upfield shift of the acetal carbon C-8 (δ 102.7 for **1**; δ 94.1 for **2**). This strongly indicates that **2** is in fact the lactol analogue of **1**, with the structure as proposed. The cascade of dipolar coupling previously seen in **1**, i.e., H-3 ↔ H-4 ↔ 5-OCH_3 ↔ H-6 and H-8 ↔ 9-OCH_3, is also observed here. However, in our course to determine its absolute stereochemistry, the compound failed to yield the CD spectra of either the parent compound or its cinnamate derivative, and Mosher's analysis proved inapplicable because of the pseudoaxial orientation of the 8-OH group. Here, the stereochemistry of the compound shown was thus arbitrarily proposed to be similar to that of **1**, i.e., possessing *S* configuration.

The molecular formula of dioscoreanone (**3**)[3c] was proposed to be $C_{16}H_{12}O_5$, as deduced from the ^{13}C NMR and HR-FAB mass spectra. The presence of two non-carboxylate carbonyls, six olefins, and three ring moieties was deduced from its unsaturation degree of 11.

The tetrasubstituted naphthalene ring system B and C was constructed by connecting two *ortho* aromatic protons (δ 8.06, d, J = 8.5 Hz, H-10; and 7.92, d, J = 8.5 Hz, H-9) to two aromatic singlets at δ 9.13 (H-5) and 7.24 (H-8), via the analysis of the HMBC spectrum; i.e., C-4a (δ 125.7) and C-8a (δ 135.1) to H-5 and H-10, and C-4b (δ 126.3) to H-8 and H-9 (see Table 2). Also by means of HMBC spectrum, a methoxyl (δ 4.76, s) and a hydroxyl group were placed onto C-6 (δ 151.6) and C-7 (δ 149.0), respectively.

Table 2. NMR Spectral Data of **3** (500 MHz for ^1H) in 1:1 Mixture of $CDCl_3$ and CD_3OD

position	^1H (mult, J in Hz)[a]	^{13}C	HMBC (C → H)
1		181.4	H-3, H-10
2		158.6	H-3, 2-OCH_3
3	6.11 (s)	111.3	
4		189.3	H-3
4a		125.7	H-5, H-10
4b		126.3	H-3, H-8, H-9
5	9.13 (s)	106.4	
6		151.6	H-5, H-8, 6-OCH_3
7		149.0	H-5, H-8
8	7.24 (s)	110.1	H-9
8a		135.1	H-5, H-10
9	7.92 (d, 8,5)	132.3	H-8
10	8.06 (d, 8,5)	121.0	
10a		129.4	H-9
2-OCH_3	3.93 (3H, s)	56.3	
6-OCH_3	4.12 (3H, s)	56.1	

[a] Unless stated otherwise, each proton signal was integrated as one proton.

The characteristic quinone chemical shifts of C-1 (δ 181.4) and C-4 (δ 189.3) suggest that ring A is a *p*-quinone moiety, of which a methoxyl group (δ 3.93, s, 3-OCH_3) was placed next to an aromatic methine (δ 6.11, s, H-2). Connection of the naphthalene unit, B and C, to the quinone ring A led to

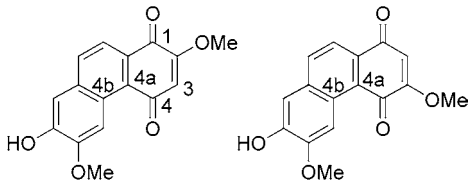


Figure 4. Possible structures of compound **3**, based on the HMBC correlation from C-1 and C-4 to H-3.

two possible structures, **C** and **D** (Figure 4). Here, we propose that structure **C** is the most plausible. The four-bond HMBC correlation, i.e., C-4b → H-3, although rare, is possible especially when facilitated by a zigzag path only seen with structure **C**. This was strongly confirmed by the dipolar coupling between 2-OCH_3 and H-3 and between H-5 and 6-OCH_3. Dioscoreanone (**3**) is therefore proposed as a new member of the 1,4-phenanthraquinone family as shown.

The cytotoxicity of all three compounds was determined using the SRB assay.[4] The target cell lines here were large-cell lung carcinoma COR-L23, colon adenocarcinoma LS-174T, breast adenocarcinoma MCF-7, and noncancer human keratinocyte SVK-14 (Table 3). Among the three compounds, **1** is slightly active against only MCF-7, whereas **2** exhibits the best potency, especially against MCF-7 and COR-L23, as well as the best selective discrimination among normal and cancer cells. On the other hand, **3**, although active at a similar magnitude, does not show selectivity as good as that of **2**.

In summary, the chemical investigation and cytotoxicity determination of *D. membranacea* rhizome supported the indigenous knowledge from traditional medicine. The naphthofuranoxepin skeleton of **1** and **2** is clearly unprecedented in nature. Their ring systems, however, while novel and fascinating, might possibly originate from a rather simple heptaketide precursor. In fact, the postulation of heptaketide precursor can be extended to the 1,4-phenanthraquinone as well. The potent and selective cytotoxicity demonstrates the strong potential of the plant, as well as of the compounds in the same series, as a new source in cancer chemotherapy.

Table 3. Cytotoxicity of **1**, **2**, and **3** (IC_{50} in μg/mL) Compared with Vincristine SO_4 (VS; IC_{50} in nM) as Positive Standard[a]

	targeted cell lines			
compounds	COR-L23	LS-174T	MCF-7	SVK-14
VS	1.38 ± 0.16	2.67 ± 0.14	1.65 ± 0.13	na
1	42.4 ± 1.40	41.9 ± 0.80	27.4 ± 0.06	>100
2	1.59 ± 0.57	5.26 ± 1.95	0.92 ± 0.10	43.5 ± 0.40
3	2.89 ± 0.13	9.96 ± 0.20	3.76 ± 2.50	16.5 ± 0.40

[a] Significant difference between normal and each cancer cell lines, $P < 0.05$).

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Supporting Information Available: Detailed description of experimental procedures, NMR (1D and HMBC) and CD spectra, and crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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