Supporting information

Dioscorealides and dioscoreanone, novel cytotoxic naphthofuranoxepins and 1,4-phenanthraquinone from *Dioscorea membranacea* Pierre.

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Supporting information

Experiment procedure

General. ^1H and ^{13}C NMR experiments were performed on a Varian Unity Inova 500 spectrometer (500 MHz for ^1H ; 125 MHz for ^{13}C), and reported in δ scale referring to solvent signals as indicated in Tables 1 and 2. UV spectra were obtained from a Hewlett Packard 8452A Diode array spectrometer. IR spectra were recorded on an Equinox55 (**1** and **2**) or a Jasco IR-810 spectrometer (**3**). EI mass spectra, both HR- and LR-, were obtained from a JEOL JMS-AX505W spectrometer. HR-FAB mass spectra was measured on KRATOS MS890MS spectrometer. CD experiment was conducted on an AVIV 17DS instrument. Melting points were determined on a Büchi 520 melting point apparatus and were uncorrected. Optical rotations were obtained from a Perkin Elmer 341 polarimeter, and reported as specific rotation.

Plant material. The rhizome of *D. membranacea* Pierre was collected from Chumphon Province, Thailand, in March 1999. The plant was kindly identified by Dr. Tippun Sdakorn of the Department of Forestry, Ministry of Agriculture and Cooperation, and by Mr. Cherdsak Thapyai of Faculty of Pharmaceutical Sciences, Naresuan University. The authentic specimen is now deposited at the herbarium of the Department of Forestry, Bangkok, Thailand (voucher number SKP A062001003).

Extraction and isolation. The dried-ground rhizome of *D. membranacea* (5 kg) was exhaustively percolated in EtOH to yield 170 g of crude extract. A 10-g aliquot of this extract was then subjected to a vacuum chromatography over a SiO_2 column with gradient CHCl_3 -MeOH (CHCl_3 to 50% MeOH in CHCl_3 to MeOH) as eluting solvent.

Monitored by cytotoxicity, the most active subfraction was further fractionated over a SiO₂ column (20% CHCl₃ in hexane), affording compounds **1** (11 mg, $3.7 \times 10^{-3}\%$), **2** (31 mg, $10.5 \times 10^{-3}\%$), and **3** (8 mg, $2.7 \times 10^{-3}\%$), all as crystals from MeOH.

Crystal and molecular structure Dioscorealide A

Crystal data

$C_{17}H_{14}O_6$

$M_r = 314.293$

Orthorhombic

$P2_12_12_1$

$a = 6.6975 (3) \text{ \AA}$

$b = 14.1282 (13) \text{ \AA}$

$c = 15.0059 (11) \text{ \AA}$

$\alpha = 90.00^\circ$

$\beta = 90.00^\circ$

$\gamma = 90.00^\circ$

$V = 1419.9 (2) \text{ \AA}^3$

$Z = 4$

$D_x = 1.470 \text{ Mg m}^{-3}$

fine-focus sealed tube

Mo $K\alpha$ radiation

$\lambda = 0.71073$

Cell parameters from 1443 reflections

$\theta = 2.910\text{--}26.373^\circ$

$\mu = 0.112 \text{ mm}^{-1}$

$T = 298 \text{ K}$

prism

Colourless

Crystal source: *Dioscorea membranacea* Pierre

Data collection

KappaCCD

Absorption correction: none

2652 measured reflections

2641 independent reflections

2389 observed reflections

Criterion: $>2\sigma(I)$

$\theta_{\max} = 26.33^\circ$

$h = -7 \rightarrow 7$

$k = -16 \rightarrow 16$

$l = -18 \rightarrow 18$

Refinement

Refinement on F^2

fullmatrix least squares refinement

$R(\text{all}) = 0.0463$

$R(\text{gt}) = 0.0395$

$wR(\text{ref}) = 0.1063$

$wR(\text{gt}) = 0.0993$

$S(\text{ref}) = 1.030$

2641 reflections

209 parameters

0 restraints

Only coordinates of H atoms refined

$\Delta\sigma_{\max} = 0.001$

$\Delta\rho_{\max} = 0.110 \text{ e \AA}^{-3}$

$\Delta\rho_{\min} = -0.119 \text{ e \AA}^{-3}$

Extinction correction: *SHELXL*

$F_c^* = kF_c [1 + 0.001 \times F_c^2 \lambda^3 / \sin(2\theta)]^{-1/4}$

Extinction coefficient = 0.069 (10)

Atomic scattering factors from

International Tables Vol C Tables

4.2.6.8 and 6.1.1.4

Data collection: KappaCCD

Cell refinement: HKL Scalepack (Otwinowski & Minor 1997)

Data reduction: Denzo and Scalepack (Otwinowski & Minor, 1997)

Program(s) used to solve structure: *SIR97* (Cascarano al., *Acta Cryst.*, 1996, A52, C-79)

Program(s) used to refine structure: *SHELXL-97* (Sheldrick, 1997)

Table 1. *Fractional atomic coordinates and equivalent isotropic thermal parameters ($\text{\AA}^2$)*

$$U_{eq} = 1/3 \sum_i \sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j.$$

	x	y	z	U_{eq}	Occ
O4	0.3373 (2)	0.03083 (12)	0.84851 (9)	0.0486 (4)	1
O3	0.8884 (2)	0.12312 (13)	0.68159 (10)	0.0554 (5)	1
O5	0.4181 (3)	-0.11710 (12)	0.91113 (10)	0.0522 (4)	1
C8A	0.3541 (3)	0.03918 (16)	1.04454 (12)	0.0413 (5)	1
O2	0.3316 (2)	0.05521 (11)	1.13616 (9)	0.0476 (4)	1
C1B	0.5372 (3)	0.08304 (15)	1.01587 (13)	0.0391 (5)	1
O6	0.0700 (3)	-0.05505 (14)	1.04268 (10)	0.0573 (5)	1
O1	0.5254 (3)	0.11513 (13)	1.24538 (9)	0.0598 (5)	1
C6A	0.5305 (3)	0.06329 (16)	0.85049 (13)	0.0420 (5)	1
C5	0.8134 (4)	0.11679 (17)	0.76599 (13)	0.0450 (5)	1
C1	0.5039 (4)	0.09896 (17)	1.16703 (13)	0.0465 (5)	1
C3	0.9086 (3)	0.17037 (16)	1.00497 (14)	0.0449 (5)	1
C3B	0.6240 (3)	0.09246 (15)	0.93041 (13)	0.0395 (5)	1
C2	0.8204 (4)	0.16195 (16)	1.08655 (14)	0.0460 (5)	1
C8	0.2283 (3)	-0.01723 (16)	0.99944 (13)	0.0423 (5)	1
C3A	0.8150 (3)	0.13669 (15)	0.92519 (13)	0.0416 (5)	1
C6	0.6229 (3)	0.07442 (17)	0.76969 (13)	0.0459 (5)	1
C4	0.9090 (3)	0.14790 (16)	0.84117 (14)	0.0448 (5)	1
C1A	0.6323 (3)	0.11764 (15)	1.09069 (12)	0.0415 (5)	1
C9	1.0821 (4)	0.16558 (19)	0.67193 (16)	0.0558 (6)	1
C7	0.2746 (3)	-0.04597 (17)	0.90490 (13)	0.0451 (5)	1
C11	-0.0653 (4)	-0.1149 (2)	0.99787 (18)	0.0687 (8)	1
C10	0.4476 (5)	-0.1676 (2)	0.82887 (19)	0.0704 (8)	1
H12	1.0586	0.1999	1.0009	0.054	1
H14	0.8957	0.1873	1.1468	0.055	1
H17	0.5416	0.0511	0.7063	0.055	1
H18	1.0523	0.1772	0.8441	0.054	1
H20A	1.0679	0.2378	0.6936	0.067	1
H20B	1.0874	0.1629	0.6092	0.067	1
H20C	1.1779	0.1221	0.7114	0.067	1
H21	0.1546	-0.0753	0.8768	0.054	1
H22A	-0.1326	-0.0773	0.9379	0.082	1
H22B	-0.1643	-0.1332	1.0381	0.082	1
H22C	0.0079	-0.1659	0.9769	0.082	1
H23A	0.3141	-0.1959	0.8116	0.085	1
H23B	0.5140	-0.1281	0.7807	0.085	1
H23C	0.5413	-0.2221	0.8417	0.085	1

Table 2. *Anisotropic displacement parameters ($\text{\AA}^2$)*

	U_{11}	U_{12}	U_{13}	U_{22}	U_{23}	U_{33}
O4	0.0380 (8)	-0.0037 (7)	-0.0031 (6)	0.0669 (11)	0.0087 (7)	0.0409 (7)
O3	0.0563 (11)	-0.0041 (9)	0.0119 (6)	0.0685 (11)	0.0003 (7)	0.0413 (8)
O5	0.0521 (10)	0.0038 (7)	0.0007 (7)	0.0555 (10)	-0.0074 (7)	0.0491 (8)
C8A	0.0417 (12)	0.0011 (9)	0.0024 (8)	0.0456 (12)	-0.0005 (8)	0.0366 (9)
O2	0.0521 (9)	-0.0045 (7)	0.0074 (6)	0.0531 (9)	-0.0042 (6)	0.0377 (7)
C1B	0.0414 (11)	0.0018 (8)	-0.0010 (7)	0.0387 (11)	0.0011 (8)	0.0372 (9)
O6	0.0468 (10)	-0.0173 (8)	0.0078 (7)	0.0738 (12)	-0.0036 (8)	0.0512 (8)
O1	0.0776 (13)	-0.0130 (9)	0.0042 (7)	0.0635 (12)	-0.0057 (7)	0.0382 (8)
C6A	0.0365 (11)	0.0007 (8)	-0.0010 (8)	0.0491 (13)	0.0018 (9)	0.0404 (10)
C5	0.0458 (12)	0.0024 (9)	0.0062 (8)	0.0488 (13)	0.0053 (9)	0.0404 (10)
C1	0.0574 (14)	-0.0037 (10)	0.0004 (9)	0.0414 (12)	-0.0011 (8)	0.0407 (10)
C3	0.0420 (12)	-0.0031 (9)	-0.0032 (9)	0.0464 (13)	0.0034 (9)	0.0462 (11)
C3B	0.0369 (11)	0.0035 (8)	0.0018 (7)	0.0438 (11)	0.0035 (8)	0.0377 (10)
C2	0.0494 (13)	-0.0024 (10)	-0.0049 (9)	0.0476 (13)	-0.0006 (9)	0.0410 (11)
C8	0.0376 (12)	-0.0003 (8)	0.0032 (8)	0.0468 (13)	0.0019 (9)	0.0424 (10)
C3A	0.0429 (12)	0.0019 (8)	0.0008 (8)	0.0404 (11)	0.0005 (8)	0.0415 (10)
C6	0.0427 (12)	-0.0004 (9)	0.0006 (8)	0.0548 (14)	0.0033 (9)	0.0403 (10)
C4	0.0423 (13)	-0.0006 (9)	0.0031 (8)	0.0479 (13)	0.0011 (9)	0.0442 (11)
C1A	0.0463 (12)	-0.0002 (9)	-0.0001 (8)	0.0402 (11)	0.0005 (8)	0.0379 (10)
C9	0.0599 (15)	-0.0052 (12)	0.0186 (11)	0.0546 (15)	0.0009 (10)	0.0528 (13)
C7	0.0405 (12)	-0.0037 (9)	-0.0003 (8)	0.0533 (13)	0.0006 (10)	0.0414 (10)
C11	0.0599 (17)	-0.0288 (14)	0.0071 (13)	0.081 (2)	-0.0078 (14)	0.0647 (15)
C10	0.0751 (19)	0.0010 (15)	0.0139 (14)	0.0707 (19)	-0.0244 (13)	0.0656 (16)

Table 3 . *Geometric parameters* (Å, °)

O4—C6A	1.373 (3)	C3—C2	1.365 (3)
O4—C7	1.439 (3)	C3—C3A	1.433 (3)
O3—C5	1.366 (2)	C3B—C3A	1.425 (3)
O3—C9	1.437 (3)	C2—C1A	1.408 (3)
O5—C7	1.394 (3)	C8—C7	1.508 (3)
O5—C10	1.439 (3)	C3A—C4	1.418 (3)
C8A—C8	1.343 (3)	C3—H12	1.0890
C8A—O2	1.401 (2)	C2—H14	1.0950
C8A—C1B	1.440 (3)	C6—H17	1.1441
O2—C1	1.389 (3)	C4—H18	1.0460
C1B—C1A	1.381 (3)	C9—H20A	1.0749
C1B—C3B	1.415 (3)	C9—H20B	0.9420
O6—C8	1.353 (3)	C9—H20C	1.0670
O6—C11	1.410 (3)	C7—H21	0.9970
O1—C1	1.206 (2)	C11—H22A	1.1380
C6A—C6	1.370 (3)	C11—H22B	0.9341
C6A—C3B	1.414 (3)	C11—H22C	0.9270
C5—C4	1.370 (3)	C10—H23A	1.0131
C5—C6	1.410 (3)	C10—H23B	1.0159
C1—C1A	1.457 (3)	C10—H23C	1.0120
C6A—O4—C7	120.96 (16)	C1B—C1A—C1	107.61 (19)
C5—O3—C9	116.95 (19)	C2—C1A—C1	130.07 (18)
C7—O5—C10	113.23 (18)	O5—C7—O4	112.42 (17)
C8—C8A—O2	121.52 (19)	O5—C7—C8	105.84 (16)
C8—C8A—C1B	129.74 (18)	O4—C7—C8	114.22 (19)
O2—C8A—C1B	108.37 (18)	C2—C3—H12	118.9
C1—O2—C8A	108.05 (16)	C3A—C3—H12	119.0
C1A—C1B—C3B	120.9 (2)	C3—C2—H14	120.8
C1A—C1B—C8A	107.62 (17)	C1A—C2—H14	121.4
C3B—C1B—C8A	131.43 (19)	C6A—C6—H17	119.2
C8—O6—C11	120.77 (18)	C5—C6—H17	121.3
C6—C6A—O4	116.40 (18)	C5—C4—H18	126.2
C6—C6A—C3B	121.1 (2)	C3A—C4—H18	114.5
O4—C6A—C3B	122.20 (18)	O3—C9—H20A	106.6
O3—C5—C4	124.8 (2)	O3—C9—H20B	96.8
O3—C5—C6	113.40 (19)	H20A—C9—H20B	110.1
C4—C5—C6	121.79 (19)	O3—C9—H20C	104.3
O1—C1—O2	120.6 (2)	H20A—C9—H20C	115.6
O1—C1—C1A	131.4 (2)	H20B—C9—H20C	120.6
O2—C1—C1A	108.02 (16)	O5—C7—H21	106.6
C2—C3—C3A	122.1 (2)	O4—C7—H21	107.4
C6A—C3B—C1B	124.0 (2)	C8—C7—H21	110.1
C6A—C3B—C3A	118.58 (18)	O6—C11—H22A	110.6
C1B—C3B—C3A	117.39 (18)	O6—C11—H22B	108.3
C3—C2—C1A	117.8 (2)	H22A—C11—H22B	111.1
C8A—C8—O6	118.97 (18)	O6—C11—H22C	106.8
C8A—C8—C7	120.33 (19)	H22A—C11—H22C	107.7
O6—C8—C7	120.39 (19)	H22B—C11—H22C	112.3
C4—C3A—C3B	119.75 (18)	O5—C10—H23A	107.1
C4—C3A—C3	120.7 (2)	O5—C10—H23B	113.4
C3B—C3A—C3	119.51 (18)	H23A—C10—H23B	114.9
C6A—C6—C5	119.47 (19)	O5—C10—H23C	107.4
C5—C4—C3A	119.2 (2)	H23A—C10—H23C	107.2
C1B—C1A—C2	122.32 (19)	H23B—C10—H23C	106.4

Name of sample: DMCS6
observed proton experiment
Pulse Sequence: s2pul

^1H NMR spectrum of **1** (500 MHz; CDCl_3)

Name of sample: DMCS6
Observed carbon experiment
Pulse Sequence: s2pu1

dmc-s1

Pulse Sequence: s2pul

¹H NMR spectrum of **2** (500 MHz; DMSO-*d*₆)

Name of sample:dmc-s1

Observed carbon experiment

exp16 s2pul

SAMPLE		DEC. & VT	
date	May 9 2000	dfrq	499.822
solvent	DMSO	dn	H1
file	exp	dpwr	38
ACQUISITION			
sfrq	125.692	dm	0
tn	C13	dmf	yyy
at	1.300	dmf	11768
np	65024	dseq	w
sw	25000.0	dres	1.0
fb	14000	homo	n
bs	64	temp	30.0
tpwr	54	PROCESSING	
pw	6.0	lb	1.00
d1	4.000	wfile	ft
nt	0	proc	not used
nt	4000	fn	f
ct	4000	math	
clock	n	werr	
gain	60	wexp	
l1	n	wbs	
l1	n	wnt	
dp	y		
hs	nn		
DISPLAY			
sp	1520.3		
wp	21329.4		
vs	3120		
sc	0		
sc	0		
h2mm	250		
ls	85.32		
ls	500.00		
rfl	5615.4		
rfl	4369.5		
th	12		
ins	100.000		
nm	cdc		
ph

Name of sample: DMSC7
observed proton experiment
solvent: CDCl₃+CD₃OD
Pulse Sequence: s2pu1

Name of sample:DMCS7

observed carbon experiment

exp4 s2pul

date	May 28 2001	dfreq	499.777
solvent	CDCl3	dn	H1
file	exp	dpwr	38
	ACQUISITION	dof	0
sfrq	125.683	dm	yyy
tn	1.301	dmm	w
at	78464	dseq	11768
np	30165.9	dres	1.0
sw	17000	homo	n
fb	8	PROCESSING	
bs	54	lb	0.50
tpwr	6.0	wtfile	
pw	1.000	proc	ft
d1	2513.4	fn	not used
tof	10000	math	f
nt	8060		
ct		werr	n
alock		wexp	n
gain	60	wbs	n
flags		wnt	n
il		y	y
in		nn	nn
dp			
hs			
sp	-659.6		
wp	30165.9		
vs	481		
sc	0		
wc	250		
hzmnm	120.66		
is	500.00		
rfl	659.6		
rfp	0		
th	3		
ins	100.000		
nm	cdc	ph

^{13}C NMR spectrum of **3** (125 MHz; $\text{CDCl}_3\text{-CD}_3\text{OD}$)

UV and CD spectra of **1**, **2** and dioscorealide A cinnamate.

note; **1** and - - - **2** (both in CH_3OH); — dioscorealide A cinnamate (in CHCl_3)