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Resveratrol Tetramers from *Vatica diospyroides*

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Vatdiospyroidol (**1**), a novel cytotoxic resveratrol tetramer, was isolated from the stems of *Vatica diospyroides* Sym. (Dipterocarpaceae) by bioassay-guided fractionation monitored with a human oral epidermoid carcinoma (KB) cell line. Another novel resveratrol tetramer, vaticaphenol A (**2**), was obtained as a noncytotoxic constituent, along with the known compounds, bergenin, betulin, betulinic acid, mangiferonic acid, and (*E*)-resveratrol 3-*O*-*β*-D-glucopyranoside. The structures of compounds **1** and **2** were elucidated by spectral analysis, including 1D and 2D NMR experiments, and by molecular modeling.

Introduction

The genus *Vatica* L. consists of over 60 species, which are small-to-medium-sized trees distributed primarily in Kalimantan and the Malay Peninsula.[1] Species in this genus grow in the drier areas of tropical evergreen forests and are found up to 1600 m in altitude.[2] *Vatica* belongs to the largest subfamily Dipterocarpoideae of the Dipterocarpaceae.[1−3] Plants of this subfamily are known to elaborate mainly resveratrol oligomers,[3−18] sesquiterpenoids,[19−22] and triterpenoids.[18−25] Resveratrol oligomers from plants in the family Dipterocarpaceae exhibit diverse biological activities, including antibacterial,[8,10,12] antifungal,[6,17] and anti-HIV effects.[4]

As a part of an ongoing collaborative search for novel antineoplastic agents of plant origin, an ethyl acetate extract of the stems of *Vatica diospyroides*, collected in Thailand, exhibited significant cytotoxic activity against a number of human cancer cell lines. This plant has not been investigated previously and therefore was subjected to detailed laboratory investigation, involving bioassay-guided chromatographic fractionation monitored by the KB cell line. We report herein a novel resveratrol tetramer, vatdiospyroidol (**1**), as a cytotoxic principle of the ethyl acetate extract of the stems of *V. diospyroides*. Another novel resveratrol tetramer, vaticaphenol A (**2**), was isolated as a noncytotoxic constituent. Five known compounds, bergenin, betulin, betulinic acid, mangiferonic acid, and resveratrol 3-*O*-*β*-D-glucopyranoside were isolated as noncytotoxic constituents of *V. diospyroides* when evaluated against the panel of cancer cell lines represented. The structures of **1** and **2** were elucidated using 2D NMR techniques such as ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR, ^{1}H−^{13}C HMQC, and ^{1}H−^{13}C HMBC, and their relative stereostructures have been proposed using

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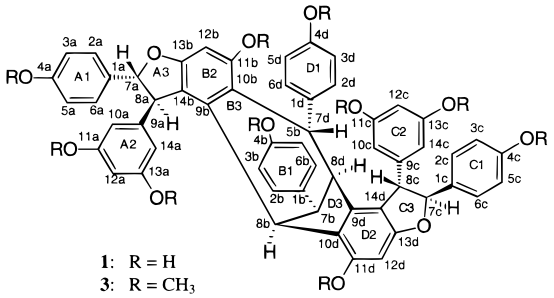
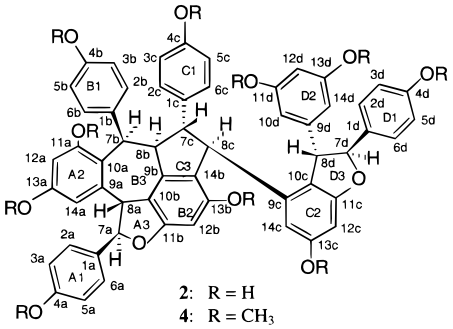
the $^1H-^1H$ NOESY NMR technique and computer-aided molecular modeling. The structural characterization of **1** and **2** and the cytotoxic evaluation of the *V. diospyroides* constituents are presented in this paper. This is the first report of cytotoxicity against a cancer cell line by a resveratrol oligomer.



1: R = H
3: R = CH_3



2: R = H
4: R = CH_3

Results and Discussion

Compound **1**, obtained as a minor constituent, was deduced as having an elemental formula of $C_{56}H_{42}O_{12}$ by negative HRFABMS (*m/z* $[M - H]^-$ 905.2638). A strong hydroxyl absorption band was observed at 3361 cm^{-1} in the IR spectrum. The UV spectrum of **1** displayed an absorption maximum at 285 nm, consistent with the presence of one or more nonconjugated phenyl rings. The ^1H NMR spectrum of **1** showed eight ortho-coupled aromatic signals from four *p*-hydroxyphenyl groups at δ_H 6.45/6.35, 6.65/7.01, 6.83/7.24, and 6.84/7.23. They were correlated to the ^{13}C NMR signals at δ_C 129.4/115.5 and 115.9 (C-3b and C-5b showed different ^{13}C NMR signals), 115.5/129.9, 116.1/128.5, and 116.0/128.1, respectively, in the $^1H-^{13}C$ HMQC NMR spectrum of **1**, indicating that this compound is a resveratrol tetramer.[3−18,26−31] Characteristic benzylic proton signals typical of a diaryl-dihydrobenzofuran moiety were observed at δ_H 4.56/5.61 and 4.93/5.42 in the ^1H NMR spectrum of **1** and showed one-bond correlations with the ^{13}C NMR resonances at δ_C 57.7/94.1 and 56.8/94.1, respectively, in the $^1H-^{13}C$ HMQC NMR spectrum, thereby indicating the presence of two diaryl-dihydrobenzofuran units in the molecule. Four aliphatic methine functionalities appeared at δ_H 3.10 (H-8d), 3.38 (H-7b), 4.00 (H-8b), and 4.17 (H-7d),

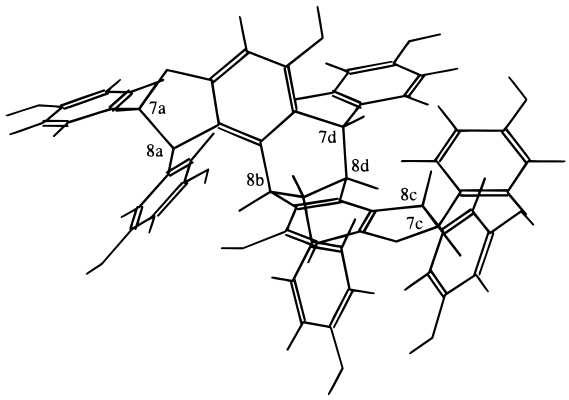


Figure 1. Energy-minimized stereostructure of vatdiospyroidol (**1**) (MM^+ calculation using the HyperChem 4.0 molecular modeling program).

which correlated with the ^{13}C NMR resonances at δ_C 51.0 (C-8d), 49.6 (C-7b), 47.5 (C-8b), and 45.5 (C-7d), respectively. The carbon connectivities between the two diaryl-dihydrobenzofuran units in **1** were deduced from detailed analysis of the cross-peaks in the HMBC spectrum which correlated with these four aliphatic protons (Table 1). Further analysis of the HMBC NMR spectrum, together with the $^1H-^1H$ COSY NMR data, led to the structural skeleton proposed for **1**. To determine the relative stereochemistry of **1**, the NOESY NMR spectrum was analyzed with the assistance of computer-aided molecular modeling. From these data, the energy-minimized stereostructure of **1** (see Figure 1) showed dihedral angles of 76.15°, 82.70°, and 77.93° for H-7d/H-8d, H-7b/H-8d, and H-7b/H-8b, respectively, which corresponded to the expectation of a very small coupling constant in each case from the vicinal Karplus correlation graph.[32] These computational calculations were compared to the actual coupling constants observed in the ^1H NMR spectrum for H-7d (δ_H 4.17, brs), H-8d (δ_H 3.10, brs), H-7b (δ_H 3.38, s), and H-8b (δ_H 4.00, s), which all occurred as singlets with very small or no vicinal coupling at all observed. In the NOESY NMR spectrum of the molecule, H-8d exhibited a cross-peak with the coincident H-2b and H-6b signals. The latter signal, in turn, was found to exhibit NOE correlations with H-8b and H-7b, indicating that all of these protons were located in close proximity (Table 1). Proton-8d showed a NOE correlation with H-2d(6d), indicating the cis configuration of H-8d and ring D1. Protons-2d(6d) also correlated with H-7b in the NOESY spectrum of **1**, providing further evidence for the α orientation of ring D1. Protons 8a and 8b could be proposed as having a cis orientation to each other from the observation of a mutual cross-peak in the NOESY NMR spectrum, whereas H-8d and H-8c were assigned as trans due to the lack of any observed NOE correlation between them. In addition, the configurations of both H-7a/H-8a and H-7c/H-8c were determined as trans from the cross-peaks corresponding to H-7a/H-10a(14a), H-8a/H-2a(6a), H-7c/H-10c(14c), and H-8c/H-2c(6c) in the NOESY spectrum. As a result, structure **1** was proposed for the novel tetrastilbenoid, vatdiospyroidol. The structure was supported further by the methylation of the 10 phenolic hydroxyls present in **1** to form **3**. All of the ^1H

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Table 1. NMR Data for Vatdiospyroidol (1) (in Acetone-d_6)

carbon	δ_C	δ_H (mult., J Hz)	$^1H-^1H$ COSY	$^1H-^1H$ NOESY	$^1H-^{13}C$ HMBC
1a	134.2 (s)				
2a(6a)	128.1 (d)	7.23 (d, 8.5)	H-3a(5a)	H-7a, H-8a	C-2a(6a), C-3a(5a), C-4a, C-7a
3a(5a)	116.0 (d)	6.84 (d, 8.5)	H-2a(6a)	OH-4a	C-1a, C-3a(5a)
4a	158.1 (s)				
7a	94.1 (d)	5.42 (d, 6.5)	H-8a	H-2a(6a), H-10a(14a)	C-1a, C-2a(6a), C-8a, C-9a, C-13b
8a	56.8 (d)	4.93 (d, 6.5)	H-7a	H-2a(6a), H-10a(14a)	C-1a, C-7a, C-9a, C-10a(14a), C-13b, C-14b
9a	148.3 (s)				
10a(14a)	107.4 (d)	6.40 (d,2.0)		H-8a, H-7a, OH-11a (13a), H-8b	C-8a, C-12a, C-10a(14a), C-11a(13a)
11a(13a)	159.9 (s)				
12a	101.8 (d)	6.36 (t, 2.0)		OH-11a (13a)	C-10a(14a), C-11a(13a)
1b	134.2 (s)				
2b(6b)	129.4 (d)	6.35 (d, 8.4)		H-7b, H-8b, H-8d	C-2b(6b), C-3b, C-4b, C-5b, C-7b
3b	115.5 (d)[a]	6.45 (d, 8.4)[c]		OH-4b	C-1b, C-3b, C-4b, C-5b
5b	115.9 (d)	6.45 (d, 8.4)[c]			
4b	155.7 (s)				
7b	49.6 (d)	3.38 (s)		H-2b(6b), H-8b, H-2d(6d)	C-1b, C-2b(6b), C-8b, C-7d, C-8d, C-9d, C-10d
8b	47.5 (d)	4.00 (s)		H-8a, H-10a (14a), H-2b(6b), H-7b	C-1b, C-7b, C-9b, C-10b, C-14b, C-8d, C-9d, C-10d, C-11d
9b	144.0 (s)				
10b	114.6 (d)				
11b	157.8 (s)				
12b	95.8 (d)	6.16 (s)		OH-11b	C-10b, C-11b, C-14b
13b	159.8 (s)[b]				
14b	117.8 (s)				
1c	133.4 (s)				
2c(6c)	128.5 (d)	7.24 (d, 8.5)	H-3c(5c)	H-7c, H-8c	C-3c(5c)
3c(5c)	116.1 (d)	6.83 (d, 8.5)	H-2c(6c)	OH-4c	C-1c, C-3c(5c), C-4c
4c	158.1 (s)				
7c	94.1 (d)	5.61 (d, 7.7)	H-8c	H-2c(6c), H-8c, H-10c(14c)	C-1c, C-2c(6c), C-8c, C-9c, C-13d
8c	57.7 (d)	4.56 (d, 7.7)	H-7c	H-2c(6c), H-7c, H-10c(14c)	C-1c, C-7c, C-9c, C-10c(14c), C-13d, C-14d
9c	145.9 (s)				
10c(14c)	108.1 (d)	6.55 (d, 2.5)		H-7c, H-8c, OH-11c(13c)	C-8c, C-11c(13c), C-12c
11c(13c)	159.8 (s)[b]				
12c	102.2 (d)	6.32 (t, 2.5)		OH-11c(13c)	C-10c(14c), C-11c(13c)
1d	138.3 (s)				
2d(6d)	129.9 (d)	7.01 (d, 8.5)	H-3d(5d)	H-7b, H-7d, H-8d	C-2d (6d), C-3d(5d), C-4d, C-7d
3d(5d)	115.5 (d)[a]	6.65 (d, 8.5)	H-2d(6d)	OH-4d	C-1d, C-3d(5d), C-4d
4d	156.0 (s)				
7d	45.5 (d)	4.17 (brs)		H-2d(6d), H-8d, OH-11b	C-7b, C-9b, C-10b, C-11b, C-1d, C-2d(6d), C-8d
8d	51.0 (d)	3.10 (brs)		H-2b(6b), H-2d(6d), H-7d	C-1b, C-7b, C-8b, C-10b, C-1d, C-7d, C-9d C-10d
9d	143.1 (s)				
10d	128.2 (s)				
11d	153.4 (s)				
12d	95.9 (d)	6.12 (s)		OH-11d	C-10d, C-11d, C-13d, C-14d
13d	160.9 (s)				
14d	118.5 (s)				
OH-4a		8.42 (s)		H-3a(5a)	C-3a(5a), C-4a
OH-11a(13a)		8.24 (s)		H-10a(14a), H-12a	C-10a(14a), C-11a(13a), C-12a
OH-4b		7.79 (s)		H-3b(5b)	C-3b, C-4b, C-5b
OH-11b		7.50 (s)		H-12b, H-7d	C-10b, C-11b, C-12b
OH-4c		8.44 (s)		H-3c(5c)	C-3c(5c), C-4c
OH-11c(13c)		8.32 (s)		H-10c(14c), H-12c	C-10c(14c), C-11c(13c), C-12c
OH-4d		7.95 (s)		H-3d(5d)	C-3d(5d), C-4d
OH-11d		8.22 (s)		H-12d	C-10d, C-11d, C-12d

[a–c]Overlapping signals.

and ^{13}C NMR signals of decamethylvatdiospyroidol (**3**) were assigned unambiguously from the $^1H-^1H$ COSY, $^1H-^1H$ NOESY, $^1H-^{13}C$ HMQC, and $^1H-^{13}C$ HMBC NMR spectral data.

A negative molecular ion $[M - H]^-$ at m/z 905.2632 was observed in the HRFABMS of compound **2**, suggesting a molecular formula of $C_{56}H_{42}O_{12}$. In the IR spectrum of **2**, a strong absorption band at 3366 cm^{-1} indicated the presence of several hydroxyl groups, and its UV spectrum showed an absorption maximum at 284 nm, consistent with there being one or more substituted phenyl rings in the molecule. The ^1H NMR spectrum of **2** exhibited four sets of ortho-coupled aromatic signals at δ_H 6.50/6.40, 6.69/7.16, 6.77/7.19, and 6.78/7.22, resulting from the presence of four *p*-hydroxyphenyl groups, which showed one-bond connectivities with ^{13}C NMR resonances at δ_C 129.2/115.3 and 115.4 (C-3c and C-5c showed different ^{13}C NMR signals), 115.9/130.6, 116.0/128.2, and 115.8/130.1, respectively, in the $^1H-^{13}C$ HETCOR NMR spectrum. These data were in accordance with compound **2** also being a resveratrol tetramer. Six signals for meta-coupled aromatic protons appeared at δ_H 6.27 (H-12a), 6.28 (H-12d), 6.11 (H-14a), 6.19 (H-12c), 6.47 (H-14c), and 6.10 (H-10d and H-14d), and showed cross-peaks with the ^{13}C NMR signals at δ_C 101.5, 102.1, 105.6, 96.4, 95.5, 106.9, and 107.3, respectively, in the HETCOR spectrum of **2**. Four aliphatic methine groups at δ_H 4.43/5.76 and 4.68/5.37 were correlated to the ^{13}C NMR resonances at δ_C 48.8/90.3 and 57.4/94.5 and were attributed to two diaryl-dihydrobenzofuran moieties. Another four aliphatic ^1H NMR signals, at δ_H 3.10 (H-8b), 4.09 (H-7c), 4.55 (H-8c), and 5.20 (H-7b), exhibited one-bond connec-

Table 2. NMR Data for Vaticaphenol A (2) (in Acetone-d_6)

carbon	δ_C	δ_H (mult., J Hz)	$^1H-^1H$ COSY	$^1H-^1H$ NOESY	$^1H-^{13}C$ HMBC
1a	130.7 (s)				
2a(6a)	130.1 (d)	7.22 (d, 8.5)		H-7a, H-8a, H-14a	C-2a(6a), C-4a, C-7a
3a(5a)	115.8 (d)	6.78 (d, 8.5)		OH-4a	C-1a, C-3a(5a), C-4a
4a	158.5 (s)				
7a	90.3 (d)	5.76 (d, 11.0)	H-8a	H-2a(6a), H-14a	C-2a(6a), C-8a, C-9a
8a	48.8 (d)	4.43 (d, 11.0)	H-7a	H-2a(6a), H-2b(6b)	C-1a, C-7a, C-9a, C-10b
9a	141.7 (s)				
10a	124.4 (s)				
11a	155.6 (s)				
12a	101.5 (d)	6.27 (d, 1.8)		OH-11a	C-10a, C-11a, C-13a, C-14a
13a	156.6 (s)				
14a	105.6 (d)	6.11 (d, 1.8)		H-2a(6a), OH-13a	C-8a, C-10a, C-12a, C-13a
1b	133.4 (s)				
2b(6b)	130.6 (d)	7.16 (d, 8.3)		H-7b, H-7c	C-2b(6b), C-4b, C-7b
3b(5b)	115.9 (d)	6.69 (d, 8.3)		OH-4b	C-1b, C-3b(5b), C-4b
4b	155.8 (s)				
7b	36.9 (d)	5.20 (t, 3.0)		H-2b(6b), H-8b, H-2c(6c), OH-11a	C-9a, C-10a, C-11a, C-1b, C-2b(6b), C-8b, C-9b
8b	53.1 (d)	3.10 (brd, 11.5)	H-7c	H-7b, H-2c(6c)	C-7b, C-9b
9b	143.1 (s)				
10b	115.6 (s)				
11b	158.7 (s)				
12b	96.4 (d)	6.04 (s)		OH-13b	C-10b, C-11b, C-13b, C-14b
13b	154.8 (s)				
14b	122.1 (s)				
1c	131.3 (s)				
2c(6c)	129.2 (d)	6.40 (d, 8.5)		H-7b, H-8b, H-7c, H-8c	C-2c(6c), C-4c, C-7c
3c(5c)	115.3 (d), 115.4 (d)	6.50 (d, 8.5)		OH-4c	C-1c, C-2c(6c), C-3c(5c), C-4c
4c	156.2 (s)				
7c	57.5 (d)	4.09 (t, 11.5)	H-8b, H-8c	H-2b(6b), H-2c(6c), H-14c	C-7b, C-8b, C-1c, C-2c(6c), C-8c, C-9c
8c	49.1 (d)	4.55 (d, 11.5)	H-7c	H-2c(6c), H-10d(14d)	C-9b, C-13b, C-14b, C-1c, C-7c, C-9c, C-10c, C-14c
9c	141.6 (s)				
10c	123.3 (s)				
11c	161.5 (s)				
12c	95.5 (d)	6.19 (d, 2.0)		OH-13c	C-10c, C-11c, C-13c, C-14c
13c	159.3 (s)				
14c	106.9 (d)	6.47 (d, 2.0)		H-7c, OH-13c	C-8c, C-10c, C-12c, C-13c
1d	134.5 (s)				
2d(6d)	128.2 (d)	7.19 (d, 8.8)		H-7d, H-8d	C-2d(6d), C-4d, C-7d
3d(5d)	116.0 (d)	6.77 (d, 8.8)		OH-4d	C-4d, C-1d, C-3d(5d)
4d	157.9 (s)				
7d	94.5 (d)	5.37 (d, 5.5)		H-2d(6d), H-10d(14d)	C-10c, C-11c, C-1d, C-2d(6d), C-8d, C-9d
8d	57.4 (d)	4.68 (d, 5.5)		H-2d(6d), H-10d(14d), OH-13b	C-9c, C-10c, C-11c, C-1d, C-7d, C-10d(14d)
9d	147.9 (s)				
10d(14d)	107.3 (d)	6.10 (brs)		H-8c, H-7d, H-8d, OH-11d(13d)	C-8d, C-10d(14d)
11d(13d)	159.7 (s)				
12d	102.1 (d)	6.28 (t, 2.5)		OH-11d(13d)	C-10d(14d), C-11d(13d)
OH-4a		8.61 (s)			C-3a(5a), C-4a
OH-11a(4b)		8.28 (s)		H-12a, H-3b(5b), H-7b	C-10a, C-11a, C-3b(5b), C-4b
OH-13a		8.16 (s)		H-14a	C-12a, C-13a, C-14a
OH-13b		7.61 (s)		H-12b, H-8d	C-12b, C-13b, C-14b
OH-4c		8.02 (s)		H-3c(5c)	C-3c(5c), C-4c
OH-13c		8.28 (s)		H-12c, H-14c	C-12c, C-13c, C-14c
OH-4d		8.50 (s)		H-3d(5d)	C-3d(5d), C-4d
OH-11d(13d)		8.12 (s)		H-12d	C-10d(14d), C-11d(13d), C-12d

tivities in the HETCOR spectrum with the ^{13}C NMR signals at δ_C 53.1 (C-8b), 57.5 (C-7c), 49.1 (C-8c), and 36.9 (C-7b), respectively. $^1H-^1H$ COSY and $^1H-^{13}C$ HMBC NMR techniques (Table 2) were used to determine the carbon skeleton between the two diaryl-dihydrobenzofuran moieties of **2**. The relative stereochemistry of **2** was proposed on the basis of the analysis of the NOESY NMR spectrum and the computer-aided energy-minimized stereostructures obtained (see Figure 2). The H-7b signal at δ_H 5.20 showed a cross-peak with H-8b at δ_H 3.10, indicating a cis orientation for these two protons. This was supported by a computer-aided calculation for the dihedral angle (72.35°) between H-7b (α) and H-8b (α), which would result in a coupling constant with a small value according to the vicinal Karplus correlation graph. This was consistent with the data obtained from the ^1H NMR spectrum which displayed a $J_{7b,8b}$ of 3.0 Hz. In turn, the dihedral angle between H-8b (α) and H-7c (β) was computed as 165.03°, which corresponds to a J value of 11.5 Hz in the vicinal Karplus correlation graph.[32] The signals for H-8b and H-7c at δ_H 3.10 (1H, brd, J = 11.5 Hz) and 4.09 (1H, t, J = 11.5 Hz), respectively, did not show any NOE correlation with each other in the NOESY NMR spectrum of **2**, supporting their trans configuration. Similarly, H-7c and H-8c were also assigned as trans according to their coupling constants (J = 11.5 Hz) and the absence of a NOE cross-peak in the NOESY spectrum. Further evidence was provided by the NOE correlation between H-7c and H-14c, indicating β orientation of the ring C2. Ring D2 was proposed as having an α

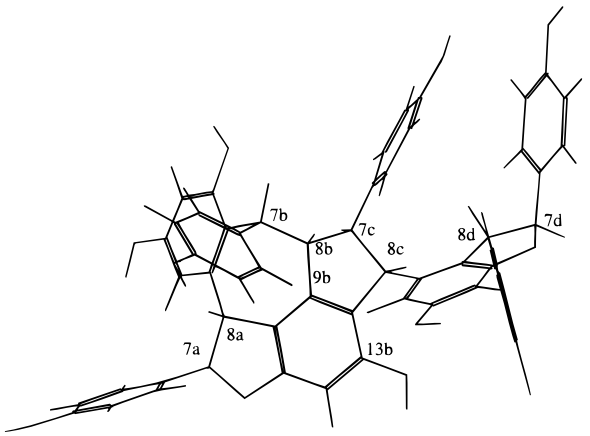
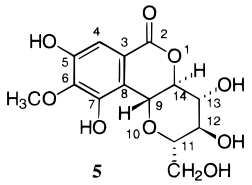


Figure 2. Energy-minimized stereostructure of vaticaphenol A (**2**) (MM^+ calculation using the HyperChem 4.0 molecular modeling program).

orientation by the NOE correlation observed between H-8c and H-10d(14d). The vicinal coupled aliphatic protons [H-7a (δ_H 5.76, 1H, d, J = 11.0 Hz)/H-8a (δ_H 4.43, 1H, d, J = 11.0 Hz) and H-7d (δ_H 5.37, 1H, d, J = 5.5 Hz)/H-8d (δ_H 4.68, 1H, d, J = 5.5 Hz)] from the two diaryl-dihydrobenzofuran rings were assigned as trans since the NOESY spectrum showed correlations of H-8a/H-2a(6a), H-7d/H-10d(14d), and H-8d/H-2d(6d). The large coupling constant for H-7a/H-8a (J = 11.0 Hz) possibly results from being adjacent to a seven-membered ring, and the coupling constant observed was consistent with reported data for this functionality.[33] The coupling constant of H-7d and H-8d (5.5 Hz) was typical for the trans protons in a diaryl-dihydrobenzofuran ring not connected to another ring.[34] Other NOESY NMR data, as summarized in Table 2, are in accordance with the proposed stereostructure of compound **2**.

Further proof for the structure of compound **2** was obtained on the methylation of its 10 hydroxyl groups. Decamethylvaticaphenol A (**4**) exhibited a molecular ion peak at *m/z* 1046 in its LREIMS, consistent with an elemental formula of $C_{66}H_{62}O_{12}$. Unambiguous assignments for the ^1H and ^{13}C NMR signals for **4** were performed using COSY, NOESY, HMQC, and HMBC NMR experiments, supporting the stereochemistry of compound **2** as proposed. However, compound **4** displayed a positive optical rotation value (+33°), whereas **2** showed a negative value (−29°), and there were a number of Cotton effect differences observed between these two compounds in their CD spectra. In the $^1H-^1H$ NOESY NMR spectrum of **4**, a correlation between H-8c and H-8d was observed, whereas compound **2** revealed a cross-peak between H-8c and H-10d(14d) in its NOESY spectrum. These observations suggested that the single bond between C-8c and C-9c of compound **2** was slightly rotated by methylation without a change of configuration at C-8c, which may have caused changes in the optical rotation and CD spectrum of **4** relative to **2**. A possible explanation for these discrepancies was obtained from observed differences between **2** and **4** in the ^1H NMR values for H-2c and H-6c, and H-3c and H-5c. Thus, the ^1H NMR signal for H-2c and H-6c (δ_H 6.40) of **2** appeared at a more shielded region than H-3c and H-5c (δ_H 6.50) of **2**, whereas the methylated product **4** displayed the H-2c and H-6c signal (δ_H 6.58) in a more deshielded region than H-3c and H-5c (δ_H 6.90). These chemical shift differences are consistent with a change in the steric environment of ring C1 of **2** on its methylation to **4**.

A known resveratrol monomer, (*E*)-resveratrol 3-*O*-β-D-glucopyranoside[35] was isolated from the ethyl acetate extract of *V. diospyroides* in the present investigation. This is the first report of a resveratrol monomer from a plant of the Dipterocarpaceae, providing some evidence that the tetrastilbenes are biosynthesized from resveratrol monomers. A known isocoumarin, bergenin (**5**),[36,37] was also isolated, and its detailed ^1H NMR assignments and a minor revision of the ^{13}C NMR assignments for C-13 (δ_C 73.7) and C-14 (δ_C 79.8) were carried out from the observation of the HMBC NMR correlations between the hydroxyl protons and their two- and three-bonded carbons. Three triterpenoids, betulin,[38] betulinic acid,[38,39]



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and mangiferonic acid[40,41] were identified by comparison of spectral data with literature values. The presence of betulinic acid was predicted using a published LC-MS dereplication method,[42] in which the ethyl acetate extract of the stems of *V. diospyroides* was evaluated using the ZR-75-1 (human hormone-dependent breast cancer) assay, although this cell line was not finally chosen for activity-guided fractionation. The masses found in the wells with biological activity were at *m/z* 456, 460, and 470, of which that at *m/z* 456 corresponded to the molecular ion of betulinic acid.[38,39]

Biological Activity. All of the isolates and methylated derivatives obtained in the present investigation were evaluated for their cytotoxic activity against several human cancer cell lines.[43] Compound **1** (vatdiospyroidol) was found to be the only active principle of the ethyl acetate extract of *V. diospyroides* stems and displayed its most potent cytotoxicity against the oral epidermoid carcinoma (KB, EC_{50} 1.0 μg/mL), colon cancer (Col2, EC_{50} 1.9 μg/mL), and breast cancer (BC1, EC_{50} 3.8 μg/mL) cell lines in the in vitro tumor cell panel. This appears to be the first occasion in which significant cytotoxic activity against a human cancer cell line has been reported for a

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resveratrol tetramer. Compound **3** (decamethylvatdiospyroidol) did not show any significant activities in the cell lines tested in this study, thereby pointing out that the hydroxyl groups in compound **1** play an important role in mediating the cytotoxic activity of **1**. All other compounds were found to be inactive in the human cancer cell lines tested. It is to be noted that betulinic acid was demonstrated in our earlier work to be selectively inhibitory to melanoma cells and also showed highly effective tumor growth inhibition in athymic mice bearing human melanoma.[44] However, the presently used cancer cell panel did not include a melanoma cell line.

Experimental Section

General Methods. ^{1}H NMR spectra were obtained at 500 MHz, and ^{13}C NMR spectra were run at 90.8 or 125 MHz. TMS was used as internal standard. ^{1}H−^{1}H COSY, ^{1}H−^{1}H NOESY, and ^{1}H−^{13}C HETCOR NMR experiments were run at 300 MHz and ^{1}H−^{13}C HMQC and ^{1}H−^{13}C HMBC NMR experiments were recorded at 500 MHz. Energy-minimized stereostructures were obtained using HyperChem 4.0 software produced by Hypercube Inc. (Waterloo, Ontario, Canada).

Plant Material. The stems of *V. diospyroides* Sym. were collected at Nong Thong Wildlife Sanctuary in Kiansaa, Surat Thani, Thailand in May 1993 and were identified by one of us (T.S.). A voucher specimen (A2531) has been deposited in the Field Museum of Natural History, Chicago, IL.

Extraction and Isolation. The dried stems (1.5 kg) of *V. diospyroides* were extracted three times with MeOH (3 × 7.5 L) overnight at room temperature. The solvent was evaporated in vacuo to afford a concentrated MeOH extract, which was then diluted with H_2O (0.9 L) to give an aqueous MeOH solution (1.0 L). The aqueous solution was defatted with hexanes (2 × 1.0 L) and subsequently partitioned with EtOAc (3 × 1.2 L). The combined EtOAc layers were concentrated to dryness in vacuo to provide a residue (D001, 59.4 g), and the aqueous fraction was dried to give extract D002 (40 g). The EtOAc extract (D001), exhibited significant cytotoxic activity against several human cancer cell lines, while the aqueous fraction was inactive. The EtOAc extract of *V. diospyroides* stems was evaluated by dereplication analysis, using a previously established protocol, with published chromatographic conditions,[42] with the ZR-75-1 (human hormone-dependent breast cancer) cell line used to monitor cytotoxic activity.[43] For chromatography, the extract (59.4 g) was mixed with silica gel (60 g, 70−230 mesh), and subjected to vacuum-liquid chromatography (VLC) [glass column (15 × 15 cm) packed with dried silica gel (560 g, 70−230 mesh), using $CHCl_3$−MeOH (19:1 to 4:1, gradient mixtures) and finally MeOH for elution, with cytotoxicity monitored using a human oral epidermoid carcinoma (KB) cell line. Of six major fractions obtained by VLC (F003−F008), fractions F004 and F005 (eluted with $CHCl_3$−MeOH, 10:1 to 17:3), showed significant cytotoxic activity against the KB cell line (EC_{50} 6.9 and 7.8 μg/mL, respectively).

Bergenin was precipitated from fractions F004 and F005 in MeOH, and recrystallized from MeOH as cubic white crystals (700 mg, 0.064% w/w). After bergenin was separated from F004, the residue (9.0 g) was mixed with silica gel (9.0 g, 70−230 mesh), dried, and subjected to flash column chromatography [glass column (4 × 40 cm) packed with silica gel (80 g, 230−400 mesh)], eluted with $CHCl_3$−MeOH (17:3 to 1:1, gradient mixtures) and MeOH, to afford fractions F019−F028. Fraction F026 (5 g, eluted with $CHCl_3$−MeOH, 5:1) was further separated by flash column chromatography using hexane−acetone (1:1), affording fractions F045−F061. Fraction F053 (650 mg) was subjected to additional flash column chromatography using $CHCl_3$−MeOH−H_2O (7:1:0.01) as eluent, producing fractions F062−F068. F066 (32 mg) showed a single spot with R_f 0.32 using hexane−acetone−MeOH (3:3:1) as the TLC solvent system. However, this fraction displayed two spots [major spot at R_f 0.25 (**2**); minor spot at R_f 0.35 (**1**)] by reversed-phase TLC (RP-18) developed with MeOH−H_2O (1:1). Fraction F071 (480 mg), which showed a similar TLC profile to fraction F066, was obtained from F005 by the same column chromatographic procedure used for F066. Fraction F066 was dissolved in MeOH (1 mL) and subjected to semi-preparative HPLC [column YMC Pack, ODS-AQ, 250 × 20 mm I.D., C_{18}, S-5 μm, 120 Å; guard column YMC Guard Pack, ODS-AQ, 50 × 20 mm I.D.; solvent MeOH−H_2O, 4.5:5.5; flow rate 4.5 mL/min) to afford the two pure isolates **1** (t_R 40 min) and **2** (t_R 50 min). Repetition of this method with fraction F071 provided additional amounts of compounds **1** and **2** (17 mg, 0.0012% w/w, and 90 mg, 0.006% w/w, respectively). F082, fractionated from F005 (10.0 g; eluted with hexane−acetone, 1:1), was further separated into fractions F087−F092 by column chromatography [glass column (3 × 40 cm) packed with silica gel (150 g, 230−400 mesh) slurry] using $CHCl_3$−MeOH−H_2O (20:3:0.01) as the solvent system. Fraction F089 (0.055 g) was subjected to repeated reversed-phase (RP-18) preparative TLC using MeOH−H_2O (6:5) as the developing solvent system to afford (*E*)-resveratrol 3-*O*-β-D-glucopyranoside (5.0 mg, 0.0004% w/w). Fraction F003 (6 g), mixed with silica gel (5 g, 70−230 mesh), was subjected to flash column chromatography (glass column 4 × 40 cm; packed with silica gel 230−400 mesh) and eluted with hexane-acetone (10:1 to 1:1, gradient mixtures), and finally washed with MeOH to afford fractions F009−F018. Betulin (3.1 mg, 0.0002% w/w) was isolated from fraction F033 by precipitation in MeOH. Betulinic acid (5.4 mg, 0.0004% w/w) was obtained as a white powder from fraction F035 in MeOH. Fraction F040 was washed with MeOH to afford mangiferonic acid (5.8 mg, 0.0004% w/w).

Vatdiospyroidol (1): white amorphous powder (H_2O); mp > 300 °C (dec); $[\alpha]_D$ −67° (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 298 (s, 3.9), 285 (4.0), 278 (s, 4.0), 239 (4.4) nm; IR (film) ν_{max} 3361, 2922, 2359, 2339, 1613, 1514, 1454, 1366, 1235, 1161, 1078, 1001, 833, 693, 540 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6), see Table 1; ^{13}C NMR (125 MHz, acetone-d_6), see Table 1; FABMS (70 eV) *m/z* $[MH]^+$ 907 (2), 906 (1), 448 (1), 337 (11), 315 (11), 253 (9), 225 (10), 207 (11), 192 (14), 175 (19), 128 (16), 119 (38), 117 (41), 115 (58), 101 (100); ESIMS *m/z* $[M - H]^-$ 905.6, $[M/2 - H]^-$ 452.5; HRFABMS *m/z* $[M - H]^-$ 905.2638 (calcd for $C_{56}H_{41}O_{12}$, 905.2598).

Vaticaphenol A (2): white amorphous powder (H_2O); mp > 300 °C (dec); $[\alpha]_D$ −29° (*c* 0.10, MeOH); $[\alpha]_D$ −27° (*c* 0.10, acetone); CD (*c* 0.12 μM, MeOH) $\Delta\epsilon_{293}$ +4.58, $\Delta\epsilon_{282}$ +19.40, $\Delta\epsilon_{240}$ −90.54, $\Delta\epsilon_{229}$ +6.61, $\Delta\epsilon_{221}$ −12.57, $\Delta\epsilon_{213}$ −8.05; UV (MeOH) λ_{max} (log ϵ) 284 (4.2), 241 (4.3) nm; IR (film) ν_{max} 3366, 2926, 1615, 1511, 1454, 1362, 1337, 1242, 1173, 1007, 833 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6), see Table 2; ^{13}C NMR (75 MHz, acetone-d_6), see Table 2; FABMS (70 eV) *m/z* $[MH]^+$ 907 (1), 906 (1), 335 (1), 313 (1), 256 (1), 207 (5), 191 (4), 189 (2), 166 (2), 150 (2), 149 (100), 148 (1), 133 (17), 129 (13), 127 (2), 116 (1), 115 (3); HRFABMS *m/z* $[M - H]^-$ 905.2632 (calcd for $C_{56}H_{41}O_{12}$, 905.2598).

Bergenin (5): physical and spectral data were comparable with literature values;[36,37] ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.20 (1H, ddd, J = 9.8, 9.3, and 5.9 Hz, H-12), 3.44 (1H, ddd, J = 11.5, 7.0, and 5.2 Hz, H-16a), 3.57 (1H, ddd, J = 9.8, 7.0, and 2.0 Hz, H-11), 3.65 (1H, dt, J = 9.3 and 5.7 Hz, H-13), 3.78 (3H, s, OCH_3), 3.84 (1H, ddd, J = 11.5, 5.2, and 2.0 Hz, H-16b), 4.00 (1H, t, J = 9.3 Hz, H-14), 4.92 (1H, t, J = 5.2 Hz, OH-16), 4.98 (1H, d, J = 9.3 Hz, H-9), 5.44 (1H, d, J = 5.9 Hz, OH-12), 5.66 (1H, d, J = 5.7 Hz, OH-13), 6.99 (1H, s, H-3), 8.45 (1H, s, OH-6), 9.76 (1H, s, OH-4); ^{13}C NMR (75 MHz, DMSO-d_6) δ 59.9 (q, C-15), 61.1 (t, C-16), 70.7 (d, C-12), 72.1 (d, C-9), 73.7 (d, C-13), 79.8 (d, C-14), 81.8 (d, C-11), 109.5 (d, C-3), 116.0 (s, C-7), 118.1 (s, C-8), 140.6 (s, C-5), 148.1 (s, C-6), 151.0 (s, C-4), 163.4 (s, C-2).

Betulin: physical and spectral data were comparable with literature values.[38]

Betulinic acid: physical and spectral data were comparable with literature values.[38,39]

Mangiferonic acid: physical and spectral data were comparable with literature values.[40,41]

(*E*)-Resveratrol 3-*O*-β-D-glucopyranoside: physical and spectral data were comparable with literature values.[35]

Decamethylvatdiospyroidol (3). Compound **1** (7 mg) was treated with dimethyl sulfate (0.1 mL) and potassium carbonate (100 mg) in dried acetone (7 mL) under reflux for 24 h. Compound **3** (decamethylvatdiospyroidol, 3.4 mg) was purified by preparative TLC with hexanes−EtOAc (1:1, R_f 0.45): white powder (MeOH), mp 137−138 °C; $[\alpha]_D$ −23° (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 298 sh (3.9), 283 (4.0), 276 sh (4.0), 231 (4.8) nm; IR (film) ν_{max} 2918, 2849, 2353, 1614, 1568, 1516, 1456, 1250, 1157, 1101, 1068 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 3.10 (1H, brs, H-8d), 3.34 (1H, s, H-7b), 3.44 (3H, s, OCH_3-11b), 3.58 (3H, s, OCH_3-11d), 3.62 (3H, s, OCH_3-4b), 3.70 (3H, s, OCH_3-4d), 3.80 (9H, s, OCH_3-4c, OCH_3-11c and OCH_3-13c), 3.82 (9H, s, OCH_3-4a, OCH_3-11a and OCH_3-13a), 4.06 (1H, s, H-8b), 4.15 (1H, brs, H-7d), 4.76 (1H, d, J = 7.8 Hz, H-8c), 4.93 (1H, d, J = 6.3 Hz, H-8a), 5.56 (1H, d, J = 6.3 Hz, H-7a), 5.74 (1H, d, J = 7.8 Hz, H-7c), 6.20 (2H, d, J = 8.5 Hz, H-2b and H-6b), 6.25 (1H, s, H-12d), 6.37 (1H, s, H-12b), 6.45 (2H, d, J = 8.5 Hz, H-3b and H-5b), 6.49 (1H, t, J = 2.0 Hz, H-12c), 6.52 (1H, t, J = 2.5 Hz, H-12a), 6.55 (2H, d, J = 2.5 Hz, H-10a and H-14a), 6.72 (2H, d, J = 8.5 Hz, H-3d and H-5d), 6.73 (2H, d, J = 2.0 Hz, H-10c and H-14c), 6.93 (2H, d, J = 8.5 Hz, H-3c and H-5c), 6.99 (2H, d, J = 8.5 Hz, H-3a and H-5a), 7.02 (2H, d, J = 8.5 Hz, H-2d and H-6d), 7.34 (2H, d, J = 8.5 Hz, H-2a and H-6a), 7.37 (2H, d, J = 8.5 Hz, H-2c and H-6c); ^{13}C NMR (90 MHz, acetone-d_6) δ 45.4 (d, C-7d), 46.9 (d, C-8b), 49.5 (d, C-7b), 51.5 (d, C-8d), 55.1 (q, OCH_3-4b), 55.2 (q, OCH_3-4d), 55.3 (q, OCH_3-11d), 55.5 (q, OCH_3-4c, interchangeable with OCH_3-4a, OCH_3-11c and OCH_3-13a, interchangeable with OCH_3-11a and OCH_3-13a), 55.6 (q, OCH_3-4a, interchangeable with OCH_3-4c, OCH_3-11a and OCH_3-13a, interchangeable with OCH_3-11c and OCH_3-13c, OCH_3-11b), 57.4 (d, C-8a and C-8c), 92.3 (d, C-12d), 92.6 (d, C-12b), 93.7 (d, C-7c), 93.9 (d, C-7a), 99.3 (d, C-12a), 99.5 (d, C-12c), 106.7 (d, C-10a and C-14a), 107.6 (d, C-10c and C-14c), 113.8 (d, C-3b and C-5b), 113.9 (d, C-3d and C-5d), 114.6 (d, C-3c and C-5c), 114.7 (d, C-3a and C-5a), 116.1 (s, C-10b), 117.2 (s, C-14b), 119.0 (s, C-14d), 128.0 (d, C-2a and C-6a), 128.2 (d, C-2c and C-6c), 128.9 (d, C-2b and C-6b), 129.3 (s and d, C-10d, C-2d and C-6d, respectively), 134.2 (s, C-1c), 134.9 (s, C-1b), 135.0 (s, C-1a), 139.9 (s, C-1d), 141.9 (s, C-9d), 143.2 (s, C-9b), 145.7 (s, C-9c), 147.6 (s, C-9a), 155.9 (s, C-11d), 158.4 (s, C-4d), 158.5 (s, C-4b), 160.2 (s, C-13b), 160.4 (s, C-4a, C-4c, C-11b), 161.4 (s, C-13d), 162.3 (s, C-11a and C-13a, C-11c and C-13c); $^1H-^1H$ NOESY correlations H-3a(5a)/OCH_3-4a, H-8a/H-10a(14a), H-10a(14a)/OCH_3-11a(13a), H-2b(6b)/H-8b,H-8d, H-3b(5b)/OCH_3-4b, H-7b/H-2d(6d), H-8b/H-2b(6b), H-12b/OCH_3-11b, H-3c(5c)/OCH_3-4c, H-8c/H-10c(14c), H-10c(14c)/OCH_3-11c(13c), H-12c/OCH_3-11c(13c), H-2d(6d)/H-8d,H-7b,H-7d, H-3d(5d)/OCH_3-4d, H-7d/H-8d, H-8d/H-2b(6b), H-12d/OCH_3-11d; $^1H-^{13}C$ HMBC correlations H-2a(6a)/C-2a(6a),C-4a,C-7a, H-3a(5a)/C-1a,C-3a(5a), H-7a/C-2a(6a),C-9a, H-8a/C-1a,C-7a,C-9a,C-10a(14a),C-13b,C-14b, H-10a(14a)/C-8a,C-10a(14a),C-11a(13a),C-12a, H-12a/C-10a(14a),C-11a(13a), H-2b(6b)/C-2b(6b),C-4b,C-7b, H-3b(5b)/C-1b,C-3b(5b),C-4b, H-7b/C-1b,C-2b(6b),C-8b,C-7d,C-8d,C-9d,C-10d, H-8b/C-1b,C-7b,C-9b,C-10b,C-14b,C-8d,C-9d,C-10d, H-12b/C-10b,C-11b,C-14b, H-2c(6c)/C-2c(6c),C-4c,C-7c, H-3c(5c)/C-1c,C-3c(5c),C-4c, H-7c/C-2c(6c),C-9c, H-8c/C-1c,C-7c,C-9c,C-10c(14c),C-13d,C-14d, H-10c(14c)/C-8c,C-10c(14c),C-11c(13c),C-12c, H-12c/C-10c(14c),C-11c(13c), H-2d(6d)/C-2d(6d),C-4d,C-7d, H-3d(5d)/C-1d,C-3d(5d),C-4d, H-7d/C-7b,C-9b,C-10b,C-1d,C-2d(6d),C-8d, H-8d/C-1b,C-7b,C-8b,C-10b,C-7d, H-12d/C-10d,C-11d,C-13d,C-14d, OCH_3-4a/C-4a, OCH_3-11a(13a)/C-11a(13a), OCH_3-4b/C-4b, OCH_3-11b/C-11b, OCH_3-4c/C-4c, OCH_3-11c(13c)/C-11c(13c), OCH_3-4d/C-4d, OCH_3-11d/C-11d; EIMS (70 eV) *m*/*z* 1046 (100), 1016 (1), 928 (2).

Decamethylvaticaphenol A (4). Compound **2** (80 mg) was treated with dimethyl sulfate (0.8 mL) and potassium carbonate (0.8 g) in dried acetone (50 mL) under reflux for 24 h. The product was extracted with ethyl acetate and water, to remove dimethyl sulfate. The ethyl acetate-soluble extract was dried and purified by flash column chromatography using hexanes−EtOAc (2:1) affording **4** (decamethylvaticaphenol A, 40 mg): white powder (MeOH); mp 158−161 °C; $[\alpha]_D$ +33° (*c* 0.10, $CHCl_3$); $[\alpha]_D$ +29° (*c* 0.10, acetone); CD (*c* 0.10 μM, $CHCl_3$) $\Delta\epsilon_{299}$ +8.87, $\Delta\epsilon_{292}$ −6.29, $\Delta\epsilon_{280}$ +48.24, $\Delta\epsilon_{260}$ −14.04, $\Delta\epsilon_{255}$ −3.83, $\Delta\epsilon_{248}$ −81.91, $\Delta\epsilon_{238}$ +17.33, $\Delta\epsilon_{232}$ −0.45; UV (MeOH) λ_{max} (log ϵ) 284 (4.5), 247 (4.6) nm; IR (film) ν_{max} 3001, 2934, 2836, 1607, 1512, 1462, 1248, 1198, 1130, 1036, 829, 754 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 3.20 (3H, s, OCH_3-13b), 3.37 (1H, brd, J = 11.5 Hz, H-8b), 3.64 (3H, s, OCH_3-11a), 3.67 (3H, s, OCH_3-13a), 3.74 (3H, s, OCH_3-4b), 3.76 (3H, s, OCH_3-4a), 3.77 (12H, s, OCH_3-4c, OCH_3-13c, OCH_3-11d and OCH_3-13d), 3.79 (3H, s, OCH_3-4d), 4.06 (1H, dd, J = 11.5 and 10.0 Hz, H-7c), 4.36 (1H, d, J = 10.0 Hz, H-8c), 4.46 (1H, d, J = 12.0 Hz, H-8a), 4.57 (1H, d, J = 4.5 Hz, H-8d), 5.15 (1H, d, J = 3.5 Hz, H-7b), 5.39 (1H, d, J = 4.5 Hz, H-7d), 5.93 (1H, d, J = 12.0 Hz, H-7a), 6.23 (1H, s, H-12b), 6.27 (2H, brs, H-10d and 14d), 6.27 (1H, brs, H-14a), 6.30 (1H, t, J = 2.3 Hz, H-12d), 6.41 (1H, d, J = 2.0 Hz, H-12c), 6.49 (1H, d, J = 2.3 Hz, H-12a), 6.50 (1H, d, J = 2.0 Hz, H-14c), 6.52 (2H, d, J = 8.5 Hz, H-3c and H-5c), 6.58 (2H, d, J = 8.5 Hz, H-2c and H-6c), 6.79 (2H, d, J = 8.7 Hz, H-3b and H-5b), 6.90 (2H, d, J = 8.6 Hz, H-3a and H-5a), 6.96 (2H, d, J = 8.5 Hz, H-3d and H-5d), 7.20 (2H, d, J = 8.7 Hz, H-2b and H-6b), 7.32 (2H, d, J = 8.5 Hz, H-2d and H-6d), 7.38 (2H, d, J = 8.6 Hz, H-2a and H-6a); ^{13}C NMR (90 MHz, acetone-d_6) δ 37.3 (d, C-7b), 49.3 (d, C-8a), 51.6 (d, C-8c), 52.4 (d, C-8b), 55.1 (q, OCH_3-13a), 55.2 (q, OCH_3-4d), 55.4 (q, OCH_3-4a and OCH_3-4b), 55.5 (q, OCH_3-4c, OCH_3-13c, OCH_3-11d, and OCH_3-13d), 55.7 (q, OCH_3-13b), 57.0 (q, OCH_3-11a), 58.0 (d, C-8d), 58.7 (d, C-7c), 90.5 (d, C-7a), 93.5 (d, C-7d), 93.7 (d, C-12b), 94.0 (d, C-12c), 98.1 (d, C-12a), 99.8 (d, C-12d), 105.3 (d, C-14a, C-10d and C-14d), 105.8 (d, C-14c), 114.07 (d, C-3c and C-5c), 114.11 (d, C-3b and C-5b), 114.7 (d, C-3d and C-5d), 114.8 (d, C-3a and C-5a), 115.7 (s, C-10b), 123.4 (s, C-10c), 124.5 (s, C-14b), 127.3 (d, C-2d and C-6d), 127.5 (s, C-10a), 128.9 (d, C-2c and C-6c), 130.1 (d, C-2a and C-6a), 130.5 (d, C-2b and C-6b), 131.7 (s, C-1a), 132.7 (s, C-1c), 133.8 (s, C-1b), 135.8 (s, C-1d), 141.1 (s, C-9a), 142.8 (s, C-9b), 143.2 (s, C-9c), 147.6 (s, C-9d), 158.0 (s, C-13b), 158.6 (s, C-11a and C-4b), 158.8 (s, C-4c), 159.3 (s, C-11b), 159.6 (s, C-13a), 160.3 (s, C-4d), 161.0 (s, C-4a), 161.6 (s, C-11c), 161.9 (s, C-13c, C-11d and C-13d); $^1H-^1H$ COSY correlations H-7a/H-8a, H-8b/H-7c, H-7c/H-8c, H-7d/H-8d; $^1H-^1H$ NOESY correlations H-2a(6a)/H-7a,H-8a,H-14a, H-3a(5a)/OCH_3-4a, H-7a/H-14a, H-8a/H-2b(6b), H-12a/OCH_3-11a,OCH_3-13a, H-14a/OCH_3-13a, H-2b(6b)/H-7c,H-14c, H-3b(5b)/OCH_3-4b, H-7b/H-8b, H-8b/H-2c(6c), H-12b/OCH_3-13b, H-2c(6c)/H-7c,H-8c, H-3c(5c)/OCH_3-4c, H-7c/H-14c, H-8c/H-8d, H-12c/OCH_3-13c, H-14c/OCH_3-13c, H-2d(6d)/H-8d, H-3d(5d)/OCH_3-4d; $^1H-^{13}C$ HMBC correlations H-2a(6a)/C-2a-(6a),C-4a,C-7a, H-3a(5a)/C-1a,C-3a(5a),C-4a, H-7a/C-2a(6a),C-8a,C-9a, H-8a/C-7a,C-9a,C-10b, H-12a/C-10a,C-11a,C-13a,C-14a, H-14a/C-8a,C-10a,C-12a,C-13a, H-2b(6b)/C-2b(6b),C-4b, H-3b(5b)/C-1b,C-3b(5b),C-4b, H-7b/C-9a,C-11a,C-1b,C-2b(6b),C-8b,C-9b, H-12b/C-10b,C-11b,C-13b,C-14b, H-2c(6c)/C-2c(6c),C-4c,C-7c, H-3c(5c)/C-1c,C-3c(5c), H-7c/C-7b,C-8b,C-1c,C-2c(6c),C-9c, H-8c/C-14b,C-1c,C-9c,C-14c, H-12c/C-10c,C-11c,C-14c, H-14c/C-8c,C-10c,C-12c,C-13c, H-2d(6d)/C-2d(6d),C-4d,C-7d, H-3d(5d)/C-1d,C-3d(5d),C-4d, H-7d/C-11c,C-1d,C-2d(6d),C-9d, H-8d/C-9c,C-10c,C-11c,C-1d,C-7d,C-9d, H-10d(14d)/C-8d,C-10d(14d), OCH_3-4a/C-4a, OCH_3-11a/C-11a, OCH_3-13a/C-13a, OCH_3-4b/C-4b, OCH_3-13b/C-13b, OCH_3-4c/C-4c, OCH_3-13c/C-13c, OCH_3-4d/C-4d, OCH_3-11d(13d)/C-11d(13d); EIMS *m*/*z* (70 eV) $[M]^+$ 1046.

Cytotoxicity Testing. All compounds obtained in this study were tested in a panel of human cancer cell lines using established protocols.[43] A human oral epidermoid carcinoma (KB) cell line was used to monitor the fractionation of active constituents from the MeOH extract of *V. diospyroides*.

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Supporting Information Available: HMBC NMR spectra of compounds **1**−**5**, HETCOR NMR spectrum of **2**, and HMQC NMR spectra of **1**, **3**, and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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