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Biologically Active Dichapetalins from *Dichapetalum gelonioides*

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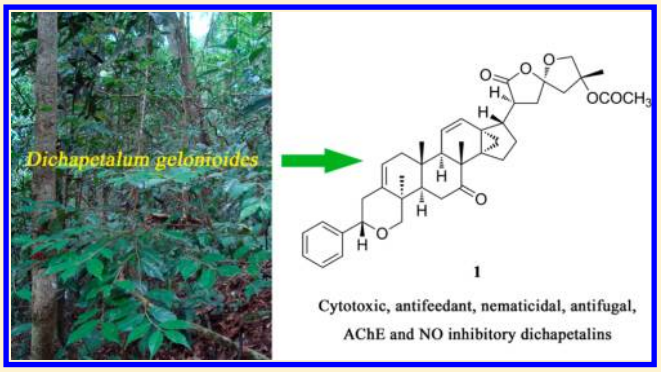
Supporting Information

ABSTRACT: A phytochemical investigation of the toxic tropical plant *Dichapetalum gelonioides* led to the isolation and identification of 14 new dichapetalins (**1**−**14**) and the known dichapetalins A (**15**) and K (**16**). The structures of the new compounds were determined by analyses of their NMR, MS, electronic circular dichroism, and X-ray diffraction data. The esterification at C-25 by 4-hydroxyphenylpropanoic acid and the hydroxylation at C-2′ are unique in this unusual class of natural products. In addition to the known cytotoxicity, an array of biological activities, including antifeedant, nematicidal, antifungal, and NO and AChE inhibitory activities, were observed for this class of compounds. These findings suggested that dichapetalin hybrid triterpenoids as a class have broad biologically active cellular functions including defense against insect herbivores and pathogens.



The genus *Dichapetalum* Thouars comprises approximately 100 shrub and small tree species primarily found in tropical and subtropical areas. Some African species are used as folk medicines for viral hepatitis and jaundice;[1] however, some species are poisonous to livestock. These toxins contain fluorinated compounds, such as fluorocarboxylic acids.[2,3] Previous studies[4−6] of the nonfluorinated chemical constituents of this genus led to the isolation of phenylpyranotriterpenoid dichapetalins A−L from the bark of Philippine *Dichapetalum gelonioides* and dichapetalin M from the roots of African *D. madagascariense*. In addition, dichapetalins N−S were isolated from *D. mombuttense*, *D. zenkeri*, and *D. leucosia*.[7] Dichapetalins have a characteristic hybrid structural feature: a C_6C_2 unit connected to a dammarane or a 14,30-cyclodammarane skeleton with variable C-17 side chains containing lactone, spirolactone, lactol, acetal, or furan moieties. The bioassay results indicated that the dichaptelins displayed potent cytotoxicity against different human cancer cell lines. Dichapetalin M exhibited potent cytotoxicity against human colorectal carcinoma (HCT116) and human melanoma (WM 266-4) cell lines (EC_{50} = 9.9 and 78 nM, respectively).[7]

D. gelonioides and *D. hainanense* are found in China and are mainly distributed in the south. The former has been used as an insecticide and raticide, and it has been named dushuzi (ratbane) in Chinese. We investigated the stems and leaves of *D. gelonioides* collected from Xishuangbanna of Yunnan Province to determine their chemical constituents and biological activities. Consequently, 14 new phenylpyranotriterpenoids (**1**−**14**), as well as the known dichapetalins A (**15**) and K (**16**), were isolated and identified. The various observed biological activities included cytotoxic, antifeedant, nematicidal, antifungal, and AChE and NO inhibitory activities for this special class of natural products.

RESULTS AND DISCUSSION

Compound **1** was isolated as colorless needles and has a molecular formula of $C_{40}H_{48}O_7$, as determined from its ^{13}C NMR spectroscopic data and HREIMS molecular ion at m/z 640.3390. The IR spectrum showed typical absorptions at 1767, 1725, and 1709 cm^{-1} for the carbonyl groups. In the ^1H NMR spectrum (Table 1), an acetoxy methyl (δ_H 2.03) and four tertiary methyl groups at δ_H 1.17, 1.28, 1.31, and 1.69 were observed. Two pairs of AB doublets were observed at δ_H 3.55 (J = 10.6 Hz), 3.81 (J = 10.6 Hz), 3.97 (J = 10.3 Hz), and 4.32 (J = 10.3 Hz), indicating the presence of two oxygenated methylene groups. Three olefinic proton resonances appeared at δ_H 5.40 (dd, J = 10.0, 2.4 Hz), 5.45 (d, J = 6.8 Hz), and 6.26 (dd, J = 10.0, 2.8 Hz). In the low-field region, the five resonances between δ_H 7.26 and 7.37 indicated the presence of a monosubstituted phenyl ring. The ^{13}C NMR spectrum showed 40 carbon resonances that were classified using DEPT-90 and DEPT-135 experiments into five methyl, 10 methylene, including two oxymethylene, 13 methine, including eight

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−5 (δ_H [ppm], J [Hz])[a]

no.	1[b,c]	2[d,e]	3[d,e]	4[b,e]	5[b,e]
1a	2.62 m	2.23 dd (16.0, 7.2)	2.24 dd (16.0, 7.0)	2.26 m	2.23 m
1b	2.33 m	1.77 br d (16.0)	1.76 br d (16.0)	1.79 m	1.73 m
2	5.45 d (6.8)	5.44[f]	5.50 d (7.0)	5.52 d (7.0)	5.47[f]
5	1.77 dd (14.8, 2.5)	1.71 d (12.9)	1.82 dd (14.9, 2.8)	1.83 dd (14.8, 2.3)	1.80 d (14.8)
6a	2.63 m	4.71 dd (12.9, 4.5)	2.80 m	2.81 m	2.79 m
6b	2.35 m		2.27 m	2.30 m	2.23 m
9	1.93 m	2.11 m	2.07 m	2.08 m	2.05 m
11	5.40 dd (10.0, 2.4)	5.45[f]	5.43 dd (10.0, 2.5)	5.45 dd (10.0, 2.1)	5.47[f]
12	6.26 dd (10.0, 2.8)	6.46 dd (10.0, 3.0)	6.42 dd (10.0, 3.0)	6.32 dd (10.0, 2.6)	6.40 dd (10.0, 2.3)
15a	2.13 m	2.11 m	2.08 m	2.08 m	2.05 m
15b	1.94 m	1.99 m	1.96 m	1.97 m	1.95 m
16a	1.66 m	1.65 m	1.62 m	1.70 m	1.56 m
16b	1.05 m	1.47 m	1.43 m	1.26 m	1.16 m
17	2.51 m	2.49 m	2.47 m	2.47 m	2.42 m
18	1.17 s (3H)	1.25 s (3H)	1.21 s (3H)	1.20 s (3H)	1.19 s (3H)
19	1.28 s (3H)	1.40 s (3H)	1.33 s (3H)	1.34 s (3H)	1.32 s (3H)
20	3.31 m	3.05 dd (10.3, 5.6)	3.05 dd (10.2, 5.5)	3.08 dd (10.0, 7.2)	3.28 m
22α	2.39 m	4.32 d (10.2)	4.30 m	2.57 m (2H)	2.38 m
22β	2.19 m				2.33 m
24α		2.41 d (14.1)	2.41 d (14.1)	2.83 d (14.0)	2.63 (14.0)
24β	2.59 m (2H)	2.36 d (14.1)	2.36 d (14.1)	2.43 d (14.0)	2.50 (14.0)
26α	4.32 d (10.3)	3.94 d (8.6)	3.94 d (8.6)	4.18 d (9.7)	4.23 d (10.0)
26β	3.97 d (10.3)	3.84 d (8.6)	3.85 m	4.08 d (9.7)	3.91 d (10.0)
27	1.69 s (3H)	1.42 s (3H)	1.42 s (3H)	1.59 s (3H)	1.62 s (3H)
28	1.31 s (3H)	1.50 s (3H)	1.29 s (3H)	1.30 s (3H)	1.27 s (3H)
30a	1.17 m	1.31 d (6.4)	1.24 d (6.3)	1.27 m	1.20 m
30b	0.79 d (6.1)	1.05 d (6.4)	0.99 d (6.3)	0.89 d (5.9)	0.86 d (5.9)
2′a	3.81 d (10.6)	4.26 d (10.6)	3.83 d (10.8)	3.85 d (10.7)	3.83 d (10.7)
2′b	3.55 d (10.6)	3.96 d (10.6)	3.56 d (10.8)	3.58 d (10.7)	3.55 d (10.7)
5′a	2.62 m	2.59 m	2.54 m	2.55 m	2.53 m
5′b	2.25 m	2.29 m	2.30 m	2.30 m	2.28 m
6′	4.30 m	4.38 d (11.7)	4.34 m	4.35 m	4.32 d (12.0)
2″,6″	7.37 (2H)[f]	7.43 d (2H, 7.4)	7.42 d (2H, 7.3)	7.43 d (2H, 7.3)	7.41 d (2H, 7.4)
3″,5″	7.35 (2H)[f]	7.26 t (2H, 7.7)	7.26 t (2H, 7.3)	7.36 t (2H, 7.5)	7.34 t (2H, 7.4)
4″	7.26[f]	7.35 t (7.4)	7.34 t (7.3)	7.26 t (7.3)	7.27 t (7.4)
2‴a				2.70 m	2.66 m
2‴b				2.66 m	2.59 m
3‴				5.05 m	5.00 m
5‴, 9‴				7.26 d (2H, 8.5)	7.22 d (2H, 8.2)
6‴, 8‴				6.81 d (2H, 8.5)	6.79 d (2H, 8.2)

[a]Resonance for acetoxy group of **1**: δ_H 2.03 s (3H); resonances for hydroxy groups: **2**: δ_H 4.04 d (4.6, 6-OH), 4.64 d (7.8, 22-OH); **3**: δ_H 4.60 d (8.0, 22-OH); **4**: δ_H 8.35 s (7‴-OH); **5**: δ_H 8.33 s (7‴-OH). [b]Recorded at 400 MHz. [c]Measured in $CDCl_3$. [d]Recorded at 600 MHz. [e]Measured in acetone-d_6. [f]Resonances overlapped in the same column.

olefinic methine and an oxymethine, and 12 quaternary carbons, including two olefinic, a ketocarbonyl (δ_C 213.4), an acetal (δ_C 113.3), and two ester carbonyl carbons (δ_C 170.4 and 177.3). Three characteristic resonances at δ_C 13.8 (t), 31.4 (s), and 35.6 (s) evidenced the presence of a cyclopropane moiety. These data suggested that **1** was a phenylpyranotriterpenoid, which is characteristic for the *Dichapetalum* species.[4] Comparing the NMR spectra of **1** with those of dichapetalin P, a phenylpyranotriterpenoid isolated from *D. zenkeri* with a spiroketal moiety,[7] revealed that the two compounds were similar. One of the oxygenated methines in dichapetalin P was replaced by a methylene (δ_C 34.2) group in **1**, and this methylene was assigned to C-22 using the HMBC cross-peaks from H_2-22 (δ_H 2.19 and 2.37) to C-17, C-21, and C-23, indicating the absence of oxygenation at C-22 in **1** (Figure 1).

The ROESY spectrum of **1** suggested that its relative configuration was similar to that of dichapetalin P.[7] The correlations of Me-18 with Me-19 and H-17 revealed their β-orientation. The correlations of H-5 with H-9 and Me-28 and of H-9 with H-30 revealed that all of these protons were α-oriented (Figure 2). A single crystal of **1** was obtained from a mixture of MeOH/water (6:1), and X-ray diffraction via molybdenum radiation (CCDC 961416) permitted definition of the relative configuration and confirmed the overall structure (Figure 3). The absolute configuration of **1** was determined from its electronic circular dichroism (ECD) spectrum. The negative Cotton effect for its C-7 ketone n→π* transition at 294 nm ($\Delta\varepsilon$ = −5.09) revealed the *R* configuration at C-8, the negative Cotton effect for its γ-lactone n→π* transition at 232 nm ($\Delta\varepsilon$ = −5.30) indicated the *R* configuration at C-23, and the positive Cotton effects for its benzene π→π* transition at

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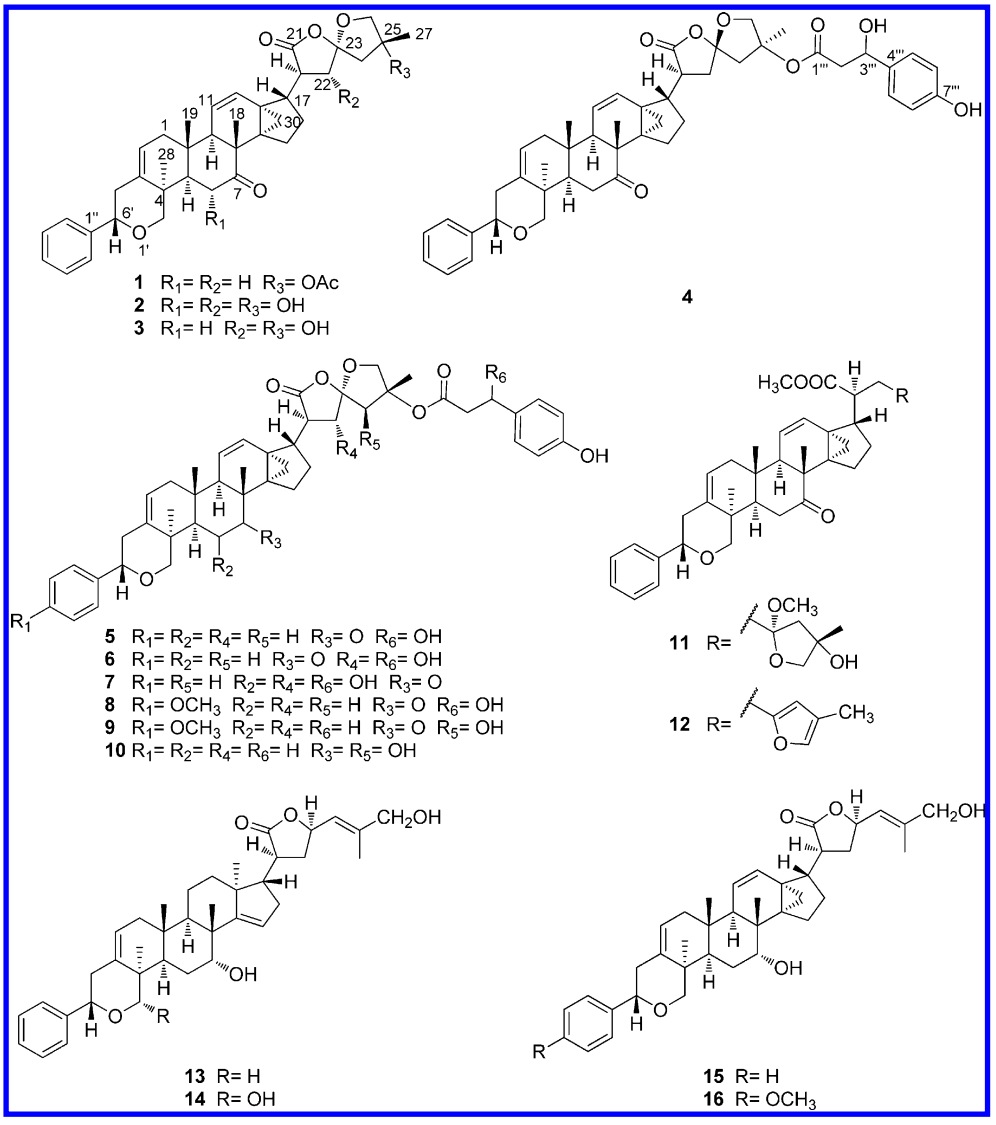

Figure 1. Structures of compounds **1**–**16** from *D. gelonioides*.

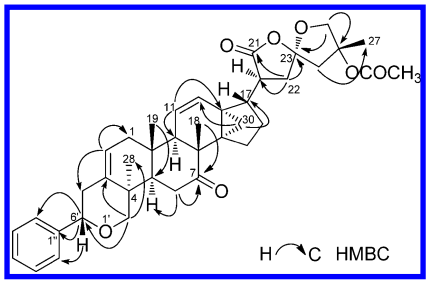

Figure 2. Selected HMBC correlations of 22-deoxydichapetalin P (**1**).

215 nm ($\Delta\varepsilon$ = +16.70) provided evidence of the *S* configuration at C-6′. The ECD data were consistent with those of dichapetalins A–D,[4] indicating that the absolute configuration of the cyclodammarane ring of **1** was identical to those of dichapetalins A–D.[4,7,8] Consequently, the structure of compound **1** was determined, and this compound was named 22-deoxydichapetalin P (Figure 1).

Compound **2** was obtained as a white, amorphous solid. Its molecular formula was $C_{38}H_{46}O_8$ according to the ^{13}C NMR spectroscopic and HREIMS data (found *m*/*z* 630.3207; calcd for 630.3193). The ^{1}H and ^{13}C NMR spectroscopic data

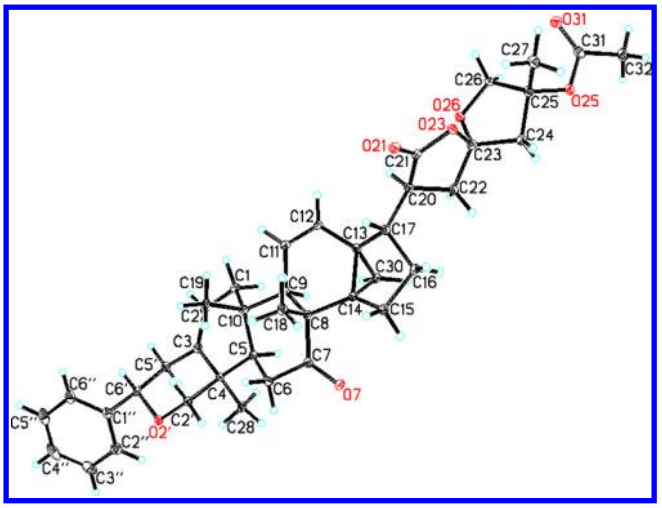

Figure 3. X-ray crystallographic structure of 22-deoxydichapetalin P (**1**).

(Tables 1 and 4) were similar to those of **1**, suggesting that **2** was also a phenylpyranotriterpenoid. Two methylene groups in

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Table 2. ^{1}H NMR Spectroscopic Data of Compounds 6−10 (Acetone-d_6, δ_H [ppm], J [Hz])[a]

no.	6[b]	7[c]	8[c]	9[d]	10[c]
1a	2.26 m	2.23 m	2.22 m	2.23 m	2.15 m
1b	1.78 m	1.76 m	1.71 m	1.74 m	1.69 m
2	5.52 d (7.0)	5.43 m	5.45 d (6.5)	5.47 m	5.44[e]
5	1.83 dd (14.4, 2.8)	1.70 d (12.9)	1.77 m	1.83 dd (14.8, 2.8)	2.06 m
6a	2.81 m	4.70 d (12.9)	2.78 m	2.82 m	2.81 m
6b	2.29 m		2.27 m	2.30 m	2.57 m
7					3.91 br s
9	2.07 m	2.11 m	2.02 m	2.05 m	2.09 m
11	5.45 dd (10.0, 2.5)	5.44 m	5.43 br d (10.1)	5.45 dd (10.1, 2.1)	5.44[e]
12	6.44 dd (10.0, 3.0)	6.45 d (10.0)	6.37 dd (10.0, 2.3)	6.37 m (10.1, 2.5)	6.29 dd (10.0, 2.6)
15a	2.10 m	2.10 m	2.03 m	2.05 m	2.00 m
15b	1.98 m	2.00 m	1.93 m	1.95 m	1.71 m
16a	1.64 m	1.64 m	1.53 m	1.56 m	1.58 m
16b	1.43 m	1.49 m	1.12 m	1.18 m	1.16 m
17	2.49 m	2.50 m	2.43 m	2.44 m	2.51 m
18	1.21 s (3H)	1.25 s (3H)	1.17 s (3H)	1.22 s (3H)	0.91 s (3H)
19	1.35 s (3H)	1.39 s (3H)	1.29 s (3H)	1.33 s (3H)	1.11 s (3H)
20	3.05 dd (10.2, 5.6)	3.03 dd (10.0, 5.3)	3.27 m	3.26 m	3.27 m
22α			2.33 m	2.26 m	2.24 m
22β	4.36 d (10.2)	4.35 m	2.36 m	2.43 m	2.43 m
24α	2.81 m	2.79 d (14.8)	2.61 d (14.8)	4.17 d (7.1)	4.17 d (6.9)
24β	2.49 m	2.48 d (14.8)	2.50 d (14.8)		
26α	4.21 d (9.7)	4.19 d (9.7)	4.22 d (10.0)	4.18 d (10.0)	4.18 d (10.5)
26β	4.09 d (9.7)	4.07 d (9.7)	3.90 d (10.0)	4.04 d (10.0)	4.05 d (10.5)
27	1.67 s (3H)	1.64 s (3H)	1.60 s (3H)	1.50 s (3H)	1.51 s (3H)
28	1.30 s (3H)	1.49 s (3H)	1.25 s (3H)	1.27 s (3H)	1.26 s (3H)
30a	1.25 d (5.9)	1.29 d (5.8)	1.19 m	1.22 m	1.19 m
30b	1.00 d (6.3)	1.04 d (6.4)	0.85 d (5.8)	0.85 d (5.9)	0.89 d (5.5)
2′a	3.86 d (10.8)	4.26 d (10.6)	3.79 m	3.81 d (10.8)	3.76 d (10.7)
2′b	3.58 d (10.8)	3.95 d (10.6)	3.50 d (10.7)	3.52 d (10.8)	3.55 d (10.7)
5′a	2.56 m	2.56 m	2.53 m	2.56 m	2.50 m
5′b	2.30 m	2.26 m	2.22 m	2.26 m	2.22 m
6′	4.34 m	4.37 m	4.24 m	4.25 d (9.2)	4.28 d (9.5)
2″, 6″	7.43 d (2H, 7.6)	7.42 d (2H, 7.6)	7.31 d (2H, 8.6)	7.32 d (2H, 8.6)	7.40 d (2H, 7.5)
3″, 5″	7.36 t (2H, 7.6)	7.34 t (2H, 7.4)	6.88 d (2H, 8.6)	6.89 d (2H, 8.6)	7.33 t (2H, 7.5)
4″	7.29 t (7.6)	7.26 t (7.4)			7.25 t (7.2)
2‴	2.64 m (2H)	2.58 t (7.5, 2H)	2.58, 2.66 m	2.59 t (7.5, 2H)	2.57 t (7.5, 2H)
3‴	5.05 m	5.03 m	5.00 m	2.80 m, 2.05 m	2.81 t (7.5, 2H)
5‴, 9‴	7.25 d (2H, 8.5)	7.22 d (2H, 8.3)	7.20 d (2H, 8.5)	7.04 d (2H, 8.5)	7.05 d (2H, 8.3)
6‴, 8‴	6.81 d (2H, 8.5)	6.78 d (2H, 8.3)	6.77 d (2H, 8.5)	6.74 d (2H, 8.5)	6.75 d (2H, 8.3)

[a]Resonances for hydroxy groups: **6**: δ_H 4.43 d (9.6, 22-OH), 4.51 d (4.3, 3‴-OH), 8.35 s (7‴-OH); **7**: δ_H 4.03 d (4.5, 6-OH), 4.42 d (9.6, 22-OH), 4.48 d (4.4, 3‴-OH), 8.32 s (7‴-OH); **8**: δ_H 4.66 d (4.3, 3‴-OH), 8.81 s (7‴-OH); **9**: δ_H 4.84 d (6.9, 24-OH), 8.31 s (7‴-OH); **10**: δ_H 3.32 m (7-OH), 8.24 s (7‴−-OH); resonances for methoxy groups: **8**: δ_H 3.76 s (3H, 4″-OCH_3); **9**: 3.77 s (3H, 4″-OCH_3). [b]Recorded at 600 MHz. [c]Recorded at 400 MHz. [d]Recorded at 500 MHz. [e]Resonances overlapped in the same column.

1 were replaced by two oxygenated methines (δ_C 72.1 and 73.7) in **2**. In the HMBC spectrum of **2**, the oxygenated methine proton at δ_H 4.71 correlated with C-5 and the C-7 carbonyl carbon, while the oxymethine proton at δ_H 4.32 showed cross-peaks with C-17, C-20, and C-23, indicating oxygenation at C-6 and C-22. In addition, the absence of the *O*-acetyl resonances in the NMR spectra of **2**, coupled with the shielding of C-25 (from δ_C 85.6 in **1** to δ_C 77.4 in **2**), suggested a C-25 hydroxy group in **2** in place of the acetoxy group in **1**. Comparing **2** and dichapetalin M, a phenylpyranotriterpenoid with a spiroketal moiety isolated from the roots of *D. madagascariensis*,[6] revealed that **2** differed from dichapetalin M in the absence of the acetyl group at C-25. The ROESY spectrum of **2** exhibited similar correlations to those of dichapetalin M, indicating that the relative configuration of those stereogenic centers in **2** remained unchanged. The ECD profile of **2** suggested that its absolute configuration was similar to that of **1**. Therefore, the structure of compound **2** was defined as 25-de-*O*-acetyldichapetalin M (Figure 1).

Compound **3** was assigned a molecular formula of $C_{38}H_{46}O_7$, as determined by its ^{13}C NMR spectroscopic and HREIMS data (found m/z 614.3254; calcd for 614.3244). A comparison of the 1D and 2D NMR spectroscopic data (Tables 1 and 4) of **3** with those of **2** and dichapetalin P[7] revealed that **3** differed from **2** through deoxygenation at C-6, and **3** differed from dichapetalin P through de-*O*-acetylation at C-25. In the HMBC spectrum of **3**, the long-range correlations from the methylene protons at δ_H 2.27 and 2.80 (H_2-6) to C-7 confirmed the above inference, which was also supported by the significant shielding of C-25 (dichapetalin P: δ_C 85.3; **3**: δ_C 77.6). The ROESY

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Table 3. ^{1}H NMR Spectroscopic Data of Compounds 11−14 (δ_H [ppm], J [Hz])[a]

no.	11[b,c]	12[b,c]	13[c,d]	14[b,e]
1a	2.21 m	2.19 m	1.98 dd (16.2, 6.9)	2.02 m
1b	1.79 m	1.74 m	1.71 m	1.91 m
2	5.51 d (6.8)	5.49 d (6.9)	5.43 d (6.9)	5.54 d (6.1)
5	1.83 m	1.81 dd (14.8, 2.6)	2.05 m	2.51 m
6a	2.81 m	2.79 m	1.81 m	2.52 m
6b	2.27 m	2.27 m	1.73 m	1.27 m
7			3.97[f]	4.24 m
9	2.07 m	2.04 m	2.11 dd (12.0, 7.5)	2.44 m
11	5.39 d (10.0)	5.92 dd (10.0, 2.9)	1.62, 1.70 m	1.52, 1.61 m
12	5.93 dd (10.0, 2.6)	5.38 dd (10.0, 2.5)	1.42, 2.21 m	1.59, 2.39 m
15a	2.06 m	2.07 m	5.50 m	5.48 m
15b	1.93 m	1.94 m		
16a	1.87m	1.89 m	2.29 m	2.19 m
16b	1.14 m	1.23 d (9.4)	2.06 m	2.12 m
17	2.15 m	2.23 m	2.21 m	2.48 m
18	1.18 s (3H)	1.16 s (3H)	1.12 s (3H)	1.17 s (3H)
19	1.33 s (3H)	1.31 s (3H)	1.07 s (3H)	1.18 s (3H)
20	2.36 m	2.63 m	2.91 m	2.99 m
22a	2.37 m	2.83 m	2.49 m	2.41m
22b	1.60 m	2.77 m	1.86 m	1.91 m
23	1.91 m	5.87 s	5.21 m	5.34 m
24	2.11 m, 1.90 m	9.70 s	5.50 m	5.99 d (8.6)
26a	3.78 d (9.0)	7.12 s	3.97[f]	4.32 m
26b	3.73 d (9.0)			
27	1.30 s (3H)	1.91 s (3H)	1.71 s (3H)	1.84 s (3H)
28	1.31 s (3H)	1.28 s (3H)	1.26 s (3H)	1.65 s (3H)
30a	1.27 d (6.0)	1.27 d (6.0)	1.09 s (3H)	1.25 s (3H)
30b	0.86 d (6.0)	0.87 d (6.0)		
2′a	3.84 d (10.7)	3.83 d (10.7)	3.77 d (10.7)	5.55 m
2′b	3.57 d (10.7)	3.55 d (10.7)	3.57 d (10.7)	
5′a	2.55 br t (11.8)	2.53 br t (11.8)	2.52 m	2.75 m
5′b	2.28 m	2.27 m	2.22 m	2.29 dd (12.9, 2.1)
6′	4.34 d (11.4)	4.31 d (11.5)	4.27 d (11.6)	4.69 d (11.5)
2″, 6″	7.41 d (2H, 7.6)	7.40 d (2H, 7.4)	7.40 d (2H, 7.6)	7.66 d (2H, 7.4)
3″, 5″	7.36 t (2H, 7.6)	7.33 t (2H, 7.4)	7.25 t (2H, 7.6)	7.36 t (2H, 7.4)
4″	7.26 t (2H, 7.3)	7.25 t (7.2)	7.33 t (7.3)	7.44 t (7.5)

[a]Resonances for methoxy groups: **11**: δ_H 3.65 s (3H, 21-OCH$_3$), 3.11 s (3H, 23-OCH$_3$); **12**: δ_H 3.59 s (3H, 21-OCH$_3$). [b]Recorded at 400 MHz. [c]Measured in acetone-d_6. [d]Recorded at 500 MHz. [e]Measured in pyridine-d_6. [f]Resonances overlapped in the same column.

spectrum and ECD curve indicated that the absolute configuration of this compound was similar to those of **1** and **2**. Accordingly, compound **3** was identified as 25-de-*O*-acetyldichapetalin P (Figure 1).

Compounds **4** and **5** were isolated as white, amorphous solids, both possessing a molecular formula of $C_{47}H_{54}O_9$ according to their ^{13}C NMR spectroscopic and HREIMS data (m/z 762.3741 and 762.3786, respectively). The analyses of their NMR, MS, and IR spectra indicated that **4** and **5** were a pair of stereoisomers with structures similar to that of **1** except for the C-25 moiety. An unusual β-hydroxy(4-hydroxyphenyl)-propanoyloxy group was deduced using the ^{1}H and ^{13}C NMR chemical shifts and the ^{1}H−^{1}H COSY, HSQC, and HMBC correlations. This group was attached to C-25 of **4** and **5** instead of the acetoxy group in **1**. A comparison of the ^{13}C NMR data of **4** with those of **5** indicated that C-16, C-17, C-20, C-21, C-22, C-23, C-24, C-25, C-26, and C-27 were shifted by $\Delta\delta_C$ +1.9, +1.0, +1.6, −0.9, +0.9, +0.5, +0.2, −1.0, +0.6, and +1.0, respectively, while only slight or no changes were evident for the remaining carbon resonances. This effect was presumably due to the opposing configurations at C-23 in **4** and **5** after comparison with dichapetalin C-23 stereoisomers reported from *Phyllanthus acutissima*.[9] In the ROESY spectrum of **5**, correlations of H-24 with H-22β and Me-27 were evident, indicating that the C-24 methylene occupied the β-position of the spiroketal ring system. In the ROESY spectrum of **4**, however, the correlation of H-24 with H-20 indicated that the C-24 methylene occupied an α-position. Therefore, the relative configuration of C-23 was established as *S** in **4** and *R** in **5**. A mild alkaline hydrolysis of **5** with K_2CO_3 in MeOH yielded methyl 3-hydroxy-3-(4-hydroxyphenyl)propanoate[10] with a specific rotation of $[\alpha]^{16}_D$ = +11, comparable to that of (*R*)-3-hydroxy-3-phenylpropanoic acid ($[\alpha]^{22}_D$ = +22.4),[11] suggesting that the absolute configuration of C-3‴ was *R*. The ECD spectra of compounds **4** and **5** were similar to those of **1**−**3** but were not distinguishable. Therefore, compounds **4** and **5** were tentatively characterized as shown in Figure 1 and were named dichapetalins T and U, respectively.

Compound **6** possessed a molecular formula of $C_{47}H_{54}O_{10}$ according to its ^{13}C NMR spectroscopic and HREIMS (m/z 778.3705) data. Typical resonances were apparent for the phenylpyranotriterpenoid class, as observed in the ^{1}H and ^{13}C NMR (including DEPT) spectra (Tables 2 and 4). A comparison of the 1D NMR spectra of **6** with those of **5** revealed that one of the methylenes was absent in **6** while an oxymethine (δ_H 4.36; δ_C 72.7) was present. This oxymethine was attributed to hydroxylation at C-22, based on the HMBC correlations from the resonance at δ_H 4.36 to C-17, C-20, and C-23. Because the C-22 hydroxy groups in **2**, **3**, and dichapetalins M and P are all α-oriented, the relative configuration of 22-OH was also proposed to be α, as confirmed by the ROESY correlations of 22-OH with H-20 and H-24α. The ECD spectrum showed similar Cotton effects to those observed for **1**−**5**, revealing the same absolute configurations for its cyclodammarane ring system. Therefore, the structure of compound **6** was elucidated as shown in Figure 1, and this compound was named dichapetalin V.

Compound **7** was obtained as a white, amorphous solid. Its ^{13}C NMR spectroscopic and HREIMS data indicated a molecular formula of $C_{45}H_{54}O_{11}$ (m/z 794.3624). The NMR spectra closely resembled those of **6** except that an oxygenated methine (δ_H 4.70 and δ_C 72.0) in **7** replaced a methylene in **6**. An HMBC experiment led to the placement of this oxymethine at C-6 due to the correlations from the resonance at δ_H 4.70 to C-5 and the carbonyl carbon (C-7). The ROEs of H-6 with Me-18 and Me-19 indicated that 6-OH was in an α-orientation. Therefore, the structure of compound **7** was established as 6α-hydroxydichapetalin V (Figure 1).

Compounds **8** and **9** were obtained as white, amorphous solids, each having a molecular formula of $C_{48}H_{56}O_{10}$ according to their ^{13}C NMR spectroscopic and HREIMS data (m/z 792.3863 and 792.3893, respectively). Inspection of their 1D and 2D NMR spectra indicated that **8** and **9** were a pair of phenylpyranotriterpenoid isomers similar to **5**. However, unlike

Table 4. ^{13}C NMR Spectroscpoic Data of Compounds 1−7 (δ_C [ppm])[a]

no.	1[b,c]	2[d,e]	3[d,e]	4[b,e]	5[b,e]	6[d,e]	7[b,e]
1	39.9 t	40.4 t	40.3 t	40.3 t	40.3 t	40.4 t	40.3 t
2	117.5 d	117.9 d	118.7 d	118.5 d	118.7 d	118.7 d	117.9 d
3	139.7 s	142.0 s	140.6 s	140.5 s	140.5 s	140.5 s	141.7 s
4	38.9 s	38.8 s	39.2 s	39.6 s	39.6 s	39.6 s	40.5 s
5	53.2 d	59.4 d	53.7 d	53.7 d	53.7 d	53.9 d	59.4 d
6	35.6 t	72.1 d	36.3 t	36.1 t	36.2 t	36.2 t	72.0 d
7	213.4 s	214.3 s	212.9 s	212.8 s	212.8 s	212.9 s	214.6 s
8	47.0 s	47.3 s	48.9 s	48.7 s	48.8 s	48.8 s	47.6 s
9	52.3 d	52.4 d	52.8 d	52.6 d	52.6 d	52.8 d	52.4 d
10	36.1 s	37.7 s	39.6 s	36.9 s	36.9 s	36.9 s	37.6 s
11	120.3 d	120.4 d	120.6 d	120.7 d	121.1 d	120.6 d	120.3 d
12	131.2 d	133.3 d	133.1 d	132.6 d	132.3 d	133.1 d	133.1 d
13	31.4 s	32.6 s	32.4 s	32.4 s	32.4 s	32.4 s	32.5 s
14	35.6 s	37.4 s	37.0 s	36.5 s	36.6 s	36.9 s	36.2 s
15	26.7 t	27.5 t	27.7 t	27.5 t	27.7 t	27.7 t	27.5 t
16	23.3 t	24.3 t	24.2 t	25.7 t	23.8 t	24.0 t	24.2 t
17	41.1 d	41.8 d	41.8 d	43.2 d	42.2 d	41.8 d	41.7 d
18	16.9 q	17.5 q	17.3 q	17.1 q	17.2 q	17.3 q	17.4 q
19	17.7 q	18.4 q	18.0 q	17.9 q	17.9 q	18.0 q	18.3 q
20	41.0 d	47.9 d	47.3 d	43.3 d	41.7 d	47.1 d	46.9 d
21	177.3 s	175.4 s	175.7 s	176.9 s	177.8 s	175.1 s	174.9 s
22	34.2 t	73.7 d	73.7 d	35.8 t	34.9 t	72.7 d	72.6 d
23	113.3 s	113.5 s	113.6 s	114.6 s	114.1 s	112.8 s	112.8 s
24	49.3 t	48.8 t	48.8 t	50.0 t	49.8 t	46.4 t	46.4 t
25	85.6 s	77.4 s	77.6 s	85.2 s	86.2 s	85.4 s	85.2 s
26	78.0 t	81.4 t	81.4 t	79.1 t	78.5 t	79.0 t	78.8 t
27	22.5 q	23.9 q	23.9 q	23.5 q	22.5 q	22.5 q	22.4 q
28	23.7 q	26.9 q	24.0 q	24.0 q	24.0 q	24.0 q	26.8 q
30	13.8 t	15.0 t	15.1 t	14.4 t	14.3 t	15.1 t	15.0 t
2′	71.9 t	72.0 t	72.6 t	72.5 t	72.5 t	72.6 t	71.9 t
5′	40.5 t	41.2 t	41.6 t	41.4 t	41.4 t	41.4 t	41.1 t
6′	81.8 d	81.9 d	82.4 d	82.3 d	82.3 d	82.4 d	81.8 d
1″	142.2 s	144.1 s	144.0 s	143.8 s	143.8 s	143.9 s	144.1 s
2″, 6″	125.7 d	126.6 d	126.6 d	126.5 d	126.6 d	126.5 d	126.4 d
3″, 5″	128.5 d	128.1 d	128.1 d	129.0 d	129.0 d	129.2 d	128.9 d
4″	127.6 d	129.1 d	129.1 d	128.0 d	128.0 d	128.2 d	127.9 d
1‴				171.3 s	171.3 s	171.4 s	171.3 s
2‴				45.8 t	45.8 t	45.9 t	45.8 t
3‴				70.7 d	70.9 d	70.9 d	70.9 d
4‴				136.0 s	136.0 s	136.1 s	136.2 s
5‴, 9‴				128.0 d	128.0 d	128.1 d	127.8 d
6‴, 8‴				115.7 d	115.7 d	115.8 d	115.7 d
7‴				157.5 s	157.6 s	157.9 s	157.8 s

[a]Resonances for acetoxy group of **1**: δ_C 170.4 s, 21.9 q. [b]Recorded at 100 MHz. [c]Measured in $CDCl_3$. [d]Recorded at 150 MHz. [e]Measured in acetone-d_6.

5, both compounds **8** and **9** showed ^{1}H and ^{13}C NMR resonances for a methoxy group, indicating that **8** and **9** were methoxylated at C-4″, as has been observed in dichapetalins J and K.[5] This inference was confirmed by the HMBC correlations from the methoxy protons to C-4″. However, **8** and **9** differed in their oxymethine resonances (**8**: δ_H 5.00 and δ_C 70.9; **9**: δ_H 4.17 and δ_C 80.7), which were assigned to C-3‴ in **8** and C-24 in **9** by their respective COSY, HSQC, and HMBC data. The similar coupling patterns and ROEs observed for **8** and **9** compared to those of **5**−**7** suggested a similar relative configuration for the basic skeletons of **8** and **9**. The C-3‴ configuration in **8** should be the same as those in **4**−**7** from a biosynthesis perspective. The 24-OH in **9** was β-oriented according to the correlation between 24-OH and Me-27 in its ROESY spectrum. The ECD spectra of **8** and **9** revealed that the absolute configurations of the cyclodammarane rings of these compounds were identical to those in **1**−**7**. Consequently, the structures of compounds **8** and **9** were identified as shown in Figure 1, and these compounds were named 22-deoxy-4″-methoxydichapetalin V and dichapetalin W, respectively.

The ^{13}C NMR spectroscopic and HREIMS data for compound **10** revealed a molecular formula of $C_{47}H_{56}O_9$ (*m*/*z* 764.3924). The absence of methoxy resonances and the presence of resonances for mono- and *para*-substituted phenyl rings in **10** suggested that C-4″ remained nonoxygenated. In addition, an oxygenated methine (δ_H 3.91 and δ_C 72.2) rather than a carbonyl group was assigned to C-7. This assignment

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Table 5. ^{13}C NMR Spectroscpoic Data of Compounds 8−14 (δ_C [ppm])[a]

no.	8[b,c]	9[c,d]	10[b,c]	11[b,c]	12[b,c]	13[c,d]	14[b,e]
1	40.4 t	40.2 t	40.7 t	40.2 t	40.3 t	40.4 t	40.9 t
2	118.4 d	118.5 d	118.6 d	118.5 d	118.5 d	119.0 d	121.0 d
3	140.6 s	140.7 s	141.0 s	140.2 s	140.5 s	140.8 s	140.5 s
4	36.8 s	39.7 s	39.0 s	39.6 s	39.8 s	39.0 s	44.7 s
5	53.7 d	53.7 d	44.3 d	53.7 d	53.6 d	45.4 d	45.7 d
6	36.2 t	36.2 t	37.2 t	36.2 t	36.2 t	24.7 t	28.5 t
7	213.4 s	212.5 s	72.2 d	212.8 s	212.6 s	72.6 d	72.8 d
8	49.0 s	48.6 s	36.9 s	48.4 s	48.8 s	44.7 s	44.0 s
9	52.8 d	52.6 d	46.5 d	52.4 d	52.5 d	41.5 d	41.2 d
10	36.9 s	36.9 s	36.5 s	36.8 s	36.9 s	37.9 s	37.1 s
11	121.1 d	121.1 d	123.1 d	120.9 d	121.0 d	16.9 t	16.8 t
12	132.2 d	132.2 d	130.7 d	132.6 d	132.4 d	33.6 t	33.5 t
13	32.2 s	36.8 s	30.8 s	33.1 s	33.0 s	47.3 s	47.2 s
14	35.6 s	36.5 s	36.8 s	39.0 s	38.8 s	161.5 s	161.2 s
15	27.6 t	27.6 t	25.3 t	29.1 t	28.6 t	119.8 d	118.8 d
16	24.0 t	23.8 t	23.6 t	27.7 t	27.7 t	32.8 t	32.7 t
17	42.3 d	42.4 d	42.3 d	46.3 d	46.2 d	55.5 d	54.9 d
18	17.2 q	17.1 q	18.1 q	17.1 q	17.1 q	27.7 q	27.5 q
19	17.9 q	17.8 q	18.6 q	17.8 q	17.8 q	16.6 q	16.6 q
20	41.7 d	40.8 d	40.7 d	46.7 d	49.4 d	41.4 d	41.1 d
21	178.0 s	177.5 s	177.6 s	176.2 s	175.8 s	178.2 s	178.8 s
22	34.8 t	31.8 t	31.8 t	36.6 t	30.2 t	35.7 t	35.4 t
23	114.2 s	110.9 s	110.9 s	110.5 s	154.8 s	75.4 d	75.2 d
24	49.6 t	80.7 d	80.8 d	51.1 t	109.3 d	122.6 d	122.1 d
25	86.2 s	85.8 s	85.8 s	77.6 s	121.1 s	142.7 s	143.5 s
26	78.6 t	77.0 t	77.1 t	81.1 t	138.7 d	67.0 t	66.6 t
27	22.7 q	19.0 q	18.9 q	25.9 q	9.70 q	14.2 q	14.2 q
28	24.1 q	24.0 q	24.2 q	24.0 q	24.0 q	24.4 q	20.0 q
30	14.3 t	14.4 t	16.6 t	14.6 t	14.6 t	20.4 q	20.4 q
2′	72.5 t	72.5 t	73.1 t	72.5 t	72.6 t	73.2 t	98.6 d
5′	41.3 t	41.3 t	41.7 t	41.3 t	41.4 t	42.0 t	41.7 t
6′	82.0 d	82.0 d	82.3 d	82.3 d	82.4 d	82.3 d	78.3 d
1″	135.9 s	135.9 s	145.0 s	143.5 s	143.9 s	144.2 s	143.5 s
2″, 6″	127.9 d	127.8 d	126.5 d	126.5 d	126.5 d	126.5 d	126.5 d
3″, 5″	114.3 d	114.3 d	128.9 d	128.9 d	129.0 d	127.8 d	127.7 d
4″	159.9 s	159.9 s	127.9 d	128.0 d	128.0 d	129.0 d	128.7 d
1‴	171.4 s	172.8 s	173.4 s				
2‴	45.6 t	37.2 t	37.1 t				
3‴	70.9 d	30.7 t	30.6 t				
4‴	135.6 s	132.0 s	132.0 s				
5‴, 9‴	127.9 d	130.0 d	130.1 d				
6‴, 8‴	115.9 d	116.0 d	116.0 d				
7‴	157.7 s	156.4 s	156.7 s				

[a]Resonances for methoxy groups: **8**: δ_C 55.5 q (4″-OCH_3); **9**: δ_C 55.4 q (4″-OCH_3); **11**: δ_C 51.1 q (21-OCH_3), 48.4 q (23-OCH_3); **12**: δ_C 51.6 q (21-OCH_3). [b]Recorded at 100 MHz. [c]Measured in acetone-d_6. [d]Recorded at 125 MHz. [e]Measured in pyridine-d_6.

was confirmed by the ^3J HMBC correlation from Me-18 to C-7 although no HMBC correlations were observed for H-7 due to the broadened resonance. The ROE correlation of H-7 with Me-18 revealed an α-orientation for 7-OH. The ECD spectrum showed a positive Cotton effect at 218 nm and a negative Cotton effect at 235 nm, suggesting that the absolute configurations at C-6′ and C-23 were *S* and *R*, respectively.[4] Compound **10** was therefore established as depicted in Figure 1 and was named 4"-demethoxy-7-dihydrodichapetalin W.

Compound **11** was obtained as a colorless oil, and its ^{13}C NMR spectroscopic and HREIMS (*m*/*z* 644.3704) data indicated a molecular formula of $C_{40}H_{52}O_7$. The ^{1}H and ^{13}C NMR (including DEPT) spectra of **11** exhibited the typical signals for a phenylpyranotriterpenoid skeleton, including four tertiary methyl groups, two double bonds, a monosubstituted phenyl ring, and a cyclopropane moiety. However, two methoxy groups were present in **11**. A comparison of the 1D and 2D NMR spectra of **11** with those of dichapetalin G,[4] a phenylpyranotriterpenoid that contains a tetrahydrofuran ring in its C-17 side chain, showed that the two compounds were similar apart from the C-7 position. The C-7 oxymethine functionality in dichapetalin G was oxidized to a carbonyl group (δ_C 212.8) in **11**, as verified by the long-range correlations from H-5, H_2-6, and Me-18 to the carbonyl carbon in the HMBC spectrum of **11**. The two methoxy groups were located at C-21 and C-23 based on the HMBC experiment. The relative configuration of **11** resembled that of dichapetalin G from their similar coupling patterns and ROESY spectra. The positive

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Cotton effect at 216 nm and the negative Cotton effects at 232 and 292 nm in the ECD spectra of **11** suggested that the absolute configurations at C-6′, C-20, and C-8 were *S*, *S*, and *R*, respectively.[4] Therefore, compound **11** was named 7-dehydrodichapetalin G (Figure 1).

The NMR, MS, and IR spectra of compound **12** indicated that it was also a phenylpyranotriterpenoid with a molecular formula of $C_{39}H_{46}O_5$, according to its ^{13}C NMR spectroscopic and HREIMS data (*m*/*z* 594.3325). Unlike compounds **1**−**11**, the C-23 acetal resonance was absent in the ^{13}C NMR spectrum of **12**, while the presence of a 2,4-disubstituted furan ring (δ_H 7.12 and 9.70 s; δ_C 109.3, 121.1, 138.7, and 154.8) was evident. The 1D and 2D NMR spectra revealed that the structure of **12** was similar to that of dichapetalin E.[4] Similar to **11**, the hydroxy group at C-7 in dichapetalin E was oxidized to a carbonyl group in **12**. The ROESY spectrum and ECD data indicated that its absolute configuration was identical to **11**. The structure of compound **12** was accordingly established as 7-dehydrodichapetalin E (Figure 1).

Compounds **1**−**12** are all phenylpyranotriterpenoids containing a cyclopropane moiety. However, the characteristic resonances for this functional group were replaced by those of a tertiary methyl group in the ^1H and ^{13}C NMR spectra of compound **13**. Compound **13** was obtained as colorless fine needles with a molecular formula of $C_{38}H_{50}O_5$, according to its ^{13}C NMR spectroscopic and HREIMS (*m*/*z* 586.3636) data. Its ^1H and ^{13}C NMR spectra were similar to those of the 14,30-*seco*-phenylpyranotriterpenoid, dichapetalin Q,[7] except for the C-21 resonance. The γ-lactone carbonyl group in **13** replaced the lactol moiety in dichapetalin Q. The location of the γ-lactone moiety between C-21 and C-23 was defined on the basis of the HMBC correlation from H-23 to C-21. In the ROESY spectrum of **13**, Me-30 showed correlation with H-9, indicating an α-orientation for Me-30. The relative configurations of the other stereogenic centers of **13** were the same as those of dichapetalin Q, according to the ROESY experiment. The positive Cotton effect at 217 nm ($\Delta\varepsilon$ = +14.02) in the ECD spectrum of **13** indicated that the absolute configuration of C-23 was *R*.[4] Compound **13** was named 21-dehyrodichapetalin Q (Figure 1).

Compound **14**, obtained as colorless, fine needles, had a molecular formula of $C_{38}H_{50}O_6$ according to its ^{13}C NMR spectroscopic data and the HREIMS molecular ion at *m*/*z* 602.3578. The 1D and 2D NMR spectra showed that the basic skeleton and the substituents of **14** were similar to those of **13**. The difference between these two compounds was that a hemiacetal group (δ_H 5.55 and δ_C 98.6) in **14** replaced the oxymethylene (δ_H 3.57 and 3.77; δ_C 73.2; C-2′) in **13**. Long-range HMBC correlations were observed from the hemiacetal proton at δ_H 5.55 to C-4 and C-28. In the ROESY spectrum of **14**, a correlation between H-2′ and H-6′ indicated an α-oriented 2′-OH group. The absolute configuration of **14** was supported by the positive Cotton effect ($\Delta\varepsilon$ = +10.99) at 218 nm in its ECD spectrum.[4] Consequently, compound **14** was assigned as 2′α-hydroxy-21-dehydrodichapetalin Q (Figure 1). This compound is the first dichapetalin isolated from the *Dichapetalum* genus with hydroxylation at C-2′.

Two known phenylpyranotriterpenoids were also isolated and were identified as dichapetalins A and K by comparing their observed and reported spectroscopic data.[4,5] Thus far, 19 dichapetalins have been isolated and identified from different *Dichapetalum* species, including eight dichapetalins with a spiroketal moiety (dichapetalins M, P, and S and acutissimatriterpenes A−E).[6,7,9] The current report adds 14 more members (including 10 spiroketal-containing compounds) to this special subclass of phenylpyranotriterpenoids. Interestingly, this is the first report of dichapetalins with two different *p*-hydroxyphenyl propionyloxy esters, and surprisingly, these substituents occurred exclusively at C-25 despite the hydroxylation at other positions.

Dichapetalins exhibited strong or selective in vitro cytotoxicities against different human tumor cell lines.[4−6] The cytotoxicity of compounds **5**, **6**, **9**, and **12** against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) was evaluated. Compound **6** exhibited broad and significant cytotoxicities against all five tumor cell lines tested (Table 6) and was even superior to cisplatin against the A-549 and MCF-7 cell lines, with IC_{50} values of 3.0 and 3.5 μM, respectively (cisplatin: IC_{50} = 8.3 and 16.1 μM, respectively). In contrast, compound **5** showed selective cytotoxicities and inhibited only the A-549 and MCF-7 cell lines. In addition, moderate and somewhat selective cytotoxicities for compound **12** were observed, but these were broader than those for **5** (Table 6). However, compound **9** did not affect any of the tumor cell lines. Modifications of the C-17 side chain might change both the spectrum and potency of the cytotoxicity by the dichapetalins, while *p*-methoxylation of the C-6′ phenyl ring may significantly reduce their cytotoxicity.

Table 6. Cytotoxic Activity of Compounds from *D. gelonioides*[a,b]

compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
5	>40	>40	26.8	8.2	>40
6	5.1	18.7	3.0	3.5	21.0
9	>40	>40	>40	>40	>40
12	10.2	24.8	17.6	21.0	>40
cisplatin	1.1	7.3	8.3	16.1	14.7

[a]IC_{50} (μM). [b]HL-60 = human promyelocytic leukemia cell line; SMMC-7721 = human hepatocellular carcinoma cell line; A-549 = human alveolar basal epithelial cell line; MCF-7 = human breast adenocarcinoma cell line; SW480 = human colon adenocarcinoma cell line.

Owing to the structural novelty of dichapetalins and the toxicity of *D. gelonioides*, a plant used as an insecticide and a raticide, compounds **1**, **5**, **8**−**13**, and **15** were tested for their feeding deterrent activity against beet armyworm (*Spodoptera exigua*). Most compounds showed potent deterrence toward this insect (Table 7), particularly compounds **11**, **13**, and **15** (EC_{50} values of 3.1, 3.4, and 3.1 μg/cm^2, respectively). These compounds were nearly as potent as commercial neem oil (1% azadirachtin; EC_{50} = 2.7 μg/cm^2). In addition, the tetrahydrofuran ring in the C-17 side chain (compound **11**) and the hydroxy group at C-7 (compounds **10**, **13**, and **15**) seemed to be correlated with the strong antifeedant activity, while *p*-methoxylation of the C-6′ phenyl ring (compounds **8**, **9**, and **16**) greatly diminished their antifeedant activity.

Compounds **1**, **3**, **5**, **8**−**10**, **12**, **13**, **15**, and **16** were tested for their nematicidal effect against *Panagrellus redivivus*. All compounds were generally toxic toward the nematode, but less so than avermectin (Table 7). Compounds **3** and **10** were more active than others, causing 46.3 ± 3.6% and 61.8 ± 5.7% mortality, respectively, at 100 μg/mL over 72 h. The reasons for the variations in toxicity exhibited by compounds with similar structures remain unclear.

Table 7. Antifeedant Activity and Nematicidal Effect of Compounds from *D. gelonioides*

compound	antifeedant activity[a]	nematicidal activity[b]
1	11.7	7.0 ± 2.6
3	ND	46.3 ± 3.6
5	78.7	20.5 ± 8.2
8	>100	4.8 ± 0.4
9	28.9	7.8 ± 1.5
10	9.8	61.8 ± 5.7
11	3.1	ND
12	>100	2.5 ± 1.0
13	3.4	3.0 ± 0.7
15	3.1	15.3 ± 4.8
16	ND	4.4 ± 0.5
positive control[c]	2.7	91.8 ± 2.8
Nnegative control[d]	>100	7.1 ± 0.9

[a]EC_{50} ($\mu g/cm^2$). [b]Mortality rate (%). [c]Neem oil (1% azadirachtin) and avermectin (10 $\mu g/mL$) were used as positive controls for antifeedant and nematicidal assays, respectively. [d]Acetone and DMSO were used as negative controls for antifeedant and nematicidal assays respectively. ND = not determined.

Compounds **1**, **5**, and **15** were tested for the inhibition of fungal growth in four strains of pathogenic fungi, *Colletotrichum gloeosporioides*, *C. musae*, *Fusarium oxysporum* f. sp. *niveum*, and *Rhizoctonia solani*. Compound **5** significantly inhibited the growth of all three fungal strains (Table 8); the diameters of the

Table 8. Diameters of Antifungal Inhibitory Zones (mm) of Compounds from *D. gelonioides*[a]

compound	*Colletotrichum gloeosporioides*	*C. musae*	*Fusarium oxysporum* f. sp. *niveum*	*Rhizoctonia solani*
1	10.1 ± 1.0	13.8 ± 2.1	11.5 ± 0.5	NA
5	12.4 ± 1.1	12.8 ± 2.4	12.8 ± 0.3	13.8 ± 1.0
15	NA	12.8 ± 0.8	NA	10.5 ± 0.02
nystatin	14.5 ± 1.4	16.6 ± 4.4	12.0 ± 0.9	14.7 ± 0.6

[a]NA = not active.

inhibition zones ranged from 12.4 ± 1.1 to 13.8 ± 1.0 mm at 50 μg/disk. The growth inhibition was almost as potent as nystatin (zone diameters: 12.0 ± 0.9 to 16.6 ± 4.4 mm). Compounds **1** and **15** were also active, but were less potent than **5**. These results suggested that the antifungal activity of dichapetalins might be modulated by C-17 side chain modifications. The differential sensitivity of fungal species or simple assay systems might explain why no antifungal activity was found for dichapetalins A–H.[4]

Dichapeltin A (**15**) potently inhibited microphage nitric oxide (NO) production with an IC_{50} value of 0.02 μM (Table 9), which was 20-fold higher than that of the positive control

Table 9. Acetylcholinesterase and Nitric Oxide Inhibitory Activities of Compound 15

test	NO inhibition [IC_{50} (μM)]	AChE inhibition (%)
15[a]	0.02	28.6 ± 0.7
positive control[b]	0.1	59.9 ± 2.2

[a]No cytotoxicity under the NO detection dosage was observed. [b]MG132 (proteasome inhibitor) and tacrine were used as positive controls for NO and AChE assays, respectively.

(MG132 (IC_{50} = 0.1 μM)). No cytotoxicity for **15** was observed at this dosage. In addition, compound **15** was a weak acetylcholinesterase (AChE) inhibitor (Table 9).

In addition to the previously known cytotoxicity, an array of biological activities including antifeedant, nematicidal, antifungal, and NO and AChE inhibitory activities were observed for the dichapetalin class of compounds. The antifeedant, nematicidal, and antifungal activities suggested that the dichapetalins might serve as defensive compounds that allow plants to survive in the complex tropical environment. Additional in-depth characterizations of the pharmacological characteristics and mechanisms of action for these biological activities are in progress.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting points were determined using an XRC-1 micromelting point apparatus and are reported uncorrected. Optical rotations were measured on a Horiba-SEAP-300 spectropolarimeter. UV spectrometric data were obtained on a Shimadzu-210A double-beam spectrophotometer. IR spectra were collected using a Bruker-Tensor-27 spectrometer with KBr pellets. The NMR experiments were carried out on either a Bruker AM-400, DRX-500, or Avance III 600 spectrometer with TMS as an internal standard. The EIMS and HREIMS were measured on a Waters Autospec Premier P776 mass spectrometer. The ESIMS were determined on an API Qstar Pulsar 1 instrument. The X-ray analysis was conducted with a Bruker SMART APEX CCD crystallography system. Semipreparative HPLC was performed on an Agilent 1200 series instrument equipped with a quaternary pump, a vacuum degasser, an autosampler, a thermostatic column compartment, and a diode array detector. Column chromatography was performed on 200- to 300-mesh silica gel (Qingdao Marine Chemical Factory, P. R. China), Sephadex LH-20 (GE Healthcare Bio-Xciences AB), or MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). TLC spots were visualized under UV light and by dipping into 15% H_2SO_4 in EtOH followed by heating. All solvents including petroleum ether (60–90 °C) were distilled before use.

Plant Material. The stems and leaves of *D. gelonioides* were collected in a tropical rain forest in Xishuangbanna, Yunnan Province, China, in October 2011 and were identified by Prof. Hong Wang at Xishuangbanna Tropic Botanic Garden, Chinese Academy of Sciences. An authentic specimen (KIB20111202) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Air-dried and pulverized branches and stems from *D. gelonioides* (27.2 kg) were extracted with MeOH (45 L × 3, each 24 h) at room temperature. After evaporating the solvents in vacuo at 50 °C, 350.5 g of a residue was obtained. This residue was suspended in H_2O (2 L) and extracted with EtOAc (2 L × 4). The EtOAc-soluble extract (163.4 g) was chromatographed using a silica gel column with $CHCl_3/Me_2CO$ (from 10:0 to 0:10, v/v) to give fractions A–E. Fraction B (43.0 g, $CHCl_3/Me_2CO$, 9:1) was subjected to MCI gel column chromatography with $MeOH/H_2O$ (from 7:3 to 10:0) to afford four subfractions, B1–B4. Subfraction B2 (3.1 g) was further chromatographed on a silica gel column using petroleum ether/Me_2CO (5:1, v/v) to give crude **1**; pure **1** (387 mg) was obtained through recrystallization in acetone. Fraction C (26.0 g, $CHCl_3/Me_2CO$, 8:2) was also subjected to MCI gel column chromatography with $MeOH/H_2O$ (from 7:3 to 10:0) to afford five subfractions, C1–C5. Subfractions C3 and C4 (18.3 g) were combined and repeatedly chromatographed on silica gel and Sephadex LH-20 columns to yield **4** (24 mg), **5** (151 mg), **11** (47 mg), **12** (36 mg), and two mixtures containing of **2** and **3** (75 mg) and **6** and **7** (50 mg), respectively. The former mixture was purified using reversed-phase semipreparative HPLC using 85% MeOH in H_2O as eluent (flow rate: 3 mL/min; column: ZORBAX SB-C_{18}, 5 μm, 9.4 × 250 mm; detection: UV 210 nm; retention times: 9.5 and 10.7 min, respectively) to yield **2** (2.4 mg) and **3** (10 mg). In a similar way, the

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latter mixture was purified using 87% MeOH in H_2O as eluent to yield **6** (2.5 mg) and **7** (6 mg) at 10 and 12 min, respectively. Fraction D (14.2 g, $CHCl_3/Me_2CO$, 7:3, early eluents) was further fractionated by MCI gel column chromatography eluting with a gradient of MeOH/H_2O (from 7:3 to 10:0) to provide five subfractions (D1−D5). Subfractions D2 and D3 (8.5 g) were combined and chromatographed over two silica gel columns (petroleum ether/EtOAc, 10:1; petroleum ether/2-propanol, 35:1) before being purified by a Sephadex LH-20 column to yield **8** (98 mg), **9** (26 mg), **10** (15 mg), and **15** (701 mg). Fraction E (16.8 g, $CHCl_3/Me_2CO$, 7:3, late eluents) was subjected to MCI gel column chromatography with a MeOH/H_2O gradient (from 5:5 to 0:10) to yield six subfractions (E1−E6). Subfractions E2 and E3 (2.5 g) were combined and separated using a silica gel column with $CHCl_3$/MeOH (30:1) before being purified with a Sephadex LH-20 column to afford **13** (33 mg) and **16** (68 mg).

The air-dried and powdered leaves of *D. gelonioides* (5.8 kg) were extracted with MeOH (15 L × 3, each 24 h) at room temperature. The extract was concentrated and partitioned between EtOAc and H_2O to generate 252 g of EtOAc-soluble extract. The EtOAc extract was subjected to silica gel column chromatography and eluted with a gradient solvent of $CHCl_3/Me_2CO$ (from 10:0 to 0:10) to give five fractions, A−E. Fraction B (9.4 g, $CHCl_3/Me_2CO$, 8:2) was chromatographed over MCI gel with an eluent of MeOH/H_2O (from 6:4 to 10:0) to afford six subfractions, B1−B6. Subfraction B3 (2.1 g) was chromatographed on a silica gel column and eluted with petroleum ether/Me_2CO (4:1) before being fractionated with a Sephadex LH-20 column to afford 238 mg of crude **1** and 78 mg of crude **5**. After recrystallization in acetone, **1** (160 mg) was obtained, and **5** (32 mg) was purified using silica gel column chromatography (petroleum ether/EtOAc as eluent, 11:5). Fraction C (6.2 g, $CHCl_3/Me_2CO$, 8:2) was subjected to MCI gel column chromatography with MeOH/H_2O (from 6:4 to 10:0) to afford six subfractions, C1−C6. Subfraction C5 (1.8 g) was chromatographed further on a silica gel column using petroleum ether/2-propanol (12:1) to afford crude **14**. Pure **14** (15.8 mg) was obtained via reversed-phase semipreparative HPLC using 85% MeOH in H_2O (retention time: 10.0 min). Fraction E (5.5 g, $CHCl_3/Me_2CO$, 7:3) was repeatedly chromatographed on silica gel and Sephadex LH-20 columns before being recrystallized from acetone to afford **15** (355 mg) and **16** (59 mg).

*22-Deoxydichapetalin P (**1**):* colorless needles (acetone); mp 218−220 °C; $[\alpha]^{21}_D$ −1 (*c* 0.1, acetone); ECD $\Delta\varepsilon_{198}$ +38.84, $\Delta\varepsilon_{215}$ +16.70, $\Delta\varepsilon_{232}$ −5.30, $\Delta\varepsilon_{294}$ −5.09 (7.50×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 205 (3.63), 216 (3.54), 287 (2.04) nm; IR (KBr) ν_{max} 2948, 1767, 1725, 1709, 1355, 1259, 1131, 907, 696 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 4; EIMS *m/z* 640 $[M]^+$ (76), 580 (33), 484 (35), 427 (37), 209 (32), 105 (75), 95 (91), 91 (100); HREIMS *m/z* 640.3390 $[M]^+$ (calcd for $C_{40}H_{48}O_7$, 640.3400).

Crystal data of compound **1** (molybdenum radiation): $C_{40}H_{48}O_7$, *M* = 640.78 g mol^{-1}, colorless needle crystal, size 0.04 × 0.08 × 0.84 mm, orthorthombic, space group $P2_12_12_1$, *a* = 6.2690(13) Å, *b* = 10.626(2) Å, *c* = 49.544(10) Å, α = 90°, β = 90°, γ = 90°, *V* = 3300.4(12) $Å^3$, *T* = 100(2) K, *Z* = 4, *d* = 1.290 mg/m^3, *F*(000) = 1376, reflections collected/unique 32 731/8155 [*R*(int) = 0.0997], completeness θ_{max} = 99.3%, 429 parameters, 0 restraints, $R1_{obs}$ = 0.0584, $wR2_{obs}$ = 0.1065, $R1_{all}$ = 0.0936, $wR2_{all}$ = 0.1248, GOF = 1.026, Absolute structure parameter 0.2(11), largest difference peak and hole = 0.278 and −0.278 e $Å^{-3}$. The crystal structure of **1** was solved by the direct method using the program SHELXS-97 (G. M. Sheldrick, SHELXS97 and SHELXL97, University of Gottingen, Germany, 1997) and subsequent Fourier difference techniques and refined anisotropically by full-matrix least-squares on F^2 using SHELXL-97 (G. M. Sheldrick, SHELXTL, version 6.10, Bruker AXS Inc., Madison, WI, USA, 2000). Crystallagraphic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (CCDC 961416). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

*25-De-O-acetyldichapetalin M (**2**):* white, amorphous solid; $[\alpha]^{22}_D$ −16 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{205}$ +9.19, $\Delta\varepsilon_{218}$ +13.52, $\Delta\varepsilon_{235}$ −0.96, $\Delta\varepsilon_{290}$ −3.41 (4.29×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.78) nm; IR (KBr) ν_{max} 3443, 2955, 2923, 2871, 1786, 1703, 1629, 1452, 1376, 1280, 1073, 1011, 817, 753, 695 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 4); ESIMS *m/z* 653 $[M + Na]^+$; HREIMS *m/z* 630.3207 (calcd for $C_{38}H_{46}O_8$, 630.3193).

*25-De-O-acetyldichapetalin P (**3**):* white, amorphous solid; $[\alpha]^{22}_D$ −2 (*c* 0.4, MeOH); ECD $\Delta\varepsilon_{196}$ +32.92, $\Delta\varepsilon_{217}$ +13.10, $\Delta\varepsilon_{232}$ −5.86, $\Delta\varepsilon_{294}$ −4.38 (1.12×10^{-3} M, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.44), 218 (3.42), 265 (2.28) nm; IR (KBr) ν_{max} 3432, 2960, 2924, 1787, 1708, 1631, 1433, 1099, 967 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 4); ESIMS *m/z* 637 $[M + Na]^+$; HREIMS *m/z* 614.3254 (calcd for $C_{38}H_{46}O_7$, 614.3244).

*Dichapetalin T (**4**):* white, amorphous solid; $[\alpha]^{21}_D$ −36 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{199}$ +14.13, $\Delta\varepsilon_{218}$ +5.78, $\Delta\varepsilon_{231}$ −3.27, $\Delta\varepsilon_{292}$ −1.93 (1.76×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.77), 217 (3.71), 276 (2.64) nm; IR (KBr) ν_{max} 3432, 2959, 1776, 1732, 1708, 1615, 1600, 1517, 1373, 1271, 1168, 1073, 1025, 836, 700 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 4); ESIMS *m/z* 785 $[M + Na]^+$; HREIMS *m/z* 762.3741 (calcd for $C_{47}H_{54}O_9$, 762.3768).

*Dichapetalin U (**5**):* white, amorphous solid; $[\alpha]^{15}_D$ +2 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{200}$ +14.07, $\Delta\varepsilon_{216}$ +6.47, $\Delta\varepsilon_{233}$ −1.52, $\Delta\varepsilon_{292}$ −1.57 (1.60×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.75), 217 (3.69), 276 (2.69) nm; IR (KBr) ν_{max} 3432, 2959, 2928, 1781, 1729, 1709, 1615, 1600, 1450, 1277, 1128, 1059, 1024, 836 cm^{-1}; ^1H and ^{13}C NMR (see Tables 1 and 4); ESIMS *m/z* 785 $[M + Na]^+$; HREIMS *m/z* 762.3786 (calcd for $C_{47}H_{54}O_9$, 762.3768).

*Alkaline Hydrolysis of **5**.* Compound **5** (10 mg) was dissolved in MeOH (5 mL) and hydrolyzed with K_2CO_3 (36 mg) at room temperature for 30 min. After dilution with H_2O (50 mL), the mixture was extracted with EtOAc (2 × 50 mL). The organic layer was concentrated, and the residue was purified by HPLC using 85% MeOH in H_2O (flow rate: 3 mL/min; column: Hypersil BDS-C_{18}, 5 μm, 10 × 250 mm; detection: UV 210 nm; retention time: 4.1 min) to afford 1.2 mg of methyl 3*R*-hydroxy-3-(4-hydroxyphenyl)-propanoate.[10,11] Methyl 3*R*-hydroxy-3-(4-hydroxyphenyl)propanoate: $[\alpha]^{16}_D$ +11 (*c* 0.1, MeOH); ^1H NMR (400 MHz, acetone-d_6) δ 7.20 (2H, d, *J* = 8.5 Hz), 6.77 (2H, d, *J* = 8.5 Hz), 5.01 (1H, m), 3.60 (3H, s), 2.68−2.55 (2H, m); EIMS *m/z* 196 $[M]^+$ (10), 178 (25), 147 (42), 123 (100).

*Dichapetalin V (**6**):* white, amorphous solid; $[\alpha]^{22}_D$ +14 (*c* 0.2, MeOH); ECD $\Delta\varepsilon_{198}$ +14.89, $\Delta\varepsilon_{218}$ +6.19, $\Delta\varepsilon_{233}$ −2.20, $\Delta\varepsilon_{295}$ −1.73 (1.72×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.85), 217 (3.77), 276 (2.75) nm; IR (KBr) ν_{max} 3432, 2958, 2922, 1789, 1728, 1708, 1629, 1618, 1517, 1372, 1100 cm^{-1}; ^1H and ^{13}C NMR (see Tables 2 and 4); ESIMS *m/z* 801 $[M + K]^+$; HREIMS *m/z* 778.3705 (calcd for $C_{47}H_{54}O_{10}$, 778.3717).

*6α-Hydroxydichapetalin V (**7**):* white, amorphous solid; $[\alpha]^{22}_D$ +17 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{198}$ +12.72, $\Delta\varepsilon_{218}$ +5.80, $\Delta\varepsilon_{233}$ −1.88, $\Delta\varepsilon_{290}$ −1.89 (1.58×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.77), 217 (3.71), 276 (2.66) nm; IR (KBr) ν_{max} 3441, 2976, 2925, 1788, 1717, 1706, 1707, 1615, 1600, 1450, 1382, 1270, 1100, 963 cm^{-1}; ^1H and ^{13}C NMR (see Tables 2 and 4); ESIMS *m/z* 817 $[M + Na]^+$; HREIMS *m/z* 794.3624 (calcd for $C_{47}H_{54}O_{11}$, 794.3666).

*22-Deoxy-4″-methoxydichapetalin V (**8**):* white, amorphous solid; $[\alpha]_D^{22}$ +4 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{202}$ +13.15, $\Delta\varepsilon_{225}$ +3.30, $\Delta\varepsilon_{239}$ −0.78, $\Delta\varepsilon_{293}$ −1.47 (1.45×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.77), 225 (3.76), 275 (2.89) nm; IR (KBr) ν_{max} 3432, 2957, 2923, 1781, 1731, 1708, 1614, 1516, 1246, 1173, 1129, 1077, 1059, 1026, 835 cm^{-1}; ^1H and ^{13}C NMR (see Tables 2 and 5); ESIMS *m/z* 815 $[M + Na]^+$, HREIMS *m/z* 792.3863 (calcd for $C_{48}H_{56}O_{10}$ 792.3873).

*Dichapetalin W (**9**):* white, amorphous solid; $[\alpha]^{16}_D$ +26 (*c* 0.1, MeOH); ECD $\Delta\varepsilon_{202}$ +13.27, $\Delta\varepsilon_{227}$ +2.87, $\Delta\varepsilon_{239}$ −1.00, $\Delta\varepsilon_{296}$ −1.60 (1.61×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 201 (3.91), 225 (3.87), 277 (2.96) nm; IR (KBr) ν_{max} 3441, 2957, 2925, 1775, 1713, 1614, 1516, 1449, 1372, 1249, 1174, 1126, 1098, 1026, 828 cm^{-1}; ^1H and ^{13}C NMR (see Tables 2 and 5); ESIMS *m/z* 815 $[M + Na]^+$; HREIMS *m/z* 792.3893 (calcd for $C_{48}H_{56}O_{10}$, 792.3873).

*4″-Demethoxy-7-dihydrodichapetalin W (**10**):* white, amorphous solid; $[\alpha]^{15}_D$ +29 (*c* 0.1, acetone); ECD $\Delta\varepsilon_{198}$ +8.12, $\Delta\varepsilon_{218}$ +5.04, $\Delta\varepsilon_{235}$ −1.06 (1.45×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 204

(3.81), 217 (3.75), 279 (2.75) nm; IR (KBr) ν_{max} 3432, 2957, 2932, 2873, 1782, 1722, 1615, 1516, 1283, 1267, 1126, 1025, 912, 700 cm^{-1}; ^1H and ^{13}C NMR (see Tables 2 and 5); ESIMS m/z 787 $[M + Na]^+$; HREIMS m/z 764.3924 (calcd for $C_{47}H_{56}O_9$, 764.3924).

*7-Dehydrodichapetalin G (**11**):* colorless oil; $[\alpha]^{22}_D$ −8 (*c* 0.1, acetone); ECD $\Delta\varepsilon_{196}$ +24.50, $\Delta\varepsilon_{216}$ +10.62, $\Delta\varepsilon_{232}$ −3.21, $\Delta\varepsilon_{292}$ −2.21 (5.23×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.60), 216 (3.49), 290 (2.25) nm; IR (KBr) ν_{max} 3440, 2952, 2932, 2875, 1726, 1710, 1635, 1452, 1436, 1375, 1281, 1166, 1074, 1027, 700 cm^{-1}; ^1H and ^{13}C NMR (see Tables 3 and 5); EIMS m/z 644 $[M]^+$ (2), 594 (51), 498 (20), 427 (17), 209 (21), 145 (45), 105 (60), 95 (100); HREIMS m/z 644.3704 (calcd for $C_{40}H_{52}O_7$, 644.3713).

*7-Dehydrodichapetalin E (**12**):* white, microcrystalline solid (acetone); mp 160−178 °C; $[\alpha]^{21}_D$ −17 (*c* 0.1, acetone); ECD $\Delta\varepsilon_{196}$ +22.87, $\Delta\varepsilon_{215}$ +10.10, $\Delta\varepsilon_{231}$ −3.64, $\Delta\varepsilon_{295}$ −2.51 (3.76×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 206 (3.58), 217 (3.52), 290 (2.36) nm; IR (KBr) ν_{max} 2956, 2926, 2875, 1733, 1710, 1628, 1451, 1435, 1374, 1288, 1164, 1074, 700 cm^{-1}; ^1H and ^{13}C NMR (see Tables 3 and 5); EIMS m/z 594 $[M]^+$ (37), 498 (10), 145 (32), 105 (35), 95 (100); HREIMS m/z 594.3325 (calcd for $C_{39}H_{46}O_5$, 594.3345).

*21-Dehyrodichapetalin Q (**13**):* colorless, fine needles (acetone); mp 194−197 °C; $[\alpha]^{22}_D$ −35 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{217}$ +14.02 (1.63×10^{-4} M, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.79) nm; IR (KBr) ν_{max} 3582, 3474, 2976, 2957, 2934, 2904, 2871, 2851, 1756, 1628, 1450, 1381, 1323, 1278, 1184, 1141, 1064, 1018, 997, 948, 832, 761, 699 cm^{-1}; ^1H and ^{13}C NMR (see Tables 3 and 5); ESIMS m/z 609 $[M + Na]^+$; HREIMS m/z 586.3636 (calcd for $C_{38}H_{50}O_5$, 586.3658).

*2′α-Hydroxy-21-dehydrodichapetalin Q (**14**):* colorless, fine needles (acetone); mp 193−203 °C; $[\alpha]^{22}_D$ −37 (*c* 0.2, acetone); ECD $\Delta\varepsilon_{218}$ +10.99 (1.99×10^{-4} M, MeOH); UV (MeOH), λ_{max} (log ε) 203.6 (3.83), 254 (2.57) nm; IR (KBr) ν_{max} 3443, 2967, 2934, 2872, 2854, 1760, 1632, 1450, 1384, 1305, 1183, 1063, 1001, 698 cm^{-1}; ^1H and ^{13}C NMR (see Tables 3 and 5); ESIMS m/z 625 $[M + Na]^+$; HREIMS m/z 602.3578 (calcd for $C_{38}H_{50}O_6$, 602.3607).

Cytotoxicity Assay. The cytotoxicity of compounds against a panel of human cancer cell lines was evaluated by the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] assay according to the manufacturer's instructions (Promega, Madison, WI, USA). All cells were cultured in RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere with 5% CO_2. A 100 μL aliquot of the adherent cells was seeded into each well of the 96-well cell culture plates and allowed to adhere for 12 h before adding the test compounds; the suspended cells were seeded just before adding the drug with an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compounds (dissolved in DMSO and diluted by DMEM medium) at a series of concentrations in triplicate for 48 h while using cisplatin as positive control and the culture solution as negative control. After treatment, cell viability was detected, and the cell growth curve was graphed. The data were obtained from experiments carried out in triplicate. The IC_{50} values were calculated by Reed and Muench's method.[12]

Antifeedant Assay. Beet armyworms (*Spodoptera exigua*) were purchased from the Pilot-Scale Base of Bio-Pesticides, Institute of Zoology, Chinese Academy of Sciences. A modified dual-choice bioassay was performed for antifeedant test as previously described.[13] The larvae were reared on an artificial diet under a controlled photoperiod (light:dark, 12:8 h) and temperature (25 ± 2 °C). The larvae were starved for 3−4 h before each bioassay. Fresh leaf disks were cut from *Brassica chinensis*, using a cork borer (1.1 cm in diameter). The treated leaf disks were painted with 10 μL of the test compound in acetone, and control leaf disks were treated with the same amount of acetone. After air drying, the tested and control leaf disks were set in alternating position in the same Petri dish (90 mm in diameter), with moistened filter paper at the bottom. Two-thirds of the instars were placed at the center of the Petri dish. Five replicates were run for each treatment. After feeding for 24 h, the areas of leaf disks consumed were measured. The antifeedant index (AFI) was calculated according to the following formula: AFI = $[(C - T)/(C + T)] \times 100$, where C and T represent the control and treated leaf areas consumed by the insect. The insect antifeedant potency of the test compound was evaluated in terms of the EC_{50} value, which was determined by probit analysis for each insect species.

Nematicidal Assay. The nematodes (*Panagrellus redivivus*) were provided by the Key Laboratory for Microbial Resources, Yunnan University. A nematicidal assay was carried out according to established protocols.[14] The nematodes were separated from the culture medium using the Baermann funnel technique. The test compounds were dissolved in DMSO and diluted with sterilized H_2O containing 0.3% (v/v) Tween-20 to prepare a 100 μg/mL solution. The same amount of DMSO dissolved in H_2O containing 0.3% (v/v) Tween-20 was used as the negative control, while 10 μg/mL avermectin was used as the positive control. Aliquots (100 μL) of the sample solutions were added to 24-well plates. A 900 μL aliquot of the nematode suspension was then transferred to each well and gently mixed. All plates were incubated at 28 °C. Nematode mortality was assessed at 72 h of exposure using a light binocular microscope, and four fields in each well were selected at random for counting. The nematodes were scored as dead when their bodies were straight and they failed to move upon physical stimulus with a hair. The toxicity was evaluated according to the mean corrected percentage of dead nematodes. All tests were repeated three times, and the mean corrected mortality rate (M%) was assessed using the following formula:

$$M(\%) = \frac{(N_t - N_{ts})}{N_t} \times 100\% - \frac{(N_c - N_{cs})}{N_c} \times 100\%$$

N_t is the total number of nematodes in the test; N_{ts} is the number of surviving nematodes in the test; N_c is the total number of total nematodes in the control; N_{cs} is the number of surviving nematodes in the control.

Antifungal Assay. *Colletotrichum gloeosporioides*, *C. musae*, *Fusarium oxysporum* f. sp. *niveum*, and *Rhizoctonia solani* were provided by the Department of Plant Pathology, South China Agriculture University. A previously described paper disk diffusion method[15] was used for the antifungal assay. The test fungi were subcultured in potato dextrose medium and incubated at 28 °C. Sterile liquid potato dextrose agar media was mixed with freshly homogenized fungus at 40 °C, poured into Petri dishes (90 mm diam), and solidified. Aliquots (10 μL) of MeOH containing 50 μg of the test samples or nystatin (positive control) were applied to the 9 mm paper disks. A disk with methanol was used as the negative control. The plates were then incubated at 28 °C for 2 d, and the inhibition zone diameters were measured. The tests were conducted in triplicate.

Nitric Oxide Inhibition Assay. The NO inhibitory activity was determined using the Griess reagent assay for NO production as previously described[16] with slight modifications. Briefly, the murine macrophage RAW264.7 cell line was cultured in Dulbecco's modified Eagle's medium. The compound was added in the presence of lipopolysaccharide (1 μg/mL) before the cultured cells were centrifuged; the supernatants were used to measure the NO production with an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay for cell viability. MG132 (proteasome inhibitor) was used as the positive control.

Acetylcholinesterase Inhibition Assay. AChE inhibitory activity was assayed according to a spectrophotometric method[17] with slight modifications. The test compound was dissolved in DMSO. The reaction mixture (200 μL total) containing phosphate buffer (pH 8.0), test compound (50 μM), and acetyl cholinesterase (0.02 U/mL) was incubated for 20 min (30 °C). The reaction was initiated by adding 40 μL of a solution containing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (0.625 mM) and acetylthiocholine iodide (0.625 mM). The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 s for 1 h. Tacrine was used as a positive control at a concentration of 0.333 μM. The reactions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = $[(E - S)/E] \times 100$

(E is the activity of the enzyme without the test compound, and S is the activity of the enzyme with the test compound).

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR (including DEPT-90 and DEPT-135) spectra of compounds **1**−**14**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published ASAP on March 5, 2014, with an error to compound **10** in the Results and Discussion and Experimental Section. The corrected version was reposted on March 24, 2014.