cis-Clerodane-Type Furanoditerpenoids from *Tinospora crispa*

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Phytochemical and cytotoxicity investigations on organic solvent extracts of the aerial parts of *Tinospora crispa* have led to the isolation of 15 *cis*-clerodane-type furanoditerpenoids. Of these, nine compounds (**1**−**9**) were found to be new. Spectroscopic assignments of a previously reported compound, borapetoside A (**13**), were revised on the basis of HMQC and HMBC correlations. No discernible activity was observed when compounds **10**−**13** were subjected to evaluation in cytotoxicity assays against human prostate cancer (PC-3) and the normal mouse fibroblast (3T3) cell lines.

Tinospora crispa (Willd.) Hook. f. & Thomson [syn.: *Tinospora crispa* (L.) Miers. ex Hook. f. & Thomas, *Menispermum crispum* L., *Tinospora nudiflora* Kurz., *Tinospora rumphii* Boerl.] is a tree belonging to the family Menispermaceae. This species is used widely in Thailand, Malaysia, and Indonesia as a bitter tonic for the treatment of jaundice, rheumatism, urinary disorders, and intermittent fever.[1,2] *Tinospora* species are among the most valuable herbs in Ayurvedic medicine and are well known for their antiallergic, antidiabetic, anti-inflammatory, antioxidant, antispasmodic, hepatoprotective,[3−5] and immunomodulatory activities.[6] A number of clerodane-type furanoditerpenoids have also been reported with activity against human oral epidermoid, medulla, and colon cancer cell lines.[7] Several studies have been conducted on the constituents of the genus *Tinospora*, and a variety of compounds have been isolated including furanoditerpene lactones, steroids, flavonoids, lignans, alkaloids, and phenolics.[8] Among them, clerodane-type furanoditerpenes are commonly found in the genus *Tinospora*.[5−8]

In the present investigation, nine new (**1**−**9**) and six known (**10**−**13**, borapetosides F and G) clerodane-type furanoditerpene lactones have been isolated and characterized.[8,10−12] Moreover, the ^{1}H and ^{13}C NMR chemical shifts for Me-19 and Me-20 in compound **13** were also reassigned. Compounds **10**−**13** were tested for their cytotoxic activities.

Results and Discussion

The air-dried aerial parts of *T. crispa* were powdered and extracted with 80% aqueous methanol. The methanol extract (280 g) was partitioned sequentially into *n*-hexane, EtOAc, *n*-BuOH, and water. The ethyl acetate and *n*-butanol fractions were subjected to repeated silica gel, Diaion HP-20, Sephadex LH-20, and ODS RP-18 column chromatography, while final purification was carried out by preparative HPLC, to afford compounds **1**−**13** and borapetosides F and G. The known compounds **10**−**13** were identified as borapetosides B, C, D, and A, respectively, along with borapetosides F and G, on the basis of the comparison of their physical and spectroscopic data with those reported in the literature.[8,11,12]

Compounds **1**−**9** were identified as clerodane-type furanoditerpenes on the basis of their characteristic spectroscopic features.[13] The EIMS of these compounds exhibited a characteristic fragmentation at *m/z* 81 as a result of bond fission at C-11/C-12. Fragment ion peaks at *m/z* 94 and 95 were due to cleavage of C-9/C-11 bond. The UV spectra showed three absorption bands in a range of 200−225 nm. The IR spectra (KBr) indicated stretching frequencies for O−H (3551−3390 cm^{-1}), C═O (1745−1650 cm^{-1}), and furan ring (1504−1438 cm^{-1}) functionalities. The ^{1}H and ^{13}C NMR spectra (Tables 1−4) of compounds **1**−**9** showed the presence of two angular methyl groups at Me-19 (δ_H 1.06−1.66/δ_C 19.4−29.7) and Me-20 (δ_H 0.83−1.35/δ_C 23.3−29.9), a methoxycarbonyl (δ_H 3.75−3.87/δ_C 52.2−53.4), and a γ- or δ-lactone at C-8/C-12 or C-18/C-6, respectively. The presence of a furan ring was supported by the signals between δ_C 125.8−126.8 (C-13), 109.4−109.9 (δ_H 6.50−6.55, C-14), 144.7−145.0 (δ_H 7.48−7.50, C-15), and 141.3−141.6 (δ_H 7.58−7.60, C-16). The configurations of D-glucose and D-xylose, where present, were determined by acid hydrolysis followed by GLC analysis. The A/B ring junction in compounds **1**−**9** was deduced to be *cis* on the basis of the ^{13}C NMR chemical shifts of the angular methyl (Me-19), which were found to be in the range δ 19.4−29.7, while in a *trans* disposition, they would appear at δ 11−19.[14] The B/C ring fusion was determined on the basis of the ^{13}C NMR chemical shift for C-11 (< δ 45.0 for *cis*-fused rings and > δ 45.0 for *trans*-fused rings).[15]

Compound **1** was obtained as a colorless, gummy solid from the butanolic extract of *T. crispa*. Its HRESIMS showed a $[M + H]^+$ peak at *m/z* 685.2701, corresponding to the molecular formula $C_{32}H_{45}O_{16}$ (calcd 685.2708). The UV spectrum showed absorptions characteristic for a clerodane-type furanoditerpene. Stretching bands at 3420, 1717, and 1507−1440 cm^{-1} in the IR spectrum indicated the presence of OH, ester carbonyl, and aromatic ring functionalities, respectively. The ^{1}H and ^{13}C NMR spectra of **1** showed the characteristic signals for two angular methyl groups (Me-19 and Me-20), a methoxycarbonyl, and a furan ring. A singlet at δ 6.40 ($J_{3,2}$ = 3.7 Hz, H-3) was assigned to the C-3 proton of the trisubstituted double bond. The connectivities of the methoxycarbonyl at C-4 and a δ-lactone between C-8/C-12 were deduced from the HBMC correlations between H-3 (δ_H 6.40, d, $J_{3,2}$ = 3.7 Hz) and C-18 (δ_C 169.4) and between H-7 (δ_H 1.47) and C-17 (δ_C 178.4), respectively. The presence of a δ-lactone was also supported by the relatively upfield chemical shift of C-17 (δ_C 178.4), due to its larger ring size. In γ-lactones, due to angular strain, C-17 resonates above 180 ppm.[16] The HMBC correlation of H-12 (δ_H 5.53, dd, J = 6.0 Hz, J = 5.2 Hz) and C-13 indicated the substitution of a furan moiety at C-12, while the $^{1,3}J$ cross-peaks of H-12 and C-17/C-9 further supported the presence of a six-membered lactone between C-8 and C-12.[12] The presence of a disaccharide moiety (β-glucopyranosyl-(1→6)-α-xylopyranose) was inferred by two sets of signals. The first set resonated at δ 4.42 (d, J = 7.7 Hz, H-1′, δ_C 106.3), 3.22 (t, J = 8.3 Hz, H-2′), 3.37−3.44 (3H, overlap, H-3′ to H-5′), 3.89 (dd, J = 11.3 Hz, J = 3.7 Hz,

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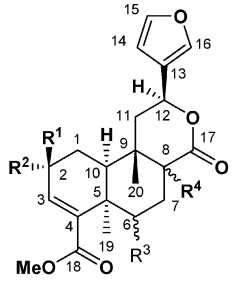
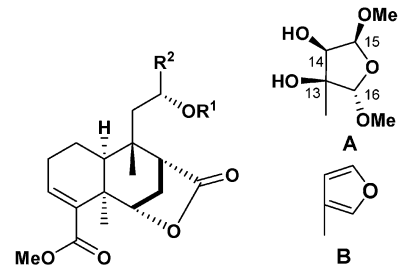
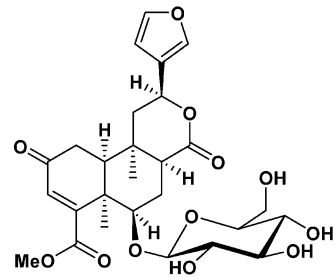
Chart 1



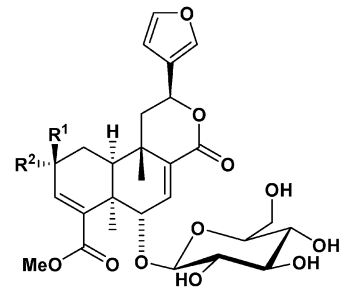
	R^1	R^2	R^3	R^4
1	H	OH	β-{β-D-glucopyranosyl-(1-6)-α-D-xylopyranose}	α-H
2	H	OH	β-(β-D-glucopyranosyl)	β-H
3	=O		β-(β-D-glucopyranosyl)	α-H
10	H	OH	α-(β-D-glucopyranosyl)	α-H
11	H	H	β-(β-D-glucopyranosyl)	β-H



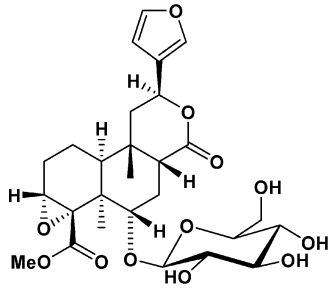
	R^1	R^2
4	H	A
12	β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl	B



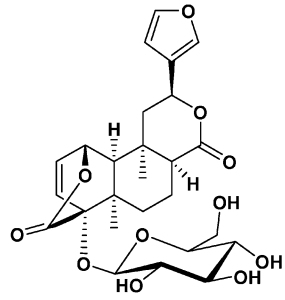
5



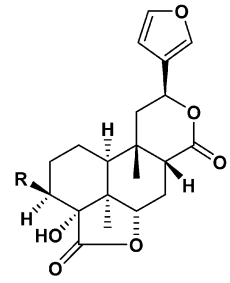
	R^1	R^2
6	H	OH
7		=O



8



9



13: R = β-D-glucopyranosyl

H-6′a), and 3.67 (dd, J = 11.3 Hz, J = 1.4 Hz, H-6′b), corresponding to a β-glucopyranosyl moiety at C-6. The second set of ^{1}H NMR saccharide signals appeared at δ 4.78 (d, J = 3.6 Hz, H-1″, δ_C 100.8), 3.58 (t, J = 9.1 Hz, H-2″), 3.31−3.45 (2H, overlap, H-3″ to H-4″), and 3.52 (m, H-5″), assigned to an α-xylopyranose, connected to β-glucopyranosyl at C-6′ through C-1″. This was deduced on the basis of the more downfield oxymethylene signal of C-6′ (δ_C 67.7) in comparison to the signal of C-5″ (δ_C 63.1).

In the NOESY spectrum of compound **1**, cross-peaks were observed between H-2/Me-20, H-12/H-10, H-8/Me-19, H-10/Me-19, and H-6/H-10, which suggested the orientation of H-2, Me-20, and the furan ring as β and those of H-6, H-8, H-10, H-12, and Me-19 as α. On the basis of these data, the structure of compound **1** was deduced as (2*R*,5*R*,6*R*,8*S*,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-{β-D-glucopyranosyl-(1→6)-α-D-xylopyranosyl}-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester.

Compound **2** was obtained from the butanolic extract of the plant as a colorless, gummy solid using recycling HPLC (GS-320). Its molecular formula was deduced as $C_{27}H_{37}O_{12}$ on the basis of the molecular ion peak at *m/z* 553.2215, corresponding to the $[M + H]^+$ peak in the HRESIMS. The ^{1}H and ^{13}C NMR spectra of **2** showed signals similar to those of compound **1**, except for the absence of signals for the xylose moiety. Along with this, differences were found in the chemical shifts of H-6 (δ_H 4.53, δ_C 78.4), H-10 (δ_H 2.56, δ_C 42.0), H-12 (δ_H 5.77, δ_C 71.3), and Me-20 (δ_H 1.09, δ_C 29.4). This indicated a possible difference in the relative configuration of compound **2**. The relative configurations at C-2, C-5, C-6, C-8, C-9, C-10, and C-12 were determined from the NOESY spectrum, in which correlations were seen between H-2/Me-20, H-8/Me-20, H-12/H-10, H-10/Me-19, and H-6/H-10, indicating that H-2, Me-20, and H-8 are β-oriented, while H-6, H-10, H-12, and Me-19 are α-oriented. On the basis of these arguments, the compound was deduced as (2*R*,5*R*,6*R*,8*R*,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester.

Compound **3** was obtained from the butanolic extract of *T. crispa*. Its molecular formula was deduced as $C_{27}H_{34}O_{12}$ on the basis of

Table 1. ^{1}H NMR Data (CD_3OD) of Compounds **1**−**3**

	1[a]	**2**[a]	**3**[b]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	2.29, m	2.25, m	2.93, dd (18.9, 6.5)
	1.99, m	2.00, m	2.55, d (18.9)
2	4.51, td (8.3, 3.7)	4.49, td (8.3, 3.8)	
3	6.40, d (3.7)	6.59, d (3.8)	6.26, s
6	4.57, brd (3.0)	4.53, brd (4.6)	4.39, brd (2.6)
7	2.27, m	2.67, ddd (14.0, 4.6, 1.5)	2.39, dt (14.4, 3.2)
	1.47, t (13.1)	1.73, dd (14.0, 6.5)	1.56, dd (14.4, 11.7)
8	2.25, m	2.33, dd (6.5, 1.5)	3.48, dd (11.7, 2.2)
10	3.45, m	2.56, brd (6.02)	2.65, d (6.5)
11	2.00, m	2.20, dd (14.6,12.4)	2.10, dd (14.0, 6.2)
	2.03, m	1.65 dd (14.6, 1.5)	1.95, dd (14.0, 11.1)
12	5.53, dd (6.0, 5.2)	5.77, brd (12.4)	5.53, dd (11.1, 6.2)
14	6.51, brs	6.53, brs	6.50, d (1.0)
15	7.48, brs	7.48, dd (1.5, 1.0)	7.50, t (1.5)
16	7.60, s	7.60, brs	7.58, brs
19	1.55 s	1.55, s	1.65, s
20	0.96, s	1.09, s	0.83, s
MeOOC−	3.75, s	3.75, s	3.85, s
1′	4.42, d (7.7)	4.41, d (7.5)	4.44, d (7.6)
2′	3.22, t (8.3)[c]	3.17, dd (7.9, 7.5)	3.27, m
3′	3.37, m	3.30, m[c]	3.37, m[c]
4′	3.39, m	3.39, dd (9.4, 9.2)	3.37, m
5′	3.44, m	3.20, m[c]	3.29, m[c]
6′	3.89, dd (11.3, 3.7)	3.80, dd (11.9, 2.5)	3.80, dd (11.9, 2.3)
	3.67, dd (11.3, 1.4)	3.70, dd (11.9, 3.9)	3.70, dd (11.9, 4.2)
1″	4.78, d (3.6)		
2″	3.58, t (9.0)[c]		
3″	3.31, m		
4″	3.45, m		
5″	3.52, m		

[a] Measured at 500 MHz. [b] Measured at 300 MHz. [c] Signals are interchangeable in each column.

Table 2. ^{1}H NMR Data (CD_3OD) of Compounds **5**, **8**, and **9**

	5[a]	**8**[b]	**9**[b]
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	2.89, dd (16.8, 6.6)	1.70, m	5.30, brd (4.6)
	2.61, dd (16.8,5.3)	1.57, m	
2		2.04, m	6.61, dd (7.2, 4.6)
		1.90, m	
3	6.42, s	3.35, m	6.81, brd (7.2)
6	4.43, d (3.4)	4.58, dd (9.5, 9.3)	1.94, m
			1.49, m
7	2.80, dd (14.3, 3.4)	2.62, m	2.55, m
	1.79, dd (14.3, 6.4)	2.59, m	2.08 m
8	2.40, dd (6.4, 1.5)	2.60, m	2.57, m
10	2.92, m	1.70, brs	1.81, brs
11	2.20, dd (14.8, 1.5)	2.22, dd (14.8, 1.8)	2.39, dd (14.7, 4.1)
	1.68, dd (14.8,12.1)	1.80, dd (14.8, 11.8)	1.98, m
12	5.78, dd (12.1, 1.5)	5.50, brd (11.2)	5.56, dd (12.2, 4.1)
14	6.54, d (1.0)	6.52, d (1.0)	6.55, d (1.0)
15	7.49, t (1.3)	7.50, t (1.5)	7.50, brs
16	7.60, brs	7.59, brs	7.59, brs
19	1.66, s	1.30, s	1.06, s
20	0.98, s	1.35, s	1.20, s
MeOOC−	3.85, s	3.78, s	
1′	4.42, d (7.7)	4.34, d (7.6)	4.71, d (6.1)
2′	3.18, dd (8.9, 7.7)	3.27, m	3.38, m
3′	3.31, m[c]	3.37, m[c]	3.38, m[c]
4′	3.38, t (9.3)	3.37, m	3.38, m
5′	3.22, m[c]	3.29, m[c]	3.29, m[c]
6′	3.81, dd (11.8, 1.9),	3.80, dd (11.8, 1.9)	3.81, dd (11.9, 2.1)
	3.70, dd (11.8, 4.5)	3.70, dd (11.8, 4.5)	3.65, dd (11.9, 5.2)

[a] Measured at 600 MHz. [b] Measured at 300 MHz. [c] Signals are interchangeable in each column.

the peak at m/z 551.2133, corresponding to $[M + H]^+$ in the HRESIMS. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 3) displayed singlets for the two methyl groups (Me-19 and Me-20), a methoxycarbonyl, and a furan ring characteristic of a furanoditerpene. A detailed analysis of the NMR data for compound **3** showed it to be similar to compound **2**, namely, a furanoditerpene δ-lactone glucosylated at C-6 (Tables 1 and 3). However, the C-2 position in compound **3** was oxidized as was inferred from the presence of an additional carbonyl group at δ_C 200.3. The HMBC correlations of H-1 (δ_H 2.55), H-10 (δ_H 2.65), and H-3 (δ_H 6.26) with C-2 further supported the keto functionality in compound **3**. The relative configuration in compound **3** was deduced on the basis of NOESY correlations between H-6/Me-19, H-8/H-10, H-8/H-6, H-10/Me-19, and H-12/H-10. These showed Me-20, the β-glucopyranosyl at C-6, and the furan ring at C-12 to be β-oriented, with H-8, H-10, H-12, and Me-19 all α-oriented. On the basis of the above discussion, compound was assigned as (5*R*,6*R*,8*S*,9*R*,10*R*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester.

Compound **4** gave the molecular formula $C_{23}H_{34}O_{10}$, as deduced from its positive-ion HRESIMS at m/z 471.2312 $[M + H]^+$. The IR spectrum showed the presence of a γ-lactone carbonyl at 1745 cm^{-1}. The ^{1}H and ^{13}C NMR spectra exhibited a downfield oxymethine signal at δ_H 5.57 (δ_C 84.1) for H-6. The upfield oxymethine signal at δ_H 3.84 (δ_C 69.0) was assigned to H-12. The HMBC correlations of H-6 (δ_H 5.57), H-7 (δ_H 2.27 and 2.18), and H-8 (δ_C 2.43) with C-17 (δ_C 180.0, C═O) indicated the presence of a γ-lactone. This was also supported by the downfield chemical shift of C-17 (δ_C 180.5) due to ring strain.[16] The presence of a tetrahydrofuran moiety was inferred from the signals at δ 82.0, 76.2, 111.7, and 109.5, corresponding to C-13, C-14, C-15, and C-16, respectively.[9] The long-range ^3J correlations of H-12 (δ_H 3.84) with C-9 (δ_C 39.1) and C-13 (δ_C 82.0) along with its COSY correlations with the H-11 methylene protons (δ_H 1.69 and 1.59) indicated a linkage between ring B and the tetrahydrofuran moiety through C-11 and C-12. The ^{1}H NMR spectrum of compound **4** also showed two methoxy singlets at δ_H 3.47 (δ_C 56.4) and 3.41 (δ_C 54.8). The location of these methoxy substituents at C-l5 (δ_C 111.7) and C-16 (δ_C 109.5) was deduced from HMBC correlations (Figure 1). On the basis of a comparison with the literature data for known related compounds,[9] it was deduced that C-13 and C-14 are hydroxylated in compound **4**. The relative configuration at various centers in compound **4** was deduced from NOESY and ROESY experiments (Figure 1). Cross-peaks were observed between H-10/Me-19, H-8/Me-20, H-12/Me-20, H-8/H-16, H-12/H-16, and H-14/H-15, indicating that H-10, Me-19, the γ-lactone ring, H-14, and H-15 are α-oriented, while H-8, H-12, Me-20, the methylene bridge at C-7, the C-13 hydroxy group, and H-16 are all β-oriented (Figure 1). On the basis of these data, the structure of **7** was identified as methyl (2*R*,7*S*,8*S*)-8-[(2*S*)-2-(3,4-dihydroxy-2,5-dimethoxytetrahydro-3-furanyl)-2-hydroxyethyl]-2,8-dimethyl-10-oxo-11-oxatricyclo[7.2.1.$0^{2,7}$]dodec-3-ene-3-carboxylate and named, trivially, rumphiol E.

Compound **5** displayed an $[M + H]^+$ peak at m/z 551.2171 in the HRESIMS, corresponding to the molecular formula $C_{27}H_{35}O_{12}$. The ^{1}H and ^{13}C NMR data of **5** were similar to compound **3**, except for slight differences in chemical shifts and splitting patterns, with differences found for C-8 (δ_H 2.40, δ_C 45.4), C-11 (δ_H 2.20, 1.18, δ_C 42.4), and C-20 (δ_H 0.98, δ_C 27.9). In addition to these, H-12 in compound **5** appeared more deshielded, resonating at δ_H 5.78. The NOESY spectrum of compound **5** showed correlations between H-6/Me-19, H-6/H-8, H-10/Me-19, H-8/Me-20, and H-12/H-10. These indicated that the β-glucopyranosyl at C-6 and the furan ring at C-12 are β-oriented, while H-6, H-8, H-10, H-12, Me-19, and Me-20 are α-oriented. Therefore, it was concluded that compound **5** is a C-9-epimer of compound **3**. Compound **5** was deduced to have the structure (5*R*,6*R*,8*S*,9*R*,10*S*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester.

Compound **6** was isolated as a colorless gum. Its HRESIMS showed a $[M + Na]^+$ peak at m/z 573.1980, corresponding to the molecular formula $C_{27}H_{34}O_{12}Na$. The glycosidic nature of **6** was inferred from the EIMS aglycon peak at m/z 388 due to the loss of 162 amu, corresponding to a hexose sugar unit. The ^{1}H and ^{13}C NMR spectra of **6** showed the presence of two angular methyl

Table 3. ^{13}C NMR Data (CD_3OD) of Compounds **1**−**3**, **5**, **8**, and **9**

	1[a]	**2**[b]	**3**[a]	**5**[c]	**8**[a]	**9**[c]
position	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.	δ_C mult.
1	27.3, CH_2	29.1, CH_2	36.2, CH_2	36.2, CH_2	16.3, CH_2	74.8, CH
2	64.6, CH	64.9, CH	200.3, qC	200.8, qC	22.3, CH_2	131.7, CH
3	140.7, CH	141.7, CH	133.4, CH	134.2, CH	60.3, CH	132.5, CH
4	140.3, qC	139.7, qC	156.7, qC	155.7, qC	63.2, qC	87.5, qC
5	42.7, qC	42.0, qC	44.0, qC	43.4, qC	40.9, qC	40.0, qC
6	80.8, CH	78.4, CH	79.6, CH	77.9, CH	78.5, CH	27.1, CH_2
7	29.3, CH_2	29.7, CH_2	28.0, CH_2	29.9, CH_2	30.6, CH_2	18.5, CH_2
8	51.7, CH	46.6, CH	49.8, CH	45.4, CH	49.3, CH	45.2, CH
9	38.3, qC	35.9, qC	38.5, qC	36.4, qC	35.6, qC	36.2, qC
10	42.1, CH	42.0, CH	41.4, CH	40.6, CH	39.9, CH	47.4, CH
11	46.2, CH_2	43.2, CH_2	45.2, CH_2	42.4, CH_2	42.0, CH_2	42.3, CH_2
12	71.9, CH	71.3, CH	71.7, CH	71.3, CH	71.3, CH	72.7, CH
13	125.9, qC	126.9, qC	125.8, qC	126.8, qC	126.6, qC	126.6, qC
14	109.9, CH	109.6, CH	109.8, CH	109.6, CH	109.4, CH	109.6, CH
15	144.9, CH	144.7, CH	145.0, CH	144.8, CH	144.9, CH	145.0, CH
16	141.6, CH	141.3, CH	141.5, CH	141.3, CH	141.3, CH	141.4, CH
17	178.4, qC	177.2, qC	177.5, qC	176.4, qC	176.9, qC	176.7, qC
18	169.4, qC	169.1, qC	168.3, qC	168.0, qC	172.2, qC	175.2, qC
19	29.7, CH_3	28.7, CH_3	28.3 CH_3	27.4, CH_3	19.4, CH_3	24.6, CH_3
20	23.8, CH_3	29.4, CH_3	23.3, CH_3	27.9, CH_3	29.9, CH_3	28.1, CH_3
MeOOC−	52.5, CH_3	52.4, CH_3	52.2, CH_3	53.2, CH_3	53.4, CH_3	
1′	106.3, CH	105.8, CH	106.2, CH	105.9, CH	104.9, CH	101.5, CH
2′	75.5, CH[d]	75.4, CH	75.6, CH	75.4, CH	75.4, CH	75.1, CH
3′	78.1, CH	78.0, CH[d]	78.1, CH[d]	77.9, CH[d]	78.0, CH[d]	78.3, CH[d]
4′	71.2, CH	70.6, CH	71.3, CH	70.6, CH	71.7, CH	71.2, CH
5′	76.5, CH[d]	77.2, CH[d]	77.8, CH[d]	77.3, CH[d]	77.8, CH[d]	78.0, CH[d]
6′	67.7, CH_2	62.3, CH_2	62.3, CH_2	62.3, CH_2	62.6, CH_2	62.5, CH_2
1″	100.8, CH					
2″	75.6, CH[d]					
3″	74.0, CH					
4″	71.6, CH					
5″	63.1, CH_2					

[a] Measured at 125 MHz. [b] Measured at 100 MHz. [c] Measured at 150 MHz. [d] Signals are interchangeable in each column.

groups, a methoxycarbonyl, and a furan ring. The proton signals resonating at δ_H 6.40 (δ_C 138.5, C-3; δ_C 141.5, C-4) and 6.94 (δ_C 140.9, C-7; δ_C 137.0, C-8) were assigned to two trisubstituted double bonds. The HBMC correlations of H-3 (δ_H 6.40) with C-18 and of H-7 (δ_H 6.94) with C-17 suggested the occurrence of a methoxycarbonyl at C-4 and a δ-lactone between C-8 and C-12. The HMBC correlations of H-12 (δ_H 5.24) and C-13 indicated the presence of a furan moiety at C-12. The spectroscopic data of **6** were closely related to those of the known compound borapetoside F,[11] with an additional hydroxymethine signal at δ_H 4.51 (δ_C 64.0) assigned to the oxygenated methine carbon at C-2. The presence of a β-glucopyranosyl moiety was inferred from the NMR signals at δ_C 105.7 (δ_H 4.41, $J = 7.6$ Hz) and supported by the MS fragment ion at *m*/*z* 389 in the FABMS, due to the loss of 162 amu. The relative configuration in compound **6** (Figure 2) was deduced on the basis of a ROESY experiment. The correlations obtained showed that H-10, Me-19, H-12, the hydroxy group at C-2, and the β-glucopyranosyl moiety at C-6 are all α-oriented, while Me-20, H-6, H-2, and the furan ring at C-12 are β-oriented (Figure 1). On the basis of these data, the structure of **6** was deduced as (2*R*,5*R*,6S,9*S*,10*S*,12*S*)-15,16-epoxy-2-hydroxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester.

Compound **7** has the molecular formula $C_{27}H_{33}O_{12}$, as deduced from its HRESIMS, with the $[M + H]^+$ peak at *m*/*z* 549.1976. Its spectroscopic data suggested structural features similar to compound **6**, except for the oxygenation at C-2, as inferred from an additional signal at δ_C 200.0. The ^{13}C NMR spectrum of compound **7** showed signals for two quaternary carbons (C-5 and C-9), two secondary carbons (C-1 and C-11), two oxygenated methine groups (C-6 and C-12), and two trisubstituted double bonds (C-3/C-4 and C-7/C-8). The relative configuration in compound **7** was deduced on the basis of NOESY correlations, which were observed between H-10/Me-19, H-12/Me-19, and Me-20/H-6, and indicated that H-10, H-12, and Me-19 all are β-oriented, while H-6 and Me-20 are α-oriented. On the basis of these data, the new compound **7** was assigned the structure (5*R*,6S,9*S*,10*S*,12*S*)-15,16-epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester.

Compound **8** displayed a $[M + H]^+$ peak at *m*/*z* 553.2255 in the HRESIMS, corresponding to the molecular formula $C_{27}H_{37}O_{12}$. The ^1H and ^{13}C NMR spectra of compound **8** showed characteristic signals for a clerodane-type furanoditerpenoid [δ_H 1.30 (δ_C 19.4), 1.35 (δ_C 29.9), and 3.78 (δ_C 53.4) assigned to Me-19, Me-20, and CO_2Me-18, respectively]. The signals for the furan moiety appeared at δ_H 6.52 (δ_C 109.4), 7.50 (δ_C 144.9), and 7.59 (δ_C 141.3) in addition to the ^{13}C NMR signal at δ_C 126.6 (C-13). The resonances at δ 4.34 (d, $J = 7.6$ Hz, H-1′), 3.80 (dd, $J = 11.8$ Hz, $J = 1.9$ Hz, H-6′a), and 3.70 (dd, $J = 11.8$ Hz, $J = 4.5$ Hz, H-6′b) were assigned to a β-glucopyranosyl moiety at C-6 (δ_C 78.5) of the clerodane-type aglycon skeleton. In turn, the signals at δ 3.25 (overlap, H-3) were assigned to the proton of a epoxide ring between C-3 and C-4 (δ_C 60.3/δ_C 63.2). The HMBC spectrum showed correlations of H-3 with C-1, C-2, and the epoxy-substituted methine carbon C-3 (δ_C 60.3). The protons of the ester methoxy at δ_H 3.78 exhibited HMBC correlations with quaternary carbons at C-4 (δ_C 63.2), C-5 (δ_C 40.9), C-18 (δ_C 172.2), and C-3 (δ_C 60.3). Furthermore, the methine signal at δ_H 4.58 (H-6) gave correlations with C-5, C-6, and C-7 (δ_C 40.9, 78.5, and 30.6, respectively). The relative configuration in compound **8** was determined on the basis of the NOESY spectrum, which showed cross-peaks between H-3/H-6, H-6/H-8, H-6/Me-20, H-8/Me-20, H-10/Me-19, and H-12/H-10, thus indicating that H-3, the methoxycarbonyl group at C-4, Me-20, H-6, H-8, and the furan ring at C-12 are all β-oriented, while the epoxide ring (C-3/C-4), Me-19, the D-glucopyranosyl, and H-12 are α-oriented. On the basis of these observations, the structure of compound was proposed as (3*R*,4*R*,5*R*,6*S*,8*R*,9*S*,10*S*,12*S*)-15,16-

Table 4. NMR Data of Compounds **4**, **6**, and **7** (**4** in $CDCl_3$; **6**, **7** in CD_3OD)

	4		**6**		**7**	
position	δ_C mult.[a]	δ_H (J in Hz)[d]	δ_C mult.[b]	δ_H (J in Hz)[d]	δ_C mult.[b]	δ_H (J in Hz)[d]
1	16.3, CH_2	1.96, m	30.8, CH_2	2.09, m	36.5, CH_2	2.67, m
		1.94, m		1.93, m		
2	24.0, CH_2	2.40, brd (5.4)	64.0, CH	4.35, m	200.0, qC	
		2.30, m				
3	142.8, CH	7.03, dd (3.8, 3.6)	138.5, CH	6.40, d (3.5)	133.4, CH	6.31, s
4	133.8, qC		141.5, qC		156.3, qC	
5	38.9[e], qC		43.5, qC		43.0, qC	
6	84.1, CH	5.57, brd (6.0)	81.1, CH	4.52, d (2.4)	75.6, CH	4.77, m
7	29.4, CH_2	2.27, m	140.9, CH	6.94, d (2.4)	136.2, CH	6.77, d (4.4)
		2.18, m				
8	48.2, CH	2.43, brd (5.4)	137.0, qC		138.6, qC	
9	39.1[e], qC		38.2, qC		38.2, qC	
10	44.3, CH	1.41, dd (5.2, 2.1)	46.5, CH	2.06, m	46.7, CH	2.63, m
11	39.7, CH_2	1.69, dd (15.5, 7.5)	45.1, CH_2	2.40, dd (14.6, 3.3)	45.6, CH_2	2.22, dd (14.3, 3.7)
		1.56, m		2.04, m		2.06, dd (14.3, 11.7)
12	69.0, CH	3.84, brd (7.5)	72.4, CH	5.24, dd (11.4, 3.3)	72.9, CH	5.21, dd (11.7, 3.7)
13	82.0, qC		125.3, qC		125.1, qC	
14	76.2, CH	4.23, brs	109.7, CH	6.53, d (1.0)	109.7, CH	6.51, brs
15	111.7, CH	4.97, d (2.7)	145.0, CH	7.51, t (1.5)	145.0, CH	7.50, t (1.4)
16	109.5, CH	4.90, s	141.5, CH	7.61, brs	141.5, CH	7.59, s
17	180.5, qC		171.2, qC		172.4, qC	
18	166.7, qC		172.0, qC		168.6, qC	
19	27.0, CH_3	1.32, s	23.9, CH_3	1.45, s	27.1, CH_3	1.69, s
20	19.9, CH_3	1.16, s	27.4, CH_3	1.12, s	27.4, CH_3	1.03, s
MeOOC−	51.6, CH_3	3.71, s	52.3, CH_3	3.76, s	53.3, CH_3	3.86, s
MeO-15	56.4, CH_3	3.47, s				
MeO-16	54.8, CH_3	3.41, s				
1′			105.7, CH	4.41, d (7.6)	105.8, CH	4.51, d (7.5)
2′			75.5, CH	3.17, dd (7.5, 7.9)	75.7, CH	3.23, t (7.7)
3′			77.5, CH[e]	3.30, m	78.0, CH[e]	3.33, m
4′			71.3, CH	3.39, dd (9.4, 9.2)	71.5, CH	3.32, m
5′			78.1, CH[e]	3.20, m	78.1, CH[e]	3.33, m
6′			62.5, CH_2	3.85, dd (12.0, 2.0)	62.6, CH_2	3.85, dd (11.7, 1.1)
				3.70, dd (12.0, 4.8)		3.70, dd (11.7, 4.0)

[a] Measured at 75 MHz. [b] Measured at 100 MHz. [c] Measured at 300 MHz. [d] Measured at 500 MHz. [e] Signals are interchangeable in each column.

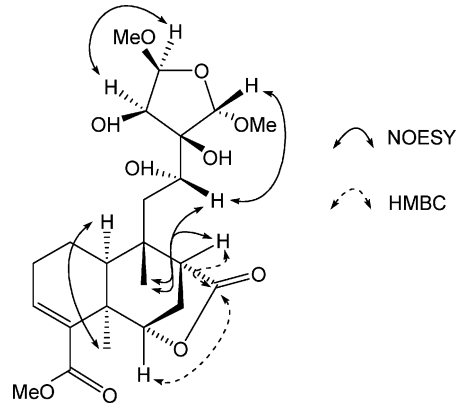


Figure 1. Key NOESY and HMBC interactions of compound **4**.

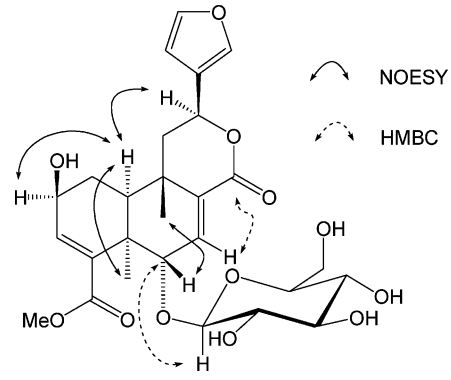


Figure 2. Key NOESY and HMBC interactions of compound **6**.

epoxy-3,4-epoxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester.

The positive-ion HRESIMS of compound **9** showed a $[M + H]^+$ peak at *m*/*z* 521.2043, corresponding to the molecular formula $C_{26}H_{34}O_{11}$. In the ^1H NMR spectrum, a broad doublet at δ_H 5.30 (δ_C 74.8) was due to coupling of H-1 and H-2 (δ_H 6.61). Protons of a disubstituted olefin (C-2/C-3) appeared as a double doublet at δ_H 6.61 (δ_C 131.7, C-2) and a doublet at δ_H 6.81 (δ_C 132.5, C-3). In the ^{13}C NMR spectrum, the quaternary carbons resonating at δ_C 87.5, 176.7, and 175.2 were assigned to C-4, C-17, and C-18, respectively. The ^1H and ^{13}C NMR spectra also showed signals for a monosubstituted furan moiety at C-12 (δ_C 72.7), a β-glucopyranosyl unit at C-4, and two angular methyls at C-5 (δ_C 40.0) and C-9 (δ_C 36.2).[10−12] The HMBC spectrum showed correlations of the methine carbon (δ 74.8) with protons at δ_H 6.61 (H-2), 6.81 (H-3), and 1.81 (H-10). The connectivity of the β-glucopyranosyl unit at C-4 was inferred from the correlation of the anomeric proton (H-1′, δ_H 4.71) with the quaternary carbon at C-4 (δ_C 87.5). The relative configuration of compound **9** was determined on the basis of NOESY interactions. The correlations between H-1/H-10, H-10/Me-19, H-8/Me-20, H-8/H-10, and H-12/H-10 indicated that H-1, H-10, Me-19, H-8, Me-20, H-12, and β-D-glucopyranosyl are all α-oriented, while the lactone ring at C-1/C-4 and furan ring at C-12 are β-oriented. Therefore, compound **9** was deduced to be (1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*S*)-15,16-epoxy-4-*O*-(β-D-glucopyranosyl)-cleroda-2,13(16),14-triene-17(12),18(1)-diolide.

Analysis of the ^{13}C NMR spectrum of borapetoside A (**13**) (Table 5) revealed that certain assignments were different from those reported, particularly the chemical shifts of Me-19 and Me-20.[17] This suggested a different configuration at the A/B ring junction. In order to resolve this ambiguity, the 2D NMR data of **13** were

Table 5. NMR Data of Compound **13** (CD_3OD)

position	δ_C mult.[a]	δ_H (J in Hz)[b]	HMBC
1	19.6, CH_2	1.73, m, 1.61, m	1, 2, 3, 9, 10, 19
2	28.3, CH_2	2.1, m, 1.70, m	1, 3, 4, 10, 18
3	80.0, CH	4.01, brs	1, 1′, 2, 3, 4, 5, 18
4	81.6, qC		
5	47.3, qC		
6	76.6, CH	5.0, dd (11.8, 4.4)	3, 5, 6, 7, 8, 19
7	26.3, CH_2	2.31, m, 2.22 m	5, 6, 7, 8, 9, 17, 20
8	48.1, CH[c]	2.74, dd (12.4, 5.6)	6, 7, 9, 10, 11, 12, 17, 20
9	36.1, qC		
10	48.3, CH[c]	1.87, m	1, 2, 5, 9, 10, 11, 19
11	45.1, CH_2	2.37, m, 1.98, m	9, 10, 11, 12, 13, 17, 20
12	72.3, CH	5.87, dd (11.8, 4.4)	9, 11, 12, 13, 14, 16, 17
13	126.1, qC		
14	109.5, CH	6.52, d (1.0)	12, 13, 14, 15, 16
15	145.1, CH	7.50, dd (1.6, 1.6)	13, 14, 15, 16
16	141.6, CH	7.62, brs	13, 14, 15, 16
17	175.9, qC		
18	180.0, qC		
19	18.1, CH_3	1.21, s	4, 5, 6, 10, 18, 19
20	33.3, CH_3	1.10, s	1, 8, 9, 10, 11
1′	105.0, CH	4.30, d (7.7)	1′, 3, 5′
2′	75.1, CH	3.14, m	
3′	77.7, CH[c]	3.32, m	
4′	71.4, CH	3.35, m	
5′	78.0, CH[c]	3.23, m	
6′	62.0, CH_2	3.81, dd (11.9, 2.0) 3.67, dd (11.9, 5.1)	

[a] Measured at 100 MHz. [b] Measured at 300 MHz. [c] Signals are interchangeable in each column.

studied carefully. The HMQC and HMBC spectra supported the structure reported previously,[17] but the assignments of Me-19 and Me-20 need to be revised. The proton signal at δ_H 1.21 (δ_C 18.2) was assigned to Me-20, and δ_H 1.10 (δ_C 33.3) was due to Me-20 on the basis of HMQC and HMBC correlations (Table 5).

Compounds **10**−**13** were assayed for cytotoxicity against cancer PC-3 (human prostrate) and normal 3T3 (mouse fibroblast) cell lines. The 50% inhibitory concentrations (IC_{50}) in the case of both cell lines were >10 μM. These results are in accordance with the reported cytotoxic activity of crude dichloromethane extract of certain *Tinospora* species, for which no cytotoxic effects have been observed.[1−4]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter in methanol, unless otherwise stated. UV spectra were recorded on a Shimadzu UV240 spectrophotometer in MeOH solution, and IR spectra were recorded as KBr disks on a JASCO A-302 spectrophotometer. 1D NMR spectra were recorded on a Bruker Avance spectrometer, operating at either 400 (^{1}H NMR) or 100 (^{13}C NMR) MHz, unless otherwise stated. The chemical shifts values are given in δ (ppm), referenced to the residual solvent signal ($CD_3OD/CDCl_3$), while coupling constants (J) were measured in Hz. 2D NMR spectra were taken on a Bruker AMX 500 NMR spectrometer. Electron-impact mass spectra (EIMS) were taken at 70 eV on a Finnigan MAT-112 or MAT-312 instrument. Fast-atom bombardment mass spectra (FABMS) were measured as a glycerol matrix on a JEOL HX-110 mass spectrometer. TLC was performed on precoated silica gel plates (DC-Alugram 60 UV254 of E. Merck), and the spots were observed first under UV light (254 nm) and then stained with ceric(IV) sulfate spray reagent and heated until the appearance of a color. Diaion HP-20 (Mitsubishi Chemical Industries, Tokyo, Japan), ODS C_{18} (63-212 μm, Wako Pure Chemical Industries, Ltd., Japan), polyamide-6 DF (Riedel-De Haen AG), and silica gel (E. Merck, 230−400 μm mesh) were used as adsorbents. Recycling preparative HPLC separation was performed on a JAI LC-908W instrument (Japan Analytical Industry) with YMC ODS H-80, L-80, and GS-320 columns (YMC, Ltd., Kyoto, Japan).

Plant Material. *Tinospora crispa* was collected from the herbal garden of the Laboratory of Natural Products (LHS), University of Putra Malaysia (UPM), in May 2003. The plant was identified by Mr. Shamsul Khamis, a Resident Botanist at LHS, and a specimen (SK 1537/08) was deposited at the Herbarium of the Institute of Bioscience, UPM.

Extraction and Isolation. The dried plant material (16 kg) was extracted three times with distilled methanol, and the methanol extracts were combined and evaporated on a rotary evaporator. The crude methanolic extract (ca. 284 g) was then partitioned successively by solvent−solvent fractionation into four major fractions, *n*-hexane (60.31 g), ethyl acetate (69.43 g), *n*-butanol (72.61 g), and water (81.65 g). The butanolic extract (72.61 g) was loaded onto a Diaion HP-20 resin column and eluted with 100% water, water−methanol (3:1), water−methanol (2:1), water−methanol (1:1), water−methanol (1:2), and then 100% methanol, to yield six fractions altogether. The second and fourth fractions from the HP-20 column were combined and further fractionated into six subfractions using a ODS column and eluted with 100% water, water−methanol (3:1, 2:1, 1:1, 1:2), and 100% methanol. The subfractions were passed through a Sephadex LH-20 column again to obtain five subfractions with 100% water, water−methanol (2:1, 1:1, and 1:2), and 100% methanol sequentially. All of the subfractions thus obtained were purified by recycling HPLC (LC-908 on L-80 and GS-320 columns), with water−methanol (1:1) and methanol, respectively, as eluent, to obtain compounds **1** (2.7 mg, L-80, 5 mL/min, t_R = 34 min), **2** (3.8 mg, GS-320, 5 mL/min, t_R = 35 min), **3** (3.2 mg, L-80, 4.5 mL/min, t_R = 44 min), **5** (4.1 mg, GS-320, 3 mL/min, t_R = 54 min), **6** (3.7 mg, GS-320, 4 mL/min, t_R = 32 min), **7** (1.1 mg, L-80, 4 mL/min, t_R = 30 min), **8** (2.9 mg, L-80, 4 mL/min, t_R = 37 min), **9** (4.3 mg, L-80, 4 mL/min, t_R = 26 min), **10** (883 mg, L-80, 3 mL/min, t_R = 38 min), **11** (1.15 g, L-80, 4 mL/min, t_R = 72 min), **12** (125 mg, GS-320, t_R = 44 min), **13** (862 mg, GS-320, 5 mL/min, t_R = 38 min), borapetoside F (29 mg, L-80, 4 mL/min, t_R = 70 min), and borapetoside G (3.1 mg, L-80, 4 mL/min, t_R = 36 min), respectively.

The ethyl acetate fraction (69.43 g) was subjected to column chromatography (silica gel) and eluted by using gradients of *n*-hexane/acetone and *n*-hexane/ethyl acetate. Altogether, 15 fractions were collected. On further column chromatography of fraction 4 (26 mg), using 10% acetone−ethyl acetate as the mobile phase on normal-phase silica gel, compound **7** (2.0 mg) was obtained as a white solid.

Hydrolysis of Compounds 1−9. Compounds **1**−**9** (1 mg) were separately dissolved in 10% aqueous HCl (1 mL) at 90 °C for 4−5 h and cooled to room temperature. The reaction mixture was concentrated in vacuo, and each residue was subjected to TLC and GLC analysis in order to confirm the nature of the sugar moiety in the hydrolysate. TLC on silica gel developed with *n*-BuOH−Me_2CO−H_2O (4:5:1) indicated glucose at R_f 0.33. The residue was trimethylsilylated with 0.1 mL of silylating agent (pyridine−trimethylchlorosilane, 5:1, by vol.) for 30 min at room temperature and subjected to GLC (1.5% silicon SE-30, 3 mm ×2 m, column temp 150 °C, N_2 1.0 kg/cm^2), which showed the presence of D-glucose with t_R 10.4 min, $[\alpha]_D^{22}$ +51.4 (*c* 0.02, H_2O). Compound **1** showed the presence of D-xylose with t_R 4.3 min, $[\alpha]_D^{22}$ +19.1 (*c* 0.02, H_2O), in addition to D-glucose.

(2*R*,5*R*,6*R*,8*S*,9*S*,10*S*,12*S*)-15,16-Epoxy-2-hydroxy-6-*O*-{β-D-glucopyranosyl-(1→6)-α-D-xylopyranosyl}-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (1): colorless, gummy solid; $[\alpha]_D^{27}$ −35.0 (*c* 0.056, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.68), 214 (4.03), 209 (4.27) nm; IR (KBr) ν_{max} 3420, 1717, 1637 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 3; EIMS *m*/*z* 373 (3), 133 (64), 119 (100), 95 (19), 94 (24), 91 (58), 81 (29), 73 (61), 60 (35), 55 (30); HRESIMS *m*/*z* 685.2701 $[M + H]^+$ (calcd for $C_{32}H_{45}O_{16}$, 685.2708).

(2*R*,5*R*,6*R*,8*R*,9*S*,10*S*,12*S*)-15,16-Epoxy-2-hydroxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (2): amorphous, colorless, gummy solid; $[\alpha]_D^{27}$ −22.7 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 223 (3.85), 217 (4.21), 201 (4.37) nm; IR (KBr) ν_{max} 3409, 1709, 1670 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 3; EIMS *m*/*z* 389 (13), 373 (37), 357 (20), 341 (27), 205 (31), 119 (70), 97 (29), 95 (66), 94 (64), 93 (23), 91 (68), 81 (100), 61 (53), 60 (60), 55 (62); HRESIMS *m*/*z* 553.2245 $[M + H]^+$ (calcd for $C_{27}H_{37}O_{12}$, 553.2280).

(5*R*,6*R*,8*S*,9*R*,10*R*,12*S*)-15,16-Epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (3): amorphous, colorless, gummy solid; $[\alpha]_D^{27}$ −33.3 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.55), 211 (4.55), 203 (4.55) nm; IR (KBr) ν_{max} 3426, 1725, 1673 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD), see Tables 1 and 3; EIMS *m*/*z* 550 (2), 388 (9), 371 (21), 356 (12), 203 (16), 167 (21), 133 (39), 119 (27), 95 (60), 94 (100), 93 (23), 91 (55), 81 (83),

73 (58), 61 (36), 60 (58), 55 (56); HRESIMS *m/z* 551.2133 $[M + H]^+$ (calcd for $C_{27}H_{35}O_{12}$, 551.2123).

Methyl (2*R*,7*S*,8*S*)-8-[(2*S*)-2-(3,4-dihydroxy-2,5-dimethoxytetrahydro-3-furanyl)-2-hydroxyethyl]-2,8-dimethyl-10-oxo-11-oxatricyclo[7.2.1.0^{2,7}]dodec-3-ene-3-carboxylate (rumphiol E) (4): amorphous, colorless solid; $[\alpha]_D^{22}$ +27.5 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 242 (2.32) nm; IR (KBr) ν_{max} 3551, 3505, 3394, 1745, 1701 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$), see Table 4; EIMS *m/z* 307 (94), 275 (47), 203 (14), 185 (21), 119 (16), 85 (100), 95 (8), 94 (95), 93 (29), 81 (12); HRESIMS *m/z* 471.2312 $[M + H]^+$ (calcd for $C_{23}H_{35}O_{10}$, 471.2225).

(5*R*,6*R*,8*S*,9*R*,10*S*,12*S*)-15,16-Epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (5): amorphous, colorless, gummy solid; $[\alpha]_D^{27}$ +260.8 (*c* 0.046, MeOH); UV (MeOH) λ_{max} (log ε) 225 (3.72), 216 (4.35), 205 (4.42) nm; IR (KBr) ν_{max} 3421, 1718, 1674 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Tables 2 and 3; HRESIMS *m/z* 549.2171 $[M + H]^+$ (calcd for $C_{27}H_{35}O_{12}$, 549.2123).

(2*R*,5*R*,6*S*,9*S*,10*S*,12*S*)-15,16-Epoxy-2-hydroxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester (6): amorphous, colorless solid; $[\alpha]_D^{22}$ +10.9 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 208 (3.82) nm; IR (KBr) ν_{max} 3430, 1725, 1664 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 4; EIMS *m/z* 388 (9), 356 (6), 218 (38), 147 (45), 119 (43), 95 (64), 94 (60), 81 (71), 55 (100); FABMS *m/z* 551 $[M + H]^+$, 573 $[M + Na]^+$, 389 $[M - 162]^+$, 371 $[M - 180]^+$; HRESIMS *m/z* 573.1980 $[M + Na]^+$ (calcd for $C_{27}H_{34}O_{12}Na$, 573.1948).

(5*R*,6*S*,9*S*,10*S*,12*S*)-15,16-Epoxy-2-oxo-6-*O*-(β-D-glucopyranosyl)-cleroda-3,7,13(16),14-tetraen-17,12-olid-18-oic acid methyl ester (7): amorphous, colorless solid; $[\alpha]_D^{22}$ −14.2 (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.70), 201 (3.81) nm; IR (KBr) ν_{max} 3425, 1723, 1667 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 4; EIMS *m/z* 386 (19), 354 (9), 309 (6), 216 (19), 202 (30), 145 (46), 135 (37), 95 (50), 94 (70), 81 (69), 73 (100); HRESIMS *m/z* 549.1976 $[M + H]^+$ (calcd for $C_{27}H_{33}O_{12}$, 547.1967).

(3*R*,4*R*,5*R*,6*S*,8*R*,9*S*,10*S*,12*S*)-15,16-Epoxy-3,4-epoxy-6-*O*-(β-D-glucopyranosyl)-cleroda-3,13(16),14-trien-17,12-olid-18-oic acid methyl ester (8): amorphous, gummy solid; $[\alpha]_D^{27}$ +135.1 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 222 (4.46), 203 (4.65) nm; IR (KBr) ν_{max} 3447, 1714, 1635 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Tables 2 and 3; EIMS (70 eV) *m/z* (%) 373 (7), 341 (2), 323 (2), 205 (6), 194 (100), 119 (16), 95 (17), 94 (19), 93 (7), 91 (28), 81 (19), 73 (24), 61 (10), 60 (18), 59 (9), 55 (33); HRESIMS *m/z* 553.2255 $[M + H]^+$ (calcd for $C_{27}H_{37}O_{12}$, 553.2280).

(1*R*,4*S*,5*R*,8*S*,9*R*,10*S*,12*S*)-15,16-Epoxy-4-*O*-(β-D-glucopyranosyl)-cleroda-2,13(16),14-triene-17(12),18(1)-diolide (9): amorphous, colorless, gummy solid; $[\alpha]_D^{27}$ −87.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 224 (3.46), 213 (4.82), 207 (4.82) nm; IR (KBr) ν_{max} 3420, 1751, 1716 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Tables 2 and 3; EIMS *m/z* 314 (34), 281 (3), 252 (3), 220 (13), 206 (39), 134 (41), 121 (88), 119 (16), 108 (98), 107 (39), 97 (23), 95 (50), 94 (56), 93 (30), 91 (66), 81 (100), 73 (86), 60 (32), 55 (46); HRESIMS *m/z* 521.2043 $[M + H]^+$ (calcd for $C_{26}H_{34}O_{11}$, 521.2017).

Cytotoxicity Assays. Cytotoxicity of the compounds was evaluated in a 96-well flat-bottomed microplate by using a standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay.[18] For this purpose, PC-3 cells (human prostate cancer) and 3T3 cells (mouse fibroblasts) were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal bovine serum (FBS), 100 IU/mL pencillin, and 100 μg/mL streptomycin in a 25 cm^3 flask, and kept in a 5% CO_2 incubator at 37 °C. Exponentially growing cells were harvested, counted with hemocytometer, and diluted with DMEM. Cell cultures at the concentration of 3×10^4 cells/mL were prepared and introduced (100 μL/well) into 96-well plates. After overnight incubation, the medium was removed and 200 μL of fresh medium was added with different concentrations of compounds (1−100 μM). After 72 h, 50 μL of MTT (2 mg/mL) was added to each well and incubated further for 4 h. Subsequently, 100 μL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 540 nm, using a microplate ELISA reader (Spectra Max Plus, Molecular Devices, CA). The cytotoxicity was recorded as the concentration causing 50% growth inhibition for both PC-3 and 3T3 cells. Doxorubicin (IC_{50} 0.912 ± 0.12 μM) and cycloheximide (IC_{50} 0.26 ± 0.04 μM) were used as positive control for the PC-3 and 3T3 cell lines, respectively.

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Supporting Information Available: The ^1H, ^{13}C NMR, COSY, HMBC, HMQC, and NOESY/ROESY spectra of new compounds **1**−**9**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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