New Cembranes from *Cleome spinosa*[⊥]

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Examination of the aerial portions of *Cleome spinosa* yielded five new cembranes, named cleospinols A (**1**), B (**3**), C (**4**), and D (**5**), and the 3′-hydroxy-*iso*-pentan-10-oate ester of cleospinol A (**2**). The cleospinols were determined to be derivatives of 10,13-dihydroxy-4,12-dimethyl-1-(1-methylethenyl)-11(*E*)-cyclotetradecene on the basis of spectroscopic data interpretation.

Cleome spinosa Jacq. (Capparaceae) is a tough-stemmed herb that is widely distributed throughout tropical climates and especially within the West Indies.[1] The plant, called "the spider flower", is widely used as a garden ornamental[2] and has no reported therapeutic uses. Previous investigations of a methanol extract of the plant resulted in the isolation of three long-chain unsaturated polyprenols: nonaprenol, decaprenol, and undecaprenol.[3−6] No work has been reported on the less polar extracts of the green or dried plant.

Our present investigation of *C. spinosa* led to the isolation of the rare flavone flindulatin (**6**), in addition to four new cembranes, designated as cleospinols A (**1**), B (**3**), C (**4**), and D (**5**). A fifth cembrane was also isolated, and while also new, it was found to be but an esterified derivative of cleospinol A (**2**). The cleospinols are all derivatives of 10*R*,13*S*-dihydroxy-4,12-dimethyl-1-(1-methylethenyl)-11(*E*)-cyclotetradecene. The stereochemistry of the hydroxyl groups accommodated by C-10 and C-13 were deduced from T-ROESY NMR experiments and were verified by Mosher's method.[7]

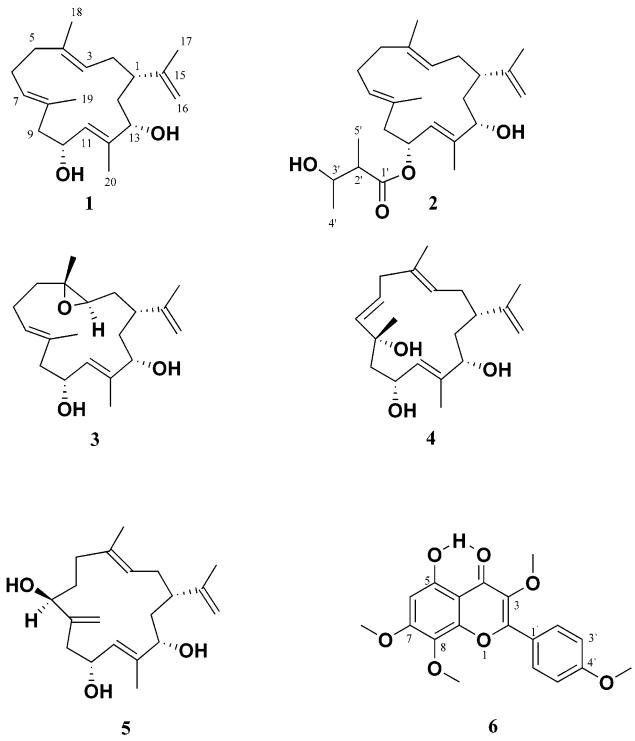
The isolation of cembranoid diterpenes from *Cleome* species is not new, as cleomolide and three cembranoic acids were isolated previously from *C. viscosa*.[8−10] In earlier work cleomolide was found in an extract of *C. icosandra*.[11] Diterpenoids with the cembrane skeleton are known to display diverse biological functions[12−16] including anti-HIV activity,[17] neuroprotection,[18] and inhibition of brain Na^+, K^+, and ATPase.[19] Some show potent cytotoxicity against a number of human cancer cell lines, including leukemia, melanoma, breast, and colon carcinomas.[20−22]

Results and Discussion

The petroleum ether extract of *C. spinosa* was chromatographed on silica gel with increasing concentrations of EtOAc in petroleum ether to give **6** (5-hydroxy-3,7,8,4′-tetramethoxyflavone), which was identified by comparison of its physical and spectral properties with those reported in the literature.[23] Compounds **1** and **2** were also isolated.

Data from the HREIMS of **1** suggested a molecular formula of $C_{20}H_{32}O_2$ (*m*/*z* 304.2402) that coincided with five degrees of unsaturation within the molecule. With four carbon−carbon double bonds present, as inferred from the ^{13}C NMR spectrum (δ_C 110.7−148.5), compound **1** was clearly monocyclic. HSQC, HMBC, and $^1H-^1H$ COSY data (Table 1) further indicated that the ring was comprised of 14 carbon atoms and incorporated three trisubstituted double bonds, each of which had a methyl substituent (C-18, -19, and -20). The configurations of these double bonds were determined to be *E* on the basis of the lack of a T-ROE between each of the relevant methyl groups and the corresponding olefinic proton of the double bond to which that methyl was attached (Table 1). Also useful for these particular assignments were the resonance values for C-18, -19, and -20 (16.32, 16.35, and 18.3 ppm, respectively) that were fairly typical of *E*-trisubstituted double bonds of polyisoprenoids.[24] The fourth double bond present was terminal (δ_C 110.7 and 148.5) and formed part of an isopropenyl substituent that HMBC data firmly placed at C-1. Two methine hydroxy functions were also noted within the structure of **1** (65.4 and 73.3 ppm; ν_{max} 3353 cm^{-1}) and were found to occupy the allylic positions of C-10 and C-13, respectively.

The stereochemical assignments of the isopropenyl group as well as the C-10 and C-13 hydroxyls posed a significant challenge in light of the flexible nature of such a large ring.



[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of the Research Triangle Institute for their pioneering work on bioactive natural products.

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Table 1. ^{1}H, ^{13}C, and 2D NMR Data for Compound **1** ($CDCl_3$, 500/125 MHz, 298 K)

carbon	δ_C	δ_H (multiplicity) *J*	HMBC	^{1}H−^{1}H COSY	T-ROE
1	45.0 (CH)	1.79 (m) 5.0		2*S*, 2*R*	
2	33.6 (CH_2)	1.98 *pro R* (m) 6.5	1, 3		
		1.90 *pro S* (ddd) 1.8, 2.7, 5.0	3, 5, 14		
3	122.5 (CH)	5.06 (t) 7.0	2, 5, 18	2*R*, 5*R*, 18	19
4	135.5 (C)		2, 18		
5	38.2 (CH_2)	2.19 *pro R* (d) 7.5	3, 6, 7, 18	6*R*	6, 7, 18
		2.03 *pro S* (d) 2.0			
6	24.8 (CH_2)	2.35 *pro R* (m) 12.5	5, 7, 9		3
		2.06 *pro S* (d) 1.5			7
7	128.2 (CH)	5.00 (dt) 1.6, 9.5			
8	130.4 (C)		6, 9, 10, 18		
9	48.6 (CH_2)	2.46 *pro S* (d) 7.0	7, 10, 19	10	10
		2.22 *pro R* (d) 7.5	7, 11, 19	6	7, 11
10	65.4 (CH)	4.55 (ddd) 3.7, 8.2, 10.4	9, 11	9, 20	9, 19, 20
11	127.2 (CH)	5.59 (dd) 1.4, 8.3	9, 13, 10, 20	10, 13, 20	14
12	144.3 (C)		14, 10, 20		
13	73.3 (CH)	3.84 (dd) 4.0, 10.1	1, 11, 14, 20	14, 20	17, 20
14	39.3 (CH_2)	1.76 *pro R* (m) 6.0	1, 2, 13		3, 16, 17
		1.74 *pro S* (m) 6.0	13		3, 11
15	148.5 (C)		2, 14, 16, 17		
16	110.7 (CH_2)	4.76 (sextet) 1.5	1, 17	17	17
		4.72 (q) 0.6	1, 17	17	13, 20
17	19.1 (CH_3)	1.71 (dd) 0.8, 1.5	1, 16		2, 18
18	16.32 (CH_3)	1.56 (bs)	3, 5	2, 5	1, 2, 5
19	16.35 (CH_3)	1.62 (bs)	7, 9	5, 9	6, 9, 20
20	18.3 (CH_3)	1.82 (d) 1.4	11, 13		

The distinct vicinal ^{1}H−^{1}H couplings observed in the ^{1}H NMR spectrum, however, strongly indicated that the molecule preferred a single conformation. The T-ROESY correlations of the olefinic protons of the three endocyclic double bonds as well as those of the methyl groups were especially useful in determining the conformation of the molecule. The *pro-S* H-14 proton (δ 1.74) had observed couplings with the protons resonating at δ 5.06 (H-3) and 5.59 (H-11), which suggested that these protons were all oriented toward the center of the molecule. H_R-14 (δ 1.76), however, was coupled to the olefinic proton of the isopropenyl group resonating at δ 4.72, which, in turn, experienced enhancements when H-13 (δ 3.84) and H-20 (δ1.82) were irradiated. These observations, along with the observed T-ROE between H-13 and H-20, were consistent with an *S*-configuration of the C-13 hydroxyl (Table 1).

The H-19 methyl (δ 1.62) protons were coupled to H-3 (but not to H-11), and this suggested that while the C-19 methyl was also oriented toward the center of the molecule, it was resident on the face opposite that to which the H-11 proton was oriented. Further T-ROE interactions between the H-19 protons and H_R-6 (δ 2.35), H-10 (δ 4.55), and H-20 supported the proposed conformation of the ring in addition to highlighting the *R*-configuration of the C-10 hydroxyl. The enhancement observed for H-10 upon irradiation of H_R-9 (δ 2.46) and the correlations of H-7 (δ 5.00) and H-11 with H_S-9 (δ 2.22) was consistent with this assignment. Finally, the $\Delta\delta$ [δ of the protons in the (*S*)-MTPA ester − δ of the protons in the (*R*)-MTPA ester] values of +0.15, +0.32, −0.15, and −0.15 obtained for H_R-9, H_S-9, H_R-14, and H_S-14, respectively, from the analysis of the *R* and *S* Mosher's derivatives of **1** agreed[7] with the identification of cleospinol A (**1**) as 10*R*,13*S*-dihydroxy-4,8,12-trimethyl-1-(1-methylethenyl)-3(*E*),7(*E*),11(*E*)-cyclotetradecatriene.

The ^{13}C NMR spectrum of **2** was markedly similar to that of **1** except for five additional resonances: two methyls (δ 14.2 and 20.8), two methines (δ 47.0 and 69.6), and a nonprotonated carbon (175.1 ppm) (Table 2). The 2D NMR experiments on **2** also produced very similar results, indicating that both **1** and **2** were very closely related. In addition, further T-ROESY data of **2** indicated that the conformation of the 14-membered ring was the same as that of **1**. The presence of an ester group in the structure of **2** was evident from the IR spectrum (ν_{max} 1718, 1180 cm^{-1}), and the placement of this functionality on C-10 was inferred from the long-range couplings observed for the H-10 (δ 5.70) and H_R-9 (δ 2.31) protons with the carbonyl resonating at δ_C 175.1. The protons resonating at δ 1.17 were assigned to H-5′ on the basis of the two-bond couplings they experienced with the carbonyl. The T-ROE observed between H_R-9 (δ 2.31) and H-2′ (δ 2.40) suggested that the carbon chain of the parent acid was partially suspended above the molecule. Compound **2** was identified as the 3-hydroxy-2-methylbutanoate ester of **1**.

The acetone extract of *C. spinosa* yielded the additional compounds **3**−**5**. HREIMS data of **3** suggested a molecular formula of $C_{20}H_{32}O_3$ (*m*/*z* 320.2344), and consequently the molecule possessed five orders of unsaturation. Only three of these were accounted for by carbon−carbon double bonds (111.0−147.7 ppm), which meant that compound **3** was bicyclic. Standard 2D NMR techniques were again used to establish connectivities, and these indicated that the three double bonds were located at C-7 (δ 127.5 and 131.5), C-11 (δ 127.2 and 142.6), and C-15 (δ 111.0 and 147.7). The appearance of a sharp band at ν_{max} 1037 cm^{-1} in the IR spectrum revealed the other ring to be from an epoxide function. HMBC data indicated that the epoxide was accommodated by carbons 3 and 4 through correlations of H-1 (δ 1.96) and H_R-2 (δ 1.81) with the methine signal at 61.9 (C-3), and of H-5 (δ 1.30) and H-18 (δ 1.23) with the nonprotonated carbon resonating at δ 60.9 (C-4). The ^{1}H−^{1}H COSY correlation between H_R-2 (δ 1.81) and H-3 (δ 2.89) also justified this placement. T-ROESY correlations around the molecule indicated that the conformation of the large ring of **3** was similar to that of **1** as exemplified by parallel enhancements of H-3 (δ 2.89) and H-11 (δ 5.61) upon irradiation of H_S-14 (δ 1.91). This suggested that **3** was simply derived from **1** by the epoxidation of the C-3 double bond. As such, the stereochemistries of the isopropenyl group and the C-10 and -13 hydroxyls were as they appeared in the structure of **1**. The *R*-configuration of oxygen-bearing C-3 was supported by observed T-ROEs between H-3 and H_R-2 (δ 1.81), H_S-5 (δ 1.30), and H-11. Further correlations between the methyl protons at δ 1.23

Table 2. ^{1}H and ^{13}C Data for Compounds **2**, **3**, and **5** ($CDCl_3$, 500/125 MHz, 298 K)

	2		**3**		**5**	
carbon	δ_C	δ_H (multiplicity) *J*	δ_C	δ_H (multiplicity) *J*	δ_C	δ_H (multiplicity) *J*
1	45.0 (CH)	1.81 (m) 15.0	41.1 (CH)	1.96 (m) 13.0	46.1 (CH)	1.88 (m) 20.0
2	33.6 (CH_2)	1.99 (m) 15	35.2 (CH_2)	1.81 (m) 20.0	33.7 (CH_2)	2.10 (m) 10.0
		1.91 (m) 20		1.28 (m) 5.0		1.91 (m) 25.0
3	122.7 (CH)	5.07 (t) 7.1	61.9 (CH)	2.89 (dd) 3.7, 9.7	125.8 (CH)	5.25 (m) 8.5
4	135.4 (C)		60.9 (C)		134.6 (C)	
5	38.2 (CH_2)	2.18 (m) 5.0	38.3 (CH_2)	2.10 (m) 20.1	34.9 (CH_2)	2.39 (dd) 2.3, 9.7
		2.05 (m) 15.0		1.30 (m) 10		2.09 (m) 12.0
6	24.8 (CH_2)	2.34 (d) 12.5	23.7 (CH_2)	2.13 (m) 21.3	31.9 (CH_2)	1.87 (m) 12.0
		2.03 (m) 5.0		1.33 (d) 5.0		1.84 (m) 12.0
7	129.1 (CH)	5.03 (d) 9.8	127.5 (CH)	5.09 (t) 5.8	70.0 (CH)	3.91 (d) 8.6
8	129.5 (C)		131.5 (C)		148.3 (C)	
9	45.1 (CH_2)	2.38 (m) 5	48.0 (CH_2)	2.46 (d) 11.9	41.6 (CH_2)	2.66 (dd) 2.3, 10.0
		2.31 (m) 10.0		2.27 (dd) 10.1, 13.5		2.36 (dd) 5.5, 10.0
10	68.8 (CH)	5.70 (ddd) 3.8, 10.0, 12.7	65.7 (CH)	4.622 (m) 12.1	68.4 (CH)	4.55 (td) 2.5, 5.9
11	122.8 (CH)	5.54 (d) 8.6	127.2 (CH)	5.61 (dd) 1.0, 8.4	127.0 (CH)	5.50 (d) 5.9
12	146.5 (C)		142.6 (C)		142.6 (C)	
13	73.3 (CH)	3.847 (dd) 3.6, 9.5	73.1 (CH)	3.86 (dd) 4.0, 10.1	74.4 (CH)	3.88 (dd) 3.5, 5.6
14	39.6 (CH_2)	1.74 (m) 8.5	39.8 (CH_2)	1.91 (m) 20.0	39.0 (CH_2)	1.77 (s)
		1.73 (m) 3.3		1.85 (dt) 4.7, 15.0		1.72 (m) 12.0
15	148.3 (C)		147.7 (C)		148.7 (C)	
16	110.7 (CH_2)	4.78 (t) 1.8	111.0 (CH_2)	4.70 (t) 1.6	110.8 (CH_2)	4.83 (m) 20.0
		4.73 (bs)		4.615 (d) 1.4		4.80 (m) 20.0
17	19.1 (CH_3)	1.72 (bs)	18.2 (CH_3)	1.67 (d) 0.5	19.0 (CH_3)	1.74 (s)
18	16.29 (CH_3)	1.56 (s)	16.83 (CH_3)	1.23 (s)	16.2 (CH_3)	1.65 (s)
19	16.25 (CH_3)	1.66 (s)	16.63 (CH_3)	1.64 (s)	112.9 (CH_2)	5.22 (s)
						5.07 (s)
20	18.2 (CH_3)	1.78 (d) 1.5	17.8 (CH_3)	1.81 (d) 1.5	17.3 (CH_3)	1.78 (s)
1′	175.1 (C)					
2′	47.0 (CH)	2.40 (m) 10.0				
3′	69.6 (CH)	3.854 (dd) 2.5, 6.6				
4′	20.8 (CH_3)	1.21 (d) 6.3				
5′	14.2 (CH_3)	1.17 (d) 8.2				

Table 3. ^{1}H, ^{13}C, and 2D NMR Data for Compound **4** ($CDCl_3$, 500/125 MHz, 298 K)

carbon	δ_C	δ_H (multiplicity) *J*	HMBC	$^{1}H-^{1}H$ COSY	T-ROE
1	45.3 (CH)	1.97 (m) 18.5	2, 14, 16, 17	3, 14	3, 16
2	33.0 (CH_2)	2.08 *pro S* (m) 15.0	1, 14		
		2.03 *pro R* (m) 18.5	1, 14		
3	124.7 (CH)	5.19 (tq) 1.3, 7.8	2, 5, 18	2*S*, 2*R*	
4	135.7 (C)		5, 6, 18		
5	41.2 (CH_2)	2.75 *pro S* (ddd) 1.3, 5.8, 16.5	3, 6, 7, 18	3, 6, 7	3
		2.68 *pro R* (dd) 6.2, 15.4	3, 6, 7		
6	125.0 (CH)	5.83 (ddd) 6.0, 6.4, 15.8	5, 7		19
7	138.1 (CH)	5.66 (dt) 1.4, 16.3	5, 6, 19		6, 19
8	73.7 (C)		7, 6, 9, 10, 19		
9	47.4 (CH_2)	2.23 *pro S* (q) 7.1	7, 10, 11, 19		
		1.78 *pro R* (d) 2.1, 5.7	7, 10, 11		
10	67.8 (CH)	4.71 (ddd) 2.0, 7.0, 8.6	9, 11	9, 11	9, 19
11	127.9 (CH)	5.84 (m) 8.0	20		
12	140.8 (C)		10, 13, 14, 20		
13	74.6 (CH)	3.88 (dd) 5.2, 7.6	1, 11, 14, 20	14	11
14	37.8 (CH_2)	1.86 *pro S* (dq) 7.1, 7.5	1, 2, 13	1	3, 11
		1.65 *pro R* (dd) 1.0, 5.0	1, 2, 13		13, 16, 17
15	149.2 (C)		1, 14, 17		
16	110.4 (CH_2)	4.76 (sextet) 1.4	1, 17		13, 20
		4.74 (sextet) 0.8			
17	19.5 (CH_3)	1.73 (d) 0.5	1, 16		2
18	16.7 (CH_3)	1.64 (d) 1.0	3, 5	3	2, 6
19	30.5 (CH_3)	1.31 (s)	7, 9		9, 20
20	15.8 (CH_3)	1.75 (s)	11, 13	10, 11, 13	1, 10, 13

(H-18) and H-20 (δ 1.81) were used to expose the *E*-nature of the C-3 epoxide. This assignment was also consistent with the principle that methyl signals of *E*-trisubstituted epoxides appear at ~17 ppm, while those of *Z*-trisubstituted epoxides occur downfield at ~25 ppm.[24]

The molecular formula of **4**, $C_{20}H_{32}O_3$ (*m*/*z* 320.2355), supported the inference that the compound was a triol (ν_{max} 3363 cm^{-1}). Like **1**, four of the five inherent degrees of unsaturation were attributed to the presence of four double bonds based on the ^{13}C NMR spectrum. Data obtained from 2D NMR experiments (Table 3) revealed that **4** was also a 14-membered macrocycle and had the same basic structure as **1** with two hydroxyls at positions 10 and 13, an isopropenyl at C-1, and carbon−carbon double bonds at positions 3 and 11. The steroechemistry was identical to that of **1** on the basis of T-ROESY data (Table 3). The third endocyclic double bond was, however, found at C-6. Its position was clearly established by HMBC from the two bond couplings observed between both H-5 protons (δ 2.68 and 2.75) and the carbon resonating at δ 125.0, and this

was reinforced by the $^1H-^1H$ COSY correlation observed between H_S-5 (δ 2.75) and both H-6 (δ 5.83) and H-7 (δ 5.66). This double bond was assigned an *E*-configuration on the basis of the large coupling constant (15.8 Hz) between its constituent protons. The olefinic H-6 proton, as implied by ROEs with H-3 and H_S-5 (Table 3), was pointed toward the core of the ring. The third OH group was located at C-8 (δ 73.7), as revealed by HMBC couplings of this carbon to the H-6 (δ 5.83), H-7 (δ 5.66), H-9 (δ 1.78 and 2.23), H-10 (δ 4.71), and H-19 (δ 1.31) resonances. The conformation of the ring in **4** differed somewhat from that of **1**. The T-ROEs between H_S-14 (δ 1.86), H-3 (δ 5.19), and H-11 (δ 5.84) showed that these protons were oriented toward the center of the molecule and pointed acutely toward its upper face. The protons on the lower face, i.e., H-10 and H-20 (δ 1.75), were strongly coupled to the H-19 protons, as were H-7 and H_S-9 (δ 2.23). These results, in addition to the lack of a ROE between the latter two protons (H-7 and H_S-9), could only occur if C-8 possessed an *S*-stereochemistry, with the hydroxyl function pointing outward from the molecule. Further T-ROESY data were consistent with the structure proposed (Table 3).

The structure of **5** ($[C_{20}H_{32}O_3-H_2O]^+$; HREIMS *m/z* 302.2246: $[C_{20}H_{32}O_3]^+$; EIMS *m/z* 320; ν_{max} 3376 cm^{-1}) differed from that of **4** in that the third hydroxyl group now occupied position C-7, while the third double bond was a terminal one between C-8 and C-19. The T-ROESY couplings around the molecule revealed that the conformation of the ring of **5** was similar to that of **4**, and this proved quite useful in ascribing an *S*-stereochemistry to the third hydroxyl based on enhancements observed for H-3 (δ 5.25) and H-11 (δ 5.50) when H-7 (δ 3.91) was irradiated.

Formation of both cleospinols C (**4**) and D (**5**) is thought to initially involve the epoxidation of the 7,8 double bond of cleospinol A (**1**) to form the *R*-epoxide. Formation of **4** was then achieved by the loss of a proton from the C-6 methylene upon the opening of this epoxide, while alternatively, it is the loss of the proton from the C-19 methyl group that would give rise to **5**. The proposed intermediary 7,8-epoxide of cleospinol A (**1**) was not isolated from this plant, however.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Optical rotations were obtained using a Perkin-Elmer 241 MC polarimeter. IR data were acquired on a Perkin-Elmer FTIR Paragon 1000 instrument using KBr disks or NaCl plates. ^1H and ^{13}C NMR data were obtained on Varian Unity 500 and Bruker AC200 spectrometers using $CDCl_3$ as solvent with TMS as internal standard. EIMS were recorded at 70 eV on a VG 70-250S mass spectrometer. Column chromatography was performed with Kieselgel silica (40−63 μm). Both (*R*)- and (*S*)-MTPA-Cl were obtained from Aldrich.

Plant Material. Leaves and stems of *Cleome spinosa* were collected in April 2000 in Yallahs, St. Thomas, Jamaica. A voucher specimen (accession no. 34683) was lodged in the Botany Herbarium, UWI, Mona, Kingston, Jamaica.

Extraction and Isolation. Fresh aerial portions of *C. spinosa* (12.7 kg) were chopped and extracted with petroleum ether (2 × 15 L) over 6 days. The solvent was removed in vacuo to yield a dark green gum (82.6 g). The remaining plant material was dried at 40 °C and milled. The resulting powder was extracted twice with acetone (11 L), which was concentrated under reduced pressure (79.5 g). The petroleum ether extract was subjected to flash chromatography using petroleum ether, then increasing concentrations of EtOAc in petroleum ether to give three main fractions, A−C. Fraction A contained nothing of interest. Chromatography of fraction B (20.5 g) with 3% EtOAc in petroleum ether yielded a yellow solid, 5-hydroxy-3,7,8,4′-tetramethoxyflavone (**6**) (33 mg). Purification of fraction C (10.3 g), by chromatography using 5% EtOAc in petroleum ether, led to the isolation of significant quantities of **1** (5.3 g). Compound **2** (34.3 mg) was also obtained. The acetone extract was subjected to flash chromatography using increasing concentrations of EtOAc in petroleum ether to produce four main fractions, D−G. Fraction D (11.3 g) was largely composed of **1** and was not further analyzed. Chromatography (20% EtOAc in CH_2Cl_2) of E (10.2 g) achieved the separation of **3** (25.2 mg) and **4** (15 mg). Fraction F (7.1 g) was analyzed in 20% EtOAc in petroleum ether to give **5** (7 mg). Fraction G (20 g) was not investigated further.

Cleospinol A (1): oil; $[\alpha]^{25}_D$ +79.2° (*c* 15.0, $CHCl_3$); IR (NaCl) ν_{max} 3353, 1643, 1439, 1377 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* 304 $[M]^+$ (9), 303 (37), 286 (14), 204 (19), 135 (26), 108 (59), 99 (73), 80 (73), 70 (100), 69 (91); HREIMS *m/z* 304.2406 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1. (*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (22 mg) was added to a solution of **1** (10 mg) in dry py (5 mL), and the solution was stirred overnight at room temperature. The reaction mixture was diluted with EtOAc, adsorbed onto silica gel, and chromatographed with 2% EtOAc in petroleum ether to give the bis(*R*)-MTPA ester of **1** (1.8 mg) as an oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 5.91 (1H, ddd, J = 3.4, 9.3, 11.5 Hz, H-10), 5.30 (1H, dd, J = 1.4, 9.0 Hz, H-11), 4.91 (1H, t, J = 7.3 Hz, H-7), 4.85 (1H, m, H-16), 4.83 (1H, m, H-13), 4.78 (1H, bs, H-16), 2.39 (1H, m, H-9), 2.38 (1H, m, H-6), 2.15 (1H, m, H-5), 2.07 (1H, dd, J = 11.0, 13.0 Hz, H-9), 2.03 (1H, m, H-6), 2.02 (1H, m, H-2), 1.98 (1H, m, H-5), 1.90 (3H, d, J = 1.4 Hz, H-20), 1.87 (1H, m, H-2), 1.82 (1H, m, H-14), 1.80 (1H, m, H-14), 1.80 (1H, m, H-1), 1.72 (3H, s, H-17), 1.49 (3H, s, H-19), 1.48 (3H, s, H-18).

The bis(*S*)-MTPA ester of **1** (3.0 mg) was prepared in the same manner as above from (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (22 mg) and was also obtained as a yellow oil: ^1H NMR ($CDCl_3$, 500 MHz) δ 5.91 (1H, ddd, J = 3.2, 8.8, 11.9 Hz, H-10), 5.56 (1H, dd, J = 1.0, 8.9 Hz, H-11), 5.04 (1H, m, H-7), 5.02 (1H, m, H-13), 4.98 (1H, m, H-3), 4.85 (1H, t, J = 1.5 Hz, H-16), 4.76 (1H, bs, H-16), 2.54 (1H, d, J = 13.0 Hz, H-9), 2.39 (1H, m, H-9), 2.37 (1H, m, H-6), 2.20 (1H, m, H-5), 2.06 (1H, m, H-6), 2.03 (1H, m, H-2), 2.00 (1H, m, H-5), 1.86 (1H, m, H-2), 1.84 (3H, d, J = 1.4 Hz, H-20), 1.80 (1H, m, H-1), 1.71 (3H, s, H-17), 1.68 (3H, s, H-19), 1.67 (1H, m, H-14), 1.63 (1H, m, H-14), 1.53 (3H, s, H-18).

Cleospinol A, 3′-hydroxy-2′-methylbutan-10-oate (2): oil; $[\alpha]^{25}_D$ +15.0° (*c* 4.8, $CHCl_3$); IR (NaCl) ν_{max} 3417, 1718, 1645, 1447, 1377, 1263, 1180 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 287 $[M - C_5H_9O_2]^+$ (10), 286 (38), 285 (30), 284 (18), 268 (25), 202 (20), 160 (35), 148 (62), 146 (43), 134 (58), 120 (69), 108 (100), 101 (35), 92 (68), 80 (74), 68 (73), 57 (15); HREIMS *m/z* 287.2375 $[M - C_5H_9O_2]^+$ (calcd for $C_{25}H_{40}O_4$, 404.2927).

Cleospinol B (3): oil; $[\alpha]^{25}_D$ +51.3° (*c* 18.0, $CHCl_3$); IR (NaCl) ν_{max} 3382, 1645, 1445, 1037 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 320 $[M]^+$ (9), 319 (23), 302 (58), 284 (25), 204 (19), 162 (31), 148 (91), 134 (51), 106 (56), 96 (57), 94 (100), 70 (94); HREIMS *m/z* 320.2344 (calcd for $C_{20}H_{32}O_3$, 320.2351).

Cleospinol C (4): needles (acetone); mp 96−98 °C; $[\alpha]^{25}_D$ +15.6° (*c* 12.0, $CHCl_3$); IR (NaCl) ν_{max} 3363, 1643, 1438, 1372 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* 320 $[M]^+$ (2), 302 (77), 301 (64), 284 (93), 266 (37), 200 (45), 174 (40), 160 (53), 146 (57), 122 (62), 120 (80), 108 (91), 106 (93), 94 (100); HREIMS *m/z* 320.2355 (calcd for $C_{20}H_{32}O_3$, 320.2351).

Cleospinol D (5): oil; $[\alpha]^{25}_D$ −9.8° (*c* 6.4, $CHCl_3$); IR (NaCl) ν_{max} 3376, 1708, 1645, 1447, 1377 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* 320 $[M]^+$ (5), 316 (29), 302 (32), 300 (36), 284 (29), 206 (22), 188 (24), 162 (34), 148 (51), 134 (52), 118 (46), 106 (67), 94 (100), 68 (69); HREIMS *m/z* 302.2246 $[M - H_2O]^+$ (calcd for $C_{20}H_{32}O_3$, 320.2351).

Flindulatin (6): yellow needles ($CHCl_3$); mp 170−172 °C [lit.[23] mp 168−169 °C]; UV (EtOH) λ_{max} (log ϵ) 268 (4.31), 348

(4.22); IR (KBr) ν_{max} 3552, 1742, 1652, 1593, 1455, 1376, 1262, 1188, 1160 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 12.50 (1H, s, OH-5), 8.17 (2H, t, J = 2.5 Hz, H-2′,6′), 7.05 (2H, t, J = 2.5 Hz, H-3′,5′), 6.42 (1H, s, H-6), 3.95 (3H, s, OCH_3-7), 3.914 (3H, s, OCH_3-8), 3.905 (3H, s, OCH_3-4′), 3.87 (3H, s, OCH_3-3); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 179.0 (C, C-4), 161.8 (C, C-4′), 158.3 (C, C-7), 157.3 (C, C-5), 155.8 (C, C-2), 150.2 (C, C-9), 138.6 (C, C-3), 130.2 (CH, C-2′,6′), 128.8 (C, C-8), 123.0 (C, C-1′), 114.2 (CH, C-3′,5′), 105.3 (C, C-10), 95.4 (CH, C-6), 61.6 (CH_3, OCH_3-8), 60.11 (CH_3, OCH_3-3), 56.3 (CH_3, OCH_3-7), 55.4 (CH_3, OCH_3-4′).

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