New Antiviral Cassane Furanoditerpenes from *Caesalpinia minax*†

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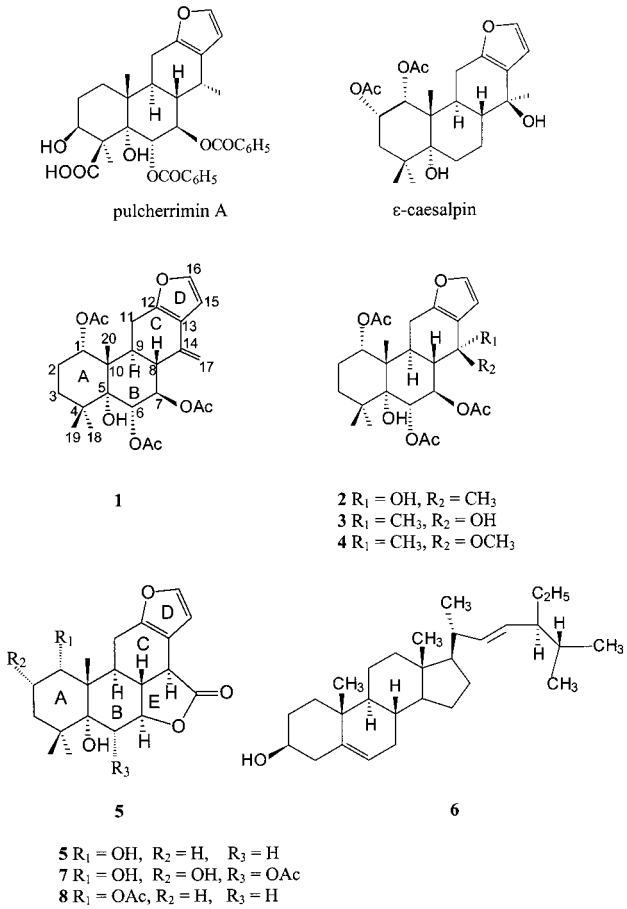
A bioassay-guided study led to the isolation of five new cassane furanoditerpenes, designated as caesalmin C (**1**), D (**2**), E (**3**), F (**4**), and G (**5**), along with stigmasterol (**6**) from the seeds of *Caesalpinia minax*. The ^{1}H and ^{13}C NMR spectra were completely assigned by using a combination of 2D NMR analyses. The structures of all five furanoditerpenes were confirmed by X-ray analyses. The structure of **6** was verified by X-ray analysis for the first time. The bioassay results showed that the anti-Para3 virus activity of tetracyclic furanoditerpenoids **1**−**4** is more potent than that of the furanoditerpenoid lactone **5**, which is in turn better than **6**. As the major components of the plant possess significant potent activity, it may be feasible to develop new antiviral agents from this source.

Cassane furanoditerpenes are characterized by a molecular skeleton constructed from the fusion of three cyclohexane rings and a furan ring. Diterpenes of this kind, e.g., pulcherrimin A, are mostly distributed in the genera *Caesalpinia*[1−12] and *Pterodon*.[13,14] In most cases, furanoditerpenoids from the former genus possess a C-5 hydroxyl group, and this is the major difference between the furanoditerpenoids from these two genera. The structural determinations of previously reported furanoditerpenes were based mostly on spectroscopic methods. However, the absolute configurations of pulcherrimin A,[15] ϵ-caesalpin,[16] and caesaldekarin A[17] were established by CD spectral correlation, X-ray analyses using the anomalous dispersion method, and comparison of the NMR data of the MTPA derivatives, respectively (Scheme 1).

Caesalpinia minax Hance is a plant belonging to the *Caesalpinia* genus of Fabaceae and finds use in Chinese folk medicine for the treatment of the common cold, fever, and dysentery.[18] In the course of our ongoing search for antiviral agents from natural sources, the ethanol (95%) extract of the seeds of this plant was found to show in vitro anti-Para3 (Parainfluenza virus type 3) activity. Previous investigation of the chloroform fraction of this extract resulted in the isolation of two new, but inactive, furanoditerpenoid lactones, caesalmin A (**7**) and B (**8**).[19] Subsequent study of the active brown precipitate fraction has now led to the isolation of five new cassane furanoditerpenoids, namely, caesalmin C (**1**), D (**2**), E (**3**), F (**4**), and G (**5**), along with stigmasterol (**6**). This paper describes their isolation, structure elucidation, and bioactivity. The proton and carbon assignments as well as the stereochemistry of all five furanoditerpenes were determined by 2D NMR and confirmed by X-ray analyses. The structure of **6** was established by comparing its physical data with the literature and was verified by X-ray crystallography for the first time (Scheme 1).

Scheme 1. Chemical Formulas of Some Cassane Furanoditerpenes and Stigmasterol



Results and Discussion

The molecular formula of compound **1** was established by HRLSIMS as $C_{26}H_{34}O_8$ (*m*/*z* [MH]$^+$ 475.2323, calcd 475.2322). The IR spectrum exhibited absorptions typical of hydroxyl (3556 cm^{-1}) and ester (1743 cm^{-1}) functionalities. The ^{1}H NMR spectrum showed the presence of three tertiary methyl groups at δ 1.16 (6H, s, H-18 and H-19) and 1.31 (3H, s, H-20), three acetoxyl groups at δ 2.08 (3H, s, H-22), 1.97 (3H, s, H-24), and 2.10 (3H, s, H-26), and three oxymethine resonances associated with acetoxyl groups at δ 5.59 (2H, br s, H-6 and H-7 overlap) and 4.89 (1H, br s, H-1). The 1,2-disubstituted furan ring was evident from low-field doublets at δ 6.41 (1H, d, J = 2 Hz,

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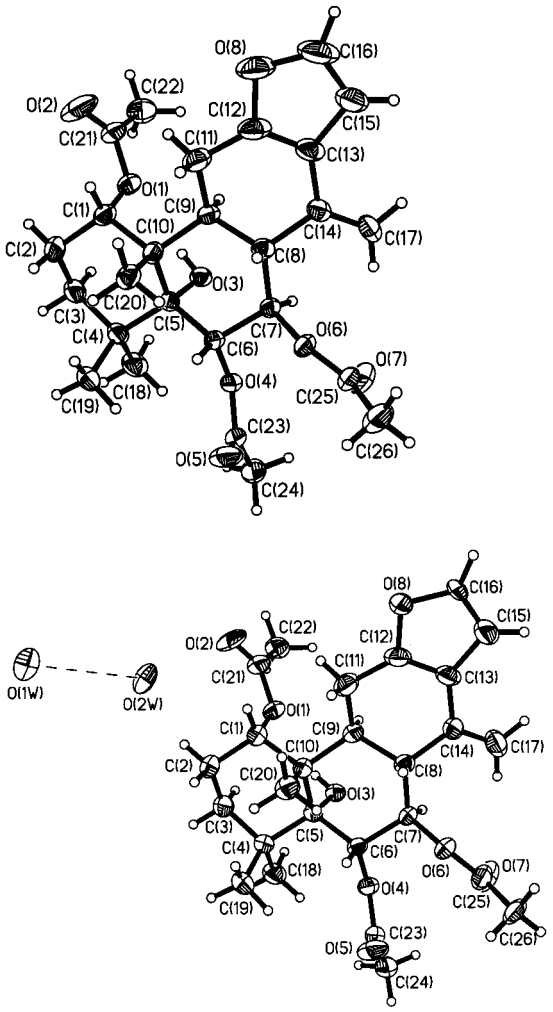

Figure 1. Molecular structure of **1** in its anhydrous form and monohydrate, showing the atom-labeling scheme.

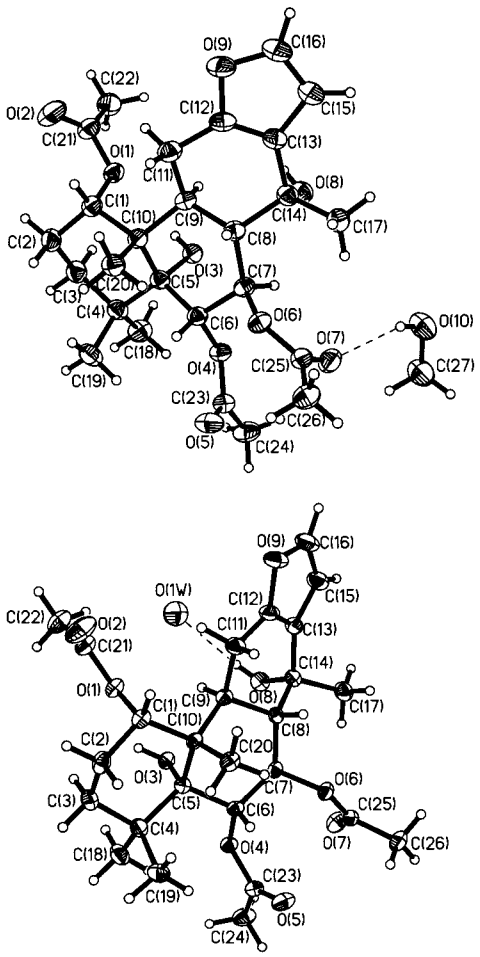

Figure 2. Molecular structure of **2** in its 1:1 methanol solvate and monohydrate, showing the asymmetric unit in each case. The intermolecular hydrogen bonds in the asymmetric units are indicated by dashed lines.

H-15) and 7.23 (1H, d, $J = 2$ Hz, H-16). The exo-methylene protons were revealed by the signals at δ 5.09 (1H, br s, H-17β) and 4.95 (1H, br s, H-17α). In addition to the signals for three acetoxyl groups (δ 169.09 and 21.43; δ 170.79 and 21.35; δ 170.19 and 21.75), the ^{13}C NMR and DEPT spectra of caesalmin C showed 20 carbon signals for three methyl groups (δ 30.70, 25.03, and 17.05), four olefinic carbon atoms of the furan ring (δ 150.49, 119.76, 106.50, and 141.98), four carbon atoms bearing oxygen (δ 75.04, 79.25, 75.24, and 75.59), three methylene carbons (δ 22.02, 32.30, and 23.20), two exocyclic double-bonded carbon atoms (δ 138.44 and 105.60) that are conjugated with the furan ring, two other methine carbons at δ 41.57 and 37.97, and two quaternary carbon atoms at δ 38.50 and 44.48. The full assignments and connectivities were determined by ^{1}H−^{1}H COSY, HMQC, and HMBC. The HMQC spectrum revealed that the exo-methylene protons are directly attached to the carbon resonating at δ 105.60 (C-17, t). In the HMBC spectrum, H-17 showed correlations to C-8, C-13, C-14, and C-15, which further supported the location of the exocyclic double bond between C-14 and C-17 and conjugated with the furan ring. In addition, the HMBC spectrum showed the following key correlations: H-1→C-3, C-5, C-10, C-20, and C-21; H-6→C-7; H-7→C-6; H-15→C-12, C-13, and C-16; H-16→C-12, C-13, and C-15, H_3-20→C-1, C-5, C-9, and C-10, and both H_3-18 and H_3-19 are correlated to C-3, C-4, and C-5.

The molecular structure and relative configuration of compound **1** were established unambiguously by X-ray crystallographic analysis as 5α-hydroxy-1α,6α,7β-triacetoxyvouacapan-14(17)-ene (Figure 1). It is of interest that compound **1** exists both in an anhydrous form and as a monohydrate. The anhydrous form was crystallized from methanol as colorless blocks, and the hydrated form was crystallized from acetone as colorless plates. The two crystal forms belong to the same system, but the unit cell of the hydrate has a bigger volume (+73.4 Å^3). The asymmetric unit of the hydrate consists of an independent molecule of **1** and a water molecule disordered over two positions, which are connected by a hydrogen bond O-1w···O-2w (2.862 Å). The molecules in both crystals take very nearly the same conformation, and their bond distances, bond angles, and torsion angles are listed in Table S1 (see Supporting Information). Perspective views of the molecular structures of **1** and the monohydrate are presented in Figure 1. Ring A exists in the chair conformation with a mean torsion angle of 51.6(4)° (53.2(4)° for the hydrate). Ring B adopts a conformation markedly different from that of a normal chair, and this is indicated by the small torsion angle C-6−C-7−C-8−C-9 = 36.6(5)° (39.5(5)° for the hydrate). The distortion of ring B can be attributed to strain caused by the fusion to unsaturated ring C, which exists in a twisted half-chair conformation with C-8 and C-9 displaced 0.310 Å above and 0.478 Å below (0.420 and 0.343 Å for the hydrate) the best plane of the remaining four atoms. The furan ring D is planar; however it is slightly tilted by 1.1° (2.4° for the hydrate) with respect to the plane of C-11 to C-14.

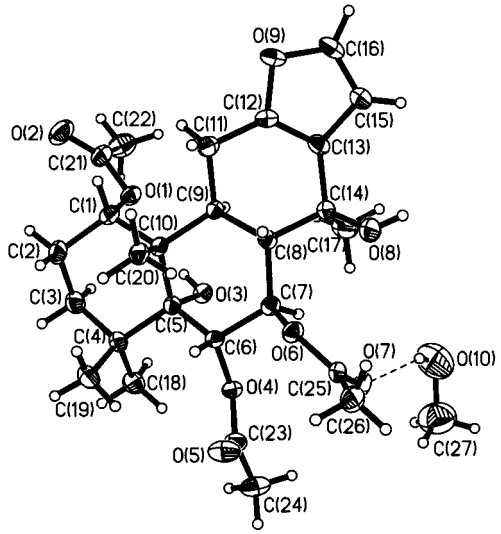

Figure 3. Molecular structure of **3** in its 1:1 methanol solvate, showing the asymmetric unit.

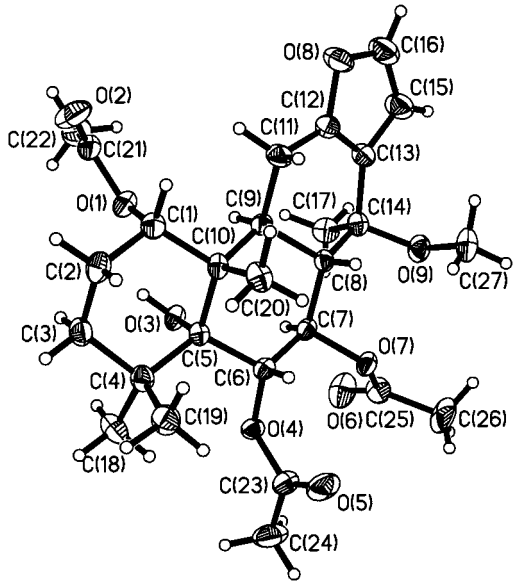

Figure 4. Molecular structure of **4** showing the atom-labeling scheme.

Compound **2** was obtained as colorless crystals. Assignment of the molecular formula $C_{26}H_{36}O_9$ was based on HRLSIMS (*m/z* $[MH]^+$ 493.2431, calcd 493.2427). The ^1H and ^{13}C NMR spectra of **2** are similar to those of **1**; however, the exo-methylene group in **1** (δ_H: 5.09 and 4.95, 1H each, br s, δ_C: 138.44 and 105.60) is replaced by a methyl and a hydroxyl group in **2** (δ_H: 1.38, 3H, s, δ_C: 76.40 and 31.11). There are two possible arrangements of the methyl and hydroxyl groups at C-14, i.e., β-methyl, α-hydroxyl or α-methyl, β-hydroxyl. To establish the stereochemistry of **2**, a single-crystal X-ray analysis was undertaken. Interestingly, **2** crystallizes as a methanol solvate and a monohydrate with similar conformation. (The bond distances, bond angles, and torsion angles for **2** in the two crystals are listed in Table S2.) Perspective views of the molecular structures of the methanol solvate and the hydrate are presented in Figure 2. Therefore, the structure of **2** was established as 5α,14α-dihydroxy-1α,6α,7β-triacetoxyvouacapane.

Compound **3** was shown to possess a molecular formula of $C_{26}H_{36}O_9$ by HRLSIMS (*m/z* $[MH]^+$ 493.2402, calcd 493.2427). The NMR spectra of **3** are similar to those of **2**, except that the signal for H-9 in **3** is shifted to higher field

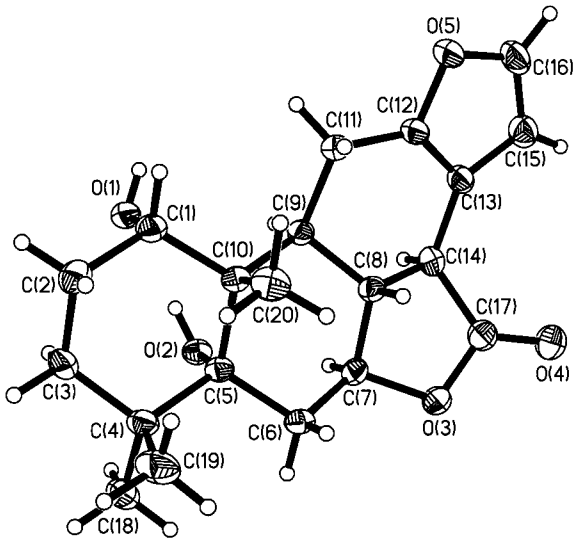

Figure 5. Molecular structure of **5**, showing the atom-labeling scheme.

(−0.35 ppm). A significant NOE (10.2%) at H-9 was observed when the methyl attached to C-14 was irradiated, thus confirming the proposed stereochemistry at C-14 as β-OH. This is consistent with the H-9α configuration that has been observed for all the furanoditerpenes previously isolated from *Caesalpinia*. Thus, the above data suggested that **3** is a stereoisomer of **2**, with the only difference being the relative configuration at C-14. The R_f value on TLC (0.50 for **3** and 0.54 for **2**, *n*-hexane:acetone = 3:2) indicated that the polarity of **3** is slightly larger than **2**. The configuration of **3** was confirmed by X-ray crystallographic analysis. A perspective view of the molecular structure of **3** is presented in Figure 3. Thus, the structure of **3** was established as 5α,14β-dihydroxy-1α,6α,7β-triacetoxyvouacapane.

The molecular formula of **4** was assigned as $C_{27}H_{38}O_9$ on the basis of HRLSIMS (*m/z* $[MH]^+$ 507.2578, calcd 507.2583). The ^1H and ^{13}C NMR spectra of **4** are similar to those of **3** except for the appearance of a methoxy group with δ_H 3.00 (3H, s, H-27) and δ_C 49.60(C-27, s), which was also substantiated by its EIMS (*m/z* 475 $[M - 31]^+$). The chemical shift and coupling constants of H-9 were nearly the same as **3**, which suggested that the configuration of C-14 is the same as **3**. The stereochemistry was verified by X-ray diffraction analysis, which disclosed two crystallographically independent molecules in an asymmetric unit. (The bond distances, bond angles, and torsion angles of the two molecules are listed in Table S3.) Therefore, the structure of **4** was established as 5α-hydroxy-14β-methoxy-1α,6α,7β-triacetoxyvouacapane. Compound **4** is not an artifact of the isolation procedure from **3** because no methanol was used. A perspective view of the molecular structure is presented in Figure 4.

Compound **5** gave a $[MH]^+$ peak at *m/z* 347.1854 (HRLSIMS) for the molecular formula $C_{20}H_{26}O_5$. Development of a reddish purple color with the Ehrlich reagent is indicative of its furanoid structure. The IR (KBr) spectrum shows characteristic absorption bands at 3594 and 1792 cm^{-1} attributable to hydroxyl and saturated γ-lactone functionalities, being consistent with a furanoditerpene lactone. Although the ^1H NMR and ^{13}C spectra of **5** are unlike those of the above four furanoditerpenes, they are similar to those of caesalmin B (**8**) except for the absence of signals for the acetyl group of **8** (δ_H: 2.11, 3H, s, δ_C: 170.65 and 20.61) and the high-field nature of H-1 and C-1 (**5**: δ_H 3.78 and δ_C 73.05; **8**: δ_H 4.88 and δ_C 75.11). This

Table 1. ^{1}H NMR Data of Compounds **1**−**5** (300 MHz, in $CDCl_3$)

position	**1**	**2**	**3**	**4**	**5**
1	4.89, 1H, d, 3	4.88, 1H, s	4.84, 1H, br s	4.86, 1H, s	3.78, 1H, br s
2	1.73, 1H, m, 2-H$^\alpha$	1.68, 1H, m, 2-H$^\alpha$	1.73, 1H, m, 2-H$^\alpha$	1.73, 1H, m, 2-H$^\alpha$	1.66, m, 2-H$^\alpha$
	1.90, 1H, m, 2-H$^\beta$	1.87, 1H, m, 2-H$^\beta$	1.86, 1H, m, 2-H$^\beta$	1.87, 1H, m, 2-H$^\beta$	2.01, H, m, 2-H$^\beta$
3	1.11, 1H, m, 3-H$^\alpha$	1.08, 1H, m, 3-H$^\alpha$	1.08, 1H, m, 3-H$^\alpha$	1.08, 1H, m, 3-H$^\alpha$	1.16, H, m,3-H$^\alpha$
	1.76, 1H, m, 2-H$^\beta$	1.74, 1H, m, 3-H$^\beta$	1.76, 1H, m, 3-H$^\beta$	1.77, 1H, m, 3-H$^\beta$	1.96, 1H, m, 3-H$^\beta$
6	5.59, 1H, br s	5.40, 1H, br s	5.51, 1H, d, 8.4	5.44, 1H, d, 9.0	1.73, 1H, dd, 11.3, 11.7
7	5.59, 1H, br s	5.86, 1H, br s	5.64, 1H, dd, 8.4, 8.8	5.75, 1H, dd, 9.0, 9.6	4.69, 1H, ddd, 10.8, 11.3, 5.1
8	2.82, 1H, br s	2.28, 1H, dd, 5.5, 15.6	2.27, 1H, dd, 5.4, 15.9	2.27, 1H, dd, 5.7, 16.5	2.32, 1H, dd, 5.1, 12.9
9	2.72, 1H, dd, 5.5, 11.0	3.00, 1H, ddd, 4.6, 5.5, 15.6	2.65, 1H, ddd, 5.4, 6.3, 16.0	2.68, 1H, ddd, 4.5, 5.7, 16.5	3.15, 1H, ddd, 8.1, 8.9, 12.9
11	2.41, 1H, dd, 4.5, 16.0, 11-H$^\alpha$	2.43, 2H, m	2.46, 2H, m	2.41, 2H, m	2.58, 1H, dd, 8.9, 17.2, 11- H$^\alpha$
					2.74, 1H, dd, 8.1, 17.2, 11- H$^\beta$
	2.60, 1H, dd, 11.0, 16.0, 11-H$^\beta$				
14					3.24, 1H, br d, 13.2
15	6.41, 1H, d, 2.0	6.42, 1H, d, 2.0	6.38, 1H, d, 2.0	6.23, 1H, d, 1.5	6.61, 1H, d, 1.5
16	7.23, 1H, d, 2.0	7.28, 1H, d, 2.0	7.21, 1H, d, 2.0	7.23, 1H, d, 1.5	7.30, 1H, d, 1.5
17	4.95, 1H, br s, 17-H$^\alpha$	1.38, 3H, s	1.48, 3H, s	1.49, 3H, s	
	5.09, 1H, br s, 17-H$^\beta$				
18	1.16, 3H, s	1.13, 3H, s	1.13, 3H, s	1.15, 3H, s	1.04, 3H, s
19	1.16, 3H, s	1.14, 3H, s	1.14, 3H, s	1.14, 3H, s	1.06, 3H, s
20	1.31, 3H, s	1.28, 3H, s	1.26, 3H, s	1.28, 3H, s	1.09, 3H, s
1-OAc	2.08, 3H, s	2.03, 3H, s	2.06, 3H, s	2.06, 3H, s	
6-OAc	1.97, 3H, s	1.99, 3H, s	1.95, 3H, s	1.98, 3H, s	
7-OAc	2.10, 3H, s	2.08, 3H, s	2.07, 3H, s	2.08, 3H, s	
14-OCH_3				3.00, 3H, s	

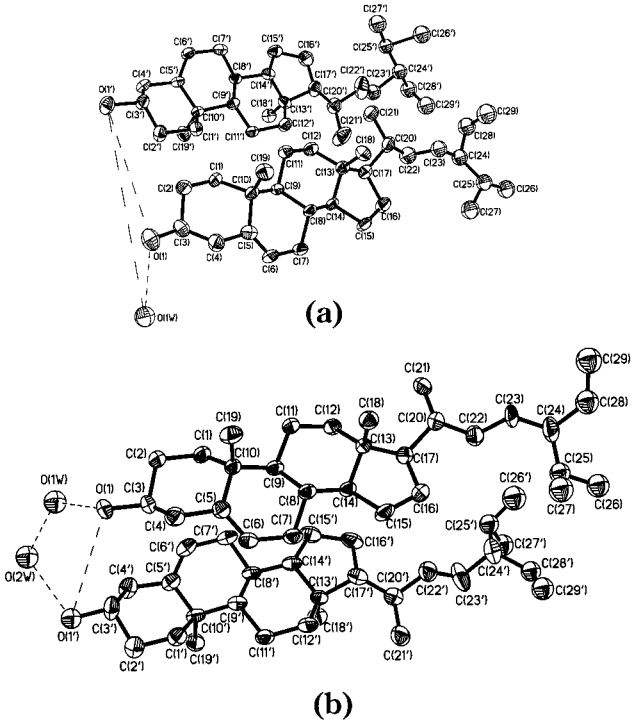

Figure 6. Molecular structure of **6** with atom-labeling scheme, showing the asymmetric unit of its (a) orthorhombic hemihydrate and (b) monoclinic monohydrate. Hydrogen atoms were omitted for clarity.

Table 2. ^{13}C NMR Data of Compounds **1**−**5** (75 MHz, in $CDCl_3$)[a]

position	**1**	**2**	**3**	**4**	**5**
1	75.04 d	69.29 d	74.33 d	73.70 d	73.05 d
2	22.02 t	22.71 t	22.58 t	22.82 t	30.23 t
3	32.30 t	32.84 t	32.75 t	32.37 t	31.06 t
4	38.50 s	39.14 s	39.04 s	38.52 s	40.01 s
5	79.25 s	79.42 s	79.70 s	79.06 s	83.39 s
6	75.24 d	74.63 d	75.59 d	75.69 d	26.60 t
7	75.59 d	76.40 d	76.29 d	76.29 d	81.81 d
8	41.57 d	44.90 d	45.12 d	39.39 d	45.35 d
9	37.97 d	34.43 d	36.31 d	36.36 d	33.52 d
10	44.48 s	47.53 s	48.16 s	45.12 s	47.20 s
11	23.20 t	23.29 t	23.19 t	22.30 t	21.77 t
12	150.49 s	150.12 s	148.07 s	150.97 s	152.65 s
13	119.76 s	123.55 s	125.99 s	121.83 s	114.57 s
14	138.44 s	76.40 s	73.09 s	78.81 s	41.97 d
15	106.50 d	108.30 d	107.94 d	107.81 d	108.47 d
16	141.98 d	142.20 d	142.55 d	142.34 d	142.08 d
17	105.60 t	31.11 q	31.11 q	24.42 q	175.56 s
18	30.70 q	29.40 q	25.50 q	30.24 q	28.74 q
19	25.03 q	25.48 q	25.44 q	24.79 q	25.26 q
20	17.05 q	17.95 q	17.64 q	17.01 q	17.79 q
1-OAc	169.09 s	169.88 s	169.61 s	170.93 s	
	21.43 q	22.14 q	21.95 q	20.23 q	
6-OAc	170.79 s	171.28 s	171.06 s	171.29 s	
	21.35 q	22.34 q	22.01 q	20.42 q	
7-OAc	170.79 s	172.20 s	171.25 s	171.29 s	
	21.75 q	22.34 q	21.16 q	20.82 q	
14-OCH_3				49.60 q	

[a] The NMR data were assigned on the basis of DEPT, H−H COSY, HMQC, and HMBC.

information indicated that **5** is the 1-hydroxy analogue of **8**. The molecular structure of **5** was confirmed by X-ray analysis as 1α,5α-dihydroxyvouacapano-7β,17β-lactone. A perspective view of the molecular structure is presented in Figure 5.

All five furanoditerpenes have similar stereochemistry. The three six-membered rings A, B, and C are fused as a *trans*−*anti*−*trans* system. The configurations at the chiral centers have been established as follows: OAc-1, OH-5, H-8, H-9, and CH_3-10 are at the axial position and OAc-6 and OAc-7 are at the equatorial position. The conformation of the individual rings can be deduced from the torsion angles and least-squares planes. Cyclohexane ring A exists in a chair conformation, while ring B is more flexible: in **1**, **2**, and **3** it exists in a twisted chair conformation, whereas in **4** it is fixed in a chair conformation because of the steric hindrance between the 7-OAc and 14-OCH_3 groups. In **5**, ring B is fixed in the chair conformation by fusion to the lactone ring E. Ring C in all five furanoditerpenes exists in a twisted half-chair conformation due to fusion to the planar furan ring D. The lactone ring of **5** exists in an envelope. All furanoditerpenoids isolated so

Table 3. Crystallographic Data of Compounds **1**−**6**

	1	**1**·H_2O	**2**·CH_3OH	**2**·H_2O
CCDC deposit no.	160425	160426	160427	160428
color/shape	colorless/block	colorless/plate	colorless/block	colorless/plate
cryst dimens (mm^3)	0.40 × 0.60 × 0.80	0.14 × 0.35 × 0.66	0.38 × 0.44 × 0.60	0.20 × 0.40 × 0.60
chem formula	$C_{26}H_{34}O_8$	$C_{26}H_{34}O_8$·H_2O	$C_{26}H_{36}O_9$·CH_3OH	$C_{26}H_{36}O_9$·H_2O
fw	474.53	492.53	524.59	508.55
temp, K	293(2)	293(2)	293(2)	293(2)
cryst syst	orthorhombic	orthorhombic	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
unit cell dimens	a = 9.993(1) Å	a = 9.9290(8) Å	a = 10.921(2) Å	a = 21.2009(8) Å
	b = 12.553(5) Å	b = 12.601(1) Å	b = 11.175(2) Å	b = 10.8654(4) Å
	c = 21.287(3) Å	c = 21.930(1) Å	c = 21.584(4) Å	c = 11.1099(4) Å
volume, $Å^3$	2670.3(12)	2743.7(4)	2634.2(9)	2559.2(1)
Z	4	4	4	4
density(calcd), g/cm^3	1.180	1.188	1.323	1.320
abs coeff, mm^{-1}	0.087	0.089	0.100	0.101
diffractometer/scan	Bruker P4/ω	Bruker P4/ω	Rigaku IP/ω	Bruker SMART1000 CCD/ω
θ range for data collection, deg	1.88 to 26.00	2.25 to 25.02	1.89 to 25.53	1.92 to 25.02
no. of reflns measd	3807	15348	8353	14 177
no. of ind reflns	3592	4823	4665	4517
no. of obsd reflns	2451	3178	4287	4054
no. of data/restrains/params	3592/0/309	4823/0/325	4665/0/339	4517/0/321
goodness of fit on F^2	1.043	1.070	1.073	1.023
final R indices [$I > 2\sigma(I)$]	0.0586	0.0711	0.0495	0.0451
R indices (all data)	0.0936	0.1051	0.0573	0.0501

	3·CH_3OH	**4**	**5**	**6**·1/2H_2O	**6**·H_2O
CCDC deposit no.	160 429	160 430	160 431	160 432	160 433
color/shape	colorless/block	colorless/needle	colorless/plate	colorless/plate	colorless/plate
cryst dimens (mm^3)	0.30 × 0.40 × 0.70	0.15 × 0.20 × 0.76	0.08 × 0.14 × 0.70	0.08 × 0.14 × 0.70	0.10 × 0.40 × 0.60
chem formula	$C_{26}H_{36}O_9$·CH_3OH	$C_{27}H_{38}O_9$	$C_{20}H_{26}O_5$	$C_{29}H_{50}O_1$·1/2H_2O	$C_{29}H_{50}O_1$·H_2O
fw	524.59	506.58	346.41	422.69	431.69
temp, K	293(2)	293(2)	293(2)	293(2)	293(2)
cryst syst	orthorhombic	orthorhombic	monoclinic	orthorhombic	monoclinic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$	$P2_1$
unit cell dimens	a = 10.711(1) Å	a = 6.8458(9) Å	a = 9.0784(7) Å	a = 9.574(1) Å	a = 10.268(1) Å
	b = 11.668(5) Å	b = 24.119(3) Å	b = 8.6720(7) Å	b = 74.144(8) Å	b = 7.6293(7) Å
	c = 20.971(4) Å	c = 32.941(4) Å	c = 10.9335(9) Å	c = 7.5420(8) Å	c = 35.392(4) Å
			β = 94.997(2)°		β = 94.402(2)°
volume, $Å^3$	2620.7(13)	5439.0(12)	857.50(12)	5353.6(10)	2764.3(5)
Z	4	8	2	8	4
density(calcd), g/cm^3	1.330	1.237	1.342	1.044	1.030
abs coeff, mm^{-1}	0.101	0.092	0.095	0.062	0.062
diffractometer/scan	Bruker P4/ω	Bruker SMART 1000 CCD/ω	Bruker SMART 1000 CCD/ω	Bruker SMART 1000 CCD/ω	Bruker SMART 1000 CCD/ω
θ range for data collection, deg	1.94 to 24.99	1.05 to 28.05	1.87 to 25.03	1.65 to 25.19	1.99 to 25.02
no. of reflns measd	3379	38 148	4693	30 100	14 920
no. of ind reflns	3180	13 096	2525	9541	7952
no. of obsd reflns	2331	6142	1964	4559	4158
no. of data/restrains/params	3180/0/337	13096/0/634	2525/0/228	9541/0/476	7952/1/494
goodness of fit on F^2	1.061	0.921	0.971	1.009	0.948
final R indices [$I > 2\sigma(I)$]	0.0598	0.0654	0.0375	0.1254	0.0997
R indices (all data)	0.0900	0.1524	0.0538	0.2167	0.1569

far from *Caesalpinia*-related species have a β-oriented methyl group at C-10 and an α-oriented hydroxyl group at C-5. The absolute configurations of pulcherrimin A,[15] ϵ-caesalpin,[16] and caesaldekarin A,[17] which share the same carbon skeleton with compounds **1**−**5**, are well established. Considering the biogenetic relationship in the furanoditerpenoids, the absolute configurations of **1**, **2**, **3**, **4**, and **5** can be assigned by analogy as shown in Scheme 1.

Stigmasterol, compound **6**, is widely distributed in nature. However, this is the first report of its isolation from this genus. Its structure was determined by co-TLC with an authentic sample and comparing its physical data with those previously reported in the literature.[20] Furthermore, the structure was verified by X-ray analysis for the first time. Interestingly, **6** has been crystallized in two unreported crystal forms: one is orthorhombic from acetone solution and the other is monoclinic from methanol/chloroform. The asymmetric unit of the orthorhombic form consists of two independent molecules of **6** and one water molecule connected by hydrogen bonds O-1···O-1′ (2.900 Å, $-x$+3/2, $-y$+1, z−1/2), O-1′···O-1w (2.841 Å, x, y, z+1), and O-1···O-1w (3.089 Å). The asymmetric unit of the monoclinic form consists of two independent molecules of **6** and two water molecules connected by hydrogen bonds O-1···O-1′ (2.842 Å, 1−x, y+0.5, 1−z) and O-1···O-1w (2.776 Å), O-1···O-1w (2.837 Å, 1−x, y−0.5, 1−z), O-1′···O-2w (2.886 Å), and O-1w···O-2w (2.875 Å). The molecules in both kinds of crystals take similar conformations in the steroidal skeleton. (The bond distances, bond angles, and torsion angles of the molecules in both kinds of crystals are listed in Table S4.) Rings A and C exist in the chair conformation with mean torsion angles of 53.8(8)° and 54.4(8)° each for the orthorhombic form and 53.6(7)° and 54.2(7)° each for the monoclinic form. Ring B adopts the

Table 4. Antiproliferation Activity of Compounds **1**−**6** (DMSO) against the Para3 Virus

compound	IC_{50} (μg/mL)	TC_{50} (μg/mL)	TI
1	8.2	196.3	23.9
2	9.6	182.4	19.1
3	10.3	165.0	16.0
4	7.8	136.5	17.5
5	14.8	44.7	3.0
6	37.5	136.6	3.6
ribavirin	2.6	62.5	24.0

twisted half-chair conformation due to the double bond between C-5 and C-6. Five-membered ring D exists in the envelope conformation with C-13 displaced 0.657 and 0.664 Å for orthorhombic and monoclinic forms, respectively, from the least-squares plane of the remaining four atoms. However, bending of the side chain is different; for example, the torsion angle C-23−C-24−C-28−C-29 takes the value 71(3)° and −87(3)° each for the orthorhombic form and −59(2)° and −76.1(14)° each for the monoclinic form. The principal source of this flexibility is the rotation about the C-23−C-24 single bond. Perspective views of the molecular structures of the two crystal forms are given in Figure 6a,b.

Compounds **1**−**6** were evaluated for their effects on the proliferation of the Para3 virus (Table 4). Furanoditerpenoids **1**−**5** showed significant activity against the Para3 virus, with IC_{50} values ranging between 7.8 and 14.8 μg/mL. However, compound **5**, which is the only furanoditerpenoid lactone, is highly toxic, with a TI value of 3.0. It is noteworthy that the TI value of compound **1** is almost the same as that of ribavirin, which serves as a positive control in the bioassay. Compound **6** shows moderate activity against the Para3 virus. It can be concluded that the anti-Para3 virus activity of tetracyclic furanoditerpenoids is better than that of the furanoditerpenoid lactone, which in turn is superior to stigmasterol. Since the major components of the seeds of *Caesalpinia minax* Hance possess such potent activity, it may be feasible to develop new antiviral agents from this medicinal plant.

Experimental Section

General Experimental Procedures. ORD was recorded on a Perkin-Elmer 341 polarimeter in MeOH solution. Melting points were determined using a Fisher Scientific instrument and were uncorrected. The UV spectra were obtained on a Beckman DU650 spectrophotometer in MeOH. IR spectra were recorded on a Nicolet Impact 420 FT-IR spectrometer. All NMR spectra were obtained on a Bruker-300 MHz spectrometer in $CDCl_3$ solution, and the δ values are in ppm and are referenced to either the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signal. EIMS and ESIMS were recorded on a Finnigan MAT TSQ 7000 instrument. HRLSIMS measurements were made on an APEX 47e FTMS spectrometer.

Plant Material. The seeds of *Caesalpinia minax* Hance (Ku-shi-lian in Chinese) were collected in Guangxi Province, People's Republic of China, in September 1999. The seeds were identified at the Institute of Chinese Medicine, The Chinese University of Hong Kong, where a voucher specimen (No. cm-99) is preserved.

Extraction and Isolation. The dried ground seeds (10 kg) were extracted with 95% ethanol, and solvent was evaporated in vacuo to give a brown syrup (892 g). The extract was suspended in distilled water and extracted with hexane. The aqueous layer was placed under room temperature to give a brown precipitate (48 g). After filtration, the residue was extracted successively with chloroform, ethyl acetate, and *n*-butanol to afford six fractions, which were evaluated for inhibition of the Para3 virus. The active precipitate fraction was chromatographed over silica gel repeatedly and preparative TLC to give **1** (140 mg), **2** (200 mg), **3** (130 mg), **4** (160 mg), **5** (16 mg), and **6** (45 mg). The ^1H and ^{13}C NMR data of **1**−**5** are shown in Tables 1 and 2, respectively.

Compound 1: colorless crystals; mp 129−130 °C for anhydrous and 121−122 °C for monohydrate; $[\alpha]_D^{20}$ +51.2° (*c* 0.25 in CH_3OH); IR (KBr) ν_{max} 3556, 1743 cm^{-1}; UV λ_{max}^{MeOH} (log ϵ) 215(3.80), 244(3.78) nm; EIMS *m*/*z* $[M]^+$ 474(2), 414(19), 294(21), 276(100), 261(14), 251(11), 225(9), 209(7), 197(8), 174(7), 139(4), 97(5), 43(4); HRLSIMS *m*/*z* $[MH]^+$ 475.2323, calcd for $C_{26}H_{34}O_8$, requires 475.2322.

Compound 2: colorless crystals; mp 192−193 °C for methanol solvate and 178−180 °C for hydrate; $[\alpha]_D^{20}$ +65.9° (*c* 0.2 in CH_3OH); IR (KBr) ν_{max} 3556, 1729 cm^{-1}; UV λ_{max}^{MeOH} (log ϵ) 230(3.74) nm; EIMS *m*/*z* $[M]^+$ 492(3), 477(7), 435(6), 375(7) 354(49), 336(9), 312(19), 294(100), 279(31), 251(13), 225(10), 211(16), 198(13), 174(10) 135(7), 97(6); HRLSIMS *m*/*z* $[MH]^+$ 493.2431, calcd for $C_{26}H_{36}O_9$, requires 493.2427.

Compound 3: colorless crystals; mp 135−136 °C; $[\alpha]_D^{20}$ +22.3° (*c* 0.2 in CH_3OH); IR (KBr) ν_{max} 3570, 1729 cm^{-1}; UV λ_{max}^{MeOH} (log ϵ) 228(3.68) nm; ESIMS *m*/*z* $[M + Na]^+$ 515, HRLSIMS *m*/*z* $[MH]^+$ 493.2402, calcd for $C_{26}H_{36}O_9$, requires 493.2427.

Compound 4: colorless crystals; mp 173−174 °C; $[\alpha]_D^{20}$ +27.6° (*c* 0.25 in CH_3OH); IR (KBr) ν_{max} 3570, 1743 cm^{-1}; UV λ_{max}^{MeOH} 220 (log ϵ) (log ϵ 3.75) nm; EIMS *m*/*z* $[M]^+$ 506(23), 475(18), 354(100), 336(14), 312(22), 294(82), 276(30), 251(16), 225(25), 211(35), 198(13), 174 (11), 138(49), 109(5); HRLSIMS *m*/*z* $[MH]^+$ 507.2578, calcd for $C_{27}H_{38}O_9$, requires 507.2583.

Compound 5: colorless needlelike crystals, mp 168−170 °C, $[\alpha]_D^{20}$ +50.5° (*c* 0.2 in CH_3OH); ESIMS *m*/*z* $[M - 1]^-$ 345(4), $[M + Ac]^-$ 405(68); UV λ_{max}^{MeOH} nm 218 (log ϵ) (3.80); IR (KBr) 3594, 1792 cm^{-1}; HRLSIMS *m*/*z* $[MH]^+$ 347.1854, calcd for $C_{20}H_{26}O_5$, requires 347.1851.

Single-Crystal X-ray Analysis. The X-ray intensities of **1**, **1**·H_2O, and **3**·CH_3OH were conducted on a Bruker P4 four-circle diffractometer, those of **2**·CH_3OH were measured on a Rigaku IP diffractometer, and those of **2**·H_2O, **4**, **5**, **6**·1/2H_2O, and **6**·H_2O were measured on a Bruker SMART1000 CCD diffractometer. Their structures were solved by direct methods (SHELXS-97) and refined by full-matrix least-squares on F^2. In the structure refinements, non-H atoms were refined with anisotropic temperature factors. H atoms bonded to carbons were placed on geometrically calculated positions, and positions for H atoms bonded to oxygen were determined from difference Fourier syntheses and included in the calculation of structure factors with isotropic temperature factors. The crystallographic data of compound **1**−**6** are shown in Table 3. Among these crystal structures, both the orthorhombic and monoclinic forms of compound **6** have a very long unit-cell axis, the crystals occur as very thin plates, and the diffraction patterns are weak. Furthermore, molecule **6** has a long side chain which shows disorder to a large extent, and thus the *R* indices of the hydrates of **6** are higher than usual. In addition, the water molecule is disordered over two positions in the asymmetric unit of **1**·H_2O, leading to relatively higher final *R* indices.

Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. The CCDC numbers are shown in Table 3. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. [fax: (+44)1223-336033; e-mail: deposit@ccdc.cam.ac.uk].

Anti-Para3 Assay. The antiviral experiments were performed in 96-well microtiter plates using the procedures described previously.[21−22] Ribavirin was used as a positive control, and an infection control was made in the absence of samples. Para3 virus suspension of the same quality was added to the monolayers of hep-2 cells, and the cytopathogenic effect (CPE) was observed under an inverted microscope. The concentration that reduced CPE by 50% with respect to virus control was defined as IC_{50}. The concentration that showed 50% cytotoxic effect was defined as TC_{50}. The selectivity against the Para3 virus was characterized by the therapeutic index (TI) = TC_{50}/IC_{50}.

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Supporting Information Available: Bond distances, bond angles, and torsion angles for compound **1** in **1** and **1**·H_2O, compound **2** in **2**·CH_3OH and **2**·H_2O, two molecules in the asymmetric unit of compound **4**, and compound **6** in **6**·1/2H_2O and **6**·H_2O are listed in Tables S1, S2, S3, and S4, respectively. This material is available free of charge at http://pubs.acs.org.

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