Five New Cassane-Type Diterpenes from *Caesalpinia crista*

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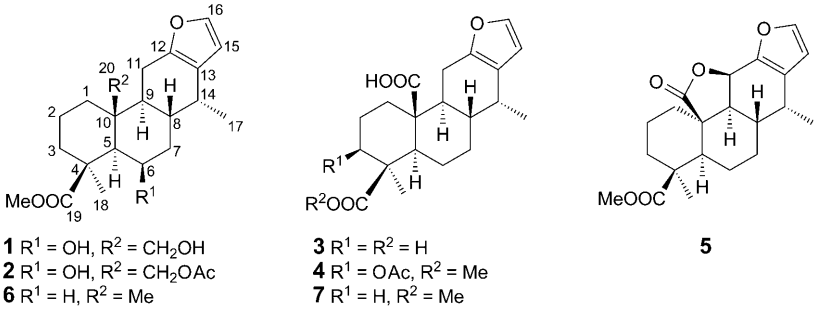
Five new cassane-type diterpenes, caesalpinista A (**1**), caesalpinista B (**2**), caesaljapin B (**3**), caesaljapin C (**4**), and caesalpinilinn (**5**) were isolated from the MeOH extract of the seeds of *Caesalpinia crista*. Their structures were elucidated by the analysis of their 1D- and 2D-NMR spectra.

Introduction. – *Caesalpinia crista* L. (Fabaceae) is a well-known medicinal plant widely distributed in tropical and subtropical regions of Southeast Asia. This plant is locally known as 'Ka-Lain' in Myanmar, and its seeds are used as an anthelmintic, antipyretic, anti-inflammatory, and antimalarial agent [1]. In Indonesia, it is known as 'Bagore', and a decoction of its roots has been used as a tonic and for the treatment of rheumatism and backache [2]. As a member of the genus *Caesalpinia*, it is a rich source of cassane-type furanoditerpenes and is reported to have antimalarial [3][4], antiviral [5], and anticancer activities [6]. We have chemically investigated the seeds of *Caesalpinia crista* which resulted in the isolation of five new cassane diterpenes (**1**–**5**; see *Fig. 1*). In this paper, we report the structure elucidation of these cassane-type diterpenes.



1 R^1 = OH, R^2 = CH_2OH
2 R^1 = OH, R^2 = CH_2OAc
6 R^1 = H, R^2 = Me

3 R^1 = R^2 = H
4 R^1 = OAc, R^2 = Me
7 R^1 = H, R^2 = Me

5

Fig. 1. *The structures of compounds* **1**–**7**

Results and Discussion. – Compound **1** was isolated as a colorless amorphous solid and its molecular formula was determined to be $C_{21}H_{30}O_5$ by HR-EI-MS. The IR

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absorptions at 3423 and 1718 cm^{-1} indicated the presence of OH and CO groups, respectively. The ^{1}H-NMR spectrum (*Table 1*) of **1** displayed signals corresponding to two H-atoms of a 1,2-disubstituted furan ring (δ(H) 7.24, 6.22), a sharp *singlet* due to a MeOCO group (δ(H) 3.71), one tertiary Me group (δ(H) 1.57), and one Me *doublet* signal at δ(H) 0.90 (*d*, $J = 6.9$). The ^{13}C-NMR spectrum (*Table 2*) of **1** showed four olefinic C-atoms (δ(C) 149.6, 140.6, 122.4, and 109.9) and two O-substituted C-atoms (δ(C) 69.1, 64.6), together with one ester CO C-atom (δ(C) 179.6). The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) were similar to those of deoxycaesaldekarin C (**6**) [7], except that a *multiplet* signal of CH_2(6) (δ(H) 1.56) was replaced by a CH−O group at δ(H) 3.89 and one tertiary Me group at δ(H) 0.93 (Me(20)) was replaced by an CH_2O group at δ(H) 4.25 and 3.60 (each *d*, $J = 12.6$). In the HMBC and HSQC spectra, we observed the correlations of the CH−O group (δ(H) 3.89) with C(5) (δ(C) 51.3) and C(7) (δ(C) 39.6), as well as of the CH_2O group (δ(H) 4.25, 3.62) with C(10) (δ(C) 41.2), which indicated the CH−O group was located at C(6) and the CH_2O group was at C(20).

Table 1. *^{1}H-NMR Data* (500 MHz) *of* **1**–**3** *in $CDCl_3$ or CD_3OD.* δ(H) in ppm, *J* in Hz[a]).

	1[b])	**2**[b])	**3**[c])
CH_2(1)	1.94 (*d*, $J = 12.9$), 1.08–1.20 (*m*)	2.42 (*d*, $J = 12.7$), 0.98–1.02 (*m*)	2.17–2.21 (*m*), 1.00–1.04 (*m*)
CH_2(2)	1.48–1.55 (*m*), 1.32–1.45 (*m*)	1.60–1.64 (*m*), 1.40–1.46 (*m*)	1.88–1.92 (*m*), 1.60–1.64 (*m*)
CH_2(3)	1.73–1.88 (*m*), 1.58–1.62 (*m*)	1.76–1.82 (*m*), 1.64–1.68 (*m*)	1.80–1.84 (*m*), 1.57–1.61 (*m*)
H−C(5)	2.02 (br. *s*)	1.98 (br. *s*)	1.98 (br. *s*)
H−C(6) or CH_2(6)	3.89 (br. *s*)	3.98 (br. *s*)	2.16–2.20 (*m*), 1.20–1.24 (*m*)
CH_2(7)	1.62–1.66 (*m*)	1.68–1.72 (*m*)	2.74–2.78 (*m*), 1.40–1.44 (*m*)
H−C(8)	2.42–2.52 (*m*)	2.18–2.24 (*m*)	2.46 (*d*, $J = 13.4$)
H−C(9)	1.68–1.72 (*m*)	1.72–1.76 (*m*)	1.58–1.62 (*m*)
CH_2(11)	2.72–2.76 (*m*), 2.52–2.60 (*m*)	2.75 (*dd*, $J = 16.4, 6.1$), 2.50 (*dd*, $J = 16.4, 11.1$)	2.68 (*dd*, $J = 16.4, 6.1$), 2.10 (*dd*, $J = 16.4, 11.1$)
H−C(14)	2.68–2.72 (*m*)	2.58–2.64 (*m*)	2.57–2.61 (*m*)
H−C(15)	6.22 (*s*)	6.20 (*s*)	6.18 (*s*)
H−C(16)	7.24 (*s*)	7.25 (*s*)	7.22 (*s*)
Me(17)	0.90 (*d*, $J = 6.9$)	0.98 (*d*, $J = 6.9$)	0.98 (*d*, $J = 7.4$)
Me(18)	1.57 (*s*)	1.58 (*s*)	1.08 (*s*)
CH_2(20)	4.25 (*d*, $J = 12.6$), 3.60 (*d*, $J = 12.6$)	4.98 (*d*, $J = 13.5$), 4.30 (*d*, $J = 13.5$)	
COOMe	3.71 (*s*)	3.70 (*s*)	
OAc		2.04 (*s*)	

[a]) Assignments were made using HSQC and HMBC data. [b]) In $CDCl_3$. [c]) In CD_3OD.

The relative configuration of **1** was determined on the basis of ROESY correlations. The ROESY correlations of Me(18) (δ(H) 1.57) with H−C(5) (δ(H) 2.02) and H−C(6) (δ(H) 3.89), of CH_2(20) (δ(H) 4.25, 3.60) with H_{ax}−C(2) (δ(H) 1.32–1.45)

Table 2. *^{13}C-NMR Data* (125 MHz) *of* **1**–**5** *in $CDCl_3$ or CD_3OD.* δ(C) in ppm.

	1[a])	**2**[a])	**3**[b])	**4**[a])	**5**[a])
C(1)	38.6	34.8	37.6	33.5	33.3
C(2)	18.9	18.6	21.1	22.8	18.0
C(3)	39.6	38.2	38.6	77.0	37.0
C(4)	48.6	48.2	49.2	52.1	47.2
C(5)	51.3	51.1	51.7	50.2	45.6
C(6)	69.1	69.6	25.0	24.5	26.5
C(7)	39.6	40.0	31.9	29.8	29.5
C(8)	32.8	31.2	37.7	35.2	38.4
C(9)	45.5	45.4	45.3	43.4	47.0
C(10)	41.2	41.1	50.1	47.8	50.2
C(11)	22.5	22.9	25.6	24.3	68.2
C(12)	149.6	149.3	150.1	147.8	144.7
C(13)	122.4	121.9	124.3	122.7	130.4
C(14)	31.4	31.0	33.2	31.1	31.7
C(15)	109.9	109.5	111.0	109.5	109.2
C(16)	140.6	140.4	142.3	140.7	143.8
C(17)	17.2	17.8	18.0	17.1	15.1
C(18)	18.8	18.5	16.4	10.5	16.9
C(19)	179.6	179.2	182.4	175.7	178.9
C(20)	64.6	64.6	179.1	180.1	175.9
COO*Me*	52.4	52.2		52.3	52.0
O*C*OMe		170.9		170.2	
OCO*Me*		21.2		21.0	

[a]) Recorded in $CDCl_3$ at 125 MHz. [b]) Recorded in CD_3OD at 125 MHz.

and H_{ax}–C(11) (δ(H) 2.52–2.60) indicated that rings *A* and *B* have a chair conformation with a *trans*-fused ring junction. On the other hand, ROESY correlations of H–C(6) (δ(H) 3.89) with H–C(5) (δ(H) 2.02) and Me(18) (δ(H) 1.57) indicated that the OH substituent at C(6) was β-oriented and that the Me(18) was α-oriented (*Fig. 2*). From these spectral evidences, the structure of **1** was determined and named as caesalpinista A.

Compound **2** showed the molecular ion peak at *m*/*z* 404.2199 ($C_{23}H_{32}O_6^+$; calc. 404.2190) in the HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*) revealed that **2** had the same cassane-type carbon skeleton as **1**. The only difference was the presence of an additional AcO group (δ(H) 2.04 and δ(C) 170.9, 21.2). The CH_2(20)–O group of **2** was shifted downfield from δ(H) 4.25 and 3.60 (each *d*, J = 12.6) to δ(H) 4.98 and 4.30 (each *d*, J = 13.5) as a result of the acetylation of the OH group. In addition, a significant HMBC between CH_2(20) and the AcO CO group (δ(C) 170.9) further confirmed the location of the AcO group at C(20). Thus, the structure of **2** was determined and named as caesalpinista B.

Compound **3** was deduced as $C_{20}H_{26}O_5$ by HR-EI-MS analysis (M^+, *m*/*z* 346.1780; calc. 346.1784). The ^{1}H- and ^{13}C-NMR spectral data (*Tables 1* and *2*) of **3** were closely related to those of caesaljapin (**7**) [8]. The only difference between them was the lack of a MeOCO group at C(19). Therefore, the structure of **3** was determined and named as caesaljapin B.

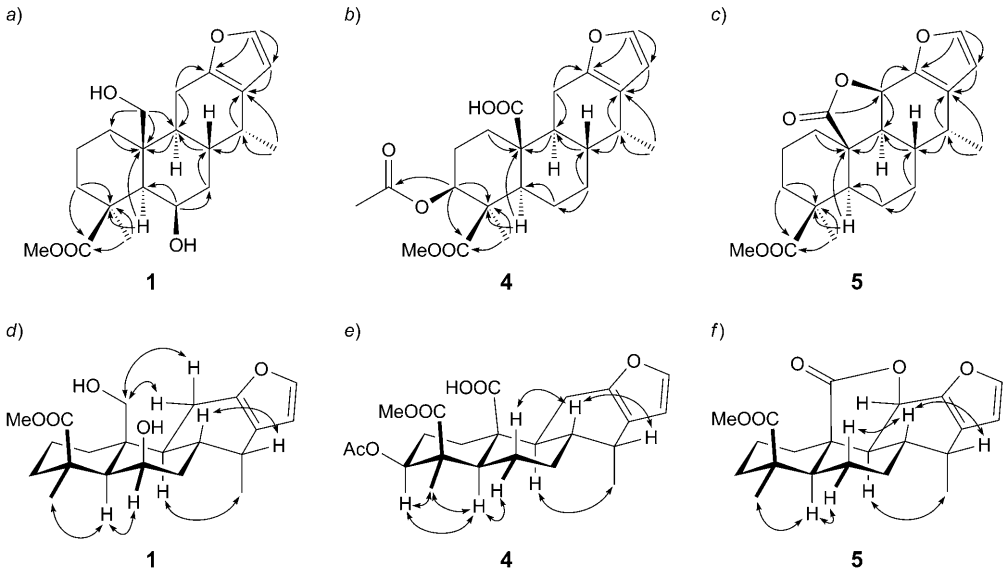

Fig. 2. *Major HMBC data of compounds a*) **1**, *b*) **4**, *and c*) **5**; *important ROESY cross-peaks of compounds d*) **1**, *e*) **4**, *and f*) **5**

Compound **4** was isolated as a colorless amorphous solid and its molecular formula was determined to be $C_{23}H_{30}O_7$ by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectral data (*Tables 3* and *2*) also revealed the same cassane-type skeleton as caesaljapin (**7**). The ^{1}H-NMR spectral data exhibited a CH–O group at δ(H) 5.21 (*dd*, $J = 9.7, 7.0$) and an

Table 3. *^{1}H-NMR Data* (500 MHz) *of* **4**–**5** *in* $CDCl_3$. δ (H) in ppm, J in Hz[a])

	4	**5**
CH_2(1)	2.55 (*d*, $J = 13.6$), 1.20–1.25 (*m*)	2.50 (*d*, $J = 13.1$), 1.44–1.50 (*m*)
CH_2(2)	2.28–2.36 (*m*), 1.00–1.10 (*m*)	1.64–1.68 (*m*), 1.52–1.58 (*m*)
H–C(3) or CH_2(3)	5.21 (*dd*, $J = 9.7, 7.0$)	1.68–1.72 (*m*)
H–C(5)	1.94 (br. *s*)	2.16 (*dd*, $J = 7.6, 4.7$)
CH_2(6)	1.88–1.92 (*m*)	1.36–1.42 (*m*)
CH_2(7)	1.70–1.78 (*m*), 1.35–1.45 (*m*)	1.58–1.64 (*m*), 1.42–1.46 (*m*)
H–C(8)	2.18–2.22 (*m*)	1.78–1.88 (*m*)
H–C(9)	1.60–1.68 (*m*)	2.06 (*dd*, $J = 12.0, 3.6$)
CH_2(11) or H–C(11)	2.80 (*dd*, $J = 16.3, 5.9$), 2.10–2.15 (*m*)	5.38 (*d*, $J = 3.6$)
H–C(14)	2.60–2.68 (*m*)	2.62–2.68 (*m*)
H–C(15)	6.15 (*s*)	6.25 (*s*)
H–C(16)	7.18 (*s*)	7.38 (*s*)
Me(17)	0.98 (*d*, $J = 6.9$)	0.98 (*d*, $J = 7.2$)
Me(18)	1.13 (*s*)	1.52 (*s*)
COOMe	3.68 (*s*)	
OAc	1.98 (*s*)	

[a]) Assignments were made using HSQC and HMBC data.

AcO group (δ(H) 1.98). The CH–O group showed HMBC correlations with C(4) (δ(C) 52.1), C(18) (δ(C) 10.5), C(19) (δ(C) 175.7), the AcO group (δ(C) 170.2), and C(2) (δ(C) 22.8), confirming an AcO group at C(3). The ROESY correlations of Me(18) (δ(H) 1.13) with H–C(5) (δ(H) 1.94) and H–C(3) (δ(H) 5.20) indicated the AcO group at C(3) to be in β-axial orientation (*Fig. 2*). Thus, the structure of **4** was determined and named as caesaljapin C.

Compound **5** showed the molecular ion peak at *m/z* 358.1886 ($C_{21}H_{26}O_5^+$; calc. 358.1881) in HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectral data (see *Tables 3* and *2*) also revealed that **5** had the similar cassane-type skeleton as caesaljapin. The ^{1}H-NMR spectral data exhibited a CH–O group at δ(H) 5.38 (*d*, $J = 3.6$). The CH–O group showed HMBC correlations with C(9) (δ(C) 47.0), C(12) (δ(C) 144.7), C(13) (δ(C) 130.4), and C(20) (δ(C) 175.9), confirming that the location of the CH–O group was at C(11) and it was linked with C(20) by an ester bond. The configuration at C(11) was determined as β-OH by the cross-peak between H–C(9) (δ(H) 2.06, *dd*, $J = 12.0, 3.6$) and H–C(11) (δ(H) 5.38, *d*, $J = 3.6$) in ROESY experiments and the small coupling constant between them (*Fig. 2*). Thus, compound **5** was determined and named as caesalpinilinn.

Accordingly, as a result of this investigation, the structures of five new compounds from *Caesalpinia crista* were identified.

Experimental Part

General. All solvents used were of chemical grade (*Shanghai Chemical Plant*). TLC: precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): silica gel (SiO_2; 200–300 mesh); *MCI Gel CHP20P* (75–150 μm; *Mitsubishi Kasei Chemical Industries*); C_{18} reverse-phased SiO_2 (20–45 μm, *Fuji Silysia Chemical Ltd.*); *Sephadex LH-20* (*Pharmacia*). Optical rotations: *Perkin-Elmer* model *341* polarimeter. IR Spectra: *Bio-Rad-FT-IR* spectrophotometer, ν in cm^{-1}. NMR spectra: *Bruker AMX-500* spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C); conventional pulse sequences for ROESY, HSQC and HMBC; 200 ms mixing time for ROESY; chemical shifts δ in ppm, J in Hz; $CDCl_3$ and CD_3OD solns. HR-EI-MS: positive mode; *Bruker Atex III* spectrometer.

Plant Material. The seeds of *Caesalpinia crista* were collected in Zhejiang Province, P. R. China in May, 2006. A voucher specimen of the plant was identified by Mr. *Jin-Gui Shen* and deposited at the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Materia Medica, Shanghai.

Extraction and Isolation. The dried and powered seeds of *Caesalpinia crista* (10.0 kg) were extracted successively with MeOH at r.t. (3 × 5 l) overnight. The conc. extract was partitioned between $CHCl_3$ and H_2O. Evaporation of $CHCl_3$ left a dark residue (150 g). The residue was subjected to *MCI* gel *CHP 20P* CC, eluted with MeOH/H_2O (30:70, 70:60, 90:50, 100:0) to yield four subfractions (*Fr. A–D*). *Fr. B* (35 g) was subjected to SiO_2 (200–300 mesh), eluted with hexane/acetone (10:1, 5:1, 3:1, 1:1, acetone) to yield three subfractions (*Fr. B-1–B-3*). *Fr. B-1* (3.6 g) was chromatographed by *RP-18* flash CC, eluted with MeOH/H_2O (40:60) to afford compounds **1** (18 mg) and **2** (15 mg). *Fr. B-2* (0.8 g) was passed through a *Sephadex LH-20* column, eluted with MeOH to give **5** (13 mg). *Fr. B-3* (7.1 g) was subjected to *MCI* gel *CHP 20P* CC, eluted with MeOH/H_2O (60:40 and 70:30) to afford compound **3** (98 mg) and **4** (12 mg).

Caesalpinista A (= *Methyl (4*S*,4a*R*,5*R*,6a*S*,7*R*,11a*S*,11b*S*)-1,2,3,4,4a,5,6,6a,7,11,11a,11b-Dodecahydro-5-hydroxy-11b-(hydroxymethyl)-4,7-dimethylphenanthro[3,2-*b*]furan-4-carboxylate*; **1**). White amorphous powder. $[\alpha]_D^{20} = +0.076$ ($c = 0.105$, MeOH). IR (KBr): 3423, 2929, 1718, 1637, 1072. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. HR-ESI-MS: 362.2093 (M^+, $C_{21}H_{30}O_5^+$; calc. 362.2089).

Caesalpinista B (= *Methyl (4*S*,4a*R*,5*R*,6a*S*,7*R*,11a*S*,11b*S*)-11b-[(Acetoxy)methyl]-1,2,3,4,4a,5,6,6a,7,11,11a,11b-dodecahydro-5-hydroxy-4,7-dimethylphenanthro[3,2-*b*]furan-4-carboxylate*; **2**). White amorphous powder. $[\alpha]_D^{20} = +0.114$ ($c = 0.12$, MeOH). IR (KBr): 3416, 2921, 1746, 1638, 1062. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*. HR-ESI-MS: 404.2199 (M^+, $C_{23}H_{32}O_6^+$; calc. 404.2190).

Caesaljapin B (= *(4*S*,4a*S*,6a*S*,7*R*,11a*S*,11b*S*)-2,3,4,4a,5,6,6a,7,11,11a-Decahydro-4,7-dimethylphenanthro[3,2-*b*]furan-4,11b(1*H*)-dicarboxylic Acid*; **3**). White amorphous powder. $[\alpha]_D^{20} = +0.104$ ($c = 0.12$, MeOH). IR (KBr): 3411, 2968, 2927, 2862, 2642, 2239, 2077, 1693, 1646, 1454, 1276, 1242, 1053, 738. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. HR-ESI-MS: 346.1780 (M^+, $C_{20}H_{26}O_5^+$; calc. 346.1784).

Caesaljapin C (= *(3*S*,4*R*,4a*R*,6a*S*,7*R*,11a*S*,11b*S*)-3-(Acetyloxy)-2,3,4,4a,5,6,6a,7,11,11a-dodecahydro-4-(methoxycarbonyl)-4,7-dimethylphenanthro[3,2-*b*]furan-11b(1*H*)-carboxylic Acid*; **4**). White amorphous powder. $[\alpha]_D^{20} = +0.084$ ($c = 0.105$, MeOH). IR (KBr): 3428, 2956, 2931, 2869, 1735, 1646, 1448, 1369, 1249, 1143, 1028. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 3* and *2*. HR-ESI-MS: 418.1992 (M^+, $C_{23}H_{30}O_7^+$; calc. 418.1985).

Caesalpinilinn (= *Methyl (4*S*,4a*S*,6a*S*,7*R*,10b*R*,12a*R*,12b*S*)-2,3,4,4a,5,6,6a,7,10b,12b-Decahydro-4,7-dimethyl-12-oxo-1*H*-phenanthro[5,4a-*bc*:6,7-*b*']difuran-4-carboxylate*; **5**). White amorphous powder. $[\alpha]_D^{20} = +0.003$ ($c = 0.1$, MeOH). IR (KBr): 3434, 2935, 2863, 1762, 1724, 1452, 1738, 1253, 1124, 1072, 937. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 3* and *2*. HR-ESI-MS: 358.1886 (M^+, $C_{21}H_{26}O_5^+$; calc. 358.1881).

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