Cassane-Type Diterpenoids from the Seeds of *Caesalpinia magnifoliolata*

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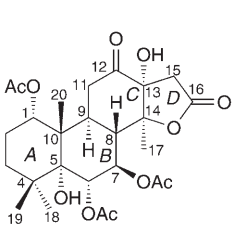
Two novel cassane-type diterpenes, named magnicaesalpin (**1**) and neocaesalpin O (**2**), together with three known compounds, caesalmin D (**3**), caesalmin E (**4**), and neocaesalpin L (**5**), were isolated from the MeOH extract of seeds of *Caesalpinia magnifoliolata* METC. Their structures were determined by spectroscopic methods, (1D- and 2D-NMR, HR-EI-MS) and X-ray analysis, as well as by comparison of their spectral data with those of related compounds.

Introduction. – *Caesalpinia magnifoliolata* METC. is widely distributed throughout the tropical and subtropical regions of Southeast Asia. The roots of the plant, which is called 'dayeyunshi' in China, are used in Chinese folk medicine for the treatment of rheumatism. It is known that the genus *Caesalpinia* is a rich source of cassane diterpenoids, and the molecular skeleton of this kind of diterpenoids is made up by three fused cyclohexane rings and a furan ring. Some of these diterpenoids have been found to display anti-inflammatory, anti-analgesic [1], radical growth regulation [2], and anticancer activities [3]. As a continuation of our study on this plant genus [4], we now report the isolation of two new cassane-type diterpenoids, magnicaesalpin and neocaesalpin O (**1** and **2**), along with the known caesalmins D, E [5], and L [4] (**3**, **4**, and **5**) from the seeds of *C. magnifoliolata* METC.

Results and Discussion. – Magnicaesalpin (**1**) was obtained as a colorless, optically active solid ($[\alpha]_D^{20} = +48$ ($c = 0.20$, MeOH)). Its HR-EI-MS showed the M^+ ion peak at m/z 524.2255, corresponding to the molecular formula $C_{26}H_{36}O_{11}$ with 9 degrees of unsaturation. The IR spectrum indicated the presence of C=O (1747 and 1790 cm^{-1}) and OH (3446 cm^{-1}) functions. From the ^{1}H- and ^{13}C-NMR data (*Table*), comparison with those of neocaesalpin L (**5**), the 2D-NMR experiments (*Fig. 1*), and by X-ray-analysis (*Fig. 2*), the structure of **1** was finally determined as 1α,6α,7β-triacetoxy-5α,13α-dihydroxy-12-oxocassane-16,14-lactone. This type of a γ-lactone was found for the first time among cassane diterpenes obtained from the genus *Caesalpinia*.

The ^{1}H-NMR spectrum of **1** showed signals of four Me groups at δ(H) 1.11, 1.12, 1.30, and 1.45, three AcO groups at δ(H) 2.06 (*s*), 2.06 (*s*), and 2.11 (*s*), and three

[1]) Both authors contributed equally to this work.



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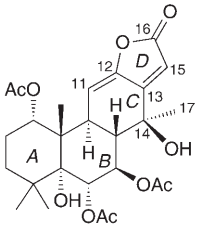

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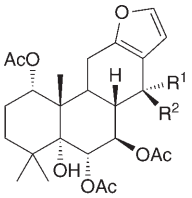

3 R^1 = OH, R^2 = Me
4 R^1 = Me, R^2 = OH

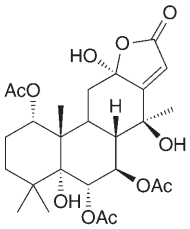

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Table. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, $CDCl_3$) *of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

	1		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
H–C(1)	4.78 (*s*)	74.3 (*d*)	5.03 (*s*)	73.8 (*d*)
H_α–C(2)	1.69–1.77 (*m*)[a])	22.2 (*t*)	1.72–1.80 (*m*)[a])	22.2 (*t*)
H_β–C(2)	1.80–2.01 (*m*)[a])		2.00–2.07 (*m*)[a])	
H_α–C(3)	1.13–1.18 (*m*)[a])	32.0 (*t*)	1.08–1.12 (*m*)[a])	32.0 (*t*)
H_β–C(3)	1.69–1.77 (*m*)[a])		1.69–1.76 (*m*)[a])	
C(4)		38.3 (*s*)		38.4 (*s*)
C(5)		79.0 (*s*)		79.0 (*s*)
H–C(6)	5.42 (br. *s*)	74.6 (*d*)	5.49 (br. *s*)	74.1 (*d*)
H–C(7)	5.72 (br. *s*)	71.3 (*d*)	5.66 (br. *s*)	71.5 (*d*)
H–C(8)	2.13–2.24 (*m*)[a])	47.5 (*d*)	2.27 (br. *s*)[a])	47.7 (*d*)
H–C(9)	2.30–2.50 (*m*)[a])	35.6 (*d*)	3.27 (*d*, J = 10.9)[a])	38.1 (*d*)
C(10)		44.7 (*s*)		44.8 (*s*)
CH_2(11)	2.30–2.50 (*m*)[a])	36.1 (*t*)		
H–C(11)			5.52 (*s*)	107.9 (*d*)
C(12)		206.0 (*s*)		149.7 (*s*)
C(13)		93.9 (*s*)		163.0 (*s*)
C(14)		83.1 (*s*)		72.2 (*s*)
CH_2(15)	3.12 (*d*, J = 17)[a]), 2.57 (*d*, J = 17)[a])	42.7 (*t*)		
H–C(15)			6.03 (*s*)	110.4 (*d*)
C(16)		171.6 (*s*)		168.9 (*s*)
Me(17)	1.45 (*s*)	12.8 (*q*)	1.46 (*s*)	22.2 (*q*)
Me(18)	1.11 (*s*)	24.4 (*q*)	1.06 (*s*)	29.6 (*q*)
Me(19)	1.12 (*s*)	30.2 (*q*)	1.08 (*s*)	23.4 (*q*)
Me(20)	1.30 (*s*)	16.7 (*q*)	1.18 (*s*)	18.7 (*q*)
*Ac*O–C(1)	2.11 (*s*)	21.0 (*q*), 169.0 (*s*)	2.08 (*s*)	21.0 (*q*), 168.7 (*s*)
*Ac*O–C(6)	2.06 (*s*)	21.0 (*q*), 170.0 (*s*)	1.97 (*s*)	21.0 (*q*), 170.1 (*s*)
*Ac*O–C(7)	2.06 (*s*)	21.4 (*q*), 170.4 (*s*)	2.04 (*s*)	21.3 (*q*), 170.2 (*s*)
HO–C(4)	2.60 (br. *s*)[a])		2.94 (*s*)[a])	
HO–C(13)	4.28 (*s*)[a])			
HO–C(14)			2.45 (*s*)[a])	

[a]) Assignment confirmed by COSY, HMBC, and HMQC experiments.

oxygenated CH groups at δ(H) 4.78 (*s*), 5.42 (br. *s*), and 5.72 (br. *s*). According to the ^{13}C-NMR and DEPT spectral data, **1** contained seven Me, four CH_2, and five CH groups (including three oxygenated CH units (δ(C) 74.3, 74.6, and 71.3)), five quaternary C-atoms (including three oxygenated quaternary C-atoms), and five C=O groups (including three AcO groups, a ketone, and a γ-lactone). By comparison of the spectroscopic data of **1** with those of neocaesalpin L (**5**), **1** has the same substructure as **5** concerning the *A* and *B* rings. The signals in the ^{1}H- and ^{13}C-NMR spectra for this fragment were almost identical to the counterpart of **5**, and this fragment (**1a**) was further confirmed by ^{1}H,^{1}H-COSY (H–C(1)/H–C(2), H–C(2)/H–C(3), and H–C(8)/H–C(9)), and HMBC (Me(18)/C(5), Me(19)/C(5), Me(20)/C(10), Me(20)/C(9), H–C(1)/C(9)) (*Fig. 1*). The remaining correlations in the HMBC spectrum of compound **1** are as follows: H–C(11)/C(10), H–C(11)/C(8), H–C(11)/C(12), H–C(15)/C(13), H–C(15)/C(14), H–C(15)/C(16), H–C(17)/C(13), H–C(17)/C(14), H–C(17)/C(8), HO–C(13)/C(12), HO–C(13)/C(13), HO–C(13)/C(15) (*Fig. 1*). The above-mentioned HMBC correlations led to the establishment of fragment **1b**. Thus, the most likely structure of **1** was determined to be 1α,6α,7β-triacetoxy-5α,13α-dihydroxy-12-oxocassane-16,14-lactone.

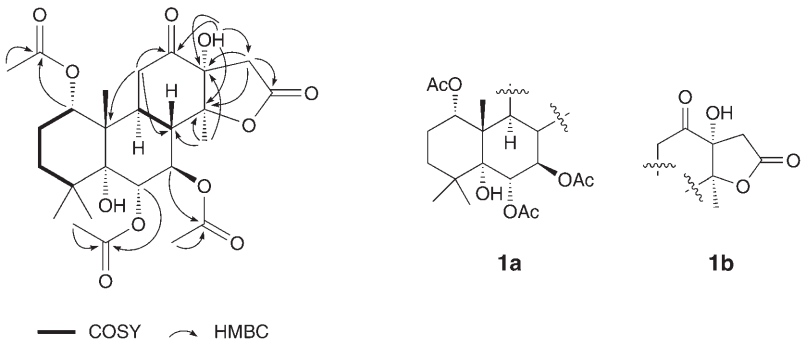

Fig. 1. *Key correlations in the HMBC and NOESY plots and fragment structures of magnicaesalpin* (**1**)

Compared to the typical type of 16,12-olide, like caesalmin D (**3**), caesalmin E (**4**), neocaesalpin L (**5**), this type of 16,14-olide has not been described so far among cassane diterpenes obtained from the genus *Caesalpinia*. Furthermore, the signal for C(17) (12.8 ppm) was uncommonly shifted upfield. Therefore, solid evidence, such as X-ray diffraction, was needed to validate the above deduction. Fortunately, after many attempts with different solvents, a colorless crystal of compound **1** was obtained from petroleum ether/AcOEt. The analysis of the single-crystal X-ray diffraction of the compound established *inter alia* the relative configuration at C(13) and C(14) (OH and Me(17) in α orientation) (*Fig. 2*).

Neocaesalpin O (**2**) was isolated as a colorless optically active solid ($[\alpha]_D^{20} = +27$ ($c = 0.20$, MeOH)). The molecular formula of **2** was determined as $C_{26}H_{34}O_{10}$ by HR-EI-MS at m/z 506.2156 (M^+). The UV absorption maximum at 276 nm (log $\varepsilon = 4.25$) indicated that it had an α,β-butenolide ring, conjugated with an additional C=C bond. This type of conjugated α,β-butenolide ring was found only in neocaesalpin D and I [6][7] among cassane diterpenes obtained from the genus *Caesalpinia*. The IR

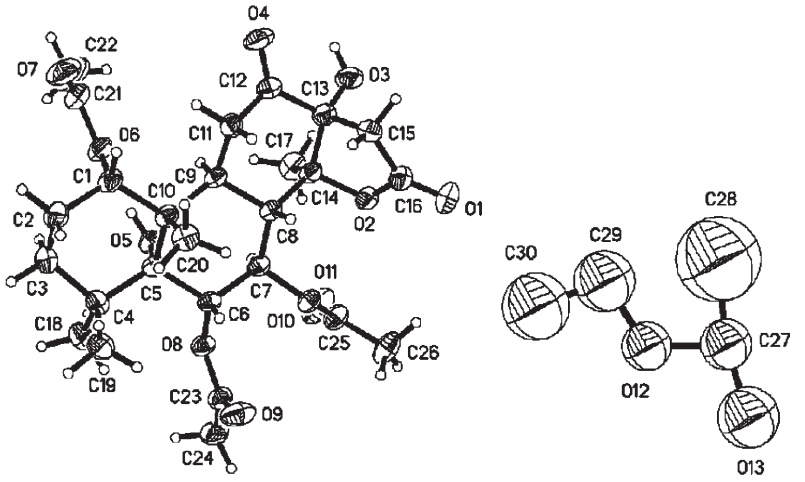

Fig. 2. *X-Ray structure of* **1** *showing relative configuration*

spectrum indicated the presence of C=O (1749 and 1782 cm^{-1}) and OH (3448 cm^{-1}) functions. On the basis of ^{1}H- and ^{13}C-NMR data (*Table*), comparison with the spectra of neocaesalpin L and neocaesalpin I [7], and 2D-NMR experiments (*Fig. 3*), neocaesalpin O (**2**) was identified as 1α,6α,7β-triacetoxy-5α,14β-dihydroxycassa-11,13(15)-diene-16,12-lactone.

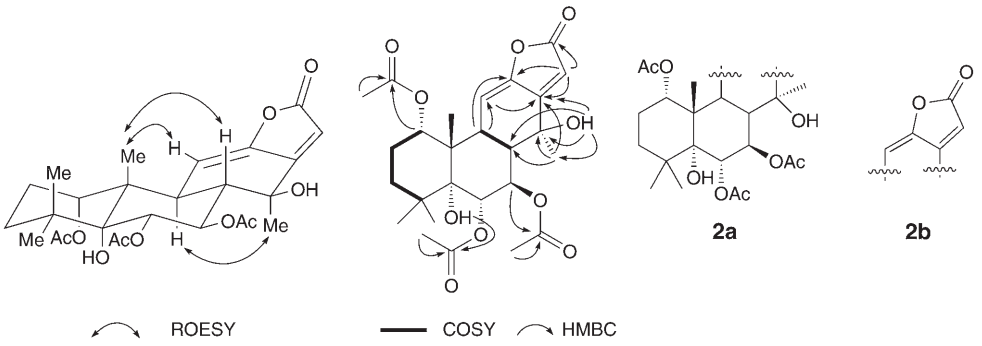

Fig. 3. *Key correlations in the COSY, HMBC, and ROESY plots and fragment structures of neocaesalpin O* (**2**)

The ^{1}H-NMR spectrum of **2** showed signals of four Me groups at δ(H) 1.06, 1.08, 1.18, and 1.46, three AcO groups at δ(H) 1.97 (*s*), 2.04 (*s*), and 2.08 (*s*), three CH−O groups at δ(H) 5.03 (*s*), 5.49 (br. *s*), and 5.66 (br. *s*), and two CH= groups at δ(H) 5.52 (*s*) and 6.03 (*s*). In the ^{13}C-NMR spectrum, the signals of two CH=, two C=, four C=O groups (including three AcO groups and a γ-lactone), three CH−O groups, and two oxygenated quaternary C-atoms were present, together with signals of four Me, two CH_2, and two CH groups, as well as two additional quaternary C-atoms. Just as in the

case of **1**, comparison of the spectroscopic data of **2** with those of neocaesalpin L (**5**) showed, that **2** has the same fragment **2a** as **5** containing the rings *A* and *B*, and C(17) (*Fig. 3*). Parts of the ^{1}H- and ^{13}C-NMR spectra were almost identical to the counterpart of **5**, and this fragment was further confirmed by ^{1}H,^{1}H-COSY (H–C(1)/H–C(2), H–C(2)/H–C(3), and H–C(8)/H–C(9)), and HMBC (Me(17)/C(14), Me(17)/C(8), Me(20)/C(10), Me(20)/C(9), H–C(1)/C(9), HO–C(14)/C(14), HO–C(14)/C(17), HO–C(14)/C(8)) (*Fig. 3*). The remaining signals of the ^{1}H- and ^{13}C-NMR spectra were almost identical to the counterpart of neocaesalpin I containing the unsaturated part and ring *D* (**2b**) (*Fig. 3*). This fragment was further confirmed by HMBC (H–C(11)/C(12), H–C(11)/C(13), H–C(15)/C(13), H–C(15)/C(14), H–C(15)/C(16)) (*Fig. 3*). The above considerations led to the establishment of fragments **2a** and **2b**. Thus, **2** was determined as 5α,14β-dihydroxy-1α,6α,7β-triacetoxy-11,13(15)-cassadien-16,12-olide. This was confirmed by the following correlations in the HMBC spectrum, H–C(17)/C(13), H–C(17)/C(14), H–C(17)/C(8), H–C(9)/C(11), H–C(9)/C(12), and HO–C(14)/C(13). There were strong correlations of Me(17) (δ(H) 1.46 (*s*)) with H–C(9) (δ(H) 3.27 (*d*, $J = 10.9$)) in the ROESY plot (*Fig. 3*). This indicated that Me(17) was α-oriented.

Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai, P. R. China). Column chromatography (CC): SiO_2 *H* (200–300 mesh; *Qingdao Marine Chemical Ltd.*, Qingdao, P. R. China), *MCI* gel *CHP 20P* (75–150 μm; *Mitsubishi Chemical Ind.*, Tokyo, Japan), TLC: SiO_2 GF_{254} (*Yantai Huiyou Inc.*, Yantai, P. R. China). UV Spectra: *Shimadzu UV-2450* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Perkin-Elmer 16 PC* FT-IR spectrophotometer; in cm^{-1}. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra: *Bruker AMX-400* spectrometer; δ in ppm, *J* in Hz, with TMS as internal standard. HR-EI-MS and EI-MS: *Finnigan/MAT 90/95* sector-field mass spectrometer; in *m*/*z*.

Plant Material. The seeds of *C. magnifoliolata* were collected from Dali City, Yunnan Province, P. R. China, and identified by Prof. *Xiao-Kuang Ma* (Department of Pharmaceutical Chemistry, Dali College). A voucher specimen was deposited at the Shanghai Research Center for Modernization of Traditional Chinese Medicine, Shanghai Institute of Material Medica, Shanghai.

Extraction and Isolation. The powdered seeds (5.0 kg) of *Caesalpinia magnifoliolata* were extracted with MeOH at r.t., which afforded a dark residue (624 g) after evaporation under reduced pressure. The residue was suspended in dist. H_2O and extracted successively with petroleum ether (PE) and $CHCl_3$. The $CHCl_3$ extract (72.5 g) was subjected to CC (SiO_2 *H*, 200–300 mesh; PE/acetone 15:1, 10:1, 5:1, 3:1, 1:1): five fractions (*Fr. A–E*). *Fr. B* (4.2 g) was subjected to CC (SiO_2 *H*, 200–300 mesh; PE/AcOEt 8:1) to provide **3** (10.7 mg) and **4** (56.4 mg). *Fr. C* (5.2g) was subjected to CC (*MCI* gel *CHP 20P*; MeOH/H_2O 40:60, 50:50, 60:40, 70:30, 80:20, and 100:0): six subfractions (*Fr. C.1–C.6*). *Fr. C.2* was resubjected to CC (SiO_2 *H*, 200–300 mesh; $CHCl_3$/MeOH 50:1) to provide **2** (12.3 mg). *Fr. D* (4.9 g) was subjected to CC (*MCI* gel *CHP 20P*; MeOH/H_2O 50:50, 60:40, 70:30, 80:20, and 100:0): five subfractions (*Fr. D.1–D.5*). *Fr. D.2* was resubjected to CC (SiO_2 *H*, 200–300 mesh; $CHCl_3$/MeOH 40:1) to provide **1** (76.2 mg) and **5** (107.3 mg).

Magnicaesalpin (= *1α,6α,7β-Triacetoxy-5α,13α-dihydroxy-12-oxocassane-16,14-lactone*; *(3a*S*,3b*S*,4*R*,5*S*,5a*R*,9*S*,9a*S*,9b*S*,11a*R*)-5a,11a-Dihydroxy-3a,6,6,9a-tetramethyl-2,11-dioxohexadecahydrophenanthro[1,2-*b*]furan-4,5,9-triyl Triacetate*; **1**): Colorless powder. M.p. 209–211° (MeOH). $[\alpha]_D^{20} = +48$ ($c = 0.20$, MeOH). IR (KBr): 3560, 3446, 1790, 1747, 1377, 1244, 1032, 951. ^{1}H- and ^{13}C-NMR: *Table.* HR-EI-MS: 524.2255 (M^+, $C_{26}H_{36}O_{11}^+$; calc. 524.2258).

Neocaesalpin O (=*1α,6α,7β-Triacetoxy-5α,14β-dihydroxycassa-11,13(15)-diene-16,12-lactone*; *(1*S*,4a*R*,5*S*,6*R*,7*S*,11a*S*,11b*S*)-1,2,3,4,4a,5,6,6a,7,9,11a,11b-Dodecahydro-4a,7-dihydroxy-4,4,7,11b-tetramethyl-9-oxophenanthro[3,2-*b*]furan-1,5,6-triyl Triacetate*; **2**): Colorless powder. M.p. 159–162°

(MeOH). $[\alpha]_D^{20} = +27$ ($c = 0.20$, MeOH). UV (MeOH): 276 (4.25). IR (KBr): 3448, 1782, 1749, 1375, 1236, 1032. ^{1}H- and ^{13}C-NMR: *Table*. HR-EI-MS: 506.2156 (M^+, $C_{26}H_{34}O_{10}^+$; calc. 506.2152).

X-Ray Crystal-Structure Analysis of **1**[2]). Colorless cubic crystals of **1** were obtained by recrystallization in PE/AcOEt. Crystal dimensions: $0.488 \times 0.397 \times 0.251$ mm. Crystal system: orthorhombic. Formula $C_{30}H_{44}O_{13}$; M_r 612.65. Space group $P2_12_12_1$ with unit cell dimensions $a = 10.781(6)$, $b = 12.712(7)$, $c = 23.524(14)$ Å; $\alpha = \beta = \gamma = 90.0°$; $V = 3224(3)$ Å^3; $Z = 4$; and $\rho_{calc} = 1.262$ mg m^{-3}. A total of 19065 reflections were collected to a maximum 2θ value of 54.00° by using the Φ/ω scan technique at 293(2) K. The structure was solved by using direct methods and was refined by means of the full-matrix least-squares procedure. The collection data were reduced by using the Saint program [8], and the empirical absorption correction was performed by using the Sadabs program [9]. All non-H-atoms were given anisotropic thermal parameters. The H-atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R = 0.0796$, $wR = 0.2090$ for 3941 observed reflections ($I > 2\sigma(I)$) and 360 variable parameters.

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[2]) CCDC-670490 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ, UK; fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).