Three New Cassane-Type Diterpenes from *Caesalpinia minax*

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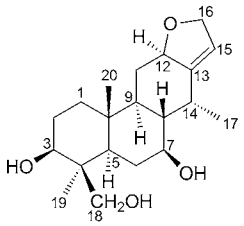
Three new cassane-type diterpenes, 14-dehydroxy-12,16-dihydrocaesaldekarin L (**1**), 1-deacetyl-12-ethoxyneocaesalpin N (**2**), and 1-deacetylneocaesalpin N (**3**), together with two known cassane-type diterpenes, neocaesalpin A and neocaesalpin L, were isolated from the EtOH extract of the twigs and leaves of *Caesalpinia minax*. Their structures were elucidated by spectroscopic methods, as well as by comparison of their spectral data with those of related compounds.

Introduction. – Due to the characteristic cassane diterpenoids and homoisoflavonoids they contain, the plants of *Caesalpinia* have attracted wide interest. The cassane diterpenoids and homoisoflavonoids display restricted occurence in the plants, and their structures and bioactivities are diverse [1][2]. The seeds of *Caesalpinia minax* HANCE, called '*ku-shi-lian*', have long been used as Chinese folk medicine for the treatment of common cold, influenza, fever, rheumatism, and dysentery [3]. Previously, it was demonstrated that 'ku-shi-lian' is a rich source of cassane-type diterpenes [4]. In the course of our studies on novel cassane-type diterpenes, three new representatives, 14-dehydroxy-12,16-dihydrocaesaldekarin L (**1**; *Fig. 1*), 1-deacetyl-12-ethoxyneocaesalpin N (**2**), and 1-deacetylneocaesalpin N (**3**), along with two known cassane-type diterpenes, neocaesalpin A [5] (**4**) and neocaesalpin L [6] (**5**), were isolated from the twigs and leaves of *C. minax*. Herein, we report the structure elucidation of these new diterpenes.



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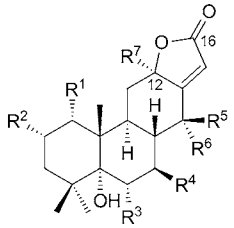

2 $R^1 = R^4 = OH$, $R^2 = R^3 = R^6 = H$, $R^5 = CO_2Me$, $R^7 = EtO$
3 $R^1 = R^4 = R^7 = OH$, $R^2 = R^3 = R^6 = H$, $R^5 = CO_2Me$
4 $R^1 = R^2 = AcO$, $R^3 = R^4 = H$, $R^5 = R^7 = OH$, $R^6 = Me$
5 $R^1 = R^3 = R^4 = AcO$, $R^2 = H$, $R^5 = R^7 = OH$, $R^6 = Me$

Fig. 1. *Structures of compounds* **1**–**5**

Results and Discussion. – 14-Dehydroxy-12,16-dihydrocaesaldekarin L (**1**; *Fig. 1*) was obtained as colorless amorphous solid. Its HR-EI-MS showed the M^+ ion peak at m/z at 336.2306, corresponding to the molecular formula $C_{20}H_{32}O_4$ with five degrees of unsaturation. The IR absorption at 3440 cm^{-1} indicated the presence of OH groups. The ^{1}H-NMR spectrum (*Table 1*) of **1** displayed signals due to a secondary and two tertiary Me, two CH_2O, three CH–O groups, and an olefinic H-atom. Moreover, the ^{13}C-NMR spectrum (*Table 2*) exhibited signals of two olefinic C-atoms (δ(C) 143.7, 114.5), three Me (δ(C) 14.6, 14.1, 11.9), two CH_2O (δ(C) 69.6, 67.5), four CH_2, three CH–O (δ(C) 73.0, 76.5, 76.5), four CH groups, and two quaternary C-atoms. These spectral data suggested that the structure of compound **1** was similar to that of the known compound caesaldekarin L [7], except for the absence of the OH group at C(14), and the presence of a dihydrogenated furan ring between C(12) and C(16) in **1**. The above conclusion was confirmed by the HMBCs from H–C(15) (δ(H) 5.66) to C(16) (δ(C) 69.6), C(12) (δ(C) 76.5), and C(14) (δ(C) 40.8), and from H–C(14) (δ(H) 2.44) to C(12), C(13) (δ(C) 143.7), C(15) (δ(C) 114.5), C(17) (δ(C) 14.6), and C(8) (δ(C) 39.6). The ROESY correlations of H–C(12) (δ(H) 4.69) with H–C(9) δ(H)

Table 1. *^{1}H-NMR Data* (500 MHz) *of* **1** (in $CDCl_3$), *and* **2** *and* **3** (in CD_3OD). δ in ppm, J in Hz.

H-Atom	**1**	**2**	**3**
CH_2(1) or H–C(1)	1.72 (*td*, J = 13.0, 3.5), 1.12 (*m*)	3.72 (br. *s*)	3.74 (br. *s*)
CH_2(2)	1.62 – 1.57 (*m*), 1.87 (*m*)	2.04 – 2.08 (*m*), 1.63 (*d*, J = 13.5)	2.04 – 2.08 (*m*), 1.65 – 1.68 (*m*)
H–C(3) or CH_2(3)	3.67 (*dd*, J = 11.5, 4.5)	2.02 – 2.05 (*m*), 1.04 (*m*)	2.00 – 2.05 (*m*), 1.08 (*m*)
H–C(5)	1.27 – 1.30 (*m*)		
CH_2(6)	1.58 – 1.63 (*m*), 1.35 – 1.37 (*m*)	1.92 (*dd*, J = 13.5, 5.5), 1.65 (*d*, J = 13.5)	1.98 (*dd*, J = 13.5, 5.0), 1.65 – 1.68 (*m*)
H–C(7)	4.72 (*m*)	3.80 (*td*, J = 10.5, 5.5)	3.85 (*td*, J = 10.5, 5.0)
H–C(8)	1.55 – 1.62 (*m*)	1.97 – 2.02 (*m*)	2.00 – 2.05 (*m*)
H–C(9)	1.32 – 1.35 (*m*)	2.81 (*td*, J = 12.5, 3.5)	2.88 (*td*, J = 12.5, 3.5)
CH_2(11)	1.58 – 1.62 (*m*), 1.35 – 1.38 (*m*)	2.60 (*dd*, J = 12.5, 3.5), 1.38 (*t*, J = 12.5)	2.51 (*dd*, J = 12.5, 3.0), 1.44 (*t*, J = 12.5)
H–C(12)	4.69 (*m*)		
H–C(14)	2.44 (*dq*, J = 7.5, 4.5)	3.19 (*dd*, J = 10.5, 2.0)	3.35 – 3.33 (*m*)
H–C(15)	5.66 (*m*)	5.79 (*d*, J = 2.0)	5.69 (*d*, J = 1.5)
CH_2(16)	4.63 (*d*, J = 16.0), 4.31 (*d*, J = 16.0)		
H–C(17)	0.98 (*d*, J = 7.5)		
CH_2(18) or Me(18)	3.70 (*d*, J = 10.5), 3.30 (*d*, J = 10.5)	0.99 (*s*)	1.02 (*s*)
Me(19)	0.76 (*s*)	1.04 (*s*)	1.07 (*s*)
Me(20)	0.88 (*s*)	0.97 (*s*)	1.00 (*s*)
CH_2O–C(12)		3.69 (*qd*, J = 7.0, 2.0), 3.44 (*qd*, J = 7.0, 2.0)	
MeCH_2O–C(12)		1.22 (*t*, J = 7.0)	
MeO–C(17)		3.77 (*s*)	3.76 (*s*)

Table 2. *^{13}C-NMR Data* (125 MHz) *of* **1** (in $CDCl_3$), *and* **2** *and* **3** (in CD_3OD). δ in ppm.

C-Atom	**1**	**2**	**3**
C(1)	37.3 (*t*)	72.7 (*d*)	72.9 (*d*)
C(2)	26.9 (*t*)	27.0 (*t*)	26.8 (*t*)
C(3)	73.0 (*d*)	31.0 (*t*)	31.1 (*t*)
C(4)	42.2 (*s*)	39.5 (*s*)	39.5 (*s*)
C(5)	47.0 (*d*)	80.7 (*s*)	80.8 (*s*)
C(6)	30.7 (*t*)	37.2 (*t*)	37.3 (*t*)
C(7)	76.5 (*d*)	73.0 (*d*)	72.7 (*d*)	
C(8)	39.6 (*d*)	49.3 (*d*)	49.5 (*d*)
C(9)	45.7 (*d*)	36.7 (*d*)	37.6 (*d*)
C(10)	36.4 (*s*)	44.2 (*s*)	44.2 (*s*)
C(11)	20.9 (*t*)	35.7 (*t*)	37.1 (*t*)
C(12)	76.5 (*d*)	109.0 (*s*)	106.8 (*s*)
C(13)	143.7 (*s*)	167.4 (*s*)	168.6 (*s*)
C(14)	40.8 (*d*)	50.4 (*d*)	50.1 (*d*)
C(15)	114.5 (*d*)	116.4 (*d*)	114.8 (*d*)
C(16)	69.6 (*t*)	171.5 (*s*)	172.1 (*s*)
C(17)	14.6 (*q*)	173.6 (*s*)	173.9 (*s*)
C(18)	67.5 (*t*)	28.5 (*q*)	28.5 (*q*)
C(19)	11.9 (*q*)	25.2 (*q*)	25.2 (*q*)
C(20)	14.1 (*q*)	17.7 (*q*)	17.6 (*q*)
CH_2O–C(12)		60.3 (*t*)	
$MeCH_2O$–C(12)		15.6 (*q*)	
*Me*O–C(17)		52.7 (*q*)	52.6 (*q*)

1.32 – 1.35 and H–C(17) (δ(H) 0.98) indicated that H–C(12) was α-oriented (*Fig. 2*). Thus, the structure of **1** was determined as depicted in *Fig. 1*.

1-Deacetyl-12-ethoxyneocaesalpin N (**2**) was obtained as colorless amorphous solid. Its HR-EI-MS showed the M^+ ion peak at *m*/*z* at 438.2245, corresponding to the

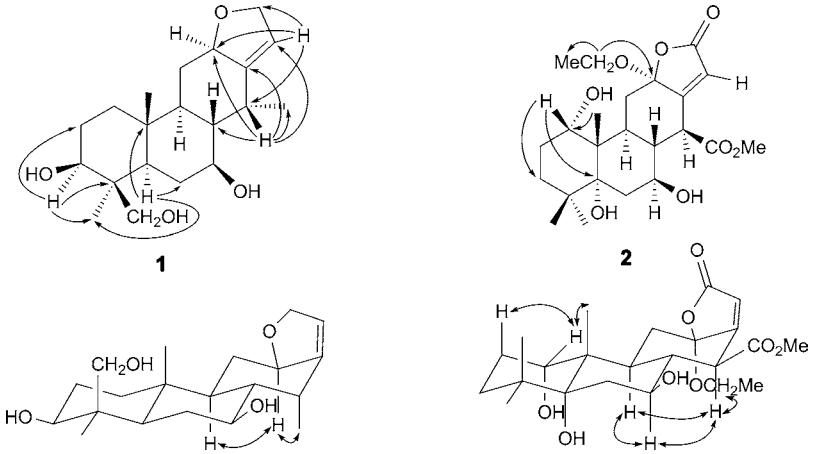

Fig. 2. *Key HMBCs* (H → C) *and NOESY* (H ↔ H) *correlations of* **1** *and* **2**

molecular formula $C_{23}H_{34}O_8$ with seven degrees of unsaturation. The IR absorptions at 3488, 3262, and 1746 cm^{-1} indicated the presence of OH and C=O groups. The ^{1}H-NMR spectrum (*Table 1*) of **2** showed three Me *singlet*s at δ(H) 0.97, 0.99, and 1.04, one Me *triplet* at δ(H) 1.22, one sharp *singlet* at δ(H) 3.77 due to a MeO group, and signals of one CH_2O group at δ(H) 3.69 (*td*, $J = 7.0$, 2.0, 1 H) and 3.44 (*td*, $J = 7.0$, 2.0, 1 H), of two CH–O groups at δ(H) 3.72 and 3.80, and of an olefinic CH group at δ(H) 5.79. The ^{13}C-NMR spectrum (*Table 2*) of **2** exhibited signals of five Me (including one MeO (δ(C) 52.7)), five CH_2 (including one CH_2O (δ(C) 60.3)), six CH (including one olefinic CH (δ(C) 116.4)), and two CH–O groups (δ(C) 72.7 and 73.0)), of seven quaternary C-atoms (including one olefinic quaternary C-atom (δ(C) 167.4)), one O-bearing quaternary C-atom (δ(C) 80.7), one hemiketal quaternary C-atom (δ(C) 109.0)), and of two C=O groups (including one ester C=O group (δ(C) 173.6) and a γ-lactone (δ(C) 171.5)). The ^{1}H- and ^{13}C-NMR data were almost identical to those of neocaesalpin N [5], except that AcO group at C(1) of neocaesalpin N was replaced by a OH group in **2**, and OH group at C(12) of neocaesalpin N was replaced by an EtO group in **2**. The HMBCs (*Fig. 2*) of H–C(1) (δ(H) 3.72) with C(3) (δ(C) 31.0), C(10) (δ(C) 44.2), and C(5) (δ(C) 80.7), as well as of MeCH_2O (δ(H) 3.69, 3.44) with *Me*CH_2O (δ(C) 15.6) and C(12) (δ(C) 109.0) indicated that the OH group was located at C(1), and EtO group was at C(12). The NOESY correlations of H–C(14) (δ(H) 3.19) with H–C(7) (δ(H) 3.80), H–C(9) (δ(H) 2.81) and MeCH_2O indicated that the 7-OH group was β-oriented, and EtO group was in an α-position. NOESY Correlation of H–C(1) (δ(H) 3.72) with Me(20) (δ(H) 0.97) revealed the α-orientation of OH at C(1). Thus, the structure of **2** was determined.

We strongly suspect that compound **2** was an artifact of the isolation, arising from the acetalization of compound **3** (see below) with the EtOH used during the isolation procedure. When compound **2** was dissolved in EtOH under acidic conditions, and the solutions was mixed with silica gel and placed in an oil bath at 70° for 24 h, compound **3** was detected in the solution by TLC with **3** as control. Thus, compound **2** should be an artifact product during the isolation procedure.

1-Deacetylneocaesalpin N (**3**) was obtained as colorless amorphous solid. Its HR-EI-MS showed the M^+ ion peak at m/z at 410.1935, corresponding to the molecular formula $C_{21}H_{30}O_8$ with seven degrees of unsaturation. The IR spectrum indicated the presence of OH (3432 cm^{-1}) and C=O (1745 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR data (*Tables 1* and *2*) were closely similar to those of **2**. The only difference between them was that the EtO group of **2** was replaced by a OH group in **3**. Therefore, the structure of **3** was determined as depicted in *Fig. 1*.

Experimental Part

General. All solvents used were of industrial grade. TLC: Precoated silica-gel GF_{254} plates (*Qingdao Marine Chemical Factory*). Column chromatography (CC): silica gel (SiO_2; 100–200 or, 200–300 mesh; *Qingdao Marine Chemical Factory*) and *Sephadex LH-20* (*GE Healthcare*). Optical rotations: *Horiba-SEAP*-300 spectropolarimeter. IR Spectra: *Bruker Tensor 27* FT-IR polarimeter; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX-AV-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-EI-MS: *AutoSpec Premier P776* spectrometer; in m/z. ESI-MS: *API QSTAR* time-of-flight (TOF) spectrometer; in m/z.

Plant Material. The materials were collected from Xishuangbanna, Yunnan Province, P. R. China, in June 2010, and identified by *Jing-Yun Cui*, Xishuangbanna Botanical Garden, Chinese Academy of Sciences. A voucher specimen was deposited with the Department of Chemistry and Chemical Engineering, Yunnan Normal University.

Extraction and Isolation. The powdered twigs and leaves (5 kg) of *Caesalpinia minax* were extracted with EtOH at r.t. to afford a dark residue (385 g) after evaporation under reduced pressure. The residue was dissolved in H_2O and extracted with AcOEt. The AcOEt extract (150 g) was subjected to CC (SiO_2 (100–200 mesh); petroleum ether/AcOEt 10:1, 5:1, 3:1, and 1:1): ten fractions, *Frs. A–J. Fr. E* (2.3 g) was subjected to CC (SiO_2; petroleum ether/acetone 5:1, 2:1): four subfractions, *Frs. E1–E4*. *Fr. E2* was resubjected to CC (SiO_2, $CHCl_3$/acetone 10:1, petroleum ether/acetone 4:1; *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to provide compound **1** (6.1 mg). *Fr. H* (8.2 g) was subjected to CC (SiO_2; petroleum ether/acetone 3:1): three subfractions, *Frs. H1–H3*. *Fr. H2* was resubjected to CC (SiO_2; petroleum ether/acetone 3:1, $CHCl_3$/MeOH 50:1, and petroleum ether/AcOEt 3:2; *Sephadex LH-20*; $CHCl_3$/MeOH 1:1) to provide compound **2** (12.6 mg). *Fr. I* (21.8 g) was separated by CC (SiO_2; $CHCl_3$/MeOH 30:1): six subfractions, *Frs. I1–I6*. *Fr. I5* was purified by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1; SiO_2; $CHCl_3$/acetone 3:1) to provide compounds **4** (123.9 mg), **3** (18.3 mg), and **5** (24.5 mg).

14-Dehydroxy-12,16-dihydrocaesaldekarin L (= *(3*S*,4*S*,4a*R*,6*S*,6a*S*,7*R*,10a*R*,11a*S*,11b*R*)-4-(Hydroxymethyl)-4,7,11b-trimethyl-1,2,3,4,4a,5,6,6a,7,9,10a,11,11a,11b-tetradecahydrophenanthro[3,2-*b*]furan-3,6-diol*; **1**). Colorless solid. $[\alpha]_D^{23} = -19.5$ ($c = 0.10$, $CHCl_3$). IR (KBr): 3440, 2922, 2851, 1631, 1040. ^{1}H and ^{13}C-NMR ($CDCl_3$): *Tables 1* and *2*, resp. ESI-MS: 359 ($[M+Na]^+$). HR-EI-MS: 336.2306 (M^+, $C_{20}H_{32}O_4^+$; calc. 336.2301).

1-Deacetyl-12-ethoxyneocaesalpin N (= *Methyl (1*S*,4a*R*,6*S*,6a*R*,7*S*,10a*R*,11a*S*,11b*S*)-10a-Ethoxy-1,4a,6-trihydroxy-4,4,11b-trimethyl-9-oxo-1,2,3,4,4a,5,6,6a,7,9,10a,11,11a,11b-tetradecahydrophenanthro[3,2-*b*]furan-7-carboxylate*; **2**). Colorless solid. $[\alpha]_D^{24.6} = -47.6$ ($c = 0.18$, MeOH). IR (KBr): 3488, 3262, 2958, 1746, 1651, 1086. ^{1}H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*, resp. ESI-MS: 461 ($[M+Na]^+$). HR-EI-MS: 438.2245 (M^+, $C_{23}H_{34}O_8^+$; calc. 438.2254).

1-Deacetylneocaesalpin N (= *Methyl (1*S*,4a*R*,6*S*,6a*R*,7*S*,10a*R*,11a*S*,11b*S*)-1,4a,6,10a-tetrahydroxy-4,4,11b-trimethyl-9-oxo-1,2,3,4,4a,5,6,6a,7,9,10a,11,11a,11b-tetradecahydrophenanthro[3,2-*b*]furan-7-carboxylate*; **3**). Colorless solid. $[\alpha]_D^{24.8} = -34.2$ ($c = 0.20$, MeOH). IR (KBr): 3432, 2951, 1745, 1651, 1036. H- and ^{13}C-NMR (CD_3OD): *Tables 1* and *2*. ESI-MS: 433 ($[M+Na]^+$). HR-EI-MS: 410.1935 (M^+, $C_{21}H_{30}O_8^+$; calc. 410.1941).

REFERENCES

[1] R. Maurya, M. Ravi, S. Singh, P. P. Yadav, *Fitoterapia* **2012**, *83*, 272.
[2] H.-B. Jiang, J. Huang, M.-J. Guo, P. Zou, X.-Q. Tian, *Acta Pharm. Sin.* **2007**, *42*, 118.
[3] State Administration of Traditional Chinese Medicine Chinese Materia Medica Editorial Committee, 'The Chinese Materia Medica', Shanghai Scientific and Technical Publishers, Shanghai, **1999**, *4*, 374.
[4] M. Wu, Y.-F. Wang, M.-L. Zhang, C.-H. Huo, M. Dong, Q.-W. Shi, H. Kiyota, *Chem. Biodiversity* **2011**, *8*, 1370.
[5] T. Kinoshita, M. Kaneko, H. Noguchi, I. Kitagawa, *Heterocycles* **1996**, *43*, 409.
[6] D.-M. Li, L. Ma, G.-M. Liu, L.-H. Hu, *Chem. Biodiversity* **2006**, *3*, 1260.
[7] D. L. Lyder, S. R. Peter, W. F. Tinto, S. M. Bissada, S. McLean, W. F. Reynolds, *J. Nat. Prod.* **1998**, *61*, 1462.

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