A New Alkaloid from the Seeds of *Sophora alopecuroides* L.

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Alopecurin A, an alkaloid with an unprecedented skeleton, was isolated from the seeds of *Sophora alopecuroides* L. The absolute configuration and structure of this compound was identified as (3*S*,12*R*)-3-hydroxy-1,7-diazatricyclo[10.4.0.0^{3,7}]heptadecane-11,16,17-trione (=(7*S*,15a*R*)-decahydro-7-hydroxy-6*H*-7,11-methano-4*H*-pyrido[1,2-*a*][1,7]diazacyclododecine-4,15,16(12*H*)-trione). The structure and absolute configuration was elucidated by spectroscopic methods, mainly HR-ESI-TOF-MS, IR, 1D-NMR (^{1}H- and ^{13}C-NMR), 2D-NMR (COSY, NOESY, HSQC, HMBC), and particularly X-ray crystal-diffraction and CD spectral analysis.

Introduction. – *Sophora alopecuroides* L. (Fabaceae) is an important traditional Chinese herbal plant, namely Ku-Dou-Zi in Chinese. It is widely distributed in the northwest of China, especially in Xinjiang. The seeds of *S. alopecuroides* L. are used for the treatment of eczema, acute pharyngolaryngeal infection, sore throat, acute dysentery, and gastrointestinal hemorrhage [1–3].

Phytochemical investigations of this plant have revealed that there exist more than twenty chemical compounds, belonging to alkaloids, flavonoids, volatile oils, organic acids, amino acids, proteins, and saccharides. Among these chemical constituents, the principal bioactive constituents of *S. alopecuroides* L. are quinolizidine alkaloids, which have been shown to exhibit sedative, depressant, analgesic, antipyretic, and cardiotonic [4], and especially antitumor activities [3] and to improve immunity [5]. Two types of quinolizidine alkaloids, matrine-type and pyridone quinolizidine bases, are found in *Sophora* species and can be used for the identification of *Sophora* species in commercial preparations [6].

Currently, there is much interest in exploiting bioactive constituents from *S. alopecuroides* L. In this article, we report the isolation and structural elucidation of a new alkaloid, named alopecurin A. Furthermore, our previous study investigated the content of alopecurin A and its dynamic accumulation in *S. alopecuroides* L. [7]. To the best of our knowledge, this is the first report on the isolation of this alkaloid featuring a new skeleton.

Results and Discussion. – Alopecurin A[1]) (*Fig. 1*) was isolated as colorless prisms and showed the molecular formula $C_{15}H_{22}N_2O_4$ as determined by HR-ESI-TOF-MS

[1]) Arbitrary atom numbering and *von Baeyer* name, respectively; for the systematic name, see *Exper. Part.* A convenient *von Baeyer* name would be (1*S*,8*R*)-1-hydroxy-3,13-diazatricyclo[11.3.1.0^{3,8}]-heptadecane-4,9,17-trione.

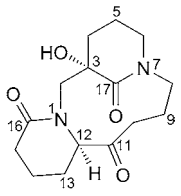

Fig. 1. *Alopecurin A*[1]), *isolated from* Sophora alopecuroides L.

(*m/z* 589.3260 ($[2M+H]^+$, $C_{30}H_{45}N_4O_8^+$) and 295.1655 ($[M+H]^+$, $C_{15}H_{23}N_2O_4^+$)). Combined with the NMR data, a degree of unsaturation of six was established. The IR absorptions revealed the presence of OH (3283 cm^{-1}), C=O (1706 cm^{-1}), and conjugated-amide C=O (1623 cm^{-1}) functionalities, besides stretching vibrations of C–H (2954, 2926, and 2855 cm^{-1}). The ^{13}C-NMR spectra (*Table 1*) exhibited 15 C-atom signals, which were classified by their chemical shifts and the HSQC spectrum as three C=O groups (δ(C) 171.1 (C(16)), 172.3 (C(17)), and 208.3 (C(11))), one sp^3 quaternary C-atom, and one sp^3 CH and ten sp^3 CH_2 groups. Among them, three sp^3 CH_2 groups (δ(C) 47.8 (C(8)), 48.3 (C(6)), and 55.0 (C(2))) were ascribed to bearing an N-atom, while the sp^3 CH group (δ(C) 66.1 (C(12))) was ascribed to bearing an N-atom, and the sp^3 quaternary C-atom (δ(C) 72.4 (C(3))) was substituted by an O-atom. The ^{1}H-NMR spectra of alopecurin A (*Table 1*) resolved some H-atom signals, which were classified by their chemical shifts and the HSQC spectrum as one OH group (δ(H) 2.95 (br. *s*, OH–C(3))), an sp^3 CH group (δ(H) 4.39 (br. *d*, H–C(12))), and three sp^3 CH_2 groups (δ(H) 4.60 (*td*, J = 13.2, 3.0 Hz, 1 H–C(8)) and 2.57 (*m*, 1 H–C(8)); δ(H) 3.25 (*m*,

Table 1. *^{1}H- and ^{13}C-NMR, HMBC, and ^{1}H,^{1}H-COSY Data ($CDCl_3$) of Alopecurin A*[1])

Position	δ(C)[a])	δ(H)[b])	HMBC	^{1}H,^{1}H-COSY
CH_2(2)	55.0	4.50, 3.12 (2*d*, each J = 13.2)	C(4), C(12), C(3), C(17)	
C(3)	72.4			
CH_2(4)	29.1	1.81–1.87, 1.75–1.80 (2*m*)	C(5), C(6), C(2), C(3)	CH_2(5)
CH_2(5)	18.4	1.81–1.87, 1.65–1.74 (2*m*)		CH_2(4), CH_2(6)
CH_2(6)	48.3	3.20–3.28 (*m*), 3.00 (*d*, J = 10.8)	C(5), C(4), C(17)	CH_2(5)
CH_2(8)	47.8	4.60 (*td*, J = 13.2, 3.0), 2.45–2.57 (*m*)	C(13), C(9), C(10), C(6), C(17), C(11)	H–C(9)
CH_2(9)	25.3	1.75–1.80, 1.65–1.74 (2*m*)	C(10), C(8)	CH_2(8), CH_2(10)
CH_2(10)	36.5	2.65–2.74 (*m*), 2.15 (*ddd*, J = 2.4, 6.6, 15.6)	C(9), C(8), C(11)	H–C(9)
C(11)	208.3			
H–C(12)	66.1	4.39 (br. *d*, J = 4.2)	C(13), C(14), C(2), C(16), C(11)	CH_2(14)
CH_2(13)	16.4	2.58–2.62, 1.55–1.63 (2*m*)	C(16), C(11), C(15), C(14)	CH_2(14), CH_2(15)
CH_2(14)	24.1	1.91–1.98, 1.75–1.80 (2*m*)	C(12), C(13)	CH_2(13)
CH_2(15)	30.4	2.32–2.41, 2.45–2.57 (2*m*)	C(13), C(14), C(10), C(16)	CH_2(13)
C(16)	171.1			
C(17)	172.3			
OH–C(3)		2.95 (br. *s*)		

[a]) Recorded at 150 MHz. [b]) Recorded at 600 MHz

1 H–C(6)) and 3.00 (*d*, *J* = 10.8 Hz, 1 H–C(6)); δ(H) 4.50 (*d*, *J* = 13.2 Hz, 1 H–C(2)) and 3.12 (*d*, *J* = 13.2 Hz, 1 H–C(2)); δ(C) 47.8, 48.3, and 55.0, resp.). The HMBC spectra (*Fig. 2*) demonstrated long-range correlations of δ(H) 4.39 (H–C(12)), 2.32–2.41 (1 H–C(15)), and δ(H) 2.58–2.62 (1 H–C(13)) with the C=O C-atoms (δ(C) 171.1 (C(16))), which confirmed the presence of a δ-lactam. In addition, δ(H) 4.60 (1 H–C(8)) and 3.20–3.28 (1 H–C(6)) showed a long-range correlation with the quaternary C-atoms at δ(C) 172.3 (C(17)), indicating that there is a second δ-lactam ring. Furthermore, in the HMBC spectrum, the cross-peaks δ(H) 1.80 (H–C(4))/δ(C) 72.4 (C(3)), 54.9 (C(2)), and 18.4 (C(5)), and δ(H) 4.50 and 3.12 (CH_2(2))/δ(C) 72.4 (C(3)) and 171.1 (C(16)) suggested that the two δ-lactam rings were connected by the C(2)–C(3) bond. The long-range correlations δ(H) 4.60 (1 H–C(8)), 4.39 (H–C(12)), 2.65–2.75 (1 H–C(10)), and 2.58–2.62 (1 H–C(13))/δ(C) 208.3 (C(11)), δ(H) 2.65–2.75 (1 H–C(10))/δ(C) 25.3 (C(9)), and δ(H) 4.60 (1 H–C(8))/δ(C) 36.5 (C(10)) suggested that the acyl group, a linear C_4-fragment, was connected to C(12) (δ(C) 66.1). Concomitantly, the cross-peaks δ(H) 4.60 (1 H–C(8))/δ(C) 48.3 (C(6)) and 172.3 (C(17)) suggested that the other end of this C_4-fragment was connected to N(7). Thus, the constitutional formula of alopecurin A, possessing an unprecedented skeleton, was established as shown in *Fig. 1*.

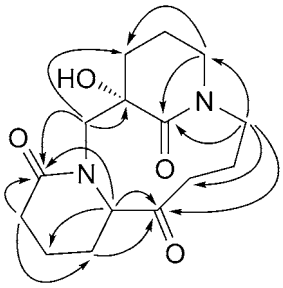

Fig. 2. *Key HMBC features of alopecurin A*

The relative configuration of alopecurin A was supported by its 1D- and 2D-NMR spectra (^{1}H- and ^{13}C-NMR, HSQC, HMBC, and ^{1}H,^{1}H-COSY) and confirmed by single-crystal X-ray analysis (*Fig. 3*). The absolute configuration of alopecurin A was determined by a CD spectrum (*Fig. 4*). A negative *Cotton* effect was observed at *ca.* 295 nm and a positive *Cotton* effect at *ca.* 220 nm. Application of *Wu*'s rule [8] led to the assignment of the (3*S*,12*R*) configuration. Based on the aforementioned results, the structure of alopecurin A (*Fig. 3*) was thus shown to be (3*S*,12*R*)-3-hydroxy-1,7-diazatricyclo[10.4.0.1^{3,7}]heptadecane-11,16,17-trione[1]). We gave the name alopecurin A to this novel metabolite.

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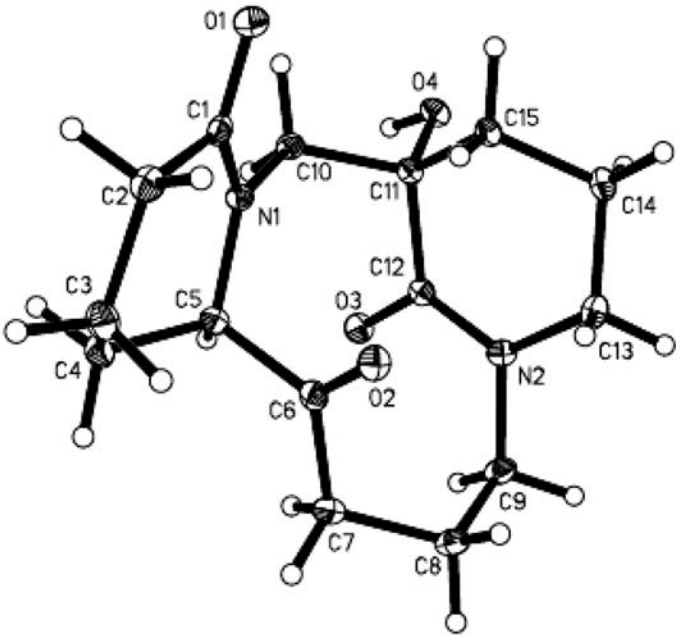

Fig. 3. *X-Ray structure of alopecurin A.* Arbitrary atom numbering.

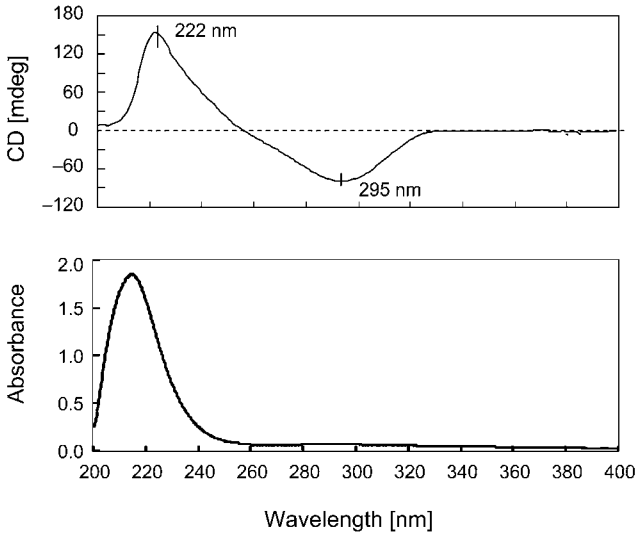

Fig. 4. *CD and UV Spectra of alopecurin A*

Experimental Part

General. All solvents used were of anal. grade (*Tianjin Chemical Plant*, Tianjin, P. R. China). Column chromatography (CC): silica gel (SiO_2, 200–300 mesh) and neutral aluminium oxide (100–200

mesh; *Shanghai Wusi Chemical Co., Ltd.*, Shanghai, P. R. China). TLC: precoated SiO_2 GF_{254} plates (*Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China). M.p.: *X-6* apparatus; uncorrected. CD Spectra: *MOS-405* CD spectrometer. UV Spectra: *Shimadzu-UV-2049* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet-Nexus-470* FT-IR spectrometer; KBr disks; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker-Avance-600* and *Bruker-Avance-150* spectrometer; in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-TOF-MS: *Waters-LCT-Premier-XE* time-of-flight mass spectrometer; in *m*/*z*.

Plant Material. The seeds of *S. alopecuroides* L. were collected in Shihezi (Xinjiang, China). The plants were authenticated by Prof. *Yong Tan*, School of Pharmacy, Shihezi University, P. R. China. A voucher specimen (No. 20070816006) was deposited with the School of Pharmacy, Shihezi University.

Extraction and Isolation. The air-dried seeds of *S. alopecuroides* L. (3 kg) were extracted with 5% aq. NaOH soln. (diffusion 24 h, 3 times) to give the residue of the crude extract, which was extracted again by 5% aq. H_2SO_4 soln. Then the extract from the 5% aq. H_2SO_4 soln. was applied to a cation-exchange-resin column and eluted with 3% NH_3/95% EtOH. The eluate was finally treated with 80% hydrazine hydrate/active charcoal to yield 201 g of crude alkaloids. The crude alkaloids were subjected to CC (neutral alumina, petroleum ether/acetone 100 : 0 → 0 : 100): *Fractions 1 – 7. Fr. 5* was separated by CC (neutral alumina, petroleum ether/acetone) and recrystallization: alopecurin A (90 mg).

Alopecurin A[1]) (=*(7*S,15a*R*)*-Decahydro-7-hydroxy-6*H*-7,11-methano-4*H*-pyrido[1,2-*a*][1,7]-diaza-cyclodecine-4,15,16(12*H*)-trione)*. Colorless prisms ($CHCl_3$). M.p. 258 – 259°. IR (KBr): 3283 (OH),

Table 2. *Crystallographic Data for Alopecurin A*

Empirical formula	$C_{15}H_{22}N_2O_4$
M_r	294.35
Temperature [K]	113(2)
Wavelength [Å]	0.71073
Crystal system	orthorhombic
Space group	*P*2(1)2(1)2(1)
Unit cell dimensions:	
a [Å]	10.016(3)
b [Å]	11.697(3)
c [Å]	11.807(3)
V [Å^3]	1383.2(7)
Z	4
Calculated density [Mg/m^3]	1.413
Absorption coefficient [mm^{-1}]	0.103
F(000)	632
Crystal size [mm]	$0.24 \times 0.20 \times 0.18$
θ Range for data collection	2.45 to 27.88°
Limiting indices	$-13 \le h \le 11,\ -15 \le k \le 15,\ -15 \le l \le 15$
Reflections collected, unique	14377, 3308
R_{int}	0.0755
Completeness to $\theta = 27.88$	99.8%
Absorption correction	Multi-scan
Max. and min. transmission	0.9817 and 0.9758
Refinement method	Full-matrix least-squares on F^2
Data, restraints, parameters	3308, 0, 195
Goodness-of-fit on F^2	1.073
Final *R* indices ($I > 2\sigma(I)$)	$R_1 = 0.0542$, $wR_2 = 0.1236$
R Indices (all data)	$R_1 = 0.0579$, $wR_2 = 0.1244$
Absolute structure parameter	1.6(14)
Extinction coefficient	0.65(2)
Largest diff. peak and hole [e Å^{-3}]	0.405; −0.538

2954, 2926, 2855; 1706 (C=O), 1623 (lactam), 1489, 1405, 1326, 1279, 1200, 1073. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-TOF-MS: 589.3260 ($[2M+H]^+$, $C_{30}H_{45}N_4O_8^+$; calc. 589.3237), 295.1655 ($[M+H]^+$, calc. 295.1658).

Crystal Data and Structure Refinement for Alopecurin A (*Table 2*). Crystal data were obtained with a *Bruker-Smart CCD* detector and graphite-monochromated MoK_α radiation (λ 0.71073 Å) at 113 K and operating in the φ–ω scan mode. The structure was solved by direct methods with SHELXS-97 [9] and refined by full-matrix least-squares calculations on F^2 with SHELXL-97 [10]. CCDC-818659 contains the supplementary crystallographic data for alopecurin A. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/data_request/cif.

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