Dahurelmusin A, a Hybrid Peptide-Polyketide from *Elymus dahuricus* Infected by the *Epichloë bromicola* Endophyte

Qiu-Yan Song,[†] Hai-Tao Yu,[‡] Xing-Xu Zhang,[†] Zhi-Biao Nan,[*,†] and Kun Gao[*,§]

[†] *State Key Laboratory of Grassland Agro-ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, China*

[‡] *Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Lanzhou, 730070, China*

[§] *State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou 730000, China*

[*] Corresponding authors,
E-mail: zhibiao@lzu.edu.cn (Z. B. Nan), npchem@lzu.edu.cn (K. Gao).

Contents of supporting information:

Page

S3 MATERIALS AND METHODS

S6 Crystal data and structure refinement for dahurelmusin A.

S7 ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S7 ^{13}C NMR spectrum (100 MHz, $CDCl_3$) of dahurelmusin A.

S8 DEPT spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S8 COSY spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S9 HMQC spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S9 HMBC spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S10 NOESY spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.

S10 HRESIMS of dahurelmusin A.

S11 UV and CD spectra of dahurelmusin A (in MeOH).

S11 IR spectrum of dahurelmusin A.

MATERIALS AND METHODS

General Experimental Procedures. X-ray crystallography was performed on a Bruker Smart CCD diffractometer (Bruker Ltd, Karlsruhe, Germany) using graphic-monochromated Cu Kα radiation. Optical rotations were determined with a Perkin-Elmer 341 polarimeter (Perkin-Elmer Ltd, Waltham, MA). Circular dichroism was determined with an Olis DSM 1000 spectrometer (Olis, Atlanta). Column chromatography was performed with a macroporous resin (HP-20) (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (200−300 mesh) (Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan), and RP-18 (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was performed with silica gel GF_{254} plates (10−40 μm) (Qingdao Marine Chemical Factory, China). IR spectrum was obtained with a Nicolet FT-IR-360 spectrometer (Thermo Nicolet Inc., Waltham, MA). UV spectrum was obtained with a Shimadzu UV-260 spectrophotometer (Shimadzu Instruments Co., Ltd, Tokyo, Japan). ^{1}H and ^{13}C NMR spectra were determined with a Bruker AM-400BB (400 MHz) spectrometer (Bruker Ltd, Karlsruhe, Germany); values of δ in ppm are relative to TMS; values of *J* are given in Hz. HRESIMS was performed with a Bruker APEX-II mass spectrometer (Bruker Ltd, Karlsruhe, Germany), and results are presented in terms of *m/z*.

Plant Materials. The endophyte in *Elymus dahuricus* (*E. dahuricus*) was first reported in China by Li et al. and was examined using the aniline blue coloring (0.1% aqueous) method.[1] The endophyte-infected *E. dahuricus* was further studied by our research group in 2007 and was determined as *Epichlo ë* sp..[2] In 2015, the fungus was identified as *Epichlo ë bromicola* (*E. bromicola*) by our laboratory.[3] We sowed the seeds of *E. bromicola*-infected *E. dahuricus* (E+) in pots at the Yuzhong Campus of Lanzhou University, China. Thirty-two plots (2 treatments × 16 replicates) were randomly built. The area of the plot was 16 m^2 (4 × 4) with 6 lines of 10 listed (40 cm apart), consisting of 60 plants in each plot. A voucher specimen (no. 20130924-01) has been deposited at Grassland Protection Academy of State Key Laboratory of

Grassland Agro-ecosystems of Lanzhou University.

Extraction, Isolation, and Purification Process. The air-dried and powdered leaves and stems of E+ (4.0 kg) was extracted three times (each time for 7 days) with methanol (MeOH) at room temperature and filtered to yield a filtrate, which were evaporated under reduced pressure, and partitioned with ethyl acetate (EtOAc) and *n*-BuOH, respectively. The EtOAc partition (25.0 g) was applied to macroporous resin column chromatography (HP-20, 2 L) and eluted with gradient mixtures of H_2O-EtOH (70:30, 50:50, 10:90) to give three fractions (labeled as Fr-A–Fr-C). Fr-B was purified by column chromatography on an RP-18 column (30 × 2 cm) (800 mL, H_2O/MeOH, 1:1) and underwent recrystallization from acetone to produce metabolite **1** (9.2 mg).

Dahurelmusin A, **1**, was a colorless crystal with $[\alpha]_D^{17}$-15 (*c* 0.50 in MeOH). The melting point was 180-181 ℃. HRESIMS indicated *m/z* 377.2047 $[M + Na]^+$ (calculated for $[C_{18}H_{31}O_5N_2Na]$ 377.2047). IR parameters (νKBr_{max}) were 3319, 1719, 1701, and 1687 cm^{-1}.

Insecticidal Assay. The insecticidal activities of compound **1** against *Rhopalosiphum padi* (*R. padi*) and *Brevicoryne brassicae* (*B. brassicae*) were evaluated by leaf-dip method using a previously reported procedure.[4] The assay was repeated in an air-conditioned room at 25 ± 2 ℃. Each tested sample was dissolved in acetone at a concentration of 2 g/L and diluted with distilled water containing TW-80 (0.1 mg/L) to obtain a required concentration. For comparative purposes, the positive control (imidacloprid) was tested under the same conditions. Water containing TW-80 (0.1 mg/L) was used as control. Leaf disks (5 cm × 3 cm) were cut from fresh corn leaves and then dipped into the test solution for 3 s. After air-drying the treated leaf disks and 30 *R. padi* and *B. brassicae* were placed individually into the disks. The mortalities were evaluated 3 days after treatment. All the assays were repeated in triplicate. The LC_{50} values were obtained using the SPSS program (version 22.0). The insects, *R. padi* and *B. brassicae* were provided by Institute of Plant Protection, Gansu Academy of Agricultural Sciences.

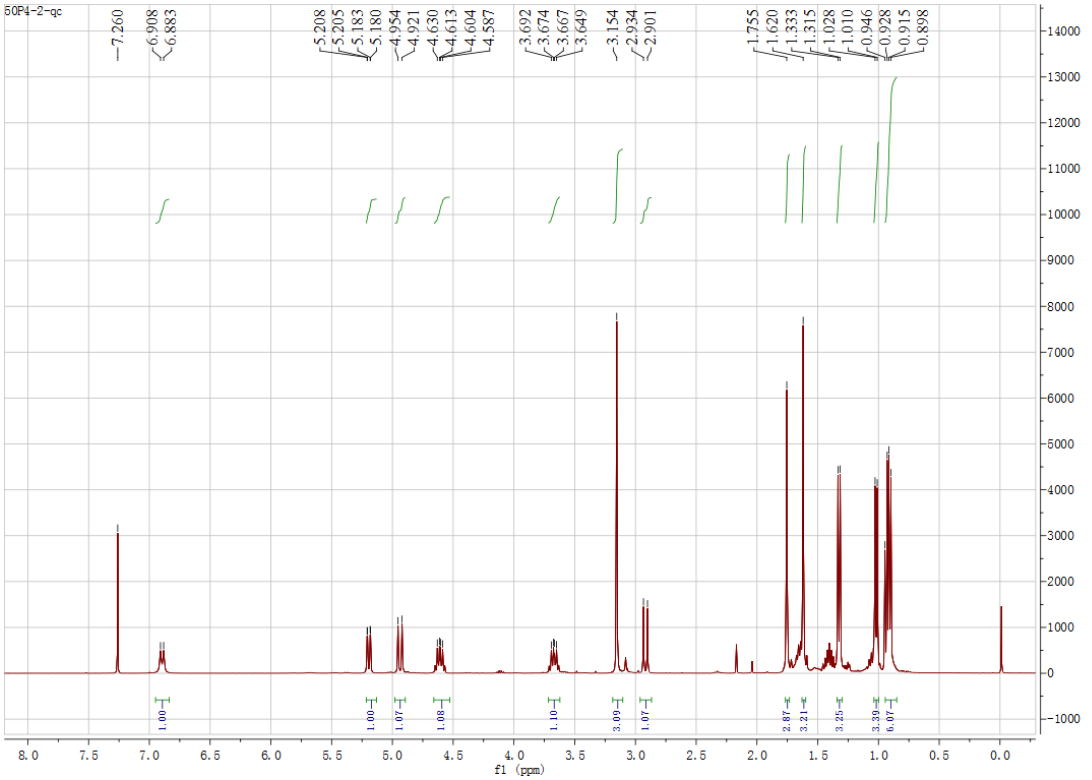
The LC_{50} values of imidacloprid against *R. padi* and *B. brassicae* were 0.078 ±

0.005 and 0.018 ± 0.003 mM, respectively.

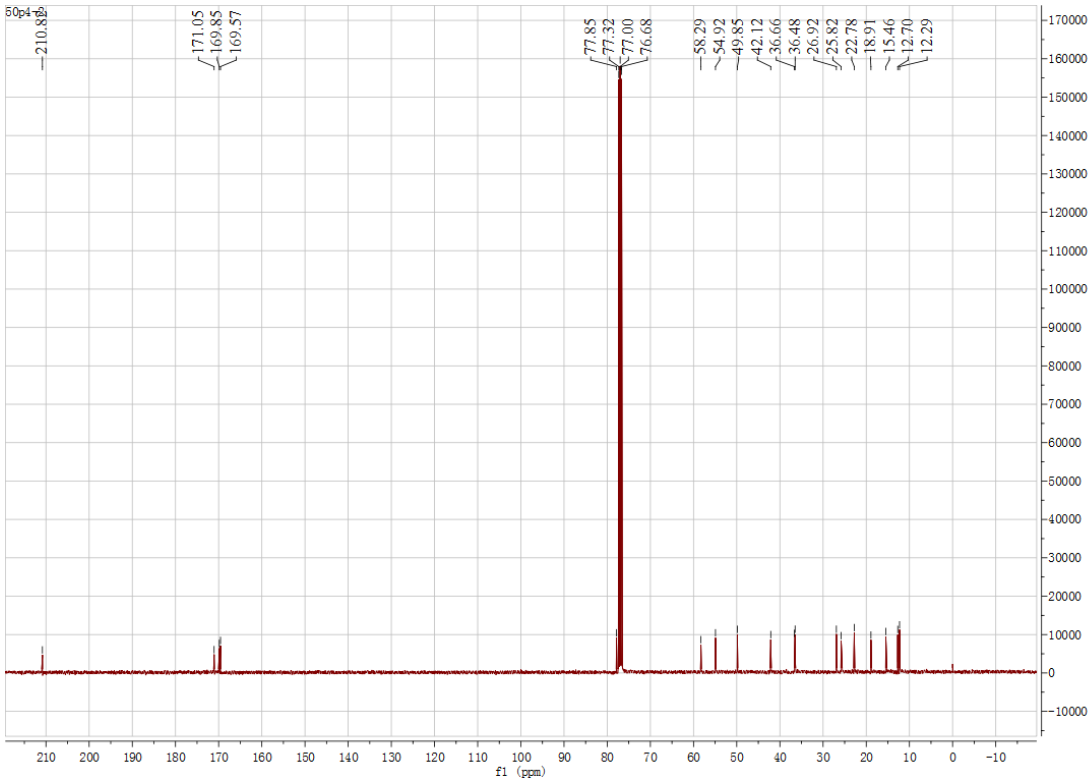
(1) Nan, Z. B.; Li, C. J. Roles of the grass-*Neotyphodium* association in pastoral agriculture systemsn. *Acta Ecol. Sin.* **2004**, *24*, 605-616.
(2) Zhang, Y. P.; Nan, Z. B. Distribution of epichloe ¨ endophytes in Chinese populations of *Elymus dahuricus* and variation in peramine levels. *Symbiosis* **2007**, *43*, 13-19.
(3) Song, H.; Nan, Z. B. Origin, divergence, and phylogeny of asexual *Epichlo ë* endophyte in *Elymus* species from Western China. *Plos One* **2015**, *10*, e0127096/1-13.
(4) Ma, Q. Q.; Liu,Y. X.; Zhang, P. X.; Li, Y. Q.; Xiong, L. X.; Wang, Q. M. Design, synthesis, and biological evaluation of various *α*-substituted benzylpyrroles based on the structures of insecticidal chlorfenapyr and natural pyrrolomycins. *J. Agric. Food Chem.* **2014**, *62*, 6072-6081.

Crystal data and structure refinement for dahurelmusin A.

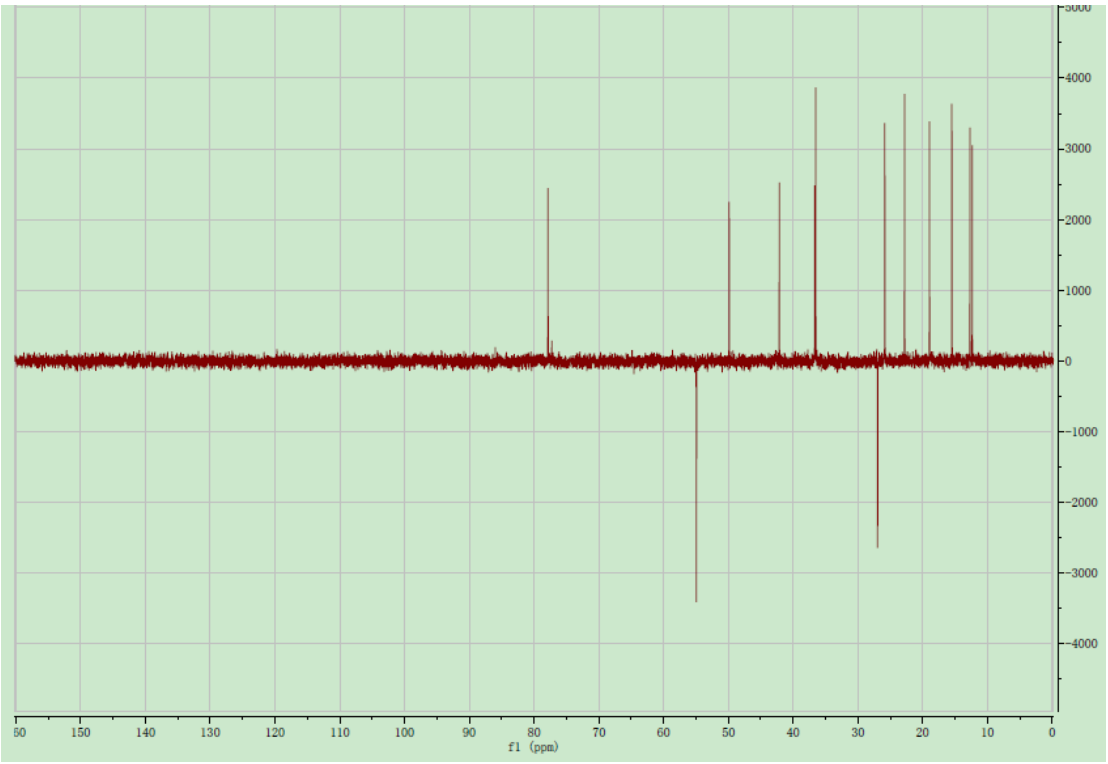
project	**data**
Empirical formula	$C_{18}H_{30}N_2O_5$
Formula weight	354.44
Temperature/K	273.77(10)
Crystal system	tetragonal
Space group	$P4_3$
a/Å	10.0307(16)
b/Å	10.0307(16)
c/Å	19.782(3)
α/ °	90
β/ °	90
γ/ °	90
Volume/Å^3	1990.4(7)
Z	4
ρ_{calc}g/cm^3	1.183
μ/mm^{-1}	0.704
F(000)	768.0
Crystal size/mm^3	0.12 × 0.06 × 0.04
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	8.816 to 139.07
Index ranges	$-12 \leq h \leq 9$, $-11 \leq k \leq 11$, $-23 \leq l \leq 12$
Reflections collected	3431
Independent reflections	2374 [R_{int} = 0.0235, R_{sigma} = 0.0466]
Data/restraints/parameters	2374/1/233
Goodness-of-fit on F^2	0.977
Final R indexes [I>=2σ (I)]	R_1 = 0.0527, wR_2 = 0.1239
Final R indexes [all data]	R_1 = 0.0866, wR_2 = 0.1508
Largest diff. peak/hole / e Å^{-3}	0.12/-0.16
Flack parameter	-0.1(3)



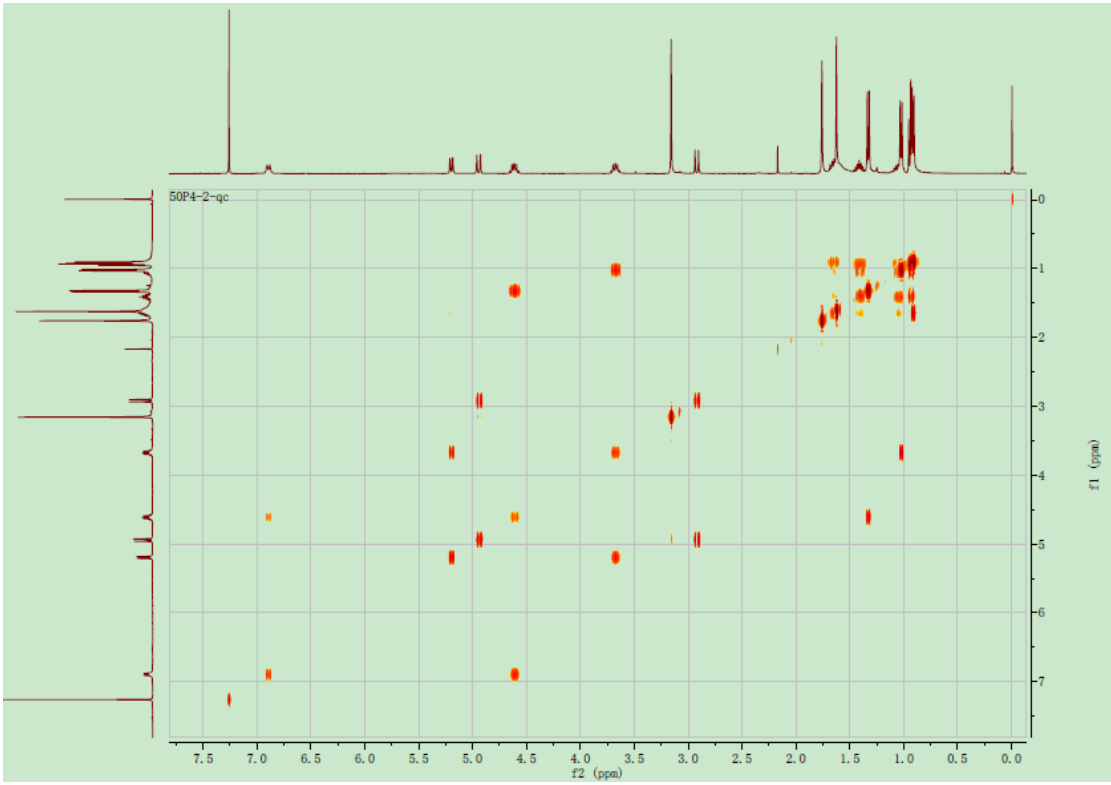
^{1}H NMR spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.



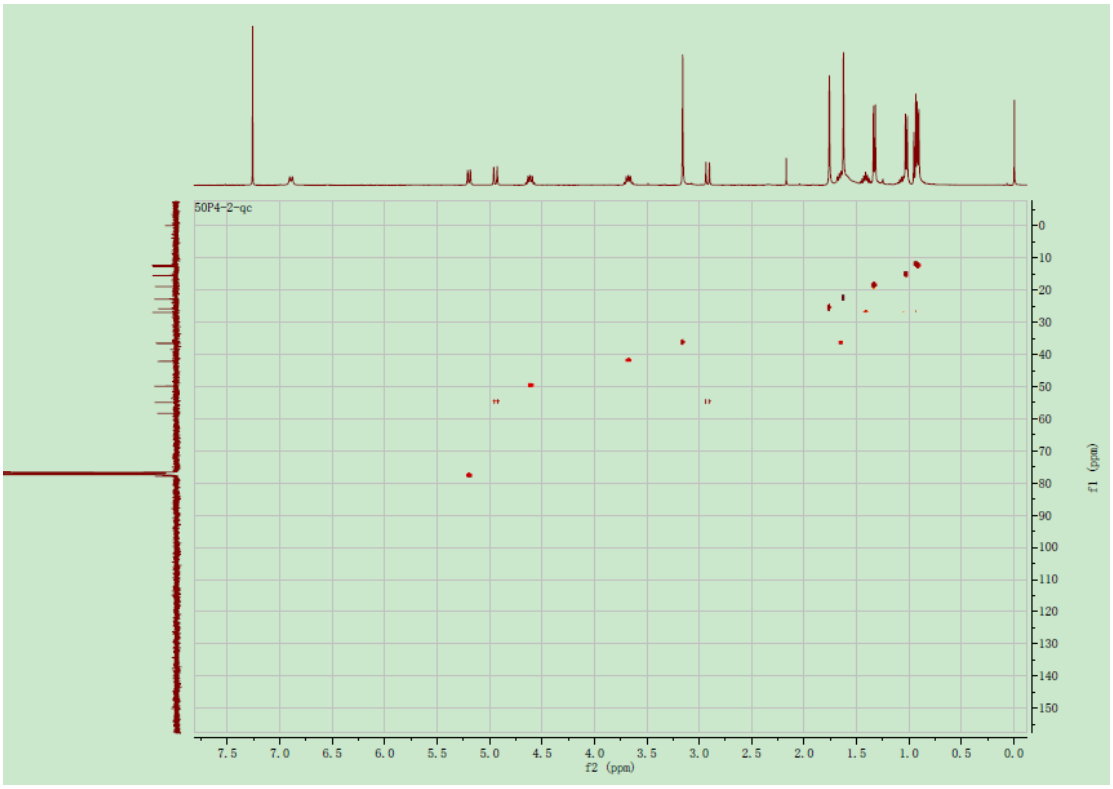
^{13}C NMR spectrum (100 MHz, $CDCl_3$) of dahurelmusin A.



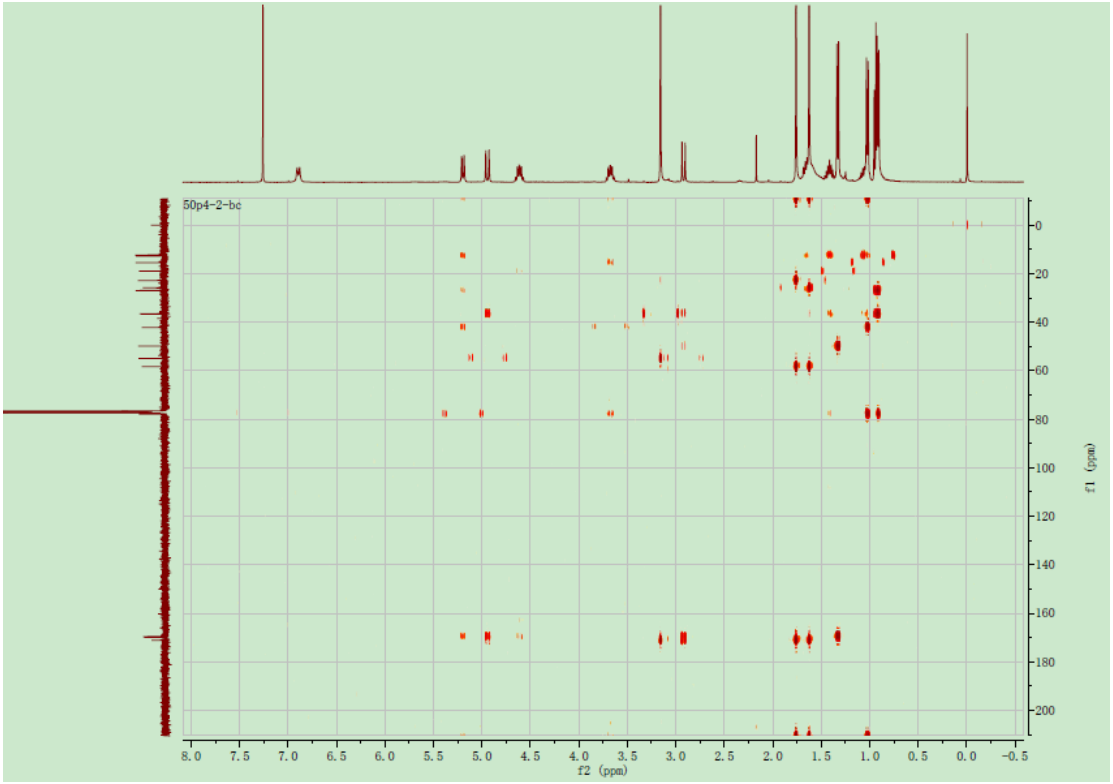
DEPT spectrum of dahurelmusin A.



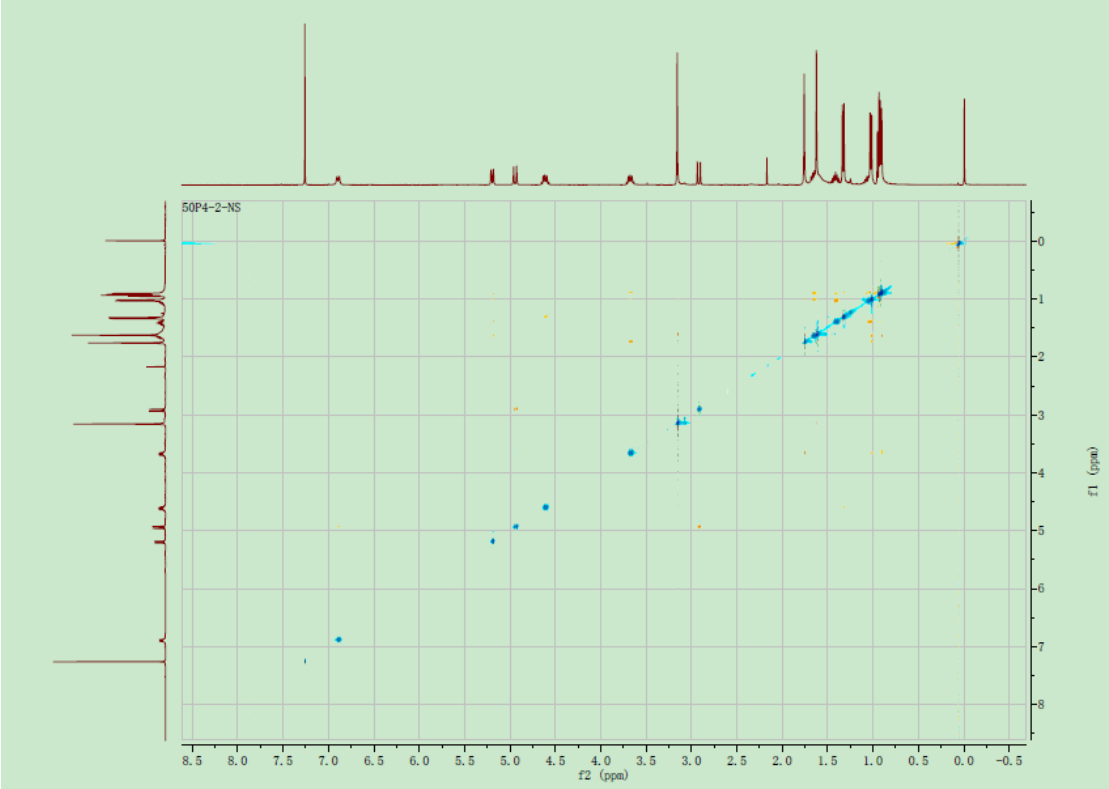
COSY spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.



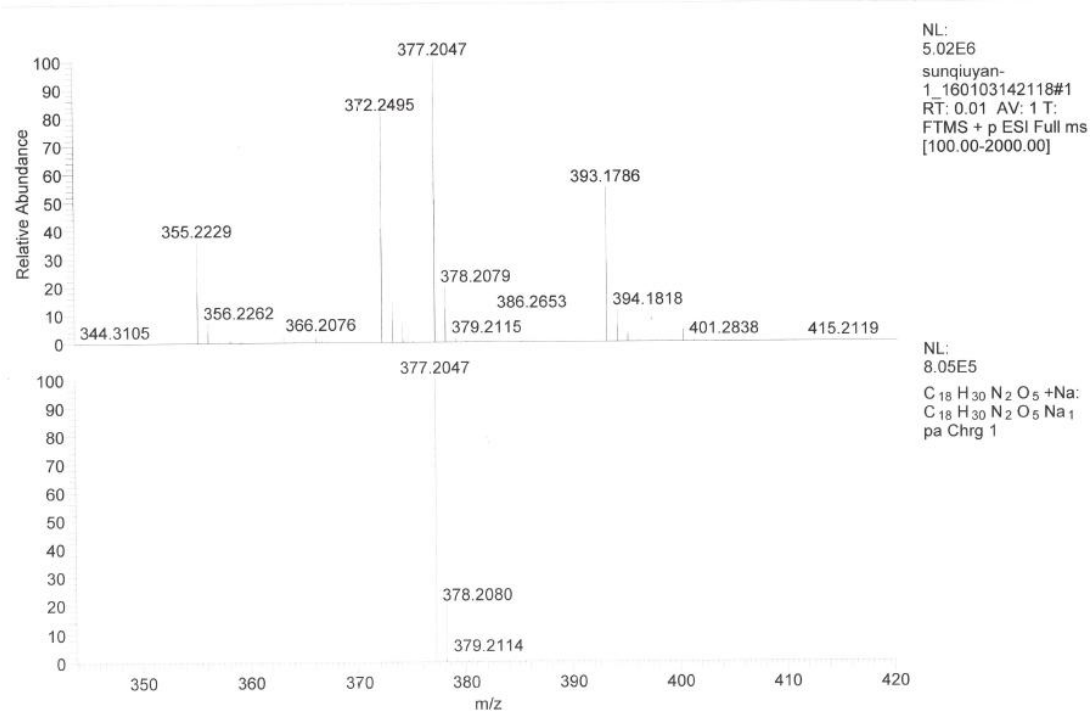
HSQC spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.



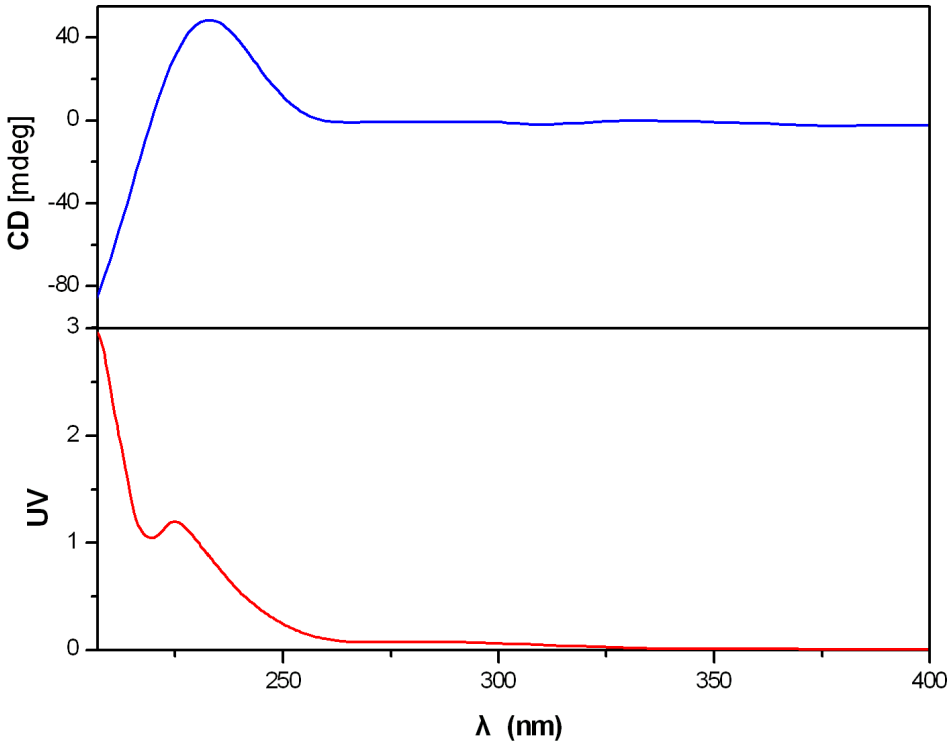
HMBC spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.



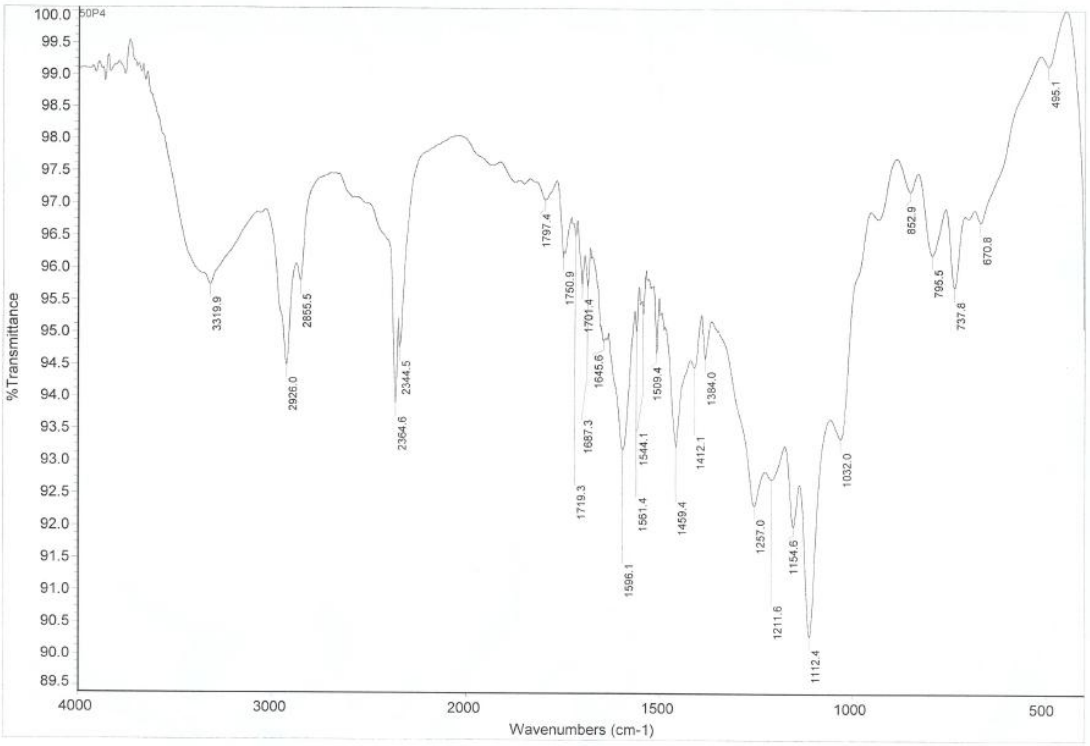
NOESY spectrum (400 MHz, $CDCl_3$) of dahurelmusin A.



HRESIMS of dahurelmusin A.



UV and CD spectra of dahurelmusin A (in MeOH).



IR spectrum of dahurelmusin A.