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RESEARCH ARTICLE

Analysis of sparteines in the seeds of *Ammopiptanthus mongolica*

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Abstract Seven alkaloids were isolated from the seeds of *Ammopiptanthus mongolica* by thin layer chromatography and silica gel column chromatography, and the chemical structures of five alkaloids, 17-oxosparteine, β-isosparteine, 3α-hydroxysparteine, sparteine, and 3β-hydroxysparteine were identified by ^{1}H nuclear magnetic resonance (NMR) and electron ionization mass spectrum (EIMS).

Keywords *Ammopiptanthus mongolicus* (Maxim) Cheng f., alkaloids, sparteine, ^{1}H NMR, electron ionization mass spectrum (EIMS)

1 Introduction

Ammopiptanthus mongolicus is distributed mainly in Mongolia, Russia, East Asia and Inner Mongolia, as well as in Ningxia and Gansu in China. It is an endemic, evergreen and broadleaf plant in the deserts of central Asia, usually growing in the desert, arid slopes and piedmont zones (Fu et al., 1993). As an ancient tertiary relict and a rare and endangered plant of China, it has important value in the study of paleogeography, paleoclimate, plant phylogeny and biodiversity change of desert areas. It is also a precious material for study, extraction and transfer of special chemical constituents of plants (alkaloid and highly active antifreeze protein) (Fu, 1989). *A. mongolicus* has strong drought, cold and salt resistance. So it is an excellent species in sand-control afforestation and landscaping (Liu et al., 1995; Jia et al., 2005). The seed of *A. mongolicus* can be used to extract special industrial oils, while the branch and leaf can be used as traditional Chinese medicine, functioning in dispelling "wind", stimulating blood flow and relieving pain. In addition, the external application can treat chilblain and chronic rheumatoid arthritis. However, *A. mongolicus* is a poisonous plant. Its branch and leaf contain several kinds of strong alkaloids, such as yellow flower lignin. Livestocks rarely eat *A. mongolicus* because excess ingestion will lead to death (Fu, 1989; Li et al., 2004), which is, on the other hand, advantageous for the protection and population reproduction of the plant and landscape use. In the 1960s and 70s, there were many reports from the former USSR about the alkaloids in *A. mongolicus* (Ma, 1989). In 1994, Xu et al. (1994) separated three alkaloids from the leaves of *A. mongolicus*. However, the study of the alkaloids in the seeds of *A. mongolicus* has rarely been reported. In the present research, sparteines in the seeds of *A. mongolicus* were determined by extraction and separation, thin layer chromatography, mass spectrometry and nuclear magnetic resonance (NMR).

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2 Materials and methods

2.1 Test materials

2.1.1 Collection of test samples

From October to November 2004, mature pods were collected from the natural population of *A. mongolicus* in Shapotou in the Ningxia Hui Autonomous Region. Five kilograms of seeds were collected, dried and grinded for reserve.

2.1.2 Experimental instrument and reagents

The APEX II FT-ICRMS high-resolution mass spectrometer was supplied by the German Bruker Daltonnics Co. The AV 400 NMR spectrometer was produced by the German Bruker Co. Silica gel thin-layer chromatography with GF254 was produced by Qingdao City Shibeiqu Sea Products Plant Desiccants Factory. Shuttle methyl cellulose sodium (CMCNa) was purchased from Shanghai Chemical Reagents Co. of the China Pharmaceutical

Corporation Group. Sparteine was purchased from SIGMA Co.. All the other reagents were of analytical grade and were produced domestically.

2.2 Methods

2.2.1 Sample preparation

Five hundred gram seed powders of *A. mongolicus* were extracted with methanol in 70°C water bath for six times. The methanol solutions were complicated and decompressed for solvent recovery. The extracts were obtained and evaporated to a constant weight, and then they were treated with hydrochloric acid. After that, acid liquid was obtained, and was extracted for several times with chloroform to remove part of the pigments. The chloroform was discarded. The pH was adjusted to 9–11. Then the acid liquid was extracted with chloroform again until no alkaloid could be detected from the chloroform solution. The latter was then complicated and decompressed for chloroform recovery and thus, the samples were obtained.

2.2.2 Thin layer chromatography test

Samples were soluted by methanol, spotted to the prepared silica gel GF254 plates, saturated for 5–10 min, and developed by the upward method. When the developing solvent ran to places 1–2 cm from the front of the gel plate, the plate was taken out and the solvent evaporated. The fluorescence was observed under a 254 nm ultraviolet lamp, and the Dragendorff agent was used for color development. The spots' *Rf* values were calculated using the standard sample as control.

2.2.3 Silica gel column chromatography

The brown extract was mixed with 200–300 mesh silica gel, put into a chromatographic column for chromatography, using chloroform : methanol = 8 : 1; 6 : 1; 4 : 1; 2 : 1; 1 : 1 for gradient elutions. The eluents were decompressed and concentrated. Every 50 mL of the eluents was collected as a share and a total of 122 shares was collected.

2.2.4 Identification of chemical structure of the samples

NMR and mass spectrometry were used to identify the chemical composition of the eluent.

3 Results

3.1 Extraction results

A total of 3.42 g of dark brown extract was used to analyze the components in it: w (N) = 5.46%; w (C) = 56.04%; w (H) = 7.90%. The extract was dissolved with 1 mol/L HCl and filtered. The residue was discarded and the acid liquid was collected. The acid liquid was extracted for several times with chloroform to remove the pigment. Then, the chloroform was discarded. The pH value was adjusted to 9–11. The acid liquid was extracted with chloroform again until no alkaloid could be detected from the chloroform solution. Then, the chloroform solutions were complicated and decompressed for chloroform recovery and the samples were obtained.

3.2 Thin layer chromatography test results

Using chloroform : methanol (2 : 1), central ethane : chloroform (8 : 2) and chloroform : *n*-butyl alcohol (7 : 3) as developing solvents, five spots were obtained from the chloroform-methanol system, with the best effect. All the spots were orange-red, indicating the presence of alkaloids.

3.3 Silica gel column chromatography results

One hundred and twenty-two shares of eluents were isolated by repeated column chromatography with chloroform : methanol (8 : 1–1 : 1) columns. The points with close *Rf* values were complicated. Combined with silica gel thin-layer chromatography, 7 points were obtained, including S1, S2, S3, S4, S5, S6, S7, and S8. The contents of S2, S5, and S6 were small, and it was difficult to identify their structures.

3.4 Results of NMR spectrum and mass spectrometry

S1 was a light yellow oil. When it was developed by chloroform : petroleum ether : methanol = 25 : 5 : 9, it was at the front of the solvent. When it was developed using 10 mL pure chloroform added with 5 drops of methanol three times, the $Rf = 0.58$.

NMR ^{1}H NMR, 300 MHz, $CDC1_3$, $\delta \times 10^6$: 2.62, 4.57 (2H, 2-H); 2.60 (2H, 3-H); 2.03 (2H, 4-H); 1.78, 1.86 (2H, 5-H); 3.31 (1H, 6-H), 2.06 (1H, 7-H); 1.30, 2.31 (2H, 8-H);

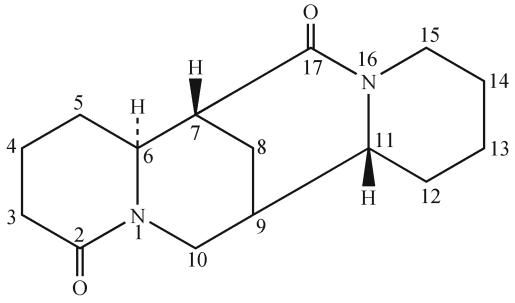
Table 1 Thin layer chromatography test results (*Rf* value)

sample	developing solvent		
	chloroform : methanol	central ethane : chloroform	chloroform : *n*-butyl alcohol
	2 : 1	8 : 2	7 : 3
total alkaloids	0.12, 0.17, 0.24, 0.48, 0.64	0.16, 0.32	0.09, 0.14, 0.42
sparteine	0.12	0.16	0.14

1.83 (1H, 9-H); 2.20 (1H, 11-H); 1.30, 1.60 (2H, 12-H); 1.25, 1.82 (2H, 13-H); 1.60, 1.86 (2H, 10-H); 2.41 and 2.77 (2H, 15-H).

EIMS, m/z (R.I), m/z M^+262 (9.5), 248 (3.0), 234 (1.0), 219 (1.6), 206 (1.7), 176 (2.0), 164 (4.6), 10 (28.9), 136 (9.7), 122 (7.9), 110 (21.3), 97 (26.8), 84 (27.3), 68 (18.9) and 55 (100).

The main peak above was uniform with the main peak of 17-oxosparteine (Murakoshil, 1977; Ning, 2000), but contained a small amount of m/z 277, 256, 212 and 180 impurities. Its molecular structure is as follows:

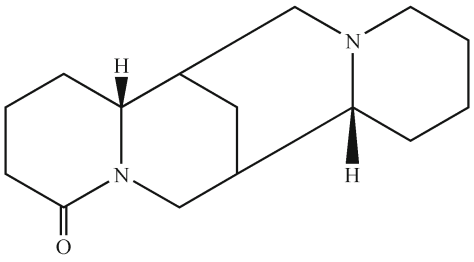


S3 was also a light yellow oil. When it was developed by chloroform : petroleum ether : methanol = 25 : 5 : 9, the $Rf = 0.05$. When sprayed with Dragendroff, it became orange-red in color.

NMR ^{1}HNMR, 300 MHz, $CDCl_3$, $\delta \times 10^6$: 2.10 (2H, 3-H); 1.87 (2H, 4-H); 1.83, 2.02 (2H, 5-H); 3.65 (1H, 6-H); 2.11 (1H, 7-H), 1.25, 2.25 (2H, 8-H); 1.67 (1H, 9-H); 2.72, 4.65 (2H, 10-H); 2.76 (1H, 11-H); 1.62, 1.67 (2H, 12-H); 1.22, 1.83 (2H, 13-H); 1.62, 1.83 (2H, 14-H), 2.29, 2.91 (2H, 15-H); 2.42 and 3.32 (2H, 17-H).

MS EIMS, m/z M^+, 248 (16.4), 247 $(M-1)^+$ (27.30), 233 (1.0), 205 (2.8), 193 (3.3), 165 (3.5), 149 (27.8), 136 (38.2), 135 (21.0), 122 (8.3), 110 (19.3), 98 (22.9), 84 (100), 67 (21.0), 55 (82) and 41 (64.7).

The main peak above was uniform with the main peak of β-isosparteine (Wei and Zhao, 2000; Zhao et al., 2004), but contained a small amount of m/z 262 and 256 impurities. Its molecular structure is as follows:

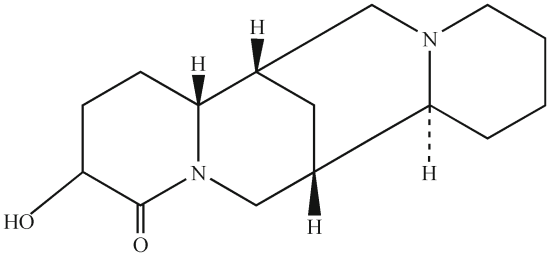


S4 was again a light yellow oil. When it was developed by chloroform : petroleum ether : methanol = 25 : 5 : 9, the $Rf = 0.28$. When sprayed with Dragendroff, it became orange-red in color.

NMR ^{1}H NMR, 300 MHz, $CDCl_3$, $\delta \times 10^6$: 4.22 (1H, 3-H); 2.03 (2H, 4-H); 1.59, 1.67 (2H, 5-H); 3.40 (1H, 6-H); 2.31 (1H, 7-H), 1.37, 2.34 (2H, 8-H); 1.35 (1H, 9-H), 2.62, 4.26 (2H, 10-H); 2.02 (1H, 11-H); 1.37, 1.40 (2H, 12-H); 1.25, 1.42 (2H, 13-H); 1.21, 1.25 (2H, 14-H); 2.34, 2.36 (2H, 15-H); 2.62 and 2.65 (2H, 17-H).

MS EIMS, m/z M^+, 264 (13.0), 247 (0.4); 246 (0.2), 149 (17.3), 136 (10.2) and 135 (100).

The main peak above was uniform with the main peak of 3α-hydroxysparteine (Ning, 2000; Wei and Zhao, 2000; Yu, 1989). Its molecular structure is as follows:

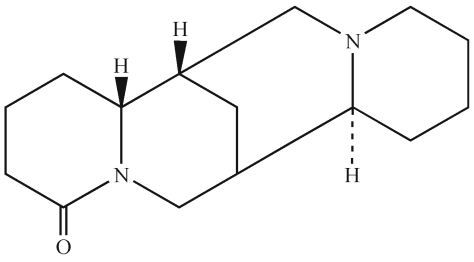


S7 was again a light yellow oil, and it had the largest content. When it was developed by chloroform : petroleum ether : methanol = 25 : 5 : 9, the $Rf = 0.49$. When sprayed with Dragendroff, it became bright red in color.

NMR ^{1}H NMR, 300 MHz, $CDCl_3$, $\delta \times 10^6$: 1.85 (2H, 3-H); 1.89 (2H, 4-H); 1.75, 1.97 (2H, 5-H); 3.36 (1H, 6-H); 2.03 (1H, 7-H); 1.47, 2.33 (2H, 8-H); 1.75 (1H, 9-H); 2.55, 4.55 (2H, 10-H), 2.01 (1H, 11-H); 1.52, 1.63 (2H, 12-H), 1.37, 1.75 (2H, 13-H); 1.42, 1.69 (2H, 14-H), 2.30, 2.78 (2H, 15-H); 2.38 and 3.34 (2H, 17-H).

MS EIMS, m/z M^+248 (43.6), $(M-1)^+$247 (28.8), 233 (2.7), 205 (5.3) 191 (13.6) 165 (5.3), 149 (59.4), 136 (100), 122 (11.9), 110 (26.1), 98 (30.0), 84 (22.0), 67 (19.3), 55 (64.3) and 41 (50.7).

The above results were uniform with isosparteine (Yu et al., 1989; Zhang and Li, 1997). Its molecular structure is as follows:

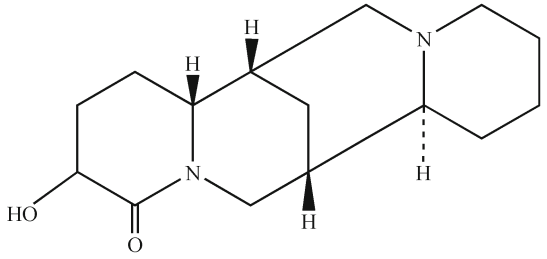


S8 was a yellow oil. When it was developed by chloroform : petroleum ether : methanol = 25 : 5 : 9, the

$Rf = 0.13$. When sprayed with Dragendroff, it became orange-red in color.

MS EIMS, m/z: M^+, 264 (7.0), 247 (0.4), 246 (0.3), 149 (33.4), 136 (7.5) and 135 (66.7).

These results were uniform with 3β-hydroxysparteine (Ning, 2000; Wei and Zhao, 2000; Zhang and Li, 1997). Its molecular structure is as follows:



The content of S8 was too low to be identified by ^{1}HNMR.

4 Discussion

Seven alkaloids were isolated from the seeds of *Ammopiptanthus mongolica* by thin layer chromatography and silica gel column chromatography, and the chemical structures of five alkaloids, 17-oxosparteine, β-isosparteine, 3α-hydroxysparteine, sparteine and 3β-hydroxysparteine were identified by ^{1}H NMR and EIMS.

The effect of sparteine on the respiratory system is similar to that of nicotine. Sparteine can stimulate respiration and consequently accelerate heart rate and raise the blood pressure. With the disappearance of the respiratory stimulatory effect, all the other changes return to normal. The excitatory effect of sparteine is stronger than lobeline. Sparteine also has the effect of pressurization on the cerebral cycle, which can be inhibited by PGE1 and PGE2 and enhanced by indomethacin. Clinically, it is used in emergency treatment for reflex respiratory apnea caused by choking agents, cyanide agents and anaesthetics, respiratory and cardiovascular failure caused by infectious diseases, as well as shock, collapse and neonatal asphyxia. It has a strong inhibitory effect on the ovum production of spruce budworms. The toxicity of sparteine is milder than nicotine. At small doses it can stimulate respiration and raise blood pressure, whereas at high doses it can suppress respiration and circulation, and even lead to death. The LD50 to mice is 18 mg/kg (Wu and Geriletu, 2004).

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