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Phytochemical study on *Berberis sibirica* Pall.

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Abstract: From the aerial parts (700g) of *berberis sibirica* pall. 6 isoquinoline alkaloids of protoberberine, protopine, benzophenantridine and proaporphine type were isolated. The known alkaloids (-)-tetrahydropseudocoptisine, pseudoprotopine, (+)-chelidonine and (+)-glaziovine are new for the family berberidaceae. from the aerial part ii (3.9 kg) 14 isoquinoline alkaloids of aporphine, proaporphine, protoberberine, protopine, benzylisoquinoline, bisbenzylisoquinoline, proaporphine-benzylisoquinoline and simple isoquinolin type were isolated and identified. The aporphine alkaloid 1-o-methylisotebaidine and simple isoquinoline dehydrocorypalline have been found for the first time in the family of berberidaceae. From the roots of *b. sibirica* 10 isoquinoline alkaloids of protoberberine, benzylisoquinoline, bisbenzylisoquinoline, aporphine-benzylisoquinoline and proaporphine-benzylisoquinoline type were isolated. 1,10-di-o-methylpakistanine has been reported for the first time as a natural alkaloid. The known alkaloids (-) -isothalidezine and (+)-armepavine have been found for the first time in the family berberidaceae. all structures were determined by physical and spectral data.

Key words: *berberis sibirica* pall., 21 bisoquinoline alkaloids, 1,10-di-o-methylpakistanine

Introduction

Berberidaceae is a large family of flowering plants divided into 15 genera. The family contains about 570 species, of which the majority (about 450) belongs to the biggest genus in this family - *Berberis* L. [1]. The *Berberis* species have been deeply investigated because of biological active compounds, namely isoquinoline alkaloids, containing inside [2,3]. The genus *Berberis* is represented by two species in Mongolian flora. *Berberis sibirica* Pall. is wide spread in Central and North Mongolia - Gobi and Altai regions. In the traditional medicine the species is used as antidote and antipyretic remedy, as well as for rheumatism and excessive menstruation [4].

Experimental

Materials and methods. GENERAL. UV: SESIL CE 8020, MeOH. IR: Bruker IFS113V, KBr. MS: Hewlett Packard MSD 5973, 70 eV. ^{1}H NMR, ^{13}C NMR and 2D experiments: Bruker DRX-250, in $CDCl_3$, with TMS as internal standard. Optical rotation: Perkin-Elmer 241, MeOH. Vacuum liquid chromatography (VLC): silica gel (Merck, Kieselgel 60, 70-230 mesh). Column chromatography (CC): neutra alumina (Merck, Aluminiumoxid 90, act. II-III Brockmann, 70-230 mesh). PTLC: Kieselgel

GF_{254}. Visualization for TLC: Dragendorff's reagent.

Plant material. *Berberis sibirica* Pall. aerial part I (700 g) was collected in August 2003 during the time of fruiting near the lake "Terkhiin Tsagaan nuur", province Arkhangai, Central Mongolia. 700 gr plant material was not enough and we collected more plant material in 2005. The sample of aerial part II (3.9 kg) and roots (2.2 kg) were collected in August 2005 during the time flowering near Khorgo mountain in Arkhangai province. The plant materials were identified by prof. Ch. Sanchir, Institute of Botany, Mongolian Academy of Sciences and the voucher specimen is deposited in the Herbarium Fund of the same Institute.

Extraction and isolation. Air dried and aerial parts (0.7 kg and 3.9 kg) and roots (2.2 kg) were worked up separately, by the same manner. They were extracted exhaustively with 95% EtOH at room temperature. The combined EtOH extracts were evaporated under reduced pressure, acidified with 5% HCl to pH 1-2 and left overnight at room temperature. Insoluble non-alkaloid materials were removed by filtration and the filtrate was subjected to n-hexane extraction to eliminate the rest of the non-alkaloid substances. Thus purified the acidic solution was made alkaline with 25% NH_4OH to pH 9-10 and extracted with $CHCl_3$. The combined $CHCl_3$ extracts were dried (anh. Na_2SO_4) and evaporated under reduced pressure to give crude mixtures of tertiary alkaloids – from the aerial part I Fraction A (1.29 g), aerial part II Fraction B (20.7 g) and from the roots Fraction R (13.91 g).

Fraction A was worked up by CC on neutra alumina, eluting with n-hexane:EtOAc of increasing polarity (7:1, 5:1, 3:1, 1:1, EtOAc) and finally with pure MeOH. 8 combined alkaloid fractions (A1 – A8) enriched in individual alkaloids were obtained. A2 (5:1) was subjected to PTLC with mobile phase petroleum:$CHCl_3$:Me_2CO:MeOH (4:4:1:1) and the alkaloid **7** (8.36 mg) was isolated. A3 (5:1) was subjected to PTLC with mobile phase petroleum:$CHCl_3$:Me_2CO:MeOH (4:4:1:1) and the alkaloids **9** (2.37 mg) and **14** (25.20 mg) were isolated. A4 (3:1) and A5 (3:1) were subjected to PTLC with mobile phase petroleum:$CHCl_3$:Me_2CO:MeOH (4:8:1:2) and the alkaloids **6**(6.84 mg) and **10** (30.00 mg) were isolated, respectively. A7 and A8 (pure MeOH) were subjected to PTLC with mobile phase petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) and the alkaloid **1** (5.00 mg) was isolated. A1 (7:1) and A6 (EtOAc) were in a small quantity (less than 10 mg) not enough for further isolation and characterization of pure compounds.

Fraction B was worked by VLC on silica gel, elutin with 1,2-dichlorethane:MeOH of increasing polarity (5:1, 3:1 and pure MeOH) and six combined alkaloid fractions (BS1-BS6) were obtained. Fraction BS1 (15 g) was worked up by CC on neutral aluminia, eluting with n-hexane:EtOAc of increasing polarity (5:1, 3:1, 1:1, EtOAc) and finally with pure MeOH. Four combined fractions (BS1-1 – BS1-4) enriched in individual alkaloids were obtained.

BS1-1 (3:1)(115.0 mg) was subjected to PTLC with Mph1and the alkaloids **12** (3.30 mg) and **11** (4.70 mg) were isolated. BS 1-2 (1:1) (1.9 g) was worked up by CC on neutral alumin, eluting with n-hexane:EtOokAc on increasing polarity (7:1, 5:1, 3:1, 1:1, EtOAc). Eight combined fractions (BS 1-2-1-BS 1-2-8) were obtained. BS 1-2-1 (7:1) (18.50 mg) was subjected to PTLC with Mph1 and the alkaloid **5** (10.0 mg) was isolated. BS 1-2-2 (5:1) was subjected to PTLC with Mph1 and the alkaloid **12** Mph1 and the alkaloid **13** (4.70 mg) and **9** (3.80 mg) were isolated. BS 1-2-4 (5:1) (350.0 mg) (50.0 mg from it) was subjected to PTLC with Mph1 and the alkaloid **11** (13.50 mg) was isolated. BS 1-2-5 (3:1) (61.70 mg) was subjected to PTLC with Mph1 and the alkaloids **11** (8.40 mg) and **6** (19.80 mg) were isolated. BS 1-2-6 (3:1) (87.10 mg) was subjected to PTLC with Mph2 and alkaloids **11** (5.20 mg), **6** (22.00 mg) and **18** (14.10 mg) were isolated. BS 1-2-7 (1:1) (81.10 mg) was subjected to PTLC with Mph2 and the alkaloid **6** (30.0 mg) was isolated. BS 1-2-8 (EtOAc) (100.0 mg) was subjected to PTLC with Mph **11** and the alkaloid **6** (13.70 mg), **16** (7.40 mg) and **8**

(11.90 mg) were isolated. BS 1-3 (1:1 and EtOAc) (650.0 mg) was worked up in the same manner, as S1-2 and four combined fractions (B.S1-3-1- B.S.1-3-4) were obtained. These fractions were separately subjected to PTLC with Mph1 and Mph2 and the same alkaloids, as in B.S.1-2 were isolated.

B.S.1-4 (MeOH) (2.0 g) (200.00 mg from it) was subjected to PTLC with Mph4 and the alkaloids **15** (11.00 mg) and **1** (2.70 mg) were isolated.

Fraction B.S.2 (5.2 g) was worked up by CC on neutra alumina, eluting with n-hexane5 EtOAc of increasing polarity (3:1, 1:1, EtOAc) and finally with pure MeOH. Four combined fractions (B.S.2-1- B.S.2-4) enriched in individual alkaloids were obtained.

B.S.2-1 (1:1) (66.50 mg) was subjected to PTLC with Mph1 and the alkaloids **5** (2.7 mg) and **6** (3.60mg) were isolated.

B.S.2-2 (EtOAc) (113.50 mg) was subjected to PTLC with Mph1 and the alkaloids **11** (10.20 mg) and **6** (5.60mg) were isolated.

B.S.2-3 (EtOAc) (102.00 mg) was subjected to PTLC with Mph2 and the alkaloids **11** (6.90 mg) and **16** (11.50 mg) were isolated.

B.S.2-4 (MeOH) (2.0 g) (100.00 mg from it) was subjected to PTLC with Mph3 and the alkaloids **15** (24 mg) and **3** (15.60 mg) were isolated.

Fraction B.S.3 (300.00 mg) was subjected to PTLC with Mph6 and the alkaloids **15** (25.40 mg), **3** (17.30 mg), **2** (19.80 mg) and 4 (11.10mg) were isolated.

Fraction B.S.4 (25.00 mg) was subjected to PTLC with Mph3 and the alkaloid **3** (5.50 mg) was isolated.

Fraction B.S.5 (100 mg) was subjected to PTLC with Mph3 and the alkaloids **15** (20.00 mg) and **3** (9.00 mg) were isolated.

Fraction B.S.6 (50 mg) was subjected to PTLC with Mph3 and the alkaloids **15** (1.90 mg), **3** (1.10 mg) and **2** (2.00 mg) were isolated.

Fraction R was separatbed by VLC on silica gel, eluting with 1,2-dichloroethane:MeOH of increasing polarity (5:1, 3:1, 1:1 and pure MeOH) and combined alkaloid fractions (R1 – R6) were obtained. R1 was in a small quantity (less than 30 mg) not enough for further isolation and characterization of pure compounds. R2, R3 and R4 were separately worked up by CC on neutra alumina, eluting with n-hexane:EtOAc of increasing polarity (7:1, 5:1, 3:1, 1:1, EtOAc) and finally with pure MeOH to afford fractions enriched in individual alkaloids. Elution of R2 with n-hexane:EtOAc (7:1, 5:1 and 3:1), followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (4:4:1:1) yielded **5** (29.80 mg) and **6** (29.30 mg). Further elution of R2 with pure MeOH, followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) yielded **1** (13.37 mg). Alkaloid **1** was identified by TLC with standard and it was **Berberine** we did not isolate all of berberine alkaloid in pure. Elution of R3 with n-hexane:EtOAc (3:1), followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (4:4:1:1) gave **18** (4.88 mg). Further elution of R3 with n-hexane:EtOAc (1:1), followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (4:8:1:2) gave **8** (3.12 mg), **20** (10.20 mg) and **17** (3.63 mg). Further elution of the same fraction with pure MeOH, followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) gave **1** (12.08 mg). Elution of R4 with n-hexane:EtOAc (3:1), followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (4:8:1:2) afforded **16** (17.68 mg). Further elution of R4 with n-hexane:EtOAc (1:1), followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (4:8:1:2) afforded **8** (2.05 mg) and **20** (9.30 mg). Further elution of R4 with EtOAc, followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) afforded **19** (8.29 mg). Further elution of the same fraction with pure MeOH, followed by PTLC purification with petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) afforded **1** (8.12 mg). R5 and R6 were directly subjected to PTLC with running phase petroleum:$CHCl_3$:Me_2CO:MeOH (2:8:1:3) and the alkaloids **20** (5.50 mg) and **21** (6.95 mg) were isolated.

1,10-Di-O-methylpakistanine (**21)**: Amorphous solid. UV λ_{max} nm (log ε): 270 sh

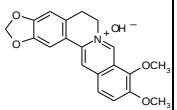
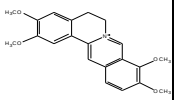
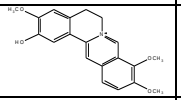
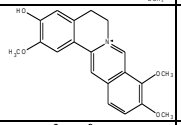
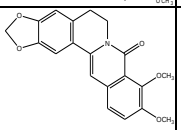
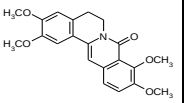
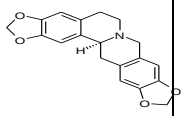
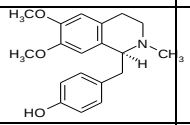
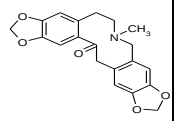
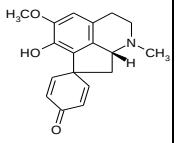
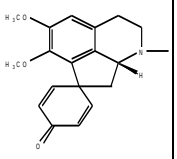
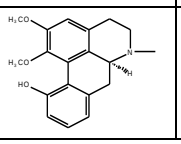
(4.23), 280 (4.30), 302 (4.10). IR ν_{max} cm^{-1}: 2858, 2800, 1595. EI MS: *m/z* (%) = 636 (2) $[M]^+$, 430 (10), 340 (6), 324 (15), 296 (5), 206 (100). ^1H and ^{13}C NMR in Table 1.

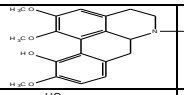
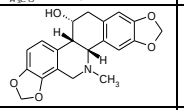
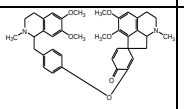
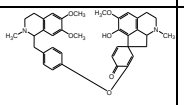
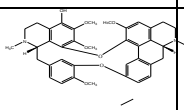
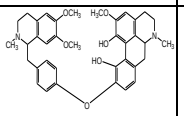
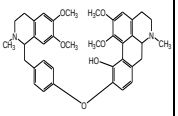
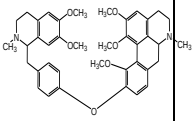
Results and discussion

The structures of the known alkaloids from the aerial part I: **7**, **9**, **14**, **6**, **10**, and **1**, earial part II: 1**, 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 15, 16** and **18**, from the roots: **5**, **18**, **8**, **20**, **17**, **16**, **19**, and **21** are determined by comparison of its ^1H NMR, EI MS, UV and IR data with those of authentic samples (Table 1) [6-18].

1,10-Di-O-methylpakistanine (**21**) was reported a natural new alkaloid and the data was reported in the international scientific journal [5]. The MS fragmentation pattern of 1,10-di-O-methylpakistanine (**21**) was characteristic of an aporphine-benzylisoquinoline dimmer. The weak molecular ion at *m/z* 636 was observed in EI MS. The base peak at *m/z* 206 represented the rings A' and B' in the benzylisoquinoline moiety of the molecule. The peaks at *m/z* 296 and *m/z* 324 corresponded to the benzylisoquinoline and aporphine parts of the dimmer, respectively. The fragments at *m/z* 430 $[M-206]^+$ and *m/z* 340 $[M-296]^+$ were also present in the same spectrum. The ^1H NMR of **21** exhibited singlets at δ 2.50 and δ 2.54 for two NCH_3 groups, as well as sharp singlets at δ 3.64, 3.75, 3.84, 3.85 and 3.91 for five OCH_3 groups. The comparison of ^1H NMR data of **21** with those of 1-O-methylpakistanine (**20**) showed that spectrum of **21** contained one additional signal at δ 3.91 for one OCH_3 group, which is not present in spectrum of **20**. In addition, by comparison with ^1H NMR spectrum of pakistanine (**19**), two more OCH_3 resonances were observed in ^1H NMR spectrum of **21**. ^1H NMR spectrum of **21** also displayed singlets at δ 6.11, 6.53 and 6.71 for four aromatic protons and two doublets at δ 6.97 and δ 7.09 with *J*=8.6 Hz for aromatic protons in ring C'. The most downfield signal in the same spectrum at δ 8.12 is for the aporphine proton H-11 (Table 2). The described spectral data of **21** closely resemble those reported for the synthetic alkaloid [17]. The carried out DEPT, NOESY, HMQC and HMBC experiments confirmed the proposal structure of **21** (Table 2).

Table 1. Alkaloids from *Berberis sibirica* Pall.

№	Name of alkaloid	Molecular mass, weigh t	Structure	Part of plant
1	Berberine $C_{20}H_{18}NO_4$	336 36.27 mg		AP I Root AP II
2	Palmatine $C_{21}H_{22}NO^+_4$	352 23.85 mgb		AP II
3	Columbamine $C_{20}H_{20}NO^+$	338 39.5 mg		AP II
4	Jatrorrhizine $C_{20}H_{20}NO^+_4$	338 11.10 mg		AP II
5	8-Oxoberberine $C_{20}H_{17}NO_5$	351 42.5 mg		AP I Root AP II
6	8-Oxopalmatine $C_{21}H_{21}NO_5$	367 124.0 mg		AP I Root AP II
7**	(-)-Tetrahydro-pseudocoptisine $C_{19}H_{17}NO_4$	323 8.36 mg		AP I
8**	(+)-Armepavine $C_{19}H_{23}NO_3$	313 15.02 mg		AP II Root
9**	Pseudoprotopine $C_{20}H_{19}NO_3$	353 6.17 mg		AP I AP II
10**	(+)-Glaziovine $C_{18}H_{19}NO_3$	297 30.0 mg		AP I
11	Pronuciferine $C_{19}H_{21}NO_3$	311 55.07 mg		AP II
12**	1-O-methylisotebaidine $C_{19}H_{21}NO_3$	311 8.0 mg		AP II

13	Isocorydine $C_{20}H_{23}NO_4$	341 4.70 mg		AP II
14**	(+)-Chelidonine $C_{20}H_{18}NbO_4$	353 25.20 mg		AP I
15**	Dehydrocorypalline	62.3 mg		AP II
16	Pakistanamine $C_{38}H_{42}O_6N_2$	36.58 mg		AP II Root
17	Valdivianine $C_{37}H_{40}O_6N_2$	608 3.63		Root
18**	Isothalidezine $C_{38}H_{42}N_2O_7$	638 4.87mg+ 14,10 mg		Root AP II
19	Pakistanine $C_{37}H_{40}N_2O_6$	608 8.29		Root
20	1-O-Methylpakistanine $C_{38}H_{42}N_2O_6$	622 25.0		Root
21*	1,10-diO-Methyl-pakistanineb $C_{39}H_{44}N_2O$	636 6.95		Root

*-Natural new alkaloid

**-First alkaloid in the family of Berberidaceae

Table 2. ^{1}H and ^{13}C NMR data for 1,10-di-O-methylpakistanine (**21**)

Position	δ H (J [Hz])	δ C[a] and HMQC
1	3.75, s	145.0, s
1a	-	129.3, s
1b	-	127.5, s or 128.6, s
2	3.85, s	152.2, s
3	6.53, s	111.3, d
3a	-	128.6, s or 127.5, s
4	2.57-2.71, m	29.3, t
5	2.99-3.07, m	53.2, t
6	2.50, s	-
6a	3.81-3.82, m	62.4, d
7	2.75-2.93, m	34.5, t
7a	-	128.2, s
8	6.71, s	117.3, d
9	-	145.9, s
10	3.91, s	155.9, s
11	8.12, s	116.6, d
11a	-	126.6, s
1’	3.87-3.88, m	64.8, d
2’	2.54, s	-
3’	2.75-2.93, m; 3.14-3.30, m	46.8, t
4’	2.57-2.71, m; 2.75-2.93, m	24.9, t
4’a	-	125.7, s
5’	6.53, s	111.3, d
6’	3.84, s	147.7, s
7’	3.64, s	146.6, s
8’	6.11, s	111.1, d
8’a	-	128.6, s
α'	2.75-2.93, m; 3.14-3.30, m	40.8, t
1’’	-	135.1, s
2’’	7.09, d (8.6)	131.3, d
3’’	6.97, d (8.6)	118.4, d
4’’	-	155.1, s
5’’	6.97, d (8.6)	118.4, d
6’’	7.09, d (8.6)	131.3, d
1-O$\underline{C}H_3$	-	60.3, q
2-O$\underline{C}H_3$	-	55.9, q
10-O$\underline{C}H_3$	-	60.4, q
6’-O$\underline{C}H_3$	-	55.8, q
7’-O$\underline{C}H_3$	-	55.7, q
6-N$\underline{C}H_3$	-	43.9, q
2’-N$\underline{C}H_3$	-	42.4, q

[a] Multiplicities of the carbon atoms are determined by DEPT experiment

Conclusions

21 isoquinoline alkaloids were isolated and identified from *Berberis sibirica* Pall. 1 was a new natural alkaloid and named monpakistanine by us. 8 alkaloids were isolated for the first time in the family of Berberidaceae. High biological active alkaloid berberine, 8-oxopalmatine, 8-oxoberberine, pronuciferine and dehydrocorypalline were predominant alkaloids of *Berberis sibirica* ant and it is showing a proof that this plant is being used widely in Mongolian traditional medicine.

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