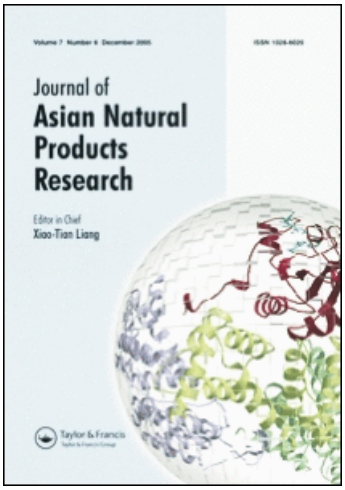

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Ginsenine, a new alkaloid from the berry of *Panax ginseng* C. A. Meyer

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Ginsenine, a new alkaloid from the berry of *Panax ginseng* C. A. Meyer

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A new indole alkaloid, ginsenine, with a seven-membered lactam unit, was isolated from the berry of *Panax ginseng*. Its structure was established on the basis of extensive NMR (^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H COSY, DEPT, HMQC, HMBC), IR, and ESI-MS analysis.

Keywords: *Panax ginseng*; Araliaceae; Indole alkaloid; Ginsenine

1. Introduction

Panax ginseng C. A. Mey. (Araliaceae) is distributed and cultivated in the Changbai Mountains, northeast China. Its fruit, with a high ginsenoside content [1–5] and evident pharmacological activity [6–16], has been studied extensively, together with research and development on other parts of the Ginseng plant with the exception of the root. We report here the isolation and structure elucidation of a new alkaloid from the berry of *Panax ginseng*.

2. Results and discussion

Fresh berries of *Panax ginseng* C. A. Mey. were immersed in water, the seeds removed, and then kneaded by hand to afford a syrupy solution. The syrup solution was centrifuged and the supernatant was concentrated *in vacuo* to give a residue. The residue was then chromatographed over Diaion-101 resin successively eluting with H_2O and 15% EtOH. Removal of EtOH *in vacuo* gave an aqueous solution that was extracted with n-BuOH to afford n-BuOH extracts. The latter extracts were further purified on a polyamide column and reversed-phase HPLC chromatography to afford the compound ginsenine.

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Ginsenine was isolated as a white powder. $[\alpha]_D^{20}$ − 13.3 (*c* 0.36, MeOH). EIMS showed a molecular ion at *m/z* 230 with significant fragment peaks at *m/z* 215 $[M-NH]^+$, 169, 157, 115. HRSIMS (positive) measurements (see Experimental section) gave the molecular formula $C_{13}H_{14}N_2O_2$. The ^{13}C-NMR spectrum showed a total of 13 carbon resonances, in agreement with the molecular formula. The UV spectrum showed absorption maxima at 221, 272, 278, and 289 nm ($\log \epsilon = 3.42$, 2.75, 2.74 and 2.63, respectively) due to an indole chromophore, which is a characteristic diagnostic of an unsubstituted moiety in the aromatic ring of an indole ring [17]. The ^{1}H-NMR spectrum indicated the presence of *N*H as a singlet at δ 11.10, and four aromatic protons [δ 7.45 (1H, d, $J = 8.0$ Hz), 7.34 (1H, d, $J = 8.0$ Hz), 7.09 (1H, ddd, $J = 1.0$, 7.5, 8.0 Hz), 7.00 (1H, ddd, $J = 1.0$, 7.5, 8.0 Hz)], indicating the presence of an indole skeleton of an unsubstituted moiety in the aromatic ring. On the basis of the ^{13}C-NMR, DEPT, HSQC, and HMBC spectra of ginsenine, δ 132.2 s, 106.8 s, 126.1 s, 118.0 d, 118.8 d, 121.4 d, 111.2 d, and 136.4 s were assigned to C-2, C-8, C-9, C-10, C-11, C-12, C-13, and C-14 (figure 1 and table 1), respectively.

The IR spectrum showed absorption bands at 3369 (broad), 3295, 1644 and 1385 cm^{-1}, assignable to −OH, *N*H, secondary lactam carbonyl and $-CH_3$ functions [18], respectively. Comparison of the ^{1}H- and ^{13}C-NMR spectra of ginsenine with those of tronocarpine isolated from *Tabernaemontana corymbosa* [19] revealed a seven-membered lactam unit incorporated in the molecule of ginsenine (figure 1). The ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H COSY, and HSQC spectral data revealed the presence of $-CH_2CH(OH)$ and $-CH(CH_3)NH$ structural moieties, in which δ_H 3.16 (1H, ddd, $J = 2.5$, 4.8, 15.2 Hz, H_b-7), 2.78 (1H, ddd, $J = 2.5$, 11.8, 15.2 Hz, H_a-7), and δ_C 23.2 are assigned to $-CH_2CH(OH)$, δ_H 3.61 (1H, dd, $J = 4.8$, 11.8 Hz, H-6) and δ_C 57.6 are assigned to $-CH_2CH(OH)$, δ_H 8.76 (1H, br s) is assigned to $-CH_2CH(OH)$, δ_H 4.52 (1H, d, $J = 6.5$ Hz) and δ_C 49.0 are assigned to $-CH(CH_3)NH$, δ_H 1.62 (3H, d, $J = 6.5$ Hz) and δ_C 17.0 are assigned to $-CH(CH_3)NH$, and δ_C 169.4 is assigned to −CH−CO−NH−. In the HMBC spectrum, the correlations from H-3 (δ 4.52) to C-2 (δ 132.2) and C-8 (δ 106.8), and from H-7 (δ 3.16) to C-8 (δ 106.8), C-9 (δ 126.1), and C-2 (δ 132.2) suggest that a seven-membered lactam moiety is fused to the indole portion at C-2 and C-8, as shown in figure 1. Likewise, the correlations from H-15 (δ 1.62) to C-3 (δ 49.0) and C-2 (δ 132.2), and from H-7 (δ 3.61) to C-7 (δ 23.2), C-8 (δ 106.8), and C-5 (δ 169.4) indicates that C=O is connected between −CH(OH) and −NH. In NOESY the interaction between H-3 (δ 4.52) and H-6 (δ 3.61) and H_a-7 (δ 2.78) allowed the assignment of the relative configuration (figure 1, **1a** and **1b**) of the methyl and hydroxyl groups in ginsenine to be the *cis* orientation. Further, the mass fragmentation ions in the EI-MS spectrum support the above structure analysis (see Experimental section).

On the basis of the above evidence, the structure of the new compound we call ginsenine was determined to be **1a** or **1b** (figure 1). The structure of ginsenine represents a new

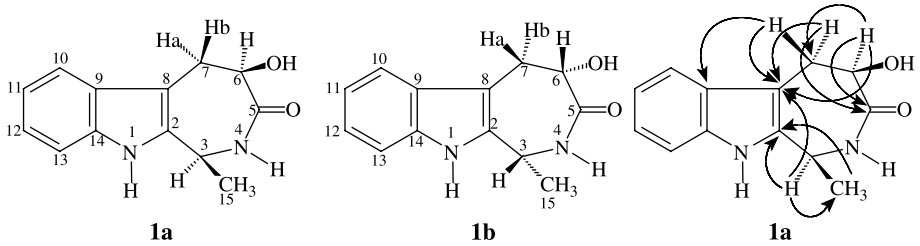

Figure 1. Structures and the key HMBC correlations of ginsenine (**1a** or **1b**).

Table 1. NMR spectral data for ginsenine (^{1}H: 500 MHz; ^{13}C: 125 MHz, DMSO-d_6).

No.	δ_C	δ_H *mult (J=Hz)*	*HMBC (C → H)*
N_1		11.10 s	
2	132.2 s		N_1-H, H-3, H-7, H-15
3	49.0 d	4.52 d (6.5)	H-15
5	169.4 s		H-6, H-7
6	57.6 d	3.61 dd (4.8, 11.8)	H-7
7	23.2 t	3.16 ddd (2.5, 4.8, 15.2)	H-6
		2.78 ddd (2.5, 11.8, 15.2)	
8	106.8 s		N_1-H, H-10, H-3, H-6, H-7
9	126.1 s		N_1-H, H-10, H-11, H-13
10	118.0 d	7.45 d (8.0)	H-11, H-12
11	118.8 d	7.00 ddd (2.0, 7.5, 8.0)	H-10, H-12, H-13
12	121.4 d	7.09 ddd (2.0, 7.5, 8.0)	H-10, H-11, H-13
13	111.2 d	7.34 d (8.0)	H-11, H-12
14	136.4 s		N_1-H, H-10, H-12
15	17.0 q	1.62 d (6.5)	H-3
OH		8.76 s	

monoterpenoid indole alkaloid skeleton and is probably related biogenetically to tryptophan and indole-3-lactic acid or indole-3-pyruvic acid.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured using a Perkin-Elmer 243B polarimeter. IR spectra were recorded on a Thermo Nicolet Nexus 470 FT-IR spectrometer with KBr pellets, and UV spectra were acquired on a Varian Cary 300 UV–VIS spectrometer. NMR spectra were obtained using a Varian INOVA-500 spectrometer with DMSO-d_6 at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts are given in δ relative to TMS as internal standard. EI-MS were obtained with a Finnigan TRACE 2000 and HR-SI-MS was performed on a Bruker Daltonics APEX-II FT-ICRMS spectrometer.

The semi-prepared HPLC system consisted of an Agilent 1100 pump and a VWD spectrophotometric detector, all purchased from Agilent. An Agilent ZORBAX SB-C_{18} column (250 × 9.4 mm I.D., 5 μm) was used. Separation was obtained using MeOH–H_2O (18:82) as eluent. The flow-rate was kept constant at 2.0 ml min^{-1}.

The macroporous resin Diaion-101 was produced by Nankai University, China, and the polyamide was produced by the biochemistry factory of Zhejiang Luqiao, China.

3.2 Plant material

Fresh berries of *Panax ginseng* were collected from Changbai county, Jilin province, China, in August 2002. A voucher specimen has been deposited in the herbarium of the College of Traditional Chinese Medicinal Material, Jilin Agriculture University. The plant was identified by Professor Xianggao Li.

3.3 Extraction and isolation

The fresh berries (10 kg) of *Panax ginseng* were immersed in water (50 l), the seeds removed and kneaded by hand to afford a syrupy solution. The syrup solution was centrifuged to obtain

the supernatant. After evaporation of the aqueous solution *in vacuo*, the residue (480 g) was chromatographed over a Diaion-101 resin column, eluting with H_2O (30 l) and then 15% EtOH (30 l). Removal of the EtOH from the 15% EtOH fraction under reduced pressure gave an aqueous solution, which was dried by freezing. The frozen residue (30 g) was dissolved in n-BuOH and then filtered. The insoluble residue was dissolved in water and filtered, and the solution was chromatographed on a column of the polyamide by elution with distilled water. The water eluate was concentrated *in vacuo*, and then extracted with n-BuOH saturated with H_2O. The extract was concentrated *in vacuo* to remove the n-BuOH, and the residue was dried by freezing. The frozen residue (2 g) was further purified by reversed-phase semi-preparative HPLC on a C_{18} column (250 × 9.4 mm I.D., 5 μm), eluting with MeOH–H_2O (18:82), and the flow-rate was maintained at 2 ml min^{-1} to afford pure ginsenine (12 mg).

3.4 Ginsenine (1a or 1b)

The compound was obtained as a white powder. $[\alpha]_D^{20}$ − 13.3 (*c* 0.36, MeOH). IR ν_{max}^{KBr} (cm^{-1}): 3369 (broad, *N*H), 3295 (–OH), 2983, 2922, 2766, 2673, 2497, 1644 (lactam C=O), 1577, 1454, 1385 (CH_3), 954, 813, 740, 698, 592. UV λ_{max}^{MeOH} (log ε) (nm): 221 (3.42), 272 (2.75), 278 (2.74), 289 (2.63). EI-MS *m/z*: 231 $[M + H]^+$, 215 $[M–NH]^+$, 185, 183, 169 (100%), 157, 143, 130, 129, 115, 101, 77. HR-SI-MS *m/z* 231.1128 $[M + H]^+$ (calcd for $C_{13}H_{15}N_2O_2$ [M + H], 231.1133). ^{1}H-MR (500 MHz, DMSO-d_6) δ: 11.10 (1H, s, N_1-H), 8.76 (1H, br s, OH), 7.45 (1H, d, *J* = 8.0 Hz, H-10), 7.34 (1H, d, *J* = 8.0 Hz, H-13), 7.09 (1H, ddd, *J* = 2.0, 7.5, 8.0 Hz, H-12), 7.00 (1H, ddd, *J* = 2.0, 7.5, 8.0 Hz, H-11), 4.52 (1H, d, *J* = 6.5 Hz, H-3), 3.61 (1H, dd, *J* = 4.8, 11.8 Hz, H-6), 3.16 (1H, ddd, *J* = 2.5, 4.8, 15.2 Hz, H_b-7), 2.78 (1H, ddd, *J* = 2.5, 11.8, 15.2 Hz, H_a-H), 1.62 (3H, d, *J* = 6.5 Hz, H-15). ^{13}C-NMR (125 MHz, DMSO-d_6), see table 1.

References

[1] S.X. Xu, G.G. Zhang, Y.J. Chen, X.S. Yao. *J. Shenyang Pharm. Univ.*, **5**, 59 (1988).
[2] Y.Q. Zhao, C.L. Yuan, H.R. Lu. *China J. Chin. Mater. Med.*, **16**, 678, 704 (1991).
[3] Y.Q. Zhao, C.L. Yuan, H.R. Lu. *China J. Chin. Mater. Med.*, **18**, 296, 319 (1993).
[4] M. Yu, Y.Q. Zhao. *Chin. Tradit. Herbs Drugs*, **33**, 404 (2002).
[5] J.Y. Wang, X.G. Li, Y.N. Zheng, X.W. Yang. *J. Asian Nat. Prod. Res.*, **6**, 289 (2004).
[6] L.J. Yin, Q. Liu, C.Z. Li. *Zhongchengyao*, **16**, 34 (1994).
[7] Y. Li, X.M. Cui, L. Pan, Z.B. Li, C.D. Ji, K.Q. Du. *J.N. Bethune Univ. Med. Sci.*, **24**, 452 (1998).
[8] X.F. Yu, S.C. Qu, H.L. Xu, D.Y. Sui, Y.P. Chen, X.Y. Ma. *J. Jilin Univ. (Med. Ed.)*, **29**, 573 (2003).
[9] H.L. Xu, X.F. Yu, S.C. Qu, D.Y. Sui. *Renshen Yanjiu*, **2**, 2 (2003).
[10] C.C. Han, H.L. Xu, X.F. Yu, S.C. Qu, D.Y. Sui. *Renshen Yanjiu*, **2**, 5 (2003).
[11] A.S. Attele, Y.P. Zhou, J.T. Xie, J.A. Wu, L. Zhang, L. Dey, W. Pugh, P.A. Rue, K.S. Polonsky, C.S. Yuan. *Diabetes*, **51**, 1851 (2002).
[12] L. Dey, J.T. Xie, A. Wang, J. Wu, S.A. Maleckar, C.S. Yuan. *Phytomed.*, **10**, 600 (2003).
[13] B. Feng, B. Wang, Y.P. Li. *J.N. Bethune Univ. Med. Sci.*, **22**, 21 (1996).
[14] A.P. Lu, G.H. Cheng, Y.P. Li, B. Feng. China *J. Gerontol.*, **17**, 242 (1997).
[15] Y.M. Yao, Y.Y. Zhang, G.J. Chen, L.H. Zou. *Acta Chin. Med. Pharm.*, **30**, 14 (2002).
[16] L.H. Zou, Y.Y. Zhang, Y.M. Yao. *Acta Chin. Med. Pharm.*, **30**, 34 (2002).
[17] L. Huang, D.Q. Yu. *Application of Ultraviolet Spectrum in Organic Chemistry*, Part II, pp. 132–137, Science Press, Beijing (1998).
[18] J.X. Xie, J.B. Chang, X.M. Wang. *Application of Infrared Spectrum in Organic Chemistry and Medicinal Chemistry*, pp. 307–372, Science Press, Beijing (2001).
[19] T.S. Kam, K.M. Sim, T.M. Lim. *Tetrahedron Lett.*, **41**, 2733 (2000).