Three New Pteridines, Hirudinoidines A – C, from *Hirudo nipponica* WHITMAN

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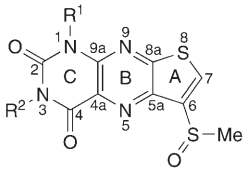
Three new pteridines, hirudinoidines A – C (**1** – **3**, resp.), with novel structural features were isolated from *Hirudo nipponica* WHITMAN, and their structures were elucidated on the basis of extensive spectroscopic analyses and by comparison of their spectral data with those of related metabolites. The configuration of **1** was unequivocally confirmed by single-crystal X-ray diffraction (*Fig. 2*).

Introduction. – *Leech* has been extensively studied for their anticoagulant compounds which maintain the blood in a fluid state during the uptake and in their guts. In the previous studies, most investigations on the bioactive agents from leech were focused on its proteases, because of its excellent pharmacodynamic activities in anticoagulation [1]. *Hirudo nipponica* WHITMAN, a Chinese medicine used commonly for activating blood circulation to dissipate blood stasis [2], is less studied. Only six trigalactosylceramides [3] and six neutral glycosphingolipids [4] from *H. nipponica* have been reported. In the present investigation, three new pteridines, hirudinoidines A – C with novel structural features were obtained. This paper deals with the isolation, structure elucidation, and NMR assignments of these new compounds.

Results and Discussion. – By a series of column chromatography, on silica gel and *Sephadex LH-20*, a 95% EtOH extract of air-dried *H. nipponica* afforded three new pteridines named hirudinoidines A – C (**1** – **3**, resp.). The identification of **1** – **3** was accomplished by the spectroscopic data.



Hirudinoidine A (**1**) $R^1 = R^2 = Me$
Hirudinoidine B (**2**) $R^1 = Me$, $R^2 = H$
Hirudinoidine C (**3**) $R^1 = R^2 = H$

Hirudinoidine A (**1**) was isolated as yellow prisms. The IR spectrum indicated that **1** possessed amide C=O (1722 and 1674 cm^{-1}) and S=O groups (1047 cm^{-1}). According to the element analysis, it contains N (17.76%), C (42.70%), H (2.85%), and S (19.84%). Its molecular formula was determined as $C_{11}H_{10}N_4O_3S_2$ by HR-ESI-MS (*m*/*z*

311.0277 ($[M+H]^+$, $C_{11}H_{11}N_4O_3S_2^+$; calc. 311.0272)). The detailed analysis of the ^{1}H- and ^{13}C-NMR (*Table*), HMBC, HMQC, and X-ray data, and comparison with literature data established the structure of **1** as 1,3-dimethyl-6-(methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione.

Table. *^{1}H-* (500 MHz) *and ^{13}C-NMR* (125 MHz) *Data of* **1**–**3**[a])[b])

Position	**1** ($CDCl_3$)		**2** ((D_6)DMSO)		**3** ((D_6)DMSO)	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
Me–N(1) or H–N(1)	3.57 (*s*)	29.2	3.53 (*s*)	28.4	11.78 (*s*)	–
C(2)	–	150.4	–	149.7	–	149.4
Me–N(3) or H–N(3)	3.80 (*s*)	29.9	12.09 (*s*)	–	12.11 (*s*)	–
C(4)	–	145.2	–	146.6	–	146.4
C(4a)	–	125.6	–	127.1	–	126.2
C(5a)	–	140.7	–	139.4	–	140.1
C(6)	–	139.0	–	137.8	–	137.9
H–C(7)	8.34 (*s*)	131.0	8.56 (*s*)	131.3	8.51 (*s*)	131.0
C(8a)	–	159.5	–	157.9	–	158.4
C(9a)	–	159.6	–	159.4	–	160.3
6-MeS(O)	3.14 (*s*)	41.8	3.07 (*s*)	40.7	3.06 (*s*)	40.7

[a]) TMS was used as an internal standard. [b]) Assignments based on HSQC and HMBC experiments.

The ^{1}H-NMR spectrum displayed signals due to two *N*-Me groups (δ(H) 3.57 (*s*), 3.80 (*s*)), one *S*-Me (δ(H) 3.14 (*s*)), and one vinyl group (δ(H) 8.34 (*s*)) (*Table*). The ^{13}C-NMR and HSQC spectra exhibited the signals indicating that **1** has three Me groups, and seven groups sp^2-quaternary and one vinyl C-atoms [5] (*Table*). Detailed analysis of the HSQC and HMBC spectra showed that **1** had the same A, B, and C ring structures as 1,3-dimethyl-7-phenylthieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione synthesized by *Gulevskaya et al.* [6]. The ^{1}H- and ^{13}C-NMR spectra displayed resonances for three Me groups (δ(H) 3.14 and δ(C) 41.8, δ(H) 3.57 and δ(C) 29.2, and δ(H) 3.80 and δ(C) 29.9), one aromatic H-atom (δ(H) 8.34 and δ(C) 131.0). All protonated C-atoms and their H-atoms were assigned by the HSQC experiments. The structure elucidation was assisted by analyses of the HMBC experiments. The correlations in the HMBC experiments between H of Me–N(1) at δ(H) 3.57 with C(2) at δ(C) 150.4 and C(9a) at δ(C) 159.6; and H of Me–N(3) at δ(H) 3.80 with C(2) and C(4) at δ(C) 145.2 confirmed the structure of the ring C, between H–C(7) at δ(H) 8.34 with C(6) at δ(C) 139.0, C(5a) at δ(C) 140.7, and C(8a) at δ(C) 159.5, and in addition, between H of MeS(O) at δ(H) 3.14 with C(6) secured the structure of ring A (*Fig. 1*). In principle, the way how the five-membered S heterocyclic ring is fused to the ring B cannot be unambiguously established by the HMBC data (the S-atom could also be looking down when the molecule is drawn as shown in *Fig. 1*, both ways are compatible with the observed HMBC correlations, but not with ^{13}C chemical shifts). Since there are no correlations between C(8a) and C(5a), and the left part of the molecule, also C(5a) and C(8a) could be swapped. To resolve that ambiguity, the X-ray structure is required. From these observations, hirudinoidine A (**1**) was considered to have the structure as shown, which was confirmed by a single-crystal X-ray-analysis (*Fig. 2*).

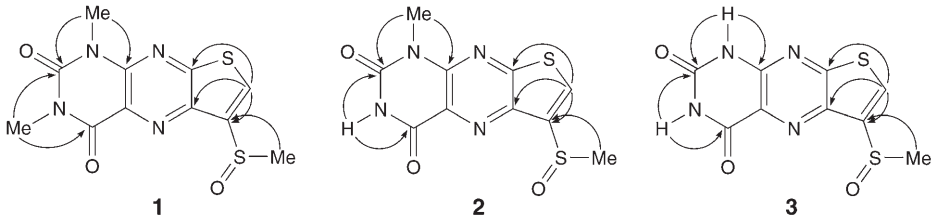


Fig. 1. *Key HMBC correlations* (H → C) *of* **1**–**3**

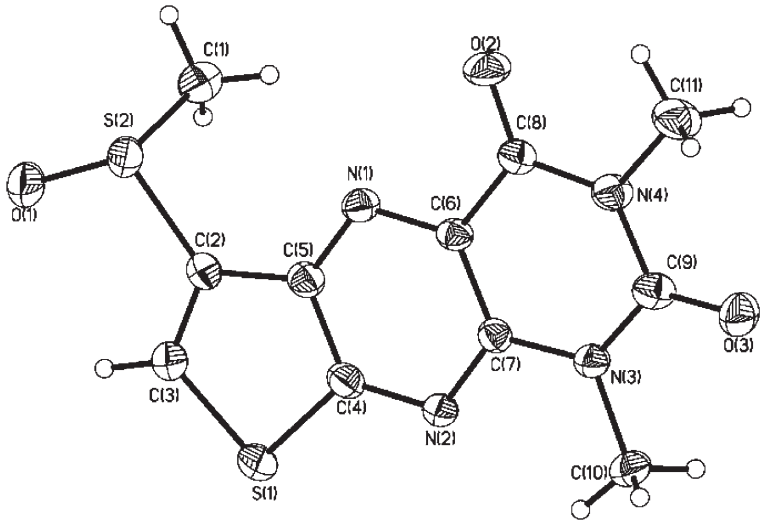


Fig. 2. *X-Ray crystal structure of Hirudinoidine A* (**1**). Arbitrary atom numbering.

Hirudinoidine B (**2**) was isolated as an amorphous solid. The IR spectrum exhibited absorption bands for amide (3533 cm^{-1}), C=O (1722 and 1689 cm^{-1}), and S=O groups (1047 cm^{-1}). Its molecular formula was deduced to be $C_{10}H_8N_4O_3S_2$ from the $[M+H]^+$ peak at *m*/*z* 297.0071 ($C_{10}H_9N_4O_3S_2^+$; calc. 297.0085) in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra (*Table*) of compounds **1** and **2** were similar, suggesting that compound **2** also possesses the main thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione skeleton as **1**. In the HMBC spectrum, the same correlations were observed for both compounds, indicating (*Fig. 1*) the same connectivity of the A, B, and C rings in **2**, as determined for compound **1**. Analysis of the ^{1}H- and ^{13}C-NMR, together with HSQC and HMBC data of **2**, showed that the only difference in **2** was the H-atom at N(3), which was substituted by a Me group in **1**. The HMBC correlations between H of H–N(3) at δ(H) 12.09 with C(4) at δ(C) 146.6 and C(2) at δ(C) 149.7; between H of Me–N(1) at δ(H) 3.53 with C(2) and C(9a) at δ(C) 159.4 confirmed the presence of an H-atom at N(3). Accordingly, the planar structure of hirudinoidine B (**2**) was established as 1-methyl-6-(methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione.

Hirudinoidine C (**3**) was obtained as an amorphous solid. IR Absorptions were observed at 3404, 3151, 1701, 1705, and 1072 cm^{-1}, suggesting the presence of amide, C=O, and S=O groups in **3**. Its molecular formula was determined to be $C_9H_6N_4O_3S_2$ from the $[M+H]^+$ peak at *m/z* 282.9946 ($C_9H_7N_4O_3S_2^+$; calc. 282.9954) in the HR-ESI-MS. The ^{1}H-NMR spectrum (*Table*) revealed signals corresponding to MeS(O) at δ(H) 3.06 (*s*), one vinyl H-atom at δ(H) 8.51 (*s*), and two amide H-atoms at δ(H) 12.08 (*s*) and δ(H) 11.78 (*s*). Analysis of the HSQC and HMBC (*Fig. 1*) indicated that the data of **3** highly resembled those of **1** and **2**, with the main differences residing in C ring. In comparison with **1** and **2**, there was no Me group in the ring C of **3**; furthermore, the structure of the ring C of **3** was confirmed by the HMBC correlations: H of H–N(3) at δ(H) 12.11 with C(4) at δ(C) 146.4 and C(2) at δ(C) 149.4; H of H–N(1) at δ(H) 11.78 with C(2) and C(9a) at δ(C) 160.3. Thus, hirudinoidine C (**3**) was established as 6-(methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione.

The three new pteridines, hirudinoidines A–C (**1**–**3**, resp.), isolated from *Hirudo nipponica* WHITMAN possess characteristic structural features. To the best of our knowledge, the main framework of the three new compounds as thieno[3,2-*g*]pteridine was also synthesized by *Pfleiderer* and co-workers [7][8]; however, this is the first report on the isolation of the compounds with these novel structural features from a natural source.

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Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai). *Sephadex LH-20* (*Amersham Biosciences*), silica gel (100–200 mesh and 200–300 mesh; *Qingdao Haiyang Chemical Co. Ltd.*, Qingdao) were used for column chromatography (CC). Precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Co. Ltd.*, Qingdao) were used for TLC. UV Spectra: *Beckman DU 640* spectrophotometer; λ_{max} in nm (log ε). IR Spectra: *Shimadzu FT/IR 8900* spectrophotometer; KBr pellets; $\tilde{\nu}_{max}$ in cm^{-1}. NMR Spectra: *Bruker ACF-500* spectrometer at 300 K with TMS as internal standard. The ^{1}H chemical shifts in $CDCl_3$ and in DMSO were referenced to the residual $CHCl_3$ resonance at 7.26 ppm and DMSO at 3.28 ppm, resp., and the ^{13}C chemical shifts in $CDCl_3$ and in DMSO were referenced to the solvent resonance at 76.9 ppm and 39.7 ppm, resp. HR-ESI-MS: *Wiff Agilent TOF* mass spectrometer; in *m/z* (rel. %). Elemental analysis: *Elementar Vario ELIII*.

Animal Material. Hirudo nipponica WHITMAN were collected from Shuqian county of Jiangsu Province, P. R. China, in September 2004, and identified by Dr. *You-bin Li* (Jiangsu Provincial Institute of Traditional Chinese Medicine, P. R. China). A voucher specimen has been deposited in Laboratory of Phytochemistry, Jiangsu Provincial Institute of Traditional Chinese Medicine (accession No.: 2004-09-08).

Extraction and Isolation. The air-dried powder of the animal (2 kg) was extracted exhaustively with circumfluent 95% EtOH at 80°, and the crude extract (434 g) was subsequently extracted successively with petroleum ether, $CHCl_3$, and BuOH. The BuOH-soluble fraction (65 g) was separated by silica gel (100–200 mesh) CC ($CHCl_3$/MeOH 50:1 to 0:1) to give six *Fractions A–F. Fr. A* (12 g) was then separated on a *Sephadex LH-20* column ($CHCl_3$/MeOH, 60:40) to afford six *Fractions A1–A6*. Hirudinoidine A (**1**; 25 mg) was obtained by recrystallisation from *Fr. A6* ($CHCl_3$/MeOH 60:40). *Fr. B* (8 g) was also chromatographed on a *Sephadex LH-20* column ($CHCl_3$/MeOH 50:50) to give seven *Fractions B1–B7. Fr. B5* (0.8 g) was subjected to CC (silica gel (200–300 mesh); $CHCl_3$/MeOH 30:1 to 20:1) to give three *Fr. B5a–B5c. Fr. B5b* (0.1 g) was purified over *Sephadex LH-20* column ($CHCl_3$/MeOH 50:50) to afford hirudinoidine B (**2**; 13 mg). *Fr. D* (7 g) was chromatographed (silica gel (200–

300 mesh); $CHCl_3$/MeOH 20:1 to 5:1) to afford four *Fractions D1–D4. Fr. D3* (1.1 g) was chromatographed (*Sephadex LH-20* column; $CHCl_3$/MeOH 40:60) to give five *Fractions D3a–D3e. Fr. D3c* (0.5 g) was further separated by CC (*Sephadex LH-20* column; $CHCl_3$/MeOH 40:60) to yield hirudinoidine C (**3**; 18 mg).

*1,3-Dimethyl-6-(methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione* (**1**). Yellow crystals. UV (MeOH): 365 (0.55), 254 (1.68), 250 (1.68), 227 (1.03). IR: 1722, 1674, 1550, 1531, 1506, 1289, 1221, 1047, 804, 748. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS (pos.): 333 (100, $[M+Na]^+$), 311 (84, $[M+H]^+$), 643 (18, $[2M+Na]^+$). ESI-MS (neg.): 309 (100, $[M-H]^-$). HR-ESI-MS: 311.0277 ($[M+H]^+$, $C_{11}H_{11}N_4O_3S_2^+$; calc. 311.0272).

*1-Methyl-6-(methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione* (**2**). Ginger powder. UV (MeOH): 363 (0.45), 248 (1.20). IR: 3533, 3180, 1722, 1689, 1548, 1531, 1485, 1383, 1047, 858, 804. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS (pos.): 319 (100, $[M+Na]^+$), 297 (75, $[M+H]^+$), 615 (30, $[2M+Na]^+$). HR-ESI-MS: 297.0071 ($[M+H]^+$, $C_{10}H_9N_4O_3S_2^+$; calc. 297.0085).

*6-(Methylsulfinyl)thieno[3,2-*g*]pteridine-2,4(1*H*,3*H*)-dione* (**3**). Ginger powder. UV (MeOH): 362 (0.40), 358 (0.39), 244 (1.12). IR: 3404, 3151, 1701, 1705, 1624, 1564, 1450, 1359, 1072, 804. ^{1}H- and ^{13}C-NMR: see *Table*. ESI-MS (pos.): 305 (100, $[M+Na]^+$), 283 (85, $[M+H]^+$), 597 (20, $[2M+Na]^+$). HR-ESI-MS: 282.9946 ($[M+H]^+$, $C_9H_7N_4O_3S_2^+$; calc. 282.9954).

X-Ray Crystal Structure of **1**[1]). Formula $C_{11}H_{10}N_4O_3S_2$; M_r 310.35 g/mol; crystal size: $0.30\times0.10\times0.10$ mm; crystal system: monoclinic; space group $P21/c$; unit-cell dimensions: $a=8.1970(16)$, $b=8.2960(17)$, $c=19.643(4)$ Å, $\alpha=90.00$, $\beta=98.04(3)$, $\gamma=90.00°$, $V=1322.6(5)$ Å^3; $Z=4$; $D_x=1.559$ mg/m^3; $F(000)=640$, $T=293(2)$ K. Diffraction data of **1** were collected with an *Enraf-Nonius CAD4* diffractometer, using MoK_α radiation ($\lambda=0.71073$ Å) and the $\omega-2\theta$ scan mode. The total number of reflections measured was 2777, of which 2591 were unique and 1724 observed, $I>2\sigma(I)$. Final indices: $R_f=0.0676$, $R_w=0.1551$ ($w=1/[\sigma^2(F_o^2)+(0.06\,P)^2+5\,P]$ where $P=(F_o^2+2\,F_c^2)/3$) for observed reflections, and $R_1=0.1105$, $wR_2=0.2088$ for all reflections (2591). The structure was solved with SHELXTL-97, and refined by means of full-matrix least-squares on F^2.

REFERENCES

[1] K. H. Strube, B. Kroger, S. Bialojan, M. Otte, J. Dodt, *J. Biol. Chem.* **1993**, *268*, 8590.
[2] States Pharmacopoeia Commission, 'Chinese Pharmacopoeia', Chinese Chemical Industry Press, Beijing, 2005, p. 57.
[3] N. Noda, R. Tanaka, K. Miyahara, T. Sukamoto, *Chem. Pharm. Bull.* **1996**, *44*, 895.
[4] N. Noda, R. Tanaka, M. Nishi, S. Inoue, K. Miyahara, *Chem. Pharm. Bull.* **1993**, *41*, 1366.
[5] S. Anandan, S. Latha, P. Maruthamuthu, *J. Photochem. Photobiol., A* **2002**, *150*, 167.
[6] A. V. Gulevskaya, D. V. Shee, A. F. Pozharskii, *Russ. Chem. Bull.* **2003**, *52*, 1403.
[7] R. H. Baur, W. Pfleiderer, *Isr. J. Chem.* **1986**, *27*, 81.
[8] A. H. Khaled, W. Pfleiderer, *Pteridines* **1996**, *7*, 113.

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[1]) The crystallographic data of **1** has been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*) as supplementary publication number CCDC-663287. Copies of the data can be obtained, free of charge, at http://www.ccdc.cam.ac.uk/data_request/cif.