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Two new alkaloids and active anti-hepatitis B virus constituents from *Hypserpa nitida*

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Abstract—Two new alkaloids, hypserpanines **A** and **B** (**1**, **11**), together with eleven known compounds, phenolbetain (**2**), acutumine (**3**), acutumidine (**4**), dechloroacutumine (**5**), dauricumine (**6**), dauricumidine (**7**), pronuciferine (**8**), glaziovine (**9**), *S*-reticuline (**10**), magnoflorine (**12**) and laurifoline(**13**), were isolated from *Hypserpa nitida* Miers. (Menispermaceae) and chemically elucidated through spectral analyses. All the isolated alkaloids were evaluated for their anti-HBV activities in vitro using the HBV transfected Hep G2.2.15 cell line. The most active compound, dauricumidine (**7**), exhibited an IC_{50} value of 0.450 mM (SI = 4.13) on hepatitis B virus (HBV) surface antigen (HBsAg) secretion of the Hep G2.2.15 cell line.

The hepatitis B virus (HBV) belongs to the family of hepadnaviruses (hepatotropic DNA viruses) and causes acute and chronic infections of the liver and is responsible for 1.2 million deaths annually.[1] Although anti-HBV drugs now available have improved the quality of the lives of HBV patients, development of drug resistance has prompted the search for new anti-HBV agents. Natural compounds, because of their structural diversity, provide a large opportunity for screening anti-HBV agents.[2–4]

Hypserpa nitida Miers. (Menispermaceae), distributed in China and south Asia, is a folk medicinal herb used to treat inflammation.[5] Following the course of our continuous search for anti-HBV active compounds from natural sources,[6] it is suggested that the 90% EtOH extract of *H. nitida* showed IC_{50} values of 0.445 and 0.925 mg/mL against HBV surface antigen (HBsAg) and HBV e antigen (HBeAg), respectively, and so far, to the best of our knowledge, there was no report about the chemical constituents of *H. nitida*, which prompted us to investigate active anti-HBV components from the title plant.

In the present study, column chromatographic separation[7] of the 90% EtOH extract of *H. nitida* yielded two new alkaloids hypserpanines **A** and **B** (**1, 11**) and eleven known compounds: phenolbetain (**2**),[8] acutumine (**3**),[9,10] acutumidine (**4**),[9,10] dechloroacutumine (**5**),[11] dauricumine (**6**),[9] dauricumidine (**7**),[9] pronuciferine (**8**),[12,13] glaziovine (**9**),[13] *S*-reticuline (**10**),[14,15] magnoflorine (**12**)[16,17] and laurifoline (**13**)[18,19] (Fig. 1). Structures of these compounds were elucidated based on spectral analyses and comparison of the spectral data with those reported in literatures. Herein, we reported the structural elucidation of two new alkaloids and anti-HBV activities of the isolated compounds.

Hypserpanine **A** (**1**) was obtained as white amorphous powder. The EIMS spectrum[20] of **1** showed ion peaks at *m/z* at 438 (M^+, isotope peak), 439, 440 and 403 $[M-Cl]^+$, indicating that compound **1** had one chlorine atom in the molecular formula. The molecular formula of compound **1** was assigned as $C_{22}H_{32}N_2O_5Cl$ (*m/z* 439.2003, $[M+H]^+$, calcd 439.1999) by positive HRE-SIMS.[20] IR absorption at 3418 cm^{-1} suggested that

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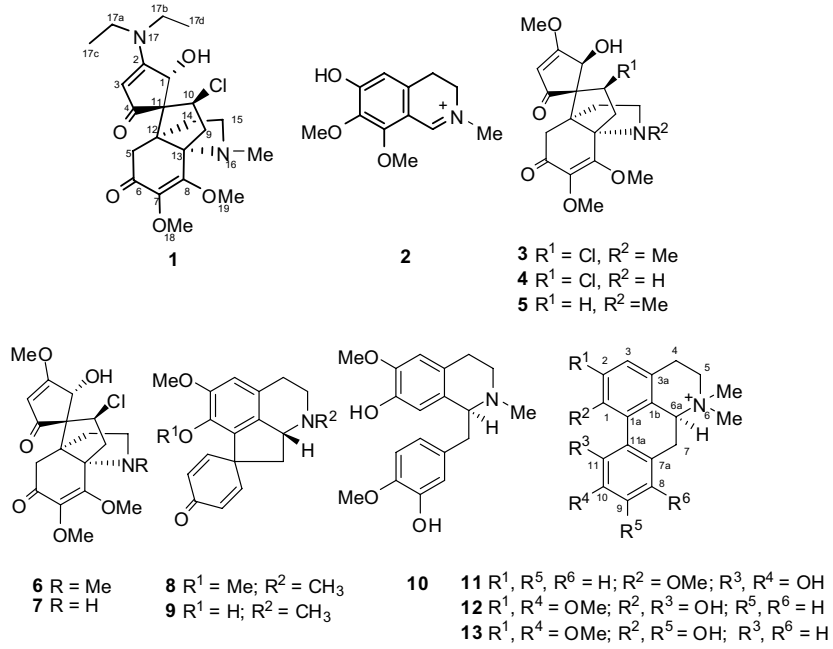

Figure 1. Structures of compounds **1**–**13**.

compound **1** contained hydroxyl group. Furthermore, its EIMS fragmentation pattern was similar to that of acutumine (**3**) or dauricumine (**6**),[21] suggesting that **1** was an acutumine or dauricumine analogue.

The ^{1}H NMR spectrum of **1** (Table 1) exhibited characteristic signals due to core structure of acutumine at δ 5.02 (H-3), δ 4.63 (H-10) and δ 4.60 (H-1) as those of **3** and **6**. The signals of two methoxyl groups (δ 4.07, H-19; δ 3.66, H-18) and one *N*-methyl (δ 2.32, H-16) were in agreement with those of compound **6** (Table 1), but one methoxyl signal at δ 3.70 (C-17 in compound **6**) disappeared, two aliphatic methyl signals triplet at 1.18 (H-17c) and δ 1.24 (H-17d) together with two heteroatom-connecting methylene signals at δ 3.58 (H-17b) and 3.26 (H-17a) were observed. The ^{13}C NMR data for compound **1** were comparable to those of **6** and this indicated an identical core structure. In fact, the major differences between **1** and **6** were the absence of one methoxyl signal at δ 58.3 (C-17 in compound **6**) and the presence of additional two aliphatic methyl signals (δ 11.3, C-17c; δ 14.4, C-17d) and two methylene signals (δ 44.5, C-17a; δ 45.1, C-17b). The above mentioned NMR data, together with the mass spectrometric information,[20] implied that one methoxyl group in **3** or **6** was replaced by a diethylamino group in **1**, which was supported by HMBC correlations (Fig. 2) from the protons at δ 3.58 (H-17b), 3.26 (H-17a) and 5.02 (H-3) to the carbon at δ 172.5 (C-2). In addition, the connectivity patterns between the proton at δ 3.58 (H-17b) and 14.4 (C-17d), δ 3.26 (H-17a) and δ 11.3 (C-17c) were observed, implying that compound **1** was a 2-diethylamino substituted analogue.

The stereochemistry of **1** was established based on ROESY correlation (Fig. 2) between H-10 and H-14, indicating that C-10 was *S*-configuration, which was in accordance with that of **3** or **6**. Moreover, H-1 displayed no correlation with H-14, suggesting that an α-OH was located at C-1. Comparatively, the ROESY spectrum of acutumine (**3**) showed strong correlation between H-1 and H-14, accordingly, the configuration of C-1 was suggested and the structure of **1** was elucidated and is shown in Figure 1.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **1** in $CDCl_3$ (δ in ppm, *J* in Hz)

Position	Compound **1**	
	δ ^{1}H	δ ^{13}C
1	4.60 s	72.6 d
2		172.5 s
3	5.02 s	102.2 d
4		197.2 s
5	2.72 m 2.14 d (15.5 Hz)	47.1 t
6		193.5 s
7		138.2 s
8		159.8 s
9	2.96 t (12.3 Hz) 2.34 m	39.8 t
10	4.63 dd (12.0, 6.9 Hz)	58.6 d
11		66.3 s
12		52.2 s
13		73.2 s
14	2.64 m 1.58 m	39.1 t
15	2.72 m overlapped 2.34 m overlapped	51.3 t
16	2.32 s	35.9 q
17		
17a	3.26 m	44.5 t
17b	3.58 m	45.1 t
17c	1.18 t (7.0 Hz)	11.3 q
17d	1.24 t (7.0 Hz)	14.4 q
18	3.66 s	60.2 q
19	4.07 s	60.6 q

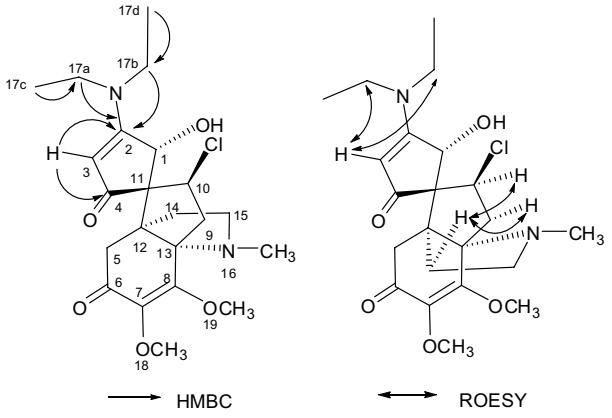

Figure 2. Key 2D NMR correlations of compound **1**.

Hypserpanine **B** (**11**) was isolated as amorphous powder and exhibited a molecular formula of $C_{19}H_{22}NO_3$ by positive HRESIMS (*m*/*z* 314.1757, $[M+2]^+$, calcd 314.1756). ^{13}C NMR data of compound **11** (Table 2) showed the presence of four sp^2 methines (C-2, C-3, C-8 and C-9), eight sp^2 quaternary carbons (C-1, C-1a, C-1b , C-3a, C-7a, C-10, C-11 and C-11a), three sp^3 methylenes (C-4, C-5, C-7), one sp^3 methine (C-6a) and three methyl groups. The ^1H NMR spectrum (Table 2) showed four aromatic protons, with two AB systems (δ 7.00 and 6.73, both d, $J = 8.4$ Hz; δ 7.07 and 6.69, both d, $J = 8.8$ Hz). Based on ^{13}C NMR data, the presence of two AB systems and the shifts of the two *N*-methyl groups (δ 3.14 and 3.06), the basic skeleton of **11** was established as those of magnoflorine (**12**) and laurifoline (**13**).

Table 2. ^1H and ^{13}C NMR spectroscopic data of **11** in CD_3OD (δ in ppm, *J* in Hz)

Position	Compound **11**	
	δ ^1H	δ ^{13}C
1		147.8 s
2	7.00 d (8.4 Hz)	113.2 d
3	6.73 d (8.4 Hz)	119.9 d
3a		122.1 s
4	2.98 m	23.9 t
	3.14 overlapped	
5	3.75 dt (13.2, 6.4 Hz)	55.4 t
	3.40 overlap	
6a	5.05 br d (5.2 Hz)	70.6 d
7	3.40 overlapped	37.4 t
	3.22 m	
7a		129.1 s
8	7.07 d (8.8 Hz)	131.4 d[a]
9	6.69 d (8.8 Hz)	116.6 d[b]
10		157.9 s
11		144.9 s
11a		120.3 s
11b		116.6 s
11c		131.4 s
1-OMe	3.87 s	56.6 q
2-OMe		
10-OMe		
N-Me	3.14 s	54.0 q
N-Me	3.06 s	51.7 q

[a] The ^{13}C NMR signal was overlapped with that of C-11c.
[b] The ^{13}C NMR signal was overlapped with that of C-11b.

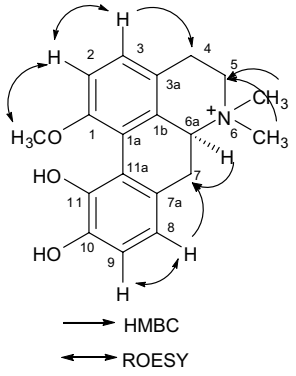

Figure 3. Key 2D NMR correlations of compound **11**.

The HMBC correlations (Fig. 3) from H-3 (δ 6.73) to C-4 (δ 23.9), H-8 (δ 7.07) to C-7 (δ 37.4) and the ROESY correlation between 1-OMe (δ 3.87) and H-2 (δ 7.00) revealed that the methoxyl group must be located in position C-1 and the two hydroxyl groups in positions C-10 and C-11. Besides, compound **11** has a positive optical rotation,[22] thus, C-6a was proposed to be *S*-configuration[23] and the structure was identified and is shown in Figure 1.

The anti-HBV activities of compounds **1**–**13** were evaluated in vitro using the Hep G2.2.15 cell line stably transfected with the HBV genome as reported previously.[6] Anti-HBV activity, cytotoxicity and selectivity index (SI) are summarized in Table 3. The following is to be noted regarding the anti-HBV activity data with the tested compounds. (i) The alkaloids with acutumine core structure such as hyperpanine **A** (**1**), acutumidine (**4**) and dauricumine (**6**), have inhibitory action on the production of HBsAg with IC_{50} values of 1.415, 2.023 and 1.312 mM ($2 > SI > 1$). The most active acutumine alkaloid, dauricumidine (**7**), shows anti-HBsAg activity

Table 3. Anti-HBV activity, cytotoxicity and selectivity index of compounds **1**–**13**[a]

Compounds	CC_{50} (mM)	HBsAg[b]		HBeAg[c]	
		IC_{50} (mM)	SI[d]	IC_{50} (mM)	SI
1	2.226	1.415	1.57	>2.870	<1
2	2.181	2.367	<1	>4.525	<1
3	>2.989	>2.989	—	>2.98	—
4	>2.888	2.023	>1.31	>2.888	—
5	>2.926	>2.926	—	>2.926	—
6	2.193	1.312	1.67	>3.291	<1
7	1.859	0.450	4.13	>3.854	<1
8	0.050	0.042	1.19	>4.180	<1
9	0.007	0.008	<1	3.694	<1
10	1.909	2.143	<1	>2.994	<1
11	>3.615	>3.615	—	>3.615	—
12	>4.114	>4.114	—	>4.114	—
13	>3.684	>3.684	—	>3.684	—
3TC[e]	30.0	11.7	2.56	25.9	1.16

[a] All values are means of two independent experiments.
[b] HBsAg, HBV surface antigen.
[c] HBeAg, HBV e antigen.
[d] $SI = CC_{50}/IC_{50}$.
[e] 3TC: Lamivudine, an antiviral agent used as positive control.

with IC_{50} of 0.450 mM (SI = 4.13), while its *N*-methylated derivative dauricumine (**6**) only shows IC_{50} values of 1.312 (SI = 1.67). Thus, it is suggested that the NH group in **6** and **7** is necessary for potent activity against HBsAg. (ii) The proaporphine alkaloids, pronuciferine (**8**) and glaziovine (**9**), exhibit high inhibitory potential against HBsAg with IC_{50} value of 0.042 and 0.008 mM, respectively, which are significantly smaller than that of the positive control 3TC (IC_{50} = 11.7 mM). But unfortunately, these two compounds are cytotoxic in Hep G2.2.15 cells, as a result, compound **8** only shows a SI value of 1.19. (iii) The quaternary aporphine alkaloids **11–13** are inactive to HBsAg and HBeAg, but the cytotoxicities of these compounds in Hep G2.2.15 cells are obviously lower than those of proaporphine alkaloids **8** and **9**. (iv) All the tested alkaloids show no anti-HBeAg activity.

In conclusion, alkaloids isolated from *H. nitida* were structurally diverse. Anti-HBV evaluation of these compounds suggested that acutumine and proaporphine were active to inhibit HBsAg in vitro for the first time, which provided candidates for the study of this medicinal plant.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.08.027.

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7. The dried whole plants (14 kg) of *Hypserpa nitida* were powdered and extracted with 90% EtOH (2 × 50 L) under reflux for 2 h. The extract was concentrated under *vacuum* to give a residue which was treated with H_2SO_4 (2%, w/w) adjusting pH to 1–2. The acidic solution was extracted with $CHCl_3$. The water solution was then basified with aqueous ammonia (25%, w/w) to pH 9–10 and extracted with $CHCl_3$. The basic $CHCl_3$ extract was evaporated to give a residue (10 g) which was directly chromatographed over a silica gel column (200 g, 200–300 mesh) with gradient elution with $CHCl_3$–MeOH (100:0, 95:5, 90:10, 80:20 v/v) to afford seven fractions (Frs. A–G). Fr. C (1.2 g) was separated by silica gel column chromatography (50 g, 200–300 mesh) eluted with $CHCl_3$–MeOH (97:3) to two sub-fractions [Frs. C1–C2]. Fr. C1 (130 mg) was subjected to Sephadex LH-20 column (MeOH) repeatedly to obtain compounds **1** (23 mg) and **6** (35 mg). Fr. C2 (100 mg) was submitted to Sephadex LH-20 column (MeOH) repeatedly to give compound **8** (20 mg). Fr. D (650 mg) was purified over silica gel column (30 g, 200–300 mesh) and eluted with $CHCl_3$–MeOH (96:4) to produce compound **3** (453 mg). Fr. E (1.2 g) was applied on silica gel column (60 g, 200–300 mesh) and eluted with $CHCl_3$–MeOH (95:5) to yield three sub-fractions [Fr. E1–E3]. Fr. E1 (20 mg) and Fr. E3 (39 mg) were further purified on Sephadex LH-20 column (MeOH) repeatedly to get compounds **9** (7 mg) and **7** (21 mg), respectively. Fr. E2 (345 mg) was further purified by silica gel column (15 g, 200–300 mesh) eluted with EtOAc–MeOH–H_2O (85:15:1.5) to afford compound **5** (160 mg). Fr. F (650 mg) was rechromatographed on silica gel (30 g, 200–300 mesh) eluted with $CHCl_3$–MeOH (95:5) to provide compound **4** (67 mg). Fr. G (1.2 g) was chromatographed on silica gel (60 g, 200–300 mesh) eluted with $CHCl_3$–MeOH–H_2O (90:10:1) to obtain three sub-fractions [Frs. G1–G3], Fr. G3 (120 mg) was then rechromatographed on silica gel column (15 g, 200–300 mesh) and eluted with petroleum ether–$CHCl_3$–MeOH–H_2O (20:70:10:1) to give compounds **2** (10 mg) and **10** (12 mg). The basic aqueous layer after extracting with $CHCl_3$ was adjusted to pH 7 and dried to give a residue. The residue was refluxed with EtOH and filtered. The filtrate was evaporated to dryness to give a residue (435 g) which was directly chromatographed over silica gel column (1200 g, 200–300 mesh) and eluted with $CHCl_3$–MeOH–NH_4OH (70:30:3) to produce three fractions (Frs. H–J). Fr. I (8 g) was applied on MCI CHP 20P (MeOH–H_2O, 50:50) and Toyopearl HW-40 C column (MeOH) repeatedly to yield compounds **11** (14 mg), **12** (385 mg) and **13** (48 mg).
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20. Hypserpanine **A** (**1**): $[\alpha]_D^{17}$ −135° (*c* 0.32; pyridine). UV λ_{max}^{MeOH} nm (log ε): 286 (4.47); IR (cm^{-1}, KBr): 3418, 1666, 1571, 1224, 1093; positive HRESIMS *m/z*: 439.2003 $[M+H]^+$ (14) ($C_{22}H_{33}N_2O_5Cl$ calcd 439.1999), 440.1765 $[M+1+H]^+$ (4), 441.1973 $[M+2+H]^+$ (5); EIMS (70 eV) *m/z*: 440 $[M+2]^+$ (1.5), 439 $[M+1]^+$ (1), 438 $[M]^+$ (4), 403 (10), 209 (100), 194 (14), 181 (5), 166 (23), 150 (15); for ^1H and ^{13}C NMR data, see Table 1.
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22. Hypserpanine **B** (**11**): $[\alpha]_D^{21}$ 2.3° (*c* 0.74; methanol); UV λ_{max}^{MeOH} nm (log ε): 204.6 (4.65), 226.8 (4.19); IR (cm^{-1}, KBr): 3418, 1614, 1516, 1501, 1443, 1287, 1245; positive HRESIMS *m/z*: $[M+2]^+$ 314.1757, ($C_{19}H_{22}NO_3$ cacld 314.1756).; EIMS (70 eV) *m/z*: 313 $[M+H]^+$ (7), 58 (100); for ^1H and ^{13}C NMR data, see Table 2.
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