THREE NEW PURINIUM DERIVATIVES, HETEROMINES A, B, AND C FROM *HETEROSTEMMA BROWNII*

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Abstract---- From the aerial parts of *Heterostemma brownii* Hay., three new purinium derivatives, heteromines A, B, and C were isolated. Their structures were elucidated as 6-methoxy-7,9-dimethyl-2-dimethylamino-purinium chloride, 6-methoxy-7,9-dimethyl-2-methylaminopurinium chloride, and 2-amino-6-methoxy-7,9-dimethylpurinium chloride on the basis of spectroscopic and chemical methods.

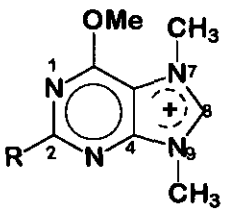
Heterostemma brownii Hay. (Asclepiadaceae) is a climber species, native to Wen-Sun mountains of Taipei Hsien. It has been used as folk medicine for the treatment of tumors. It is also used as an expelling dampness and detoxifying agent.[1] Previous phytochemical investigation on this plant has involved the isolation of flavonoids, flavonoid glycosides, adenine and uridine.[2] In the course of our research for higher polar components, we have investigated a 60% MeOH extract of the aerial parts of *H. brownii*. The extract was chromatographed on Diaion HP-20, and the fraction of 50-80% MeOH eluents was further purified repeatedly with Diaion HP-20 and Sephadex LH-20. Three new water soluble components, heteromines A, B, and C, were isolated and identified as 6-methoxy-7,9-dimethyl-2-dimethylaminopurinium chloride, 6-methoxy-7,9-dimethyl-2-methylaminopurinium chloride, and 2-amino-6-methoxy-7,9-dimethylpurinium chloride, respectively. This paper deals with the structural elucidation of three new purine derivatives.

Heteromine A (**1a**) was obtained as colorless needles (from MeOH) mp 225-227°C. Elemental

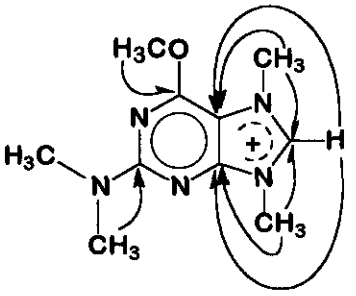
Table 1 ^{1}H and ^{13}C nmr data (δ-value) for **1a**, **1b**, and **1c** (300 MHz and 75 MHz, DMSO-d_6, TMS as an internal standard).

H	1a	1b	1c	C	1a	1b	1c
8	9.34s	9.37s	9.63s	2	159.6	160.8	161.6
7-CH_3	3.99s	3.98s	3.99s	4	151.9	151.9	152.0
9-CH_3	3.78s	3.77d	3.75s	5	104.0	104.5	104.6
N-CH_3	3.20s	2.84d		6	157.7	157.9	158.3
		(4.3)		8	140.6	140.1	140.4
O-CH_3	4.10s	4.03s	4.04s	7-CH_3	35.9	35.8	35.8
N-H		7.76q	7.32br s	9-CH_3	30.9	30.9	31.0
				N-CH_3	37.0	28.0	
				O-CH_3	54.5	54.4	54.4

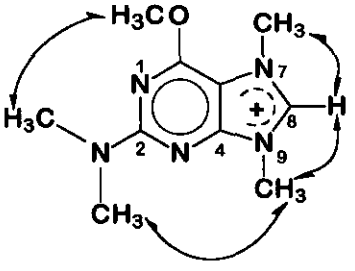
Figures in parantheses are coupling constants in Hz.



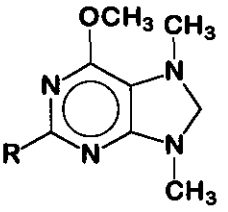
1a R=$N(CH_3)_2$
b R=$NHCH_3$
c R=NH_2



2 (HMBC)



3 (NOESY)



4a R=$N(CH_3)_2$
b R=$NHCH_3$
c R=NH_2

analysis indicated a molecular formula $C_{10}H_{16}N_5OCl$. The EI-ms of **1a** exhibited the (M^+-Cl-1) peak at m/z (%) 221 (64) and fragment ion peaks at m/z (%) 207 (100), 192 (52), 178 (35), 163 (57), 136 (38), and 123 (12). The uv absorption bands presented at λ_{max}^{MeOH} (log ε) 254 (3.62) and 315 (3.40). Reaction of **1a** with methanolic $AgNO_3$, white AgCl precipitated promptly. The evident suggested that **1a** is a quaternary ammonium chloride. The ^1H nmr spectrum (Table 1) of **1a** exhibited signals for a methoxy group (δ 4.10, s), a dimethylamino group [δ 3.20 (6H, s)], two methyl groups attached on two quaternary amines (δ 3.78 and 3.99), and a typical purinium base H-8 (δ 9.34).[3] The ^{13}C nmr data of **1a** (Table 1)[3] assigned by ^1H-^{13}C COSY also confirmed the shown structure. The ^1H and ^{13}C correlation *via* J^2 and J^3 (HMBC) of **1a** was described as structure (**2**). From the above result, the structure of **1a** can be assigned as 7,9-dimethylpurinium chloride with two substitutions, methoxy and dimethylamino groups, may be located at C-6 and C-2 positions or reversal. The NOESY result exhibited in structure (**3**) suggested that the structure has methoxy and dimethylamino groups linked to C-6 and C-2 positions, respectively. Compound (**1a**) can be reduced by sodium borohydride in water solution and afforded a product (**4a**) [mp 43-44°C, δ 4.26 (2H, s, H-8)] which can be dissloved in less polar solvent. The evidence proved heteromine A (**1a**) is a quaternary ammonium compound unambiguously.

Heteromine B (**1b**) was obtained as colorless needles (from MeOH), mp 229-231°C. It is a quaternary ammonium chloride due to giving AgCl precipitation as reaction with $AgNO_3$. The molecular formula $C_9H_{14}N_5OCl$ was deduced from elementary analysis. Ms fragment ion peaks expressed at 207 [$(M\text{-}Cl\text{-}1)^+$, 87 %], 193 (100%), 178 (28%), 163 (42%), 150 (16%), and 136 (20%). The uv absorption bands presented at λ_{max}^{MeOH} (log ε) 249 (3.57) and 306 (3.48) nm, and ^1H nmr spectrum (Table 1) exhibited signals at δ 2.84 (3H, d, J=4.3 Hz), 3.77, 3.99 and 4.04 (each 3H, s), 7.76 (1H, q, J=4.3 Hz, -NH), and 9.37 (1H, s, H-8). Using the ^1H-^{13}C COSY experiment, ^{13}C nmr data of **1b** were assigned as in Table 1. By the comparison of physical data with heteromine A (**1a**), heteromine B (**1b**) can be elucidated as 6-methoxy-7,9-dimethyl-2-methylamino-

purinium chloride. As reacted with sodium borohydride in water solution, heteromine B (**1b**) was reduced to a product (**4b**) [mp 84-85°C; δ 4.30 (2H, s, H-8)]. The product showed less polar than **1b**. The third quaternary ammonium compound is heteromine C (**1c**), colorless needles (from MeOH), mp 268-270°C. Basis on the elemental analysis and ms spectrum, molecular formula $C_8H_{12}N_5OCl$ was deduced for **1c**. It also has two maxima absorption bands at λ_{max}^{MeOH} (log ε) 245 (3.60) and 296 (3.59)nm in its uv spectrum. ^1H- (Table 1) and ^{13}C- nmr (Table 1) data of **1c** are similar to heteromine A (**1a**) and B (**1b**). The assignment of nmr data also utilized ^1H-^{13}C COSY and HMBC expeiments. Only difference is that no methyl group attached to amino group in **1c**. Therefore, the structure of **1c** can be assigned as 2-amino-6-methoxy-7,9-dimethylpurinium chloride. Reduction of **1c** with sodium borohydride in water also yielded 7,8-dihydropurine derivative (**4c**) [mp 121-122°C; δ 4.28 (2H, s, H-8)]. Compounds (**1a** and **1b**) showed inhibitory effect on K562 and HL-60 cell lines at the concentration of 10^{-6}M.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 781 spectrophotometer. ^1H and ^{13}C nmr spectra were run on a Bruker AM 300 at 300 MHz and 75 MHz in DMSO-d_6 or $CDCl_3$ solution with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ-value and coupling constants (*J*) are given in hertz (Hz). EI-ms and uv spectra were taken on a JEOL JMS-100 spectrometer and Hitachi U-3200 spectrophotometer, respectively.

Extraction and Isolation

The aerial parts of *Heterostemma browniii* (5.0 kg), collected in April 1991 in Wen-Sun mountains, Taipei Hsien, was extracted with 60% MeOH (80 l x 3) at 50°C, overnight. The extract (954 g) was subjected to Diaion HP-20 column chromatography, and eluted with H_2O-MeOH gradient solvent system. The fraction eluted from 50-80% aqueous methanol, was rechromatographed on Diaion HP-20 and Sephadex LH-20, respectively. The MeOH eluent on

Sephadex LH-20 column yielded heteromine A (**1a**) (146 g), B (**1b**) (86 g) and C (**1c**) (16 g) in that eluting order.

Heteromine A (**1a**): Ir (KBr) ($\upsilon_{cm^{-1}}$): 1640, 1605, 1555, 1505, 1385, 1355, 1320, 1145; ^1H and ^{13}C nmr (DMSO-d_6): Table 1; *Anal.* Calcd for $C_{10}H_{16}N_5OCl$: C, 46.60; H, 6.26; N, 27.17; Found: C, 46.41; H, 6.30; N, 27.08; Exact mass for $C_{10}H_{15}N_5O$ required 221.1276; Found 221.1280.

Heteromine B (**1b**): Ir (KBr) ($\upsilon_{cm^{-1}}$): 3450, 3200, 1640, 1610, 1500, 1385, 1338, 1140; ^1H and ^{13}C nmr (DMSO-d_6): Table 1; *Anal.* Calcd for $C_9H_{14}N_5OCl$: C, 44.36; H, 5.79; N, 28.74; Found: C, 44.28; H, 5.85; N, 28.64; Exact mass for $C_9H_{13}N_5O$ required 207.1120; Found 207.1123.

Heteromine C (**1c**): Ir (KBr) ($\upsilon_{cm^{-1}}$): 3400-3200, 1640, 1610, 1500, 1395, 1335, 1145; ms m/z (%): 193 [M-Cl-1)$^+$, 100], 179 (97), 164 (23), 150 (12), 137 (22), 123 (13), 95(16); ^1H and ^{13}C nmr (DMSO-d_6): Table 1; *Anal.* Calcd for $C_8H_{12}N_5OCl$: C, 41.84; H, 5.27; N, 30.49; Found: C, 41.91; H, 5.30; N, 30.59; Exact mass for $C_8H_{11}N_5O$ required 193.0963; Found 193.0963.

Reduction of 1a, 1b, or 1c with Sodium Borohydride in Water

Excess of sodium borohydride was added in small portion to a solution of **1a**, **1b** or **1c** (each of 30 mg) in 5 ml of H_2O, and the reaction mixture was allowed to stand for 20 min. Then the reaction mixture was extracted with ethyl acetate (10 ml x 3), and gave the 7, 8-dihydropurine (**4a**), (**4b**) or (**4c**) (each of 22 mg) after purification on SiO_2 column chromatography.

6-Methoxy-7,9-dimethyl-2-dimethylamino-7,8-dihydropurine (**4a**): Mp 43-44°C; ir (KBr) ($\upsilon_{cm^{-1}}$): 2786, 1620, 1585, 1497, 1054, 774; ms m/z (%): 223 (M$^+$, 100), 222 (14), 209 (12), 208 (13); *Anal.* Calcd for $C_{10}H_{17}N_5O$: C, 53.79; H, 7.67; N, 31.37; Found: C, 53.87; H, 7.68; N, 31.29; ^1H nmr ($CDCl_3$) δ 2.66, 2.80, 3.06, 3.06, and 3.85 (each 3H, s) and 4.26 (2H, s, H-8); ^{13}C nmr ($CDCl_3$) δ 30.3 (9-$\underline{C}H_3$), 37.1 (N$\underline{C}H_3$), 37.1 (N$\underline{C}H_3$), 40.7 (7-$\underline{C}H_3$), 52.3 (O$\underline{C}H_3$), 78.4 (C-8), 106.5 (C-5), 154.8 (C-4), 159.2 (C-6), 163.6 (C-2).

6-Methoxy-7,9-dimethyl-2-methylamino-7,8-dihydropurine (**4b**): Mp 84-85°C; ir (KBr) ($\upsilon_{cm^{-1}}$): 3312, 2792, 1619, 1516, 1407, 1386, 1262, 1189, 1082, 773; ms m/z (%): 209 (M$^+$, 100), 208

(43), 194 (7), 177 (3); *Anal.* Calcd for $C_9H_{15}N_5O$: C, 51.66; H, 7.23; N, 33.47; Found: C, 51.57; H, 7.24; N, 33.58; ^{1}H nmr ($CDCl_3$) δ 2.67, 2.79, and 3.86 (each 3H, s), 2.90 (3H, d, *J*=5.1 Hz), and 4.28 (2H, s, H-8), 4.76 (1H, br s, -N<u>H</u>); ^{13}C nmr ($CDCl_3$) δ 28.7 (N<u>C</u>H$_3$), 30.2 (9-<u>C</u>H$_3$), 40.6 (7-<u>C</u>H$_3$), 52.6 (O<u>C</u>H$_3$), 78.3 (C-8), 107.8 (C-5), 154.9 (C-4), 159.5 (C-6), 163.6 (C-2).

2-Amino-6-methoxy-7,9-dimethyl-7,8-dihydropurine (**4c**): Mp 121-122°C; ir (KBr) ($\upsilon_{cm^{-1}}$): 3361, 2785, 1620, 1587, 1367, 1248, 1103, 1053, 777; ms m/z (%): 195 (M^+, 100), 194 (73), 180 (5), 163 (7), 138 (9); *Anal.* Calcd for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.88; Found: C, 49.17; H, 6.74; N, 35.95; ^{1}H nmr ($CDCl_3$) δ 2.71, 2.81, and 3.84 (each 3H, s), 4.36 (2H, s, H-8), 4.48 (2H, br s, -N<u>H</u>$_2$); ^{13}C nmr ($CDCl_3$) δ 30.2 (9-<u>C</u>H$_3$), 40.3 (7-<u>C</u>H$_3$), 52.9 (O<u>C</u>H$_3$), 78.3 (C-8), 108.9 (C-5), 154.5 (C-4), 158.5 (C-6), 163.5 (C-2).

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