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PURINIUM DERIVATIVES WITH ANTITUMOR ACTIVITIES FROM *HETEROSTEMMA ALATUM* WIGHT

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Abstract – Heteromine I (**1**), a new purinium derivative, together with six known compounds, were isolated from the aerial parts of *Heterostemma alatum* Wight. The structure of **1** has been established as 2-methylamino-6-methoxy-7,9-dimethyl-8-purinone by means of spectroscopic analysis. Two puriniums, heteromines J (**2**) and K (**3**) were isolated as natural products for the first time. Heteromine D (**4**) showed strong cytotoxicity against HL-60 cell line with IC_{50} of 4.04 nmol/mL.

Heterostemma alatum Wight (Asclepiadaceae) is a liana that is distributed over the south and the southwest of China, Nepal, and India. Its roots or whole plant were used as a Chinese folk medicine for expelling dampness and detoxifying.[1] A few phytochemical studies on plants of the genus *Heterostemma* reported the isolation of steroids, fatty acids, flavonoids, flavonoid glycosides, adenines, uridines, puriniums and pyrimidines from *H. brownii* [2-4] and *H. alatum*.[5] Further exploration of the title plant led to the isolation of a new purinium derivative, heteromine I (**1**), two first obtained natural puriniums, heteromines J (**2**) and K (**3**), and four know compounds. The structure of **1** is elucidated as 2-methylamino-6-methoxy-7,9-dimethyl-8-purinone on the basis of spectroscopic analysis. Those known

compounds are identified to be 2-dimethylamino-6-methoxy-7,9-dimethyl-8-purinone (**2**),[4] 6-methoxy-9-methyl-2-dimethylaminopurinium (**3**),[4,6,7] 11-oxo-α-amyrin acetate,[8] α-amyrin acetate,[9] sitgmast-4-ene-1,3-dione,[10] and stigmast-4-en-3-one.[11] An assay on cytotoxicity showed that heteromine D (**4**) had strong cytotoxicity against HL-60 cell line with IC_{50} of 4.04 nmol/mL in contrast with 6-mercaptopurine of 3.96 nmol/mL.

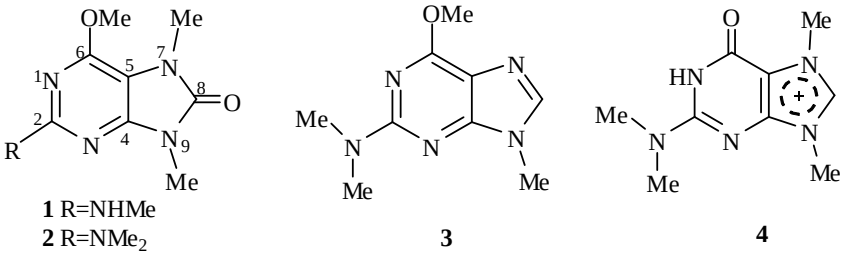

Figure 1. Structures of compounds **1–4**

Heteromine I (**1**) was isolated as white amorphous powders. The molecular formula was determined to be $C_9H_{13}N_5O_2$ based on the pseudomolecular ion peak $[M+Na]^+$ at *m/z* 246.0959 in the HR-ESI-MS. The IR spectrum exhibited absorptions at 3385 (N-H), 1703 (C=O), 1635 (C=C), 1608 (C=N), and 1294 (-NH-CH_3) cm^{-1}. The UV appeared the maximum absorptions at 205, 250 and 298 nm. The above evidences indicated a purinium derivative.[3,4] The ^{1}H-NMR spectrum of **1** showed signals for a methylamino group (δ 2.78, 3H, br s), two methyl groups attached on two quaternary amines (δ 3.18 and 3.31, each s), and a typical methoxy group (δ 3.92, s). The four methyls were easily assigned by the HMBC and ROESY cross-peaks (Figure 2), the structure of heteromine I (**1**) was assigned as 2-methyl-amino-6-methoxy-7,9-dimethyl-8-purinone.

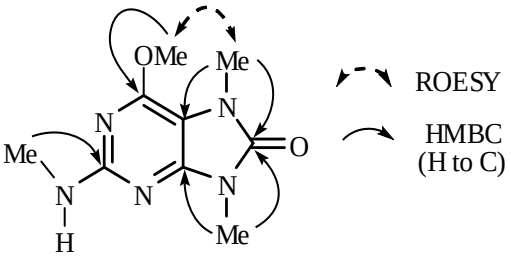

Figure 2. The 2D NMR correlations of **1**

Compounds **2** and **3**, named heteromines J and K, were obtained from plant resource for the first time. As an analogue of **1**, **2** was easily assigned its ^{1}H and ^{13}C signals in contrast with those of **1**. The original assignments for C-2 (δ 153.6) and C-8 (δ 157.9) in the literature[4] were reversed and revised. **2** and **3** had been reported as derivatives from heteromine A under treatment of $NaBH_4$, following air oxidation.[4] **3** was also a key intermediate in the course of synthesis of heteromine A.[6,7]

All puriniums isolated from the title plant, including **1-3** and heteromines A, B, C, D (**4**), and F, were evaluated their cytotoxicities against tumor cell line HL-60 (human promyelocytic leukemia cell). The results showed that **4** had a comparable cytotoxic activity (IC_{50} = 4.04 nmol/mL) with that of 6-MP (6-mercaptopurine, IC_{50} = 3.96 nmol/mL).

EXPERIMENTAL

General Experimental Procedures: Optical rotations were measured using a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Nicolet Magna 750 FTIR (KBr) spectrometer. ESI-MS and HR-ESI-MS were obtained on an Esquire 3000plus and a Q-TOF-Ultima mass spectrometer, respectively. NMR spectra were recorded on a Bruker AM-400 NMR spectrometers, the chemical shift values are reported in ppm (δ) and coupling constants (J) are given in Hz. Silica gel (200-300, 400 mesh) and precoated plates of silica gel (HSGF-254) (Qingdao Haiyang Chemical Group Co., Qingdao) were used for column chromatography (CC) and TLC, respectively.

Plant Material: The aerial parts of *H. alatum* were collected in Xishuangbanna County, Yunnan Province, China, in July 2006. The plant were identified by Prof. J.-Y. Cui of Xishuangbanna Tropical Botanical Garden, CAS, China. A voucher specimen (No. 2006-64) was deposited in the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation: The dried aerial parts of *H. alatum* (5.0 kg) was extracted with 95% EtOH (25 L×3, each 3 d) at room temperature. The solvents were evaporated under reduced pressure to give 524 g residue. The concentrated extract was suspended in H_2O (3 L) and partitioned successively with petroleum ether (PE), $CHCl_3$, EtOAc to provide the PE- (80 g), $CHCl_3$- (50 g), and EtOAc-soluble fraction (30 g). The EtOAc-soluble fraction (30 g) was subjected to CC of silica gel eluted with EtOAc-acetone (1:0 → 0:1) to afford Fr.E1–Fr.E8. Fr.E6 (2.1 g) was repeatedly chromatographed on silica gel columns with EtOAc-acetone (10:1–5:1) to give **1** (11 mg), **2** (27 mg) and **3** (35 mg). By the similar procedures, 11-oxo-α-amyrin acetate (255 mg), α-amyrin acetate (50 mg), sitgmast-4-ene-1,3-dione (40 mg), and stigmast-4-en-3-one (9 mg) were obtained from the $CHCl_3$-soluble fraction.

Assay of Cytotoxic Activity: HL-60 cells (ATCC, Rockville, MD) were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum at 37 °C in humidified air with 5% CO_2. Cytotoxicity in vitro was measured by the MTT method. Briefly, cells (1×10^4 per well) were seeded into 96-well plates and exposed to various concentrations of test compounds. After treatment for 72 h, 10 μL MTT at 5 mg/ml was added to each well. The cells were then incubated at 37 °C for another 4 h and then 10% sodium dodecyl sulfate (SDS) in 0.01 M HCl was added to each well. The absorbance was detected at 570 nm with a microplate reader (KLx808, Bio-Tek, USA). Cytotoxicity was expressed as a percentage of that of the control culture, and IC_{50} was given as the concentration (nmol/mL) required for 50% inhibition of cell growth.

Heteromine I (1): White amorphous powders. UV (MeOH) λ_{max}: 205, 250, 298 nm; IR (KBr) ν_{max}: 3385, 2945, 1703, 1635, 1608, 1562, 1504, 1398, 1294, 1196, 1132, 771, 582 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.92 (6-Me, 3H, s), 3.31 (7-Me, 3H, s), 3.18 (9-Me, 3H, s), 2.78 (2-Me, 3H, br s); ^{13}C-NMR

(DMSO-d_6, 100 MHz) δ: 158.1 (C-2), 152.9 (C-6), 152.7 (C-8), 150.8 (C-4), 98.6 (C-5), 53.1 (6-Me), 29.0 (7-Me), 28.1 (2-NMe), 26.0 (9-Me); ESI-MS m/z 234 ($[M+H]^+$), 246 ($[M+Na]^+$), 469 ($[2M+Na]^+$); HR-ESI-MS *m/z*: 246.0959 (calcd. for $C_9H_{13}N_5O_2Na$: 246.0967).

Heteromine J (2): White amorphous powders. UV (MeOH) λ_{max}: 205, 250, 298 nm; IR (KBr) ν_{max}: 2929, 1618, 1574, 1462, 1390, 1329, 1298, 1261, 1068, 787, 638 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.94 (6-Me, 3H, s), 3.43 (7-Me, 3H, s), 3.31 (9-Me, 3H, s), 3.11 (2-Me_2, 6H, s); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 157.8 (C-2), 153.5 (C-8), 153.3 (C-6), 150.9 (C-4), 98.7 (C-5), 52.9 (6-Me), 29.2 (7-Me), 37.1 (2-NMe_2), 26.2 (9-Me); ESI-MS *m/z*: 238 ($[M+H]^+$), 260 ($[M+Na]^+$), 497 ($[2M+Na]^+$).

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