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ALKALOIDS FROM *PIPER PUBERULLUM*

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Key Word Index—*Piper puberullum*; Piperaceae; piperlactam S; piperine S; puberullumine; piplartine dimer; piplartine; 8,9-dihydropiplartine.

Abstract—Two new alkaloids named piperlactam S and piperine S have been isolated from the stem and leaves of *Piper puberullum*, in addition to puberullumine and three known pyridone alkaloids. The structures of these compounds were determined on the basis of spectral analyses. The known compounds were identified as piplartine dimer, piplartine and 8,9-dihydropiplartine and obtained for the first time from this species.

INTRODUCTION

Piper is a large genus of herbs or somewhat woody climbers found in the warm, humid regions of the world. In China there are more than 60 species distributed mainly in the southeast and southwest regions, and Taiwan province. Some of them, such as *P. kadsura* (Choisy) Ohwi, *P. hancei* Maxim and *P. puberullum* (Benth.) Maxim. are widely used in Chinese herbal medicine for the treatment of arthritic conditions.

In recent years, *Piper* species have received considerable attention because of their reputation for producing lignans with PAF antagonist activity [1] and cytotoxic alkaloids [2–4]. Previous workers on *P. puberullum* reported the presence of lignans [3]. As part of a chemical study on this species, six alkaloids have been isolated, including two new compounds and one new natural product.

RESULTS AND DISCUSSION

An ethanol extract of the stems and leaves was extracted with CH_2Cl_2 in a Soxhlet. The dichloromethane fraction was further purified and six alkaloids were obtained, two of which are new compounds (**1** and **2**), in addition to one new natural product (**3**).

Compound **1** gave yellow needles, mp 242–244°. The HR mass spectrum established the molecular formula $C_{17}H_{13}NO_4$, $[M]^+$ *m/z* 295.0850 (calc. $[M]^+$ 295.0843). Its UV (MeOH) spectrum was characteristic of an aristolactam sharing absorptions at 195, 206, 232, 248, 274, 284, 315 and 380 nm [6]. The absorption bands at 3260, 1695 and 1655 cm^{-1} in its IR (KBr) spectrum revealed the presence of hydroxyl and lactam carbonyl groups.

The ^1H NMR (DMSO-d_6, 500 MHz) spectrum of (**1**) revealed six aromatic protons, a phenolic hydroxyl and two methoxyl singlets with quite similar chemical shifts. The signals at δ 9.14 (1H, *m*), 7.16 (2H, *m*), 8.02 (1H, *m*) suggested four adjacent aromatic protons at C-5, -6, -7 and -8, respectively [3]. To further confirm the substitution pattern, four NOE difference experiments were conducted. Irradiation of H-5 (δ 9.14) resulted in very significant enhancement of a methoxyl signal at δ 4.03. These results indicated that the methoxyl at δ 4.03 was located at C-4. Irradiation of the hydroxyl proton signal at δ 10.44 resulted in enhancement of the methoxyl signal at δ 4.03 (MeO-4) and the aromatic proton signal at δ 7.66. Irradiation of the aromatic proton singlet at δ 7.66 resulted in enhancement of the hydroxyl proton at δ 10.44, indicating the presence of the hydroxyl group (δ 10.44) at C-3 and an aromatic proton (δ 7.66) at C-2, respectively. Irradiation of the aromatic proton signal at δ 7.39 resulted in enhancement of the H-8 signal (δ 8.02) and another methoxyl signal at δ 4.06. These data indicated the aromatic proton at δ 7.39 to be located at C-9 and the presence of MeO-N. From its HMQC and HMBC spectral data, all of the carbon signals were assigned (see Experimental). Thus, taking into consideration all the above data and analyses, the structure of compound **1** was elucidated as 10-amino-3-hydroxy-4-methoxy-*N*-methoxyphenanthrene-1-carboxylic acid lactam, named as piperlactam S. In

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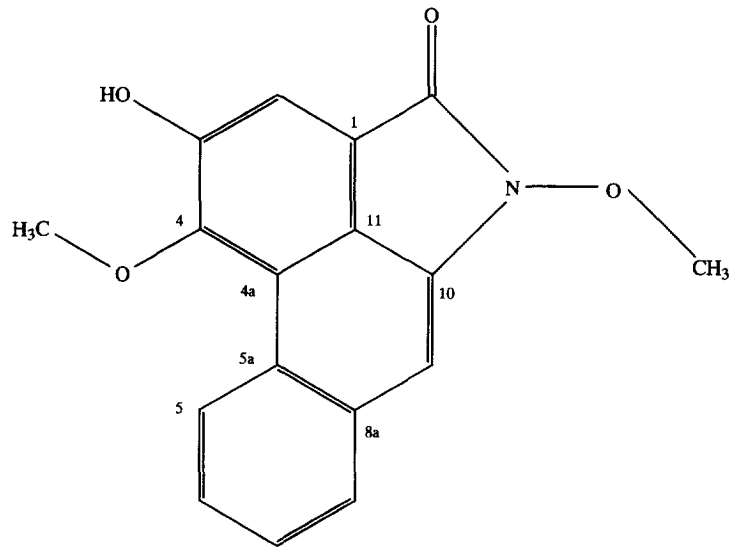

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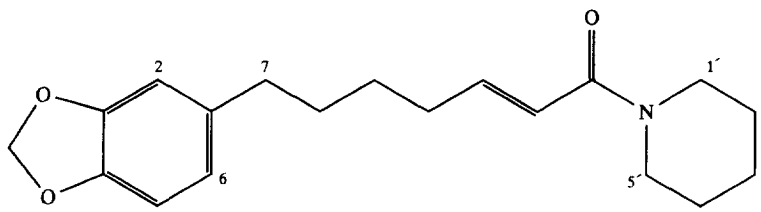

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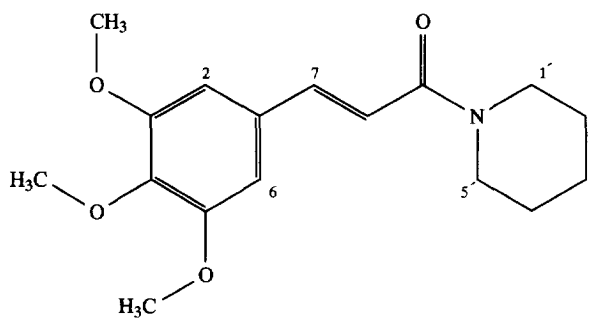

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general, aristolactams have no *N*-oxy function and the aromatic methyoxyl carbon signals are located at δ 55.0–60.0; the carbon signal of MeO-N is at δ 64.5. This is the first report of a naturally occurring *N*-oxygenated methoxy aristolactam [7].

Compound **2** also gave yellow needles, mp 52–54°, positive to Dragendorff's reagent. The HR mass spectrum established the formula $C_{19}H_{25}NO_3$, $[M]^+$ *m/z* 315.1828 (calc. $[M]^+$ 315.1833) and UV (MeOH) showed 201, 232 and absorptions at 380 nm. The IR (KBr) spectrum of (**2**) showed tertiary amide carbonyl absorption bands at 1670 and 1610 cm^{-1}, as well as a methylenedioxy group at 1250, 1035 and 925 cm^{-1}. The downfield proton signals in the ^1H NMR ($CDCl_3$, 500 MHz) spectrum indicated the presence of a piperidine ring [8]. The signals at δ 6.64 (1H, *d*, $J = 1.7$ Hz) 6.70 (1H, *d*, $J = 7.7$ Hz) and 6.58 (1H, *dd*, $J = 7.7$, 1.7 Hz) were assigned to three aromatic protons at

C-2, -5 and -6. The signals at δ 6.81 (1H, *dt*, J = 15.1, 7.0 Hz) and 6.22 (1H, *dt*, J = 15.1, 1.5 Hz), indicated the presence of two *trans*- olefinic protons adjacent to a carbonyl; the singlet at δ 5.87 was ascribed to two protons of the methylenedioxy group. The EI-mass spectrum gave a $[M]^+$ at m/z 315 (56.8%), and the other significant peaks m/z (%) 135 (100), 180 (46.4), 166 (93.0), 152 (6.9), 138 (62.9), 112 (36.3) and 84 (46.3), corresponding with the fragment of $C_8H_7O_2$ (benzenyl rearrangement) $C_{11}H_{18}NO$ $[M-135]^+$, $C_{10}H_{16}NO$ $[180\text{-}CH_2]^+$, $C_9H_{14}NO$ $[166\text{-}CH_2]^+$, $C_8H_{12}NO$ $[152\text{-}CH_2]^+$, $C_5H_{10}NCO$ and $C_5H_{10}N$ (piperidine ring), respectively. Combining its ^1H NMR, ^{13}C NMR [$(CD_3)_2O$], ^1H–^1H COSY and ^{13}C–^1H COSY spectra, compound **2** was elucidated as *N*-piperidyl-7-(3,4-methylenedioxy heptenyl)-*trans*-2-heptenamide, named as piperine S; all of the proton and carbon signals were assigned (see Experimental) [9, 10].

Compound **3** was obtained as needles, mp 94–96°, giving a positive Dragendorff's reaction. HR-mass spectrometry gave the formula $C_{17}H_{23}NO_4$, $[M]^+$ m/z 305.1631 (calc. $[M]^+$ 205.1626). The IR (KBr) spectrum of (**3**) showed an amide carbonyl at 1640 cm^{-1}. The ^1H NMR ($CDCl_3$, 500 MHz) spectrum showed three broad multiplets at δ 3.59 (4H, *m*, 1′,5′-H), 1.65(2H, *m*, 3′-H) and 1.58 (4H, *m*, 2′,4′-H), indicating the presence of a piperidine ring. The signal at δ 6.70 (2H, *brs*) was assigned to the two same aromatic protons, 2- and 6-H. The singlets at δ 3.85 (6H, *s*) and 3.83 (3H, *s*) were attributed to the 3-, 5- and 4-methoxyl groups, respectively. The doublets at δ 7.52 (1H, *d*, J = 15.5 Hz) and 6.75 (1H, *d*, J = 15.5 Hz) were due to two *trans*-olefine protons adjacent to a carbonyl. In the EI-mass spectrum, besides the $[M]^+$ at m/z 305 (67.3%), other main peaks at m/z (%) 84 (34.2), 221 (82.2) and 222 (100) corresponded with the fragments of $C_5H_{10}N$ (piperidine ring), $((Me)_3C_6H_2—CH=CH_2—CO$ $[M-84]^+)$ and $((Me)_3C_6H_2—CH=CH—CO—H)$, respectively. On the basis of the above spectral data and analyses, the structure of (**3**) was established to be *N*-piperdyl-3-(3,4,5-trimethyoxyl heptenyl)-*trans*-2-heptenamide [8, 9]. Although compound **3** is reported as a synthetic product [11], this is the first report of it occurring in nature. We suggest the name puberullumine for this component. Its spectral data are also reported for the first time.

EXPERIMENTAL

All mps are uncorr. ^1H and ^{13}C NMR were obtained with TMS as int. standard. UV: MeOH. IR: KBr. Chromatographic separation was carried out on silica gel, TLC on silica gel G.

Plant material. Stems and leaves of *P. puberullum* were collected from Hainan province, China, in August 1992. Voucher specimens are kept in the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, China.

Extraction and isolation. Air-dried stems and leaves (6.5 kg) were extracted with 95% EtOH, repeatedly. The EtOH extract was re-extracted with CH_2Cl_2 in a Soxhlets and 95 g of dichoromethane were obtained. The CH_2Cl_2 fr. was chromographed on a silica gel column using CH_2Cl_2–EtOAc and 500 ml frs were collected. Frs containing similar components as judged by TLC were combined into 10 frs. Fr. 4 was repeatedly rechromatographed on silica gel using petrol–Me_2CO and petrol–EtOAc to furnish compounds **1** (40 mg), **2** (65 mg), **3** (120 mg) and **5** (70 mg). Rechromatography of fr. 7 and fr. 5 (silica gel, petrol–EtOAc) gave compounds **4** (150 mg) and **6** (50 mg).

Identification of components. Piplartine dimer (**4**): Needles, mp 270–272° (lit. [12] 269–272°); UV, IR, ^1H NMR and ^{13}C NMR in agreement with lit. [12]. *Piplartine* (**5**). Pale yellow needles, mp 114–116° (lit. [12] 123–124°), identified by spectral comparison (UV, IR, ^1H NMR, ^{13}C NMR and MS) with lit. [2, 13]. 8,9-*Dihydropiplartine* (**6**). Needles, mp 65–66°; UV, IR, ^1H NMR and MS as previously reported [14]. ^{13}C NMR ($CDCl_3$, 125M Hz): δ 175.5 (C-7), 165.3 (C-2), 153.1 (C-12, 14), 145.2 (C-4), 136.7 (C-13), 136.2 (C-10), 125.6 (C-3), 105.4 (C-11, 15), 60.8 (MeO-13), 56.0 (MeO-12, 14), 41.0, 40.8 (C-6, C-8), 31.5 (C-9), 24.6 (C-5).

Piperlactam S (**1**). Yellow needles, mp 242–244°. $[\alpha]_D^{20}$ −10.3. HR MS $[M]^+$ m/z 295.0850. UV λ_{max}^{MeOH} nm: 195, 206, 232, 248, 274, 284, 315, 380. IR ν_{max}^{KBr} cm^{-1}: 3260 (br. OH), 1695 (C=O), 1615, 1600, 1490, 1310, 990. ^1H NMR (DMSO-d_6, 500 MHz): δ 7.66 (1H, *s*, H-2), 9.14 (1H, *m*, H-5), 7.67 (2H, *m*, H-6, 7), 8.02 (1H, *m*, H-8), 7.39 (1H, *s*, H-9), 10.44 (1H, *s*, HO-3), 4.03 (3H, *s*, MeO-4), 4.06 (3H, *s*, MeO-N). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 118.0 (C-1), 114.1 (C-2), 152.4 (C-3), 149.4 C-4), 120.3 (C-4a), 126.9 (C-5), 126.4 (C-5a), 126.0 (C-6), 127.6 (C-7), 129.3 (C-8), 131.9 (C-8a), 102.8 (C-9), 134.1 (C-10), 117.1 (C-11), 162.1 (C=O), 59.6 (MeO-4), 64.5 (MeO-N). EI-mass spectrum m/z (vol. int.): 295 (51.9) $[M]^+$, 280 (100) $[M-Me]^+$, 265 (37.2) [280-Me, $M-CH_2O]^+$, 250 (11.8) $[280-CH_2O]^+$, 237 (6.5) $[265\text{-}CO]^+$, 222 (16.6) $[250\text{-}CO]^+$, 193 (9.2), 177 (6.2), 165 (21.6), 151 (4.4), 138 (6.9).

Piperine S (**2**). Yellow needles, mp 52–54°. HR-mass spectrum $[M]^+$ m/z 315.1828. UV λ_{max}^{MeOH} nm: 202, 232 (sh), 280. IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O), 1610 (C=C), 1505, 1490, 1440, 1250, 1035, 920, 810. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.64 (1H, *d*, J = 1.7 Hz H-2), 6.70 (1H, *d*, J = 7.7 Hz, H-5), 6.58 (1H, *dd*, J = 7.7, 1.7 Hz, H-6), 2.51 (2H, *t*, J = 7.5 Hz, H-7), 1.55 (6H, *m*, H-8, 2′, 4′), 1.46 (2H, *m*, H-9), 2.19 (2H, *m*, H-10), 6.81 (1H, *dt*, J = 15.1, 7.0 Hz, H-11), 6.22. (1H, *dt*, J = 15.1, 1.5 Hz, H-12), 3.50 (4H, *m*, H-1′, 5′), 1.61 (2H, *m*, H-3′), 5.87 (2H, *s*, OCH_2O). ^{13}C NMR ($CD_3)_2O$, 125 MHz: δ 137.2 (C-1), 109.4 (C-2), 148.4 (C-3), 146.4 (C-4), 108.6 (C-5), 121.8 (C-6, C-12), 35.8 (C-7), 31.8 (C-8), 28.6 (C-9), 32.5 (C-10), 145.5 (C-11), 47.1 (C-1′), 27.2 (C-2′), 25.2 (C-3′), 26.3 (C-4′), 43.8 (C-5′), 101.5 (OCH_2O), 165.6 (C=O). EI-mass spectrum m/z (vol. int.): 315 (56.8) $[M]^+$, 230

(7.1), 202 (28.0), 180 (46.4) $[C_{11}H_{18}NO(M-135)]^+$, 166 (93.0) $[C_{10}H_{16}NO(180-CH_2)]^+$, 152(6.9) $[C_9H_{14}NO$ $(166-CH_2)]^+$, 138 (62.9) $[C_8H_{12}NO$ $(152-CH_2)]^+$, 135 (100) $[C_8H_7O_2$ (benzenyl rearrangement)$]^+$, 112(36.3) $[C_5H_{10}NCO]^+$, 84 (46.3) $[C_5H_{10}N$ (piperidine ring)$]^+$.

Puberulumine (**3**). Needles, mp 94–96°. HR MS $[M]^+$ m/z 305.1631. UV λ_{max}^{MeOH} nm: 200, 224, 296. IR ν_{max}^{KBr} cm^{-1}: 1640 (C=O), 1595, 1580, 1500, 1415, 1340, 1120, 1005, 815. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.70 (2H, *brs*, H-2, 6), 7.52 (1H, *d*, J = 15.5 Hz, H-7), 6.75 (1H, *d*, J = 15.5 Hz, H-8), 3.59 (4H, *m*, H-1′, 5′], 1.65 (2H, *m*, H-3′), 1.58 (4H, *m*, H-2′, 4′), 3.85 (6H, *s*, MeO-3,5), 3.83 (3H, *s*, MeO-4). ^{13}C NMR ($(CD_3)_2O$, 125 MHz): δ 132.1 (C-1), 105.4 (C-2,6), 154.5 (C-3,5), 140.5 (C-4), 142.6 (C-7), 118.3 (C-8), 47.2 (C-1′), 27.5 (C-2′), 25.3 (C-3′), 26.4 (C-4′), 43.7 (C-5′), 56.5 (MeO-3,5) 60.6 (MeO-4), 165.7 (C=O). EI-mass spectrum m/z (vol. int): 305 (67.3) $[M]^+$, 222 (100) 221 (822.2), 206 (6.3), 194 (23.7), 193 (10.2), 179 (26.3), 163 (9.1), 147 (6.5), 84 (34.2).

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