Two Rare Alkaloids from *Pratia nummularia*

Li-Kang Ho[1], *Jun-Chih Ou*[2], *Mei-Ling Sun*[2], *and Chang-Ming Sun*[2,3]

[1] Department of Pharmacology, National Yang-Ming University, Taipei 112, Taiwan, Republic of China
[2] National Research Institute of Chinese Medicine, Taipei Hsien, 231 Taiwan, Republic of China
[3] Address for correspondence

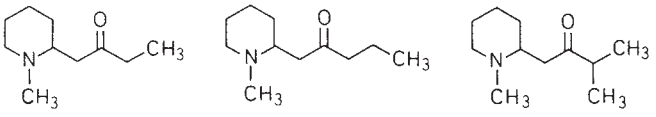
Received: March 1, 1995; Revision accepted: May 6, 1995

Abstract

Two rare alkaloids, 1-(2-*N*-methylpiperidyl)-butan-2-one (**1**) and 1-(2-*N*-methylpiperidyl)-pentan-2-one (**2**), were identified from the medicinal plant *Pratia nummularia*. The structure of **1** was elucidated by means of spectroscopic methods. Compound **2** was established by spectral comparison with synthetic material.

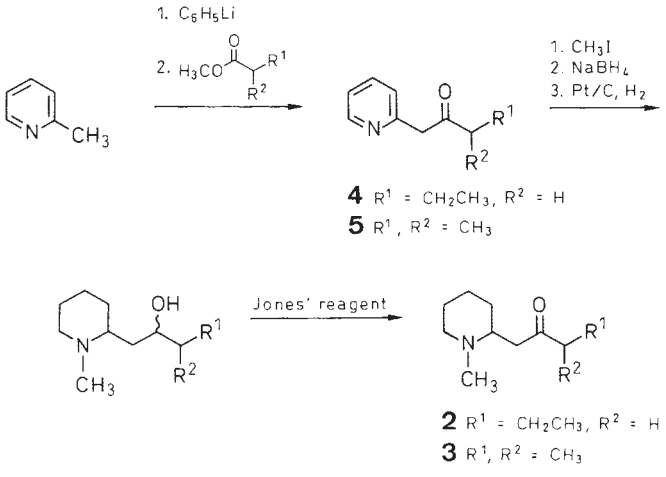
The medicinal plant *Pratia nummularia* (Lam.) A. Br. et Asch. (Campanulaceae) is used as folk medicine for the treatment of diabetes (1), irregular menstrual cycles (1, 2), rheumatism (2) and inflammation (1, 3) in Taiwan. It is also used as traditional remedy against malaria and tumour by aboriginal residents (4).

Previously it was noted that this plant contains alkaloids (5) but their structures have not been identified. In our study of the alkaloidal contents of the plant, we identified two rare, naturally-occurring piperidine alkaloids, 1-(2-*N*-methyl-piperidyl)-butan-2-one (**1**) and 1-(2-*N*-methylpiperidyl)-pentan-2-one (**2**). The structure of the major alkaloid **1** with a molecular formula of $C_{10}H_{17}NO$ (HR-EI-MS, m/z = 169.1468, Δm = 1 mmu) was elucidated by ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY 2D-NMR spectroscopic methods. The optical rotation of **1** ($[\alpha]_D^{22}$ = 0°) indicated the presence of a racemic mixture.



1 **2** **3**

The quantity of the minor alkaloid was small and its structure was studied from GC and GC-MS techniques by comparison with synthetic samples. The GC trace of the first fraction collected from column chromatography displayed the retention times (R_t) for **1** and the minor alkaloid as 7.9 min and 10.3 min, respectively. A GC-MS analysis of the minor alkaloid showed a molecular ion peak at m/z = 183 and the GC-HR-MS results indicated the formula as $C_{11}H_{19}NO$ (m/z = 183.1624, Δm = 1 mmu). The mass spectrum of this alkaloid gave a base peak at m/z = 98. Other ion peaks at m/z = 112, 70, and 42 were also noted. These fragment ions suggested that the minor alkaloid was very likely to have the same skeleton as **1** with an additional methyl group situated at C-9 or C-10. Therefore, isomers **2** and **3** were synthesized for comparison with the minor alkaloid. A modified Büchi method (6) was used to prepare **2** and **3** from the corresponding 1-(2-pyridyl)-pentan-2-one (**4**) and 3-methyl-1-(2-pyridyl)-butan-2-one (**5**), respectively. The stepwise methylation, reduction, hydrogenation and oxidation of **4** and **5** gave the desired alkaloids **2** and **3**, respectively (Scheme **1**). In the GC analysis, the retention time of **3** (R_t = 9.2 min) was shorter than **2** (R_t = 10.3 min). Comparison of **2** with the minor alkaloid by GC-MS showed them to be identical.



4 R^1 = CH_2CH_3, R^2 = H
5 R^1, R^2 = CH_3

2 R^1 = CH_2CH_3, R^2 = H
3 R^1, R^2 = CH_3

Scheme 1 Synthesis of alkaloids for comparison.

Both **1** and **2** were previously found in the *Sedum* species (Crassulaceae) by GC-MS analysis (7), but are here found for the first time in the Campanulaceae.

Materials and Methods

General

Optical rotation was measured with JASCO DIP-370 instrument; ^{1}H- and ^{13}C-NMR spectra were determined in $CDCl_3$ on a Bruker AC-300 spectrometer operating at 300 and 75 MHz, respectively; a Jeol SX102 spectrometer equipped with a Shimadzu GC-14A chromatograph was used for GC-MS analysis. The GC separations were effected on an SGE fused silica BP5, 0.25 μm capillary column (25 m × 0.22 mm i.d.) with injector temperature at 180 °C, oven temperature at 150 °C, and FID temperature at 220 °C with nitrogen at 0.45 ml/min as carrier gas. The general synthetic methods used in this work were based on a published procedure (6).

Planta Med. 61 (1995) 567–568

Plant material, extraction, and isolation

P. nummularia was collected in Taichung, central Taiwan. A whole plant voucher specimen was identified by Jun-Chih Ou and deposited in our institute (NRICM-Taichung ou s.n. 1992). The fresh plant material (10 kg) was cut into small pieces and extracted with 95 % EtOH for 36 h at 60 °C. The extract was concentrated in vacuo to 1/10 of its original volume, acidified with 6 N HCl to pH 4, and partitioned with diethyl ether. The aqueous layer was subsequently basified with concentrated NH_4OH solution to pH 9 and extracted with diethyl ether to give the crude alkaloids. The alkaloids were subjected to a basic aluminium oxide column (2.5 × 50 cm) and eluted with hexane/$CHCl_3$/CH_3CN, 88 : 10 : 2, 2.5 ml/min. The first fraction (720–800 ml) was collected as a mixture of the major (**1**) and minor alkaloids, 35 mg. The latter was identified as structure **2** by spectral comparison with synthetic material. The second fraction (800–1040 ml) gave pure **1**, 540 mg.

1-(2-N-Methylpiperidyl)-butan-2-one (**1**): Pale yellow oil; $[\alpha]_D^{20}$: 0.0° ($CHCl_3$, *c* 0.5); R_f = 0.31 ($CHCl_3$, Al_2O_3); 0.36 (10 % CH_3OH/$CHCl_3$, silica gel), detection Dragendorff reagent; ^{1}H-NMR: δ = 2.74 (1H, dd, *J* = 11 Hz, 5 Hz, H-6_{ax}), 2.72 (1H, dd, *J* = 16 Hz, 5 Hz, H-7), 2.50 (1H, m, H-2), 2.41 (2H, dq, *J* = 7.5 Hz, 2 Hz, H-9), 2.31 (1H, dd, *J* = 16 Hz, 7 Hz, H-7), 2.15 (3H, s, N-CH_3), 2.09 (1H, dd, *J* = 11 Hz, 3 Hz, H-6_{eq}), 1.63 (1H, m, H-4_{eq}), 1.59 (1H, m, H-3_{eq}), 1.54 (2H, m, H-5), 1.25 (1H, m, H-4_{ax}), 1.22 (1H, m, H-3_{ax}), 1.01 (3H, t, *J* = 7.5 Hz, H-10); ^{13}C-NMR: δ = 210.8 (C-8), 59.0 (C-2), 56.1 (C-6), 46.1 (C-7), 43.2 (N-CH_3), 37.0 (C-9), 31.9 (C-3), 25.6 (C-5), 23.5 (C-4), 7.7 (C-10); EI-MS: *m/z* (rel. int. %) = 169 (M^+, 58), 154 (9), 140 (4), 112 (48), 98 (100), 70 (84), 42 (81).

Preparation of 1-(2-pyridyl)-alkan-2-ones

To a solution of 2-methylpyridine (12 ml, 12 mmol) in THF (250 ml) was gradually added a solution of phenyllithium in cyclohexane-ether (7.5 ml, 1.8 M, 13.5 mmol) with stirring at –76 °C under nitrogen. The reaction mixture was kept stirring for 30 min and methyl butyrate (or methyl isobutyrate) (12 mmol) in THF (50 ml) was added. The cooling bath was then removed and the reaction mixture was stirred for another 1 h. Work-up in the usual manner gave a pale yellow oil that was purified by silica gel chromatography to afford the desired compound.

1-(2-Pyridyl)-pentan-2-one (**4**): 41 % yield; ^{1}H-NMR: δ: 8.53 (1H, m), 7.69 (1H, dd, *J* = 7.5 Hz, 7.5 Hz), 7.25 (1H, d, *J* = 7.5 Hz), 7.21 (1H, m), 3.95 (2H, s), 2.51 (2H, t, *J* = 7.3 Hz), 1.59 (2H, sextet, *J* = 7.3 Hz), 0.87 (3H, t, *J* = 7.3 Hz); ^{13}C-NMR: δ = 207.0, 154.6, 149.0, 136.6, 124.1, 121.8, 51.9, 44.4, 16.8, 13.4.

3-Methyl-1-(2-pyridyl)-butan-2-one (**5**): 43 % yield; ^{1}H-NMR: δ = 8.53 (1H, ddd, *J* = 0.7 Hz, 1.7 Hz, 5.0 Hz), 7.72 (1H, ddd, *J* = 1.7 Hz, 7.5 Hz, 7.5 Hz), 7.28 (1H, d, *J* = 7.5 Hz), 7.23 (1H, ddd, *J* = 1.0 Hz, 5.0 Hz, 7.5 Hz), 4.05 (2H, s), 2.78 (1H, sep, *J* = 7.0 Hz), 1.13 (6H, d, *J* = 7.0 Hz); ^{13}C-NMR: δ = 210.7, 154.9, 149.1, 136.3, 124.1, 121.6, 49.7, 40.6, 17.9.

Preparation of 1-(2-N-methylpiperidyl)-alkan-2-ones

The above pyridyl ketone (2 mmol) was mixed with CH_3I (excess) to give the light brown solid, in quantitative yield, which was purified by recrystallisation from CH_2Cl_2. The crystalline solid (150 mg) was reduced with $NaBH_4$ (excess) in CH_3OH (20 ml) at room temperature with stirring for 2 h. After the work-up of the reaction mixture, the intermediate was hydrogenated in ethanol over Adams catalyst at room temperature overnight. Filtration and removal of the ethanol gave a light yellow oil, which was then reacted with Jones' reagent in acetone. The work-up methods proceeded in the usual manner; then after purification by silica gel chromatography the desired compound was obtained.

1-(2-N-methylpiperidyl)-pentan-2-one (**2**): Pale yellow oil; 60 % yield; ^{1}H-NMR: δ = 2.77 (1H, dd, *J* = 11 Hz, 5 Hz, H-6_{ax}), 2.76 (1H, dd, *J* = 16 Hz, 5 Hz, H-7). 2.55 (1H, m, H-2), 2.50 (2H, t, *J* = 7.5 Hz, H-9), 2.34 (1H, dd, *J* = 16 Hz, 7 Hz, H-7), 2.19 (3H, s, N-CH_3), 2.14 (1H, dd, *J* = 11 Hz, 4 Hz, H-6_{eq}), 1.62–1.56 (6H, m, H-3_{eq}, H-4_{eq}, H-5 and H-10), 1.36–1.24 (2H, H-3_{ax} and H-4_{ax}), 0.90 (3H, t, *J* = 7 Hz, H-11); ^{13}C-NMR: δ = 208.5 (C-8), 59.5 (C-2), 56.3 (C-6), 45.6 (C-7), 45.4 (N-CH_3), 42.1 (C-9), 30.8 (C-3), 24.1 (C-5), 23.0 (C-4), 17.1 (C-10), 13.6 (C-11); EI-MS: *m/z* (rel. int. %) = 183 (M^+, 11), 112 (10), 98 (100), 70 (47), 42 (40).

1-(2-N-methylpiperidyl)-3-methylbutan-2-one (**3**): Pale yellow oil; 64 % yield; ^{1}H-NMR: δ = 2.78 (1H, m, H-6_{ax}), 2.76 (1H, dd, *J* = 16 Hz, 5 Hz, H-7), 2.56 (1H, m, H-2), 2.54 (1H, dq, *J* = 6.5 Hz, 2 Hz, H-9), 2.38 (1H, dd, *J* = 16 Hz, 7 Hz, H-7), 2.15 (3H, s, N-CH_3), 2.10 (1H, dd, *J* = 11 Hz, 4 Hz, H-6_{eq}), 1.65–1.50 (4H), 1.35–1.10 (2H), 1.04 (6H, d, *J* = 6.5 Hz, H-10 and H-11); ^{13}C-NMR: δ = 213.5 (C-8), 58.8 (C-2), 56.3 (C-6), 44.2 (C-7), 43.3 (N-CH_3), 41.5 (C-9), 32.0 (C-3), 25.7 (C-5), 23.6 (C-4), 18.0 and 18.1 (C-10, C-11).

Acknowledgements

This project was financially supported by the grant from the National Science Council (Grant No. NSC 82-0208-M-077-004), ROC.

References

[1] Chiu, N. Y. (1987) Alpine Medicinal Plants of Taiwan, pp. 152, Southern Materials Center, Inc., Taiwan.
[2] Kao, M. T. (1985) Popular Herbal Remedies of Taiwan (1), pp. 162, Southern Materials Center, Inc., Taiwan.
[3] Kan, W.-S. (1975) Pharmaceutical Botany, pp. 547, National Research Institute of Chinese Medicine, Taiwan.
[4] Liu, T. S. (1952) A List of Taiwan Economical Plants, pp. 39, Taiwan Provincial Museum, Taipei, Taiwan.
[5] Chen, C. S., Ramstad, E., Koo, W. Y. (1963) Kuo Li Taiwan Da Hsueh Yi Hsueh Yuan Yen Chiu Pao Kao 9, 48–51; (1965) Chem. Abstr. 63, 4659f.
[6] Büchi, J., Kracher, F., Schmidt, G. (1962) Helv. Chim. Acta 45, 729–737.
[7] Stevens, J. F., Hart, H. 't., Hendriks, H., Malingré, T. M. (1992) Phytochemistry 31, 3917–3924.