Natural Product Sciences
4(3) : 148 ~ 152 (1998)

Alkaloids from Amaryllidaceae III. Alkaloids from the Bulbs of *Pancratium maritimum*

Bilge Sener*[1], Sakine Könükol[1], Cornelis Kruk[2], and Upendra K. Pandit[2]

[1]Dept. of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara-Turkey
[2]Organic Chemistry Laboratory, University of Amsterdam,
Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands .

Abstract – The extract from the bulbs of *Pancratium maritimum* L. afforded 12 alkaloids belonging to the skeletally six different groups of the Amaryllidaceae alkaloids. In this paper, the isolation and identification of (–)-N-demethylgalanthamine (**1**), (+)-tazettine (**2**) and (–)-2-O-demethylmontanine (**3**) are described. Their structures have been determined by using extensive spectroscopic techniques. This is the first report describing the occurrence of **1** and **3** in this plant.

Key words – *Pancratium maritimum*, alkaloids, Amaryllidaceae, spectral data.

Introduction

Continuing our studies on the alkaloids from Turkish medicinal plants, a project has been developed which aims to search, identify and evaluate new biologically active compounds from the uninvestigated Amaryllidaceae plants growing in Turkey. To date, a total of some 180 Amaryllidaceae alkaloids have been described (Cordell, 1981). The Amaryllidaceae alkaloids have shown a variety of biological activities such as antitumor, antiviral, immunostimulant, antimalarial and activity on the central nervous system (Martin, 1987). Some members of the Amaryllidaceae plants have been employed in Turkish folk medicine against cancer.

As a part of our systematic studies, we have in the initial phase of our work investigated the alkaloid content of *Pancratium maritimum* of Turkish origin. The bulbs of this plant have afforded 12 compounds belonging to the skeletally six different groups of the Amaryllidaceae alkaloids. Three of these groups namely, lycorine-, lycorenine- and crinane- types have been reported in our previous papers (Sener *et al.*, 1993 and 1994). In this paper, the isolation and structure elucidation of the galanthamine-, pretazettine- and montanine- types are presented.

*Author for correspondence.

Experimental

Plant material – The bulbs of *Pancratium maritimum* L. (Amaryllidaceae) were collected from the vicinity of Inkum, the cost of The Black Sea, Turkey, during the flowering stage. The plant was identified by Prof. Dr. B. Sener and a voucher specimen was kept in the Herbarium of the Faculty of Pharmacy, Gazi University, Ankara.

Instrumentation – The UV spectra were recorded in MeOH on a 8450 UV/VIS Hewlett-Packard spectrophotometer. The IR spectra were taken in KBr pellets on a Perkin-Elmer 281 B infrared spectrophotometer. The optical rotations were measured in $CHCl_3$ on a Polartronic Universal Australian Standard K-157 spectropolari-

meter. The ^{1}H- and ^{13}C-NMR spectra were run on a Bruker WM 250 NMR spectrometer. ^{2}H resonance of the solvent ($CDCl_3$) was used for field frequency lock. The ^{13}C spectra using APT and DEPT techniques were obtained from the same solutions on a Bruker 250, operating at 62.8 MHz. This spectrophotometer was also used for the 2D ^{1}H-^{13}C correlation experiments. The mass spectra were obtained on V.G. Micromass 2 AB-HF 9Q spectrometer, coupled to a V.G. 11/250 Data System.

Extraction, isolation and purification – Air-dried and powdered bulbs (3.5 kg) of *P. maritimum* were extracted with EtOH by percolation at room temperature and the extract was concentrated to a crude gum. The gum was acidified with 5% HCl and extracted with chloroform (fraction A, 5.73 g). The acidic phase was made basic with 10% NH_4OH and extracted with chloroform. The combined chloroform extracts were dried over anhydrous sodium sulphate. Thereafter, the solution was evaporated to dryness *in vacuo* leaving a brown residue (fraction B, 8.38 g). The large quantity of lycorine found in the fractions A and B were obtained as white crystals (5.96 g) from MeOH. The mother liquors of fraction A and B, after removal of lycorine, were evaporated and subjected to column chromatography over silica gel. Elution was achieved using chloroform and chloroform-methanol mixtures of increasing polarity. Fractions (25 ml each) were collected and examined by using tlc. Final purification was achieved using prep. tlc. These procedures resulted in the isolation of the following alkaloids:

(–)-N-Demethylgalanthamine (**1**): Fraction A-5 was chromatographed on prep. tlc to give **1** as white crystals m.p. 158°C from chloroform (14.6 mg). In addition 20.2 mg of **1** was obtained by using prep. tlc of the residue of fraction B-3. $[\alpha]_D^{26}=-78°$ (c. 0.3, $CHCl_3$). UV ($CHCl_3$): λ_{max} 278.6 nm. IR ($CHCl_3$): 3600 (N-H), 2900, 1610, 1595, 1420, 1030 cm^{-1}. EIMS m/z (rel. int.): 273 (M^+, 100), 272 (M-1, 68), 256 (5), 242 (6), 230 (M-43, 24), 202 (M-71, 17).

(+)-Tazettine (**2**): Fraction B-1 was rechromatographed on prep. tlc to afford **2** (9.6 mg). $[\alpha]_D^{26}=+148.5°$ (c. 0.04, $CHCl_3$). EIMS m/z (rel. int.): 331 (M^+, 65), 316 (M-15, 24), 298 (M-15-18, 20), 247 (M-84, 100).

(–)-2-O-Demethylmontanine (**3**): Fractions B-6 and B-7 were chromatographed on prep. tlc to give **3** (80 mg). In addition 76.4 mg of 3 were obtained as white prismatic crystals m. p. 269°C from fraction B-6. $[\alpha]_D^{26}=-76°$ (c. 0.2, MeOH). UV (MeOH): λ_{max} 241.5 and 292. 5 nm. IR (KBr): 3400-3300 (O-H), 1505, 1490, 1440 and 1300 cm^{-1}. EIMS m/z (rel. int.): 287 (M^+, 100), 286 (M-1, 18), 270 (M-OH, 19), 243 (17), 214 (13), 185 (15).

Results

Pancratium is a small genus but is widespread in temperate regions. In Turkey, there is only one species, *P. maritimum* L. which is called "Kumzambagi" in Turkish (Baytop *et al.*, 1984). According to the procedure described in the experimental section, 12 alkaloids have been isolated using column chromatography and preparative tlc. Of these, nine alkaloids have been reported previously (Şener *et al.*, 1993-1994). It is useful to summarize these here as follows:

i. (–)-lycorine, (–)-3β-11α-dihydroxy-1,2-dehydrocrinane and (–)-crinine were found to be the major alkaloids of the bulbs.

ii. (–)-3β-11α-dihydroxy-1,2-dehydrocrinane and (–)-8-hydroxy-9-methoxycrinine were identified as new crinane-type alkaloids from this plant.

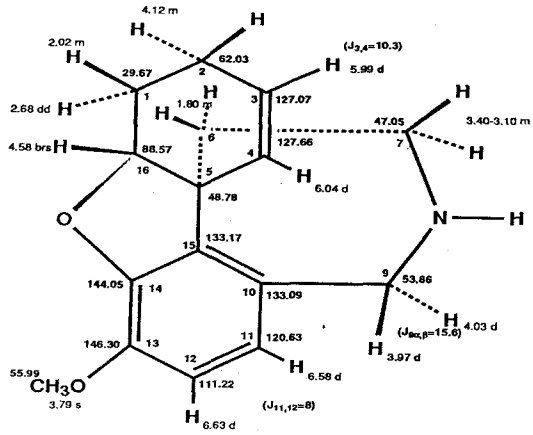
iii. The known alkaloids, namely, (–)-crinine, (+)-buphanisine, (–)-6α-hydroxy-3β-methoxy-1,2-dehydrocrinane* and (–)-6β-

*6α- and 6β- are present in equilibrium in solution. They may not be present as such in the plant.

hydroxy-3β-methoxy-1,2-dehydrocrinane* were demonstrated for the first time in *P. maritimum*. These compounds are also new for the genus.

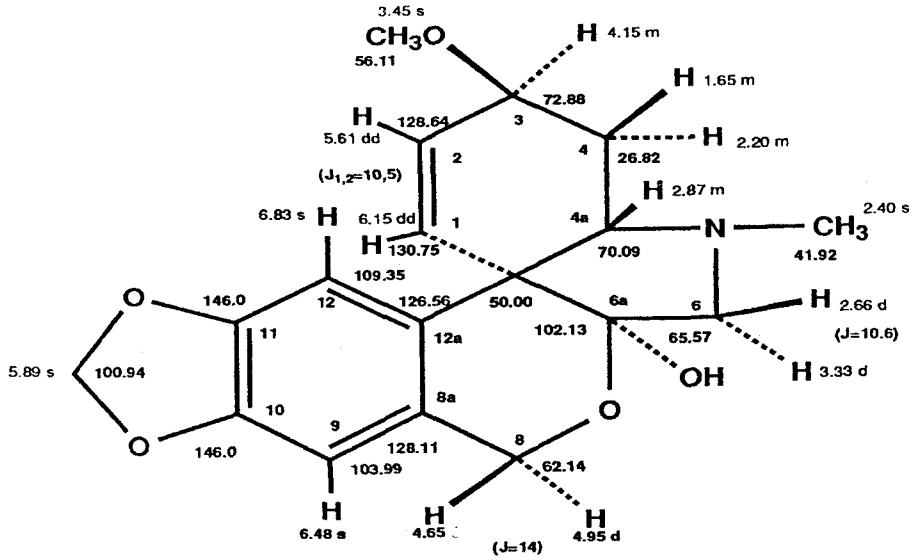
In addition to the above mentioned alkaloids, three other alkaloids, representing the galanthamine-, pretazettine- and montanine-skeletons have been identified. These are discussed in sequence.

Alkaloid **1** was isolated as white needles with a prominent UV absorption maximum at 278.6 nm. The chemical shift assignments in the ^{1}H-NMR spectrum summarized around the structure of **1** were confirmed by spin-spin decoupling as well as Homo-COSY experiments. The chemical shifts in the ^{13}C-NMR spectrum were assigned on the basis of APT experiment. The EIMS of alkaloid **1** showed the molecular ion as the base peak at m/z 273 and exhibited the characteristic galanthamine-type fragmentation pattern (Bastida *et al.*, 1990). Comparison of the spectral data with those reported in the literature (Bastida *et al.*, 1987-1990 and Kobayashi *et al.*, 1976), led to the identification of alkaloid **1** as (−)-N-demethyl-galanthamine (=norgalanthamine).

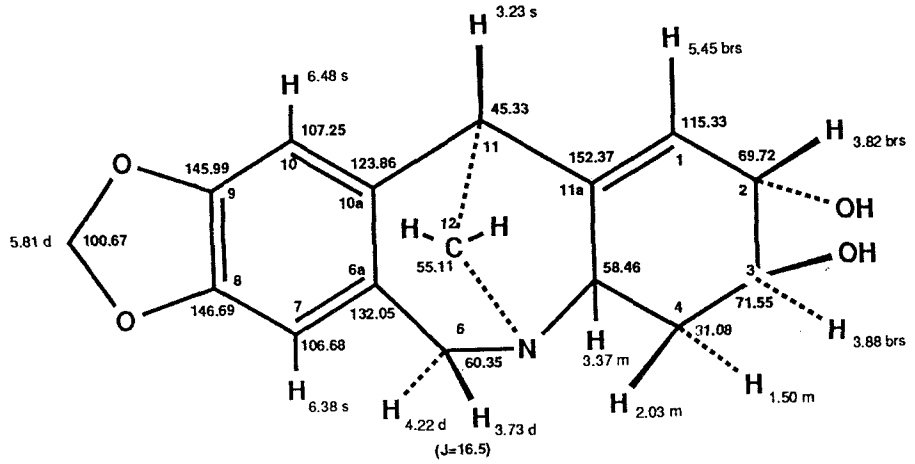


1

Preparative tlc of the fraction B-1 afforded alkaloid **2** as a minor compound. The ^{1}H-NMR assignments given on the structure **2** were confirmed by application of Homo-COSY experiments. The chemical shifts of the C- atoms in the ^{13}C-NMR spectrum were obtained by APT and using Het-COSY experiments. The mass spectrum of **2** exhibited the characteristic pretazettine- type fragmentation pattern with a base peak at m/z 247 and the molecular ion at m/z 331 (Ghosal *et al.*, 1984). From the spectroscopic data obtained by us and the data reported



2



3

in the literature (Ghosal *et al.*, 1984), the alkaloid **2** has been identified as (+)-tazettine which was firstly characterized from *Narcissus tazetta* (Southon *et al.*, 1989).

Fractions B_6 and B_7 gave alkaloid **3** as a pure compound in the form of white prisms. The chemical shifts in the ^{1}H-NMR spectrum of **3**, given on its structure, were determined by nOe and Homo-COSY experiments. The assignments of the ^{16}C-atoms were obtained by a combination of DEPT, APT and Hetero-COSY experiments. The mass spectrum of **3** showed the molecular ion as the base peak at m/z 287. These findings coupled with the data reported in the literature (Wildman *et al.*, 1968 and Clark *et al.*, 1975) helped to identify alkaloid **3** as (−)-2-O-demethylmontanine. This is the first report describing the occurrence of (−)-N-demethylgalanthamine and (−)-2-O-demethylmontanine in *P. maritimum*.

Acknowledgement

This research was supported by a NATO Grant (Nr. 910743).

References

Bastida, J., Viloadomat, F., Llabres, J. M., Codina, C., Feliz, M. and Rubiralta, M., Alkaloids from Narcissus confusus. *Phytochemistry* **26**(5), 1519-1524 (1987).

Bastida, J., Viloadomat, F., Llabres, J. M., Quiroga, S., Codina, C., and Rubiralta, M., Narcissus nivalis. A New Source of Galanthamine. *Planta Medica* **56**, 123-124 (1990).

Baytop, T. and Mathew, B., The Bulbous Plants of Turkey, B. T. Batsford Ltd., London, 1984.

Clark, R. C. and Warren, F. L., Alkaloids of the Amaryllidaceae Brunvigine. *Tetrahedron* **31**, 1855-1859 (1975).

Cordell, G. A., Introduction to Alkaloids, John Wiley and Sons, New York, 1981.

Ghosal, S., Kumar, Y. and Singh, S. K., Glucosyloxy Alkaloids from Pancratium maritimum. *Phytochemistry* **23**(5), 1167-1171 (1984).

Kobayashi, S., Ishikawa, H., Kihara, M., Shing, T. and Uyeo, S., Isolation of N-Demethylgalanthamine from the Bulbs of Crinum asiaticum L. var. japonicum (Amaryllidaceae). *Chem. Pharm. Bull.* **24**(10), 2553-2555 (1976).

Martin, S. F., The Amaryllidaceae Alkaloids, Brossi, A. (ed.), *The Alkaloids*, Vol. 30, p. 251-375, Academic Press, New York, 1987.

Sener, B., Könükol, S., Kruk, C. and Pandit, U. K., Alkaloids from Amaryllidaceae I. Alkaloids of Lycorine and Lycorenine Class from Pancratium maritimumL. *Arch. Pharm.* (Weinheim) **326**, 61-62 (1993).

Sener, B., Könükol, S., Kruk, C. and Pandit, U. K., New Crinine-Type Alkaloids from Pancratium maritimum L. Growing in Turkey. *Nat. Prod.*

Lett. **1**(4), 287-291 (1993).

Sener, B., Könükol, S., Kruk, C. and Pandit, U. K., Alkaloids from Amaryllidaceae II. Alkaloids of Crinine Class from Pancratium maritimum. *J. Chem. Soc. Pak.* **16**(4), 275-279 (1994).

Southon, I. W. and Buckingham, J., (eds.), Dictionary of Alkaloids, Chapman and Hall, Ltd., New York, 1989.

Wildman, W. C. and Warren, F. L. *Tetrahedron* **31**, 1855-1859 (1975).

(Accepted June 10, 1998)