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Further alkaloids from the flowers of *Pancratium maritimum*

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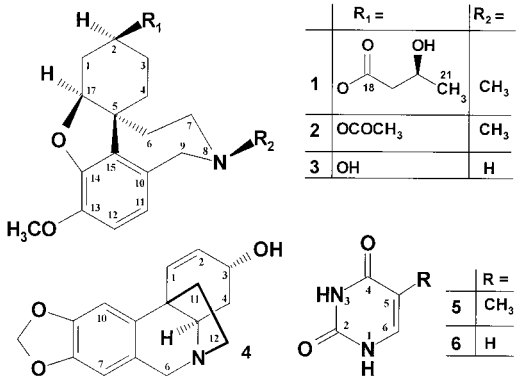
The ethanolic extract of the fresh flowers of *Pancratium maritimum* L. yielded a new alkaloid ester pancritamine (**1**), together with the alkaloids acetyllycoramine (**2**), (−)-*N*-demethyllycoramine (**3**) and crinine (**4**). Additionally, two nitrogenous bases, thymine (**5**) and uracil (**6**) were isolated and identified. The structure and stereochemistry of pancritamine have been determined by spectroscopic analyses and by application of 2D-NMR techniques as well as CD study. The ^{1}H and/or ^{13}C NMR spectra of acetyllycoramine and (−)-*N*-demethyllycoramine have now been completely characterized and revised.

1. Introduction

The Amaryllidaceae alkaloids have been studied extensively because of the variety of their structures and biological activities and also from the biosynthetic viewpoint [1]. However, flowers of the Amaryllidaceae plants have not attracted much attention from phytochemists [2]. In a previous paper on the alkaloidal composition of the fresh flowers of *Pancratium maritimum* L. we reported on the isolation and characterization of four alkaloids [3]. As a continuation of our efforts, we describe here the characterization of a new alkaloid ester from an ethanolic extract of the flowers of the same plant, pancritamine (**1**) as well as the alkaloids acetyllycoramine (**2**), (−)-*N*-demethyllycoramine (**3**) and crinine (**4**). Moreover, the nitrogenous bases thymine (**5**) and uracil (**6**), were isolated and characterized by means of spectroscopic methods.



2. Investigations, results and discussion

The name pancritamine is proposed for the optically active new alkaloid ester **1**. Its EIMS shows a molecular ion peak at m/z 375 and the HREIMS shows the $[M]^{+}$ at m/z 375.2045 which is consistent with the molecular formula of $C_{21}H_{29}NO_5$. The base peak was observed at m/z 374 for the $[M-1]^{+}$ fragment. Further consecutive elimination of CH_2, CHO and C_2H_3O fragments from the ester side chain afforded important fragment ions at m/z 360, 331 and 288, respectively. In the CD spectrum, pancritamine displays two negative Cotton effects at 234 and 280 nm, which are in agreement in sign and shape with those of lycoramine and acetyllycoramine [3, 4]. These findings suggested that **1** has a lycoramine-type skeleton. The ^{1}H NMR spectrum of **1**, recorded in methanol-d_4, shows resonances for 28 protons and is repoorted in Table 1. From the contour plot of the COSY ^{1}H-^{1}H- and HMQC experiments, the existence of five spin-coupling systems could be traced through the molecule of **1**; (i) the protons H_{17}, 2 H_1, H_2, 2 H_3 and 2 H_4, (ii) the protons 2 H_6 and 2 H_7, (iii) the protons at C_9, (iv) the aromatic protons H_{11}, H_{12} and OMe_{13} and finally (v) the protons of the ester side chain 2 H_{19}, H_{20} and 3 H_{21}. The first spin coupling system could be traced from the resonating triplet for H_{17} at δ 4.29 (J = 3.5 Hz) to the protons at C_1, further cross-peaks in the COSY spectrum were also observed from the resonating multiplet of H_2 at δ 5.10 to the methylene protons of C_1 and C_3, respectively. Finally, geminal as well as vicinal couplings between the protons at C_3 and C_4 were also observed. At this point the chain of the coupling is interrupted suggesting that C_5 is a quaternary carbon. The second spin-system was securely assigned starting from the strong coupling between the protons at C_6, H_{7a} and H_{7b}, respectively, in addition to the geminal coupling (J = 14.5 Hz) between the protons at C_7. The NMe, the resonating singlet of which appeared at δ 2.36, showed interruption of the coupling at this point within the seven-membered hetero ring. Due to the position of the methylene protons at C_9 between the NMe and the aromatic moiety, their resonances appeared at δ 3.98 and 3.62 as an AB system (J = 14.8 Hz) constituting the third spin system. The unambiguous assignments of H_{11} and H_{12} within the fourth spin-coupling system, were done by the observation of two doublets at δ 6.63 and 6.73 (J = 8.0 Hz) and the long-range cross peak in the COSY spectrum of the resonance at δ 6.73 (H_{12}) with the signal of the methoxyl group at δ 3.82 (OMe_{13}), respectively. Moreover, the strong cross-peaks in the 2D-NOESY ^{1}H-^{1}H spectrum (Fig.) between H_{12} and OMe_{13} as well as between H_{11} and the signals of the protons at C_9 confirmed these assignments. Finally, the last spin-system could be traced through the ester side chain. The ester moiety was identified as 3-hydroxybutyryl group based on resonating ^{1}H signals at δ 2.44, 4.13 (m) and 1.19 (d, J = 6.3 Hz) for 2 H_{19}, H_{20} and 3 H_{21}, respectively. The position of the acid moiety was assigned to be bounded

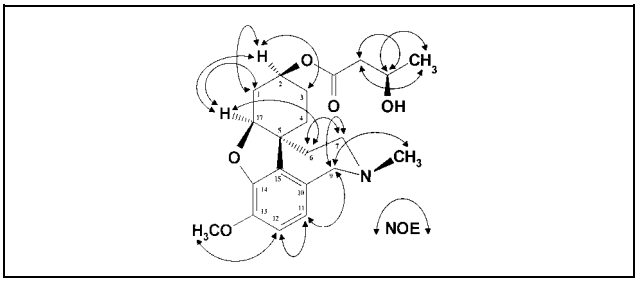


Fig.: Important ^{1}H-^{1}H NOESY cross-peaks observed for **1**

Table 1: Proton chemical shift assignments of compounds 1–3

^{1}H	**1***		**2****	**3***	**2/3**
	δ (ppm)	J (Hz)	δ (ppm)	δ (ppm)	J (Hz)
1a	2.44***		2.51 brd	2.23 m	16.3
1b	2.04 ddd	16.5, 4.0, 3.5	2.10–1.86 m***	2.05–1.96 m***	
2	5.10 m		5.07 m	4.05 m	
3a	1.95 dd	12.4, 3.2	2.10–1.86 m***	1.87–1.69 m****	
3b	1.86–1.77 m***		1.39 m	1.56 m	
4a	1.86–1.77 m***		1.83–1.59 m****	2.05–1.96 m***	
4b	1.61 m		1.83–1.59 m****	1.87–1.69 m****	
6a	1.86–1.77 m***		2.10–1.86 m***	2.05–1.96 m***	
6b	1.86–1.77 m***		1.83–1.59 m*	1.87–1.69 m***	
7a	3.18 ddd	14.5, 14.5, 2.0	3.26 ddd	3.50–3.20*****	14.5, 14.5, 2.0
7b	3.02 ddd	14.5, 2.0, 2.0	3.05 ddd	3.50–3.20*****	14.5, 2.0, 2.0
$NC\underline{H}_3$	2.36 s		2.35 s		
9a	3.98 d	14.8	4.07 d	4.00 d	15.0
9b	3.62 d	14.8	3.64 d	3.89 d	15.0
11	6.63 d	8.0	6.56 d	6.63 d	8.2
12	6.73 d	8.0	6.65 d	6.71 d	8.2
$OC\underline{H}_3$	3.82 s		3.85 s	3.81 s	
17	4.29 t	3.5	4.35 t	4.31 t	3.3
19	2.44***		2.02 s		
20	4.13 m				
21	1.19 d	6.3			

* Recorded in CD_3OD; ** Recorded in $CDCl_3$; ***, **** In each column partially overlapped signals; ***** Partially overlapped with methanol-signal

to C_2 on the basis of the deshielded resonance of H_2 at δ 5.10 in comparison with its chemical shift in compound **3**, which has a free OH at C_2 (Table 1).

The ^{13}C NMR chemical shifts of **1** were assigned from analysis of DEPT, HMQC and HMBC. These experiments revealed resonances for 3 quartets, 7 triplets, 5 doublets and 6 singlets, respectively. Unambiguous assignments of the protonated carbon resonances of **1**, given in Table 2, were assigned from DEPT and HMQC experiments. Unequivocal assignments of the non-protonated carbon resonances were made possible from HMBC spectrum. In the HMBC of **1** (Table 2), cross-peaks ($^3J_{CH}$) from the signal at δ 6.73 (H_{12}) with the signals resonating at δ 128.94 (C_{10}) and 147.99 (C_{14}), were observed, confirming the assignments of these signals. Moreover H_{11} showed cross-peaks of the $^3J_{CH}$ type with the carbons at δ 145.60 (C_{13}) and 137.08 (C_{15}), respectively. Cross-peaks of both $^3J_{CH}$ and $^2J_{CH}$ types were also observed through the ester side chain from $2\,H_{19}$ to the signals at δ 172.87 (C_{18}), 65.60 (C_{20}) and 23.28 (C_{21}), respectively.

To assign and confirm the relative stereochemistry of **1**, a 2D-NOESY ^{1}H-^{1}H experiment (Fig.) was performed. For example, important cross-peaks between H_{17} and H_2, between H_{17} and $2H_6$ were observed suggesting the α-configuration of these protons and positioning the ester moiety at C_2 in the β-configuration, respectively. Further NOESY cross-peaks were shown in the Fig.

Acetyllycoramine (**2**) showed ^{1}H and ^{13}C NMR resonances in agreement with previously reported data [4], but we report here for the first time the unequivocal assignments of both ^{1}H and ^{13}C NMR spectra using 2D NMR techniques. The assignment of the carbons C_1, C_3, C_4 and C_6 of acetyllycoramine was corrected based on the data obtained from COSY, DEPT and HMQC spectra (Table 2).

(−)-*N*-demethyllycoramine (**3**) was first reported as one of the alkaloidal constituents of *Hymenocalis rotata* [5]. The authors identified the alkaloid through its MS, m.p., $[\alpha]_D$ and an incomplete ^{1}H NMR data. We report for the first time the complete ^{1}H NMR data and new ^{13}C NMR data (Tables 1 and 2) for the alkaloid.

Crinine (**4**) was identified through its m.p., $[\alpha]_D$, EIMS and ^{1}H NMR data, which were in agreement with those recently reported by Viladomat et al. for crinine [6].

Thymine (**5**) with a molecular formula of $C_5H_6N_2O_2$ as deduced from CIMS and ^{13}C NMR data. Its ^{1}H NMR spectrum revealed signals due to two strongly chelated NH, H_6 and Me_5 at δ 10.83 as a broad singlet, δ 7.19 (q, J = 1.3 Hz) and δ 1.72 (d, J = 1.3 Hz), respectively. Its ^{13}C NMR spectrum revealed the presence of 3 singlets for C_2, C_4 and C_5, one doublet for C_6 and one quartet for Me_5, respectively. The ^{13}C NMR data of **5** were in agreement with those reported for thymine [7].

Table 2: Carbon chemical shift assignments of compounds 1–3*

^{13}C	**1****		**2*****	**3****
	δ (ppm)	HMBC with H	δ (ppm)	δ (ppm)
1	29.83 t		29.14 t	32.79 t
2	69.44 d		67.41 d	65.85 d
3	33.02 t		31.59 t	27.91 t
4	24.94 t		24.01 t	25.25 t
5	47.69 s		46.73 s	48.24 s
6	25.38 t		24.84 t	37.23 t
7	55.38 t		53.39 t	47.73 t
$NC\underline{H}_3$	42.98 q		40.56 q	
9	61.32 t		59.50 t	53.59 t
10	128.94 s	H-12	128.77 s	130.56 s
11	122.79 d	H-9	121.59 d	121.89 d
12	133.13 d		111.15 d	112.86 d
13	145.60 s	H-11, $OC\underline{H}_3$	144.22 s	145.76 s
$O\underline{C}H_3$	56.78 q		55.99 q	56.69 q
14	147.99 s	H-12	146.71 s	148.39 s
15	137.08 s	H-11	135.05 s	137.31 s
17	89.61 d	H-1a, H-6	88.29 d	90.46 d
18	172.87 s	H-19 ($^2J_{CH}$)	170.92 s	
19	45.36 t	$C\underline{H}_3$-21	21.43 q	
20	65.60 d	H-19 ($^2J_{CH}$), $C\underline{H}_3$-21 ($^2J_{CH}$)		
21	23.28 q	H-19		

* Carbon multiplicities and signal assignments from HMQC and DEPT spectra; Long-range connectivities in HMBC spectra are given; Long-range couplings were via $^3J_{CH}$ unless otherwise noted in parentheses. The HMBC spectrum was recorded with the long-range delay optimized for 8 Hz

** Recorded in CD_3OD

*** Recorded in $CDCl_3$

Uracil (**6**) with a molecular formula of $C_4H_4N_2O_2$, as deduced from CIMS and ^{13}C NMR data. Its ^1H NMR spectrum showed in addition to the deshielded signals of the chelated NH protons at δ 10.91 and 10.85, two doublets at δ 7.34 and 5.42 ($J_{5,6} = 6.6$ Hz) for H_6 and H_5, respectively. The ^{13}C NMR spectrum showed resonances for two singlets (C_2 and C_4) and two doublets (C_5 and C_6) and is comparable with those reported for uracil [7].

To the best of our knowledge, this is the first report of thymine and uracil in the Amaryllidaceae. The occurrence of such nitrogenous bases is noteworthy in the view of chemotaxonomy of the genus *Pancratium* and the family Amaryllidaceae.

3. Experimental

3.1. Plant material, apparatus and methods

The flowers of *P. maritimum* were collected from plants cultivated at the campus of the Suez Canal University at Ismailia during the flowering period in August 1997. Samples were identified and authenticated by Dr. N. El-Hadidy, Prof. of Taxonomy, Cairo University and a voucher specimen (No. PM1) is deposited at the Herbarium of the Faculty of Pharmacy, Suez Canal University.

M.p.'s were uncorr. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. NMR spectra were conducted on Bruker AM-400 at 400 MHz (for ^1H NMR) and at 100 MHz (for ^{13}C NMR). Inverse heteronuclear correlations were performed with the sequences of Bax and Summers for HMBC [8] and Bax and Subramanian for HMQC [9]. MS were performed on Finnigan MAT-312 at 70 eV. MPLC was conducted on LiChroprep® Si 60 (40–63 µm, Merck). TLC was performed on pre-coated silica gel 60 F_{254} and RP-18 F_{254s} (0.2 mm, Merck). Spots of the alkaloids on the chromatograms were detected under UV (254 nm) and by spraying with Dragendorff's reagent.

3.2. Extraction and isolation

The fresh flowers of *P. maritimum* (3.5 kg) were crushed and macerated with EtOH (3 × 10 liters) for 48 h at room temperature. The combined extracts were evaporated and concentrated under reduced pressure to 500 ml, diluted with 200 ml H_2O, then extracted with $CHCl_3$ (4 × 400 ml). The residue of the combined $CHCl_3$-extracts (3 g) was then subjected to flash CC on silica gel, eluted with $CHCl_3$–MeOH (9 : 1) to give three main alkaloid-containing fractions. F-1 (500 mg) contained **5** and **6**. F-2 (420 mg) contained compounds **1** and **2**, while **3** and **4** were contained in F-3 (120 mg).

3.3. Purification of compounds **1**–**6**

F-1 was subjected to MPLC (LiChroprep Si 60), eluted with EtOAC–MeOH (8 : 2, 1 ml/min) and fractions of 2 ml were collected. Fractions 21–27 contained **5** (30 mg), while fractions 29–33 contained **6** (20 mg). F-2 (500 mg) was subjected to chromatography on MPLC (LiChroprep Si 60), eluted with $CHCl_3$–MeOH (8 : 2, 1 ml/min); fractions of 2 ml were collected. Fractions 66–73 were combined, evaporated and recrystallized from MeOH to afford **2** (17 mg). Fractions 90–93 (70 mg) contained an impure **1**, which was further purified on MPLC (LiChroprep Si 60) using $CHCl_3$–MeOH (85 : 15, 1 ml/min), furnishing 40 mg of the new alkaloid **1**. F-3 (120 mg) was chromatographed on MPLC (LiChroprep Si 60), eluted with $CHCl_3$–MeOH (85 : 15, 1 ml/min); fractions of 2 ml were collected. Fractions 40–44 yielded upon crystallization 7 mg of **3**, while **4** (4 mg) was obtained from the fractions 85–110 upon crystallization.

3.4. Identification of pancritamine **(1)**

Obtained as colorless oil; $C_{21}H_{29}NO_5$; $[\alpha]_D^{22} = -62.9°$ (MeOH, c 0.7); EIMS, m/z (Rel. int. %) 375 $[M]^+$ (77), 374 $[M - H]^+$ (100), 360 (7), 331 (3), 288 (9), 272 (27), 244 (15), 228 (20), 214 (26), 202 (20), 187 (9), 174 (8), 159 (6), 128 (6), 115 (11), 77 (10), 45 (77), 43 (76), HREIMS: calculated for $C_{21}H_{29}NO_5$: 375.4638, found 375.2045; CD: $\Delta\varepsilon_{234} - 2.292$, $\Delta\varepsilon_{280} - 0.655$ (MeOH; c 4.554×10^{-4}); for ^1H NMR: see Table 1; for ^{13}C NMR: see Table 2.

3.5. Identification of acetyllycoramine **(2)**

Obtained as colorless needles from MeOH; $C_{19}H_{25}NO_4$; M.p. 93-5°; $[\alpha]_D^{22} = -87°$ (MeOH, c 0.1); EIMS, m/z (Rel. int. %) 331 $[M]^+$ (44), 330 $[M - H]^+$ (100), 288 (14), 274 (17), 270 (25), 232 (12), 174 (19); for ^1H NMR: see Table 1; for ^{13}C NMR: see Table 2.

3.6. Identification of (–)-N-demethyllycoramine **(3)**

Obtained as colorless prisms from MeOH; $C_{16}H_{21}NO_3$; M.p. 124-5°; $[\alpha]_D^{22} = -37.6°$ (MeOH, c 0.09); EIMS, m/z (Rel. int. %) 275 $[M]^+$ (100), 274 $[M - H]^+$ (90), 228 (10), 188 (20), 175 (9), 159 (10), 128 (5), 77 (6); for ^1H NMR: see Table 1; for ^{13}C NMR: see Table 2.

3.7. Identification of crinine **(4)**

Obtained as colorless needles from acetone; $C_{16}H_{17}NO_3$; M.p. 209-11°; $[\alpha]_D^{22} = -10.5°$ (MeOH, c 0.1); IR, EIMS and ^1H NMR data were in agreement with literature data [6].

3.8. Identification of thymine **(5)**

Amorphous powder; $C_5H_6N_2O_2$; CIMS (iso-butane) m/z (Rel. int. %) 127 $[M + H]^+$ (13), 113 $[M - CH_3 + H]^+$ (100); ^1H NMR (400 MHz, DMSO-d_6) δ 10.83 and 10.85 (each 1 H, brs, disappear with D_2O, 2 × NH), 7.19 (1 H, q, $J_{6,Me} = 1.3$ Hz, H_6), 1.72 (3 H, d, $J_{Me,6} = 1.3$ Hz, Me_5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.8 (s, C_5), 151.4 (s, C_2), 137.6 (s, C_4), 107.6 (d, C_6), 11.7 (q, Me_5).

3.9. Identification of uracil **(6)**

Amorphous powder; $C_4H_4N_2O_2$; CIMS (iso-butane) m/z (Rel. int. %) 225 $[2M + H]^+$ (25), 113 $[M + H]^+$ (100); ^1H NMR (400 MHz, DMSO-d_6) δ 10.91 (2 H, brs, disappear with D_2O, 2 × NH), 7.34 (1 H, d, $J_{5,6} = 6.6$ Hz, H_6), 5.42 (1 H, d, $J_{6,5} = 6.6$ Hz, H_5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2 (s, C_4), 151.4 (s, C_2), 142.0 (d, C_6), 100.1 (d, C_5).

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