# Pyrrolizidine Alkaloids from *Onosma leptantha*

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Three new pyrrolizidine alkaloids, leptanthine (**2**), its corresponding *N*-oxide **1**, and *N*-oxide of 3′-*O*-acetylechihumiline (**3**), were isolated from the aerial parts of *Onosma leptantha*, together with two known alkaloids of the same type, echihumiline (**5**) and echihumiline *N*-oxide (**4**). Their structures and configurations were determined by chemical and spectroscopic methods, especially 1D and 2D NMR analyses, including long-range $^{1}H,^{15}N$ correlations at natural abundance.

**Introduction.** – The family Boraginaceae is known for its high content of hepatotoxic pyrrolizidine alkaloids. *Onosma leptantha* HELDR., a perennial herb with lignified base and cushion-like growth habit, belongs to this family and can be found in southern Greece [1]. Until now, ten species of *Onosma* are reported to be endemic in Greece, and this study is the first phytochemical investigation of such a species. Investigation of the MeOH extract of the aerial parts of *Onosma leptantha* has led to the isolation and structural elucidation of the novel pyrrolizidine alkaloids **1**–**3**. Besides these new compounds, the pyrrolizidine alkaloids **4** and **5** [2] are reported for the first time from these species.

**Results and Discussion.** – The crude MeOH extract of the aerial parts of *O. leptantha* was suspended in 2.5% aqueous HCl solution and extracted with $Et_2O$. The aqueous layer was basified with 25% aqueous $NH_3$ solution and extracted with $CH_2Cl_2$. The remaining aqueous phase contained the *N*-oxides as well as any polar free bases. The organic layer was subjected to extraction with 2.5% aqueous HCl solution, which, after basification, was extracted again with $CH_2Cl_2$. The remaining organic phase contained the less-polar free bases. The two alkaloid-containing phases were subjected to various types of liquid chromatography, and compounds **1**–**5** (*Fig.*), the structures of which were elucidated by IR, mass, and NMR spectrometry, were finally obtained.

Compound **1**, which was obtained as a solid, gives a deep-purple spot on TLC after spraying with sulfuric acid/vanillin reagent. Its molecular formula $C_{15}H_{25}NO_7$ was determined by HR-FAB-MS. The IR spectrum exhibits absorptions at 3200–2800 (OH), 1740 (C=O), and 1630 $cm^{-1}$ (C=C), as well as at 1246, 1125, and 1014 $cm^{-1}$ (C–O).

The structure of **1** was established by $^{1}H$- and $^{13}C$-NMR (*Tables 1* and *2*), COSY, HMQC, $^{1}H,^{13}C$-HMBC, and $^{1}H,^{15}N$-HMBC data as 2,3-dihydroxy-2-(1-hydroxyethyl)-3-methylbutanoic acid (2,3,5,7a-tetrahydro-1-hydroxy-4-oxido-1*H*-pyrrolizin-7-yl)methyl ester, for which we propose the name leptanthine *N*-oxide. Leptanthine *N*-oxide (**1**) has not been previously described in the literature. The $^{13}C$-NMR spectrum of **1** (*Table 2*) displays 15 C-signals, which were assigned by HMQC and DEPT-135° experiments to the resonances

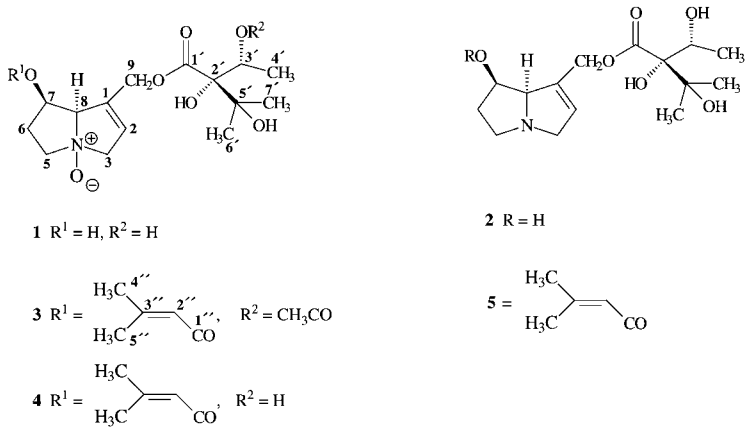


Fig. 1. *Structure of alkaloids* **1**–**5**. Arbitrary numbering[1]).

Table 1. *$^1$H-NMR* ($CD_3OD$, 400 MHz) *Data of Alkaloids* **1**–**3**[1]). $\delta$ in ppm, $J$ in Hz.

| | **1** | **2** | **3** | | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|
| H–C(2) | 6.05 (*d*, *J* = 1.5) | 5.90 (br. *s*) | 6.02 (br. *s*) | H–C(3′) | 4.22 (*q*, *J* = 6.0) | 4.20 (*q*, *J* = 6.0) | 5.46 (*q*, *J* = 6.0) |
| $H_a$–C(3) | 4.65 (*d*, *J* = 16.0) | 3.90 (*m*) | 4.37 (br. *d*, *J* = 15.0) | Me(4′) | 1.28 (*d*, *J* = 6.0) | 1.29 (*d*, *J* = 6.0) | 1.34 (*d*, *J* = 6.0) |
| $H_b$–C(3) | 4.85 (*m*) | 4.40 (*d*, *J* = 15.0) | 4.69 (*m*) | Me(6′) | 1.25 (*s*) | 1.28 (*s*) | 1.25 (*s*) |
| | | | | Me(7′) | 1.31 (*s*) | 1.32 (*s*) | 1.35 (*s*) |
| $H_a$–C(5) | | | | MeCO | | | 1.98 (*s*) |
| $H_b$–C(5) | 4.10–4.00 (*m*) | 3.90 (*m*) | 3.90–3.75 (*m*) | H–C(2″) | | | 5.67 (br. *s*) |
| $H_a$–C(6) | 2.23 (*m*) | 2.20 (*m*) | 2.20 (*m*) | Me(4″) | | | 1.92 (br. *s*) |
| $H_b$–C(6) | 2.63 (*m*) | | 2.78 (*m*) | Me(5″) | | | 2.18 (br. *s*) |
| H–C(7) | 4.83 (*m*) | 4.65 (*m*) | 5.76 (*m*) | | | | |
| H–C(8) | 5.20 (*m*) | 4.95 (*m*) | 4.83 (*m*) | | | | |
| $H_a$–C(9) | 4.92 (br. *d*, *J* = 14.0) | 4.90 (*m*) | 4.72 (*m*) | | | | |
| $H_b$–C(9) | 4.98 (br. *d*, *J* = 14.0) | | | | | | |

of four quaternary, four CH, four $CH_2$, and three Me C-atoms. The chemical shifts of the ring C-atoms are in close agreement with the values reported for other monoesters of retronecine *N*-oxide alkaloids [3], the values for C(6) at $\delta$ 36.2 and C(7) at $\delta$ 71.2 being the most typical[1]) (retronecine = (1*R*,7a*R*)-2,3,5,7a-tetrahydro-1-hydroxy-1*H*-pyrrolizine-7-methanol). Furthermore, C(3) ($\delta$ 78.0), C(5) ($\delta$ 69.7), and C(8) ($\delta$ 96.8) are distinctively deshielded, due to the O-atom at N(4). The signals at $\delta$ 124.0 and 133.7 indicate the presence of a C=C bond located between C(1) and C(2). The values of the ring protons were assigned by combination of data from $^{13}$C, $^1$H, and HMQC spectra and are similar to those of acyclic diesters of retronecine *N*-oxide alkaloids [4], except for H–C(7), which is less deshielded due to the absence of an ester moiety at OH–C(7) [5]. The $^1$H,$^{15}$N-HMBC plot reveals the correlation between N(4) ($\delta$ 152) and H–C(2) ($\delta$ 6.05), H–C(7) ($\delta$ 4.83), and $H_a$–C(6) ($\delta$ 2.23). The chemical shifts of the echimidinic acid moiety of **1** (*Tables 1* and *2*) were assigned by comparison with the reported data [4][6] (echimidinic acid = 2,3-dihydroxy-2-(1-hydroxyethyl)-3-

[1]) Arbitrary numbering; for systematic names, see *Exper. Part.*

Table 2. *$^{13}$C-NMR* ($CD_3OD$, 50 MHz) *Data of Alkaloids* **1**–**3**[1]). $\delta$ in ppm.

| | **1** | **2** | **3** | | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|
| C(1) | 133.7 (*s*) | 133.7 (*s*) | 133.0 (*s*) | C(4′) | 19.1 (*q*) | 18.0 (*q*) | 16.1 (*q*) |
| C(2) | 124.0 (*d*) | 124.6 (*d*) | 124.9 (*d*) | C(5′) | 75.2 (*s*) | 74.2 (*s*) | 74.0 (*s*) |
| C(3) | 78.0 (*t*) | 61.7 (*t*) | 79.4 (*t*) | C(6′) | 27.0 (*q*) | 26.0 (*q*) | 27.3 (*q*) |
| C(5) | 69.7 (*t*) | 54.9 (*t*) | 70.5 (*t*) | C(7′) | 26.3 (*q*) | 25.4 (*q*) | 26.4 (*q*) |
| C(6) | 36.2 (*t*) | 36.3 (*t*) | 33.9 (*t*) | Me | | | 21.6 (*q*) |
| C(7) | 71.2 (*d*) | 70.0 (*d*) | 73.3 (*d*) | C=O | | | 172.0 (*s*) |
| C(8) | 96.8 (*d*) | 79.9 (*d*) | 95.6 (*d*) | C(1″) | | | 166.8 (*s*) |
| C(9) | 62.5 (*t*) | 61.6 (*t*) | 63.0 (*t*) | C(2″) | | | 116.2 (*d*) |
| C(1′) | 175.5 (*s*) | 174.8 (*s*) | 174.7 (*s*) | C(3″) | | | 161.9 (*s*) |
| C(2′) | 86.1 (*s*) | 85.1 (*s*) | 85.6 (*s*) | C(4″) | | | 28.0 (*q*) |
| C(3′) | 71.2 (*d*) | 70.2 (*d*) | 74.8 (*d*) | C(5″) | | | 21.0 (*q*) |

methylbutanoic acid). The echimidinic acid moiety is esterified at OH–C(9), as suggested by the HMBC spectrum showing a signal which corresponds to a $^3J$ coupling of H–C(9) ($\delta$ 4.92 and 4.98) with C(1′) ($\delta$ 175.5)[1]).

Compound **2** is a light brown oil exhibiting a deep-purple spot on TLC after spraying with sulfuric acid/vanillin reagent. Its molecular formula $C_{15}H_{25}NO_6$ was determined by HR-FAB-MS. The IR spectrum shows absorptions at 3200–2800 (OH), 1734 (C=O), and 1638 $cm^{-1}$ (C=C), as well as at 1259, 1117, and 1016 $cm^{-1}$ (C–O). Compound **2** was identified by $^1$H- and $^{13}$C-NMR (*Tables 1* and *2*), COSY, HMQC, $^1$H,$^{13}$C-HMBC, and $^1$H,$^{15}$N-HMBC data as 2,3-dihydroxy-2-(1-hydroxyethyl)-3-methylbutanoic acid (2,3,5,7a-tetrahydro-1-hydroxy-1*H*-pyrrolizin-7-yl)methyl ester and the name leptanthine, in accordance with that of its *N*-oxide **1**, is proposed. Leptanthine (**2**) has not been previously described in the literature.

The $^{13}$C-NMR spectrum of **2** (*Table 2*) is almost identical to that of **1**, except for the signals of C(3) ($\delta$ 61.7), C(5) ($\delta$ 54.9), and C(8) ($\delta$ 79.9), which are directly attached to N(4) and, thus, appear at higher field compared to the corresponding signals of the *N*-oxide **1**, whereas the other ring C-atoms rest intact. The same trend can be noted for the ring H-atoms, although not so clearly. The absence of the *N*-oxide moiety is also confirmed by the $^1$H,$^{15}$N-HMBC spectrum, which reveals the correlation of N(4) at $\delta$ 71.1, a resonance value that is characteristic of a tertiary amine [7][8], and H–C(2) at $\delta$ 5.90. Therefore, the $^1$H,$^{15}$N-HMBC correlation at natural abundance can be used as a rapid diagnostic method for the distinction of pyrrolizidine alkaloids or the corresponding *N*-oxides.

Compound **3** is an off-white oil that gives a deep-purple spot on TLC after spraying with sulfuric acid/vanillin reagent. The HR-FAB-MS indicates the molecular formula $C_{22}H_{33}NO_9$, and the IR spectrum exhibits absorptions at 3200–2800 (OH), 1728 (C=O), and 1649 $cm^{-1}$ (C=C), as well as at 1243, 1133, and 1020 $cm^{-1}$ (C–O). By considering $^1$H- and $^{13}$C-NMR (*Tables 1* and *2*), COSY, HMQC and $^1$H,$^{13}$C-HMBC data, **3** was identified as 2-[1-(acetyloxy)ethyl]-2,3-dihydroxy-3-methylbutanoic acid (2,3,5,7a-tetrahydro-1-[(3-methyl-1-oxobut-2-enyl)oxy]-4-oxido-1*H*-pyrrolizin-7-yl)-methyl ester or 3′-*O*-acetylechihumiline *N*-oxide, a compound that has not been previously described in the literature.

The $^{13}$C-NMR spectrum of **3** (*Table 2*) displays 22 C-signals, which were assigned by HMQC and DEPT-135° experiments to the resonances of seven quaternary, five CH, four $CH_2$ and six Me C-atoms. The chemical

shifts of the ring protons as well as those of the ring C-atoms are in close agreement with the values reported for other acyclic diesters of retronecine *N*-oxides [4]. The $^1$H-NMR (*Table 1*) shows the presence of a senecioic ester moiety (senecioic acid = 3-methylbut-2-enoic acid), more specifically, an olefinic proton at $\delta$ 5.67, which has a $^4J$ coupling with Me(4″) ($\delta$ 1.92) and Me(5″) ($\delta$ 2.18) in the COSY plot [8][1]). The senecioate moiety is also confirmed by the $^{13}$C-NMR signals at $\delta$ 116.2 and 161.9 for the olefinic C(2″) and C(3″), and at $\delta$ 28.0 and 21.0 for C(4″) and C(5″), respectively [9] (*Table 2*). The chemical shifts of the echimidinic acid moiety (*Tables 1* and *2*) were assigned as above by comparison with reported data [2], except for a downfield shift of H–C(3′) to $\delta$ 5.46, in comparison to alkaloid **2**. That, along with the appearance of a *s* at $\delta$ 1.98 in the $^1$H-NMR spectrum, suggests the presence of an acetyloxy group at C(3′). The $^{13}$C-NMR spectrum confirms the presence of the acetyl group, since extra signals appear at $\delta$ 172 (C=O) and 21.6 (*Me*C=O [10]), whereas its location is confirmed by the HMBC spectrum that shows a signal corresponding to the $^3J$ coupling of H–C(3′) with Me*C*=O.

The structures of the known compounds echihumiline *N*-oxide (**4**) and echihumiline (**5**) have been established by *El-Shazly et al.* [4].

## Experimental Part

*General*. Column chromatography (CC): silica gel *60*, 70–230 mesh (*Merck*). Vacuum liquid chromatography (VLC): silica gel *60 H* (*Merck*). Flash chromatography (FC): silica gel *60*, 230–400 mesh (*Merck*). Reversed-phase medium-pressure liquid chromatography (MPLC): *RP-18* silica gel *60*, 230–400 mesh (*Merck*). TLC: precoated silica gel $F_{254}$ plates; detection at 254 and 365 nm and by the sulfuric acid/vanillin and *Draggendorff* reagents. Reversed-phase TLC: precoated *RP-18* silica gel $F_{254S}$ plates; detection at 254 and 365 nm and by the sulfuric acid/vanillin reagent. Optical rotations: *Perkin-Elmer 341* polarimeter. IR Spectra: *Perkin-Elmer Paragon-500* spectrophotometer; $\tilde{\nu}$ in cm$^{-1}$. $^1$H-NMR, COSY, NOESY, $^1$H,$^{13}$C HMBC, HMQC, and $^1$H,$^{15}$N HMBC [11]: *Bruker DRX-400* spectrometer; at 400 MHz. $^{13}$C-NMR, DEPT: *Bruker AC-200* spectrometer; at 50 MHz. HR-FAB-MS: *AEI MS-902* spectrometer.

*Plant Material*. Aerial parts of *Onosma leptantha* were collected from the mountain Taygetos (Greece) in July 1999 at an altitude of 1800 m, and identified by Dr. *T. Constantinidis*, Lecturer at the Department of Botany of the Agricultural University of Athens.

*Extraction and Isolation*. Plant material (854 g) was shade-dried and pulverized to a fine powder, which was exhaustively extracted with MeOH by cold maceration. The extract was evaporated, and the residue (44.8 g) was subjected to the usual procedure of successive extractions for the isolation of alkaloids [12][13] yielding an aq. and an org. phase. The aq. phase residue was submitted to VLC ($CH_2Cl_2$/MeOH gradients), to give ten fractions, four of which were subjected to reversed-phase MPLC ($H_2O$/MeOH gradients) or CC ($CH_2Cl_2$/MeOH gradients) yielding alkaloids **1**–**4**. Alkaloid **5** was isolated from the org. layer by means of FC ($CH_2Cl_2$/MeOH gradients).

*Leptanthine* N-*Oxide* (=*2,3-Dihydroxy-2-(1-hydroxyethyl)-3-methylbutanoic Acid (2,3,5,7a-Tetrahydro-1-hydroxy-4-oxido-1*H*-pyrrolizin-7-yl)methyl Ester*; **1**): Amorphous powder (30 mg). $[\alpha]_{25} = +16.5$ ($c = 0.20$, MeOH). IR (MeOH soln., $CaF_2$ crystal): 3200–2800, 1740, 1630, 1246, 1125, 1014. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-FAB-MS: 332.1641 ($C_{15}H_{26}NO^+$; calc. 332.1641).

*Leptanthine* (=*2,3-Dihydroxy-2-(1-hydroxyethyl)-3-methylbutanoic Acid (2,3,5,7a-Tetrahydro-1-hydroxy-1*H*-pyrrolizin-7-yl)methyl Ester*; **2**): Light brown oil (26 mg). $[\alpha]_{25} = -2.0$ ($c = 0.20$, MeOH). IR (MeOH soln., $CaF_2$ crystal): 3200–2800, 1734, 1638, 1259, 1117, 1016. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-FAB-MS: 316.1681 ($C_{15}H_{26}NO_6^+$; calc. 316.1681).

*3′*-O-*Acetylechihumiline* N-*Oxide* (=*2-[1-(Acetyloxy)ethyl]-2,3-dihydroxy-3-methylbutanoic Acid (2,3,5,7a-Tetrahydro-1-[(3-methyl-1-oxobut-2-enyl)oxy]-4-oxido-1*H*-pyrrolizin-7-yl)methyl Ester*; **3**): Off-white oil (18 mg). $[\alpha]_{25} = +35.0$ ($c = 0.20$, MeOH). IR (MeOH soln., $CaF_2$ crystal): 3200–2800, 1728, 1649, 1243, 1133, 1020. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. HR-FAB-MS: 456.2155 ($C_{22}H_{34}NO_9^+$; calc. 456.2155).

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