Turk J Chem
26 (2002) , 195 – 199.

Pyrrolizidine Alkaloids from *Symphytum sylvaticum* Boiss. subsp. *sepulcrale*. (Boiss. & Bal.) Greuter & Burdet var. *sepulcrale* and *Symphytum aintabicum* Hub. - Mor. & Wickens

Semra KURUCU, Murat KARTAL
Department of Pharmacognosy, Faculty of Pharmacy,
University of Ankara, 06100 Ankara-TURKEY
Muhammad Iqbal CHOUDARY
H.E.J. Research Institute of Chemistry,
University of Karachi, 75270 Karachi-PAKISTAN
Gülaçtı TOPCU
Department of Analytical Chemistry, Faculty of Pharmacy,
İstanbul University, TK-34452 İstanbul-TURKEY

Received 16.03.2001

Pyrrolizidine alkaloid (Echimidine-N-oxide) was isolated from *Symphytum sylvaticum* Boiss. subsp. *sepulcrale* (Boiss. & Bal.) Greuter & Burdet var. *sepulcrale* and pyrrolizidine alkaloid (Echimidine) was isolated from *Symphytum aintabicum* Hub. - Mor. & Wickens. The structures of the isolated compounds were elucidated based on IR, EIMS, ^{1}H, and ^{13}C NMR analysis and also on 2D NMR (COSY, HMBC, HMQC) experiments.

Key Words: *Symphytum sylvaticum, Symphytum aintabicum*, Pyrrolizidine alkaloids.

Introduction

There are 17 *Symphytum* species (*Boraginaceae*) growing in Turkey and 8 of these are endemic[1−3]. *Symphytum officinale* L (Comfrey) is applied topically in the treatment of inflammatory disorders, especially in Europe. It is considered useful in several skin complications such as chronic wounds, burns, sores, eczema and leg ulcers [4,5]. There are reports of hepatotoxicity attributed to pyrrolizidine alkaloids present in comfrey preparations[6]. They represent a serious health risk not only to livestock and other animals, but sometimes also to human populations which may be exposed to them either through contamination of foodstuff or in herbal teas or medicines[7]. In this report, we describe the separation and structural elucidation of two alkaloids from two endemic *Symphytum* species growing in Turkey. This is the first phytocemical investigation carried out on pyrrolizidine alkaloids of these two endemic *Symphytum* species.

Experimental

Materials and Methods

Symphytum sylvaticum Boiss. subsp. *sepulcrale* (Boiss. & Bal.) Greuter & Burdet var. *sepulcrale* were collected from Rize-Ikizdere, 1200-1400 m, during the flowering stage in July 1993. The plant specimen has been deposited at the herbarium of the Faculty of Pharmacy, Ankara University, Ankara-TURKEY (AEF 17908).

Symphytum aintabicum Huber-Morath & Wickens were collected from Gaziantep-Acaroba village, during the flowering stage, in June 1993 (AEF 17910).

Extraction and Isolation

Four and a half kilograms of dried-powdered root of *Symphytum sylvaticum* subsp. *sepulcrale* var. *sepulcrale* was macerated with ethanol at room temperature by occasional stirring. The mixture was filtered and evaporated in vacuo to a gummy residue. The extraction procedure was repeated for 12 days in the same manner and combined extracts were evaporated under vacuum at 50°C. The pH of the residue (333.61 g) was adjusted to 3 with 2 N HCl and partitioned with Et_2O. In order to reduce pyrrolizidine alkaloids, zinc dust was added to the aqueous acid layer, which was then stirred for 24 h and filtered. The alkaloids were extracted with chloroform from the basified aqueous solution (pH=9) using ammonia. Evaporation of the chloroform yielded a crude red-brown oil (9.39 g).

The same extraction procedure was used for 1 kg of dried-powdered whole plant of *Symphytum aintabicum*. The alkaloid fraction after evaporation of chloroform was obtained from *Symphytum aintabicum* (4.50 g).

The alkaloids were separated using column chromatography over silica gel (Merck-9385). First elution was started with chloroform and then polarity was increased with methanol. One hundred millilitre fractions were collected. The fractions were combined according to TLC results. The alkaloid fractions 95-105 from the roots of *Symphytum sylvaticum* subsp. *sepulcrale* var. *sepulcrale* yielded **1**, and 170-187 from *Symphytum aintabicum* yielded **2**. Further purification of the alkaloid mixture was achieved by preparative TLC on 0.5 mm silica gel plates (Merck-7748). The alkaloids were obtained using $CHCl_3/CH_3OH/CH_3COCH_3$, 10:7.5:2.5 for **1** (R_f 0.25), and $CHCl_3/CH_3OH/CH_3COCH_3$ (20:5:3) for **2** (R_f 0.35) as eluent and detected by Dragendorff reagent.

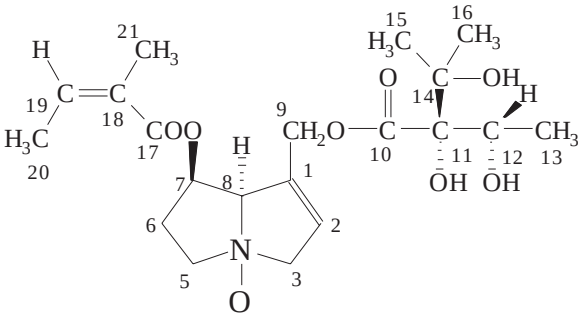
Results and Discussion

Since authentic specimens of pyrrolizidine alkaloids were unavailable and many configurational isomers are possible for each basic skeleton, the alkaloids were characterized by IR, ^{1}H NMR, ^{13}C NMR spectroscopy (Table) and EIMS spectrometry. Complete attribution was performed on the basis of 2D NMR (COSY, HMBC, HMQC) experiments. The structures of **1** and **2** (Figure) were identified by comparison of their spectral data with those reported in literature[8−19].

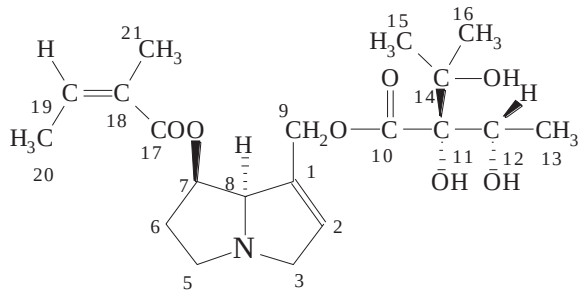
Table ^{13}C NMR assignments of compounds **1-2**[a]

Carbon	1	2
1	132.22	133.04
2	122.95	127.78
3	77.58	62.14
5	69.10	53.65
6	32.69	34.32
7	72.27	73.24
8	93.47	75.68
9	60.53	61.65
10	174.45	174.26
11	84.92	83.39
12	70.05	69.67
13	18.62	18.43
14	73.24	73.49
15	26.38	26.07
16	24.82	24.81
17	165.80	166.62
18	126.53	127.15
19	140.83	139.70
20	15.86	15.76
21	20.28	20.44

[a]Taken in $CDCl_3$



Echimidine-N-oxide (1)



Echimidine (2)

Figure Structures of isolated compounds.

The ^{1}H NMR spectrum of **1** showed the characteristic signals of a echimidinic acid ester at δ 4.17 (1H, q, J=6.4 Hz, H-12), δ 1.26 (1H, d, J=6.4 Hz, H-13), δ 1.29 (3H, s, H-15) and δ 1.20 (3H, s, H-16)[8,9,10,16,18]. This was proven by the ^{13}C NMR shift values of C-14 (δ 73.24)[11,16,17,18,19]. An olefinic proton at δ 6.15 (1H, dq, J=1.5/7.2 Hz, H-19), coupling with H-20 at δ 1.96 (3H, dq, J=1.5/7.2 Hz) and H-21 at δ 1.80 (3H, quintet, J=1.5 Hz), indicated an angelic acid ester moiety. The angelic acid ester moiety was also confirmed in the ^{13}C NMR spectrum[16−19] by the signal at 140.83 which belongs to the olefinic C-19.The mass spectrum of **1** was also identical to that of N-oxides of Echimidine[12,16,18]. In the region of δ 2.6-4.6 the of ^{1}H NMR spectra, there is a significant difference between the N-oxides of the pyrrolizidine alkaloids and the corresponding free bases[13,14]. Characteristics patterns in the deshielding of hydrogens in the retronecine moiety were recognizable in all N-oxides. The chemical shifts of the necine base hydrogens of the retronecine moiety are important parameters for the analysis of related compounds[15]. Spectral data for the necic base (retronecine) in **1** differing from **2** could be explained by its N-oxide.

Echimidine-N-Oxide (7-*O*-Angelyl-9-*O*-echimidinyl retronecine-N-oxide) (**1**), white amorphous compound (250 mg). IR (KBr) $\gamma_{max.}$ 3200-3400 cm^{-1}(OH), 2800-2950 cm^{-1} (-CH), 1700-1730 cm^{-1}(C=O), 1640 cm^{-1}(C=C), 1340-1440 cm^{-1}(-CH$_3$), 1220-1240 cm^{-1}(C-O). EIMS: m/z (%)= 413 [M]$^+$(1.44), 295.2 (3.56), 220 (52.67), 117 (76.47), 100 (67.38), 55.2 (100). ^{1}H NMR (CDCl$_3$, 400 MHZ): δ= 5.92 (1H, s, H-2), 4.62 (1H, d, J=16.2 Hz, H-3a), 4.50 (1H, d, J=16.2 Hz, H-3b), 3.97 (1H, ddd, J=1.6/6.5/11.4 Hz, H-5a), 3.68 (1H, ddd, J=5.6/11.6/12 Hz, H-5b), 2.85 (1H, m, H-6a), 2.22 (1H, dddd, J=1.6/5.6/13.2, H-6b), 5.79 (1H, ddd, J=1.6/5.5/7.5 Hz, H-7), 5.30 (1H, d, J=5.5 Hz, H-8), 4.83 (1H, d, J=13.7 Hz, H-9a), 4.69 (1H, d, J=13.7 Hz, H-9b), 4.17 (1H, q, J=6.4 Hz, H-12), 1.26 (1H, d, J=6.4 Hz, H-13), 1.29 (3H, s, H-15), 1.20 (3H, s, H-16), 6.15 (1H, dq, J=1.5/7.2 Hz, H-19), 1.96 (3H, dq, J=1.5/7.2 Hz, H-20), 1.80 (3H, quintet, J=1.5 Hz, H-21).

Echimidine (7-*O*-Angelyl-9-*O*-echimidinyl retronecine) **(2)** was a light brown gummy compound (35 mg). IR (KBr) $\gamma_{max.}$ 3290-3440 cm^{-1}(OH), 2850-2940 cm^{-1}(-CH), 1680-1720 cm^{-1}(C=O), 1560 cm^{-1}(C=C), 1360-1460 cm^{-1}(-CH3), 1250-1280 cm^{-1}(C-O). EIMS: m/z (%)= 398.2 [M+1]$^+$(100), 369.3 (15.1), 220.1 (10), 135 (18.5), 111 (33.5), 109 (47.5). ^{1}H NMR (CDCl$_3$, 200 MHZ): δ= 5.82 (1H, s, H-2), 4.03 (1H, d, J=16 Hz, H-3a), 3.41 (1H, d, J=16 Hz, H-3b), 3.45 (1H, m, H-5a), 2.70 (1H, dd, J=1.5/16 Hz, H-5b), 2.12 (1H, m, H-6a), 2.12 (1H, m, H-6b), 5.46 (1H, ddd, J=1.5/5.0 Hz, H-7), 4.75 (1H, br s, H-8), 4.88 (1H, d, J=13.5 Hz, H-9a), 4.61 (1H, d, J=13.5 Hz, H-9b), 4.14 (1H, q, J=6.5 Hz, H-12), 1.24 (1H, d, J=7 Hz, H-13), 1.21 (3H, s, H-15), 1.18 (3H, s, H-16), 6.07 (1H, dq, J=1.5/7.2 Hz, H-19), 1.90 (3H, dq, J=1.5/7.0 Hz, H-20), 1.78 (3H, quintet, J=1.5 Hz, H-21).

Information in detail on the work-up procedure and copies of the original spectra are obtainable from the correspondence author.

References

1. P.H. Davis, "**Flora of Turkey and The East Aegean Islands"**, Vol. 6, pp. 378-386, Edinburgh University Press, Edinburgh, 1978.
2. P.H. Davis, R.R. Mill, K. Tan, "**Flora of Turkey and The East Aegean Islands (Supplement)"**, Vol.10, pp. 186-189, Edinburgh University Press, Edinburgh,1988.

3. M. Kartal, **"Research on the active constituents of *Symphytum sylvaticum* Boiss. subsp. *sepulcrale* (Boiss. & Bal.) Greuter & Burdet var. *sepulcrale"*** Ph.D. Thesis in Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey, 1997.
4. R.F. Weiss, "**Herbal Medicine**", pp. 334-335, Beaconsfield Publishers Ltd., Beaconsfield, England, 1991.
5. D.D. Buchman, "**Herbal Medicine-The Natural Way to Get Well and Stay Well"**, pp. 3-8, 128, 158, Gramerey Publishing Co., New York, 1980.
6. J.E.F. Reynolds, " **Martindale - The Extra Pharmacopoeia"**, 30th Edition, pp. 1358, Pharmaceutical Press, London, 1993.
7. A.R. Mattocks, "**Chemistry and Toxicology of Pyrrolizidine Alkaloids"**, Academic Press, London, 1986.
8. T. Furuya, M. Hikichi, **Phytochemistry**, 10, 2217-2220, 1971.
9. E. Roeder, B. Rengel, **Phytochemistry**, 29(2), 690-693, 1990.
10. A. Ulubelen, F. Öcal, **Phytochemistry**, 16, 499-500, 1977.
11. E. Roeder, **Phytochemistry**, 29(1), 11-29, 1990.
12. E. Pedersen, E. Larsen, **Organic Mass Spectrometry**, 4, 249-256, 1970.
13. C.F. Asibal, J.A. Glinski, L.T. Gelbaum, L.H. Zalkow, **J. of Natural Products,** 52(1), 109-118, 1989.
14. C.C.J. Culvenor, J.A. Edgar, J.L. Frahn, L.W. Smith, A. Ulubelen, S. Doğanca, **Australian of Chemistry,** 28, 173-178, 1975.
15. H.J. Segall, J.L. Dallas, **Phytochemistry**, 22(5), 1271-1273, 1983.
16. T. Sarg, S. El-Dahmy, E. Abdel Aziz, A. Abdel Ghani, **Fitoterapia**, LXIII (5), 466-468, 1992.
17. E. Roeder, T. Bourauel, V. Neuberger, **Phytochemistry**, 31(11), 4041-4042, 1992.
18. A. El-Shazly, M. Abdel-All, A. Tei, M. Wink, **Z. Naturforsch.**, 54c, 295-300, 1999.
19. E. Roeder, H. Wiedenfeld, P. Stengl, **Arch. Pharm.**, 315, 87-89, 1982.