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# BETAINES AND FREE PROLINE WITHIN THE *ACHILLEA MILLEFOLIUM* GROUP

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**Key Word Index**—*Achillea millefolium* group; Asteraceae; Compositae; L-proline; stachydrine; betonicine; betaine; choline

**Abstract**—From above ground parts of *Achillea collina* L., proline, stachydrine, betonicine, betaine and choline were isolated as the major nitrogen containing compounds. The TLC screening of 11 different species belonging to *A. millefolium* group showed qualitatively identical betaine patterns but quantitative differences were observed. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

As known from folk medicine various species of the *Achillea millefolium* group are widely used as a herbal bile remedy [1]. About 37 years ago, Pailer and Kump isolated quaternary amino acid derivatives from a sample not specified with respect to a certain taxon within the *A. millefolium* group [2, 3]. According to their bipolar structure and therefore similarity to the human biliary salts those compounds may contribute to the cholagogic effects. Recently, remarkable differences in the essential oil and sesquiterpenoid pattern of several taxa of the *A. millefolium* group have been found [4–7]. Therefore, this investigation was performed to obtain more information about the variation of betaines within the *A. millefolium* aggregate.

## RESULTS AND DISCUSSION

From air-dried above ground parts of *A. collina*, five compounds (**1**–**5**) were isolated, all of them giving the typical Dragendorff reaction. Compounds **2**–**4**, pre-identified by TLC, corresponded to stachydrine (**2**), betonicine (**3**) and betaine (**4**), already isolated from *Achillea* by Pailer and Kump [2, 3]. The spectral data for **1** and **2** were nearly identical. The lack of the signals from the *N*-methyl-groups, as well as the other further signals similar to those of **2**, indicated that **1** was the amino acid proline. Compound **5** showed all the characteristics of choline. Comparison of the NMR data and optical rotation of all of the isolated compounds showed good agreement with literature data.

In a TLC screening, 46 individuals of 11 different *Achillea* species were examined with regard to their betaine pattern, eight species belonging to the *A. millefolium* group (*A. roseo-alba*, *A. asplenifolia*, *A. setacea*, *A. ceretanica*, *A. pratensis*, *A. collina*, *A. millefolium* and *A. pannonica*) and three species which might belong to this group (*A. crithmifolia*, *A. nobilis*, and *A. sibirica*) [8]. Flower heads and leaves were analysed separately. Semi-quantitative evaluation was performed after spraying with Dragendorff reagent. Results are given in Table 1.

All the extracts showed qualitatively identical betaine patterns. Quantitative variations were observed; however, **3** represented the major compound in all the extracts. Within one species the leaves showed amounts of betaines higher than those of flower heads. Within the period of vegetation the concentration of betaines in the flower heads strongly increased; in the leaves the concentration remained constant.

The diploid *A. roseo-alba*, *A. asplenifolia* and *A. setacea* contain **2** in a remarkable amount next to **3**, whereas **1**, **4** and **5** were found in very low concentrations. The tetraploid *A. ceretanica* showed almost equal amounts of **1** and **3**, and smaller amounts of **2**, **4** and **5**. The other tetraploid *Achillea* species, *A. collina* and *A. pratensis*, as well as the octoploid *A. pannonica* showed a pattern similar to the diploid species. Only the hexaploid *A. millefolium* showed **1**, **2**, **4** and

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Table 1. Betaine pattern of 11 different *Achillea* species

| Species | Samples | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| *A. roseo-alba* | 4 | + | ++ | +++ | + | + |
| *A. setacea* | 4 | + | ++ | +++ | + | + |
| *A. asplenifolia* | 2 | + | ++ | +++ | + | + |
| *A. collina* | 4 | + | ++ | +++ | + | + |
| *A. ceretanica* | 4 | ++(+) | + | +++ | + | + |
| *A. pratensis* | 6 | + | ++ | +++ | + | + |
| *A. millefolium* | 4 | + | + | ++(+) | + | + |
| *A. pannonica* | 6 | + | ++ | +++ | + | + |
| *A. crithmifolia* | 4 | + | ++ | +++ | + | + |
| *A. nobilis* | 4 | + | + | +++ | + | + |
| *A. sibirica* | 4 | + | + | ++(+) | + | + |

**5** in equal concentrations, and **3** as the major compound.

The two other *Achillea* species, *A. nobilis* and *A. sibirica*, which generally are not included in the *A. millefolium* group also have a pattern similar to the hexaploid species. *Achillea crithmifolia* shows a pattern equal to that of the diploid species. These results indicate that betaines are ubiquitous within the *A. millefolium* group. The TLC spectra of betaines may serve as an additional tool for differentiation within the *A. millefolium* group.

## EXPERIMENTAL

*Plant material. Achillea* samples were collected in different parts of Austria. Vouchers are deposited in the Herbarium of the Institute of Pharmacognosy, University of Vienna. *Achillea roseo-alba* (Carinthia, 1991), *A. asplenifolia* (Rust, 1991), *A. setacea* (Retz, 1995), *A. collina* (Korneuburg, 1991), *A. ceretanica* (cultivated at the University of Vienna, 1995), *A. pratensis* (Salzburg, 1992), *A. millefolium* (Salzburg, 1995), *A. pannonica* (Oberweiden, 1995), *A. crithmifolia* (cultivated at the University of Vienna, 1994), *A. nobilis* (cultivated at the University of Vienna, 1995) and *A. sibirica* (cultivated at the University of Vienna, 1994).

*Isolation of betaines.* Above ground parts of air dried flowering plants (60 g) of *A. collina* were extracted with 40% MeOH at room temp. After removal of the solvent under red. pres. at 30° a brown, oily residue was obtained. CC over silica gel 60 (Merck) was performed using $CH_2Cl_2$–MeOH (5:3) containing 0.5 parts of $H_2O$ with gradually decreasing pH from 3 to 1.8 (the pH of the $H_2O$ was obtained by addition of TFA). This procedure yielded 5 impure compounds, which were re-chromatographed using the same method. Removal of sugars and other neutral compounds over cation exchange material (Dowex 50WX8) yielded chromatographically pure compounds: 80 mg **1**, 13 mg **2**, 115 mg **3** and 23 mg **4**. Compound **5** was not purified over cation exchange resin, because it was strongly bound to the resin and not eluted with $NH_3$. Due to some difficulties during purification the isolated amounts do not represent the concns in the drug material. With respect to the loss during isolation the total amount of betaines was *ca* 0.4%.

*Thin layer chromatography.* CC was monitored by TLC using silica gel 60 (Merck) plates (10 × 10 cm) and $CH_2Cl_2$–MeOH–concd $NH_3$ (5:4:1) as mobile phase. Betaines were detected as orange (**3**), red (**4**) or purple (**1**, **2**, **5**) spots by spraying with Dragendorff reagent [35 ml $H_2SO_4$, 20 ml HOAc, 1.7 g Bi(III)$NO_3$, 100 ml 40% KI sol, $H_2O$ to 1 l).

*Structural elucidation.* $^1H$, $^{13}C$ and 2D NMR were recorded in $CD_3OD$ on a Varian 600 MHz, with TMS as int. standard.

$^1H$ *NMR data.* Compound **1**: $^1H$ ($\delta$): 3.96 ($\alpha$-H, *dd*, $J_{\alpha\beta'} = 6.3$ Hz, $J_{\alpha\beta} = 8.7$ Hz), ABXY-system ($\nu_A = 3.37$, $\nu_B = 3.22$, $\delta\delta'$-H, $J_{\delta\delta'} = 14.6$ Hz, $J_{\delta\gamma} = 7.0$ Hz, $J_{\delta\gamma'} = 4.5$ Hz, $J_{\delta'\gamma} = 7.3$ Hz, $J_{\delta\gamma'} = 4.3$ Hz), ABXY-system ($\nu_A = 2.28$, $\nu_B = 2.10$, $\beta\beta'$-H, $J_{\beta\beta'} = 13.2$ Hz, $J_{\beta\gamma} = 7.3$ Hz, $J_{\beta\gamma'} = 2.9$ Hz), 1.97 ($\gamma\gamma'$-H, *m*); **2**: $^1H$ ($\delta$): 4.02 ($\alpha$-H, *m*), 3.68, 3.50 ($\delta\delta'$-H, *m*), 3.31 (Me, *s*), 3.14 (Me, *s*), 2.49, 2.32 ($\beta\beta'$-H, *m*), 2.13 ($\gamma\gamma'$-H, *m*); **3**: $^1H$ ($\delta$): 4.59 ($\gamma$-H, *m*), 4.24 ($\alpha$-H, *dd*, $J_{\alpha\beta} = 9.2$ Hz, $J_{\alpha\beta} = 8.6$ Hz), ABX-system ($\nu_A = 4.00$, $\nu_B = 3.41$, $\delta\delta'$-H, $J_{\delta\delta'} = 12.3$ Hz, $J_{\gamma\delta} = 7.4$ Hz), 3.39 (Me, *s*), 3.14 (Me, *s*), ABXY-system ($\nu_A = 2.65$, $\nu_B = 2.33$, $\beta\beta'$-H, $J_{\beta\beta'} = 15.1$ Hz, $J_{\beta\gamma} = 8.3$ Hz); **4**: $^1H$ ($\delta$): 4.00 (2H, *s*), 3.28 (9H, *s*); **5**: $^1H$ ($\delta$): 3.99 (2H, *m*, H-2), 3.49 (2H, *m*, H-1), 3.21 (9H, *s*, H-3).

$^{13}C$ *NMR data* (*ppm*). **1**: 173.9 (C-1), 62.7 (C-2), 30.4 (C-3), 25.1 (C-4), 47.0 (C-5); **2**: 170.7 (C-1), 77.7 (C-2), 26.7 (C-3), 19.8 (C-4), 68.0 (C-5), 52.7 (C-6), 46.6 (C-7); **3**: 172.2 (C-1), 77.9 (C-2), 38.2 (C-3), 67.8 (C-4), 75.4 (C-5), 55.0 (C-6), 49.1 (C-7); **4**: 170.7 (C-1), 68.7 (C-2), 56.5 (C-3); **5**: 57.4 (C-1), 69.4 (C-2), 55.0 (C-3).

*TLC screening of individuals.* Sample: extracts with hot $H_2O$ (*ca* 100 mg/10 ml) were prepd from dried *Achillea* herbs; TLC—see above.

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