Pyrrolizidine Alkaloids from *Lithospermum canescens* Lehm.

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Seven pyrrolizidine alkaloids (PAs) have been isolated from *Lithospermum canescens* and their structures determined by spectroscopical methods. Besides the known lycopsamine, O^7-acetyl-lycopsamine and O^7-acetylintermedine four new PAs were found. Their structures are O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-rinderine = canescine), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(–)-viridifloryl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-echinatine = canescenine) and their O^{13}-acetyl-derivatives (= acetylcanescine; acetylcanescenine).

Key words: Lithospermum canescens, Pyrrolizidine Alkaloids, Canescine and Derivatives

Introduction

Lithospermum canescens Lehm., Boraginaceae (native American name: "hoary puccoon") grows in open prairies in northern USA and southern Canada. Because it contains pigments of the shikonin-type (Wiedenfeld *et al.*, 1998) it is used as a body dye by native people (Densmore, 1928).

As *L. canescens* belongs to the Boraginaceae family the presence of pyrrolizidine alkaloids (PAs) could be expected.

Based on structural aspects (double-bond in position 1,2; esterification at both necic OH-functions) PAs can show toxic effects. Toxicity occurs not only after oral administration but also after percutaneous absorption although to a smaller extent than by ingestion (Brauchli *et al.*, 1982). Thus, according to the German Federal Health Bureau regulations the sale of PA containing products for external use is restricted in Germany to a daily dose of 100 μg and a maximum use of six weeks per year (Bekanntmachung, 1992). Aerial parts of *L. canescens* were therefore investigated. Seven PAs were isolated and their structures determined by GC-mass spectroscopy and homo- as well as heteronuclear 2D-NMR correlated spectroscopy. Four of them have not been described previously. The known alkaloids belong to the retronecine-type and are O^9-(–)-viridifloryl-retronecine (= lycopsamine), its O^7-acetylderivative (= acetyllycopsamine) and O^7-acetyl-O^9-(+)-trachelanthoyl-retronecine (= acetylintermedine), The new PA show the structures of O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-rinderine), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(–)-viridifloryl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-echinatine), O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine, O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(–)-viridifloryl-heliotridine.

Based on structure-toxicity relationships (Wiedenfeld and Roeder, 1984) toxic side effects must be expected for all substances found.

Results and Discussion

Aerial parts of *L. canescens* were extracted as previously described (Roeder and Wiedenfeld, 1977; Wiedenfeld and Roeder, 1979). From the crude alkaloidal extract **1–7** were isolated (Fig. 1).

The GC-MS spectrum of **1** shows the $[M]^+$-peak at 299 indicating the fomula $C_{15}H_{25}NO_5$. Those of **2** and **3** show $[M]^+$-Peaks at 341 corresponding to the formulas $C_{17}H_{27}NO_6$. The further MS fragmentation and also the NMR data of **1–3** are as described earlier (Wiedenfeld and Roeder, 1991; Roeder *et al.*, 1982; Kelley and Seiber, 1992; Roitman, 1983).

The $[M]^+$-Peak in the GC-MS spectrum of **4** and **6** occurs at 399 indicating the molecular formulas

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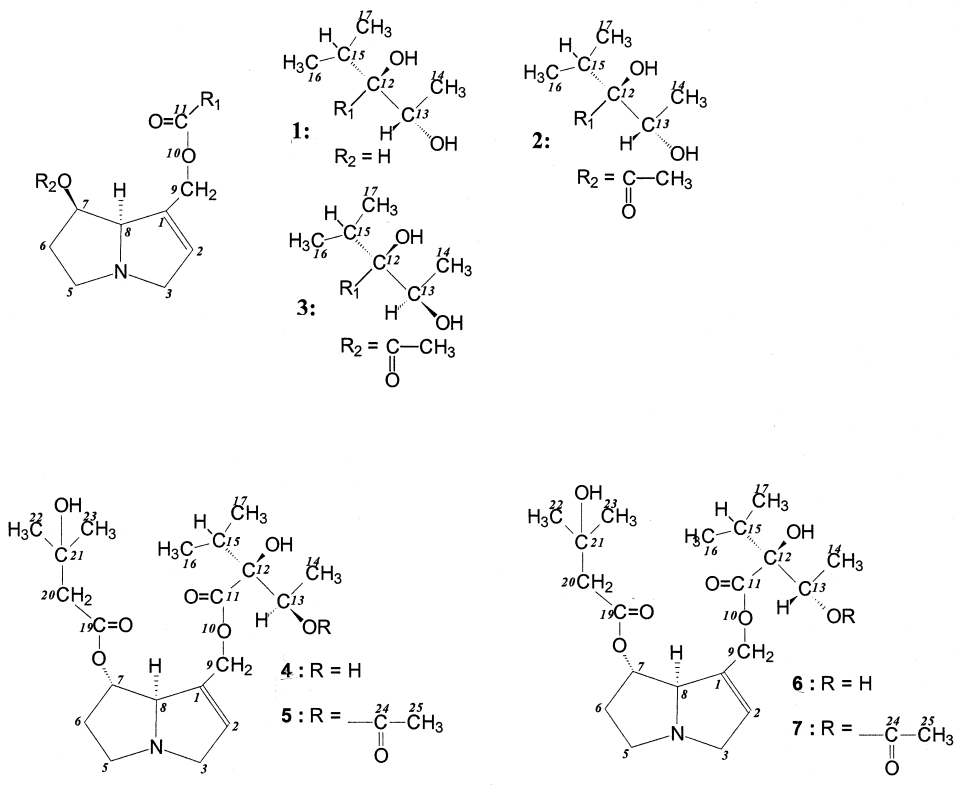

Fig. 1: Structures of the isolated Pas: Lycopsamine (**1**), O^7-acetyl-lycopsamine (**2**), O^7-acetylintermedine (**3**), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine = canescine (**4**), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(–)-viridifloryl-heliotridine = canes-cenine (**6**), O^{13}-acetylcanescine (**5**), O^{13}-acetylcanescenine (**7**).

$C_{20}H_{33}NO_7$. Loss of CH_3 leads to *m/z* 384. The ions *m/z* 355 and 338 result from $[M]^+$-C_2H_4O and further loss of OH. The cleavage of the ester function at O-9 leads to *m/z* 256 and *m/z* 238. The decay of the O^7-acid is demonstrared by an ion at *m/z* 220 (loss of OH at C-21), *m/z* 180 (loss of C_3H_7O) and cleavage of the ester function to *m/z* 136. The fragments *m/z* 136, 120, 93 and 80 are typical for retronecine or its isomer heliotridine. The ms spectra of **5** and **7** show the $[M]^+$-Peaks at 441 corresponding to the formulas $C_{22}H_{35}NO_8$. After loss of an acetyl function (indicated by *m/z* 441–426–398) the further fragmentations are similar to those of **4** and **6**; they differ only in intensities.

The ^{1}H- and ^{13}C-NMR-data of **4–7** are summarized in the Experimental part. The assignment was performed by interpretation of H,H- and C,H-correlated spectra. Important structural information is provided by the ^{13}C chemical shifts of C-6 (~ 34 ppm), C-7 (~ 74 ppm) and C-8 (~ 75 ppm) (Jones *et al.*, 1982; Mohanraj and Herz, 1982; Wiedenfeld and Roeder, 1991). These signals establish **4–7** as diesters of heliotridine. This is further confirmed by the ^{1}H and ^{13}C shift for C-9: ~ 4,7/62 ppm as well as by the ^{1}H data for C-7: ~ 5.4 ppm. The esterifying acid at O-7 is identical for all 4 PA and is characterised by the values for the methylene group C-20 (2.5 and 47 ppm), C-21 (69 ppm) and the methyl groups 22/23 (1.3 and ~ 29 ppm). These data proof the structure of a 3-hydroxy-3-methyl-butanoic ester. The NMR data for the O-9 acid in **6** are the same as in **1** leading to the structure of a (–)-viridiflorylester. **4** shows differences in the data for H-13 (4.07 instead of 3.89 ppm) and for C-16 and C-17 (17.8 and 14.2 instead of 17.2 and 17.1 ppm). The stereochemistry at C-12 can be deduced by interpretation of the shift difference of the C-9H_2 AB-system (Mohanraj and Herz, 1982; Wiedenfeld and Roeder, 1991). For this aspect values from 0–0.2 ppm indicate an

S-configuration while higher values indicate *R*-configuration. The configuration at C-13 is shown by the H-13 and C-13 data as well as by the shift differences of the C-NMR data for the methyl groups C-16/C-17 (Wiedenfeld and Roeder, 1991). Thus, the values for C-13 (4.07 and 69.5 ppm instead of 3.89 and 71.1 ppm) and C-16/C-17 (ΔHz = 3.6 instead of 0.1 ppm) give evidence for a C-12*S* and a 13*R* configuration (= (+)-trachelanthic acid). The data for **5** and **7** differ from those in **4** and **6** only in a downfield shifting of H-13 and C-13 (~ 5.2 and ~ 74 ppm) and the additional data for an acetyl group (~ 2.0 and ~ 21 ppm for CH_3 and 170 ppm for C = O) which confirm the structures as O-13-acetyl-(+)-trachelanthic and a O-13-acetyl-(−)-vridifloric acid, repectively. These data proof the structures of O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-rinderine) (**4**), O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(−)-viridifloryl-heliotridine (= O^7-(3-hydroxy-3-methyl-butanoyl)-echinatine) (**6**), O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(+)-trachelanthoyl-heliotridine (**5**), O^{13}-acetyl-O^7-(3-hydroxy-3-methyl-butanoyl)-O^9-(−)-viridifloryl-heliotridine (**7**).

For the new PAs we propose the names canescine (**4**), canescenine (**6**), acetylcanescine (**5**) and acetylcanescenine (**7**).

All isolated PAs are expected to produce toxic side effects. The PA content (GC) was about 0.02% (dry weight). Therefore, based on the German regulations for external use of preparations from PAs containing plants, application of more than 0.5 g dried plant material per day may expose the user to a health risk.

Experimental

General

NMR-spectra (Bruker AC 400) were measured in $CDCl_3$/D_6-DMSO at 400 and 100 MHz, respectively. GC-MS: GC: 150° (5 min.) – 250 °C, 10°/min; HP-1, 25 m × 0.32 mm; Inj.: 250 °C, det.: 280 °C; R_t: **1**: 12.67 min, **2**: 13.49 min, **3**: 13.88 min, **4**: 16.79 min, **5**: 17.51 min, **6**: 16.92 min, **7**: 18.14 min; MS: 220 °C; interface: 250 °C; 2000 emV.

Plant material

Plants were collected in July 2000 at Parkland Bot, Togo, Saskatchewan, Canada. A voucher specimen is deposited at the Department of Biology and Pharmaceutical Botany, Medical University of Warsaw, Poland.

Extraction and isolation

Extn. of plant material (aerial parts; 500 g) was carried out as described earlier (Roeder and Wiedenfeld, 1977; Wiedenfeld and Roeder, 1979). Prep. TLC [silica gel F_{254}, CH_2Cl_2-MeOH-NH_4OH (25%), 75:24:1 v/v/v] yielded the alkaloids as oils (6 mg **1**, 5 mg of **2** and **3**, 8 mg **5** and **7**, 10 mg **4**, 1 mg **6**).

Canescine (**4**)

GC-MS *m/z* (%): $[M]^+$ $C_{20}H_{33}NO_7$ 399 (0.41); $C_{19}H_{30}NO_7$ 384 (4.10); $C_{18}H_{28}NO_6$ 355 (0.82); $C_{18}H_{27}NO_5$ 338 (0.25); $C_{13}H_{20}NO_4$ 256 (9.84); $C_{13}H_{20}NO_3$ 238 (66.0); $C_{13}H_{18}NO_2$ 220 (21.4); $C_{10}H_{14}NO_2$ 180 (11.6); $C_8H_{10}NO$ 136 (41.6); $C_8H_{10}N$ 120 (100); C_6H_7N 93 (64.7); C_5H_6N 80 (20.1). ^{1}H NMR: δ (ppm): 5.83 (d, $J_{2,3a}$ = 1.6 Hz, 1H, H-2), 5.38 (ddd, $J_{7,8}$ = 1.9 Hz, $J_{7,6}$ = 1.8; 1.6 Hz, 1H, H-7), 4.79 (dd, $J_{9a,9b}$ = 13.9 Hz, $J_{9a,8}$ = 9.1 Hz, 1H, H-9A), 4.67 (dd, $J_{9b,9a}$ = 13.9 Hz, $J_{9,8}$ = 6.2 Hz, 1H, H-9B), 4.35 (m, 1H, H-8), 4.07 (q, $J_{13,14}$ = 6.4 Hz, 1H, H-13), 3.95 (ddd, $J_{3a,3b}$ = 11.4 Hz, $J_{3a,8}$ = 3.2 Hz, $J_{3a,2}$ = 1.6 Hz, 1H, H-3A), 3.38 (dddd, $J_{3b,3a}$ = 11.4, $J_{3b,8}$ = 3.2, $J_{3b,5a}$ = 2.0 Hz, $J_{3b,2}$ = 1.6 Hz, 1H, H-3B), 3.33 (dm, $J_{5a,5b}$ = 10.7, 1H, H-5A), 2.98 (3OH), 2.65 (ddd, $J_{5b,5a}$ = 10.7, $J_{5b,6}$ = 7.8, $J_{5b,7}$ = 1.8 Hz, 1H, H-5B), 2.45 (s, 2H, H_2-20), 2.09 (dm, $J_{6,5b}$ = 10.7, 2H, H_2-6), 2.00 (qq, $J_{15,16/17}$ = 6.8 Hz, 1H, H-15), 1.26 (s, 6H, H_3-22/23), 1.19 (d, $J_{14,13}$ = 6.4 Hz, 3H, H_3-14), 0.94 (d, $J_{16,15}$ = 6.8 Hz, 3H, H_3-16), 0.91 (d, $J_{17,15}$ = 6.8 Hz, 3H, H_3-17), ^{13}C NMR: δ (ppm): 175.1 (C-11), 171.7 (C-19), 133.0 (C-1), 127.5 (C-2), 83.4 (C-12), 75.4 (C-8), 74.4 (C-7), 69.5 (C-13), 69.1 (C-21), 62.6 (C-3), 62.3 (C-9), 53.6 (C-5), 46.9 (C-20), 34.4 (C-6), 33.1 (C-15), 29.4 (C-22), 29.2 (C-23), 17.2 (C-14), 17.2 (C-16), 17.1 (C-17).

Canescenine (**6**)

GC-MS *m/z* (%): $[M]^+$ $C_{20}H_{33}NO_7$ 399 (0.10); $C_{19}H_{30}NO_7$ 384 (1.56); $C_{18}H_{28}NO_6$ 355 (0.41);

$C_{18}H_{27}NO_5$ 338 (0.26); $C_{13}H_{20}NO_4$ 256 (9.59); $C_{13}H_{20}NO_3$ 238 (67.5); $C_{13}H_{18}NO_2$ 220 (20.6); $C_{10}H_{14}NO_2$ 180 (14.7); $C_8H_{10}NO$ 136 (45.1); $C_8H_{10}N$ 120 (100); C_6H_7N 93 (61.6); C_5H_6N 80 (20.2). ^{1}H NMR: δ (ppm): 4.74 (dd, $J_{9a,9b}$ = 12.9 Hz, $J_{9a,8}$ = 6.2 Hz, 1H, H-9A), 4.73 (dd, $J_{9b,9a}$ = 12.9 Hz, $J_{9,8}$ = 6.4 Hz, 1H, H-9B), 3.89 (q, $J_{13,14}$ = 6.6 Hz, 1H, H-13), ^{13}C NMR: δ (ppm): 71,1 (C-13), 16.0 (C-14), 17.8 (C-16), 14.2 (C-17). Further data are similar to **4**.

Acetylcanescine (**5**)

GC-MS *m/z* (%): $[M]^+$ $C_{22}H_{35}NO_8$ 441 (0.73); $C_{21}H_{32}NO_8$ 426 (2.60); $C_{18}H_{28}NO_6$ 355 (2.36); $C_{13}H_{19}NO_4$ 255 (6.26); $C_{13}H_{20}NO_3$ 238 (62.4); $C_{13}H_{18}NO_2$ 220 (18.3); $C_{10}H_{14}NO_2$ 180 (38.9); $C_8H_{10}NO$ 136 (47.2); $C_8H_{10}N$ 120 (100); C_6H_7N 93 (73.9); C_5H_6N 80 (19.9). ^{1}H NMR: δ (ppm): 5.21 (q, $J_{13,14}$= 6.4 Hz, 1H, H-13), 2.00 (s, 3H, H_3-25), ^{13}C NMR: δ (ppm): 170.4 (C-24), 72.4 (C-13), 21.2 (C-25). Further data are similar to **4**.

Acetylcanescenine (**7**)

GC-MS *m/z* (%): $[M]^+$ $C_{22}H_{35}NO_8$ 441 (1.23); $C_{21}H_{32}NO_8$ 426 (2.38); $C_{20}H_{32}NO_7$ 398 (1.56); $C_{18}H_{28}NO_6$ 355 (1.39); $C_{13}H_{19}NO_4$ 255 (5.66); $C_{13}H_{20}NO_3$ 238 (58.2); $C_{13}H_{18}NO_2$ 220 (20.3); $C_{10}H_{14}NO_2$ 180 (44.3); $C_8H_{10}NO$ 136 (43.4); $C_8H_{10}N$ 120 (100); C_6H_7N 93 (80.3); C_5H_6N 80 (22.1). ^{1}H NMR: δ (ppm): 5.17 (q, $J_{13,14}$= 6.6 Hz, 1H, H-13), 2.03 (s, 3H, H_3-25), ^{13}C NMR: δ (ppm): 170.4 (C-24), 73.7 (C-13), 21.3 (C-25). Further data are similar to **6**.

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