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, O7-acetyl-lycopsamine and O7-acetylintermedine four new PAs were found. Their structures are O7-(3-hydroxy-3-methyl-butanoyl)-O9-(+)-trachelanthoyl-heliotridine (= O7-(3-hydroxy-3-methyl-butanoyl)-rinderine = canescine), O7-(3-hydroxy-3-methyl-butanoyl)-O9(−)-viridifloryl-heliotridine (= O7-(3-hydroxy-3-methyl-butanoyl)-echinatine = canescenine) and their O13-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 PAs belong to the retronecine-type and are O9(−)-viridifloryl-retronecine (= lycopsamine), its O7-acetyl derivative (= acetylycopsamine) and O7-acetyl-O9(+) -trachelanthoyl-retronecine (= acetylintermedine). The new PA show the structures of O7-(3-hydroxy-3-methyl-butanoyl)-O9(−) -trachelanthoyl-heliotridine (= O7-(3-hydroxy-3-methyl-butanoyl)-rinderine), O7-(3-hydroxy-3-methyl-butanoyl)-O9(−) -viridifloryl-heliotridine (= O7-(3-hydroxy-3-methyl-butanoyl)-echinatine), O13-acetyl-O7-(3-hydroxy-3-methyl-butanoyl)-O9(−) -trachelanthoyl-heliotridine, O13-acetyl-O7-(3-hydroxy-3-methyl-butanoyl)-O9(−) -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 formula C₁₅H₂₅NO₅. Those of 2 and 3 show [M]⁺-Peaks at 341 corresponding to the formulas C₁₇H₂₇NO₆. 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
C_{20}H_{33}NO_{7}. Loss of CH\textsubscript{3} leads to m/z 384. The ions m/z 355 and 338 result from [M]+-C\textsubscript{3}H\textsubscript{5}O 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\textsuperscript{7}-acid is demonstrated by an ion at m/z 220 (loss of OH at C-21), m/z 180 (loss of C\textsubscript{3}H\textsubscript{7}O) 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\textsubscript{22}H\textsubscript{35}NO\textsubscript{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 \textsuperscript{1}H- and \textsuperscript{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 \textsuperscript{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 \textsuperscript{1}H and \textsuperscript{13}C shift for C-9: ~ 4.7/62 ppm as well as by the \textsuperscript{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\textsubscript{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-12S and a 13R 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 CH3 and 170 ppm for C = O) which confirm the structures as O-13-acetyl- (+)-trachelanthic and a O-13-acetyl- (−)-viridifloric acid, respectively. These data prove the structures of O7(−)- butanoyl)-O13(−)- viridifloryl-heliotridine (= O7(−)-3-hydroxy-3-methyl-butanoyl)-rinderine (4), O7(−)-3-hydroxy-3-methyl-butanoyl)-O13(−)- viridifloryl-heliotridine (5), O13(−)-acetyl-O7(−)3-hydroxy-3-methyl-butanoyl)-O13(−)- viridifloryl-heliotridine (7).

For the new PAs we propose the names canescine (4), canescenine (6), acetylcanescine (5) and acetylanesceenine (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 CDC13/D2O-DMSO at 400 and 100 MHz, respectively. GC-MS: GC: 150-player (5 min.) – 250 °C, 10°/min; HP-1, 25 m × 0.32 mm; Inj.: 250 °C, det.: 280 °C; Rf: 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 eV.

**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 F254, CH2Cl2-MeOH-NH4OH (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]+ C20H33NO7 399 (0.41); C19H32NO7 384 (4.10); C18H32NO6 355 (0.82); C18H32NO5 338 (0.25); C17H32NO4 256 (9.84); C13H28NO3 238 (66.0); C12H18NO2 220 (21.4); C10H14NO2 180 (11.6); C9H10NO 136 (41.6); C8H10N 120 (100); C6H7N 93 (64.7); C5H6N 80 (20.1). 1H NMR: δ (ppm): 5.83 (d, J2,3a = 1.6 Hz, 1H, H-2), 5.38 (dd, J7,9a = 1.9 Hz, J7,6 = 1.8 Hz, 1H, H-7), 4.79 (dd, J9a,9b = 13.9 Hz, J9a,8 = 9.1 Hz, 1H, H-9A), 4.67 (dd, J9b,9a = 13.9 Hz, J9b,8 = 6.2 Hz, 1H, H-9B), 4.35 (m, 1H, H-8), 4.07 (q, J3,3a = 6.4 Hz, 1H, H-3), 3.95 (ddd, J3a,3b = 6.4 Hz, 1H, 3a,8 = 3.2 Hz, J3a,2 = 1.6 Hz, 1H, H-3A), 3.38 (ddd, J3b,3a = 11.4 Hz, J3b,8 = 3.2, J3b,5a = 2.0 Hz, J3b,2 = 1.6 Hz, 1H, H-3B), 3.33 (dm, J5a,5b = 10.7, 1H, H-5A), 2.98 (3OH), 2.65 (ddd, J5b,5a = 10.7, J5b,6 = 7.8, J5b,7 = 1.8 Hz, 1H, H-5B), 2.45 (s, 2H, H2-20), 2.09 (dm, J6,5b = 10.7, 2H, H2-20), 2.00 (qq, J15,16/17 = 6.8 Hz, 1H, H-15), 1.26 (s, 6H, H3-22/23), 1.19 (d, J14,13 = 6.4 Hz, 3H, H3-14), 0.94 (d, J16,15 = 6.8 Hz, 3H, H3-16), 0.91 (d, J17,15 = 6.8 Hz, 3H, H3-17), 13C 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]+ C20H33NO7 399 (0.10); C19H32NO7 384 (1.56); C18H32NO6 355 (0.41);
C_{18}H_{27}NO_{5} 338 (0.26); C_{13}H_{20}NO_{4} 256 (9.59); C_{13}H_{19}NO_{3} 238 (20.6); C_{13}H_{18}NO_{2} 220 (20.2); C_{10}H_{14}NO_{2} 180 (14.7); C_{8}H_{10}NO 136 (45.1); C_{9}H_{10}N 120 (100); C_{6}H_{7}N 93 (61.6); C_{5}H_{6}N 80 (20.2). 1H 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). 13C 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.

**Acetylcanscine (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_{10}H_{14}NO_{2} 180 (39.9); C_{13}H_{18}NO_{2} 160 (47.2); C_{9}H_{10}N 120 (100); C_{6}H_{7}N 93 (73.9); C_{5}H_{6}N 80 (19.9). 1H NMR: δ (ppm): 5.21 (q, J_{13,14} = 6.6 Hz, 1H, H-13), 2.00 (s, 3H, H_{3}-25), 13C NMR: δ (ppm): 170.4 (C-24), 72.4 (C-13), 21.2 (C-25). Further data are similar to 4.

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