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Lycopsamine and cumambrin B from *Eupatorium maculatum*

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The pyrrolizidine alkaloid (PA) lycopsamine and the guaianolide cumambrin B were isolated from *Eupatorium maculatum* L. Their structures were elucidated by spectroscopical methods.

1. Introduction

Eupatorium maculatum L. (Asteraceae) (common name: Spotted Joe-Pye weed) is a plant widespread in North America and Canada. This purple flowering plant is used by indigenous Indians as a herbal medicine for treating diuretical problems and is said to have an influence on chronical renal and cystic troubles (Densmore 1974). On account of its belonging to the Eupatorieae tribe *E. maculatum* should contain pyrrolizidine alkaloids (PA) and sesquiterpene lactones. This is of importance because those PA showing a double-bond in position 1–2 of their five-membered ring system (necine) and occuring as esters (open-chain or macrocycle) show a toxic potential whereas sesquiterpene lactones can cause allergies.

From upper-earth plant material collected in Canada the PA lycopsamine and the sesquiterpene lactone of guaianolide-type cumambrin B were isolated and their structures elucidated by means of LC-MS and NMR. On account of the low PA content (less than 0.06%) and the fact that lycopsamine shows the structure of a retronecine-O^9-(−)-viridiflorine ester the plant should show a very low toxicity (Wiedenfeld et al. 2008). The guaianolide cumambrin B can lead to contact dermatitis as shown in an epicutane test model (Hansen and Schulz 1976).

2. Investigations, results and discussion

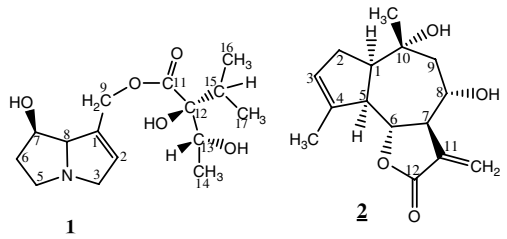
In 1963 Tsuda and Marion reported the occurrence of echinatine and trachelanthamidine in *E. maculatum* (Tsuda and Marion 1963). As PA with heliotridine as the basic moiety have not been mentioned to be contained in *Eupatorium* species to date, this plant seemed worth being reinvestigated.

From an alcoholic extract of leaf material we succeeded in isolating lycopsamine and cumambrin B by CC-flash-chromatography followed by purification of the resulting fractions by normal pressure CC.

The structures were elucidated by spectroscopical methods (LC-MS/NMR) and were established as the retronecine-O^9-(−)-viridifloryl ester (= lycopsamine) (**1**) and as (3a*R*,4*S*,6*R*,6a*R*,9a*R*,9b*R*)-4,6-dihydroxy-6,9-dimethyl-3-methylene-3a,4,5,6,6a,7,9a,9b-octahydroazuleno [4,5-*b*]furan-2(3*H*)-one (= cumambrin B) (**2**). The LC-MS (thermospray) of (**1**) showed a protonated molecular ion at m/z 300.21 which leads to the molecular mass of 299.21 and the formula of $C_{15}H_{25}NO_5$. Further structural informations were received by NMR. In this way the retronecine ester is proven by a ^{13}C shift for C-6 (about 35–36 ppm; the C-7 *S*-configurated heliotridine shows values at about 33 ppm). The structure of the ester part is elucidated by interpretation of H,H- and C,H-correlated spectra. Important structural information is provided by the ^{13}C chemical shift of C-6 (≈ 36 ppm), C-7 (≈ 71 ppm) and C-8 (≈ 78 ppm). These signals determine a retronecine-*O*-9 ester (Jones et al. 1982; Mohanraj and Herz 1982; Wiedenfeld and Roeder 1991). The stereochemistry at C-12 can be deduced by interpretation of the shift-difference of the C9–H_2 AB-system (Mohanraj and Herz 1982; Wiedenfeld and Roeder 1991). For this aspect values from 0–0.2 ppm indicate *S*-configuration, higher values the opposite one ($\Delta Hz = 0.1$ ppm in **1**). The configuration at C-13 is shown by the H-13 and C-13 data as well as by the shift differences of the ^{13}C-NMR data for the methyl groups C-16/C-17 (Wiedenfeld and Roeder 1991). Thus, the values for C-12 of 3.9 and 71 ppm as well as the ΔHz C-16/C-17 values (1.8 ppm) indicate a 12*S* 13*S* configuration (= (−)-viridifloric acid). These data agree with those already described for lycopsamine (Culvenor et al. 1980; Roeder et al. 1982; Badzies-Crombach 1989).

The EIMS for **2** showed a M^+-Peak at 264.25 leading to the molecular formula of $C_{15}H_{20}O_4$. Loss of 2 molecules H_2O led to m/z 246 and m/z 228, respectively. The NMR data show an exocyclic methylene function (AB-system 6.13 and 65.55 and 121 ppm) and a further double bond C3–C4 (C-3: 5.48 and 126 ppm; C-4: 136 ppm). The lactone group is characterized by the C-12 at 170 ppm and the downfield-shift of C-6 to 80 ppm on account of the neighboring oxygene of the lactone. The absolute configuration was deduced by the interpretation of NOE-difference measurement. Especially NOEs between C1–H and C5–H, C5–H and C7–H, C8–H and C9–H_α proved the 1*R*,5*R*,6*R*,7*R*,8*S*,10*R* configuration. These data are in accordance with those reported for cumambrin B by Romo et al. (1968) and Vajs et al. (2000).

The amounts of the contained compounds in dried and pulverized plant material were calculated to 0.06% (lycopsamine) and 0.07% (cumambrin B). On account of the



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low concentrations of the isolated compounds and due to the fact that the structural aspects for lycopsamine indicate minor toxic side-effects, a possible toxic or allergic risk should be negligible when using *E. maculatum* medicinically.

3. Experimental

3.1. General procedure

GC-MS: Fisons GC 8000 series; Trio 1000; MassLab Rel 1.27 data system; fused Silica cap. column, Permabond SE-54 CB, 50 m × 0.32 mm, 0.25 µ (Macherey & Nagel). Interface: 280 °C, source 220 °C, repeller 1.5 V, 70 eV.

LC-MS: Gynkotek Delivery System, 480; Trio 1000, quadrupole to m/z 1000; thermospray; MassLab Rel 1.27 data system; LiChrospher 60, RP-Select B 5 µm, 25 × 4 mm; mobile phase: A: acetonitril (25%) with 0.1 mol NH_4OOCCH_3; B: acetonitril (50%) with 0.1 mol NH_4OOCCH_3; ionization: TSP^+; Source 230 °C; nozzle 230 °C; plasm 20 V; repeller 163 °C; detector 450 V; full-scan.

NMR spectra (Bruker AC-400) were measured in DMSO-D_6. Chemical shifts (δ = ppm) were referenced to DMSO (2.50 and 39.43 ppm, respectively). Coupling constants in Hz.

Flash liquid chromatography (FLC): 150 × 3 cm column, packed with silicagel 60, 0.04–0.063 mm (Merck, Darmstadt, Germany). TLC: Silicagel glass plates 20 × 20 cm, 0.25 mm (Merck, Darmstadt, Germany). CC: 30 × 1 cm column silicagel 60, 0.063–0.200 mm (Merck, Darmstadt, Germany).

3.2. Plant material

The plant was collected during the summer season of 1999 in the area of Georgian Bay, Huron Lake, Canada. It was identified by Dr. Heinz Heltmann, Institute of Pharmaceutical Biology, University of Bonn; a voucher specimen is deposited in the Pharmaceutical Institute, University of Bonn.

3.3. Extraction and isolation

The dried and pulverized plant material was extracted with MeOH followed by a liquid-liquid purification with tetrachlorocarben 5, methanol 4, water 1. After reduction by Zn/HCl the resulting extract was applied on a flash-column and eluted with CH_2Cl_2–MeOH mixtures (each 500 ml from 80 : 20 to 50 : 50) and monitored by TLC CH_2Cl_2 : MeOH : NH_4OH (25%) 85 : 14 : 1; detection: Dann-Mattocks (Dann, 1960; Mattocks, 1967) as well as by GC (Roeder et al. 1990). R_f: 0.64, green-blue = **2**, R_f: 0.10, blue-violet = **1**. Final separation and purification was done using CC eluting with a CH_2Cl_2-mixture 70 : 30 yielding the compounds.

3.4. Characterization of the compounds

3.4.1. Lycopsamine (**1**)

NMR: C-1: 132.68; C-2: 129.35; 5.90 (1 H, sext, J = 1.7); C-3: 62.60; 4.02 (1 H, dm, J = 15.6, H3α)/3.46 (1 H, dm, J = 15.6, 3 Hβ); C-5: 53.91; 3.40 (1 H, m, H5α)/2.82 (1 H, ddd, J = 10.8, 8.1, 6.5, H5β); C-6: 36.21; 2.07 (1 H, m, H6α)/1.99 (1H, m, H6β); C-7: 71.25; 4.35 (1 H, m); C-8: 78.46; 4.30 (1 H, m); C-9: 62.45; 4.84 (1 H, dd, J = 13.5, 1.6, H9α)/4.74 (1 H, dd, J = 13.5, 1.6, H9β; C-11: 174.55; C-12: 83.77; C-13: 70.91; 3.98 (1 H, q, J = 6.4); C-14: 17.21; 1.27 (3 H, d, J = 6.4); C-15: 32.40; 2.14 (1 H, sept, J = 7.0); C-16: 15.98; 0.92 (3H; d, J = 7); C-17: 17.80; 0.86 (3 H, d, J = 7.0).

3.4.2. Cumambrin B (**2**)

NMR: C-1: 54.27; 2.42 (1 H, dd, J = 9.2, 8.4); C-2: 33.45; 2.15 (2 H, m, J = 9.2, 1.5); C-3: 125.68; 5.48 (1 H, sbr); C-4: 136.05; C-5: 54.49; 2.58 (1 H, dd, J = 10.9, 8.4); C-6: 79.62; 4.34 (1 H, dd, J = 10.9, 8.8); C-7: 48.11; 3.56 (1 H, dt, J = 8.8, 3.8); C-8: 63.60; 4.18 (1 H, ddd, J = 7.5, 7.2, 6.2); C-9: 42.81; 1.96 (1 H, dd, J = 14.8, 7.2, H9α)/1.76 (1 H, dd, J = 14.8, 7.5, H9β); C-10: 51.57; C-11: 142.48; C-12: 170.04; C-13: 121.28; 6.13 (1 H, d, J = 3.8, H13α)/5.55 (1 H, d, J = 3.8, H13β); C-14: 31.90; 1.11 (3 H, s): C-15: 17.47; 1.81 (d, J = 1.5); C-8 OH: 4.57 (1 H, d, J = 6.2); OH10: 4.62 (1 H, s).

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