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Pyrrolizidine alkaloids from *Ageratum houstonianum* Mill

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Dedicated with great compliments to Professor Dr. Dr. h. c. mult. H. Oelschläger on the occasion of his 80th birthday

Abstract

Four pyrrolizidine alkaloids (PA) were isolated from *Ageratum houstonianum* and their structures elucidated by spectroscopical methods. Besides the already known lycopsamine three new PA were found. Their structures are the 2*S*-2-hydroxy-2,3-dimethyl-butanoyl-O^9 as well as the O^7 esters of retronecine and the O^9 derivative of heliotridine. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Ageratum houstonianum* (Mill); Asteraceae; Eupatorieae; Pyrrolizidine alkaloids; Lycopsamine; O^9-(2*S*-2-Hydroxy-2,3-dimethyl-butanoyl)-retronecine; O^9-(2*S*-2-Hydroxy-2,3-dimethyl-butanoyl)-heliotridine; O^7-(2*S*-2-Hydroxy-2,3-dimethyl-butanoyl)-retronecine

1. Introduction

Ageratum houstonianum (Mill) (Asteraceae, tribe Eupatorieae) is a plant widespread in Mexico and southern USA. It is reported that the leaves of this plant show antifungal properties (Pandey et al., 1984). The essential oil contents chromenes and shows antimicrobiotical activities (Menut et al., 1993). Further investigations show the occurence of benzofurans (Anthonsen and Chantarasakul, 1970; Bowers, 1977; Breuer et al., 1987; Siebertz et al., 1988) as well as of flavonoids and methoxyflavones (Minonskowski and Gill, 1973, 1975; Quijano et al., 1982, 1985, 1987). In the Indian medicine of Mexico it is used traditionally. Our own ethnopharmacological studies in the zone of Tlanchinol, Hidalgo, showed that the natives use water decotions of the plant against pain and infections, especially for cleaning external wounds. There the plant is called with its indian (nahuatl) name "Micashihuil". As *A. houstonianum* belongs to the asteraceae family and the tribe eupatorieae the presence of pyrrolizidine alkaloids (PA) could be presumed.

To estimate a possible toxic effect the knowledge of the particular structures of the PA contained is necessary. Therefore aerial parts of the plant *A. houstonianum* were investigated. The plant material was collected in the above mentioned region.

Four PA were isolated and their structures determined by GC–MS and homo- as well as hetero-nuclear 2D NMR correlated spectroscopy. Three of them have not been described previously. The known alkaloid belongs to the retronecine-type and is O^9-(−)-viridifloryl-retronecine (=lycopsamine). The new PA show the structures of the retronecine-O^9- as well as retronecine-O^7-2*S*-2-hydroxy-2,3-dimethyl-butanoic ester and the heliotridine-O^9-2*S*-2-hydroxy-2,3-dimethyl-butanoic ester.

Based on the structure–toxicity relationship (Wiedenfeld and Röder, 1984) for all substances low toxic side effects must be expected.

2. Results and discussion

In our investigation, aerial parts of *A. houstonianum* were extracted as already described (Röder and Wiedenfeld, 1977; Wiedenfeld and Röder, 1979). From the crude alkaloidal extract **1**, **2**, **3** and **4** were isolated (Fig. 1).

The GC–MS spectrum of **1** shows the $[M]^+$ peak at 299 corresponding to the formula $C_{15}H_{25}NO_5$. The further MS fragmentation and also the NMR data are corresponding to those describes earlier (Wiedenfeld and Röder, 1991).

The $[M]^+$ peak in the GC–MS spectrum of **2** occurs at 269 indicating the molecular formula $C_{14}H_{23}NO_4$.

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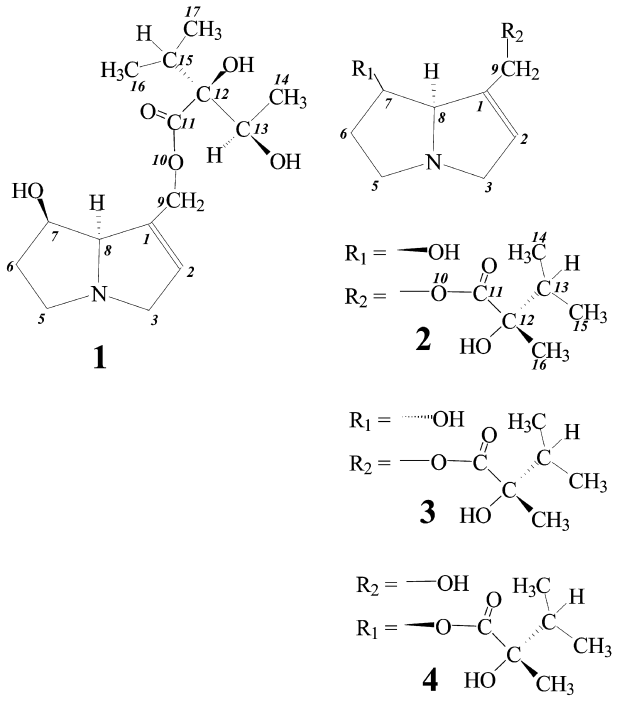

Fig. 1. Structures of the isolated compounds **1**–**4**.

Loss of OH leads to *m*/*z* 252. The ions *m*/*z* 225 and 208 result from $[M]^+ - C_3H_8$ and further loss of OH. The cleavage of the ester function leads to *m*/*z* 138. The fragments *m*/*z* 138, 120, 93 and 80 are typical for retronecine or its isomer heliotridine and are identical with those in **1**. The MS spectra of **3** and **4** are similar to that of **2**; they only differ in the intensities of their fragments.

The ^1H and ^{13}C NMR data of **2**, **3** and **4** 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 shift of C-6 (**2**: 35.7; **3**: 33.7; **4**: 34.2 ppm), C-7 (**2**: 70.1; **3**: 73.7; **4**: 74 ppm) and C-8 (**2**: 77.6; **3**: 79.5; **4**: 74.8 ppm) (Jones et al., 1982; Mohanraj and Herz, 1982; Wiedenfeld and Röder, 1991). These signals determine a retronecine-*O*-9 ester **2**, a heliotridine-*O*-9 ester **3** and a retronecine-*O*-7 ester **4**. This is further proofed by the ^1H and ^{13}C shift for C-9: 4.70/61.8 (**2**), 4.67/60.7 (**3**) and 3.97/58.2 (**4**) as well as by the ^1H data for C-7: 4.20 (**2**), 4.16 (**3**) and 5.12 (**4**). The esterifying acid is characterized by the values for C-12 (76 ppm, S), C-13 (1.80 and 35 ppm) and the methyl groups 14/15 (~0.8 and ~17 ppm) as well as for the methyl group 16 (~1.2 and ~23 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 Röder, 1991). For this aspect a value near 0 is found indicating the *S*-configuration. These data leads to the structure of a 2*S*-2-hydroxy-2,3-dimethyl-butanoic acid.

Until now, only one report can be found in literature about a PA esterified with a 6-C-acid (Hartmann et al., 1990). There, a *O*-9 and a *O*-7 ester of retronecine with 2-hydroxy-3-methyl-butyric acid is described which were formed after feeding *Creatonotus transiens* butterflies with retronecine.

Our results show that similar structures can occur natively in plant material, too. For the new PA, we propose the names retrohoustine **2**, isoretrohoustine **4** and heliohoustine **3**.

3. Experimental

3.1. General

NMR spectra were measured in $CDCl_3$/DMSO-d_6 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**: 12.94 min, **2**: 10.45 min, **3**: 10.93 min, **4**: 10.72 min; MS: 220°C; interface: 250°C; 2000 emV.

3.2. Plant material

Plants were gathered at several places near Tlanchinol, Hidalgo, Mexico, in the years 1998 and 1999. The material was identified by Enrique Ortíz Bemudes, University of Mexico, a voucher specimen (IMSS14141) was deposited at the herbarium of medicinal plants at the Mexican Institut for Social Security in Mexico City.

3.3. Isolation of alkaloids

Extn of plant material (aerial parts; 500 g) was carried out as described earlier (Röder and Wiedenfeld, 1977; Wiedenfeld and Röder, 1979). Prep. TLC [silica gel F_{254}, CH_2Cl_2–MeOH–NH_4OH (25%), 75:24:1] yielded the alkaloids as oils (4 mg **1**, 12 mg **2** and 8 mg **3** and **4**).

3.4. Retrohoustine 2

GC–MS *m*/*z* (rel. int.): $[M]^+$ $C_{14}H_{23}NO_4$ 269 (0.22); $C_{14}H_{22}NO_3$ 252 (0.65); $C_{11}H_{15}NO_4$ 225 (5.65); $C_{11}H_{14}NO_3$ 208 (1.74); $C_8H_{12}NO$ 138.04 (100); $C_8H_{10}N$ 120 (8.26); C_6H_7N 93 (68.04); C_5H_6N 80 (16.96).

^1H NMR: δ 5.74 (1H, *td*, $J_{2,3a/b}=1.7$ Hz, H-2), 4.70 (2H, *ddt*, $J_{9a,9b}=13.3$ Hz, $J_{9a/b,3a/b}=1.2$ Hz $J_{9a/b,2}=1.0$ Hz, H_2-9), 4.20 (1H, *td*, $J_{7,8}=3.3$ Hz, $J_{7,6}=1.2$ Hz, H-7), 4.10 (1H, *m*, H-8), 3.84 (1H, *dddt*, $J_{3a,3b}=15.6$ Hz, $J_{3a,8}=2.4$ Hz, $J_{3a,2}=1.7$ Hz, $J_{3a,9}=1.2$ Hz, H-3A), 3.33 (1H, *dddd*, $J_{3b,3a}=15.6$, $J_{3b,5b}=5.8$, $J_{3b,8}=1.9$ Hz, $J_{3b,2}=1.7$ Hz, H-3B), 3.17 (1H, *dt*, $J_{5a,5b}=8.5$, $J_{5a,6}=1.7$ Hz, H-5A), 3.1 (2OH), 2.66 (1H, *ddd*, $J_{5b,5a}=8.5$, $J_{5b,3b}=5.8$, $J_{5b,6}=2.9$ Hz, H-5B), 1.88 (1H, *q*, $J_{13,14/15}=6.9$ Hz, H-13), 1.86 (2H, *m*, H_2-6), 1.17 (3H, *s*, H_3-16), 0.85 (3H, *d*, $J_{15,13}=6.9$ Hz, H_3-15), 0.79 (3H, *d*,

$J_{14,13}$ = 6.9 Hz, H_3-14), ^{13}C NMR: δ 179.2 (*s*, C-11), 132.9 (*s*, C-1), 127.8 (*d*, C-2), 77.6 (*d*, C-8), 76.5 (*s*, C-12), 70.1 (*d*, C-7), 62.1 (*t*, C-3), 61.8 (*t*, C-9), 53.3 (*t*, C-5), 35.7 (*t*, C-6), 35.3 (*d*, C-13), 22.7 (*q*, C-16), 16.8 (*q*, C-14), 14.4 (*q*, C-15).

3.5. Isoretrohoustine **4**

GC–MS *m*/*z* (rel. int.): $[M]^+$ $C_{14}H_{23}NO_4$ 269 (0.28); $C_{14}H_{22}NO_3$ 252 (0.13); $C_{11}H_{15}NO_4$ 225 (3.83); $C_{11}H_{14}NO_3$ 208 (0.26); $C_8H_{12}NO$ 138.04 (100); $C_8H_{10}N$ 120 (10.0); C_6H_7N 93 (87.18); C_5H_6N 80 (26.92).

^1H NMR: δ 5.55 (1H, *dd*, $J_{2,3a/b}$ = 1.8 Hz, $J_{2,8}$ = 1.4 Hz, H-2), 5.12 (1H, *ddd*, $J_{7,8}$ = 3.3 Hz, $J_{7,6a}$ = 1.2 Hz, $J_{7,6b}$ = 1.0 Hz, H-7), 4.2 (2OH), 4.18 (1H, *m*, H-8), 3.97 (2H, *dt*, $J_{9a,9b}$ = 11.5 Hz, $J_{9a/b,3a/b}$ = 5.4 Hz, H_2-9), 3.78 (1H, *ddd*, $J_{3a,3b}$ = 14.0 Hz, $J_{3a,8}$ = 6.2 Hz, $J_{3a,9a/b}$ = 5.4 Hz, H-3A), 3.29 (1H, *ddd*, $J_{3b,3a}$ = 14.0, $J_{3b,8}$ = 6.3, $J_{3b,9a/b}$ = 5.0 Hz, H-3B), 3.10 (1H, *dd*, $J_{5a,5b}$ = 9.0, $J_{5a,6}$ = 2.7 Hz, H-5A), 2.66 (1H, *dd*, $J_{5b,5a}$ = 9.1, $J_{5b,3b}$ = 8.2, H-5B), 1.94 (2H, *m*, H_2-6), 1.78 (1H, *q*, $J_{13,14/15}$ = 6.9 Hz, H-13), 1.13 (3H, *s*, H_3-16), 0.81 (3H, *d*, $J_{15,13}$ = 6.9 Hz, H_3-15), 0.73 (3H, *d*, $J_{14,13}$ = 6.9 Hz, H_3-14), ^{13}C NMR: δ 175.3 (*s*, C-11), 139.1 (*s*, C-1), 124.4 (*d*, C-2), 76.2 (*s*, C-12), 74.8 (*d*, C-8), 74.0 (*d*, C-7), 62.7 (*t*, C-3), 58.2 (*t*, C-9), 53.1 (*t*, C-5), 34.7 (*d*, C-13), 34.2 (*t*, C-6), 23.3 (*q*, C-16), 17.2 (*q*, C-14), 15.9 (*q*, C-15).

3.6. Heliohoustine **3**

GC–MS *m*/*z* (rel. int): $[M]^+$ $C_{14}H_{23}NO_4$ 269 (1.09); $C_{14}H_{22}NO_3$ 252 (0.22); $C_{11}H_{15}NO_4$ 225 (4.78); $C_{11}H_{14}NO_3$ 208 (0.11); $C_8H_{12}NO$ 138.04 (100); $C_8H_{10}N$ 120 (5.33); C_6H_7N 93 (81.53); C_5H_6N 80 (19.02).

^1H NMR: δ 5.62 (1H, *t*, $J_{2,3a/b}$ = 1.2 Hz, H-2), 4.67 (2H, *m*(broad), H_2-9), 4.2 (2OH), 4.16 (1H, *t*, $J_{7,8}$ = 2.2 Hz, H-7), 3.98 (1H, *m*, H-8), 3.77 (1H, *ddt*, $J_{3a,3b}$ = 14.6 Hz, $J_{3a,8}$ = 6.6 Hz, $J_{3a,9}$ = 5.2 Hz, H-3A), 3.29 (1H, *dtd*, $J_{3b,3a}$ = 14.6, $J_{3b,5b}$ = 4.1, $J_{3b,8}$ = 2.0 Hz, H-3B), 3.11 (1H, *dm*, $J_{5a,5b}$ = 10.5, H-5A), 2.57 (1H, *ddd*, $J_{5b,5a}$ = 10.6, $J_{5b,3b}$ = 4.8, $J_{5b,6}$ = 1.3 Hz, H-5B), 1.78 (1H, *q*, $J_{13,14/15}$ = 6.7 Hz, H-13), 1.74 (1H, *tm*, $J_{6a,6b}$ = 6.0 Hz, H-6A), 1.62 (1H, *td*, $J_{6b,6a}$ = 6.1 Hz, $J_{6b,5}$ = 5.8 Hz, H-6B), 1.23 (3H, *s*, H_3-16), 0.83 (3H, *d*, $J_{15,13}$ = 6.7 Hz, H_3-15), 0.80 (3H, *d*, $J_{14,13}$ = 6.7 Hz, H_3-14), ^{13}C NMR: δ 175.5 (*s*, C-11), 136.5 (*s*, C-1), 124.4 (*d*, C-2), 79.5 (*d*, C-8), 77.1 (*s*, C-12), 73.7 (*d*, C-7), 61.4 (*t*, C-3), 60.7 (*t*, C-9), 53.7 (*t*, C-5), 35.2 (*d*, C-13), 33.7 (*t*, C-6), 22.8 (*q*, C-16), 17.1 (*q*, C-14), 16.1 (*q*, C-15).

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