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PYRROLIZIDINE ALKALOIDS FROM *CRITONIA MORIFOLIA*

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Key Word Index—Critonia morifolia; Asteraceae; pyrrolizidine alkaloids; morifoline; rinderine; O^{12}-acetylrinderine.

Abstract—Three pyrrolizidine alkaloids were isolated from *Critonia morifolia* and their structures elucidated by spectroscopic methods. Besides the already known rinderine and its O^{12}-acetylderivative, one with a new strucure was found. For this alkaloid (O^{9}-(+)-viridifloryl-retronecine) the name, morifoline, is proposed.

INTRODUCTION

Critonia morifolia (P.Browne) (former name: *Eupatorium morifolia*) is an endemic plant from Mexico. It can be found in the states of Vera-Cruz and Oaxaca. Traditionally, it is used by the natives for the treatment of several diseases. Ethnopharmacological reports lead to the conclusion that there should be an immunostimulating effect like that is reported for other *Eupatorium* species. As *C. morifolium* belongs to the Asteraceae and the tribe Eupatorieae, the presence of pyrrolizidine alkaloids (PA) could be presumed. The plant material was collected in the region of the Chinanteca indians, south from Tuxtepec, in the northern part of Oaxaca state. The natives use the plant material in the form of aqueous solutions (decoctions); these preparations contain only traces of PAs.

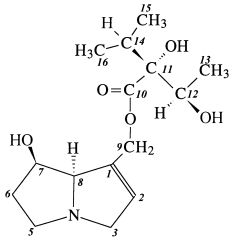
The estimation of a potentially toxic effect presupposes the knowledge of the particular structures of the PAs contained. Therefore, aerial parts of the plant *C. morifolia* were investigated. Three PA were isolated and their structures determined by GC-mass spectroscopy and homo- as well as heteronuclear 2D-NMR correlated spectroscopy. One of them has not been described previously. The two known alkaloids belong to the heliotridine-type and are *O*-9-(+)-trachelanthyl-heliotridine (= rinderine) and its *O*-12-acetylderivative [1, 2, 3, 4, 5]. The new PA shows the structure of a retronecine esterified at position C-9 with a (+) viridifloric acid.

For the new compound, the name morifoline is proposed. Based on the structure-toxicity relationship [6] for all substances, toxic side-effects must be expected.

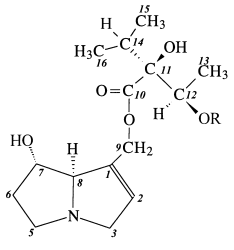
RESULTS AND DISCUSSION

In our investigation, aerial parts of *C. morifolia* were extracted as previously described [7, 8]. From the crude alkaloidal extract compounds **1**–**3** were isolated. The mass spectrum of **1** shows a $[M]^+$ at *m/z* 299.18 corresponding to the fomula $C_{15}H_{25}NO_5$ (calcd. 299.37). The fragmentation ions at *m/z* 281 $[M\text{-}H2O]^+$, 255 $[M\text{-}C_2H_4O]^+$ and 138 $[M\text{-}C_7H_{13}O_4]^+$ (C-9-O-cleavage) prove the necine ester structure of **1**. The typical fragmentation pattern between *m/z* 138 and 80 is characteristic for retronecine or its isomer heliotridine.

The same molecular formula $C_{15}H_{25}NO_5$ is also found for **2**. The fragmentation pattern of **2** differs from that of **1**, only in the relative intensities of the single fragments, so that **2** has to be an isomer of **1**. Compound **3** shows an $[M]^+$ at *m/z* 341.21 indicating the molecular formula $C_{17}H_{27}NO_6$.(calcd. 341.40).



1



2: R = H
3: R = —C(=O)—CH$_3$

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After loss of a C_2H_3O-fragment, the pattern of **3** is similar to those of **1** and **2**, which indicates that **3** has to be an acetyl derivative of **1** or **2**.

The ^1H and ^{13}C-NMR data are summarized in the Experimental. The assignment was performed by interpretation of H,H-COSY- and C,H-correlation spectra. Important structural information is provided by the chemical shift of C-6 (for **1** ^1H at 1.89 and ^{13}C at 36 ppm, for **2** and **3** ^1H at 1.85/1.76 and ^{13}C at 33 ppm) [9, 10]. These signals identify retronecine as the necine of **1** as well as heliotridine for **2** and **3**. The stereochemistry of the esterifying acid at C-9 can be deduced by interpretation of the shift-difference of the C-9H_2 AB-system [11], the ^1H-value of the C-12-proton and the shift-difference of the carbons C-15/C-16 [10]. In **1**, for the first aspect, a value of Δ0.40 ppm is found indicating the *R*-configuration at C-11. In addition, the signal of the proton at C-12 at 3.92 ppm and the ^{13}C shift-difference of C-15/C-16 ($\Delta = 0.7$ ppm) determine the "identical" configuration at C-11/C12 [10], which means *S*/*S* or *R*/*R*, signifying that **1** has to show the 11*R*,12*R*-configuration. From this follows the structure of a (+)-viridifloric acid. This acid has up to now only been described in the PAs coromandaline and heliocoromandaline, where it was identified by GC-mass spectrometry and its optical rotation after hydrolysis of the alkaloids [12, 13]. The NMR data given for the acidic part of coromandaline and heliocoromandaline are 4.01 (3.99) for C-12H, 1.27 (1.29) for C-13H_3, 2.16 for C-14H and for the C-15/C-16 methyl functions 0.94/0.89 (0.94/0.88 ppm). Compound **2** shows its C-9H_2 AB-system at 4.93 and 4.71 (=11*S* configuration), the C-12 proton at 4.04 ppm and a ^{13}C-ΔC-15/C-16 value of 0.4 ppm indicating the structure 11*S*/12*R*, leading to the conclusion that **2** has to be a (+)-trachelanthoyl-heliotridine. This compound is known as rinderine. All spectroscopic data corresponds to those already described [1, 2, 3].

Compound **3** differs from **2** only by the presence of an additional acetyl group (1.85 and 21 ppm for the methyl and 172 ppm for the C=O function). Final proof of the location of the acetoxyl group at C-12 is obtained from the downfield shift of this carbon atom (^{13}C: $\Delta = 5$; ^1H: $\Delta = 0.95$ ppm). The other NMR data of **3** correspond to those of **2**. Therefore, **3** has to be *O*-12-acetylrinderine, which was first identified by GC-mass spectrometry [4, 5].

Compound **1** is a new natural PA; the name morifoline is proposed.

EXPERIMENTAL

General

NMR were measured in $CDCl_3$/D_6-DMSO at 400 and 100 MHz, respectively. GC-MS: GC: 180°–280°C, 4°/min.; Permabond SE-54, 50 m × 0.32 mm; inj.: 280°; R_t: **1** 17.31., **2** 17.86., **3** 18.64. MS: 220°; interface: 280°; 70 eV; repeller: 1.5 V.

Plant material

Plants were gathered at their original place near Tuxtepec, Oaxaca, Mexico. The material was identified by J. L. Villasenor, University of Mexico-City, and voucher specimen is deposited at the herbarium of the Biological Institute of the UNAM, Mexico-City.

Isolation of alkaloids

Extraction of aerial parts; (350 g) was carried out as described earlier [7, 8]. Prep. TLC [silica gel F_{254}, CH_2Cl_2 -MeOH-NH_4OH (25%), 75:24:1] yielded the alkaloids as oils (7 mg **1** and 5 mg **2** and **3**).

Morifoline

(**1**). GC-MS *m*/*z* (rel. int): $[M]^+$ $C_{15}H_{25}NO_5$ 299.18 (0.10); calcd. 299.37; $C_{13}H_{21}NO_4$ 255.22 (0.13); $C_{13}H_{20}NO_4$ 254.17 (0.23); $C_8H_{14}NO_2$ 156.05 (5.28); $C_8H_{13}NO$ 139.07 (23.2); $C_8H_{12}NO$ 138.04 (77.7); $C_8H_{10}N$ 120.02 (11.3); C_6H_9N 95.01 (20.5); C_6H_8N 94.00 (71.6); C_6H_7N 92.96 (100); C_5H_6N 79.97 (30.9). ^1H NMR: δ 5.77 (1H, *s*, H-2), 4.95 (1H, *d*, $J_{9a,9b} = 12.8$ Hz, H-9A), 4.54 (1H, *d*, $J_{9b,9a} = 12.8$ Hz, H-9B), 4.22 (1H, *tm*, $J_{7,8} = 1.9$ Hz, H-7), 4.13 (1H, *dm*, $J_{8,7} = 1.9$ Hz, H-8), 3.92 (1H, *q*, $J_{12,13} = 6.6$ Hz, H-12), 3.86 (1H, *d*, $J_{3a,3b} = 15.1$ Hz, H-3A), 3.36 (1H, *ddd*, $J_{3b,3a} = 15.1$, $J_{3b,7} = 5.2$, $J_{3b,5a} = 1.9$ Hz, H-3B), 3.20 (1H, *td*, $J_{5a,5b} = 8.0$, $J_{5a,3b} = 1.9$ Hz, H-5A), 3.1 (3OH), 2.69 (1H, *ddd*, $J_{5b,5a} = 8.2$, $J_{5b,6} = 6.4$, $J_{5b,7} = 2.4$ Hz, H-5B), 2.02 (1H, *qq*, $J_{14,15/16} = 6.6$ Hz, H-14), 1.89 (2H, *dm*, $J_{6,5b} = 6.4$ Hz, H_2-6), 1.06 (3H, *d*, $J_{13,12} = 6.6$ Hz, H_3-13), 0.86 (3H, *d*, $J_{15,14} = 6.6$ Hz, H_3-15), 0.82 (3H, *d*, $J_{16,14} = 6.6$ Hz, H_3-16). ^{13}C NMR: δ 174.4 (*s*, C-10), 132.7 (*s*, C-1), 128.3 (*d*, C-2), 82.5 (*s*, C-11), 78.0 (*d*, C-8), 70.3 (*d*, C-12), 68.6 (*d*, C-7), 62.1 (*t*, C-3), 62.0 (*t*, C-9), 53.3 (*t*, C-5), 35.7 (*t*, C-6), 31.9 (*d*, C-14), 16.9 (*q*, C-15), 16.7 (*q*, C-13), 16.2 (*q*, C-16).

Rinderine

(**2**). GC-MS *m*/*z* (rel.int.): $[M]^+$ $C_{15}H_{25}NO_5$ 299.21 (0.09); calc. 299.37; $C_{15}H_{23}NO_4$ 281.16 (0.33); calc. 281.35; $C_{13}H_{20}NO_4$ 254.33 (0.28); $C_8H_{14}NO_2$ 156.10 (4.75); $C_8H_{13}NO$ 139.07 (22.8); $C_8H_{12}NO$ 138.08 (74.8); $C_8H_{10}N$ 120.06 (13.1); C_6H_9N 95.04 (24.6); C_6H_8N 94.03 (44); C_6H_7N 93.01 (100); C_5H_6N 79.99 (33.2). ^1H and ^{13}C NMR data correspond with those reported earlier within a range of 0.2 (^1H NMR) and 2 (^{13}C NMR) ppm [1, 2, 3].

O-12-acetylrinderine

(**3**). GC-MS *m*/*z* (rel.int.): $[M]^+$ $C_{17}H_{27}NO_6$ 341.21 (0.07); calcd. 341.40; $C_{15}H_{24}NO_5$ 298.38 (0.7); $C_{13}H_{21}NO_4$ 255.22 (0.32); $C_{13}H_{20}NO_4$ 254.36 (0.21); further fragmentation identical to 2 [1–3].

^1H NMR: δ 4.99 (1H, *q*, $J_{12,13} = 6.8$ Hz, H-12), 1.85

(3H, *s*, H_3-18), further data correspond to those of **2** [1, 2, 3] within a range of 0.2 ppm. ^{13}C NMR: δ 172.2 (*s*, C-17), 73.9 (*d*, C-12), 21.0 (*q*, C-18), further data corresponds to those of **2** [1, 2, 3] within a range of 2 ppm.

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