

## PYRROLIZIDINE ALKALOIDS FROM *Critonia morifolia* P.Browne

H. Wiedenfeld\* and A. Andrade Cetto\*\*

\* Pharmazeutisches Institut der Universität, An der Immenburg 4, D-53121 Bonn, F.R.G.

\*\* Depto. de Biología, Fac. de Ciencias, Universidad Autonoma de Mexico, Mexico, D.F., Mexico

**Key words Index** - *Critonia morifolia* (P.Browne), Asteraceae, Eupatorieae, pyrrolizidine alkaloids, morifoline, rinderine, O<sup>12</sup>-acetylrinderine;

**Abstract** - Three pyrrolizidine alkaloids were isolated from *Critonia morifolia* and their structures elucidated by spectroscopical methods. Besides the already known rinderine and its O<sup>12</sup>-acetyl derivative one with a new structure was found.

For this alkaloid (O<sup>9</sup>-(+)-viridifloryl-retronecine) the name morifoline is proposed.

### Introduction

*Critonia morifolia* (P.Browne) (Asteraceae, tribe Eupatorieae) (former name: *Eupatorium m.*) 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 it is reported for other *Eupatorium*-species.

As *C. morifolium* belongs to the asteraceae family 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, north part of Oaxaca state. The natives use the plant material in form as aqueous solutions (decoctions). In those preparations only traces of PA can be found.

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 helitridine-type and are O-9-(+)-trachelanthyl-heliotridine (= rinderine) and its O-12-acetyl derivative [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 already described [7,8]. From the crude alkaloidal extract **1**, **2** and **3** were isolated.

The mass spectrum of **1** shows the  $[M]^+$ -Peak at 299.18 corresponding to the formula  $C_{15}H_{25}NO_5$  (calc. 299.37). The fragmentation ions at  $m/z$  281 =  $[M]^+ - H_2O$ ,  $m/z$  255 =  $[M]^+ - C_2H_4O$  and  $m/z$  138 =  $[M]^+ - C_7H_{13}O_4$  (C-9-O-cleavage) prove the necine ester structure of **1**. The typical fragmentation pattern between  $m/z$  138 and  $m/z$  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** differ from that of **1** only in the relative intensities of the single fragments so that **2** has to be an isomer of **1**. **3** shows an  $[M]^+$ -Peak at 341.21 indicating the molecular formula  $C_{17}H_{27}NO_7$  (calc. 341.40). 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 part. 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**  $^1\text{H}$  at 1.85/1.76 and  $^{13}\text{C}$  at 33 ppm) [9,10]. These signals determine 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<sub>2</sub> AB-system [11], the  $^1\text{H}$ -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  $\Delta = 0.40$  ppm is found indicating the R-configuration at C-11. In addition the signal of the proton at C-12 at 3.92 and the  $^{13}\text{C}$  shift-difference of C-15/C-16 ( $\Delta = 0.7$  ppm) determine "identical" configuration at C-11/C12 [10], which means S/S or R/R, resulting that **1** has to show the 11R,12R configuration. From this follows for this acid the structure of a (+)-viridifloric acid. (+)-Viridifloric acid is up to now only described in the PA coromandaline and heliocoromandaline were it was identified by GC-MS 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<sub>3</sub>, 2.16 for C-14H and for the C-15/C-16 methyl functions 0.94/0.89 (0.94/0.88).

**2** shows its C-9H<sub>2</sub> AB-system at 4.93 and 4.71 (= 11S configuration), the C-12 proton at 4.04 ppm and a  $^{13}\text{C}$  -  $\Delta\text{C-15/C-16}$  value of 0.4 ppm indicating the structure 11S/12R leading to the conclusion that **2** has to be a (+)-trachelanthoyl-heliotridine. This compound is known as rinderine. All spectroscopical data correspond to those already described [1,2,3].

**3** differs from **2** only by 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 acetoxy group at C-12 is obtained from the downfield shift of this carbon atom ( $^{13}\text{C}$ :  $\Delta = 5$ ;  $^1\text{H}$ :  $\Delta = 0.95$  ppm). The other NMR data of **3** correspond to those of **2**. Therefore **3** has to be the O-12-acetyl-rinderine which was first identified by GC-MS [4,5].

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

## Experimental

*General:* NMR-spectra were measured in  $\text{CDCl}_3$ /  $\text{D}_6\text{-DMSO}$  at 400 and 100 MHz, respectively. GC-MS: GC: 180° - 280°C, 4°/min.; Permabond SE-54, 50m x 0.32mm; Inj.: 280°C; R<sub>t</sub>: **1**: 17.31 min., **2**: 17.86 min., **3**: 18.64 min.; MS: 220°C; interface: 280°C; 70 eV; repeller: 1.5V

*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 are deposited at the herbarium of the Biological Institute of the UNAM, Mexico-City.

*Isolation of alkaloids:* Extn of plant material (aerial parts; 350 g) was carried out as described earlier [7,8]. Prep. TLC [silica gel F<sub>254</sub>, CH<sub>2</sub>Cl<sub>2</sub> -MeOH-NH<sub>4</sub>OH (25%), 75:24:1] yielded the alkaloids as oils (7 mg **1** and 5 mg **2** and **3**).

*Morifoline:* GC-MS *m/z* (rel. int): [M]<sup>+</sup> C<sub>15</sub>H<sub>25</sub>NO<sub>5</sub> 299.18 (0.10); calc. 299.37; C<sub>13</sub>H<sub>21</sub>NO<sub>4</sub> 255.22 (0.13); C<sub>13</sub>H<sub>20</sub>NO<sub>4</sub> 254.17 (0.23); C<sub>8</sub>H<sub>14</sub>NO<sub>2</sub> 156.05 (5.28); C<sub>8</sub>H<sub>13</sub>NO 139.07 (23.2); C<sub>8</sub>H<sub>12</sub>NO 138.04 (77.7); C<sub>8</sub>H<sub>10</sub>N 120.02 (11.3); C<sub>6</sub>H<sub>9</sub>N 95.01 (20.5); C<sub>6</sub>H<sub>8</sub>N 94.00 (71.6); C<sub>6</sub>H<sub>7</sub>N 92.96 (100); C<sub>5</sub>H<sub>6</sub>N 79.97 (30.9).

<sup>1</sup>H NMR: δ 5.77 (1H, *s*, H-2), 4.95 (1H, *d*, *J*<sub>9a,9b</sub> = 12.8 Hz, H-9A), 4.54 (1H, *d*, *J*<sub>9b,9a</sub> = 12.8 Hz, H-9B), 4.22 (1H, *tm*, *J*<sub>7,8</sub> = 1.9 Hz, H-7), 4.13 (1H, *dm*, *J*<sub>8,7</sub> = 1.9 Hz, H-8), 3.92 (1H, *q*, *J*<sub>12,13</sub> = 6.6 Hz, H-12), 3.86 (1H, *d*, *J*<sub>3a,3b</sub> = 15.1 Hz, H-3A), 3.36 (1H, *ddd*, *J*<sub>3b,3a</sub> = 15.1, *J*<sub>3b,7</sub> = 5.2, *J*<sub>3b,5a</sub> = 1.9 Hz, H-3B), 3.20 (1H, *td*, *J*<sub>5a,5b</sub> = 8.0, *J*<sub>5a,3b</sub> = 1.9 Hz, H-5A), 3.1 (3OH), 2.69 (1H, *ddd*, *J*<sub>5b,5a</sub> = 8.2, *J*<sub>5b,6</sub> = 6.4, *J*<sub>5b,7</sub> = 2.4 Hz, H-5B), 2.02 (1H, *qq*, *J*<sub>14,15/16</sub> = 6.6 Hz, H-14), 1.89 (2H, *dm*, *J*<sub>6,5b</sub> = 6.4 Hz, H<sub>2</sub>-6), 1.06 (3H, *d*, *J*<sub>13,12</sub> = 6.6 Hz, H<sub>3</sub>-13), 0.86 (3H, *d*, *J*<sub>15,14</sub> = 6.6 Hz, H<sub>3</sub>-15), 0.82 (3H, *d*, *J*<sub>16,14</sub> = 6.6 Hz, H<sub>3</sub>-16), <sup>13</sup>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:* GC-MS *m/z* (rel.int.): [M]<sup>+</sup> C<sub>15</sub>H<sub>25</sub>NO<sub>5</sub> 299.21 (0.09); calc. 299.37; C<sub>15</sub>H<sub>23</sub>NO<sub>4</sub> 281.16 (0.33); calc. 281.35; C<sub>13</sub>H<sub>20</sub>NO<sub>4</sub> 254.33 (0.28); C<sub>8</sub>H<sub>14</sub>NO<sub>2</sub> 156.10 (4.75); C<sub>8</sub>H<sub>13</sub>NO 139.07 (22.8); C<sub>8</sub>H<sub>12</sub>NO 138.08 (74.8); C<sub>8</sub>H<sub>10</sub>N 120.06 (13.1); C<sub>6</sub>H<sub>9</sub>N 95.04 (24.6); C<sub>6</sub>H<sub>8</sub>N 94.03 (44); C<sub>6</sub>H<sub>7</sub>N 93.01 (100); C<sub>5</sub>H<sub>6</sub>N 79.99 (33.2).

<sup>1</sup>H NMR and <sup>13</sup>C NMR data correspond to those reported earlier within a range of 0.2 (<sup>1</sup>H NMR) and 2 (<sup>13</sup>C NMR) ppm [1,2,3].

*O-12-Acetylrinderine:* GC-MS *m/z* (rel.int.): [M]<sup>+</sup> C<sub>17</sub>H<sub>27</sub>NO<sub>6</sub> 341.21 (0.07); calc. 341.40; C<sub>15</sub>H<sub>24</sub>NO<sub>5</sub> 298.38 (0.7); C<sub>13</sub>H<sub>21</sub>NO<sub>4</sub> 255.22 (0.32); C<sub>13</sub>H<sub>20</sub>NO<sub>4</sub> 254.36 (0.21); Further fragmentation identical to **2** [1,2,3].

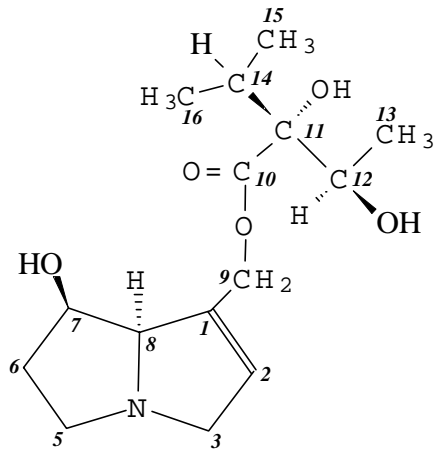
$^1\text{H}$  NMR:  $\delta$  4.99 (1H, *q*,  $J_{12,13} = 6.8$  Hz, H-12), 1.85 (3H, *s*, H<sub>3</sub>-18), further data correspond to those of **2** [1,2,3] within a range of 0.2 ppm.

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

## References

1. Asibal, C.F., Glinski, J.A., Gelbaum, L.T. and Zalkow, L.H. (1989) *J.Nat.Prod.* **52**, 109
2. Hagan, D.B. and Robins, D.J. (1990) *Fitoterapia* **LXII**, 186
3. Liu, H., Roeder, E., Chen, H.L. and Xiu, X.J. (1992) *Phytochemistry* **31**, 2573
4. Trigo, J., Witte, L., Brown, K.Jr., Hartmann, T. and Barata, L. (1993) *J.Chem.Ecol.* **19**, 669
5. Biller, A., Boppré, M., Witte L. and Hartmann, T. (1994) *Phytochemistry* **35**, 615
6. Wiedenfeld, H. and Roeder, E. (1984) *Dtsch. Apoth. Ztg.* **124**, 2116.
7. Roeder, E. and Wiedenfeld, H. (1977) *Phytochemistry* **16**, 1462.
8. Wiedenfeld, H. and Roeder, E. (1979) *Phytochemistry* **18**, 1083.
9. Jones, A.J., Culvenor, C.C.J. and Smith, L.W. (1982) *Aust. J. Chem.* **35**, 1173
10. Wiedenfeld, H. and Roeder, E. (1991) *Planta Med.* **57**, 578.
11. Mohanraj, S. and Herz, W. (1982) *J. Nat. Prod.* **45**, 328.
12. Subramanian, P.S., Mohanraj, S., Cockrum, P.A., Culvenor, C.C.J., Edgar, J.A., Frahn, J.L. and Smith, L.W. (1980) *Aust. J. Chem.* **33**, 1357
13. Mohanraj, S., Subramanian, P.S., Culvenor, C.C.J., Edgar, J.A., Frahn, J.L., Smith, L.W. and Cockrum, P.A.(1978) *J. Chem. Soc. Chem. Commun.* **1978**, 423

**Acknowledgement** - We thank Dr. Jose Louis Villasenor (National Herbarium, Inst. de Biologia, UNAM, Mexico-City, Mexico) very much for identifying the plant material.



1

