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MYRICOIDINE AND DIHYDROMYRICOIDINE, TWO NEW MACROCYCLIC SPERMIDINE ALKALOIDS FROM *CLERODENDRUM MYRICOIDES*.

SANVURA BASHWIRA and CLAUDE HOOTELE* +.

Service de Chimie Organique, Faculté des Sciences.
Université Libre de Bruxelles, B-1050 Bruxelles.

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Abstract: Two new spermidine alkaloids, myricoidine 1 and dihydromyricoidine 2 have been isolated from *Clerodendrum myricoides* (Verbenaceae) and their structures established by a study of their spectral and chemical properties.

The genus *Clerodendrum* comprises more than 500 species and varieties and is the largest genus of the family Verbenaceae[1]. While some *Clerodendrum* species were previously reported to give positive reactions for the presence of alkaloids no base was isolated or identified[2]. We now wish to report the isolation and the structure elucidation of two new alkaloids, which we have named myricoidine 1 and dihydromyricoidine 2, from *Clerodendrum myricoides*. These two bases are present in *C. myricoides* in minute amounts (ca. 10 ppm from the dried plant); they were isolated as homogeneous compounds (1: $[\alpha]_D^{22}$ +83° (c = 6, MeOH); 2: $[\alpha]_D^{22}$ +77° (c = 5.3, MeOH) by repetitive countercurrent distribution.

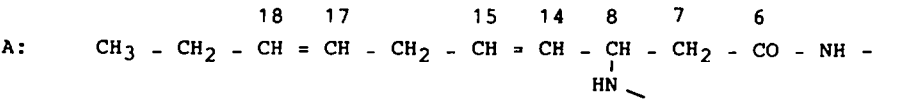
The high resolution mass spectra of myricoidine 1 and dihydromyricoidine 2 showed molecular ions (at m/z 293 and at m/z 295 respectively) corresponding to the formula $C_{17}H_{31}N_3O$ (1) and $C_{17}H_{33}N_3O$ (2). The IR spectra established the presence of a secondary amide group in both compounds. The two remaining nitrogen atoms belong to secondary amines as demonstrated by the formation of the corresponding N,N-diacetylderivatives ($M^{+\cdot}$ at m/z 377 and 379 respectively) after Ac_2O-pyridine treatment. Myricoidine has two C=C double bonds whereas dihydromyricoidine has only one. Hydrogenation of myricoidine 1 over platinum yielded tetrahydromyricoidine 3 ($M^{+\cdot}$ at m/z 297) identical in all respects (MS, NMR, rotation) with the derivative obtained in the same conditions from dihydromyricoidine 2. The two alkaloids 1 and 2 have therefore the same skeleton and must be monocyclic compounds.

+Research Associate of the National Fund for Scientific Research (Belgium).

Structure of myricoidine.

The ^{1}H and ^{13}C measurements including two-dimensional techniques (homonuclear and heteronuclear COSY) and spectrum simulation allowed the assignment of the majority of the NMR signals of 1. The relevant ^{1}H and ^{13}C parameters are listed in Tables 1 and 2 and the results of the 2D experiments are presented in Figures 1 and 2.

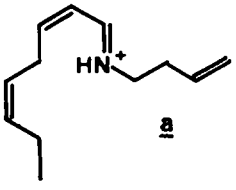
From these informations it appears that the partial sequence A is present in the acid part of the amide linkage of 1:



The vicinal coupling constants between H-14 and H-15 (J = 11 Hz) and between H-17 and H-18 (J = 11 Hz) indicate a cis-configuration for the two C=C double bonds. The chemical shifts of the hydrogens at C-7 and C-8 support the attachment of the amide carbonyl group and of a secondary amine function respectively at these positions[3].

At this stage it remains to introduce seven methylene groups and the third NH function. Incorporation of these elements in a spermidine moiety joining C-6 and C-8 with formation of a 13-membered ring accounts for all the NMR properties of the base.

The mass spectrum of 1 exhibited a medium intensity peak at m/z 178 ($C_{12}H_{20}N$; shifted to m/z 180 for 2) attributed to a and supporting the presence of four methylene groups between N-1 and N-9 as observed for celacinnine-type derivatives[4].



a

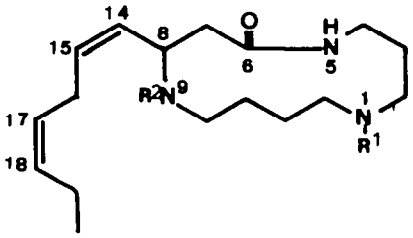
A prominent peak at m/z 276 (shifted to m/z 278 in 2, base peak) corresponds to the loss of NH_3 from the molecular ion; $M^{+\cdot}$ - 17 ions have been recorded previously for related compounds with two basic NH functions[5,6].

Structure of dihydromyricoidine.

The spectral properties of dihydromyricoidine 2 are reminiscent of those of 1. The chemical shifts and the coupling constants observed for H-8, H-14 and H-15 are nearly identical in the ^{1}H NMR spectra of the two bases and no other signal occurs in the region between 4 and 6 ppm for 2. These observations, the ^{13}C chemical shifts (Table 2) and the chemical correlation performed between 1 and 2 by hydrogenation indicate that 2 must be 17,18-dihydromyricoidine.

Myricoidine 1 and dihydromyricoidine 2 are closely related to the macrocyclic alkaloids 4 (loesenerine) and 5 recently isolated from *Maytenus loeseneri*[7] (Celastraceae). Direct comparison proved that N,N-diacetyldihydromyricoidine 6 is identical (including the absolute configuration) with acetylloesenerine.

Myricoidine and dihydromyricoidine appear to be the first polyamine alkaloids isolated from a species of the family Verbenaceae. This result constitutes one more illustration of the broad distribution of this type of alkaloids[8].



1 $R^1 = R^2 = H$
2 17,18-dihydro, $R^1 = R^2 = H$
3 14,15,17,18-tetrahydro, $R^1 = R^2 = H$
4 17,18-dihydro, $R^1 = CH_3CO$, $R^2 = H$
5 $R^1 = CH_3CO$, $R^2 = H$
6 17,18-dihydro, $R^1 = R^2 = CH_3CO$

Table 1. ^1H parameters ($CDCl_3$) of myricoidine 1.

Proton	δ (ppm)	J Hz (coupled proton)
H-4a	3.28	13.5(H-4b), 5(H-3)
H-4b	3.55	13.5(H-4a), 6(H-3)
H-5	8.62	H-4a, H-4b
H-7a	2.30	15(H-7b), 10(H-8)
H-7b	2.40	15(H-7a), 3(H-8)
H-8	3.77	9(H-14), 10(H-7a), 3(H-7b)
H-14	5.15	11(H-15), 9(H-8), 1.5(H-16)
H-15	5.50	11(H-14), 7(H-16), 1(H-8)
H-17	5.25	11(H-18), 7(H-16), 1(H-19)
H-18	5.40	11(H-17), 7(H-19), 1(H-16)
H-19	2.07	7(H-20), 7(H-18), 1(H-17)
H-20	0.97	7(H-19)

Table 2. ^{13}C chemical shifts ($CDCl_3$) of 1 and 2.

	1	2
C-2, C-10, C-13	46.0	45.9
	48.8	48.7
	49.9	49.8
C-3, C-11, C-12	27.5	27.6*
	27.7	27.7*
	28.3	28.3
C-4	39.7(39.9)+	39.7(39.9)+
C-6	171.4	171.4
C-7	42.6	42.7
C-8	52.2	52.1
C-14	131.7	131.3
C-15	130.2	132.3
C-16	26.0	27.4*
C-17	126.6	29.4
C-18	132.5	31.5
C-19	20.6	22.6
C-20	14.2	14.0

+ The presence of two signals is presumably due to the existence of cis and trans conformers around the amide linkage[9].

* Alternative.

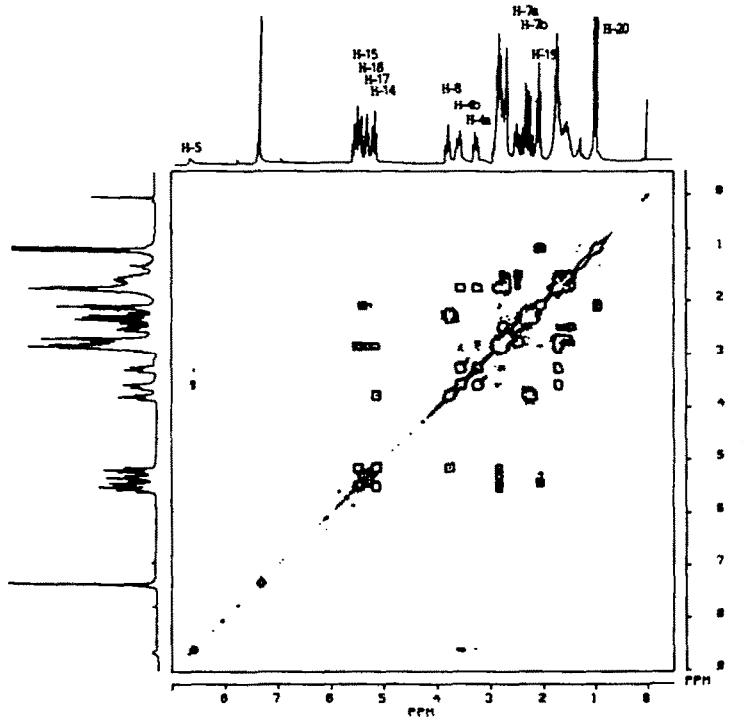


Figure 1. Homonuclear ($^1H,^1H$) correlated spectrum ($CDCl_3$) of myricoidine 1.

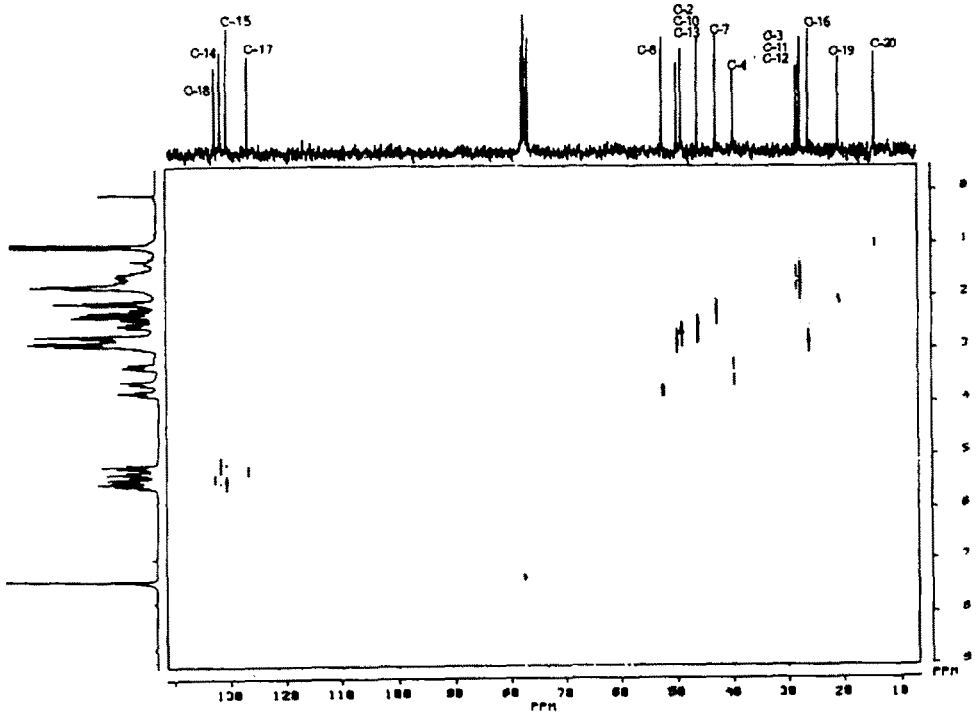


Figure 2. Heteronuclear ($^1H,^{13}C$) correlated spectrum ($CDCl_3$) of myricoidine 1

EXPERIMENTAL.

IR spectra were determined on a Perkin-Elmer 237 spectrometer.

Mass spectral data were obtained on a Micromass 7070F spectrometer.

The NMR spectra were recorded on a Bruker WM 250 apparatus, in $CDCl_3$ with TMS as internal standard. Signal assignment in the ^{13}C NMR spectra was aided by the DEPT pulse sequence.

Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

The countercurrent distributions were analyzed by measuring the optical density (420 nm) of the organic phase of each tube after basification (aq. NaOH), decantation, drying and addition of a $CHCl_3$ solution of picric acid.

Isolation of the alkaloids of *Clerodendrum myricoides*.

Dried and ground whole plants of *Clerodendrum myricoides* (15 Kg; collected near Bukavu, Zaïre) were left at room temperature in 2% aqueous HCl. After three days, the solution was filtered, basified with aqueous NaOH and extracted with $CHCl_3$. Evaporation of the solvent under reduced pressure yielded a brown residue (25 g) which was distributed between $CHCl_3$ and McIlvaine buffer pH 2.2. The organic phase was evaporated to give Fraction A1; the fraction obtained after basification of the aqueous phase, extraction with $CHCl_3$ and evaporation was partitionned again between $CHCl_3$ and McIlvaine buffer pH 7.1. Evaporation of the $CHCl_3$ phase yielded Fraction A2 (2.5 g). The aqueous phase was basified (NaOH) and extracted with $CHCl_3$; evaporation of the solvent yielded Fraction B (1.5 g) containing the polar alkaloids.

Isolation of myricoidine and dihydromyricoidine.

Fraction B (1.5 g) was subjected to a countercurrent distribution (trichloroethylene/borax-NaOH buffer pH 9.6; 60 transfers). Tubes 53-60 contained two alkaloids whose $M^{+\cdot}$ appeared at m/z 309 and 311 in the mass spectrum. The residue from tubes 11-32 and tubes 33-52 were each subjected to a further CCD in the same conditions to give homogeneous myricoidine 1 (60 mg) and dihydromyricoidine 2 (53 mg).

Myricoidine 1: (oil), $[\alpha]_D^{22}$ +83° (c = 6, MeOH); IR ($CHCl_3$): 3450, 3020, 1680 and 1560 cm^{-1}; MS: 293 ($M^{+\cdot}$, 100%; $C_{17}H_{31}N_3O$, calc.: 293.2467, found: 293.2471), 276 ($M^{+\cdot}$ - NH_3, 39; $C_{17}H_{28}N_2O$, calc.: 276.2201, found: 276.2198); 235 (18; $C_{14}H_{23}N_2O$, calc.: 235.1810, found: 235.1808), 224 (35; $C_{12}H_{22}N_3O$, calc.: 224.1763, found: 224.1762), 208 (27), 207 (29), 178 (14; $C_{12}H_{20}N$, calc.: 178.1596, found: 178.1594), 168 (20; $C_9H_{16}N_2O$, calc.: 168.1262, found: 168.1265), 164(17), 159 (19).

Dihydromyricoidine 2: (oil), $[\alpha]_D^{22}$ +77° (c = 5.3, MeOH); IR: 3020, 1680 and 1560 cm^{-1}; ^1H NMR: δ 0.89 (t, J = 7 Hz, CH_3), 3.80 (dt, J = 9, 9 and 5 Hz, H-8), 5.13 (tdd, J = 11, 9 and 1 Hz, H-14), 5.54 (tdd, J = 11, 7 and 1 Hz, H-15), 8.66 (broad signal, amide NH); MS: 295 ($M^{+\cdot}$, 37%; $C_{17}H_{33}N_3O$, calc.: 295.2622, found: 295.2613), 278 ($M^{+\cdot}$ - NH_3, 100; $C_{17}H_{30}N_2O$, calc.: 278.2358, found: 278.2356), 237 (14; $C_{14}H_{25}N_2O$, calc.: 237.1967, found: 237.1966), 221 (58; $C_{13}H_{21}N_2O$, calc.: 221.1654, found: 221.1650), 180 (16; $C_{12}H_{22}N$, calc.: 180.1752, found: 180.1753), 168 (23), 166 (21; $C_{10}H_{16}NO$, calc.: 166.1232, found: 166.1237), 155 (27; $C_8H_{15}N_2O$, calc.: 155.1184, found: 155.1184), 152 (49; $C_9H_{14}NO$, calc.: 152.1075, found: 152.1072).

Preparation of N,N-diacetyldihydromyricoidine (acetylloesenerine) and N,N-diacetylmyricoidine.

Dihydromyricoidine 2 (9 mg) was dissolved in a 1:1 acetic anhydride-pyridine mixture (0.1 mL). After standing for one night at room temperature, EtOH and $CHCl_3$ were added and the solution was evaporated to dryness; the residue was dissolved in $CHCl_3$ and the solution was washed with dil. NH_4OH, dried and evaporated to give homogeneous (TLC) N,N-diacetyldihydromyricoidine 6 (11 mg): $M^{+\cdot}$ at m/z 379; $[\alpha]_D^{22}$ +89° (c = 1.1, MeOH); ^1H NMR: δ 0.89 (t, J = 7 Hz, CH_3), 2.1 (Ac-N), the general pattern of the spectrum indicates the presence of several conformers.

Loesenerine 4 (1 mg) was acetylated in the same experimental conditions to give acetylloesenerine identical with N,N-diacetyldihydromyricoidine 6 (TLC, ^1H NMR, MS, positive rotation in methanolic solution).

Myricoidine 1 (5 mg) was acetylated in the same experimental conditions to give N,N-diacetylmyricoidine (5 mg), $M^{+\cdot}$ at m/z 377.

Tetrahydromyricoidine 3.

Myricoidine 1 (20 mg) was dissolved in EtOH (2 mL) and after addition of some drops of acetic acid was hydrogenated over Pt at room temperature under 4 atm. for one night. Filtration and evaporation of the solvent yielded tetrahydromyricoidine 3 (oil), $[\alpha]_D^{22}$ +4° (c = 0.8, MeOH); ^1H NMR: δ 0.88 (t, J = 7 Hz, CH_3), 3.3 and 3.5 (m, H-4a and H-4b), 8.59 (broad signal, amide N-H), no signal between 4 and 8 ppm; ^{13}C NMR: δ 14.7 (C-20), 23.3, 26.3, 27.6, 28.4, 28.7, 29.9, 30.3, 32.4, 34.6, (39.9 and) 40.0 (C-4), 41.5, 45.7, 49.3, 50.1, 56.3 (C-8), 172.7 (C-6); MS: 297 ($M^{+\cdot}$, 15%), 280 (16), 254 (15), 198 (100), 195 (18), 182 (16), 168 (29), 155 (27).

Tetrahydromyricoidine (oil), $[\alpha]_D^{22}$ +4.2° (c = 0.6, MeOH) was also obtained, in the same experimental conditions, from dihydromyricoidine 2; the Mass and NMR spectra are identical with those described above.

Acetylation of tetrahydromyricoidine (pyridine-acetic anhydride 1:1) yielded N,N-diacetyltetrahydromyricoidine, MS: 381 ($M^{+\cdot}$, 5%), 338 (100), 267 (5), 240 (27).

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