A New Guanidine Alkaloid from Millettia laurentii

Dieudonne Ngamga, Samuel N. Y. Fanso Free, Z. Tanee Fomum, Marie-Therese Martin, and Bernard Bodo

J. Nat. Prod., **1994**, 57 (7), 1022-1024• DOI: 10.1021/np50109a026 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 7, pp. 1022-1024, July 1994

A NEW GUANIDINE ALKALOID FROM *MILLETTIA LAURENTII*[1]

DIEUDONNE NGAMGA, SAMUEL N.Y. FANSO FREE, Z. TANEE FOMUM,*

Département de Chimie Organique, Faculté des Sciences, Université de Yaoundé, BP 812, Yaoundé, Cameroon

MARIE-THERESE MARTIN, and BERNARD BODO*

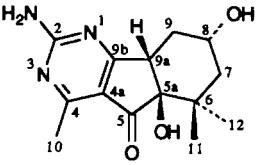
Laboratoire de Chimie, URA 401 CNRS, Muséum National d'Histoire Naturelle, 63, rue Buffon, 75005 Paris, France

ABSTRACT.—A new guanidine alkaloid, 5a,9a-dihydro-5a-hydroxymillaurine, has been isolated from the seeds of *Millettia laurentii* and its structure determined spectroscopically.

Millettia laurentii De Wild. (Leguminosae) is a tree found in the rain forest of Cameroon, North Gabon, and Zaire, and several parts of it are used in folk medicine (1). Interest in *Millettia* species arises mainly from their tendency to produce isoflavonoids and rotenoids which are responsible for their insecticidal activity (2). We previously described the isolation of alkaloids with a new skeleton, millaurine and acetylmillaurine from the seeds, and millettonine from the stem bark of *M. laurentii* (3,4). We now report the isolation and structure elucidation of a new guanidine alkaloid, 5a,9a-dihydro-5a-hydroxymillaurine [**1**] from the seeds of *M. laurentii*.

Compound **1** was purified from the EtOAc extract of the ground and defatted seeds of *M. laurentii* by Si gel cc and was obtained as a colorless solid which was further crystallized from EtOH (mp 259–260°). Eims showed a molecular ion at *m/z* 277 and hrms indicated the molecular formula $C_{14}H_{19}N_3O_3$.

The ^{13}C-nmr spectrum depicted signals for 14 carbon atoms, which represented three methyls, two methylenes, one methine, one oxymethine, and seven quaternary carbons, among which five were sp^2. The sp^2 carbon at δ_C 200.6 was assigned to a carbonyl carbon and the remaining sp^2 carbons had chemical shifts characteristic of a 2-amino-4-methyl-5,6-disubstituted pyrimidine ring, as previously described for millaurine and related compounds (3,4).



1

The ^{1}H-nmr spectrum was deceptively simple, showing only signals for one oxymethine, one methine, two methylenes, and three methyl groups. Two singlet signals for three exchangeable protons were also observed at δ_H 7.59 (2H) and 5.30 (1H), in addition to a doublet at δ_H 4.49 (1H), which was assigned to the secondary hydroxyl group. The ^{1}H-^{1}H COSY nmr spectrum indicated the oxymethine proton to be vicinal to the two methylenes (H_2-7 and H_2-9), and one of them (H_2-9) to be coupled to the remaining methine group, leading to the partial structure >CH-CH_2-CHOH-CH_2-.

The HMBC nmr spectrum (DMSO-d_6) gave information allowing the various structural units of **1** to be linked together (Table 1). Long-range correlations were observed between the carbon at δ_C 114.0 (C-4a) and both H-9a and CH_3-10. The carbonyl at δ_C 200.6 (C-5) gave long-range correlations with H-9a, and the hydroxyl at 5a. The oxygenated sp^3 quaternary carbon at δ_C 81.2 (C-5a) was correlated with H-7, H-9 and H-9a,

[1]Part 3 in the series, "The *Millettia* of Cameroon." For part 2, see Kamnaing *et al.* (4).

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for 5a,9a-Dihydro-5a-hydroxymillaurine [**1**].

Position	^{13}C[a]	^{1}H[a]		^{13}C long-range correlation (H)	^{1}H[b]	
2	165.0	—		—	—	
4	168.0	—		10; NH_2	—	
4a	114.0	—		9a; 10	—	
5	200.6	—		9a; OH (5a)	—	
5a	81.2	—		7; 9; 9a; 11; 12; OH (5a)	—	
6	36.6	—		11; 12; 9a; OH (5a)	—	
7	45.8	ax 1.11 m		9_{eq}; 11; 12; OH (8)	1.26 dd	−13.2; 8.9
	—	eq 1.73 dd	−13.2, 5.8	—	1.91 ddd	−13.2; 6.4; 1.7
8	63.6	ax 3.86 m		7_{ax}; 7_{eq}; 9_{ax}; 9_{eq}; OH (8)	4.07 dddd	11.1; 8.9; 6.4; 4.6
9	36.6	ax 1.14 m		9a; OH (8)	1.43 ddd	−13.3; 11.1; 11.0
	—	eq 2.20 m		—	2.40 dddd	−13.3; 4.6; 7.6; 1.7
9a	47.9	2.91 dd	11.2, 7.6	9_{ax}; OH (5a)	3.05 dd	11.0; 7.6
9b	184.9	—		9a; 9_{eq}	—	
10	20.7	2.41 s		—	2.51 s	
11	25.6	0.99 s		7_{eq}; 12	1.10 s	
12	26.7	0.93 s		7_{eq}; 11	0.99 s	
NH_2	—	7.59 s		—	—	
OH (C-8)	—	4.49 d	4.9	—	—	
OH (C-5a)	—	5.30 s		—	—	

[a]Spectrum determined in DMSO-d_6.
[b]Spectrum determined in CD_3OD.

methyls 11 and 12, and the hydroxyl at 5a. The quaternary carbon at δ_C 36.6 (C-6) was correlated with proton H-9a, methyls 11 and 12, and the hydroxyl group at C-5a. Finally, carbon C-9b gave long-range correlations with H-9 and H-9a.

As the ^{1}H-^{1}H coupling constant values for **1** in DMSO-d_6 solution were difficult to measure, the ^{1}H-nmr spectrum was obtained in CD_3OD solution where the signals were better resolved (Table 1). The large coupling constants of the proton at δ_H 4.07 (8_{ax}) with the protons at δ_H 1.26 (7_{ax}) and 1.43 (9_{ax}) and of this latter H-9_{ax} with the proton at δ_H 3.05 ($9a_{ax}$) indicated that they were all axial (Table 1). A *W* coupling (^4J=1.7 Hz) was observed between protons at δ_H 1.91 (7_{eq}) and 2.40 (9_{eq}) which confirmed their equatorial disposition. The results indicated that the hydroxyl group at C-8 was equatorial.

The stereochemistry (relative configuration) of the cyclohexane ring, which is in a chair conformation, was determined from coupling constant values (Table 1, CD_3OD) and nOe difference measurements (DMSO-d_6; Figure 1). Significant nOes were observed between H-$9a_{ax}$ and OH-5a (4%), H-9_{eq} (5%), Me-11 (4%) and H-8_{ax} (3%) on one hand, and between proton H-8 and H-9_{eq} (3.5%), H-7_{eq} (3.5%) and Me-11 (2%) on the other hand. The protons H-9a, H-8_{ax}, methyl-11, and the hydroxyl at C-5a were thus on the same side of the cyclohexane ring.

The structure of 5a,9a-dihydro-5a-hydroxymillaurine **1** is related to that of millaurine (3), from which it differs by a

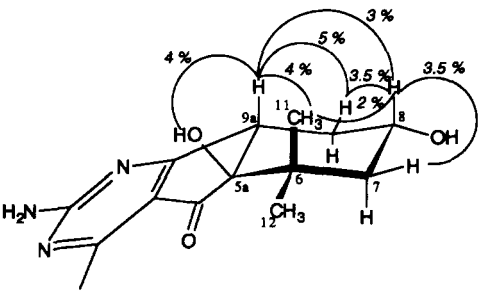


FIGURE 1. Observed NOes in **1** (DMSO-d_6).

cis-hydration of the C5a–C9a double bond. These compounds, along with millettonine (4), are the first alkaloids characterized from *Millettia* species, and have a new type of three-ring skeleton with a guanidine and a terpenic moiety. From a biosynthetic point-of-view it is clear that a part of the cyclohexanediol unit has an isoprenoid origin, and the pyrimidine moiety may arise from the ororate pathway as postulated for the unusual amino acid lathyrine (5,6).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra (^{1}H, 300.13 MHz and ^{13}C, 75.47 MHz) were performed on an AC 300 Bruker spectrometer. Ei and cims were obtained with a Nermag R 10-10 mass spectrometer.

PLANT MATERIAL.—Seeds of *Millettia laurentii* were collected in Yaoundé in May 1990. Voucher specimens identifying the sample are on deposit in the National Herbarium, Yaoundé.

EXTRACTION AND ISOLATION.—Ground seeds (28 kg) were defatted with hexane and then extracted with EtOAc to give 900 g of a residue. A portion (300 g) of this material was subjected to cc over Si gel. Elution with C_6H_6 followed by C_6H_6 containing increasing amounts of EtOAc gave from 40% EtOAc a fraction containing one major compound. This fraction was dissolved in hot EtOH and left to stand at room temperature. A colorless compound precipitated which was filtered and washed with cold EtOH to yield 5a,9a-dihydro-5a-hydroxymillaurine [**1**] (120 mg).

5a,9a-Dihydro-5a-hydroxymillaurine [**1**].—$C_{14}H_{19}N_3O_3$; colorless needles, mp 259–260° (EtOH); $[\alpha]^{21}D$ +6.8° (c=0.2, MeOH); ir (KBr) ν max 3436, 3326, 3222, 2976, 2950, 1708, 1632, 1588, 1572, 1492, 1372, 1346, 1264, 1068, 1032, 1010, 984, 974, 822, 616 cm^{-1}; eims *m/z* 277 (M^+, 17), 259 (19), 249 (10), 244 (5), 232 (8), 221 (9), 216 (11), 205 (7), 192 (23), 190 (13), 188 (8), 177 (29), 165 (66), 136 (100), 123 (25), 109 (6), 67 (43); hreims *m/z* [M^+], 277.1425 calcd for $C_{14}H_{19}N_3O_3$, 277.1426; cims (NH_3) *m/z* 278 $[M+H]^+$.

ACKNOWLEDGMENTS

We gratefully acknowledge the practical help of Mr. Jacques Yougang, formerly Director General of Forests Regeneration in Cameroon, with regard to the collection of the plant sample. We thank Dr. Jean-Paul Brouard for the mass spectra, and the Centre d'Analyses du CNRS, Lyon for hrms.

LITERATURE CITED

1. J.O. Kokwaro, "Medicinal Plants of East Africa." East Africa Literature Bureau, Nairobi, Kenya, 1976.
2. P.M. Dewick, in: "The Flavonoids: Advances in Research since 1986." Ed. by J.B. Harborne, Chapman and Hall, London, 1993, pp. 117–213.
3. D. Ngamga, S.N.Y. Fanso Free, Z.T. Fomum, A. Chiaroni, C. Riche, M.-T. Martin, and B. Bodo, *J. Nat. Prod.*, **56**, 2126 (1993).
4. P. Kamnaing, S.N.Y. Fanso Free, Z.T. Fomum, M.-T. Martin, and B. Bodo, *Phytochemistry*, in press.
5. E.G. Brown and N.F. Al-Baldawi, *Biochem. J.*, **164**, 589 (1977).
6. E.G. Brown and J. Mohamad, *Phytochemistry*, **29**, 3117 (1990).

Received 7 March 1994