



Myrciaine, a new nicotinic ester from *Myrcia blanchetiana* (Myrtaceae)

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ABSTRACT

A nicotinic ester with a new carbon skeleton named myrciaine was obtained from hexane extract of stems of *Myrcia blanchetiana* using chromatographic methods and from leaves by supercritical CO₂. The molecular structure was established on the basis of extensive NMR studies and HREIMS. Myrciaine did not display substantial activity against Gram-negative and Gram-positive bacteria neither against the fungi *Aspergillus niger* and *Cladosporium cladosporioides*. Density functional theory (DFT) methods were applied to analyze the stability of two possible conformational structures. The calculated relative energy between the optimized conformers has confirmed the proposed structure based on the experimental results.

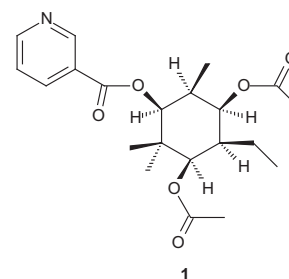
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Indigenous tribes and traditional Brazilian communities use some species of the genus *Myrcia* as astringent, against diabetes and diarrhea, as diuretic, to stanch hemorrhages, against the hypertension and ulcers of the mouth.¹ The chemical composition of non-volatile components from species of this genus is little-known. Previous studies had revealed the occurrence of eucalyptin, a C-methylated flavone, and β-amyrin from *Myrcia citrifolia*,² flavanone glycosides, acetophenone glycosides, and flavonols from *Mirabilis multiflora*.^{3,4} From the essential oils of eighteen *Myrcia* species sesquiterpenes were predominant except for *Myrcia acuminatissima* and *Mammillaria bombycina* for which the monoterpenes fraction is slightly larger^{5–8} and for *Micromeria myrtifolia* which presented a high content of monoterpenes.⁹ *Myrcia blanchetiana* is a wild shrub that occurs in campo rupestre areas (rocky fields) near Andaraí, at Chapada Diamantina, Bahia, northeastern of Brazil, and was in bloom when stems and leaves were collected. From our knowledge, in the literature there is no report on its chemical and biological properties or their traditional use.

In this work we describe the isolation and structure elucidation of a nicotinic ester with new carbon skeleton from the stems and leaves of *M. blanchetiana*. As a complement, DFT calculations at the B3LYP/6-31G(d,p) level^{10,11} were performed to investigate the conformational stability of the isolated compound. Until 2010, no

alkaloid has been isolated from Myrtaceae. In the literature only one paper describes the detection of alkaloids in *Syzygium cumini*¹² and another describes the isolation of 1,2,3,4-tetrahydro-1-methyl-β-carboline alkaloid from *Calycorectes psidiiflorus*.¹³ Thus, compound **1**, named myrciaine, is only the second alkaloid isolated from a Myrtaceae species.

The dried stems were extracted with hexane to give a brown gum (945 mg). This extract was submitted to a chromatography column on silica gel (eluent: from hexane to EtOAc; from EtOAc to MeOH) to give white crystalline 3β-acetoxi-olean-18-en-28-oic acid (76 mg) and compound **1** (42 mg) as a colorless gum [α]_D²⁵ –6.9 (c 0.53, CHCl₃). Compound **1**, was also observed as the major component of the extract obtained from leaves after a selective extraction with supercritical CO₂ at 40 °C and 2000 psi by 1 h.



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Its molecular formula, $C_{21}H_{29}NO_6$, was established on the basis of the molecular ion peak at m/z 391.1954, in HREIMS and NMR data. Its IR spectrum showed strong absorptions in 1728, 1288, and 1242 cm^{-1} indicating the presence of ester. The ^1H NMR spectrum (Table 1) showed four aromatic proton signals at δ 9.50 (s, 1H), δ 8.82 (d, 1H; 3.9 Hz), δ 8.48 (dd, 1H; 8.0 and 1.8 Hz), and δ 7.45 (dd, 1H; 8.0 and 4.8 Hz) consistent with a 3-monosubstituted-pyridine ring. The base peak at m/z 124, corresponding to ion $C_6H_6NO_2^+$ (protonated nicotinic acid) and the fragment at m/z 106, corresponding to ion $C_6H_4NO^+$ in the mass spectrum, suggest the presence of a nicotinic moiety which was confirmed by ^{13}C NMR data [δ 165.1 (COO), δ 153.9 (CH), δ 151.6 (CH), δ 138.0 (CH), δ 126.5 (C), and δ 123.8 (CH)] (Table 1). In the aliphatic region, the ^1H NMR spectrum showed three oxymethine proton signals at δ 5.33 (t, 1H; 6.6 Hz), δ 5.10 (d, 1H; 2.8 Hz), and δ 5.04 (d, 1H; 3.1 Hz) with corresponding ^{13}C NMR signals at δ 71.1, 78.5, 75.6, respectively. The signals at δ 2.11 (s, 3H) and at δ 2.13 (s, 3H) in the ^1H NMR spectrum and at δ 21.4 ($2 \times \text{CH}_3$), δ 171.1 and 171.3 in the ^{13}C NMR spectrum suggested the presence of two acetate groups. Two signals in a complex splitting pattern were observed at δ 2.32 and 1.96 which were assigned for two methine hydrogens between two oxymethine carbons. Four signals assigned to methyl groups were observed as singlet at δ 1.20, triplet at δ 0.97 (7.4 Hz) in a partial overlap with a singlet at δ 0.95 and this in a partial overlap with a doublet at δ 0.94 (6.1 Hz).

The HOMOCSY spectrum revealed the connections of the hydrogens at δ 5.10 (H-8) with δ 2.32 (H-13); δ 2.32 (H-13) with δ 5.10 (H-8), δ 5.33 (H-12) and δ 0.94 (H-18); δ 5.33 (H-12) with δ 2.32 (H-13) and δ 1.96 (H-11); δ 1.96 (H-11) with δ 5.33 (H-12), δ 5.04 (H-10) and δ 1.36 (H-14), establishing a well defined splitting pattern among these protons. The carbon skeleton of molecule and the exact positions of the nicotinoyl and acetyl groups in the aliphatic moiety were completely established by the connectivities observed in the HMBC spectrum (Table 1). The cross peaks showed by the signals of methine protons at δ 5.10 (H-8) with C-7, C-9, C-13, C-12, C-16, and C-18 and those of the signal at δ 5.04 (H-10) with C-8, C-9, C-11, C-12, C-16, C-17, and C-21 permitted to trace the carbon backbone of aliphatic moiety as well as to locate the positions of one methyl, gem-dimethyl, nicotinoyl, and one acetyl groups. The localization of the ethyl and of the

reminder acetyl groups in the molecule skeleton was obtained from the correlations between the signal at δ 5.33 (H-12) with the carbons C-10, C-11, C-13, and C-19 and of the signal at δ 0.97 (H-15) with C-11 and C-14.

The relative stereochemistry was established by careful NOE difference experiments (Fig. 1). The selective irradiation of protons allowed the establishment of spatial correlations between them, thus the correlations of H-2 and H-4 with both acetyl-Me H-20 and H-22 suggested that the nicotinoyl and acetyl groups are in the same face of molecule (α -face). The correlations among the methyl group H-16 with H-8, H-10, H-11, and H-13 indicated that these hydrogens are in the β -face of the molecule. The correlations observed between the hydrogens H-2 and H-4 with both acetyl-Me as well as the magnitudes of the coupling constants of hydrogens H-8 ($J = 2.8\text{ Hz}$), H-10 ($J = 3.1\text{ Hz}$), and H-12 ($J = 3.3\text{ Hz}$) are consistent with a molecule in a conformational arrangement where nicotinoyl and both acetyl groups are in axial. A molecular model examination demonstrated that a conformation with these groups in axial positions (Fig. 2A) is, apparently, more stable. The conformation with these groups in equatorial (Fig. 2B) is destabilized due to a strong 1,3-diaxial repulsion among C-14, C-17, and C-18. To confirm these findings, we have optimized the geometry of two different conformational structures within DFT/B3LYP/6-31G(d,p) level using the Gaussian 03 program.¹⁴ The frequency calculations indicate that both conformers correspond to minimum energy structures. The results showed that the molecular structure with these groups in the axial positions is ca. 10.4 kcal/mol more stable than the structure with nicotinoyl and both acetyl groups in the equatorial positions. The results also revealed that the exocyclic C–O bond of both acetyl and nicotinoyl groups are eclipsed due to repulsions between the carbonyls and equatorial substituents on the adjacent carbons while the carbonyls are in a synperiplanar arrangement with oxymethine hydrogen.¹⁵

Compound **1** did not display substantial activity ($IC_{50} > 100\ \mu\text{M}$) when tested against the Gram-negative bacteria *Escherichia coli* (ATCC 94863), *Salmonella choleraesuis* (ATCC 14028), and *Pseudomonas aeruginosa* (obtained of an isolate in the Faculty of Pharmacy - UFBA), Gram-positive bacteria *Streptococcus mutans* (ATCC 5175), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (ATCC 10240) or the fungi *Aspergillus niger* (ATCC 16404) and *Cladosporium cladosporioides* (IMI 178517).

A possible pathway to the biosynthesis of myrciaine was delineated in Scheme 1. The alternating oxygenation pattern of aliphatic moiety suggested that it could have been synthesized from four units of AcCoA (Scheme 1). This is a plausible assumption by the fact that some Myrtaceae species produce acetophenones.^{4,16} Subsequently, three successive methylations with SAM, reduction of carbonyl and esterifications with AcCoA and nicotinoyl-CoA led to the formation of compound **1**.

Table 1
 ^1H , ^{13}C and HMBC data for compound **1**

No.	^1H (m, J, Hz)	^{13}C	HMBC (H to C)
2	9.50 (s)	151.6	3, 4, 6, 7
3	—	126.5	—
4	8.48 (dd, 8.00; 1.8)	138.0	2, 6, 7
5	7.45 (dd, 8.00; 4.8)	123.8	3, 6
6	8.82 (d, 3.9)	153.9	2, 5
7	—	165.1	—
8	5.10 (d, 2.8)	78.5	7, 9, 12, 13, 16, 18
9	—	39.2	—
10	5.04 (d, 3.1)	75.6	8, 9, 11, 12, 16, 17, 21
11	1.96 (m)	41.7	14
12	5.33 (t, 3.3)	71.1	10, 11, 13, 19
13	2.32 (m)	34.6	18
14	1.36 (m)	21.6	10, 11, 12, 15
15	0.97 (t, 7.4)	11.9	11, 14
16	1.20 (s)	25.7	8, 9, 10, 17
17	0.95 (s)	23.3	8, 9, 10, 16
18	0.94 (d, 6.1)	14.3	8, 12, 13
19	—	171.1 ^a	—
20	2.13 (s)	21.4	19
21	—	171.3 ^a	—
22	2.11 (s)	21.4	21

Mesured at 500 MHz (^1H) and 125 MHz (^{13}C) in CDCl_3 .

^a The signals can be changed.

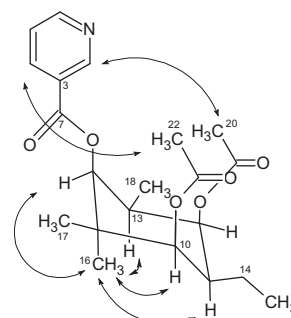


Figure 1. Some important NOE interactions of compound **1**.

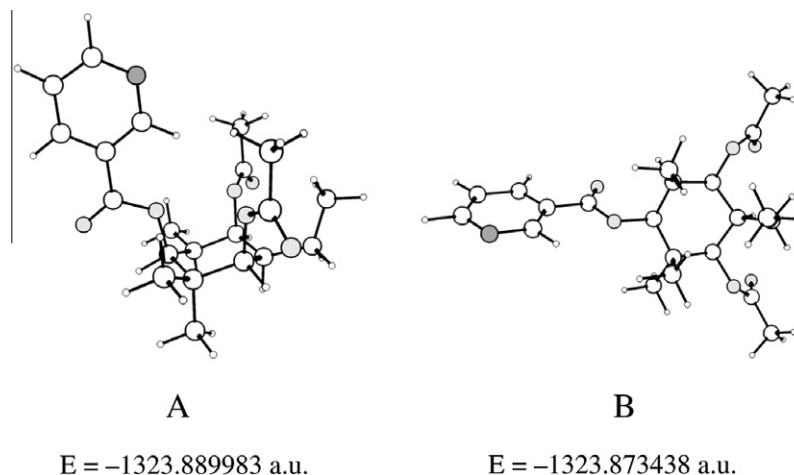
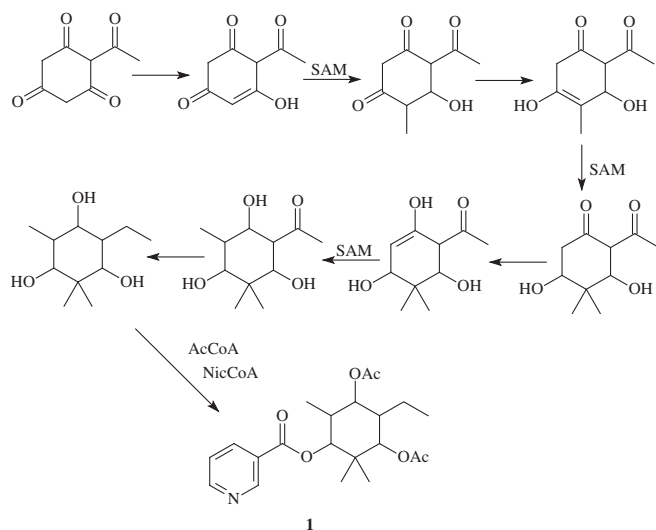


Figure 2. Optimized structures of two isomers obtained at the B3LYP/6-31G(d,p) level of theory. The calculated relative energy between isomers A and B is of 10.4 kcal/mol.



Scheme 1. Proposed biogenesis for compound 1.

Acknowledgments

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Supplementary data

Supplementary data (experimental details, 1D and 2D NMR spectra) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2012.12.117>. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

- Russo, E. M. K.; Reichelt, A. A. J.; Desa, J. R.; Furlanetto, R. P.; Moises, R. C. S.; Kasamatsu, T. S.; Chacra, A. R. *Braz. J. Med. Biol. Res.* **1990**, *23*, 11.
- Gottlieb, O. R.; Silva, M. L.; Maia, J. G. S. *Phytochemistry* **1972**, *11*, 1185.
- Matsuda, H.; Nishida, N.; Yoshikawa, M. *Chem. Pharm. Bull.* **2002**, *50*, 429.
- Yoshikawa, M.; Shimada, H.; Nishida, N.; Li, I. Y.; Toguchida, I.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 113.
- Henriques, A. T.; Sobral, M.; Bridi, R.; Vêrin, P.; Menut, C.; Lamaty, G. *J. Essent. Oil Res.* **1997**, 913.
- Zoghbi, M. D.; Andrade, E. H. A.; da Silva, M. H. L.; Carreira, L. M. M.; Maia, J. G. S. *Flavour Fragr. J.* **2003**, *18*, 421.
- Limberger, R. P.; Sobral, M.; Henriques, A. T.; Menut, C.; Bressière, J. M. *Quim. Nova* **2004**, *27*, 916.
- de Cerqueira, M. D.; Souza-Neta, L. C.; Passos, M. G. V. M.; Lima, E. O.; Roque, N. F.; Martins, D.; Guedes, M. L. S.; Cruz, F. G. *J. Braz. Chem. Soc.* **2007**, *18*, 998.
- De Cerqueira, M. D.; Marques, E. J.; Martins, D.; Roque, N. F.; Cruz, F. G.; Guedes, M. L. S. *Quim. Nova* **2009**, *32*, 1544.
- Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
- Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.
- Gowri, S. S.; Vasanth, K. *Int. J. PharmTech Res.* **2010**, *2*, 1569.
- Domingues, E. A.; Nakamura, C. V.; de Souza, M. C.; Teixeira, T. S.; Peixoto, J. L. B.; Sarragiotto, M. H.; Vidotti, G. J. *Braz. J. Pharmacogn.* **2010**, *20*, 23.
- Frisch, M. J. et al. Gaussian 03, Revisions D.01; Gaussian: Wallingford, CT, 2004.
- González-Outeiriño, J.; Nasser, R.; Anderson, J. E. *J. Org. Chem.* **2005**, *70*, 2486.
- Al-Sayed, E.; Martiskainen, O.; Bobrowska-Hagerstrand, M.; Sinkkonen, J.; Tornquist, K.; Pihlaja, K.; Ayoub, N.; Singab, A. *Nat. Prod. Commun.* **2010**, *5*, 1639.