April 7, 2008

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Dear Professor Compagnone:

This short note is to express my deepest gratitude to you and your colleges for dedicating to me, in occasion of my sixty fifth birthday, the work “New Guanidine Alkaloids from the Leaves of Verbesina peraffinis” by Reinaldo S. Compagnone, Jhorman Bermudez, Glorymar Ibáñez, Beth Díaz, María R. Garrido, Anita Israel and Alirica I. Suárez, that just appeared in Natural Product Communications, 4, 511-514 (2008).

I am delighted by this situation and send, together with my appreciation, best wishes to you and to your colleges.

Very truly yours,

Pedro Joseph-Nathan
Editorial

In Honor of the 65th Birthday of Professor Pedro Joseph-Nathan

It is my privilege and pleasure to introduce this issue, which is dedicated to Professor Pedro Joseph-Nathan, Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Apartado 14-740, México, D. F., 07000 Mexico, on the occasion of his 65th birthday.

The editors join me in paying tribute to Professor Joseph-Nathan for his outstanding contributions to various aspects of Organic Chemistry. A peculiar characteristic of Prof. Joseph-Nathan’s trajectory is the scientific cooperation among nineteen countries of Latin America, Spain and Portugal. He has coordinated and worked as an advisor in the development of research projects within the Ibero American Program of Science and Technology for Development (CYTED). He has authored more than 380 original papers, reviews, books and monographs. Professor Joseph-Nathan is internationally recognized for his outstanding research in Organic Chemistry, particularly structure establishment, chemical reactivity and synthesis of natural products, emphasizing the application of nuclear magnetic resonance to solve structural and mechanistic problems.

It is a great pleasure to honor the scientific achievements of Professor Pedro Joseph-Nathan on the occasion of his 65th birthday and to send him warm wishes from all his colleagues and friends. My thanks go to the authors and reviewers who have made this issue of *Natural Product Communications* possible and to our production department for their efforts in putting this issue in print.

On the occasion of his 65th birthday, the March and April issues of 2008 will be comprised entirely of contributions honoring Professor Joseph-Nathan.

We all express our appreciation for his excellent contributions to natural products and NMR and wish him all the best for his future.

Pawan K. Agrawal
Editor-in-Chief
New Guanidine Alkaloids from the Leaves of
Verbena peraffinis

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This paper is dedicated to Professor P. Joseph-Nathan for his 65th birthday.

Two new guanidine alkaloids, 1 and 2, were isolated from the methanol extract of the leaf of Verbena peraffinis, in addition

to four known substances: galegine, the methylamido of caffee acid, galactitol and the flavonoid 3-O-glucosyl-(1-2)-
galactoside-5,7,4'-trimethoxy-kaemperol. Their structures were determined on the basis of spectroscopic methods. The

anti hypertensive activity of the methanolic extract was assayed in rats using a model of hypertension induced by footshock.

Keywords: Verbena peraffinis, Moraceae, guanidine alkaloids, caffee amide.

Verbena is a genus of the Asteraceae with about 300 species, widespread in tropical regions of

America [1]. According to botanical sources there are twenty-one endemic species in Venezuela [2].

Members of the Verbena genus have been used in folk medicine for the treatment of disease,
external wounds, hypertension, and inflammation. Metabolites from this genus include triterpenes,
diterpenes, flavonoids and guanidine derivatives [3-7]. Five novel guanidine alkaloids isolated
from V. baracasana Fries showed a significant anti hypertensive activity [8-10]. In the

pharmaceutical market, some drugs with a guanidine moiety have been used in anti hypertensive therapy
with high efficiency [11-13].

Due to the anti hypertensive activity shown by the guanidine derivatives isolated from V. caracasana

growing in Venezuela [7-9], the aim of this research was to investigate the presence of compounds with

similar structure in V. peraffinis Blake.

From the methanol extract of the aerial parts of Verbena peraffinis, three guanidines (1, 2), and galegine, a

sugar known as galactitol, methylamido of caffee acid, and the 3-O-glucosyl-(1-2)-galactoside-5,7,4'-
trimethoxy-kaemperol, were obtained and purified by repeated chromatography on Sephadex LH-20 CC

alternating with a C-18 silica gel reverse phase phase cartridge.

Compound 1, the major metabolite isolated from the methanol extract, was obtained as a brown solid,
which exhibited in the ESI mass spectrum an ion at m/z 186.26. From this ion, its molecular composition
was deduced as C13H16N6. The 'H NMR spectrum of this compound was very simple showing only two

singlet signals, one at 6 3.07 (12 H) and the second at
δ 3.71 integrating for two protons. The 13C NMR spectrum of 1 showed resonances for only three carbons. The DEPT spectrum identified one signal belonging to a methyl group with a chemical shift at δ 53.3, characteristic of the methyl substituents of electronegative atoms such as nitrogen (N-CH3), and a second signal belonging to a methylene was distinguished at δ 61.2. Finally, the signal of a quaternary carbon at δ 159.1 suggested an imine carbon in the structure. The simplicity of the spectra indicated that the analyzed compound was a symmetrical molecule. The above inference was confirmed through the correlations obtained from the HMOC and HMBE experiments. Comparison of these data with guanidine compounds such as galegine (3) [13], also isolated in this work, indicated the presence of two guanidine units in the isolate. On the basis of the above spectral observations, compound 1 was deduced to be 1,1-bis-N1,N1'-tetr methylguanidinomethane (1).

Compound 2 was isolated as a colorless gum and the molecular ion peak at m/z 229.3461 in the HREIMS indicated its molecular formula to be C10H2N4, suggesting two degrees of unsaturation in the analyzed structure. The 13C NMR spectrum taken in D2O, and assigned with the help of a DEPT spectrum, showed the presence of four methyl groups at δ 37.3 as substituents of guanidine units and four methylenes at δ 53.3, 66.9, 70.3 and 75.1. The presence of two imine functions was evident from the signals at δ 159.6, and 158.4. The 1H NMR spectrum of 2 showed very poor resolution as a group of overlapped broad signals, possibly due to the exchange of protons with deuterium. However, this spectrum was useful, together with the HMOC and HMBE experiments, to obtain the correlations in the new guanidine compound 2. It was assumed that the compound is protonated, which explained the unusual chemical shifts for methyl and methylene carbons. The fragmentation obtained from the EI mass spectrum showed fragments (Figure 2) that supported the structure of compound 2 as 1,4-bis-N1,N1'-tetr methylguanidinobutane.

**Effects of V. peraffinis on cardiovascular parameters:** Acute stress induced by footshocks cause sympathoadrenal activation with increases in arterial pressure and heart rate [14]. I.p. administration at doses of 22.5 mg/kg (1/4 TD50) and 45.0 mg/kg (1/2 TD50) of the methanolic extract of V. peraffinis were evaluated for cardiovascular parameters, such as mean arterial pressure (MAP) and heart rate (HR). The results, as shown in Figure 3, indicated that the administered doses produce dose-dependent hypotension, because the lowest values of the MAP were obtained with the higher administered doses. Similar results were obtained for heart rate (data not shown).

**Experimental**

**General:** 1H and 13C NMR spectra were recorded on a Bruker AMX instrument operating at 500 MHz and 125 MHz, respectively. IR spectra were obtained on a FT-IR Thermo Nicolet Nexo 470 model. Electrospray ionization (ESI) mass spectrometric analyses were performed with an Applied Biosystems model 3200 Q-Trap, and electron impact (EI) ionization was performed with a Varian Saturn mass spectrometer, using 70 eV ionization conditions. Analytical thin-layer chromatography (TLC) was performed on precoated Merck plates. Column chromatography...
was carried out on silica gel (200-300 mesh) of Merck, Sephadex LH-20 (Fluaka) and RPC-18 (Merck).

**Plant material:** The aerial parts of *Verbesina perennis* were collected from the area surrounding Caracas city in March 2006. The identity of the plant material was verified by Dr Stephen Tillet of the Herbarium Victor Manuel Ovalles of the Facultad de Farmacia, Universidad Central de Venezuela, where a voucher specimen has been deposited with the number 26070.

**Extraction and isolation:** Leaves of *V. perennis* were air dried and powdered. The dried material (580.3 g) was extracted with MeOH in a Soxhlet apparatus for 24 h. The extract was evaporated and dissolved in a mixture of EtOAc/H2O 1:1. The water soluble fraction of the MeOH extract was subjected to lyophilization to obtain a brown material, which was dissolved in MeOH and filtered to obtain 21.3 g of insoluble and 45.4 g of methanol extract, which was subjected to Sephadex LH-20 column chromatography using MeOH as solvent to afford seven fractions. The fractions were further rechromatographed to obtain the pure compounds. Chromatographic purification using a RP-18 cartridge and the solvent mixture H2O: MeOH (7:3) gave: F1, galactitol (27.6 mg) [15], F2, the methylamide of caffic acid (24.9 mg) [16], F4, the 3-O-glucosyl-(1-2)-galactoside-5,7,4'-trimethoxy-caempferol (48.6 mg)[17], F5 was further separated by reverse phase using H2O: MeOH (1:1) to yield galegine (15.1 mg) [18]; and F6 was submitted to further separation with RP-18 and H2O: MeOH (1:9) to give compounds 1 and 2. All the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

**Antihypertensive assays:** [19] For this experiment, male Sprague Dawley rats (160-250g) were used and maintained in a laboratory animal unit with a 12 h light dark cycle. Water and pellet diets were supplied ad libitum. All the experiments were performed in accordance with Institutional Guidelines for the ethical care of animals. The TD50 was determined using the Probit method [20]. Thirty rats were divided into three groups; one control group was administered with saline solution, the other groups received i.p. doses of 22.5 mg/kg (1/4 TD50) and 45.0 mg/kg (1/2 TD50) of the methanolic extract. Systolic and diastolic pressure and heart rate were recorded using a tail-cuff digital pletysmograph. After 25 min of drug treatment, the animals were transferred to a Plexiglass chamber with a copper rod floor where they received mild footshocks (2.0 Hz, 100V, 5 ms, for 5 min), delivered by a Grass stimulator. All data are expressed as the means ± S.E.M. Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA). A value of p<0.05 was considered significant.

1,1-Bis-N1,N1'-tetramethylguanidinomethane (1)

RI: 0.3 (EtOAc: MeOH, 5:1).
IR (film): 3364, 1631 cm-1.
1H NMR (500 MHz, D2O) δ: 3.07 (12H, s, Me), 3.71 (2H, s, CH3).
13C NMR (125 MHz, D2O) δ: 53.3 (CH2), 61.2 (CH3), 159.1 (C=NH).
ESIMS: m/z 186.26 [M]+.

1,4-Bis-N1,N1'-tetramethylguanidinobutane (2)

1H NMR (500 MHz, D2O) δ: 1.90 (6H, brs, CH3), 2.09 (6H, brs, Me), 3.27 (4H, brs, CH3), 3.87(2H, brs, CH2).
13C NMR (125 MHz D2O) δ: 23.8 (CH2), 29.1 (CH2), 37.9 (CH3), 45.7 (CH2), 61.3 (CH3), 67.8 (CH3), 70.1 (CH3), 75.4 (CH3), 158.5 (C=NH), 159.4(C=NH).
MS (EI, 70 eV) m/z (%): 230 [M+1] (100), 215 (10), 129 (65), 129 (56).
HREIMS: m/z 229.3461 [M]+.

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**References**


