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1-Amino-3-methylcyclobutane carboxylic acid in Seeds of *Ateleia glazioviana* Baillon *(Leguminosae)*

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SUMMARY. The structure of a novel nonprotein amino acid isolated from the seeds of *Ateleia glazioviana* Baillon was established as 1-amino-3-methylcyclobutane carboxylic acid on basis of spectroscopic evidences. For the isolation, the ethanol-water extract was successively extracted with dichloromethane, ethyl acetate and n-butano. Thereafter, the amino acids were separated from the remaining water phase using a cation-exchange resin column and further submited to column chromatography on silica gel to yield 80 mg (0,02%) of the new amino acid.

RESUMEN. "Acido 1-amino-3-metilciclobutano carboxílico en Semillas de *Ateleia glazioviana* Baillon *(Leguminosae)*". Un nuevo aminoácido del tipo no proteico, el ácido 1-amino-3-metilciclobutano carboxílico, fue aislado de las semillas de *Ateleia glazioviana* Baill. extraídas con etanol 50%. El extracto alcohólico, después de ser purificado por extracción sucesiva con diclorometano, acetato de etilo y *n*-butanol, fue fraccionado en una columna con resina de intercambio iónico. La separación final fue realizada por CC con sílica gel, con un rendimiento final de 0,02%. La estructura química fue establecida a través de IR, EIMS de alta resolución y ^{13}C y ^{1}H-RMN.

INTRODUCTION

Ateleia glaziovíana is a native tree of Rio Grande do Sul, Brazil. It is popularly known as "timbó" and it has been reported to be toxic to cattle and fish and repellent to home insects [1]. In previous work, the ichthyotoxic activity of the aqueous extracts of leaves could be proven [2]; the dichloromethane and the amino acid fractions of the aqueous extract were shown to be responsible for the abortions or reabsorptions of foetusses in female rats [3]. When leaves of *Ateleia glazio-*

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KEY WORDS: 1-amino-3-methylcyclobutane Carboxylic Acid, *Ateleia glazioviana, Leguminosae,* Nonprotein amino acid, "Timbó".
PALABRAS CLAVE: Acido 1-amino-3-metilciclobutano carboxílico, Aminoácido no proteico, *Ateleia glazioviana, Leguminosae,* "Timbó".

viana were eaten by cows in different stages of gestation occurs apathy, recumbency, vulvar edema, retention of the placenta, endometritis and abortion [4]. Chemical investigations allowed the identification of rutin [5], the isoflavones afrormosin and 5-methoxyafrormosin [6], the free amino acids asparagine, leucine, valine, arginin, lysine, serine, alanine and threonine in the leaves [1]. Furthermore, the presence of three nonprotein amino acids were detected in the leaf extracts. From the seeds, which also accumulated these nonprotein amino acids, they were isolated and two of them were identified as the already known compounds δ-acetylornithine and 1-amino-1,3-dicarboxycyclobutane acid [7]. The last compound was first isolated from another species of the same genus, *Ateleia herbert-smithii* Pittier, whose seeds are ignored by at least 100 seed predators in its habitat, and considered to have a possible role in protecting seeds from insect predation [8].

The present paper describes the structure elucidation of a third nonprotein amino acid from the seeds of *Ateleia glaziovíana*.

EXPERIMENTAL

Plant material

The seeds of *Ateleia glaziovíana* were collected in Palmeira das Missões (RS, Brazil) in January 1990. A herbarium specimen is deposited in the Herbarium of the Botany Department of the Federal University of Rio Grande do Sul, Porto Alegre, Brazil (ICN 94460).

General experimental

IR spectrum was taken with a Shimadzu FT-IR, mod. 408. For TLC, precoated silica gel $60F_{254}$ (Merck) ^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AM 300 WB spectrometer (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75.5 MHz) and Bruker DMX 500 MHz (500MHz/125MHz). EIMS was obtained on a Varian MAT 44 Spectrometer; High resolution EIMS on a Varian MAT 311 Spectrometer.

Isolation

See ref. [7] for details.

ANP$_1$

White powder, Mp > 200 °C (dec.)ν_{max} KBr (cm^{-1}): 3440 br, 3100-2300 br, 1640, 1580, 1450, 1420, 1175, 1047, 860, 778, 663, 573. EIMS (direct insert), 70 eV, m/z (rel. int.): 129 (0.5 %), 111 (0.7 %), 96 (1.0 %), 87 (100 %), 84 (4%).High resolution EIMS (direct insert), 70 eV, m/z (rel. int.): 129.0791 (0.24%) for $C_6H_{11}NO_2$ (calc. 129.07897); 87.0318 (100%) for $C_3H_5NO_2$ (calc. 87.03203), 84.0 (2.7 %), 69.0 (3.5 %), 60.0 (4.4 %), 42.0 (40.3 %). ^{1}H-NMR (D_2O with DSS as internal standard): δ 1.09 (3H, *d*, *J* = 6.3 Hz, H-3), 1.91 (2H, *dd*, *J* = 11.2, 8.0 Hz), 2.45 - 2.63 (3H, *m*). ^{13}C-NMR (D_2O with DSS as internal standard): δ 24.0 (*q*, CH_3 at C-3), 25.7 (*d*, C-3), 57.4 (*s*, C-1), 180.2 (*s*, COO- at C-1).

RESULTS AND DISCUSSION

The amino acids present in 0.5 kg seed were extracted with ethanol 50% (v/v). The unpolar and phenolic compounds were eliminated by successive extraction with dichloromethane, ethyl acetate and *n*-butanol; thereafter the amino acids were separated on a cation-exchange resin column. After purification by column chromatography on silica gel [7], compound (I) was isolated as white powder (80 mg).

In comparison with the others nonprotein amino acids isolated from the seeds of Ateleia glazioviana [7], compound (I) is a minor ninhydrin-reacting component and shows a different chromatographic behavior, behaving as a neutral amino acid when chromatographed on silica gel TLC in *n*-BuOH-HOAc-H_2O (12:03:05) (Rf = 0.40). The IR spectrum was typical of a neutral amino acid and indicated no other functional groups.

The molecular formula was established as $C_6H_{11}NO_2$ by high resolution EIMS. Its ^{13}C NMR spectra contained signals for five carbon resonances. Comparison of the ^{13}C NMR spectra of 1-amino-1,3-dicarboxycyclobutane [7,8] and compound (I), indicated that both compounds are close related, being (I) also a cyclobutane derivative, as it displays one more intensive ^{13}C NMR signal for two symetrical methylene groups. In contrast to the spectra of the acidic amino acid, (I) contains only one resonance for carboxylic group (d 180.2) and an additional one for a methyl group (δ 24.0). The ^1H NMR spectrum displayed three groups of protons, a doublet at δ 1.09 (3H, J = 6.3 Hz) for the methyl group, a doublet of doublets at δ 1.91 (J = 11.2 and 8.0 Hz) and a signal with a complex pattern (3H, δ 2.4 - 2.6). H-H and H-C irradiation experiments proved the coupling between the protons at δ 1.91 and δ 2.4 - 2.6 (H_a and H_b at the methylene groups of the cyclobutane ring); the irradiation at δ 1.91 turned the triplet of the methylene carbon at δ 40.3 to a doublet; the same effect was observed by the irradiation at δ 2.5, allowing to confirm the connectivity of hydrogens at these δ values to the methylene carbons. Additionally, the irradiation at δ 2.5 turned the doublet of the methyne group at the ^{13}C NMR spectrum at δ 25.7 in to a singlet, confirming the attributions of the ^{13}C and ^1H NMR signals.

The EIMS showed the same base peak at *m/z* 87, observed for 1-amino-1,3-dicarboxycyclobutane [7,8], attributed to the fragment [H_2C=C-COOH -NH_2] and also an intensive peak at *m/z* 42 (40 %), which can be rationalized in terms of a retro cycloaddition as [H_3C-CH=CH_2]

From these data we propose for compound (I) the structure 1-amino-3-methyl-cyclobutane carboxylic acid. The compound did not show optical activity experimental; the same phenomenon was observed for the acidic amino acid, 1-amino-1,3-dicarboxycyclobutane, isolated form the seed of *Ateleia herbert-smithii* [8].The geometric position of the methyl group in compound (I) could not be proven. It is expected to be *trans* to the carboxy group, as in the acidic amino acid, whose structure was proven by x-ray analysis [8].

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