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A TETRAHYDROPROTOBERBERINE ALKALOID FROM *CROTON HEMIARGYREUS*

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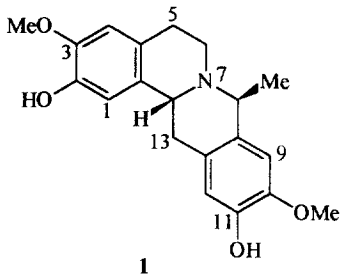
Key Word Index—*Croton hemiargyreus*; Euphorbiaceae; alkaloids; hemiargyrine; glaucine; oxoglaucine; salutaridine; norsalutaridine.

Abstract—A new tetrahydroprotoberberine alkaloid, 2,10-dihydroxi-3,10-dimethoxy-8β-methyldibenzo[a,g]quinolizidine (hemiargyrine), was isolated from *Croton hemiargyreus*. Other bases present were glaucine, oxoglaucine, salutaridine and norsalutaridine. Their structures were elucidated using spectroscopic techniques.

INTRODUCTION

A preliminary examination of Brazilian Euphorbiaceae showed that plants of the genus *Croton* were most likely to contain alkaloids [1]. One of the first to be investigated was *C. salutaris*, which was found to contain salutaridine [2] and its racemic form salutarine [3]. In the present paper, we report the isolation and characterization of five alkaloids from *C. hemiargyreus* one of which, hemiargyrine, is a new compound.



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RESULTS AND DISCUSSION

An ethanolic extract of the leaves and stems of *C. hemiargyreus* was partitioned between $CHCl_3$ and 5% aqueous HCl to separate basic material. Separation of individual substances was made by various chromatographic procedures, to afford five alkaloids of which one, a new tetrahydroprotoberbine, was the major constituent of the crude base mixture. The known alkaloids were identified as the two aporphines, glaucine and oxoglaucine, and the two morphinandienones, salutaridine and norsalutaridine, by comparison of their physical and spectroscopic properties (mp, IR, UV, mass spectrum, NMR) with literature data [2–5] or authentic samples. Oxoglaucine has been isolated from the plants of several genera [6, 7] but not previously from other *Croton* species. Structure **1** is being proposed for hemiargyrine by a combination of EI mass spectrometry, ^1H and ^{13}C NMR spectra in combination with 2D NMR techniques (long-range $^1H \times {}^1H$-COSY).

Hemiargyrine was isolated by flash column chromatography as an amorphous solid. The mass spectrum showed the general features characteristic of tetrahydroprotoberbine alkaloids [8, 9]. The $[M]^+$ at *m/z* 341 (16%) is of low intensity, while the peak at *m/z* 326 (95%) corresponds with the loss of a methyl group. The two other important peaks at *m/z* 178 (100%) and 164 (68%) result from the cleavage of the molecule into two fragments by rupture of the benzylic bonds to yield an isoquinoline fragment (rings A and B) and a benzylic fragment containing a methyl group (ring D) (Scheme 1). The mass of these fragments indicate each to have one methoxyl and one hydroxyl group attached to rings A and D, respectively.

The ^1H NMR spectrum of **1** ($CDCl_3$) showed signals for a methyl group (δ 1.36), two methoxyl groups a δ 3.83, a quartet for a methine group (δ 4.06) and four aromatic proton singlets (δ 6.75, 6.60, 6.58 and 6.54), indicating a 2,3 and 10,11 substitution pattern. The

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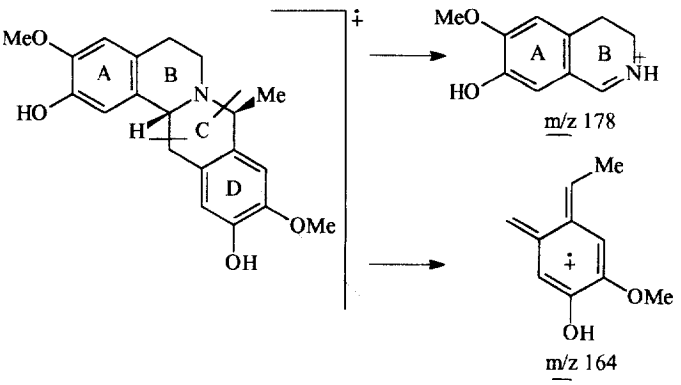

Scheme 1.

exact position of the substituents on rings A and D was deduced from the homonuclear (long range $^1H \times ^1H$-COSY) NMR spectrum. The aromatic signals at δ 6.75 and 6.60 are not coupled to any methoxyl groups but the latter is coupled to a proton in the multiplet at δ2.80 (C-13). The aromatics protons at δ 6.58 and 6.54 are coupled to the methoxyl groups at δ 3.83, but only the second signal is coupled to the methine group located at δ 4.06. This latter signal is also coupled to a methyl proton at δ 1.36, which established the position of this proton linked to C-8. According to these data, the methoxyl groups are located at positions 3 and 10 and the hydroxyl groups are at positions 2 and 11. The assignments of the ^{13}C NMR signals of **1** ($CDCl_3$) was assisted by the use of APT and comparison with data reported for other tetrahydroprotoberbine alkaloids [10, 11]. Conformational analysis was established using spectroscopic methods. The chemical shift to the low magnetic field at H-8 (δ 4.06) when compared with H-14 (δ 4.16) [12] and the ^{13}C NMR values at C-14 (δ 50.0) and the methyl group (δ 17.9), established a *trans*-configuration of these two protons [12, 13]. The positive value of the optical rotation for **1** showed that the absolute configuration at carbon 14 is *R* [14]. Since there is a *trans*-relationship between H-8 and H-14, the configuration of 8 must be *S*, which is in agreement with structure **1** presented for this molecule. Although various tetrahydroprotoberbine have been isolated from other plants this is the first one of this class to be isolated from *Croton*.

EXPERIMENTAL

Leaves and stems of *C. hemiargyreus* Muell. Arg. var. *hemiargyreus* were collected near Nova Friburgo (state of Rio de Janeiro, Brazil) and identified by Prof. Arline Souza de Oliveira. A voucher specimen has been deposited in the herbarium of the Federal University of Rio de Janeiro (number 182775), Brazil. Powdered dry leaves and stems (1.28 kg) were extracted with 95% EtOH by percolation. The EtOH extract was evapd to dryness under red. pres. and the residue taken up in 5% aq. HCl. The soln was extracted with $CHCl_3$. After alkalinization with conc. NH_4OH (pH 10), the aq. phase was extracted with $CHCl_3$ and the solvent removed *in vacuo* to yield a mixt. of alkaloids (1.3 g). The crude base mixt. was chromatographed on a column of silica gel: elution was started with CH_2Cl_2 and then continued with CH_2Cl_2–MeOH mixts. Frs (75) were collected and grouped into three principal frs after monitoring by TLC: A (122 mg), B (143 mg) and C (157 mg). Alkaloids were detected by UV and after spraying with Dragendoff's reagent. Frs were treated separately to isolate and purify their alkaloid content by flash CC and prep. TLC on silica gel. Glaucine and oxoglaucine were obtained from fr. A, the new alkaloid, hemiargyrine, from fr. B and salutaridine and norsalutaridine from fr. C.

Glaucine

CH_2Cl_2 removed this substance from the silica gel column. It was first obtained as an oil but finally recrystallized (EtOAc) mp 118–120° (lit. [15] 122°). Identified by comparison with reported spectra data [4].

Oxoglaucine

Isolated by flash CC eluted with CH_2Cl_2–$CHCl_3$ (1:1). mp 225–227° (lit. [5] 225–226°). Identified by comparison with reported spectral data [6, 7].

Hemiargyrine

Amorphous solid (mp 164–166°). $[\alpha]_D$ + 198 (*c* 0.1; MeOH). MS *m/z* (rel. int.): 341 ($[M]^+$, 16), 326 (95), 178 (100), 164 (68), 149 (21). UV nm; λ_{max} in MeOH 287, 228 (sh), 207. IR (KBr) cm^{-1}; 3450, 3250, 2980, 2848, 1640, 1600, 1518, 1450, 1255, 1123, 872. ^1H NMR (200 MHz, $CDCl_3$): δ 6.75 (1H, *s*, H-1), 6.60 (1H, *s*, H-12), 6.58 (1H, *s*, H-4), 6.54 (1H, *s*, H-9), 4.16 (1H, *ddl*, *J* = 10.6 and 4.5 Hz, H-14), 4.06 (1H, *q*, *J* = 6.8 Hz, H-8), 3.83 (6H, *s*, 2x Ar-OMe), 1.36

(3H, *d*, $J = 6.8$ Hz, 8-Me). ^{13}C NMR (50 MHz, $CDCl_3$): δ 17.9 (Me), 29.4 (C-5), 35.2 (C-15), 47.2 (C-6), 50.0 (C-14), 55.8 (OMe), 55.9 (OMe), 59.1 (C-8), 109.0 (C-9), 110.7 (C-12), 112.0 (C-4), 114.1 (C-1), 125.5* (C-4a), 126.0* (C-8a), 131.1 (C-12a), 131.4 (C-1a), 143.8** (C-11), 144.0** (C-2), 145.0 (C-3 and C-10). *, **—assignments may be interchanged.

Salutaridine

Isolated by prep. TLC using $CHCl_3$–EtOAc–MeOH (3:2:1). Identified by comparison with an authentic sample and reported spectral data [2, 16].

Norsalutaridine

Isolated by prep. TLC ($CHCl_3$–EtOAc–MeOH, 3:2:1). Identified by comparison with reported spectral data [3].

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