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Research Article

STEROIDS AND TRITERPENE FROM THE BARK OF *UNONOPSIS GUATTERIOIDES* R. E. FR. (ANONNACEAE)

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ABSTRACT

The phytochemical study of the bark of the stalk from *Unonopsis guatterioides* (Annonaceae) led to the isolation of the triterpene polycarpol, a substance with biological activities described in the literature, and a mixture of β-sitosterol and stigmasterol. Steroids are reported for the first time in this species. The substances were characterized based on the interpretation of their ^{1}H, ^{13}C NMR data and MS.

Keywords: Annonaceae, *Unonopsis guatterioides*, Polycarpol, Triterpene.

INTRODUCTION

The Neotropical genus *Unonopsis* comprises about 50 species and presents a wide distribution through the Amazon region, with some species in this genus with restricted distribution in this region, as is the case of *U. duckei*[1]. The *Unonopsis* genus is rich in aporphine alkaloids and its derivatives, being most common the oxoaporphines, azafluorenones, phenanthrenes and bisaporphine[2]. There is also available in the literature data on the composition of the essential oils of some species, such as *Unonopsis guatterioides* and *Unonopsis costaricencis*[3,4].

Among the compounds described in the literature, the one that stands out for its distribution between the species of the genus, is the triterpene polycarpol, its isolation have been reported in the species *Unonopsis glaucopetala*, *Unonopsis spectabilis*, *Unonopsis pacifica* and from roots of *Unonopsis guatterioides*[5-8]. This triterpenoid, discovered by Cave and colleagues in the barks of *Polyalthia oliveri* and *Meiocurpidium lepidotum* (Annonaceae) in the 70's [9], have some biological activities such as: antineoplastic, antitrypanosomal and antifilarial[10-12].

The species *U. guatterioides* presents as shrubs and trees that have up to 10 meters in high and approximately 20 cm in diameter. Its distribution is mainly in the Amazon region of Colombia, Venezuela, Peru and Bolivia, in Brazil the species have a wide distribution. This species is usually found in seasonally flooded forests[1]. *U. guatterioides* has characteristics that makes it special in the genus, such as its pollination by male bees from species of the Euglossine tribe instead of beetles, which is unusual in Annonaceae family[13]. Recent study has shown its alkaloidic potential, where several aporphine and oxoaporphine alkaloids were characterized[2]. This work describes the isolation of triterpene polycarpol and a mixture of the steroids stigmasterol and β-sitosterol from the barks of *U. guatterioides*.

MATERIALS AND METHODS

Botanical Material

The botanical material of *Unonopsis guatterioides* was collected in the Campus of the Federal University of the Amazon in January of 2010. The specimen was identified by the Dr. Antônio Carlos Webber from the Biology Department of Federal University of Amazonas. A voucher specimen was deposited in the herbarium of the Federal University of Amazon under the number 8249. The barks were dried, powdered and weighed.

Preparation of the hexanic extract

The powdered material (503.9 g) was macerated for three days with hexane, the extract was concentrated at reduced pressure. The procedure was repeated three times, with the solvent renewal every three days. At the end of the process, the extract was weighed, resulting in 3.8561 g of hexanic extract.

Isolation of chemical constituents

During the concentration process of the hexanic extract was observed the formation of a precipitate, this was purified by successive washings with hexane, crystallized in ethyl acetate and analyzed by T.L.C. with vanillin reagent. The crystalline solid was identified as compound **1**.(20.3 mg)

3.6651 g of extract were fractionated in separatory funnel (250 ml) under reduced pressure using silica gel (70-230 Mesh) as adsorbent and polarity gradient of hexane, ethyl acetate and methanol, resulting in five fractions. The fraction three (1,4324 g) was fractionated in open column using silica gel (70-230 Mesh) as adsorbent and polarity gradient of hexane, ethyl acetate and methanol, which resulted in eighty fractions. After evaluation by T.L.C., met the fractions 21 to 33. The new fraction appeared as a crystalline solid, which was identified as compound **2** (12 mg).

Spectroscopic characterization

The NMR spectra was registered in a ARX-200 apparatus (Bruker), operating at 200 MHz to ^{1}H NMR and 50 MHz for ^{13}C NMR and a DRX-400 (Bruker) operating at 400 MHz to ^{1}H NMR and 100 MHz for ^{13}C NMR. The samples were analyzed in $CDCl_3$. The chemical shift (δ) was expressed in ppm and the coupling constants (*J*) in Hz. The mass spectra were performed in a Ion Trap spectrometer LCQ-Fleet™ (Thermo Scientific).The ionization of the compound was made in an APCI probe in the positive mode of operation.

Polycarpol (**1**)

^{1}H-NMR ($CDCl_3$, 200 MHz) δ: 5.85 (d, J= 6.0 Hz, H-7), 5.31 (d, J= 6.0 Hz, H-11), 5.09 (t, J= 7.0 Hz, H-24), 4.31-4.24 (m, H-15), 3.29-3.21 (m, H-3), 1.69 (s, H-27), 1.61 (s, H-26), 1.01 (s, H-29), 0.98 (s, H-19), 0.94 (s, H-30), 0.88 (s, H-28), 0.88 (d, 6,2, H-21), 0.61 (s, H-18). ^{13}C-NMR ($CDCl_3$, 50MHz) δ: 146.1 (C-9), 140.8 (C-8), 131.2 (C-25), 124.9 (C-24), 121.3 (C-7), 116.0 (C-11), 78.9 (C-3), 74,7 (C-15), 51.9 (C-14), 48.9 (C-17), 48.8 (C-5), 44.3 (C-13), 40.1 (C-16), 38.7 (C-4), 38.5 (C-12), 37.4(C-10), 36.2 (C-22), 35.8 (C-20), 35.7 (C-1), 28.1(C-28), 27.7 (C-2), 25.7 (C-27), 24.8 (C-23), 22.9 (C-6), 22.8 (C-19), 15.9 (C-18), 18.4 (C-21), 17.6 (C-26), 17.1 (C-30), 15.8 (C-29). APCI-MS: *m/z* 441 $[M+H]^+$, and fragments at *m/z* 423 $[M+H-H_2O]^+$ and *m/z* 405 $[M+H-2H_2O]^+$

Stigmasterol and β-sitosterol mixture (2)

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.36 (d, J= 4.8 Hz, H-6), 5.16 (dd, J= 8.5 and 15.5 Hz, H-23), 5.00 (dd, J= 8.7 and 15.5 Hz, H-22), 3.57 (t, J= 7

Hz H-3), 1.01 (s, H-19), 0.68 (s, H-18). ^{13}C-RMN ($CDCl_3$, 100 MHz) δ: 140.7 (C-5), 33.9 and 138.3 (C-22), 26.0 and 129.2 (C-23), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 45.8 and 51.2 (C-24), 50.1 (C-9), 42.2 (C-4), 42.2 (C-13), 39.8 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 and 40.5 (C-20), 31.9 (C-7), 31.9 (C-8), 29.1 and 31.8 (C-25), 31.6 (C-2), 28.2 (C-16), 23.0 and 25.4 (C-28), 24.3 (C-15), 19.8 and 21.2 (C-26), 21.1 (C-11), 18.8 and 21.1 (C-21), 19.4 (C-19), 19.3 and 18.9 (C-27), 12.3 and 12.2 (C-29), 11.8 (C-18). In the carbons with two signals, the first one is assigned to stigmasterol and the second one to the β-sitosterol.

RESULTS AND DISCUSSION

The compound **1** was obtained as white needles. In the ^{1}H-NMR spectrum were observed the signals at δ 5.85 (d, J= 6.0 Hz) and 5.31 (d, J= 6.0 Hz) suggesting the presence of intercalated double bonds between C-7 and C-11 and another olefinic signal at δ 5.09 (t, J= 6.9 Hz, H-24). Carbinolic hydrogens were signed at δ 4,31-4,24 (m, H-15) and 3,29-3,21 (m, H-3). The ^{13}C NMR spectrum exhibited 30 signals, observing metilic signals at δ 16,1-28,4, olefinic signals at δ 146,1-116,0 and carbinolic signals at δ 78,9 and 74,7. The analysis of mass spectrum in positive mode show the protonated ion at *m/z* 441 $[M+H]^+$. The water losses observed by the ions at *m/z* 423 $[M-H_2O+H]^+$ and *m/z* 405 $[M-2H_2O+H]^+$ confirmed the presence of two carbinolic carbons observed. The obtained data in comparison with the literature led to the identification of the compound **1** as being the lanostane triterpene polycarpol[14] (Figure 1).

The compound **2** was obtained as a cristaline solid. In the ^{1}H-NMR spectrum verified the presence of signals at δ 5.36 (d, J= 4.8 Hz, H-6) indicative of vinyl hydrogen of steroid skeleton and two signals at at δ 5.16 (dd, J= 8.5 and 15.5 Hz, H-23) and 5.00 (dd, J= 8.7 and 15.5 Hz, H-22) confirming the presence of trans disubstituted double bond, beyond a carbinolic hydrogen at δ 3.57 (t, J= 7 Hz, H-3). The ^{13}C-NMR showed 39 signals pointing to a mixture of two compounds, the correct assignation of these carbons were made by comparison with literature data[15], which led to the identification of this mixture as being the steroids stigmasterol (2a) and β-sitosterol (2b) (Figure 1)

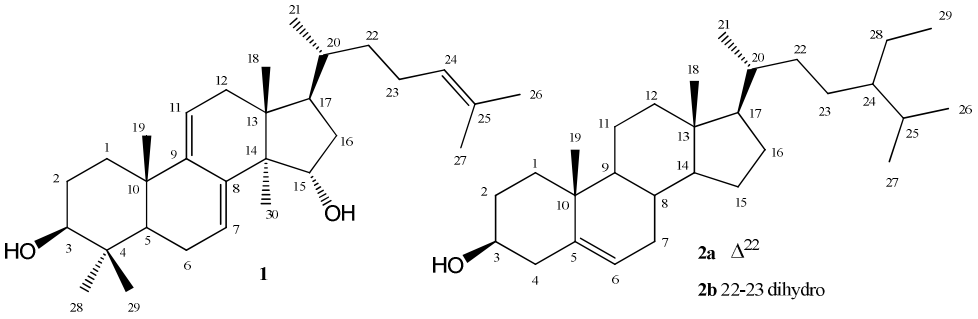

Fig. 1: Structures of the isolated compounds.

CONCLUSIONS

The isolation of the polycarpol from barks of *U. guatterioides* has considerable importance for the preservation of this species, since with the possibility of isolation from the barks, the isolation of the roots becomes unnecessary, thus avoiding the predation of the species. The isolation of the mixture of stigmasterol and β-sitosterol contributes to the phytochemical knowledge of this species.

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