ACTA
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Gram-scale isolation of isobrucein B and neosergeolide from *Picrolemma sprucei* Hook. f.

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ABSTRACT

Quassinoids neosergeolide and isobrucein B, obtained from *Picrolemma sprucei,* have proven *in vitro* antitumor, antimalarial, anthelminthic, cytotoxic, insecticide and leishmanicidal activities. There is interest in the *in vivo* pharmacological study of these natural compounds and their semi-synthetic derivatives, however, the quantities obtained in previous extraction processes have been shown to be a limiting factor for continuation of these studies. Herein, we describe a method for obtaining grams of these quassinoids whose purification relies only on recrystallization.

KEYWORDS: Caferana, Simaroubaceae, Quassinoid, Recrystallization.

Isolamento de isobruceina B e neosergeolida de *Picrolemma sprucei* Hook. f. em escala-grama

RESUMO

Os quassinóides neosergeolida e isobruceína B, obtidos de *Picrolemma sprucei*, possuem atividades antitumoral, antimalárica, anti-helmíntica, citotóxica, inseticida e anti-leishmania comprovadas em estudos *in vitro*. Há interesse no estudo farmacológico *in vivo* dessas substâncias naturais e de seus derivados semi-sintéticos, porém a quantidade obtida nos processos de extração tem se mostrado um fator limitante à continuação desses estudos. No presente trabalho, descrevemos um método para obtenção de gramas desses quassinóides cuja purificação depende apenas de cristalização fracionada.

PALAVRAS CHAVE: Caferana, Simaroubaceae, Quassinóide, Recristalização.

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Isobrucein B (**1**) (Kupchan *et al.*, 1975) and neosergeolide (**2**) (Schpector *et al.*, 1994) are bioactive secondary metabolites belonging to the class of highly oxygenated terpenoids known as quassinoids, a class which is exclusively found in the Simaroubaceae plant family (Figure 1). Their isolation on small scales has been described previously from the stems and roots of *Picrolemma sprucei* (synonym *P. pseudocoffea* Ducke) (Moretti *et al.*, 1982), one of several plant species known in the Brazilian Amazon as "caferana" (Silva *et al.*, 1977). Other species known as caferana are *Tachia guyanensis* Aubl. (Gentianaceae) and *Quiina rhytidopus* Tul. (Quiinaceae) (Corrêa, 1926; Silva *et al.*, 1977). Compound **1** was also obtained from the leaf extract of *P. sprucei* (Polonsky *et al.*, 1984). The antileukemic, antifeedant, antimalarial (Fandeur *et al.*, 1985; Andrade-Neto *et al.*, 2007), leishmanicidal and anthelmintic (Nunomura *et al.*, 2006) activities of these quassinoids have been reviewed recently (Amorim and Pohlit, 2006) and a study on the cytotoxic, larvicidal, antimalarial and hemolytic activity of **1** and **2** and two semi-synthetic derivatives has been recently published (Silva *et al.*, 2009)

Despite more than 25 years of interest on the part of chemists and pharmacologists in quassinoids from *P. sprucei*, methods for obtaining them are still both resource and time-consuming. Moreover, non-sustainable extraction directly from the Amazon forest is the available method for obtaining *P. sprucei* at present (Amorim and Pohlit, 2006). This fact limits the potential usefulness of this Amazonian medicinal plant and the quassinoids obtained from it. Studies are underway to develop sustainable production methods for this plant and its tissues (Barros and Pohlit, unpublished work). In this report, we describe a method for obtaining both **1** and **2** on gram-scales without the need for preparative chromatographic methods and using fractional recrystallization in the purification step.

Collection was performed in Silves Municipality, in Amazonas State, Brazil, in April, 2003. Voucher specimens have been previously deposited at the UFAM Herbarium (Silva 5729 & 5730). Identification was performed by Dr. Wayt Thomas as *Picrolemma sprucei* Hook. f.(Wayt Thomas, personal comm.). Roots and stems were cut into small pieces while fresh and allowed to dry in the shade and were then ground.

In a pilot-scale, stainless-steel Soxhlet extractor, powdered stems and roots were degreased by continuous extraction with hexanes. The solvent was evaporated from the marc which was then exhaustively extracted in the same apparatus with distilled hot water. The water extracts were combined and concentrated using rotatory evaporation *in vacuo* with a 50-55ºC bath. The resulting concentrated water extract was re-suspended in 700 mL of water and continually extracted with chloroform. The three concentrated chloroform extracts (totaling 42.8 g, after total evaporation) were each separately dissolved in a minimum of hot water and acetone (2:1, v/v) (Silva, 2006). Crystallization occurred upon cooling of the resulting solutions. The first chloroform extract (CF1) yielded three crops of crystals (PPT1.1, PPT1.2 and PPT1.3) and a filtrate. The second and third chloroform extracts (CF2 and CF3) yielded one crop each (PPT2 and PPT3, respectively). After recrystallization, PPT1.1, PPT1.2, PPT2 and PPT3 yielded a total of 1.75 g (0.027 %) of pure **2**. Recrystallizations of PPT1.3 yielded 1.17 g (0.018 %) of pure **1** (Scheme 1). Identification was performed by comparison of physical and spectral data with literature data (Moretti *et al.*, 1982; Vieira *et al.*, 2000). The above isolation and purification procedures were monitored by analyzing extracts, fractions and substances by normal-phase thin-layer chromatography (TLC) using ether : isopropyl alcohol (9:1) as mobile-phase. TLC plates were conveniently developed by contact with iodine vapor and illumination with UV light (254nm). The R_F values for **1** and **2** were 0.6 and 0.4, respectively under these conditions. The melting points (m.p.) obtained for **1** and **2** were 258-260.1 °C and 202-204.1 °C, respectively.

Moretti *et al.* (1982) used degreasing and exhaustive, serial hot water infusions which are a lengthy and tedious procedure, especially on a pilot scale. In our adaptation, the use of continuous liquid-solid extraction for these tandem steps provided quassinoid-rich, partially concentrated water extracts. Whereas in Moretti *et al.* (1982) the chloroform extract was chromatographed to yield **1** and **2** on milligram scales, our method obviates chromatography altogether. The key recrystallization step removes polar, water soluble substances present in the chloroform extract and provides for differential recrystallization of **1** and **2**.

This method should aid researchers interested in obtaining **1** and **2** for pharmacological studies. Cold and room temperature extraction with non-chlorinated solvents, followed by recrystallization, are under study to further develop multi-gram isolation of **1** and **2**.

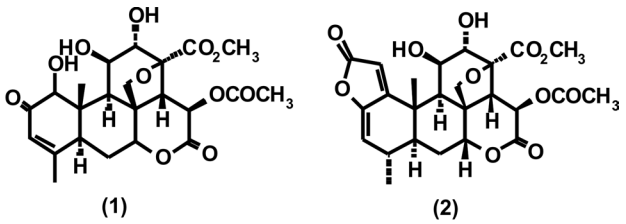
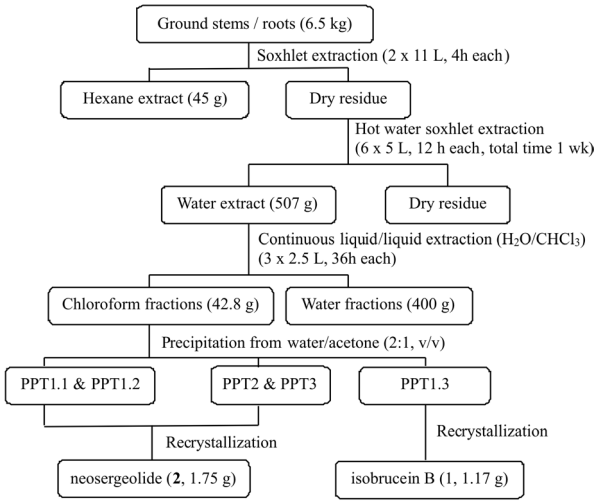


Figure 1 - Quassinoids obtained from *Picrolemma sprucei*.



Scheme 1 - Flowchart of the gram-scale process of neosergeolide and isobruceine B extraction and purification from *P. sprucei*.

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