Research Article

Uptake of Seeds Secondary Metabolites by Virola surinamensis Seedlings

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The major secondary metabolites and fatty acids occurring in the seeds of Virola surinamensis were monitored by GC-MS during germination and seedling development. The role as carbon source for seedling development was indicated considering that both classes of compounds were similarly consumed in the seeds and that no selective consumption of compounds could be detected.

1. Introduction

Several neotropical trees produce fruits with large and heavy seeds [1]. Virola surinamensis is a myristicaceous tree growing in the Amazonian flooded plains and produces seeds during the rainy season [2, 3]. Seeds are viable shortly after ripening and are adapted to be dispersed by water or by large birds such as toucans and araçás. The seedling formation can be divided in two distinct phases: seed germination and seedling development [4]. The cotyledons are hidden in the seed coat (cryptocotylar) and are storage organs of fatty material and polysaccharides that are recruited for the maintenance of seedling during its growth and development [5]. A study carried out on V. venosa revealed that the major lignans cubein and dihydrokusunokinin accumulated in the seeds were not detected in its seedlings which accumulated a polyketide instead [6]. The major constituent identified in the seedling roots was shown to be the lignan sesamin, a minor constituent in the seeds. A different result were observed with V. sebifera in which a possible translocation of hydroxytetralone lignans and a preferential accumulation of a lignan hydroxy-otobain was observed in the whole seedlings [7].

In view of the lack of systematic investigation regarding this important event in the reproduction of tropical trees, the translocation of secondary metabolites occurring in large seeds to be used as a defensive compounds in the seedlings remains as a hypothesis [8, 9].

Virola surinamensis seeds contain 15.4% of soluble tannins as a dry mass and the highest concentration of compounds with a probable defensive function yet recorded [10]. Their cotyledons are rich in triacylglycerols and free fatty acids. Phytochemical analysis of V. surinamensis seeds collected at Combu Island demonstrated the occurrence of lignoids, propiophenone, and γ-lactones in these organs [11]. Analysis of seedling leaves of V. surinamensis growing in the field, in greenhouse conditions and in micropropagated plantlets revealed the absence of lignans and the exclusive occurrence of jurueneolide C (8a) (Figure 1) [12]. Herein, we
wish to report the analyses of fatty acids and major secondary compounds in seeds of *V. surinamensis* in order to evaluate a selective consumption during the germination process.

### 2. Experimental Section

#### 2.1. General Procedures

Preparative thin-layer chromatography (prep. TLC) was carried out on silica gel GF-254 (Merck) and column chromatography (CC) on silica gel 60H (0.005–0.045 mm) (Merck). The 1H NMR (200 MHz) and 13CNMR (50 MHz) spectra of samples were recorded on a Bruker-AC 200 in CDCl3 with tetramethylsilane (TMS) as an internal standard. EIMS was obtained at 70 eV on HP 5988-A.

#### 2.2. Plant Material

Seeds of *Virola surinamensis* (Rol.) Warb. were collected in February 1995 at Combu Island (01°30′10″S; 048°27′42″W), near Belém, Pará State, Brazil. A dry voucher sample (LOPES-037) has been deposited in the SPF-Herbário do Instituto de Biociências da Universidade de São Paulo. Mature seeds were frozen for analysis or germinated as previously reported [13] and maintained at greenhouse facilities of Instituto de Química-USP.

#### 2.3. Standards Isolation

One dried seed (320 mg), after the germination process, was extracted with CH3OH (3x 50 mL). The concentrated extract (70 mg) was suspended in CH3OH/H2O (6:4) and filtered through a Millipore membrane (0.45 μm). The filtered extract was submitted to preparative on HPLC (RP-8, 10 μm, 250 × 22 mm column; CH3OH/H2O 60:40 → CH3OH 100% (50 min), 8 mL·min⁻¹, optimized conditions), followed by prep. TLC (silica gel; Hexane/EtOAc/i-PrOH or CH2Cl2/Me2CO) to yield 4-hydroxy-3-methoxypropiophenone (1, 1.6 mg) [14], galbulin (2, 5.5 mg) [15], guaiacin (3) (1.4 mg) [15], galbacin (4a, 2.0 mg) [16], galbelgin (4b, 1.0 mg) [17], calopeptin (5a, 1.6 mg) [18], veraguensin (5b, 5.0 mg) [19], 7,2′-dihydroxy-4′-methoxy-isoflavone (6, 1.5 mg) [20], α,2′-dihydroxy-4,4′-dimethoxydihydrochalcone (7, 1.8 mg) [21], juruenolide C (8a, 1.2 mg) [12], and juruenolide D (8b, 1.3 mg) [11]. All these compounds were identified by comparison of spectroscopic data with that reported in the literature.

### Table 1: Arithmetic mean of dry weight extracts and yields of *V. surinamensis* seeds.

| Seeds* | Dry weight (mg) | Extract (mg) | Yield (%) |
|--------|----------------|--------------|-----------|
| BG (n-hexane) | 1030 | 310 | 30 |
| AG (n-hexane) | 290 | 81 | 28 |
| BG (CH3OH) | 950 | 180 | 19 |
| AG (CH3OH) | 270 | 46 | 17 |

BG: seeds before germination; AG: seeds after germination.

*Number of seeds used in each experiment = 7.
n-hexane. The transesterification of oils was carried out according to a procedure described by Maia and Rodrigues-Amaya, 1993 [22]. The methyl esters were dissolved with n-hexane (2 mg·mL⁻¹), and 1 μL was injected in a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5988 mass spectrometer in the condition previously described [12, 23].

2.5. Analyses of Secondary Compounds. Individual seeds, before and after the germination process, were extracted (3x) with 20 mL of CH₃OH. The extract was concentrated to dryness and the residue dissolved with CH₂Cl₂ to obtain 2 mg·mL⁻¹ as the final concentration, and 1 μL was injected. All the analyses were performed with seven replicates in a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5988 mass spectrometer. The sample was injected (250°C) on a DB-5 column (30 m × 0.25 mm ID × 0.25 μm of film thickness). The column temperature was initially 120°C (2 min), then programmed to 230°C at 7°C·min⁻¹, kept at 230°C for 10 min, and then increased to 290°C in 15 min. The mass spectra were recorded at 70 eV. The identification of individual constituents was carried based on injection of isolated substances and comparison of their mass spectra.

2.6. Statistical Analysis. Statistical analyses were performed with the graphPad InStat software. All values were reported as means ± SEM, and were analyzed for statistical significance by two way analysis of variance followed by Student test. The minimum level of significance considered was $P < 0.05$.

### 3. Results and Discussion

Two groups of seeds of *V. surinamensis*, before germination (BG) and 6-7 months after germination (AG), were analyzed for fatty acids and major secondary metabolites. The second group (AG) showed a decrease of 30% in dry weight, but without significant changes in the extraction yield (Table 1). These results are in agreement with Durían's hypothesis, in which seeds are a nutrient storage organ to supply the seedling during the growth process [8]. The analyses of fatty acids content carried out in seeds of *V. surinamensis* before and after germination showed similar relative content of lauric acid (16%), myristic acid (70%), palmitic acid (6%), and stearic acid (8%). This result is similar to that previously reported [23], and since no preferential uptake of fatty acids could be detected, the major role of fatty material as carbon source is clearly supported (Table 2).

The secondary metabolites in both groups of seeds of *V. surinamensis* were analyzed by GC-MS. The chromatographic profile observed for both groups exhibited the predominance of galbulin (2), galbacin (4a), and veraguensin (5b) as the major compounds (Figure 2). After statistical analyses, no significant variation was observed in the relative content of monitored compounds, except to compound 1 ($P < 0.05$) (Table 2).

From *V. surinamensis*, new substances were isolated [24] and some neolignans showed allelopathic properties [25]. Recently, other neolignans showed antiinflammatory and antileishmanial activities [26, 27]. In addition, the increase of phenolic compounds was observed after elevated CO₂ submission in *V. surinamensis* [28] and a strong inhibition of CO₂ assimilation by sun exposure [29]. However, the analyses of the composition occurring in the seeds of this
species during germination and seedling processes had not been studied yet.

In summary, the germination of \textit{V. surinamensis} seeds and the seedling development are processes in which both fatty acids and secondary metabolites (lignans, isoflavonoids, and jurueneolides) are equally consumed in the seeds indicating their physiological role as energy and carbon source, or by other physiological function. In spite of the large concentration of lignans in the seeds (8.5% as dry weight basis), no specific translocation to the seedlings and no consumption of a specific compound from the seeds could be detected. The lignans could have biological importance to the seeds, but after the lignans uptake to the seedling, our results, in addition to the previous phytochemical investigations [12], reinforce the use of these compounds as energy and carbon source by the seedlings.

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