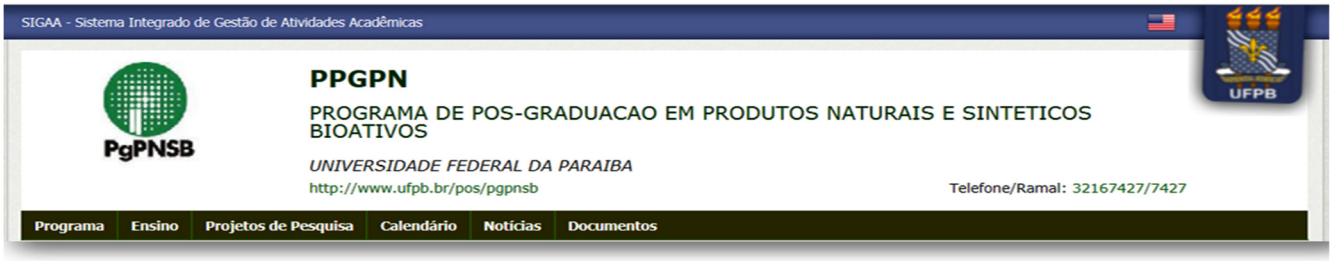

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Semisynthesis of a novel benzotriazole-trachylobane derivative from *ent*-7α-acetoxi-trachyloban-18-oic acid

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Abstract:
We describe semisynthesis of a novel benzotriazole-trachylobane derivative **(3)** starting from *ent*-7α-acetoxi-trachyloban-18-oic acid **(1)**, using a modified Steglich esterification employing EDC as the coupling agent in the presence of HOBt as equivalent of alcohol in acetonitrile solution. The natural product used as raw material, *ent*-7α-acetoxi-trachyloban-18-oic acid **(1)**, was isolated from the ethanol extract of *Xylopia langsdorffiana* stems with 1% yield. These compounds **(1** e **3)** were identified by NMR, IR besides TLC comparison with authentic sample of **(1)**.

Keywords: semisynthesis; trachylobane diterpene; *Xylopia langsdorffiana*

1. Introduction

The usage of natural products as raw material of structural or functional templates for the design and synthesis of a wide variety of novel molecules has been an important strategy of new drug design. In line with this context, the isolation of several biologically active trachylobane diterpenes with anticancer activity such as 7α-acetoxi-trachyloban-18-oic acid **(1)** [1] and mitrephorone A **(2)** [2] (Figure 1) has motivated the development of efficient functional diversification approaches via semisynthesis protocols aiming at the preparation of novel semisynthetic derivatives. The semisynthesis of derivatives from natural (bioactive) products is a strategy used for the improvement, enhancement and/or discovery of biological activities. In this perspective, we report in this communication the isolation of *ent*-7α-acetoxi-trachyloban-18-oic acid **(1)** from stems of *Xylopia langsdorffiana* and the preparation of its derivative **(2)** by semisynthesis.

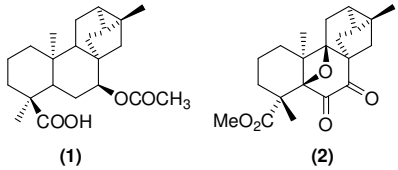

Figure 1. Biologically active trachylobanes

2. Results and Discussion

The natural product used as raw material, *ent*-7α-acetoxi-trachyloban-18-oic acid **(1)**, was isolated from the ethanol extract of *Xylopia langsdorffiana* stems with 1 % yield as described previously [3]. This compound was characterized by NMR spectroscopy besides TLC comparison with authentic sample [3].

With natural product **(1)** in hand, our initial objective was the synthesis of new molecular hybrids from 7α-acetoxi-trachyloban-18-oic acid **(1)** and paracetamol or methyl salicylate using a modified Steglich esterification [4]. Unfortunately, this was not possible from the reaction conditions we employed. Despite this, we have been able to isolate and characterize a novel derivative with the benzotriazole moiety connected to the trachylobane structural scaffolding via the ester function formed under the reaction conditions employed. This novel semisynthetic compound was called benzotriazole-trachylobane derivative **(3)**, Figure 2.

The infrared absorption spectrum showed bands in 1798 and 1729 cm -1 which are consistent with the carbonyl groups present in the molecular structure of **(3)**. Additionally, the set of signals observed at δ7.31 (dl, *J* = 8 Hz, 1H); 7.40 (t, *J* = 8 Hz, 1H); 7.54 (t, *J* = 8 Hz, 1H) and 8.04 (d, *J* = 8 Hz, 1H) in the region of aromatic hydrogens in the ^{1}H NMR spectrum were quite consistent with the presence of the core benzotriazole-type heterocycle in **(3)** [5].

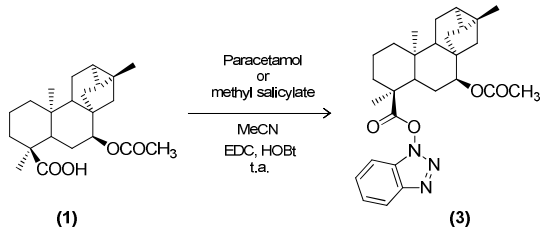

Figure 2. Benzotriazole-trachylobane derivative **(3)**

3. Materials and Methods

General Experimental Procedures

All solvents and chemicals were used as purchased without further purification. The progress of the reactions was monitored by thin layer chromatography (TLC). Column chromatography was performed over silica gel (60-230 mesh). Infrared spectra were recorded on an IR Prestige-21 FTIR (Shimadzu) spectrophotometer with 1 mg of sample in KBr plates and frequency of absorption reported in cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded on a Brucker Ascend 400 spectrometer operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR or on a Varian Mercury Spectra AC20 spectrometer operating at 200 MHz for ^{1}H NMR and 50 MHz for ^{13}C NMR. Spectra were recorded using $CDCl_3$ as the solvent and tetramethylsylane (TMS) as the internal standard, unless otherwise stated. The NMR data are presented as follows: chemical shift, in ppm, multiplicity, number of protons and *J* in Hz. Multiplicities are indicated by the following abbreviations: s (singlet), d (doublet), dd (double doublet), dl (doublet large), t (triplet), tl (triplet large), m (multiplet) and brs (broad singlet).

Plant material

The stems of *Xylopia langsdorffiana* were collected in the municipality of Cruz do Espírito Santo, State of Paraíba (PB), in Setember 2016. A voucher specimen (AGRA5541) is deposited in the herbarium Prof. Delby Fernandes de Medeiros of the Federal University of Paraiba, João Pessoa – PB, Brazil.

Extraction and isolation of **(1)**

The *ent*-7α-acetoxytrachyloban-18-oic acid **(1)** was purified from *Xylopia langsdorffiana* stems. Dried stems of *X. langsdorffiana* (2,5 kg) were exhaustively extracted with 95% EtOH (3 × 5 L) for 72 hours at room temperature The solvent was evaporated to yield a dark syrup (169,8 g), which was successively partitioned with hexane, chloroform and ethyl acetate to yield 16, 35, and 7 g of crude residue, respectively. A 4 g portion of the hexane fraction was subjected to column chromatographic separation, using hexane and hexane with increasing amounts of ethyl acetate as eluents, and the eluate was monitored by TLC. Altogether, 35 fractions of 25 mL each were collected and pooled into 11 fractions (Fr1 - Fr11). Fraction Fr2 (1,83 g) was recrystallized from hexane/Et_2O, yielding 913 g *ent*-7α-acetoxy-trachyloban-18-oic acid **(1)**. Purification of the crude material obtained from concentration of

mother liquor by column chromatography using hexane/EtOAc (6:1), yielding additionally 0,405 g of **(1)**. Futhermore, fractions Fr3, Fr4 and Fr8 were purified by trituration using hexane/CH_2Cl_2 (1:1) yielding additionally 0,182 g of **(1)**. This natural product was obtained as amorphous white solid and was identified by NMR spectroscopy besides TLC comparison with authentic sample [3].

(1) *ent*-7α-acetoxytrachyloban-18-oic acid: amorphous white solid; ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.55-0.62 (*m*, 1H); 0.85-0.90 (*m*, 2H); 0.96 (*s*, 3H); 1.1 (*s*, 3H); 1.12 (*s*, 3H); 1.25-1.32 (*m*, 2H); 1.47-1.75 (*m*); 1.84-1.98 (*m*); 2.06 (*s*, 3H); 2.13 (*d*, *J* = 4,5 Hz, 1H); 3.58 (*s*, 3H); 4.57 (*tl*, 1H).

Semisynthesis of benzotriazole-trachylobane derivative (**3**)

A mixture of the *ent*-7α-acetoxytrachyloban-18-oic acid (**1**) (50 mg; 0.14 mmol), EDC (29.5 mg; 0.15 mmol), and HOBt (21.4 mg; 0.14 mmol) in dry MeCN (2.5 mL) was stirred at room temperature for 30 minutes and then treated with paracetamol (0.14 mmol) or methyl salicylate (0.14 mmol). The mixture was stirred at room temperature for na additional 24 h. Then the solution was evaporated to dryness in vácuo. The residue was dissolved in EtOAc (10 mL) and washed with brine (2x 5 mL), 5% aqueous sodium hydroxide (2x 5 mL), and water (2 x 5 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered and concentrated. The crude material was purified by column chromatography (3x11 cm) using hexane/EtOAc (8:1) to afford 54.9 mg (77% yield) of semisynthetic derivative (**3**) as amorphous white solid.

(3) benzotriazole-trachylobane derivative: amorphous white solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.61-0.63 (*m,* 1H); 0.92 (*dd*, *J* = 8,0 e 4.0 Hz, 1H); 0.98-1.04 (*m*, 1H); 1.06 (*s*, 3H); 1.13 (*s*, 3H); 1.35-1.38 (*m*); 1.39 (*s*, 3H); 1.57-1.88 (*m*); 1.84 (*s*, 3H); 1.95-2.15 (*m*); 2.44 (*dl*, 1H); 4.80 (*t*, *J* = 4 Hz, 1H); 7.31 (*dl*, *J* = 8 Hz, 1H); 7.40 (*tl, J* = 8 Hz, 1H); 7.54 (*tl, J* = 8 Hz, 1H); 8.04 (*dl*, *J* = 8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.2 (CH_3); 16.5 (CH_3); 17.1 (CH_2); 20.3 (CH_3); 20.4 (CH); 21.3 (CH_3); 22.9 (*C_0); 23.9 (CH); 28.7 (CH_2); 32.5 (CH_2); 37.3 (CH_2); 37.8 (CH_2); 37.9 (*C_0); 42.7 (CH); 44.3 (*C_0); 44.5 (CH_2); 47.8 (*C_0); 48.6 (CH); 77.3 (CH); 108.4 (CH); 120.5 (CH); 124.9 (CH); 128.7 (CH); 128.8 (*C_0); 143.6 (*C_0); 170;8 (*C_0); 174.4 (*C_0). *C_0 = unsubstituted carbons with H atoms. IR (KBr, cm^{-1}): 2952; 2923; 2860; 1798; 1729.

4. Conclusions

In summary, we have isolated the *ent*-7α-acetoxytrachyloban-18-oic acid **(1)** from the ethanol extract of *Xylopia langsdorffiana* stems with 1% yield. This predominant natural product was used as raw material for the semisynthesis of a novel benzotriazole-trachylobane derivative **(3)**.

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Author Contributions

Conceived and designed the experiments: J.F; M.S.S. Performed the experiments: T.A.S.; J.A.M.S. Analyzed the data: J.F.; J.F.T. Contributed reagents/materials/analysis tools: M.S.S.; J.F.T.; J.F. Wrote the paper: J.F. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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