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Two pyrolysate products from Omani frankincense smoke: First evidence of thermal aromatization of boswellic acids

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ABSTRACT

The frankincense resin of botanically-certified *Boswellia sacra* was pyrolyzed and the smoke was trapped into water using self-developed assembly. Two compounds, namely 1,2,4a,9-tetramethyl-1,2,3,4,4a,5, 6,14b-octahydropicene (**1**) and 2,9-dimethylpicene (**2**) were isolated from *n*-hexane extract of the smoke-saturated water. Their structures were determined by means of spectroscopic data including ESIMS, ^{1}H NMR, ^{13}C NMR, and 2D NMR (COSY, HSQC, HMBC, and NOESY). Dehydration of C-3 alcohols in boswellic acids (BAs) lead to aromatization of ring A followed by dehydrogenation and demethylation to afford triaromatic derivative viz., 1,2,4a,9-tetramethyl-1,2,3,4,4a,5, 6,14b-octahydropicene (**1**) and finally penta-aromatic derivative viz., 2,9-dimethylpicene (**2**). Compounds **1** and **2** were screened for their antiproliferative effects on MDA-MB-231 breast cancer cells. It was found that these pyrolysate products were capable of inhibiting cancer cell growth. However, this growth inhibitory effect was less when compared to their precursor AKBA (3-acetyl-11-keto-β-boswellic acid). The antiproliferative activity is inversely proportional to loss of functional groups.

1. Introduction

The chemistry as well as the pharmacological activities of the frankincense resin was well documented in the literature [1]. The rapid publications demonstrate the importance of this precious resin and that its role has exceeded its religious and social importance. However, the resin is not used in its natural form rather burned in homes, temples and churches to produce smoke "incense". In spite of the large literature describing the chemistry and biology of frankincense, there is a paucity of literature describing the pyrolysis of frankincense resin that is when the resin comes into direct contact with the hot charcoal. This fact is not unexpected due to the difficulty to capture the smoke of frankincense and convert it into an extractable form. Pailer et al. [2–4] detected some volatiles of *Boswellia carterii* using GC–MS. Basar [5] studied in her Ph.D. thesis the pyrolysis of four frankincense species included *B. carterii*, *Boswellia frereana* Birdw, *Boswellia serrata*, *Boswellia rivae*, and *Boswellia neglecta* using hot charcoal. We decided not to adopt this method due to possible confusing hallucinogenic or carcinogenic products, in particular polyaromatic hydrocarbons (PAHs) in charcoal.

Since frankincense is mostly burned at homes, churches, and temples to produce incense which is ethnopharmacologically known as antidepressant, a study to reveal the chemical transformations will be of high significance. We herein report the first isolated pyrolysis products named 1,2,4a,9-tetramethyl-1,2,3,4,4a,5, 6,14b-octahydropicene (**1**) and 2,9-dimethylpicene (**2**) from *Boswellia sacra* smoke through a self-developed assembly. Furthermore we are also reporting the anticancer activity of 1,2,4a,9-tetramethyl-1,2,3,4,4a,5, 6,14b-octahydropicene (**1**), 2,9-dimethylpicene (**2**) and 3-acetyl-11-keto-β-boswellic acid (AKBA, **3**) against breast cancer cells (MDA-MB-231 cells).

2. Experimental

2.1. General experimental procedure

Optical rotations were measured on a KRUSS P P3000 polarimeter (A. Kruss Optronic, Germany). IR spectra were recorded on a Bruker, ATR-Tensor 37 spectrophotometer. ESI-MS was recorded on Waters Quattro Premier XE Mass Spectrometer (Waters, Milford,

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Fig. 1. Assembly used to produce the smoke.

MA). The ^{1}H and ^{13}C NMR spectra were recorded on Bruker NMR spectrometers operating at 600 MHz (150 MHz for ^{13}C). The chemical shifts values are reported in ppm (δ) units and the coupling constants (J) are given in Hz. The purification of the final product was carried out by using recycling preparative High Performance Liquid Chromatography (HPLC) by JAI using silica column and using $CHCl_3$ as solvent. For TLC, pre-coated aluminum sheets (silica gel 60F-254, E. Merck) were used. Visualizations of the TLC plates were achieved under the UV light at 254 and 366 nm, and also by spraying with the ceric sulfate reagent.

2.2. Plant material

The frankincense samples of *B. sacra* were collected from the southern part of Oman and were also supplied by a trustful partner (Mr. Saleh Al-Amri, Ministry of Agriculture and Marine Wealth). All these samples were authenticated by Dr. Sayed Gilani (botanist), Department of Biological Sciences and Chemistry, University of Nizwa, the Sultanate of Oman. A voucher specimen (No: BSHR-01/2012) was deposited in the herbarium of the Department of Biological Sciences and Chemistry.

2.3. Production of plant derived smoke

The air-dried ground material (500 g) of Hougari regular (HR) grade frankincense was placed into a round bottom flask connected with a trap via a teflon pipe by using self-developed smoke-traping assembly (Fig. 1). The flask was heated using a heating mantle till smoke is produced. The smoke passed through the pipe and trapped in water. The dissolved smoke was extracted first with *n*-hexane to produce *n*-hexane fraction. Then the aqueous layer was extracted with ethyl acetate and *n*-butanol.

2.4. Extraction and isolation

The smoke solution (1 l) was extracted with *n*-hexane three times to obtain a crude gummy extract (6.1 g). The *n*-hexane fraction was chromatographed on a silica column using *n*-hexane, dichloromethane in *n*-hexane (various polarities), and finally pure dichloromethane by gradually increasing the polarity to give different sub-fractions. The 1,2,4a,9-tetramethyl-1,2,3,4,4a,5,6,14b-octahydropicene (**1**, 110 mg) was obtained as greenish crystals which was recrystalized from pentane/chloroform to afford white crystals. The sub-fraction obtained using *n*-hexane as a mobile phase was further purified through recycling preparative HPLC using 1:9 (ethyl acetate/hexane) system as the mobile phase in a silica column at a constant flow rate of 3.5 ml/min and give 2,9-dimethylpicene (**2**, 5.4 mg).

2.4.1. 1,2,4a,9-Tetramethyl-1,2,3,4,4a,5,6,14b-octahydropicene (**1**)

White crystal; MP: 220 °C; $[\alpha]^{25}_{D}$ +21 (*c* 0.006, *n*-hexane); IR (KBr): 2949, 2906, 2860, 2840, 1593, 833, 814, and 794 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε) 308 (2.92), 313 (2.93) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.55 d (*J* = 7.0 Hz, 1*H*, H-1), 8.44 d (*J* = 8.4 Hz, 1*H*, H-11), 8.01 d (*J* = 9.0 Hz, 1*H*, H-7), 7.95 d (*J* = 9.0 Hz, 1*H*, H-6), 7.50 t (*J* = 7.0 Hz, 1*H*, H-2), 7.39 d (*J* = 7.0 Hz, 1*H*, H-3), 7.31 d (*J* = 8.4 Hz, 1*H*, H-12), 3.40 dd (*J* = 9.0, 18.0 Hz; 1H, H-15α), 3.18 dd (*J* = 9.0, 18.0 Hz, 1*H*, H-15β), 2.73 s (3*H*, 23-Me), 2.45 m (1*H*, H-16α), 2.10 d (*J* = 10.0 Hz, 1*H*, H-18), 1.60 m (1*H*, H-22α), 1.55 m (2*H*, H-22β, H-21α), 1.46 m (1*H*, H-21β), 1.38 m (1*H*, H-16β), 1.20 m (1*H*, H-20), 1.16 m (1*H*, H-19), 0.94 d (*J* = 6.0 Hz, 3*H*, 26-Me), 0.89 d (*J* = 6.0 Hz, 3*H*, 25-Me), 0.78 s (3*H*, 24-Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 137.8 (C-13), 134.5 (C-4), 131.6 (C-12), 130.9 (C-5), 130.8 (C-14), 130.7 (C-10), 129.9 (C-9), 129.0 (C-8), 127.0 (C-3), 125.9 (C-2), 122.3 (C-6), 121.9 (C-7), 120.9 (C-1), 118.8 (C-11), 55.2 (C-18), 41.9 (C-19), 40.1 (C-22), 39.1 (C-20), 32.4 (C-17), 30.9 (C-21), 27.9 (24-Me), 27.4 (C-16), 23.4 (C-15), 20.9 (26-Me), 19.8 (23-Me), 17.8 (25-Me); ESIMS: *m*/*z* 365 $[M+Na]^+$ ($C_{26}H_{30}Na$).

2.4.2. 2,9-Dimethylpicene (**2**)

Amorphous powder; IR (KBr): 1590, 830, 815, and 795 cm^{-1}; UV (CH_2Cl_2) λ_{max} (log ε) 238 (3.1), 276 (3.0), 311 (2.93), 317 (2.91), 332 (3.17) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.92 br s (1*H*, H-11), 8.91 br s (1*H*, H-12), 8.80 d (*J* = 9.0 Hz, 1*H*, H-7); 8.74 d (m, 2*H*, H-1, H-16), 8.62 br s (1*H*, H-19), 8.21 d (*J* = 9.0 Hz, 1*H*, H-6), 7.98 d (*J* = 9.0 Hz, 1*H*, H-15), 7.89 d (*J* = 8.4 Hz, 1*H*, H-22), 7.60 t (*J* = 7.2 Hz, 1*H*, H-2), 7.49 m (2*H*, H-3, H-21), 2.82 s (3*H*, 24-Me), 2.67 s (3*H*, 23-Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ 136.5 (C-4), 134.8 (C-20), 130.7 (C-8), 130.5 (C-14), 129.6 (C-13, C-17), 128.9 (C-9), 128.6 (C-5), 128.4 (C-22), 128.3 (C-3), 128.2 (C-10), 128.1 (C-18), 127.5 (C-21), 127.2 (C-15), 126.3 (C-2), 123.4 (C-6), 122.7 (C-19), 121.7 (C-11), 121.6 (C-12), 121.4 (C-7), 121.3 (C-1), 120.6 (C-16), 22.9 (Me-23), 19.8 (Me-44); ESIMS: *m*/*z* 307 $[M+H]^+$ ($C_{24}H_{19}$).

2.5. Anticancer activity

2.5.1. Cell line and reagents

Breast cancer cell line MDA-MB-231 was maintained in DMEM (Invitrogen, Carlsbad, CA, USA). The media was supplemented with 10% fetal bovine serum (FBS) and 1% antimycotic antibiotic (Invitrogen, Carlsbad, CA, USA). Cells were cultured in a 5% CO_2—humidified atmosphere at 37 °C. Stock solutions of Doxorubicin compounds **1** and **2**, and AKBA (**3**) were made in DMSO at a final concentration of 2 mg/ml and were always made fresh just prior to experiments.

2.5.2. Cell growth inhibition studies by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cells were seeded at a density of 1×10^5 cells per well in 96-well microtiter culture plates. After overnight incubation, normal growth medium was removed and replaced with either fresh medium (untreated control) or different concentrations of respective compounds in growth medium diluted from a 2 mg/ml stock. After 24 h of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated further for 4 h at 37 °C. Upon termination, the supernatant was aspirated and the MTT formazan, formed by metabolically viable cells, was dissolved in a solubilization solution containing DMSO (100 μl) by mixing for 5 min on a gyratory shaker. The absorbance was measured at 540 nm on an Ultra Multifunctional