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Isolation and characterization of incensole from *Boswellia dalzielii* stem bark

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Abstract

Boswellia dalzielii is the West African species of the frankincense genus. It is used in ethnomedicine to treat gastrointestinal disorders, rheumatism and various diseases of microbial origin. The dried pulverized bark material was extracted with 50% ethanol by percolation. Column chromatography, gel filtration and preparative thin-layer chromatography (TLC) of the ethyl acetate fraction yielded incensole, a cembrane diterpenoid. Its structure was established by nuclear magnetic resonance (NMR) spectroscopy, including 2-D NMR experiments (reported here for the first time), and fast atom bombardment mass spectrometry (FAB-MS). Tests for antimicrobial and antioxidant (free-radical scavenging) activities revealed that while the extracts and fractions demonstrated strong antioxidant and antimicrobial activities, incensole was only moderately active.

Keywords: *Boswellia dalzielii*; Incensole; Diterpenoid; Antimicrobial activity; Antioxidant activity;

Introduction

Boswellia dalzielii Hutch (family Burseraceae) is a common deciduous tree in the Sudan savanna, growing up to 12m high. Its stem bark is pale brown and smooth, peeling off in thin ragged papery patches. The slash is reddish brown, exuding a whitish fragrant resin. It is the West African species of the frankincense-producing plants - *B. carteri*, *B. frereana* and *B. serrata*. It is used, among others, to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments (Burkill, 1985; Evans, 1989). However, in spite of its numerous applications in traditional medical practice in the sub region, the West African species is less known and studied compared to its more popular congeners. Phytochemical screening of the plant revealed the absence of alkaloids (Baoua *et al.*, 1976), while saponins, tannins, flavonoids, cardiac glycosides, steroids and terpenes were shown to be present (Alemika and Oluwole, 1991; Adelakun *et al.*, 2001). In addition, the aqueous (dialyzed) extract of the dried gum resin from Cameroon has been shown to possess anti-inflammatory activity in male rats (Duwiejua *et al.*, 1993). The methanol and aqueous extracts also showed broad-spectrum antibacterial and antifungal activities (Ntiejumokwu and Alemika, 1991; Adelakun *et al.*, 2001). The present study

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therefore aimed at investigating the chemical constituents of the stem bark of the plant.

The method of bioactivity-guided fractionation was employed in order to isolate bioactive compounds. Since many of the plant's known ethnomedical uses are related to the treatment of diseases caused by microbes, antimicrobial tests were carried out. Also since it is now believed that free radicals are responsible for many diseases such as cancer, artherosclerosis, rheumatoid arthritis, and diabetes mellitus, among others (Speroni *et al.*, 1998; Thabrew *et al.*, 1998; Toda and Shirataki, 1998; Burits and Bucar, 2000), the test for free-radical scavenging (antioxidant) activity was considered relevant.

Experimental

General procedures. Thin-layer Chromatography (TLC) was carried out on Si gel 60 F_{254} Merck®. Accelerated Gradient Chromatography - AGC (Bæckström, 1993), a form of Medium Pressure Liquid Chromatography (MPLC) was carried out on columns packed with Si gel 60, 0.040-0.063mm Merck®. The MPLC workstation was from Bæckström Separo Ab, Sweden. Sephadex LH-20 for gel filtration was a product of Pharmacia®. NMR was carried out on a Brucker 400 MHz spectrometer.

Plant material. The stem bark of *Boswellia dalzielii* was collected around Jos, Nigeria. The plant was authenticated by comparing with voucher specimens deposited at the Herbaria of the Forestry Research Institute of Nigeria (FRIN), Ibadan and Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Extraction and isolation. The dried, pulverized bark (1.14 kg) was extracted with 50% ethanol by percolation at room temperature for 48h, yielding a crude extract (151 g) which was separately partitioned between H_2O and CH_2Cl_2, EtOAc, *n*-BuOH. The EtOAc fraction (5.15 g) was chromatographed on Si gel employing *n*-hexane-CH_2Cl_2-EtOAc-MeOH gradient. The fractions eluted with hexane, up to CH_2Cl_2/ EtOAc (23:2) were bulked together, subjected to further column chromatography followed by prep TLC to give a colourless oil characterized as incensole (51mg).

Antimicrobial tests. Screening of extracts, fractions and pure compounds against typed organisms was carried out by agar diffusion cup-plate method (B.P. 1988). The bioautography technique involved an overlay of developed TLC plates with inoculated agar (Onawunmi, 1997).

Test for antioxidant (radical scavenging) activity. Extracts, fractions and isolated compounds were tested. The tests were carried out by running the TLC of the samples (in duplicate). Ascorbic acid was spotted along to serve as positive control. One chromatogram was sprayed with β-carotene (0.1% w/v in MeOH or EtOH) while the other was sprayed with 1,1-diphenyl-2-picrylhyrazyl i.e. DPPH (0.1% w/v in MeOH or EtOH). When the plate sprayed with β-carotene is irradiated with UV light at 366nm for 15 minutes, antioxidant spots appear yellow on a bleached background. For the DPPH-sprayed plate, antioxidant spots appear yellow against a purple background (Burits and Bucar, 2000).

Results and Discussion

Bioactivity-guided fractionation and isolation. From 1.14 kg of dried plant material, 151 g of crude extract was obtained, indicating a yield of 13.2 %. The fractionation process is represented in Fig. 1. Results of the antimicrobial screening of the extract and fractions (Table 1) show that the organic layers (CH_2Cl_2, EtOAc and *n*-BuOH) were by far more active than the aqueous layers (mother liquor). In addition, this was most pronounced in the EtOAc/ H_2O partition. This means that the antimicrobial constituents of the crude extract resides largely in the organic phase, with the EtOAc fraction being the most

active. Hence the EtOAc fraction was further investigated following the principle of bioactivity-guided fractionation. Similarly, as fractionation progressed, only active sub-fractions were further examined for the isolation of active compounds. Incensole did not show any antimicrobial activity in the cup-plate agar diffusion method probably due to its poor solubility in the solvent system used. It, however, showed moderate activity against Gram-positive bacteria (*Staph. aureus* and *B. subtilis*) by the method of bioautography. It is therefore likely that other compound(s) contribute more significantly to the antimicrobial/ antioxidant activities of the plant.

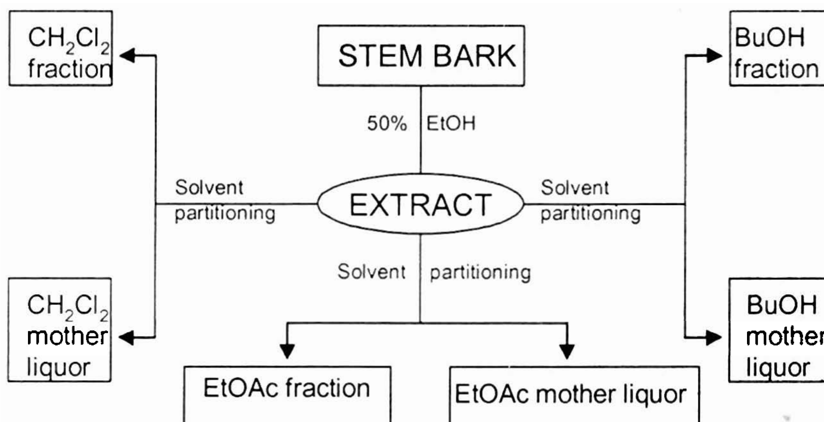


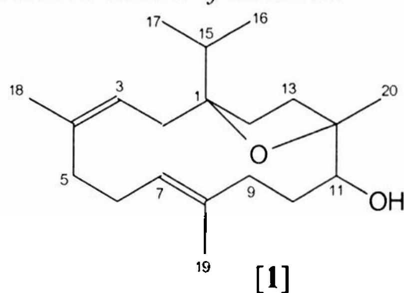
Fig. 1: Extraction and preliminary fractionation of *B. dalzielii* stem bark

TABLE 1: Zones of inhibition* of *Boswellia dalzielii* extract and fractions against selected typed organisms.

Samples	1	2	3	4	5	6	7	8
E. coli NCTC 10418	3.8	0.0	7.0	0.0	0.0	0.0	0.0	9.8
Staph. aureus NCTC 6571	10.5	2.8	14.0	2.5	3.5	4.8	0.0	16.0
Ps. aeruginosa ATCC 10145	2.3	0.0	6.5	0.0	0.0	0.0	0.0	0.0
B. subtilis NCTC 8236	7.8	3.3	9.8	3.8	4.5	4.0	0.0	12.5
C. pseudotropicalis NCYC 6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	8.0
Concn. (mg/mL)	40.0	40.0	40.0	40.0	40.0	40.0		1.0

* Mean of duplicate readings; diameter, mm, less cup size. 1= n-BuOH fraction; 2= n-BuOH mother liquor; 3= EtOAc fraction; 4= EtOAc mother liquor; 5= CH_2Cl_2 mother liquor; 6= *B. dalzielii* crude extract; 7= solvent [$MeOH/H_2O$ (2:1)]; 8=streptomycin

Characterization of incensole



[1]

Appearance/ solubility: Colourless to pale yellow oil; soluble in CH_2Cl_2, EtOAc and Me_2CO.

NMR (400 MHz; $CDCl_3$): ^1H, ^{13}C and 2-D NMR experiments (COSY, HETCOR, COLOC, and NOESY) – see Table 2.

FAB-MS - (Matrix: m-nitrobenzyl alcohol + Na): m/z 306 (62%), m/z 289 (51.5%), m/z 273 (13.5%), 263 (26.5%).

The ^{13}C NMR of this compound showed twenty peaks comprising five methyl, seven methylene, and four each of methine and quaternary carbon signals. The mass spectrum, with a molecular ion peak at m/z 306 ($C_{20}H_{34}O_2$), suggests that this 20-carbon

compound is a diterpenoid. From the molecular formula, the Double Bond Equivalent (DBE) of this compound is 4. Since the ^{13}C NMR shows the presence of 4 different olefinic C resonances (comprising two methylene – δ 122.20, 125.55 and two quaternary signals – δ 134.62, 134.69) there should be two double bonds in the structure. The remaining two DBE can therefore be ascribed to two rings.

The ^{1}H NMR spectrum indicates the presence of: two olefinic protons (poorly resolved signals at δ 5.1 on C-3 and δ 5.08 on C-7); a proton attached to an oxygenated carbon (δ 3.30, *d*, J = 10Hz on C-11); three methyl groups (δ 1.068, 1.505 and 1.626 all singlets with the two most downfield being attached to unsaturated carbon atoms); an isopropyl group (two superimposed doublets at δ 0.91, J = 6.5Hz) as well as some overlapping, poorly resolved methylene signals in the upfield region of the spectrum. As shown in Table 2 the HETCOR spectrum confirms one-bond correlation of ^{13}C with ^{1}H resonances. In addition to the COLOC assignments which indicate long range correlations, shown in Table 2, the following correlations were also observed: H-16,17 x C-1,15; H-18 x C-3,4,5; and H-19 x C-7,8,9. The long range C-H correlation deduced from the COLOC spectrum, H-H COSY coupling and NOESY, prove that compound is the macrocyclic diterpenoid: 1,12-epoxy-3,7-cembradien-11-ol, known as incensole [1].

Table 2: NMR data on incensole

Position	δ_C (ppm)	DEPT	δ_H (ppm) / HETCOR	COLOC	H-H COSY	NOESY
1	88.99	C		H-16/17; H-2		
2	32.76	CH_2	2.00, 2.20		H-3	H-16/17
3	122.20	CH	5.10	H-2; H-18	H-2	H-11
4	134.69	C		H-18;		
5	39.04	CH_2	2.10	H-6; H-18	H-6	H-18
6	25.26	CH_2	2.18	H-5	H-5; H-7	H-19
7	125.55	CH	5.08	H-9; H-19	H-6	
8	134.62	C		H-9; H-19		
9	34.08	CH_2	1.97, 2.12	H-11; H-19	H-10	H-10
10	31.13	CH_2	1.32, 1.87	H-9	H-9; H-11	H-9; H-20
11	75.79	CH	3.30	H-9; H-20	H-10	H-13
12	84.58	C		H-10; H-20		
13	36.77	CH_2	1.75, 2.02	H-14; H-20	H-14	H-11; H-14
14	31.02	CH_2	1.57, 1.83	H-13	H-13	H-13
15	35.23	CH	1.90	H-2; H-16/17	H-16/17	H-16/17
16	18.43*	CH_3	0.90		H-15	H-2; H-14
17	18.50*	CH_3	0.91		H-15	H-2; H-14
18	16.57	CH_3	1.505		H-3	H-5
19	18.60	CH_3	1.62		H-7	H-6
20	21.10	CH_3	1.06		H-11	H-10; H-13

N.B.: * assignments are interchangeable.

Further confirmation of the structure was obtained from FAB-MS data. Apart from the molecular ion at m/z 306, a few fragment ions also appear. These include: m/z 289 – corresponding to the loss of OH group (17 mass units) from the molecular ion; m/z 263 – corresponding to the loss of an isopropyl group (43 mass units) from the molecular ion; and m/z 273 which corresponds to the loss of OH group and O atom (33 mass units) from the molecular ion. This latter fragment confirms the presence of an oxygen bridge.

Due to the nature of the matrix, many peaks also appear in the spectrum. Those that can be readily identified include: m/z 329 – molecular ion + Na; m/z 442 – due to ion at m/z 289+ *m*-nitrobenzylalcohol (MNOBA); and m/z 426 – due to ion at m/z 273 + MNOBA.

The ^{1}H NMR data for this compound agree with that obtained by earlier workers (Corsano and Nicoletti, 1967), who isolated and characterized the same compound, incensole, from the neutral fraction of frankincense, produced by *Boswellia carteri*. Its chemical synthesis (Kato, 1976) and ^{13}C NMR spectral data (Gacs-Baitz, *et al.*, 1978) have also been reported. It is also noteworthy that the 2-D NMR data for incensole is being reported here for the first time.

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