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Article · July 2016

DOI: 10.1080/22311866.2016.1250676

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Journal of Biologically Active Products from Nature

ISSN: 2231-1866 (Print) 2231-1874 (Online) Journal homepage: http://www.tandfonline.com/loi/tbap20

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To cite this article: Balogun Olaoye S., Ajayi Olukayode S. & Agberotimi Bukola J. (2016) A Cytotoxic Indole Alkaloid from Alstonia boonei, Journal of Biologically Active Products from Nature, 6:4, 347-351, DOI: 10.1080/22311866.2016.1250676

To link to this article: http://dx.doi.org/10.1080/22311866.2016.1250676

Published online: 24 Nov 2016.

Taylor & Francis
Taylor & Francis Group

ISSN Print: 2231-1866
ISSN Online: 2231-1874

A Cytotoxic Indole Alkaloid from *Alstonia boonei*

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Received 20 November 2015; accepted in revised form 16 October 2016

Abstract: Stem bark of *Alstonia boonie* (1.24 kg) was exhaustively extracted with 95 % aqueous methanol. The crude extract was defatted with n-hexane and thereafter subjected to extensive chromatography to yield Alstiboonine (1) which was fully elucidated and characterised using 2D NMR analysis. Alstiboonine, crude extract and the n-hexane fraction were subjected to brine shrimps lethality test at varying concentrations. The result showed that all the test samples exhibited significant cytotoxicity.

Key words: Indole alkaloids; *Alstonia boonie*; Alstiboonine; cytotoxicity.

Introduction

Alstonia boonei De Wild belongs to the family Apocynaceae. The specie of *Alstonia boonie* is indigenous to Africa and it has rich folkloric usages [1,5]. The fresh bark of *A. bonnie* could be used for herbal preparations against snake, rat, or scorpion poison. The bark decoction is also used with other preparations in the treatment of fractures or dislocation, jaundice, asthma, malaria, bacterial infections, rheumatism and arthritic pain [8]. Some of these therapeutic claims by the traditional health practitioners have been scientifically validated [9]. The genus Alstonia is a veritable source of a number of bioactive alkaloids. Indole alkaloids such as echitamine, echitamidine, voacangine, akuammidine, Nα-formylechitamidine and Nα-formyl-12-methoxyechitamidine have been isolated from *A. boonie* [3,6], while an array of picraline-type alkaloids, alstiphyllanines A-O have been reported from *A. macrophylla* [2,4]. This study aimed at further profiling the secondary metabolites of *A. boonie*.

Materials and methods

The stem bark of *Alstonia boonei* was collected in premises of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria in April, 2013 and was authenticated by Mr. A. S. Gabriel, a taxonomist at the Department of Botany of the same institution. The air-dried stem barks (1.20 kg) were pulverized, extracted with 95 % aqueous methanol and concentrated *in vacuo* at 37°C using rotary evaporator to give 56 g of crude extract which was further defatted with n-hexane to yield 48 g extract.

The defatted extract (25 g) was subjected to column chromatography usig silica gel stationary phase and gradient of dichloro-methane and methanol as eluting solvents afforded a total of 83 fractions pooled to 10 sub-fractions (A1-A10) based on TLC experiment. Sub-fraction A5 (0.44 g) eluted by 0.25 % methanol in dichloromethane had a better TLC profile and therefore was further chromatographed on sephadex LH 20 and eluted with gradient mixture of chloroform and methanol to give 10 fraction bulked into 3 sub-fractions (B1-B3). B2 (119 mg) eluted by 50 % chloroform in methanol showed few unresolved bands on TLC using app-ropriate developing solvents, therefore it was separated on a small silica gel column and using dichloromethane, ethyl acetate and methanol in an increasing order of po-

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larity to afford compound **1** (8 mg).

IR ν_{max} (cm^{-1})

3421 (O-H), 1640 (C=C), 1376 (C-H), 1210 (C-O), 1036 (C-N).

^{1}H NMR (DMSO, 600 MHz):

δ 4.39 (1H, s, H-3), δ 5.18 (1H, m, H-5), δ 3.77 (1H, m, H-6a), δ 3.38 (1H, d, *J* = 10.9 Hz, H-6b), δ 7.73 (1H, d, *J* = 7.9 Hz, H-9), δ 6.77 (1H, m, H-10), δ 7.11 (1H, m, H-11), δ 6.75 (1H, m, H-12), δ 2.02 (1H, m, H-14a), δ 2.26 (1H, m, H-14b), δ 3.59 (1H, t, H-15), δ 3.75 (1H, m, H-17a), δ 3.87 (1H, m, H-17b), δ 1.78 (3H, d, *J* = 6.8 Hz, H-18), δ 5.74 (1H, q, *J* = 6.8 Hz, H-19), 4.25 (1H, d, *J* = 15.0 Hz, H-21), δ 4.41 (1H, m, *J* = 15.0 Hz, H-21), δ 6.33 (NH), δ 3.28 (3H, s, *N* (4)-Me), δ 3.74 (3H, s, COOMe)

^{13} C NMR (DMSO, 150 MHz)

δ 110.5 (C-2), δ 64.8 (C-3), δ 99.7 (C-5), δ 41.2 (C-6), δ 51.9 (C-7), δ 135.2 (C-8), δ 126.7 (C-9), δ 119.5 (C-10), δ 129.7 (C-11), δ 128.8 (C-12), δ 147.4 (C-13), δ 31.2 (C-14), δ 34.5 (C-15), δ 60.7 (C-16), δ 64.7 (C-17), δ 14.7 (C-18), δ 128.9 (C-19), δ 132.0 (C-20), δ 61.7 (C-21), δ 173.1 (C-22), δ 51.9 (*O-Me*), δ 55.7 (*N*(4)-*Me*) .

Cytotoxicity assay

The brine shrimp lethality assay was used for the evaluation of cytotoxicity of the crude extract, defatted fraction and the isolated compound. Little quantity of the brine shrimps (*Artemia salina* Leach) eggs was sprinkled into a crucible containing seawater and the crucible was left half covered to allow partial illumination. After a period of about 48 hours the egg hatched into matured *nauplii*. To each solution of 1000, 100 and 10 µg/mL of the test sample, 10 matured *nauplii* were added. Distilled water was used in place of the test samples for negative control. All the experiments were carried out in triplicate. Numbers of survivors were noted after 24 hours and the LC_{50} was computed at 95 % confidence limit using Finney programme.

Results and discussion

Compound 1 was obtained as brown amorphous solid. The IR spectrum showed diagnostic absorption bands for stretches of O-H, C=C, C-H, C-O (ester) and C-N (2^{o} amine) at 3421, 1640, 1376, 1210 and 1036 cm^{-1} respectively. The ^{13}C NMR spectrum showed 22 signals which were differentiated by DEPT experiments into quaternary, methine, methylene, methyl and heteroatom-linked carbons. The chemical shifts of the carbons are typical of a picraline-type skeleton with the presence an ethylidene side chain [δ_C 14.7 (C-18)], ethylidene carbons [δ_C 128.9 (C-19) & 132 (C-20)], ten sp^3 carbons [δ_C 110.5 (C-2), 64.8 (C-3), 99.7 (C-5), 41.2 (C-6), 51.9 (C-7), 31.2 (C-14), 34.5 (C-15), 60.7 (C-16), 64.7 (C-17), 61.7 (C-21)], two amino carbons [δ_C 30.5 (*N*-1) & 55.8 (*N*-4)], aromatic carbons [δ_C 135.2 (C-8), 126.7 (C-9), 119.5 (C-10), 129.7 (C-11), 128.8 (C-12), 147.4 (C-13)], a methoxy carbon at 51.7 ppm and the carbonyl carbon at 173.1 ppm.

The ^{1}H NMR data showed the presence of following diagnostic proton peaks: aromatic protons (δ_H 6.75-7.73), ethylidene side chain [δ_H 1.78 (3H, d, *J* =6.8 Hz), olefinic [δ_H 5.75, 1H, q, *J* =6.8 Hz), methyl ester [δ_H 3.75, (3H, s)], methyl of quaternary amine [δ_H 3.28, (3H, s) and 2^{o} amine [δ_H 6.33, (1H, s)]. The characteristic chemical shifts and couplings of protons at position 19 and 18 were similar to those of ajmaline and picraline type alkaloids [2,4]. Also, the prominent singlets at δ_H 3.74 and 3.28 were indicative of protons of *O* and *N*-linked methyl groups respectively and have been reported for most alstiphyllanine alkaloids which are of picraline-type [2,4].

On comparison of the NMR spectroscopic data of compound 1 with that of alstiphyllannine B isolated from *Alstonia macrophylla* [4], a close match was observed, except for few discrepancies arising from the differences in the chemical moiety at position 1, 10, & 17 and nature of deuterated solvent used in the experiments (Table 1). Unlike the NMR spectrum of compound 1, alstiphyllanine B (Figure 1) has signals representing aromatic system and carbonyl of ester due to its dimethoxybenzoate moiety at position 17. Also, the signals of *N*-methyl and methoxy at position 1 and 10 respectively found in alstiphyllanine B were not observed in the spectrum of compound 1. The amino proton resonating as

Table 1 Comparison of NMR data of Alstiphyllanine B and Compound 1

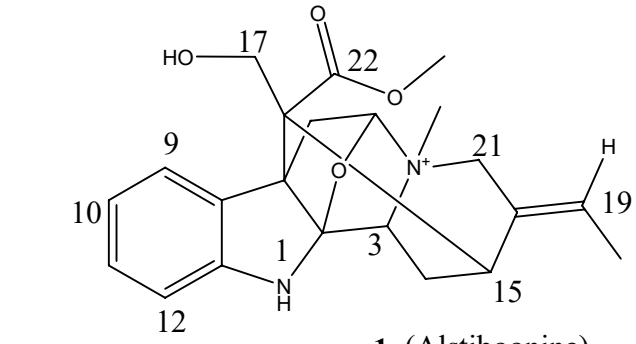
No.	^{13}C*	^{13}C	DEPT	^{1}H* (multiplicity, *J*)	^{1}H(multiplicity, *J*)
2	113.4	110.5			
3	64.8	64.8	CH	4.30 (brs)	4.39 (1H, brs)
5	99.2	99.7	CH	5.43 (brs)	5.18 (1H, m)
6a & b	41.5	41.2	CH_2	3.55 (d, 15.4), 3.30 (d, 13.9)	3.77 (m), 3.38 (d, 10.9)
7	51.8	51.9			
8	134.4	135.2			
9	116.4	126.7	CH	7.03 (s)	7.73 (1H, d, 7.9)
10	156.4	119.5	CH		6.77 (1H, m)
11	112.3	129.7	CH	6.38 (d, 8.0)	7.11 (1H, m)
12	111.2	128.8	CH	6.58 (d, 8.0)	6.75 (1H, m)
13	144.7	147.4			
14a & b	31.0	31.2	CH_2	2.28 (d, 15.3), 2.50 (d, 15.3)	2.02 (m), 2.26 (m)
15	34.3	34.5	CH	3.67 (brs)	3.59 (1H, t)
16	57.2	60.7			
17a & b	68.1	64.7	CH_2	4.07 (m), 4.91 (m)	3.75 (m), 3.87 (m)
18	14.9	14.7	CH_3	1.74 (d, 6.1)	1.78 (3H, d, 6.8)
19	127.4	128.9	CH	5.76 (m)	5.74 (1H, q, 6.8)
20	128.8	132.0			
21a & b	58.1	61.7	CH_2	4.32 (m), 4.36 (m)	4.25 (15.0), 4.41 (15.0)
22	172.5	173.1			
23	166.0				
NH					6.33 (brs)
N(1)Me		30.5		3.00 (s)	
N(4)Me	54.8	55.7	CH_3	3.28 (s)	3.28 (s)
COOMe	52.8	51.9	CH_3	3.79 (s)	3.74 (s)
10-O-Me	55.5			3.34 (s)	
3'-O-Me	56.3			3.88 (s)	
4'-O-Me	56.5			3.88 (s)	
1'	112.4				
2'	113.3			7.10 (s)	
3'	149.9				
4'	154.8				
5'	111.7			6.94 (d, 8.2)	
6'	124.7			7.24 (d, 8.2)	

*NMR data of alstiphyllanine B [4].

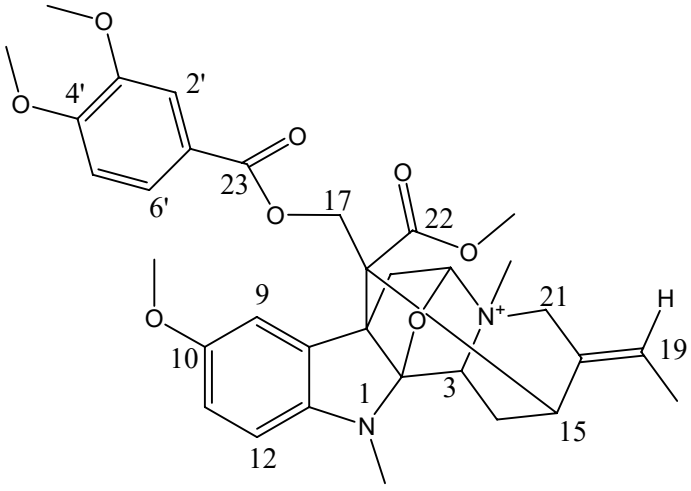
singlet at δ_H 6.33 for compound 1 was absent in alstiphyllanine B. The ^{1}H-^{1}H correlation spectroscopy (COSY) showed cross peaks for aromatic protons C-9 to C-12, ethylidene protons C-19 and C-18 and protons at C-14 and C-15. The compound was fully elucidated and characterized by 2D NMR as alstiboonine (Figure 1). However, the stereochemistry of the compound was not established because NOESY experiment was not carried out.

The brine shrimp lethality test indicated LD_{50} of 194.50, 161.66 and 39.72 µg/mL for crude ex-

tract, defatted extract and Alstiboonine respectively. According to Khaled [7], plants having LC_{50} values >1000 ppm are considered inactive while plants having LC_{50} values < 200 ppm (extract) and < 5 ppm (pure compound) are considered highly active. Thus, both the extracts and Alstiboonine are considered active.



1 (Alstiboonine)



Alstiphyllanine B

Figure 1. Structure of Compound 1 (Alstiboonine) and Alstiphyllanine B

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