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Isolation and characterization of nicotinic alkaloid from *Caesalpinia bonduc*

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ABSTRACT

Caesalpinia bonduc a medicinal plant belonging to the family caesalpiniaceae has been reported to possess a number of pharmacological activities. Phytochemical investigation have led to the isolation of constituents such as alkaloids, phytosterols, amino acids, phenolic acids, sugars, minerals, flavonoids and terpenoids. The objective of the present study was to isolate and characterize phytoconstituents from the leaf extract of the plant. The methanol extract of the leaf was fractionated by solvent-solvent extraction into petroleum ether, chloroform and ethyl acetate soluble fractions. Column chlomatography of petroleum ether fraction gave seven fractions (A-G). Column chromatography and TLC analysis of fraction A afforded two white crystals (1 and 2). The compounds (1 and 2) were characterised as 3-carboxylethyl pyridine and 3-carboxylbutyl pyridine respectively on the basis of spectroscopic (ID and 2D NMR, MS and IR) analyses.

KEYWORDS: *Ceasalpinia bonduc;* phytoconstituents; chromatography

INTRODUCTION

Herbal medicine has been used worldwide for the treatment, control and management of a variety of ailments since prehistoric times [1-4]. Prior to the nineteenth century, plant medicines were administered mostly in their crude forms as infusions, tinctures, decoctions, syrups or applied externally as ointments and herbal washes [1, 5]. However, during the late nineteenth and early twentieth century, scientists began isolating, purifying and identifying the active ingredients from medicinal plant extracts. This endeavour led to the discovery of some of the most important drugs that are still widely used in modern medicine [6-9]. For example, morphine isolated from opium poppy (Papaver somniferum) is a powerful narcortic analgesic; quinine isolated from Cinchona bark is an effective anti-malarial drug; taxol (isolated from Taxus brevifolius) and vincristine (isolated from Catharanthus rosesus) are highly effective against certain types of cancer and serpentine (isolated from the root of the Indian plant Rauwolfia serpentina) is used in the treatment of hypertension [5, 7, 10, 9]

In addition to the biologically active plant-derived natural products mentioned above, many other plant derived natural products have served as "lead compounds" for the design, synthesis and development of novel drug compounds [2, 7, 10, 11]

Caesalpiniabonduc(L.) Roxb(family: caesalpiniaceae) is a shrub that grows in tropical part of the world [12]. The plant is well known for its medicinal uses as antimalarial, anthelminthic, antiproliferation and antirheumatic [13,1,15]. Phytochemical investigation has led to the isolation of compounds from different parts of the plant. Some of the compounds isolated from this plant include bonducellin, caesaldekarin C, caesalpinin, flavonol and caesalmin. To add to this wealth of phytochemicals, herein we report the isolation of two nicotinic alkaloids from the leaf extract of the plant.

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MATERIALS AND METHODS

General experimental procedure

The NMR spectra were recorded on a Bruker Avance 400FT spectrometer (for 1H-NMR) and 75 FT spectrometer (for 13C-NMR). Chemical shifts were expressed in parts per million (ppm) using TMS as internal standard. The IR spectra were determined on a Thermo Nicolet 470 FTIR spectrometer. Melting point was determined using Thomas HOOVER capillary melting point apparatus. Column chromatography was done using silica gel (200-400 mesh), TLC analyses were performed on precoated silica gel 60 F254 plates. The plates were visualized under UV (254 and 366 nm) and by spraying with Dragendorf reagent and vanillin sulphuric acid and drying.

Plant collection and preparation

Fresh leaves of *Caesalpinia bonduc* were collected in June 2011 from Igueben in Igueben Local Government Area of Edo State. It was identified and authenticated by Ugbogu O.A. and Shasanya O.S. of the Forestry Research Institute of Nigeria (FRIN), Ibadan. A Specimen number 109493 was given and the plant specimen was deposited at the FRIN Herbarium. The fresh leaves were air dried and the dried material was grounded into powder using an electric blender (pye Unicam, Cambridge, England) and stored in an airtight container until further use.

Extraction and isolation

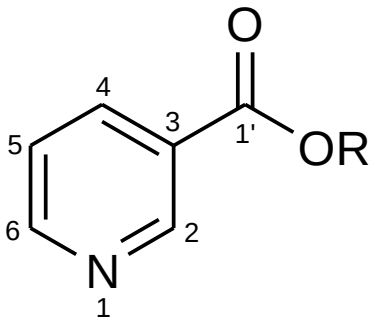
Dried leaves (3.8 kg) were extracted with 15 L of methanol by cold maceration for 72 hours. Dried methanol extracts were obtained after removing the solvent by evaporation under reduced pressure. The extract (268 g) was suspended in 200 mL methanol and 50 mL distilled water and extracted with 15 L of petroleum ether. The ether insoluble portion was partitioned successively into 7.5 L of chloroform and 5 L of ethyl acetate.

The petroleum ether fraction (29 g) was subjected to silica gel column chromatography eluting with petroleum ether and increasing polarity with ethyl acetate to obtain seven fractions (A-G). Fraction A (7.6 g) was chromatographed on silica gel column eluting with petroleum ether:ethyl acetate (9:1, 8:2, 7:3 up to 100% ethyl acetate). Compounds 1 and 2 crystallised out of solution and were obtained as white crystalline solid in petroleum ether:ethyl acetate mixture (4:6) and (3:7) for compound 1 and 2 respectively. After recrystallisation in petroleum ether, the compounds were characterised by spectroscopic analyses (NMR, MS and IR).

RESULTS AND DISCUSION

Compound 1 (3-carboxylethyl pyridine)

White crystalline solid (2.2 mg); m.p. 223 - 225°C, MS: m/z = 151.2 $[M]^+$.
IR (KBr) cm-1 2980.5 (aromatic CH), 1720.3 (CO), 1593.7 (C = C), 1476.4 (C= N).
1H-NMR (DMSO-d6) δH: 9.20 (s, H-2, 1H), 8.80 (d, H-6, J = 4.0 Hz, 1H), 8.30 (d, H–4, J = 2.0 Hz, 1H), 7.12 (dd, H-5, J = 6.2 Hz, 7.9 Hz, 1H), 4.20 (m, H-2', 2H), 1.12 (t, H-3', 3H).
^{13}CNMR (DMSO-d6) δc: 164.9 (C-1'), 153.1 (C-2), 150.6 (C-6), 126.1 (C-3), 136.8 (C-4), 123.1 (C-5), 60.9 (C-2'), 14.0 (C-3').



1 R = CH_2CH_3

2 R = $CH_2CH_2CH_2CH_3$

Compound 2 (3-carboxylbutyl pyridine)

White crystalline solid (2.0 mg), m.p. = 124 - 125°C, MS: 178.0 [M-H]+. IR (KBr) cm-1: 1720.3 (CO), 2958.3 (aromatic C-H), 1590 (C=C), 1497 (C=N).
1H-NMR (DMSO-d6) δH: 9.20 (s, H-2, IH), 8.80 (d, H-4, J = 4.0 Hz, IH), 8.30 (d, H-6, J = 2.0 Hz, IH), 7.50 (dd, H-5, J = 6.2, 7.9 Hz, IH), 4.40 (t, H-2', 2H), 1.70 (m, H-3', 2H), 1.30 (m, H-4', 2H), 0.99 (t, H-5', 3H).

13C-NMR (DMSO-d6) δc: 165.0 (C-1'), 153.0 (C-6), 150.6 (C-2), 136.8 (C-5), 126.2 (C-3), 123.1 (C-4), 65.0 (C-2'), 30.5 (C-3'), 19.0 (C-4'), 13.5 (C-5').

Table 1: NMR data of compounds 1 and 2 ($CDCl_3$, 250 and 300 MHz for ^1H and 62.5 MHz for ^{13}C)

Position	1		2	
	^{13}C	^1H (multiplicity, J in Hz)	^{13}C	^1H (multiplicity, J in Hz)
1	----	----	----	----
2	153.1	9.20 (s)	153.0	9.20 (s)
3	126.1	----	126.2	
4	136.8	8.30 (d, 2.0)	123.1	8.80 (d, 4.0)
5	123.1	7.12 (dd, 6.2, 7.9)	136.8	7.50 (dd, 6.2, 7.9)
6	150.6	8.80 (d, 4.0)	150.6	8.30 (d, 2.0)
1'	164.9	----	165.0	---
2'	60.9	4.20 (m)	65.0	4.40 (t)
3'	14.0	1.12 (t)	30.5	1.70 (m)
4'	**----**	----	19.0	1.30 (m)
5'	----	----	13.5	0.99 (t)

Fractionation of petroleum ether extract of *Ceasalpinia bonduc* leaf on silica gel column chromatography gave seven fractions (A-G). Purification of Fraction A on silica gel column eluting with petroleum ether and increasing polarity with ethyl acetate yielded compounds 1 (2.2 mg) and 2 (2.0 mg) both crystallising out of solution in petroleum ether:ethyl acetate (4:6 and 3:7). The compounds were isolated as white crystals with melting point of 223 - 225°C and 124 - 125°C for 1 and 2 respectively. Chemical reactions with Lassaigne's and Ninhydrin reagents, suggested that the isolated compounds were nitrogenous.

Compound 1 was isolated as a white crystalline solid. The IR spectrum showed a strong absorption at 1720.3 cm-1 suggesting the presence of a carbonyl function. The weak absorption at 2980.5 cm-1 was assigned for aromatic C-H stretching. Aliphatic C-H stretching was also found at 2930 cm-1. An absorption peak of medium intensity at 1593.7 cm-1 was also observed indicating an aromatic C=C stretching. In addition, the quite intense band at 1476.4 cm-1 indicated the existence of aromatic C=N stretching. Mass spectra analysis of 1 gave the molecular ion [M]+ at m/z 151.2 with characteristic fragment ions observed at m/z: 123 [M-CO]+, 106 [M-OC2H5]+, and 78 [C5H4N]+. 1H-NMR spectrum of 1 showed the presence of 6 proton signals. Four aromatic protons at δH 9.20 (s), 8.80 (d, J = 4 Hz), 8.30 (d, J = 2 Hz), 7.60 (dd, J = 6.2 Hz, 7.9 Hz). The peak at 4.20 ppm indicates the presence of methylenic protons (-CH2) in highly deshielded environment. The peak at 1.12 ppm is indicative of methyl protons (-CH3). The 13C-NMR spectrum of 1 showed eight carbon signals of which 5 signals accounted for the pyridine moiety. The peaks at 153.1 ppm and 150.6 ppm were assigned to carbons (C-2 and C-6) adjacent to the nitrogen atom in the pyridine ring. The peak at 164.9 ppm indicates an ester carbonyl. The peaks at 123.1 and 14.0 ppm were assigned to the least deshielded carbon in the pyridine moiety and the aliphatic chain respectively. The DEPT-135 spectrum showed six signals at δc 153.1, 150.6, 136.8, 123.1, 60.9 and 14.0 indicating the presence of three aromatic methine carbons, one methylene carbon in a de-shielded environment and one methyl carbon.

Compound 2 was isolated as white crystalline solid. The MS of 2 showed m/z 178 for the molecular ion [M-H]+ with characteristic fragments ions at m/z 164 [M-CH_3]+, 106 [M-OC4H9]+ and 78 [C5H4N]+. The

1H and 13C-NMR spectra data of 2 was similar to that of 1 except for the extra two signals in the aliphatic region. 1H-NMR spectrum of 2 revealed the presence of two CH_2 multiplet and a CH2 triplet at 1.30, 1.70 and 4.40 ppm respectively. The DEPT-135 spectrum showed four aromatic carbon signals at δ 153.0, 150.6, 136.8 and 123.1 ppm, three methylene carbons at 65.0, 30.5 and 19.0 and one methyl carbon at 13.5 ppm.

On the basis of the spectral data and by comparison with pure authentic samples, compounds 1 and 2 were characterised as 3-carboxylethyl pyridine and 3-carboxylbutyl pyridine respectively. This is the first report of the isolation of these compounds from *Ceasalpinia bonduc*.

COMPETING INTERESTS

The authors declare no competing interests.

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