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CHARACTERISATION OF INDOLE ALKALOIDS FROM SEEDS OF ERYTHRINA POEPPIGIANA (FABACEAE)

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Keywords: *hypaphorine, erysodine, Erythrina poeppigiana, Fabaceae, indole alkaloids , antioxidant.*

Abstract

Two known alkaloids, hypaphorine (1) and erysodine (2) were isolated for the first time from the seeds of Erythrina poeppigiana, together with one known sterol, the stigmasterol (3). The structures of isolated compounds were elucidated with various spectroscopic methods such as 1D- and 2D- NMR, IR, UV, Mass. the complete 13C-NMR data of hypaphorine (1) were also reported. The compounds (1) and (2) were tested to examine their DPPH radical scavenging and enzyme inhibition activities. Compound (2) exhibited the good antioxidant activity with the IC50 of 29.5 µg/mL while compound (1) exhibited moderate antioxidant activity with the IC50 of 59.8 µg/mL. Compound (2) had a weak Urease Inhibition activity with a IC50 > 200 µg/mL.

Introduction

Medicinal plants have been used since several years in traditional medicines for the treatment of many diseases. This situation has pushed researchers to screen a variety of plants for their biological activities and their chemical constituents (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017). It is this context that we carried out the study of *Erythrina poeppigiana,* a Brazilian medicinal plant of the family of Fabaceae.

Erythrina poeppigiana is a tree reaching up to 35 m high and which occurs in tropical America, Panama, Bolivia, Brazilia, Peru, Africa and Asia (Little, 1964).

Flowers of this species are used as spices in some meals and salads. The roots extract are used to stun fishes and capture them easily (Little, 1964).

A survey of the literature revealed the isolation and identification of a number of isoflavonoids as the erypoegin I isolated from specie roots (Tanaka et al., 2003), the érysubine F isolated from species roots and exhibiting the antibacterial activity (Tanaka et al., 2002a; 2002b), a number of alkaloids as the époxyde de 8-oxo-α-érythroïdine isolated from the specie wood (Tanaka et al., 2001), a number of arylbenzofurannes as erypoegin F (Tanaka et al., 2003). The present study deals with the isolation and identification of two indole alkaloids (isolated for the first time from this species) and one known sterol from the seeds of *Erythrina poeppigiana*. Furthermore, the antioxidant and enzyme inhibition activities were studied for this plant.

Materials and methods

Plant material

Seeds of *Erythrina poeppigiana* were collected at Rio de Janeiro in Brazil in 2009 and identified by Mr. Nana Victor of the National Herbarium, Yaoundé, Cameroon.

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Extraction and isolation of compounds

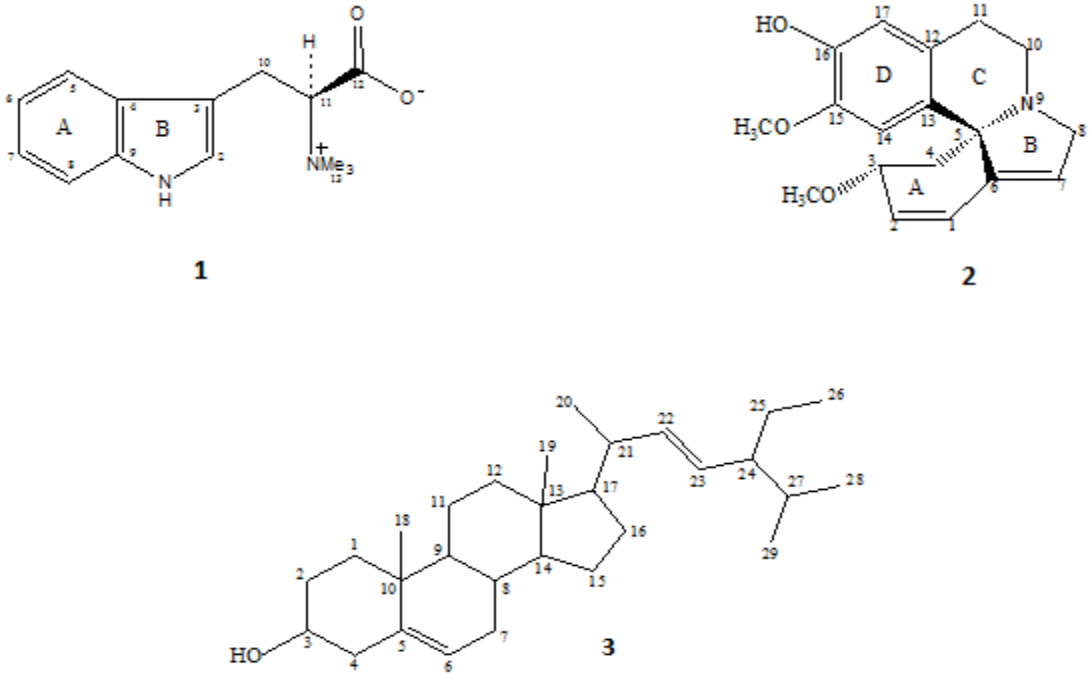
The air-dried and powdered seeds (5.5 kg) of *Erythrina poeppigiana* were separately extracted with MeOH at room temperature for 72 h. After evaporation under reduced pressure, 1000.0 g of brown crude material were obtained. The crude extract (975 g) was extracted with n-Hexane (4 x 500 ml), with CH_2Cl_2 (3 x 500 ml) and with ethyl acetate. The extracts were concentrated under reduced pressure to afford 75.0, 15.0 and 37.5 g of n-hexane, dichloromethane and ethyl acetate extracts respectively. The acetate fraction was purified by column chromatography over silica gel 60 (230-400 mesh) using a gradient system of n-hexane, ethyl acetate and MeOH. All together, 108 subfractions (250 ml each) were collected and pooled on the basis of TLC analysis, leading to seven main fractions (A-H). Fraction B (5.0 g) was chromatographed over silica gel 60H column with a mixture of n-hexane-acetate (6:4) to yield stigmasterol **(3)** (15 mg). Fraction F (5.0 g) was chromatographed over silica gel 60H column with a mixture of acetate-methanol (8:2) to yield erysodine **(2)** (16.0 mg). Fraction H (5.0 g) was chromatographed over silica gel 60H column with a mixture of acetate-methanol (6:4) to yield hypaphorine **(1)** (20 mg)**.**

hypaphorine (1): obtained as a colourless crystal (20 mg). m.p. 254-255 ^oC optical activity $[\alpha]_D^{20}$ + 140^o (c 1.00, H_2O). ^1H NMR (600 MHz, MeOD) and ^{13}C-NMR (600 MHz, MeOD): Table 1. FAB-MS at m/z 247.1 for $(M+H)^+$ (calc for $C_{14}H_{18}O_2N_2$)

erysodine (2): obtained as an brown amorphous solide (16.0 mg). m.p. 204-205 ^oC. MS(IE) at m/z 299.1 (calc for $C_{18}H_{21}O_3N$)

stigmasterol (3): obtained as a white powder (15 mg). m.p. 170 ^oC. MS(IE+): m /z 412 $[M]^+$ (calc for $C_{29}H_{48}O$)

Figures:



1 2 3

Fig.1. Structure of compounds 1-3

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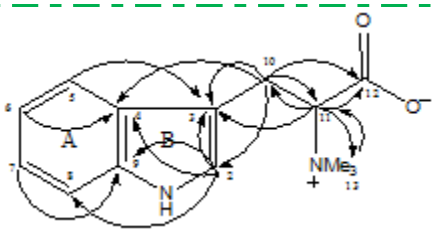

Fig. 2. Selected HMBC correlations of 1

Apparatus

Optical rotations were determined on a JASCO P-1030 polarimeter. UV and IR spectra were obtained on JASCO Ubest-35 and JASCO FT/IR-230 spectrometer, respectively. 1H- and 13C-NMR spectra were recorded on Bruker AMX-600 spectrometers. The 3.35 and 49.8 ppm resonances of residual CD_3OD were used as internal references for 1H and 13C-NMR spectra, respectively. FAB mass spectra were measured on a JEOL HX-110 spectrometer using a glycerol matrix. All solvents, except those used for bulk extraction are AR grade. Silica gel 60 F254 for thin layer chromatography (TLC) was used for column chromatography. Glass and aluminium supported silica gel 60 F254 plates were used for TLC. TLC spots were visualized under UV light (254 and 365 nm) followed by spraying with Dragendorff's reagent for alkaloid detection.

Procedure: DPPH radical scavenging activity and Urease inhibition assay

DPPH radical scavenging activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) (Gulcin *et al*, 2005).The solution of DPPH of 0.3 mM was made in ethanol. Five microlitres of each sample of different concentrations (62.5 μg - 500 μg) were mixed with 95 μl of DPPH solution in ethanol. The mixture was distributed in a 96 well plate and incubated in dark at 37 °C for 30 min. The absorbance at 515nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined in comparison with the methanol treated control (Eqn. 2).

Butylated hydroxyanisole (BHA) was used as a standard.

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Ac - As}}{\text{Ac}} \times 100 \quad \text{(Eqn. 2)}$$

Where Ac and As respectively account for the absorbances of control (DMSO treated) and sample.

Urease inhibition assay

Reaction mixtures with 25 µl of enzyme (Jack bean Urease bought from Sigma) solution and 55 µl of buffers containing 100 mM urea were incubated with 5 µl of test compounds (compounds 1-2) (1 mM concentration) at 30°C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as previously described by Weatherburn in 1967 (Weatherburn, 1967) with little modification (Pervez et al., 2016). Briefly, 45 µl each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µl of alkali reagent (0.5% w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µl. The results (change in absorbance per min) were processed by using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M LiCl). Percentage inhibitions were calculated from the formula (Eqn. 1):

Urease inhibition (%) = 100 – ($OD_{test\ well}$ /$OD_{control}$) x 100 (Eqn. 1)

Where OD stands for Optical Density

Thiourea was used as the standard inhibitor of urease.

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Results and discussion

Identification of compounds

The methanol extract from the seeds of *Erythrina poeppigiana* was subjected to repeated column chromatography on silica gel and three compounds were isolated: two known indole alkaloids hypaphorine **(1)** (Preeyanuch et al., 2015; Sook et al., 2012) and erysodine **(2)** (Juma et Majinda, 2004) and one known sterol, the stigmasterol **(3)** (Huang et Chiang, 2004).

Compound **1** was obtained as a colourless crystal, m.p. 254-255 ^{o}C optical activity $[\alpha]_D$ + 140^{o} (c 1.00, H_2O). It molecular formula was established as $C_{14}H_{18}O_2N_2$ from FAB-MS data [m/z 247.1 for $(M+H)^+$], indicating seven degrees of insaturation.

The infrared spectrum showed absorption bands characteristic of NH (3399.8 cm^{-1}), carboxylic carbonyl (1660.2 cm^{-1}) and CO (1091.5 cm^{-1}) groups. The UV spectrum of **1** was characteristic of an indole chromophore. Analysis of the 13C NMR (Table 1) in combination with DEPT and HSQC experiments revealed the presence of 12 signals including one aminomethyl (δ_c 52.8) one sp3 methylene (δ_c 24.6), one sp3 methine (δ_c 80.4) five sp2 methines (δ_c 112.4, 119.0, 120.0, 122.6, and 125.2) and four quaternary carbons (δ_c 108.9, 128.3 and 138.1) among which one carboxylic carbonyl (δ_c 172.0). The ^{1}H NMR spectrum (table 1) showed the presence of the four aromatic vicinal protons (δ_H 7,62 , 7.03, 7.09 and 7.32) of disubstitued benzene ring, of three protons in AAB system at δ_H 3.45 (H-10) and δ_H 3.96 (H-11) and of three methyl protons in singlet at δ_H 3.31, attributed to $N^+(CH_3)_3$ group. The ^{1}H–^{1}H COSY spectrum revealed connectivities (Fig. 3) of C-5 to C-8 and C-10 to C-11. HMBC correlations (Fig. 2) of H-2 to C- 3 (δ_c 108.9), H-5 to C-3 (δc 108.9), H-6 to C-4(δc 128.3) and H-7 to C-9 (δ_c 138.1) indicated the presence of a β -carboline ring (rings A and B). HMBC correlations of H_2- 10 to C-12 (δ_c 172.0), H-11 to C-10(δ_c 24.6), H3-13 to C-11 (δc 80.4) and H-11to C-13 (δc 52.8), revealed the connectivity of the $N^+(CH_3)_3$ group to C_α-11(δ_c 80.4) of the propanoate. HMBC of H_2 -10 to C-3 (δ_c 108.9), H-2 to C-3 (δ_c 108.9) and H-2 to C-8 (δ_c 112.4) with NOESY experiments which exhibited proximity to H_2 -10 and H-5 revealed the connectivity of C-3 (δ_c 108.9) to C_β-10 of the propanoate. Thus, compound **1**was established as known hypaphorine, α-N,N,N-trimethyltryptophanium betaine (Hakima et al., 2008) **(fig.1)**.

DPPH radical scavenging activity and Urease inhibition assay

DPPH radical scavenging activity

Compounds **(1-2)** were investigated using DPPH radical scavenging activity. It was noticed that all tested compounds exhibited the absorbance at 515nm. Compound **(2)** exhibited the smallest absorbance with the IC_{50} of 29.5 µg/mL, while compound (1) exhibited the highest absorbance with the IC_{50} of 59.8 µg/mL and as compared to the reference, BHA with IC_{50} value of 44.2 µg/mL (Table 2). This is the first report of the DPPH radical scavenging activity of these two compounds**.**

Urease inhibition assay

Compounds **(1-2)** were also investigated using Urease inhibition assay. It was noticed that compound **(1)** exhibited no Urease inhibition. Compound **(2)** had a weak response in the Urease Inhibition with a IC_{50} > 200 µg/mL and as compared to the reference, thiourea with IC_{50} value of 21.6 µg/mL (Table 2).

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Tables:

Table1: The ^{1}H NMR and ^{13}C NMR spectra data of compound 1(MeOD, 600 MHz for ^{1}H, 600 MHz for ^{13}C) data of compound 1

Position	^{1}H NMR	^{13}C NMR
1	-	-
2	7.19 (1H, s)	125.2
3	-	108. 9
4	-	128.3
5	7,62 (1H, d, J=8.0 Hz)	119.0
6	7.03 (1H, t, J= 7.5 Hz)	120.0
7	7.09 (1H, t, J= 7.5 Hz)	122.6
8	7.32 (1H, d, J=8.0 Hz)	112.4
9	-	138.1
10	3.45 (2H, d, J=7.2 Hz)	24.6
11	3.96 (1H, t, J=7.2 Hz)	80.4
12	-	172.0
13	3.31 (9H, s)	52.8

Table 2: Determination of DPPH radical scavenging and enzyme inhibition activities

Compound	Antioxidant IC_{50} (µg/mL)	Urease Inhibition IC_{50} (µg/mL)
1	59.8 ± 0.33	Nil
2	29.5 ± 0.23	> 200
BHA	44.2 ± 0.09	-
Thiourea		21.6 ± 0.12

Conclusion

Two known indole alkaloids hypaphorine **(1)** and erysodine **(2)** and one known sterol, the stigmasterol have been isolated from the seeds of *Erythrina poeppigiana*. The compounds **(1)** and **(2)** were tested to examine their DPPH radical scavenging and enzyme inhibition activities. Compound **(2)** exhibited the good antioxidant activity with the IC_{50} of 29.5 µg/mL while compound **(1)** exhibited moderate antioxidant activity with the IC_{50} of 59.8 µg/mL. Furthermore, Compound **(2)** had a weak Urease Inhibition activity with the IC_{50} > 200 µg/mL while compound **(1)** exhibited no Urease inhibition activity. These results suggest that compounds **(1)** and **(2)** could have a supportive role in the pharmaceutical field towards the development of new drugs against cancers.

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