Isolation and Spasmolytic Evaluation of New Alkaloids from *Dichrostachys cinerea* (L.) Wight et Arn. (Fabaceae)

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**ABSTRACT**

*Dichrostachys cinerea* (L.) Wight et Arn. (Fabaceae) root bark is used in Ivorian Traditional Medicine to treat asthma, which is a respiratory disorder characterized by inflammation and the restriction of tracheal muscles obstructing the air circulation. The tracheal relaxant effect of a crude aqueous-alcoholic extract of the plant root bark was previously shown. For the present study, alkaloids were isolated from the same extract and investigated *ex vivo* in C57Bl/6j mice isolated trachea contracted with carbachol 1 µM, in comparison with a reference bronchodilator, i.e. salbutamol. Two extraction procedures allowed isolating 2 Alkaloids that monodimensional and bi-dimensional nuclear magnetic resonance (NMR) and mass specters allowed identifying a pyrolidine structure type nucleus with a long bi-hydroxyled alkyl chain. Alkaloid 1, carrier of a sugar, is a glycoside of Alkaloid 2. Both alkaloids induced similar spasmolytic effects, but Alkaloid 1 was more effective than Alkaloid 2 at 9 × 10⁻⁶ M (p < 0.01), 3 × 10⁻⁵ M, and 9 × 10⁻⁵ M (p < 0.001). Salbutamol induced its spasmolytic effect in a different way, and its maximal effect E_max (less than 30%) was obtained at 9 × 10⁻⁶ M, while E_max of both alkaloids (100%) was obtained at 3 × 10⁻⁴ M.

**Keywords:** Alkaloids Isolated; Plant; Spasmolytic; Asthma

1. Introduction

*Dichrostachys cinerea* (L.) Wight et Arn. (Fabaceae), among others numerous plants, is commonly used, alone or in association with other plants, in African Traditional Medicine. The ethnobotanic inquiries state that the roots of this plant are astringent and used in rheumatism, urinary calculi and renal troubles [1]; in Togo, the root decoction is administered by oral route in abscesses [2]. In Ivory Coast, whereas the root decoction is used as mouthwash in case of tooth decays by people of the North, people of the South use it to treat asthma [3]. As asthma is a respiratory disorder which is essentially characterized by the restriction of tracheal muscles obstructing the air circulation [4], the tracheal relaxant effect of a crude aqueous-alcoholic extract of the plant root bark was previously shown [5]. Besides, bisnordihydrotoxiferine, a tertiary indole alkaloid isolated from the root of *Strychnos divaricans* was shown to antagonize acetylcholine-induced contractions in rat uterus and in guinea-pig ileum [6]; other alkaloids were also shown to have spasmolytic effects on guinea-pig isolated trachea contracted by carbachol, histamine, or KCl [7]. For the present study, alkaloids were isolated from the root bark extract and investigated *ex vivo* in mice isolated trachea in comparison with a reference bronchodilator, i.e. salbutamol.

2. Materials and Methods

2.1. Plant Material

The roots of *D. cinerea* were collected in September 2009 in the South-East of Ivory Coast in bushes near Grand-Bassam. The plant was authenticated by Professor Aké-Assi Laurent, a taxonomist at the Centre National de Floristique (Ivory Coast), in comparison with identified specimens and vouchers were deposited there. The barks
were removed from the roots, washed in distilled water, air-dried at air-conditioning temperature (18°C) for two weeks, and pulverized. Dehydration yield was 55.2%.

2.2. Extraction Procedure
The research for alkaloids in the crude extract was made by reactions of characterization in tube with the reactive of Valsen-Mayer, then on Thin Layer Chromatography plates revealed by the reactive of Dragendorff.

After that, two ways were used to isolate alkaloids from the crude aqueous-alcoholic extract of the plant root bark: direct division and division after acidification then alkanisation (Figure 1).

2.3. Pharmacological Tests
Alkaloids isolated from *D. cinerea* root bark were performed on mice isolated trachea pre-contracted with carbachol 1 µM in the aim to evaluate their spasmolytic effect.

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**Figure 1. Pure alkaloids extraction scheme.**
2.3.1. Preparation of Solutions
Alkaloids isolated, as well as the reference product (salbutamol), were extemporaneously dissolved and serially diluted in distilled water (3 × 10⁻⁵, 3 × 10⁻⁴, 3 × 10⁻³ and 3 × 10⁻² M).

2.3.2. Chemicals
Salbutamol and carbachol were purchased from Sigma-Aldrich Chemicals, France; all salts (NaCl, KCl, KH₂PO₄, MgSO₄, CaCl₂ and NaHCO₃) and d-glucose from Pro-lab.

2.3.3. Animals
All experiments were performed in accordance with the European Community guidelines in the care and use of animals (86/609/CEE, CE Off. J. no. L358, 18th December 1986), the European Ethics Committee (CREEA Ile-de-France Sud) guidelines, and the French Decree no. 87 - 848 (J Off République Française, 20 October 1987: pp 12245-12248). Experiments were performed on male C57BL/6J mice at 7 - 8 weeks of age (25 - 30 g; Elevage Janvier, Le Genest Saint Isle, France).

2.3.4. Preparation of Trachea Rings and ex Vivo Procedure
Mice were anesthetized with pentobarbital (60 mg/kg i.p.). The upper respiratory tract and associated alimentary tissue were rapidly excised and placed in ice-cold Krebs bicarbonate solution containing: NaCl 117 mM, KCl 5.36 mM, NaHCO₃ 25 mM, KH₂PO₄ 1.03 mM, MgSO₄ 0.57 mM, CaCl₂ 2.5 mM, D-glucose 11.1 mM. The tracheas were dissected free from surrounding tissue and cut into 2-mm length segments, which were suspended isometrically between 2 stainless steel hooks in organ chambers containing 5 mL Krebs bicarbonate solution at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. Isometric tension was recorded in real time by a force-displacement transducer connected to the PowerLab® data acquisition system controlled by the Chart® version 5 data analysis software (AD Instruments, Bella Vista, Australia). The rings were stretched in a stepwise manner to a resting value of 0.6 g for at least 1 hour. Tracheal rings were then challenged with 10⁻⁵ M carbachol (Sigma-Aldrich Chemicals, France) to evaluate their functional integrity. After a 45-minutes washout period, tracheal preparations were precontracted with a submaximal concentration of carbachol (10⁻⁴ M). After stabilization of the contraction, cumulative additions of the different products and of distilled water as control vehicle were performed.

2.3.5. Data Analysis and Statistics
Chemically, isolated components’ structure has been analyzed using monodimensional NMR (¹H, ¹³C), bi-dimen-

sional NMR (DEPT, COSY, HMBC, HSQC, NOESY), and mass (ESI, APCI, high resolution) specters.

Biologically, the relaxant response of the tracheal rings was expressed as percentage of the precontractile tone induced by carbachol. The effect of vehicle (distilled water) was systematically subtracted from the effect of the products.

Results are expressed as mean ± standard errors of the mean (S.E.M) of 6 experiments. Eₘₐₓ is the maximal relaxation obtained and CEX is the concentration of product which induces X% relaxation. Data were analyzed with Sigmamplot® software by an unpaired Student’s t-test or by one way analysis of variance (ANOVA) followed by Holm-Sidak or Bonferroni-test, with criterion set for statistical significance at p < 0.05.

3. Results and Discussion
The analysis of the root bark extract highlighted the presence of an alkaloid fraction (tests in reactive of Valsen-Mayer and in reactive of Dragendorff were positive) which purification allowed obtaining 49.2 mg of total alkaloids, purification yield being 0.0164%.

The division allowed isolating 2 majority pure products. Their monodimensional and bi-dimensional nuclear magnetic resonance specters (NMR) allowed identifying a new structure in alkaloids’ serial: pyrolidin with a bi-hydroxylated long alkyl chain (Figures 2 and 4) confirmed by mass spectrometry. These compounds are called Alkaloid 1 (4.2 mg; y = 0.0014%), Alkaloid 2 (29.7 mg; y = 0.0099%) and Alkaloid 2 bis (15.3 mg; y = 0.0051%), alkaloids 2 and 2 bis being identical compounds isolated by two different ways.

These data allow proposing the fact that Alkaloid 1, carrier of a sugar, is a glycoside of Alkaloid 2 (baptized JEMIGRACINE by our care).

Not aromatic, these isolated alkaloids probably arise from lysine metabolism [8].

Both alkaloids 1 and 2 induced almost similar regular spasmyolytic effects (Eₘₐₓ value in Table 1). However, Alkaloid 1 was more effective than Alkaloid 2 at 9 × 10⁻⁶ M (p < 0.01), 3 × 10⁻⁵ M and 9 × 10⁻⁵ M (p < 0.001) just like indicated on Figure 3 and in Table 1 (lowest EC₂₅ and EC₅₀ for alkaloid 1), probably because of the sugar component which could make the cell entry easier.
Alkaloid 1 104.5 ± 0.9 2.22 ± 0.25 5.22 ± 0.77
Alkaloid 2 104.2 ± 1.8 4.32 ± 0.41 9.27 ± 0.8
Salbutamol 28.1 ± 3.2 6.87 ± 0.22 n.d

Figure 3. Relaxant effects of alkaloids isolated from *D. cine-rea* and salbutamol; **: statistically significance p ≤ 0.01.

Figure 4. Structure of alkaloid 2 = [12-(1-méthylpyrrolidin-2-yl) dodecane-1,5-diol].

Table 1. E_{max}, EC_{50} and EC_{25} of isolated alkaloids and salbutamol.

![Diagram showing relaxation effect of alkaloids and salbutamol](image)

The E_{max} value is the percentage of relaxation obtained at the maximal tested concentration. The EC_{x} value is defined as the concentration of extract that induces x% relaxation. n.d is not determined value.

4. Conclusion

This study allowed isolating new structures of alkaloids from plants. Those new components exerted a good *ex vivo* activity on contracted trachea. Nevertheless, further investigations will be required to completely identify these components, and to confirm their real potency with *in vivo* bronchodilatation tests.

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