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New nitro-benzo[*c*]phenanthridine and indolopyridoquinazoline alkaloids from *Zanthoxylum atchoum*

Nouveaux alcaloïdes nitro-benzo[c]phénanthridines et indolopyridoquinazolines de Zanthoxylum atchoum

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ABSTRACT

The first phytochemical investigation of the roots of *Zanthoxylum atchoum* has led to the isolation of two new nitro-benzo[*c*]phenanthridine alkaloids 6-nitronitidine (**1**) and 6-nitro-8-methoxy-7,8-dihydronitidine (**2**), two new salts of indolopyridoquinazoline alkaloids 3-hydroxy-8,13-dihydro-14-methyl-5-oxo-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-14-ium (**3**) and its zwitterionic form 3-phenolate-8,13-dihydro-14-methyl-5-oxo-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-14-ium (**4**) along with 18 (**5**–**22**) known compounds. Their chemical structures were elucidated by spectroscopic analysis including 1D and 2D NMR and MS techniques. This is the first report of the nitro group on the biosynthesis of the natural benzo[*c*]phenantridine alkaloids. Compound **2** exhibited potent antibacterial activity against *Staphylococcus aureus* of $MIC_{50} = 4\ \mu g \cdot mL^{-1}$.

Mots clés :
Zanthoxylum atchoum
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Indolopyridoquinazolines

RÉSUMÉ

La première étude phytochimique des racines de *Zanthoxylum atchoum* a conduit à l'isolement de deux nouveaux alcaloïdes nitro-benzo[*c*]phénanthridine, le 6-nitronitidine (**1**) et 6-nitro-8-méthoxy-7,8-dihydronitidine (**2**), de deux nouveaux sels d'indolopyridoquinazoline, le 3-hydroxy-8,13-dihydro-14-méthyl-5-oxo-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-14-ium (**3**), et sa forme zwittérionique, le 3-phénolate de 8,13-dihydro-14-méthyl-5-oxo-7*H*-indolo [2',3':3,4]pyrido[2,1-*b*]quinazolin-14-ium (**4**), et de

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18 composés connus (**5**–**22**). Leurs structures chimiques ont été élucidées par analyse des techniques spectroscopiques 1D et 2D-RMN et SM. C'est le premier rapport d'un groupement nitro dans la biosynthèse de benzo[*c*] phénantridines naturelles. Le composé (**2**) montre une puissante activité antibactérienne contre *Staphylococcus aureus*, avec une CMI50 = 4 μg·mL^{-1}.

1. Introduction

Zanthoxylum atchoum (Aké Assi), P.G. Waterman (Rutaceae) is an endemic straggling shrub distributed in humid forests of southern Ivory Coast. The plant is used to treat amenorrhea in traditional medicine. There has been no report of other phytochemical study and biological value of *Zanthoxylum atchoum* so far. In our continuous search for chemical bioactive constituents of Ivorian *Zanthoxylum* species [1], we investigated the methanol extract of *Z. atchoum*.

The present study deals with the isolation and structural elucidation of two new benzophenanthridine alkaloids, 6-nitronitidine (**1**) and 6-nitro-8-methoxy-7,8-dihydronitidine (**2**), and of two new indolopyridoquinazoline alkaloids, 3-hydroxydehydroevodiamine (**3**) and its zwitterionic form 3-hydroxylatedehydroevodiamine (**4**), along with 18 (**5**–**22**) known compounds.

This is the first report of a nitro group in the biosynthesis of new natural benzo[*c*]phenantridine alkaloids. We also report in the ^{1}H NMR experiences an unusual hydrogen–deuterium exchange of the 8-methoxy hydrogens (CD_3OD solvent) in compound **2**. The antibacterial activities of compounds **1**-**4** were evaluated against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*.

2. Experimental

2.1. Apparatus

NMR spectra were recorded in CD_3OD using a Bruker Avance DRX-500 spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz), and 2D-NMR experiments were performed using Bruker's standard microprograms (XWIN-NMR version 2.6 software). HR–ESI–MS and EI experiments were obtained using a micromass Q-TOF micro instrument (Manchester, UK) and water-micromass GCT (UK). Ultraviolet spectra were recorded in MeOH on a Philips PU 8720 spectrophotometer. Infrared spectra were measured using a Nicolet Avatar 320 FT-IR spectrometer. Chromatography was performed on silica gel 60 (63–200 μm, Merck). Preparative glass-backed TLC plates, coated with silica gel 60 F254 (Merck) were used. TLC spots were visualized under UV light (254 and 365 nm) followed by spraying with Dragendorff's reagent for alkaloids or with 50% H_2SO_4 for the detection of other compounds.

2.2. Plant material

The plant was identified and collected by Prof. Aké Assi in April 2003 in Yapo (Agboville), Ivory Coast. A voucher specimen (Aké Assi 14820) was deposited in the National Herbarium of Floristic Center of University HFB Cocody-Abidjan.

2.3. Extraction and isolation

A total of 920 g of air-dried powdered roots of *Zanthoxylum atchoum* were successively extracted with petroleum ether and methanol for 48 h. After removal of the solvent, the petroleum ether and methanol extracts were repeatedly chromatographed on silica gel to give 22 compounds (**1**–**22**). The petroleum ether extract (2 g) was chromatographed over a silica gel column using a gradient system of cyclohexane/chloroform (5:5 to 0:1) and then chloroform/methanol (1:0 to 9:1) to give: **19** (102 mg), **8** (4 mg) and **22** (406 mg) (in $C_6H_{12}/CHCl_3$: 5:5), **21** (15 mg) (with 100% $CHCl_3$) and **20** (60 mg) (eluted with $CHCl_3/CH_3OH$: 99:1). The methanol extract (5 g) was fractionated into seven fractions (F1–F7) by vacuum liquid chromatography on silica gel using a gradient of mixtures of $C_6H_{12}/CHCl_3$ (1:0 to 0:1) and $CHCl_3$/MeOH (9:1 to 5:5).

The 100% $CHCl_3$ fraction F4 (1.3 g) was subjected to silica gel column chromatography (CC) eluting with an increasing gradient of $C_6H_{12}/CHCl_3$ (1:0 to 0:1) and $CHCl_3$/MeOH (1:0 to 99:1) to give nine compounds. The $C_6H_{12}/CHCl_3$ fractions gave **11** (32 mg) ($C_6H_{12}/CHCl_3$, 7:3), **10** (15 mg) ($C_6H_{12}/CHCl_3$, 5:5), **6** (107 mg) and **17** (158 mg) (100% $CHCl_3$). The $CHCl_3$/MeOH fractions yielded compounds **9** (12 mg) ($CHCl_3$/MeOH, 199:1), **7** (10 mg), **18** (23.4 mg) ($CHCl_3$/MeOH, 99:1) and **14** (10 mg) obtained by preparative TLC protocol (CH_3OH/NH_4NO_2, 9:1).

The fractions F5 + F6 (1.8 g) ($CHCl_3$/MeOH, 8:2) were subjected to CC on silica gel, eluting with $CHCl_3$/MeOH (1:0 to 5:5) to afford 60 subfractions (A1 to A60).

Fractions A25–A28 (68 mg, $CHCl_3$/MeOH, 95:5) were separated by preparative TLC eluting with CH_3OH/NH_4NO_2 9:1 to give **16** (16 mg) and **13** (9 mg).

Fractions A30–A33 (80 mg, $CHCl_3$/MeOH, 9:1) gave **15** (10 mg).

Fractions A35–A40 (160 mg, $CHCl_3$/MeOH, 85:15) gave **12** (102 mg) and **5** (14 mg) by preparative TCL (CH_3OH/NH_4NO_2 9:1).

Fractions A42–A45 (110 mg, $CHCl_3$/MeOH, 8:2) gave **1** (10 mg) and **2** (8 mg) by preparative TCL (CH_3OH/NH_4NO_2 9:1).

Fractions A48–A58 (98 mg, $CHCl_3$/MeOH, 7:3) afforded **3** (9 mg) and **4** (5 mg) by preparative TCL (CH_3OH/NH_4NO_2 9:1).

2.3.1. 2,3-(methylenedioxy)-6-nitro-10,11-(dimethoxy)-7-methylbenzo[c]phenanthridinium or (6-nitronitidine) (1)

Yellow powder; UV (MeOH) λ_{max}: 231, 266, 312 nm; IR (KBr): 3408, 2924, 1613, 1521, 1496, 1351, 1277, 1036 cm^{-1}; ^{1}H (CD_3OD + TFA, 500 MHz) and ^{13}C NMR