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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
Rutaceae
Alcaloïdes
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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

Table 1
^1H (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data for compounds **1** and **2**.

Position	**1**		**2**	
	δ_C ppm	δ_H ppm [mult, J (Hz)]	δ_C ppm	δ_H ppm [mult, J (Hz)]
1	106.0	8.17 (s)	101.0	7.71 (s)
2[a]	153.5		152.0	
3[a]	154.0		152.5	
4	108.0	7.68 (s)	106.0	7.33 (s)
4a	132.0		130.0	
5	128.0	8.63 (s)	121.0	7.99 (s)
6	144.0		144.0	
8	155.0	9.82 (s)	92.0	5.25 (s)
8a	122.0		121.0	
9	110.0	7.93 (s)	112.0	7.13 (s)
10	155.0		150.0	
11	160.0		150.0	
12	105.0	7.41 (s)	110.0	6.85 (s)
13	119.0		119.0	
12a	133.0		127.0	
14	138.0		141.0	
14a	123.0		130.5	
OCH_2O	103.0	6.33 (s)	104.0	6.15 (s)
10-OCH_3	58.0	4.07 (s)	57.0	3.93 (s)
11-OCH_3	58.0	4.12 (s)	57.0	3.79 (s)
8-OCH_3			55.0	3.50 (s)
N-CH_3	53.0	4.93 (s)	40.0	2.68 (s)

[a] Interchangeable, ^1H and ^{13}C recorded in CD_3OD.

(CD_3OD + TFA, 125 MHz) data see Table 1; EIMS *m/z* 393 $[M]^+$; Tandem MS/MS 393: *m/z* 393$[M]^+$, 346 $[M–NO_2–H]^+$, 332 $[M–NO_2–CH_3]^+$, 318 $[M–NO_2–OCH_3 + 2H]^+$, 301 $[M–NO_2–OCH_3–CH_3]^+$; HRESIMS *m/z* 394.1348 $[M + H]^+$ (calcd. for $C_{21}H_{18}N_2O_6$, 394.1347).

2.3.2. 2,3-methylenedioxy-6-nitro-8,10,11-trimethoxy-7-methylbenzo[c]phenanthridine or 6-nitro-8-methoxy-7, 8-dihydronitidine (2)

Yellow powder; UV (MeOH) λ_{max}: 271, 293, 307, 328; IR (KBr): 3408, 1615, 1502, 1470, 1384, 1284, 1209, 1036 cm^{-1}; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz) data see Table 1; ESIMS: *m/z* 871 $[M + Na + M]^+$, 447 $[M + Na]^+$, 393[M- OCH_3]$^+$; HRESIMS *m/z* 425.1349 $[M + H]^+$ (calcd. for $C_{22}H_{21}N_2O_7$, 425.1343).

2.3.3. 3-hydroxy-8,13-dihydro-14-methyl-5-oxo-7H-indolo[2′,3′:3,4]pyrido[2,1-b]quinazolin-14-ium or 3-hydroxydehydroevodiamine (3)

Yellow powder; UV (MeOH) λmax: 214, 245, 330, 368, 381 nm; IR (KBr): 3063, 2920, 1692 cm^{-1}; EI *m/z*: 318 $[M]^{+\cdot}$; ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz); data see Table 2. Elementary analysis: C 62.36%, H 5.40%, N 9.01% for $C_{19}H_{16}N_3O_2$.

2.3.4. 3-phenolate-8,13-dihydro-14-methyl-5-oxo-7H-indolo[2′,3′:3,4]pyrido[2,1-b]quinazolin-14-ium or 3-hydroxylatedehydroevodiamine (4)

Orange powder; EI *m/z*: 317 $[M]^{+\cdot}$, ^1H (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz); data see Table 2.

2.3.5. (–)-Evodiamine (6)

Yellow brilliant crystal; $[\alpha]_D = -522^\circ$(C= 0.54, CH_3OH),–642° (C = 0.67, $CHCl_3$); UV (MeOH) λ_{max}: 225, 272, 284, 293 nm; IR (KBr): 3582, 3269, 3063, 2920, 1637, 1605, 1480, 1424, 1306, 1031, 745 cm^{-1}; ESI *m/z*: 304 [M + H]; ^1H ($CDCl_3$, 500 MHz): 2.50 (*N*-CH_3, *s*), 2.98 (H-8, *m*), 3.30 (H-7b, *ddd*, 15.7, 10.7, 5), 4.89 (H-7a, *ddd*, 10.7, 5, 2.6), 5.92 (H-13b, *s*), 7.15 (H-1, *d*, 8), 7.21 (H-10, *t*, 7.5), 7.23 (H-3, *t*, 8), 7.27 (H-11, *t*,7.2), 7.43 (H-12, *d*, 8.0), 7.49 (H-2, *t*, 8), 7.60 (H-9, *d*, 8.2), 8.13 (H-4, *d*, 8), 8.31 (NH, *s*), ^{13}C NMR ($CDCl_3$, 125 MHz): 20.1 (C-8), 37.2 (CH_3), 39.5 (C-7), 68.8 (C-13b), 111.3 (C-12), 113.6 (C-8a), 118.9 (C-9), 120.0 (C-10), 122.4 (C-1), 123.0 (C-11), 123.7 (C-4a), 124.1 (C-3), 126.2 (C-9a), 128.2 (C-13a), 128.9 (C-4), 133 (C-2), 136.7 (C-12a), 150.6 (C-14a), 164.7 (C-5).

Table 2
^1H and ^{13}C NMR data of compounds **3** and **4**. δ in ppm, *J* in Hz.

Position	**3**		**4**	
	δ_C	δ_H (mult, J)	δ_C	δ_H (mult, J)
1	121.2	7.96 (d, *J* = 9.7)	120.2	7.65 (d, *J* = 9.2)
2	126.3	7.55 (dd, *J* = 9.7, 2.8)	130.5	7.22 (dd, *J* = 9.2, 3)
3	159.8		170.4	
4	113.0	7.72 (d, *J* = 2.8)	114.8	7.32 (d, *J* = 3)
4a	122.0		121.7	
5	159.4		159.7	
7	43.6	4.58 (t, *J* = 6.7)	43.5	4.50 (t, *J* = 7.0)
8	20.0	3.33 (t, *J* = 6.7)	20.1	3.34 (t, *J* = 7.0)
8a	131.8		129.8	
9	122.4	7.85 (d, *J* = 8.2)	122.0	7.85 (d, *J* = 8.2)
9a	125.2		125.4	
10	123.2	7.30 (t, *J* = 7.7)	122.8	7.26 (t, *J* = 7.5)
11	130.2	7.56 (t, *J* = 7.7)	129.2	7.48 (t, *J* = 7.5)
12	114.2	7.65 (d, *J* = 8.4)	114.2	7.63 (d, *J* = 8.4)
12a	143.1		147.8	
13a	121.9		121.9	
13b	149.6		146.5	
14a	133.9		128.9	
NCH_3	41.1	4.40 (s)	40.8	4.35 (s)

^1H recorded at 500 MHz in CD_3OD and ^{13}C recorded at 125 MHz in CD_3OD.

2.4. Antibacterial assays

The assay for antibacterial activity against standard strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (29212), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) was performed using the liquid microdilution growth inhibition method (Grare et al., 2006) [2]. Antibacterial agents used for positive controls were vancomycin for Gram+ and imipenem for Gram–strains. The MICs of test compounds were determined as described in the previous study by Yao-Kouassi et al. (2008) [3].

3. Results and discussion

The root bark of *Z. atchoum* was extracted successively with petroleum ether and methanol. The extracts were chromatographed on silica gel to afford 22 compounds **1**–**22**. Structural elucidation of these compounds was performed using spectroscopic methods, especially 1D and 2D-NMR, HR-EI MS, UV and IR.

Compound **1** was isolated as a yellow powder. The UV spectrum showed absorptions at 271, 293, 309, 328 and 390 nm, which are characteristic of the benzo[*c*]phenanthridine skeleton [4]. The HRESIMS spectrum displayed the

molecular ion $[M+H]^+$ at *m/z* 394,1348 (calcd for 394.1347), which, in combination with ^1H and ^{13}C NMR spectroscopy and IR data, indicated molecular formula $C_{21}H_{17}N_2O_6$.

The ^1H-NMR spectrum (Table 1) of **1** revealed only ten singlet signals due to six aromatic proton resonances at δ_H 8.17 (1H, s, H-1), 7.68 (1H, s, H-4), 8.63 (1H, s, H-5), 9.82 (1H, s, H-8), 7.93(1H, s, H-9), and 7.41(1H, s, H-12), one methylenedioxy group at 6.33 (2H, s, O–CH_2–O) and three methyl groups assignable to two methoxy groups at 4.07 (3H, s, 10-OCH_3), 4.12 (3H, s, 11-OCH_3) and one N-methyl group at 4.93. In agreement with the above, the ^{13}C NMR of compound **1** showed resonances for 21 C-atoms, including 11 quaternary carbons, six methines, one methylene and three methyl groups (Table 1).

The ^1H NMR and ^{13}C NMR signals of compound **1** were closely related to those of nitidine (**8**), except for the absence of one aromatic proton and the presence of one more quaternary carbon, suggesting that **1** was certainly an *ortho*-pentasubstituted benzo[*c*]phenanthidine [4]. Total assignments of protons H-1, H-4, H-5, H-8, H-9, H-12 and of the methyl groups were clearly obtained from the NOESY and HMBC experiments (Fig. 1). The location of the N-methyl group and of proton H-1 were secured by the NOE interaction between H-1 at δ_H 8.17 and N-Me at δ_H 4.93; in addition, both protons and the deshielded proton at δ_H 9.82 (H-8) showed long-range connectivity with the signal at δ_C 138.0 (C-14). The HMBC interactions between the protons at δ_H 6.33, 8.17 and 7.68 (H-4) and carbons at δ_C 153.5 (C-2) and 152.9 (C-3) suggested that the methylenedioxy group was connected to C-2 and C-3 on the ring D. Furthermore, the NOESY spectrum displayed a significant cross-peak between H-4 and the proton at δ_H 8.63, which was unequivocally assigned to H-5, and the proton H-9 at δ_H 7.93 was assigned by its cross-peak between H-8. Thus H-12 was assigned to δ_H 7.41, confirming its long-range correlations with C-8a at δ_C 122.0 and C-13 at δ_C 119.0. The location of the two methoxy groups at C-10 and C-11 were established by HMBC correlations of protons at δ_H 4.07 to C-10 (δ_C 155.0) and 4.12 to C-11 (δ_C 160.0), and were confirmed by the NOESY correlations between H-9/Me-10 and H-12/Me-11. The HMBC correlations between H-5 and C-6 (δ_C 144.0) suggested that a heteroatom, presumably a nitro group, was certainly attached to C-6. This was in accordance with the upfield shift of C-6 and consequently inferred that **1** was a 2,3,6,10,11-*ortho*-pentasubstituted benzophenanthridine alkaloid. The IR spectrum of **1** showed two strong bands at 1521 and 1351 cm^{-1} which can be ascribed to N-O stretching of the nitro group. In addition, the presence of the nitro group was confirmed by the fragmentation MS/MS of the molecular ion $[M]^+$ at *m/z* 393, which gave a series of ions at *m/z* 346, 332, 318 and 301, from which the loss of a nitro group was respectively deduced as follows: $[M–NO_2–H]^+$, $[M–NO_2–CH_3]^+$, $[M–NO_2–OCH_3+2H]^+$ and $[M–NO_2–OCH_3–CH_3]^+$. Thus, compound **1** was characterized as 2,3-(methylenedioxy)-6-nitro-10,11-(dimethoxy)-7-methylbenzo[*c*]phenanthridinium, to which we gave the trivial name 6-nitronitidine. This is the first occurrence of a natural benzylisoquinoline alkaloid with a nitro group on the C ring of the benzo[*c*]phenanthidine nucleus isolated from *Zanthoxylum atchoum*. The presence of the nitro group seems to be scarce in the plant kingdom, regardless of special phenanthrene derivatives mentioned in the *Aristolochia* genus [5]. Furthermore, several examples of C-6 substituted on the C ring of benzophenanthridines do exist, but it concerns 6-*O*-alkyl compounds in which the C ring is not completely aromatic [6,7].

Compound **2** was obtained as a yellow amorphous solid. Its molecular formula was deduced as $C_{22}H_{20}N_2O_7$ by positive-ion mode HRESIMS $[M+H]^+$ at *m/z* 425.1349 (calcd for 425.1349). Comparison of the NMR data (Table 1) of **2** to those of **1** indicated a slight modification of the iminium bond C=N at C-8. Instead of the aromatic methine proton at C-8, the signals detected were those of the methine proton at δ_H 5.25 (1H, s, H-8) and of a methoxy group at δ_H 3.50 (3H, s, 8-OCH_3) in the ^1H-NMR spectrum. The location of this methoxy group was confirmed by the NOE correlation between the –OMe and H-8. Compound **2** seems to be the pseudo base of **1** and was characterized as 6-nitro-8-methoxy-7,8-dihydronitidine (Fig. 2).

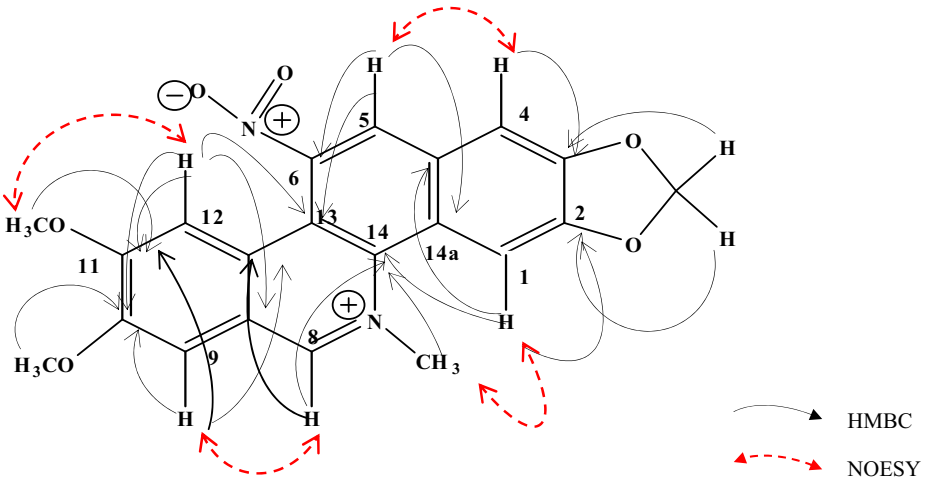

Fig. 1. (Color online.) Selected HMBC and NOESY correlations for compound **1**.

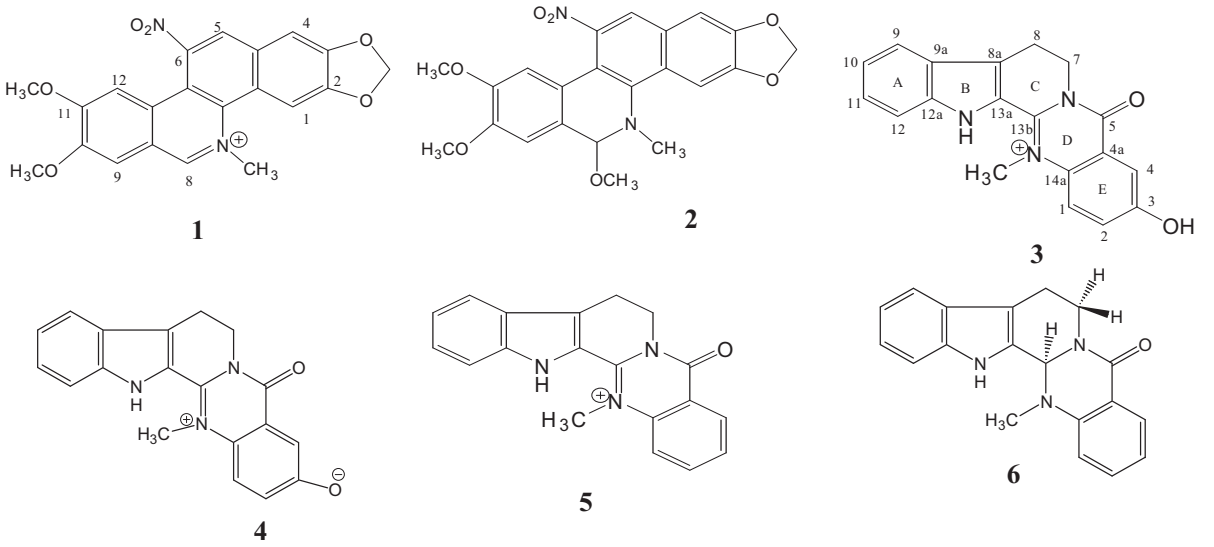

Fig. 2. Structures of compounds **1**–**6**.

This kind of product is well known among the quaternary benzo[*c*]phenanthridine alkaloid-free bases and detailed structural and NMR studies have already been reported by Dostal et al. [8,9]. Surprisingly enough, the signal of this methoxy group at δ_H 3.5 was gradually vanished within 24 h, whereas the signal of the residual solvent (MeOH) at δ_H 3.38 was increased (Fig. 3). Fig. 3 shows the ^{1}H-NMR spectrum of **2**, in which t_1 and t_3 represent respectively the beginning and the end of the experiments. It is remarkable that the protons of the 8-OCH_3 group were H–D exchanged during the ^{1}H-NMR experiments by the deuterated solvent (CD_3OD) (Fig. 3). This situation made very difficult the NMR interpretation because of the bewildering assignment of the 8-OCH_3 or OH group in benzophenanthridine pseudo-base compounds. In our study, deuterium retention of the 8-OCH_3 to 8-OCD_3 was confirmed by mass spectra with 3 amu more than in **1**.

Compound **3** was isolated as a yellow amorphous solid. Our EI mass spectra evidenced the positive ion $[M]^+$ at *m/z* 318, which is consistent with the molecular formula $C_{19}H_{16}N_3O_2$. The IR spectrum of **3** implied the presence of hydroxyl and NH groups (3000–3063 cm^{-1}) and of the amide carbonyl group (1692 cm^{-1}). The UV spectrum showed absorptions bands at 250, 330 and 380 nm, with a bathochromic shift observed in alkaline medium at 417 nm, suggesting the presence of a phenol group. The ^{1}H-NMR spectrum of **3** showed four aromatic protons of an 1,2-*ortho*-disubstituted phenyl ring at δ_H 7.85 (1H, d, J = 8.2 Hz, H-9), 7.30 (1H, t, J = 7.7, H-10), 7.56 (1H, t, J = 7.7 Hz, H-11), 7.65 (1H, d, J = 8.4 Hz, H-12), three aromatic proton signals at δ_H 7.96 (1H, d, J = 9.7 Hz, H-1), 7.55 (1H, dd, J = 9.7, 2.8 Hz, H-2) and 7.72 (1H, d, J = 2.8 Hz, H-4), revealing the presence of an ABX system in a 1,3,4-trisubstituted phenyl ring, one methyl singlet group at δ_H 4.40 (3H, s, N-CH_3), and two methylene groups at δ_H 4.58 (2H, t, J = 6.7 Hz, H-7) and 3.33 (2H, t, J = 6.7 Hz, H-8). All these data readily help us to identify a typical 7, 8-dihydropyridoquinazoline nucleus [10,11]. The ^{13}C-NMR spectrum of **3** showed 19 carbon signals due to eight quaternary carbons, seven methines, two methylenes, N-methyl and carbonyl groups. The ^{1}H and ^{13}C NMR spectra (Table 2) of **3** were similar to those of dehydroevodiamine (**5**) [12], except for the increase of chemical shift of C-3. According to the preceding data, **3** was suggested to be a 3-hydroxy analog of **5** and similar to those described by Li et al., 2001 [13]. The protons H-9 and H-1 were assigned by the NOESY spectrum, which showed respectively cross-peaks between H-9/H-8 and H-1/N–CH_3 (Fig. 4). The location of the hydroxy at C-3 was confirmed by the HMBC experiment, which showed correlations between the protons H-1, H-2 and H-4 and C-3 (δ_C 159.8) (Fig. 4). Thus, on the basis of spectral evidence, the structure of compound **3** was elucidated as a salt of 3-hydroxy-8,13-dihydro-14-methyl-5-oxo-7*H*-indolo[2',3':3,4]pyrido[2,1-*b*]quinazolin-14-ium, to which we gave the trivial name of 3-hydroxydehydroevodiamine.

Compound **4** was obtained as an orange amorphous solid. A molecular ion peak at *m/z* $[M]^+$ 317 suggested its molecular formula $C_{19}H_{15}N_3O_2$, which was one proton less than in that of **3**.

Furthermore, the NMR data of **4** were similar to those of **3**. However, the ^{13}C NMR spectrum of **4** showed the presence of a deshielding C-3 at δ_C 170.4 instead of 159.8; this means that **4** is the zwitterionic 3-phenolate form of **3**, named 3-phenolatedehydroevodiamine (Fig. 2).

The known compounds (**5**–**23**) were readily identified, from their spectral data and by comparison with reported corresponding compounds in the literature, as dehydroevodiamine (**5**), evodiamine (**6**) [14], decursinol (**7**) [15], asarinin (**8**) [16], oxynitidine (**9**), norchelerythrine (**10**) [17], tridecanonchelerythrine (**11**) [18], nitidine (**12**), chelerythrine (**13**), methoxychelerythrine (**14**), fagaridine (**15**), methoxyfagaridine (**16**), arnottianamide (**17**), pellitorine (**18**), sesamin (**19**), skimmianine (**20**), stigmasterol (**21**), and lupeol (**22**). Compounds **12**–**23** were previously found in other Ivorian *Zanthoxylum* species [1]. We

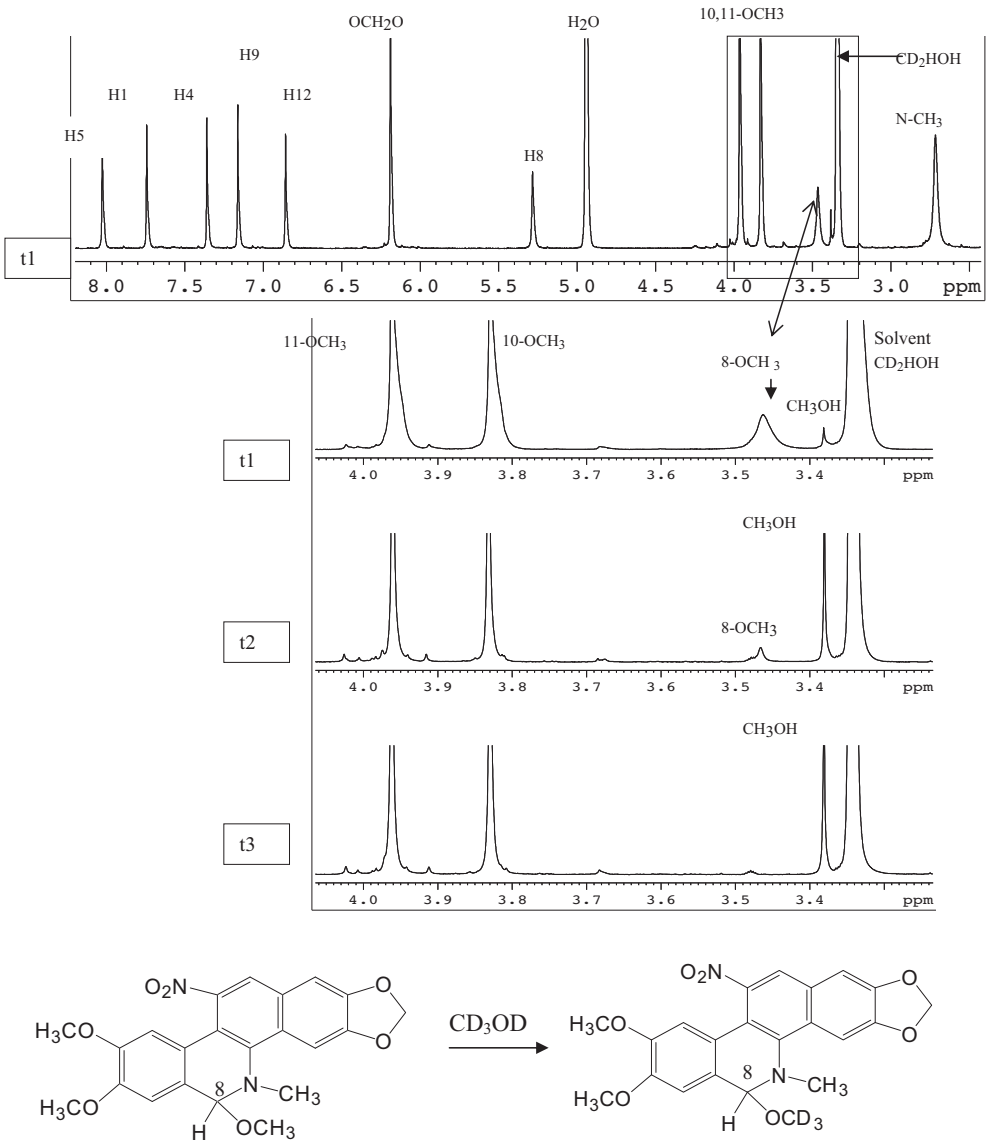

Fig. 3. H-D exchanged in the ^{1}H-NMR experiments by CD_3OD solvent.

describe the structural elucidation and configuration of 13b αH of compound **6** which displayed negative values: $[\alpha]_D = -522^\circ$ (*c* 0.54 in CH_3OH) and -642° (*c* 0.67 in $CHCl_3$) for optical rotation, like that of the synthesized one [19]. Nevertheless, the isolated evodiamine described in literature was optically inactive or displayed positive optical rotation values [20–22].

The isolated new compounds (**1**–**4**) were evaluated for their antibacterial activities against four pathogenic microorganisms, *E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*; the results are summarized in Table 3. Among them, compound **2** was the most active one, with MICs of 4, 8, 16, and 32 μg·mL^{-1} against *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*, respectively (Table 3). Compound **1** exhibited mild activity against these four bacteria with MICs of 32 μg·mL^{-1}. This result suggested that the pseudo base of these isolated nitro benzophenanthridine is more active than the ammonium quaternary **1**. A similar antimicrobial effect was observed with sanguinarine and chelerythrine and their pseudo bases [23].

Table 3
Antibacterial activity of isolated compounds **1**–**4**.

Test materials	MIC μg·mL^{-1}			
	S. aureus	*E. coli*	*E. faecalis*	*P. aeruginosa*
1	32	32	32	32
2	4	32	8	16
3	64	64	64	64
4	64	128	64	128

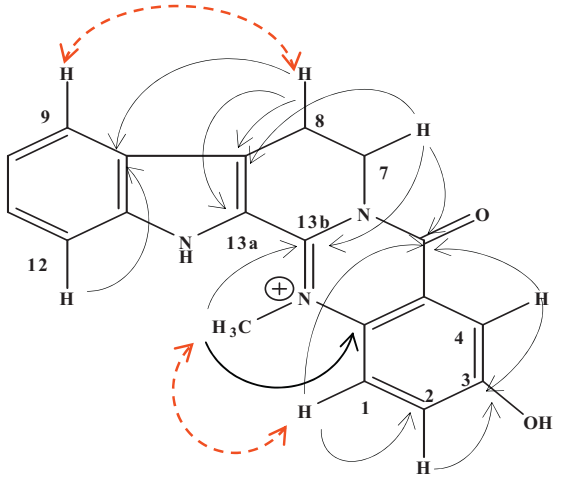

Fig. 4. (Color online.) Selected HMBC and NOESY correlations for compounds **3**.

4. Conclusions

This work constituted the first phytochemical study of *Z. acthoum*. The methanolic extract of the roots led to the isolation of 11 benzophenanthridines, four indolopyridoquinazoline, one furanoquinoline alkaloids, two lignans, one coumarin, one amid, one triterpene, and one phytosterol. Among them, 6-nitronitidine (**1**), 6-nitro-8-methoxy-7,8-dihydronitidine (**2**), 3-hydroxydehydroevodiamine (**3**), and 3-phenolatedehydroevodiamine (**4**) were characterized as new compounds. This paper reports the first natural nitro group on the benzophenanthridine nucleus. This work allowed us to understand the fickle behavior of benzophenanthridine bases towards solvents, in particular deuterium retention of the OCH_3 to OCD_3, which made difficult the interpretation of the spectra. The antibacterial activities against two gram-positive (*S. aureus* and *E. faecalis*) and two gram-negative (*E. coli* and *P. aeruginosa*) strains for compounds **1**–**4** were tested. Compound **2** showed strong antibacterial activity against *S. aureus* at the concentration of MIC_{50} 4 $\mu g \cdot mL^{-1}$.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.crci.2015.01.005.

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