*Available free online at www.medjchem.com*

*Mediterranean Journal of Chemistry* **2014**, 3(5), 1013-1020

# A New Cycloheptapeptide from *Zanthoxylum mezoneurispinosum* Aké Assi (Rutaceae)

**Philomène A. Yao-Kouassi [1,2,*], Catherine Caron[1†], Emma Aké-Assi [3], Agathe Martinez [1], Elisabeth Le Magrex-Debar [4], Sophie C. Gangloff [4] and Monique Zèches-Hanrot [1]**

[1] Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, CPCBAI, Bâtiment 18, Moulin de la Housse, BP 1039, 51687 Reims Cedex, France.

[2]Laboratoire de Chimie Organique Biologique, Université FHB Cocody-Abidjan, 22 BP 582, Abidjan, Côte d'Ivoire.

[3]Laboratoire de Botanique, Université FHB Cocody-Abidjan, 22 BP 582, Abidjan, Côte d'Ivoire.

[4]Laboratoire de Microbiologie, EA "Biomatériaux et Inflammation en site osseux" UFR de Pharmacie, 1, Rue du Maréchal Juin, 51096 Reims Cedex, France.

**Abstract**: A new cycloheptapeptide named akeassimezorine **1** and twelve known compounds: methoxy-chelerythrine **2**, chelerythrine **3**, nitidine **4**, methoxyfagaridine **5**, jatrorrhizine **6**, tembetarine **7**, *N*-methylatanine **8**, magnoflorine **9**, skimmianine **10**, sesamine **11**, hesperidine **12**, and lupeol **13** were isolated from the roots of *Zanthoxylum mezoneurispinosum* Aké Assi (Rutaceae). The structures of those compounds were elucidated on the basis of one- and two-dimensional NMR and HR-ESI-MS analyses. Antibacterial activity of compound **1** was evaluated against pathogenic agents. This study constituted the first phytochemical investigation of the roots of *Zanthoxylum mezoneurispinosum.*

**Keywords**: *Zanthoxylum mezoneurispinosum*; Rutaceae; Alkaloids; Cycloheptapeptide.

## Introduction

The genus *Zanthoxylum* belongs to the family of Rutaceae, with more than 250 species in the World and widely distributed throughout tropical and subtropical regions[1]. There are 9 species in Ivory Coast. *Z. mezoneurispinosum* is an endemic straggling shrub found in the South along the coast. It is well known that the species of this genus produced a significant variety of molecules such alkaloids, terpenes, coumarins, amides, lignans and flavonoids[2]. The important presence of secondary metabolites in this genus explained its extensively used in traditional medicine and numerous studies. Also, *Zanthoxylum* has been used traditionally in ethno medicines to treat different troubles such as: headache, cough, rheumatism, hemorrhoids, stomachache, boils, tooth-ache, gonorrhea, diarrhea[3]; the stern bark of the roots is used as an anti-sickling agent[4]. Modern pharmacological and biological studies have demonstrated that the extracts or isolated compounds from genus *Zanthoxylum* had antibacterial, anti-cancer, antiparasitary, anti-inflammatory, antileishmanial and antioxidant, activities[5-13].

**Corresponding author: Philomène A. Yao-Kouassi*
*E-mail address: kouassiap@yahoo.fr*
DOI: http://dx.doi.org/10.13171/mjc.3.5.2014.10.10.16.08

The purpose of this work is to study the phytochemical composition of *Z. mezoneurispinosum*. Herein, we report the isolation and structural elucidation of a new 21-membered cyclopeptide**1**, along with twelve known compounds (**2-13**). The antibacterial test of compound **1** was evaluated against *Enterococcus faecalis*, *Escherichia Coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. To our best knowledge, this is the first phytochemical report of *Z. mezoneurispinosum.* The cyclopeptides of *Zanthoxylum* genus holds our attention because rarely described or almost inexistent in this genus. The cyclopeptides were reported from the marine cyanobacteria or from some higher seed plants[14-17]. Recently cyclopeptides have been described in the leaves of *Zanthoxylum rigidum*[18] and in fungus of *Zanthoxylum leprieurii*[19]. The new molecule did not exhibit significant antibacterial activity against *S. aureus, P. aeruginosa, E. Coli and E. faecalis.*

## Experimental Section

### *General*

Optical rotation was measured with an electronic Polarimeter Perkin Elmer 241. IR spectra measures were taken with a spectrometer Nicolet Avatar 320 FT-IR. UV spectra were obtained by using a Philips PU 8720 UV/VIS spectrophotometer. $^{1}H$ and $^{13}C$-NMR spectra were recorded with Bruker Avance DRX-500 spectrometer operating at 500 MHz for $^{1}H$, and 125 MHz for $^{13}C$. Coupling constants were expressed in Hz. The high resolution mass (HRESIMS) and ESIMS (positive-ion mode)were recorded using Micromass ESI-Q-TOF micro-instrument (Manchester, UK). Column chromatography (CC) was performed on silica gel (SiO2) 60 (63-200 μm, Merck), or on Sephadex LH-20 gel (Amersham Pharmacia, Sweden). Vacuum Liquid Chromatography (VLC) was carried out on silica gel (63-200 μm, Merck). Analytical and preparative TLC were performed on precoated kieselgel 60 $F_{254}$ plates 250 μm (Merck) using as eluents $CHCl_3/CH_3OH$ or $CH_3OH/NH_4NO_3$ and detected by spraying with Dragendorff or 5% ethanolic $H_2SO_4$ reagent followed by heating. HPLC data were obtained on a Dionex apparatus, RP-18 column Dionex (4.6 x 50 mm, 5μm).

### *Materials*

The roots of *Zanthoxylum mezoneurispinosum* were collected in Ivory Coast (near Irobo region of Grand-Lahou) in July 2005 by Prof. L. Aké Assi. A voucher specimen (AA21009) is deposited in the Herbarium of CNF of University FHB Cocody-Abidjan.

### *Extraction and Isolation*

The air-dried powdered roots of *Z. mezoneurispinosum* (980 g) were successively macerated (15L, 48 h) and extracted with petroleum ether and methanol at room temperature and evaporated under reduced pressure. The methanol extract (18 g) was subjected to vacuum liquid chromatography (VLC) eluting with a gradient system of $CHCl_3$/MeOH (100/0, 95/5, 90/10, 80/20, 50/50) to give seventeen fractions 1-17 (96 mL each).

Fraction 1, (275 mg, 100% $CHCl_3$), gave **13** (198 mg).

Fractions 2-3, (324 mg, $CHCl_3$/MeOH, 95/5), gave **11** (56.5 mg) and **10** (25 mg).

Fraction 5, (1.8 g, $CHCl_3$/MeOH, 95/5), gave **2** (502.8 mg).

Fractions 8-9, (52 mg, $CHCl_3$/MeOH, 90/10), were purified by preparative TLC using $CH_3OH/NH_4NO_3$ (90/10) as eluent to give**3** (8 mg) and **5** (5.4 mg).

Fractions 11-12, (84 mg, $CHCl_3$/MeOH, 90/10), were purified by preparative TLC using $CH_3OH/NH_4NO_3$ (90/10) as eluent to afford compounds **6** (8.6 mg) and **7** (6.7 mg).
Fraction 13, (225 mg, $CHCl_3$/MeOH, 80/20), yielded compound **4** (68.7 mg).
Fraction 14, (610.2 mg, $CHCl_3$/MeOH, 80/20), was further subjected to silica gel column chromatography (CC) eluted with a gradient of $CHCl_3$/MeOH to afford thirty subfractions 1-30, subfractions 19-22 (175 mg, $CHCl_3$/MeOH, 80/20) gave **12** (119.3) mg and **8** (10 mg).
Fraction 15, (303 mg, $CHCl_3$/MeOH, 80/20), was applied on silica gel on Sephadex LH-20 CC ($CHCl_3$/MeOH 20:80) to yield compounds **1** (48 mg) and **9** (73.7 mg).

***Hydrolysis and amino acid derivatization***

A sample of compound **1** (2 mg) was hydrolyzed with 6 N HCl (2 mL) at 110°C for 12 h. The acidic solution was evaporated to dryness under reduced pressure and redissolved in 100 µL of $H_2O$. The hydrolysated mixture was treated with 1% of 1-fluoro-2,4-dinitrophenyl-5-L-alaninamide FDAA[20] in acetone (200 µL) and 1M $NaHCO_3$ (40 µL), at 35° for 1h. HCl (2M, 20µL) was added and concentration to dryness. The residue of amino acids was dissolved in MeOH and analyzed by reversed-phase HPLC (column: Dionex 4.6 x 50 mm C18, 5µm) eluted with a binary gradient of $H_20$ with 0.025% TFA (A) and MeCN (B) from 25 to 80% B in A in 30 min, at a flow-rate 1 mL/min at 25°C and detection by UV (340 nm). Comparison of $t_R$ values to those of the standard derivative amino acids established that all amino acids in **1** were L-configuration. Standards $t_R$[(L-Asp (8.0 min), D-Asp (11.1 min), L-Arg (9.1 min), D-Arg (11.9 min), Gly (12.5 min), L-Pro (14.5 min), D-Pro (18.4 min), L-Phe (20.0 min), D-Phe (23 min)]; amino acids in **1**$t_R$[(L-Asp (8.0 min), L-Arg (9.1 min), Gly (12.5 min), L-Pro (14.5 min), L-Phe (20.0 min)].
*Akeassimezorine**1** Cyclo(L-phenylalanyl-L-asparagyl-L-prolyl-L-arginyl-L-phenylalanyl-glycylglycyle)*: pale yellow powder, $[\alpha]^{25}_D$ -59,6 (*c* 0.50, MeOH); HR-ESIMS 776.3853 ($[M+H]^+$, $C_{37}H_{49}N_{11}O_8$, calcd. 776.3844); IR (KBr): 3340, 1225, 1663,1544 and 772 $cm^{-1}$; UV(MeOH) $\lambda_{max}$: 281, 323 nm; NMR $^1H$ and $^{13}C$-NMR: see the Table 1.

***Antibacterial Assays***

The antibacterial activity of **1** against four enteric pathogenic microorganism: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 was evaluated by using the liquid microdilution growth inhibition method[21]. The MIC of test compound was determined as described before by Yao-Kouassiet al.[22]. The assays were performed three times. Compound **1** did not exhibit inhibition activity against the four tested microorganisms at a concentration of 128µg/mL.

## Results and Discussion

Column chromatography of the MeOH extract from dried roots of *Z. mezoneurispinosum* led to the isolation and structure elucidation of a new heptacyclopeptide, which we gave the trivial name akeassimezorine **1**. Compound **1**, as well as secondary metabolites **2-10**, gave a very faint positive coloration with Dragendorff's reagent. The known compounds **2-13** were readily identified, from their spectral data and by comparison with reported corresponding compounds in the literature[23-27] as methoxychelerythrine **2**, chelerythrine **3**, nitidine **4**, methoxyfagaridine **5**, jatrorrhizine **6**, *N*-methylatanine **7**, (+)-tembetarine **8**, (+)-magnoflorine **9**, skimmianine **10**, sesamine **11**, hesperidine **12**, and lupeol **13**.

Compounds **2-4**, and **10-13** were previously isolated from the methanolic extract of *Z. psammophilum*[28]. The structure of **1** was elucidated based on the combination of various NMR ($^1$H and $^{13}$C NMR, COSY, HSQC, HMBC, and ROESY) spectroscopic and mass spectrometric (HR-EI-MS) techniques.

Compound **1** was isolated as pale yellow powder, optically active $[\alpha]^{25}_D$-59,6 (*c* 0.50, MeOH) and exhibited a quasi-molecular ion peak in positive HRESIMS at m/z 776.3853 $[M+H]^+$ (calcd. for 776.3844) which was consistent with the molecular formula $C_{37}H_{49}N_{11}O_8$.

The IR spectrum of **1** displayed the absorptions characteristic of NH or OH (3340 cm$^{-1}$), amide carbonyl (1663 cm$^{-1}$) and imine (1544 cm$^{-1}$) groups. The $^{13}$C- and $^1$H-NMR spectra (table 1), combined with HSQC data showed 37 C-atom signals, which were ascribed to eleven methylenes, fifteen methines and eleven quaternary C-atoms. Quaternary C-atoms were assigned to eight CO ($\delta_C$ 173.4, 171.7, 171.4, 171.3, 170.8, 170.6, 170.1 and 170.0 ppm), one CN ($\delta_C$ 157.5 ppm) and two aromatics C (138.3, 137.4 ppm). The $^1$H-NMR spectrum (in DMSO) showed ten NH (9.29, 9.20, 8.58, 8.44, 8.24, 8.18, 8.00, 7.91, 7.87 and 7.48 ppm) groups suggesting that **1** might be a peptide. Seven spin systems on the TOCSY spectrum were showed from Hα and six from NH of each amino acid suggesting the presence of one unit of proline. One isolated spin system (NH) was assigned to $NH_2$ at 8.18 and 8.44 ppm.

The amino acid residues were identified by COSY, TOCSY and HMBC spectra as two glycines (Gly), two phenylalanines (Phe), one proline (Pro), one arginine (Arg) and one asparagine (Asn) (table 1). The sequence of these amino acid residues was assigned by HMBC and ROESY spectra fig. 1 and 2). The connectivity of the carbonyl residue (i) and the NH proton residue (i+1) on the HMBC spectrum was established as follows (figure 1): δ (ppm) 170.6 (Phe$^1$)with 8.24NH (Asn$^2$); 171.3 (Pro$^3$) with 7.91 NH (Arg$^4$); 171.7 (Arg$^4$) with 7.48 NH (Phe$^5$);170.8 (Phe$^5$) with 7.87 NH (Gly$^6$); 170.1 (Gly$^6$) with 8.58 NH (Gly$^7$) and 170 (Gly$^7$) with 9.29 NH (Phe$^1$), which gave a sequence of linear heptapeptide: [-Pro$^3$-Arg$^4$-Phe$^5$-Gly$^6$-Gly$^7$-Phe$^1$-Asn$^2$-].

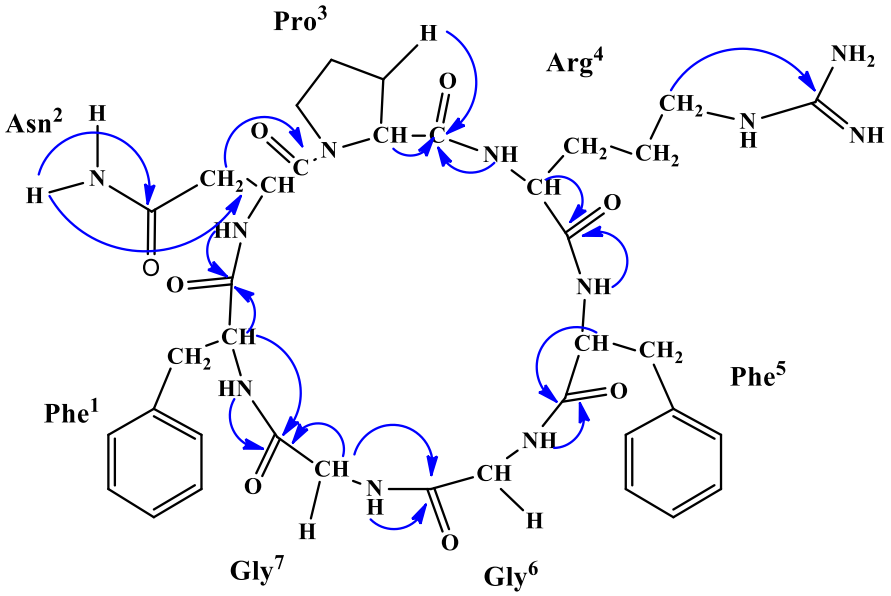


**Figure 1.** Important HMBC correlations of **1**

Table 1: NMR data for akeassimezorine (**1**) (in DMSO-$d_6$,$^1$H: 500 MHz, $^{13}$C: 125 MHz)

| Position | | $\delta_C$ (ppm) | $\delta_H$ (ppm) mult. (*J* in Hz) | HMBC ($^{13}$C-$^1$H) | ROESY ($^1$H-$^1$H) |
|---|---|---|---|---|---|
| Phe[1] | C=O | 170.6 | | | |
| | NH | | 9.29 d (8.4) | 170 Gly[7] | 3.76 Hα Gly[7] |
| | CH(α) | 55.9 | 4.23 ddd (11.6, 8.4, 3.6) | 170.6 | |
| | $CH_2$(β) | 36.2 | 3.19 m, 2.83 dd (14, 11.6) | 55.9, 138.3 | |
| | 1’ | 138.3 | | | |
| | 2’6’ | 128.9 | 7.16-7.19 d (9) | 36.2 | 4.23, 3.19, 2.83 |
| | 3’ 5’ | 128.3 | 7.27 m | | |
| | 4’ | 126.4 | 7.27 m | | |
| Asn[2] | C=O | 173.4 | | | |
| | C=O | 171.4 | | | |
| | NH | | 8.24 d (9) | 170.6 phe[1] | 3.15 |
| | $NH_2$ | | 8.18 br s, 8.44 br s | 173.4 | 2.72 (NH 8.44) |
| | CH(α) | 47.2 | 5.00 ddd (12.5, 9, 4) | 38.5 | |
| | $CH_2$(β) | 38.5 | 3.15 m, 2.72 dd (12.5, 4 ) | 173.4, 171.4 | |
| Pro[3] | C=O | 171.3 | | | |
| | CH(α) | 61.6 | 4.12 dd (8.7, 6.2) | 171.3 | |
| | $CH_2$(β) | 29.5 | 2.20 m, 1.85 dd (12, 6.2) | 171.3 | |
| | $CH_2$(γ) | 24.4 | 1.97 m | 61.6 | |
| | $CH_2$(δ) | 47.8 | 3.99 dd (8.9, 6), 3.73 m | 24.4 | 5.00 Hα Asn[2] |
| Arg[4] | C=O | 171.7 | | 4.47 Phe[5] | |
| | C=N | 157.5 | | | |
| | NH-Cα | | 7.91 d (8.9) | 171.3 Pro[3] | 4.12 Hα Pro[3] |
| | NH | | 9.20 br s | | |
| | NH | | 8.00 br s | | |
| | $NH_2$ | | - | | |
| | CH(α) | 53.9 | 4.01 td (8.9, 6) | | |
| | $CH_2$(β) | 28.1 | 1.45 td (10.3, 6), 1.3 m | 171.7 | |
| | $CH_2$(γ) | 25.1 | 1.20 m, 1.10 m | | |
| | $H_2$(δ) | 40.1 | 2.95 m, 2.88 m | 157.5 | |
| Phe[5] | C=O | 170.8 | | 3.47, 4.28 Gly[6] | |
| | NH | | 7.48 d (9.3) | 171.7 Arg[4] | 7.91 NH Arg[4] |
| | CH(α) | 54.9 | 4.47 ddd (11, 9.3, 4) | 170.8 | |
| | $CH_2$(β) | 38.8 | 3.22 br t (4), 2.57 dd (14, 11) | 54.9, 137.4 | |
| | 1’ | 137.4 | | | |
| | 2’ 6’ | 128.9 | 7.16-7.19 d (9) | 38.8 | 4.47, 3.22, 2.57 |
| | 3’ 5’ | 128.3 | 7.27 m | | |
| | 4’ | 126.4 | 7.27 m | | |
| Gly[6] | C=O | 170.1 | | 3.76, 3.44 Gly[7] | |
| | NH | | 7.87 t (8.5) | 170.8 Phe[5] | 3.47 Hα Gly[6] |
| | $CH_2$ | 41.3 | 4.28 dd (16.6, 8.5) | 170.1 | |
| | | | 3.47 dd (16.6, 2.2) | 170.1 | |
| Gly[7] | C=O | 170 | | 4.23 Hα Phe[1] | |
| | NH | | 8.58 t (8.5) | 170.1 Gly[6] | 3.47 Hα Gly[6] |
| | $CH_2$ | 43.3 | 3.76 m, 3.44 m | 170 | |

The amide protons $NH_2$ of $Asn^2$ located on the TOCSY spectrum, showed a cross-peak between the carbonyl group at $\delta_C$ 173.4 ppm and Cβ at $\delta_H$ 2.72 ppm of $Asn^2$ in the HMBC spectrum. Finally the carbonyl group at 171.4 ppm involved in the peptide ring was identified by its correlations with Hβ and Hα respectively at 2.72 and 5.00 ppm of $Asn^2$. Furthermore this carbonyl group was not correlated to an amide proton, suggesting its connection with $Pro^3$.

The ROESY correlations (figure 2) between the amide proton $NH_{(i+1)}$ and each adjacent amino acid residue $H\alpha_{(i,)}$ supported the proposed sequence and the ROESY cross-peak between H-δof $Pro^3$ and Hα of $Asn^2$, allowed us to propose for **1**, the following cyclic sequence of cyclo (-$Phe^1$-$Asn^2$-$Pro^3$-$Arg^4$-$Phe^5$-$Gly^6$-$Gly^7$-). In addition the difference of $^{13}C$ NMR chemical shifts between Cβ and Cγ of $Pro^3$ showed that the amide bond in the proline residue was *trans*[29,30].

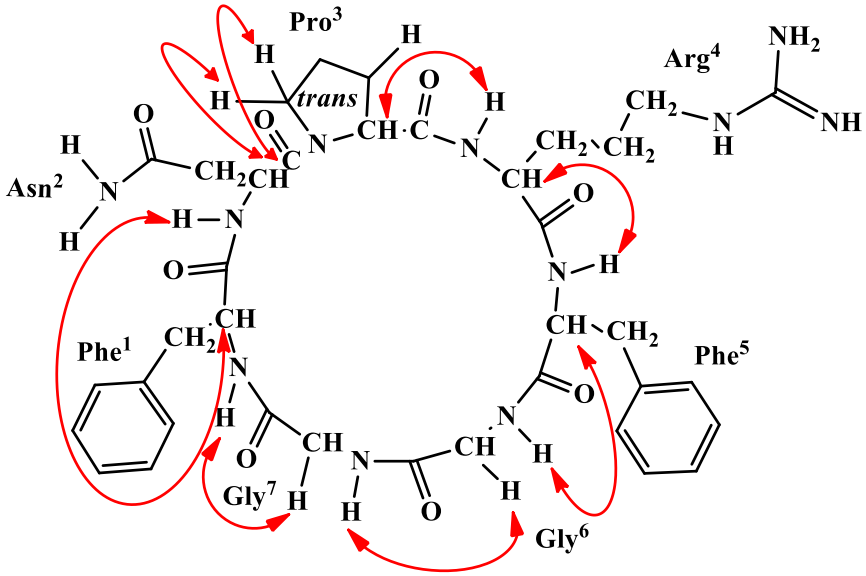


**Figure 2**. Selected ROESY $^1H$-$^1H$ correlations of **1**

The structure of **1** was further confirmed by ESI-MS/MS experiments of a pseudo-molecular ion at *m/z* 776 $[M+H]^+$. Important ion peaks at*m/z* 205,212, 262, 289,376, 523 and 662 were observed and were respectively assigned to ($Phe^1$-$Gly^7$), ($Asn^2$-$Pro^3$), ($Phe^1$-$Gly^7$-$Gly^6$), ($Arg^4$-$Phe^5$), ($Asn^2$-$Phe^1$-$Gly^7$-$Gly^6$), ($Asn^2$-$Phe^1$-$Gly^7$-$Gly^6$-$Phe^5$) and ($Phe^1$-$Asn^2$-$Pro^3$-$Arg^4$-$Phe^5$). The two main sequential cleavages at *m/z* 523 and 662 confirmed the cyclic structure of **1**as showed in figure 3.

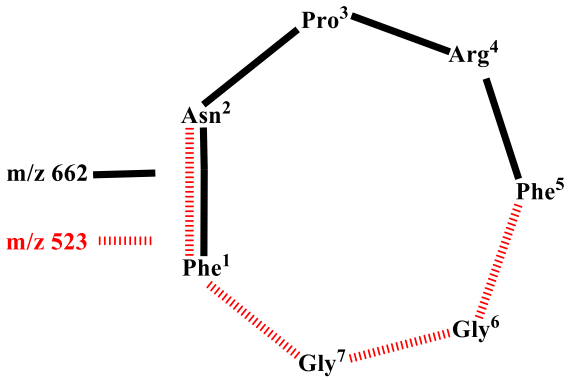


**Figure 3.** ESI-MS-MS fragment analysis of **1**

The absolute configuration of chiral amino acids in akeassimezorine (**1**) was determined by HPLC analysis of the acid hydrolysate derivatized with Marfey's reagent, and comparison to appropriate amino acid standards confirmed the L-configuration of Arg, Asn, Phe and Pro. Therefore, the structure of **1** was *cyclo(L-phenylalanyl-L-asparagyl-L-prolyl-L-arginyl-L-phenylalanylglycylglycine)*. The new compound **1** was evaluated for its antibacterial activity against four enteric pathogenic microorganisms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*. Cyclopeptide alkaloids have been reported to have various and interesting biologic activities[31]. However this compound was inactive against the four tested microorganisms at the concentration of 128µg/mL. The assessment of the toxicity of compound **1** on *Artemiasalina* showed total mortality (100%) at the concentration of 100 µg/mL. Anti-cancer activity of compound **1** will be examined in the near future.

## Conclusion

This was the first report on the phytochemical study of *Zanthoxylum mezoneurispinosum*. The methanolic extract of the roots of *Z. mezoneurispinosum* was thus carried out and led to isolation of a new heptacyclopeptide alkaloid akeassimezorine **1** along with twelve known compounds including alkaloid, lignan, flavonoid and triterpenoid secondary metabolites. Compound **1** did not show inhibition at a concentration of 128 µg/mL against *Enterococcus faecalis*, *Escherichia Coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

### Acknowledgements

This work is dedicated to Professor L. Aké Assi who passed away in 14th January 2014.
The authors thank Professor C. Lavaud and Dr J. M. Nuzillard for fruitful discussions and the Government of Ivory Coast for granting the scholarship to P. A.Yao-Kouassi.

### References

1- D. J. Mabberly; The plant book: a portable dictionary of the higher plant. Cambridge University Press, **1987**, 707.
2- M. J. Cheng, I. L. Tsai and I. S. Chen, J. Chin. Chem. Soc., **2003**, 50, 1241-1246.
3- E. J. Adjanohoun, Contribution aux études ethnobotaniques et floristiques au Togo, Paris, France, A.C.C.T, **1986**, 671.
4- I. Elekwa, M. O. Monanu and E. O. Anosike, Biokemistri, **2005**, 17, 7-12.
5- O. M. Adegbolagun and O. O. Olukemi, Afr. J. Pharm. Pharmaco, **2010**, 4, 145-150.
6- O. J. Patiño, J. A. Prieto, J. M. Lozano, L. Lesmes and L. E. Cuca, Rev. Cubana Fram. **2011**, 45, 431-438.
7- N. Verma and R. L. Khosa, IJBB, **2010**, 47, 124-127.
8- J. Arrieta, B. Reyes, F. Calzada, R. Cedillo-Rivera and A. Navarrete, Fitoterapia, **2001**, 72, 295-297.
9- P. A. Olounlade, E. V. Azando, M. S. Hounzangbe-Adote, T. B. Ha, E. Leroy, C. Moulis, N. Fabre, J. F. Magnaval, H. Hoste and A. Valentin, Parasitol. Res., **2012**, 110, 1427-1433.

10- J. J. Chen, Y. H. Lin, S. H. Day, T. L. Hwang and I. S. Chen, Food Chem., **2011**, 125, 282-287.
11- F. Hiroyuki, K. Marii, M. Y. Kanami, S. Setsuko, S. Motoyoshi, I. Tsutomu, K. Fumiyuki and K. Nobuo, Chem. Pharm. Bull., **2010**, 8, 1047-1050.
12- F. Batool, S. S. Mubashir, J. B. T. Rocha, A. S. Hussain, Z. S. Saied and S. A. Dilnawad, PJB**, 2010**, 42, 4299-4311.
13- L. Xia, J. You, G. Li, Z. Sun and Y. Suo, J. Am. Oil Chem. Soc., **2011**, 88, 1029-1036.
14- L. T. Tan, Phytochemistry, **2007**, 68, 954-979.
15- K. Tidgewell, N. Engene, T. Byrum, J. Media, T. Doi, F. A. Valeriote and W. H. Gerwick, Chem. Bio. Chem., **2010**, 11, 1458-1466.
16- A. Wélé, Y. Zhang, C. Caux, J. P. Brouard, L. Dubost, C. Guette, J. L. Pousset, M. Badiane and B. Bodo, J. Chem. Soc. Perkin Trans I, **2002**, 2712-2718.
17- A. Wélé, Y. Zhang, I. Ndoye, J. P. Brouard, J. L. Pousset and B. Bodo, J. Nat. Prod., **2004**, 67, 1577-1579.
18- A. N. T. Ribeiro, R. L. Da Silva, T. de S. P. Junior, N. R. Castro and G. M. A. De Carvalho, Helv. Chim. Acta, **2012**, 95, 935-939.
19- M. F. Talontsi, P. Facey, K. M. D. Tatong, T. M. Islam, H. Frauendorf, S. Draeger, A. Von Tiedemann and H. Laatsch, Phytochemistry, **2012**, 83, 87-94.
20- P. Marfey, Carlsberg Res. Commun., **1984**, 49, 591-596.
21- M. Grare, M. Mourer, J. B. Regnouf-de-Vains, C. Finance and R. E. Duval, Pathol. Biol., **2006**, 54, 470-476.
22- A. P. Yao-Kouassi, A. A. Magid, B. Richard, A. Martinez, M. J. Jacquier, C. Caron, D. E. Le Magrex, C. S. Gangloff, C. A. Ahiboand M. Zèches-Hanrot, J. Nat. Prod., **2008**, 71, 2073-2076.
23- N. Keawpradub, S. Dej-Adisaiand S. Yuenyongsawad,Songklanakarin J. Sci. Technol., **2005**, 27 (Suppl.2), 455-467.
24- H. Mozammel, B. Sufia, S. Hossain, A. Monira, M. H. Choudhury, A. Nuruddin and H. Rashedul, Pharmacogn. Mag., **2013**, 9, 76-80.
25- J. A Swinehart and F. R. Stermitz, Phytochemistry, **1980**, 19, 1219-1223.
26- J. M. Barbosa-Filho, E. V. L. Da-Cunha, M. L. Cornélio, C. D. Da Silva and A. I. Gray, Phytochemistry, **1997**, 44, 959-961.
27- E. Hisatomi, M. Matsui, A. Kobayashi and K. Kubota, J. Agric. Food Chem., **2000**, 48, 4924-4928.
28- A. P. Yao-Kouassi, A. Martinez, D. F. Malan, D. E. Le Magrex, C. S. Gangloff, C. Caron, C. A. Ahibo and M. Zèches-Hanrot, Int. J. Biol. Chem. Sci., **2014**, 8, 377-385.
29- D. E. Dorman and F. A. Bovey, J. Org. Chem., **1973**, 38, 2379-2383.
30- K. Wüthrich, M. Billeter and W. Braun, J. Mol. Biol., **1984**, 180, 715-740.
31- N. H. Tan and J. Zhou, Chem. Rev., **2006**, 106, 840-895.