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Novel Cytotoxic Cycloheptapeptide from the Latex of *Jatropha integerrima*

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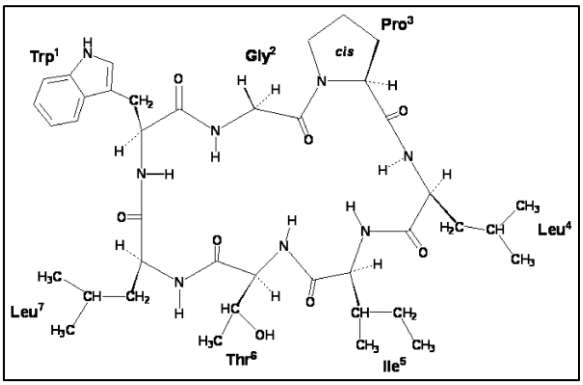
ABSTRACT

A new cycloheptapeptide, integerrimide C (1), which exhibited significant cytotoxic activity against tumoral KB cells (IC_{50} 1.7 µM), was isolated from the ethyl acetate extract of the latex of Jatropha integerrima. The structure was elucidated on the basis of the MS/MS fragmentation using a Q-TOF mass spectrometer equipped with an ESI source, chemical degradation and extensive one and two dimensional nuclear magnetic resonance.

Keywords: Cyclopeptide; *Jatropha integerrima*; Euphorbiaceae; Integerrimide C

INTRODUCTION

Cyclopeptides find many applications in the fields of biotechnology, among which pharmaceutical applications. Natural cyclopeptides and diterpenes from *Jatropha* and *Annona* species have potent biologic activities [1-5, 8, 10, 11, 14, 15, 17-20]. Previously phytochemical studies on *J. integerrima* (Euphorbiaceae) reported on the isolation of integerrimides A and B which exhibited significantly inhibited neurite outgrowth in neuronal cell culture [12]. Continuing our investigation from the latex of this specie, we here report on the isolation by HPLC and the structural elucidation, based on tandem mass spectrometry and 2D NMR, of a new cycloheptapeptide named integerrimide C (1) with the known cyclic peptides previously described [12]. This compound found to be cytotoxic against tumoral KB cells with an IC_{50} 1.7 µM.



EXPERIMENTAL SECTION

General experimental procedures

The melting point was determined on a Büchi melting point B-545 apparatus. The optical rotation was measured with a Perkin-Elmer model 341 polarimeter, and the $[\alpha]_D^{22}$ values is given in deg. $cm^2\ g^{-1}$. Infrared and ultra-violet spectra were recorded on a spectrometer Philips PU 8720. ^{1}H-NMR and 13C NMR spectra were recorded on a Bruker Avance 400 spectrometer, operating at 400.13 MHz using DMSO-d_6 as solvent. Mass spectra were recorded on an API Q-STAR PULSAR *i* ms (Applied Biosystem). For the CID spectra, the collision energy was 40-60 eV and the collision gas was nitrogen. Thin layer chromatography was performed on a pre-coated TLC plates (Merk, silica 60 F_{254}), by spraying with ninhydrin and chlorine/*o*-tolidine reagents.

Absolute configuration of amino acids

Solution of integerrimide C containing 1 mg of cyclopeptide, in 6N HCl (1ml) was heated at 110°C for 24 hours in sealed tubes. Free amino acids residues were methylated and the methylated amino acids were analysed by CPG [17-20]. Comparison of relative retention time (min) values with those of standards amino acids was used: Gly (14.6), DL-Ile (16.2, 16.9), DL-Leu (18.1, 19.2), DL-Trp (34.2, 36.4), DL-Pro (18.0, 18.2) and DL-Thr (14.5, 15.2).

Plant material

Jatropha integerrima (Euphorbiaceae) is a small tree which use as ornamental plant in Dakar [9]. This plant was authenticated by Doctor William Diatta (Laboratory of botanic and pharmacognosy, faculty of medicine, pharmacy and odontology, UCAD, DAKAR, SENEGAL). The latex of *J. integerrima* was collected in October 2015 by incision of the stems of the plant and was immediately conserved at 10°C.

Extraction and isolation

Details of the methodology of isolation and purification of cyclic peptides are described in the last papers [17-20]**.** The latex of *Jatropha integerrima* (Euphorbiaceae) was dissolved in 200 ml of distilled water. This solution was introduced in phial decanters ant treated with 250 ml of ethyl acetate. After energetic agitation the organic (ethyl acetate) extract containing cyclopeptides was concentrated to dryness. This operation was repeated three times. Then, the ethyl acetate extract was dissolved in 3 ml of methanol and chromatographed successively on Sephadex LH-20 and silica gel (Kieselgel 60 H Merck) columns, and finally purified by isochratic reversed phase HPLC (Kromasil C_{18}, 250 x 7.8 mm, 5 µm, AIT France; flow rate 2 mL/min, detection 220 nm) using MeOH/H_2O : 72/28 to give integerrimide C (t_R 14.0 min), integerrimide A (t_R 18.8 min) and integerrimide B (t_R 20.5 min).

Bioassays

In a 3-day cytotoxicity bioassay, integerrimide C exhibited significant activity in vitro against the KB (human nasopharyngeal carcinoma) cell culture system, with an IC_{50} 1.7 µM. Doxorubicine (IC_{50} 0.02 µM) was used as the positive control. Details of the assays procedure expressed as IC_{50} (µM) are described in the literature [6].

RESULTS AND DISCUSSION

Isolation and structural determination

The latex of *Jatropha integerrima* (50 ml) was treated with water and ethyl acetate (EtOAc) was added to this solution. The EtOAc-soluble fraction (280 mg) was purified by exclusion chromatography, silica gel column chromatography, and C_{18} reversed-phase HPLC to yield a new cycloheptapeptide, integerrimide C (**1**) (7.2 mg) with two known cyclopeptides, integerrimides A and B [12]. This compound showed principals IR absorption maximum at 3320, 1650 cm^{-1} and UV absorption at 240, 255, 262 nm. Positive reaction with chlorine/*o*-tolidine reagent suggested it was a peptide and the absence of coloration of its TLC spot with ninhydrin, that it was cyclic. Analysis of the total acidic hydrolyzate, after derivatization, indicated the presence of Gly (1), Ile (1), Leu (2), Pro (1), Thr (1) and Trp (1). The acids were converted into the *n*-propyl esters of their N-trifluoroacetyl derivatives, analyzed by gas chromatography on a chiral capillary column, and their relative retention time compared with those of standards indicated that all the chiral amino acids were *L*.

The molecular weight M = 780 was deduced from the positive ESI-Q-TOF mass spectrum where the protonated molecule MH^+ and the adduct ion $[M+Na]^+$ and $[M+K]^+$ were observed at *m/z* 781, 803 and 819, respectively. In the high-resolution mass spectrum, the protonated molecule MH^+ at *m/z* at 781.4516 corresponding to the molecular formula $C_{40}H_{60}N_8O_8$ was in agreement with the above amino acid composition in a cyclic heptapeptide. The CID experiment on the protonated molecular $[M+H]^+$ ion of integerrimide C at *m/z* 781.4516

allowed the structure determination. The ring opening occurred at the Trp[1]-Gly[2] amide bond level, and a series of adjacent acylium ions (b) at *m/z* 668, 567, 454, 341 and 241 was generated. The successive loss of, Leu/Ile, Thr, Leu/Ile, Leu/Ile, Pro, was observed, yielding to the N-terminal dipeptide Trp-Gly at *m/z* 241 (Figure 1). A second series of ions was depicted at *m/z* 640, 539, 426, 313 and 216 which were assigned to adjacent (a_n) series related to the above b_n series. At *m/z* 763 was observed an abundant ion originating from the loss of a molecule of water from the protonated molecular ion $[M+H]^+$. These results suggested the structure [H-Trp[1]-Gly[2]-Pro[3]-Leu/Ile[4]-Leu/Ile[5]-Thr[6]-Leu/Ile[7]]$^+$ for the linearized peptide ion derived from this compound, but with ambiguity related to the respective location of Leu and Ile.

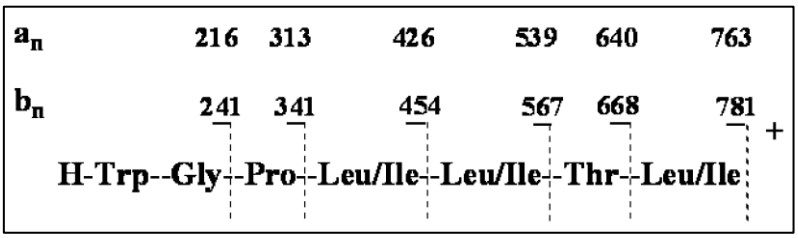


Figure 1: MS/MS fragmentation of the protonated integerrimide C (1) ion

The 1H NMR spectrum of (**1**) in DMSO-d_6 solution showed a main stable conformational state (>90%) for which seven amide protons were clearly depicted, as well as the presence of seven carbonyl groups in the 13C NMR spectrum, in agreement with a heptapeptide structure including one proline residue and one residue of tryptophan. The presence of the tryptophan residue in the sequence is not usual in the kind of cyclic peptide. The amino acid spin systems of the different residues were identified using scalar spin-spin couplings determined from the 1H-1H COSY and TOCSY experiments which indicated clearly the presence of two residues of leucine and one residue of isoleucine [7, 21, 22]. The 13C assignments of the protonated carbons were obtained from the proton-detected heteronuclear HSQC spectrum and combined with the HMBC experiment optimized for long-range *J*-value of 7 Hz, for the nonprotonated carbons. This experiment allowed the carbonyl groups to be assigned. Carbonyl carbons of Trp[1], Pro[3], Leu[4], Thr[6] and Ile[7] were easily identified from their intra-residue ^3J correlations with β protons, those of Gly[2] and Ile[5] from the intra-residue connectivities with the α protons. The sequence determination was obtained on the basis on the HMBC experiment. In this heteronuclear methodology the structure of 1 was carried out from the connectivities between the carbonyl of residue (i) with the amide and/or α protons of residue (i+1). The principal $^3J_{CH}$, CO(i) to NH(i+1) correlations from Trp[1] to Leu[7] shown in Figure 2, were observed on the HMBC spectrum. The HMBC correlation between the α proton of the Pro and the carbonyl of the Gly[2] indicated that the proline residue is at position 3. In the other hand the strong ^3J connectivities between the carbonyl of Pro[3] and the amide protons of Leu, and between the carbonyl of Ile and the proton amide of Thr[6] indicated that this leucine residue is at position 4 and the isoleucine residue is at position 5. The localisation of the second residue of leucine was determined by connectivities between the carbonyl of Trp[1] and the ββ protons of Leu and between the carbonyl of Thr[6] and the amide proton of Leu at position 7.

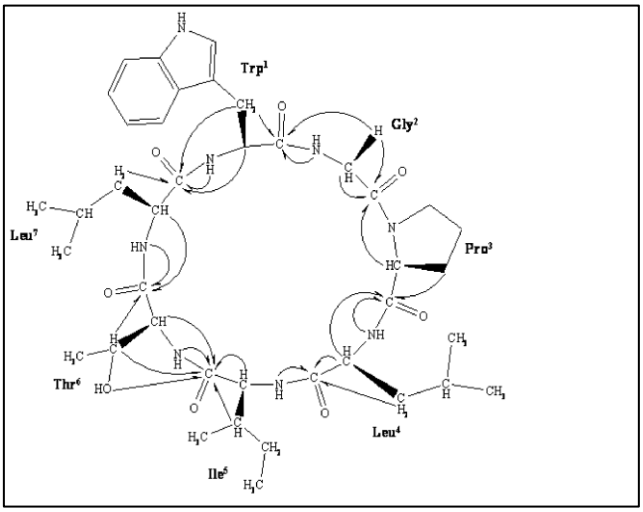


Figure 2: Sequence of integerrimide C (1): principal correlations from the HMBC spectrum

Other connectivities are in full agreement with the structure deduced from the MS-MS mass spectrometry study. The NOESY spectrum depicted strong and medium sequential NOE $d_{NN(i,i+1)}$ interactions between Trp^1 and Gly^2 and from Leu^4 to Leu^7. Sequential $d_{\alpha N(i,i+1)}$ connectivities between Trp^1 and Gly^2, and from Leu^4 to Leu^7 was also identified to confirm the structure of integerrimide C (Figure 3). In addition, strong NOE correlations were observed between the α protons of Gly^2 and Pro^3 indicates that Gly^2-Leu^3 amide bond is in *cis* configuration. This stereochemistry is further confirmed by the γ carbon ^{13}C chemical shift of the Pro^3 at 21.0 ppm [7].

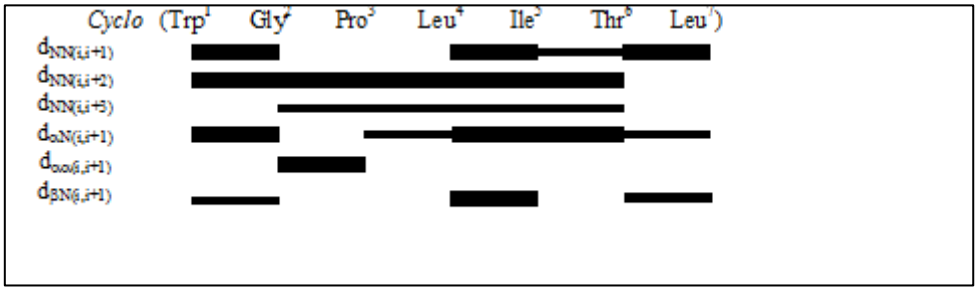


Figure 3: Diagrammatic representation showing the pattern of strong and medium connectivities involving the NH, □ and □ protons for integerrimide C (1) in DMSO-d_6 solution

CONCLUSION

This study confirmed the chemical richness and variety of biological of the latex of *Jatropha* species. 1H-NMR and 13C-NMR spectra and MS-MS mass spectra are very useful to determine the structure of cyclic peptide witch revealed significant cytotoxic activity against tumoral KB cells. This result clearly indicate that the potential benefit of medicinal plants depends on this anticancer compound.

Spectral data

Integerrimide C **(1)**-Colourless solid, Mp 187-188°C (MeOH); $[\alpha]_D^{22}$ -65° (c 0.1, MeOH) ; IR (KBr) cm^{-1}: 3320 and 1650. UV λ_{max} (MeOH) nm (ε): 240, 255, 262; 1H NMR (DMSO-d_6, 400Mz, 298K, TMS): 4.44 (1H, m, Trp^1-Hα), 3.30 (1H, dd, 7.0, 13.8, Trp^1-Hβ), 3.12 (1H, dd, 7.4, 13.8, Trp^1-Hβ'), 10.87 (NH, m, 8.3, Trp^1-1'), 7.09 (1H, m, Trp^1-2'), 7.64 (1H, m, Trp^1-4'), 6.93 (1H, d, 8.3, Trp^1-5'), 7.06 (1H, m, Trp^1-6'), 7.30 (1H, m, Trp^1-7'), 8.00 (1H, d, 8.0, Trp^1-NH), 11.40 (1H, s, Trp^1-OH), 4.30 (1H, dd, 18.0, 6.3, Gly^2-Hα), 3.55 (1H, dd, 18.0, 5.3, Gly^2-Hα'), 8.15 (1H, dd, 5.3, 6.9, Gly^2-NH), 4.65 (1H, d, 8.0, Pro^3-Hα), 1.81 (1H, m, Pro^3-Hβ), 1.60 (1H, m, Pro^3-Hβ'), 1.15 (1H, m, Pro^3-Hγ), 0.47 (1H, m, Pro^3-Hγ'), 3.10 (1H, m, Pro^1-Hδδ'), 4.03 (1H, m, Leu^4-Hα), 1.53 (1H, m, Leu^4-Hβ), 1.43 (1H, m, Leu^4-Hβ'), 1.60 (1H, m, Leu^4-γH), 0.78 (3H, m, Leu^4-δH), 0.88 (3H, d, 5.8, Leu^4-δ'CH_3), 7.68 (1H, d, 5.1, Leu^4-NH), 4.30 (1H, dd, 9.2, 9.7, Ile^5-Hα), 1.75 (1H, m, Ile^5-Hβ), 1.15 (1H, m, Ile^5-γH), 0.90 (1H, m, Ile^5-γ'H), 0.83 (3H, d, 6.7, Ile^5-δCH_3), 7.40 (1H, m, Ile^5-NH), 5.25 (1H, dq, 8.5, 6.8, Thr^6-Hα), 1.38 (3H, d, 6.8, Thr^6-βCH_3), 8.62 (1H, d, 8.6, Thr^6-NH), 5.80 (1H, t, 5.7, Thr^7-OH), 3.93 (1H, m, Leu^7-Hα), 1.75 (1H, m, Leu^7-Hβ), 1.60 (1H, m, Leu^7-Hβ'), 1.50 (1H, m, Leu^7-γH), 0.85 (3H, m, Leu^7-δH), 0.80 (3H, d, 5.8, Leu^7-δ'CH_3), 8.08 (1H, d, 7.7, Leu^7-NH). 13C NMR (DMSO-d_6, 400 MHz, 298K, TMS): 170.9 (Trp^1-CO), 54.8 (Trp^1-Cα), 27.3 (Trp^1-Cβ), 123.5 (Trp^1-C2'), 110.4 (Trp^1-C3'), 118.3 (Trp^1-C4'), 118.3 (Tyr^7-C5'), 120.9 (Trp^1-C6'), 111.2 (Trp^1-C7'), 136.1 (Trp^1-C8'), 127.1 (Trp^1-C9'), 169.5 (Gly^2-CO), 48.4 (Gly^2-Cα), 173.8 (Pro^3-CO), 60.1 (Pro^3-Cα), 30.2 (Pro^3-Cβ), 21.0 (Pro^3-Cγ), 45.7 (Pro^3-Cδ),172.5 (Leu^4-CO), 53.0 (Leu^4-Cα), 40.5 (Leu^4-Cβ), 24.1 (Leu^4-Cγ), 22.7 (Leu^4-Cδ-CH_3), 21.9 (Leu^4-Cδ-CH_3), 170.5 (Ile^5-CO), 55.8 (Ile^5-Cα), 37.4 (Ile^5-Cβ), 24.1 (Ile^5-Cγ), 15.4 (Ile^5-Cδ-CH_3), 11.6 (Ile^5-Cγ-CH_3), 169.8 (Thr^6-CO), 54.4 (Thr^6-Cα), 18.4 (Thr^6-Cβ), 170.2 (Leu^7-CO), 53.6 (Leu^7-Cα), 39.5 (Leu^7-Cβ), 24.1 (Leu^7-Cγ), 22.4 (Leu^7-Cδ-CH_3), 21.0 (Leu^7-Cδ-CH_3). ESI-QTOF, *m/z* : 819 $[M+K]^+$, 803 $[M+Na]^+$, 781 $[M+H]^+$; ESI-QTOF MS/MS on *m/z* 781 $[M+H]^+$ (ce 40 eV) *m/z* (%): 781 (100), 763 (38), 753 (24), 694 (21), 668 (76), 654 (24), 640 (15), 623 (12), 599 (10), 581 (13), 567 (39), 555 (23), 553 (6), 539 (40), 537 (8), 535 (15), 527 (6), 454 (80), 440 (32), 426 (60) 422 (10), 417 (21), 396 (34), 394 (3), 374 (63), 341 (75), 325 (56), 313 (25), 297 (31), 241 (19), 226 (16), 244 (16), 216 (15), 183 (14), 155 (3), 86 (20), 70 (78).

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REFERENCES

[1] W-F Altei; D-G Picchi; B-M Abissi; G-M Giesel; OJr Flausino; M Reboud-Ravaux; H Verli; E Jr. Crusca; E-R Silveira; E-M Cilli; V-S Bolzani. *Phytochemistry* **2014**, *107*, 91-96.
[2] C Auvin-Guette; C Baraguey; A Blond; J-L Pousset; B Bodo. *J. Nat. Prod*. **1997**, 60, 1155-1157.
[3] C. Baraguey; C Auvin-Guette; A Blond; F Cavelier; F Lezenven; J-L Pousset; B Bodo. *J. Chem. Soc.; Perkin Trans. 1* **1998**, 3033-3039.
[4] C Baraguey; A Blond; I Correia; J-L Pousset; B Bodo; C Auvin-Guette. *Tetrahedron Lett*. **2000**, 41, 325-29.
[5] C Baraguey; A Blond; F Cavelier; J-L Pousset; B Bodo; C Auvin. *J. Chem. Soc., Perkin Trans*., **2001**, *1*, 2098-2103.
[6] FR Chang; JL Chen; HF Chiu; MJ Wu; YC Wu. **1998**, *47*, 1057-1061.
[7] DE Douglas; FA Bovey. *J. Org. Chem.* **1973**, *38*, 2379-2383.
[8] SFAJ Horsten; AJJ Van der Berg; JJ Kettenes-van den Bosch; BR Leeflang; RP Labadie. *Planta Med*.**1996**, 6246-6250.
[9] J Kerharo; G Adam. ed. *Vigot Frères*, Paris, **1974.**
[10] S Kosasi; WG Van der Sluis; R Boelens; LA Hart; RP Labadie. *FEBS Lett*. **1989**, 256, 91-96.
[11] C-M Li; N-H Tan; H-L Zheng; Q Mu; X-J Hao; Y-N He; J Zhou. *Phytochemistry*, **1999**, *50*, 1047-1052.
[12] W Mongkolvisut; S Sutthivaiyakit; H Leutbecher; S Mika; I. Klaiber; W Möller; H Rösner; U. Beifuss; J. Conder. *J. Nat. Prod*. **2006**, 69, 1435-144.
[13] S Sutthivaiyakit; W Mongkolvisut; P Ponsitipiboon; S Prabpai; P Kongsaeree; S Ruchirawat; C Mahidol. *Tetrahedron Lett*. **2003**, 44, 3637-3640.
[14] AJJ Van der Berg; SFAJ Horsten; JJ Kettenes-van den Bosch; BH Kroes; CJ Beukelman; BR Leeflang; R.P. Labadie. *FEBS Lett*. **1995**, 358, 215-218.
[15] AJJ Van der Berg; SFAJ Horsten; JJ Kettenes-van den Bosch; CJ Beukelman; BH Kroes; BR Leeflang; RP Labadie. *Phytochemistry* **1996**, 42, 129-133.
[16] G Wanger; A Kumar; K Wüthrich. *Eur. J. Biochem.* **1981**, *114*, 319-375.
[17] A Wélé; Y Zhang; C Caux; J-P Brouard; L Dubost; C Guette; J-L Pousset; M Badiane; B Bodo. *J. Chem. Soc., Perkin Trans.* **2002**, *1*, 2712-2718.
[18] A Wélé; C Landon; H Labbé; F Vovelle; Y Zhang; B Bodo. *Tetrahedron* **2002**, *60*, 405-414.
[19] A Wélé; Y Zhang; J-P Brouard; J-L Pousset; B Bodo. *Phytochemistry* **2005**, *66*, 2376-2380.
[20] A Wélé; Y Zhang; L Dubost; J-L Pousset; B Bodo. *Chem. Pharm. Bull.* **2006**, *66*, 693-696.
[21] K Wüthrich; G Wider; G Wagner; W Braun. *J. Mol. Biol.* **1982**, *155*, 311-319.
[22] K Wüthrich; M Billeter; W Braun. *J. Mol. Biol.* **1984**, *180*, 715-740.
[23] YL Yang; KF Hua; PH Chuang; SH Wu; KY Wu; FR Chang; YC Wu. *J Agric Food Chem*. **2008**, *56*, 38**6**-92.