Compounds with myorelaxant activity from the leaves of Mareya micrantha (Benth.) Müll. Arg. (Euphorbiaceae)

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Abstract

Mareya micrantha (Euphorbiaceae) is an Ivorian medicinal plant widely used in gastrointestinal disorders. So as to understand and identify metabolites possibly implicated in the activity of the plant on intestinal muscles, this study has been undertaken. From the crude ethanolic extract EE of the leaves, hexanic (EH), chloromethylenic (EC), ethyl acetatic (EA) sub-extracts were prepared as well as the hydroethanolic residual phase dried (Ar) and their effects on the isolated duodenum of rabbits were determined. If the sub-extracts EH, EA and EB, but also Ar, confirmed the myostimulant activity of the crude extract EE, this was not the case of EC which showed myorelaxant activity. This sub-extract, subjected to purification, led to the isolation of three major triterpenoids type (Euphorbiaceae) occupies an important place. Its leaves are used as an antidote and oxytocic substances Naturelles (LCOSN), UFR Sciences des Structures de la Matière et Technologie, Univ. FHB, BP 34 Abidjan, Côte d'Ivoire, West Africa

Keywords: Mareya micrantha, myorelaxant activity, 29-norcucurbitacins, maremicranthine, euphorbiaceae

1. Introduction

Gastrointestinal diseases are disorders that affect any section of the gastrointestinal tract, from the esophagus to the rectum, and the accessory digestive organs liver (gall, bladder and pancreas). These encompass acute, chronic, recurrent or functional disorders and cover a wide range of diseases, including inflammatory bowel disease and functional dyspepsia that cause more than 8 million deaths per year worldwide [1,2]. Gastrointestinal disorders are one of the main causes of childhood morbidity and mortality. Undernourished infants and children under the age of 5 are the most affected. This constitutes a permanent threat to socio-economic development in developing countries, particularly in sub-Saharan Africa [2]. In addition to the difficulties of access to treatment, we are witnessing the appearance of more and more forms of resistance to existing therapies of the bacteria and parasites responsible for these infections. The treatment and search for new therapeutic agents remains a challenge. A potential gastrointestinal agent can show its effect by increasing or decreasing gastrointestinal motility. Nowadays, medicinal plants play a key role in the treatment of several pathologies such as gastrointestinal disorders [3-5].

In Côte d’Ivoire, 761 medicinal plants and 1,421 medicinal recipes have been identified for the treatment of many diseases [6]. Among these plants, Mareya micrantha (Benth.) Müll. Arg. (Euphorbiaceae) occupies an important place. Its leaves are used as an antiedote and oxtotic drug, and against wounds, ulcers, sprains, rheumatism, coughs and gastrointestinal disorders such as constipation, diarrhea and ascites [7-9]. In addition, recent works have shown that the leaves have antibacterial and antifungal activities [10, 11].

The present study is carried out to understand and identify metabolites possibly implicated in the activity of leaves of the plant on intestinal muscles. Phytochemical survey of the plant reveals the presence of cucurbitacin from the leaves [12]. These compounds have been suspected in the toxicity of the plant [8, 13].
2. Experimental
2.1 General Methods
The optical rotations were measured on a PolAAR 32 polarimeter (Optical Activity Ltd., Ramsey, UK) equipped with a sodium lamp (589 nm) and a 1 dm microcell. The IR spectra were recorded on a Bruker Vector 22 spectrometer (Champs-sur-Marne, France). UV spectra are recorded in MeOH using a Jenway 7315 spectrometer (Staffordshire, UK). Column chromatography were carried out on silica gel (Merck, 40-230 mesh) or Sephadex® LH-20 (Pharmacia). Thin layer chromatography (TLC) were carried out on aluminum plates coated with silica gel 60 F254 (Merck) and revealed under UV light (254 and 366 nm) and with vanillin-H$_2$SO$_4$, Liebermann (Acetydihydrinde-H$_2$SO$_4$) or Fast Blue B. The $^1$H and $^13$C NMR spectra (COSY, HSQC, HMBC and NOESY), were recorded in the CD$_2$OD on a Bruker AC-400 spectrometer (Champs-sur-Marne, France) operating at 400 MHz for $^1$H spectra and 100 MHz for $^{13}$C. Low resolution mass spectra, AP Crab, and ESIMS, were acquired using a Bruker Esquire LC-00040 spectrometer (Champs-sur-Marne, France). HRESIMS spectra were recorded with a Bruker Esquire LC-00040 spectrometer (Champs-sur-Marne, France). The leaves were crushed using a Retsch machine (Illkirch-Graffenstaden, France).

2.2. Plant Material
The plant material constituted by the leaves of Mareya micrantha were collected in May 2017 in Yakassé-Mé (5°48′40″N, 3°57′04″W), in the Department of Adzopé in the south-east of Côte d’Ivoire. It was authenticated at the Centre National de Floristique (CNF) of Félix Houphouët Boigny University, Abidjan (Côte d’Ivoire) where a specimen (OAT-Mm-2017) was deposited. The leaves were dried at laboratory temperature (approximately 25 °C) for two weeks.

2.3. Extraction and Isolation of Compounds
Dried powdered leaves (3 kg) of Mareya micrantha was suspended, at room temperature, in 10 L of ethanol (EtOH) in a 25 L canister of Pyrex type (SGDG) surmounted by a Heidolph type mechanical stirrer (RZR 2020) for $3 \times 24$ h, followed by a successive filtration on fabric, cotton and on Whatman N° 4 paper (Schleicher & Schuell). Thus, the filtrates are mixed and concentrated using Heidolph Rotavapor® to provide the total ethanolic crude extract of the leaves (EE, 671.5 g, 22.4%). A fraction of this extract (400 g) was suspended in 600 mL of EtOH/H$_2$O (1:3, v/v) to be successively extracted with hexane (4 × 300 mL), dichloromethane (4 × 300 mL), ethyl acetate (4 × 300 mL) and n-butanol (4 × 300 mL) to yield hexanic (HE, 63.6 g, 15.9%), chloromethylene (EC, 32.1 g, 8.0%), ethyl-acetate (EA, 52.2 g, 13.0%) and butanolic (EB, 109.0 g, 27.3%) sub-extracts. The residual hydroethanolic phase evaporated to dryness provided the residual aqueous fraction (Ar, 68.0 g, 17.0%).

Based on bioguided fractionation outcomes, EC fraction was selected for further fractionation (Figures S8–S9). A 25 g aliquot of this sub-extract was fractionated on a silica gel column using a CHCl$_3$/MeOH elution gradient (99:1 to 90:10) to yield nineteen fractions (F1 to F19). Fraction F4 (0.6 g) was further chromatographed on a silica gel using EtOAc/MeOH (95:5) to provide five sub-fractions (F41 to F45). Sub-fraction F42 (296 mg), in crystals form, was first washed several times with $n$-hexane and then with dichloromethane before being purified on silica gel (CHCl$_3$/CH$_2$Cl$_2$/EtOAc, 30:60:10) and Sephadex LH-20 (CH$_2$Cl$_2$/MeOH, 2:1) columns, yielding compounds 1 (6.6 mg) and 2 (55.8 mg).

Fraction F7 (6.3 g) was chromatographed on a silica gel column using an elution gradient (EtOAc/MeOH, 95:5 to 90:10) to provide six fractions (F71 to F76). Sub-fraction F72 (3.5 g) was purified by successive chromatographies on silica gel (CH$_2$Cl$_2$/EtOAc, 80:20; CH$_2$Cl$_2$/Isopropanol, 90:10), and on Sephadex® LH-20 gel (CH$_2$Cl$_2$/MeOH, 2:1) to give compound 2 (56.6 mg). Purified by successive chromatographies on silica gels (n-C$_3$H$_7$/EtOAc/MeOH, 2:7:1; EtOAc/MeOH, 9:1), and on Sephadex® LH-20 gel (CH$_2$Cl$_2$/MeOH, 2:1), the subfraction F74 led to compound 3 (29.7 mg).

2.3.1 Spectral data of Maremnicranthine A (1)
Orange crystals; [α]$_D$ + 9.7 (c 1.50, MeOH); UV $λ_{max}$ (nm): 282, 230; IR $ν_{max}$ (cm$^{-1}$): 3313, 2925, 1662, 1615, 1484, 1243, 1019; For $^1$H and $^{13}$C NMR (CD$_2$OD) data, see Table 1; HR-ESIMS $m/z$ 543.2946 [M + H]$^+$ (calcd for C$_3$H$_2$O$_8$N, 543.2958).

2.4. Biological Assays
Smooth muscle activity tests were carried out according to the experimental method described by Mea et al. [14]. The rabbits used for the tests, Oryctolagus cuniculus (Leporidae) species, were brought from farms located in the town of Bingerville (5°21′20″N, 3°53′07″W), near Abidjan. The experimental protocol followed the principles and guidelines published by the National Institutes of Health for care and use of laboratory animals. Briefly, the rabbits required adaptive feeding for at least one week at 26 ± 4 °C under a relative humidity of 60% with a photoperiod of 12/24 and a fasting period of 24-48 h before the experiments. The experimental device consisted of an insulated organ tank containing a physiological reference solution at pH 7.4 of Mac Ewen glucose medium composed of NaCl (80 g/L), KCl (20 g/L), CaCl$_2$ (20 g/L), Na$_2$HPO$_4$ (15 g/L), NaHCO$_3$ (40 g/L), MgCl$_2$ (10 g/L) and glucose (2 g/L). This tank, oxygenated by an aquarium bubbler and immersed in a thermostatic water bath, was surmounted by a bottle containing the physiological glucose solution which allowed its supply from a serpentine immersed in a thermostatic water bath. The Mac Ewen's glucose medium arrival at the organ tank was controlled by a central tap. Fragments of duodenum, taken from the fasting rabbits, after a laparotomy, were kept alive in the glucose medium arrival at the organ tank was controlled by a central tap. Fragments of duodenum, taken from the fasting rabbits, after a laparotomy, were kept alive in the glucose medium composed of NaCl (80 g/L), KCl (20 g/L), CaCl$_2$ (20 g/L), Na$_2$HPO$_4$ (15 g/L), NaHCO$_3$ (40 g/L), MgCl$_2$ (10 g/L) and glucose (2 g/L). This tank, oxygenated by an aquarium bubbler and immersed in a thermostatic water bath, was surmounted by a bottle containing the physiological glucose solution which allowed its supply from a serpentine immersed in a thermostatic water bath. The Mac Ewen's glucose medium arrival at the organ tank was controlled by a central tap. Fragments of duodenum, taken from the fasting rabbits, after a laparotomy, were kept alive in the glucose-containing Mac Ewen solution, oxygenated and kept at a constant temperature of 38 °C.

For the experiment, fragments of the duodenum (about 3 cm) were mounted in the insulated organ tank at one end and the other end was connected to a lever system carrying a recording stylus. This stylus allowed the recording of contractile movements of the isolated intestine on recording paper, coated with smoke black, fixed on a cylinder which made a rotary movement at constant speed by a motor. A calibration of the recording pen indicated that, under our experimental conditions, a 24 mm drop in height corresponded to a 1 g force (10 mN), or 1 gram force (1 gF), exerted by the intestine.

For the tests, a stock solution of concentration 10 mg/mL of each of the evaluated substances (extract or isolated compound) was made up in the Mac Ewen's glucose medium. Dilutions of each of these stock solutions were prepared and their effects on the contractile activity of the isolated rabbit duodenum were measured.
To delineate the underlying mode of action, ethylene diamine tetra-acetic acid (EDTA, SIGMA, USA) and ethylene glycol (β-aminoethyl ether) N,N,N’,N’-tetra-acetic acid (EGTA, SIGMA, USA) were used at final concentrations of 10⁻⁴ mg/mL to chelate residual calcium from the modified Mac Ewen's glucose medium i.e. lacking of calcium (0 Ca²⁺ medium).

Values were expressed as the average including the standard error over the average. The variance analysis (Anova) of the Turkey-Kramer multiple comparison test was used to appreciate the significance of the observed differences. The statistical value was considered significant if \( p < 0.05 \) (\( n = 3 \)). Paint® and Microsoft Picture Softwares® were used to display the trace of intestinal contractions (black on a light blue background).

3. Results and Discussion

The crude ethanolic extract (EE) of *Mareya micrantha* leaves was partitioned with solvents of increasing polarities to give hexanic (EH), chloromethenylenic (EC), ethyl acetate (EA) and butanolic (EB) sub-extracts as well as the residual aqueous fraction (Ar). The extracts EE, EH, EA and EB, but also Ar, at a dose of 10 µg/mL, showed myostimulant activity with an increase in the basic tone of 0.63 ± 0.12, 0.38 ± 0.07, 0.41 ± 0.16, 0.40 ± 0.15 and 0.62 ± 0.07 gF, respectively. On spontaneous rhythmic contractions, the extracts EE, EB and EH, but also Ar, induced a decrease effect on the amplitudes of 62.50 ± 7.21, 97.77 ± 27.33, 33.33 ± 22.22 and 50 ± 0.00%, respectively. This positive inotropic effect was comparable to that obtained with aqueous extracts from the leaves of the plant [15,16].

At the dose of 10 µg/mL, the sub-extract EC only showed myorelaxant activity with a decrease in the amplitude of rhythmic contractions of 69.23 ± 7.60%, and a non-significant decrease (\( p > 0.05 \)) of the basic tone (Figure S9). This activity paved the way for the chromatographic fractionation of this fraction. Compounds 1-3, responsible for the activity, have been isolated. The structures of these compounds were essentially established on the basis of spectroscopic data (MS, UV, IR, NMR). Compounds 2 and 3, already known, were identified as 29-nor-1,2,3,4,5,10-dehydro-3,15a,20β-trihydroxy-16α-acetyl-11,22-dioxo-cucurbit-23-ene 2-O-β-D-glucopyranoside (2) and 29-nor-1,2,3,4,5,10-dehydro-3,15a,20β-trihydroxy-16α-acetyl-3,11,22-trioxo-cucurbit-4,23-diene 3-O-β-D-glucopyranoside (3), recently isolated from the leaves of the plant [12].

Compound 1, isolated as orange-colored crystals, gave positive reactions with Fast Blue B and the Liebermann reagent, respectively indicative of phenolic and triterpene compounds. Its molecular formula, C₃₈H₄₅O₉, was deduced from its HRESIMS [M+H]⁺ peak at m/z 543.2946 (Calcd for C₃₈H₄₅O₉ 543.2958). This mass differed from 2 by the loss of a C₃H₅O₅ unit (m/z 162.0528) corresponding to its osidic unit, tentatively identifying 1 as its aglycone. The ¹H NMR spectrum of 1 (Table 1) was in agreement with this hypothesis. It highlighted signals of aromatic proton at \( δ_H 6.29 \) (s), trans-disposed ethylenic protons resonating at \( δ_H 6.96 \) (1H, dd, \( J = 15.5 \) and 7.1 Hz) and at \( δ_H 6.70 \) (1H, d, \( J = 15.5 \) Hz), five methines at \( δ_H 5.39 \) (1H, t, \( J = 7.7 \) Hz), 4.09 (1H, d, \( J = 8.0 \) Hz), \( δ_H 2.63 \) (1H, d, \( J = 7.3 \) Hz), 2.50 (1H, d, \( J = 7.1 \) Hz) and 2.30 (1H, d, \( J = 3.1 \) Hz), three diastereotropic pairs of methyl protons at \( δ_H 2.70 \) (1H, H₂, d, \( J = 14.0 \) Hz)/ 3.05 (1H, H₂, d, \( J = 14.0 \) Hz), at \( δ_H 2.59 \) (1H, H₂, m)/2.82 (1H, H₂, dd, \( J = 18.7 \) and 9.4 Hz) and at \( δ_H 2.20 \) (1H, H₂, m)/2.53 (1H, H₂, m), and seven methyl groups at \( δ_H 2.09 \) (3H, s), 1.90 (3H, s), 1.38 (3H, s), 1.35 (3H, s), 1.09 (6H, d, \( J = 6.5 \)), 1.06 (3H, s) and 0.97 (3H, s). The ¹³C NMR spectrum of 1 (Table 1), in agreement with all these assignments, highlighted 31 carbon signals whose chemical shifts were very similar to those of the genin skeleton of 2 except for the signals at \( δ 110.3 \), 142.9 and 143.8 ppm which hinted a shielding of carbons C-1, C-2 and C-3 following the loss of the osidic unit in 2 as was the case for most of these cucurbitacins with aromatic A nucleus [17-19]. The HSQC, COSY and HMBC spectra data confirmed this tentative structure. On the COSY spectrum, correlations were observed between signals of methylene H-6 (\( δ_H 2.59 \) (H-6a)/2.82 (H-6b)) and those of methylene H-7 (\( δ_H 2.20 \) (H-7a)/2.52 (H-7b), this latter correlating with the methine detected at \( δ_H 2.30 \) (H-8). We also observed a second spin system consisting of \( δ_H 5.39 \) (H-16), 4.09 (H-15) and 2.62 (H-17) ppm. In addition, the methine signal at \( δ_H 2.50 \) ppm (H-25) also showed a COSY crosspeak with the methyl group resonating at \( δ_H 1.09 \) ppm (H-26/H-27) and 6.96 (H-24), the latter also correlating with that at \( δ_H 6.70 \) ppm (H-23). The thorough analysis of the HMBC spectrum (Figure 2), allowed to assign all quaternary carbons, thus confirming the proposed structure. The stereochemistry of compound 1 was finally established by a careful analysis of the data of its NOESY spectrum (Figure 2), but also by analogy with 2. On the basis of these spectral data, the structure of compound 1 was established as 29-nor-1,2,3,4,5,10-dehydro-3,15α,20β-trihydroxy-16α-acetyl-11,12-dioxocucurbit-23-ene (Figure 1). To our knowledge, the cucurbitacins identified to date in Euphorbiaceae plants, consisting of 23,24-dihydro cucurbitacin A and cucurbitacins A, B, E and L [12,13]. Cucurbitacins obtained from *Mareya micrantha* display an atypical oxidation at C-15, rarely observed in this group [17,20]. As a result, we proposed for compound 1 the trivial name of maremicranthine A.

Norcucurbitacins 1-3 were then evaluated in a single dose of 10 µg/mL to establish their effects on the activity of duodenal smooth muscle. The obtained results (Figure S10) showed that all molecules caused some decreases in the amplitude not only of spontaneous rhythmic contractions but also of the basic tone. However, at this dose, these molecules had no significant effect (\( p > 0.05 \)) on the frequency of rhythmic contractions.

Compound 2, the majority compound of the EC sub-extract revealed the most important inhibition effects on the amplitude of the rhythmic contractions and on the basic tone of the isolated intestine of rabbit (Figure S11), and was selected for the rest of the study. When administrated at increasing doses of 10⁻² to 10⁻¹ µg/mL, compound 2 led to a dose-dependent decrease in the amplitude of spontaneous rhythmic contractions of the isolated duodenum fragments of rabbit (Figure S12). At doses lower than 10⁻² µg/mL, no significant effect could be reported even on intestinal contractions, and above 10⁻¹ µg/mL, these effects remained constant. The maximum myorelaxant effects obtained for the 10⁻¹ µg/mL dose corresponded to a reduction in the amplitude of the contractions of 89.23 ± 0.08% (\( p<0.01 \)). Figure 3 shows the decrease in the amplitude of the rhythmic contractions of the duodenum as a function of the concentration of compound 2 which determined, for this molecule, an IC₅₀ value of the intestinal rhythmic contractions of 26 µg/mL (36.9 µM).

In order to understand the mode of action of 2, recordings were made in calcium-free media with or without the EDTA or EGTA. Indeed, calcium, as an intracellular messenger, steps in the activation of contractile proteins of smooth muscles [21,22], while EDTA and EGTA are substances able to
chelate extracellular and intracellular calcium. We have found that in a physiological environment lacking calcium, very slight rhythmic contractions of the intestine appeared. After adding compound 2 at a dose of 10 µg/mL, the rhythmic contractions were completely inhibited with a relatively small drop in basic tone (Figure S13-C). Furthermore, in media containing one of the calcium chelators, there was either a quasi-inhibition of the rhythmic contractions of the duodenum (case of EDTA), and the addition of compound 2 led to a total inhibition of the intestinal rhythmic contractions (Figure S13-D), or a total disappearance of duodenal rhythmic contractions (case of EGTA). In this case, adding 2 had no effect (Figure S13-E). These results showed that in an extracellular medium poor in calcium ions or lacking of calcium, compound 2 had muscle relaxant effects reinforced by total inhibition of rhythmic contractions. This could be explained by the fact that this molecule inhibited extracellular and/or intracellular Ca$^{2+}$. Therefore, it could act by preventing the entry of calcium into the cell by blocking the calcium channel as demonstrated by Fleckenstein. Given that compound 2 no longer had an effect on intestinal contractions in the presence of EGTA, the obtained data suggested that this molecule relaxed duodenal smooth muscle of rabbits by blocking both extracellular and intracellular calcium.

The myorelaxant properties of the sub-extract EC and its molecules on the duodenal smooth muscle justified the inhibitory effects on the motor skills of this muscle. These data confirmed the traditional use of the leaves in the management of gastrointestinal disorders.

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**Fig 1:** Compounds 1–3 isolated from leaves of *Mareya micrantha*

**Fig 2:** Important 2D correlations of compound 1

**Fig 3:** Decrease in the amplitude of rabbit’s intestinal contractions according to compound 2 concentration (n = 3; ** p < 0.01; *** p < 0.001; compared to control recordings)
Data of compounds 2 and 3 [12]

29-Nor-1,2,3,4,5,10-dehydro-3,15α, 20β-tri-hydroxy-16α-acetyl-11,22-dioxo-cucurbita-23-ene 2-O-β-D-gluco- pyranoside (2): Whitish crystalline powder; $[\alpha]_{D}^{25} = -10.8$ ($c$ 1.42, MeOH); UV $\lambda_{max}$ (nm): 280.6, 231.5; IR $\nu_{max}$ (cm$^{-1}$): 3313, 3295, 2925, 1662, 1615, 1484, 1243, 1019; $^1$H NMR (400 MHz, CD$_2$OD): 6.97 (1H, ddd, J = 15.4 and 7.1 Hz, H-24), 6.73 (1H, s, H-1), 6.68 (1H, d, J = 15.4 Hz, H-23), 5.40 (1H, t, J = 7.7 Hz, H-16), 4.60 (1H, d, J = 7.3 Hz, H-3), 4.10 (1H, d, J = 8.2 Hz, H-15), 4.01 (1H, dd, J = 12.1 and 2.3 Hz, H-6b), 3.89 (1H, dd, J = 12.1 and 4.3 Hz, H-6a), 3.55 (1H, t, J = 4.3 Hz, H-4), 3.50 (1H, t, J = 4.1 Hz, H-2), 3.48 (1H, t, J = 4.4 Hz, H-3), 3.37 (1H, m, H-5), 3.05 (1H, d, J = 14.4 Hz, H-12b), 2.86 (1H, dd, J = 18.6 et 9.2 Hz, H-6b), 2.71 (1H, d, J = 14.3 Hz, H-12a), 2.66 (1H, m, H-6a), 2.63 (1H, d, J = 7.3 Hz, H-17), 2.56 (1H, m, H-7b), 2.52 (1H, m, H-25), 2.34 (1H, d, J = 6.3 Hz, H-8), 2.23 (1H, m, H-7a), 2.12 (3H, s, H-28), 1.90 (3H, s, H-18), 1.385 (3H, s, H-21), 1.376 (1H, s, H-19), 1.09 (6H, d, J = 6.5 Hz, H-26/H-27), 0.94 (3H, s, H-29); $^1$C NMR (100 MHz, CD$_2$OD): 216.4 (C-1), 204.4 (C-22), 172.1 (C-1’), 156.8 (C-24), 144.9 (C-3), 144.6 (C-2), 131.4 (C-10), 129.8 (C-5), 124.9 (C-4), 122.7 (C-23), 113.4 (C-1), 105.9 (C-1’), 79.5 (C-20), 78.1 (C-5’), 77.7 (C-3’), 75.6 (C-1’), 74.8 (C-2’), 74.0 (C-16), 70.9 (C-4’), 62.1 (C-6’), 54.9 (C-17), 52.2 (C-9), 51.9 (C-12), 51.7 (C-14), 48.1 (C-13), 43.9 (C-8), 32.6 (C-25), 29.2 (C-19), 24.9 (C-6), 24.5 (C-21), 21.75 (C-27), 21.72 (C-26), 20.9 (C-19), 20.6 (18), 19.4 (C-7), 12.3 (C-29), 11.5 (C-28); HR-ESIMS m/z 705.3485 [M + H]$^+$ (calfed for C$_{37}$H$_{59}$O$_{13}$, 705.3486).

Nor-2β, 15α, 20β-tri-hydroxy-16α-acetyl-3,11,22-trioxy-cucurbita-4,23-diene 2-O-β-D-glucopyranoside (3): Whitish amorphous powder; $[\alpha]_{D}^{25} = -25.2$ ($c$ 0.52, MeOH); UV $\lambda_{max}$ (nm): 245: IR $\nu_{max}$ (cm$^{-1}$): 3321, 2929, 1679, 1615, 1246, 1027; $^1$H NMR (400 MHz, CD$_2$OD): 7.00 (1H, dd, J = 15.4 and 7.0 Hz, H-24), 6.76 (1H, d, J = 15.4 Hz, H-23), 5.42 (1H, t, J = 7.6 Hz, H-16), 4.52 (1H, d, J = 7.6 Hz, H-1’), 4.43 (1H, dd, J = 14.4 and 5.4 Hz, H-2), 4.11 (1H, d, J = 8.1 Hz, H-15), 3.92 (1H, d, J = 6.0 Hz, H-6a’), 3.69 (1H, dd, J = 11.7 and 6.0 Hz, H-6b’), 3.42 (1H, m, H-3’), 3.31 (1H, m, H-2’), 3.30 (2H, m, H-4’/H-5’), 3.27 (1H, m, H-10), 3.21 (1H, d, J = 16.6 Hz, H-12b), 2.72 (2H, m, H-12a/17), 2.62 (1H, m, H-6), 2.57 (1H, m, H-25), 2.28 (1H, m, H-8), 2.20 (1H, m, H-1b), 2.17 (1H, m, H-7b), 2.00 (1H, m, H-7a), 1.94 (3H, m, H-2’), 1.85 (1H, m, H-1a), 1.82 (3H, s, H-28), 1.42 (3H, s, H-21), 1.39 (1H, s, H-1a), 1.14 (6H, d, J = 6.7 et 2.3 Hz, H-26/H-27), 1.13 (3H, s, H-18), 1.10 (3H, s, H-19), 1.11 (3H, s, H-29); $^1$C NMR (100 MHz, CD$_2$OD): 215.9 (C-11), 204.5 (C-22), 200.1 (C-3), 172.0 (C-1’), 159.9 (C-5), 156.9 (C-24), 132.0 (C-4), 122.1 (C-23), 105.1 (C-1’), 80.1 (C-2), 79.6 (C-20), 78.2 (C-5’), 78.1 (C-3’), 76.0 (C-15), 75.7 (C-2’), 73.8 (C-16), 71.5 (C-4’), 62.9 (C-6’), 55.9 (C-17), 52.4 (C-12), 51.5 (C-14), 50.1 (C-9), 46.3 (C-13), 45.9 (C-8), 41.4 (C-10), 33.2 (C-25), 32.7 (C-1), 28.9 (C-6), 24.5 (C-21), 23.3 (19), 21.8 (C-26/C-27), 21.6 (C-27), 21.1 (C-18), 20.9 (C-2’), 13.4 (C-29), 11.2 (C-28); HR-ESIMS m/z 707.3647 [M + H]$^+$ (calfed for C$_{37}$H$_{59}$O$_{13}$, 707.3643)
Fig S1: $^1$H NMR (400 MHz in CD$_3$OD) spectrum of compound 1

Fig S2: $^{13}$C NMR (100 MHz in CD$_3$OD) spectrum of compound 1

Fig S3: $^1$H-$^1$H COSY spectrum of compound 1
Fig S4: $^1$H-$^{13}$C HSQC spectrum of compound 1

Fig S5: $^1$H-$^{13}$C HMBC spectrum of compound 1

Fig S6: $^1$H-$^1$H NOESY spectrum of compound 1
Fig S7: ESI-MS spectrum of compound 1

Fig S8: Pharmacological effects of the extracts on the contractile activity of the isolated rabbit’s duodenum

A: Normal recording of intestinal contractions,

B to G: Normal recording (before arrows) and effect of extracts at a concentration of 10 µg/mL (after arrows) on contractions of isolated rabbit’s duodenum
Fig S9: Effects of extracts in the amplitude of rhythmic contractions (A) and on the basal tone (B) of isolated rabbit’s duodenum (n = 3; * p < 0.05; ** p < 0.01; *** p < 0.001 compared to normal recordings)

Fig S10: Pharmacological effects of the isolated compounds (1–3) on the contractions of the isolated rabbit’s duodenum A to C: Normal recording (before arrows) and effects of the isolated molecules (after arrows) on contractions of isolated rabbit’s duodenum.
Fig S11: Effects of isolated molecules in the amplitude of rhythmic contractions (A) and on the basal tone (B) of isolated rabbit’s duodenum (n = 3; * p < 0.05; ** p < 0.01; *** p < 0.001 compared to the effects of isolated molecules)

Fig S12: Pharmacologic effects of compound 2 on contractions of isolated rabbit’s duodenum
A: Normal recording of intestinal contractions,
B to F: Normal recording (before arrows) and effects of compound 2 (after arrows) on contractions of isolated rabbit’s duodenum
**Fig S13:** Effects of compound 2 on contractions of isolated rabbit’s duodenum in physiological solution devoid of calcium, with or without EDTA or EGTA

A: Normal recording of intestinal contractions

B: Normal recording (before arrow) and effects of compound 2 at a concentration of 10 µg/mL on intestinal contractions.

C to E: Control recording (before arrows) and effects of compound 2 at a concentration of 10 µg/mL on intestinal contractions (after arrows) in 0 Ca$^{2+}$ (C), 0 Ca$^{2+}$ + EDTA $10^{-5}$ mg/ml (D) and 0 Ca$^{2+}$ + EGTA $10^{-5}$ mg/ml (E) in physiological solution.

### 4. Conclusion

*Mareya micrantha* is an Ivorian medicinal plant which is known for its myostimulant activities on the intestinal smooth muscle. If the crude ethanolic extract EE of the leaves and some of its sub-extracts (EH, EA, EB and Ar) confirmed the known myostimulant activity of the plant on the isolated duodenum of rabbit, this was not the case for the chloromethylene sub-extract EC which only showed myorelaxant activity. From this sub-extract, three triterpenoids type 29-norcucurbitacin were isolated and characterized, including one previously undescribed one. The peculiarity of these cucurbitacins was their singularly unusual oxidation to C-15. In addition, these molecules reduced rhythmic contractions and lowered the basic tone. This myorelaxant activity on the intestinal muscle, initially attributed to their fraction, could result in the blocking of both extracellular and intracellular calcium.

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