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Isoquinoline alkaloids from *Monodora crispata* Eng. and Diels, *Monodora myristica* Gaertn. and *Monodora tenuifolia* Benth. (Annonaceae)

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ABSTRACT: Three compounds namely (-)-xylopinidine (**1**), (-)-*N*-methylarmeparvine (**2**) and (+)-menispermine (**3**) were respectively isolated for the first time from the leaves of *Monodora crispata*, *Monodora myristica* and *Monodora tenuifolia* (Annonaceae). Their structures were established according to their spectral data (NMR, MS, IR and UV).

KEY WORDS: *Monodora, crispata, tenuifolia*, aporphine, benzyltetrahydroisoquinoline, Annonaceae

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I. INTRODUCTION

Monodora crispata (Eng. and Diels), *Monodora myristica* Gaertn. and *Monodora tenuifolia* Benth. belong to the Annonaceae's family. The genus *Monodora* comprises sixteen (16) recognized species, all being small trees confining to tropical eastern and western Africa forests[**1**,**2**,**3**,**4**,**5**].This genus is a sister clade of *Isolona* [**6**]. It is now regrouped along with eight (8) others in the monophyletic Monodoreae Baill. tribe, within the large Annonoideae Raf. Subfamily [**1**]. *Monodora* is largely used in traditional medicine to treat various diseases. To the best of our knowledge, there is no use for *M. crispata* in traditional medicine in Côte d'Ivoire. However, the stem bark of *M. myristica* is used in the treatment of abdominal pain, fever, headache, hemorrhoids, febrile diseases, constipation and Buruli ulcer [**7**]. Recent studies have shown that the *Monodora tenuifolia* seed extract possesses several biological effects such as antidiarrhoeal, antioxidant and antimicrobial activities. In traditional medicine practice, the plant is used mainly to treat dysentery, diarrhoea, dermatitis and toothache. It is also used as vermifuge. The reported antioxidant potential of the plant makes it important in the management of stress induced conditions such as depression [**8**].

Concerning its chemical composition, *Monodora* genus is a reach source of bioactive compounds like alkaloids[**6**,**9**,**10**], sesquiterpenes, monoterpenes, diterpenes, indole[**11**] and essential oils[**12**,**13**,**14**]. *M. crispata* is proposed as possibly endangered species. Eleven aporphine alkaloids, (-)-mocrispatine, (-)-pallidine, (-) *N*-méthylarmépavine, (+)-magnoflorine, (+)-menispermine, (-)-anolobine, (+)-anolobine, (+)-listeferine, (+)-réoméroline, (+)-laurotetanine and (+)-corydine were isolated and identified from the leaves of *M. crispata*. The essential oil of the leaves of *M. myristica* has been studied and showed as major components: *β*-caryophyllene, *α*-humulene, α-copaene, α-pinene, α-cubebene and γ-cadinene. As for the fruits, they revealed the presence of α-phellandrene, p-cymene, α-pinene, myrcene, germacreneD-4-ol, spathulenol, limonene, β-phellandrene, δ-cadinene and α-thujene. Finally, the seeds contained α-phellandrene, α-pinene, myrcene and limonene. α-

phellandrene, p-cymene, α-pinene and limonene isolated from this plant have shown interesting antimicrobial activity [**15**].

In *M. tenuifolia*, laurelliptin (norisoboldine) appeared as the predominant alkaloid isolated in the leaves [**9**].

We report here, the isolation and characterization of three known isoquinoline alkaloids from the leaves of *M. crispata*, *M. myristica* and *M. tenuifolia.* To our knowledge, they were isolated for the first time from these species.

II. MATERIAL AND METHODS

2.1. General

The NMR spectra were recorded on a Brüker Advance-300 operating at 300 MHz, using TMS as internal standard. Chemical shifts were quoted in *d* ppm and coupling constant *J* was measured in Hertz (Hz). One-dimensional ^{1}H and ^{13}C spectra were acquired under standard conditions. Currently, $^{1}H–^{1}H$ homonuclear (COSY, NOESY) and $^{1}H–^{13}C$ heteronuclear (HSQC, HMBC) correlation techniques were routinely applied in field of constitutional analysis. These techniques were recorded on a Brücker Avance-400 operating at 400 MHz. Column chromatography was performed on silica gel (Kieselgel 60, particle size 0.040–0.063 mm) and Sephadex®LH-20. TLC was run on silica gel pre-coated glass plates (Merck silica gel 60 F254). Spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and phosphomolibdic acid. This operation was followed by a heating.

ESIMS were obtained with ITQ 900 spectrometer using an Agilent DB-5HT (30 x0.32 x 0.1) column. Gas chromatography was performed on TRACE GC ULTRA Thermo Scientific instrument. HR-ESIMS were run on a TOF LCT Premier WATERS coupling with HPLC Alliance 2695 (Waters) and also with microTOFqBrüker. IR spectra were measured on a Brüker Vector 22.

Polarimeter Optical rotations were recorded on an Optical Activity PolAAr 32. Polarimeter using a sample concentration of 10 mg/ml, unless otherwise specified.

2.2. Plant material

The leaves of *M.crispata*, *M. myristica* and *M. tenuifolia* were collected in August 2015 in Adiopodoumé (South of Côte d'Ivoire). They were identified by Pr. IpouIpou Joseph (Centre National de Floristique-Université Félix Houphouët-Boigny). A voucher specimen (n° MC-KABLAN-Diapodoumé2015-2; n° MM-KABLAN-Diapodoumé2015-2 and n° MT-KABLAN-Diapodoumé2015-2) is deposited at the Herbarium of the Botanic Laboratory (Université Félix Houphouët-Boigny).

2.3. Isolation

Air-dried pulverized leaves of *M. crispata* (2000 g) were three times defatted with petroleum ether and successively extracted with CH_2Cl_2, AcOEt and MeOH. The collected fractions were evaporated under reduced pressure to yield 9.9 g of petroleum ether, 7.6 g of CH_2Cl_2, 5.8g of AcOEt and 3.0 g of MeOH extracts. The MeOH extract was fractionated over silica gel column chromatography, eluting with hexane-methanol gradient systems. Twelve fractions (F-1 to F-12) were obtained. Fraction F-4 was purified using repeated Sephadex® LH-20 [CH_2Cl_2: MeOH (1:1)] and column chromatography on silica gel [AcOEt: MeOH (95:5)] to yield 6.2 mg of compound **1**(Fig.1).

The leaves of *M. myristica* (1500 g) were submitted to similar extraction steps, yielding petroleum ether (9.9 g), CH_2Cl_2 (5.8 g), AcOEt (3.2g) and MeOH (2.1 g) extracts. The MeOH extract was chromatographed over silica gel column chromatography, eluting with hexane-methanol gradient systems. Twenty fractions (F-1' to F-20') were obtained. Fraction F-15' was purified using repeated Sephadex® LH-20 (CH_2Cl_2: MeOH (2:1)) and column chromatography on silica gel (AcOEt: MeOH (90:10)), to yield 6.2 mg of compound **2** (Fig.1).

The leaves of *M. tenuifolia* (1700 g) were submitted to similar extraction steps, yielding petroleum ether (12.7 g), CH_2Cl_2 (6.1 g), AcOEt (3.1 g) and MeOH (1.9 g) extracts. The MeOH extract was fractionated on silica gel column chromatography, eluting with AcOEt-methanol gradient systems. Seven fractions (F-1'' to F-7'') were obtained. Fraction F-6'' was purified on column chromatography of Sephadex® LH-20 [CH_2Cl_2/MeOH (2:1) and CH_2Cl_2/MeOH (1:1)] to yield 7.3 mg of compound **3** (Fig.1).

2.4. Identification of compounds 1, 2 and 3

(-)-xylopinidine (**1**): Amorphous brown solid; ^{1}H and ^{13}C NMR (400 MHz) data in Table 1, $[\alpha]_D^{24}$ (O) = -21.2; c 6.8 mg/ml in MeOH; IR ($CHCl_3$): υ_{max} (cm^{-1}) = 2921 ; 1518 ; 1261; UV (MeOH): λ_{max} (nm) = 221.4; 268.5 ; 301.6; ESI-MS (*m/z*) = 344.0 $[M]^+$.

(-)-*N*-méthylarmeparvine (**2**): Amorphous brown solid; ^{1}H and ^{13}C NMR (400 MHz) data in table 1, $[\alpha]_D^{21}$ (O) = -32.0; c 6.0 mg/ml in MeOH; IR ($CHCl_3$): υ_{max} (cm^{-1}) = 2920 ; 2336; 1260 ; 718; UV (MeOH): λ_{max} (nm)

= 221.4; 268.5 ; 301.6; HR-ESI-MS (*m/z*) = 328.1946 $[M]^+$ (molecular formula $C_{20}H_{26}NO_3$; calc. 328.1947 mDa = 0.1), SMIE : *m/z* (%) 328 $[M]^+$(17%), 283$[M\text{-}H\text{-}N(CH_3)_2]^+$(22%), 268$[M\text{-}H\text{-}N(CH_3)_2\text{-}CH_3]^+$(13%), 252$[M\text{-}H\text{-}N(CH_3)_2\text{-}CH_3\text{-}OH]^+$(22%), 237$[M\text{-}H\text{-}N(CH_3)_2\text{-}2CH_3\text{-}OH]^+$(8%).

(+)-menispermine (**3**): Amorphous brown solid; ^1H and ^{13}C NMR (400 MHz) data in table 2, $[\alpha]_D^{21}$ (O) = +22.0; c 0.67 mg/ml in MeOH; IR ($CHCl_3$): υ_{max} (cm^{-1}) = 2839 ; 1604 ; 1277 ; 1105; 705; UV (MeOH): λ_{max} (nm) = 221.4; 265.0 ; 304.0; HR-ESI-MS (*m/z*) = 356.1862 $[M]^+$ (molecular formula $C_{21}H_{26}NO_4$; calc. 356.1864 mDa = 0.2), SMIE : *m/z* (%) 356 $[M]^+$(16%), 325$[M\text{-}OCH_3]^+$(5%), 311$[M\text{-}H\text{-}N(CH_3)_2]^+$(92%), 296$[M\text{-}CH_3\text{-}H\text{-}N(CH_3)_2]^+$(100%), 279$[M\text{-}CH_3\text{-}H\text{-}N(CH_3)_2 - OH]^+$(25%).

III. RESULT AND DISCUSSION

Compound **1** was isolated as colorless amorphous powder. Its ESIMS showed a peak at *m/z* 344.0 $[M]^+$ corresponding to the formula $C_{20}H_{26}NO_4$. The UV, ^1H and ^{13}C NMR spectra of **1** (Table 1) resembled those of (+)-tembetarine, suggesting that **1** was a 6,7,12,13-tetrasubstituted tetrahydrobenzylisoquinoline alkaloid. Measuring its various 2D-NMR spectra provided further support for the structure of this compound. All methyl, methylene and methine protons and carbons were assigned from analysis of the ^1H-^1H COSY, HSQC and HMBC spectra. The presence of two singlet protons (δ 6.83 and δ 5.96 ppm) (Table 1) and an HMBC correlation from one of the protons (δ_H 5.96 ppm) to C-1 (δ_C 74.0 ppm), and from another proton (δ_C 6.83 ppm) to C-4 (δ_C 24.4 ppm) revealed that they were bound to C-5 and C-8, respectively. An HMBC correlation was also observed between H-9α (δ 2.84 ppm) and two aromatic carbons (δ 117.8 and δ 122.4 ppm) (Fig. 1). The presence of three aromatic protons combined with C-11, C-14 and C-15 was noted having an ABX spin system. Therefore, two methoxyl and two hydroxyl groups are substituents at C-6, C-7, C-12 and C-13. From the HMBC and NOESY experiments, signals at δ_H 3.86 and δ 3.85 ppm were assigned to two methoxyl groups on C-7 and C-13, respectively; hence two hydroxyl groups must bind to C-6 and C-12. Accordingly, the structure of compound **1** was elucidated as that of (-)-xylopinidine (**1**) (Fig. 1). This was confirmed by its optical rotation (sodium D line) which was negative, indicating that it has either a very optical rotation. Its physical and spectral data are consistent to those reported by literature [**16**]. To our knowledge, it was isolated for the first time from *M. crispata*.

Compound **2** was isolated as an amorphous brown powder. Its UV spectrum showed maximum absorption bands at λ_{max} 221.4, 268.5 and 301.6 nm. HR-ESI-MS showed the pseudo-molecular ion fragment $[M]^+$ at *m/z* 328.1946. So, its molecular formula was deduced to be $C_{20}H_{26}NO_3$ (calc. 328.1947 mDa = 0.1).The ^{13}C NMR spectrum of **2** (Table 1) exhibited characteristic signals at δ_C 113.3 ppm (C-5), 112.4 ppm (C-8), 38.2 ppm (C-9) and 74.3 ppm (C-1) corresponding to a 6,7,13-trisubstituted benzyltetrahydroisoquinoline alkaloid [**6**,**10**,**17**]. The significant differences between compounds **1** and **2** were observed on the ring C. The ^1H NMR spectrum of **2** (Table 1) exhibited characteristic signals of four aromatic protons at δ_H 6.75 ppm (H-11, H-15) and 6.87 ppm (H-12, H-14). The HMBC correlations confirmed the position of two methoxyl groups on the aromatic ring B and one hydroxyl group on the aromatic ring C (Fig. 1).In consequence, compound **2** was identified as *N*,*N*-dimethylatedbenzyltetrahydroisoquinoline [**6**]. The absolute configuration of the asymmetric carbon C-1 was also determined according to its $[\alpha]^D$ value ($[\alpha]_D^{21}$(O)= -32.0) to be *R* form. The compound **2** was identified as (-)-*N*-methylarmeparvine (Fig. 1). It was already isolated from *M. crispate* and *M. brevipes* [**6**].

Compound **3** was isolated as an amorphous brown powder. Its HR-ES-MS spectrum exhibited pseudo-molecular ion fragment $[M]^+$ at *m/z* 356.1862 $[M]^+$; calc. 356.1864 mDa = 0.2). Therefore, the molecular formula was determined to be $C_{21}H_{26}NO_4$. The ^1H and ^{13}C spectra of **3** (Table 2) were characteristic of 1,2,10,11-tetrasubstitutedaporphinealkaloids [**16**].The ^1H NMR spectrum showed two adjacent aromatic protons at δ_H7.27ppm (1H, *d*, J= 8.4 Hz) and 7.12 ppm (1H, *d*, J= 8.4 Hz), and one isolated aromatic proton at δ_H 7.00 ppm(1H, *s*), which were ascribed to the hydrogens of A and D rings of the aporphine nucleus. This spectrum also exhibited characteristic signals of three methoxyl groups at δ_H3.71ppm (3H, s), 3.93 ppm (3H, s) and 3.93ppm (3H, s) attributed respectively to methoxy groups at positions 1, 2 and 10. The signals at δ_H 3.77 ppm (3H, s), 2.82 ppm (3H, s) and 4.52 ppm (H-6a) indicated the presence of *N*,*N*-dimethyl groups in the molecule. The NMR data were similar to those of magnoflorine, except for the methoxyl group at position C-1 and the hydroxyl group at position C-11 for compound **3**. So, compound **3** was identified as a 1,2,10,11-substituted aporphine alkaloid[**6**]; precisely a *N*,*N*-dimethyl aporphine alkaloid. The ^{13}C-NMR data together with a HSQC experiment indicated the presence of twenty one carbons in **3**, comprising twelve aromatic carbons, two methyl groups,three methoxyl groups, three methylenes and one methine. The absolute configuration of the asymmetric carbon C-6a was also determined according to its $[\alpha]_D$ value ($[\alpha]_D^{21}$ (O)= +22.0) to be ***S*** form. The compound **3** was identified as (+)-menispermine (Fig. 1). It was already isolated in *Xylopia parviflora* [**16**], *Nandina domestica* [**18**] and *Anamirta cocculus*[**19**]. To our knowledge, it was isolated for the first time from *Monodora tenuifolia*.

IV. CONCLUSION

The phytochemical investigation of the leaves from *M. crispata*, *M. myristica* and *M. tenuifolia* leaded to the isolation and identification of two *N,N*-dimethylatedbenzyltetrahydroisoquinoline and one aporphine alkaloid derivative. Their complete structures were established according to their spectroscopic (^{1}H and ^{13}C NMR, COSY, HSQC, HMBC, UV and IR) and spectrometric (ESI-MS) data. The *N,N*-dimethylatedbenzyltetrahydroisoquinoline derivatives were identified as (-)-xylopinidine (**1**) and (-)-*N*-methylarmeparvine (**2**) . The aporphine alkaloid was identified as (+)-menispermine (**3**). Their structures were in agreement with those reported by literature.

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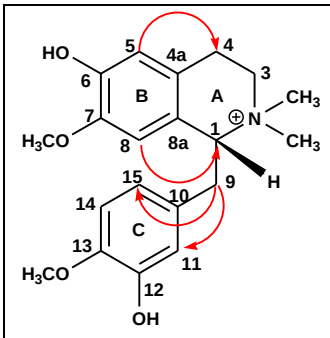
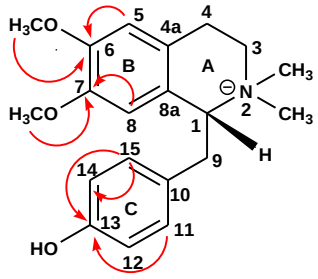
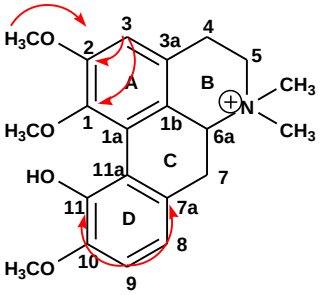


(-)-xylopinidine (**1**)



(-)-*N*-methylarmeparvine (**2**)



(+)-menispermine (**3**)

HMBC Correlations ( )

Figure 1: Isoquinoline alkaloids isolated from *M. crispata*, *M. myristica* and *M. tenuifolia* (Annonaceae).

Table 1: ^{1}H and ^{13}C NMR spectral data of xylopinidine (1) and *N*-méthylarmeparvine (2)

N°	1		2	
	^{13}C	^{1}H	^{13}C	^{1}H
1	74.0	4.67, 1H,*m*	74.3	4.61,1H, *dd* (*J* = 10.8, 3.2)
3	56.1	3.61,1H$_a$, *m*	55.7	3.67,1H$_a$, *dd* (*J* = 12.4, 4.0)
	56.1	3.77,1H$_b$,*m*	55.7	3.84,1H$_b$, *m*
4	24.4	3.16,1H$_a$, *m*	24.4	3.24 ; 1H$_a$; *m*
	24.4	3.16,1H$_b$,*m*	24.4	3.25 ; 1H$_b$; *m*
4a	120.5	-	121.8	-
5	116.2	6.83,1H,*s*	113.3	6.86,1H,*s*
6	147.7	-	150.9	-
7	146.2	-	148.3	-
8	112.7	5.96,1H,*s*	112.4	5.71,1H,*s*
8a	123.9	-	123.1	-
9α	38.7	2.84,1Hα,*m*	38.2	2.89,1Hα ,*t* (*J* = 12.4)
9β	38.7	3.58,1Hβ,*m*	38.2	3.67,1Hβ,*dt* (*J* = 12.4,4.0)
10	129.3	-	127.1	-
11	117.8	6.57,1H,*s*	132.5	6.75,1H, *d* (*J* = 8.4)
12	148.4	-	116.6	6.87,1H, *d* (*J* = 8.4)
13	149.7	-	158.1	-
14	112.4	6.84,1H,*d* (*J* = 8.4)	116.6	6.87,1H, *d* (*J* = 8.4)
15	122.4	6.51,1H,*dd* (*J* = 8.4,2.0)	132.5	6.75,1H, *d* (*J* = 8.4)
O-CH$_3$(C-6)	-	-	56.4	3.84,3H,*s*
O-CH$_3$(C-7)	56.4	3.86,3H,*s*	55.9	3.40,3H,*s*
O-CH$_3$(C-13)	56.4	3.85,3H,*s*	-	-
N-(CH$_3$)$_1$	52.8	3.43,3H,*s*	51.4	3.17,3H,*s*
N-(CH$_3$)$_2$	51.6	3.04,3H,*s*	53.0	3.46,3H,*s*

Table 2: ^{1}H and ^{13}C NMR spectral data of (+)-menispermine (3)

N°	1	
	^{13}C	^{1}H
1	144.8	-
1a	122.0	-
1b	121.2	-
2	151.9	-
3	112.0	7.00, 1H, *s*
3a	120.4	-
4	24.7	3.08,1H$_\alpha$,*d* (*J* = 3.6)
	24.7	3.41,1H$_\beta$,*d* (*J* = 3.6)
5	62.7	3.77,1H$_\alpha$,*m*
	62.7	3.83,1H$_\beta$,*m*
6a	70.6	4.52,1H,*dd* (*J* = 14.8;4.0)
7	31.4	2.82, 1H$_\alpha$,*m*
	31.4	3.43, 1H$_\beta$,*m*
7a	127.4	-
8	125.7	7.27,1H,*d* (*J* = 8.4)
9	113.4	7.12,1H,*d* (*J* = 8.4)
10	154.2	-
11	146.0	-
11a	126.0	-
O-CH$_3$(C-1)	62.2	3.71 ,3H,*s*
O-CH$_3$(C-2)	56.6	3.93,3H,*s*
O-CH$_3$(C-10)	56.6	3.93,3H,*s*
N-(CH$_3$)$_1$	43.9	3.05,3H,*s*
N-(CH$_3$)$_2$	54.3	3.37,3H,*s*

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