Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2021; 10(4): 66-69
Received: 15-05-2021
Accepted: 22-06-2021

Seri Seri Chardin
Maitre-Assistant 1 Laboratoire de constitution et réaction de la matière (LCRM), Unité de Formation et de Recherches des sciences des Structures de la Matière et de Technologie (UFR-SSMT), Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Dade Joël Maxime Eric
Maitre-Assistant 1 Laboratoire de constitution et réaction de la matière (LCRM), Unité de Formation et de Recherches des sciences des Structures de la Matière et de Technologie (UFR-SSMT), Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Ramiarantsoa Harisolo
Maitre de Conférences 1 Laboratoire de constitution et réaction de la matière (LCRM), Unité de Formation et de Recherches des sciences des Structures de la Matière et de Technologie (UFR-SSMT), Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Corresponding Author:
Seri Seri Chardin
Maitre-Assistant 1 Laboratoire de constitution et réaction de la matière (LCRM), Unité de Formation et de Recherches des sciences des Structures de la Matière et de Technologie (UFR-SSMT), Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire

Isolation and identification of Anthraquinones from the roots of *Morinda Morindoides* (Rubiaceae)

Seri Seri Chardin, Dade Joël Maxime Eric and Ramiarantsoa Harisolo

DOI: https://doi.org/10.22271/phyto.2021.v10.i4a.14210

Abstract
Morinda Morindoides is a widely used plant in the traditional Ivorian pharmacopoeia, for the treatment of many microbial infections such as those due to amoeba, fungi, and is also used to treat diarrhea and rheumatic pain. The chemical composition of this species is very varied and includes numerous anthraquinones. In this article, we describe the isolation of a new Anthraquinone, 4-hydroxy-1-methoxy-2-methylanthraquinone (1) *M Morindoides* in conjunction with the known anthraquinones (2), 2-methylanthraquinone (3), Soranjidiol (4), Damnacanthol (5), Damnacanthal (6), Rubiadin-1-methyl-ether (7). The structures were elucidated through spectroscopic studies including 2D-NMR experiments (HSQC, HMBC, COSY, and NOESY).

Keywords: *Morinda Morindoides*, Rubiaceae, 4-hydroxy-1-methoxy-2-methylanthraquinone, anthraquinones

Introduction
Morinda Morindoides is a very popular medicinal plant in many African countries. This plant is used against diarrhoea, amoebiasis, rheumatic pains and fungi [1, 2]. In Ivory Coast, the aqueous decoctions of leaves or roots are widely used for the treatment of malaria [3]. Many traditional uses were confirmed by reports on biological studies [4-8]. The ethyl acetate extract showed significant plasmodial and antidiarrheal activity [6, 9, 10]. In this present paper, we report the isolation and identification of the Anthraquinone, 4-hydroxy-1-methoxy-2-methylanthraquinone (1) from a natural source which was identical to a known synthetic product [11, 12].

Experimental
General: Melting points were determined with a Büchi B-545 and were uncorrected. The optical rotation was measured on a Schmidt-Haensch Polartronic HH8 polar meter. The UV spectra were obtained by using a Philips PU 8720 spectrophotometer. The IR spectra were recorded on a Bruker Vector 22 FT-IR spectrometer. The EIMS were recorded on Varian MAT-312 mass spectrometer. The HREIMS were measured on a Micro mass Q-TOF micro instrument (Manchester, UK). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in DMSO-d_6 on a Bruker Avance DRX-400 spectrometer with TMS as internal standard. Column chromatography and gel permeation were run on Merck silica gel 60 and Sephadex LH 20. Analytical TLC was carried out on 0.25 mm thick layer of silica gel percolated on aluminium foil (Merck GF254). Spots on chromatograms were detected by observing under UV light (254 nm) and were further visualized by spraying with a vanillin-H_2SO_4.

Plant material: Roots of *Morinda Morindoides* were collected in July 2009 in Saïoua, in the West of Ivory Coast. The plant was identified by Prof. Aké Assi of the University of Cocody-Abidjan. A voucher specimen (ZG N° 116) was deposited at the "Centre National de Floristique" of the University of Cocody-Abidjan. The collected plant materials were dried and the dry samples were crushed and stored at 10°C until use.

Extraction and isolation: Dried and powdered roots of *Morinda Morindoides* were extracted three times with 80% aqueous ethanol. After filtration, the combined extracts were concentrated at 40°C in the rotary evaporator under reduced pressure. The resulting residue was suspended in water and successively partitioned with petroleum ether, ethyl acetate and n-butanol. The ethyl acetate extract (15.23 g) was subjected to silica gel column chromatography

with a gradient of AcOEt in Et_2O (from 100:0 to 0:100). Five fractions (F_1 to F_5) were obtained were these dried in the rotary evaporator. The fraction F_3 contained yellow crystals; their recrystallization from Et_2O gave the compound 1 (70 mg). The fraction F_4 was repeatedly separated on Sephadex LH-20 columns elated with $CHCl_3$/MeOH (1:1) to yield three compounds 2 (24 mg), 3 (47 mg) and 4 (42 mg). The fraction F_5 was eluted repeatedly on Sephadex LH-20 chromatography column with $CHCl_3$/MeOH (7:3) to yield three compounds 5 (47 mg), 6 (39 mg) and 7 (45 mg).

4-hydroxy-1-methoxy-2-methylanthraquinone (1): Yellow amorphous powder (70 mg); MP 176-178°C; UV (MeOH): λ_{max} (nm) (log ε) 202 (1.04), 278 (1.35); IR(KBr): ν_{max} cm^{-1}: 3308, 1671, 1565, 1446, 1414, 1335, 1300; HR-ESI-MS: m/z $[M+Na]^+$ 291.0630 (calcd for $C_{16}H_{12}O_4Na$: 291.0633); ^{1}H-NMR, ^{13}C-NMR (see Table 1).

Results and discussion

Chromatographic separations on silica gel of the ethyl acetate extract of the roots of *Morinda Morindoides* have led to the isolation of Anthraquinone 1. Six other compounds were isolated: Rubiadin (2) [13, 14], 2-methylanthraquinone (3) [15, 16], Soranjidiol (4) [13, 14], Damnacanthol (5) [14], Damnacanthal (6) [13, 14, 17] and rubiadin-1-methyl-ether (7) [13, 14]. The structure of compound 1 is presented in Figure 1.

Compound 1 was obtained as a yellow amorphous powder. Its UV and IR spectra were typical of those of anthraquinones [18-20]. The positive ion mode HRESIMS gave a pseudo molecular ion peak at m/z 291.0633 $[M+Na]^+$, consistent with a molecular formula $C_{16}H_{12}O_4Na$. The NMR spectra data supported the presence of Anthraquinone skeleton. The ^{1}H NMR spectra gave a singlet at δ_H 13.10 ppm assigned to a chelated hydroxy proton at C-4. Five aromatic protons exhibited as two doublets of doublet at δ_H 8.25 (1H, J = 7.5 and 1.5 Hz, H-8) and δ_H 8.16 (1H, J = 7.5 and 1.5 Hz; H-5), two triplets of doublet at δ_H 7.84 (1H, J = 7.5 and 1.5 Hz; H-7) and δ_H 7.76 (1H, J = 7.5 and 1.5 Hz; H-6) and a singlet at δ_H 7.20 ppm (H-2). These five protons suggested that A ring was unsubstituted and B ring trisubstituted. A Methoxy group appeared as a singlet at δ_H 3.86 ppm and a methyl group as a singlet at δ_H 2.42 ppm. The ^{13}C NMR spectrum showed 16 carbon signals: 12 aromatic carbons, one methoxy (δ_C 61.1 ppm), one methyl (δ_C 16.5 ppm), and two carbonyls (δ_C 183.5 and 182.1 ppm) characteristic of anthraquinones. In the HMBC spectrum (Figure 2), the signal of the hydroxyl proton (δ_H 13.10 ppm) correlated with those of C-3, C-4 and C-4a. The cross peaks between the signal of the methyl protons group and those of C-1 (δ_C 153.5), C-2 (δ_C 127.6) and C-3 (δ_C116.1) afforded the positions of the methyl and the methoxyl groups at C-2 and C-1. The assignments of C-3, C-5, C-6, C-7 and C-8 were determined from the HSQC correlations. The structure of the compound 1 was identified as 4-hydroxy-1-methoxy-2-methyl Anthraquinone (named morindin). It was identical to a synthetic Anthraquinone obtained by the reaction of phtalide anions with Quinone monoacetals [11] and the reaction of 3-cyano-1(*3H*)-isobenzofuranone with *N*-methyl-*p*-methoxy semi Quinone animal [12].

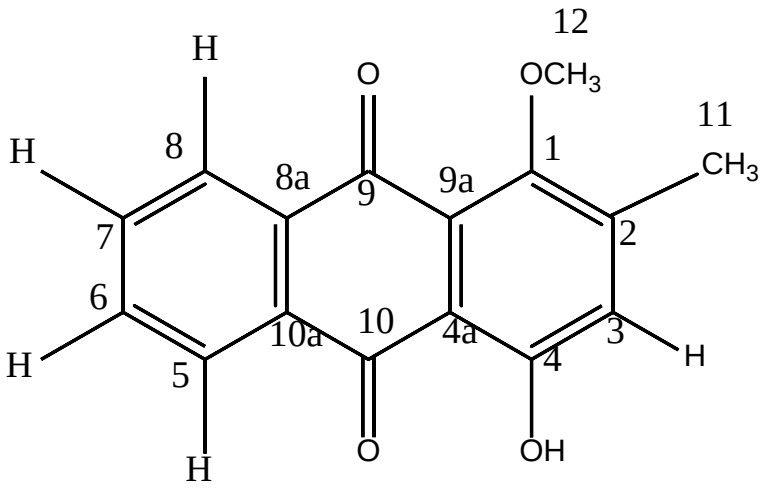

Fig 1: Chemical Structure of compound 1

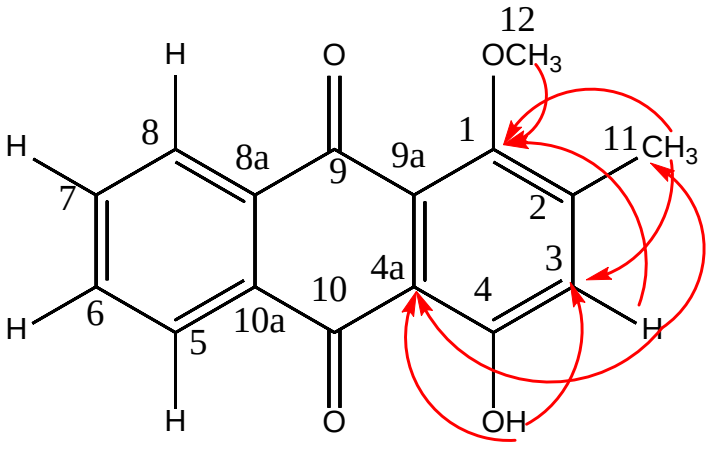

Fig 2: Structure and Key HMBC (H ⟶ C) correlations of compound 1

Table 1: NMR spectral data of compound 1 in DMSO-d_6

Position	δ_C	δ_H [(m, J (Hz)]	COSY	HMBC [b]
1	153.48 (q) [a]	-		
2	127.63 (q)	-		
3	116.03 (t)	7.20 (s)		1, 2, 4, 4a, 11
4	160.08 (q)	-		
4a	132.20 (q)	-		
5	123.82 (t)	8.16 (dd:7.5;1.5)	H-6	6, 7, 8a, 10, 10a
6	133.81 (t)	7.76 (td:7.5;1.5)	H-5, H-7	5, 7, 8, 10a
7	134.78 (t)	7.84 (td:7.5;1.5)	H-6, H-8	5, 6, 8, 8a
8	126.52 (t)	8.25 (dd:7.5;1.5)	H-7	6, 7, 8a, 9, 10a
8a	145.96 (q)	-		
9	182.16 (q)	-		
9a	127.02 (q)	-		
10	183.45 (q)	-		
10a	135.14 (q)	-		
11	16.48 (p)	2.42 (s)		1, 2, 3
12	61.07 (p)	3.86 (s)		1
4-0H	-	13.10 (s)		3, 4, 4a

From a chemotaxonomic viewpoint, similar 1, 2, 4-trisubstituted B-ring anthraquinones, such as 1 were obtained in *Prismatomeris Connata* [21, 22], *Digitlis lanata* [23] and *Tectona grandis* [24] belonging respectively to Rubiaceae, Scrophulariaceae and Verbenaca family. Rubiadin (2) was previously isolated from the roots of *M. citrifolia* [14], *M. angustifolia* [25] and *M. Elliptica* [26]. 2-methylanthraquinone (3) was isolated from the roots of *M. umbellata* [27] and *M. officinalis* [28]. Soranjidiol (4) was isolated from the roots of *M. citrifolia* [14] and *M. elliptica* [26]. Damnacanthol (5) was identified from the roots of *M. umbellata* [27] and *citrifolia* [14]. Damnacanthol (6) was obtained from the roots of *M. citrifolia* [14] and *M. elliptica* [26]. Rubiadin-1-methyl-ether (7) was isolated from roots and stems of *M. umbellata* [27], *M. citrifolia* [14] and *M. elliptica* [24]. This is the first report of the occurrence of morindin (1) as a new natural Anthraquinone, though it has artificially prepared from 3-cyano-1(*3H*)-isobenzofuranone and *N*-methyl-*p*-methoxy semi quinone animal [12]. They are to our knowledge describe here for the first time in *M. Morindoides* roots. Therefore, they could be used to establish a relationship between these species.

Conclusion

The results of this study, obtained for the first time from *Morinda Morindoides* roots, led to the isolation and characterization of seven anthraquinones (morindin, rubiadin, 2-methylanthraquinone, Soranjidiol, Damnacanthol, Damnacanthol and rubiadin-1-methyl-ether). The complete attribution of the structures was established by inspection of NMR spectral data (^{1}H and ^{13}C) and by mass spectrometry. However, one compound, morindin was isolated for the first time from a natural source. This compound has a hydroxyl group attached at 4-position, a methoxy group attached at the1-position and a methyl group attached at the 2-position of Anthraquinone. Anthraquinones are well known in the literature for their broad biological therapeutic. The results of our study could explain the biological activity of *Morinda Morindoides* Roots.

Acknowledgement

The authors would like to thank the Service Cultural Action and Cooperation of France Ambassy in Ivory Coast for financing. They are grateful to Prof. Andre AMBLES, Director of LSRSN and in memoriam of Prof. Jean Marie COUSTARD, both from the University of Poitiers, France l for their kind support of this work. The authors also thank the staff of the CRMPO of the University of Rennes 1, France, for the high-resolution mass spectrometry, and in memoriam of Prof. Aké-Assi Laurent of the University of Cocody-Abidjan, Ivory Coast, for identification of the plant samples.

References

1. Kambu K. Elements de Phytothérapie Comparée, Plantes Médicinales Africaines. CRP-Kinshasa, 1990, 20–62.
2. Adjanohoun EJ, Aké AL. Abidjan CNF, Contribution au recensement des plantes médicinales de Côte d'Ivoire 1979, 258.
3. Zirihi GN. Contribution au recensement, à l'identification et à la connaissance de quelques espèces végétales utilisées en médicine traditionnelle chez les Bétés du département d'Issia. Thèse de Doctorat du troisième cycle, Université de Cocody, Côte d'Ivoire, 1991, 179.
4. Tona L, Kambu K, Mesia K, Cimanga K, Apres S, De Bruyne T *et al.* Biological screening of traditional preparations from some medicinal plants used as antidiarrheal in Kinshasa, Congo. Phytomedicine, 1999;6:59–66., DOI: 10.1016/S0944-7113(99)80036-1
5. Tona L, Mesia K, Ngimbi NP, Chrimwami B, Okond'akhoka A, Cimanga K *et al.* In vivo antimalarial activity of Cassia occidentalis, Morinda morindoides and Phyllantus niruri. Ann. Trop. Med. Parasitol 2001;95:47–57., DOI: 10.1080/00034983.2001.11813614
6. Tona L, Cimanga RK, Mesia K, Musuamba CT, De Bruyne T, Apres S *et al.* In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants in the Democratic Republic of Congo, J Ethnopharmacol 2004;93:27–32., DOI: 10.1016/j.jep.2004.02.022
7. Cimanga K, Hermans N, Apers S, Van Miert S, Van den Heuvel H. Complement inhibiting iridoids from Morinda Morindoides J Nat Prod. 2003;66:97–102., DOI: 10.1021/np020215h
8. Cimanga K, Kambu K, Tona L, Hermans N, Apers S, Totté J *et al.* Citotoxicity and in vitro susceptibility of Entamoeba Histolytica to Morinda Morindoides leaf extracts and its isolated constituents, J Ethnopharmacol 2006;107:83–90., DOI: 10.1016/j.jep.2006.02.010
9. Zirihi GN, Mambu L, Guédé GF, Bodo B, Grellier P. In vitro antiplasmodial activity and citotoxicity of 33 West African plants used for treatment of malaria, J. Ethnopharmacol. 2005;98:281–285., DOI: 10.1016/j.jep.2005.01.004
10. Meite S, N'Guessan JD, Bahi C, Yapi HF, Djaman AJ, Guede Guina F. Antidiarrheal activity of the ethyl acetate extract of Morinda Morindoides in rats Trop, J. Pharm. Res. 2009;8:201–207., DOI: 10.4314/tjpr.v8i3.44533
11. Russell R, Warrener RN. The regiospecific preparation of 1, 4-dioxygenated anthraquinones: a new route to islandicin, digitopurrrrpone, and Madeirin, J. Chem. Soc. Chem Commun. 1981;3:108–110. DOI: 10.1039/C39810000108
12. Nicolaou KC, Min L, Pengxi C, Akshay AS. Practical Synthesis of p - and o - Amino - and Methoxyphenolic Anthraquinones Angew Chem. Int. Ed. 2015;54:12687-12691., DOI: 10.1002/anie.201507007
13. Adesogan EK. Anthraquinones and anthraquinols from Morinda lucida: the biogenetic significance of oruwal and oruwalol. Tetrahedron. 1973;29:4099–4102. DOI:10.1016/0040-4020(73)80244-3
14. Kamiya K, Hamabe W, Tokuyama S, Hirano K, Satake T, Kumamoto-Yonezawa Y *et al.* Inhibitory effect of anthraquinones isolated from the Noni (Morinda

citrifolia) root on animal A-, B- and Y-families of DNA polymerases and human cancer cell proliferation Food Chem. 2010;118:725–730.
DOI: 10.1016/j.foodchem.2009.05.053
15. Bani TSG and Sunil KT. Acalyphamide, a new amide and other and chemical constituents of Acalypha indica Linn, Indian J. Chem (B). 1981;20(11):974–977.
16. Ramadevi D, Ganapaty S. Phytochemical examination of Corchorus aestuans (Titiaceae) capsule Int J Pharmacogn Phytochem Res. 2012;4(2):54-58.
17. Thomson RH. Naturally occurring quinones, 2e Ed., Academic Press, London and New York, 1971, 734.
18. Murti VVS, Seshadri TR, Sivakumaran S. Chemical components from Rubia Iberica, Indian J. Chem 1972;10:246-247.
19. Itokawa H, Mihara K, Takeya K. Studies on a novel anthraquinone and its glycosides isolated from Rubia cordifolia and R Akane Chem Pharm Bull 1983;31(7):2353., DOI: 10.1248/cpb.31.2353
20. Tessier AM, Dalaveau P, Champion B. Nouvelles anthraquinones des raciness de Rubia cordifolia, Planta Med. 1981;41:337-343., DOI: 10.1055/s-2007-971724.
21. Wang CX, Zhao SN, Feng SX, Zhang XP, Chenb T. Two New Anthraquinones from the Roots of Prismatomeris Connata Nat Prod Commun. 2016;11(4):481-482.
DOI: 10.1177/1934578X1601100415
22. Feng SX, Hao J, Chen T, Qiu SXS. A New Anthraquinone and Two New Tetrahydroanthraquinones from the Roots of Prismatomeris connata Helv Chim Act 2011;94 :1843-1849., DOI : 10.1002/hlca.201100108
23. Furuya T, Kojima H, Katsuta T. 3-methylpurpurin and anthraquinones from callus tissue of Digitalis lanata. Phytochemistry. 1972;11:1073-1076.
DOI: 10.1016/S0031-9422(00)88455-1
24. Sandermann W, Simatuang MH. A new quinones from teak (Tectona Grandis), Naturwissenschaften. 1965;52:262, DOI: 10.1007/BF00602933
25. Xiang M, Song Q, Zhang H, Guo S. Antimicrobial anthraquinones form Morinda Angustifolia Fitoterapia. 2008;79:501-504, DOI: 10.1016/j.fitote.2008.04.008.
26. Ismail N, Ali A, Aimi N, Kitajima M, Takayama H, Lajis N. Anthraquinones from Morinda elliptica Phytochemistry. 1997;45(8):1723-1725.
27. Burnett A, Thomson R. Anthraquinones in two Digitalis species Phytochemistry. 1968;7:1423.
28. Yoshikawa M, Yamaguchi S, Nishisaka H, Yamahara J, Murakami N. Chemical Constituents of Chinese natural medicine, Morindae Radix, the dried roots of Morinda officinalis How: Structures of Morindoides and morofficinaloside Chem Pharm Bull. 1995;43:1462-1465., DOI: 10.1248/cpb.43.1462.