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A new ketosteroid from *Morinda morindoides* (Rubiaceae)

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Abstract
The chemical investigations of the roots of *Morinda morindoides* led to the isolation of a new ketosteroid 20(*R*) 24(*R*) 2β-hydroxy-ethylcholest-4-en-3-one. The structure was elucidated through the spectral studies including UV, MS, 1D-NMR (^{1}H and ^{13}C NMR) and 2D-NMR (HSQC, HMBC, COSY, NOESY) experiments.

Keywords: *Morinda morindoides*, Rubiaceae, ketosteroid, 20(*R*) 24(*R*)-2β-hydroxy-ethylcholest-4-en-3-one

1. Introduction
Morinda morindoides is a very popular medicinal plant in Africa: it is used in the African tradition for the diarrhoea, amoebiasis, rheumatic pains and fungus [1-2]. In Ivory Coast, the leaves and roots aqueous decoctions are widely used for the treatment of malaria [3]. Many traditional uses were confirmed by reported biological studies [4-8]. The petroleum ether and ethyl acetate extracts showed significant antiplasmodial and antidiarrheal activities [6, 9-10]. Previously, *Morinda* species revealed the presence of flavonoids [11-12], anthraquinones [13], sterols [14] and iridoids [15-19]. Nine iridoid glucosides [7, 20] and a ketosteroid [21] have been isolated from *Morinda morindoides*.
In this paper, we report the isolation and structural determination of the ketosteroid 20(*R*) 24(*R*) 2β-hydroxy-ethylcholest-4-en-3-one (1).

2. Materials and Methods
2.1 General
Melting points were determined with a Büchi B-545.
The optical rotation was measured on a Schmidt-Haensch POLARTRONIC HH8 polarimeter.
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 Micromass Q-TOF micro instrument (Manchester, UK).
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ on a Bruker Avance DRX-400 spectrometer with TMS as internal standard.
Column chromatography and gel permeation were run on Merck silica gel 60.
Analytical TLC was carried out on 0.25 mm thick layer of silica gel precoated 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-sulphuric acid solution (1g in 250 mL of MeOH, 10 mL of H_2SO_4 and 25 mL of CH_3COOH).

2.2 Plant material
Roots of *Morinda morindoides* were collected in July 2009 in Saïoua, west of Ivory Coast. The plant was identified by Prof. Aké Assi of the University Félix Houphouët-Boigny of Cocody-Abidjan. A voucher specimen (ZG n°116) was deposited at the "Centre National de Floristique" of the Félix Houphouët-Boigny University, Cocody-Abidjan.

2.3 Extraction and isolation
Dried and powdered roots of *Morinda morindoides* were extracted with 80% of ethanol three times. The combined extracts were concentrated under reduced pressure.

The obtained residue was suspended in water and successively partitioned with petroleum ether, ethyl acetate and n-butanol.

The petroleum extract was fractionated on a silica gel column and eluated with CH_2Cl_2/EtOAc in linear gradient form. The fraction obtained with CH_2Cl_2/EtOAc (6:4) was rechromatographed on a silica gel column using the mixture CH_2Cl_2/EtOAc (8:2) as eluent. Compound 1 (10mg) was isolated as a white amorphous powder.

2.4 Spectral data

20(*R*) 24(*R*)-2β-hydroxy-ethylcholest-4-en-3-one (1).

White amorphous powder (10 mg); mp 180-182 °C; Rf value 0.55 (CH_2Cl_2/EtOAC, 1:3); $[\alpha]_D^{27}$ +32.88 (c 0.4, MeOH); UV (MeOH): λ_{max}(nm) (log ε) 205.9 (1.21), 237.3 (1.57); IR (KBr): ν_{max} cm^{-1}: 3410, 2933, 2869, 1685, 1458, 1385, 1033; EIMS *m/z* (rel. int.): 428 (100) $[M]^+$, 414 (68), 413 (20), 385 (17), 384 (19), 383 (16), 370 (18), 355 (28), 343 (14), 315 (14), 287 (40) $[M\text{-}SC]^+$, 269 (35), 256 (85), 245 (57), 227 (41), 213 (63), 199 (35), 185 (56), 149 (50) $[M\text{-}SC\text{-}138]^+$ (fission of the 6,7 and 9,10 allylic bonds); HR-ESI-MS: *m/z* $[M+Na]^+$ 451.3559 (calcd for $C_{29}H_{48}O_2Na$: 451.3552); ^1H and ^{13}C-NMR (see table 1).

3. Results and Discussion

Chromatographic separation on silica gel of the petroleum extract of the roots of *Morinda morindoides* gave a white amorphous powder. The compound 1 showed a positive reaction with Liebermann Burchard test. The ^{13}C NMR spectrum displayed 29 signals indicating the presence of a steroid. The IR spectrum exhibited characteristic absorption band of hydroxyl (OH) at 3410 cm^{-1} and α, β-unsaturated carbonyl group at 1685 cm^{-1}. Other frequencies at 2933, 2869, 1458 and 1385 cm^{-1} were due to aliphatic C-H, particularly cyclic $(CH_2)n$.

The HR-ESI-MS of compound 1 revealed a pseudo molecular ion peak $[M+Na]^+$ at *m/z* 451.3559 (calcd 451.3552) corresponding to the molecular formula $C_{29}H_{48}O_2$. The NMR data agreed with those reported for steroids having 24-ethylcholestane side chain [22-23]. The ^1H NMR spectrum showed an equatorially oriented oxymethine [δ 4.36 (1H, dd, J = 2.9, 2.5 Hz, H-2)], a trisubstituted double bond (δ 5.83, s, H-4) and two tertiary methyl groups [δ 0.77, 0.94 (each s)]. The ^{13}C NMR spectrum confirmed the presence of the ketone (δ 200.5, C-3) conjugated with the trisubstituted double bond [δ 168.4 (C) and 126.3 (CH)], and the hydroxyl group [δ 73.3 (CH)]. Additionally the resonances of six methyls, seven methines, ten methylenes and two quaternary carbons were shown (Table 1). Most of ketosteroids belonging to cholestane series have their enone moiety situated in the A ring [24-25]. The HMBC correlations between the signal of the carbonyl group at C-3 and those of the olefinic proton H-4 and the oxymethine proton at δ 4.36 (H-2) justified the position of OH at C-2. Furthermore, the partial structure -CHOH-CH_2- deduced from the ^1H-^1H COSY spectrum confirmed the locations of the hydroxyl group and the enone moiety in the A ring of the steroid.

The attachment of the side chain at C-17 was supported by the correlation between H-17 and both C-18 and C-21 in the HMBC spectrum. The same HMBC spectrum showed correlations between the signal of C-1 and those of H-19, H-9 and H-2; C-4 and H-6; C-18 and H-12, H-14 and H-17; and between C-13 and H-14 and H-15.

The ^1H-^1H COSY correlations between H-6 and H-7, H-7 and H-8, H-8 and H-9 completed the assignment of the rings A and B. The correlations between H-14 and H-8, H-12 and H-11 in the same COSY spectrum allowed to assign the C-ring. The D-ring assignment was determined through correlations between H-15 and H-16, and H-16 and H-17 in the same spectrum.

These NMR data allowed to establish the structure of compound 1 (Fig 1). This structure was further confirmed by the fragments obtained from its EIMS. In this spectrum, the peak at *m/z* 428 corresponded to the molecular ion $[M]^+$. The peak at *m/z* 385 suggesting a loss of propyl group (C_3H_7), while that at *m/z* 287 corresponding to the loss of $C_{10}H_{21}$ group. This group ($C_{10}H_{21}$) suggested the presence of a steroid with saturated side chain. The peak at *m/z* 384 could be obtained from the fragment at *m/z* 385 or by retro Diels-Alder cleavage from the molecular ion ($[M]^+$, *m/z* 428). This fragment certified that the hydroxyl (OH) group was in α-position of the carbonyl and confirmed the location of the double bond at C4-C5. The fragment at *m/z* 383 was due to the transfer of a proton from the fragment at *m/z* 384 (Fig 2).

The relative orientations of the protons were confirmed by the NOESY spectrum data. In particular the cross-peaks between H-8 and both H-18 and H-19, and between H-18 and H-20, indicated their spatial proximity. The lack of correlation between H-19 and H-9, and correlation between H-9 and H-14, and H-14 and H-17 indicated the α-orientation of these protons and established the relative stereochemistry of C-17. The *R* configuration of the C-24 ethyl group was deduced by comparison of the ^{13}C chemical shifts of the side chain with those of cholestane sterols [26-27]. So compound 1 was identified as 20(*R*) 24(*R*)-2 β-hydroxy-24-ethylcholest-4-en-3-one (Fig 1).

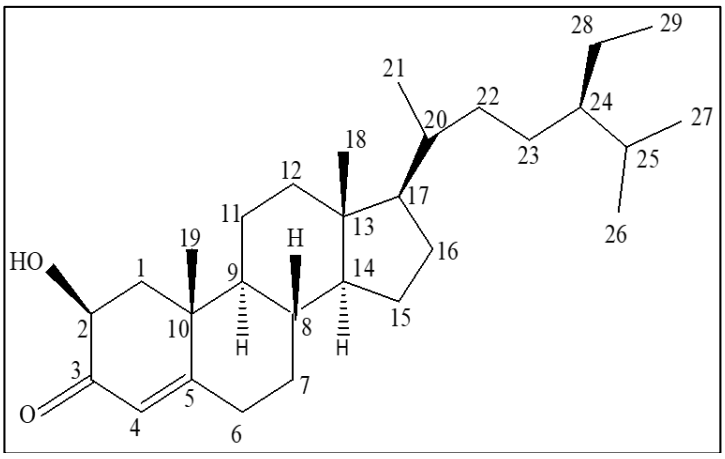

Fig 1: Chemical structure of compound 1

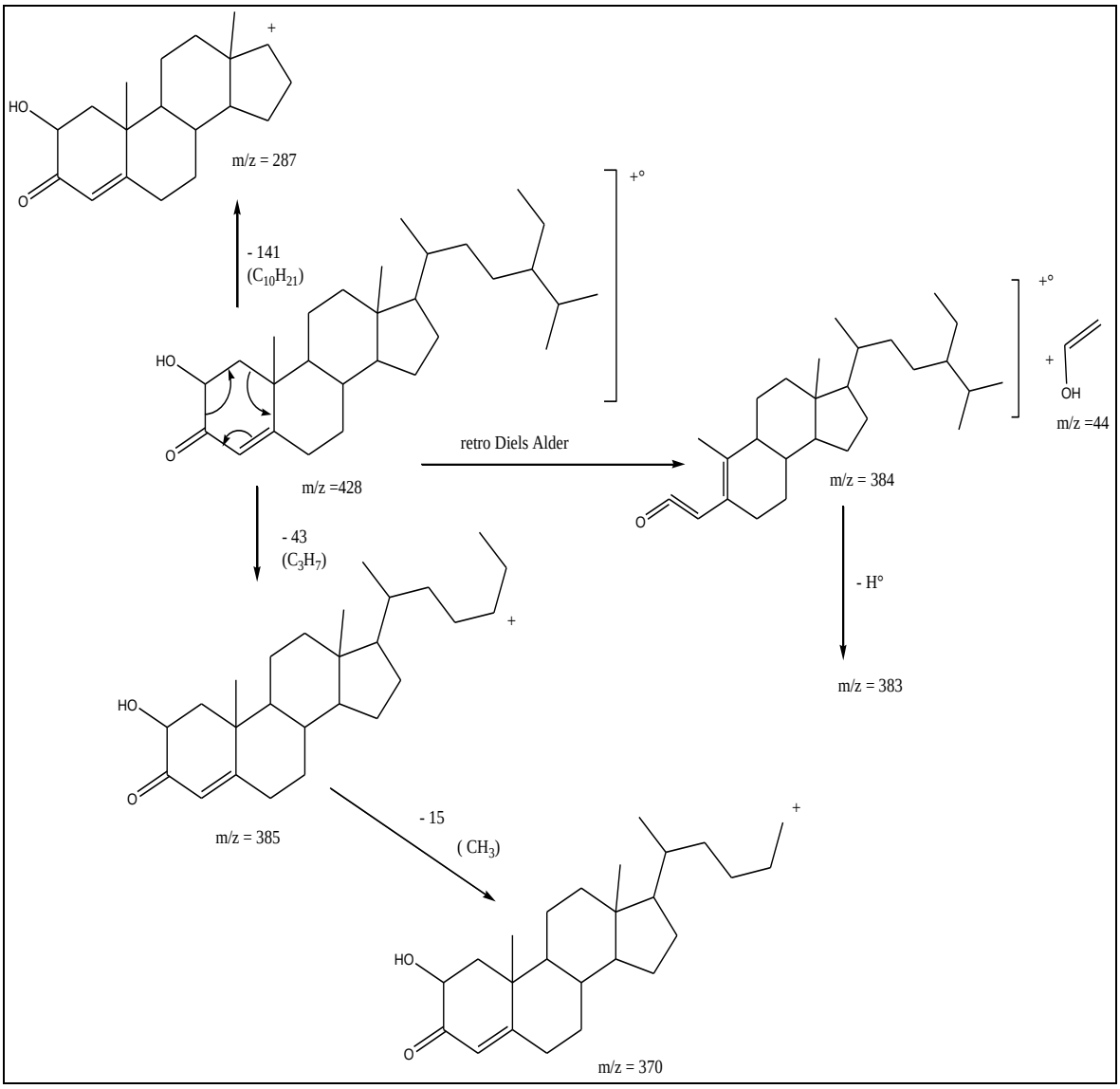

Fig 2: Mass fragmentations of compound 1

Table 1: ^{1}H (400MHz) and ^{13}C (100MHz) NMR spectral data of compound 1 in $CDCl_3$

Position	δ_C	δ_H [m, J (Hz)] a	b	HMBC[b]
1	39.5	2.02(m)	1.16(m)	H_2; H_9; H_{19}
2	73.3	4.36 (dd, 2.5; 2.9)		H_{1a}; H_4
3	200.5	-	-	H_2; H_4
4	126.3	5.83(s)		H_2; H_{6a} ; H_{6b}
5	168.4	-	-	H_{1a}; H_{7b}; $H_{6b;}$ H_{19}
6	34.2	2.52(m)	1.28(m)	H_4; H_{7a} ; H_{7b}
7	33.8	2.41(m)	1.38(m)	H_4; H_{14}
8	36.1	1.38(m)		H_{7b}; H_{11a}; H_{14b}; H_{15}
9	53.6	1.01(m)		H_{1a}; H_{7b}; H_8; H_{12a}; H_{12b}; H_{19}
10	38.5	-	-	H_{1a}; H_4; H_{6a}; H_{6b}; H_8; H_9; H_{11a};
11	20.9	1.88(m)	1.28(m)	H_9 ; H_{12b}
12	39.4	2.04(m)	1.61(m)	H_{11a}; H_{14}; H_{17}; H_{18}
13	42.5	-	-	H_{12a}; H_{14}; H_{15b}; H_{18}
14	55.8	1.19(m)		H_{7b}; H_9; H_{12a}; H_{15b}; H_{16b}; H_{18}
15	24.1	1.95(m)	1.34(m)	H_{14}; H_{16b}; H_{17}
16	28.9	1.69(m)	1.31 (m)	H_{14} ; H_{15b}; H_{17}
17	55.9	1.03(m)		H_{12a}; H_{12b}; H_{14}; H_{15b}; H_{16b}; H_{18};H_{20};H_{21}
18	12.2	0.77(s)		H_{12b};H_{14};H_{17}
19	18.7	0.94(s)		H_{1a};H_4; H_9
20	36.1	1.38(m)		H_{21};H_{23b}
21	18.9	0.93 (d, 6.4)		H_{17}; H_{20}
22	34.2	2.52(m)	2.40(m)	H_{17};H_{20};H_{21};H_{24}
23	25.9	1.18(m)		H_{28b}
24	45.8	0.77(m)		H_{23}; H_{26}; H_{27}; H_{28b}; H_{29}
25	29.1	1.95(m)		H_{23}; H_{24}; H_{26}; H_{27}

26	19.5	0.92 (d, 6.4)		H_{24}; H_{27}
27	19.8	0.82(d, 6.4)		H_{24}; H_{26}
28	23	1.25(m)	1.15(m)	H_{23};H_{24}; H_{29}
29	12.2	0.78(t, 6.8)		H_{24}; H_{28}

4. Conclusion

The chemical study of the roots of *Morinda morindoides* resulted in the isolation of a new ketosteroid. It has an alcohol function at C2, a ketone function at C3, a double bond between C4-C5 and no double bond between C22-C23. The biological study of this compound will be carried out subsequent of this work.

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6. Dedication

This publication is dedicated in memory of Prof. Jean-Marie COUSTARD, who supervised this work and passed away at the end of August 2013.

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