

Available online on 15.10.2026 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Isolation of two cassane-type diterpenoids from the root bark of *Erythrophleum ivorense* A. Chev. (Fabaceae)

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Article Info:



Article History:

Received 11 July 2026
Reviewed 20 Aug 2026
Accepted 13 Sep 2026
Published 15 Oct 2026

Cite this article as:

Kablan RJF, Kabran AF, Kablan ALC, Konan DJ, Camara Y, Attioua KB, Isolation of two cassane-type diterpenoids from the root bark of *Erythrophleum ivorense* A. Chev. (Fabaceae), Journal of Drug Delivery and Therapeutics. 2026; 16(10):1-6 DOI: <https://doi.org/10.22270/jddt.v16i10.8014>

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Abstract

Erythrophleum ivorense is a large tree found in the tropical forests of Africa, used to treat certain skin conditions or injuries. This plant species is known for its high content of cassane-type diterpenoids, compounds known for their diverse biological activity. Extraction by maceration in ethanol was followed by successive purifications via silica gel column chromatography, Sephadex® LH-20 gel and preparative HPLC-UV. Structural determination was carried out on the basis of comprehensive spectroscopic data, including one- and two-dimensional nuclear magnetic resonance (¹H, ¹³C, COSY, HSQC, HMBC, NOESY) as well as high-resolution mass spectrometry (HR-ESI-MS). Two cassane-type diterpenoids, namely erythroivorensin and erythrodine, were isolated. The results confirm that cassane-type diterpenoids are chemotaxonomic markers of the genus *Erythrophleum*.

Keywords: *Erythrophleum ivorense*, cassane-type diterpenoids, erythroivorensin, erythrodine, NMR.

INTRODUCTION

The genus *Erythrophleum* (family Fabaceae, subfamily Caesalpinioideae) comprises several species of tropical trees widely distributed in sub-Saharan Africa¹⁻⁵, Asia⁶⁻⁸, and Australia⁹⁻¹¹. Species of the genus *Erythrophleum* have been used as digitalis-like drugs due to their various effects on the heart. Medicinal plants of this genus also show good results when used as tonics and blood circulation stimulants, as well as emetics, anesthetics, anthelmintics, antimalarials, analgesics, disinfectants, and for treating dermatitis, convulsions, inflammations, heart disorders, venom poisoning, headaches, edema, gangrenous wounds, and rheumatism. This article presents an overview of isolated compounds of this kind, along with their biological evaluations. The bibliographic

sources used for this article come from the literature published from the 1960s to the present¹².

The genus *Erythrophleum* contains diverse classes of compounds, including alkaloids, terpenoids, phytosterols, saponins, flavonoids, and their derived glycosides. Numerous *in vitro* and *in vivo* pharmacological results, such as cytotoxicity, antioxidant, antibacterial, anticonvulsant, anti-inflammatory, anticancer, anti-angiogenic, sedative, cardiac, and other toxic activities, indicate that extracts, fractions, and isolated compounds from *Erythrophleum* species can be valuable resources for the development of new natural medicines. Phytochemically, *Erythrophleum* is characterized by an abundance of cassane-type diterpenoids with nitrogenous (amine and amide) or non-nitrogenous functional groups. These secondary

metabolites possess a fused perhydrophenanthrenic tricyclic skeleton and exhibit a broad spectrum of biological activities: regulation of cardiac inotropy, anti-inflammatory, antimicrobial activities and strong cytotoxicity towards multiple tumor cell lines¹¹.

Among them, *Erythrophleum ivorense* A. Chev. (known as Tali) is traditionally used in African folk medicine to treat various ailments (pain, skin infections, parasites) and has historically been used as a trial and ambush poison due to its high toxicity^{13,14}.

Although the trunk bark of *E. ivorense* has been the subject of several studies isolating major compounds such as cassain and cassaidin, the root bark remains a less explored plant matrix, potentially rich in novel minor derivatives. The objective of this study is to isolate and elucidate the structure of two new diterpenoids, non-nitrogenous cassanes from root bark: erythroivorenin and erythroline.

1. MATERIALS AND METHODS

1.1. Equipment

1.1.1. Plant material

The root bark of *E. ivorense* (Fabaceae) was collected in Agboville, southern Côte d'Ivoire, in December 2024. It was identified and authenticated at the National Floristic Center of Félix Houphouët-Boigny University. The bark of roots growing above ground was scraped with a machete, dried in the laboratory in the shade, and ground using an electric grinder. The resulting powder was used for the extractions.

1.1.2. Technical Equipment

MassHunter software (Agilent Technologies) was used for the acquisition and analysis of spectrometric data obtained by high-performance liquid chromatography coupled with electrospray ionization mass spectrometry (HPLC-ESI-MS). The analyses were performed using an Agilent Quadrupole Time-of-Flight (Q-TOF) mass spectrometer, specifically a Q-TOF 6200 (TOF Series/6500 Series Q-TOF, software version B.08.00, build B8058.0).

1.2. Methods

1.2.1. Extraction

Erythrophleum ivorense root bark (700 g) was pulverized and then subjected to solid-liquid extractions at room temperature. This operation was performed three times over 24 hours in distilled ethanol (7 L). After filtration, the different solutions were evaporated using a rotary evaporator (Rotavapor, Sartorius Stedim Biotech), then dried to give 34.3 g of crude extract. A portion of this ethanolic extract (15 g) was dissolved in 50 mL of MeOH, then alkalized with a few drops of NH₄OH (25%), and then made up to 100 mL of CH₂Cl₂. To the resulting solution, 100 mL of 1% sulfuric acid was added, and then it was counter-extracted with distilled CH₂Cl₂ (100 mL x 4) to give, after evaporation under reduced pressure, the dichloromethane extract (ESD). The supernatant phase was first alkalized with NH₄OH at pH 10, then counter-extracted with distilled AcOEt (100 mL x 4) to give, after

evaporation using a rotary evaporator, the ethyl acetate extract (ESA). The aqueous phase is dried to give the aqueous extract (EAq).

1.2.2. Thin-layer chromatography (TLC)

Thin-layer chromatography analyses were performed on aluminum plates (Silicagel 60 F254, Merck, Germany). After development in glass tanks, the plates were observed under ultraviolet light (254 nm and 366 nm). The UV lamp used was a Vilber Lourmat™ (230 V, 50 Hz, France).

1.2.3. Flash chromatography

A device with an isocratic and gradient pumping system, equipped with a double-piston pump was used. The 330 g, 120 g, and 24 g cartridges from the Grace Resolv® Silica Flash Silica series were used. These cartridges have the advantage of effectively replacing glass columns.

1.2.4. Silica gel column chromatography (CC)

Chromatographic column separations were performed taking into account the mass of the sample (fraction or extract) to be fractionated and its chromatographic profile (TLC). The column diameter and height were determined based on the sample mass. Indeed, for good separation, the mass of silica in the column is equal to 10 times the mass of the sample to be purified⁷. In this study, the stationary phase used in the various TLC separations was silica gel 60 (40–63 µm, Chromagel, Merck).

1.2.5. Infrared (IR) Spectrometry

The infrared spectra of our samples were recorded on a Perkin Elmer type 257 spectrometer. The samples were dissolved in MeOH or in CHCl₃. The liquid is deposited between two pellets of single-crystal sodium chloride (NaCl).

1.2.6. Mass Spectrometry (MS)

Mass spectra of the isolated compounds were obtained by electrospray in positive mode on an Agilent 6530 Q-TOF-MS mass spectrometer equipped with an ESI source. The samples were dissolved in analytical-grade methanol (HPLC).

1.2.7. Analysis of compounds by nuclear magnetic resonance (NMR)

Spectra were recorded on a Bruker Avance 3 HD 400 spectrometer (¹H, 400 MHz; ¹³C, 100 MHz), equipped with a direct multinuclear probe with gradients, an inverse multinuclear probe with gradients, an HF probe, a 1.7 mm H, C, N microprobe, and a 25-sample autosampler. Chemical shifts are expressed in ppm (parts per million) using TMS (tetramethylsilane) as the chemical shift reference. Coupling constants (J) are expressed in Hertz (Hz). The standard pulse sequence programs provided by Bruker were used to perform the two-dimensional experiments: COSY (²J_{H-H}, ³J_{H-H}), HSQC (¹J_{C-H}), HMBC (²J_{C-H}, ³J_{C-H}, and ⁴J_{C-H}), and NOESY.

1.2.8. Fractionation and purification of the constituents of the ESD extract

The 2.15 g methylene chloride extract (ESD) was fractionated by flash chromatography using a cartridge

containing 10 g of silica gel. Elution was carried out in a MeOH/CH₂Cl₂ mixture following an elution gradient of 0:100 to 50:50, v/v. Fractions G1, G2, and G3 were retained based on their TLC profiles. These fractions were then purified by HPLC. Fraction G1 was purified using the H₂O + 0.1% HCOOH/ACN system (80:20 to 50:50, v/v) to obtain compound ESD2. Fraction G2 was purified using the H₂O + 0.1% HCOOH/ACN system (80:20 to 20:80, v/v) to obtain compound ESD4.

2. RESULTS AND DISCUSSION

2.1. Results

2.1.1. Structural elucidation of ESD2

The compound (ESD2) was obtained as a white, amorphous solid soluble in methanol. Its IR spectrum shows the absorption band of the OH bond of the carboxylic acid at a maximum frequency of 3289 cm⁻¹, that of the C=O bond of the α,β unsaturated carboxylic acid at a maximum frequency of 1696 cm⁻¹, and the vibrations followed by stretching of the CH bond of the methyl (CH₃) and methylene (CH₂) groups at maximum frequencies of 2945, 2922, and 2867 cm⁻¹. The C=C double bonds give absorption bands at maximum frequencies of 1690, 1633, and 1594 cm⁻¹. The HRESI-QTOF-MS⁺ of the ESD2 compound indicates the peak of the pseudomolecular ion [M+H]⁺ at *m/z* 303.2321, resulting in a molecular mass of 302.2243 g/mol. This

value corresponds to the empirical formula C₂₀H₃₀O₂ (calculated mass 302.2246). ¹H and ¹³C NMR spectra were obtained in deuterated methanol. The presence of an α,β unsaturated carboxylic acid group is confirmed by the ¹³C NMR peak at 174.2 ppm. The general shape of the proton and carbon-13 spectra suggests the presence of a compound with three polysubstituted rings, as in the case of cassanes diterpenes. Comparison of the spectral data of ESD2 with those in the literature allowed it to be identified as a known molecule: cassan-13,15-diene-17-oic acid or erythroivorensin (Figure 1). This molecule was previously isolated from the seeds of the species *E. ivorens* by Armah et al.⁴ but not from root bark. The chemical shift assignments are given in Table I.

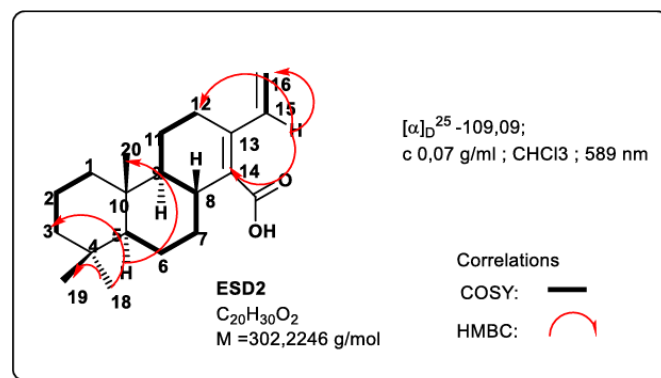


Figure 1: Structure of erythroivorensin

Table I: NMR chemical shift of compound ESD2 (MeOD)

Atom number	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm; m; J, Hz)	COSY $^1\text{JH-H}$	HMBC	NOESY
1	39.8	1.79 (1H; d; 12.7); 0.9 (1H; m)	H-2	H-3; H-5; H-20	-
2	19.9	1.49 (1H; m); 1.66 (1H; d; 12.5)	H-1; H-3	-	-
3	43.3	1.21 (1H; m); 1.43 (1H; m)	H-2	H-1; H-5; H-18, H-19	-
4	34.2	-	-	H-2; H-6	-
5	56.7	0.94 (1H; m)	H-6	H-3; H-7; H-9; H-18; H-19; H-20	H-9; H-18
6	23.0	1.39 (1H; m); 1.71 (1H; m)	H-5	H-8	-
7	33.1	1.09 (1H; m); 1.95 (1H; m)	H-6; H-8	H-5; H-9	-
8	38.6	2.42 (1H; m)	H-7; H-9	H-6; H-11	H-19; H-20
9	54.5	0.95 (1H; m)	H-8; H-11	H-5; H-7; H-12; H-20	H-5; H-18
10	37.9	-	-	H-2; H-8; H-11	-
11	22.0	1.15 (1H; m); 1.88 (1H; m)	H-9; H-12	H-8	-
12	26.6	2.15 (1H; m); 2.45 (1H; m)	H-11	H-9	-
13	138.6	-	-	H-8; H-11; H-16	-
14	134.2	-	-	H-7; H-9; H-12; H-15	-
15	136.6	6.72 (1H; dd; 17.2; 11.0)	H-16	H-12	-
16	114.2	5.07 (1H; d; 11.0); 5.28 (1H; d; 17.2)	H-15	-	-
17	174.2	-	-	H-8	-
18	33.9	0.87 (3H; s)	-	H-3; H-5; H-19	H-5; H-9
19	22.1	0.87 (3H; s)	-	H-3; H-5; H-18	H-8; H-20
20	14.6	0.90 (3H; s)	-	H-1; H-5; H-9	H-8; H-19

2.1.2. Structural elucidation of ESD4

The compound ESD4 was obtained as an amorphous solid and is soluble in methylene chloride. Its HRESI-QTOF-MS⁺ indicates the peak of the pseudomolecular ion [M+H]⁺ at *m/z* 301.2130, resulting in a molecular mass of 300.2052 g/mol. This value corresponds to the empirical formula C₂₀H₂₈O₂ (calculated mass 300.2089). Its IR spectrum indicates the presence of a single carbonyl group across the absorption band at $\mu_{\max}$ 1730 cm⁻¹. The methyl (CH₃) and methylene (CH₂) groups give absorption bands at $\mu_{\max}$ 2947, 2922, and 2844 cm⁻¹. Unlike all the compounds we analyzed, ESD4 is the only one for which ¹H and ¹³C NMR spectra were obtained in deuterated chloroform (this molecule not being soluble in methanol). This suggests that it probably does not have a free OH group in its structure. Its NMR spectra are similar to those of compound ESD2. Indeed, the ¹³C NMR spectrum shows the presence of two double bonds (C=C) with peaks at δ_c 134.6 and 136.4. At 134.9 and 115.5 ppm, and on the proton spectrum, the signals concentrated in the δ_H range of 0.8–2.017 ppm indicate the presence of protons (CH₃, CH₂, and CH) carried by a system with at least three rings. The carbonyl at δ 174.7 ppm (C=O) belongs to an ester group, and the alcohol portion of this ester gives the peak at 79.1 ppm (HC-O). On the HSQC spectrum, the proton at δ 3.20 ppm is found to be carried by this carbon. The HMBC spectrum of ESD4 shows that this carbon at δ 79.1 ppm correlates in ²J_{CH}

with the protons at δ 1.68 ppm (H-6 α), 1.38 ppm (H-6 β), and 2.47 ppm (H-8). Also, the carbonyl at δ 174.7 ppm correlates in ³J_{CH} with the protons at δ 3.20 ppm (H-7) and 2.47 ppm (H-8). These correlations allowed the ester group to be attached to the carbon chain. The stereochemistry of this molecule was established using correlations observed in the NOESY spectrum. Indeed, proton H-7 (δ_H 3.20) correlates with protons H-5 (δ_H 0.89), H-9 (δ_H 0.96), and H-18 (δ_H 0.84), which are behind the plane; it is therefore deduced that it is also in the same plane. Based on the above analyses, the compound ESD4 was formally identified as cassan-15-ene- [7,17]- γ -lactone, or erythrodine (Figure 2). This structure is isolated for the first time in *Erythrophleum ivorense*. The chemical shift assignments are given in Table II.

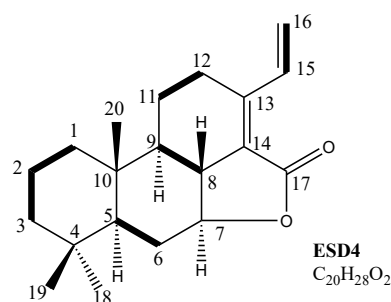


Figure 2: Structure of Cassan-15-ene- [7,17]- γ -lactone or Erythrodine

Table II: NMR chemical shift of compound ESD4 (CDCl₃)

Atom number	$\delta^{13}\text{C}$ (ppm)	$\delta^1\text{H}$ (ppm; m; J, Hz)	COSY ¹ J _{H-H}	HMBC	NOESY
1	38.6	1.78 (1H; d; 12.7); 0.92 (1H; m)	H-2	H-3; H-5; H-20	-
2	19.0	1.46 (1H; m); 1.60 (1H; d; 12.5)	H-1; H-3	-	-
3	42.2	1.16 (1H; m); 1.41 (1H; m)	H-2	H-1; H-5; H-18, H-19	-
4	33.3	-	-	H-2; H-6	-
5	55.4	0.89 (1H; m)	H-6	H-3; H-7; H-9; H-18; H-19; H-20	H-7; H-9; H-18
6	21.8	1.38 (1H; m); 1.68 (1H; m)	H-5	H-8	-
7	79.1	3.20 (1H; m)	H-6; H-8	H-5; H-9	H-5; H-9; H-18
8	37.4	2.47 (1H; m)	H-7; H-9	H-6; H-11	H-19; H-20
9	52.9	0.96 (1H; m)	H-8; H-11	H-5; H-7; H-12; H-20	H-5; H-7; H-18
10	36.8	-	-	H-2; H-8; H-11	-
11	21.8	1.15 (1H; m); 1.84 (1H; dd; 12.6; 5.1)	H-9; H-12	H-8	-
12	26.1	2.16 (1H; m); 2.45 (1H; d; 12.3)	H-11	H-9	-
13	136.4	-	-	H-8; H-11; H-16	-
14	134.6	-	-	H-7; H-9; H-12; H-15	-
15	134.9	6.83 (1H; dd; 17.3; 11.2)	H-16	H-12	-
16	115.5	5.15 (1H; d; 11.2); 5.33 (1H; d; 17.3)	H-15	-	-
17	174.7	-	-	H-7; H-8	-
18	33.2	0.84 (3H; s)	-	H-3; H-5; H-19	H-5; H-7; H-9
19	21.5	0.83 (3H; s)	-	H-3; H-5; H-18	H-8; H-20
20	14.9	0.87 (3H; s)	-	H-1; H-5; H-9	H-8; H-19

2.2. Discussion

Phytochemical analysis of *Erythrophleum ivorense* A. Chev. root bark led to the purification and isolation of erythroivorensin and erythrodine. Although these two cassane-type diterpenoids had previously been isolated from the root barks of a related species, *Erythrophleum suaveolens* (Guill. & Perr.) Brenan this is the first isolation from the root bark of *E. ivorense*. It confirms the close phylogenetic and metabolic relationship between *E. ivorense* and *E. suaveolens*, two major species of the genus *Erythrophleum* in Africa¹⁵⁻²⁰. It demonstrates that the biosynthetic pathway of cassanic diterpenes (particularly derivatives functionalized at the A/B nuclei and the C-13 ester chain) is conserved within the root system of these two taxa. It highlights that the chemical profile of *E. ivorense* root bark shares key biomarkers with other members of the genus *Erythrophleum*, while also paving the way for the search for species-specific quantitative or stereochemical variations.

Cassane-type diterpenes of the genus *Erythrophleum* share structural similarities with cardenolides, notably their ability to inhibit the Na⁺/K⁺-ATPase pump. Comparison of the biological activity of erythroivorensin and erythrodine suggests that:

The presence of an amino or amidated group on the side chain significantly increases affinity for membrane receptors. The degree of hydroxylation on the tricyclic ring plays a crucial role in modulating selective cytotoxicity against tumor cells.

3. CONCLUSION

The phytochemical study carried out on the root bark of *Erythrophleum ivorense* enabled the purification and complete structural characterization of two cassane-type diterpenoids: erythroivorensin and erythrodine. The combined use of modern chromatographic techniques and high-resolution multidimensional NMR spectroscopy ensured the elucidation of their structures and relative stereochemistry. These results enrich the chemotaxonomy of the genus *Erythrophleum* and confirm the underestimated molecular diversity of the underground parts of this plant. Further *in vitro* and *in vivo* pharmacological evaluations are planned to clarify the cytotoxic mechanisms of action and assess the therapeutic potential of these molecules as lead compounds in anticancer or cardioprotective pharmacology.

Acknowledgement: The authors wish to express their profound gratitude to Mr. TERE Henri for the collection of the plants. They also thank the staff of UFR SSMT at Université Félix HOUPHOUËT-BOIGNY for their technical support, as well as the inhabitants of the Agboville region, whose ethnobotanical knowledge proved to be of great richness.

Conflict of Interest: The authors declare that they have no conflict of interest in relation to this manuscript.

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