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ERYTHROSUAVINE, A NEW DITERPENIC ALKALOID FROM *ERYTHROPHLEUM SUAVEOLENS* (GUILL. & PERR.) BRENAN

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ABSTRACT. From the chloroform extract of the stem bark of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan, a new diterpenic alkaloid (Erythrosuavine) has been isolated and characterized as N-acetyl-9,11-dehydro-6-ketocassaidinyl-3-acetate **1**. The structure of the above compound was elucidated from one and two-dimensional ^{1}H and ^{13}C NMR spectra as well as from ^{1}H-^{1}H COSY, HMQC and HMBC correlations.

KEY WORDS: N-acetyl–9,11-dehydro–6-ketocassaidinyl-3-acetate, *Erythrophleum suaveolens*, Erythrosuavine

INTRODUCTION

The study of *Erythrophleum* alkaloids has attracted the attention of scientists since the years 1930s, and several of these alkaloids have been isolated and described so far [1]. *Erythrophleum suaveolens* is the species widespread in Cameroon, the bark decoction of which is used as emetic, anti-inflammatory and analgesic. This decoction is also used to dress wounds and to treat chicken pox and gangrenous sores and as ordeal poison [2]. As part of our systematic search for anti-fungal and antibacterial agents from natural source, we investigated the different parts of this plant material. The bio-guided fractionation of the stem bark extract of *E. suaveolens* for the above activity led to the obtention of the chloroform extract which exhibited a significant anti-fungal activity against *Candida albicans*. Further purification of this fraction led to the isolation of a new diterpenic alkaloid, erythrosuavine which was characterized as N-acetyl-9,11-dehydro-6-ketocassaidinyl-3-acetate **1**. The present paper deals with the structure elucidation of this compound.

RESULTS AND DISCUSSION

The chloroform extract of the crude mixture, obtained from the stem bark of *Erythrophleum suaveolens* according to the alkaline standard procedure [3], was purified by chromatographic techniques to furnish the new diterpenic alkaloid **1**.

Compound **1** was obtained as white crystals from methanol (m.p. 173-174 °C) and gave positive response for both Draggendorf's and Mayer's reagents, suggesting its alkaloidic nature. The UV spectrum (λ_{max} nm, log ε): (254, 3.98) and (366, 4.14) was compatible with the presence of a substituted α,β-unsaturated ketone, while the IR spectrum showed bands due to hydroxyl (3408 cm^{-1}), ester carbonyl (1724 cm^{-1}), amide carbonyl (1591 cm^{-1}), conjugated C=C (1653 cm^{-1}), ester C-O (1151 cm^{-1}) and amide C-N (1131 cm^{-1}). The EIMS exhibited the molecular ion (M^{+}) at *m/z* 489 which was also the base peak, with other significant fragment peaks at *m/z* 374 (result of a McLafferty rearrangement from the α,β-unsaturated ketone [4, 5]), 208 (fragmentation of 7-hydroxyl-6-keto side chain [4, 6]). These EIMS measurements gave the

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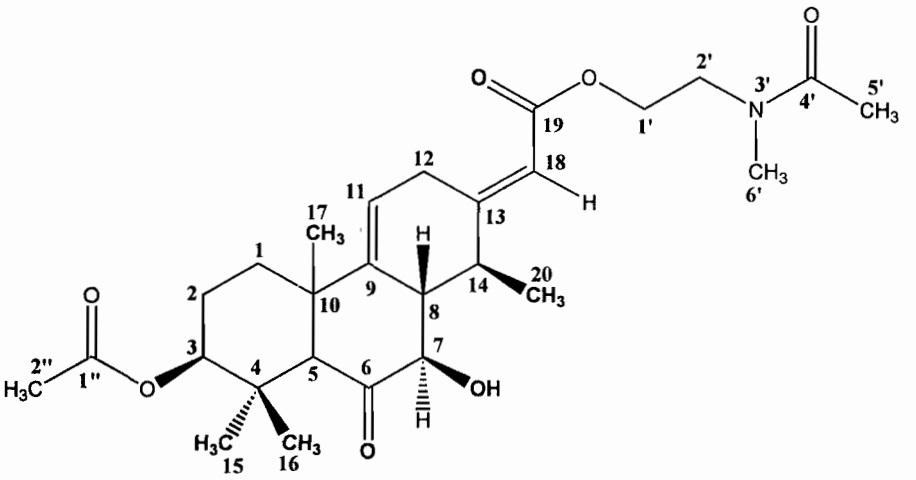
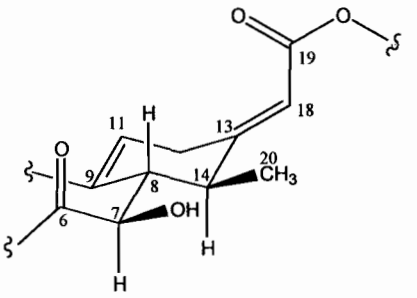
molecular formula $C_{27}H_{39}NO_7$ requiring nine degrees of unsaturation. The ^{13}C NMR spectrum (Table 1) revealed the presence of 27 carbon atoms among which four carbonyls and four sp^2 carbons of two trisubstituted double bonds (δ 115.1, 116.6, 157.3 and 158.0). Among the four carbonyls, two were those of acetyl groups (δ 166.8 and 169.1), one of an ester (δ 170.5) and the last one was that of a ketonic function (δ 210.6). The remaining three degrees of unsaturation indicated that **1** had a tricyclic diterpenic moiety. Its ^1H NMR spectrum (Table 1) showed the presence of two olefinic protons, two acetyl methyl groups, four 3-proton singlets and two oxygenated methine protons. The interpretation of these ^1H and ^{13}C spectral data by means of ^1H-^1H COSY, HMQC and HMBC experiments allowed full assignment of all the NMR signals and led to structure **1**.



1

In fact, the examination of the ^1H NMR spectrum of compound **1** (Table 1) revealed the presence of four signals at δ 0.81 (3H, s, H-17); 0.98 (3H, s, H-15); 1.24 (3H, d, J = 7.1 Hz, H-20) and 1.38 (3H, s, H-16) which could be assigned to C-10, C-4 (α), C-14 and C-4 (β) methyl groups, respectively. The above spectrum also revealed the presence of two olefinic proton signals at δ 5.70 (1H, dd, J = 1.2 and 2.4 Hz) and 5.92 (1H, s). The signal at δ 5.92 was assigned to the olefinic proton H-18 and showed four correlations with carbons at δ 24.8 (C-12), 40.3 (C-14), 157.3 (C-13) and 170.5 (C-19). The presence of the 13,18-double bond has been known to be characteristic of various *Erythrophleum* diterpenic alkaloids [1, 4, 6, 7]. The second olefinic proton signal at δ 5.70 is unusual and was found to be located at C-11 position on the following basis: from the ^1H NMR spectral data, the signal appeared as a doublet of doublets due to the coupling with the methylene protons at C-12. In addition from the HMBC spectral data, the above proton showed ^2J coupling with C-9 (δ 158.0) and C-12 (δ 24.8). Also a ^3J coupling was visible between H-7 (δ 3.92) and C-9; H-14 (δ 2.83) and C-9 (see Scheme 3). The down-field effect observed on H-8 (δ 3.51) which appeared as a doublet (J = 9.9 Hz) compared to similar derivatives corroborated the presence of the above 9,11-double bond as it is drawn in Scheme 1. Furthermore, the hydroxyl group as well as the ketonic function were located at C-7 and C-6 position, respectively. In fact, from the HMBC experiment, the signal at δ 3.92 (H-7) showed three cross peaks with the signals at δ 40.3 (C-14), 51.1 (C-8) and 210.6 (C-6). The presence of

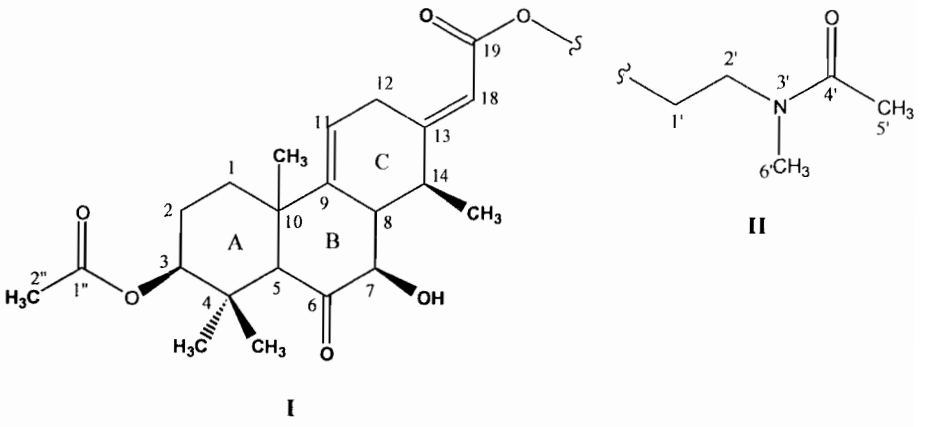
the hydroxyl group geminated to H-7 as well as that of the ketonic function at C-6 can be seen as the consequence of the down-field effect on the above chemical shift. Because H-7 showed a *trans*-antiperiplanar coupling with H-8, H-7 is α-axial oriented and accordingly, the hydroxyl geminated to it was located in β-equatorial position. In addition, the β-orientation of the hydroxyl group could explain the observed deshielding effect on the C-20 methyl proton which appeared in **1** as a doublet (J = 7.1 Hz) at δ 1.24 and also confirm the β-configuration of this C-20 methyl group located on C-14. A similar situation was found in many diterpenic alkaloid derivatives as cassaidine [8].



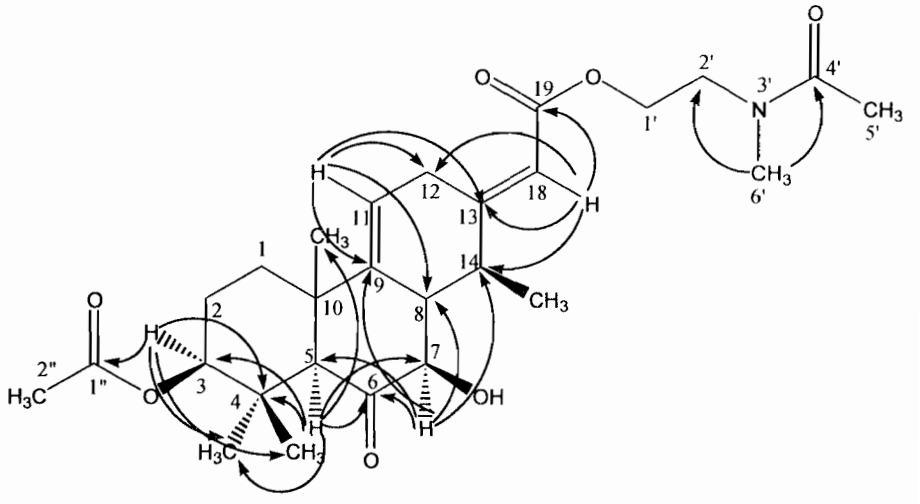
Scheme 1. Spatial representation of the antiperiplanar coupling between H-7 and H-8, β-orientation of 7-hydroxyl and C-14 methyl groups in compound **1**.

The methine carbon at δ 63.1 was assigned to C-5, as it was linked to the proton at δ 2.20 from HMQC correlation. The above proton showed on the HMBC spectrum, a ^{2}J coupling with the C-6 carbonyl group (responsible for its observed deshielding effect) and with C-4 (δ 37.0); ^{3}J couplings of H-5 with C-17 (δ 15.5), C-16 (δ 28.0), C-15 (δ 17.4), C-9 (δ 158.0) and C-3 (δ 78.9) were also observed. Moreover, the first acetyl group was located at 3β-position because of the deshielding effect and of the coupling pattern of the proton H-3 geminated to it which appeared at δ 4.48 (dd, J = 5.0 and 10.5 Hz). The β-configuration of the acetyl group can also be explained on the basis of the biogenetic pathway of terpenoid formation [3]. All the above results defined the three ring systems of **1**, similar to those characteristic of the A, B and C-rings of cassaine derived diterpernes (Substructure **I**) with acetylated hydroxyl group at C-3, a hydroxyl at C-7, a carbonyl at C-6 and an ester at C-18 [4, 6-9]. It is noteworthy that the *trans* configuration of the $\Delta^{13,18}$ double bond as well as the β-orientation of the C-7 hydroxyl and C-14 methyl groups in **1** are in good accord with the works of Hauth *et al.* [8], Turner *et al.* [9] and of Clarke and co-workers [7]. The structure of the remaining side chain was characterized from the following data. The two methylene proton signals at δ 3.59 (t, J = 5.0 Hz, H-2') and 3.81 (t, J = 5.0 Hz, H-1') were clearly visible on the ^{1}H-^{1}H COSY spectrum where they appeared as an isolated spin system. The corresponding carbon resonances at δ 51.6 (C-2') and 62.2 (C-1') and the observed correlations on the HMBC experiment revealed the hetero-atoms linked: N-CH_2 and O-CH_2, respectively. The correlations between H-2' and C-4' (δ 169.1), H-1' and C-2' (δ 51.6); C-19 (170.5) were observed. The 3-protons singlet signals at δ 2.18 and 3.09 were assigned to H-5' and H-6' on the side chain, respectively (Substructure **II**).

In view of the above evidence, the structure of compound **1** was found to be N-acetyl-9,11-dihydro-6-ketocassaidinyl-3-acetate, which to our knowledge has not yet been described in the literature.



Scheme 2. Substructures **I** and **II**.



Scheme 3. Observed HMBC correlations of Compound **1**.

EXPERIMENTAL

General. Melting points are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR at 75 MHz in $CDCl_3$ using TMS as internal standard. EIMS were obtained at 70 eV. Silica gel 60 (Fluka, 230-400 mesh) was used for CC and precoated silica gel plates (Merck, siliea gel 60 F_{254}, 0.25 mm) were used for TLC.

Plant material. Erythrophleum suaveolens (Guill.& Perr.) Brenan was collected in the Yaounde zone (Centre Province, Cameroon) in June 2000. A Voucher specimen (N° 2644/SRFK) is deposited at the Cameroon National Herbarium.

Extraction, isolation and characterization. Air-dried and finely powdered stem bark (2 kg) was firstly extracted in hexane (10 L) for 3 days to give the hexane extract (15 g). The residual air-dried powder was treated with 10% NH_4OH and further extracted in $CHCl_3$ for 24 hours. After filtration and evaporation at reduced pressure, the crude material (90 g) was treated with 5% HCl solution (3 L). The filtrate was in turn extracted several times with $CHCl_3$ and dried ($MgSO_4$); solvent evaporation furnished a fluffy extract (7 g). Half of this solid was chromatographed on silica gel (60 g) using $CHCl_3$-MeOH mixtures of increasing polarity and collecting 100 mL fractions. Identical fractions from TLC obtained upon elution with $CHCl_3$-MeOH (0.5:9.5) were combined and the solvent removed. The semi-solid thereby obtained was further purified on preparative chromatography using $CHCl_3$ as elution solvent to give a solid (35 mg).

N-acetyl-9,11-dehydro-6-ketocassaidinyl-3-acetate ($C_{27}H_{39}NO_7$). Solid, m.p. 173-174 °C. UV (λ_{max} nm, log ε): (254, 3.98) and (366, 4.14). IR ν_{max} (cm^{-1}): 3408, 1724, 1953, 1591, 1151, 1131. EIMS *m/z* (rel. int.): 489 ($[M]^+$, 100), 473 (18), 414 (58), 398 (12), 390 (7), 374 (3), 208 (21), 149 (9), 135 (6), 121 (10) and 107 (11). ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Table 1. ^1H and ^{13}C NMR data* with observed HMBC correlations of compound **1**.

	δ_H (ppm)	δ_C (ppm)	HMBC
1	1.21 m	26.4	3, 10
2	1.66 m	24.8	3
3	4.48 (dd, J = 5.0 and 10.5 Hz)	78.9	1", 4, 5, 15, 16
4		37.0	
5	2.20 s	63.1	3, 4, 6, 7, 15, 16, 17
6		210.6	
7	3.92 (d, J = 9.9 Hz)	76.4	5, 6, 8, 9, 14
8	3.51 (dd, J = 5.4 and 9.9 Hz)	51.1	7, 14
9		158.0	
10		43.4	
11	5.70 (t, J = 1.2 and 2.4 Hz)	116.6	8, 9, 12
12	2.01 m and 1.99 m	24.8	13, 14
13		157.3	
14	2.83 m	40.3	9, 13, 18, 20
15	0.98 s	28.0	3, 4, 5
16	1.38 s	17.4	3, 4
17	0.81 s	15.5	5, 9
18	5.92 s	115.1	12, 13, 14, 19
19		170.5	
20	1.24 (d, J = 7.1 Hz)	14.2	8, 13, 14
1'	3.81 (t, 5.0 Hz)	62.2	2', 19
2'	3.59 (t, 5.0 Hz)	51.6	1', 4'
4'		169.1	
5'	1.91 s	27.9	4'
6'	3.09 s	40.2	2', 4'
1"		166.8	
2"	2.18	20.7	1"

* Assignments based on COSY, HMQC and HMBC.

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REFERENCES

1. Clarke, R.L. *Phytochemistry* **1971**, 10, 851.
2. Hutchinson, J.; Dalziel, J.M. *Flora of West Tropical Africa*, 2nd ed., Crown Agents: London; **1958**.
3. Bruneton, J. *Pharmacognosie, Phytochimie, Plantes Medicinales*, Lavoisier Technique et Documentation: Paris; **1993**.
4. Friedrich-Fiechtl, J.; Spiteller, G. *Chem. Ber.* **1971**, 104, 3535.
5. McLafferty, F.W. *Spectrographie de Masse*, Ediscience: Paris; **1969**.
6. Lindwall, O.; Sandberg, F.; Thorsen, R. *Tetrahedron Letters*, **1965**, 47, 4203.
7. Clarke, R.L.; Daum, S.J.; Shaw, P.E.; Kullnig, R.K. *J. Am. Chem. Soc.* **1966**, 88, 5865.
8. Hauth, H.; Stauffacher, D.; Niklaus, P.; Melera, A. *Helv. Chim. Acta* **1965**, 48, 1087.
9. Turner, R.B.; Buchardt, O.; Herzog, E.; Morin, R.B.; Riebel, A.; Sanders, J.M. *J. Am. Chem. Soc.* **1966**, 88, 1766.