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ISOFLAVONES AND ALKALOIDS FROM THE STEM BARK AND SEEDS OF *ERYTHRINA SENEGALENSIS**

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Key Word Index—*Erythrina senegalensis*; Leguminosae; stem bark; seeds; erysenegalenseins L and M; erysodine; glucoerysodine; isoflavonoids; alkaloids.

Abstract—Two new isoflavones, erysenegalensein L, 5,6′,4′-trihydroxy-8-(2‴-hydroxy-3‴-methylbut-3‴-enyl)-2″,2″-dimethylpyrano [5″,6″,6,7] isoflavone and erysenegalensein M, 5,4′-dihydroxy-8-(2‴-hydroxy-3‴-methylbut-3‴-enyl)-2″,2″-dimethylpyrano [5″, 6″: 6, 7] isoflavone have been isolated from the stem bark of the Cameroonian medicinal plant *Erythrina senegalensis*. The seeds of this plant afforded erysodine, glucoerysodine and hypaphorine. Their structures were determined by the usual spectroscopic methods, 2D NMR techniques and were confirmed by chemical reactions.

INTRODUCTION

Erythrina senegalensis is a medicinal plant widely distributed in the subtropical and tropical regions. Its stem bark is used in decoction in folk medicine among many tribes in Africa [1]. Its seeds are well known for alkaloidal compounds whose main physiological property is a curare-like action [2]. Recently, we have isolated from the stem bark some new neutral isoflavones with hydroxylated and epoxidized side-chains [3–5]. As a continuation of our studies on this species, we now report the isolation and structural elucidation of two new isoflavones, erysenegalensein L (**1**) and erysenegalensein M (**2**) from the stem bark along with the alkaloids, erysodine (**5**), glycoerysodine (**6**) and hypaphorine (**7**) from the seeds.

RESULTS AND DISCUSSION

Silica gel chromatography of the methylene chloride extract of the stem bark of E. senegalensis afforded two novel compounds (**1**) and (**2**). Silica gel chromatography of the methanol extract of its seeds gave known alkaloids (**5**, **6** and **7**) whose 2D ^{13}C–^{1}H NMR are reported here for the first time.

Compound **1**, erysenegalensein L, was obtained as a yellow sticky oil. Its molecular formula, $C_{25}H_{24}O_7$, was deduced by CI/NH_3 *m/z* 437 $[M + H]^+$ and EI mass spectrometry which showed the $[M]^+$ at *m/z* 436. It gave positive $FeCl_3$ (blue) and Mg–HCl (reddish) tests suggesting the presence of an isoflavonoid or flavonoid skeleton [6]. Its IR spectrum showed vibration bands at 3440 (free OH) and 1640 (C=O chelated) cm^{-1}. The ^{1}H NMR showed a singlet signal at δ7.84, characteristic of H-2 of an isoflavone which was also supported by the UV spectrum indicating maximum absorption at 286 nm. The bathochromic shift observed by adding $AlCl_3$ ($\Delta\lambda$ = 8 nm) and HCl ($\Delta\lambda$ = 14 nm) suggested the presence of a free hydroxyl group at C-5 which chelated with the carbonyl function at C-4 [6]. This hypothesis was then confirmed by its ^{1}H NMR spectrum (Table 1) which showed a singlet signal at δ12.57 exchangeable with D_2O. The ^{1}H NMR spectrum also indicated the presence of a 2-hydroxy-3-methylbut-3-enyl group by signals of two benzylic protons shown as two double doublets at δ3.02 (1H, *dd*, *J* = 8.5, 15.0 Hz, H-1‴) and 3.10 (1H, *dd*, *J* = 2.6, 15.0 Hz, H-1‴); the doublet of doublets at δ4.38 was assigned to the methine proton (H-2‴, *J* = 2.6, 8.5 Hz), the two doublets at δ4.87 (1H, *J* = 1.0 Hz) and 4.99 (1H, *J* = 1.0 Hz) corresponded to the two protons at the *gem*-position, while the singlet at δ1.90 was assigned to the methyl protons. This five-carbon hydroxylated unit was confirmed by the ion fragment at 365 $[M - 71]^+$ in the EI mass spectrum. Furthermore the ^{1}H NMR spectrum indicated two doublets at δ5.65 (1H, *J* = 10.0 Hz, H-3″) and 6.75 (1H, *d*, *J* = 10.0 Hz, H-4″), one singlet at δ1.51 (6H, 2 × Me) assigned to protons of a dimethylchromene moiety which was then confirmed by the ion fragment at *m/z* 421 $[M - 15]^+$; the ion at 350 $[M - 71 - 15]^+$ also confirmed the presence of one dimethylchromene [7] and one 2-hydroxy-3-methylbut-3-enyl group [3]. The lack of

*Part 32 in the series '*Erythrina* studies'; for Part 31 see ref. [16].

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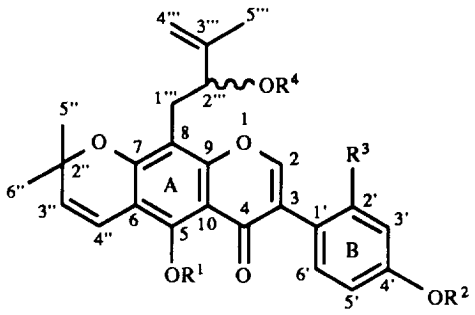
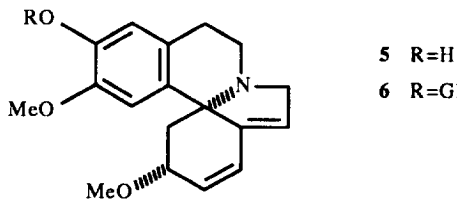
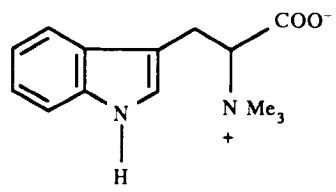


	R^1	R^2	R^3	R^4
1	H	H	OH	H
2	H	H	H	H
3	Ac	Ac	OAc	Ac
4	Ac	Ac	H	Ac



5 R=H
6 R=Glc



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chemical shift at 5.90–6.10 ppm suggested the absence of protons at the H-6 and H-8 positions [6]. Therefore, the two groups mentioned above were linked to the ring A moiety. This was confirmed by the EI mass spectrum which showed ions at *m/z* 231, 216 and 134, resulting from the further RDA cleavage of the ions at *m/z* 421 $[M - 15]^+$, 365 $[M - 71]^+$ and 350 $[M - 71 - 15]^+$ (Scheme 1). The ion at *m/z* 134 suggested that ring B carried two hydroxyl groups. Their assignments to the C-2′ and C-4′ positions was deduced from the ^{1}H NMR spectrum which showed three aromatic protons in an ABX system at δ6.56 (1H, *d*, J = 2.6 Hz, H-3′), 6.38 (1H, *dd*, J = 8.2, 2.6 Hz, H-5′) and 6.9 (1H, *d*, J = 8.2 Hz, H-6′) and from the ^{13}C NMR spectrum (Table 1) which showed chemical shifts at δ158.5 (C-2′) and 156.7 (C-4′) consistent with *meta*-dihydroxyl groups on ring **B** [8]. It remained then to establish unambiguously the positions of the 2-hydroxy-3-methylbut-3-enyl and dimethylchromene groups on ring A.

In order to decide on their orientations, ^{13}C–^{1}H (HETCOR) and ^{13}C–^{1}H (COLOC) NMR techniques were used [9]. The COLOC spectrum showed correlations between H-2 (δ7.84) and C-9 (δ154.0), H-1‴ (δ3.10) and C-9 (δ154.0), H-1‴ (δ3.10) and C-7 (δ157.9), H-1‴ (δ3.10) and C-8 (δ104.3). These correlations confirmed that the five-carbon hydroxylated group was linked to the C-8 position. Furthermore, the ^{1}H NMR spectra of (**1**) and its tetraacetate (**3**) were compared. The chemical shift variations observed for protons H-3″ ($\Delta\delta$ = − 0.11) and H-4″ ($\Delta\delta$ = + 0.27) permitted us to conclude that the dimethylchromene fused at a linear position [10] and consequently the 2-hydroxy-3-methylbut-3-enyl unit linked at C-8. Thus **1** is 5,2′,4′-trihydroxy-8-(2′-hydroxy-3‴-methylbut-3‴-enyl)-2″,2″-dimethylpyrano [5″,6″:6,7] isoflavone and is named erysenegalensein L.

Compound **2**, erysenegalensein M, was obtained as thick yellow oil. Its molecular formula, $C_{25}H_{24}O_6$, was deduced by CI/NH_3 *m/z* 421 $[M + H]^+$ and EI mass spectrometry which showed the molecular ion at *m/z* 420. Its UV (λ_{max} 285 nm) and ^{1}H NMR (δ = 7.83, 1H, H-2) spectra were characteristic of an isoflavone [6]. The downfield signal at δ = 13.00, exchangeable with D_2O, indicated the presence of chelated hydroxyl group at C-5. This was supported by the bathochromic shifts observed after adding $AlCl_3$ ($\Delta\lambda$ = 9 nm) and HCl ($\Delta\lambda$ = 14 nm). The IR spectrum showed maximum absorption bands at 3430 (free OH) and 1640 (C=O chelated) cm^{-1}. The ^{1}H NMR spectrum showed signals corresponding to one 2-hydroxy-3-methylbut-3-enyl unit at δ3.03 (1H, *dd*, J = 15.0, 8.7 Hz, H-1‴), 3.12 (1H, *dd*, J = 15.0, 2.5 Hz, H-1‴), 4.40 (1H, *dd*, J = 8.7, 2.5 Hz, H-2‴), 4.85 (1H, *d*, J = 1.0 Hz, H-4‴), 4.98 (1H, *d*, J = 1.0 Hz, H-4‴) and 1.88

Table 1. ^1H and ^{13}C NMR assignments of erysenegalensein L (**1**) and erysenegalensein M (**2**)

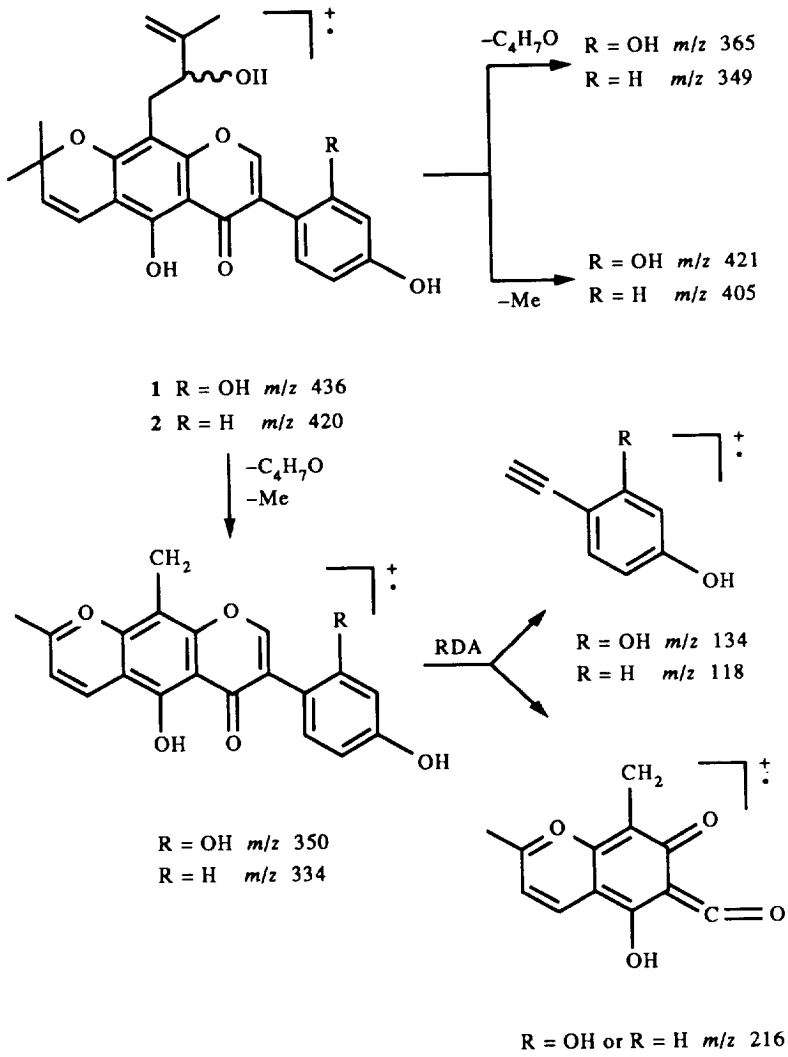
	1 ($CDCl_3$)		**2** $CDCl_3$	
Attribution	^{13}C	^1H (J Hz)	^{13}C	^1H (J Hz)
2	155.1	7.84 *s*	154.5	7.83 *s*
3	122.4		122.3	
4	182.1		181.9	
5	154.9		154.6	
6	105.6		105.4	
7	157.9		158.0	
8	104.3		104.2	
9	154.0		154.1	
10	105.3		105.1	
1′	111.6		123.2	
2′	158.5		130.2	7.32 *d*, J = 8.8
3′	106.0	6.46 *d*, J = 2.6	115.6	6.85 *d*, J = 8.8
4′	156.7		157.0	
5′	108.7	6.38 *dd*, J = 2.6, 8.2	115.4	6.85 *d*, J = 8.8
6′	130.7	6.91 *d*, J = 8.2	130.1	7.32 *d*, J = 8.8
2″	78.7		78.5	
3″	128.1	5.65 *d*, J = 10.0	128.2	5.64 *d*, J = 10.0
4″	115.5	6.75 *d*, J = 10.0	115.4	6.74 *d*, J = 10.0
5″	28.4	1.51 *s*	28.5	1.52 *s*
6″	28.4	1.51 *s*	28.5	1.52 *s*
1‴	28.8	3.02 *d*, J = 8.5, 15.0	28.9	3.03 *dd*, J = 8.7, 15.0
		3.10 *dd*, J = 2.6, 15.0		3.12 *dd*, J = 2.5, 15.0
2‴	75.5	4.38 *dd*, J = 2.6, 8.5	75.6	4.40 *dd*, J = 2.5, 8.7
3‴	146.8		146.4	
4‴	111.0	4.87 *d*, J = 1.0	110.8	4.85 *d*, J = 1.0
		0.99 *d*, J = 1.0		4.98 *d*, J = 1.0
5‴	18.0	1.90 *s*	17.8	1.88 *s*
5-OH	—	12.57 *s**		13.00 *s**
4′-OH		8.70 *s**		8.45 *s**
2‴-OH		5.40 *s**		5.30 *s**

*Exchanged on deuteration.

(3H, *s*, Me). This was confirmed by the EI spectrum which indicated an ion fragment at m/z 349 $[M - 71]^+$ [3]. Chemical shifts assigned to the protons of one dimethylchromene moiety were exhibited in the ^1H NMR spectrum at δ5.64 (1H, *d*, J = 10.0 Hz, H-3′), 6.74 (1H, *d*, J = 10.0 Hz, H-4″) and 1.52 (6H, *s*, 2 × Me). This was confirmed by the ion fragment at m/z 405 $[M - 15]^+$ [7]. Since there were no signals at 5.90–6.10 ppm characteristic of protons at C-6 and C-8, the two groups mentioned above were linked on ring A. This was supported by the ions at m/z 263, 216 and 118 resulting from the further RDA cleavage of the ions m/z 405 $[M - 15]^+$ and 349 $[M - 71]^+$ (Scheme 1). The fragment at m/z 118 belonged to ring B which carried one hydroxyl group; its assignment to the C-4′ position was done on the basis of the ^1H NMR spectrum which showed two doublets of four aromatic protons in an AA′BB′ system at δ7.32 (2H, *d*, J = 8.8 Hz, H-2′, H-6′) and 6.85 (2H, *d*, J = 8.8 Hz, H-3′, H-5′) (Table 1).

In order to establish the unambiguous positions of the dimethylchromene and the 2-hydroxy-3-methylbut-3-enyl groups on ring A, the 2D ^{13}C–^1H (HETCOR) and (COLOC) techniques were used as for **1**. The COLOC spectrum showed correlations between H-2 (δ7.83) and C-9 (δ154.1), H-1‴ (δ3.12) and C-9 (δ154.1), H-1‴ (δ3.12) and C-7 (δ158.10), H-1‴ (δ3.12) and C-8 (δ104.2). This confirmed clearly that the 2-hydroxy-3-methylbut-3-enyl linked at the position C-8 and consequently the chromone fused at the linear position. Furthermore, the comparison of the ^1H NMR spectra of (**2**) and its triacetate (**4**) indicated chemical shift variations for protons H-3″ ($\Delta\delta$ = − 0.12) and H-4″ ($\Delta\delta$ = + 0.26) and permitted the conclusion that the dimethylchromene fused at a linear position [10]. Thus, **2** is 5,4′-dihydroxy-8-(2″-hydroxy-3‴-methylbut-3‴-enyl)-2″,2″-dimethylpyrano [5″,6″:6,7] isoflavone and is named erysenegalensein M.

Erysodine (**5**), glucoerysodine (**6**) and hypaphorine (**7**) have been isolated from a number of different *Erythrina* species [11–13] and are now described for the first time from *E. senegalensis*. Very little treatment of the 2D ^1H and ^{13}C of *Erythrina* alkaloids has been reported in the literature [14, 15]. In this paper we report for the first time the complete assignments of ^1H and ^{13}C NMR chemical shifts using the 2D NMR (HETCOR and COLOC) techniques [9] (see experimental). These results have permitted us to confirm the ^{13}C NMR chemical shift attributions of (**5**) and (**6**) as previously reported in the literature [15].



Scheme 1.

EXPERIMENTAL

General. All mps. uncorr. Silica gels 60C (5–40 μm), 60H (20–40 μm) and 60 (70–230 mesh) were used for CC under compressed air (30 mbar) whilst silica gel 60 F254 or 1500/LS254 was used for TLC and prep. TLC. All NMR exps were performed at 300 and 75 MHz, respectively. Samples were dissolved in $CDCl_3$ or DMSO-d_6 and chemical shifts were referenced to int. TMS for ^1H NMR and to deuterated solvents for ^{13}C NMR. For the 2D NMR COLOC experiments, an optimized sequence has been developed for correlation via long-range coupling, notably $^3J_{C-H}$ [9].

Plant material. E. senegalensis (DC) stem bark and seeds were collected at Foumban, West Cameroon in April 1988. Herbarium specimens documenting the collection are described at the National Herbarium, Yaounde.

Extraction and isolation. Dried and ground stem bark of *E. senegalensis* (17 kg) were extracted with MeOH and evapd. The crude MeOH extract was then re-extracted with CH_2Cl_2 extract. Repeated CC on silica gel (using cyclohexane, CH_2Cl_2, EtOAc and MeOH) and TLC permitted the regrouping of the resulting fr. into series A–G. Further purification of series E (62–g) by CC and prep. TLC (solvents: CH_2Cl_2–MeOH, 19:1) gave **1** (16 mg) and **2** (20 mg).

Dried and ground seeds of *E. senegalensis* (510 g) were also extracted with MeOH and evapd to give 145 g MeOH extract. Repeated CC on silica gel (using cyclohexane, CH_2Cl_2, EtOAc and MeOH) and TLC afforded **5** (15 mg, cyclohexane–EtOAc, 1:1), **6** (3 g, CH_2Cl_2–MeOH, 17:3) and **7** (4 g, CH_2Cl_2–MeOH, 17:3).

Compound **1**. $[\alpha]_D^{20}$ + 19.0° ($CHCl_3$; *c* 1.308). IR ν_{max}^{KBr} 3440, 3300, 1640 cm^{-1}; UV [MeOH] nm (log ε): 286 (4.20); + [$AlCl_3$] nm (log ε): 294 (4.09); + [HCl] nm (log ε): 300 (4.09). CI/NH_3 MS *m/z* 437 $[M + H]^+$; EI-MS (probe) 70 eV, *m/z* (rel. int.): 436 $M]^+$ (30), 421 (50), 365 (100), 350 (10), 279 (10), 231 (10), 216 (12), 134 (20), 96 (25), 74 (45). ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1. Tetra-acetate (**3**). Gum. CIMS (NH_3, probe) 90 eV, *m/z* (rel. int.) 605 $[M + H]^+$ (100).

^{1}H NMR (300 MHz, $CDCl_3$) δ7.80 (1H, *s*, H-2), 7.24 (1H, *d*, J = 7.0 Hz, H-6'), 7.08 (1H, *d*, J = 1.2 Hz, H-3'), 6.98 (1H, *dd*, J = 7.0 Hz, J = 1.5 Hz, H-5'), 6.48 (1H, *d*, J = 9.0 Hz, H-4''), 5.76 (1H, *d*, J = 9.0 Hz, H-3''), 5.00 (1H, *br*, H-4'''), 4.88 (1H, *br*, H-4'''), 4.42 (1H, *t*, J = 6.0 Hz, H-2'''), 3.00 (2H, *dd*, J = 6.0 Hz, 3.0 Hz, H-1'''), 2.40 (3H, *s*, Ac), 2.29 (3H, *s*, Ac), 2.18 (3H, *s*, Ac), 2.10 (3H, *s*, Ac), 1.91 (3H, *s*, Me), 1.50 (6H, *s*, 2 × Me).

Compound **2**. Thick yellow oil; α_D^{20} + 24.0° ($CHCl_3$; *c* 1.405). IR ν_{max}^{KBr} 3430, 3300, 1640 cm^{-1}; UV [MeOH] nm (log ε): 285 (4.15); + [$AlCl_3$] nm (log ε): 294 (4.10); + [HCl] nm (log ε): 299 (4.10). CI/NH_3 MS *m/z* 421 [M + H]$^+$; EI-MS (probe) 70 eV, *m/z* (rel. int.): 420 [M]$^+$ (25), 405 (35), 349 (100), 334 (10), 263 (8), 231 (10), 216 (7), 118 (15), 80 (15), 80 (20), 58 (40). ^{1}H (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1. Tetra-acetate (**4**). Gum. CIMS (NH_3, probe) 90 eV, *m/z* (rel. int.) 547 [M + H]$^+$ (100). ^{1}H NMR (300 MHz, $CDCl_3$) δ7.78 (1H, *s*, H-2), 7.39 (2H, *d*, J = 8.5 Hz, H-2', H-6'), 6.82 (2H, *d*, J = 8.5 Hz, H-3'. H-5'), 6.49 (1H, *d*, J = 9.0 Hz, H-4''), 5.76 (1H, *d*, J = 9.0 Hz, H-3''), 4.99 (1H, *br*, H-4'''), 4.86 (1H, *br*, H-4'''), 4.46 (1H, *t*, J = 6.0 Hz, H-2'''), 3.02 (2H, *dd*, J = 6.0, 3.0 Hz, H-1'''), 2.42 (3H, *s*, Ac), 2.30 (3H, *s*, Me), 2.12 (3H, *s*, Ac), 1.90 (3H, *s*, Me), 1.50 (6H, *s*, 2 × Me).

Erysodine (**5**). Shiny needle crystals, mp 200–202°. CI/NH_3MS *m/z* 300 [M + H]$^+$. ^{1}H NMR (300 MHz, $CDCl_3$), δ6.57 (1H, *dd*, J = 10.0, 2.0 Hz, H-1), 6.00 (1H, *d*, J = 10.0 Hz, H-2), 3.98 (1H, *m*, H-3), 2.50 (1H, *m*, H-4), 1.80 (1H, *m*, H-4), 5.58 (1H, *d*, J = 2.0 Hz, H-7), 3.50 (1H, *m*, H-8), 3.60 (1H, *m*, H-8), 3.32 (1H, *d*, J = 11.3 Hz, H-10), 2.80 (1H, *d*, J = 7.0 Hz, H-10), 2.85 (1H, *d*, J = 13.0 Hz, H-11), 2.50 (1H, *d*, J = 13.0 Hz, H-11), 6.70 (1H, *s*, H-14), 6.80 (1H, *s*, H-17), 3.30 (3H, *s*, MeO), 3.70 (3H, *s*, MeO). ^{13}C NMR (75 MHz, $CDCl_3$), δ125.0 (C-1), 131.4 (C-2), 76.0 (C-3), 41.4 (C-4), 66.8 (C-5), 142.2 (C-6), 123.0 (C-7), 56.9 (C-8), 43.8 (C-10), 23.8 (C-11), 127.2 (C-12), 130.7 (C-13), 108.4 (C-14), 144.1 (C-15), 144.7 (C-16), 114.5 (C-17), 55.8 (OMe).

Glucoerysodine (**6**). Yellow crystals, mp 166–168°. CI/NH_3MS *m/z* 462 [M + H]$^+$. ^{1}H NMR (300 MHz, DMSO-d_6), δ6.57 (1H, *dd*, J = 10.0, 2.0 Hz, H-1), 6.00 (1H, *d*, J = 10.0 Hz, H-2), 3.92 (1H, *m*, H-3), 2.40 (1H, *m*, H-4), 1.60 (1H, *m*, H-4), 5.57 (1H, *d*, J = 2.0 Hz, H-7), 3.50 (1H, *m*, H-8), 3.40 (1H, *m*, H-8), 3.32 (1H, *d*, J = 11.3 Hz, H-10), 2.80 (1H, *d*, J = 7.5 Hz, H-10), 2.85 (1H, *d*, J = 13.0 Hz, H-11), 2.50 (1H, *d*, J = 15.0 Hz, H-11), 6.75 (1H, *s*, H-14), 6.85 (1H, *s*, H-17), 3.28 (3H, *s*, MeO), 3.60 (3H, *s*, MeO). 4.85 (1H, *d*, J = 7.0 Hz, H-1'), 3.25 (1H, *m*, H-2'), 3,32 (1H, *m*, H-3'), 3.15 (1H, *m*, H-4'), 3.28 (1H, *m*, H-5'), 3.45 (1H, *d*, J = 3.0 Hz, H-6'), 3.60 (1H, *d*, J = 3.0 Hz, H-6'), ^{13}C NMR (75 MHz, DMSO-d_6), δ126.3 (C-1), 132.4 (C-2), 76.9 (C-3), 42.6 (C-4), 67.2 (C-5), 143.0 (C-6), 124.2 (C-7), 57.3 (C-8), 44.0 (C-10), 24.3 (C-11), 127.8 (C-12), 133.9 (C-13), 111.3 (C-14), 146.4 (C-15), 147.8 (C-16), 116.9 (C-17), 56.8 (OMe), 56.9 (OMe), 101.4 (C-1'), 74.4 (C-2'), 78.1 (C-3'), (70.9 (C-4'), 78.3 (C-5'), 61.9 (C-6').

Hypaphorine (**7**). Shiny transparent crystals, mp > 260°. CI/NH_3 MS *m/z* 247 [M + H]$^+$. All IR, MS, ^{1}H and ^{13}C NMR spectral data were in agreement with literature [14].

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REFERENCES

1. Dalziel, J. M. (1937) *The Useful Plants of West Tropical Africa*, pp. 612–615. Crown Agents for Colonies, London.
2. Dyke, S. F. and Quessy, D. (1981) in *The Alkaloids–Chemistry and Physiology* (Rodrigo, R. G. A., ed.), Vol. 18, pp. 1–17. Academic Press, New York.
3. Wandji, J., Fomum, Z. T., Tillequin, F., Sequin, E. and Koch, M. (1994) *Phytochemistry* **35**, 245.
4. Wandji, J., Fomum, Z. T., Tillequin, F., Skaltsounis, A. L. and Koch, M. (1994) *Planta Med.* **60**, 178.
5. Wandji, J., Fomum, Z. T. Tillequin, F., Baudouin, G. and Koch, M. (1994) *Phytochemistry* **35**, 1573.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
7. Takayama, M., Fukai, T., Hano, Y. and Nomura, T. (1992) *Heterocycles* **33**, 405.
8. Agrawal, P. K. (1989) *Carbon-13 NMR of Flavonoids*, pp. 5–230. Elsevier, Amsterdam.
9. Croasmum, W. R. and Carlson, M. K. (1987) in *Two Dimensional NMR Spectroscopy: Applications for Chemists and Biochemists*, pp. 199–201. VCH Publishers, New York.
10. Arnone, A., Cardillo, G., Merlini, L. and Mondelli, R. (1967) *Tetrahedron Letters* 4201.
11. Lapière, C. (1949) *Bull. Soc. Chim. Biol.* **31**, 862.
12. Lapière, C. (1951) *J. Pharm. Belg.* **6**, 71.
13. Millington, D. S., Steinman, D. H. and Rinehart, K. L. Jr, (1974) *J. Am. Chem. Soc.* **96**, 1906.
14. Sarragiotto, M. H., Filho, H. L. and Marsaioli, A. J. (1981) *Can. J. Chem.* **59**, 2771.
15. Chawla, A. S., Chunchatprasert, S. and Jackson, A. H. (1983) *Organic Magnetic Resonance* **21**, 39.
16. Nkengfack, A. E., Vardamides, J. C., Meyer, M. and Fomum, Z. T. (1994) *Phytochemistry*, (in press).