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Prenylated isoflavonoids from *Erythrina senegalensis* ☆

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Abstract

Two prenylated isoflavonoids, erysenegalensein N, 5,7,2′,4′-tetrahydroxy-6-(2″-hydroxy-3″-methylbut-3″-enyl)-8-(γ,γ-dimethylallyl) isoflavone and erysenegalensein O, 5,2′,4′-trihydroxy-6-(γ,γ-dimethylallyl)-3‴-hydroxy-2‴, 2‴-dimethyldihydropyrano[5‴,6‴;8,7]isoflavone, in addition to six known compounds were isolated from the stem bark of *Erythrina senegalensis* (Leguminosae). Their structures were elucidated by spectroscopic methods. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Erythrina senegalensis*; Leguminosae; Prenylated isoflavonoids; Erysenegalensein N and O

1. Introduction

The genus *Erythrina* (Leguiminosae) contains numerous species distributed in tropical and subtropical regions (Bever, 1986). Extracts of leaves, barks and roots of the genus *Erythrina* have a significant history of medicinal use for treatment of diseases such as female infertility, stomach pain and gonorrhea (Irvine, 1961). In recent years, many studies have been carried out on the neutral compounds of this genus. As a continuation of studies on the non-alkaloidal components of *Erythrina senegalensis* (Fomum, Ayafor & Wandji, 1985; Wandji, Fomum, Tillequin, Baudouin & Koch, 1994; Wandji et al., 1990), we now report the isolation and structural elucidation of two prenylated isoflavonoids named erysenegalensein N (**1**) and O (**2**), together with six known isoflavonoids, erysenegalensein D (**3**), 8-prenylleutone (**4**), auriculatin (**5**), derrone (**6**), alpinumisoflavone (**7**) and 6,8-diprenylgenistein (**8**).

2. Results and discussion

Column chromatography (silica gel, ODS-18, Sephadex LH-20, HPLC) of the CH_2Cl_2 extract of the stem bark of *Erythrina senegalensis* afforded two compounds (**1** and **2**). Compound **1**, erysenegalensein N, was obtained as a pale yellow oil, $[\alpha]_D^{20} = -3.3$ (MeOH; c = 0.8). The molecular formula of **1** was established as $C_{25}H_{26}O_7$ by HR-EIMS, *m*/*z* 438.1661 (438.1678 calcd. for $C_{25}H_{26}O_7$).

A brown color in the $FeCl_3$ test and maximum absorption at 268 nm in UV spectrum (MeOH) suggested the presence of an isoflavonoid or flavonoid skeleton. In the ^1H NMR spectrum, the singlet observed at δ 8.02 ppm was characteristic of the isoflavone skeleton. It also showed a doublet at δ 3.38 ppm (2H, *J* = 6.5 Hz, H-1‴), a triplet at δ 5.16 ppm (1H, *J* = 6.5 Hz, H-2‴) and two singlets at 1.63 ppm (Me-*trans*) and 1.76 ppm (Me-*cis*), which indicated the pre-

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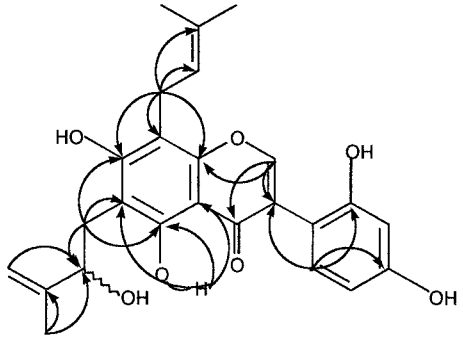
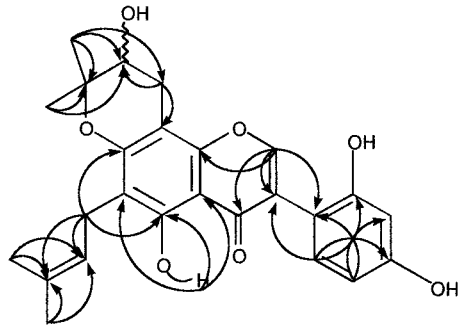


Erysenegalensein N (**1**)



Erysenegalensein O (**2**)

Fig. 1. Significant correlations observed in the HMBC spectra of erysenegalensein N (**1**) and erysenegalensein O (**2**).

sence of a prenyl group. The presence of a five-carbon hydroxylated unit linked to an aromatic ring was identified by two benzylic proton signals at δ 2.84 ppm (1H, *dd*, $J=8.0$, 14.5 Hz, H-1″), 2.94 ppm (1H, *dd*, $J=2.0$, 14.5 Hz, H-1″) and an oxymethine proton at δ 4.23 ppm (1H, *d*, $J=8.0$ Hz, H-2″). Two singlets at δ 4.74 ppm (1H) and 4.88 ppm (1H) were assigned to two *gem*-olefinic protons. The ^{1}H and ^{13}C NMR spectral data of **1** were similar to those of the co-isolated erysenegalensein D (**3**). Furthermore, the EIMS data of **1** and **3** showed the same ion fragments at *m/z* 367 $[M—C_4H_7O]^+$, 311 $[M—C_4H_7O–C_4H_8]^+$, 177 and 134 resulting from the *retro*-Diels-Alder (RDA) cleavage of the more stable ion at *m/z* 311 (Wandji, Fomum, Tillequin, Seguin & Koch, 1994). From these data, we concluded that **1** and **3** had the same skeleton except for the position of the prenyl units at C-6 and C-8 of **1** and **3**. Thus, in order to deduce their positions, heteronuclear multiple-bond correlation (HMBC) experiments were used. Long-range couplings of **1** from H-1″ (δ 2.84 and 2.94) to C-5 (156.93), C-6 (109.10), C-7 (161.72) and C-2″ (74.69), from 5-OH (δ 13.38) to C-5, C-6 and C-10 (103.79), from H-1‴ (δ 3.38) to C-7, C-8 (106.17), C-9 (153.20), C-2‴ (122.51) and C-3‴ (130.65) indicated that the unsubstituted prenyl group is situated at C-8, while the hydroxylated prenyl group is linked to the C-6 position on the contrary position of **3** (Fig. 1). From the above spectroscopic data, compound **1** was determined to be a new isoflavone, 5,7,2′,4′-tetrahydroxy-6-(2″-hydroxy-3″-methylbut-3″-enyl)-8-(γ,γ-dimethylallyl)isoflavone, and was named erysenegalensein N (**1**).

Compound **2**, erysenegalensein O, was obtained as a pale yellow oil, and has a molecular formula of $C_{25}H_{26}O_7$ as deduced from its HR-EIMS data which showed a molecular ion peak at *m/z* 438.1668 (calcd. 438.1678). Its UV spectrum ($\lambda_{max}=268$ nm in MeOH) and its ^{1}H NMR spectrum were characteristic of an isoflavone. Mass spectral fragments at *m/z* 395 $[M—43]^+$, 383 $[M—55]^+$ and ^{1}H NMR signals at δ 3.27 ppm (2H, *d*, $J=7.2$ Hz, H-1″), δ 5.20 ppm (1H, *t*, H-2″), 1.66 ppm (3H, *s*) and 1.79 ppm (3H, *s*) were consistent with the presence of a 3,3-dimethylallyl group. There were also chemical shifts indicating the presence of a 3-hydroxy-2,2-dimethyldihydro-pyran group (Wandji, Fomum, Tillequin, Skaltsounis & Koch, 1994), namely the geminal dimethyl proton peaks at δ 1.28 and 1.36 ppm, one oxymethine at δ 3.78 ppm (1H, *t*, $J=6.2$ Hz, H-3‴), and two diastereotopic proton peaks at δ 2.64 ppm (1H, *dd*, $J=7.0$, 16.5 Hz, H-4‴) and 2.98 ppm (1H, *dd*, $J=5.2$, 16.5 Hz, H-4‴). Thus, in order to decide the positions on ring A to which the prenyl and dihydropyran units are attached, HMBC and heteronuclear multiple quantum coherence (HMQC) experiments were used. Long-range couplings of H-1″ of the prenyl units of **2** (δ 3.27) to C-5 (155.67), C-7 (156.41), C-2″ (121.96) and C-3″ (130.52), also from 5-OH (δ 13.10) to C-5, C-6 (111.01) and C-10 (104.83) indicated that the prenyl group is linked to C-6 of ring A. Furthermore, the long-range couplings from H-6‴ (δ 1.28) of the dihydropyran ring to C-2‴ (78.64), C-3‴ (66.76), C-4‴ (24.99) supported that the dihydropyran ring is linked at C-8 position (Fig. 1). From the above spectroscopic data, compound **2** was elucidated to be a new isoflavone, 5,2′,4′-trihydroxy-6-(γ,γ-dimethylallyl)-3‴-hydroxy-2‴,2‴-dimethyldihydropyrano [5‴,6‴;8,7]isoflavone, and was named erysenegalensein O.

3. Experimental

3.1. General

HR-EIMS spectra were obtained on VG Autospec Ultma mass spectrometer. IR spectra were run on Bomen MB-100, and UV spectra on a Beckman Du-64 instrument. All NMR experiments were performed on a Varian Unity 500 spectrometer (^{1}H: 500 MHz, ^{13}C: 125 MHz). Samples were run in DMSO-*d*6 and chemical shifts expressed in ppm. HPLC apparatus were

Table 1
^1H and ^{13}C NMR spectral data of erysenegalensein N (**1**) and erysenegalensein O (**2**)

	(**1**) DMSO-*d*6		(**2**) DMSO-*d*6	
Positions	^1H	^{13}C	^1H	^{13}C
1	–	–	–	–
2	8.18, *s*	154.93	8.23, *s*	155.21
3	–	119.90	–	120.49
4	–	180.50	–	180.68
5	–	156.93	–	155.67
6	–	109.10	–	111.01
7	–	161.72	–	156.41
8	–	106.17	–	98.54
9	–	153.20	–	152.78
10	–	103.79	–	104.83
1′	–	108.88	–	108.73
2′	–	156.36	–	156.30
3′	6.39, *s*	102.61	6.38, *s*	102.53
4′	–	158.43	–	158.56
5′	6.28, *d*, *J*=8.0	106.16	6.31, *d*, *J*=8.2	106.18
6′	6.97, *d*, *J*=8.0	132.04	7.00, *d*, *J*=8.2	132.05
1″	2.84, *dd*, *J*=8.0, 14.5 2.94, *dd*, *J*=2.0, 14.5	28.88	3.27, *d*, *J*=7.2	21.05[a]
2″	4.23, *d*, *J*=8.0	74.69	5.20, *t*, *J*=7.2	121.96
3″	–	147.05	–	130.52
4″	4.74, *s* 4.88, *s*	109.74	1.79, *s*	17.74
5″	1.76, *s*	18.05	1.66, *s*	25.28
1‴	3.38, *d*, *J*=6.5	21.36	–	–
2‴	5.16, *t*, *J*=6.5	122.51	–	78.64
3‴	–	130.65	3.78, *t*, *J*=6.2	66.76[a]
4‴	1.76, *s*	17.63	2.64, *dd*, *J*=7.0, 16.5 2.98, *dd*, *J*=5.2, 16.5	24.99[a]
5‴	1.63, *s*	25.39	1.36, *s*	25.51
6‴	–	–	1.28, *s*	20.87
5-OH	13.38, *s*	–	13.10, *s*	–
2′-OH	9.35, *s*	–	9.41, *s*	–
4′-OH	9.35, *s*	–	9.35, *s*	–

[a] Assignment of these carbons was used by HMQC.

consisted of Shimadzu model LC-6AD pump, SPD-10A UV detector and C-R6A integrator. For preparative-scale HPLC conditions were as follows: column: J′ sphere ODS H-80 (S—4 μm), i.d. 20 × 150 mm; mobile phase: 75% MeOH (v/v); flow rate: 4 ml/min.

3.2. Plant materials

The stem bark of *Erythrina senegalensis* (DC) was collected at Foumban, west Cameroon, in April 1988. Herbarium specimens documenting the collection have been deposited at the National Herbarium, Yaounde.

3.3. Extraction and isolation

Dried and ground stem bark of *Erythrina senegalensis* (17 kg) was extracted with MeOH. The evaporated MeOH extract was then re-extracted with CH_2Cl_2 to give 580 g of extract. Successive column chromatography followed by TLC analysis gave several fractions labeled in series as A–G. Series G (20 g) was subjected to Sephadex LH-20 (Sigma Co.) column chromatography with $CHCl_3$-MeOH-n-hexane (2:1:3) to give three fractions (I–III). Fraction I (912 mg) was rechromatographed on ODS-RP18 (70 ~ 230 mesh, YMC Co.) with MeOH-H_2O (6:4 → 8:2, stepwise) to give **4** (137 mg) and **5** (230 mg). Fraction II (120 mg) was further purified by HPLC (YMC-Pack ODS-AM, i.d. 6.0 × 250 mm) to afford the three related compounds, **1** (67 mg), **2** (4.7 mg) and **3** (4.2 mg). Finally, the fraction III (150 mg) was subjected to ODS RP-18 with MeOH-H_2O (8:2) to give **6** (25 mg), **7** (13 mg) and **8** (8 mg).

3.3.1. Compound *1*

Pale yellow oil (67 mg), $[\alpha]_D^{20} = -3.3$ (MeOH; c = 0.8). UV (MeOH) λ_{max} 268 nm. IR ν_{max} 3325, 1620, 1431, 1079 cm^{-1}. HR-EIMS *m*/*z* 438.1661 (calcd. for $C_{25}H_{26}O_7$ 438.1678), EIMS *m*/*z* 438 $[M]^+$ (10), 367 (63), 311 (100), 293 (13), 177 (19), 134 (6). ^1H and ^{13}C NMR spectral data are listed in Table 1.

3.3.2. Compound *2*

Pale yellow oil (4.7 mg). UV (MeOH) λ_{max} 268 nm. HR-EIMS *m*/*z* 438.1668 (calcd. for $C_{25}H_{26}O_7$ 438.1678), EIMS *m*/*z* 438 $[M]^+$ (89), 423 (11), 395 (92), 383 (100), 323 (29), 311 (66), 293 (16), 231 (16), 217 (19). ^1H and ^{13}C NMR spectral data are listed in Table 1.

3.3.3. Erysenegalensein D (*3*)

Yellow oil (4.2 mg). UV (MeOH) λ_{max} 268 nm. IR ν_{max} 3400, 1652, 1558, 1097 cm^{-1}. HR-EIMS *m*/*z* 438.1619 (calcd. for $C_{25}H_{26}O_7$ 438.1678), EIMS *m*/*z* 438 $[M]^+$ (10), 367 (48), 311 (100), 293 (14), 177 (27), 167 (22), 149 (73), 134 (8). ^1H and ^{13}C NMR spectral data were in agreement with literature values (Wandji et al., 1994).

3.3.4. 8-Prenylleutone (*4*)

Yellow oil (137 mg). HR-EIMS *m*/*z* 422.1969 (calcd. for $C_{25}H_{26}O_6$ 422.1787) EIMS; *m*/*z* 422 $[M]^+$ (100), 379 (69), 367 (14), 351 (36), 323 (37), 311 (86), 217 (11). ^1H and ^{13}C NMR spectral data were in agreement with literature values (Nkengfack, Sanson, Fomum & Tempesta, 1989).

3.3.5. Auriculatin (*5*)

Yellow powder (230 mg). HR-EIMS *m*/*z* 420.0306 (calcd. for $C_{25}H_{24}O_6$ 420.1573); EIMS *m*/*z* 420 $[M]^+$ (55), 405 (100), 377 (18), 365 (20). ^1H and ^{13}C NMR

spectral data were in agreement with literature values (Taylor et al., 1986).

*3.3.6. Derrone (**6**)*

White needles (25 mg). EIMS *m/z* 336 $[M]^+$ (18), 321 (100), 203 (36), 135 (11), 118(25). The differences between **6** and **7** were determined by ^{13}C-^{1}H COSY spectroscopy. δ 6.24 ppm (*s*, 1H, Ar–H) was shifted from δ 99.36 ppm on this compound. ^{1}H NMR spectral data were in agreement with literature values (Chibber & Shapma, 1980).

*3.3.7. Alpinumisoflavone (**7**)*

Yellow oil (13 mg). EIMS *m/z* 336 $[M]^+$ (21), 321 (100), 161 (9), 152 (4). In the ^{13}C-^{1}H COSY spectrum, δ 6.54 ppm (*s*, 1H, Ar–H) was shifted from δ 94.73 ppm on this compound. ^{1}H and ^{13}C NMR spectral data were in agreement with literature values (Agrawal, 1989; Khalid & Waterman, 1983).

*3.3.8. 6,8-Diprenylgenistein (**8**)*

Yellow powder. (8 mg). EIMS *m/z* 406 $[M]^+$ (51), 363 (45), 351 (33), 307 (70), 295 (100), 217 (4), 189 (9). ^{1}H and ^{13}C NMR spectral data were in agreement with literature values (Fomum, Ayafor, Ifeadike, Nkenfack & Wandji, 1986).

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Appendix A

Prenylated isoflavonoids from *Erythrina senegalensis*

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Two prenylated isoflavonoids named erysenegalensein N and O, together with six known compounds, were isolated from the stem bark of *Erythrina senegalensis* (Leguminosae).

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