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SIGMOIDINS J AND K, TWO NEW PRENYLATED ISOFLAVONOIDS FROM *ERYTHRINA SIGMOIDEA*[1]

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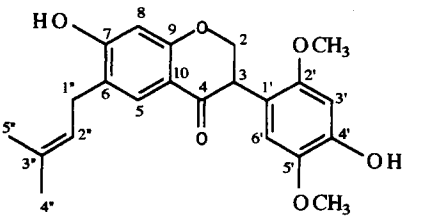
ABSTRACT.—In addition to the two known compounds neorautenol [**2**] and erythrinassinate B [**4**], two new compounds, an isoflavanone named sigmoidin J [**1**] and a coumestan derivative named sigmoidin K [**3**], have been isolated and characterized from the root bark of the Cameroonian medicinal plant *Erythrina sigmoidea*. Their structures have been established as 7,4′-dihydroxy-2′,5′-dimethoxy-6-(γ,γ-dimethylallyl)isoflavanone [**1**] and 3,9-dihydroxy-2,10-(γ,γ-dimethylallyl) coumestan [**3**], respectively, by spectroscopic techniques and from chemical evidence.

The legume subfamily Papilionoideae is a rich source of alkaloids, coumarins, terpenoids, and flavonoids (1–4). We have recently undertaken the investigation of several *Erythrina* species from which we have isolated a wide range of flavonoids (2,5–7) some of which possess pronounced antibacterial, antifungal (5,8), antiarrhythmic, and muscle relaxant properties (9). In previous papers (10,11), we have reported the isolation and characterization of one novel coumestan (4-hydroxycoumestrol) and two novel isoflavanones (sigmoidins H and I) along with seven known flavonoids (isoflavones, pterocarpans, a flavanone, and a chalcone) from the root bark of *Erythrina sigmoidea* Hua, a species used in Cameroonian traditional medicine for the treatment of several conditions such as female infertility, stomach pain, and gonorrhea (12). In a continuation of this work, we now describe the isolation and structure elucidation of two new compounds, an isoflavanone derivative, sigmoidin J [**1**] and a coumestan, sigmoidin K [**3**], from the root bark of *Erythrina sigmoidea*. These compounds were isolated along with the known



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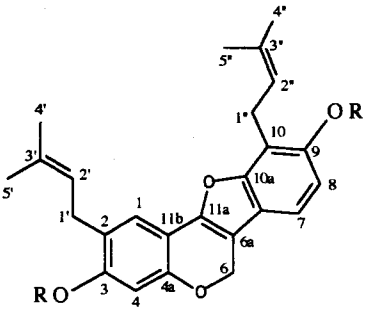

3 R=H
3a R=Ac

neoautenol [**2**] (13) erythrabyssin II [**6**] (3,14), erythrinassinate B [**4**] (15), and neobavaisoflavone [**5**] (16).

A crude MeOH extract of the powdered root bark of *E. sigmoidea* was suspended in H_2O and successively partitioned into *n*-hexane, CH_2Cl_2 and *n*-BuOH-soluble extracts. The CH_2Cl_2 and hexane extracts were found to be active against *Staphylococcus aureus*, a Gram-posi-

[1]Part 30 in the series "*Erythrina* Studies." For part 29, see Wandji *et al.* (26).

tive bacterial species, when tested using a streak-dilution technique (17). The CH_2Cl_2 extract, on chromatographic separation, afforded a novel isoflavanone named sigmoidin J [**1**] along with the known pterocarpan neorautenol [**2**] (13). Also, Si gel chromatography of the hexane extract yielded the novel coumestan derivative, sigmoidin K [**3**], in addition to the known erythrinassinate B [**4**] (15), neobavaisoflavone [**5**], and erythrabyssin II [**6**] (10,14,16), all previously isolated from a CH_2Cl_2 extract of the root bark of *E. sigmoidea* (10). The known compounds **2** and **4–6** were identified by direct comparison of their physical and spectral data (mmp, ir, uv, ^{1}H- and ^{13}C-nmr) with authentic samples and corresponding published values (13–16). Bioassays on purified neobavaisoflavone [**6**] showed that it exhibited significant in vitro antibacterial activity against *S. aureus* with an MIC value of 3.2 μg/ml (10) and antifungal activity against *Aspergillus fumigatus* and *Cryptococcus neoformans* with MIC values of 50 μg/ml (11).

Sigmoidin J [**1**], mp 262°, $[\alpha]^{22}D$ 0°, was obtained as green prisms from hexane/EtOAc. Its molecular formula, $C_{22}H_{24}O_6$, was assigned from the hrms which showed a molecular ion at *m/z* 384.1563 (calcd 384.1573). Its ir spectrum exhibited absorption bands at 3415 (free OH) and 1648 cm^{-1} (conjugated carbonyl). In the ^{1}H-nmr spectrum in Me_2CO-d_6 (Table 1), signals characteristic for isoflavanones were found. In particular, the AMX spin system at δ 4.37 (dd, J=10.8 and 5.4 Hz), δ 4.50 (t, J=11.5 Hz) and at δ 4.16 ppm (dd, J=11.9 and 5.4 Hz) are typical for equatorial H-2, axial H-2, and H-3β, respectively, in the isoflavanone skeleton (18). The uv spectrum (λ max 279 and 319 nm) and HETCOR nmr experiments supported this concept by correlating these nmr protons with the ^{13}C-nmr signals at δ 71.7 and δ 48.1 ppm due to C-2 and C-

TABLE 1. ^{1}H- and ^{13}C-Nmr Assignments for Sigmoidin J [**1**].[a]

Position	δ ^{13}C	δ ^{1}H [multiplicity, J (Hz)]	LR-HETCOR Coupling to C	NOESY Coupling to H
2	71.7 t	eq 4.37 (dd, 10.8, 5.4)	3, 4, 9, 1′	—
		ax 4.50 (dd, 11.5)	3, 4, 9, 1′	2′-OMe
3	48.1 d	4.16 (dd, 11.9, 5.4)	2, 4, 1′, 2′	6′
4	191.6 s	—	—	—
5	128.6 d	7.61 (s)	4, 7, 9, 1″	1″, 2″
6	123.6 s	—	—	—
7	162.8 s	—	—.	—
8	102.7 d	6.39 (s)	6, 7, 9, 10	—
9	162.5 s	—	—	—
10	115.3 s	—	—	—
1′	115.1 s	—	—	—
2′	153.2 s	—	—	—
3′	101.2 d	6.54 (s)	1′, 2′, 4′, 5′	2′-OMe
4′	147.5 s	—	—	—
5′	141.9 s	—	—	—
6′	115.5 d	6.73 (s)	3, 1′, 2′, 4′, 5′	3, 5′-OMe
1″	28.1 t	3.25 (d, 7.2)	5, 6, 7, 2″, 3″	5, 4″
2″	123.1 d	5.32 (m)	1″, 4″, 5″	5, 5″
3″	132.7 s	—	—	—
4″	25.8 q	1.71 (s)	2″, 3″, 5″	2″
5″	17.7 q	1.69 (s)	2″, 3″, 4″	—
2′-OMe	56.4 q	3.69 (s)	2′	2a, 3′
5′-OMe	57.1 q	3.71 (s)	5′	6′

[a]Measured at 500 MHz (^{1}H nmr) and 125.7 MHz (^{13}C nmr) in Me_2CO-d_6. Chemical shifts are expressed in ppm.

3, respectively (Table 1). The signal at δ 191.6 was assigned to C-4 (18,19). The presence of two methoxy groups and one *C*-fused prenyl group was inferred from two 3H singlets at δ 3.69 and δ 3.71 ppm and a set of signals at δ 5.32 (1H, m, H-2″), 3.25 (2H, d, J=7.2 Hz, H-1″), 1.71 (3H, s, H-4″) and 1.69 ppm (3H, s, H-5″) in the ^{1}H-nmr spectrum of **1**. Furthermore, four aromatic protons formed two para-coupled paired signals with four one-proton singlets at δ 7.61, 6.73, 6.54, and 6.39 ppm. Long-range coupling from δ 7.61 to C-4 is consistent with a proton occurring at position 5. This proton also showed coupling to C-1″ in the prenyl group and to carbons at δ 162.8 and δ 162.5 ppm, which is consistent with the presence of a prenyl group at position 6 and a hydroxyl group at position 7. ^{1}H-^{1}H NOESY nmr correlations (Table 1) from the δ 7.61 resonance to the prenyl group confirmed this structure. The aromatic proton at δ 6.39 was assigned to position 8 due to the observation of long-range coupling with the aromatic carbons in ring A. The methoxyl protons at δ 3.69 as well as H-3 showed long-range coupling to the aromatic carbon at δ 153.2 ppm, and the proton at δ 6.73 was long-range coupled to C-3. This is consistent only with a methoxyl group occurring at position 2′ and a hydrogen at position 6′. This inference was also confirmed by the NOESY spectrum. The 2′-methoxyl protons also exhibited nOe effects with the signal at δ 6.54 (H-3′). The δ 3.71 methoxyl group gave NOESY coupling to H-6′ but not to H-3′ which argues for a methoxyl group at position 5′ and a hydroxyl group at position 4′.

The structure of compound **1** was also confirmed by its eims which showed a molecular ion at *m/z* 384, an A-ring fragment with one hydroxyl and one prenyl at *m/z* 205, and a prominent B-ring fragment with one hydroxyl and two methoxyl substituents at *m/z* 180, formed by the retro-Diels-Alder cleavage. The A-ring fragment also gave rise to one ion at *m/z* 149 consistent with the loss of C_4H_8, and the B-ring fragment lost CH_3 to form an ion at *m/z* 165. From all of the above spectroscopic observations, sigmoidin J [**1**] was assigned as 7,4′-dihydroxy-2′,5′-dimethoxy-6-(γ,γ-dimethylallyl)isoflavanone.

Sigmoidin K [**3**], mp 95°, obtained as brown prisms from hexane/CH_2Cl_2, gave a positive reaction in the $FeCl_3$ test. Mass spectral analysis of compound **3** was not fruitful, as neither eims nor fabms gave any useful spectral information. The ir spectrum of **1** showed strong absorption bands at 3400 (free OH), 1720 (carbonyl), 1615, 1510, 1490 (aromatic absorption), and 1265 cm^{-1} (ether function). Acetylation of sigmoidin K [**3**] with Ac_2O in pyridine afforded a diacetate derivative [**3a**], indicating the presence of two hydroxyl groups in **3**. The structure of compound **3** was deduced mainly from the nmr spectral data of the diacetate **3a** (Table 2). HMQC and HMBC nmr experiments enabled the identification of two separate aromatic sub-structures, each containing one acetyl group ortho- to a prenyl group. One of these rings had two para-hydrogens, and the other had two ortho-hydrogens. The singlet proton at δ 7.82 ppm gave an HMBC correlation to the carbon at δ 160.0, and the proton at δ 7.95 in the other ring gave an HMBC correlation to the carbon at δ 105.5. The remaining carbon at δ 157.8 did not show any long-range coupling at all. The chemical shifts of these three carbons and the uv maximum at 345 nm could be explained for a coumarin-like ring system with oxygen substitution at the β-carbon and aromatic substitution at the α-carbon to the carbonyl group (20,21). This led to the conclusion that **3** is a coumestan, and that the signals at δ 157.8, δ 105.5, and δ 160.0 correspond to C-6, C-6a, and C-11a, respectively.

The proton signal at δ 7.82 was assigned to H-1, since it gave an HMBC correlation to C-11a. This proton also correlates to one of the prenyl groups,

TABLE 2. ^{1}H- and ^{13}C-Nmr Assignments for Sigmoidin K Diacetate [**3a**].[a]

Position	δ ^{13}C	δ ^{1}H [multiplicity, *J* (Hz)]	LR-HETCOR Coupling to C	NOESY Coupling to H
1	122.1 d	7.82 (s)	4a, 11a, 1′	1′, 2′
2	131.2 s	—	—	—
3	151.5 s	—	—	—
4	111.8 d	7.24 (s)	2, 4a, 11b	—
4a	152.4 s	—	—	—
6	157.8 s	—	—	—
6a	105.5 s	—	—	—
6b	121.3 s	—	—	—
7	119.2 d	7.95 (d, 8.4)	6a, 9, 10a	8
8	120.4 d	7.15 (d, 8.3)	6b, 9, 10	7
9	147.5 s[b]	—	—	—
10	119.0 s	—	—	—
10a	154.6 s[b]	—	—	—
11a	160.0 s	—	—	—
11b	110.6 s	—	—	—
1′	28.3 t	3.35 (d, 7.1)	1, 2, 3, 2′, 3′	1, 2′, 5′
2′	120.3 d	5.30 (m)	—	1, 1′, 4′
3′	134.7 s	—	—	—
4′	25.8 q	1.82 (s)	2′, 3′, 5′	2′
5′	17.9 q	1.75 (s)	2′, 3′, 4′	1′
1″	23.8 t	3.63 (d, 7.2)	9, 10, 10a, 2″, 3″	2″, 5″
2″	120.3 d	5.25 (m)	—	1″, 4″
3″	133.2 s	—	—	—
4″	25.7 q	1.72 (s)	2″, 3″, 5″	2″
5″	17.8 q	1.90 (s)	2″, 3″, 4″	1″
Ac-Me	20.8 q	2.37 (s)	Ac-CO	—
Ac-Me	20.8 q	2.37 (s)	Ac-CO	—
Ac-CO	168.6 s	—	—	—
Ac-CO	169.5 s	—	—	—

[a]Chemical shifts (δ, $CDCl_3$) are in ppm relative to TMS (multiplicity, *J* in Hz) at 500 MHz (^{1}H nmr) and 125.7 MHz (^{13}C nmr).

[b]Interchangeable.

which indicates that the prenyl group is ortho- to H-1. This was confirmed by the NOESY correlation between H-1 and H_2-1′.

The other part of the coumestan exhibited two vicinal protons at δ 7.95 and δ 7.15 ppm, one hydroxyl group and one prenyl group. The proton at δ 7.95 gave an HMBC coupling to C-6a and was therefore assigned to C-7. The H-7 signal also gave HMBC correlations to δ 147.5 and δ 154.6 (C-9 and C-10a), both oxygenated aromatic carbons, indicating that C-7 and the carbon bearing the prenyl group were oriented in a para- manner. This was confirmed by HMBC correlations between H_2-1″ and C-9, C-10a and the signal at δ 119.0 (which consequently must be C-10), placing the prenyl group at C-10, in addition to the HMBC correlations between H-8 and C-10 as well as δ 121.3 (which consequently must be C-6b). From the above spectroscopic studies, sigmoidin K [**3**] was assigned as 3,9-dihydroxy-2,10-(γ,γ-dimethylallyl) coumestan.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mps were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature in MeOH. Ir spectra were recorded on a Nicolet 20 DBX spectrophotometer and uv spectra with a Shimadzu UV-220 spectrophotometer. Mass spectra were measured with a JMS-DX 300 mass spectrometer. ^{1}H- and ^{13}C-nmr

spectra were performed on a Varian Gemini-500 instrument equipped with a 5 mm ^1H and ^{13}C probe, operating at 500.13 MHz and 125.77 MHz, respectively. Samples were run in DMSO-d_6, Me_2CO-d_6, or $CDCl_3$ and chemical shifts were referenced to internal TMS (0.00 ppm) for ^1H nmr and to deuterated solvents for ^{13}C nmr. For ^1J C-H correlations, the HETCOR sequence according to Bax and Morris (22) was used and for long-range correlation, the COLOC technique (23) was used. Inverse heteronuclear correlations were carried out using the sequences of Bax and Subramanian for HMQC (24) and Bax and Summers for HMBC (25).

PLANT MATERIAL.—*Erythrina sigmoidea* Hua (Fabaceae) root bark was collected at Foumban in western Cameroon, in June 1992. Voucher material documenting the collection was identified by the Director of the National Herbarium, Yaoundé, Cameroon, and is on deposit there.

EXTRACTION AND ISOLATION.—Dried ground root bark (10 kg) was extracted in a Soxhlet with MeOH. The residue left after the removal of solvent (900 g) was dissolved in MeOH-H_2O (1:1). The aqueous solution was extracted successively with *n*-hexane, CH_2Cl_2, and *n*-BuOH. Evaporation of these various extracts under reduced pressure gave 46 g (0.4%) of a hexane extract and 200 g (2%) of a CH_2Cl_2 extract. The *n*-BuOH extract consisted mainly of tannins. The hexane and CH_2Cl_2 extracts were examined during this investigation.

The hexane extract was subjected to cc over Si gel (350 g) packed in hexane. Gradient elution was effected with hexane, hexane/EtOAc, and EtOAc mixtures. A total of 400 fractions of about 200 ml per fraction was collected and combined on the basis of tlc analysis.

Combined fractions 56–77, eluted with hexane-EtOAc (19:1), were evaporated, and the residue (3.5 g) was crystallized from hexane/EtOAc to give erythrinassinate B [**4**] as colorless crystals (2.5 g), R_f 0.70 [C_6H_{12}-Me_2CO (9:1)], mp 76° [lit. (15) 80–81°]. Fractions 231–238, eluted with hexane-EtOAc (7:1), were rechromatographed on Si gel (petroleum ether/EtOAc gradient) to yield erythrabyssin II [**5**] as whitish crystals (80 mg), R_f 0.46 [C_6H_{12}-Me_2CO (7:3)], mp 156° [lit. (14) 152–153°]. Fractions 327–368, eluted with hexane-EtOAc (3:1), were evaporated and the residue (100 mg) was easily crystallized from petroleum ether/EtOAc to afford sigmoidin K [**3**]. Fractions 366–373, eluted with hexane-EtOAc (7:3), were further purified by flash chromatography on Si gel with hexane-EtOAc (8:2) to give, after crystallization from CH_2Cl_2/MeOH, neobavaisoflavone [**6**] as a white powder (30 mg), R_f 0.28 [C_6H_{12}-Me_2CO (7:3)], mp 198–199° [lit. (16) 195–196°].

Sigmoidin K [**3**].—Brown crystals (35 mg) exhibited R_f 0.31 [C_6H_{12}-Me_2CO (7:3)], mp 95°; uv λ max (MeOH) (log ε) 247 (3.55), 257 (3.58), 345 (3.61) nm; ir ν max (KBr) 3400, 1720, 1615, 1510, 1490, and 1265 cm^{-1}; ^1H nmr and ^{13}C nmr, see Table 3.

Sigmoidin K diacetate [**3a**].—Sigmoidin K [**3**] (18 mg) was dissolved in pyridine (1.1 ml) and 1.3 ml of Ac_2O was added. The reaction mixture was kept at room temperature overnight and worked up in the usual manner to afford the diacetate **3a** as an oil. This exhibited: ir ν max 1765, 1760, 1725, 1610, 1520, 1490, and 1265 cm^{-1}; ^1H and ^{13}C nmr in $CDCl_3$, see Table 2; hreims *m/z* 488.1834 (calcd for $C_{29}H_{28}O_7$, 488.1835); eims 70 eV *m/z* [M^+] 488 (89), 446 (94), 404 (100), 348 (65), 293 (62).

Part of the viscous CH_2Cl_2 extract (100 g) was column chromatographed on Si gel (900 g) packed in CH_2Cl_2 and eluted with the following gradient: CH_2Cl_2, CH_2Cl_2/MeOH mixtures, and MeOH. A total of 150 fractions of about 200 ml per fraction was collected and combined on the basis of tlc. The combined fractions 5–9, eluted

TABLE 3. ^1H- and ^{13}C-Nmr Data of Sigmoidin K [**3**].

Position	δ ^{13}C	δ ^1H
1	121.5 d	7.60 (s)
2	126.3 s	—
3	157.8 s	—
4	102.4 d	6.93 (s)
4a	152.8 s	—
6	158.9 s	—
6a	102.2 s	—
6b	114.6 s	—
7	117.6 d	7.51 (d, 8.4)
8	113.6 d	6.97 (d, 8.3)
9	154.6 s	—
10	111.9 s	—
10a	154.0 s	—
11a	159.3 s	—
11b	103.9 s	—
1′	27.2 t	3.30[b]
2′	121.5 d	5.30 (t, 7.5)
3′	132.9 s	—
4′	25.5 q	1.77 (s)
5′	17.6 q	1.71 (s)
1″	22.3 t	3.57 (d, 7.4)
2″	120.7 d	5.30 (t, 7.4)
3″	131.4 s	—
4″	25.5 q	1.66 (s)
5″	17.5 q	1.89 (s)

[a]Chemical shifts (δ, DMSO-d_6) are in ppm relative to TMS (multiplicity, *J* in Hz) at 500 MHz (^1H nmr) and 125.7 MHz (^{13}C nmr).

[b]This signal was concealed, but appeared in a COSY experiment.

with CH_2Cl_2 were concentrated to give a yellow sticky oil (16 g). This oil was subjected to repeated cc on Si gel eluted with *n*-hexane and increasing concentrations of EtOAc in hexane, yielding two fractions, A and B. Fraction A (8 g), eluted with a mixture of hexane-EtOAc (4:1) was rechromatographed over Si gel. Elution of the column with hexane-EtOAc (17:3) afforded neorautenol [**2**] (11 mg) as yellow needles, R_f 0.62 [C_6H_{12}-Me_2CO (7:3)], mp 202° [lit. (13), 198–200°]. Fraction B (3 g), eluted with hexane-EtOAc (3:1) was subjected to cc over Si gel. Elution of the column with hexane-EtOAc (4:1) yielded sigmoidin J [**1**] (20 mg).

Sigmoidin J [**1**].—Green crystals from hexane/EtOAc, exhibited R_f 0.33 [C_6H_{12}-Me_2CO (7:3)], mp 262°, $[\alpha]^{22}D$ 0° (c=1.0, MeOH); uv λ max (MeOH) (log ε) 279 (4.30), 319 nm (3.93); ir ν max (KBr) 3380, 2953, 1648, 1565, 1525, 1465, 1263, 1062 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; eims (probe) 70 eV *m/z* $[M]^+$ 384 (38), 205 (48), 180 (100), 165 (25), 149 (11); hreims *m/z* found 384.1563 (calcd for $C_{22}H_{24}O_6$, $[M]^+$ 384.1573).

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