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A NEW PRENYLATED ISOFLAVONE AND LONG CHAIN ESTERS FROM TWO *ERYTHRINA* SPECIES[1]

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ABSTRACT.—Two new compounds, a long chain ester **1** of *p*-coumaric acid and a prenylated isoflavone, senegalensin [**5**], 5,4′-dihydroxy-8-(γ,γ-dimethylallyl)-[5″-(hydroxyisopropyl) dihydrofurano (2″,3″:6.7)] isoflavone, in addition to a known long chain ester **2** of ferulic acid, have been isolated and characterized from the stem bark of the Cameroonian medicinal plant *Erythrina senegalensis*. Another known compound **3**, a long chain ester of ferulic acid, was isolated from *Erythrina excelsa*. The structures of all the compounds were determined by spectroscopic techniques.

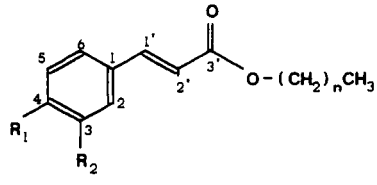
As a continuation of our studies on the non-alkaloidal components of Cameroonian medicinal plants of *Erythrina* species (1–7), we now report the isolation and structural elucidation of two novel compounds: a long chain ester **1** of *p*-coumaric acid and a prenylated isoflavone that is named senegalensin [**5**]. The two known esters **2** and **3** of ferulic acid (8) were also isolated.

RESULTS AND DISCUSSION

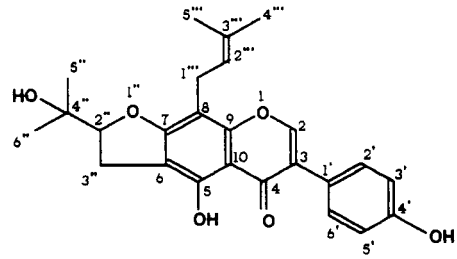
Si gel chromatography of the $CHCl_3$ extract of the stem bark of *Erythrina senegalensis* DC. (Leguminosae) afforded two novel compounds **1** and **5**.

Compound **1** (60 mg) was obtained as a white amorphous powder from EtOH, mp 94°. Its molecular formula, $C_{39}H_{68}O_3$, was assigned from elemental analysis and eims, which showed a molecular ion at *m/z* 584. It gave a positive phenol test (green-brown with $FeCl_3$), and its ir spectrum showed absorption bands at 3500 (free OH), 1700 (C=O), 1670 (C=C), 1600, 1510, 1460 (aromatic), 1260, 1160 (C-O), and 715 cm^{-1} [-$(CH_2)_x$-]. Compound **1** is therefore a long chain aromatic ester with one unsaturation on its side chain. Its uv spectrum in MeOH showed λ max 235 (log ε 4.04) and 325 (log ε 4.05) which is very similar to that of erythrinasinate [**4**], a cinnamic ester previously isolated from the same species (3). Compound **1** is therefore a long chain ester of a substituted cinnamic acid.

This skeleton of **1** was supported by the ^{13}C-nmr spectrum (see Experimental)



1 R_1=OH, R_2=H, n=29
2 R_1=OH, R_2=OMe, n=27
3 R_1=OH, R_2=OMe, n=25
4 R_1=OMe, R_2=OH, n=27



5

[1]Part 18 in the series "Erythrina studies." For part 17, see Nkengfack *et al.* (7).

which showed peaks at δ 169.3 (C-3′) due to the carbonyl group of an ester function and δ 144.4 (C-1′) and 110.5 ppm (C-2′) due to the side chain C-C double bond. Further confirmation of this skeleton came from the mass spectrum of **1**, which showed, besides the molecular ion, significant fragment peaks at *m/z* 164 and 147, both characteristic of a hydroxy-substituted cinnamic moiety (8). The former ion (*m/z* 164) led us to conclude that the hydrocarbon chain linked to the hydroxy-substituted cinnamic moiety contains 30 carbon atoms.

In the ^{1}H nmr in $CDCl_3$, a typical AA′BB′ system at δ 7.43 (2H, d, J = 8 Hz) and δ 6.85 (2H, d, J = 8 Hz) showed the presence of four aromatic protons corresponding to H-2, H-6, H-3, and H-5, respectively. This shows clearly that the aromatic ring has no other substituents besides the hydroxy function, which is at the C-4 position. The methyl at δ 0.93 and the methylene protons at δ 1.25 (56H) and δ 4.19 (2H) show clearly that the hydrocarbon side-chain is triacontyl. Compound **1** is therefore the novel ester, *n*-triacontyl 4-cinnamate.

Pearl and Beyer (9) have shown that esters of substituted cinnamic acids are active against microorganisms. The three esters reported here and the ester **4** were tested (by Panlabs in Taiwan) against six strains of Gram positive bacteria, five strains of Gram negative bacteria, the protozoan *Trichomonas foetus*, and the mammalian pathogens *Candida albicans* and *Trichophyton mentagrophytes*, but no activity was indicated.

Senegalensin [**5**] (70 mg) was obtained as yellow needles from hexane/CH_2Cl_2, mp 150°. Its molecular formula, $C_{25}H_{26}O_6$, was assigned from the hrms, which showed a molecular ion at *m/z* 422.1715 (calcd 422.1729). Its ir spectrum exhibited absorption bands at 3450 (free OH), 3280 (chelated), and 1642 cm^{-1} (conjugated carbonyl). In the ^{1}H nmr in Me_2CO-d_6, a 1H singlet at δ 8.14 ppm was characteristic of an isoflavone and assignable to H-2. This skeleton was also supported by its uv spectrum (278 nm) and color tests: positive to $FeCl_3$ (greenish-brown) and negative to Mg-HCl. The downfield signals at δ 13.50 and 8.60 ppm confirmed the presence of a chelated hydroxyl at C-5 (δ 13.50) as well as the presence of one free phenolic group. Major mass spectral fragments at *m/z* 379 $[M-43]^+$ and *m/z* 367 $[M-55]^+$ and ^{1}H-nmr signals at δ 1.65 (3H,s), 1.77 (3H, s), 3.38 (2H, d, J = 7.2 Hz, H-1‴), and 5.28 ppm (1H, t, J = 7.2 Hz, H-2‴) were consistent with the presence of a 3,3-dimethylallyl group. Mass fragments at *m/z* 363 $[M-59]^+$, 321 $[M-(59+43)]^+$, 307 $[M-(59+55)]^+$, and 59 were indicative of an hydroxy isopropyl-dihydrofuran substituent (10,11). The presence of such substituent was confirmed by the ^{1}H-nmr spectrum which showed a *gem*-dimethyl signal at 1.28 ppm, one methine proton near an oxygen atom at δ 4.84 (1H, t, J = 12 Hz, H-2″), and two diastereotopic protons at δ 3.25 (1H, dd, J = 9.5 and 4.5 Hz, H-3″) and 3.30 (1H, dd, J = 9.5 and 4.5 Hz, H-3″). The isopropyl-dihydrofuran skeleton was also supported by the ^{13}C-nmr spectrum (Table 1) which showed peaks for C-2″, C-3″, and C-4″ at δ 92.2, 27.5, and 71.4 ppm, respectively.

Furthermore, a typical AA′BB′ system at δ 7.44 (2H, d, J = 8.5 Hz) and δ 6.90 (2H, d, J = 8.5 Hz) showed the presence of four aromatic protons in ring B corresponding to H-2′ and H-6′, while the lack of any signal at 5.90–6.10 ppm established that there were no aromatic protons in ring A. This showed clearly that the hydroxy isopropyl-dihydrofuran unit of **5** was fused to ring A on which a 3,3-dimethylallyl group is located.

It remained for us to establish unambiguously the position of the free prenyl group on ring A of **5** and to see if the fusion of the hydroxy isopropyl-dihydrofuran unit is linear or angular. An nmr technique based on ^{13}C-^{1}H long range spin-spin coupling, selective INEPT (12,13) (also called INAPT), and the 2D-HMBC (Heteronuclear Multiple Bond Connectivity) short and long range heteronuclear experiments (14) were used to verify the prenyl attachment. When H-1‴ was irradiated, polarization transfer

TABLE 1. ^{1}H-nmr Data and ^{13}C-nmr Assignments for Senegalensin [**5**].

Atom	^{1}H (δ, Me_2CO-d_6)	*J* (Hz)	^{13}C (δ, Me_2CO-d_6)	^{13}C (δ, $CDCl_3$)
1	—	—	—	—
2	8.14 s	—	153.6	155.8
3	—	—	123.8	123.8
4	—	—	181.6	181.6
5	—	—	165.7	165.7
6	—	—	103.9	103.9
7	—	—	160.7	160.7
8	—	—	107.7	107.7
9	—	—	151.8	151.9
10	—	—	106.1	106.1
1′	—	—	123.2	123.4
2′	7.44 d	8.5	131.2	130.3
3′	6.90 d	8.5	115.8	115.5
4′	—	—	158.2	160.1
5′	6.90 d	8.5	115.8	115.5
6′	7.44 d	8.5	131.2	130.3
1″	—	—	—	—
2″	4.85 t	—	92.2	91.0
3″	3.25 dd 3.30 dd	9.5 and 4.5 9.5 and 4.5	27.5	27.7
4″	—	—	71.4	71.4
5″	1.28 s	—	26.2	26.2
6″	1.28 s	—	24.9	24.0
1‴	3.38 d	7.2	22.4	21.9
2‴	5.28 t	7.2	122.5	121.5
3‴	—	—	131.9	131.9
4‴	1.65 s	—	25.8	25.7
5‴	1.77 s	—	17.9	18.0
5-OH	13.50 s	—	—	—
4′	8.60 s	—	—	—
4″	1.54 b	—	—	—

was seen at C-1‴ (δ 22.4), C-2‴ (δ 122.5), C-3‴ (δ 131.9), C-7 (δ 160.7), and C-9 (δ 151.8). When H-2″ was irradiated, we observed polarization transfer to C-2″ (δ 92.2), C-3″ (δ 27.5), and C-7 (δ 160.7). Irradiation of H-3″ showed enhancement through polarization transfer to C-3″ (δ 27.5), C-4″ (δ 71.4), and C-5 (δ 165.7), while polarization transfer to C-2 (δ 153.6), C-3 (δ 123.8), C-4 (δ 181.6), and C-9 (δ 151.8) was observed when H-2 was irradiated. These results showed clearly that the free prenyl group was attached at the C-8 position in ring A and the fusion of the hydroxy isopropyl-dihydrofuran ring is linear.

Confirmation of the prenyl attachement on ring A was given by the eims of compound **5** which showed, besides the molecular ion peak (*m/z* 422) and fragment ion peaks at *m/z* 367 and 379, other significant ion peaks at *m/z* 306, 278, 263, 249, 117, 116, 59, 55, and 43. Ions peaks at *m/z* 306 and 116 arose from RDA cleavage followed by hydrogen transfer. The former ion (*m/z* 306) resulted from the ring A moiety and showed that this ring possessed the isoprenyl group and dihydrofuran ring. On the other hand, the latter ion (*m/z* 116) arose from the B ring moiety and confirmed that this ring, in conjunction with the AA′BB′ ^{1}H-nmr spin pattern, had a hydroxyl group at position C-4′ and no isoprenyl group. Further confirmations of structure **5** came from a NOESY (15) experiment which showed intense nOe between H-2″ and H-3″, H-1‴ and H-5‴, H-1‴ and H-2‴, H-2‴ and H-4‴, H-2 and H-2′, and H-2 and H-3′. ^{13}C-nmr spectral data (see Table 1) were also in accord with structure **5**. From the above

spectroscopic studies, senegalensin [**5**] was deduced to be 5,4′-dihydroxy-8-(γ,γ-dimethylallyl)-[5″-(hydroxyisopropyl) dihydrofurano (2″,3″:6,7)] isoflavone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All mp's were determined on a Kofler hot-stage apparatus and are uncorrected. Mass spectra are obtained with a Kratos MS-25 with a DS-55 data system. Ir spectra were run on a Nicolet 20 DBX and uv spectra were run on Beckman 25 spectrometer. Si gel GF_{254} (Merck) and Si gel 60 (70–230 mesh) (ASTM) (Merck) were used for tlc and cc, respectively. Some nmr experiments were performed on a Nicolet NT-300 WB spectrometer equipped with a 5-mm ^1H and ^{13}C probe operating at 300.06 and 75.46 MHz, respectively, while other spectra were recorded on a Variant 90 MHz spectrometer. Samples were run in 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. Selective INEPT experiment used the pulse sequence by Bax (13,14). A decoupler field strength $\gamma H_2 = 25$ Hz was used to generate a selective 90° proton pulse = 10 ms.

The polarization transfer delays, Δ_1 and Δ_2, were optimized for $J_{CH} = 6$ Hz. Between 600 and 2000, 16K acquisitions were signal-averaged in double precision mode and processed in floating point mode with standard Nicolet software.

PLANT MATERIAL.—*E. senegalensis* stem bark was collected at Abagana, Nigeria, in March 1985. *Erythrina excelsa* Bak. stem bark was collected at Mbalmayo, Cameroon, in April 1984. Herbarium specimens documenting the collection are deposited at the National Herbarium, Yaounde.

EXTRACTION AND ISOLATION.—Dried, ground, stem bark of *E. senegalensis* (14 kg) was successively extracted with hexane and $CHCl_3$. Some of the $CHCl_3$ extract (90 g) was chromatographed over a Si gel (900 g) column and eluted with varying proportions of hexane/EtOAc. Tlc analysis permitted the grouping of fractions that had the same compounds. Fractions 60–62 (450 mg) were a mixture of three compounds. Selective crystallization from a mixture of hexane/EtOAc gave compound **1** (60 mg) and compound **2** (170 mg).

Fractions 93–123 (10 g) were combined and rechromatographed on Si gel (300 g) and eluted with hexane-EtOAc (17:3) to give a fraction that contained mainly two compounds. This fraction was rechromatographed on Si gel (200 g) and eluted with hexane-EtOAc (17:3). Fractions 47–55 contained one main compound. These fractions were combined and further chromatographed on Si gel (200 g), eluting with hexane-EtOAc (17:3), to give a solid which was recrystallized from hexane/CH_2Cl_2 to give compound **5** (70 mg) as light yellow needles, mp 150°.

Dried ground stem bark of *E. excelsa* was extracted with *n*-hexane to give 50 g of extract which was chromatographed on Si gel (360 g) eluting with hexane/EtOAc in varying proportions. Tlc analysis permitted the regrouping of fractions 14–60 which was a mixture of two compounds. The mixture was rechromatographed on Si gel (200 g) and eluted with hexane-Et_2O (9:1) to give a solid which was recrystallized from MeOH-hexane to give compound **3** (300 mg) as a white powder.

Compound **1**.—White powder (EtOAc): mp 94°; ir ν max 3500, 1700, 1670, 1600, 1510, 1460, 1280, 1160, 715 cm^{-1}; uv λ max (MeOH) (log ε) 235 (4.04), 325 nm (4.05); ^1H nmr (90 MHz, $CDCl_3$) δ 0.93 (3H, t, $J = 6.7$ Hz, Me), 1.25 [56H, b, $(CH_2)_{28}$], 4.19 (2H, t, 7.5 Hz, OCH_2CH_2R), 6.29 (1H, d, $J = 16.0$ Hz, =CH), 6.85 (2H, d, $J = 8.0$ Hz, H-3 and H-5), 7.00 (1H, s, exchangeable D_2O, OH), 7.43 (2H, d, $J = 8$ Hz, H-2 and H-6), 7.63 (1H, d, $J = 16.0$ Hz, CH=); ^{13}C nmr (22.5 MHz, $CDCl_3$) δ 169.3 (C-3′), 152.3 (C-4), 144.4 (C-1′), 129.9 (C-2 and C-6), 127.5 (C-1), 115.9 (C-3 and C-5), 110.5 (C-2′), 64.6 (C-1″), 31.9 (C-28″), 30.8 (C-5″↦C-26″), 29.6 (C-4″), 29.4 (C-27″), 29.3 (C-3″), 26.1 (C-2″), 22.6 (C-29″), 14.0 (C-30″). Found C 80.11, H 11.64; calcd for $C_{39}H_{68}O_3$, C 80.14, H 11.64. Eims *m/z* $[M]^+$ 584 (30), 557 (100), 396 (20), 178 (50), 164 (67), 147 (50), 120 (30).

Compound **2**.—White powder (hexane/EtOAc): mp 80°; ir ν max 3525, 1720, 1640, 1600, 1170, 710 cm^{-1}; uv λ max (EtOH) (log ε) 230 (4.03), 320 (4.05); ^1H nmr (90 MHz, $CDCl_3$) δ 0.93 (3H, t, $J = 6.7$ Hz, Me), 1.25 [52H, b, $(CH_2)_{26}$], 4.15 (2H, t, $J = 7.5$ Hz, OCH_2CH_2-R), 6.28 (1H, d, $J = 16.0$ Hz, =CH), 7.61 (1H, d, $J = 16.0$ Hz, CH=), 7.04 (1H, d, $J = 1.1$ Hz, H-2), 6.93 (1H, d, $J = 8.0$ Hz, H-5), 7.07 (1H, dd, $J = 1.1$ and 8.1 Hz, H-6), 3.90 (3H, s, OMe); eims *m/z* $[M + 1]^+$ 587, 559, 509, 481, 453, 425, 194, 177, 97, 83, 63, 57, 43.

Compound **3**.—White powder: mp 66.5°; ir ν max 3525, 1705, 1655, 1460, 1270, 1180, 720 cm^{-1}; uv λ max (EtOAc) (log ε), 234 (4.03), 325 (4.06); ^1H nmr (90 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.7$ Hz, Me), 1.26 [48, s, $(CH_2)_{24}$], 3.89 (3H, s, OMe), 4.19 (2H, t, $J = 7.5$ Hz, OCH_2CH_2R), 6.28 (1H, d, $J = 16.0$ Hz), 6.98 (1H, s, exchangeable D_2O, OH), 6.94 (1H, d, $J = 8.1$ Hz, H-5), 7.04 (1H, d, $J = 1.3$ Hz, H-2), 7.07 (1H, dd, $J = 8.1$ and 1.3 Hz, H-6), 7.61 (1H, d, $J = 16$ Hz, CH=).

Senegalensin [**5**].—Light yellow needles: mp 150°; ir ν max 3450, 3280, 1642, 1627, 1445 cm^{-1}; uv λ max (MeOH) 278 nm (log ϵ 4.17); ^1H nmr (300 MHz, CD_3COCD_3) see Table 1; ^{13}C nmr (75.45 MHz, Me_2CO-d_6 and $CDCl_3$) see Table 1; eims *m/z* $[M]^+$ 422 (78), 407 (24), 389 (92), 379 (72), 367 (100), 363 (22), 349 (20), 335 (23), 321 (21), 307 (55), 306 (37), 295 (58), 278 (30), 263 (24), 249 (20), 117 (15), 116 (33), 59 (60), 55 (80), 43 (85); hrms calcd for $C_{25}H_{26}O_6$ *m/z* $[M]^+$ 422.1729, found 422.1715.

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