Structural Elucidation of New Flavanones Isolated from *Erythrina abyssinica*

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Three new prenylated flavanones, abyssinin I (**1**), II (**2**), and III (**3**), have been isolated from the African medicinal plant *Erythrina abyssinica*, together with six known flavanones. The structural elucidation of **1**−**3** by spectroscopic studies is described.

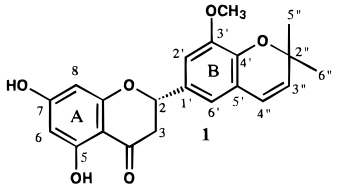
Erythrina abyssinica DC. (Leguminosae) is a tree, usually highly branched with rounded spreading crown, 2−15 m tall, which is distributed throughout the savanna of Eastern Africa.[1] This plant is an important folk medicine in Kenya. The bark has been used in folk remedies for treatment of trachoma and elephantiasis and the roots for malaria and syphilis.[2] From the root of this plant, Nakanishi and co-workers isolated some pterocarpans and flavanones and a chalcone, some of which possessed important biological activities.[3]

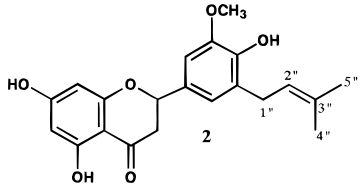
When we examined HPLC profiles of the constituents of stem bark extract of *E. abyssinica*, many peaks were found that could not be attributed to compounds already reported. We therefore tried to obtain the compounds by preparative HPLC and isolated three new prenylated flavanones named abyssinin I (**1**), II (**2**), and III (**3**) along with abyssinone V, sigmoidin A, B, C, and F, and sigmoidin B 4′-(methyl ether). This paper deals with the structure elucidations of the three new flavanons isolated from the stem bark of *E. abyssinica*.

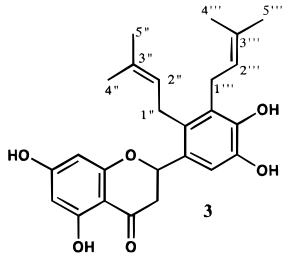
Results and Discussion

The Et_2O-soluble fraction of the MeOH extract of stem bark of *E. abyssinica* was separated by a combination of chromatographic procedures to yield nine compounds. All of the compounds displayed similar UV spectra (λ_{max} 285−290 nm, λ sh 325 nm), typical of compounds having a flavanone skeleton.[4] This was supported by ^{1}H NMR spectra that showed resonances for the two H-3 and the H-2 protons of characteristic flavanones. Furthermore, ^{1}H NMR spectra showed *meta*-coupled or singlet H-6 and H-8 protons for ring A (δ 5.94−5.98) and a chelated 5-hydroxyl proton at δ 12.17−12.19. The EIMS spectra of all compounds exhibited a fragment ion at *m/z* 153 consistent with retro-Diels−Alder fragmentations[5] and consistent with a 5,7-dihydroxyflavanone skeleton. This conclusion was also supported by ^{13}C NMR spectra. Further structural features were determined by ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HMQC, HMBC, and 2D NOESY spectra.

The first new compound (**1**) was obtained as colorless crystals from Et_2O/hexane, mp 220 °C (dec), $[\alpha]_D$ −23.5°.







HREIMS revealed a molecular formula of $C_{21}H_{20}O_6$. ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) were very similar to those of sigmoidin C,[6] except that a methoxyl signal was evident in **1**. The ^{1}H NMR spectrum showed signals of vinylic protons at δ 5.77 (1H, d, J = 10 Hz) and δ 6.42 (1H, d, J = 10 Hz), suggesting a ring closure of the prenyl unit. Thus, **1** is a 5,7-dihydroxyflavanone bearing a methoxyl group and a 2,2-dimethylpyran unit in ring B. The HMBC spectrum of **1** (Figure 1) showed that the protons at δ 7.09 and δ 6.87, which were *meta* coupled with each other, were correlated to C-2 (δ 80.08); thus, these protons were assigned to H-2′ and H-6′. Therefore, it was found that the 3′, 4′, and 5′ of the ring B were replaced with a methoxyl and a 2,2-dimethylpyran group. Further, the location of these groups was described as structure **1** since a correlation between the proton at δ 6.87 (H-6′) and C-4 (δ 122.95) of the 2,2-dimethylpyran ring (C-4″) was observed. This was also deduced from a 2D NOESY spectrum, which showed cross peaks between H-6′ and H-4″, and

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Table 1. ^{1}H NMR Chemical Shift Values (δ) of Abyssinin I (**1**), II (**2**), and III (**3**)[a]

	compound		
proton	**1**	**2**	**3**
2	5.43 (dd, J = 3.0, 13.0)	5.42 (dd, J = 3.0, 13.0)	5.59 (dd, J = 3.0, 13.0)
3ax	3.22 (dd, J = 13.0, 17.0)	3.22 (dd, J = 13.0, 17.0)	3.08 (dd, J = 13.0, 17.0)
3eq	2.75 (dd, J = 3.0, 17.0)	2.72 (dd, J = 3.0, 17.0)	2.63 (dd, J = 3.0, 17.0)
6	5.96, 5.98 (each 1H, d, J = 2.0)	5.95, 5.96 (each 1H, d, J = 2.5)	5.94, 5.95 (each 1H, d, J = 2.5)
8			
2′	7.09 (d, J = 2.0)	7.06 (d, J = 2.0)	
6′	6.87 (d, J = 2.0)	6.92 (d, J = 2.0)	7.00 (s)
1″		3.36 (br d, J = 7.5)	3.33, 3.40 (each 1H, m)
2″		5.35 (m)	5.03 (m)
4″		1.72 (br s)	1.65 (br s)[b]
5″		1.70 (br s)	1.66 (br s)[b]
1‴			3.44 (br d, J = 6.5)
2‴			5.12 (m)
4‴			1.75 (br s)
5‴			1.67 (br s)[b]
3″	5.77 (d, J = 10.0)		
4″	6.42 (d, J = 10.0)		
5″			
6″	1.43 (6H, s)		
OMe	3.84 (s)	3.89 (s)	
5-OH	12.18 (s)	12.19 (s)	12.17 (s)
7-OH	9.70 (br s)	9.62 (br s)	9.58 (br s)
		7.59 (br s)	7.28 (br s)
other OH			8.43 (br s)

[a] Spectra were recorded at 499.84 MHz in Me_2CO-d_6. Coupling constants (J) are given in Hz. [b] May be interchanged.

Table 2. ^{13}C NMR Chemical Shift Values (δ) of Abyssinin I (**1**), II (**2**), and III (**3**)[a]

	compound		
carbon	**1**	**2**	**3**
2	80.08	80.49	77.35
3	43.57	43.65	43.55
4	197.24	197.37	197.65
5	165.38	165.37	165.37
6	96.91	96.82	96.83
7	167.39	167.38	167.31
8	95.91	95.88	95.85
9	164.37	164.45	164.73
10	103.25	103.27	103.18
1′	131.99	130.47	128.11
2′	112.55	108.56	131.07
3′	149.50	148.06	128.78
4′	143.39	145.28	144.77
5′	122.72	128.41	143.46
6′	118.03	121.21	112.03
1″		28.92	27.81
2″		123.54	125.19
3″		132.57	131.53
4″		17.86	18.10[b]
5″		25.89	25.85[c]
1‴			26.09
2‴			124.38
3‴			131.36
4‴			18.06[b]
5‴			25.75[c]
2″	77.13		
3″	132.16		
4″	122.95		
5″	28.13		
6″	28.13		
OMe	56.61	56.51	

[a] Spectra were recorded at 125.7 MHz in Me_2CO-d_6. [b, c] May be interchanged

between H-2′ and methoxyl protons, respectively (Figure 1). Thus, the structure of **1** was found to be 5,7-dihydroxy-2′,2′-dimethyl-8′-methoxy-[2,6′-bi-2*H*-1-benzopyran]-4(3*H*)-one.

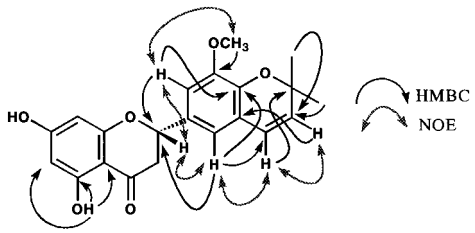

Figure 1. Significant correlations observed in the HMBC and NOESY spectra of abyssinin I (**1**).

Two maxima of positive [330 nm ($\Delta\epsilon$ +1.3)] and negative [286 nm ($\Delta\epsilon$ −6.4)] Cotton effect were present in the CD spectrum of **1**. This agreed with reported data for 2(*S*)-flavanones.[7] Therefore, the absolute stereochemistry of **1** is 2*S* as illustrated. Compound **1** was named abyssinin I.

The second new compound (**2**) was isolated as a white amorphous solid, [α_D] 0°. The molecular formula was determined to be $C_{21}H_{22}O_6$ by HREIMS. ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) were very similar to that of sigmoidin B 4′-(methyl ether).[8,9] Compound **2** showed the following signals relating to ring B in the ^{1}H NMR spectrum: (1) signals due to a 3-methylbut-2-enyl (prenyl) unit, (2) a signal due to a methoxyl group, (3) a signal due to a hydroxyl group, and (4) signals of two *meta*-coupled aromatic protons. Thus, **2** is a 5,7-dihydroxyflavanone bearing prenyl, methoxyl, and hydroxyl groups in ring B. The locations of these substituents were determined by HMBC (Figure 2). The 3′, 4′, and 5′ substitution pattern for ring B was elucidated as ^3J interactions between C-2 (δ 80.49), and two aromatic protons belonging to ring B, H-2′ and H-6′, were observed. A methoxyl group was not at C-4′ but C-3′, because the correlation between methoxyl protons and C-4′ was not observed but C-3′ was observed.

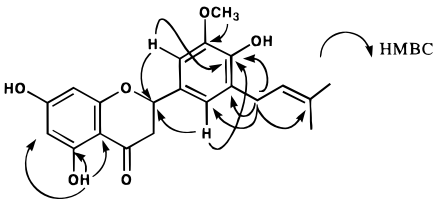

Figure 2. Significant correlations observed in the HMBC spectrum of abyssinin II (**2**).

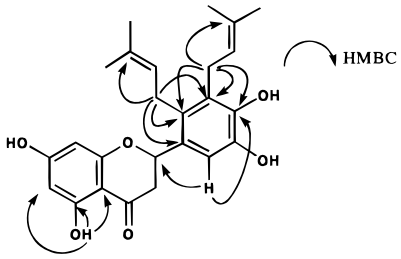

Figure 3. Significant correlations observed in the HMBC spectrum of abyssinin III (**3**).

Furthermore, connectivities from methylene protons of the prenyl unit to C-4′ and C-6′ were observed, suggesting that the prenyl unit was located at C-5′. Therefore, a hydroxy group was at C-4′. Consequently, the structure of **2** was determined to be 5,7,4′-hydroxy-3′-methoxy-5′-prenylflavanone. Compound **2** was isolated as a racemate and named abyssinin II.

The structure of **2** had once been reported as a compound isolated from *Erythrina berleroana*[8] and also from *Erythrina sigmoidea*,[10] but later, the structure had been revised.[9] Thus, this is actually the first report of the isolation of **2**. Recently, synthesis of **2** via condensation of 2-hydroxy-4,6-bis(methoxymethoxy)acetophenone with 3-methoxy-4-(methoxymethoxy)-5-prenylbenzaldehyde followed by cyclization and demethoxymethylation was reported.[11]

The third new compound (**3**) was isolated as a white amorphous solid, $[\alpha]_D$ 0°, and had a molecular formula of $C_{25}H_{28}O_6$ by HREIMS. The ^{1}H NMR spectrum of **3** (Table 1) showed signals due to two prenyl groups, two hydroxyl groups, and an isolated aromatic proton concerning to ring B. Accordingly, **3** is a 5,7-dihydroxyflavanone with four substituents, two prenyl and two hydroxyl groups, on ring B. The relative positions of these groups were determined on the basis of the HMBC spectrum (Figure 3). The correlation between C-2 (δ 77.35) and an isolated aromatic proton at δ 7.00 indicated that this proton was present at C-6′. Hence, the 2′, 3′, 4′, and 5′ substitution pattern for ring B was proved. The methylene protons (δ 3.33 and δ 3.40) of a prenyl unit were correlated with the aromatic carbons at δ 128.11 (C-1′), δ 128.78 (C-3′), and δ 131.07 (C-2′), whereas the methylene protons (δ 3.44) of another prenyl unit were correlated with the aromatic carbons at δ 128.78 (C-3′), δ 131.07 (C-2′), and δ 144.77 (C-4′, chemical shift indicated an oxygenated carbon). Further, no correlations between the methylene protons of either prenyl unit and C-6′ were recognized. Therefore, it was apparent that the two prenyl groups were adjacent to each other and at the C-2′ and C-3′ positions, and the two hydroxyl groups at the C-4′ and C-5′ positions of ring B, respectively. Thus, the structure of **3** was determined to be 5,7,4′,5′-hydroxy-2′,3′-prenylflavanone. Compound **3** was also racemic and named abyssinin III. The six remaining compounds were identified as sigmoidin C,[6] sigmoidin B,[6] sigmoidin B 4′-(methyl ether),[8,9] sigmoidin A,[6,12] sigmoidin F,[13] and abyssinone V,[3,14] respectively.

In conclusion, we have isolated three new flavanones (**1**−**3**), named abyssinin I, II, and III, along with six known flavanones from the stem bark of *E. abyssinica*. Five of these that have a 4′-hydroxy group, including **2** and **3**, were isolated as racemates. The remaining four compounds, including **1**, were isolated as optically active, and these had a 2*S* configuration from their CD spectra. It was assumed that the compounds having a 4′-hydroxy group were racemized at C-2 during the isolation process.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanaco MP-500D micro melting point apparatus and are uncorrected. IR spectra were obtained on a Hitachi 270-30 IR spectrometer, and UV spectra were measured with a photodiode array detector (Model 990J, Waters). EIMS were taken with a Hitachi M-4100 spectrometer. Optical rotations and CD spectra were measured with a JASCO DIP-370 digital polarimeter and a J-500C spectropolarimeter. All NMR experiments were performed on a Varian VXR-500 spectrometer equipped with 5 mm ^{1}H and ^{13}C probes operating at 499.84 and 125.7 MHz, respectively. Chemical shifts were referenced to internal TMS. HPLC conditions for analytical HPLC were as follows: column, Cosmosil 5C18-AR (5 μm, ODS-type), 150 × 6 mm i.d. (Nacalai Tesque); mobile phase (A) 0.1 M ammonium acetate and (B) MeOH [A/B = 70/30 to 50/50, 15 min, 50/50 to 10/90, 75 min, 1.0 mL/min]. For preparative-scale HPLC as follows: column, Cosmosil 5C18-AR, 250 × 20 mm i.d. (Nacalai Tesque); mobile phase, A/B = 70/30 to 50/50, 30 min, 50/50 to 10/90, 150 min, 9.0 mL/min. Preparative TLC was carried out on Si-gel plates (Merck, Art 5744 Kieselgel 60 F_{254}).

Plant Material. Stem bark of *E. abyssinica* was collected in the Meru district of Kenya. The samples were identified and authenticated by Mr. G. M. Mungai, Eastern African Herbarium, P.O. Box 45166, Nairobi, Kenya. Voucher specimens are deposited at Kobe Pharmaceutical University and at Eastern African Herbarium.

Extraction and Isolation. Powdered bark (100 g) of *E. abyssinica* was extracted with MeOH twice and then with 80% MeOH once to give 14.6 g of crude extract. The crude extract was defatted with hexane and then extracted with Et_2O repeatedly to give 4.5 g of Et_2O-soluble fraction. This was dissolved in MeOH and submitted to HPLC. On analytical-scale HPLC, seven major peaks and a number of small peaks were observed. Further isolation of the compounds was carried out by preparative HPLC, and seven major peaks were collected. MeOH was evaporated from the collected effluent, Et_2O was poured into the remaining aqueous solution, and the compounds were then extracted with Et_2O. After evaporation of Et_2O, pale yellow solids were obtained. Preparative TLC of the Et_2O extract from each peak ($CHCl_3$:Me_2CO = 9:1 for **2**, sigmoidin C, sigmoidin B 4′-(methyl ether), sigmoidin A, sigmoidin F, and abyssinone V or $CHCl_3$:MeOH = 10:1 for **1**, **3**, and sigmoidin B) was performed for final

purification. The first eluate peak afforded sigmoidin C (R_f 0.44, 86 mg). The second afforded sigmoidin B (R_f 0.61, 238 mg) and **1** (R_f 0.86, 17 mg). The third afforded sigmoidin B 4′-(methyl ether) (R_f 0.41, 84 mg) and **2** (R_f 0.58, 162 mg). After that, in order of elution, peaks yielded sigmoidin A (R_f 0.62, 610 mg), sigmoidin F (R_f 0.72, 173 mg), **3** (R_f 0.52, 138 mg), and abyssinone V (R_f 0.78, 92 mg), respectively.

Abyssinin I (1): colorless crystals; mp 220 °C (dec) (Et_2O/hexane); $[\alpha]_D$ −23.5° (*c* 0.33, MeOH); CD (*c* 1.82 × 10^{-4} M, MeOH) nm ($\Delta\epsilon$) 370 (0), 330 (+1.3), 312 (0), 286 (−6.4), 266 (0); IR (KBr) ν_{max} 3360 (br, OH), 1646 (C═O), 1588, 1492, 1464, 1404, 1382, 1344, 1274, 1186, 1152, 1084, 1064, 852, 832, 790, 756, 732 cm^{-1}; EIMS *m/z* $[M]^+$ 368 (36.7), 353 (100), 201 (17.5), 153 (2.7); HREIMS $[M]^+$ 368.1264 (368.1258 calcd for $C_{21}H_{20}O_6^+$); ^1H and ^{13}C NMR (Me_2CO-d_6, 499.84 and 125.7 MHz) see Tables 1 and 2.

Abyssinin II (2): white amorphous solid; $[\alpha]_D$ 0° (*c* 0.78, MeOH); IR (KBr) ν_{max} 3460 (br, OH), 1642 (C═O), 1604, 1502, 1466, 1344, 1298, 1186, 1162, 1088, 1066, 846, 748, 548 cm^{-1}; EIMS *m/z* $[M]^+$ 370 (87.8), 205 (100), 153 (25.5); HREIMS $[M]^+$ 370.1409 (370.1414 calcd for $C_{21}H_{22}O_6^+$); ^1H and ^{13}C NMR (Me_2CO-d_6, 499.84 and 125.7 MHz) see Tables 1 and 2.

Abyssinin III (3): white amorphous solid; $[\alpha]_D$ 0° (*c* 0.69, MeOH); IR (KBr) ν_{max} 3440 (br, OH), 1644 (C═O), 1458, 1386, 1346, 1302, 1184, 1160, 1096, 1070, 834, 788 cm^{-1}; EIMS *m/z* $[M]^+$ 424 (40.1), 368 (35.9), 355 (28.2), 153 (100); HREIMS $[M]^+$ 424.1881 (424.1884 calcd for $C_{25}H_{28}O_6^+$); ^1H and ^{13}C NMR (Me_2CO-d_6, 499.84 and 125.7 MHz) see Tables 1 and 2.

Sigmoidin C: white amorphous solid; $[\alpha]_D$ −21.6° (*c* 0.62, MeOH); CD (*c* 1.75 × 10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 330 (+1.65), 312 (0), 285 (−8.0), 268 (0); IR (KBr) ν_{max} 3420 (br, OH), 1644 (C═O), 1466, 1280, 1162 cm^{-1}; EIMS *m/z* $[M]^+$ 354 (52.7), 339 (100), 153 (13); HREIMS $[M]^+$ 354.1129 (354.1102 calcd for $C_{20}H_{18}O_6^+$); ^1H and ^{13}C NMR spectra are in close agreement with literature values.[6]

Sigmoidin B: white amorphous solid; $[\alpha]_D$ 0° (*c* 0.63, MeOH); IR (KBr) ν_{max} 3436 (br, OH), 1644 (C═O), 1454, 1306, 1162 cm^{-1}; EIMS *m/z* $[M]^+$ 356 (99.5), 191 (100), 153 (47.5); HREIMS $[M]^+$ 356.1242 (356.1258 calcd for $C_{20}H_{20}O_6^+$); ^1H and ^{13}C NMR spectra are in close agreement with literature values.[6]

Sigmoidin B 4′-(methyl ether): white amorphous solid; $[\alpha]_D$ −29° (*c* 1.29, MeOH); CD (*c* 1.74 × 10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 328 (+3.1), 307 (0), 288 (−11.5), 259 (0); IR (KBr) ν_{max} 3420 (br, OH), 1644 (C═O), 1466, 1278, 1162 cm^{-1}; EIMS *m/z* $[M]^+$ 370 (100), 205 (62.5), 153 (33); HREIMS $[M]^+$ 370.1420 (370.1414 calcd for $C_{21}H_{22}O_6^+$). ^1H and ^{13}C NMR spectra are in close agreement with literature values.[8,9]

Sigmoidin A: white amorphous solid; $[\alpha]_D$ 0° (*c* 0.38, MeOH); IR (KBr) ν_{max} 3450 (br, OH), 1640 (C═O), 1446, 1294, 1166 cm^{-1}; EIMS *m/z* $[M]^+$ 424 (36.5), 368 (40.6), 153 (100); HREIMS $[M]^+$ 424.1880 (424.1884 calcd for $C_{25}H_{28}O_6^+$); ^1H and ^{13}C NMR spectra are in close agreement with literature values.[6,12]

Sigmoidin F: white amorphous solid; $[\alpha]_D$ −42.6° (*c* 0.77, MeOH); CD (*c* 1.66 × 10^{-4} M, MeOH) nm ($\Delta\epsilon$) 370 (0), 330 (+1.0), 318 (0), 286 (−8.2), 267 (0); IR (KBr) ν_{max} 3450 (br, OH), 1644 (C═O), 1464, 1272, 1160 cm^{-1}; EIMS *m/z* $[M]^+$ 422 (83.9), 407 (46.0), 366 (57.7), 153 (100); HREIMS $[M]^+$ 422.1739 (422.1728 calcd for $C_{25}H_{26}O_6^+$); ^{13}C NMR (Me_2CO-d_6, 125.7 MHz) δ 197.55 (C-4), 167.40 (C-7), 165.32 (C-5), 164.62 (C-9), 143.95 (C-3′), 140.66 (C-4′), 131.56 (C-3″), 131.46 (C-3‴), 130.24 (C-1′), 127.80 (C-2′), 124.13 (C-2″), 122.79 (C-4‴), 120.12 (C-5′), 115.96 (C-6′), 103.10 (C-10), 96.88 (C-6), 95.87 (C-8), 77.66 (C-2‴), 77.08 (C-2), 43.32 (C-3), 28.10, 27.95 (C-5‴, C-6‴), 25.83 (C-5″), 25.33 (C-1″), 18.00 (C-4″). ^1H NMR spectra are in close agreement with literature values.[13] ^{13}C NMR spectral data are reported for the first time.

Abyssinone V: white amorphous solid; $[\alpha]_D$ −0° (*c* 0.48, MeOH); IR (KBr) ν_{max} 3440 (br, OH), 1646 (C═O), 1472, 1160 cm^{-1}; EIMS *m/z* $[M]^+$ 408 (82.5), 243 (100), 153 (36.0); HREIMS $[M]^+$ 408.1924 (408.1935 calcd for $C_{25}H_{28}O_5^+$). ^1H and ^{13}C NMR spectra are in close agreement with literature values.[3,14]

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