

Minor Flavanones from *Erythrina abyssinica*

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Four new prenylated flavanones, abyssinone-V 4'-methyl ether (**1**) and abyssinoflavanones IV (**2**), V (**3**), and VI (**4**), have been isolated as minor flavanones from the African medicinal plant, *Erythrina abyssinica*, together with a known flavanone, sigmoidin D. The structure elucidation of compounds **1–4** by spectroscopic studies is described.

Erythrina abyssinica DC. (Leguminosae) is an important medicinal plant in Kenya; its bark has been used in folk remedies for the treatment of trachoma and elephantiasis.¹ Nakanishi and co-workers isolated pterocarpan, flavanones, and a chalcone from the roots, of which some possessed antimicrobial activity and inhibited platelet aggregation.² In a previous report³ we isolated and identified abyssinins I, II, and III as novel compounds in the stem bark of *E. abyssinica*. We have now examined the minor flavonoid constituents of this plant and have elucidated the structures of four new flavanones (**1–4**), which are reported herein.

Results and Discussion

The Et₂O-soluble fraction of the MeOH extract of the stem bark of *E. abyssinica* contained flavanones as deduced from the absorption spectra (λ_{\max} 285–290 nm, λ_{sh} 325 nm)⁴ obtained by HPLC. Furthermore, LC-APIMS measurements gave the molecular weight of each peak. This fraction was further separated to yield five compounds of interest. The ¹H NMR spectra (Table 1) of these compounds showed resonances for the two H-3 (δ 2.62–2.75 and δ 3.14–3.23) protons and the H-2 (δ 5.36–5.75) proton, characteristic of flavanones.⁵ These ¹H-NMR spectra also showed *meta*-coupled or singlet H-6 and H-8 protons for ring A (δ 5.95–5.99) and a chelated 5-hydroxyl proton (δ 12.17–12.20). The EIMS spectra exhibited a fragment ion at *m/z* 153 consistent with a *retro*-Diels–Alder fragmentation⁶ of a 5,7-dihydroxyflavanone skeleton. Support was provided by ¹³C NMR (Table 2). In their CD spectra, these flavanones showed positive maxima near 330 nm and negative maxima near 285–290 nm, in accordance with data reported for 2(*S*)-flavanones.⁷ Further structural features were determined from their ¹H–¹H COSY, ¹³C–¹H COSY, HMQC, HMBC, and 2D NOESY spectra. One known compound from this fraction was identified as sigmoidin D, isolated previously from *E. sigmoidea*.⁸

Compound **1** was obtained as an optically active, white amorphous solid with a molecular formula of C₂₆H₃₀O₅ as determined by HREIMS. Its ¹H- and ¹³C-

NMR spectra resembled those of abyssinone V^{2,3} with the exception of a methoxyl group (δ 3.75 in the ¹H NMR and δ 61.21 in the ¹³C NMR). An HMBC correlation (Figure 1) between H-2 (δ 5.45) and a C–H carbon (δ 127.04) attributable to C-2' (and C-6') was observed. Also, an HMBC correlation was observed between the methoxyl protons and a quaternary carbon (δ 157.40), which was assigned as C-4' by HMBC correlations from H-2' (δ 7.23) and H₂-1''. These data suggest that **1** is abyssinone-V 4'-methyl ether. Although abyssinone V was racemic,^{2,3} **1** showed a negative optical rotation similar to other flavanones isolated from *E. abyssinica*, indicating a 2*S* configuration. As shown in our previous report,³ racemization at C-2 occurs readily when a hydroxyl group is present at C-4' in ring B.

Compound **2**, abyssinoflavanone IV,⁹ was obtained as an optically active, white amorphous solid with a molecular formula of C₂₂H₂₀O₆ as determined by HREIMS. The presence of a 3-methylbut-2-enyl (prenyl) unit was apparent from the ¹H- and ¹³C-NMR spectra. Three aromatic protons, a singlet (δ 7.45) and two coupled doublets (δ 6.89, 7.81, *J* = 2.0 Hz), were apparent in addition to H-6 and H-8. A singlet proton was assigned to C-6' because of observed HMBC correlations (Figure 1) between this proton and C-2 (δ 77.58), and between H-2 (δ 5.75) and C-6' (δ 111.29). HMBC correlations from H₂-3 (δ 2.75, 3.23) and H-2, respectively, led to the identification of C-1' (δ 134.19) and C-2' (δ 123.35). The prenyl group was attached to C-2', because of HMBC correlations between H₂-1'' (δ 3.65) and C-1' and C-3' (δ 140.49). Although the coupling constant of the doublet proton signal was small (*J* = 2.0 Hz), the ¹H–¹H COSY spectrum clearly indicated a vicinal disposition. From H-6' an HMBC correlation was observed to a carbon at δ 145.01, which was attributed to C-4'. HMBC correlations between both doublet protons and C-4' suggested the presence of a five-membered aromatic ring. The small vicinal coupling constant and the lowfield chemical shifts of H-2''' (δ 7.81), C-4' (δ 145.01), and C-2''' (δ 146.32) could be ascribed to a furan ring fused to a benzene. A hydroxyl group was attached to C-3' (δ 140.49). From the negative $[\alpha]_D$ value and the CD curve, the 2*S* configuration was evident for **2**.

Compound **3**, abyssinoflavanone V, was obtained as an optically active, pale yellow amorphous solid with a molecular formula of C₂₅H₂₈O₇ as determined by HRE-

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Table 1. $^1\text{H-NMR}$ Chemical Shift Values (δ) of Abyssinone-V 4'-methyl ether (**1**) and Abyssinoflavanones IV (**2**), V (**3**), and VI (**4**)

proton	compound			
	1	2	3	4^a
2	5.45 (dd, $J = 12.5, 3.5$)	5.75 (dd, $J = 13.0, 3.0$)	5.59 (dd, $J = 13.5, 2.5$)	5.45 (dd, $J = 13.0, 3.0$) 5.46 (dd, $J = 13.0, 3.0$)
3ax	3.14 (dd, $J = 17.0, 12.5$)	3.23 (dd, $J = 17.0, 13.0$)	3.16 (dd, $J = 17.0, 13.5$)	3.19 (dd, $J = 17.0, 13.0$) 3.21 (dd, $J = 17.0, 13.0$)
3eq	2.74 (dd, $J = 17.0, 3.5$)	2.75 (dd, $J = 17.0, 3.0$)	2.62 (dd, $J = 17.0, 2.5$)	2.70 (dd, $J = 17.0, 3.0$) 2.72 (dd, $J = 17.0, 3.0$)
6, 8	5.95, 5.96 (each 1H, d, $J = 2.0$)	5.98, 5.99 (each 1H, d, $J = 2.5$)	5.96, 5.97 (each 1H, d, $J = 2.5$)	5.95–5.98 (2H) ^b
2'	7.23 (s)			
6'	7.23 (s)	7.45 (s)	6.85 (s)	6.71 (s), 6.73 (s)
1''	3.39 (br d, $J = 7.0$)	3.65 (br d, $J = 6.5$)	3.47 (br d, $J = 7.0$)	
2''	5.31 (m)	5.18 (m)	5.14 (m)	
3''				4.62 (dd, $J = 9.0, 3.5$) 4.64 (dd, $J = 9.0, 3.5$)
4''	1.74 (br s)	1.71 (br s)	1.67 (br s)	3.22–3.38 (2H) ^b
5''	1.72 (br d, $J = 1.0$)	1.65 (br s)	1.63 (br s)	1.23 (br s), 1.23 (br s)
6''				1.26 (br s), 1.26 (br s)
1'''	3.39 (br d, $J = 7.0$)			
2'''	5.31 (m)	7.81 (d, $J = 2.0$)		
3'''		6.89 (d, $J = 2.0$)		
4'''	1.74 (br s)			1.82 (t-like, $J = 7.5$) 2.76–2.80 ^b
5'''	1.72 (br d, $J = 1.0$)			
6'''				1.31 (br) 1.34 (br)
OMe	3.75 (s)			
5-OH	12.17	12.20	12.18	12.19
7-OH				9.63

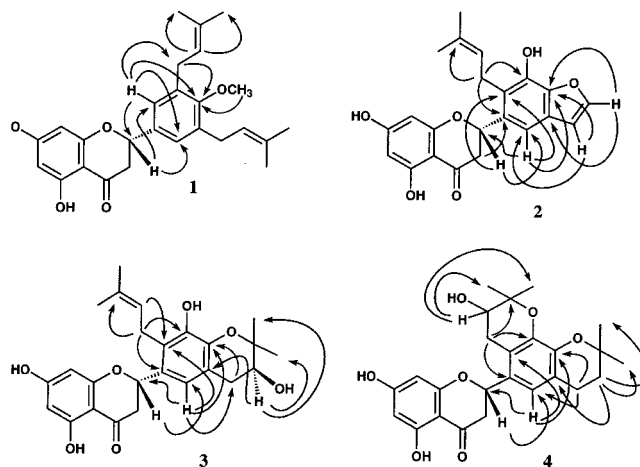
^a Because **4** was the mixture of two diastereomers, two sets of signals were observed in most cases. ^b Due to the proximity of chemical shift values (δ) of the diastereomers, determination of spin coupling values (J) was not possible.

Table 2. $^{13}\text{C-NMR}$ Chemical Shift Values (δ) of Abyssinone-V 4'-methylether (**1**), and Abyssinoflavanones IV (**2**), V (**3**), and VI (**4**)

carbon	compound			
	1	2	3	4^a
2	79.9	77.6	77.2	78.7, 78.6
3	43.5	43.7	43.4	42.4, 42.3
4	196.8	197.4	197.5	197.32, 197.28
5	165.3	165.3	165.3	165.3
6	97.0	97.0	96.9	96.9, 96.8
7	168.1	167.6	167.8	167.3
8	96.0	95.9	95.9	95.8, 95.7
9	164.2	164.6	164.7	164.4
10	103.0	103.1	102.9	103.3, 103.2
1'	135.5	134.2	129.6	126.4, 126.2
2'	127.0	123.4	124.51 ^b	125.6, 125.5
3'	135.7	140.5	144.1	149.3, 149.2
4'	157.4	145.0	141.5	140.2, 140.1
5'	135.7	127.7	118.9	121.9, 121.8
6'	127.0	111.3	118.7	119.6, 119.4
1''	29.1	25.0	25.0	
2''	123.9	124.6	124.46 ^b	71.49, 71.48
3''	133.0	131.6	131.2	91.02, 90.96
4''	17.9	18.0	18.0	30.6, 30.5
5''	25.9	25.8	25.8	25.6
6''				26.2, 26.1
1'''	29.1			
2'''	123.9	146.3	78.8	75.03, 75.00
3'''	133.0	108.1	70.0	33.6
4'''	17.9		31.9	23.1, 23.0
5'''	25.9		20.6	26.8, 26.7
6'''			26.0	27.5, 27.4
OMe	61.2			

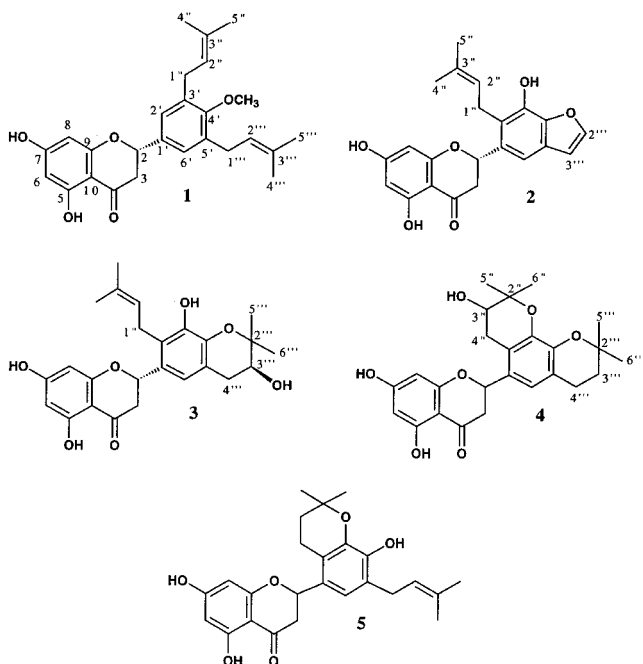
^a Because **4** was the mixture of two diastereomers, two sets of signals were observed. ^b Values may be interchanged.

IMS. The ^1H - and ^{13}C -NMR spectral data resembled those of sigmoidin D, with the presence of a 2,2-dimethylpyran ring and two hydroxyl groups being evident. Additionally, the presence of a prenyl group was inferred. From H-2 an HMBC correlation (Figure 1) to C-6' (δ 118.65) was observed, and a singlet proton

**Figure 1.** Significant correlations observed in the HMBC spectra of Abyssinone-V 4'-methyl ether (**1**) and Abyssinoflavanones IV (**2**), V (**3**), and VI (**4**).

(δ 6.85) was assigned as H-6'. The prenyl group was attached to C-2' as evidenced by HMBC correlations from H₂-1'' to C-1', and from H-2'' to C-2'. An HMBC correlation between H-6' and C-4''' (δ 31.85) was observed, and the H₂-4''' (δ 2.73 and 3.01) signals could be therefore identified. A methine proton (δ 3.82) was assigned as H-3''' from the ^1H - ^1H COSY spectrum. Because C-3''' (δ 70.02) is a methine carbon, a hydroxyl group could be placed at C-3''', similar to sigmoidin D. The oxygen atom of the 2,2-dimethylpyran ring was connected to C-4' (δ 141.5). The remaining hydroxyl group was bonded to C-3' (δ 144.11). Compound **3** was therefore assigned as the 2'-prenyl derivative of sigmoidin D. The Cotton curve of **4** (two maxima at 257 and 242 nm) was very similar to that of sigmoidin D, from which the absolute configuration was deduced to be 2*S*,3'''*S*.⁸

Compound **4**, abyssinoflavanone VI, was obtained as an optically inactive, pale-yellow amorphous solid with a molecular formula of $C_{25}H_{28}O_7$ as determined by HREIMS. This was a mixture of labile diastereomers that interconverted on standing in solution. In both the ^{13}C - and 1H -NMR spectra of **4**, most signals were split into two in a 1:1 ratio. Compound **4** exhibited a pair of singlet aromatic protons (δ 6.71, 6.73) that were assigned to C-6' by an HMBC correlation (Figure 1) from H-2. The placement of two 2,2-dimethylpyran rings, to one of which a hydroxyl group was attached, was determined in the following manner. The $-CH_2-CH_2-$ and $-CH_2-CHOH-$ moieties were elucidated from the $^1H-^1H$ COSY spectra and chemical shift values. An HMBC correlation between the above-mentioned methylene protons (δ 2.76–2.80) and C-6' was observed. Consequently, the 2,2-dimethylpyran ring possessing no hydroxyl group was attached to C-4' and C-5', and another 2,2-dimethylpyran ring possessing a hydroxyl group was attached to C-2' and C-3'. As a result of HMBC correlations from H-4'' (δ 3.22–3.38) and H-3''' (δ 4.62, 4.64), the structure of **4** was elucidated. It is evident that when asymmetric carbons (C-2, and C-3'') racemize, diastereomers are formed. It is probable that **4** is an artifact from **5** because the prenyl group may react with an adjacent hydroxyl group to form a 2,2-dimethyldihydropyran ring.⁵ In fact, the formation of minute amounts of similar artifacts during our purification procedures was observed in the case of sigmoidin B and abyssinin II. Compound **5**, however, was not isolated in the present study. Moreover, LC-APIMS give no evidence for **5** in the Et_2O -soluble fraction. No m/z 440 peak was apparent by LC-APIMS except for **3** and **4**. It is clear that even though **5** is originally present in the raw plant, it is highly unstable and changed rapidly into **4** during the extraction procedure.



Experimental Section

General Experimental Procedures. IR spectra, optical rotations, and CD spectra were measured with

a Shimadzu FT-IR 8200 spectrometer, a JASCO DIP-370 polarimeter, and a JASCO J-500 spectropolarimeter, respectively. 1H -, ^{13}C -, and various 2D NMR spectra were obtained using a Varian VXR 500 instrument. EIMS were measured with a Hitachi M-4100 spectrometer. A Waters HPLC apparatus equipped with a 990 photodiode-array detector was used. Cosmosil 5C18-AR (5 μm , ODS-type, Nacalai Tesque, Kyoto, Japan) columns of small (150 \times 6 mm i.d.) and large (250 \times 20 mm i.d.) size were used for analysis including LC-MS and preparative HPLC, respectively. Two preparative HPLC steps were performed for purification. The first step was carried out using a solvent system composed of (a) aqueous 0.2 M sodium perchlorate, 60% perchloric acid (1000:0.2) and (b) MeCN, under the following gradient conditions: a/b = 70/30 to 10/90, 240 min, 9 mL/min. The second step was performed with a solvent system composed of (c) 0.1 M ammonium acetate and (d) MeOH, under the following conditions: c/d = 70/30 to 50/50, 30 min, 50/50 to 10/90, 150 min, 9.0 mL/min. LC-MS was performed with a Hitachi M-1000 apparatus equipped with an atmospheric pressure ionization (API) module as the LC-MS interface. Protonated molecular ions ($[M + H]^+$) were of very high relative intensity when the drift voltage was chosen as -30 eV under the positive ionization mode with the latter solvent system (c/d = 70/30 to 50/50, 15 min, 50/50 to 10/90, 75 min, 1.0 mL/min). Preparative TLC was carried out on Si gel plates (Merck, art. 5744 Kieselgel 60 F₂₅₄).

Plant Material. The stem bark of *E. abyssinica* was collected (October 1990) in the Meru district of Kenya. The samples were identified by Mr. G. M. Mungai, Eastern African Herbarium, P.O. Box 45166, Nairobi, Kenya. Voucher specimens are deposited at Kobe Pharmaceutical University, Kobe, Japan, and the Eastern African Herbarium, Nairobi, Kenya.

Extraction and Isolation. Powdered bark (226 g) of *E. abyssinica* was extracted with MeOH three times to give 32.3 g of crude extract. After being defatted with hexane, the residue was suspended with H_2O and extracted with Et_2O three times. The Et_2O -soluble fraction (10.6 g) was dissolved in MeOH, and 300-mg aliquots were submitted to the first-step HPLC procedure and divided into 58 fractions (each 20 mL). Among these, five fractions, 15, 37, 38, 39, and 56, were further purified. From each fraction, MeCN was evaporated, and the residual aqueous layer was extracted with Et_2O . After evaporation of Et_2O , the residue was submitted to the second-step HPLC procedure. MeOH was evaporated from the collected effluent, the aqueous layer was extracted with Et_2O , and compounds were obtained after evaporation of Et_2O . Each compound was finally purified by preparative TLC ($CHCl_3$ - Me_2CO , 9:1). Thus, from fractions 15, 38, 39, and 56, sigmoidin D (R_f 0.25, 23.2 mg), abyssinoflavanone IV (**2**; R_f 0.69, 8.3 mg), abyssinoflavanone V (**3**, R_f 0.41, 16.9 mg), and abyssinone-V 4'-methyl ether (**1**; R_f 0.55, 10.4 mg), were obtained, respectively. Fraction 37 comprised two peaks whose retention times in HPLC were close to each other with similar peak areas. Also, by TLC, two spots (R_f 0.53 and 0.50) appeared. Preparative TLC was carried out to purify these; however, they mutually interconverted promptly during the evaporation of solvent.

Thus, abyssinoflavanone VI (**4**; 20.0 mg) was obtained as an equilibrated 1:1 mixture.

Abyssinone-V 4'-methyl ether (1): obtained as a white amorphous solid; yield 0.0097%; $[\alpha]_D -33^\circ$ (*c* 0.43, MeOH); CD (*c* 1.53×10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 329 (+1.3), 309 (0), 287 (-5.2), 256 (0), 248 (+0.4); IR (KBr) ν_{\max} 3450 (br, OH), 1641 (C=O), 1385, 1161, 1088 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EIMS *m/z* [M^+] 422 (20), 201 (29), 153 (51), 69 (98), 41 (100); HREIMS *m/z* [M^+] 422.2082 (422.2092 calcd for $\text{C}_{26}\text{H}_{30}\text{O}_5^+$).

Abyssinoflavanone IV (2): obtained as white amorphous solid; yield 0.0058%; $[\alpha]_D -53^\circ$ (*c* 0.40, MeOH); CD (*c* 1.57×10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 328 (+1.5), 310 (0), 288 (-7.1), 258 (0), 250 (+0.5); IR (KBr) ν_{\max} 3425 (br, OH), 1639 (C=O), 1456, 1275, 1161 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EIMS *m/z* [M^+] 380 (65), 324 (72), 212 (47), 153 (100); HREIMS *m/z* [M^+] 380.1251 (380.1258 calcd for $\text{C}_{22}\text{H}_{20}\text{O}_6^+$).

Abyssinoflavanone V (3): obtained as pale-yellow amorphous solid; yield 0.022%; $[\alpha]_D -56^\circ$ (*c* 0.75, MeOH); CD (*c* 1.35×10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 329 (+1.5), 316 (0), 290 (-8.0), 267 (0), 257 (+2.0), 246 (0), 242 (-0.6); IR (KBr) ν_{\max} 3450 (br, OH), 1639 (C=O), 1456, 1275, 1161 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EIMS *m/z* [M^+] 440 (76), 384 (47), 273 (21), 153 (100); HREIMS *m/z* [M^+] 440.1825 (440.1834 calcd for $\text{C}_{25}\text{H}_{28}\text{O}_7^+$).

Abyssinoflavanone VI (4): obtained as pale-yellow amorphous solid; yield 0.0094%; $[\alpha]_D 0^\circ$ (*c* 0.75, MeOH); IR (KBr) ν_{\max} 3425 (br, OH), 1641 (C=O), 1454, 1161, 1121 cm^{-1} ; ^1H - and ^{13}C -NMR data, see Tables 1 and 2; EIMS *m/z* [M^+] 440 (100), 381 (77), 153 (28); HREIMS *m/z* [M^+] 440.1821 (440.1833 calcd for $\text{C}_{25}\text{H}_{28}\text{O}_7^+$).

Sigmoidin D: obtained as white amorphous solid; yield 0.010%; $[\alpha]_D -30^\circ$ (*c* 0.30, MeOH); CD (*c* $1.88 \times$

10^{-4} M, MeOH) nm ($\Delta\epsilon$) 380 (0), 330 (+1.9), 313 (0), 292 (-7.2), 265 (0), 254 (+1.0), 242 (+0.1); IR (KBr) ν_{\max} 3450 (br, OH), 1639 (C=O), 1458, 1277, 1163, 1088 cm^{-1} ; EIMS *m/z* [M^+] 372 (100), 207 (18), 153 (34); HREIMS *m/z* [M^+] 372.1229 (372.1208 calcd for $\text{C}_{20}\text{H}_{20}\text{O}_7^+$). ^1H - and ^{13}C -NMR spectra were in agreement with literature values.⁸

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References and Notes

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- (9) In an earlier report (ref 3), three flavanones were named as abyssinins I–III; however, the name abyssinin was used previously for a bufadienolide isolated from *Bersama abyssinica* (Kubo, I.; Matsumoto, T. *Tetrahedron Lett.* **1984**, 4601–4604). To avoid further confusion, we have named three of the new compounds in this study as abyssinoflavanones IV, V, and VI.

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