Trypanocidal Diarylheptanoids from *Aframomum letestuianum*

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Three new diarylheptanoids, (4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one, letestuianin A (**1**), (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one, letestuianin B (**2**), and 1,7-bis(4-hydroxyphenyl)heptan-3,5-dione, letestuianin C (**3**), as well as the known (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one (**5**) were isolated from *Aframomum letestuianum*. The known flavonoids 3-acetoxy-5,7,4′-trihydroxyflavanone, 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone, 7-methoxy-3,5,4′-trihydroxyflavone, and 3,3′,4′,5,7-pentahydroxyflavan were also obtained from this plant. Their structures were determined using a combination of 1D and 2D NMR techniques. The four diarylheptanoids were tested for growth inhibitory activity in vitro versus bloodstream forms of African trypanosomes. IC_{50} values in the range of 1−3 μg/mL were found for compounds **3** and **5**.

The genus *Aframomum* K. Schum belongs to the economically and medicinally important family Zingiberaceae. It is represented in Cameroon by over 20 species of rhizomatous herbs.[1] All of them are widely used locally in ethnodietary and in folk medicinal preparations as well as for cultural and spiritual purposes.[2] In our previous research on this genus, we reported the isolation and characterization of several flavonoids and labdane diterpenes.[3−5] In continuation of our work on this genus and as part of our efforts to discover new antiparasitic drug leads from Cameroonian medicinal plants[6] we have investigated the seeds of *Aframomum letestuianum* and herein report the isolation of four diarylheptanoids. Three are new compounds to which we have given the trivial names letestuianin A (**1**), letestuianin B (**2**), and letestuianin C (**3**). The fourth is the previously reported (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one (**5**).[7] In addition, the known flavonoids 3-acetoxy-5,7,4′-trihydroxyflavanone,[4] 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone,[4] 7-methoxy-3,5,4′-trihydroxyflavone,[5] and 3,3′,4′,5,7-pentahydroxyflavan[8] were isolated in large quantitities. The trypanocidal activity of the diarylheptanoids is presented.

Results and Discussion

A sample of the air-dried powdered seeds of *A. lestestuianum* was extracted with MeOH−CH_2Cl_2 and subjected to sequential extraction with hexane and CH_2Cl_2. Bioassay-guided fractionation and purification of the CH_2Cl_2-soluble fractions led to the isolation of four diarylheptanoids and four flavonoids. The structures of the compounds were elucidated by spectroscopic techniques, and comparison with literature data revealed that three of the isolated diarylheptanoids are new compounds.

Compound **1** was obtained as a yellowish oil. The EIMS spectrum showed a molecular ion peak at *m*/*z* 340 with 100% intensity, compatible with the molecular formula $C_{20}H_{20}O_5$. The IR spectrum showed important absorption bands at ν_{max} 3363 (OH) and 1633 cm^{-1} (C=C−C=O). The ^1H NMR spectrum revealed the presence of a *para*-disubstituded benzene ring characterized by signals at δ 7.52 (2H, d, J = 8.5 Hz) and 6.88 (2H, d, J = 8.5 Hz); a 1,3,4-trisubstituted benzene ring [δ 6.85 (H-2″, d, J = 2.0 Hz), 6.72 (H-5″, d, J = 8.4 Hz), and 6.68 (H-6″, dd, J = 8.4, 2.0 Hz)]; a pair of *trans* olefinic protons at δ 7.53 (H-7, d, J = 15.9 Hz) and 6.53 (H-6, d, J = 15.9 Hz); a methoxy signal at δ 3.80 (s); and two methylenes at δ 2.85 and 2.67 (each triplet, J = 8.1 Hz). This was in sound agreement with the ^{13}C NMR spectrum (Table 1), which showed signals attributed to a carbonyl at δ 199.9 (C-3) and a hydroxylated olefinic carbon at δ 178.5, which with subsequent HMBC cross correlation peak with the *trans* olefinic protons as well as with H-4 (δ 5.81) was attributed to C-5. Three oxygenated sp^2 carbon atoms were also observed at δ 145.9, 148.3, and 160.5. A judicious analysis of the $^1H-^1H$ COSY data of **1** implied connectivities of H-7 to H-6, H-2 to H-1, H-2′ to H-3′ and H-6′, H-5′ to H-3′ and H-6′, and H-6″ to H-2″ and H-5″. The correlations observed in the NOESY and HMBC spectra attached the methoxy group at position C-3″ rather than C-4″, and pertinent correlation peaks were observed between the OMe group (δ 3.80) and H-2″ (δ 6.85) in the NOESY spectrum and between the OMe protons and C-3″ in the HMBC spectrum. The stereochemistry of the C-6/C-7 double bond bond is *E* as judged by the coupling constant between the two protons (J = 15.9 Hz), and that of the C-4 double bond is *Z*, as a clear NOESY correlation peak was observed between H-4 and H-6. Further analysis of HMBC and NOESY spectra led to the assignment of all carbons and protons, and the structure of compound **1** is (4*Z*,6*E*)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one. The trivial name letestuianin A was given to this new diarylheptanoid.

Compound **2** was obtained as yellow needles (CH_2Cl_2), mp 179−180 °C. The EIMS of **2** showed a molecular ion peak at *m*/*z* 370 compatible with the molecular formula $C_{21}H_{22}O_6$. The IR spectrum showed absorption bands due

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Table 1. ^{13}C (125 MHz) and ^{1}H NMR (500 MHz) Data for Compounds **1**, **2**, and **3**

	1[a]		**2**[b]		**3**[c] (major component)	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	31.7	2.85 t (8.1)	30.4	2.77 t (8.0)	29.7	2.71 s
2	42.7	2.67 t (8.1)	41.3	2.65 t (8.0)	46.4	2.71 s
3	199.5		199.2		206.5	
4	101.0	5.81 s	100.2	5.90 s	57.4	3.52 s
5	178.5		177.9		206.5	
6	120.8	6.53 d (15.9)	119.7	6.63 d (15.9)	46.4	2.71 s
7	140.6	7.53 d (15.9)	140.2	7.45 d (15.9)	29.7	2.71 s
1′	127.8		126.3		133.0	
2′	130.9	7.52 d (8.5)	111.0	7.25 d (2.0)	130.4	6.98 d (8.5)
3′	116.9	6.88 d (8.5)	148.0		116.3	6.68 d (8.5)
4′	160.5		149.2		156.7	
5′	116.9	6.88 d (8.5)	115.7	6.78 d (8.0)	116.3	6.68 d (8.5)
6′	130.9	7.52 d (8.5)	123.0	7.13 dd (8.0, 2.0)	130.4	6.98 d (8.5)
1″	133.4		131.6		133.0	
2″	112.9	6.85 d (2.0)	112.5	6.77 d (2.0)	130.4	6.98 d (8.5)
3″	148.3		147.4		116.3	6.68 d (8.5)
4″	145.9		144.7		156.7	
5″	115.8	6.72 d (8.4)	115.3	6.64 d (7.9)	116.3	6.68 d (8.5)
6″	121.6	6.68 dd (8.4, 2.0)	120.3	6.59 dd (7.9, 2.0)	130.4	6.98 d (8.5)
OMe′			55.7	3.78 s		
OMe″	56.3	3.80 s	55.5	3.71 s		

[a] Spectra recorded in acetone-d_6. [b] Spectra recorded in DMSO-d_6. [c] Spectra recorded in CD_3OD.

to hydroxyl group(s), enone, and aromatic ring(s) functionalities at ν_{max} 3436, 1631, and 1602 cm^{-1}, respectively. The ^{1}H and ^{13}C NMR data (Table 1) of **2** were closely related to those of compound **1**. The only significant differences compared to **1** are that both aromatic systems are 1,3,4-trisubstituted and the presence of an additional methoxy group in **2**. Once more the NOESY spectrum was useful for the determination of the position of the methoxy groups on the aromatic rings as well as for the *Z* conformation of one of the double bond. Important correlation peaks were observed between the OMe at δ 3.71 and the proton at δ 6.77 (d, $J = 2.0$ Hz) as well as the OMe at δ 3.78 and the proton at δ 7.25 (d, $J = 2.0$ Hz). Together with COSY and HMBC data the structure (4*Z*,6*E*)-5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-4,6-dien-3-one was determined for compound **2**, and it was given the trivial name letestuianin B.

Compound **3** was obtained as a pale yellow oil. The EIMS spectrum of **3** showed a molecular ion peak at *m/z* 312 compatible with the molecular formula $C_{19}H_{20}O_4$. The IR spectrum showed absorption bands at ν_{max} 3407, 1630, 1613, 1515, and 828 cm^{-1} closely related to those of **1** and **2**. The 1D NMR spectra suggested the presence of two components, in a 3:7 ratio. For the major component, the ^{1}H NMR spectrum indicated the presence of a *para*-disubstituted benzene ring [δ 6.98 (2H, d, $J = 8.5$ Hz) and 6.68 (2H, d, $J = 8.5$ Hz)] and two methylenes appearing as singlet at δ 2.71. An isolated proton appeared at δ 3.52 as a singlet. The intensity of the latter signal was very low due to exchange with deuterium from the methanol solvent used for NMR experiments. These data account only for nine protons, and the fact that only eight carbon signals appeared in its ^{13}C NMR spectrum suggests that **3** is symmetric with two identical benzene rings. The data for the major component were compatible only with the 1,3-diketone shown in Figure 1, and as expected, this is in equilibrium with an enol tautomer. Typical signals for the enol appeared in the ^{1}H and ^{13}C NMR spectra, for example a proton signal at δ 4.58 (H-4) and carbon signals at δ 194.7 (C-3) and 100.0 (C-4), but to confirm this tautomeric equilibrium, compound **3** was treated with a mixture of pyridine−Ac_2O (1:1) to give the acetylated derivative **4**. The analysis of the ^{1}H NMR spectrum of **4** revealed the presence of a 1,4-disubstituted benzene ring, showing that

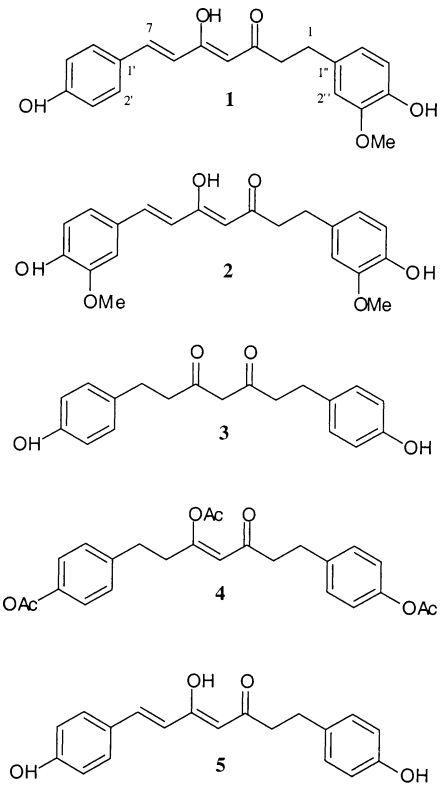

Figure 1.

the symmetric nature of the molecule had been distorted. An olefinic signal was also observed in **4** at δ 5.42 in replacement of the methylene signal that was present at δ 3.52 in **3**. The presence of three acetate functions was characterized by shifts at δ 2.22 (6H, s) and 2.10 (3H, s). The analysis of the ^{13}C NMR spectrum of **4** with signals at δ 169.5, 169.7, and 170.0 confirmed the three acetate functions. A conjugated carbonyl function was also observed at δ 193.2. All the above information showed that **4** was the enol form of **3**. Further analysis of HMBC, COSY, and NOESY spectra of the nonacetylated and acetylated derivative led to the characterization of compound **3** as 1,7-bis(4-hydroxyphenyl)heptan-3,5-dione, consequently named letestuianin C.

Table 2. Antitrypanocidal Activities of *Aframomum letestuianum* Diarylheptanoids

	IC_{50} (μg/mL)		
		KETRI *T. b. rhodesiense* KETRI isolates	
compound	Lab110 EATRO *T. b. brucei*	243	243 As 10−3
1	>100		
2	67	>100	>100
3	1.4	2.3	2.6
5	2.6	2.8	1.3
melarsoprol	0.002	0.0005	0.005
pentamidine	0.0006	0.0005	0.004

Previous studies on the genus *Aframomum* have, up to date, reported the presence of only two major classes of natural products, diterpenoids and flavonoids. To the best of our knowledge, **1**, **2**, **3**, and **5** are the first diarylheptanoids reported from this important genus, although they are common in the sister genera *Alpinia*[9−11] and *Curcuma*.[12−14] The four diarylheptanoids obtained were assayed for trypanocidal activity, tested against bloodstream forms of *Trypanosoma b. brucei* and *Trypanosoma b. rhodesiense* isolates grown in vitro in 24-well plates. Coulter counts were made daily, and the IC_{50} values determined after 48 h are given in Table 2. Compound **1** was not growth inhibitory below 100 μg/mL. Compound **2** gave an IC_{50} value of 67 μg/mL with the *T. b. brucei* isolate but >100 μg/mL with *T. b. rhodesiense* isolates. Compounds **3** and **5**, however, were highly effective in the range 1−3 μg/mL for all isolates tested. Interestingly, the additional methoxy group in **1**, compared to **5**, makes it inactive. Corresponding IC_{50} values for the trypanocides melarsoprol and pentamidine were ~300−5000-fold lower; however, lack of sufficient material prevented us from testing these compounds in vivo in a mouse model infection.

Experimental Section

General Experimental Procedures. Melting points were recorded with a Reichter microscope and are uncorrected. The UV and IR spectra (KBr) were recorded with a Shimadzu UV-3001 and a Jasco FT-IR spectrophotometer, respectively. ^{1}H NMR and ^{13}C NMR were recorded in $CDCl_3$, acetone-d_6, DMSO-d_6, or CD_3OD using a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in parts per million relative to tetramethylsilane (TMS, δ = 0), while the coupling constants (J) are given in Hz. COSY, HMQC, and HMBC experiments were recorded with gradient enhancements using sine-shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}$ = 145 Hz and $^nJ_{CH}$ = 10 Hz. The raw data were transformed and the spectra evaluated with the standard Bruker UXNMR software. The positive EI (70 eV) and CI mass spectra were recorded with a JEOL SX102 spectrometer. Column chromatography was run on Merck Si gel 60 and gel permeation on Sephadex LH-20. TLC analyses were carried out on Si gel GF_{254} precoated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C, or by visualizing with a UV lamp at 254 and 366 nm.

Plant Material. The seeds of *A. letestuianum* were collected from Abong-bang, East Province, Cameroon, in December 1998. Mr. Paul Mezili, a retired botanist of the Cameroon Herbarium, authenticated the plant material. Voucher specimens (BUD 0391) were deposited at the Herbarium of the Botany Department of the University of Dschang.

Extraction and Isolation. The air-dried powdered seeds of *A. letestuianum* (2 kg) were macerated with a mixture (1:1) of MeOH−CH_2Cl_2 (4 L) overnight and evaporated in vacuo to yield a crude extract (150.5 g). This crude extract was dissolved in 80% MeOH (600 mL) and extracted hexane (3 × 500 mL). The aqueous MeOH was further diluted with water to 60%MeOH and extracted with CH_2Cl_2 (3 × 500 mL). Vacuum concentration yielded CH_2Cl_2 extract (36.5 g) and hexane extract (28.0 g), which contained mostly fats. Subjection of the CH_2Cl_2 extract to column chromatography over silica gel eluting with a CH_2Cl_2−hexane gradient followed by acetone−CH_2Cl_2 afforded three major fractions, I [500 mg, CH_2Cl_2−hexane (6:4)], II [16.0 g, CH_2Cl_2−hexane (8:2) and acetone−CH_2Cl_2 (1:9)], and III [2.1 g, acetone−CH_2CL_2 (2:8)]. Subjecting fraction I to repeated column chromatography on silica gel eluted with a CH_2Cl_2−hexane gradient and further purification by gel permeation chromatography on Sephadex LH-20 (MeOH) afforded compounds **1** (24 mg), **2** (10.4 mg), and 7-methoxy-3,5,4′-trihydroxyflavanone (5.5 mg). Subjection of fraction II (7.5 g) to gel permeation chromatography on Sephadex LH-20 (MeOH) gave additional amount of **1** (15 mg), 3-acetoxy-5,7,4′-trihydroxyflavanone (3.5 g), 3-acetoxy-7-methoxy-5,4′-dihydroxyflavanone (1.8 g), and a mixture of two main products (350 mg), which was further purified by countercurrent chromatography (CCC) eluting head to tail with hexane−ethyl acetate−MeOH−H_2O (4:6:5:5) and reversing the flow after 3 h to obtain compounds **3** (179 mg) and **5** (86 mg). Treatment of fraction III on a silica gel column eluted with MeOH−CH_2Cl_2 gradient followed by gel permeation on Sephadex LH-20 (MeOH−CH_2Cl_2, 1:1) afforded 3,3′,4′,5,7-pentahydroxyflavan (139 mg) and a mixture of nonresolved compounds.

(4*Z*,6*E*)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)hepta-4,6-dien-3-one, letestuianin A (1): yellowish oil; UV (MeOH) λ_{max} (log ϵ) 380 (3.2) and 283 (3.9) nm; IR (KBr) ν_{max} 3363, 2937, 1633, 1583, 1514, 1431, 831, and 790 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 340 [M]$^+$ (100), 322 (10), 189 (30), 147 (70), 137 (55), 107 (18); HREIMS *m*/*z* 340.1304 (calcd for $C_{20}H_{20}O_5$, 340.1311).

(4*Z*,6*E*)-5-Hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-hepta-4,6-dien-3-one, letestuianin B (2): shiny yellow needles (CH_2Cl_2−hexane); mp 179−180 °C; UV (MeOH) λ_{max} (log ϵ) 374 (2.9) and 288 (3.4) nm; IR (KBr) ν_{max} 3436, 1631, 1602, 1511, 1280, 1202, 1028, and 814 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 370 [M]$^+$ (44), 352 (16), 219 (18), 177 (63), 137 (100), 44 (25); HREIMS *m*/*z* 370.1411 (calcd for $C_{21}H_{22}O_6$, 370.1416).

1,7-Bis(4-hydroxyphenyl)heptan-3,5-dione, letestuianin C (3): yellowish oil; UV (MeOH) λ_{max} (log ϵ) 279 (3.4) and 224 (2.4) nm; IR (KBr) ν_{max} 3407, 1623, 1613, 1515, 1462, 1385, 1243, 828 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 312 [M]$^+$ (34), 191 (10), 120 (20), 107 (100), 77 (10); HREIMS *m*/*z* 312.1358 (calcd for $C_{19}H_{20}O_4$, 312.1361).

Acetylation of Letestuianin C (3). Compound **3** (25 mg) was dissolved in a (1:1) mixture of pyridine−Ac_2O (4 mL) and the reaction mixture left at room temperature overnight. The product was concentrated with addition of toluene and purified on a silica gel column (hexane−EtOAc, 9:1) to give 5-acetoxy-1,7-bis(4-acetoxyphenyl)hepta-4-en-3-one (**4**) (26 mg) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.10 (Ac), 2.22 (2 × Ac), 2.52 (4H, t, J = 7.6 Hz, H-2, H-6), 2.81 (4H, t, J = 7.6 Hz, H-1, H-7), 5.42 (H-4, s), 6.90 (4H, m, H-2′, H-6′, H-2″, H-6″), 7.23 (4H, m, H-3′, H-5′, H-3″, H-5″); ^{13}C NMR $CDCl_3$, 125 MHz) δ 31.2 (C-1, C-7), 40.3 (C-2, C-6), 100.1 (C-4), 121.9 (C-3″, C-5″), 122.0 (C-3′, C-5′) 129.7 (C-2″, C-6″), 129.8 (C-2′, C-6′), 138.5 (C-1″), 138.6 (C-1′), 149.4 (C-4′, C-4″), 179.1 (C-5), 193.2 (C-3).

Biological Assay. Assays for inhibition of trypanosomal growth were conducted as previously described.[15,16] Bloodstream-form trypanosomes were cultured in modified IMDM with 20% horse serum at 37 °C. Drug studies were done in duplicate in 24-well plates (1 mL/well) with final inhibitor concentrations of 0.1, 1, 10, 25, and 100 μg/mL. Wells were inoculated with 10^5 trypanosomes, and one-half the volume of each well was changed daily. After 48 h, the parasite number was determined in a Model Z1 Coulter counter and IC_{50} values were calculated from semi-log plots. Assays were done two or more times, using widely spaced concentration curves initially, followed by curves of closely spaced values to obtain the IC_{50} value.

Compounds were dissolved in 100% dimethyl sulfoxide and diluted in medium, so that the dimethyl sulfoxide concentration never exceeded 0.3%, a noninhibitory concentration.

Strains used were *Trypanosoma b. brucei* Lab 110 EATRO and Kenya Trypanosomiasis Research Institute (KETRI) isolates *Trypanosoma b. rhodesiense* 243 and 243 As 10-3.[15,16]

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