Four Diphenylpropanes and a Cycloheptadibenzofuran from *Bussea sakalava* from the Madagascar Dry Forest[1]

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Investigation of the endemic Malagasy plant *Bussea sakalava* for antiproliferative activity against the A2780 ovarian cancer cell line led to the isolation of the four new diphenylpropanes **1**−**4** and the new cycloheptadibenzofuran **5**; compound **5** has a previously unreported natural product skeleton. The structure elucidation of these compounds was based on the analysis of their 1D and 2D NMR and mass spectroscopic data. Compounds **1**−**5** were tested for antiproliferative activity against the A2780 human ovarian cancer cell line.

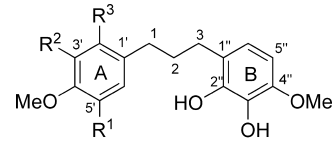
In our continuing search for biologically active natural products from tropical rainforests as part of an International Cooperative Biodiversity Group (ICBG) program, we obtained an ethanol extract from the roots of a plant identified as *Bussea sakalava* Du Puy & R. Rabev. (Fabaceae) from Madagascar. This extract showed moderate antiproliferative activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 10 μg/mL. The extract was selected for examination on the basis of this activity and the absence of previous phytochemical studies of the species.

Previous studies on the genus *Bussea* indicated the presence of azetidine-2-carboxylic acid and 3-hydroxyproline in seeds of different *Bussea* species,[2,3] and the cytotoxicity and high trypanocidal activity of a methanol extract of stem bark of *Bussea occidentalis* have been reported.[4]

Fractionation of a dichloromethane fraction of an ethanol extract of *B. sakalava* by C-18 open-column and high-performance liquid chromatography (HPLC) yielded four new diphenylpropanes named bussealins A−D (**1**−**4**) and a cycloheptadibenzofuran derivative named bussealin E (**5**). Herein we report the structural elucidation of these new compounds and their antiproliferative properties against the A2780 human ovarian cancer cell line.

Results and Discussion

Bussealin A (**1**) was obtained as an off-white, amorphous solid. Its positive ESIMS revealed a pseudomolecular ion peak at *m/z* 321.1338 $[M + H]^+$ corresponding to the molecular formula $C_{17}H_{21}O_6$. The IR spectrum showed absorptions of OH (3367 cm^{-1}) and aromatic groups. The ^1H NMR spectrum (Table 1) exhibited a singlet at δ_H 6.18 (2H, s) corresponding to a pair of aromatic protons of an A_2 system, two aromatic doublets [δ_H 6.50 (d, $J = 8.4$) and 6.38 (d, $J = 8.4$)] of an AB system, two OCH_3 groups [δ_H 3.75 (s) and 3.78 (s)], and a multiplet and two triplet methylene groups at δ_H 1.79 (2H, m), 2.52 (2H, t, $J = 7.7$), and 2.41 (2H, t, $J = 7.7$), respectively. The ^{13}C NMR spectrum of **1** exhibited signals for 17 carbons, including three methylene carbons (δ_C 36.5, 33.0, and 30.6), two OCH_3 groups (δ_C 56.5 and 60.8), and 12 aromatic carbons assignable to two isolated aromatic rings. Six of the aromatic carbons were oxygenated, as shown by their deshielded carbon chemical shifts (Table 1), and were consistent with the molecular formula. The above data suggested that **1** had a diphenyl propane skeleton. The complete ^1H and ^{13}C NMR assignments and the connectivities were determined from analysis of a combination of COSY, HMQC, and HMBC data. Three mutually coupled methylene groups were revealed by the cross-peaks observed in the COSY spectrum. In the HMBC spectrum, H-1 (δ_H 2.41) showed correlations with C-2 (δ_C 33.0), C-3 (δ_C 30.6), C-1′ (δ_C 140.2), and C-2′ and C-6′, both of which had the same chemical shifts (δ_C 108.7). The A_2 substitution pattern of the A ring of **1** was established by HMBC correlations from the signal at δ_H 6.18 (H-2′ and H-6′) to C-1 (δ_C 36.5), C-1′ (δ_C 140.2), C-3′ (δ_C 151.3), C-4′ (δ_C 134.7), and C-6′ and C-2′ (δ_C 108.7), as well as the correlation from one OCH_3 group at δ_H 3.75 to C-4′ (δ_C 134.7). The proton substitutions on the B ring were assigned on the basis of the ^3J HMBC correlations between H-3 (δ_H 2.52) and C-6″ (δ_C 120.5) and between H-5″ (δ_H 6.38) and C-1″ (δ_C 123.4). Moreover, the H-5″ proton showed HMBC correlations to C-6″ (δ_C 120.5), C-4″ (δ_C 147.8), and C-3″ (δ_C 134.9). The location of the remaining OCH_3 group was at C-4″, as deduced from the HMBC correlation between the signal at δ_H 3.78 and that of C-4″. On the basis of the molecular formula of **1**, the remaining four OH groups were located at C-2″ (δ_C 144.7), C-3″ (δ_C 134.9), C-3′ (δ_C 151.3), and C-5′ (δ_C 151.3). Bussealin A is thus assigned the structure 3′,5′,2″,3″-tetrahydroxy-4′,4″-dimethoxy-1,3-diphenylpropane (**1**).



1 $R^1 = R^2 = OH$, $R^3 = H$
2 $R^1 = OH$, $R^2 = H$, $R^3 = OMe$
3 $R^1 = R^3 = H$, $R^2 = OH$
4 $R^1 = R^3 = OMe$, $R^2 = H$

Bussealin B (**2**) was obtained as an off-white, amorphous solid. Its positive ESIMS revealed a pseudomolecular ion peak at *m/z* 335.1512 $[M + H]^+$ corresponding to the molecular formula $C_{18}H_{23}O_6$. The ^1H NMR spectrum (Table 1) showed two singlets of an AX system at δ_H 6.58 (s) and 6.60 (s), two aromatic doublets of an AB system at δ_H 6.51 (d, $J = 8.4$) and 6.39 (d, $J = 8.4$), three OCH_3 groups [δ_H 3.76 (s), 3.80 (s), and 3.83 (s)], and one multiplet and two triplet methylene groups at δ_H 1.76 and 2.54 (t, $J = 7.8$) and 2.50 (t, $J = 7.8$). Inspection of the ^1H and ^{13}C NMR spectra of **2** revealed close similarities with those of **1**, except for

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Table 1. ^{1}H and ^{13}C NMR Data for Bussealin A−D (**1**−**4**)[a]

	1		**2**		**3**		**4**	
position	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C	^{1}H (*J*, Hz)	^{13}C
1	2.41 t (7.9)	36.5	2.50 t (7.8)	30.4	2.49 t (7.9)	36.1	2.55 t (7.7)	30.4
2	1.79 m	33.0	1.76 m	31.9	1.81 m	33.2	1.79 m	31.8
3	2.52 t (7.7)	30.6	2.54 t (7.8)	30.7	2.54 t (7.7)	30.6	2.55 t (7.7)	30.6
1′		140.2		124.7		137.2		124.3
2′	6.18 s	108.7		152.2	6.64 d (2.0)	116.5		153.3
3′		151.3	6.58 s	99.4		147.3	6. 61 s	99.6
4′		134.7		147.2		147.0		149.1
5′		151.3		141.0	6.79 d (8.2)	112.9		144.1
6′	6.18 s	108.7	6.60 s	117.9	6.60 dd (8.2, 2.0)	120.6	6.75 s	116.3
1″		123.4		123.7		123.5		123.6
2″		144.7		144.7		144.7		144.7
3″		134.9		134.9		135.0		134.9
4″		147.8		147.8		147.8		147.8
5″	6.38 d (8.4)	103.8	6.39 d (8.4)	103.8	6.39 d (8.4)	103.9	6.39 d (8.4)	103.9
6″	6.50 d (8.4)	120.5	6.51 d (8.4)	120.4	6.50 d (8.5)	120.5	6.51 d (8.3)	120.4
2′-OMe			3.76 s	56.8			3.78 s	56.6
4′-OMe	3.75 s	60.8	3.83 s	57.0	3.80 s	56.6	3.82 s	56.8
5′-OMe							3.76 s	57.6
4″-OMe	3.78 s	56.5	3.80 s	56.6	3.80 s	56.6	3.80 s	56.9

[a] In CD_3OD; δ (ppm) 500 MHz for ^{1}H and 125 MHz for ^{13}C; multiplicities; *J* values (Hz) in parentheses.

the presence of an additional OCH_3 signal and the chemical shifts of the AX system of ring A. The fact that the chemical shifts of the carbons of ring B of compounds **1** and **2** were superimposable (Table 1) indicated the presence of a 2″,3″-dihydroxy-4″-methoxyphenyl group in **2**. Interpretation of HMBC and NOESY experiments allowed us to determine the location of the OCH_3 groups to be at 2′, 4′, and 4″. The two singlet aromatic protons on ring A were assigned according to the observation of ^3J HMBC correlations from H-6′ (δ_H 6.60) to C-1 (δ_C 30.4) and from H-3′ (δ_H 6.58) to C-1′ (δ_C 124.7). Moreover, the proton signal of H-1 (δ_H 2.50) showed HMBC correlations with C-1′ (δ_C 124.7), C-6′ (δ_C 117.9), and the methoxylated carbon at C-2′ (δ_C 152.2). This indicated that the third OCH_3 group must be at C-4′ or C-5′. NOESY correlations from H-3′ (δ_H 6.58) to 2′-OMe (δ_H 3.76) and to 4′-OMe (δ_H 3.83) established the location of the methoxy group at C-4′ and the hydroxy group at C-5′. The structure of bussealin B was thus assigned as 5′,2″,3″-trihydroxy-2′,4′,4″-trimethoxy-1,3-diphenylpropane.

Bussealin C (**3**) was obtained as an off-white, amorphous solid. Its positive ESIMS revealed a pseudomolecular ion peak at *m/z* 305.1384 $[M + H]^+$ corresponding to the molecular formula $C_{17}H_{21}O_5$. Its ^{1}H NMR and ^{13}C NMR spectra (Table 1) indicated that **3** is also a diphenylpropane with a 2″,3″-dihydroxy-4″-methoxyphenyl group substituted at C-3. The 1,3,4-trisubstituted A ring was determined by the proton coupling constants and HMBC correlations from H-2′ (δ_H 6.64) and H-6′ (δ_H 6.60) to C-1 (δ_C 36.1) and COSY correlations between H-5′ (δ_H 6.79) and H-6′ (δ_H 6.60). Furthermore, the HMBC spectrum showed a ^3J correlation from H-6′ to the methoxylated carbon at C-4′ (δ_C 147.0), which was confirmed by NOESY correlations between H-5′ (δ_H 6.79) and 4′-OMe (δ_H 3.80). The above data coupled with the molecular formula led to assignment of the structure of bussealin C as 3′,2″,3″-trihydroxy-4′,4″-dimethoxy-1,3-diphenylpropane.

Bussealin D (**4**) was obtained as an off-white, amorphous solid. The positive ESIMS exhibited a pseudomolecular ion peak at *m/z* 349.1648 $[M + H]^+$ corresponding to the molecular formula $C_{19}H_{25}O_6$. The ^{1}H NMR and ^{13}C NMR spectra (Table 1) indicated that **4** had the same tetrasubstituted B ring with an OCH_3 group at C-4″ as in compounds **1**−**3**. In its ^{1}H NMR spectrum, the coupling patterns and the locations of the aromatic proton resonances of ring A were very similar to those of **2**. The presence of three OCH_3 groups and the substitution pattern of ring A of compound **4** were deduced by interpretation of the 1D and 2D NMR data. The HMBC spectrum of **4** showed correlations from H-1 (δ_H 6.79) to C-1′ (δ_C 124.3), C-6′ (δ_C 116.3), and the methoxylated carbon at C-2′ (δ_C 153.3). Furthermore, a clear ^3J long-range correlation from the singlet proton H-3′ (δ_H 6.61) to C-1′ (δ_C 124.3) was also observed. Thus, the two remaining OCH_3 groups were determined to be at C-4′ (δ_C 149.1) and C-5′ (δ_C 144.1). The structure of bussealin D was thus determined to be 2″,3″-dihydroxy-2′,4′,5′,4″-tetramethoxy-1,3-diphenylpropane.

The positive ESIMS of bussealin E (**5**) displayed a pseudomolecular ion peak at *m/z* 331.1181 $[M + H]^+$ corresponding to the molecular formula $C_{18}H_{19}O_6$. The ^{1}H NMR spectrum in $CDCl_3$ showed signals for a singlet aromatic proton at δ_H 6.70, two OH groups (δ_H 5.75 and 5.69), three OCH_3 groups at δ_H 4.24, 4.24, and 4.01, and three methylene groups as multiplets at δ_H 3.13, 3.12, and 2.17. The ^{13}C NMR spectrum of **5** exhibited 18 signals, assigned to three methylene (δ_C 35.5, 28.7, and 24.3), three OCH_3 (δ_C 60.8, 60.8, and 61.7), and 12 aromatic carbons of two isolated aromatic rings. Seven of the aromatic carbons were oxygenated, based on their deshielded chemical shifts (Table 2). The 10 degrees of unsaturation implied by the molecular formula $C_{18}H_{18}O_6$ required two additional rings. Interpretation of ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra allowed assignment of the locations of the functionalities present in **5**. In the COSY spectrum, the three methylene groups were mutually coupled. The assignment of a singlet aromatic proton was substantiated by the observation of HMBC correlations from H-1 (δ_H 6.70) to C-10 (δ_C 35.5), C-3b (δ_C 118.2), and two oxygenated aromatic carbons at C-2 (δ_C 146.5) and C-3 (δ_C 129.7). HMBC correlations from the signal at δ_H 5.69 to C-1 (δ_C 110.1), C-2 (δ_C 146.5), and the methoxylated carbon at C-3 (δ_C 129.7) were observed, substantiating the location of a hydroxy group at C-2. The other hydroxy group was assigned to position 7 on the basis of the observation of HMBC correlations from the signal at δ_H 5.75 to the carbon signals at C-6 (δ_C 136.5), C-7 (δ_C 142.3), and C-7a (δ_C 115.0). In addition, the signal at δ_H 5.75 showed NOESY correlations to H-8 (δ_H 3.13) and 6-OMe (δ_H 4.01). These observations required that the remaining OCH_3 group be placed at C-5. Furthermore, the HMBC correlations observed from H-10 (δ_H 3.12) to C-1 (δ_C 110.1), C-10a (δ_C 131.7), C-3b (δ_C 118.2), C-8 (δ_C 28.7), and C-9 (δ_C 24.3) confirmed the location of the cycloheptadiene ring. The above data confirmed the cycloheptadibenzofuran skeleton of **5**. Assignments of the ^{13}C NMR signals of C-3a, C-4a, and C-4b were made by comparing the measured data with those calculated by ACD/ChemSketch version 11.01. The calculated shifts were in excellent agreement with the observed values and were all within the standard deviation of the software (5 ppm), except for C-7a. Therefore, the structure of **5**

Table 2. ^{1}H and ^{13}C NMR Data for Bussealin E (**5**)

position	^{1}H[a]	^{13}C[a]	^{13}C[b]	^{1}H[c]
1	6.70 s	110.1	110.0	6.59 s
2		146.5	149.1	
3		129.7	131.3	
3a		146.2	148.7	
3b		118.2	113.3	
4a		140.7	139.7	
4b		120.6	117.4	
5		135.6	137.0	
6		136.5	137.9	
7		142.3	145.1	
7a		115.0	109.7	
8	3.13 m	28.7	28.9	3.08 m
9	2.17 m	24.3	24.2	2.12 m
10	3.12 m	35.5	34.5	3.07 m
10a		131.7	128.6	
2-OH	5.69 s			
3-OCH$_3$	4.24 s	60.8	61.5	4.18 s
5-OCH$_3$	4.24 s	60.8	61.6	4.08 s
6-OCH$_3$	4.01 s	61.7	61.0	3.90 s
7-OH	5.75 s			

[a] In $CDCl_3$; δ (ppm) 600 MHz for ^{1}H and 150 MHz for ^{13}C; multiplicities. [b] Calculated using ACD/ChemSketch version 11.01. [c] In CD_3OD; δ (ppm) 600 MHz for ^{1}H; multiplicities.

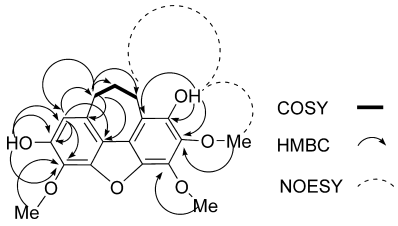
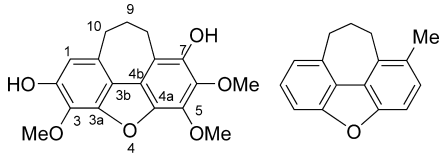


Figure 1. COSY, HMBC, and NOESY correlations of **5**.

was assigned as 9,10-dihydro-2,7-dihydroxy-3,5,6-trimethoxy-8*H*-cyclohepta[*klm*]dibenzofuran.



5 **6**

It is noteworthy that bussealin E is the first cycloheptadibenzofuran isolated from natural sources, and the cycloheptadibenzofuran skeleton is rare among synthetic compounds. The only simple synthetic compound with this ring system is 9,10-dihydro-1-methyl-8*H*-cyclohepta[*klm*]dibenzofuran (**6**) and its 8-keto derivative.[5]

The presence of diphenylpropanes in *B. sakalava* suggests that bussealin E is biosynthesized by oxidative coupling of an appropriate precursor diphenylpropane. This could be followed by nucleophilic attack from a phenolate anion on a carbonyl group followed by dehydration to afford the new cycloheptadibenzofuran skeleton (**5**) as indicated in Scheme 1.

The bioactivity of diphenylpropanes has been widely studied. The diphenylpropane broussonin A inhibited respiratory syncytial-virus (RSV) more effectively than the standard antiviral drug ribavirin,[6] and its antiaromatase activity has also been evaluated.[7] Broussonin B moderately inhibited chymotrypsin-like activity of the proteasome.[8] The anti-inflammatory,[9,10] antifungal,[11] antivascular,[12] antiadipogenic,[13] and anti-hCNT3 (human concentrative nucleoside transporter 3)[14] activities of diphenylpropane analogues have also been reported. Since there have been no previous studies on the properties of diphenylpropanes on human ovarian cancer cells, we investigated the antiproliferative activity of diphenylpropanes **1**−**4** against the A2780 human ovarian cancer cell line. Bussealins A−D (**1**−**4**) showed only weak antiproliferative activities, with IC_{50} values of 36, 24, 36, and 40 μM, respectively. Bussealin E (**5**), with a new chemical skeleton, was also tested against the A2780 cell line, but it also exhibited only weak activity, with an IC_{50} value of 45 μM. The new skeleton of bussealin E thus does not appear to confer any novel antiproliferative activity beyond that which is normal for diphenylpropanes.

Experimental Section

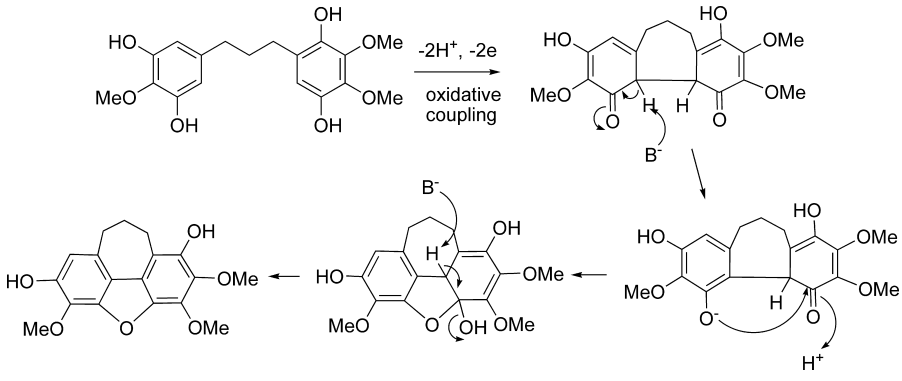
General Experimental Procedures. UV and IR spectra were measured on a Shimadzu UV-1201 spectrophotometer and a MIDAC M-series FTIR spectrophotometer, respectively. NMR spectra were recorded in CD_3OD or $CDCl_3$ on either JEOL Eclipse 500 or Bruker Avance 600 spectrometers. The chemical shifts are given in δ (ppm), and coupling constants (J) are reported in Hz. Mass spectra were obtained on an Agilent 6220 LC-TOF-MS. HPLC was performed on a Shimadzu LC-10AT instrument with a semipreparative C18 Varian Dynamax column (5 μm, 250 × 10 mm).

Antiproliferative Bioassays. Antiproliferative activities were obtained at Virginia Polytechnic Institute and State University against the drug-sensitive A2780 human ovarian cancer cell line as previously described, except that the samples were added in 1 μL of 100% DMSO per well instead of 20 μL of 1:1 DMSO−H_2O; paclitaxel (IC_{50} 0.017 μM) was used as a positive control.[15] The A2780 cell line is a drug-sensitive ovarian cancer cell line.[16]

Plant Material. A sample of root of *Bussea sakalava* Du Puy & R. Rabev. (Fabaceae) was collected on January 25, 2007, near Ambolobozobe, Madagascar, at coordinates 12°31′26″ S, 49°31′29″ E, at an elevation of 20 m. Its assigned collection number is Rakotonandrasana et al. 1079. The genus *Bussea* Harms is a small genus including seven species (five from tropical Africa and two from Madagascar). *B. sakalava* is endemic to deciduous forest from western to northern Madagascar. The hardwood of this species is used in construction and as firewood.[17] Voucher specimens have been deposited at the Parc Botanique and Zoologique de Tsimbazaza and at the Centre National d'Application des Recherches Pharmaceutiques in Antananarivo, Madagascar; the Missouri Botanical Garden in St. Louis, Missouri; and the Muséum National d'Histoire Naturelle in Paris, France.

Extraction and Isolation. Dried roots of *B. sakalava* (275 g) were ground in a hammermill, then extracted with ethanol by percolation

Scheme 1. Possible Biosynthesis of Cycloheptadibenzofuran **5** in *B. sakalava*



for 24 h at room temperature to give the crude extract MG 4273 (14.4 g), of which 3.0 g was shipped to Virginia Polytechnic Institute and State University for bioassay-guided isolation. Sample MG 4273 (IC_{50} 9.6 μg/mL, 2.1 g) was suspended in aqueous MeOH (MeOH−H_2O, 9:1, 100 mL) and extracted with hexane (3 × 100 mL portions). The aqueous layer was then diluted to 60% MeOH (v/v) with H_2O and extracted with CH_2Cl_2 (3 × 150 mL portions). The hexane extract was evaporated in vacuo to leave 227 mg with an IC_{50} value of 19 μg/mL. The 102.9 mg of residue from the CH_2Cl_2 extract had an IC_{50} of 10 μg/mL. The aqueous MeOH extract (1.7 g) was inactive. The CH_2Cl_2 extract was selected for fractionation using an SPE cartridge over C-18, and two fractions were collected. Fractions I and II (70.2 and 26.8 mg) had IC_{50} values of 8.6 and 15 μg/mL, respectively. Fraction I was separated by C-18 HPLC (65% MeOH−H_2O), and compounds **1** (3.3 mg, t_R 12.5 min), **2** (1.7 mg, t_R 18.6 min), **3** (2.0 mg, t_R 22.0 min), **4** (1.1 mg, t_R 29.5 min), and **5** (1.1 mg, t_R 26.5 min) were isolated.

3′,5′,2″,3″-Tetrahydroxy-4′,4″-dimethoxy-1,3-diphenylpropane (1): off-white amorphous solid; UV (MeOH) λ_{max} nm (log ε) 218 (4.40), 267 (3.69), 294 (3.52); IR ν_{max} cm^{-1} 3367, 1648, 1450, 1115, 1024; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 321.1338 $[M + H]^+$ (calcd for $C_{17}H_{21}O_6$, 321.1338).

5′,2″,3″-Trihydroxy-2″,4′,4″-trimethoxy-1,3-diphenylpropane (2): off-white, amorphous solid; UV (MeOH) λ_{max} nm (log ε) 214 (4.25), 229 (sh) (4.10), 290 (3.59) nm; IR ν_{max} cm^{-1} 3332, 1599, 1444, 1095, 1032; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 335.1512 $[M + H]^+$ (calcd for $C_{18}H_{23}O_6$, 335.1495).

3′,2″,3″-Trihydroxy-4′,4″-dimethoxy-1,3-diphenylpropane (3): off-white, amorphous solid; UV (MeOH) λ_{max} nm (log ε) 208 (4.15), 267 (3.54), 289 (3.47) nm; IR ν_{max} cm^{-1} 3338, 1656, 1450, 1115, 1024; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 305.1384 $[M + H]^+$ (calcd for $C_{17}H_{21}O_5$, 305.1389).

3′,2″,3″-Trihydroxy-4′,4″-dimethoxy-1,3-diphenylpropane (4): off-white, amorphous solid; UV (MeOH) λ_{max} nm (log ε) 210 (4.21), 229 (sh) (4.01), 289 (3.48) nm; IR ν_{max} cm^{-1} 3350, 1602, 1450, 1115, 1026; ^1H NMR (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD), see Table 1; ESIMS *m*/*z* 349.1648 $[M + H]^+$ (calcd for $C_{19}H_{25}O_6$, 349.1651).

9,10-Dihydro-2,7-dihydroxy-3,5,6-trimethoxy-8*H*-cyclohepta[*k-lm*]dibenzofuran (5): off-white, amorphous solid; UV (MeOH) λ_{max} nm (log ε) 218 (4.25), 270 (3.78), 294 (3.73), 316 (3.47)) nm; IR ν_{max} cm^{-1} 3332, 1567, 1449, 1115, 1024; ^1H NMR (600 MHz, CD_3OD and $CDCl_3$) and ^{13}C NMR (150 MHz, CD_3OD), see Table 2; ESIMS *m*/*z* 331.1181 $[M + H]^+$ (calcd for $C_{18}H_{19}O_6$, 331.1182).

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Supporting Information Available: ^1H, ^{13}C, COSY, HMBC, HMQC, and NOESY spectra of bussealins A−E (**1**−**5**). This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Biodiversity Conservation and Drug Discovery in Madagascar, Part 44. For Part 43, see: Cao, S.; Hou, Y.; Brodie, P.; Miller, J. S.; Randrianaivo, R.; Rakotobe, E.; Rasamison, V. E.; Kingston, D. G. I. *Chem. Biodiversity* 2010, 10.1002/cbdv.201000061.
(2) Evans, C. S.; Bell, E. A. *Phytochemistry* **1978**, *17*, 1127–1129.
(3) Watson, R.; Fowden, L. *Phytochemistry* **1973**, *12*, 617–622.
(4) Freiburghaus, F.; Kaminsky, R.; Nkunya, M. H. H.; Brun, R. *J. Ethnopharmacol.* **1996**, *55*, 1–11.
(5) Cagniant, P.; Bellinger, N.; Cagniant, D. *C. R. Acad. Sci., Sér. C* **1973**, *277*, 383–385.
(6) Bae, G.; Yu, J. R.; Lee, J.; Chang, J.; Seo, E. K. *Chem. Biodiversity* **2007**, *4*, 2231–2235.
(7) Lee, D.; Bhat, K. P. L.; Fong, H. H. S.; Farnsworth, N. R.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **2001**, *64*, 1286–1293.
(8) Tsukamoto, S.; Wakana, T.; Koimaru, K.; Yoshida, T.; Sato, M.; Ohta, T. *Biol. Pharm. Bull.* **2005**, *28*, 1798–1800.
(9) Leu, Y. L.; Hwang, T. L.; Chung, Y. M.; Hong, P. Y. *Chem. Pharm. Bull.* **2006**, *54*, 1063–1066.
(10) Sawle, P.; Moulton, B. E.; Jarzykowska, M.; Green, C. J.; Foresti, R.; Fairlamb, I. J. S.; Motterlini, R. *Chem. Res. Toxicol.* **2008**, *21*, 1484–1494.
(11) Lopes, N. P.; Kato, M. J.; Yoshida, M. *Phytochemistry* **1999**, *51*, 29–33.
(12) Ducki, S.; Rennison, D.; Woo, M.; Kendall, A.; Chabert, J. F. D.; McGown, A. T.; Lawrence, N. J. *Bioorg. Med. Chem.* **2009**, *17*, 7698–7710.
(13) Youn, U. J.; Lee, Y. S.; Jeong, H.; Lee, J.; Nam, J. W.; Lee, Y. J.; Hwang, E. S.; Lee, J. H.; Lee, D.; Kang, S. S.; Seo, E. K. *J. Nat. Prod.* **2009**, *72*, 1895–1898.
(14) Gupte, A.; Buolamwini, J. K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 917–921.
(15) Cao, S.; Brodie, P. J.; Miller, J. S.; Randrianaivo, R.; Ratovoson, F.; Birkinshaw, C.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. I. *J. Nat. Prod.* **2007**, *70*, 679–681.
(16) Louie, K. G.; Behrens, B. C.; Kinsella, T. J.; Hamilton, T. C.; Grotzinger, K. R.; McKoy, W. M.; Winker, M. A.; Ozols, R. F. *Cancer Res.* **1985**, *45*, 2110–2115.
(17) Du Puy, D. J.; Labat, J.-N.; Rabevohitra, R.; Villiers, J.-F., Bosser, J.; Moat, J. *The Leguminosae of Madagascar*; Royal Botanical Gardens: Kew, 2002; pp 22−24.

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