Six New Isoflavones and a 5-Deoxyflavonol Glycoside from the Leaves of *Ateleia herbert-smithii*

Nigel C. Veitch,* Polly S. E. Sutton, Geoffrey C. Kite, and Helen E. Ireland†

Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K.

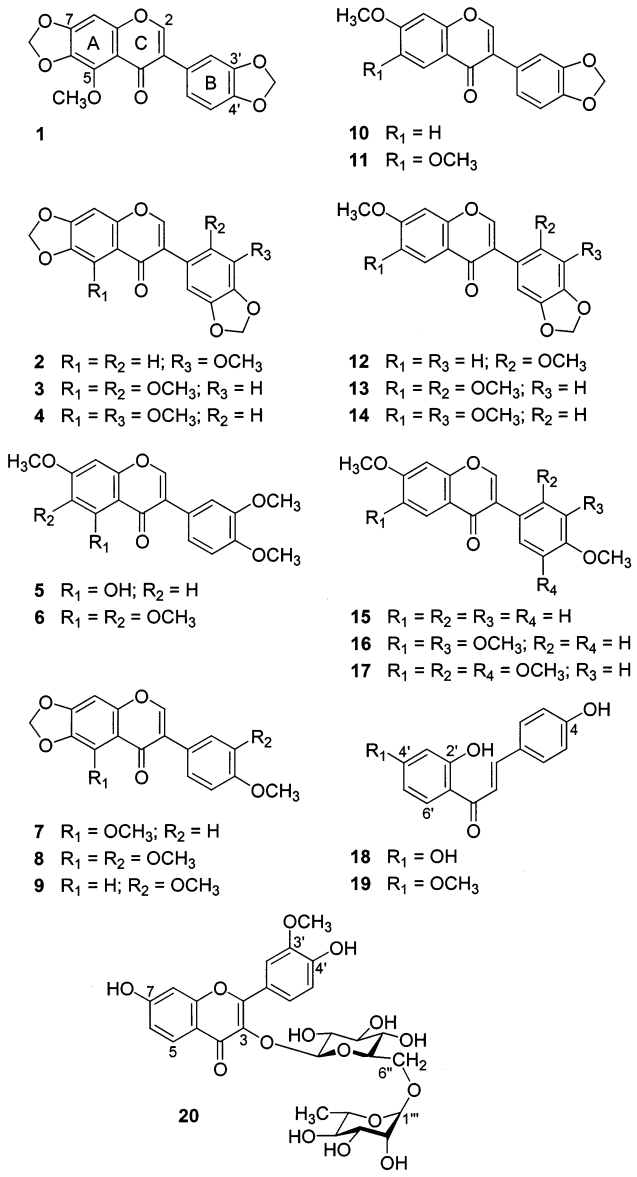
Received September 13, 2002

Six new isoflavones, 5-methoxy-6,7:3′,4′-bis(methylenedioxy)isoflavone (**1**), 3′-methoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**2**), 5,2′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**3**), 5,3′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**4**), 5-hydroxy-7,3′,4′-trimethoxyisoflavone (**5**), and 5,6,7,3′,4′-pentamethoxyisoflavone (**6**), were obtained from diethyl ether extracts of the leaves of *Ateleia herbert-smithii* together with 11 known isoflavones and two chalcones. Four of the isoflavones (**1**−**4**) are characterized by a unique bis-methylenedioxyl substitution pattern. A new flavonol glycoside, 5-deoxyisorhamnetin 3-*O*-α-L-rhamnopyranosyl(1‴ → 6″)-*β*-D-glucopyranoside (**20**), and three known flavonol 3-*O*-glycosides were obtained from aqueous methanol extracts of leaves of the same species. Spectroscopic methods were used to determine the structures of the compounds. The significance of their occurrence in *A. herbert-smithii* is discussed from both biosynthetic and taxonomic viewpoints.

Ateleia herbert-smithii Pittier (Leguminosae) is an uncommon tree, 6−12 m in height, found in seasonally dry tropical forests of Colombia, Costa Rica, and Nicaragua.[1,2] The seeds and leaves of *A. herbert-smithii* contain the unusual nonprotein amino acids 2-azabicyclo[2.1.1]hexane 1-carboxylic acid (2,4-methanoproline), 1-amino-1,3-cyclobutanedicarboxylic acid (2,4-methanoglutamic acid), and 1-amino-3-(hydroxymethyl)cyclobutanecarboxylic acid,[3] but little is known about other aspects of the phytochemistry of this species. In a survey of the flavonoid constituents of leaf material of *Ateleia* species cultivated at the Royal Botanic Gardens Kew it was noted that *A. herbert-smithii* contained a large number of isoflavones together with chalcones and flavonol *O*-glycosides. This paper describes the isolation and identification of 17 isoflavones, two chalcones, and four flavonol *O*-glycosides from this species. Of these, 5-methoxy-6,7:3′,4′-bis(methylenedioxy)isoflavone (**1**), 3′-methoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**2**), 5,2′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**3**), 5,3′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (**4**), 5-hydroxy-7,3′,4′-trimethoxyisoflavone (**5**), 5,6,7,3′,4′-pentamethoxyisoflavone (**6**), and 5-deoxyisorhamnetin 3-*O*-α-L-rhamnopyranosyl(1‴→6″)-*β*-D-glucopyranoside (**20**) are reported for the first time.

Results and Discussion

Diethyl ether extracts of fresh leaves of *A. herbert-smithii* yielded **1**−**17** as white to off-white crystalline substances after fractionation by preparative TLC and semipreparative HPLC. The purification process was monitored by analytical HPLC coupled to diode array detection, and the compounds were recognized as isoflavones from their characteristic UV spectra.[4] Their structures and molecular formulas were obtained using NMR spectroscopy and high-resolution mass spectrometry, respectively. ^{1}H and ^{13}C NMR resonance assignments were made by acquiring 1D ^{1}H, 1D ^{13}C, DEPT, DQFCOSY, HSQC, and HMBC datasets as required. Specific assignments of methoxy groups were confirmed for both new and known compounds using site-selective NOE[5a] or ROE[5b] pulse sequences, as these data are frequently absent from earlier published lists of ^{1}H NMR assignments of isoflavones.



* To whom correspondence should be addressed. Tel: +44 208 332 5312. Fax: +44 208 332 5310. E-mail: n.veitch@rbgkew.org.uk.

† Current address: The National Botanic Garden of Wales, Middleton Hall, Llanarthne, Carmarthenshire, SA32 8HG, U.K.

10.1021/np020425u CCC: $25.00
Published on Web 01/01/2003

Table 1. ^{1}H NMR Chemical Shift Assignments (δ) and Coupling Constant Data for Compounds **1**−**6** in $CDCl_3$

proton	**1**	**2**	**3**	**4**	**5**	**6**
2	7.76 (s)	7.91 (s)	7.75 (s)	7.77 (s)	7.88 (s)	7.82 (s)
5		7.61 (s)				
6					6.39 (d, 2.2)	
8	6.63 (s)	6.87 (s)	6.63 (s)	6.63 (s)	6.41 (d, 2.2)	6.69 (s)
2′	7.08 (d, 1.7)	6.80 (d, 1.5)		6.77 (d, 1.5)	7.11 (d, 2.0)	7.18 (d, 2.0)
3′			6.60 (s)			
5′	6.84 (d, 8.0)				6.93 (d, 8.3)	6.91 (d, 8.3)
6′	6.94 (dd, 8.0, 1.7)	6.72 (d, 1.5)	6.83 (s)	6.69 (d, 1.5)	7.04 (dd, 8.3, 2.0)	7.02 (dd, 8.3, 2.0)
OCH_2O-6,7	6.06 (s)	6.11 (s)	6.06 (s)	6.07 (s)		
OCH_2O-3′,4′	5.97 (s)					
OCH_2O-4′,5′		6.00 (s)	5.93 (s)	5.98 (s)		
OCH_3-5	4.08 (s)		4.06 (s)	4.08 (s)		3.961 (s)
OCH_3-6						3.92 (s)
OCH_3-7					3.87 (s)	3.959 (s)
OCH_3-2′			3.71 (s)			
OCH_3-3′		3.94 (s)		3.92 (s)	3.92 (s)	3.92 (s)
OCH_3-4′					3.91 (s)	3.90 (s)
OH-5					12.84 (s)	

The molecular formula of **1** was determined to be $C_{18}H_{12}O_7$ by HRESIMS. A characteristic resonance for H-2 of an isoflavone was observed at δ_H 7.76 (1H, s, δ_C 150.4 by HSQC) in the ^{1}H NMR spectrum of **1**.[6] This assignment was confirmed by long-range connectivities to δ_C 175.3 (C-4), 154.7 (C-9), and 125.7 (C-1′) in the corresponding HMBC spectrum. A second singlet (1H) corresponding to H-8 of the A-ring (δ_H 6.63, δ_C 93.3) and three coupled B-ring multiplets at δ 7.08 (1H, d, J = 1.7 Hz, H-2′), 6.94 (1H, dd, J = 8.0, 1.7 Hz, H-6′), and 6.84 (1H, d, J = 8.0 Hz, H-5′) comprised the remaining aromatic resonances. The assignment of H-8 was supported by long-range HMBC connectivities to δ_C 154.7 (C-9), 152.9 (C-7), 135.7 (C-6), and 113.9 (C-10). The ^{1}H NMR spectrum also included resonances for one methoxy group at δ 4.08 (3H, s, δ_C 61.3) and two methylenedioxy groups at δ 6.06 (2H, s, δ_C 102.2) and 5.97 (2H, s, δ_C 101.1). Long-range HMBC connectivities from the methylenedioxy protons at δ 6.06 to δ_C 152.9 (C-7) and 135.7 (C-6) allowed this substituent to be assigned to C-6 and C-7 of the A-ring. The second methylenedioxy group was assigned to C-3′ and C-4′ of the B-ring on the basis of the long-range connectivities from δ 5.97 and H-2′, H-5′, and H-6′ to these carbons (both δ_C 147.6). A weak ^4J(^{1}H, ^{13}C) HMBC connectivity from H-8 to δ_C 141.8 (a similar connectivity was observed from H-8 to C-4) and a strong ^3J(^{1}H, ^{13}C) HMBC connectivity from the methoxy protons to the same carbon resonance confirmed that the location of the methoxy substituent was at C-5. Thus, **1** was identified as 5-methoxy-6,7:3′,4′-bis(methylenedioxy)isoflavone.

The molecular formula of **2** was also $C_{18}H_{12}O_7$ by HRESIMS, and its ^{1}H NMR spectrum indicated that it contained the same number of substituents as **1**, with a single methoxy group at δ 3.94 (3H, s, δ_C 56.7) and two methylenedioxy groups at δ 6.11 (2H, s, δ_C 102.6) and 6.00 (2H, s, δ_C 101.6). The ^{1}H and ^{13}C NMR chemical shift values of the first methylenedioxy group at δ_H 6.11 and the observation of two 1H singlets at δ_H 7.61 (δ_C 103.1) and 6.87 (δ_C 97.9) characteristic for H-5 and H-8, respectively, indicated that this substituent was located at C-6 and C-7 of the A-ring. The remaining aromatic resonances in the ^{1}H NMR spectrum comprised H-2 at δ 7.91 (1H, s, δ_C 151.9 by HSQC) and two *meta*-coupled protons at δ 6.72 and 6.80 (both 1H, d, J = 1.5 Hz) assigned to the B-ring. Site selective excitation of the methoxy resonance at δ 3.94 gave only a single ROE correlation with the B-ring proton at δ 6.80. This indicated that the methoxy substituent must be located between this proton (H-2′) and the second methylenedioxy group at C-4′ and C-5′. Compound **2** was therefore identified as 3′-methoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone.

The molecular formula of **3**, determined to be $C_{19}H_{14}O_8$ by HRESIMS, suggested the presence of an additional methoxy substituent compared to **1** and **2**. This was confirmed by the ^{1}H NMR spectrum, which comprised resonances for two methoxy groups at δ 4.06 (3H, s, δ_C 61.3) and 3.71 (3H, s, δ_C 56.9) and two methylenedioxy groups at δ 6.06 (2H, s, δ_C 102.1) and 5.93 (2H, s, δ_C 101.3). The assignment of a 1H singlet at δ_H 6.63 (δ_C 93.4) to H-8 was confirmed from an identical pattern of long-range HMBC connectivities to those seen in **1**. The A-ring of **3** was therefore characterized by 5-methoxy-6,7-methylenedioxyl substitution. Of the remaining aromatic proton resonances, that at δ 7.75 (1H, s, δ_C 152.3) was assigned to H-2 and two 1H singlets at δ 6.60 (δ_C 95.4) and 6.83 (δ_C 111.5) were assigned to the B-ring. The B-ring protons (which must be *para*-related) both showed long-range correlations to the oxygen-bearing carbons (δ_C 141.2 and 148.3) of the remaining methylenedioxy group. Selection of the methoxy resonance at δ 3.71 in a 1D NOE experiment gave a NOE connectivity to the proton at δ 6.60. These data indicated a B-ring substitution pattern of 2′-methoxy-4′,5′-methylenedioxyl and specific assignments of the protons at δ 6.60 and 6.83 to H-3′ and H-6′, respectively. This was also supported by the long-range connectivities observed from H-6′ (δ_H 6.83) to C-3 (δ_C 122.4) and C-2′ (δ_C 153.0) in the HMBC spectrum. Compound **3** was therefore identified as 5,2′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone.

The molecular formula of $C_{19}H_{14}O_8$ determined for **4** by HRESIMS and the observation of two methoxy and two methylenedioxy resonances in its ^{1}H NMR spectrum suggested that it might be an isomer of **3**. The resonances at δ 4.08 (3H, s, δ_C 61.3), 6.07 (2H, s, δ_C 102.2), and 6.63 (1H, s, δ_C 93.2) were similar to those of the A-ring of **3** (Tables 1 and 2) and characteristic of a 5-methoxy-6,7-methylenedioxyl substitution pattern. Long-range connectivities observed in the HMBC spectrum from H-8 to C-6 (δ_C 135.6), C-7 (δ_C 152.9), C-9 (δ_C 154.7), and C-10 (δ_C 113.9) were as expected. Likewise the resonances for two *meta*-coupled protons at δ 6.77 and 6.69 (both 1H, d, J = 1.5 Hz), a methoxy group at 3.92 (3H, s, δ_C 56.8), and methylenedioxy group at 5.98 (2H, s, δ_C 101.5) were similar to those of the B-ring of **2** and characteristic of a 3′-methoxy-4′,5′-methylenedioxyl substitution pattern. A NOE connectivity was observed between the 3′-methoxy protons at δ 3.92 and the B-ring proton at δ 6.77, allowing it to be assigned to H-2′. Useful long-range connectivies were observed in the HMBC

Table 2. ^{13}C NMR Chemical Shift Assignments (δ) for Compounds **1**, **3**, **4**, and **6** in $CDCl_3$

carbon	1	3	4	6
2	150.4	152.3	150.6	150.7
3	125.5	122.4	125.5	125.6
4	175.3	175.2	175.2	175.2
5	141.8	141.7	141.8	153.1
6	135.7	135.7	135.6	140.7
7	152.9	152.7	152.9	157.8
8	93.3	93.4	93.2	96.1
9	154.7	154.8	154.7	154.6
10	113.9	114.1	113.9	113.7
1′	125.7	112.8	126.1	124.8
2′	110.1	153.0	109.3	112.9
3′	147.6	95.4	143.5	148.9
4′	147.6	148.3	135.4	149.2
5′	108.3	141.2	148.8	111.3
6′	122.6	111.5	103.6	121.3
OCH_2O-6,7	102.2	102.1	102.2	
OCH_2O-3′,4′	101.1			
OCH_2O-4′,5′		101.3	101.5	
OCH_3-5	61.3	61.3	61.3	62.1
OCH_3-6				61.5
OCH_3-7				56.3
OCH_3-2′		56.9		
OCH_3-3′			56.8	56.0
OCH_3-4′				56.0

[a] Spectra acquired in $CDCl_3$ at 100 MHz and 30 °C.

spectrum from H-2′ to C-3 (δ_C 125.5), C-3′ (δ_C 143.5), and C-4′ (δ_C 135.4) and from H-6′ to C-3 (δ_C 125.5), C-4′ (δ_C 135.4), and C-5′ (δ_C 148.8). The remaining proton resonance in the ^1H NMR spectrum, a 1H singlet at δ 7.77 (δ_C 150.6), was assigned to H-2. Compound **4** was therefore identified as 5,3′-dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone.

The unusual bis(methylenedioxy)isoflavones **1**−**4** have not been reported previously in the literature. Methylenedioxyl substitution in both A- and B-rings of isoflavones is rare, although substitution by one group is relatively common, particularly at C-6/C-7 of the A-ring or at either C-3′/C-4′ or C-4′/C-5′ of the B-ring.[7] Only one example of bis-methylenedioxyl substitution of isoflavones is known from the literature, that of 7,8:3′,4′-bis(methylenedioxy)-isoflavone (maximaisoflavone A) from the roots of *Tephrosia maxima* (L.) Pers. (Leguminosae).[8]

Compound **5** was one of the least abundant isoflavones isolated from leaves of *A. herbert-smithii*. Its molecular formula was determined to be $C_{18}H_{16}O_6$ by HRESIMS. The ^1H NMR spectrum of **5** contained an exchangeable resonance at δ 12.84 (1H, s) characteristic of a OH-5 group and three methoxy resonances at δ 3.87 (3H, s, δ_C 56.0), 3.91 (3H, s, δ_C 56.2), and 3.92 (3H, s, δ_C 56.2). A 1H singlet at δ 7.88 (δ_C 153.2) was assigned to H-2, and two *meta*-coupled protons at δ 6.39 (1H, d, J = 2.2 Hz, δ_C 98.5) and 6.41 (1H, d, J = 2.2 Hz, δ_C 92.6) were assigned to H-6 and H-8 of the A-ring, respectively. The remaining aromatic proton resonances comprised three coupled multiplets at δ 7.11 (1H, d, J = 2.0 Hz, δ_C 112.7), 6.93 (1H, d, J = 8.3 Hz, δ_C 111.5), and 7.04 (1H, dd, J = 8.3, 2.0 Hz, δ_C 121.7) assigned to H-2′, H-5′, and H-6′ of the B-ring, respectively. The specific assignments of the methoxy groups were obtained by site selective excitation of their proton resonances in 1D XSROESY experiments.[5b] Thus ROE connectivities detected between δ 3.87 and both H-6 and H-8, between δ 3.91 and H-5′, and between δ 3.92 and H-2′ allowed assignment to OCH_3-7, OCH_3-4′, and OCH_3-3′, respectively. Compound **5** was therefore confirmed to be 5-hydroxy-7,3′,4′-trimethoxyisoflavone, an isoflavone that has not been reported previously in the literature.

The ^1H NMR spectrum of **6** contained five singlet 3H resonances between δ 3.96 and 3.90 characteristic of methoxy groups. These data and the molecular formula of $C_{20}H_{20}O_7$ determined for **6** by HRESIMS indicated that this compound was a pentamethoxylated isoflavone derivative. The remaining resonances in the ^1H NMR spectrum comprised a 1H singlet at δ 7.82 (δ_C 150.7) assigned to H-2, a second 1H singlet at δ 6.69 (δ_C 96.1) assigned to H-8, and three coupled multiplets at δ 7.18 (1H, d, J = 2.0 Hz, δ_C 112.9), 6.91 (1H, d, J = 8.3 Hz, δ_C 111.3), and 7.02 (1H, dd, J = 8.3, 2.0 Hz, δ_C 121.3) assigned to H-2′, H-5′, and H-6′ of the B-ring, respectively. Specific assignments of the five methoxy groups were obtained using a combination of NOE and HMBC data. Thus, NOE connectivies between H-8 and δ 3.959 (δ_C 56.3), H-2′ and δ 3.92 (δ_C 56.0), and H-5′ and δ 3.90 (δ_C 56.0) allowed assignment to OCH_3-7, OCH_3-3′, and OCH_3-4′, respectively. Long-range connectivies from H-8 and a second methoxy resonance at δ 3.92 (δ_C 61.5) to δ_C 140.7 (C-6) gave the assignment of OCH_3-6. The remaining methoxy resonance at δ 3.961 (δ_C 62.1) was assigned to OCH_3-5. Compound **6** was therefore identified as 5,6,7,3′,4′-pentamethoxyisoflavone. Several pentamethoxyisoflavone derivatives have been reported previously in the literature, but all are characterized by two methoxy groups in the A-ring (either 5,7 or 6,7 substitution) and three methoxy groups in the B-ring (either 2′,3′,4′, 2′,4′,5′, or 3′,4′,5′ substitution).[7] Compound **6** appears to be the first example of a pentamethoxyisoflavone derivative in which the A-ring is substituted by three methoxy groups.

The structures of the remaining isoflavones **7**−**17** were identified independently using the procedures adopted for **1**−**6** as the known compounds 5,4′-dimethoxy-6,7-methylenedioxyisoflavone (irisolone methyl ether) (**7**), 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (iriskumaonin methyl ether) (**8**), 3′,4′-dimethoxy-6,7-methylenedioxyisoflavone (**9**), 7-methoxy-3′,4′-methylenedioxyisoflavone (pseudobaptigenin methyl ether) (**10**), 6,7-dimethoxy-3′,4′-methylenedioxyisoflavone (fujikinetin methyl ether) (**11**), 7,2′-dimethoxy-4′,5′-methylenedioxyisoflavone (cuneatin methyl ether) (**12**), 6,7,2′-trimethoxy-4′,5′-methylenedioxyisoflavone (milldurone) (**13**), 6,7,3′-trimethoxy-4′,5′-methylenedioxyisoflavone (**14**), 7,4′-dimethoxyisoflavone (**15**), 6,7,3′,4′-tetramethoxyisoflavone (**16**), and 6,7,2′,4′,5′-pentamethoxyisoflavone (**17**). All of these isoflavones are reported for the first time in *A. herbert-smithii*. The occurrence of **7** and **8** in this legume genus is of particular interest, as these compounds have been reported previously only in Iridaceae, **7** from *Iris tingitana* Boiss. & Reut.[9a] and **8** from *I. germanica* L.[9b] and *I. tingitana*.[9a] Compounds **9**−**17** are constituents of a small number of taxa in Leguminosae subfamily Papilionoideae comprising *Calopogonium mucunoides* Desv. (**10**),[10] *Cordyla africana* Lour. (**11**, **13**, **14**, **16**, and **17**),[11] *Dalbergia lanceolaria* L.f. subsp. *paniculata* (Roxb.) Thoth. (**13**),[12a] *D. miscolobium* Benth. (**15**),[12b] *Glycyrrhiza pallidiflora* Maxim. (**15**),[13] *Mildbraediodendron excelsum* Harms (**13**, **14**, and **17**) (published as "*M. excelsa* Harms"),[14] *Millettia dura* Dunn (**13**),[15] *Pterodon apparicioi* Pedersoli (**12**, **13**, **15**−**17**),[16a,b] *P. emarginatus* Vogel (**11**, **13**, **16**, and **17**) (published as "*P. polygalaeflorus* (Benth.) Benth."[16c] and "*P. pubescens* (Benth.) Benth."[16d]), *Tephrosia maxima* (**12**),[17] and *Xanthocercis zambesiaca* (Baker) Dumaz-le Grand (**9**).[18] These isoflavones were obtained from heartwood of the species concerned with the exception of *Dalbergia lanceolaria* subsp. *paniculata* and *Millettia dura* (seed), *Glycyrrhiza pallidiflora* (root), and *Tephrosia maxima* (aerial parts and roots). Full ^1H (and for most compounds, ^{13}C) NMR spectral assignments for **9**−**17** are given in the Experimental Section where data published previously in the literature are incomplete or require revision.

The diethyl ether extract of fresh leaves of *A. herbert-smithii* also yielded two chalcones (**18** and **19**) as yellow crystalline solids after preparative TLC and semipreparative HPLC. These were identified as the known compounds 4,2′,4′-trihydroxychalcone (isoliquiritigenin) and 4,2′-dihydroxy-4′-methoxychalcone (isoliquiritigenin 4′-methyl ether) using UV, MS, and NMR data. Isoliquiritigenin is widespread in the Leguminosae, but the 4′ methyl ether has only been reported from two sources, *Caesalpinia pulcherrima* (L.) Sw. (Leguminosae subfamily Caesalpinioideae) and *Xanthorrhoea australis* R.Br. (Xanthorrhoeaceae).[7]

Analysis of an aqueous MeOH extract (H_2O−MeOH, 1:1) of *A. herbert-smithii* leaves by HPLC coupled to diode-array detection and LC-MS revealed the major components to be three flavonol *O*-glycosides. Following scale-up to semipreparative HPLC and analysis of the purified compounds by NMR spectroscopy their structures were confirmed as the 3-*O*-rutinosides (α-L-rhamnopyranosyl(1‴→6″)-*β*-D-glucopyranosides) of kaempferol, quercetin, and isorhamnetin. A minor component (**20**) eluting between quercetin 3-*O*-rutinoside and kaempferol 3-*O*-rutinoside had a UV spectrum (λ_{max} 247, 348 nm) with a pronounced low-wavelength shoulder to band I at 315 nm typical of a 5-deoxyflavonol 3-*O*-glycoside.[4a] APCI-MS (positive mode) of **20** gave $[M + H]^+$ at *m*/*z* 609 and fragment ions at *m*/*z* 463 and 301, consistent with loss of a deoxyhexosyl moiety alone $[(M + H) - (146)]^+$ and with a hexosyl moiety $[(M + H) - (146 + 162)]^+$, respectively. HRESIMS of **20** confirmed a molecular formula of $C_{28}H_{32}O_{15}$. These preliminary data suggested that **20** might be a 5-deoxyflavonol 3-*O*-rutinoside.

Scale-up to semipreparative HPLC followed by final separation of the 5-deoxy compound from quercetin 3-*O*-rutinoside by analytical HPLC yielded **20** as a yellow solid. The ^{1}H NMR spectrum of **20** comprised resonances for a methoxylated flavonol aglycone and a disaccharide. No exchangeable 1H singlet characteristic of a OH-5 group was detected, as expected. Coupled aromatic proton resonances at δ 7.64 (1H, d, J = 8.6 Hz, δ_C 125.5), 6.49 (1H, br d, J = 8.6 Hz, δ_C 119.0), and 6.33 (1H, br s, δ_C 101.4) were assigned to H-5, H-6, and H-8 of the A-ring, respectively. Similarly, the remaining aromatic resonances at δ 7.82 (1H, d, J = 2.0 Hz, δ_C 113.1), 6.83 (1H, d, J = 8.5 Hz, δ_C 115.1), and 7.54 (1H, dd, J = 8.5, 2.0 Hz, δ_C 121.9) were assigned to H-2′, H-5′, and H-6′ of the B-ring, respectively. The downfield shift of H-2′ and the ROE connectivity detected between this proton and the methoxyl group at δ 3.82 indicated that the latter was located at the C-3′ position. Thus the aglycon of **20** was identified as 5-deoxyisorhamnetin (3,7,4′-trihydroxy-3′-methoxyflavone), in agreement with the fragment ion at *m*/*z* 301 (corresponding to the protonated aglycon) detected by APCI-MS (positive mode). The two anomeric proton resonances of the disaccharide at δ 5.13 (1H, d, J = 6.9 Hz, δ_C 103.4) and 4.44 (1H, br s, δ_C 100.7) were used in conjunction with DQF-COSY and HSQC data to assign the ^{1}H and ^{13}C NMR resonances of the sugars and identify them as *β*-glucopyranose and α-rhamnopyranose, respectively.[19] The downfield shift of *β*-Glc C-6″ to δ_C 66.7 and the ROE connectivities detected between α-Rha H-1‴ and *β*-Glc 6″-CH_2 characterized the interglycosidic linkage between these sugars as (1‴ → 6″) and the disaccharide as rutinose. The absolute configurations of D for *β*-Glc and L for α-Rha were assumed as those naturally occurring in flavonoid glycosides. Thus, compound **20** was identified as 5-deoxyisorhamnetin 3-*O*-α-L-rhamnopyranosyl(1‴→6″)-*β*-D-glucopyranoside (5-deoxyisorhamnetin 3-*O*-rutinoside), a new 5-deoxyflavonol glycoside.

The occurrence of compounds **1**−**20** in *A. herbert-smithii* is of interest from both biosynthetic and taxonomic viewpoints. With the exception of **5**, all the isoflavones described from this species are characterized by substitution patterns involving only methoxy and methylenedioxy groups in various combinations. The occurrence of 5-deoxyisoflavones (**2**, **9**, **10**−**17**) is a characteristic feature of Leguminosae subfamily Papilionoideae, while the presence of 5-deoxyflavonols (**20**) is a general feature of the Leguminosae.[20] However, *A. herbert-smithii* is also rich in 5-oxyisoflavones (**1**, **3**−**8**), among which are five new compounds (**1**, **3**−**6**) and two known compounds not previously recorded in legume taxa (**7** and **8**). Of these, 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (iriskumaonin methyl ether) (**8**) is also the most abundant isoflavone in leaves of *A. herbert-smithii*. The co-occurrence of chalcones in *A. herbert-smithii* is not unexpected, as they are the biosynthetic precursors of isoflavones.[20b,c] The 6′-deoxychalcone liquiritigenin (4,2′,4′-trihydroxychalcone, **18**) is the precursor of 5-deoxyisoflavones, and its 4′-methyl ether (4,2′-dihydroxy-4′-methoxychalcone, **19**) is thus a potential precursor of 5-deoxy-7-methoxyisoflavones (note that the 6′ and 4′ positions of chalcones are equivalent to the 5 and 7 positions of isoflavones, respectively, due to the different numbering systems adopted for these compounds). The chalcone precursor of 5-hydroxyisoflavones, 4,2′,4′,6′-tetrahydroxychalcone (naringenin chalcone), was not found to accumulate in *A. herbert-smithii* leaves. However, chalcone isomerases (which catalyze the formation of the (*2S*)-flavanone precursors of isoflavones from chalcones) that show substrate specificity for both 6′-deoxy- and 6′-hydroxychalcones are known from legumes.[21]

The flavonoid chemistry of *A. herbert-smithii* is also relevant to the current debate about the systematic position of *Ateleia* in Leguminosae.[1d,22] Considered to be one of a number of genera that are transitional between subfamilies Caesalpinioideae and Papilionoideae, *Ateleia* has most recently been placed in the basal papilionoid legume tribe Swartzieae.[23] In some earlier treatments the genus was placed in the basal papilionoid legume tribe Sophoreae.[24] Evidence has also been presented from floral morphology that supports placing the Swartzieae (as traditionally circumscribed) in subfamily Caesalpinioideae.[25] More recently, nucleotide sequence data obtained from the chloroplast *trnL* intron for the majority of genera in Swartzieae and Sophoreae indicated that both tribes were nonmonophyletic and that *Ateleia* was a member of a clade at the base of the Papilionoideae.[22c] The presence of isoflavones in *A. herbert-smithii* is a chemical character associated with subfamily Papilionoideae (isoflavonoids are not found in subfamily Caesalpinioideae), and the fact that only simple derivatives are synthesized is consistent with the basal position of *Ateleia* in the subfamily. Comparative chemical data for other genera in Swartzieae (as defined by Polhill)[23] are limited at present to *Aldina*,[26] *Cordyla*,[11] *Mildbraediodendron*,[14] *Swartzia*,[27] and *Zollernia*.[28] The presence of isoflavonoids in all five genera supports recent chloroplast *trnL* intron nucleotide sequence data that places them in subfamily Papilionoideae.[22c] Some interesting variations in the isoflavonoid chemistry of these genera are evident from the published data.[11,14,26−28] For example, *Cordyla africana* synthesizes simple 5-deoxy- and 5-oxyisoflavones similar to those of *Ateleia herbert-smithii*, whereas only simple 5-deoxyisoflavones have been reported from *Mildbraediodendron excelsum*. In contrast, the isoflavonoid chemistry of *Aldina*, *Swartzia*, and *Zollernia* is dominated by the production of pterocarpans and coumestans (*Swartzia*

only).[26−28] The biosynthesis of these compounds requires the presence of an isoflavone 2′-hydroxylase to produce 2′-hydroxyisoflavone precursors from which ring closure to the C-4 position of the C-ring can be effected.[20b] Although this 2′-hydroxylase activity is clearly present in *A. herbert-smithii* (as a requirement for the biosynthesis of **3**, **4**, **12**, **13**, and **17**), the 2′-position is effectively blocked by the action of 2′-*O*-methyltransferases, and no further transformation occurs. At a more detailed level, the unique bis-(methylenedioxy)isoflavones **1**−**4** identified in *A. herbert-smithii* may be useful chemical characters both for investigating species relationships within *Ateleia* and for more extensive generic studies. Likewise the unusual 5-deoxyflavonol glycoside (**20**), which preliminary survey work shows to be of limited distribution within *Ateleia*, may also be a valuable chemical character for further investigation.

Experimental Section

General Experimental Procedures. UV spectra were recorded either on a Shimadzu UV-1601 spectrophotometer or online by HPLC coupled to diode array detection (Waters 996 photodiode array detector). ^{1}H NMR (500 and 400 MHz) and ^{13}C NMR (125 and 100 MHz) spectra were recorded in either DMSO-d_6, $CDCl_3$, or CD_3OD with the residual solvent resonances of DMSO-d_6 used as an internal reference for this solvent at $\delta_{H/C}$ 2.50/39.5 and TMS used as an internal reference for both $CDCl_3$ and CD_3OD. Standard pulse sequences and parameters were used for the experiments. High-resolution ESI-MS (negative or positive mode) and APCI-MS (positive mode) were obtained on a Bruker Apex II instrument using internal calibrants. Low-resolution APCI-MS (positive mode) were obtained with a quadrupole ion-trap instrument (Thermo Finnigan LCQ) using a vaporizer temperature of 550 °C, sheath and auxiliary nitrogen gas pressures of 80 and 20 psi, a needle current of 5 μA, and a heated capillary temperature of 150 °C. Samples were introduced by direct infusion or by using an HPLC system with a Merck LiChrospher 100RP-18 (250 × 4.0 mm i.d.; 5 μm particle size) column and a 20 min linear gradient of 25−100% MeOH in 1% aqueous HOAc at 1 mL/min. Analytical and semipreparative HPLC were carried out using a Waters LC600 pump and a 996 photodiode array detector. A Merck LiChrospher 100RP-18 (250 × 4.0 mm i.d.; 5 μm particle size) column with a gradient elution program (solvent A = MeOH−HOAc−H_2O (18:1:1), solvent B = HOAc−H_2O (2:98); A = 25%, B = 75% at t = 0 min; A = 100% at t = 20 min; A = 100% at t = 25 min and A = 25%, B = 75% at t = 26 min) operating at a flow rate of 1 mL/min was used routinely for analytical HPLC. An identical LiChrospher column but with 10 mm i.d. was used for semipreparative HPLC, and a flow rate of 4.5 mL/min was adopted. The column temperature was maintained at 30 °C in both applications. Preparative TLC was carried out using Si gel UV_{254} plates (Machery-Nagel, 20 × 20 × 0.1 cm).

Plant Material. Leaf material of *A. herbert-smithii* was collected from a specimen growing under glasshouse conditions at the Royal Botanic Gardens, Kew (accession number 1997-6547). This specimen was grown from seed taken from *Hughes 816* (K) (voucher specimen lodged in the Herbarium, Royal Botanic Gardens, Kew).

Extraction and Isolation. Fresh leaf material of *A. herbert-smithii* (typically 10−150 g fresh weight) was dipped in Et_2O for 10 min, and the leaves were discarded. Filtration and rotary evaporation of the resulting extract gave a residue that was redissolved in MeOH, applied to preparative TLC, and developed in 11% MeOH in $CHCl_3$. Three prominent bands, I (R_f 0.73−0.82, dark purple under UV light at 254 nm, **1**−**17**), II (R_f 0.55−0.59, yellow, **19**), and III (R_f 0.31−0.37, yellow, **18**) were obtained, the components of which were eluted with MeOH to give fractions I−III, respectively. Use of analytical HPLC coupled to diode-array detection indicated that fraction I contained isoflavonoids and that both fractions II and III contained chalcones. Purification of II and III by semipreparative HPLC afforded the chalcones **19** (3.1 mg) and **18** (3.4 mg), respectively, as yellow crystalline solids (yields from 65 g of fresh leaves). The isoflavonoid constituents of fraction I were separated further by preparative TLC using hexane−ethyl acetate (1:1) to give six bands (dark purple under UV light at 254 nm). The components of each band were eluted with MeOH to give fractions I.1−I.6, which were purified subsequently by semipreparative HPLC to afford **1**−**17** as white to off-white crystalline substances. Compounds **10** (0.7 mg) and **15** (0.7 mg) were obtained from fraction I.1 (R_f 0.49−0.56), **1** (2.8 mg), **9** (0.5 mg), and **11** (1.8 mg) from fraction I.3 (R_f 0.28−0.40), and **4** (1.0 mg), **7** (0.4 mg), **13** (1.6 mg), and **14** (0.5 mg) from fraction I.4 (R_f 0.28−0.40) (yields quoted from 65 g of fresh leaves). Likewise **2** (0.1 mg), **5** (0.1 mg), and **12** (0.3 mg) were obtained from fraction I.2 (R_f 0.40−0.49) and **8** (0.8 mg) and **17** (0.5 mg) from fraction I.6 (R_f 0.08−0.12) (yields quoted from 12 g of fresh leaves). Compounds **3** (2.2 mg), **6** (3.8 mg), **8** (3.9 mg), and **16** (5.5 mg) were obtained from fraction I.5 (R_f 0.12−0.22) (yields quoted from 150 g of fresh leaves).

Extraction of 5 g of freeze-dried ground leaf material of *A. herbert-smithii* for 24 h in MeOH−H_2O (1:1) followed by filtration and rotary evaporation gave a residue that was redissolved in a few milliliters of the same solvent, further diluted with distilled H_2O, and applied to a C_{18} SepPak column for sample cleanup. After column washing with MeOH−H_2O (1:9) a fraction containing mainly flavonoids (as confirmed by analytical HPLC with diode-array detection) was eluted from the SepPak column with a few milliliters of MeOH (chlorophyll and other pigments were retained on the column). A fraction (t_R = 11.8 min) containing the 5-deoxyflavonol glycoside (**20**) was obtained by semipreparative HPLC using a gradient method with A = MeOH and B = H_2O; A = 25% at t = 0 min, A = 100% at t = 20 min, A = 100% at t = 24 min, A = 25% at t = 25 min (initial conditions). This was contaminated by quercetin 3-*O*-rutinoside (rutin), and analytical HPLC with isocratic elution with MeOH−H_2O (37:63) was used to separate the two components giving rutin (t_R = 13.4 min) and **20** (t_R = 16.0 min, 1.1 mg).

5-Methoxy-6,7:3′,4′-bis(methylenedioxy)isoflavone (1): UV (MeOH) λ_{max} 265, 295 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS m/z 341.0655 $[M + H]^+$ (calcd for $C_{18}H_{13}O_7$, 341.0656).

3′-Methoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (2): UV (MeOH) λ_{max} 266, 322 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) (assignment of nonquaternary C atoms by HSQC) δ 151.9 (C-2), 109.3 (C-2′), 103.5 (C-6′), 103.1 (C-5), 102.6 (OCH_2O-6,7), 101.6 (OCH_2O-4′,5′), 97.9 (C-8), 56.7 (OCH_3-3′); HRESIMS m/z 341.0657 $[M + H]^+$ (calcd for $C_{18}H_{13}O_7$, 341.0656).

5,2′-Dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (3): UV (MeOH) λ_{max} 260, 304 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS m/z 371.0763 $[M + H]^+$ (calcd for $C_{19}H_{15}O_8$, 371.0761).

5,3′-Dimethoxy-6,7:4′,5′-bis(methylenedioxy)isoflavone (4): UV (MeOH) λ_{max} 269, 321 (sh) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS m/z 371.0762 $[M + H]^+$ (calcd for $C_{19}H_{15}O_8$, 371.0761).

5-Hydroxy-7,3′,4′-trimethoxyisoflavone (5): UV (MeOH) λ_{max} 262, 292 (sh), 334 (sh) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz) (assignment of nonquaternary C atoms by HSQC) δ 153.2 (C-2), 121.7 (C-6′), 112.7 (C-2′), 111.5 (C-5′), 98.5 (C-6), 92.6 (C-8), 56.2 (OCH_3-3′ and -4′), 56.0 (OCH_3-7); HRESIMS m/z 329.1019 $[M + H]^+$ (calcd for $C_{18}H_{17}O_6$, 329.1020).

5,6,7,3′,4′-Pentamethoxyisoflavone (6): UV (MeOH) λ_{max} 261, 287 (sh), 317 (sh) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; HRESIMS m/z 373.1281 $[M + H]^+$ (calcd for $C_{20}H_{21}O_7$, 373.1282).

5,4′-Dimethoxy-6,7-methylenedioxyisoflavone (7): UV (MeOH) λ_{max} 264, 324 nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.77 (1H, s, H-2), 7.47 (2H, d, J = 8.8 Hz, H-2′,6′), 6.94 (2H, d, J = 8.8 Hz, H-3′,5′), 6.63 (1H, s, H-8), 6.06 (2H, s, OCH_2O), 4.08

(3H, s, OCH_3-5), 3.83 (3H, s, OCH_3-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4 (C-4), 159.6 (C-4′), 154.8 (C-9), 152.8 (C-7), 150.2 (C-2), 141.8 (C-5), 135.6 (C-6), 130.4 (C-2′,6′), 125.4 (C-3), 124.2 (C-1′), 114.0 (C-10), 113.9 (C-3′,5′), 102.2 (OCH_2O), 93.3 (C-8), 61.3 (OCH_3-5), 55.4 (OCH_3-4′); HRESIMS *m/z* 327.0863 $[M + H]^+$ (calcd for $C_{18}H_{15}O_6$, 327.0863).

5,3′,4′-Trimethoxy-6,7-methylenedioxyisoflavone (8): UV (MeOH) λ_{max} 264, 288 (sh), 329 (sh) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.78 (1H, s, H-2), 7.18 (1H, d, J = 2.0 Hz, H-2′), 7.00 (1H, dd, J = 8.3, 2.0 Hz, H-6′), 6.89 (1H, d, J = 8.3 Hz, H-5′), 6.62 (1H, s, H-8), 6.05 (2H, s, OCH_2O), 4.08 (3H, s, OCH_3-5), 3.91 (3H, s, OCH_3-3′), 3.89 (3H, s, OCH_3-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4 (C-4), 154.7 (C-9); 152.8 (C-7), 150.4 (C-2), 149.1 (C-4′), 148.8 (C-3′), 141.8 (C-5), 135.5 (C-6), 125.4 (C-3), 124.7 (C-1′), 121.4 (C-6′), 113.9 (C-10), 113.0 (C-2′), 111.2 (C-5′), 102.2 (OCH_2O), 93.2 (C-8), 61.2 (OCH_3-5), 56.1 (OCH_3-3′), 56.0 (OCH_3-4′); HRAPCIMS *m/z* 357.0970 $[M + H]^+$ (calcd for $C_{19}H_{17}O_7$, 357.0969).

3′,4′-Dimethoxy-6,7-methylenedioxyisoflavone (9): UV (MeOH) λ_{max} 260, 323 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.94 (1H, s, H-2), 7.62 (1H, s, H-5), 7.21 (1H, d, J = 2.0 Hz, H-2′), 7.05 (1H, dd, J = 8.2, 2.0 Hz, H-6′), 6.93 (1H, d, J = 8.2 Hz, H-5′), 6.87 (1H, s, H-8), 6.11 (2H, s, OCH_2O), 3.93 (3H, s, OCH_3-3′), 3.91 (3H, s, OCH_3-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4 (C-4), 153.6 (C-9), 152.7 (C-7), 151.3 (C-2), 149.2 (C-4′), 148.9 (C-3′), 146.3 (C-6), 124.6 (C-1′), 124.5 (C-3), 120.8 (C-6′), 119.6 (C-10), 112.4 (C-2′), 111.0 (C-5′), 102.5 (C-5), 101.8 (OCH_2O), 97.4 (C-8), 55.8 (OCH_3-3′ and -4′); HRESIMS *m/z* 327.0863 $[M + H]^+$ (calcd for $C_{18}H_{15}O_6$, 327.0863).

7-Methoxy-3′,4′-methylenedioxyisoflavone (10): UV (MeOH) λ_{max} 250 (sh), 262 (sh), 294 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.21 (1H, d, J = 8.9 Hz, H-5), 7.91 (1H, s, H-2), 7.10 (1H, d, J = 1.7 Hz, H-2′), 6.99 (1H, dd, J = 8.9, 2.4 Hz, H-6), 6.98 (1H, dd, J = 8.1, 1.7 Hz, H-6′), 6.87 (1H, d, J = 8.1 Hz, H-5′), 6.85 (1H, d, J = 2.4 Hz, H-8), 5.99 (2H, s, OCH_2O), 3.92 (3H, s, OCH_3-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.7 (C-4), 164.1 (C-7), 158.0 (C-9), 152.2 (C-2), 147.7 (C-3′ and C-4′), 127.9 (C-5), 125.8 (C-1′), 125.1 (C-3), 122.4 (C-6′), 118.4 (C-10), 114.6 (C-6), 109.8 (C-2′), 108.4 (C-5′), 101.2 (OCH_2O), 100.2 (C-8), 55.8 (OCH_3-7); HRESIMS *m/z* 297.0759 $[M + H]^+$ (calcd for $C_{17}H_{13}O_5$, 297.0757).

6,7-Dimethoxy-3′,4′-methylenedioxyisoflavone (11): UV (MeOH) λ_{max} 262, 292 (sh), 324 (sh) nm; ^1H and ^{13}C NMR identical to literature;[16c] HRESIMS *m/z* 327.0862 $[M + H]^+$ (calcd for $C_{18}H_{15}O_6$, 327.0863).

7,2′-Dimethoxy-4′,5′-methylenedioxyisoflavone (12): UV (MeOH) λ_{max} 247 (sh), 302 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.19 (1H, d, J = 8.8 Hz, H-5), 7.88 (1H, s, H-2), 6.98 (1H, s, J = 8.8, 2.4 Hz, H-6), 6.85 (1H, d, J = 2.4 Hz, H-8), 6.83 (1H, s, H-6′), 6.62 (1H, s, H-3′), 5.95 (2H, s, OCH_2O), 3.91 (3H, s, OCH_3-7), 3.73 (3H, s, OCH_3-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.8 (C-4), 163.9 (C-7), 157.9 (C-9), 153.7 (C-2), 153.1 (C-2′), 148.5 (C-4′), 141.3 (C-5′), 127.7 (C-5), 122.3 (C-3), 118.5 (C-10), 114.2 (C-6), 112.9 (C-1′), 111.0 (C-6′), 100.9 (OCH_2O), 100.1 (C-8), 95.5 (C-3′), 57.0 (OCH_3-2′), 55.9 (OCH_3-7); HRESIMS *m/z* 327.0861 $[M + H]^+$ (calcd for $C_{18}H_{15}O_6$, 327.0863).

6,7,2′-Trimethoxy-4′,5′-methylenedioxyisoflavone (13): UV (MeOH) λ_{max} 253 (sh), 310 nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.89 (1H, s, H-2), 7.62 (1H, s, H-5), 6.88 (1H, s, H-8), 6.83 (1H, s, H-6′), 6.62 (1H, s, H-3′), 5.96 (2H, s, OCH_2O), 3.99 (3H, s, OCH_3-7), 3.98 (3H, s, OCH_3-6), 3.73 (3H, s, OCH_3-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.4 (C-4), 154.3 (C-7), 153.8 (C-2), 153.1 (C-2′), 152.3 (C-9), 148.4 (C-4′), 147.6 (C-6), 141.3 (C-5′), 121.8 (C-3), 118.0 (C-10), 113.2 (C-1′), 111.3 (C-6′), 105.2 (C-5), 101.4 (OCH_2O), 99.6 (C-8), 95.6 (C-3′), 57.0 (OCH_3-2′), 56.4 (OCH_3-6 and -7); HRESIMS *m/z* 357.0969 $[M + H]^+$ (calcd for $C_{19}H_{17}O_7$, 357.0969).

6,7,3′-Trimethoxy-4′,5′-methylenedioxyisoflavone (14): UV (MeOH) λ_{max} 268, 317 (sh) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.94 (1H, s, H-2), 7.63 (1H, s, H-5), 6.88 (1H, s, H-8), 6.84 (1H, d, J = 1.5 Hz, H-2′), 6.73 (1H, d, J = 1.5 Hz, H-6′), 6.00 (2H, s, OCH_2O), 4.00 (3H, s, OCH_3-7), 3.99 (3H, s, OCH_3-6), 3.94 (3H, s, OCH_3-3′); HRESIMS *m/z* 357.0967 $[M + H]^+$ (calcd for $C_{19}H_{17}O_7$, 357.0969).

7,4′-Dimethoxyisoflavone (15): UV (MeOH) λ_{max} 251, 262 (sh), 300 (sh) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.21 (1H, d, J = 8.9 Hz, H-5), 7.92 (1H, s, H-2), 7.50 (2H, d, J = 8.9 Hz, H-2′,6′), 6.99 (1H, dd, J = 8.9, 2.3 Hz, H-6), 6.97 (1H, d, J = 8.9 Hz, H-3′,5′), 6.85 (1H, d, J = 2.3 Hz, H-8), 3.92 (3H, s, OCH_3-7), 3.84 (3H, s, OCH_3-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.9 (C-4), 164.0 (C-7), 159.7 (C-4′), 158.0 (C-9), 152.0 (C-2), 130.2 (C-2′,6′), 127.9 (C-5), 125.0 (C-3), 124.3 (C-1′), 118.5 (C-10), 114.5 (C-6), 114.0 (C-3′,5′), 100.2 (C-8), 55.8 (OCH_3-7), 55.4 (OCH_3-4′); HRESIMS *m/z* 283.0963 $[M + H]^+$ (calcd for $C_{17}H_{15}O_4$, 283.0965).

6,7,3′,4′-Tetramethoxyisoflavone (16): UV, ^1H and ^{13}C NMR identical to literature;[16c] HRESIMS *m/z* 343.1175 $[M + H]^+$ (calcd for $C_{19}H_{19}O_6$, 343.1176).

6,7,2′,4′,5′-Pentamethoxyisoflavone (17): UV (MeOH) λ_{max} 252 (sh), 301, 317 (sh) nm; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.97 (1H, s, H-2), 7.63 (1H, s, H-5), 6.97 (1H, s, H-6′), 6.89 (1H, s, H-8), 6.63 (1H, s, H-3′), 3.99 (3H, s, OCH_3-7), 3.98 (3H, s, OCH_3-6), 3.93 (3H, s, OCH_3-4′), 3.86 (3H, s, OCH_3-5′), 3.78 (3H, s, OCH_3-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 175.5 (C-4), 154.3 (C-7), 154.1 (C-2), 152.4 (C-9), 151.9 (C-2′), 149.9 (C-4′), 147.7 (C-6), 143.3 (C-5′), 121.3 (C-3), 118.0 (C-10), 115.2 (C-6′), 112.6 (C-1′), 104.8 (C-5), 99.2 (C-8), 98.2 (C-3′), 56.7 (OCH_3-2′), 56.3 (OCH_3-5′), 56.1 (OCH_3-6 and -7), 55.9 (OCH_3-4′); HRESIMS *m/z* 373.1280 $[M + H]^+$ (calcd for $C_{20}H_{21}O_7$, 373.1282).

4,2′,4′-Trihydroxychalcone (18): UV (MeOH) λ_{max} 233 (sh), 372 nm; ^1H NMR (CD_3OD, 400 MHz) δ 7.95 (1H, d, J = 8.9 Hz, H-6′), 7.78 (1H, d, J = 15.4 Hz, H-β), 7.61 (2H, d, J = 8.7 Hz, H-2,6), 7.59 (1H, d, J = 15.4 Hz, H-α), 6.84 (2H, d, J = 8.7 Hz, H-3,5), 6.40 (1H, dd, J = 8.9, 2.4 Hz, H-5′), 6.27 (1H, d, J = 2.4 Hz, H-3′); ^{13}C NMR (CD_3OD, 100 MHz) δ 193.4 (C═O), 167.7 (C-4′/C-2′), 167.4 (C-4′/C-2′), 161.7 (C-4), 145.5 (C-β), 133.4 (C-6′), 131.8 (C-2,6), 128.0 (C-1), 118.5 (C-α), 117.0 (C-3,5), 114.5 (C-1′), 109.6 (C-5′), 104.1 (C-3′); APCI-MS (positive mode) *m/z* 257 $[M + H]^+$.

4,2′-Dihydroxy-4′-methoxychalcone (19): UV (MeOH) λ_{max} 233, 260 (sh), 299 (sh) 370 nm; ^1H NMR (CD_3OD, 400 MHz) δ 8.03 (1H, d, J = 9.0 Hz, H-6′), 7.82 (1H, d, J = 15.3 Hz, H-β), 7.62 (2H, d, J = 8.6 Hz, H-2,6), 7.61 (1H, d, J = 15.3 Hz, H-α), 6.83 (2H, d, J = 8.6 Hz, H-3,5), 6.54 (1H, dd, J = 9.0, 2.5 Hz, H-5′), 6.45 (1H, d, J = 2.5 Hz, H-3′), 3.86 (3H, s, OCH_3-4′); ^{13}C NMR (CD_3OD, 100 MHz) δ 193.9 (C═O), 167.7 (C-4′), 167.5 (C-2′), 162.9 (C-4), 146.3 (C-β), 133.0 (C-6′), 132.0 (C-2,6), 127.4 (C-1), 117.9 (C-α), 117.4 (C-3,5), 115.5 (C-1′), 108.4 (C-5′), 102.1 (C-3′), 56.2 (OCH_3-4′); APCI-MS (positive mode) *m/z* 271 $[M + H]^+$.

5-Deoxyisorhamnetin 3-*O*-α-L-rhamnopyranosyl(1‴→6″)-β-D-glucopyranoside (20): yellow solid (MeOH); UV (MeOH) λ_{max} 247, 315 (sh), 348 nm; ^1H NMR (DMSO-d_6, 500 MHz) δ 7.82 (1H, d, J = 2.0 Hz, H-2′), 7.64 (1H, d, J = 8.6 Hz, H-5), 7.54 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 6.83 (1H, d, J = 8.5 Hz, H-5′), 6.49 (1H, br d, J = 8.6 Hz, H-6), 6.33 (1H, br s, H-8), 5.13 (1H, d, J = 6.9 Hz, H-1″), 4.44 (1H, br s, H-1‴), 3.82 (3H, s, OCH_3-3′), 3.72 (1H, br d, J = 11.0 Hz, H-6″), 3.44 (1H, m, H-2‴), 3.32 (1H, m, H-6″), 3.31 (1H, m, H-3‴), 3.30 (1H, m, H-5‴), 3.26 (1H, m, H-5″), 3.24 (1H, m, H-3″), 3.23 (1H, m, H-2″), 3.09 (1H, m, H-4‴), 3.05 (1H, m, H-4″), 1.02 (3H, d, J = 6.3 Hz, CH_3-6‴); ^{13}C NMR (DMSO-d_6, 125 MHz) (assignment of nonquaternary C atoms by HSQC) δ 125.5 (C-5), 121.9 (C-6′), 119.0 (C-6), 115.1 (C-5′), 113.1 (C-2′), 103.4 (C-1″), 101.4 (C-8), 100.7 (C-1‴), 76.6 (C-3″), 75.6 (C-5″), 74.0 (C-2″), 71.6 (C-4‴), 70.4 (C-3‴), 70.0 (C-2‴), 69.7 (C-4″), 67.7 (C-5‴), 66.7 (C-6″), 55.3 (OCH_3-3′), 17.3 (C-6‴); HRESIMS *m/z* 607.1653 $[M - H]^-$ (calcd for $C_{28}H_{31}O_{15}$, 607.1657).

Acknowledgment. We thank Laurienne Tibbles and Cornelia Tan for their contributions to this project during university placements at the Royal Botanic Gardens, Kew. Access to high-field NMR facilities was provided by the MRC Biomedical NMR Centre, National Institute for Medical Research, Mill Hill, London, U.K. High-resolution MS data were provided by chemiSPEC (North of England Business and

Innovation Centre, Sunderland Enterprise Park East, Sunderland, U.K.).

References and Notes

(1) (a) Mohlenbrock, R. H. *Webbia* **1962**, *17*, 153−186. (b) Rudd, V. E. *Contr. U.S. Natl. Herb.* **1968**, *32*, 385−411. (c) Janzen, D. H. In *Advances in Legume Biology*; Stirton, C. H., Zarucchi, J. L., Eds.; *Monographs in Systematic Botany from the Missouri Botanical Garden*; 1989; Vol. 29, pp 293−376. (d) Ireland, H. E. The Taxonomy and Systematics of *Ateleia* and *Cyathostegia* (Leguminosae-Swartzieae). Ph.D. Thesis, University of Reading, U.K., 2001, 283 pp.

(2) The type specimen of *A. herbert-smithii* was collected in Colombia, but subsequent attempts at re-collection in the locality concerned have been unsuccessful. This species has also been introduced into cultivation in Honduras.

(3) (a) Bell, E. A.; Qureshi, M. Y.; Pryce, R. J.; Janzen, D. H.; Lemke, P.; Clardy, J. *J. Am. Chem. Soc.* **1980**, *102*, 1409−1412. (b) Austin, G. H.; Baird, P. D.; Chow, H.-F.; Fellows, L. E.; Fleet, G. W. J.; Nash, R. J.; Peach, J. M.; Pryce, R. J.; Stirton, C. H. *Tetrahedron* **1987**, *43*, 1857−1861. (c) Kite, G. C.; Ireland, H. *Phytochemistry* **2002**, *59*, 163−168.

(4) (a) Mabry T. J.; Markham K. R.; Thomas M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: Berlin, 1970. (b) Markham K. R. *Techniques of Flavonoid Identification*; Academic Press: London, 1982; pp 36−51.

(5) (a) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T. L.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200. (b) Gradwell, M. J.; Kogelberg, H.; Frenkiel, T. A. *J. Magn. Reson.* **1997**, *124*, 267−270.

(6) Williams, C. A.; Harborne, J. B. In *Methods in Plant Biochemistry*; Dey, P. M., Harborne, J. B., Eds.; Academic Press: London, 1989; Vol. 1, Chapter 12, p 443.

(7) Harborne, J. B.; Baxter, H. *The Handbook of Natural Flavonoids*; Wiley: Chichester, UK, 1999; Vol. 2.

(8) Kukla, A. S.; Seshadri, T. R. *Tetrahedron* **1962**, *18*, 1443−1448.

(9) (a) El-Emary, N. A.; Kobayashi, Y.; Ogihara, Y. *Phytochemistry* **1980**, *19*, 1878−1879. (b) Pailer, M.; Franke, F. *Monatsh. Chem.* **1973**, *104*, 1394−1408.

(10) Vilain, C.; Jadot, J. *Bull. Soc. R. Sci. Liège* **1976**, *45*, 468−475.

(11) Campbell, R. V. M.; Harper, S. H.; Kemp, A. D. *J. Chem. Soc. (C)* **1969**, 1787−1795.

(12) (a) Adinarayana, D.; Rao, J. R. *Indian J. Chem.* **1975**, *13*, 425−426. (b) Gregson, M.; Ollis, W. D.; Sutherland, I. O.; Gottlieb, O. R.; Magalhães, M. T. *Phytochemistry* **1978**, *17*, 1375−1377.

(13) Fukai, T.; Wang, Q. H.; Inami, R.; Nomura, T. *Heterocycles* **1990**, *31*, 643−650.

(14) Meegan, M. J.; Donnelly, D. M. X. *Phytochemistry* **1975**, *14*, 2283−2285.

(15) Ollis, W. D.; Rhodes, C. A.; Sutherland, I. O. *Tetrahedron* **1967**, *23*, 4741−4760.

(16) (a) Galina, G.; Gottlieb, O. R. *Phytochemistry* **1974**, *13*, 2593−2595. (b) Leite de Almeida, M. E.; Gottlieb, O. R. *Phytochemistry* **1975**, *14*, 2716. (c) Marques, D. D.; Machado, M. I. L.; De Carvalho, M. G.; Meleira, L. A. D.; Braz-Filho, R. *J. Braz. Chem. Soc.* **1998**, *9*, 295−301. (d) Braz-Filho, R.; Gottlieb, O. R.; Assumpção, R. M. V. *Phytochemistry* **1971**, *10*, 2835−2836.

(17) Rao, E. V.; Murthy, M. S. R.; Ward, R. S. *Phytochemistry* **1984**, *23*, 1493−1501.

(18) Harper, S. H.; Shirley, D. B.; Taylor, D. A. *Phytochemistry* **1976**, *15*, 1019−1023.

(19) Markham, K. R.; Geiger, H. In *The Flavonoids. Advances in Research since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; pp 441−497.

(20) (a) Hegnauer, R.; Grayer-Barkmeijer, R. J. *Phytochemistry* **1993**, *34*, 3−16. (b) Dixon, R. A. In *Comprehensive Natural Products Chemistry*; Sankawa, U., Ed.; Elsevier: Oxford, 1999; Vol. 1, Chapter 1.28, pp 773−823. (c) Aoki, T.; Akashi, T.; Ayabe, S. *J. Plant Res.* **2000**, *113*, 475−488.

(21) Dixon, R. A.; Blyden, E. R.; Robbins, M. P.; Van Tunen, A. J.; Mol, J. N. *Phytochemistry* **1988**, *27*, 2801−2808.

(22) Ireland, H.; Pennington, R. T.; Preston, J. In *Advances in Legume Systematics*; Herendeen, P. S., Bruneau, A., Eds.; Royal Botanic Gardens: Kew, 2000; Vol. 9, pp 217−231. (b) Pennington, R. T.; Klitgaard, B. B.; Ireland, H.; Lavin, M. In *Advances in Legume Systematics*; Herendeen, P. S., Bruneau, A., Eds.; Royal Botanic Gardens: Kew, 2000; Vol. 9, pp 233−248. (c) Pennington, R. T.; Lavin, M.; Ireland, H.; Klitgaard, B.; Preston, J.; Hu, J.-M. *Syst. Bot.* **2001**, *26*, 537−556.

(23) Polhill, R. M. In *Phytochemical Dictionary of the Leguminosae*; Bisby, F. A., Buckingham, J., Harborne, J. B., Eds.; Chapman and Hall: London, 1994; Vol. 1, pp xxxv−xlviii.

(24) Polhill, R. M. In *Advances in Legume Systematics*; Polhill, R. M., Raven, P. H., Eds.; Royal Botanic Gardens: Kew, 1981; Part 1, pp 213−230.

(25) Tucker, S. C. In *Advances in Legume Biology*; Stirton, C. H., Zarucchi, J. L., Eds.; *Monographs in Systematic Botany from the Missouri Botanical Garden*; 1989; Vol. 29, pp 59−75.

(26) Braz Filho, R.; Gottlieb, O. R.; Pinho, S. L. V.; Monte, F. J. Q.; Da Rocha, A. I. *Phytochemistry* **1973**, *12*, 1184−1186.

(27) Hegnauer, R.; Hegnauer, M. *Chemotaxonomie der Pflanzen*; Birkhäuser Verlag: Basel, 2001; Vol. XIb-2, pp 24−30.

(28) Ferrari, F.; Botta, B.; De Lima, R. A. *Phytochemistry* **1983**, *22*, 1663−1664.

NP020425U