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

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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′,5′-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-methylenedioxy 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′,5′-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 semi-preparative 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.1H and 13C NMR resonance assignments were made by acquiring 1D 1H, 1D 13C, DEPT, DQFCOSY, HSQC, and HMBC datasets as required. Specific assignments of methoxy groups were confirmed for both new and known compounds using site-selective NOE5a or ROE5b pulse sequences, as these data are frequently absent from earlier published lists of 1H NMR assignments of isoflavones.

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The molecular formula of 1 was determined to be C18H12O7 by HRESIMS. A characteristic resonance for H-2 of an isoflavone was observed at δH 7.76 (1H, s, δC 150.4 by HSQC) in the 1H NMR spectrum of 1. This assignment was confirmed by long-range connectivities to δC 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 (δH 6.63, δC 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 δC 154.7 (C-9), 152.9 (C-7), 135.7 (C-6), and 113.9 (C-10). The 1H NMR spectrum also included resonances for one methoxy group at δ 3.71 (3H, s, δC 56.9) and two methylenedioxy groups at δ 6.06 (2H, s, δC 61.3) and 6.07 (2H, s, δC 61.3), determined to be C19H14O8 (C-18). The aromatic proton resonances at δ 7.08 were assigned to H-2', δ 6.94 to H-6', δ 6.84 to H-5', δ 6.63 to H-2, δ 6.60 to H-3', and δ 6.60 to H-6' with the remaining aromatic protons being assigned by HMBC and 1D NOE experiments.

The molecular formula of 2 was also C18H12O7 by HRESIMS, and its 1H NMR spectrum indicated that it contained the same number of substituents as 1, with a single methoxy group at δ 3.94 (3H, s, δC 56.7) and two methylenedioxy groups at δ 6.11 (2H, s, δC 102.6) and 6.00 (2H, s, δC 101.6). The 1H and 13C NMR chemical shift values of the first methylenedioxy group at δH 6.11 and the observation of two 1H singlets at δH 7.61 (λC 103.1) and δH 6.87 (δC 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 1H NMR spectrum comprised H-2 at δ 7.91 (1H, s, δC 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 C19H14O8 by HRESIMS, suggested the presence of an additional methoxy substituent compared to 1 and 2. This was confirmed by the 1H NMR spectrum, which comprised resonances for two methoxy groups at δ 4.06 (3H, s, δC 61.3) and 3.71 (3H, s, δC 56.9) and two methylenedioxy groups at δ 6.06 (2H, s, δC 102.1) and 5.93 (2H, s, δC 101.3). The assignment of a 1H singlet at δH 6.63 (δC 93.4) to H-8 was confirmed by 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-methylenedioxy substitution. Of the remaining aromatic proton resonances, that at δ 7.75 (1H, s, δC 152.3) was assigned to H-2 and two 1H singlets at δ 6.60 (δC 95.4) and 6.83 (δC 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 (δC 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′-methylenedioxy 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' (δH 6.83) to C-3 (δC 122.4) and C-2' (δC 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 C19H14O9 determined for 4 by HRESIMS and the observation of two methoxy and two methylenedioxy resonances in its 1H NMR spectrum suggested that it might be an isomer of 3. The resonances at δ 4.08 (3H, s, δC 61.3), 6.07 (2H, s, δC 102.2), and 6.63 (1H, s, δC 93.2) were similar to those of the A-ring of 3 (Tables 1 and 2) and characteristic of a 5-methoxy-6,7-methylenedioxy substitution pattern. Long-range connectivities observed in the HMBC spectrum from H-8 to C-6 (δC 135.6), C-7 (δC 152.9), C-9 (δC 154.7), and C-10 (δC 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, δC 56.8), and methylenedioxy group at 5.98 (2H, s, δC 101.5) were similar to those of the B-ring of 2 and characteristic of a 3′-methoxy-4′,5′-methylenedioxy 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 connectivities were observed in the HMBC.

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Table 2. 13C NMR Chemical Shift Assignments (δ) for Compounds 1, 3, 4, and 6 in CDCl3

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* Spectra acquired in CDCl3 at 100 MHz and 30 °C.

The 1H NMR spectrum of sia maxima (L.) Pers. (Leguminosae). 8 isoflavone (maximaisoflavone A) from the roots of Tephrosia zambesiaca (seed), 11 Dalbergia laceolaria (Benth.), 14 Xanthocercis doidendron excelsum (Dunn.), 15 and Cordylophora cernua (Boiss.) Reut. 16

The unusual bis(methyleneedioxy)isoflavones 1–4 have not been reported previously in the literature. Methyleneedioxy 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. Only one example of bis-methyleneedioxy substitution of isoflavones is known from the literature, that of 7,8′,3′,4′-bis(methyleneedioxy)-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 C30H56O6 by HRMS. The 1H NMR spectrum of 5 contained an exchangeable resonance at δ 7.77 (H-2, s) characteristic of a OH-5 group and two meta-coupled protons at δ 6.91 (1H, d, J = 2.0 Hz, H-2, s) and 6.52 (1H, d, J = 2.0 Hz, H-2, s) assigned to H-6 and H-8 of the A-ring, respectively. The remaining aromatic proton resonances comprised three coupled triplets at δ 7.11 (1H, d, J = 2.0 Hz, δ 112.7), 6.93 (1H, d, J = 8.3 Hz, δ 111.3), and 7.04 (1H, d, J = 8.3, 2.0 Hz, δ 121.3) 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 XSRROESY experiments. 1b Thus ROE connectivity 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 OCH3-7, OCH3-4, and OCH3-3, 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 C29H32O7 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 150.7) assigned to H-2, a second 1H singlet at δ 6.69 (δ 96.1) assigned to H-8, and three coupled triplets at δ 7.18 (H, d, J = 2.0 Hz, δ 112.9), 6.91 (H, d, J = 8.3 Hz, δ 111.3), and 7.02 (1H, dd, J = 8.3, 2.0 Hz, δ 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 connectivities between H-8 and δ 3.959 (δ 56.3), H-2′ and δ 3.92 (δ 56.0), and H-5′ and δ 3.90 (δ 56.0) allowed assignment to OCH3-7, OCH3-3, and OCH3-4, respectively. Long-range connectivities from H-8 and a second methoxy resonance at δ 3.92 (δ 61.5) to δ 140.7 (C-6) gave the assignment of OCH3-6. The remaining methoxy resonance at δ 3.961 (δ 62.1) was assigned to OCH3-5. 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′,3′,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 (irisikmaunio 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 (fujiikinnetin 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. 1b and 1b from I. germanica L. 1b and I. tingitana. 30 Compounds 9–17 are constituents of a small number of taxa in Leguminosae subfamily Papilionoideae comprising Calo pogonium mucunoides Desv. (10), 13 Cordylia africana Lour. (11), 13, 14, 16, and 17), Dalbergia lanceolaria L.f. subsp. subsp. paniculata (Roxb.) Thoth. (13), 12a D. micrololium Benth. (15, 15), Glycyrrhiza pallidiflora Maxim. (15), 13 Mildbraediodendron excusum Harms (13, 14, and 17) (published as "M. excus Harms"), 14 Millettia dura Dunn (13), 15 Pterodon apparidic Pedersoli (12, 13, 15–17), 14b P. emarginatus Vogel (11, 13, 16, and 17) (published as "P. polygalaeflorus (Benth.) Benth."), and "P. pubescens (Benth.) Benth."), 14b Tephrosia maxima (12, 17), and Xanthocercis zambesiaca (Baker) Dumat-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, 13C) 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 semi-preparative 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 (H2O−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 semi-preparative 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 (λmax 247, 348 nm) with a pronounced low-wavelength shoulder band at 315 nm typical of a 5-deoxyflavonol 3-O-glycoside.45 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 C28H32O15. These preliminary data suggested that 20 might be a 5-deoxyflavonol 3-O-rutinoside.

Scale-up to semi-preparative 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 1H NMR spectrum of 20 comprised resonances for a methoxyflavonol 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, δc 125.5), 6.49 (1H, br s, δc 8.6 Hz, δc 119.0), and 6.33 (1H, br s, δc 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, δc 113.1), 6.83 (1H, d, J = 8.5 Hz, δc 115.1), and 7.54 (1H, dd, J = 8.5, 2.0 Hz, δc 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 anomic proton resonances of the disaccharide at δ 5.13 (1H, d, J = 6.9 Hz, δc 103.4) and 4.44 (1H, br s, δc 100.7) were used in conjunction with DQF-COSY and HSQC data to assign the 1H and 13C NMR resonances of the sugars and identify them as β-D-glucopyranose and α-L-rhamnopyranose, respectively.19 The downfield shift of β-β-GlC C-6′ to δc 66.7 and the ROE connectivities detected between α-Rha H-1″ and β-GlC 6″-CH3 characterized the interglycosidic linkage between these sugars as (1″→6″) and the disaccharide as rutinose. The absolute configurations of δ 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). Among these, 5,3′,4′-trimethoxy-6,7-methylenedioxyisoflavone (irisikumaoiin 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.20c The 6′-deoxychalcone liquirigenin (4,2′,4″-trihydroxychalcone, 18) is the precursor of 5-deoxyisoflavones, and its 4′-methyl ether (4,2′-dihydroxy-4′-methoxy-chalcone, 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″-tetrahydroxy-chalcone (narigenin 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′-hydroxy-chalcones are known from legumes.21

The flavonoid chemistry of A. herbert-smithii is also relevant to the current debate about the systematic position of Atelaea in Leguminosae.1d,22 Considered to be one of a number of genera that are transitional between subfamilies Caesalpinioideae and Papilionoideae, Atelaea 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 Atelaea was a member of a clade at the base of the Papilionoideae.22 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 Atelaea in the subfamily. Comparative chemical data for other genera in Swartzieae (as defined by Pohlili)23 are limited at present to Aldina,26 Cordyla,271 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 Atelaea 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 pterocarps and coumestans (Swartzia
only). The biosynthesis of these compounds requires the presence of an isoflavone 2′-hydroxymerase to produce 2′-hydroxyisoflavone precursors from which ring closure to the C-4 position of the C-ring can be effected. Although this 2′-hydroxymerase 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-(methyleneoxy)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-deoxyxanolonic 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). H NMR (500 and 400 MHz) or online by HPLC coupled to diode-array detection (Waters 214). 1H NMR (500 and 400 MHz) and 13C NMR (125 and 100 MHz) spectra were recorded in CDCl3 or CD3OD with the residual solvent used as an internal reference. 1H NMR data, see Table 1; 13C NMR data, see Table 2; HRESIMS m/z 341.0653 [M + H] (calcld for C19H15O8, 341.0656).

5-Methoxy-6,7,3′-4-bis(methyleneoxy)isoflavone (1): UV (MeOH) λmax 265, 295 nm; 1H NMR data, see Table 1; 13C NMR data, see Table 2; HRESIMS m/z 341.0653 [M + H] (calcld for C19H15O8, 341.0656).

5-Methoxy-6,7,3′-4-bis(methyleneoxy)isoflavone (2): UV (MeOH) λmax 266, 322 nm; 1H NMR data, see Table 1; 13C NMR (CDCl3, 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 (OCH3-6), 101.6 (OCH3-4′, 5′, 9.7 (C-8), 56.7 (OCH3-3′). HRESIMS m/z 341.0657 [M + H] (calcld for C19H15O8, 341.0656).

5-Methoxy-6,7,3′-4-bis(methyleneoxy)isoflavone (3): UV (MeOH) λmax 260, 304 nm; 1H NMR data, see Table 1; 13C NMR data, see Table 2; HRESIMS m/z 371.0763 [M + H]+ (calcld for C19H15O8, 371.0763).

5-Methoxy-6,7,3′-4-bis(methyleneoxy)isoflavone (4): UV (MeOH) λmax 269, 321 (sh) nm; 1H NMR data, see Table 1; 13C NMR data, see Table 2; HRESIMS m/z 371.0762 [M + H]+ (calcld for C19H15O8, 371.0761).

5-Methoxy-6,7,3′-4-trimethoxyisorafonosidavone (5): UV (MeOH) λmax 262, 292 (sh), 334 (sh) nm; 1H NMR data, see Table 1; 13C NMR (CDCl3, 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 (OCH3-3′ and 4′, 56.0 (OCH3-7), HRESIMS m/z 329.1019 [M + H]+ (calcld for C19H15O8, 329.1020).

5-Methoxy-6,7,3′-4-pentamethoxyisorafonosidavone (6): UV (MeOH) λmax 261, 287 (sh), 317 (sh) nm; 1H NMR data, see Table 1; 13C NMR data, see Table 2; HRESIMS m/z 373.1281 [M + H]+ (calcld for C19H15O8, 373.1282).

5-Methoxy-6,7-methylenedioxyisorafonosidavone (7): UV (MeOH) λmax 264, 324 nm; 1H NMR (CDCl3, 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), 6.02 (2H, s, OCH3O, 4.08
6.4,7-Dimethoxy-6,7-dimethylenedioxyisoflavone (15): UV (MeOH) $\lambda_{max}$ 251, 262 (sh), 300 (sh) nm; $\lambda_{max}$ (CDCl$_3$, 400 MHz) $\delta$ 8.21 (1H, d, J = 8.9 Hz, H-5), 1.92 (1H, d, J = 8.9 Hz, H-2'), 5.90 (2H, 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) $\delta$ 175.9 (C-4), 164.0 (C-7), 159.7 (C-4), 158.0 (C-19), 152.0 (C-2), 130.2 (C-2'), 127.9 (C-5), 125.0 (C-3), 124.3 (C-11), 118.5 (C-10), 111.5 (C-3'), 114.0 (C-7'), 100.2 (C-5'), 55.8 (OCH$_3$-7), 55.4 (OCH$_3$-4); HRESIMS m/z 328.0963 [M + H]$^+$ (calcd for C$_{24}$H$_{23}$O$_7$, 328.0965).

6.3,4-Tetramethoxy-6,7-dimethylenedioxyisoflavone (16): UV, $\lambda_{max}$ and $^{13}$C NMR identical to literature.$^{16}$ HRESIMS m/z 343.1175 [M + H]$^+$ (calcd for C$_{21}$H$_{19}$O$_5$, 343.1176).

6.7,2,4-5-Pentamethoxyisoflavone (17): UV (MeOH) $\lambda_{max}$ 252 (sh), 301, 317 (sh) nm; $\lambda_{max}$ (CDCl$_3$, 400 MHz) $\delta$ 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$_3$-2), 3.86 (3H, s, OCH$_3$-5), 3.78 (3H, s, OCH$_3$-4); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 175.5 (C-4), 154.3 (C-7), 154.1 (C-2), 154.2 (C-9), 151.9 (C-19), 149.9 (C-4), 147.7 (C-6), 143.3 (C-5), 121.3 (C-3), 118.0 (C-10), 115.2 (C-11), 104.8 (C-5), 99.2 (C-8), 98.2 (C-3), 56.7 (OCH$_3$-5), 56.3 (OCH$_3$-3'), 56.1 (OCH$_3$-6 and -7), 55.9 (OCH$_3$-4'); HRESIMS m/z 373.1280 [M + H]$^+$ (calcd for C$_{27}$H$_{27}$O$_{13}$, 373.1282).

4.2,4-Trihydroxychalcone (18): UV (MeOH) $\lambda_{max}$ 233, 260, 299 (sh) nm; $\lambda_{max}$ (CD$_3$OD, 400 MHz) $\delta$ 7.95 (1H, d, J = 8.9 Hz, H-6), 7.78 (1H, d, J = 15.4 Hz, H-4'), 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-4), 6.27 (1H, d, J = 2.4 Hz, H-3', 5'), 6.30 (1H, d, J = 2.0 Hz, H-2'), 6.27 (1H, d, J = 8.7 Hz, H-4); $^{13}$C NMR (CD$_3$OD, 100 MHz) $\delta$ 193.4 (C-4), 176.7 (C-4/C-2'), 167.4 (C-4/C-2'), 161.7 (C-4), 145.5 (C-1), 133.4 (C-6), 131.8 (C-2), 128.0 (C-1), 118.5 (C-α), 117.0 (C-3), 114.5 (C-1'), 109.6 (C-5), 104.1 (C-3'); APCLI-MS (positive mode) m/z 257 [M + H]$^+$.

4.2-Dihydroxy-4-methychalcone (19): UV (MeOH) $\lambda_{max}$ 233, 260, 299 (sh) nm; $\lambda_{max}$ (CD$_3$OD, 400 MHz) $\delta$ 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 = 8.0, 5.5 Hz, H-5), 6.45 (1H, dd, J = 2.5 Hz, H-3, 5), 3.68 (3H, s, OCH$_3$-5); $^{13}$C NMR (CD$_3$OD, 100 MHz) $\delta$ 193.9 (C-4), 176.7 (C-2), 167.5 (C-2'), 162.9 (C-4), 146.3 (C-β), 133.0 (C-6), 132.0 (C-2'), 127.4 (C-1), 117.9 (C-α), 117.4 (C-3), 115.5 (C-1'), 108.4 (C-5'), 102.1 (C-3'), 56.2 (OCH$_3$-4'); APCLI-MS (positive mode) m/z 271 [M + H]$^+$.

5-Decylosoramnetin 3-O-$\alpha$-rhamnopyranosyl(1′−6′)-β-D-glucopyranoside (20): yellow solid (MeOH); UV (MeOH) $\lambda_{max}$ 247, 315 (sh) nm; $\lambda_{max}$ (DMSO-d$_6$, 500 MHz) $\delta$ 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), 5.33 (1H, d, J = 6.9 Hz, H-1'), 4.44 (1H, br s, H-7'), 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) $\delta$ 125.5 (C-5), 121.9 (C-6), 119.0 (C-6), 115.1 (C-5'), 113.1 (C-2'), 103.4 (C-4'), 101.4 (C-1'), 100.7 (C-7'), 76.6 (C-3'), 75.6 (C-5'), 74.0 (C-2'), 71.6 (C-1), 70.4 (C-3'), 70.0 (C-2'), 69.7 (C-9'), 66.7 (C-5'), 56.5 (OCH$_3$-5), 17.5 (C-6'); HRESIMS m/z 607.1653 [M + H]$^+$ (calcd for C$_{33}$H$_{44}$O$_{16}$, 607.1657).

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References and Notes


(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.


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