A New Dammarane-Type Triterpene Glycoside from *Polyscias fulva*

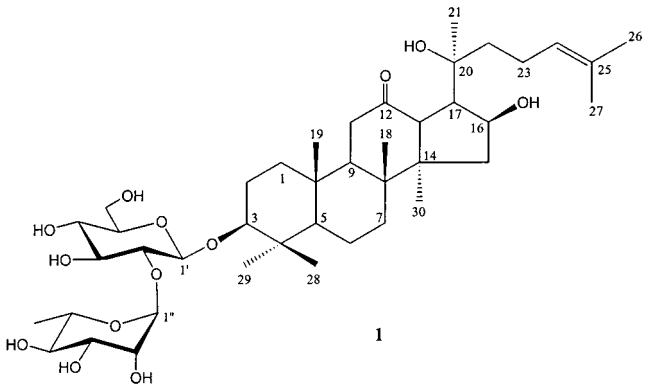
Erdal Bedir,[†] Ngeh J. Toyang,[†] Ikhlas A. Khan,*[,†,‡] Larry A. Walker,[†,§] and Alice M. Clark[†,‡]

National Center for Natural Products Research, Research Institute of Pharmaceutical Sciences, and Departments of Pharmacognosy and Pharmacology, School of Pharmacy, University of Mississippi, University, Mississippi 38677

Received July 19, 2000

A new dammarane-type triterpene glycoside, polysciasoside A (**1**), and three known compounds have been isolated from the leaves of *Polyscias fulva*. The structure of the new compound was established as 12-oxo-3β,16β,20(*S*)-trihydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

Polyscias fulva (Hiern) Harms (Araliaceae) is a tree found in the mountain forests of Cameroon and other Central African countries. It has been used in traditional medicine for its wound-healing properties.[1] No phytochemical or pharmacological investigations to support the ethnopharmacological claims of this plant have been reported. As part of our ongoing biological evaluation of West African medicinal plants, we undertook a bioactivity-guided phytochemical investigation of the aerial parts of *P. fulva*, which resulted in the isolation of a new dammarane-type triterpenoid, polysciasoside A (**1**), and three known compounds, two hederagenin-type saponins—α-hederin and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester[2,3]—and quercetin 3-*O*-β-D-glucopyranoside.[4]



1

The molecular formula of **1** was determined as $C_{42}H_{70}O_{13}$ by HRESIMS, which exhibited ion peaks at *m*/*z* 805.4943 $[M + Na]^+$ and at 783.5473 $[M + H]^+$. The IR spectrum of **1** indicated the presence of hydroxyl (3326 cm^{-1}) and olefinic (1642, 802 cm^{-1}) functionalities.

The ^1H NMR spectrum of **1** (Table 1) suggested the presence of six tertiary and two allylic-methyl groups, from signals at δ 0.90, 1.00, 1.02, 1.05, 1.08, 1.22, 1.64, and 1.68 (each s; respectively, H_3-29, H_3-19, H_3-30, H_3-21, H_3-28, H_3-18, H_3-27, and H_3-26), as well as a trisubstituted double-bond proton at δ 5.14 (1H, t, J = 7.0 Hz, H-24). Additionally, the resonances of two anomeric protons, indicative of the presence of two sugar moieties, were observed in the downfield region at δ 4.43 (d, 1H, J = 7.5 Hz, H-1′) and 5.39 (d, 1H, J = 1.1 Hz, H-1″). Acid hydrolysis of **1** followed by TLC analysis showed the presence of glucose and rhamnose. A combination of 2D DQF-COSY and TOCSY experiments allowed the sequential assignments of all proton resonances within each sugar residue, starting from the readily identifiable anomeric protons. Thus, on the basis of chemical shifts, the multiplicity of the signals, and the coupling constants, the two sugar residues were identified as α-rhamnopyranose and β-glucopyranose.[5] We favor their L and D absolute configurations, respectively, consistent with all other naturally occurring sugar residues present in the family Araliaceae. The HMQC experiments correlated each ^1H NMR sugar signal to the corresponding carbon resonance and showed the absence of any glycosylation shift for the ^{13}C NMR resonances of the rhamnopyranosyl residue, suggesting this sugar to be terminal. Additionally, in the ^{13}C NMR spectrum of **1**, C-2_{glc} was observed at δ 79.4 (d), showing the downfield shift due to glycosylation. A cross-peak observed in the HMBC spectrum of **1** between C-2_{glc} (δ 79.4, d) and H-1_{rha} (δ 5.39, d, J = 1.1 Hz) permitted the disaccharide chain to be defined as α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside.

The ^{13}C NMR spectrum of **1** showed 42 signals. After subtraction of the 12 carbon resonances of the sugar units, the remaining 30 signals were attributable to a triterpene aglycon. Detailed examination of the ^1H and ^{13}C NMR spectra suggested that **1** was a dammarane-type triterpene glycoside.[6−8] The aglycon moiety resonances were consistent with $C_{30}H_{50}O_4$, indicating the presence of six degrees of unsaturation. Three downfield carbon signals between δ 110 and 215 are due to a double bond (δ 126.4, d, C-24 and δ 132.3, s, C-25) and a carbonyl group (δ 215.0, s, C-12). Collectively, these features make up two of the six degrees of unsaturation. The covalent connectivities of the tetracyclic triterpenoid aglycon of **1** were established by analysis of the DQF-COSY, TOCSY, and HMQC spectra, which revealed the presence of five isolated spin systems (Figure 1) in the triterpene skeleton: "A" (H-3→H_2-1), "B" (H-5→H_2-7), "C" (H-9→H_2-11), "D" (H-13→H_2-15), and "E" (H-24→H_2-22). The connectivities between the partial structures (A−E) and assignment of quaternary carbon atoms in the molecule were determined via long-range correlations (Figure 1) in the HMBC spectrum.

The carbonyl group of the aglycon was affixed to C-12, on the basis of the large deshielding of C-11 and C-13 (ca. 17 and 18 ppm, respectively), and C-9 and C-14 (ca. 5 ppm) as compared to those reported for model compounds,[9] and the observation of cross-peaks in the HMBC spectrum from C-12 to H-9, H_2-11, H-13, and H-17. The attachment of the sugar chain at the C-3 hydroxyl group of the aglycon was established by means of the diagnostic glycosidation shift

* To whom correspondence should be addressed. Tel./Fax: (662) 915-7821. E-mail: rikhan@olemiss.edu.
† National Center for Natural Products Research.
‡ Department of Pharmacognosy, University of Mississippi.
§ Department of Pharmacology, University of Mississippi.

10.1021/np0003589 CCC: $20.00
Published on Web 11/23/2000

Table 1. NMR Spectral Data of Polysciasoside A (**1**) (CD_3OD)[a]

position	δ (ppm), *J* (in Hz)	δ (ppm)	position	δ (ppm), *J* (in Hz)	δ (ppm)
1	1.06 m, 1.56 m	40.4 t	23	1.54 m	42.4 t
2	1.70 m, 2.02 m	27.6 t	24	5.14 t (7.0)	126.4 d
3	3.19 dd (11.0, 4.5)	90.2 d	25		132.3 s
4		40.8 s	26	1.68 s	26.3 q
5	0.84 br. d (8.9)	58.0 d	27	1.64 s	18.1 q
6	1.57 m, 1.70 m	19.7 t	28	1.08 s	28.7 q
7	1.38 dd (2.4, 9.4), 1.58 m	35.7 t	29	0.90 s	17.3 q
8		42.0 s	30	1.02 s	18.7 q
9	1.79 dd (3.4, 12.4)	56.4 d			
10		38.9 s	1′	4.43 d (7.5)	106.0 d
11	2.18 dd (3.6, 13.2), 2.37 t (13.3)	40.9 t	2′	3.43 dd (7.4, 9.3)	79.4 d
12		215.0 s	3′	3.46 t (8.7)	79.9 d
13	3.14 d (10.5)	57.9 d	4′	3.30 t (8.6)	72.5 d
14		55.8 s	5′	3.26 m	78.0 d
15	1.28 d (13.3), 2.12 m	43.6 t	6′	3.84 dd (11.8, 2.0)	63.2 t
16	4.34 dd (4.0, 7.9)	74.5 d		3.65 dd (11.8, 4.0)	
17	2.30 dd (4.2, 10.5)	55.5 d	1″	5.39 d (1.1)	102.3 d
18	1.22 s	16.8 q	2″	3.97 dd (3.1, 1.5)	72.5 d
19	1.00 s	17.1 q	3″	3.76 dd (9.5, 3.3)	72.6 d
20		73.0 s	4″	3.39 t (9.8)	74.4 d
21	1.05 s	26.5 q	5″	3.99 m	70.4 d
22	2.12 m	24.2 t	6″	1.24 d (6.2)	18.4 q

[a] Assignments confirmed by DQF-COSY, TOCSY, HMQC, and HMBC experiments.

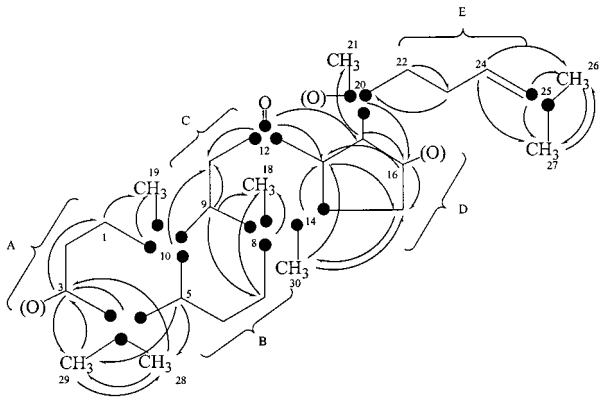


Figure 1. Spin systems (A−E) deduced from DQF−COSY, TOCSY, and HMQC spectra, and key HMBC interactions of **1**.

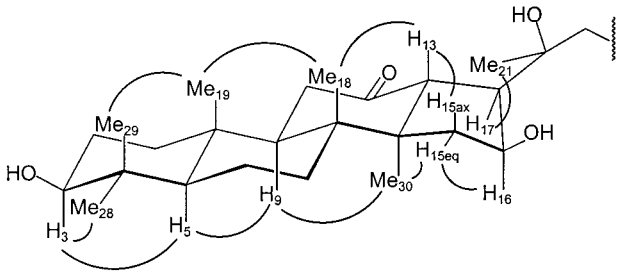


Figure 2. ROESY correlations and probable conformations of rings A−D.

of this carbon atom and was confirmed by the HMBC and ROESY spectra. In the HMBC spectrum, the anomeric proton assigned to the β-D-glucopyranosyl unit (δ 4.43, d, J = 7.5 Hz) showed a long-range correlation with the carbon at δ 90.2 (C-3), while in the ROESY spectrum the same anomeric proton exhibited a cross-peak with the proton at δ 3.19 (H-3).

The stereochemistry of **1** was resolved by a combination of 2D ROESY data and analysis of coupling constants. The cross-peaks observed in the ROESY spectrum for H-3 to H_3-28 and H-5 and for H-9 to H-5 and H_3-30 implied that these protons were cofacial (α), while observation of the key ROESY couplings from H_3-29 to H_3-19 and from H_3-18 to H_3-19 and H-13 revealed that these protons occupy the β-face of the molecule. These results also suggested that **1** has the usual A/B trans, B/C trans, and C/D trans ring junctions shown in Figure 2. Other ROESY networks from H-16 to H-15eq and from H-15eq to H_3-30 provided evidence for the β-orientation of the C-16 hydroxyl group. The orientation of 3β-OH was evident from the multiplicity of H-3, which was observed as a double doublet due to diaxial (J = 11.0 Hz) and axial−equatorial couplings (J = 4.5 Hz). This finding was also supported by the ROESY correlations among H-3, H-5, and H_3-28. ROESY correlations between H-17 and H_3-21 facilitated assignment of the C-20(*S*) configuration. On the basis of all of this evidence, the structure of polysciasoside A (**1**), was established as 12-oxo-3β,16β,20(*S*)-trihydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. As far as could be ascertained, only three 12-oxo-dammarane-type saponins have previously been isolated.[8] Compound **1** represents the first such analogue reported from the family Araliaceae.

The known compounds were identified as α-hederin,[2,3] 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester,[2,3] and quercetin 3-*O*-β-D-glucopyranoside.[4] Among the isolated compounds, only α-hederin showed antifungal activity against *Candida albicans* and *Cryptococcus neoformans*, with minimum inhibitory concentrations of 6.25 and 25 μg/mL, respectively.[10]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp operating at 589 nm. The IR spectra were recorded with an ATI Mattson Genesis Series FT−IR spectrophotometer. The 1D and 2D NMR spectra were obtained on a Bruker Avance DRX 500 FT spectrometer operating at 500 and 125 MHz, respectively. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane (TMS), and the coupling constants are in hertz (Hz, in parentheses). For the ^{13}C NMR spectra, multiplicities were determined by a distortionless enhancement by polarization transfer (DEPT) experiment. HRESIMS FT data were obtained using a Bruker BioApex FT−MS instrument in the ESI mode. TLC, precoated Si 250F plates (Baker); developing system, $CHCl_3$−MeOH−H_2O (70:30:3); visualization, 30% H_2SO_4. Column chromatography, Si gel 230−400 mesh (Merck).

Plant Material. The leaves of *P. fulva* were collected from Babanki Keku, Northwest Province of Cameroon, in December 1999. A voucher specimen verified by Dr. Clare Wirmum of Medicinal Foods and Plants Research Center, Bamenda, Cameroon, is deposited at the Heifer Project International Herbarium (HPI−NJT-025), Bamenda, Cameroon.

Extraction and Isolation. The air-dried leaves of *P. fulva* (600 g) were pulverized in a Waring blender and macerated with 4 L of MeOH at room temperature for 4 days. The extract was filtered and the filtrate concentrated in vacuo to give a greenish residue (85 g). An aliquot of this residue (80 g) was dissolved in MeOH (150 mL) and H_2O (350 mL). This solution was extracted with *n*-hexane (500 mL × 3) followed by EtOAc (500 mL × 4). The extracts were concentrated *in vacuo* to yield 18 g of a greenish oil from the *n*-hexane extraction and 26 g of a brown powder from the EtOAc extraction. The aqueous portion was lyophilized to give 32 g of brown powder. The EtOAc extract (15 g) was chromatographed on a Si gel column (400 g) using a gradient of 0−100% of MeOH in $CHCl_3$ to give 11 main fractions (fractions 1−11). Fraction 5 (1.0 g) was dissolved in MeOH (30 mL) and resulted in the formation of white crystals. The white crystals were recrystalized to yield α-hederin (628 mg). The remaining part of fraction 5 was subjected to column chromatography (Si gel, 30 g), eluted with $CHCl_3$−MeOH mixtures (10−100% MeOH). Altogether, 26 fractions were collected and pooled to four major fractions (fractions 5A-5D). Fraction 5C was crystallized in EtOAc to yield compound **1** (23 mg). Further chromatography on Si gel afforded 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin-28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (28 mg) and quercetin 3-*O*-*β*-D-glucopyranoside (42 mg).

Polysciasoside A (1): $[\alpha]^{25}_D$ −66°(*c* 0.1, MeOH); IR (KBr) ν_{max} 3326 (OH), 2913, 1642, 1592, 1414, 1123, 1075, 1040, 853, 802 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD), see Table 1; HRESIMS *m/z* 805.4943 $[M + Na]^+$, 783.5473 $[M + H]^+$.

Acid Hydrolysis of 1. A solution of **1** (3 mg) in 2 N HCl (1 mL) was refluxed for 3 h. The reaction mixture was extracted with EtOAc. After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The lyophilized residue was dissolved in pyridine (0.2 mL) and analyzed by TLC in EtOAc−*n*-BuOH−H_2O (20:70:10, v/v), together with authentic sugar samples.

Acknowledgment. We wish to acknowledge Mr. Andrew Kulie for assisting with the collection of the plant material. We are grateful to Dr. Chuck Dunbar for conducting the HRESIMS analysis. We also thank Mr. Frank Wiggers for his assistance in obtaining the 2D NMR spectra of **1**. This work was supported in part by the United States Department of Agriculture, ARS Specific Cooperative Research Agreement no. 58-6408-7-012 and the Heifer Project International.

References and Notes

(1) Burkill, H. M. *The Useful Plants of West Tropical Africa*; Royal Botanic Gardens: Kew, 1985; p 213.
(2) Kizu, H.; Kitayama, S.; Nakatani, F.; Tomimori, T. *Chem. Pharm. Bull.* **1985**, *33*, 3324−3329.
(3) Kizu, H.; Hirabayashi, S.; Suzuki, M.; Tomimori, T. *Chem. Pharm. Bull.* **1985**, *33*, 3473−3478.
(4) Harborne J. B.; Mabry T. J. *The Flavonoids: Advances in Research*; Chapman and Hall: New York, 1982; pp 72−73.
(5) Agrawal, P. K.; Jain, D. C.; Gupta, R. K.; Thakur, R. S. *Phytochemistry* **1985**, *24*, 2479−2496.
(6) Yahara, J.; Kosai, R.; Tanaka O. *Chem. Pharm. Bull.* **1977**, *25*, 2041−2048.
(7) Kusumoto, K.; Nagao, T.; Okabe, H.; Yamauchi, T. *Chem. Pharm. Bull.* **1989**, *37*, 18−22.
(8) Hu, L.; Chen, Z.; Xie, Y. *J. Nat. Prod.* **1996**, *59*, 1143−1145.
(9) Iwamoto, M.; Fujioka, T.; Okabe, H.; Mihashi, K.; Yamauchi, T. *Chem. Pharm. Bull.* **1987**, *35*, 553−561.
(10) Moulin, J. T.; Favel, A.; Elias, R.; Regli, P. *Mycoses* **1998**, *41*, 411−416.

NP0003589