A New Triterpene Saponin from *Pittosporum viridiflorum* from the Madagascar Rainforest[1]

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Received June 29, 2001

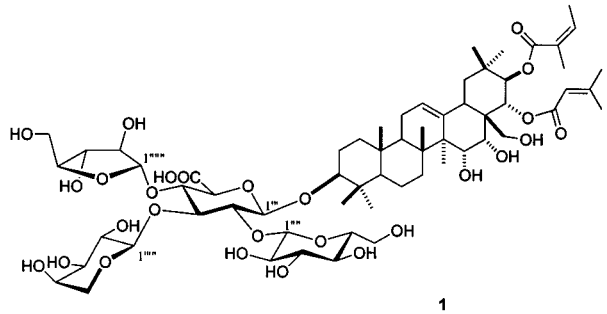
A novel triterpenoid saponin, pittoviridoside (**1**), which possesses an unusual 2,3,4-trisubstituted glycosidic linkage, has been isolated from *Pittosporum viridiflorum* using the engineered yeast strains 1138, 1140, 1353, and Sc-7 for bioactivity-guided fractionation. The structure of this compound was determined to be 3-*O*-[*β*-D-glucopyranosyl(1→2)]-[α-D-arabinopyranosyl(1→3)],[α-l-arabinofuranosyl(1→4)-*β*-D-glucuronopyranosyl-21-angeloyl-22-senecioylolean-12-en-3*β*,15α,16α,21*β*,22α,28-hexol by spectral, chemical, and GC analyses. This compound showed weak cytotoxicity against the A2780 human ovarian cancer cell line.

In recent years we have been engaged in a program to discover bioactive compounds using a mechanism-based bioassay involving genetically engineered mutants of the yeast *Saccharomyces cerevisiae*[2] along with cytotoxicity against the A2780 human ovarian cancer cell line.[3] As a part of our continuing search for potential anticancer agents, a sample of *Pittosporum viridiflorum* Sims (Pittosporaceae) collected in Madagascar as part of the plant collection program of the National Cancer Institute was subjected to testing, and its methanol extract was found to exhibit weak cytotoxicity to the A2780 human ovarian cancer cell line as well as activity in the yeast assay.

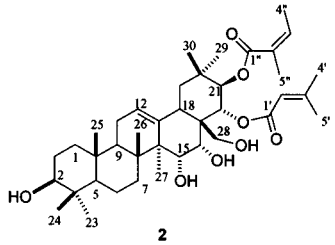
A number of investigators have examined extracts of the leaves of *Pittosporum* species, including *P. viridiflorum*, for their antimicrobial effects;[4,5] this is apparently due to a number of volatile mono- and sesquiterpenes.[6−8] Molluscicidal activity was also described.[9] A number of saponin aglycons isolated from *P. viridiflorum* have also been reported.[10] Herein we report the structure and bioactivity of a novel triterpenoid saponin, designated as pittoviridoside (**1**).

The MeOH extract of *P. viridiflorum* was subjected to solvent partitioning followed by C_{18} vacuum flash chromatography of the bioactive aqueous MeOH fraction, with the fractionation being guided by the results of A2780 cytotoxicity and yeast assays. The chromatographic fraction eluted from the C_{18} column with 70% and 80% aqueous MeOH was bioactive (IC_{12} 120, 150, 150, 140 μg/mL against the 1138, 1140, 1353, and Sc7 yeast strains) and was purified by repeated reversed-phase HPLC and Si gel preparative TLC to give the new saponin pittoviridoside (**1**).

Compound **1** was obtained as an amorphous white powder that was determined to have the composition $C_{62}H_{96}O_{27}$ by HRFABMS and ^{13}C NMR analyses. The IR spectrum showed absorptions at 3400 (OH) and 1720 (C=O of COOH and α*β*-unsaturated ester) cm^{-1}. The ^{13}C NMR spectra of this compound showed signals characteristic of three carbonyl groups and three double bonds at *δ* 176.7, 169.5, 168.3, 159.1, 143.6, 138.4, 129.5, 127.0, and 116.7. The chemical shifts of the carbonyl signals and the absorption band at 1720 cm^{-1} in the IR spectrum indicated the presence of two α,*β*-unsaturated ester functionalities. This interpretation was supported by an absorption maximum at 214 nm in the UV spectrum. In addition, the presence of a carboxylic acid functionality was indicated by a carbon signal at *δ* 176.7 in the ^{13}C NMR spectrum and an absorption maximum at 1720 cm^{-1} in the IR spectrum. The negative FABMS of **1** exhibited a quasimolecular ion peak at *m*/*z* 1271 $[M-H]^-$. Other significant fragmentation peaks visible at *m*/*z* 1139 $[(M - H) - 132]^-$ and 1109 $[(M - H)^+ - 162]^-$ corresponded to the loss of one terminal hexose and one terminal pentose unit, respectively.



1



2

Hydrolysis of **1** with 3 N HCl under reflux for 4 h afforded the aglycon, named pittoviridagenin (**2**), which showed a simplified NMR spectrum. A molecular formula of $C_{40}H_{62}O_8$ was deduced for **2** by a combination of HR-FABMS and ^{13}C NMR spectrometry. The presence of six carbon signals (*δ* 78.9, 78.0, 73.0, 72.5, 67.6, and 62.8) in the ^{13}C NMR spectrum indicated this compound to contain a number of oxygen-based functionalities. This interpreta-

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10.1021/np010327t CCC: $22.00
Published on Web 12/21/2001

tion was supported by the ^{1}H NMR spectrum, in which seven downfield signals were observed at δ 6.65 (1H, d, J = 10.2 Hz), 6.25 (1H, d, J = 10.2 Hz), 4.35 (1H, dd, J = 4.2, 4.2 Hz), 4.20 (1H, dd, J = 9.4, 4.2 Hz), 3.73 (1H, dd, J = 9.8, 4.6 Hz), 3.48 (1H, m), and 3.43 (1H, dd, J = 9.8, 4.6 Hz). Two of the seven oxygenated proton signals at δ 3.73 and 3.43 were confirmed to be due to oxymethylene protons by DEPT and HMQC experiments. In addition, a careful examination of the ^{1}H and ^{13}C NMR and IR spectra revealed that another two oxygenated protons (δ 6.65 and 6.25) were present as esters of angelic and senecic acids.

The overall structure of **2** was determined by a combination of ^{1}H COSY, TOCSY, HMQC, and HMBC experiments and by comparison of NMR spectral data with literature data.[11] The relative positions of the angeloyl and senecioyl groups were confirmed by long-range correlations of H-21 and H-22 with quaternary carbonyl carbons; H-21 correlated with C-1″ of the angeloyl group at δ 169.0 and H-22 with C-1′ of the senecioyl group at δ 166.7, respectively. Thus, the structure of pittoviridagenin was unambiguously determined as 21-angeloyl-22-senecioylolean-12-en-3β,15α,16α,21β,22α,28-hexol, or 21-angeloyl-22-senecioyl-R_1-barrigenol (**2**).

In the ^{1}H NMR spectrum of pittoviridoside (**1**), signals for four anomeric protons at δ 5.18 (1H, br s), 5.00 (1H, d, J = 7.8 Hz), 4.91 (1H, d, J = 7.4 Hz), and 4.46 (1H, d, J = 7.8 Hz) were correlated with signals for four carbons at δ 108.2, 102.9, 104.1, and 105.7 by an HMQC experiment and were diagnostic for the presence of four sugar residues. The chemical shift of C-3 at δ 91.9 indicated that the four sugars were attached to C-3 of the aglycon. The identification of each sugar unit as well as their linkages was established by chemical degradation, TLC and GC analyses, and extensive NMR experiments.

Acid hydrolysis of **1** followed by TLC analysis of the hydrolysate and direct comparison with standard sugars indicated the presence of glucose, arabinose, and glucuronic acid, and these results were confirmed by GC−MS analysis of the methyl acetates and comparison to standards. The absolute configurations of the hydrolyzed pentoses were determined by GC−MS analysis of their thiazolidine derivatives prepared with l-cysteine methyl ester hydrochloride followed by peracetylation, as previously reported.[2g,12]

Most of the protons and carbons of each sugar unit were assigned on the basis of extensive NMR ^{1}H−^{1}H COSY, TOCSY, and HMQC experiments. The assignment of the signals of C-2‴, C-3‴, and C-4‴ in the glucuronic acid moiety was difficult because the signals of H-2‴, H-3‴, and H-4‴ were overlapped almost completely in the ^{1}H NMR spectrum; these signals were thus assigned by an HMQC-COSY experiment. The cyclic structures of the monosaccharides were also determined to be D-glucuronopyranose, D-glucopyranose, L-arabinopyranose, and D-arabinofuranose (or alternatively D-arabinopyranose and L-arabinofuranose) by 1D TOCSY and HMBC data. A combination of the HMBC and 1D and 2D NOESY experiments and mass spectral data of sugar derivatives established the arrangement of these sugar residues. A long-range correlation of H-1‴ with C-3 revealed a linkage between C-1‴ of the glucuronic acid and C-3 of the aglycon. This interpretation was confirmed by a strong NOESY cross-peak between H-3 and H-1‴ and also between H-23 and H-1‴. Similarly, a long-range correlation between H-1⁗ and C-2‴ assigned the linkage between glucuronic acid and glucose with the additional confirmation of NOESY correlations between H-24 and protons of H-5⁗ and H-6⁗. The linkage between the glucuronic acid and the arabinopyranose was defined by an HMBC correlation of the anomeric proton of the arabinopyranose at δ 4.91 (H-1⁗) with C-3‴ of the glucuronic acid at δ 80.2. The linkage between the arabinofuranose and the glucuronic acid was assigned by an HMBC correlation between the anomeric proton of the arabinofuranose at δ 5.18 (H-1⁗′) and C-4‴ at δ 75.1.

Further evidence for the nature of the sugar linkages was derived from analysis of the MS fragmentation of the sugars after methylation, reduction, hydrolysis, reduction, acetylation, and GC separation (Scheme 1, Supporting Information).[13] The monosaccharides D-glucuronopyranose, D-glucopyranose, and D-arabinopyranose were confirmed to have β-, β-, and α-linkages respectively by measurement of the coupling constants of their anomeric protons, while the α-configuration of L-arabinofuranose was clarified by observation of NOESY correlations between the C-1, C-3, and C-5 protons of this sugar.

The remaining problem was to distinguish between the two possibilities of L-arabinopyranose/D-arabinofuranose and D-arabinopyranose/L-arabinofurnaose for the arabinose units; this assignment was made by interpretation of NOESY data. Correlations between H-3‴ and H-1⁗ and between H-4‴ and H-1⁗′ could be assigned unambiguously since the attachment point of each sugar was established by HMBC experiments and other key protons of arabinoses were not involved in the region around δ 3.86, where H-3‴ and H-4‴ of glucuronic acid were almost completely overlapped. A strong NOE was also observed between H-2⁗ of the arabinopyranose and H-4⁗′ of the arabinofuranose. Therefore, the absolute configurations of arabinopyranose and arabinofuranose must be defined as D and L, respectively. If these configurations were exchanged, a NOESY cross peak would not have been found between H-2⁗ of arabinopyranose and H-4⁗′ of arabinofuranose.

The structure of pittoviridoside (**1**) was thus identified unequivocally as 3-*O*-[β-D-glucopyranosyl(1→2)]-[α-D-arabinopyranosyl(1→3)]-[α-L-arabinofuranosyl(1→4)-β-D-glucuronopyranosyl-21-angeloyl-22-senecioyl-R_1-barrigenol. Although several saponins containing R_1-barrigenol and glucuronic acid have been isolated from various phytochemical sources,[9,14] **1** has two unusual structural features compared with previous isolates. In the first place, it has both D-arabinopyranose and L-arabinofuranose in the sugar portion of the molecule. The coexistence of arabinopyranose and arabinofuranose with the same absolute configuration has been reported only rarely,[15] although different compounds isolated from the same plant having arabinose units with the opposite absolute configurations have also been reported.[16] To the best of our knowledge, however, this is the first example of a nonpolymeric compound simultaneously having the same sugar unit with opposite absolute stereochemistries and different ring sizes. A second unusual feature is the presence of a consecutively 1,2,3,4-tetrasubstituted glycosidic linkage in the glucuronic acid part. This kind of functionality appears to have been reported only once previously.[15b]

Pittoviridoside **1** showed weak activity (IC_{12} 85, 80, 140, and 100 μg/mL) against the 1138, 1140, 1353, and Sc7 strains in our yeast bioassay; IC_{12} is the concentration required to inhibit the growth of a microorganism in a 12 mm diameter zone. In a cytotoxicity test using the A2780 human ovarian cancer cell line it also exhibited weak cytotoxicity (IC_{50} 10.1 μg/mL).

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Sugar Moiety for Pittoviridoside (**1**)[a]

position	^{13}C	^{1}H	position	^{13}C	^{1}H
β-D-Glc			α-D-Ara(p)		
1‴	105.7 s	4.46 (1H, d, 7.8)	1⁗	104.1 s	4.91 (1H. d, 7.4)
2‴	79.9 d	3.89 (1H, m)	2⁗	73.1 d	3.56 (1H, dd, 9.0, 7.4)[b]
3‴	80.2 d	3.87 (1H, m)	3⁗	74.5 d	3.52 (1H, dd, 9.0, 3.7)[b]
4‴	75.1 d	3.85 (1H, m)	4⁗	70.4 d	3.77 (1H, brs)[b]
5‴	78.6 d	3.68 (1H, m)	5⁗	67.5 t	3.82 (1H, m), 3.54 (1H, brd, 15.6)[b]
6‴	176.7 s				
β-D-Glc			α-L-Ara(f)		
1⁗	102.9 s	5.00 (1H, d, 7.8)	1′′′′′′	108.2 s	5.18 (1H, brs)
2⁗	76.2 d	3.20 (1H, dd, 9.1, 7.8)	2′′′′′′	81.8 d	3.97 (1H, d, 1.2)
3⁗	78.0 d	3.39 (1H, dd, 9.1, 9.0)	3′′′′′′	79.5 d	3.75 (1H, brd 5.0)[b]
4⁗	72.6 d	3.12 (1H, dd, 9.5, 9.0)	4′′′′′′	87.4 d	4.43 (1H, dt, 5.0, 4.5)
5⁗	78.2 d	3.31 (1H, m)	5′′′′′′	63.5 t[c]	3.66 (2H, m)
6⁗	63.6 t[c]	3.83 (1H, dd, 12.0, 2.0),[b] 3.58 (^{1}H, dd, 12.0, 7.2)[b]			

[a] ^{1}H and ^{13}C NMR spectra were measured in CD_3OD at 100 and 400 MHz, respectively. Assignments were made by DEPT, ^{1}H COSY, TOCSY, HMQC, and HMBC experiments. [b] Coupling constant is based on a 1D TOCSY experiment. [c] These assignments may be interchanged.

Experimental Section

General Experimental Procedures. Optical rotations were taken on a Perkin-Elmer 241 polarimeter. UV spectra were measured on a Shimadzu UV1201 spectrophotometer. IR spectra were recorded on a Perkin-Elmer FT-IR 1600 instrument. NMR spectra were recorded in CD_3OD on a JEOL Eclipse$^+$ 500 instrument at 500.1624 for ^{1}H and 125.8 MHz for ^{13}C, and Varian Unity 400 NMR instrument at 399.95 MHz for ^{1}H and 100.58 MHz for ^{13}C NMR, using standard pulse sequence programs. Mass spectral data were obtained at the Nebraska Center for Mass Spectrometry and on a VG 7070EHF mass spectrometer in the Department of Biochemistry, Virginia Polytechnic Institute and State University.

Plant Material. The plant was collected in April 1993 by staff from the Missouri Botanical Garden in Antsiranana Province in the Ankarana Reserve of Madagascar. *Pittosporum viridiflorum* is an undergrowth tree, and the sample was from a branch. The collection number was NCI # Q66V2626, MBG # 1726; the sample used was one of four parts of the plant that were collected. The plant was identified by Roy Gereau at Missouri Botanical Garden.

Yeast Bioassay. The Sc-7[2a] and 1138/1140/1353[2c] yeast bioassays were carried out as previously described.

Cytotoxicity Bioassay. The A2780 cytotoxicity assay was performed at Virginia Polytechnic Institute and State University as previously reported.[3]

Extraction and Isolation. The plant samples were dried, ground, and extracted with MeOH to give the dried methanolic extract N056221. The bioactive MeOH extract (2.71 g) described was active against the mutant 1138, 1140, 1353, and Sc7. The MeOH extract (IC_{12} 650, 700, 800, 1150 μg/mL in the 1138, 1140, 1353, and Sc7 yeast strains) was dissolved in 80% aqueous MeOH (250 mL) and extracted with *n*-hexane (300 mL × 2). The bioactive aqueous MeOH layer was diluted with H_2O to 60% aqueous MeOH and then partitioned with CH_2Cl_2 (300 mL × 2). The activity was retained in the 60% aqueous MeOH, which was fractionated between *n*-BuOH and H_2O to afford 1.62 g of *n*-BuOH-soluble bioactive extract (IC_{12} 500, 530, 640, 570 μg/mL in the 1138, 1140, 1353, and Sc7 yeast strains). Half of the *n*-BuOH fraction was subjected to C_{18} vacuum flash chromatography using gradient mixtures of MeOH and H_2O (elution order: 50, 60, 70, 80, 90% aqueous MeOH, MeOH). The active fractions (0.420 g) eluted with 70% and 80% aqueous MeOH were combined (IC_{12} 120, 150, 150, 140 μg/mL in the 1138, 1140, 1353, and Sc7 yeast strains) and separated by semipreparative reversed-phase HPLC (YMC ODS-A column, 1 cm × 25 cm, 37% aqueous MeOH, 230 nm). Final purification was done by Si gel preparative TLC with the solvent of the upper layer of *n*-BuOH−EtOH−H_2O (5:1:4) to give 13 mg of pittoviridoside (**1**).

Pittoviridoside (1): mp 276 °C (dec); $[\alpha]^{25}_D$ −19.0° (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.71); IR (KBr) ν_{max} 3400 (br), 2920, 1720, 1610, 1380, 1240, 1160, 1080, 1040, 1000 cm^{-1}; ^{1}H NMR (CD_3OD) δ 6.03 (1H, qq, 7.1, 1.5, H-3″), 5.85 (1H, d, 10.2, H-21), 5.67 (1H, m, H-2′), 5.51 (1H, d, 10.2, H-22), 5.47 (1H, t, 3.3, H-12), 3.72 (2H, m, H-15, -16), 3.26 (1H, d, 10.2, H-28), 3.17 (1H, dd, 11.8, 4.1, H-3), 2.98 (1H, d, 11.3, H-28), 2.62 (2H, m, H-18, -19), 2.11 (3H, d, 1.0, H-5′), 1.91 (3H, m, H-2, -11), 1.88 (3H, dq, 7.2, 1.5, H-4″), 1.87 (3H, d, 1.0, H-4′), 1.81 (3H, m, H-5″), 1.75 (2H, m, H-7), 1.70 (1H, m, H-2), 1.61 (1H, m, H-1), 1.57 (1H, m, H-9), 1.55 (1H, m, H-6), 1.40 (1H, m, H-6), 1.40 (3H, s, H-27), 1.07 (6H, s, H-23, -30), 1.00 (3H, s, H-26), 0.98 (3H, s, H-25), 0.96 (1H, m, H-1), 0.87 (6H, s, H-24, -29), 0.78 (1H, d, 12.0, H-5); ^{13}C NMR (CD_3OD) δ 169.5 (s, C-1″), 168.3 (s, C-1′), 159.1 (s, C-3′), 143.6 (s, C-13), 138.4 (d, C-3″), 129.5 (s, C-2″), 127.0 (d, C-12), 116.7 (d, C-2′), 91.9 (d, C-3), 79.8 (d, C-21), 74.1 (d, C-16), 73.1 (d, C-22), 68.5 (d, C-15), 62.8 (t, C-28), 56.7 (d, C-5), 49.1 (s, C-17), 48.4 (s, C-14), 48.2 (d, C-9), 47.5 (t, C-19), 42.3 (s, C-8), 41.4 (d, C-18), 40.4 (s, C-4), 40.2 (t, C-1), 37.9 (s, C-10), 37.2 (t, C-7), 36.7 (s, C-20), 29.6 (q, C-29), 28.4 (q, C-23), 27.5 (q, C-4′), 27.1 (t, C-2), 24.8 (t, C-11), 21.0 (q, C-27), 20.8 (q, C-5″), 20.4 (q, C-5′), 20.1 (q, C-30), 19.5 (q, C-6), 17.9 (q, C-26), 17.0 (q, C-24), 16.3 (q, C-25), 15.9 (q, C-4″); ^{1}H and ^{13}C NMR data for sugar moieties, see Table 1; HMBC correlations H-12/C-9, C-14; H-21/C-22, C-1″; H-22/C-30, C-1′; H-23/C-3, C-4, C-5, C-24; H-24/C-3, C-5, C-23; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-20, C-21, C-30; H-30/C-19, C-20, C-21, C-29; H-2′/C-1′, C-4′, C-5′; H-4′/C-2′, C-3′; H-5′/C-2′, C-3′, C-4′; H-4″/C-2″, C-3″; H-5″/C-2″, C-3″, C-4″, C-5″; HRFABMS *m*/*z* 1317.5857 (M − H + 2Na)$^+$ (calcd for $C_{62}H_{95}O_{27}Na_2$, 1317.5876).

Acid Hydrolysis of 1. A solution of pittoviridoside (**1**, 4.8 mg) in MeOH (2 mL) was treated with an equal volume of 3 N HCl under reflux for 4 h. After hydrolysis, the solution was neutralized with 5% Na_2CO_3 and extracted with EtOAc three times to afford an aqueous layer and organic layer containing the sugars and the aglycon, respectively. Preparative TLC of the aglycon from the organic layer on Si gel ($CHCl_3$−MeOH, 1:9) gave 1.5 mg of pittoviridagenin (**2**).

Pittoviridagenin (2): mp 240 °C (dec); $[\alpha]^{25}_D$ +22.5° (*c* 1.10, MeOH); UV (MeOH) λ_{max} (log ϵ) 211(3.86); IR (KBr) ν_{max} 3400 (br), 2960, 2920, 1720, 1700, 1650, 1560, 1460, 1420, 1380, 1230, 1160, 1080, 1070, 1040, 1000 cm^{-1}; ^{1}H NMR (pyridine-d_5) δ 6.65 (1H, d, 10.2, H-22), 6.25 (1H, d, 10.2, H-21), 5.90 (1H, br q, 7.1, H-3″), 5.57 (1H, t, 3.1, H-12), 5.22 (1H, m, H-2′), 4.35 (1H, dd, 4.2, 4.2, H-16), 4.20 (1H, dd, 9.4, 4.2, H-15), 3.73 (1H, dd, 9.8, 4.6, H-28), 3.48 (1H, m, H-3), 3.43 (1H, dd, 9.8, 4.6, H-28), 3.13 (1H, m, H-18), 3.08 (1H, m, H-19), 2.23 (1H, m, H-7), 2.12 (1H, m, H-7), 2.10 (3H, d, 1.0, H-4′), 2.05 (3H, dq, 7.2, 1.5, H-4″), 1.92 (2H, m, H-11), 1.99 (3H, m, H-5″), 1.87 (2H, m, H-2), 1.84 (3H, s, H-27), 1.78 (1H, dd, 11.2, 5.7, H-9), 1.68 (1H, m, H-6), 1.61 (1H, m, H-1), 1.51 (3H, d, 1.0, H-5′), 1.46 (1H, dd, 11.5, 2.7, H-19), 1.45 (1H, m, H-6), 1.33 (3H, s, H-29), 1.23 (3H, s, H-23), 1.11 (3H, s, H-29), 1.08 (3H, s, H-26), 1.06 (1H, m, H-1), 1.05 (3H, s, H-24), 0.96 (3H, s,

H-25), 0.95 (1H, m, H-5); ^{13}C NMR (pyridine-d_5) δ 169.0 (s, C-1″), 166.7 (s, C-1′), 156.2 (s, C-3′), 143.7 (s, C-13), 136.5 (d, C-3″), 129.2 (s, C-2″), 125.5 (d, C-12), 116.5 (d, C-2′), 78.0 (d, C-3), 78.9 (d, C-21), 73.0 (d, C-16), 72.5 (d, C-22), 67.6 (d, C-15), 62.8 (t, C-28), 55.6 (d, C-5), 48.6 (s, C-17), 47.9 (s, C-14), 47.3 (d, C-9), 46.9 (t, C-19), 41.5 (s, C-8), 40.9 (d, C-18), 39.3 (s, C-4), 39.4 (t, C-1), 37.4 (s, C-10), 36.8 (t, C-7), 36.3 (s, C-20), 29.5 (q, C-29), 28.7 (q, C-23), 28.2 (t, C-2), 26.8 (q, C-4′), 24.1 (t, C-11), 21.0 (q, C-27), 20.9 (q, C-5″), 19.9 (q, C-5′), 20.1 (q, C-30), 19.2 (q, C-6), 17.6 (q, C-26), 16.6 (q, C-24), 16.0 (q, C-25), 15.8 (q, C-4″); HMBC correlations H-12/C-9, C-14; H-21/C-22, C-1″; H-22/C-30, C-1′; H-23/C-3, C-4, C-5, C-24; H-24/C-3, C-5, C-23; H-25/C-1, C-5, C-9, C-10; H-26/C-7, C-8, C-9, C-14; H-27/C-8, C-13, C-14, C-15; H-29/C-20, C-21, C-30; H-30/C-19, C-20, C-21, C-29; H-2′/C-1′, C-4′, C-5′; H-4′/C-2′, C-3′; H-5′/C-2′, C-3′, C-4′; H-4″/C-2″, C-3″; H-5″/C-2″, C-3″, C-4″, C-5″; HRFABMS *m*/*z* 694.4319 $(M + Na)^+$ (calcd for $C_{40}H_{62}O_8Na$, 694.4342).

Sugar Analysis by TLC and GC−MS. The sugar components of the aqueous layer left after hydrolysis were analyzed by Si gel TLC, the solvent system used being $CHCl_3$−MeOH−H_2O (32:18:4, v/v). Spots were visualized by spraying with 95% EtOH−H_2SO_4−anisaldehyde (9:0.5:0.5, v/v) and heating at 120 °C for 10 min. The results were confirmed by GC−MS analysis of methyl sugar peracetates. The aqueous layer was evaporated, dissolved in 10% HCl−dry MeOH (2 mL), and heated under reflux for 4 h. After removing the solvent under reduced pressure, the residue was treated with excess acetic anhydride and pyridine. Removal of the solvent by blowing with nitrogen gave the methyl sugar peracetates, which were analyzed by GC−MS and compared with authentic methyl sugar acetate standards. The GC−MS runs were performed using a 30 m × 0.32 mm i.d. HP-5 column with an initial temperature of 75 °C for 1 min and then temperature programming to 250 °C at a rate of 10 °C/min.

Linkage Analysis. The analysis was carried out as previously described.[2g]

Stereochemical Determination of Pentoses. The determination of the absolute configuration of the pentoses was performed by Hara's method[12] as previously described.[2g] Pittoviridoside (1 mg) was placed in 0.25 mL of MeOH and 0.5 mL of 1 N HCl. This was heated at 100 °C overnight. Water and $CHCl_3$ were added, the mixture was shaken, and the water-soluble fraction was dried by rotary evaporation. This fraction was placed in 0.3 mL of pyridine, and 3 mg of cysteine methyl ester hydrochloride was added; this was heated for 2 h at 100 °C. Acetic anhydride (0.3 mL) was then added and allowed to react for a further 2 h. The sample was cooled and solvent removed by blowing off with argon to yield a mixture of thiazolidine-carboxylate derivatives, which was analyzed by GC−MS using a 30 m × 0.32 mm i.d. HP-5 capillary column connected to the VG7070EHF mass spectrometer. The injector temperature was 200 °C, and a temperature of 75 °C for 1 min and then a gradient to 250 °C at the rate of 10 °C/min was used. Individual peaks for the thiazolidine derivatives of L-arabinose (26.24 min) and D-arabinose (24.41 min) were observed.

Acknowledgment. This research was financially supported by an International Cooperative Biodiversity Grant, number U01 TW/CA-00313, from the Fogarty Center, NIH. We thank Dan Harder, Mary Merello, Thomas G. Razafindrabaeza, and Jim Miller of the Missouri Botanical Garden, and Sylvain Razafimandimbison of Parc Botanique et Zoologique de Tsimbazaza for the plant collection, the staff of CNARP and the Parc de Tsimbazaza for their help in the collection, drying, and processing of the plant samples, Dr. Gordon Cragg, Dr. David Newman, and the National Cancer Institute for making the extract available, and Dr. Rabodo Andriantsiferana and Dr. Maminirina Andriantsoa of CNARP for their assistance in obtaining approval for the use of this extract. Field work was conducted under collaborative agreements between the Missouri Botanical Garden and the Parc Botanique et Zoologique de Tsimbazaza and the Direction de la Recherche Forestière et Piscicole, FOFIFA, Antananarivo, Madagascar. We gratefully acknowledge courtesies extended by the Government of Madagascar (Direction Générale de la Gestion des Ressources Forestières) and by the Association Nationale pour la Gestion des Aires Protégées.

Acknowledgment. We thank Mr. Kim Harich for carrying out the GC−MS analyses and the Nebraska Center for Mass Spectrometry for obtaining HRFAB mass spectra. We also thank Dr. Maged Abdel-Kader and Mr. Kim Harich for assistance with the linkage analysis and Dr. Bing-Nan Zhou for helpful discussions.

Supporting Information Available: Figure 1 (NMR correlations for the sugar moieties of pittoviridoside (**1**)) and Scheme 1 (mass spectroscopic fragmentations of reduced alditol acetates from **1**). These materials are available free of charge via the Internet at http://pubs.acs.org

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NP010327T