Cytotoxic Sesquiterpene Lactones from *Vernonia pachyclada* from the Madagascar Rainforest[1]

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Bioassay-guided fractionation of the cytotoxic leaf extract of *Vernonia pachyclada* Baker led to the isolation of three new sesquiterpene lactones, designated glaucolides K−M (**1**−**3**). The structures of the new compounds were determined using 1D and 2D NMR spectroscopy, and the structure and stereochemistry of **1** were confirmed by single-crystal X-ray diffraction. Compound **3** showed moderate activity in the A2780 human ovarian cancer cell line, with an IC_{50} of 3.3 μM.

In our continuing search for bioactive molecules from the rainforests of Madagascar as part of an International Cooperative Biodiversity Group (ICBG) program,[2] an extract of the leaves of a Madagasacar collection showed moderate cytotoxicity against the A2780 human ovarian cancer cell line. This extract was selected for bioassay-guided fractionation on the basis of its cytotoxicity and its presumed botanical novelty.[3] The crude extract was purified by liquid−liquid partition, reversed-phase flash chromatography, and reversed-phased HPLC to yield three new sesquiterpene lactones designated glaucolides K−M. After this work was complete the plant was identified as *Vernonia pachyclada* Baker (Asteraceae).

V. pachyclada is known locally as Tsaboraty and is used for the treatment of wounds. Members of the Asteraceae family are well known for the production of sesquiterpenes such as the glaucolides, and their presence in the genus *Vernonia* is particularly well studied.[4−9] The genus *Vernonia* has between 500 and 1000 species, depending on taxonomic circumscription, with the majority of the species in tropical and warm parts of the Old World, but species are also found in North America. The genus is particularly rich in Madagascar, where nearly 100 species occur, the vast majority of which are endemic.[10]

Results and Discussion

Compound **1** was obtained as colorless crystals from methanol. HRFABMS indicated a molecular formula of $C_{23}H_{30}O_9$. The ^{1}H and ^{13}C NMR data (Table 1), the HMQC correlations, and an HMBC experiment (Figure 1) indicated that compound **1** was a sesquiterpene with the same atom connectivity as that of the previously reported compound 8-*O*-desacetyl confertolide-8-*O*-methacrylate.[11] However, the ^{1}H NMR data for 8-*O*-desacetyl confertolide-8-*O*-methacrylate were not in agreement with those of compound **1** when both spectra were determined in the same solvent. Since no ^{13}C NMR or optical rotation data were reported for the literature compound, these data could not be compared. The variations in the ^{1}H NMR data indicated that the compounds are not identical and suggested that the difference is a stereochemical one.

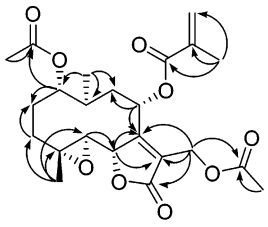


Figure 1. Key HMBC correlations for **1**.

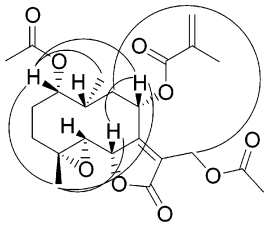
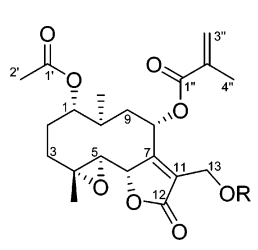


Figure 2. Key NOESY correlations for **1**.

Therefore, the only remaining problem to solve was that of the stereochemistry of compound **1**. The relative stereochemistry was determined on the basis of an analysis of NOESY correlations (Figure 2). The C-15 protons and the C-6 and C-8 protons all correlated to each other, indicating that they have syn-orientations to each other. Also observed were NOESY correlations from the C-15, the C-14, and the C-8 protons to the C-1 proton. Surprisingly, a NOESY correlation was also observed between the C-13 protons and the C-9 protons. This correlation was unexpected, as the two positions appear to be some distance apart, but in some ring conformations the two positions do appear to be close enough to produce a correlation. Thus, the relative stereochemistry of compound **1** was determined.



1 R = Ac
2 R = H

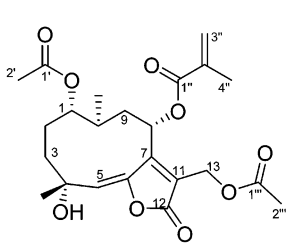


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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**–**3** in CD_3OD

	glaucolide K (**1**)		glaucolide L (**2**)		glaucolide M (**3**)	
	^{1}H[c]	^{13}C[d]	^{1}H[c]	^{13}C[d]	^{1}H[c]	^{13}C[d]
1	5.35 dd (4.5, 10.9)	72.0	5.36 dd (4.2, 11.1)	72.0	4.58 ddd (2.5, 6.7, 9.3)	79.0
2	1.82 m	27.1	1.82 m	27.1	1.82 m	29.5
	1.95[a]		1.95[a]		1.96 m	
3	1.25 ddd (7.6, 10.7, 14.0)	33.6	1.24 ddd (7.9, 10.6, 14.0)	33.7	2.13 dd (6.6, 9.5)	38.4
	2.18 ddd (2.5, 7.9, 14.0)		2.18 ddd (2.5, 7.9, 14.0)			
4		60.8		60.7		74.2
5	2.85 d (9.6)	67.2	2.79 d (9.7)	67.7	6.12 s	129.2
6	5.06 d (9.6)	82.5	5.00 d (9.7)	82.3		146.2
7		166.4		163.9		153.4
8	5.47 dd (3.2, 11.5)	69.7	5.45 dd (3.2, 11.5)	69.5	6.05 dd (6.6, 6.9)	68.8
9	2.00[a]	39.8	1.98[a]	40.1	2.34 m	39.1
	2.15 m		2.22 m			
10	1.99[a]	31.1	1.97[a]	31.0	1.94[a]	37.3
11		128.2		132.4		129.1
12		173.1		174.1		169.0
13	4.86 d (12.4)	56.5	4.38 dd (0.8, 12.0)	54.1	4.91 d (12.5)	56.6
	4.90 d (12.4)		4.41 dd (0.6, 12.0)		4.99 d (12.5)	
14	1.00 d (6.4)	14.6	0.99 d (6.2)	14.6	1.06 d (7.3)	19.4
15	1.70 s	18.3	1.70 s	18.3	1.63 s	30.2
1′		173.1		173.1		171.9
2′	2.06 s	20.8	2.05 s	20.8	2.02 s[b]	20.7
1″		167.6		167.8		167.4
2″		137.0		137.1		137.0
3″	5.70 m	127.5	5.67 m	127.3	5.72 m	127.6
	6.13 m		6.15 m		6.15 m	
4″	1.92 dd (1.0, 1.5)	18.3	1.93 dd (0.9, 1.6)	18.3	1.93 dd (1.1, 1.5)	18.3
1‴		171.9				171.9
2‴	1.99 s	20.6			2.00 s[b]	21.0

[a] Signal in overlapped region of the spectrum. [b] Signals are interchangeable. [c] 500 MHz. [d] 125 MHz.

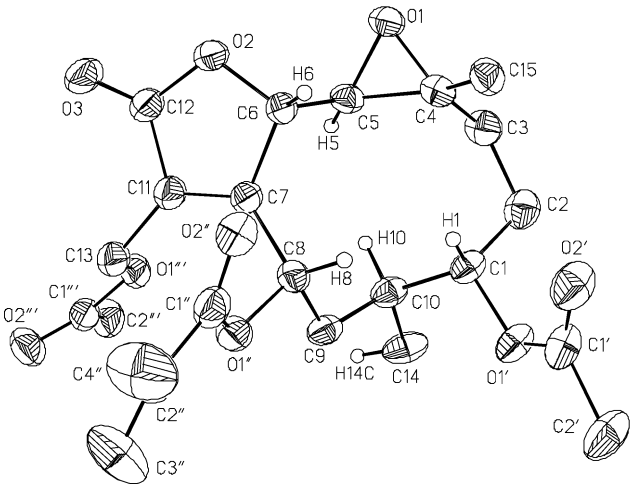

Figure 3. ORTEP diagram of **1**.

During the course of the isolation, crystals of **1** were obtained from methanol. A crystal was thus selected and analyzed by single-crystal X-ray diffraction. The results of this experiment confirmed the relative stereochemistry proposed based on the NOESY data and indicated an absolute stereochemistry of 1*S*,4*R*,5*R*,6*S*,8*S*,10*S*, as depicted (Figure 3). The relative stereochemistry for the known compound 8-*O*-desacetyl confertolide-8-*O*-methacrylate is 1*S*,4*R*,5*S*,6*S*,8*S*,10*R*,[11] so this compound and **1** differ in relative stereochemistry at C-5 and C-10. The structure was thus assigned as **1**, and the compound was named glaucolide K.

Compound **2** was obtained as a colorless amorphous solid. HRFABMS indicated a molecular formula of $C_{21}H_{28}O_8$. The ^{1}H and ^{13}C NMR spectra clearly indicated that **2** was an analogue of **1**. The only significant differences in the ^{1}H NMR data for **2** and **1** were the missing methyl signal at δ_H 1.99 and the shift of the methylene signal from δ_H 4.90 and 4.86 in **1** to δ_H 4.41 (d, J = 12.2 Hz) and 4.38 (d, J = 12.2 Hz) in **2**. Thus it appeared as though compound **2** was the C-13 deacetate of **1**. This hypothesis was supported by all of the spectral data and confirmed by acetylation of compound **2** to yield compound **1**. The structure was thus assigned as **2** and was named glaucolide L.

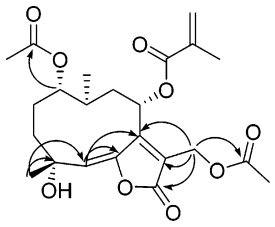

Figure 4. Key HMBC correlations for **3**.

Compound **3** was obtained as a slightly yellow amorphous solid. HRFABMS indicated a molecular formula of $C_{23}H_{30}O_9$. The ^{1}H and ^{13}C NMR spectra indicated that **3** was an analogue of **1**. No characteristic epoxide signals were observed in the ^{1}H NMR spectrum of **3**, and one additional signal was observed for a vinylic proton (δ_H 6.12, s). The ^{13}C NMR spectrum also contained two additional signals for a C–C double bond. Thus it appeared as though compound **3** was the Δ_5 4-hydroxy analogue of **1**. This hypothesis was supported by HMBC correlations (Figure 4) and the remaining spectral data. The configuration of the Δ_5 double bond was assigned as *E,* as the only NOESY correlation observed from the C-5 proton was to the C-15 protons. No correlations were observed from the C-5 proton to the protons of the 8 or 9 positions. The remaining stereocenters were based on those found in **1**. The structure was assigned as **3** and was named glaucolide M.

Compounds **1**–**3** were found to be cytotoxic in the A2780 human ovarian cancer cell line. Glaucolide M (**3**, 3.3 μM) was the most active compound and was slightly more active than glaucolide K (**1**, 5.8 μM). Glaucolide L (**2**, 24.5 μM) was the least active.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. NMR spectra were obtained on a JEOL Eclipse 500 or a Varian Inova 400 spectrometer in CD_3OD. Mass spectra were obtained on a JEOL JMS-HX-110 instrument. The chemical shifts are given in δ (ppm) with the residual CD_3OD solvent peak referenced to δ 3.30 as the internal reference, and coupling constants are reported in Hz. A Horizon flash chromatograph from BioTage Inc. was used for flash column chromatography. HPLC was performed on a Shimadzu LC-10AT or an LC-8A instrument with a C_{18} Varian Dynamax column (5 μm, 100 Å, 250 × 10 mm, or 8 μm, 100 Å, 250 × 21.4 mm, respectively).

Plant Material. The plant material used was a collection of the leaves of *Vernonia pachyclada* Baker obtained on July 13, 2003. The specimen was collected 2 km north of Fotsialanana, near Zahamena National Park, in the province of Toamasina, Madagascar, at 17°45′01″ S, 48°58′07″ E at an elevation of 320 m. Duplicates of the voucher specimens were deposited at the Missouri Botanical Garden, the Muséum National d'Histoire Naturelle, Paris, the Département des Recherches Forestières et Pisicoles, Madagascar, and the Centre National d'Applications et des Recherches Pharmaceutique, Madagascar.

Extraction and Isolation. The dried leaf material was extracted with EtOH to yield extract M1984. The crude extract (1.14 g) was partitioned between hexane (3 × 100 mL) and 90% aqueous MeOH (100 mL). The MeOH fraction was adjusted to 50% aqueous MeOH and further extracted with CH_2Cl_2 (3 × 200 mL). The CH_2Cl_2 fraction (341 mg) was the most active and was further fractionated with 65% aqueous MeOH, followed by a gradient to 100% MeOH, on a Biotage Horizon flash chromatograph over a C_{18} column. Fraction I (151 mg) was found to be the most active. Fraction I was dissolved in MeOH and stored at −20 °C to produce glaucolide K (**1**) as colorless crystals. Both the crystals and the mother liquor of fraction I were found to be active. The mother liquor of fraction I was fractionated by reversed-phase C_{18} HPLC with elution by 50% aqueous MeOH to produce 16 fractions (A−P). Fraction G contained glaucolide L (**2**, 3.5 mg, t_R 39 min), and fraction M contained additional glaucolide K (**1**, t_R 72 min), which was combined with the crystals to give 72 mg of **1**. Fraction L was refractionated on HPLC under the same conditions to give glaucolide M (**3**, 2.8 mg, t_R 67 min). Fractions L and M were found to be the most cytotoxic; the remaining fractions were too small and complex to facilitate further fractionation.

Glaucolide K (1): colorless crystals (MeOH); mp 141−142 °C; $[\alpha]^{22}_D$ −42.8° (*c* 0.90, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (4.3) nm; IR (film) ν_{max} 1770, 1726 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 451.1966 $[M + H]^+$ (calcd for $C_{23}H_{31}O_9$, 451.1968).

X-ray Crystallography of Glaucolide K (1). Large colorless rods (0.3 × 0.3 × 1.6 mm^3) of glaucolide K were crystallized from methanol by slow cooling to room temperature. The chosen crystal was cut (0.20 × 0.22 × 0.30 mm^3) and mounted on a nylon CryoLoop (Hampton Research) with Krytox Oil (DuPont) and centered on the goniometer of an Oxford Diffraction PX Ultra diffractometer equipped with an Onyx CCD detector and Cu radiation (λ = 1.54178 Å). The data collection routine, unit cell refinement, and data processing were carried out with the program CrysAlis.[12] The Laue symmetry and systematic absences were consistent with the orthorhombic space group $P2_12_12_1$. The structure was solved by direct methods and refined using SHELXTL NT.[13] The asymmetric unit of the structure comprised one crystallographically independent molecule. The final refinement model involved anisotropic displacement parameters for non-hydrogen atoms and a riding model for all hydrogen atoms. The absolute configuration was established from anomalous dispersion effects (Flack parameter = −0.19(17), 1758 Friedel pairs (74.4%) measured).[14] SHELXTL NT was used for molecular graphics generation.[15]

Summary of Experimental Information: orthorhombic crystal system; space group $P2_12_12_1$; a = 8.609(2) Å, b = 13.836(3) Å, c = 19.514(4) Å, V = 2308.2(9) $Å^3$, Z = 4. Data were collected at 100 K using Cu Kα radiation (λ = 1.54178 Å) in the theta range 4.53−66.58°, yielding 12 518 reflections of which 4120 were unique (R(int) = 0.0318). Refinement was carried out using full-matrix least squares on F^2 with final R indices of R1 = 0.0397 and wR2 = 0.1180, GOF = 1.144, Flack parameter = −0.19(17).[16]

Glaucolide L (2): colorless residue; $[\alpha]^{24}_D$ −20.3° (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 214 (3.9) nm; IR (film) ν_{max} 3433, 1762, 1733, 1722 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 409.1848 $[M + H]^+$ (calcd for $C_{21}H_{29}O_8$, 409.1862).

Glaucolide M (3): slightly yellow residue; $[\alpha]^{22}_D$ +31.3° (*c* 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.0), 286 (4.0) nm; IR (film) ν_{max} 3488, 1765, 1721 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 451.1974 $[M + H]^+$ (calcd for $C_{23}H_{31}O_9$, 451.1968).

Cytotoxicity Bioassays. The A2780 human ovarian cancer cell line assay was performed at Virginia Polytechnic Institute and State University as previously reported.[17] The three compounds had IC_{50} values of 5.8 μM (**1**), 24.5 μM (**2**), and 3.3 μM (**3**). The actinomycin D positive control had IC_{50} values of (8−24) × 10^{-4} μM under the same conditions.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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