New Bioactive Triterpenoids from *Melia volkensii*

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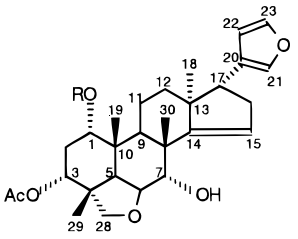
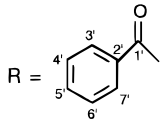
Bioactivity-directed fractionation of the root bark of *Melia volkensii* resulted in the isolation of two new natural products, meliavolkinin (**1**) and melianin C (**3**), together with two known compounds, 1,3-diacetylvilasinin (**2**) and melianin B (**4**). Jones oxidation of **4** gave compounds **3**, 23,24-diketomelianin B (**5**), and 16,23,24-triketomelianin B (**6**). The structures of the new compounds were elucidated by spectral and chemical data. Compounds **1**−**6** all showed marginal cytotoxicities against certain human tumor cell lines, while **5** showed selective cytotoxicities for the human prostate (PC-3) and pancreatic (PACA-2) cell lines with potencies comparable to those of adriamycin.

Melia volkensii Gürke (Meliaceae) is a subtropical tree found in eastern areas of Africa. A tea prepared from the bark is used to alleviate pain and is reported to be poisonous in overdoses.[1] The extracts of the seed kernels have been reported to have potential antifeedant activity against locusts.[2] Several limonoids with high antifeedant activity have been found from the fruits.[3,4] In our search for new potential anticancer constituents from plants, we have investigated the root bark of *M. volkensii*, collected from Kenya. Nine new bioactive compounds were previously found by directing the fractionation with the brine shrimp lethality test (BST).[5−8] We now report the isolation of two additional new limonoids, meliavolkinin (**1**) and melianin C (**3**), together with two known compounds 1,3-diacetylvilasinin (**2**) and melianin B (**4**). Jones oxidation of **4** afforded two semisynthetic compounds, 23,24-diketomelianin B (**5**) and 16,23,24-triketomelianin B (**6**). Compound **5** showed selective cytotoxicities for the human prostate (PC-3) and pancreatic (PACA-2) cell lines with potencies comparable to those of adriamycin.

Results and Discussion

Meliavolkinin (**1**) was isolated as colorless prisms. The molecular weight was indicated by a peak at *m/z* 575 for the $[M + H]^+$ ion and a peak at *m/z* 597 for the $[M + Na]^+$ ion in the *m*-nitrobenzyl alcohol (*m*-NBA) FABMS. High-resolution FABMS gave *m/z* 575.2996 (calcd 575.3009) for the $[M + H]^+$ ion, corresponding to the elemental formula, $C_{35}H_{42}O_7$.



1 R = 

2 R = Ac

The ^1H NMR spectrum of **1** showed considerable similarity with that of 1,3-diacetylvilasinin (**2**), a known compound,[9] indicating that the structures are closely related. The most significant difference was the absence of one of the acetate signals in the ^1H NMR spectrum of **2** and replacement by a group of benzoate signals at δ 7.0−8.2. The remaining assignments were then the placement of the hydroxy group, the acetate group, and the benzoate group to the three oxygenated carbons, C-1, C-3, and C-7. The chemical shift at δ 5.60 (H-15) suggested the presence of a free hydroxyl at C-7, since acetylation of a C-7α hydroxyl would cause a significant upfield shift of H-15.[10,11] The NOESY spectrum showed a cross peak between the methyl protons of the acetate group and the protons at the C-28 position. Thus, the acetate group was placed at the C-3 position, and the benzoate group was then placed at the C-1 position.

It was interesting to observe that the doublet of doublets at δ 8.06 (H-3′, H-7′) showed cross peaks with a doublet at δ 2.94 (H-5), a doublet of doublets at δ 2.70 (H-9), a singlet at δ 1.60 (AcO), as well as a singlet at δ 0.48 (H-18) in the NOESY spectrum. Compared with **2**, in which an acetate group was placed on C-1 instead of a benzoate group, the chemical shifts of H-5, H-9, H-18, and the protons from the acetate group in **1**, all showed anisotropic effects in the ^1H NMR spectrum from the benzene ring. The chemical shifts of H-9, H-18, and the AcO protons in **1** showed an upfield shift of 0.12, 0.35, and 0.40 ppm, respectively, while H-5 showed a downfield shift of 0.28 ppm compared with the chemical shifts from the corresponding signals in **2**. These data suggested that H-9, H-18, and the AcO on C-3 in **1** were held directly above or below the benzene ring and were shielded, whereas H-5 showed a deshielding effect from the benzene ring. Thus, **1** was identified to be **2** but with a benzoate group in the C-1 position instead of an acetate.

Melianin C (**3**) was isolated as a white powder. The molecular weight of **3** was indicated by a peak at *m/z* 621 for the $[M + H]^+$ ion in the dithiothreitol/dithioerythritol (DTT/DTE) FABMS. High-resolution FABMS gave *m/z* 659.3008 (calcd 659.2986) for the $[M + K]^+$ ion, corresponding to the elemental formula $C_{37}H_{48}O_8$.

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Table 1. ^{1}H and ^{13}C NMR Data for **1**

position	δ_C[a]	DEPT	δ_H mult	coupling (Hz)	$^{13}C/^{1}H$ correlation(s)
1	72.46	CH	4.92 t	3.0	H-3, H-19
2	27.59	CH_2	2.21 td	3.0, 16.0	
			2.28 td	3.0, 16.0	
3	71.67	CH	4.95 t	3.0	H-19, H-29
4	42.38	C			H-29
5	39.68	CH	2.94 d	12.5	H-1, H-3, H-19, H-28, H-29
6	73.94	CH	4.20 dd	3.0, 12.5	H-5, H-28
7	72.78	CH	4.23 d	3.0	H-30
8	45.98	C			H-30
9	33.55	CH	2.70 dd	7.0, 12.0	H-19, H-30
10	39.68	C			H-19
11	15.16	CH_2	1.37 m		
			1.62 m		
12	32.58	CH_2	1.50 m		H-18
			1.79 m		
13	47.23	C			H-15, H-18
14	160.3	C			H-18, H-30
15	120.5	CH	5.60 dd	1.0, 3.0	
16	34.22	CH_2	2.36 ddd	3.5, 7.0, 15.5	
			2.50 ddd	1.0, 11.0, 15.5	
17	51.51	CH	2.76 dd	7.0, 11.0	H-18
18	20.70	CH_3	0.48 s		H-12
19	15.34	CH_3	1.06 s		
20	124.4	C			H-21
21	139.6	CH	7.14 brs		H-22
22	111.0	CH	6.15 dd	1.0, 1.5	
23	142.4	CH	7.26 t	1.5	H-21, H-22
28	77.91	CH_2	3.60 d	7.5	H-29
			3.67 d	7.5	
29	19.62	CH_3	1.23 s		H-5, H-28
30	26.30	CH_3	1.12 s		
AcO	170.0	C	1.60 s		AcO
	20.43	CH_3			
1′	165.3	C			H-3′, H-7′
2′	129.8	C			
3′	129.7	CH	8.06 dd	1.5, 8.0	H-6′
4′	128.3	CH	7.35 t	8.0	H-3′, H-7′
5′	133.2	CH	7.48 tt	1.5, 7.5	H-4′
6′	128.3	CH	7.35 t	8.0	H-3′, H-5′
7′	129.7	CH	8.06 dd	1.5, 8.0	

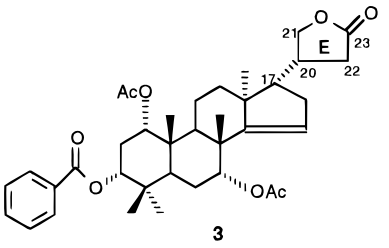
[a] The assignments were made by DEPT, COSY, HMQC (J = 140 Hz), and HMBC (J = 10 Hz).

Table 2. ^{1}H and ^{13}C NMR Data for **3**

position	δ_C[a]	DEPT	δ_H mult	coupling (Hz)	position	δ_C[a]	DEPT	δ_H mult	coupling (Hz)
1	72.62	CH	4.66 t	2.5	19	19.72	CH_3	1.02 s	
2	25.43	CH_2	2.27 td	3.0, 17.0	20	34.78	CH	2.66 s	
			2.16 m		21	72.46	CH_2	3.88 t	9.5
3	77.20	CH	4.87 t	2.5	22	34.05	CH_2	4.41 t	8.0
4	36.57	C						2.18 dd	11.5, 17.0
5	37.42	CH	2.52 dd	6.0, 9.5	23	176.60	C	2.49 dd	8.0, 17.0
6	22.73	CH	1.83 dd	3.0, 9.5					
7	75.19	CH	5.19 t	2.5	28	28.06	CH_3	0.89 s	
8	42.09	C			29	21.51	CH_3	0.98 s	
9	35.09	CH	2.63 dd	6.0, 11.5	30	26.94	CH_3	1.12 s	
10	40.30	C	1.26 m		AcO	169.69	C	1.62 s	
11	15.67	CH_2				21.01	CH_3		
12	33.79	CH_2	1.49 m		AcO	170.08	C	2.04 s	
			1.46 m			21.01	CH_3		
			1.56 m		1′	165.24	C		
13	46.52	C			2′	130.72	C		
14	158.98	C			3′	129.51	CH	8.06 m	
15	118.95	CH	5.32 dd	1.0, 3.0	4′	128.34	CH	7.41 t	7.5
16	34.74	CH_2	2.04 m		5′	133.07	CH	7.54 tt	1.5, 7.5
			2.14 m		6′	128.34	CH	7.41 t	7.5
17	57.99	CH	1.68 m		7′	129.51	CH	8.06 m	
18	16.11	CH_3	1.00 s						

[a] The assignments were made by DEPT, COSY, and HMQC (J = 125 Hz).

Compound **3** showed NMR spectral data (Table 2) suggestive of a structure similar to those of a known compound, melianin A,[5] with differences only in the side chain. In the COSY spectrum of **3**, a triplet at δ 4.41 (H-21) showed a correlation with another triplet at δ 3.88 (H-21), and they both showed correlation with a multiplet at δ 2.66 (H-20). H-20 was then additionally correlated to a doublet of doublets at δ 2.18 (H-22) and another doublet of doublets at δ 2.49 (H-22). A correlation between the two H-22 protons was also seen.



3

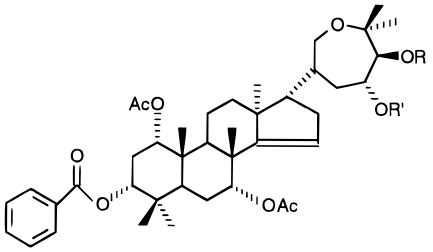
These data suggested a five-membered lactone ring for the side chain of **3**, and in fact, the ^{1}H NMR spectrum was identical with that of a semisynthetic compound obtained by Jones oxidation of melianin A.[5] Comparison of the ^{13}C NMR spectra for the two compounds, however, showed mismatches for a few signals (C-9, C-12, C-16, C-20, and C-22). By carefully analyzing the DEPT and HMQC spectra, **3** was determined to have the same structure as the semisynthetic compound, and the mismatched signals were due to the misassignments for the reported semisynthetic compound. Although this structure has been reported previously,[5] this is the first time that it has been encountered in nature.

Melianin B (**4**)[11] was isolated as colorless prisms. The molecular weight was indicated by a peak at *m*/*z* 695 for the $[M + H]^+$ ion in the DTT/DTE FABMS. High-resolution FABMS gave *m*/*z* 695.4137 (calcd 695.4159) for the $[M + H]^+$ ion, corresponding to the elemental formula $C_{41}H_{58}O_9$. The ^{1}H and ^{13}C NMR signals in the core portion of **4** were also very similar to those of melianin A,[5] indicating that the difference between the two was, again, in the side-chain portion.

In the COSY spectrum of **4**, cross peaks were evident from the oxymethylene protons at δ 3.43 (H-21) and 3.56 (H-21) to a methine proton at δ 1.85 (H-20), which, in turn, correlated to one of the methylene protons at δ 1.60 (H-22). The two protons at δ 1.60 (H-22) and δ 1.92 (H-22) were then correlated to an oxymethine proton at δ 3.79 (H-23), which further correlated to an oxymethine at δ 3.40 (H-24). Mild acetylation of **4** afforded **4a**, in which the signals at H-23 and H-24 shifted 1.36 and 1.62 ppm to lower field, respectively. This confirmed the presence of the two hydroxyl groups and their locations at C-23 and C-24.

This conclusion was further confirmed by Jones oxidation of **4** to **5** as a major product (Scheme 1). The signals for H-23 and H-24 disappeared in **5** compared with the spectrum of **4**, and two new carbonyl signals (209.2 and 212.2 ppm) were seen in the ^{13}C NMR spectrum. Besides **5**, **3** and **6** were also produced as oxidation products and were isolated. The structure of **4** was, therefore, concluded to be melianin B. Although the structure of **4** has been proposed before,[12] several ^{1}H NMR resonances were left unassigned and no ^{13}C NMR data was reported. Also, the absolute stereochemistries at C-23 and C-24 were not defined.

In our attempt to determine the absolute stereochemistry at C-23 in **4**, advanced Mosher's methodology was used. The method, using (*S*)- and (*R*)-Mosher esters [methoxy(trifluoromethyl)phenyl acetate or MTPA] introduces more or less shielding effects on different substituents of the chiral carbon, and the chemical shifts of the ^{1}H NMR spectra of these substituents in the esters change accordingly.[12−14] This method has been used successfully in the determination of the absolute stereochemistry at C-23 in melianin A.[5] In the case of **4**, although there are two secondary hydroxyl groups in the molecule, the one connected with C-24 is more sterically hindered, and, by carefully treating **4** with (*R*)- and (*S*)-methoxy(trifluoromethyl)-phenyl-acetyl chloride, only the monoesters of the hydroxyl at C-23, the (*S*)-Mosher ester **4b**, and the (*R*)-Mosher ester **4c** were obtained. The chemical shifts of the (*S*)-MTPA ester (**4b**) and the (*R*)-MTPA ester (**4c**) were assigned by careful analysis of the COSY spectra. The chemical



4	R = H	R' = H
4a	R = OAc	R' = OAc
4b	R = OH	R' = *S*-MPTA
4c	R = OH	R' = *R*-MPTA

shift difference of $\Delta\delta_H(\delta_S - \delta_R)$ changed from the core side to the C-24 hydroxyl side, from positive to negative (Table 4), which indicated that C-23 was of the *R*-configuration. Since the coupling constant between H-23 and H-24 in **4** was 9 Hz, these two protons were concluded to be trans. Upon the determination of C-23 to be *R*, the absolute stereochemistry at C-24 could also be concluded to be *R*.

Compound **5** was obtained as one of the Jones oxidation products of **4**. The FABMS spectrum of **5** showed a molecular ion peak at *m*/*z* 691 for $(M + H)^+$, which was four units less than that of **4**. This indicated that the oxidation of **4** generated a product with two

Scheme 1

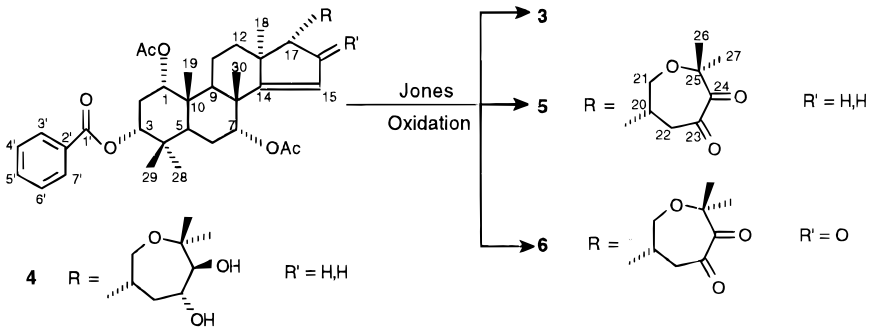


Table 3. ^{1}H and ^{13}C NMR Data of **4**

positon	δ_C[a]	DEPT	δ_H mult.	coupling (Hz)	^{13}C/^{1}H correlation(s)
1	72.64	CH	4.67 t	3.0	H-19
2	25.49	CH_2	2.15 td	3.0, 16.5	H-28, H-29
			2.27 td	3.0, 16.5	
3	77.10	CH	4.87 t	3.0	H-28, H-29
4	36.58	C			H-5, H-28, H-29
5	37.44	CH	2.51 t	8.0	H-28, H-29
6	22.83	CH_2	1.82 dd	3.0, 8.0	
7	75.59	CH	5.16 t	3.0	H-30
8	42.11	C			H-30
9	35.38	CH	2.62 dd	6.0, 12.0	H-19, H-30
10	40.27	C			H-19
11	16.09	CH_2	1.22 m		
			1.48 m		
12	34.97	CH_2	1.52 m		
			1.68 m		
13	46.25	C			H-15, H-18
14	159.4	C			H-18, H-30
15	119.2	CH	5.32 brd	2.5	
16	34.29	CH_2	1.98 m		
			2.25 m		
17	54.24	CH	1.85 m		
18	20.05	CH_3	0.99 s		
19	16.18	CH_3	0.98 s		
20	36.38	CH	1.85 m		
21	64.32	CH_2	3.43 dd	4.0, 13.0	
			3.56 dd	3.0, 13.0	
22	37.73	CH_2	1.60 m		
			1.92 m		
23	68.23	CH	3.79 ddd	3.0, 9.0, 9.0	
24	80.76	CH	3.40 d	9.0	H-26, H-27
25	76.17	C			H-26, H-27
26	22.25	CH_3	1.12 s		
27	26.38	CH_3	1.27 s		
28	28.04	CH_2	0.88 s		
29	21.46	CH_3	0.97 s		
30	26.81	CH_3	1.13 s		
AcO	169.7	C	1.62 s		
	20.99	CH_3			
AcO	170.1	C	2.03 s		
	21.0	CH_3			
1′	165.2	C			H-3′, H-7′
2′	130.7	C			H-4′, H-6′
3′	129.5	CH	8.06 dd	1.5, 8.0	
4′	128.3	CH	7.40 t	8.0	
5′	133.0	CH	7.54 tt	1.5, 8.0	H-4′
6′	128.3	CH	7.40 t	8.0	
7′	129.5	CH	8.06 dd	1.5, 8.0	

[a] The assignments were made by DEPT, COSY, HMQC (J = 140 Hz), and HMBC (J = 10 Hz).

more unsaturation units. The ^{1}H and ^{13}C NMR spectra of **5** showed great similarity as those of **4** with the exception of the signals for the seven-membered ring on the side chain. The chemical shifts at δ 3.79 for H-23 and at δ 3.40 for H-24 in the ^{1}H NMR of **4** were not seen in that of **5**. In addition, the ^{13}C NMR of **5** showed two new carbonyl signals at δ 209.2 and 212.0, and there were two oxygenated carbon signals less in **5** than that of in **4**. Therefore, these two carbonyl signals were assigned to C-23 and C-24, and **5** was identified to be 23,24-diketomelianin B.

Compound **6** was also obtained as one of the Jones oxidation products in a small amount. The CIMS spectrum of **6** gave a base peak at m/z 705 for $(M + H)^+$. In the ^{1}H NMR spectrum of **6**, the signal for H-15 showed a downfield shift and was seen at δ 5.80 as a singlet. In the ^{13}C NMR spectrum of **6**, a new carbonyl signal was seen at δ 205.5 besides the signals for C-23 and C-24. This signal had a strong correlation with H-15 in the HMBC spectrum and, therefore, was determined to be derived from C-16. Compound **6** was then identified to be 16,23,24-triketomelianin B.

Meliavolkinin (**1**) and 1,3-diacetylvilasinin (**2**) were significantly active in the brine shrimp[15] and the yellow fever mosquito[16] tests. All the compounds showed marginal cytotoxicities to certain human solid tumor cell lines,[17−21] while **5**, the oxidation product of **4**, showed selective cytotoxicities toward the prostate (PC-3) and pancreatic (PACA-2) cell lines with protencies equivalent to those of adriamycin (Table 6). Compounds showing cytotoxic ED_{50} values less than 4 μg/mL are considered significantly active in the search for new antitumor drugs, and borderline cytotoxicity may be an indication of other useful bioactivities.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus, and the thermometer was used without correction. The optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were taken in MeOH on a Beckman DU-640 spectrophotometer. IR spectra were taken on a Perkin-Elmer 1600 FTIR spectrophotometer. The 1D and 2D NMR spectra were recorded on a Varian VXR-500S, and the ^{13}C NMR spectra were recorded on a Bruker ARX-300 (300 MHz for ^{1}H; 75 MHz for ^{13}C) spectrometer. Low-resolution MS were recorded on a Finnigan 4000 mass spectrometer. The exact masses were obtained on a Kratos 50 mass spectrometer. HPLC was performed on a Dynamax software system (Rainin Instrument Co., Inc.), a Rainin HPXL solvent delivery system (two Rainin HPXL pumps), a Dynamax UV-1 variable-wavelength detector that was set at 220 nm, and Dynamax-60A 8 μm silica gel columns. Analytical TLC was carried out on silica gel plates and visualized with 5% phosphomolybdic acid in absolute ethanol followed by heating. (*R*)- and (*S*)-methoxy(trifluoromethyl)phenylacetyl chlorides were purchased from Aldrich (Milwaukee, WI).

Plant Material. The root bark of *M. volkensii* (B-644035, BRS-2-193) was collected in October 1971 from Kenya for the National Cancer Institute, National Institutes of Health, under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, U.S.D.A., Beltsville, MD, where voucher specimens are maintained.

Biological Evaluations. The extracts, fractions, and compounds isolated from the title plant were routinely evaluated for lethality to brine shrimp larvae (BST).[15] Sea salt was prepared from 3.8% (w/v) Instant Ocean artificial sea salt (Instant Ocean Co., Cincinnati, OH) in double-distilled water. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC_{50}). Crude extracts resulting in LC_{50} values of less than 200 ppm were considered significantly active. The isolated compounds were also evaluated by the yellow fever mosquito larvae (YFM) test[16] with rotenone as the positive control; pure compounds with LC_{50} values < 1 ppm are considered worthy of development as pesticides. The cytotoxicity tests against six human solid tumor cell lines [A-549 (human lung carcinoma),[17] MCF-7 (human breast carcinoma),[18]

Table 4. ^{1}H NMR Data of **4a**−**c**

	4a		**4b**[a] (*S*-MTPA)		**4c**[b] (*R*-MTPA)		
position	δ_H mult	coupling (Hz)	δ_H MVH	coupling (Hz)	δ_H MVH	coupling (Hz)	$\Delta\delta_H$[c] ($\delta_{2b} - \delta_{2c}$)
1	4.68 t	3.0	4.67 t	3.0	4.67 t	3.0	
2	2.15 td	3.0, 16.5	2.15 td	3.0, 16.5	2.15 td	3.0, 16.5	
	2.27 td	3.0, 16.5	2.28 td	3.0, 16.5	2.28 td	3.0, 16.5	
3	4.87 t	3.0	4.87 t	3.0	4.87 t	3.0	
5	2.51 t	8.0	2.51 t	8.0	2.51 t	8.0	
6	1.82 m		1.83 brd 8.0		1.83 brd	8.0	
7	5.15 t	3.0	5.16 t	3.0	5.16 t	3.0	
9	2.63 dd	6.0, 12.0	2.62 dd	6.0, 12.0	2.62 dd	6.0, 12.0	
11	1.22 m		1.22 m		1.22 m		
	1.48 m		1.48 m		1.48 m		
12	1.57 m		1.52 m		1.52 m		+0.02
	1.69 m		1.67 m		1.65 m		
15	5.29 bd 2.5		5.30 d	2.5	5.30 d	3.0	
16	1.87 m		1.86 m		1.81 m		+0.05
	2.21 m		2.25 m		2.22 m		+0.03
17	1.82 m		1.86 m		1.82 m		+0.04
18	1.01 s		0.97 s		0.94 s		+0.03
19	0.98 s		0.99 s		0.99 s		
20	1.87 m		1.97 m		1.88 m		+0.09
21	3.47 dd	5.5, 13.0	3.45 dd	6.0, 13.0	3.42 dd	6.0, 13.0	+0.03
	3.69 dd	3.5, 13.0	3.63 dd,	4.0, 13.0	3.60 dd	4.0, 13.0	+0.03
22	1.74 m		1.78 m		1.67 m		+0.11
	1.83 m		1.99 m		1.89 m		+0.10
23	5.15 td	3.5, 8.5	5.22 ddd	3.0, 9.0, 9.0	5.18 ddd	3.0, 8.0, 8.0	+0.04
24	5.02 d	9.0	3.60 d	9.0	3.62 d	9.0	−0.02
26	1.16 s		1.17 s		1.19 s		−0.02
27	1.18 s		1.23 s		1.25 s		−0.02
28	0.88 s		0.88 s		0.88 s		
29	0.97 s		0.97 s		0.97 s		
30	1.14 s		1.14 s		1.14 s		
AcO	1.62 s		1.61 s		1.61 s		
AcO	2.03 s		2.03 s		2.03 s		
AcO	2.00 s						
AcO	2.04 s						
3′	8.06 dd	1.5, 8.0	8.06 dd	1.5, 8.0	8.06 dd	1.5, 8.0	
4′	7.40 t	8.0	7.40 t	8.0	7.40 t	8.0	
5′	7.54 tt	1.5, 8.0	7.54 tt	1.5, 8.0	7.54 tt	1.5, 8.0	
6′	7.40 t	8.0	7.40 t	8.0	7.40 t	8.0	
7′	8.06 dd	1.5, 8.0	8.06 dd	1.5, 8.0	8.06 dd	1.5, 8.0	

[a] MTPA signals: δ_H 3.53 (3H, s, MeO), 7.35−7.45 (3H, m, phenyl protons), 7.50−7.58 (2H, m, phenyl protons). [b] For MTPA signals: δ_H 3.55 (3H, s, MeO), 7.36−7.44 (3H, m, phenyl protons), 7.50−7.58 (2H, m, phenyl protons). [c] Only chemical shift changes are listed in this column.

HT-29 (human colon adenocarcinoma),[19] A-498 (human kidney carcinoma),[17] PACA-2 (pancreatic carcinoma),[20] and PC-3 (prostate adenocarcinoma)[21]] were performed, in 7-day MTT tests, at the Cell Culture Laboratory, Purdue Cancer Center, using adriamycin as a positive control in each run. Generally, cytotoxicity ED_{50} values less than 20 μg/mL for crude extracts and less than 4 μg/mL for pure compounds are considered active.

Extraction and Isolation. Powdered root bark of *M. volkensii* (10 kg) was extracted by ethanol (100 L) for 7 days using a continuous extractor at 22 °C. The ethanol residue (F001, 502 g) was partitioned four times between CH_2Cl_2 and water (1:1) to give water-soluble (F002, 307.2 g), CH_2Cl_2-soluble (F003, 190 g), and interfacial (F004, 4.8 g) portions. The CH_2Cl_2 residue (F003, 190 g) was further partitioned four times between hexane and 90% methanol in water (1:1) to give 90% methanol (F005, 160 g) and hexane portions (F006, 30 g). The partitioning steps were monitored by the BST test, in which the LC_{50} values for F001−F006 were 1.50, >1000, 0.94, >1000, 0.71, and >1000 μg/mL, respectively. The methanol residue (F005, 160 g) was subjected to column chromatography (silica gel, 1350 g), eluted in a gradient fashion with hexane- CH_2Cl_2−EtOAc−MeOH; 61 fractions were collected, and the residues of each fraction were tested by the BST. Active fractions were pooled and subjected to repeated chromatography over silica gel columns and further purified by HPLC to give colorless prisms of **1** (3 mg), a white powder **2** (10 mg), a white powder **3** (5 mg), and colorless prisms **4** (60 mg).

Meliavolkinin (1): colorless prisms; mp 203−205 °C; $[\alpha]^{25}_D$ +37.5° (*c* 0.004, MeOH); UV (MeOH) λ_{max} 228 nm, log ϵ = 3.84; IR (film) cm^{-1}, 3450 OH, 2929 CH, 1733 C═O, 1716 C═O, 1275 CC(═O)O; ^{1}H and ^{13}C NMR chemical shifts are given in Table 1; FABMS (*m*NBA) *m/z* 597 ([M + Na]$^+$, 6), 575 ([M + H]$^+$, 43), 307 (92), 289 (100); HRFABMS (*m*-NBA) *m/z* 575.3009 for [M + H]$^+$ (calcd 575.2996).

Melianin C (3): colorless prisms; mp 148−152 °C; $[\alpha]^{25}_D$ −27.8° (*c* 0.018, MeOH); UV (MeOH) λ_{max} 229 nm, log ϵ = 3.73; IR (film) cm^{-1}, 2929 CH, 1779 C═O, 1725 C═O, 1277 CC(═O)-O, 1248 CC(═O)O; ^{1}H and ^{13}C NMR data are given in Table 2; FABMS (DTT/DTE) *m/z* 659 ([M + K]$^+$, 2), 621 ([M + H]$^+$, 4), 439 (14), 379 (19), 269 (11), 195 (16), 155 (48); HRFABMS (DTT/DTE) *m/z* 659.3008 for [M + K]$^+$ (calcd 659.2986).

Melianin B (4): colorless prisms; mp 190−194 °C; $[\alpha]^{25}_D$ −36.9° (*c* 0.046, MeOH); UV (MeOH) λ_{max} 229 nm, log ϵ = 3.92; IR (film) cm^{-1}, 3466 OH, 2963 CH, 1728

Table 5. ^{1}H and ^{13}C NMR Data for **5** and **6**

	5					**6**			
position	δ_C[a]	DEPT	δ_H mult	coupling (Hz)	position	δ_C[a]	DEPT	δ_H mult	coupling (Hz)
1	72.57	CH	4.66 t	2.5	1	72.28	CH	4.67 t	3.0
2	25.47	CH_2	2.27 td	3.0, 16.5	2	25.32	CH_2	2.20 td	3.0, 16.5
			2.16 m					2.29 m	
3	77.00	CH	4.87 t	2.5	3	77.20	CH	4.89 t	3.0
4	36.63	C			4	36.54	C		
5	37.37	CH	2.52 d	13.0	5	37.42	CH	2.51 dd	3.0, 13.5
6	22.72	CH_2	1.83 dd	3.0, 9.5	6	22.36	CH	1.86 m	
7	75.28	CH	5.19 t	2.5	7	74.58	CH	5.22 t	3.0
8	42.53	C			8	43.98	C		
9	35.16	CH	2.63 dd	6.0, 11.5	9	34.71	CH	2.87 dd	7.0, 11.0
10	40.25	C			10	40.21	C		
11	15.93	CH_2	1.26 m		11	15.12	CH_2	1.40 m	
			1.49 m					1.63 m	
12	34.81	CH_2	1.46 m		12	30.82	CH_2	1.88 m	
			1.56 m						
13	46.39	C			13	47.63	C		
14	159.0	C			14	192.3	C		
15	118.9	CH	5.32 dd	1.0, 3.0	15	124.0	CH	5.80 s	
16	34.43	CH_2	1.98 ddd	1.0, 10.5, 15.0	16	205.5	C		
			2.14 ddd	3.5, 7.5, 15.0					
17	56.17	CH	1.68 m		17	61.63	CH	2.00 d	6.5
18	19.63	CH_3	1.00 s		18	21.05	CH_3	1.28 s	
19	16.12	CH_3	0.98 s		19	16.02	CH_3	1.05 s	
20	36.56	CH	2.30 m		20	33.72	CH	2.43 m	
21	68.63	CH_2	3.38 dd	10.5, 12.5	21	68.59	CH_2	3.77 dd	10.5, 12.5
			4.02 dd	3.5, 12.5				3.87 dd	4.0, 12.5
22	42.10	CH_2	2.30 m		22	40.39	CH_2	2.90 dd	11.0, 16.0
			2.48 dd	12.5				3.13 dd	3.5, 16.0
23	209.1	C			23	209.2	C		
24	212.0	C			24	211.5	C		
25	80.53	C			25	80.69	C		
26	24.78	CH_3	1.31 s		26	24.51	CH_3	1.34 s	
27	20.51	CH_3	1.38 s		27	24.69	CH_3	1.38 s	
28	28.04	CH_3	0.88 s		28	28.03	CH_3	0.90 s	
29	21.48	CH_3	0.97 s		29	21.50	CH_3	1.00 s	
30	26.87	CH_3	1.12 s		30	25.95	CH_3	1.22 s	
AcO	169.7	C	1.62 s		AcO	169.2	C	2.03 s	
	21.00[b]	CH_3				20.83	CH_3		
AcO	170.1	CH_3	2.04		AcO	170.0	CH_3	2.17 s	
	21.10[b]					20.83			
1′	165.2	C			1′	165.2	C		
2′	130.7	C			2′	130.6	C		
3′	129.5	CH	8.06 m		3′	129.5	CH	8.06 m	
4′	128.3	CH	7.41 t	7.5	4′	128.4	CH	7.41 t	7.5
5′	133.1	CH	7.54 tt	1.5, 7.5	5′	133.2	CH	7.54 tt	1.5, 7.5
6′	128.3	CH	7.41 t	7.5	6′	128.4	CH	7.41 t	7.5
7′	129.5	CH	8.06 m		7′	129.5	CH	8.06 m	

[a] The assignments were made by DEPT, COSY, HMQC (J = 140 Hz), and HMBC (J = 10 Hz). [b] Chemical shifts are interchangeable.

Table 6. Bioactivities of Compounds **1**−**6**

	BST[a]	YFM[a]	A-549[b]	MCF-7[b]	HT-29[b]	A-498[b]	PC-3[b]	PACA-2[b]
1	5.37	59.70	25.36	27.84	28.07	26.88	1.02	16.73
2	10.02	29.18	3.29	2.42	3.73	3.35	2.78	3.06
3			21.85	14.47	18.05	26.52	4.73×10^{-1}	20.62
4	> 100	>100	1.68	3.29	2.15	3.67	3.93×10^{-1}	2.09
5			1.07	6.13	2.65	4.78	2.81×10^{-2}	1.30×10^{-2}
6			18.20	2.61	6.36	4.15	2.10	3.32
adr[c]			4.99×10^{-3}	2.53×10^{-1}	3.51×10^{-2}	4.24×10^{-3}	2.76×10^{-2}	1.33×10^{-2}

[a] LC_{50} values (μg/mL). [b] ED_{50} values (μg/mL). [c] Adriamycin was used as the positive control.

C═O, 1277 CC(═O)O; ^{1}H and ^{13}C NMR data are given in Table 3; FABMS (DTT/DTE) *m/z* 695 ([M + H]$^+$, 3), 453 (100); HRFABMS (DTT/DTE) *m/z* 695.4137 for [M + H]$^+$ (calcd 695.4159).

Acetylation of 4. Compound **4** (13.5 mg) was treated with 1 mL of acetic anhydride and 0.5 mL of pyridine overnight. The products were purified over a silica gel microcolumn, eluted by CH_2Cl_2:MeOH (9.5:0.5), to give **4a** (10 mg). Compound **4a**: colorless prisms; mp 174−177 °C; $[\alpha]^{25}_D$ −14.8° (*c* 0.044, MeOH); UV (MeOH) λ_{max} 229 nm, log ϵ = 3.94; IR (film) cm^{-1}, 2957 CH, 1731 CC(═O)O, 1277, 1247, 1170 CC(═O)O; ^{1}H NMR chemical shifts are given in Table 4; FABMS (DTT/DTE) *m/z* 779 ([M + H]$^+$, 7), 537 (100).

MPTA Derivatives of 4. To 1 mg of **4** in 0.5 mL of CH_2Cl_2 were sequentially added pyridine (0.2 mL), 4-(dimethylamino)pyridine (0.5 mg), and 12 mg of (*R*)- or (*S*)-α-methoxy-α-(trifluoromethyl)phenyl acetic acid (MTPA) in 1 mL of CH_2Cl_2. The mixture was left at room temperature for 4−5 h and then purified over a

microcolumn (0.6 × 6 cm) of silica gel (60−200 mesh) eluted with 3−4 mL of hexanes−CH_2Cl_2 (1:4). The elute was dried, CH_2Cl_2 (5 mL) was added, and the CH_2Cl_2 layer was washed twice with 1% $NaHCO_3$ (5 mL each) and twice with H_2O (5 mL each) and dried in vacuo to give (*S*)- and (*R*)-Mosher esters. The ^{1}H NMR chemical shifts are given in Table 4.

Jones Oxidation of 4 Leading to the Formation of 3, 5, and 6. To a solution of 40 mg (0.06 mmol) of **4** in 40 mL of acetone was added Jones reagent (5 mL) dropwise until the solution remained yellowish (Jones reagent was prepared by slowly adding a suspension of 2.0 g of CrO_3 in 2.0 mL of concentrated H_2SO_4 to 6 mL of water). The reactants were stirred for 5 h. The reaction products were separated by HPLC. Compounds **3** (2 mg, 5% yield), **5** (6 mg, 14.5% yield), and **6** (2 mg, 4.7% yield) were isolated as oxidation products. **5** was obtained as colorless prisms: mp 144−147 °C; $[\alpha]^{25}_D$ −44.1° (*c* 0.03, MeOH); UV (MeOH) λ_{max} 229 nm, log ϵ = 3.89; IR (film) cm^{-1}, 2956 CH, 1728 C=O, 1277 CC(=O)O; ^{1}H and ^{13}C NMR data are given in Table 5; FABMS (DTT/DTE) *m*/*z* 691 ([M + H]$^+$, 0.7), 449 (100). **6** was also obtained as colorless prisms: mp 151−154 °C; $[\alpha]^{25}_D$ −73.1° (*c* 0.012, MeOH); UV (MeOH) λ_{max} 231 nm, log ϵ = 4.11; IR (film) cm^{-1}, 3062 CH, 2960 CH, 1731 (broad) C=O, 1277 CC(=O)O; ^{1}H NMR and ^{13}C NMR are given in Table 5; CIMS *m*/*z* 705 ([M + H]$^+$, 100), 677 (11), 645 (8), 463 (16).

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