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# Meliavolkenin, a New Bioactive Triterpenoid from *Melia volkensii* (Meliaceae)

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**Abstract**—Meliavolkenin, a new triterpene with an apotirucallane skeleton, has been isolated from the root bark of *Melia volkensii* (Meliaceae) by bioactivity-directed fractionation using the brine shrimp lethality test. The structure has been elucidated using spectral and chemical data. The relative stereochemistries were determined by reduction and acetonide derivations, and the ring conformations were analyzed using the results of NOESY experiments. Meliavolkenin was bioactive in the brine shrimp lethality test and gave moderate cytotoxicities against three human solid tumor lines.

## Introduction

*Melia volkensii* Gurke (Meliaceae) is a subtropical tree which is distributed in the eastern region of Africa. In our search for new potential anticancer constituents from plants, we investigated the root bark for bioactive compounds. The brine shrimp lethality test (BST)[1,2] detected bioactivity and was used to direct the fractionation. In previous papers,[3–5] we reported the isolation, identification, and bioactivities of six new (meliavolin, meliavolkin, meliavolen, melianinone, meliavokensins A and B) and three known (melianin A, nimbolin B, and 3-episapilin A) natural compounds from the root bark. Further fractionation of the methanol

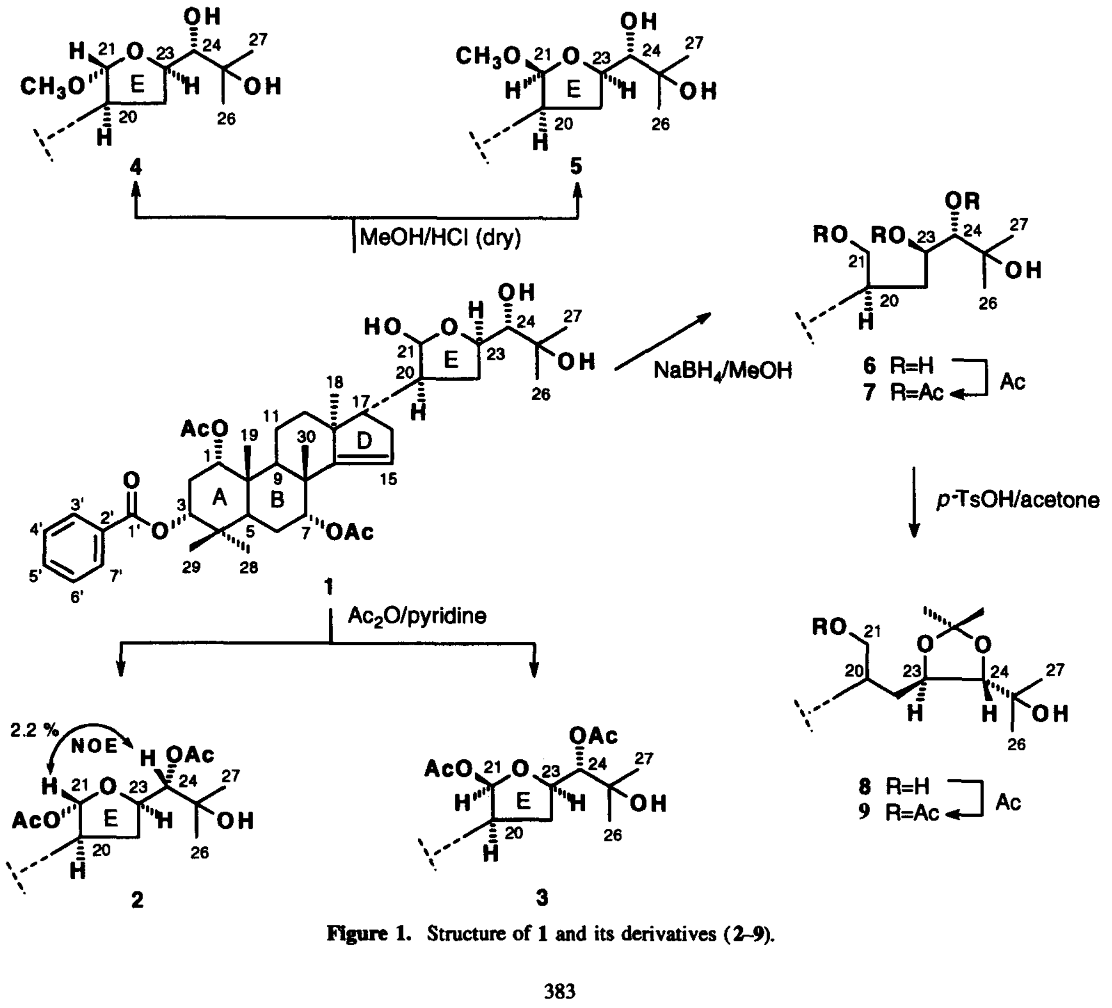

**Figure 1.** Structure of **1** and its derivatives (**2**–**9**).

soluble residue of the 95% ethanol extract has now led to a new apotirucallane type triterpene which we have named meliavolkenin (**1**) (Fig. 1). The structure of **1** has been elucidated by spectral and chemical data. The relative stereochemistries of **1** were determined by reduction and acetonide derivations, and the ring conformations were analyzed by the results of NOESY experiments. Brine shrimp lethality test data and data from comparative cytotoxicities of **1** against three human solid tumor lines are presented.

## Results and Discussion

Meliavolkenin (**1**) was isolated as a white powder. The molecular weight of **1** was indicated by a peak at m/z 733 $[M+Na]^+$ in the dithiothreitol (DTT), dithioerythritol (DTE), and sodium iodine (NaI) FABMS. High resolution FABMS gave m/z 733.3914 (calcd 733.3928) for $[M+Na]^+$, corresponding to the formula, $C_{41}H_{58}O_{10}$. The $^1H$ and $^{13}C$ NMR spectra of **1** appeared as the equilibrium mixture of anomers, in the proportion of 95:5 in chloroform-*d* solution (5 mg/0.6 mL). Only one hemiacetal carbon was observed at δ 96.66 in the $^{13}C$ NMR spectrum, but **1** formed two diacetates (**2** and **3**) when treated with acetic anhydride in pyridine; **1** also formed two methoxylates (**4** and **5**) when treated with dry hydrogen chloride and methanol. The $^1H$ NMR spectra of **1**–**5** and $^{13}C$ NMR spectra of **1**, **3**, and **5** showed that the compounds are apotirucallane type triterpenes, having 1α- and 7α-diacetates, and 3α-benzoate (Table 1). Three oxymethine signals in the $^1H$ NMR spectrum of **1** appeared at δ 4.68, 4.89 and 5.21 and were placed at C-1, C-3, and C-7, based on the comparisons of the $^1H$ and $^{13}C$ NMR spectra of **1** with those of meliavolin and meliavolen which have similar chemical shifts and splitting patterns.[3,4] These three protons were concluded to take the axial orientation because the coupling constants are of 2.5 Hz, respectively, and arise from vicinal methylene protons.

The side-chain five-membered ring of **1** was traced by cross-peaks in the COSY spectrum starting from an hydroxymethine doublet at δ 3.12 to an oxymethine triplet at δ 4.52, then to two methylene multiplets at δ 1.88 and 1.95, both of these were coupled to a methine multiplet at δ 2.09 which was connected to a hemiacetal methine doublet at δ 5.26. The same type of cross peaks can be observed with **2**–**5**. All of these data indicated that the C-21 aldehyde and C-23 hydroxyl formed an oxygenated bridge in the molecule. The side-chain conformational analyses were made with a series of NOE experiments. In the NOESY spectrum of **4** (Fig. 2), a cross-peak was found between H-21 (δ 4.78, *d*) and H-17 (δ 1.70, *m*), whereas in the NOESY spectrum of **5** (Fig. 2), cross-peaks were observed between H-21 (δ 4.72, *d*) and H-12 (δ 1.52, *m*), H-20 (δ 2.12, *m*), and H-30 (δ 1.01, *s*), which gave direct evidence that the H-17 was oriented to the β-position in **1** and its derivatives; the methoxyl in **4** is in the α-position and the methoxyl in **5** is in the β-position. A difference NOE experiment was carried out using the acetate, **2**; when a doublet at δ 6.12 was irradiated, 2.2% enhancement was observed on the doublet at δ 4.85, which indicated that the C-24 and H-21 are oriented in the same direction; thus, the C-24 hydroxyl in **1** and all of its derivatives (**2**–**5**) is in the β-position.

The relative stereochemistry of C-23 and C-24 was determined first by reduction of **1** with sodium borohydride in methanol to form **6**, in which the $^1H$ NMR spectrum showed the hydroxymethylene protons at δ 3.41(*dd*, *J* = 9.0 and 10.5 Hz) and δ 3.92 (*dd*, *J* = 2.5 and 9.0 Hz), respectively, and two hydroxymethines at δ 4.01 (*br d*, *J* = 10.0 Hz) and δ 3.09 (*d*, *J* = 10.0 Hz); the $^{13}C$ NMR spectrum showed the methylene carbon signal at δ 65.35; **6**, furthermore, formed a triacetate **7**, which indicated that the oxgenated bridge in the five-membered ring was cleaved into one primary alcohol and one secondary alcohol. The acetonide **8** was derived by treatment of **6** with *p*-toluenesulfonic acid in dry acetone. The $^1H$ NMR spectrum of **8** showed that two acetonyl methyls appeared as a singlet at δ 1.42; this singlet gave two cross-peaks to the oxymethines at δ 4.03 (*t*, *J* = 8 Hz) and δ 3.54 (*t*, *J* = 8 Hz) in the NOESY spectrum. These results indicated that the diol at C-23 and C-24 is *threo*,[6,7] since *erythro* diols generally give two separated acetonyl methyl signals.[7–9] Interestingly, in **8**, an hydroxyl group is substituted two carbons away from C-23, and acetylation of this hydroxyl influenced the acetonyl methyls, which resulted in **9**, to give two separated singlets at δ 1.36 and 1.38, respectively. The NOESY experiment of **9** showed two cross-peaks; one was between δ 4.01 and 1.38, and that connects the H-23α and the acetonyl α-methyl; the other is between δ 3.49 and 1.36, and that connects the H-24β and the acetonyl β-methyl (Fig. 3). This information proved that the C-23 and C-24 diol is in the *threo* configuration. The absolute stereochemistries of the side chain of **1** are proposed from biogenetic considerations based on the proven analyses of similar compounds, isolated from the same plant,[3,4] and other compounds which exist in the Meliaceae.[10] The apotirucallane type of triterpenes from the Meliaceae typically have 20*S* configurations; thus, C-23 should have the *R*-configuration and C-24 the *S*-configuration, as illustrated in Figure 1.

Meliavolkenin (**1**) was significantly active in the brine shrimp lethality test (**1**, BST $LC_{50}$ 0.55 μg $mL^{-1}$), and it was moderately cytotoxic against three human tumor cell lines [**1**, $ED_{50}$ 10.33, 4.30, and 0.67 μg $mL^{-1}$ in A-549 (human lung carcinoma),[11] MCF-7 (human breast carcinoma),[12] and HT-29 (human colon adenocarcinoma)[13] cells, respectively. Adriamycin, as a positive control, gave $ED_{50}$ values of $1.54 \times 10^{-2}$, $2.49 \times 10^{-1}$, and $3.99 \times 10^{-2}$ μg $mL^{-1}$, respectively, when compound **1** was tested in the same run]. Compounds showing cytotoxic $ED_{50}$ values less than 4 μg $mL^{-1}$ are considered significantly active in the search for new antitumor drugs; however, borderline cytotoxicity may be an indication of other useful bioactivities.

Table 1. $^1$H NMR spectral data of **1–5**

| Proton | Chemical shifts (δ), multiplicities, in Hz | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | | **2** | | **3** | | **4** | | **5** | |
| 1 | 4.68 | t, 2.5 | 4.67 | t, 2.5 | 4.64 | t, 2.5 | 4.69 | t, 3.0 | 4.70 | t, 3.0 |
| 2 | 2.19 | td, 2.5, 16.0 | 2.20 | td, 2.5, 16.0 | 2.18 | td, 2.5, 16.0 | 2.18 | td, 3.0, 16.0 | 2.18 | td, 3.0, 16.0 |
| | 2.30 | td, 2.5, 16.0 | 2.30 | td, 2.5, 16.0 | 2.30 | td, 2.5, 16.0 | 2.30 | td, 3.0, 16.0 | 2.30 | td, 3.0, 16.0 |
| 3 | 4.89 | t, 2.5 | 4.89 | t, 2.5 | 4.90 | t, 2.5 | 4.90 | t, 3.0 | 4.89 | t, 3.0 |
| 5 | 2.54 | t, 8.0 | 2.54 | t, 8.0 | 2.53 | t, 8.0 | 2.55 | t, 8.0 | 2.54 | t, 8.0 |
| 6 | 1.84 | m | 1.85 | m | 1.85 | m | 1.85 | m | 1.85 | m |
| | 1.84 | m | 1.85 | m | 1.85 | m | 1.85 | m | 1.85 | m |
| 7 | 5.21 | t, 2.5 | 5.19 | t, 3.0 | 5.21 | t, 3.0 | 5.20 | t, 3.0 | 5.21 | t, 3.0 |
| 9 | 2.67 | dd, 5.0, 12.0 | 2.66 | dd, 6.0, 12.0 | 2.68 | dd, 6.5, 11.5 | 2.68 | dd, 6.5, 12.0 | 2.67 | dd, 6.0, 11.5 |
| 11 | 1.30 | m | 1.24 | m | 1.26 | m | 1.30 | m | 1.25 | m |
| | 1.54 | m | 1.52 | m | 1.50 | m | 1.46 | m | 1.50 | m |
| 12 | 1.60 | m | 1.53 | m | 1.30 | m | 1.48 | m | 1.52 | m |
| | 1.68 | m | 1.66 | m | 1.53 | m | 1.72 | m | 1.60 | m |
| 15 | 5.32 | br d, 2.0 | 5.32 | br d, 2.5 | 5.33 | br d, 2.5 | 5.31 | br d, 2.5 | 5.32 | br d, 2.0 |
| 16 | 2.00 | m | 1.98 | m | 2.06 | m | 2.00 | m | 2.00 | m |
| | 2.10 | m | 2.10 | m | 2.16 | m | 2.16 | ddd, 2.0, 6.5, 15.0 | 2.11 | m |
| 17 | 1.92 | m | 1.77 | m | 1.88 | m | 1.70 | m | 1.96 | m |
| 18 | 1.13 | s | 1.14 | s | 1.14 | s | 1.14 | s | 1.13 | s |
| 19 | 1.02 | s | 1.01 | s | 1.01 | s | 1.03 | s | 1.02 | s |
| 20 | 2.09 | m | 2.44 | ddd, 3.5, 8.5, 11.5 | 2.28 | m | 2.30 | m | 2.12 | m |
| 21 | 5.26 | d, 3.0 | 6.12 | d, 3.5 | 6.05 | d, 4.0 | 4.78 | d, 3.5 | 4.72 | d, 4.0 |
| 21-OMe | | | | | | | 3.34 | s | 3.35 | s |
| 22 | 1.88 | m | 1.38 | m | 1.58 | m | 1.80 | t, 11.0 | 1.75 | m |
| | 1.95 | m | 2.07 | m | 1.96 | m | 1.91 | ddd, 5.0, 7.0, 11.0 | 1.88 | m |
| 23 | 4.52 | t, 7.5 | 4.54 | ddd, 2.5, 5.5, 10.0 | 4.47 | ddd, 4.0, 6.0, 10.0 | 4.24 | ddd, 1.0, 5.0, 9.5 | 4.42 | ddd, 2.0, 7.0, 9.0 |
| 24 | 3.12 | brd, 7.5 | 4.85 | d, 2.5 | 4.78 | d, 4.0 | 3.24 | br s | 3.17 | d, 7.0 |
| 25-OH | 2.18 | s | | | | | 2.80 | br s | 2.80 | br s |
| 26 | 1.24 | s | 1.19 | s | 1.15 | s | 1.26 | s | 1.26 | s |
| 27 | 1.28 | s | 1.31 | s | 1.29 | s | 1.30 | s | 1.26 | s |
| 28 | 0.91 | s | 0.90 | s | 0.90 | s | 0.91 | s | 0.90 | s |
| 29 | 1.00 | s | 1.00 | s | 1.00 | s | 1.00 | s | 1.00 | s |
| 30 | 1.03 | s | 1.05 | s | 1.04 | s | 1.06 | s | 1.01 | s |
| 1-AcO | 2.06 | s | 2.06 | s | 2.06 | s | 2.06 | s | 2.06 | s |
| 7-AcO | 1.65 | s | 1.64 | s | 1.63 | s | 1.66 | s | 1.65 | s |
| 21-AcO | | | 2.16 | s | 2.15 | s | | | | |
| 24-AcO | | | 2.03 | s | 2.06 | s | | | | |
| 3' | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 |
| 4' | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 |
| 5' | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 |
| 6' | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 |
| 7' | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 |

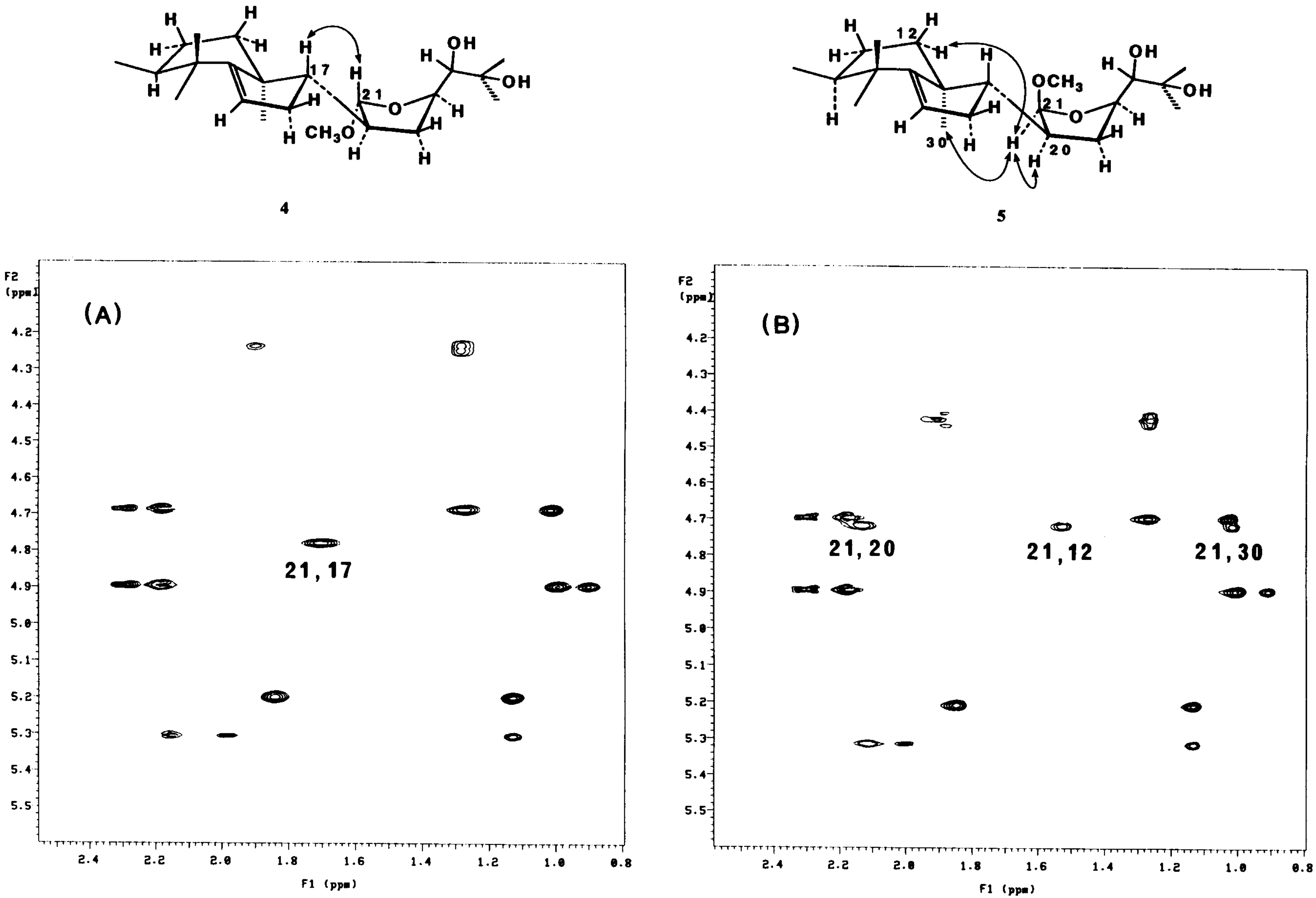

**Figure 2.** Partial NOESY spectra of **4** and **5** showing the correlation between H-21 and H-17 in A and the correlations between H-21 and H-20, H-12, and H-30 in B.

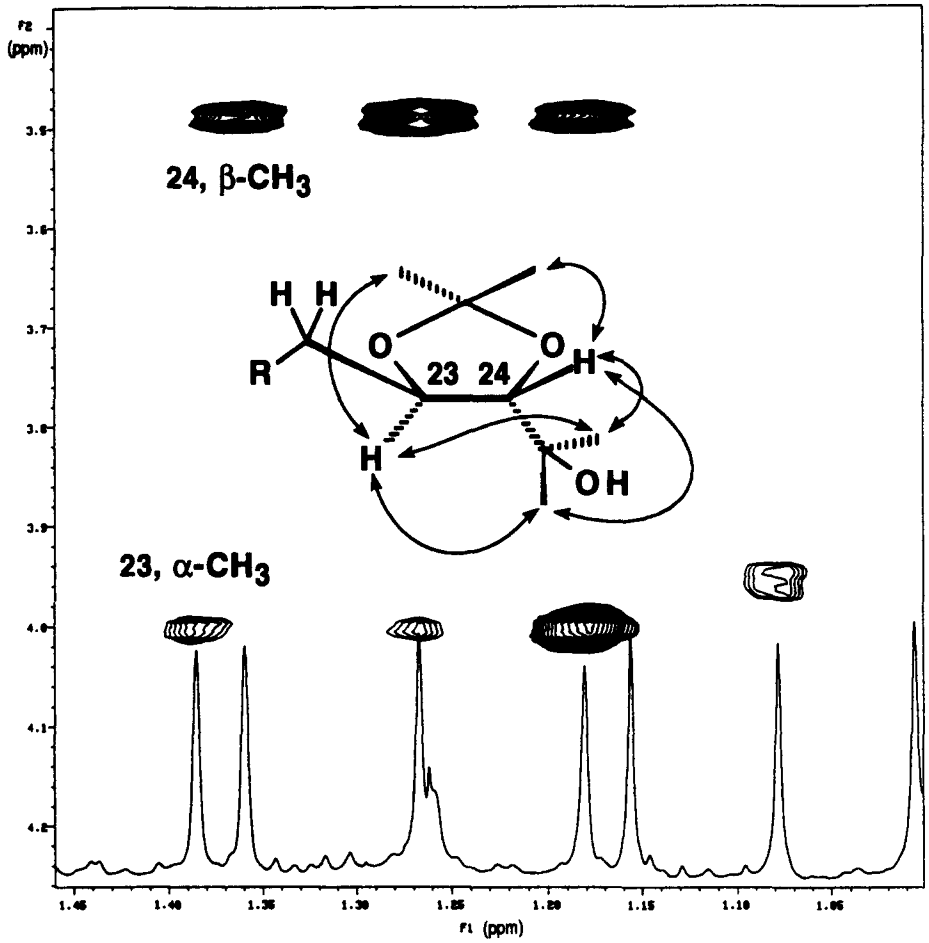

**Figure 3.** Partial NOESY spectrum of **9** showing the cross peaks between the hydroxymethines H-23 (δ 4.01) and the α-acetonyl methyl (δ 1.38), H-24 (δ 3.49) and the β-methyl (δ 1.36).

## Experimental

*General.* Mps were determined on a Mettler FP5 hot-stage apparatus and are uncorrected. The optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were taken in MeOH on a Beckman DU-7 spectrophotometer. Low resolution MS were recorded on a Finnigan 4000 mass spectrometer. The exact masses were determined on a Kratos 50 mass spectrometer through peak matching. All of the 1D and 2D NMR spectra were recorded on a Varian VXR-500S spectrometer, using Varian software systems. HPLC was performed on a Dynamax software system (Rainin Instrument Company, Inc.), a Rainin HPXL solvent delivery system (2 Rainin HPXL pumps), a Dynamax UV-1 variable wavelength detector which was set at 284 nm, Dynamax-60 A 8 μm silica gel columns (a 21.4 mm i.d. × 250 mm column and a 10 mm, i.d. × 250 mm column for separations and a 4.6 mm i.d. × 250 mm column for purity determinations). Either hexane:EtOAc (15:1) or hexane:MeOH:THF (10:0.9:0.1) were used as mobile phases.

*Plant material.* The root bark of *Melia volkensii* Gurke (B-644035, BRS-2-193) was collected in Kenya for the Natural Cancer Institute, under the auspices of Dr Robert E. Perdue, Medicinal Plant Laboratory, U.S.D.A., Beltsville, Maryland, where voucher specimens are maintained.

*Bioassays.* The extracts, fractions, and compounds isolated from the title plant were routinely evaluated for lethality to brine shrimp larvae (BST).[1,2] The cytotoxicity tests against A-549 (human lung carcinoma),[10] MCF-7 (human breast carcinoma),[11] and HT-29 (human colon adenocarcinoma)[12] cells were performed at the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive standard control.

*Extraction and isolation.* The crude residue (502 g) of the 95% EtOH extract of 10 kg of the root bark of the title plant was partitioned between $H_2O$ and $CH_2Cl_2$ to give a $H_2O$ layer and a $CH_2Cl_2$ layer; the residue of the $CH_2Cl_2$ layer was further partitioned between hexane and 10% $H_2O$ in MeOH to give a MeOH layer (*ca* 160 g dry residue) and a hexane layer. The MeOH residue was repeatedly chromatographed over silica gel columns, directed by BST activity, using gradients of hexane–$CH_2Cl_2$, $CH_2Cl_2$, and $CH_2Cl_2$–EtOAc and then purified by HPLC to give a white powder of **1** (80 mg).

*Meliavolkenin (1).* Powder, $[\alpha]^{22}_D$ –46.4 (c 0.28; $CHCl_3$); UV $\lambda_{max}^{MeOH}$: 208 nm (log ε 3.45), 228 nm (log ε 3.95); IR $\nu_{max}^{film}$ $cm^{-1}$: 3554, 2977, 1720, 1256, 1040; FABMS (DDT/DTE/NaI) m/z (%): 733 ($[M+Na]^+$, 5), 693 ($[M+H^+-H_2O]^+$, 18), 675 ($[M+H^+-2xH_2O^+$, 10), 553 ($M+H^+-2xH_2O^+-PhCOOH^+$, 5); HRFABMS (DDT/ DTE/NaI) m/z: 733.3914 for $C_{41}H_{58}O_{10}$ Na ($[M+Na]^+$) calcd 733.3928; $^1H$ and $^{13}C$ NMR (see Tables 1 and 2); COSY (500 MHz, nt 16, ni 256, d1 3.0, 2K × 2K); NOESY (500 MHz, nt 16, ni 256, mix 0.04 sec, dm 'nnn', 2K × 2K).

**Table 2.** $^{13}C$ NMR spectral data of **1**, **2**, **4**, and **6**

| | Chemical shifts (δ), multiplicities[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Carbon | 1 | | 2 | | 4 | | 6 | |
| 1 | 72.73 | CH | 72.71 | CH | 72.70 | CH | 72.62 | CH |
| 2 | 25.47 | $CH_2$ | 25.46 | $CH_2$ | 25.49 | $CH_2$ | 25.53 | $CH_2$ |
| 3 | 77.09 | CH | 77.40 | CH | 77.07 | CH | 77.07 | CH |
| 4 | 36.61 | C | 36.62 | C | 36.59 | C | 36.60 | C |
| 5 | 37.49 | CH | 37.47 | CH | 37.44 | CH | 37.42 | CH |
| 6 | 22.80 | $CH_2$ | 22.85 | $CH_2$ | 22.75 | $CH_2$ | 22.85 | $CH_2$ |
| 7 | 75.34 | CH | 75.41 | CH | 75.27 | CH | 75.58 | CH |
| 8 | 42.10 | C | 42.18 | C | 42.05 | C | 42.16 | C |
| 9 | 35.15 | CH | 35.30 | CH | 35.05 | CH | 35.48 | CH |
| 10 | 40.34 | C | 40.33 | C | 40.33 | C | 40.28 | C |
| 11 | 15.95 | $CH_2$ | 15.90 | $CH_2$ | 15.94 | $CH_2$ | 16.22 | $CH_2$ |
| 12 | 35.05 | $CH_2$ | 34.62 | $CH_2$ | 35.06 | $CH_2$ | 34.95 | $CH_2$ |
| 13 | 46.31 | C | 46.48 | C | 46.31 | C | 46.47 | C |
| 14 | 159.11 | C | 159.44 | C | 159.11 | C | 159.26 | C |
| 15 | 119.03 | CH | 118.73 | CH | 119.06 | CH | 119.43 | CH |
| 16 | 33.57 | $CH_2$ | 33.80 | $CH_2$ | 33.12 | $CH_2$ | 34.94 | $CH_2$ |
| 17 | 56.63 | CH | 56.67 | CH | 55.15 | CH | 56.07 | CH |
| 18 | 16.16 | $CH_3$ | 16.22 | $CH_3$ | 16.09 | $CH_3$ | 16.21 | $CH_3$ |
| 19 | 20.30 | $CH_3$ | 20.31 | $CH_3$ | 20.17 | $CH_3$ | 19.96 | $CH_3$ |
| 20 | 46.68 | CH | 44.82 | CH | 44.70 | CH | 40.73 | CH |
| 21 | 96.66 | CH | 100.56 | CH | 104.58 | CH | 65.35 | $CH_2$ |
| 22 | 29.95 | $CH_2$ | 32.79 | $CH_2$ | 31.45 | $CH_2$ | 37.50 | $CH_2$ |
| 23 | 78.58 | CH | 77.05 | CH | 78.82 | CH | 77.80 | CH |
| 24 | 73.63 | CH | 72.39 | CH | 72.84 | CH | 71.63 | CH |
| 25 | 74.86 | C | 76.23 | C | 76.66 | C | 73.97 | C |
| 26 | 26.95 | $CH_3$ | 27.37 | $CH_3$ | 26.38 | $CH_3$ | 27.12 | $CH_3$ |
| 27 | 26.76 | $CH_3$ | 26.77 | $CH_3$ | 26.29 | $CH_3$ | 26.40 | $CH_3$ |
| 28 | 28.11 | $CH_3$ | 28.11 | $CH_3$ | 28.08 | $CH_3$ | 28.07 | $CH_3$ |
| 29 | 21.58 | $CH_3$ | 21.53 | $CH_3$ | 21.55 | $CH_3$ | 21.48 | $CH_3$ |
| 30 | 26.70 | $CH_3$ | 26.83 | $CH_3$ | 26.92 | $CH_3$ | 26.40 | $CH_3$ |
| 1' | 165.14 | C | 165.10 | C | 165.23 | C | 165.24 | C |
| 2' | 130.67 | C | 130.70 | C | 130.74 | C | 130.73 | C |
| 3' | 129.44 | C | 129.46 | C | 129.50 | C | 129.51 | C |
| 4' | 128.23 | C | 128.23 | C | 128.29 | C | 128.30 | C |
| 5' | 132.93 | C | 132.93 | C | 132.93 | C | 133.03 | C |
| 6' | 128.23 | C | 128.23 | C | 128.29 | C | 128.30 | C |
| 7' | 129.44 | C | 129.46 | C | 129.50 | C | 129.51 | C |
| AcO | 169.68 | C | 169.60 | C | 169.75 | C | 169.73 | C |
| | 170.05 | C | 169.62 | C | 170.25 | C | 170.75 | C |
| | 21.06 | $CH_3$ | 168.84 | C | 21.03 | $CH_3$ | 21.02 | $CH_3$ |
| | 21.09 | $CH_3$ | 170.95 | C | 21.06 | $CH_3$ | 21.08 | $CH_3$ |
| | | | 20.92 | $CH_3$ | | | | |
| | | | 20.96 | $CH_3$ | | | | |
| | | | 21.11 | $CH_3$ | | | | |
| | | | 21.25 | $CH_3$ | | | | |
| MeO | | | | | 52.28 | $CH_3$ | | |

[a] By INEPT experiment.

Table 3. $^1H$ NMR spectral data of 6–9

| Proton | Chemical shifts (δ), multiplicity, in Hz | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 6 | | 7 | | 8 | | 9 | |
| 1 | 4.71 | t, 2.5 | 4.68 | t, 2.5 | 4.71 | t, 2.5 | 4.68 | t, 3.0 |
| 2 | 2.18 | td, 2.5, 16.5 | 2.18 | td, 2.5, 16.0 | 2.18 | td, 2.5, 16.0 | 2.18 | td, 3.0, 16.0 |
| | 2.30 | td, 2.5, 16.5 | 2.30 | td, 2.5, 16.0 | 2.30 | td, 2.5, 16.0 | 2.30 | td, 3.0, 16.0 |
| 3 | 4.90 | t, 2.5 | 4.90 | t, 2.5 | 4.90 | t, 2.5 | 4.90 | t, 3.0 |
| 5 | 2.54 | t, 8.0 | 2.54 | t, 7.5 | 2.54 | t, 8.5 | 2.54 | t, 8.0 |
| 6 | 1.85 | dd, 3.0, 8.0 | 1.85 | dd, 2.5, 7.5 | 1.85 | dd, 2.5, 8.5 | 1.85 | dd, 3.0, 8.0 |
| | 1.85 | dd, 3.0, 8.0 | 1.85 | dd, 2.5, 7.5 | 1.85 | dd, 2.5, 8.5 | 1.85 | dd, 3.0, 8.0 |
| 7 | 5.16 | t, 3.0 | 5.19 | t, 2.5 | 5.18 | t, 3.0 | 5.19 | t, 3.0 |
| 9 | 2.64 | dd, 5.0, 11.0 | 2.65 | dd, 6.5, 11.5 | 2.65 | dd, 5.5, 12.0 | 2.66 | dd, 6.0, 11.5 |
| 11 | 1.28 | m | 1.24 | m | 1.30 | m | 1.32 | m |
| | 1.49 | m | 1.47 | m | 1.47 | m | 1.52 | m |
| 12 | 1.68 | m | 1.64 | m | 1.65 | m | 1.52 | m |
| | 1.74 | m | 1.72 | m | 1.70 | m | 1.70 | m |
| 15 | 5.35 | br d, 3.0 | 5.32 | br d, 2.0 | 5.32 | br d, 2.5 | 5.34 | br d, 2.0 |
| 16 | 1.98 | m | 1.95 | m | 1.90 | ddd, 2.5, 10.0, 15.0 | 2.00 | m |
| | 2.26 | ddd, 3.0, 6.5, 15.0 | 2.16 | m | 2.20 | m | 2.25 | m |
| 17 | 1.64 | m | 1.70 | m | 1.74 | m | 1.71 | m |
| 18 | 1.15 | s | 1.14 | s | 1.14 | s | 1.15 | s |
| 19 | 1.00 | s | 1.00 | s | 1.00 | s | 1.00 | s |
| 20 | 1.82 | m | 1.90 | m | 1.75 | m | 2.00 | m |
| 21 | 3.41 | dd, 9.0, 10.5 | 3.68 | dd, 5.0, 11.5 | 3.47 | dd, 5.0, 11.5 | 3.96 | dd, 5.5, 11.0 |
| | 3.92 | dd, 2.5, 9.0 | 4.24 | dd, 3.5, 11.5 | 3.84 | dd, 2.5, 11.5 | 4.37 | dd, 3.0, 11.0 |
| 22 | 1.68 | m | 1.57 | m | 1.57 | m | 1.67 | m |
| | 1.74 | m | 1.71 | m | 1.81 | m | 1.67 | m |
| 23 | 4.01 | br d, 10.0 | 5.44 | dt, 1.5, 7.0 | 4.03 | t, 8.0 | 4.01 | dt, 5.5, 7.5 |
| 24 | 3.09 | d, 10.0 | 4.88 | d, 1.5 | 3.54 | t, 8.0 | 3.49 | d, 7.5 |
| 25-OH | 2.18 | s | | | | | | |
| 26 | 1.30 | s | 1.24 | s | 1.19 | s | 1.18 | s |
| 27 | 1.32 | s | 1.26 | s | 1.29 | s | 1.26 | s |
| 28 | 0.91 | s | 0.90 | s | 0.91 | s | 0.90 | s |
| 29 | 1.00 | s | 1.00 | s | 1.00 | s | 1.00 | s |
| 30 | 1.08 | s | 1.06 | s | 1.07 | s | 1.07 | s |
| 1-AcO | 2.07 | s | 2.06 | s | 2.07 | s | 2.07 | s |
| 7-AcO | 1.66 | s | 1.64 | s | 1.66 | s | 1.65 | s |
| 21-AcO | | | 2.20 | s | | | 2.03 | s |
| 23-AcO | | | 2.88 | s | | | | |
| 24-AcO | | | 2.78 | s | | | | |
| 3' | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 |
| 4' | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 |
| 5' | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 | 7.57 | tt, 1.5, 7.5 |
| 6' | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 | 7.43 | t, 7.5 |
| 7' | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 | 8.09 | dd, 1.5, 7.5 |
| Me | | | | | 1.42 | s | 1.36 | s |
| Me | | | | | 1.42 | s | 1.38 | s |

*Diacetates (2 and 3).* Treatment of **1** (20 mg) with 1 mL of acetic anhydride and 0.5 mL of pyridine overnight to give **2** (8 mg) and **3** (2 mg) after HPLC separation. Compound **2**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 817 ($[M+Na]^+$, 14), 799 ($[M+Na^+-H_2O]^+$, 8), 757 ($[M+Na^+-HOAc]^+$, 11), 553 (5); $^1H$ and $^{13}C$ NMR (see Tables 1 and 2). Compound **3**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 817 ($[M+Na]^+$, 90), 799 ($[M+Na^+-H_2O]^+$, 10), 757 ($[M+ Na^+-HOAc]^+$, 20), 702 (18), 595 (8), 553 (15); $^1H$ NMR (see Table 1).

*Monomethoxylates (4 and 5).* To the solution of **1** (25 mg) in 10 mL MeOH, 2 mL of 2% dry HCl in MeOH was added. The solution was stored at rt for 6 h. HPLC separation gave **4** (10 mg) and **5** (3 mg). Compound **4**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 747 ($[M+Na]^+$, 10), 687 ($[M+ Na^+-HOAc]^+$, 5), 251 (100); $^1H$ and $^{13}C$ NMR (see Tables 1 and 2). Compound **5**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 747 ($[M+Na]^+$, 8), 687 ($[M+ Na^+-HOAc]^+$, 2), 251 (40); $^1H$ NMR (see Table 1).

*Reduction of 1 to 6 with sodium borohydride.* Compound **1** (15 mg) was reduced with $NaBH_4$ (20 mg) in MeOH (5 mL) at rt for 10 h, and after purification by HPLC, gave **6** (8 mg). Compound **6**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 735 ($[M+Na]^+$, 80), 675 ($[M+ Na^+-HOAc]^+$, 15), 471 (20), 435 (18), 381 (100); $^1H$ and $^{13}C$ NMR (see Tables 2 and 3).

*Formation of acetonide (8) and its monoacetate (9).* A mixture of **1** (5 mg), *p*-toluenesulfonic acid (3 mg) and dry $Me_2CO$ (5 mL) was stored at rt for 6 h. The products were purified over a pipette silica gel column, eluted by hexane–EtOAc (7:3) to give **8** (2 mg). Compound **8**, white powder, FABMS (DDT/DTE/NaI) m/z (%): 775 ($[M+Na]^+$, 100), 715 ($[M+ Na^+-HOAc]^+$, 5), 660 (10), 435 (6), 360 (9); $^1H$ NMR (see Table 3). Treatment of **8** (2 mg) with acetic anhydride and pyridine gave the monoacetate **9** (1.8 mg). Compound **9**, white oil, $^1H$ NMR (see Table 2 ).

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## References

1. Meyer, B. M.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31.

2. McLaughlin, J. L. *Methods in Plant Biochemistry,* Vol. 6, p. 1, Hostettmann, K., Ed; Academic Press; London, 1991.

3. Zeng, L.; Gu, Z.-M.; Fang, X.-P.; Fanwick, P. E.; Chang, C.-J.; Smith, D. J.; McLaughlin, J. L. *Tetrahedron* **1995**, *51*, 2477.

4. Zeng, L.; Gu, Z.-M.; Fang, X.-P.; Fanwick, P. E.; Chang, C.-J.; Smith, D. J.; McLaughlin, J. L. *Heterocycles* in press.

5. Zeng, L.; Gu, Z.-M.; Fang, X.-P.; Fanwick, P. E.; Chang, C.-J.; Smith, D. J.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett* **1995**, *5*, 181.

6. Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu. Y.-M.; Smith, D. J.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1991**, *32*, 11.

7. Breen, G. J. W.; Ritchie, E.; Sidwell, W. T. L.; Taylor, W. C. *Aust. J. Chem.* **1966**, *19*, 455.

8. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Kozlowski, J. K.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 473.

9. Lobo, A. M.; Abreu, P. A.; Prabhakar, S.; Godinho, L. S.; Jones, R.; Rzepa, H. S.; Williams, D. J. *Phytochemistry* **1988**, *27*, 3569.

10. Taylor, D. A. H. *Fortschritte Chem. Org. Naturst.* **1984**, *45*, 1.

11. Giard, S. J.; Aronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl Cancer Inst.* **1973**, *51*, 1417.

12. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl Cancer Inst.* **1973**, *51*, 1409.

13. Fogh, J.; Trempe, G. *Human Tumor Cells in Vitro,* p. 115, Fogh, J. Ed.; Plenum Press, New York, 1975.

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