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A PAIR OF NEW APOTIRUCALLANE TRITERPENES, MELIAVOLKENSINS A AND B, FROM *MELIA VOLKENSII* (MELIACEAE)

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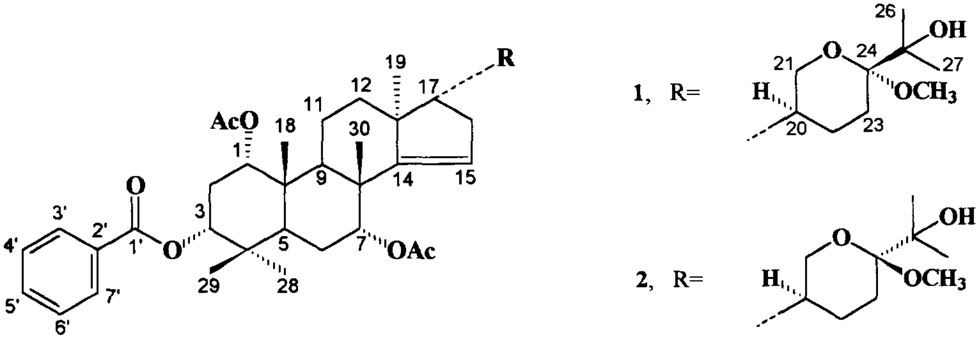
Abstract: Two new bioactive apotirucallane triterpenes, meliavolkensins A (**1**) and B (**2**), were isolated from the root bark of *Melia volkensii* (Meliaceae). These two compounds have a new type of substituted tetrahydropyran ring side chain, and their structures have been elucidated by spectroscopic analyses. **1** and **2** also show cytotoxic selectivities to a human colon tumor cell line (H-29).

From the root bark of *Melia volkensii* Gurke (Meliaceae), obtained from Kenya, seven bioactive compounds were previously isolated in our laboratory.[1,2] In our further bioactivity-directed search for antitumor compounds, two new apotirucallane triterpenes, meliavolkensins A (**1**) and B (**2**), were isolated from the same plant material, and their structures have been determined by MS, ^{1}H and ^{13}C NMR, COSY, NOESY and difference NOE experiments. **1** and **2** are the first apotirucallane triterpenes with a methoxyl substituted tetrahydropyran ring side chain attached to the C-17α positions. In addition, although **1** and **2** have the same components in their side chain, their ring conformations are different. The core moiety of **1** takes an axial orientation to its six membered tetrahydropyran ring, whereas the core moiety of **2** takes an equatorial orientation to its six membered tetrahydropyran ring (see Figure 1).



The molecular weight of meliavolkensin A (**1**, $[\alpha]^{22}_D$ -42.3, c 0.41 in CHCl$_3$) was determined by a dominant peak at m/z 707 (M-H$^-$) in the negative ion FABMS. The HRFABMS gave m/z 707.4155 for the M-H$^-$ (calcd

707.4159) corresponding to the molecular formula, $C_{42}H_{60}O_9$. The ^1H and ^{13}C NMR spectra of **1** showed signals due to seven tertiary methyls, seven methylenes, one oxymethylene, four methines, three oxymethines, and one double bond, together with two acetates and one benzoate (Tables 1 and 2). The ^{13}C NMR spectrum of **1** also showed four quaternary carbon signals in the molecule. These data suggested that **1** is a triterpene with an apotirucallane skeleton.[3, 4] Since most of the carbon signals in the core portion of **1** are quite similar to those of melianin A, meliavolin, and melianinone, which were previously isolated from the same plant, **1** was concluded to have the same substitution pattern as those compounds, i.e., **1** is a 1α, 7α-diacetoxy-3α-benzoxy-apotirucall-14-ene with a 17α side chain.

The side chain of **1** consists of a six membered tetrahydropyran ring substituted with a 2-methoxyl group and a 2-hydroxyisopropyl group. The 2-methoxyl and 2-hydroxyisopropyl groups are attached to the same acetal carbon (δ 99.57). Cross peaks between the hydroxyl singlet (δ 3.30) and the methoxyl singlet (δ 3.26) and the two isopropyl methyl singlets (δ 1.10, δ 1.22) and the methoxyl singlet (δ 3.26) were observed in

Table 1 ^1H NMR data of **1** and **2** (500 MHz, δ).

	1[a]		**1**[b]		**2**[a]		**2**[b]	
1	4.71	t, 2.5	4.61	t, 2.5	4.71	t, 2.5	4.61	t, 2.5
2	2.19	td, 2.5, 16.0	2.11	td, 2.5, 16.5	2.19	td, 2.5, 15.5	2.12	td, 2.5, 16.5
	2.30	td, 2.5, 16.0	2.26	td, 2.5, 16.5	2.30	td, 2.5, 15.5	2.30	td, 2.5, 16.5
3	4.90	t, 2.5	4.79	t, 2.5	4.90	t, 2.5	4.79	t, 2.5
5	2.54	dd, 7.0, 8.5	2.50	dd, 3.5 12.0	2.54	dd, 6.5, 9.5	2.52	dd, 2.5 12.5
6	1.85	dd, 2.5, 7.0	1.78	m	1.85	dd, 2.5, 6.5	1.82	m
	1.85	dd, 2.5, 8.5	1.81	m	1.85	dd, 2.5, 9.5	1.84	m
7	5.19	t, 2.5	5.10	t, 2.5	5.19	t, 2.5	5.11	t, 2.5
9	2.65	dd, 6.0, 11.5	2.63	m	2.65	dd, 6.5, 11.5	2.65	dd, 6.0, 11.0
11	1.28	m	1.21	m	1.28	m	1.23	m
	1.50	m	1.45	m	1.50	m	1.48	m
12	1.56	m	1.50	m	1.48	m	1.46	m
	1.76	m	1.69	m	1.68	m	1.67	m
15	5.34	br d, 2.0	5.26	br d, 2.0	5.34	br d, 2.0	5.28	br d, 2.0
16	1.93	ddd, 2.5, 11.5, 15.0	1.89	ddd, 2.0, 10.0, 15.0	1.95	ddd, 2.0, 11.5, 15.0	1.92	m
	2.25	ddd, 3.5, 7.5, 15.0	2.14	m	2.18	ddd, 4.0, 8.0, 15.0	2.14	m
17	1.76	m	1.64	m	1.56	m	1.54	m
18	1.01	s	0.97	s	1.00	s	1.00	s
19	1.06	s	1.05	s	1.11	s	1.10	s
20	2.34	m	2.25	m	2.36	m	2.37	m
21	3.90	dd, 6.0, 11.0	4.00	dd, 6.0, 11.0	3.67	t, 11.0	3.62	t, 11.0
	4.15	dd, 4.0, 11.0	4.07	dd, 4.0, 11.0	3.95	br d, 11.0	4.02	br d, 11.0
22	1.76	m	2.08	m	1.56	m	2.02	m
	2.33	m	2.10	m	2.20	m	2.10	m
23	2.35	m	2.34	dd, 9.5, 14.5	2.36	m	2.00	m
	2.72	dd, 4.0, 15.0	2.60	m	2.44	m	2.76	m
25-OH		s	3.30	s	2.98	s	3.33	s
26	1.21	s	1.10	s	1.21	s	1.13	s
27	1.32	s	1.22	s	1.31	s	1.21	s
28	0.91	s	0.85	s	0.91	s	0.87	s
29	1.00	s	0.95	s	1.00	s	0.98	s
30	1.15	s	1.10	s	1.15	s	1.11	s
1-AcO	2.06	s	2.00	s	2.06	s	2.07	s
7-AcO	1.65	s	1.57	s	1.66	s	1.58	s
3'	8.09	dd, 1.5, 7.5	8.02	d, 7.5	8.09	dd, 1.5, 7.5	8.04	d, 7.5
4'	7.43	tt, 1.5, 7.5	7.39	t, 7.5	7.43	tt, 1.5, 7.5	7.42	t, 7.5
5'	7.56	t, 7.5	7.51	t, 7.5	7.56	t, 7.5	7.55	t, 7.5
6'	7.43	tt, 1.5, 7.5	7.39	t, 7.5	7.43	tt, 1.5, 7.5	7.42	t, 7.5
7'	8.09	dd, 1.5, 7.5	8.02	d, 7.5	8.09	dd, 1.5, 7.5	8.04	d, 7.5
24-MeO	3.43	s	3.26	s	3.47	s	3.36	s

[a] In $CDCl_3$. [b]In acetone-d_6-$CDCl_3$ (1:1).

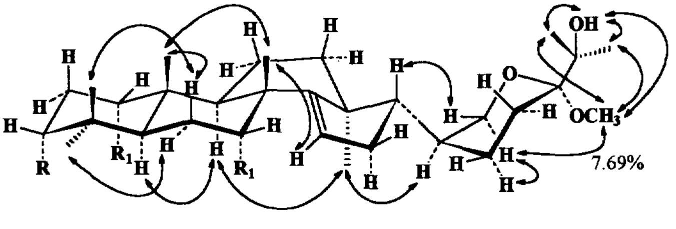
NOESY experiments (see Table 1, **1**[b]). Therefore, the side chain of **1** is a 2-methoxyl, 2-hydroxyisopropyl, 5-alkylated tetrahydropyran. This is a new substitution pattern for the apotirucallane or tirucallane types of triterpenes. Also, we found in the literature only one previously known example of this type, named 21-methyltoosindanpentol,[3] and it has a five member tetrahydrofuran ring side chain with a methoxyl attached to its 21 acetal carbon. The six membered ring moiety in **1** has a chair form, and the core moiety takes an axial orientation since both H-21 protons appeared as doublets of a doublet at δ 4.00 (*J*=6.0 and 11.0 Hz) and 4.07 (*J*=4.0 and 11.0 Hz). When one of the oxymethylenes at δ 4.00 was saturated, the methoxyl signal at δ 3.26 showed 7.69% NOE enhancement, which indicated that the methoxyl takes the α-axial orientation and the 2-hydroxyisopropyl group takes the β-equatorial orientation. In addition, cross peaks between the 19-methyl (δ 1.05) and the 20-methine (δ 2.25) were observed in the NOESY experiment and provided further evidence for the existence of the 17α side chain. The conformational analyses for **1**, which are illustrated in Figure 1, resulted from the NOESY experiments. The absolute stereochemistries of the side chain of **1** were deduced from biogenetic considerations based on the proven analyses of similar compounds.[4] Apotirucallane type triterpenes from Meliaceous plants typically have 20*S* configurations;[4] thus, C-24 should have the *R*-configuration. Therefore, the structure of **1** is proposed to be 1α, 7α-diacetoxy-3α-benzoxy-17a-20*S*-21,24-epoxy-24*R*-methoxy-apotirucall-14-ene-25-ol.

Table 2 ^{13}C NMR data of **1** and **2** (125 MHz, $CDCl_3$, δ).

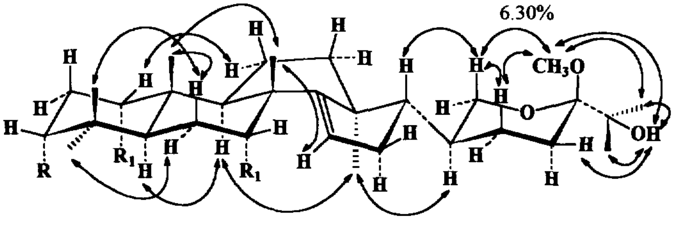
	1	**2**		**1**	**2**		**1**	**2**
1	72.64	72.63	16	34.68	34.78	AcO	169.67	169.68
2	25.51	25.51	17	55.10	56.71		170.03	170.01
3	77.25	77.11	18	20.11	20.03		20.02	20.99
4	36.60	36.60	19	16.21	16.21		21.06	21.05
5	37.44	37.44	20	38.08	40.07	1'	165.22	165.23
6	22.83	22.83	21	65.03	65.16	2'	130.73	130.73
7	75.54	75.49	22	34.29	33.70	3'	129.51	129.51
8	42.16	42.15	23	43.13	43.00	4'	128.29	128.31
9	35.44	35.41	24	100.83	99.57	5'	133.02	133.05
10	40.29	40.29	25	75.54	75.56	6'	128.29	128.31
11	16.06	16.06	26	24.99	24.80	7'	129.51	129.51
12	35.44	35.42	27	25.40	25.22	MeO	52.44	52.60
13	46.51	46.41	28	28.07	28.07			
14	159.07	159.07	29	21.48	21.48			
15	119.31	119.18	30	26.80	26.79			

Meliavolkensin B (**2**, $[\alpha]^{22}_D$ -55.7, c 0.56 in $CHCl_3$) has the same molecular formula as that of **1**, $C_{42}H_{60}O_9$, which was indicated by a peak at m/z 707.4162 for the $M-H^-$ (calcd 707.4159) in the negative ion HRFABMS. Very similar ^1H and ^{13}C NMR data showed that the difference between **2** and **1** was only in the side chain moiety. The six membered tetrahydropyran ring has a chair conformation in **2**, but the core moiety takes an equatorial orientation, since one axial proton of the oxymethylene appeared as a triplet (δ 3.67 in $CHCl_3$ and δ 3.62 in acetone-d_6) with *J*=11.0 Hz, which suggested that the H-20 takes the axial orientation. In addition, cross peaks between the axial proton (δ 3.62) at the C-20 position and the methoxyl singlet (δ 3.36) and the axial proton (δ 2.02) at the C-22 position and the methoxyl singlet were observed in the NOESY experiments. When the triplet at δ 3.62 was saturated, a 6.30% NOE enhancement for the methoxyl singlet was observed, which indicated that the methoxyl group takes the β-position. The conformational analyses for **2**, using NOESY experiments, are illustrated in Figure 1. The absolute stereochemistries of the side chain of **2** were deduced in the same way as those of **1**. Therefore, the structure of **2** is proposed to be 1α, 7α-diacetoxy-3 α-benzoxy-17α-20*S*-21,24-epoxy-24*S*-methoxy-apotirucall-14-ene-25-ol.

Melvolkensins A (**1**) and B (**2**) were moderately active in the brine shrimp lethality test (**1**, BST LC_{50} 106.6 μg/ml; **2**, BST LC_{50} 89.3 μg/ml)[5,6] and comparatively cytotoxic in 7 day MTT assays against human solid tumor cell lines [**1**, ED_{50} 13.26, 3.06, 0.49 μg/ml, **2**, ED_{50} 10.63, 2.02, 0.25 μg/ml in A-549 (human lung carcinoma),[7] MCF-9 (human breast carcinoma),[8] and HT-29 (human colon adenocarcinoma)[9] cells, respectively]. Thus, both **1** and **2** showed selective cytotoxicities toward the human colon tumor cell line (H-29). Adriamycin, as a positive control, gave ED_{50} values of 8.44×10^{-3}, 6.73×10^{-2}, and 2.61×10^{-2} μg/ml, respectively, when compounds **1** and **2** were tested in the same run.



1 R=Benzoxyl R$_1$=Acetoxyl



2 R=Benzoxyl R$_1$=Acetoxyl

Figure 1. Conformations of **1** and **2**. The arrows show the relationships suggested from NOESY experiments, and the numbers show the enhancements from difference NOE experiments.

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