ADDITIONAL BIOACTIVE TRITERPENOIDS FROM *MELIA VOLKENSII* (MELIACEAE)

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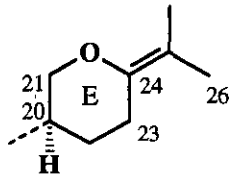
Abstract-Meliavolen (**1**) and melianinone (**2**), new triterpenes with an apotirucallane skeleton, together with two known compounds, 3-episapelin A (**5**) and nimbolin B (**6**), have been isolated from the root bark of *Melia volkensii* (Meliaceae) by using brine shrimp lethality for bioactivity-directed fractionation. The structures have been elucidated by spectral data. The relative and absolute stereochemistries of **2** were determined by correlating **2** with melianin A (**3**). The stereochemistry of **3** was determined by X-ray crystallographic analysis and Mosher methodology. The stereochemistry of **5** was also determined by Mosher methodology. The four isolated compounds, meliavolen (**1**), melianinone (**2**), 3-episapelin (**5**), and nimbolin B (**6**), were all moderately active in the brine shrimp lethality test and were significantly cytotoxic among human solid tumor cell lines with noted selectivities toward the colon cell line (HT-29).

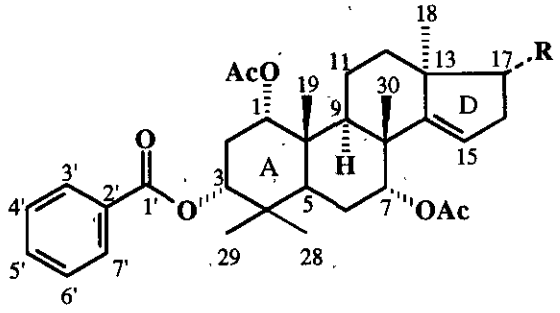
In a previous paper,[1] we reported two new bioactive triterpenes, meliavolin and meliavolkin, isolated from the root bark of *Melia volkensii* Gurke (Meliaceae) obtained from Kenya. Further fractionations of the root bark, directed by a test for brine shrimp lethality (BST),[2, 3] have yielded two new apotirucallane triterpenes, meliavolen (**1**) and melianinone (**2**), together with two known compounds, 3-episapelin A (**5**) and nimbolin B (**6**). The structures of meliavolen (**1**) and melianinone (**2**) were determined by spectral data, and the stereochemistry of **2** was determined by correlating **2** with melianin A (**3**), through oxidation. By using advanced Mosher's methodology, the absolute stereochemical structures of melianin A (**3**) and 3-episapelin (**5**) were determined. The relative stereochemistry of melianin A (**3**) was also determined by X-ray crystallographic analysis.

Meliavolen (**1**) was isolated as a white powder. The molecular weight of **1** was indicated by a peak at m/z 683 ($M+Na^+$) in the *m*-nitrobenzyl alcohol (*m*-NBA) FABms. High resolution FABms gave m/z 683.3918 (calcd 683.3923) for the $M+Na^+$, corresponding to the formula, $C_{41}H_{56}O_7$. The ^1H nmr and ^{13}C nmr spectra of **1** showed signals due to five tertiary methyls, two methyls attached to a double bond,

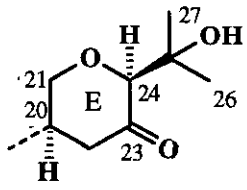
seven methylenes, one oxymethylene, four methines, three oxymethines, and two double bonds, together with two acetates and one benzoate (Tables 1 and 2). The ^{13}C nmr spectrum of **1** also showed four quaternary carbon signals in the molecule. These data suggested that **1** is a triterpene with an apotirucallane skeleton.[4, 5] The three oxymethines in **1** appeared at δ 4.70, δ 4.90 and δ 5.19 and were placed at the C-1, -3, and -7 positions based on the comparisons of the nmr spectra of **1** to those of melianin A (**3**).[1, 6] These three protons were concluded to take the equatorial orientation because the coupling constants are 2.5 Hz, respectively, and arise from vicinal methylene protons. Most carbon signals of the core of **1** are similar to those of **3** indicating the similarity of the two compounds in the core portions. These data suggested that **1** is a 1α, 7α-diacetate, 3α-benzoate-apotirucall-14-ene with a 17α-side chain.

1, R=

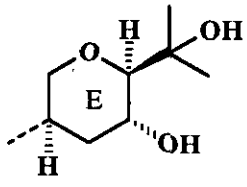


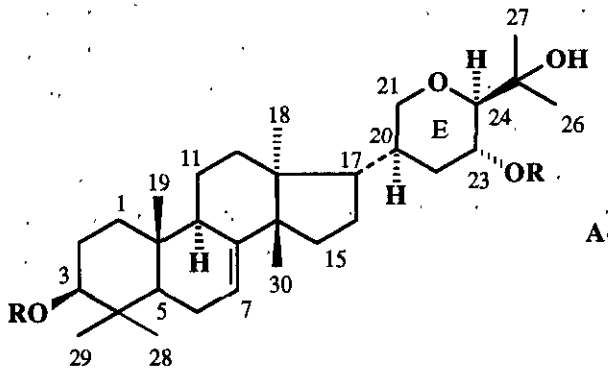


2, R=



3, R=

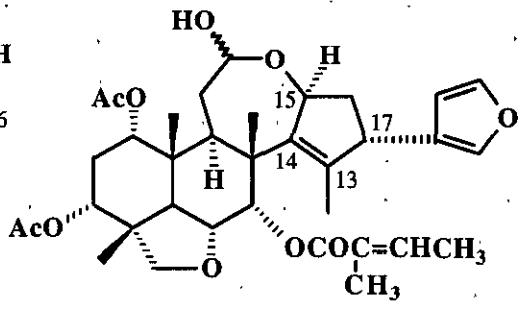




5, R=H

5a, R=*S*-MTPA

5b, R=*R*-MTPA



6

Table 1. ^{13}C-Nmr Data of **1-4**.

No.	1	2*	3*	4	No.	1	2	3	4
1	72.62 CH	72.37 CH	72.64 CH	72.60 CH	22	34.04 CH_2	43.30 CH_2	36.36 CH_2	37.47 CH_2
2	25.49 CH_2	25.43 CH_2	25.59 CH_2	25.44 CH_2	23	43.55 CH_2	212.96 C	64.36 CH	176.54 C
3	77.04 CH	77.20 CH	77.11 CH	76.97 CH	24	159.11 C	86.29 CH	86.52 CH	
4	36.59 C	36.59 C	36.64 C	36.57 C	25	144.93 C	72.64 C	74.28 C	
5	37.43 CH	37.44 CH	37.49 CH	37.42 CH	26	19.91 CH_3	24.78 CH_3	24.07 CH_3	
6	22.81 CH_2	22.79 CH_2	22.93 CH_2	22.73 CH_2	27	20.99 CH_3	26.41 CH_3	28.70 CH_3	
7	75.45 CH	75.42 CH	75.75 CH	75.17 CH	28	28.06 CH_3	28.07 CH_3	28.12 CH_3	28.07 CH_3
8	42.13 C	42.13 C	42.22 C	42.08 C	29	21.48 CH_3	21.49 CH_3	21.50 CH_3	21.52 CH_3
9	35.75 CH	35.29 CH	35.64 CH	34.73 CH	30	26.80 CH_3	26.86 CH_3	26.82 CH_3	26.93 CH_3
10	40.28 C	40.29 C	40.35 C	40.29 C	AcO	169.70 C	169.63 C	169.66 C	169.63 C
11	16.04 CH_2	15.99 CH_2	16.29 CH_2	15.68 CH_2		20.19 CH_3	21.01 CH_3	20.06 CH_3	21.02 CH_3
12	35.38 CH_2	34.50 CH_2	35.28 CH_2	35.09 CH_2	AcO	170.05 C	170.05 C	169.95 C	170.02 C
13	46.44 C	46.60 C	46.36 C	46.51 C		21.06 CH_3	21.05 CH_3	21.14 CH_3	21.02 CH_3
14	159.04 C	159.03 C	159.18 C	158.92 C	1'	165.23 C	165.23 C	165.14 C	165.23 C
15	119.28 CH	119.11 CH	119.71 CH	118.96 CH	2'	130.72 C	130.73 C	130.69 C	130.67 C
16	34.70 CH_2	34.37 CH_2	34.92 CH_2	33.79 CH_2	3'	129.50 CH	129.51 CH	129.46 CH	129.46 CH
17	56.61 CH	56.48 CH	52.30 CH	57.94 CH	4'	128.31 CH	128.30 CH	128.24 CH	128.29 CH
18	20.56 CH_3	20.08 CH_3	20.56 CH_3	16.11 CH_3	5'	133.03 CH	133.03 CH	132.85 CH	133.02 CH
19	16.18 CH_3	16.17 CH_3	16.11 CH_3	19.73 CH_3	6'	128.31 CH	128.30 CH	128.24 CH	128.29 CH
20	35.75 CH	36.44 CH	35.88 CH	33.79 CH	7'	129.50 CH	129.51 CH	129.46 CH	129.46 CH
21	70.11 CH_2	68.72 CH_2	70.00 CH_2	72.45 CH_2					

*Assignments were made by the measurements of HETCOR and COLOC experiments.

Another double bond located in the side chain of **1** was indicated by two carbon signals at δ 129.41 and 144.92; the position of the latter carbon signal showed that it was connected to an oxygen atom. Since no free hydroxyl peak was observed in the ir spectrum of **1**, this oxygen was predicted to exist as an oxygenated bridge. Two singlet methyl signals appeared at δ 2.08 and 1.80; the former methyl obviously was located in a deshielding region, that is, in the same orientation as an oxygen atom. The oxymethylene in the side chain was then suggested to be the other part of the oxygenated bridge. The absolute stereochemistries of the side chain of **1** were deduced from biogenetic considerations based on the proven analyses of similar compounds.[5] Apotirucallane type triterpenes from Meliaceous plants typically have 20*S* configurations.[5] Therefore, the structure of **1** was established as 1α, 7α-diacetoxy-3α-benzoxy-17α,20*S*,21, 24-epoxy-apotirucalla-14 (15), 24 (25)-diene.

Melianinone (**2**) was also obtained as a white powder. The molecular weight of **2** was indicated by a peak at m/z 715 ($M+Na^+$) in the *m*-NBA FABms. High resolution FABms gave m/z 715.3826 (calcd 715.3822) for the $M+Na^+$, corresponding to the formula, $C_{41}H_{56}O_9$. The ^{13}C nmr spectrum of **2** showed a similar pattern to that of **3**, i.e., the signals due to seven tertiary methyls, six methylenes, one oxymethylene, four methines, four oxymethines, one carbonyl carbon, and one double bond, together with two acetates and one benzoate. These data suggested that **2** has the same apotirucallane skeleton as that of **3**. The comparisons of ^{1}H nmr and ^{13}C nmr spectra of **2** and **3** showed that most of the signals in the core region are quite similar, indicating the same substitution pattern of the two compounds, and the difference only being in the side chain. One proton singlet at δ 3.58, correlated to one of the oxymethylene protons at δ 3.88 in the NOESY spectrum, indicated that these two protons take axial orientation

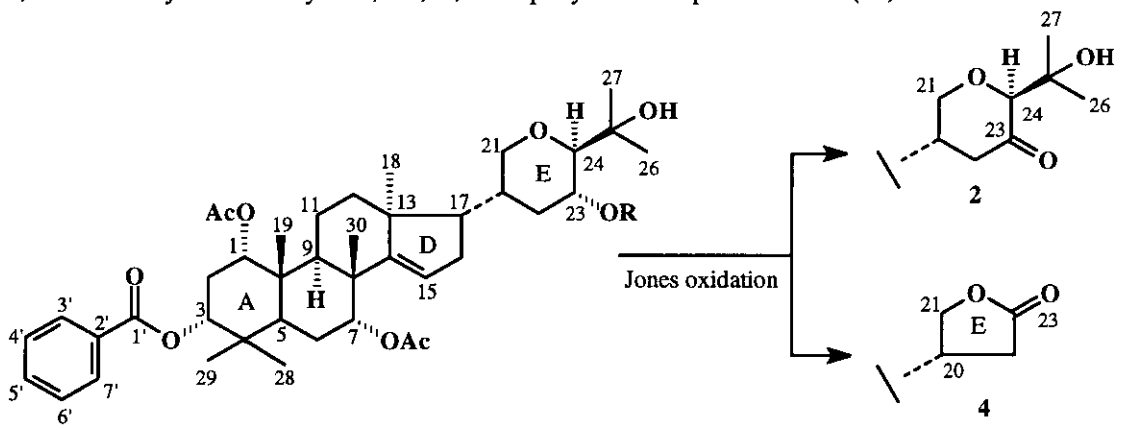
Table 2. ^{1}H Nmr Data of **1-4**.

	1*		**2****		**3****		**4**	
No.	δ_H	Coupling in Hz	δ_H	Coupling in Hz	δ_H	Coupling in Hz	δ_H	Coupling in Hz
1	4.70	t, 2.5	4.70	t, 3.0	4.75	t, 3.0	4.69	t, 2.5
2	2.20	td, 2.5, 16.0	2.18	td, 3.0, 16.0	2.18	td, 3.0, 16.0	2.18	td, 2.5, 16.0
	2.28	td, 2.5, 16.0	2.30	td, 3.0, 16.0	2.30	td, 3.0, 16.0	2.30	td, 2.5, 16.0
3	4.90	t, 2.5	4.90	t, 3.0	4.89	t, 3.0	4.90	t, 2.5
5	2.54	dd, 6.5, 8.0	2.54	dd, 7.5, 8.5	2.56	dd, 6.5, 9.5	2.54	dd, 6.0, 8.0
6	1.85	dd, 3.0, 6.5	1.85	dd, 2.5, 7.5	1.85	dd, 3.0, 6.5	1.85	dd, 3.0, 6.0
	1.85	dd, 3.0, 8.0	1.85	dd, 2.5, 8.5	1.85	dd, 3.0, 9.5	1.85	dd, 3.0, 8.0
7	5.19	t, 2.5	5.19	t, 2.5	5.19	t, 3.0	5.21	t, 2.5
9	2.64	dd, 6.5, 11.5	2.65	dd, 6.0, 12.0	2.65	dd, 7.0, 11.5	2.66	dd, 6.5, 12.0
11	1.28	m	1.26	m	1.27	m	1.28	m
	1.50	m	1.52	m	1.46	m	1.48	m
12	1.60	m	1.58	m	1.60	m	1.50	m
	1.70	m	1.75	m	1.74	m	1.60	m
15	5.34	dd, 1.5, 3.0	5.34	br d, 1.5	5.37	br d, 3.0	5.34	br d, 2.0
16	1.96	ddd, 1.5, 11.5, 15.0	1.94	ddd, 1.5, 11.0,	1.96	ddd, 3.0, 8.0, 15.0	2.05	ddd, 1.5, 11.5, 16.5
	2.22	m	2.23	15.0	2.28	m	2.16	ddd, 2.0, 7.5, 16.5
17	1.98	m	1.81	m	2.00	m	1.70	td, 7.5, 10.0
18	0.99	s	1.03	m	1.01	s	1.02	s
19	1.01	s	1.02	s	1.00	s	1.04	s
20	1.56	m	2.25	s	1.86	m	2.68	m
21	3.59	dd, 10.0, 11.0	3.88	m	3.41	dd, 5.5, 11.5	3.91	t, 9.0
	4.23	ddd, 2.0, 4.0, 11.0	3.98	dd, 5.0, 11.5	3.93	dd, 3.5, 11.5	4.44	t, 9.0
22	1.98	m	2.45	dd, 5.0, 11.5	1.52	m	2.21	dd, 8.0, 17.0
	2.32	m	2.46	d, 7.5	2.04	m	2.52	dd, 12.0, 17.0
23	2.18	m		d, 6.0	3.86	dd, 4.5, 8.5		
	2.56	ddd, 1.5, 5.0, 16.5						
24			3.58		2.88	d, 11.0		
26	2.08	s	1.23	s	1.27	s		
27	1.80	s	1.24	s	1.30	s		
28	0.91	s	0.91	s	0.91	s	0.91	s
29	1.00	s	1.00	s	1.00	s	1.00	s
30	1.16	s	1.15	s	1.17	s	1.15	s
AcO	2.07	s	1.65	s	2.06	s	2.06	s
	1.66	s	2.06	s	1.65	s	1.65	
				s				
3'	8.09	dd, 1.5, 8.0	8.09		8.09	dd, 1.5, 8.0	8.09	dd, 1.5, 8.0
4'	7.43	t, 8.0	7.43	dd, 1.5, 8.0	7.43	t, 8.0	7.43	t, 8.0
5'	7.56	tt, 1.5, 8.5	7.57	t, 8.0	7.56	tt, 1.5, 8.5	7.56	tt, 1.5, 8.5
6'	7.43	t, 8.0	7.43	tt, 1.5, 8.5	7.43	t, 8.0	7.43	t, 8.0
7'	8.09	dd, 1.5, 8.0	8.09	t, 8.0	8.09	dd, 1.5, 8.0	8.09	dd, 1.5, 8.0
				dd, 1.5, 8.0				

*The assignments were made by COSY and Relay COSY measurements. **Also by HETCOR, COLOC measurements.

along both sides of the oxygen bridge. The singlet signal at δ 3.58 also showed cross peaks to both methyl signals of the 2-hydroxyisopropyl group at δ 1.23 and 1.24 and indicated that the 2-hydroxyisopropyl group and the proton at δ 3.58 are connected to the same carbon, which is C-24. Since the proton at C-24 appeared as a singlet, the carbonyl group was deduced to be at C-23. This conclusion is supported by the COSY spectrum of **2**; the cross peaks were evident from the oxymethylene protons at δ 3.88 and 3.98 to a methine proton at δ 2.52 which further correlated to the methylene protons at δ 2.45 and 2.46. The structure and absolute stereochemistry was confirmed by conversion of **3** to **2** through oxidation. One of the oxidation (Jones reagent) products of **3** showed exactly the same ^{1}H nmr and ^{13}C nmr spectra as **2**; **4** was also produced and isolated (Figure 1). Both **2** and **3** also showed identical cd spectra, so the absolute configurations of **2** at C-20 and C-24 are of the *S*- and *S*-forms, respectively,

which are the same as those of **3** whose relative and absolute stereochemical structure was determined (see below) by X-ray crystallographic analysis and Mosher's methodology . Thus, the structure of **2** is 1α, 7α-diacetoxy-3α-benzoxy-17α,20*S*,21, 24*S*-epoxy-23-oxo-apo-tirucall-14 (15)-ene-25-ol.[7]



3, R=H
3a, R=*S*-MTPA
3b, R=*R*-MTPA

Figure 1. The oxidation of **3** by Jones reagent.

Recently, a modified Mosher methodology, developed by Ohtani and co-workers, has been successfully applied to the determination of the absolute configuration of stereogenic centers bearing an hydroxyl group.[8-10] The method, using *S*- and *R*- Mosher esters [methoxytrifluoromethylphenyl acetate or MTPA], introduces more shielded effects or less shielded effects on different substituents of the chiral carbon, and the chemical shifts of the ^{1}H nmr spectra of these substituents change accordingly. Following Ohtani's procedure, **3** and **5** were treated with *R*- and *S*-methoxytrifluoromethylphenylacetyl chloride to give the *S*- and *R*-Mosher esters (**3a** and **3b**, **5a** and **5b**). The chemical shifts of the *S*-MTPA esters (**3a** and **5a**) and the *R*-MTPA esters (**3b** and **5b**) were assigned by careful analysis of the COSY spectra. In the case

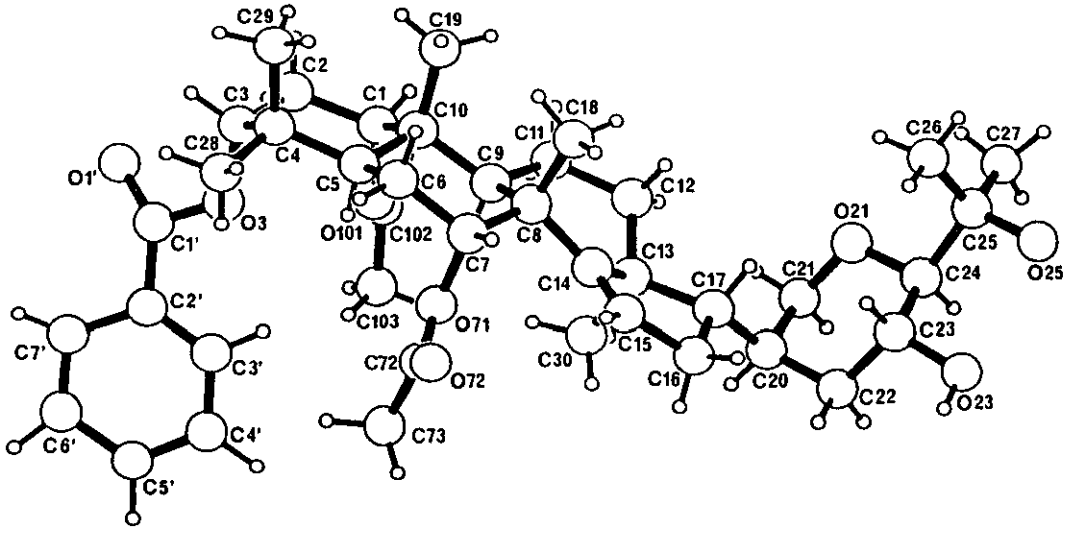


Figure 2. ORTEP plot of melianin A (**3**).

Table 3. ^{1}H Nmr Data of **3a**, **3b**, **5a**, and **5b**.

	3a* (*S*-MTPA)		**3b**** (*R*-MTPA)	Δδ***		**5a**† (*S*-MTPA)		**5b**†† (*R*-MTPA)		Δδ***
No.	δ_H	Coupling in Hz	δ_H	Coupling in Hz	(δ_{3a}-δ_{3b})	No.	δ_H	Coupling in Hz	δ_H	Coupling in Hz	(δ_{5e}-δ_{5f})
1	4.71	t, 2.5	4.71	t, 2.5		1	1.42	m	1.50	m	-0.08
2	2.16	td, 2.5, 16.0	2.16	td, 2.5, 16.0			1.52	m	1.58	m	-0.06
	2.30	td, 2.5, 16.0	2.30	td, 2.5, 16.0		2	1.64	m	1.74	m	-0.10
3	4.89	t, 2.5	4.89	t, 2.5			1.76	m	1.80	m	-0.04
5	2.54	dd, 6.5, 9.0	2.54	dd, 6.0, 9.5		3	4.72	dd, 4.0, 12.0	4.75	dd, 4.0, 11.5	-0.03
6	1.85	dd, 3.0, 6.5	1.85	dd, 3.0, 6.0		5	1.43	m	1.40	m	+0.03
	1.85	dd, 3.0, 9.0	1.85	dd, 3.0, 9.5		6	1.98	m	1.95	m	+0.03
7	5.19	t, 3.0	5.18	t, 3.0	+0.01		2.14	m	2.12	m	+0.02
9	2.64	dd, 5.5, 11.5	2.64	dd, 6.5, 12.0		7	5.27	br s	5.26	br s	+0.01
11	1.25	m	1.25	m		9	2.00	m	2.00	m	
	1.50	m	1.50	m		11	1.23	m	1.23	m	
12	1.60	m	1.60	m			1.68	m	1.70	m	
	1.70	m	1.70	m		12	1.30	m	1.30	m	
15	5.37	br d, 2.0	5.36	br d, 3.0	+0.01		1.51	m	1.51	m	
16	1.94	m	1.86	m	+0.08	15	1.52	m	1.50	m	+0.02
	2.40	m	2.35	m	+0.05		1.83	m	1.81	m	+0.02
17	2.05	m	2.05	m		16	1.52	m	1.52	m	+0.02
18	0.97	s	0.97	s			2.23	m	2.23	m	
19	1.01	s	1.01	s		17	1.98	m	1.96	m	+0.02
20	2.08	m	1.90	m	+0.18	18	0.78	s	0.78	s	
21	3.60	dd, 6.0, 11.5	3.60	dd, 5.5, 11.0	+0.01	19	0.76	s	0.72	s	+0.04
	3.90	br d, 11.5	3.87	br d, 11.0	+0.03	20	1.80	m	1.70	m	+0.10
22	1.67	m	1.60	m	+0.07	21	3.60	dd, 4.5, 11.0	3.58	dd, 4.0, 11.5	+0.02
	2.16	m	2.05	m	+0.11		3.88	dd, 3.5, 11.0	3.84	dd, 5.0, 11.5	+0.04
23	5.13	ddd, 4.5, 8.5, 8.5	5.17	ddd, 4.5, 8.5, 8.5	-0.05	22	1.66	m	1.60	m	+0.06
24	3.15	d, 8.5	3.16	d, 8.5	-0.01		2.07	m	1.96	m	+0.11
26	0.92	s	1.09	s	-0.17	23	5.19	dd, 4.5, 8..0	5.23	dd, 4.5, 7.5	-0.04
27	1.00	s	1.10	s	-0.10	24	3.14	d, 8.0	3.17	d, 7.5	-0.03
28	0.91	s	0.91	s		26	1.00	s	1.12	s	-0.12
29	1.00	s	1.00	s		27	1.01	s	1.13	s	-0.12
30	1.17	s	1.16	s		28	0.92	s	0.80	s	+0.12
AcO	2.07	s	2.07	s		29	1.01	s	0.99	s	+0.02
	1.64	s	1.64	s		30	0.88	s	0.88	s	
3'	8.09	dd, 1.5, 8.0	8.09	dd, 1.5, 8.0							
4'	7.43	t, 8.0	7.43	t, 8.0							
5'	7.56	tt, 1.5, 8.5	7.56	tt, 1.5, 8.5							
6'	7.43	t, 8.0	7.43	t, 8.0							
7'	8.09	dd, 1.5, 8.0	8.09	dd, 1.5, 8.0							

*For MTPA signals: 3.58 (3H, s, MeO), 7.39-7.42 (3 H, m, phenyl protons), 7.50-7.55 (2 H, m, phenyl protons). **For MTPA signals: 3.46 (3H, s, MeO), 7.40-7.42 (3 H, m, phenyl protons), 7.50-7.52 (3 H, m, phenyl protons). ***Only chemical shift changes were listed in this column. †For MTPA signals: 3.53, 3.58 (each 3H, s, MeO), 7.33-7.43 (6 H, m, phenyl protons), 7.53-7.60 (4 H, m, phenyl protons). ††For MTPA signals: 3.48, 3.57 (each 3H, s, MeO), 7.33-7.43 (6 H, m, phenyl protons), 7.53-7.60 (4 H, m, phenyl protons).

of **3a** and **3b**, the chemical shifts of the side chain showed $\Delta\delta_H(\delta_S$-$\delta_R)$ changes of the core side to the 2-hydroxyisopropyl side, from positive to negative (Table 3), which indicated that C-23 was of the *R*-configuration. The relative stereochemistry of the other part of the molecule was proven by X-ray crystallography (Figure 2). Thus, the absolute structure of **3** was demonstrated to be 1α, 7α-diacetoxy-3α-benzoxy-17α,20*S*,21,24*R*-epoxy-apotirucall-14(15)-ene-23*R*, 25-diol.[6, 11] In the case of **5a** and **5b**, the chemical shifts of the side chain showed $\Delta\delta_H(\delta_S$-$\delta_R)$ changes of the core side to the 2-hydroxyisopropyl side, from positive to negative (Table 3), which also indicated that its C-23 is of the *R*-configuration. The ^{1}H nmr spectrum of **5** showed that the relative configurations of the side chain tetrahydropyran ring from C-20 to C-24 are trans /trans, so the absolute configurations of **5** are concluded to be C-20*S*, -23*R*, and -24*R*. The stereochemistry of C-3 of **5** is concluded to be in the *S*-form, since the

chemical shift changes of $\Delta\delta_H(\delta_S-\delta_R)$ are negative, from H-1 and -2, and positive, from H-4 to -7 and H-28 and -29. Thus, the absolute structure of **5** was demonstrated to be 17α,20*S*-tirucall-7(8)-ene-21,24*R*-epoxy-3*S*, 23*R* , 25-triol.[12] It is interesting to note that the E-ring conformations of **1** and **2** are different, and these are illustrated in Figure 3. Both have C-20*S* configurations but assume the different ring forms. The H-20 of **1** is in the axial orientation, whereas the H-20 of **2** is in the equatorial orientation.

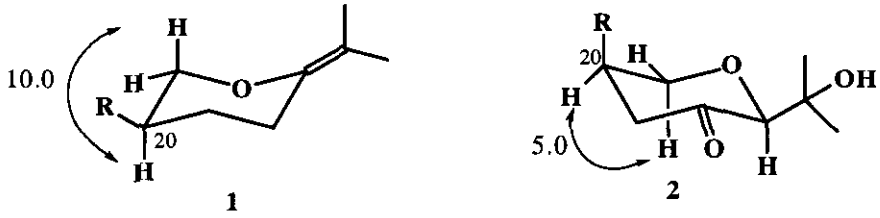


Figure 3. The E ring conformations of **1** and **2** (the arrows show coupling constants in Hz).

The four isolated compounds, meliavolen (**1**), melianinone (**2**), 3-episapelin (**5**), and nimbolin B (**6**) were all moderately active in the brine shrimp lethality test and were significantly cytotoxic among human solid tumor cell lines showing selectivities toward the colon cell line (HT-29) (Table 4).

Table 4 Bioactivities of Compounds (**1**, **2**, **4**, **5**, **6**).

	BST[a]	A-549[b]	MCF-7[b]	HT-29[b]
1	54.5 (144.4-28.7)	4.33	5.66	8.49×10^{-1}
2	88.5 (160.7-48.0)	2.51	>10	8.48×10^{-1}
4	>100	>10	>10	>10
5	154.4 (328.5-83.3)	5.98	9.77	1.46
6	20.1 (37.9-11.8)	3.12	5.90×10^{-1}	$<10^{-3}$
Adriamycin[c]	0.08 (0.069-0.091)	2.99×10^{-2}	9.91×10^{-1}	7.51×10^{-2}
azadirachtin[d]	>25	7.63	13.81	7.51

[a]LC_{50} (μg/ml) with 95% confidence interval in the parentheses. [b]ED_{50} values (μg/ml). [c]Positive antitumor control. [d]Positive pesticide control.

EXPERIMENTAL

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. Cd spectra were taken on a JASCO J600 spectropolarimeter. 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. Analytical tlc was carried out on Si gel plates (0.25 mm) and visualized with 5% phosphomolybdic acid in EtOH. Hplc was performed on a Dynamax software system (by 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, and Dynamax-60 A 8 μm silica gel columns (a 21.4 mm I. D. x 250 mm column and a 10 mm, I. D. x 250 mm column for preparative separations and a 4.6 mm I. D. x 250 mm column for purity determinations). *R*- and *S*-Methoxytrifluoromethylphenylacetyl chlorides are Aldrich products.

Plant Material

The root bark of *M. volkensii* (B-644035, BRS-2-193) was collected in Kenya for the National Cancer Institute, National Institute of Health, 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).[5,6] The cytotoxicity tests against A-549 (human lung carcinoma),[13] MCF-7 (human breast carcinoma),[14] and HT-29 (human colon adenocarcinoma)[15] cells were performed, in 7 day MTT tests, at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with Adriamycin as a positive standard control.

Isolation of Triterpenoids

Powdered root bark of *M. volkensii* (10 kg) was extracted by ethanol (100 l) for seven days using a continuous extractor at 22 °C. The ethanol residue (F001, 502 g) was partitioned between dichloromethane and water (5 l: 5 l for 4 times) to give water soluble (F002, 307.2 g), dichloromethane soluble (F003, 190 g), and interface portions (F004, 4.8 g). The dichloromethane residue (F003, 190 g) was further partitioned between hexane and 90% methanol in water (3 l: 3 l for 4 times) to give 90% methanol (F005, 160 g) and hexane portions (F006, 30 g). The partitioning steps were monitored by the BST bioassay, in which the LC_{50} values for F001 to 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 (column A, silica gel, 1350 g), washed gradiently with hexane (fr. 1-2), hexane-dichloromethane (9:1 to 1:9, fr. 3-6), dichloromethane (fr.7-9), dichloromethane-ethyl acetate (9:1 to 1:9, fr. 10-55), ethyl acetate (fr. 56-60), and methanol (fr. 61-62); 3 l portions were collected for each fraction. The residues of each fraction were tested by the BST, and the most active parts between fr. 20-27 (BST LD_{50}, 0.32-0.62 μg/ml) were combined into a pool (22.9 g). A part of this active eluent (11.5 g) was further separated by column chromatography (column B, silica gel, 150 g), eluted by hexane-dichloromethane (1:1 to 1:9), dichloromethane, and dichloromethane-ethyl acetate (9.8:0.2 to 1:9), and ethyl acetate. The 50 fractions collected were combined into eight pools (pools-1 to 8) on the basis of their similar tlc patterns. The activities of the eight pools showed BST LC_{50} values of 4.02, 0.62, 0.23, 0.27, 0.09, 0.17, and 0.37 μg/ml, respectively. Part of pool-6 (300 mg) was separated by hplc using hexane-(90% methanol in THF) 92:8 as eluent, to give **1** (2 mg), **2** (4 mg), **3** (40 mg), **4** (25 mg), and **5** (5 mg). The active fraction, fr. 16, 0.3 g (BST LC_{50}, 3.3 μg/ml,) was purified by hplc using hexane-(90% methanol in THF) 95:5 as eluent to give **6** (50 mg). The structures of the known compounds (**5**) and (**6**) were determined by the comparison of their ^1H and ^{13}C nmr spectral values to those reported in the literature.[12,16]

Meliavolen (1)

White powder, $[\alpha]^{22}_D$ - 30.1°(c 0.58 in $CHCl_3$); uv λ_{max} (MeOH): 208 nm (log ε 3.41), 228 nm (log ε 3.93); ir ν_{max} (film) cm^{-1}: 2922, 1733, 1717, 1276; FABms (*m*-NBA) m/z (%): 683 ($M+Na^+$, 11); HR-FABms m/z: 683.3918 for $C_{41}H_{56}O_7Na$ ($M+Na^+$) calcd 683.3923; ^1H and ^{13}C nmr (see Tables 1 and 2).

Melianinone (2)

White powder, $[\alpha]^{22}_D$ - 61.2°(c 0.50 in $CHCl_3$); uv λ_{max} (MeOH): 208 nm (log ε 3.43), 228 nm (log ε 3.88); ir ν_{max} (film) cm^{-1}: 3505, 2966, 1731, 1215, 1374; FABms (*m*-NBA) m/z (%): 715 ($M+Na^+$, 8); HRFABms (*m*-NBA) m/z: 715.3826 for $C_{41}H_{56}O_9$ Na ($M+Na^+$) calcd 715.3822; ^1H and ^{13}C nmr (see Tables 1 and 2). Cd (4.48 x 10^{-4} mol/l, MeOH): $[\theta]_{208.2}$ -1095.9 $[\theta]_{221.0}$ 0, $[\theta]_{229.2}$ +632.3, $[\theta]_{267.0}$ 0, $[\theta]_{304.4}$ -543.6 $[\theta]_{334.0}$ 0.

*Oxidation of **3** with Jones reagent (the formation of **2** and **4** from **3**)*

To a solution of 50 mg (0.07 mmol) of **3** in 50 ml of acetone, Jones reagent (5 ml) was added dropwise

Table 5. Position Parameters and Their Estimated Standard Deviations for Melianin A(**3**)[a]

Atom	x	y	z	B(Å^2)	Atom	x	y	z	B(Å^2)
C(1)	-0.4666(4)	0.5810(3)	0.5106(3)	4.7(1)	C(26)	0.1351(5)	0.4371(5)	0.5482(5)	7.8(2)
C(2)	-0.5470(4)	0.6364(4)	0.4886(4)	5.8(1)	C(27)	0.1575(5)	0.2952(5)	0.4812(5)	8.8(2)
C(3)	-0.5807(4)	0.6921(4)	0.5575(4)	5.7(1)	C(28)	-0.5496(5)	0.7888(4)	0.6757(4)	7.0(2)
C(4)	-0.5109(4)	0.7481(4)	0.5988(4)	5.5(1)	C(29)	-0.4845(5)	0.8229(4)	0.5408(4)	7.2(2)
C(5)	-0.4332(4)	0.6869(3)	0.6232(3)	4.4(1)	C(30)	-0.1887(4)	0.6605(4)	0.6069(4)	5.2(1)
C(6)	-0.3631(4)	0.7310(3)	0.6749(3)	4.5(1)	C(72)	-0.3633(3)	0.6253(4)	0.8445(3)	4.5(1)
C(7)	-0.3034(3)	0.6657(3)	0.7138(3)	4.0(1)	C(73)	-0.4268(5)	0.5653(5)	0.8843(4)	7.1(2)
C(8)	-0.2583(3)	0.6068(3)	0.6516(3)	4.0(1)	C(102)	-0.5198(4)	0.4379(4)	0.5279(4)	5.4(1)
C(9)	-0.3294(3)	0.5666(3)	0.5940(3)	3.7(1)	C(103)	-0.5475(5)	0.3729(4)	0.5883(5)	7.4(2)
C(10)	-0.3930(4)	0.6317(3)	0.5534(3)	4.2(1)	C(1')	-0.7003(4)	0.6312(5)	0.6330(6)	9.6(2)
C(11)	-0.2905(4)	0.5005(3)	0.5345(3)	4.8(1)	C(2')	-0.7214(4)	0.5818(4)	0.7087(5)	7.5(2)
C(12)	-0.2104(3)	0.4522(3)	0.5655(3)	4.4(1)	C(3')	-0.6608(4)	0.5338(5)	0 7477(5)	7.3(2)
C(13)	-0.2056(3)	0.4443(3)	0.6581(3)	4.1(1)	C(4')	-0.6799(6)	0.4852(6)	0.8162(6)	10.5(3)
C(14)	-0.2098(3)	0.5351(3)	0.6954(3)	4.0(1)	C(5')	-0.7636(8)	0.4888(8)	0.8451(6)	14.6(3)
C(15)	-0.1623(4)	0.5411(4)	0.7620(4)	5.4(1)	C(6')	-0.8295(5)	0.5412(6)	0.8052(7)	12.6(3)
C(16)	0.1113(4)	0.4596(4)	0.7772(4)	5.4(1)	C(7')	-0.8095(5)	0.5875(5)	0.7359(8)	12.4(3)
C(17)	-0.1155(3)	0.4146(4)	0.6931(3)	4.5(1)	O(1')	-0.7487(7)	0.6958(9)	0.6235(9)	11.9(4)
C(18)	-0.2788(4)	0.3869(4)	0.6918(4)	5.3(1)	O(101)	-0.4953(2)	0.5116(2)	0.5641(2)	4.5(8)
C(19)	-0.3501(4)	0.6860(4)	0.4860(4)	5.5(1)	O(102)	-0.5183(4)	0.4278(3)	0.4548(3)	8.7(1)
C(20)	-0.0920(4)	0.3191(4)	0.6948(4)	5.4(1)	O(21)	-0.0031(2)	0.3184(2)	0.5714(2)	5.1(8)
C(21)	-0.0779(4)	0.2804(4)	0.6098(4)	5.9(2)	O(24)	0.1442(3)	0.3141(3)	0.7491(3)	7.8(1)
C(22)	-0.0100(5)	0.3004(4)	0.7434(4)	6.6(2)	O(25)	0.2304(3)	0.3350(3)	0.6069(4)	8.7(1)
C(23)	0.0694(4)	0.3383(4)	0.7020(4)	5.5(1)	O(3)	-0.6166(2)	0.6363(3)	0.6202(3)	5.8(9)
C(24)	0.0770(4)	0.3030(4)	0.6152(4)	5.4(1)	O(71)	-0.3582(2)	0.6107(2)	0.7653(2)	3.9(7)
C(25)	0.1492(4)	0.3425(4)	0.5615(4)	7.4(2)	O(72)	-0.3240(3)	0.6801(3)	0.8804(3)	7.5(1)

[a]Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent temperature factor defined as: (4/3) * [$a^2*\beta(1,1) + b^2*\beta(2,2) + c^2*\beta(3,3) + ab(\cos\gamma)*\beta(1,2) + ac(\cos\beta)*\beta(1,3) + bc(\cos\alpha)*\beta(2,3)$]

until the solution remained yellowish. The reactants were stirred for 5 h. The reaction products were separated by hplc using 7% MeOH (containing 10% of THF) in hexane. Compound **2** (5 mg, yield 10%) and compound **(4)**(12 mg, yield 27%) were obtained at the retention times of 70 and 81 min, respectively. The ^{1}H nmr, ^{13}C nmr, and cd spectra of compound (**2**) were the same as those of melianinone (**2**). Compound (**4**) was a white powder; ^{1}H nmr, ^{13}C nmr (see Tables 1 and 2).

Table 6. Bond Angles in Degrees for Melianin A (**3**) with esds in Parentheses.

Atoms	angle	Atoms	angle	Atoms	angle
C(1)-O(101)-C(102)	116.9(5)	C(9)-C(10)-C(19)	113.1(5)	C(2')-C(3')-C(4')	122.4(9)
C(1)-C(2)-C(3)	114.8(5)	C(9)-C(11)-C(12)	115.7(5)	C(2')-C(7')-C(6')	116.0(1)
C(1)-C(10)-C(5)	108.7(5)	C(10)-C(9)-C(11)	114.3(5)	C(3')-C(2')-C(7')	123.0(1)
C(1)-C(10)-C(9)	108.8(4)	C(11)-C(12)-C(13)	114.1(5)	C(3')-C(4')-C(5')	117.0(1)
C(1)-C(10)-C(19)	105.8(5)	C(12)-C(13)-C(14)	108.5(5)	C(4')-C(5')-C(6')	121.0(1)
C(2)-C(1)-C(10)	113.9(5)	C(12)-C(13)-C(17)	115.4(5)	C(5')-C(6')-C(7')	120.0(1)
C(2)-C(3)-C(4)	114.3(6)	C(12)-C(13)-C(18)	111.5(5)	O(21)-C(21)-C(20)	110.4(5)
C(3)-O(3)-C(1')	121.6(7)	C(13)-C(14)-C(15)	111.7(5)	O(21)-C(24)-C(23)	109.5(5)
C(3)-C(4)-C(5)	107.3(5)	C(13)-C(17)-C(16)	103.1(5)	O(21)-C(24)-C(25)	105.5(5)
C(3)-C(4)-C(29)	109.8(6)	C(13)-C(17)-C(20)	120.1(5)	O(24)-C(23)-C(24)	110.0(6)
C(3)-C(4)-C(28)	108.8(6)	C(14)-C(8)-C(30)	106.3(5)	O(24)-C(23)-C(22)	107.8(6)
C(4)-C(5)-C(6)	113.7(5)	C(14)-C(13)-C(17)	99.5(5)	O(3)-C(1')-O(1')	121.0(1)
C(4)-C(5)-C(10)	116.7(5)	C(14)-C(13)-C(18)	111.1(5)	O(3)-C(1')-C(2')	112.2(8)
C(5)-C(4)-C(29)	114.0(6)	C(14)-C(15)-C(16)	111.4(5)	O(1')-C(1')-C(2')	113.0(1)
C(5)-C(4)-C(28)	109.8(5)	C(15)-C(16)-C(17)	102.3(5)	O(101)-C(1)-C(2)	108.0(5)
C(5)-C(6)-C(7)	111.0(5)	C(16)-C(17)-C(20)	114.5(6)	O(101)-C(1)-C(10)	109.2(5)
C(5)-C(10)-C(9)	107.3(4)	C(17)-C(13)-C(18)	110.2(5)	O(3)-C(3)-C(2)	108.2(5)
C(5)-C(10)-C(19)	113.1(5)	C(17)-C(20)-C(21)	113.6(6)	O(3)-C(3)-C(4)	107.1(5)
C(6)-C(5)-C(10)	111.8(5)	C(17)-C(20)-C(22)	113.0(6)	O(71)-C(72)-O(72)	125.0(6)
C(6)-C(7)-C(8)	113.6(5)	C(20)-C(22)-C(23)	111.3(6)	O(71)-C(72)-C(73)	111.0(6)
C(7)-O(71)-C(72)	119.9(5)	C(21)-C(20)-C(22)	106.3(6)	O(72)-C(72)-C(73)	124.0(6)
C(7)-C(8)-C(9)	108.5(5)	C(21)-O(21)-C(24)	113.5(5)	O(101)-C(102)-O(102)	122.8(7)
C(7)-C(8)-C(14)	110.5(5)	C(23)-C(24)-C(25)	116.1(6)	O(101)-C(102)-C(103)	111.8(6)
C(7)-C(8)-C(30)	107.7(5)	C(24)-C(25)-C(26)	116.6(6)	O(102)-C(102)-C(103)	125.4(7)
C(8)-C(14)-C(13)	120.5(5)	C(24)-C(25)-C(27)	111.1(7)	O(25)-C(25)-C(24)	107.3(6)
C(8)-C(14)-C(15)	127.6(5)	C(24)-C(23)-C(22)	109.7(6)	O(25)-C(25)-C(27)	109.3(6)
C(8)-C(9)-C(10)	115.8(4)	C(27)-C(25)-C(26)	111.3(7)	O(25)-C(25)-C(26)	106.0(7)
C(8)-C(9)-C(11)	111.9(5)	C(29)-C(4)-C(28)	107.0(6)	O(71)-C(7)-C(6)	106.6(5)
C(9)-C(8)-C(14)	109.4(4)	C(1')-C(2')-C(3')	121.7(7)	O(71)-C(7)-C(8)	107.0(4)
C(9)-C(8)-C(30)	114.5(5)	C(1')-C(2')-C(7')	116.0(1)		

X-Ray Crystallographic Analysis of Melianin A (3), Crystal Data and Data Collection and Processing.

Crystals of **3** were obtained as transparent prisms by recrystallization from MeOH. $C_{41}H_{58}O_9$, M = 694.91. Orthorhombic, a = 15.3649 (9) Å, b = 15.528 (2) Å, c = 16.304 (2) Å, V = 3889 (1) Å^3, (by least-squares refinement, using the setting angles of 25 reflections in the range $35 < \theta < 41°$, measured by the computer-controlled diagonal slit method of centering), λ (Mo-Kα) = 1.54184 Å, space group $P2_12_12_1$ (No. 19), Z = 4, Dx = 1.187 g cm^{-3}. Crystal dimensions 0.25 x 0.25 x 0.25 mm, λ (Mo-Kα) =

6.30 cm^{-1}. Enraf-Nonius CAD4 diffractometer, $\omega/2\theta$ mode with ω scan width = 0.47 + 0.15 tan θ, 2θ range 4.00-125.00 deg, take-off angle 2.95 deg, scan rate 1-16 deg min^{-1}, graphite monochromated radiation; 3361 reflections measured (*h, k, l* limits: 0 to 16, 0 to 17, 0 to 18), 3361 unique, giving 2557 with $I > 3.0\sigma(I)$. Corrections were applied for Lorentz and polarization factors, but not for absorption.

Table 7. Bond Distances in Angstroms for Melianin A (**3**) with esds in Parentheses.

Atoms	Distance	Atoms	Distance	Atoms	Distance
C(1)-C(2)	1.547(9)	C(13)-C(14)	1.535(8)	C(3')-C(4')	1.38(1)
C(1)-C(10)	1.544(8)	C(13)-C(17)	1.567(8)	C(4')-C(5')	1.37(2)
C(2)-C(3)	1.51(1)	C(13)-C(18)	1.537(8)	C(5')-C(6')	1.45(2)
C(3)-C(4)	1.54(1)	C(14)-C(15)	1.312(9)	C(6')-C(7')	1.37(2)
C(4)-C(5)	1.576(8)	C(15)-C(16)	1.509(9)	O(21)-C(21)	1.435(8)
C(4)-C(29)	1.553(9)	C(16)-C(17)	1.540(9)	O(21)-C(24)	1.443(7)
C(4)-C(28)	1.52(1)	C(17)-C(20)	1.526(9)	O(3)-C(3)	1.450(8)
C(5)-C(6)	1.529(8)	C(20)-C(21)	1.53(1)	O(3)-C(1')	1.31(1)
C(5)-C(10)	1.553(8)	C(20)-C(22)	1.52(1)	O(71)-C(7)	1.464(6)
C(6)-C(7)	1.507(8)	C(23)-C(22)	1.51(1)	O(71)-C(72)	1.313(7)
C(7)-C(8)	1.531(8)	C(24)-C(23)	1.52(1)	O(72)-C(72)	1.197(7)
C(8)-C(9)	1.570(8)	C(24)-C(25)	1.54(1)	O(101)-C(1)	1.454(7)
C(8)-C(14)	1.517(8)	C(25)-C(27)	1.51(1)	O(101)-C(102)	1.342(7)
C(8)-C(30)	1.540(8)	C(25)-C(26)	1.50(1)	O(102)-C(102)	1.203(8)
C(9)-C(10)	1.554(8)	C(102)-C(103)	1.47(1)	O(25)-C(25)	1.456(9)
C(9)-C(11)	1.533(8)	C(72)-C(73)	1.497(9)	O(24)-C(23)	1.432(8)
C(10)-C(19)	1.534(8)	C(1')-C(2')	1.49(1)	O(31)-C(1')	1.26(2)
C(11)-C(12)	1.527(9)	C(2')-C(3')	1.35(1)		
C(12)-C(13)	1.517(8)	C(2')-C(7')	1.43(1)		

Structure Analysis and Refinement.

The structure was solved by direct methods using SHELX-86. The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were located and added to the structure factor calculations, but their positions were not refined. The structure was refined in full-matrix least-squares where the function minimized was $\sum w(|Fo|-|Fc|)^2$ and the weight w is defined as per the Killean and Lawrence method with terms of 0.02 and 1.0.[17] Scattering factors were taken from Cromer and Waber.[18] Anomalous dispersion effects were included in Fc;[19] the values for $\delta f'$ and $\delta f''$ were those of Cromer and Waber.[18] Only the 1376 reflections having intensities greater than 3.0 times their standard deviation were used in the refinements. The final cycle of refinement included 469 variable parameters and converged with unweighted and weighted agreement factors $R = 0.063$, and $R_W = 0.077$. The standard deviation of an observation of unit weight was 2.12. There were 66 correlation coefficients >0.50. Plots of $\sum w(|Fo|-|Fc|)^2$ *versus* [Fo], reflection order in data collection, sin θ /λ, and various classes of indices showed no unusual trends. All calculations were performed on a VAX computer using SDP/VAX.

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