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Antimycobacterial Activities of Dehydrocostus Lactone and Its Oxidation Products

Charles L. Cantrell,[†] Isabel S. Nuñez,[†] José Castañeda-Acosta,[†] Maryam Foroozesh,[†] Frank R. Fronczek,[†] Nikolaus H. Fischer,*[,†] and Scott G. Franzblau[‡]

Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana 70803-1804, and G. W. L. Hansen's Disease Center, P.O. Box 25072, Baton Rouge, Louisiana 70894

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In an attempt to study the structural dependence of antimycobacterial activity of the guaianolide dehydrocostus lactone and its derivatives, *m*-chloroperoxybenzoic acid oxidations of dehydrocostus lactone (**1a**) were performed. Three new monoepoxides, one previously synthesized diepoxide, and two new diepoxides were obtained. Two of the monoepoxides are C-10 epimers (**3a, 3b**), while the 4(15)-monoepoxide (**2**) has the 4α-*O*-configuration. The known diepoxide (**4a**) contains a C-10 α-epoxide and a *β*-epoxide at C-4. The diepoxides **4b** and **4c**, each with a C-4 α-epoxy group, differ in the configuration of the epoxide ring at C-10. Allylic oxidation of dehydrocostus lactone (**1a**) with selenium dioxide/*tert*-butyl hydroperoxide afforded the known 3-epizaluzanin C (**1b**). The relative configurations of compounds **1b**−**4c** were established by 1D and 2D NMR techniques (^{1}H, ^{13}C, COSY, NOESY, HMQC, and HMBC) as well as comparison with literature data. The molecular structures of lactones **1b**, **4a**, and **4c** were determined by single-crystal X-ray diffraction. In radiorespirometric bioassays against *Mycobacterium tuberculosis* and *Mycobacterium avium*, dehydrocostus lactone (**1a**) exhibited minimum inhibitory concentrations of 2 and 16 μg/mL, respectively. In contrast, its monoepoxides (**2**, **3a**, and **3b**) and diepoxides (**4a**−**c**), as well as its hydrogenated derivatives and other analogues (**1b**, **1c**, **5**, and **6**), showed significantly lower activities against *M. tuberculosis*.

Worldwide, the number of tuberculosis cases is currently on the rise, and it is estimated that new infections of humans by *Mycobacterium tuberculosis* are greater than 8 million annually, and more than 3 million people will die of this disease each year.[1] This devastating pandemic is worsened by the fact that strains of *M. tuberculosis* have developed resistance to currently administered therapeutic agents,[2] creating a need for the discovery and development of new and more effective antituberculosis drugs.

In a previous paper we reported on bioassay-guided fractionations of crude plant extracts of *Borrichia frutescens* (L.) DC. (Asteraceae) with significant antimycobacterial activity. Chemical investigation of active fractions resulted in the isolation of epoxycycloartane-type triterpenes with minimum inhibitory concentrations (MICs) of 8 μg/mL.[3] In a search for new chemotypes of antimycobacterial natural products, in vitro tests against *M. tuberculosis* of a series of natural and semisynthetic guaianolide-type sesquiterpene lactones were performed. Dehydrocostus lactone (**1a**), a major constituent of *Saussurea lappa* Clark (Asteraceae),[4,5] gave MICs of 2 and 16 μg/mL against *M. tuberculosis* and *M. avium*, respectively. It was therefore of interest to learn whether epoxidation of the nonlactonic double bonds of **1a** would result in derivatives with increased activity, because antimycobacterial triterpenes from *B. frutescens* correlated well with the presence of an epoxyprenyl group.[3] Therefore, **1a** was used as a starting compound for various oxidative modifications in an attempt

* To whom correspondence should be addressed. Tel: (504) 388-2695. Fax: (504) 388-2695. E-mail: fischer@chemgate.chem.lsu.edu.
[†] Louisiana State University.
[‡] G. W. L. Hansen's Disease Center.

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Table 1. 300 MHz ^{1}H NMR Spectral Data for Compounds **2**−**4c** Performed in $CDCl_3$[a]

proton	2	3a	3b	4a	4b	4c
1	3.21 ddd (8.1, 8.1, 8.1)	2.93 ddd (4.0, 9.0, 9.0)	2.43 ddd (8.1, 8.1, 8.1)	2.85 m	2.45 ddd (8.7, 8.7, 8.7)	2.74 ddd (8.1, 8.1, 8.1)
2a	1.91 m	2.05 m	1.6−1.85[b]	1.66 m	1.70 m	1.68 m
2b	2.11 m	2.05 m	2.37 m	1.78 m	2.02 m	1.92 m
3a	1.69 m	1.86 ddd (6.5, 9.0, 14.5)	1.6−1.85[b]	1.49 m	1.60−1.78[b]	1.55−1.70[b]
3b	2.25 m	2.26 m	2.37 m	2.14 m	2.11−2.25[b]	2.02−2.12[b]
5	2.13 dd (8.1, 11.0)	2.64 dd (9.6, 9.6)	2.88 dd (9.3, 9.3)	2.69 dd (9.9, 9.9)	2.12 m	2.22 dd (8.8, 10.7)
6	4.04 dd (8.8, 11.0)	4.08 dd (9.6, 9.6)	4.12 dd (9.0, 10.2)	4.10 dd (9.7, 9.8)	4.13 dd (8.8, 11.2)	4.23 dd (9.4, 9.4)
7	2.74 m	2.86 m	2.98 m	2.85 m	2.99 m	2.74 m
8a	1.45 m	1.42 dddd (5.0, 11.9, 11.9, 13.0)	1.48 dddd (6.1, 8.6, 11.3, 13.6)	2.23 dddd (3.0, 3.0, 4.7, 13.7)	1.52 m	1.61 m
8b	2.25 m	2.22 m	2.27 dddd (4.3, 6.0, 6.0, 13.6)	1.45 m	2.29 m	2.11 m
9a	2.19 m	2.09 m	1.78 m	1.62 m	1.70 m	1.55−1.70[b]
9b	2.46 dd (6.5, 12.7)	2.56 ddd (3.5, 5.0, 14.6)	1.91 m	2.10 m	2.02 m	2.02−2.12[b]
13a	5.46 d (3.4)	5.48 d (3.1)	5.53 d (3.1)	5.53 d (3.1)	5.51 d (3.1)	5.50 d (2.9)
13b	6.17 d (3.6)	6.19 d (3.6)	6.26 d (3.1)	6.24 d (3.5)	6.22 d (3.6)	6.22 d (3.67)
14a	4.94 s	2.75 d (4.9)	2.54 d (4.7)	2.55 d (4.3)	2.63 d (4.8)	2.60 d (4.8)
14b	4.97 s	3.10 d (4.9)	2.62 dd (1.2, 4.5)	2.85 d (4.3)	2.68 d (4.8)	2.78 d (4.7)
15a	2.84 d (4.4)	5.00 s	5.02 d (1.7)	2.80 d (4.4)	2.87 d (4.6)	2.86 d (4.4)
15b	3.36 d (4.5)	5.00 s	5.26 d (1.8)	3.27 d (4.5)	3.33 d (4.4)	3.32 d (4.7)

[a] Coupling constants (Hz) are given in parentheses. [b] Overlap with other signals.

to find sesquiterpene lactones with increased antimycobacterial activity.

Results and Discussion

Dehydrocostus lactone (**1a**) was previously isolated from costus root oil in our laboratory as described earlier.[5] The reaction of a solution of **1a** in CH_2Cl_2 with 1.5 molar equivalents of *m*-chloroperbenzoic acid (*m*-CPBA) gave a mixture of reaction products that were separated by vacuum-liquid chromatography (VLC), providing the monoepoxides **3a**, **2**, and **3b** in the less polar fractions, followed by the more polar diepoxides **4a**, **4b**, and **4c**.

Inspection of the ^{1}H NMR spectrum of **3a** indicated a monoepoxide that differed from **1a** in the absence of the two olefinic methylene protons at C-14 with singlets at δ 4.82 and 4.90. Instead, two mutually coupled doublets appeared at δ 2.75 and 3.10 (J = 4.9 Hz), suggesting epoxidation at the C-10(14) position. This was supported by the ^{13}C NMR spectrum of **3a**, in which the two olefinic carbon signals (C-10 and C-14) in **1a** were replaced by signals corresponding to the oxygen-bearing C-10 (δ 65.1, s) and C-14 (δ 48.4, t) in **3a**. The stereochemistry of the epoxide ring was determined by NOESY spectroscopy with correlations being observed between H-14b and H-5α, suggesting a C-10 β-epoxide. Further ^{1}H and ^{13}C NMR assignments were made by inspection of COSY, HMQC, and HMBC spectra, and the chemical shifts obtained are summarized in Tables 1 and 2, respectively.

^{1}H and ^{13}C NMR analysis of epoxide **3b** also indicated epoxidation at C-10(14) based on reasoning similar to that described for **3a**. The stereochemistry of **3b** was confirmed by NOESY spectroscopy, which showed interactions between H-6β and both epoxide protons at C-14, suggesting a C-10 α-epoxide. The application of 2D COSY, HMQC, and HMBC permitted all assignments in the ^{1}H and ^{13}C NMR spectra, which are summarized in Tables 1 and 2, respectively.

The ^{1}H NMR spectrum of compound **2** indicated that it differed from **1a** in the absence of the olefinic methylene protons at C-15. Instead, **2** exhibited mutually coupled oxirane methylene doublets at δ 2.84 and 3.36 (J = 4.5 Hz), which were absent in **1a**. The ^{13}C NMR data also indicated that the epoxide group in **2** could be placed between the C-4(15) positions. The stereochemistry at C-4 was determined by NOESY spectroscopy, which indicated a strong NOE correlation between H-15b and H-6β. Further support for a C-4α-epoxide was provided by reaction of **2** with excess *m*-CPBA to produce diepoxides **4b** and **4c**. Because the stereochemistry of **4c** was determined by single-crystal X-ray diffraction, this conversion unambiguously established the α-epoxide stereochemistry at C-4(15) in **2**. ^{1}H and ^{13}C NMR assignments of **2** were based on inspection of COSY, HMQC, and HMBC spectra and are summarized in Tables 1 and 2, respectively.

Table 2. 75.4 MHz ^{13}C NMR Spectral Data for Compounds **2**−**4c** Performed in $CDCl_3$[a]

carbon	2	3a	3b	4a	4b	4c
1	46.8 d	46.6 d	46.5 d	43.8 d[b]	45.1 d[b]	44.4 d
2	28.4 t[b]	29.5 t[b]	25.5 t[b]	22.4 t	25.4 t	24.5 t[b]
3	31.4 t[c]	33.5 t[c]	32.2 t[c]	32.6 t	29.6 t	31.1 t
4	66.4 s	149.1 s	150.9 s	65.1 s	66.3 s	66.8 s
5	53.2 d	49.2 d	51.0 d	47.4 d[b]	52.8 d	52.8 d
6	81.8 d	82.0 d	84.1 d	81.1 d	81.1 d	81.6 d
7	45.8 d	44.9 d	44.7 d	44.5 d[b]	46.4 d[b]	47.4 d
8	29.8 t[b]	31.5 t[b]	28.8 t[b]	27.5 t	25.6 t	24.7 t[b]
9	33.1 t[c]	38.5 t[c]	33.4 t[c]	38.1 t	30.7 t	32.6 t
10	148.1 s	65.1 s	58.1 s	56.8 s	58.3 s	58.7 s
11	139.3 s	139.3 s	139.7 s	138.5 s	139.6 s	139.4 s
12	169.8 s	170.4 s	170.2 s	169.7 s	169.8 s	169.8 s
13	120.2 t	120.6 t	120.8 t	120.9 t	121.0 t	120.5 t
14	114.1 t	48.4 t	51.6 t	48.1 t[c]	50.3 t[c]	50.7 t
15	50.1 t	113.2 t	109.8 t	48.9 t[c]	54.6 t[c]	53.8 t

[a] Peak multiplicities were determined by DEPT experiments. [b,c] Data in same column are interchangeable.

In the ^{1}H NMR spectrum of compound **4a**, the olefinic H-14 and H-15 signals present in **1a** were replaced by two sets of mutually coupled oxirane proton doublets at δ 2.55 and 2.85 (J = 4.3 Hz) corresponding to H-14a and H-14b and doublets (H-15a and H-15b) at δ 2.80 and 3.27 (J = 4.4 Hz), suggesting a C-10(14),C-4(15) diepoxide. The relative stereochemistry of the epoxide groups was unambiguously determined by single-crystal X-ray diffraction.

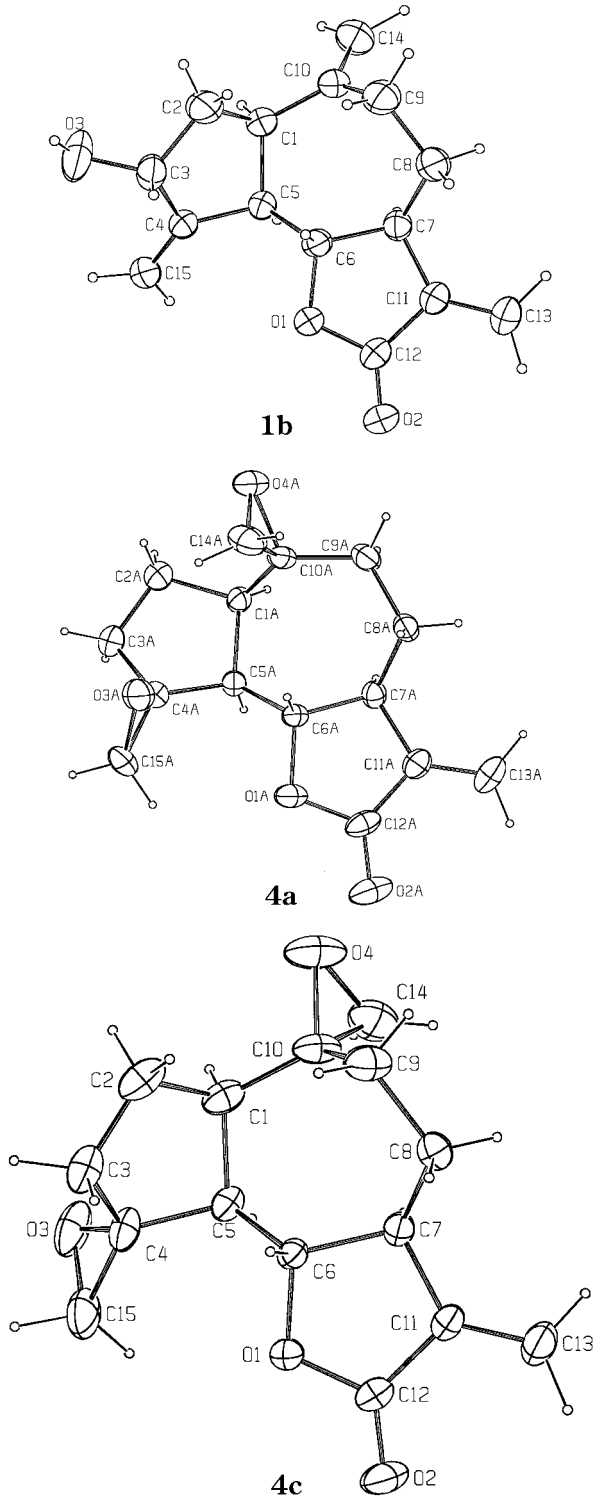


Figure 1. Molecular structure of compounds **1b**, **4a**, and **4c**.

Its molecular structure is shown in Figure 1 and the X-ray crystallographic atomic coordinates are given in Table 5. The molecular structure indicated a C-4(15) β-epoxide, while the C-10(14) epoxide oxygen is α-oriented. Compound **4a** has been previously prepared from **1a** as a mixture of C-10(14) epoxide epimers with an undefined stereochemistry of the products.[6] ^{1}H and ^{13}C NMR spectra of pure **4a** were assigned by various 2D NMR techniques, and the spectral data are reported in Tables 1 and 2, respectively.

Examination of the ^{1}H and ^{13}C NMR spectra of diepoxides **4b** and **4c** indicated that they represented C-10(14),C-4(15) isomers based on arguments made above for **4a**. The epoxide stereochemistry of compound **4b** was determined by NOESY spectroscopy with correlations observed between H-6β and H-14b as well as between H-6β and H-15b, both indicating an α-orientation for the two epoxide oxygens. The relative stereochemistry of compound **4c** was determined by single-crystal X-ray diffraction (Figure 1), which showed a C-4(15) α-epoxide and a C-10(14) β-epoxide. Therefore, the epimers **4b** and **4c** differed only in the configuration of the C-10(14) epoxide group. X-ray coordinates for **4c** are listed in Table 6 and ^{1}H and ^{13}C NMR spectral assignments by various 2D NMR techniques for **4b** and **4c** are summarized in Tables 1 and 2, respectively.

3-Epizaluzanin C (**1b**) was prepared from **1a** by a previously described method[7] using SeO_2 and *t*-BuOOH in CH_2Cl_2. The ^{1}H and ^{13}C NMR spectral data of **1b** were in full agreement with reported values.[8] Single-crystal X-ray crystallographic analysis of **1b** (Figure 1) was performed, and its atomic coordinates are listed in Table 4.

The crystal structures of **1b**, **4a**, and **4c** are illustrated in Figure 1. Lactone **1b** is essentially isomorphous with dehydrocostus lactone,[9] which lacks the OH group at C-3, despite the intermolecular hydrogen bonding by the OH group. That hydrogen bond is linear and involves the lactone carbonyl oxygen O-2 as acceptor, having an O···O distance of 2.858(2) Å and an angle about H of 179(3)°. Although the conformations of the seven-membered and lactone rings in **1b** and dehydrocostus lactone (**1a**) are nearly identical, the conformations of their other five-membered rings differ as a result of the C-3-OH substitution. While this ring in dehydrocostus lactone has a distorted half-chair conformation, it has the envelope conformation with C-5 in the flap position in **1b**.

The conformation of **4c** is similar to that of **1b**, with a twist-chair seven-membered ring having C-8 on the twist axis and a half-chair lactone. Diepoxide **4a** has two independent molecules in the asymmetric unit. Their conformations are nearly identical, but differ markedly from that of **4c**. Lactone **4a** has its seven-membered ring in a distorted twist-chair with C-5 on the twist axis, and an envelope cyclopentane ring with C-4 at the flap position.

The MICs of the guaianolides were determined in radiorespirometric bioassays against *M. tuberculosis* (H_{37}Rv), and **1a** and **1b** were also tested against *M. avium*.[3,10] The MICs against *M. tuberculosis* of dehydrocostus lactone (**1a**), its oxidative derivatives (**2**, **3a**, **3b**, and **4a**−**4c**), and hydroxylated analogues (**1b**, **1c**, **5**, and **6**) are listed in Table 7. The most active lactone **1a**, with an MIC of 2 μg/mL, is also the most lipophilic among this group of guaianolides. It is evident that the presence of a hydroxyl group at different positions of the guaianolide skeleton, as shown for **1b**, **1c**, **5**, and **6**, significantly reduces activity against *M. tuberculosis*. This might be due to the increase in polarity, which possibly reduces transport through the outer lipid layer of the mycobacterium.[10]

Because, in our previous study on cycloartane triterpenes from *Borrichia frutescens*, the presence of an epoxide group in the molecule significantly augmented the antituberculosis activity,[3] monoepoxide- and diepoxide derivatives of dehydrocostus lactone (**1a**) were tested for their activity. Compared to **1a**, the monoepoxides **2**, **3a**, and **3b** showed significantly decreased activity against *M. tuberculosis* with MICs of 32, 32, and 64 μg/mL, respectively. The more polar diepoxides **4a**, **4b**, and **4c** exhibited MICs of 64, 128, and 128 μg/mL, respectively, supporting the correlation between

Table 3. Crystal Data and X-ray Collection Parameters of **1b**, **4a**, and **4c**

compound	**1b**	**4a**	**4c**
formula	$C_{15}H_{18}O_3$	$C_{15}H_{18}O_4$	$C_{15}H_{18}O_4$
FW	246.3	262.3	262.3
crystal system	orthorhombic	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
cell constants			
a, Å	7.650(1)	6.7140(5)	8.3193(6)
b, Å	11.8057(7)	15.980(2)	10.1704(8)
c, Å	14.2153(6)	24.931(3)	15.919(3)
V, Å^3	1283.8(4)	2674.9(9)	1346.9(5)
Z	4	8	4
D_c, g cm^{-3}	1.274	1.303	1.293
μ(Cu Kα), cm^{-1}	6.7	7.3	7.3
T	24	24	22
crystal size, mm	0.11 × 0.13 × 0.50	0.18 × 0.38 × 0.60	0.15 × 0.27 × 0.55
θ limits, deg.	2−75	2−75	2−75
octants collected	$\pm h$, k, $\pm l$	h, k, l	$\pm h$, $\pm k$, $\pm l$
unique data	2603	3224	2763
obsd data	2427	2509	2427
criterion	$I > 3\sigma(I)$	$I > 3\sigma(I)$	$I > 1\sigma(I)$
variables	236	344	245
R	0.031	0.091	0.045
R_w	0.040	0.107	0.049
resid density, e$^-$ Å^{-3}	0.23	0.66	0.27
extinction	3.8(3) × 10^{-6}	1.7(2) × 10^{-6}	2.7(1) × 10^{-6}
hydrogen atoms	refined iso	calcd	refined iso

Table 4. Coordinates and Equivalent Isotropic Thermal Parameters for **1b**

atom	*x*	*y*	*z*	B_{eq} (Å^2)[a]
O-1	0.0798(1)	0.94141(7)	0.92791(7)	3.69(2)
O-2	0.2231(1)	1.09870(8)	0.96659(8)	4.89(2)
O-3	−0.0907(2)	0.51947(9)	0.8894(1)	6.45(3)
C-1	−0.2437(2)	0.7666(1)	0.78196(9)	3.57(2)
C-2	−0.2973(2)	0.6747(1)	0.8548(2)	6.06(4)
C-3	−0.1310(2)	0.6345(1)	0.9026(1)	3.97(3)
C-4	0.0138(2)	0.70896(9)	0.86393(9)	3.24(2)
C-5	−0.0674(2)	0.81357(9)	0.82126(8)	2.94(2)
C-6	−0.0930(2)	0.90910(9)	0.89195(8)	2.91(2)
C-7	−0.1695(2)	1.01729(9)	0.84877(9)	3.04(2)
C-8	−0.3672(2)	1.0285(1)	0.8588(1)	4.59(3)
C-9	−0.4654(2)	0.9187(1)	0.8368(1)	4.77(3)
C-10	−0.3871(2)	0.8494(1)	0.7588(1)	3.62(2)
C-11	−0.0640(2)	1.1091(1)	0.89462(8)	3.29(2)
C-12	0.0949(2)	1.0548(1)	0.93343(9)	3.54(2)
C-13	−0.0961(2)	1.2180(1)	0.9046(1)	4.38(3)
C-14	−0.4460(3)	0.8583(2)	0.6717(1)	5.79(4)
C-15	0.1812(2)	0.6811(1)	0.8619(1)	4.12(3)

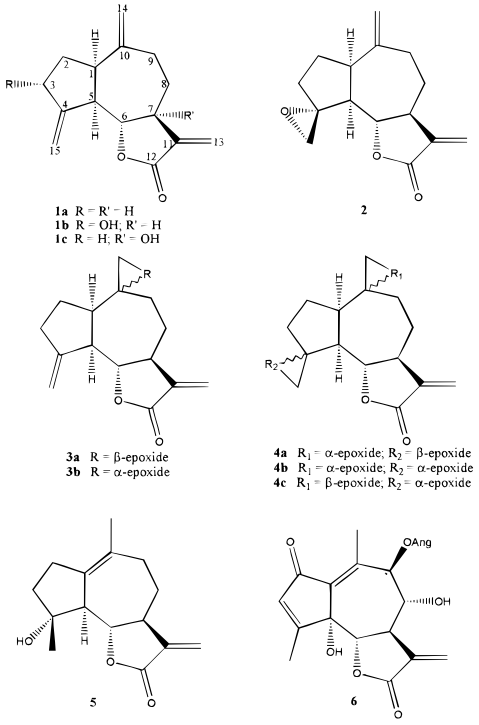
[a] $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij}a_i^*a_j^*\boldsymbol{a_i}\cdot\boldsymbol{a_j}$.

lipophilicity and antituberculosis activity within this guaianolide series.

In conclusion, the above structure−activity data suggest that the antimycobacterial activity of the tested guaianolides is not as strongly determined by the exocyclic methylene lactone moiety as by polarity factors. Activity is reduced when at least one hydroxyl group is present in the molecule (compounds **1b**, **1c**, **5**, and **6**). The successive replacement of double bonds with epoxide groups also caused a significant loss of activity, with the more polar diepoxides being less active than the monoepoxide analogues. Because the most lipophilic guaianolide **1a** gives the highest antimycobacterial activity, it can be concluded that activity is most strongly influenced by the polarity of a given molecule. Therefore, within this structurally related group of sesquiterpene lactones antimycobacterial activity is most likely controlled by the transport through the outer lipid layer of mycobacteria.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on either a Bruker ARX 300 MHz spectrometer or a Bruker AM 400 MHz spectrometer. MS were obtained on a Hewlett−Packard 5971A GC−MS. IR spectra were run on a Perkin−Elmer 1760X spectrometer as a film on KBr plates. VLC separations were carried out on TLC grade Si gel (MN Kieselgel).[11]



***m*-CPBA Oxidation of Dehydrocostus Lactone (1a).** A solution of 980 mg of dehydrocostus lactone (**1a**) in 20 mL of CH_2Cl_2 was added to a solution of 1.5 molar equivalents *m*-CPBA in 20 mL CH_2Cl_2 and stirred in an ice bath for 2 h. The reaction mixture was washed twice with 40 mL of 0.01 M NaOH solution and once with 40 mL of distilled H_2O. TLC of the reaction mixture revealed at least five products. Accordingly, the crude mixture was adsorbed onto Si gel and chromatographed on a VLC column (3.5 cm i.d.) using solvent mixtures of hexane and EtOAc of increasing polarity. Fraction 7 (100 mL hexane−EtOAc, 88:12) contained 70 mg of **3a**, and

Table 5. Coordinates and Equivalent Isotropic Thermal Parameters for **4a**

atom	*x*	*y*	*z*	B_{eq} (Å^2)[a]
O-1A	0.2190(6)	0.4585(3)	0.6081(1)	4.90(9)
O-2A	0.2238(8)	0.3525(3)	0.6657(2)	7.6(1)
O-3A	0.3605(8)	0.6486(3)	0.5576(2)	6.7(1)
O-4A	0.3271(9)	0.5685(3)	0.3922(2)	7.1(1)
C-1A	0.094(1)	0.5504(3)	0.4695(2)	4.6(1)
C-2A	0.052(1)	0.6452(4)	0.4611(3)	6.9(2)
C-3A	0.030(1)	0.6832(4)	0.5155(3)	6.8(2)
C-4A	0.155(1)	0.6263(4)	0.5512(2)	5.6(2)
C-5A	0.1081(9)	0.5395(3)	0.5322(2)	4.0(1)
C-6A	0.2498(9)	0.4712(3)	0.5502(2)	3.8(1)
C-7A	0.2090(9)	0.3858(3)	0.5241(2)	4.1(1)
C-8A	0.3402(9)	0.3713(4)	0.4751(2)	4.5(1)
C-9A	0.278(1)	0.4257(4)	0.4279(2)	5.5(2)
C-10A	0.271(1)	0.5183(4)	0.4392(2)	4.5(1)
C-11A	0.2355(9)	0.3269(4)	0.5693(3)	4.9(1)
C-12A	0.2267(9)	0.3743(4)	0.6191(2)	5.3(1)
C-13A	0.265(1)	0.2452(4)	0.5686(3)	7.2(2)
C-14A	0.459(1)	0.5651(5)	0.4375(3)	7.0(2)
C-15A	0.229(1)	0.6496(4)	0.6047(3)	6.6(2)
O-1B	0.7720(7)	0.5216(3)	0.1331(1)	5.26(9)
O-2B	0.7719(8)	0.6165(4)	0.0675(2)	7.8(1)
O-3B	0.9224(9)	0.3467(3)	0.1984(2)	7.8(1)
O-4B	0.838(1)	0.4558(4)	0.3558(2)	8.9(2)
C-1B	0.621(1)	0.4560(4)	0.2752(2)	5.6(2)
C-2B	0.595(2)	0.3635(5)	0.2893(3)	9.5(3)
C-3B	0.591(1)	0.3129(5)	0.2402(3)	8.5(2)
C-4B	0.713(1)	0.3648(4)	0.2018(2)	6.2(2)
C-5B	0.6541(9)	0.4543(4)	0.2123(2)	4.4(1)
C-6B	0.7904(9)	0.5206(4)	0.1919(2)	4.2(1)
C-7B	0.7388(9)	0.6105(4)	0.2097(2)	4.3(1)
C-8B	0.855(1)	0.6368(4)	0.2599(3)	5.8(2)
C-9B	0.785(1)	0.5910(5)	0.3107(3)	6.9(2)
C-10B	0.787(1)	0.4988(5)	0.3064(2)	5.6(2)
C-11B	0.7772(9)	0.6588(4)	0.1601(3)	4.9(1)
C-12B	0.7745(9)	0.6023(4)	0.1156(2)	5.5(1)
C-13B	0.813(1)	0.7399(5)	0.1558(4)	8.7(2)
C-14B	0.983(1)	0.4563(7)	0.3123(3)	8.6(2)
C-15B	0.794(2)	0.3340(5)	0.1524(3)	8.3(2)

[a] $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \boldsymbol{a_i} \cdot \boldsymbol{a_j}$.

fraction 8 (hexane−EtOAc, 86:14) provided 138 mg of **2**. Fraction 9 (hexane−EtOAc, 84:16) gave 18 mg **3b**, and fraction 11 (hexane−EtOAc, 4:1) contained 160 mg of crystalline **4a**. Fraction 14 (hexane−EtOAc, 3:2) contained a mixture of two diepoxides that required further purification. Crude fraction 14 was adsorbed onto Si gel and rechromatographed on a VLC column (2.3 cm i.d.) using hexane or hexane−EtOAc mixtures of increasing polarity. Fraction 14−10 (50 mL hexane−EtOAc, 77:23) contained 33 mg of **4b**, and fraction 14−11 (hexane−EtOAc, 76:24) gave 20 mg of crystalline **4c**.

Dehydrocostus lactone, 4α(15)-epoxide (2): gum; IR ν_{max} 1764 (C=O), 1637, 1258, 1138 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 246 $[M]^+$ (12), 228 $[M - H_2O]^+$ (5), 217 (10), 188 (20), 161 (24), 150 (37), 124 (64), 91 (100), 79 (67), 67 (43), 53 (81).

***m*-CPBA Oxidation of 2.** Compound **2** (40 mg) was dissolved in 5 mL of CH_2Cl_2 and added to 5 mL of CH_2Cl_2 solution containing 4 equivalents of *m*-CPBA. The reaction mixture was placed in an ice bath and stirred until TLC indicated that all **2** had reacted. The reaction was stopped after 2 h and worked up as described previously. Crude reaction products were chromatographed as indicated above to yield 16 mg of **4b** and 11 mg of **4c**.

Dehydrocostus lactone, 10β(14)-epoxide (3a): gum; IR ν_{max} 1763 (C=O), 1255, 996 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 246 $[M]^+$ (2), 228 $[M - H_2O]^+$ (7), 215 (44), 199 (9), 187 (12), 173 (14), 159 (21), 150 (99), 123 (41), 105 (45), 91 (100), 79 (76), 53 (84).

Dehydrocostus lactone, 10α(14)-epoxide (3b): gum; IR ν_{max} 1764 (C=O), 1254, 995 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 246 $[M]^+$ (2), 228 $[M - H_2O]^+$ (3), 216 (13), 200 (8), 171 (13), 159 (12), 120 (36), 105 (40), 91 (69), 80 (100), 53 (47).

Table 6. Coordinates and Equivalent Isotropic Thermal Parameters for **4c**

atom	*x*	*y*	*z*	B_{eq} (Å^2)[a]
O-1	0.5714(2)	0.7098(1)	0.39907(9)	4.80(3)
O-2	0.7116(2)	0.8958(2)	0.3942(1)	7.04(4)
O-3	0.5560(2)	0.3325(2)	0.3468(1)	8.42(4)
O-4	−0.0431(2)	0.5139(2)	0.2787(1)	9.48(5)
C-1	0.2248(3)	0.4807(2)	0.3402(1)	5.54(5)
C-2	0.2003(3)	0.3974(3)	0.4198(2)	7.53(6)
C-3	0.3615(3)	0.3791(2)	0.4603(2)	6.49(6)
C-4	0.4800(3)	0.4308(2)	0.3980(2)	5.58(5)
C-5	0.3959(2)	0.5394(2)	0.3495(1)	4.35(4)
C-6	0.4015(2)	0.6694(2)	0.3953(1)	3.75(3)
C-7	0.3146(2)	0.7833(2)	0.3526(1)	4.07(3)
C-8	0.1412(3)	0.8038(2)	0.3835(2)	5.25(4)
C-9	0.0429(3)	0.6768(3)	0.3853(2)	6.40(6)
C-10	0.0917(3)	0.5773(2)	0.3197(2)	5.99(5)
C-11	0.4261(3)	0.8959(2)	0.3666(1)	4.46(4)
C-12	0.5842(2)	0.8408(2)	0.3868(1)	4.86(4)
C-13	0.3982(3)	1.0239(2)	0.3637(2)	6.55(6)
C-14	0.0568(3)	0.6065(4)	0.2316(2)	8.23(8)
C-15	0.6526(4)	0.4132(3)	0.4015(2)	8.58(7)

[a] $B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \boldsymbol{a_i} \cdot \boldsymbol{a_j}$.

Table 7. Minimum Inhibitory Concentrations of Compounds **1a**−**6** against *Mycobacterium tuberculosis*

compound	MIC (μg/mL)	compound	MIC (μg/mL)
1a[a]	2	**4a**	64
1b	>128	**4b**	128
1c	>128	**4c**	128
2	32	**5**	50
3a	32	**6**[a]	128
3b	64		

[a] Lactones **1a** and **6** were also tested against *M. avium* and gave MICs of 16 and 128 μg/mL, respectively.

Dehydrocostus lactone, 4β(15),10α(14)-diepoxide (4a): colorless crystals (hexane−EtOAc); mp 97 °C; IR ν_{max} 1765 (C=O), 1254, 996, 867 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 262 $[M]^+$ (1), 244 $[M - H_2O]^+$ (2), 231 (35), 214 (27), 203 (13), 185 (26), 173 (17), 159 (24), 105 (47), 91 (100), 79 (79), 67 (59), 53 (80).

Dehydrocostus lactone, 4α(15),10α(14)-diepoxide (4b): gum; IR ν_{max} 1763 (C=O), 1258, 732 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 262 $[M]^+$ (1), 261 (3), 247 $[M - CH_3]^+$ (1), 244 $[M - H_2O]^+$ (2), 231 (14), 215 (7), 203 (19), 187 (13), 175 (20), 151 (29), 131 (33), 117 (44), 105 (47), 91 (100), 79 (67), 67 (54), 53 (58).

Dehydrocostus lactone, 4α(15),10β(14)-diepoxide (4c): colorless crystals (hexane−EtOAc); mp 133−135 °C; IR ν_{max} 1765 (C=O), 1258, 999 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS 70 eV *m/z* 262 $[M]^+$ (1), 244 $[M - H_2O]^+$ (2), 231 (4), 215 (5), 203 (9), 151 (19), 131 (32), 117 (34), 105 (41), 91 (100), 79 (80), 67 (76), 53 (99).

Selenium Dioxide Oxidation of Dehydrocostus Lactone (1a). Oxidation was performed as previously described, and the crude product was purified by preperative TLC to yield pure **1b**.[7] The NMR data were essentially identical with values reported in the literature,[7] and the molecular structure was unambiguously established by single-crystal X-ray diffraction.

Compounds 1c, 5, and 6. The natural sesquiterpene lactones 7α-hydroxydehydrocostus lactone (**1c**),[12] micheliolide (**5**),[13] and pumilin (**6**)[14] have been obtained previously in our laboratory.

X-ray Crystallographic Analyses.[15] Intensity data were collected for **1b**, **4a**, and **4c** on an Enraf−Nonius CAD4 diffractometer with graphite-monochromated Cu Kα radiation (λ = 1.54184 Å) by ω-2θ scans. Crystals of **4a** were of considerably lower quality than those of the other two compounds, yielding broad diffraction peaks. The resulting structure determination is of lower precision, but is sufficient to establish the molecular structure. Two octants of data were collected for **4a** and five octants for **4c**. Data reduction

included corrections for background, Lorentz, polarization, decay (for **4a**, 19%), and absorption (for **4c**) effects. Absorption corrections were based on ψ scans, which, for **4c**, indicated no change in intensity with rotation about the diffraction vector. Crystals of **1b** are isomorphous with dehydrocostus lactone; all atoms but C-3 of that structure were used as a beginning refinement model. Structures **4a** and **4c** were solved by direct methods. Refinement was by full-matrix least squares, with neutral-atom scattering factors and anomalous dispersion corrections. All nonhydrogen atoms were refined anisotropically, while H atoms were treated as specified in Table 3. Refinement of the absolute structures of **1b** and **4c** is consistent with the expected absolute configurations. Crystal data, details of data collection and refinement, and final agreement indices for the three structures are given in Table 3.

Radiorespirometric Bioassays. Bioassays were performed essentially as described previously.[3,16−18] Experiments for *M. avium* were usually completed within 5 days and those for *M. tuberculosis* in 10 days.

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