New Antimicrobial Cycloartane Triterpenes from *Acalypha communis*

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Three new cycloartane-type triterpenes, 16α-hydroxymollic (**1**), 15α-hydroxymollic (**2**), and 7*β*,16*β*-dihydroxy-1,23-dideoxyjessic acids (**3**), were isolated from the aerial parts of *Acalypha communis*. The structures of the novel triterpenes were determined by spectroscopic methods as well as chemical derivatization. These compounds were tested for their antimicrobial activity against Gram-positive and -negative bacteria. Compounds **1**−**3** exhibited moderate antimicrobial activity (MIC 8, 32, 8 *μ*g/mL, respectively) against vancomycin-resistant enterococci. In addition, compound **1** was found to be active against methicillin-resistant staphylococci. In contrast, compounds **1**−**3** were poorly active against Gram-negative bacteria. Compound **3** was tested in an in vivo model; it did not provide protection to mice infected with *Staphylococcus aureus*.

Acalypha, the fourth largest genus in the family Euphorbiaceae with about 450 species, is widespread throughout the tropics except in Hawaii and a few Pacific archipelagos.[1] Some species are widely used as traditional medicines throughout Latin America for treating skin diseases, venereal infections, headache, and inflammation and as diuretics.[2]

Despite the diversity on this genus, to date only few species have been chemically studied. The chemical investigation of *Acalypha* afforded the isolation of different classes of natural products including phenolic compounds such as flavonoids and tannins, sterols, diterpenes, and amides as well as cyanogenic glycosides.[3−10]

Acalypha communis Müll. Arg. is a wild species that grows in arid lands in Argentina, Bolivia, and Paraguay. The decoction or fresh cataplasm of this herb is used in folk medicine as a purgative and in the treatment of skin wounds.[11]

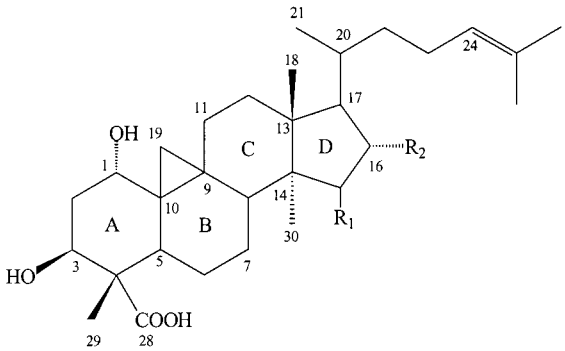
As a part of our research program to discover bioactive agents from dryland plants in Latin America,[12] a CH_2Cl_2−MeOH extract of the aerial part of *A. communis* was selected for bioassay-guided fractionation on the basis of its antimicrobial activity against *Staphylococcus aureus* and *Enterococcus faecium*. In the present investigation, we describe the identification and antimicrobial activity of three new naturally occurring cycloartane-type triterpenes.

Results and Discussion

16α-Hydroxymollic acid (**1**), 15α-hydroxymollic acid (**2**), and 7*β*,16*β*-dihydroxy-1,23-dideoxyjessic acid (**3**) were isolated and purified from *A. communis* as described in the Experimental Section.

16α-Hydroxymollic acid (**1**) was obtained as a white amorphous solid, and its molecular formula was determined as $C_{30}H_{48}O_5$ using HR-FABMS. Absorption bands at 3447−3423 and 1706 cm^{-1} in the IR spectrum indicated the presence of hydroxyl and carbonyl groups, respectively.

The ^{13}C spectrum of **1** exhibited signals of 30 carbons (Table 1). Analysis of the ^{13}C and DEPT spectra indicated the presence of six methyls, nine methylenes, eight methines, and seven tetrasubstituted carbons. The signal at *δ* 180.9 in the ^{13}C spectrum was attributed to a carbonyl carbon, while the signals at *δ* 73.4, 71.4, and 78.1 indicated the presence of carbons bearing a hydroxyl group.



	R_1	R_2
1	H	α OH
2	α OH	H

The ^1H NMR spectrum of **1** (Table 1) displayed two upfield shifted doublets as an AX system (*δ* 0.69 and 0.48 both d, $J = 4.6$ Hz) assignable to a cyclopropyl methylene group (H_2-19) characteristic of cycloartane-type triterpenes.[13−17] Signals for five tertiary (1.05, 1.18, 1.02, 1.66, 1.60) and one secondary methyl (0.93, d, $J = 6.4$) group could also be discerned in the ^1H spectrum. The signals at *δ* 4.52 (dd, $J = 12.0$, 5.4 Hz), 3.95 (dd, $J = 7.8$, 6.4 Hz), and 3.52 (t, $J = 2.7$ Hz) indicated the presence of three protons attached to carbons bearing a hydroxyl group. In addition, the signal at 5.12 (t, $J = 7.3$ Hz) indicated the presence of an olefinic proton.

The ^{13}C NMR data of **1** were similar to those of mollic acid[18−20] except that compound **1** had one additional hydroxyl group. In addition, the mass spectrum of **1** gave prominent peaks due to the sequential losses of three molecules of water, supporting the presence of three free hydroxyl groups in the molecule. The presence of three hydroxyl groups was supported by silylation of **1**.

Full assignments of the proton and carbon signals and the position of the three hydroxyl groups of **1** were

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **1**−**3** in Methanol-d_4

	1		**2**		**3**	
position	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)
1	73.4	3.52 (t, 2.4)	73.7	3.52 (br s)	32.2	1.63[a]
						1.34[a]
2	37.8	1.84[a]	37.7	1.85[a]	30.3	1.78[a]
		1.77[a]		1.78[a]		1.62[a]
3	71.4	4.52 (dd, 12.0, 5.4)	71.5	4.52 (dd, 11.9, 4.8)	76.1	4.01 (dd, 10.8, 4.4)
4	55.9		55.9		55.3	
5	38.3	2.56 (dd, 12.6, 4.8)	38.1	2.56 (dd12.8, 4.6)	43.8	2.13 (dd, 12.2, 4.1)
6	24.0	1.30[a]	24.1	1.30[a]	33.7	1.38[a]
		0.97 (dd, 12.4, 2.8)		0.97 (dd, 12.8, 1.8)		1.14 (d 11.4)
7	26.7	1.25[a]	26.5	1.63[a]	70.7	3.50 (ddd, 10.9, 8.5, 4.1)
		1.25[a]		1.16[a]		
8	50.0	1.46 (dd 11.5, 5.0)	50.7	1.58 (dd, 12.4, 4.1)	55.2	1.75 (d, 8.4)
9	21.5		22.4		21.2	
10	30.4		30.2		26.9	
11	26.8	2.47 (ddd, 14.9, 10.6, 6.4)	26.4	2.46 (ddd 14.7, 11.0, 7.3)	27.6	1.88[a]
		1.25		1.23		1.37[a]
12	33.8	1.76[a]	34.9	1.75[a]	33.8	1.63[a]
		1.66, m		1.63[a]		1.60[a]
13	48.0		51.5		47.5	
14	48.4		46.9		46.8	
15	49.0	1.85[a]	79.7	3.89 (dd, 9.6, 5.0)	50.4	2.17[a]
		1.37 (d, 13.75)				1.64[a]
16	78.1	3.95 (dd, 7.8, 6.4)	40.4	1.82[a]	73.1	4.34 (dt, 7.8, 6.0)
				1.68[a]		
17	62.6	1.61, m	51.8	1.67[a]	56.8	1.65[a]
18	19.8	1.02 (s)	18.9	1.03 (s)	18.5	1.17 (s)
19	31.4	0.69 (d, 4.6)	31.3	0.70 (d, 4.6)	28.8	0.79 (d, 4.2)
		0.48 (d, 4.6)		0.51 (d, 4.6)		0.35 (d, 4.2)
20	35.6	1.52, m	36.9	1.35[a]	31.6	1.87[a]
21	19.1	0.93 (d, 6.4)	18.7	0.88 (d, 6.4)	18.7	0.96 (d, 6.6)
22	36.7	1.79[a]	37.4	1.42, m	36.4	1.86[a]
		1.16 (dd, 13.3, 5.0)		1.04[a]		1.18[a]
23	26.5	2.13, m	25.8	2.04, m	32.7	2.16[a]
		1.91, m		1.89, m		2.00 (td 10.8, 5.4)
24	126.4	5.12 (t, 7.3)	126.1	5.08 (t, 7.3)	158.1	
25	131.8		131.9		35.0	2.28 (sep, 6.6)
26	25.9	1.66 (s)	25.9	1.67 (s)	22.5	1.02 (dd, 6.6, 1.8)
27	17.8	1.60 (s)	17.7	1.60 (s)	22.4	1.02 (dd, 6.6, 1.8)
28	180.9		181.1		180.6	
29	9.2	1.05 (s)	9.2	1.06 (s)	9.8	1.09 (s)
30	20.6	1.18 (s)	12.0	1.00 (s)	20.0	0.94 (s)
31					106.6	4.70 (br s)
						4.69 (br s)

[a] Overlapping, assignment determined by COSY, HSQC, HMBC, and ROESY.

established by the analysis of the HSQC and HMBC spectra (Table 1 and Figure 1).

The stereochemistry of **1** was determined by the analysis of the ROESY spectrum and ^{1}H−^{1}H coupling constants. The lack of any diaxial coupling of H-1 (J = 2.4 Hz) with H-2$_{ax}$ indicated that the hydroxyl group at C-1 is located in the axial position. This was supported by the correlation observed between H-1 and H-19 in the ROESY spectrum (Figure 1b). The H-3 signal was observed as a double doublet due to diaxial (J = 12.0 Hz) and axial−equatorial coupling (J = 5.4 Hz) with H-2$_{ax}$ and H-2$_{ec}$, respectively. These observations suggested the axial nature of H-3. The α configuration of the hydroxyl group at C-16 was determined on the basis of the coupling constants between H-16 and H-15$_{ax}$ (J = 7.8 Hz) for the H-16 double doublet and the ROESY correlation with H-18.

15α-Hydroxymollic acid (**2**) was isolated as white crystals and had the same molecular formula $C_{30}H_{48}O_5$ as **1**. The IR, ^{1}H NMR, and ^{13}C NMR spectra (Table 1) of **2** were almost the same as those of **1**. Differences between **1** and **2** were apparent only in the signals in ring D. Analysis of its HMBC spectrum (Figure 1a) indicated the presence of a hydroxyl group on ring D as C-15 instead of C-16, as in **1**. The stereochemistry of ring A was determined to be the same as **1**, and the configuration of the C-15 hydroxyl group was determined as α on the basis of the coupling constants (J = 9.6, 5.0 Hz) as well as the analysis of the ROESY spectrum (Figure 1b).

7β,16β-Dihydroxy-1,23-dideoxyjessic acid (**3**) was obtained as white crystals, and its molecular formula was determined as $C_{31}H_{50}O_5$. The IR spectrum of **3** showed a broad absorption band at 3446−3355 cm^{-1} and a sharp absorption band at 1706 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum of 3 (Table 1) displayed one AX system (δ 0.79 and 0.35, both d, J = 4.2 Hz) assignable to a cyclopropyl methylene group (H_2-19) and signals for three tertiary (1.17, 1.09, 0.94) and three secondary methyls (1.02, 1.02, 0.96). The signals at δ 4.01 (dd, J = 10.8, 4.4 Hz), 3.50 (ddd, J = 10.9, 8.5, 4.1 Hz), and 4.34 (dt, J = 7.8, 6.0 Hz) indicated the presence of three carbons attached to oxygen. Additionally, two singlets at 4.70 and 4.69 were assignable to terminal methylene protons.

The mass spectrum of **3** gave prominent peaks due to the sequential losses of three molecules of water, supporting the presence of three free hydroxyl groups. The presence of these groups was supported by silylation of **3**. The position of the hydroxyl groups was determined to be at C-3, C-7, and C-16 by the analysis of the HMBC spectrum (Figure 2a).

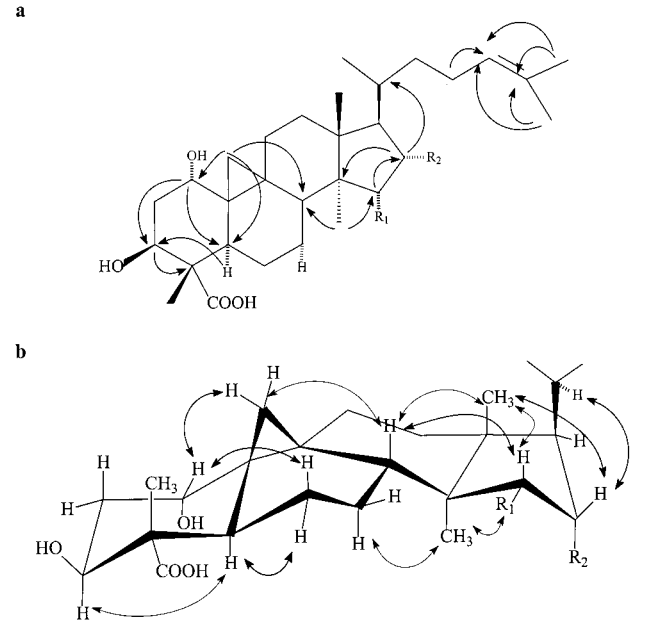

Figure 1. (a) Significant correlation observed in the HMBC spectrum of compounds **1** and **2**. (b) NOEs observed in the ROESY spectrum of compounds **1** and **2**. (**1**) R_1 = H, R_2 = OH, (**2**) R_1 = OH, R_2 = H.

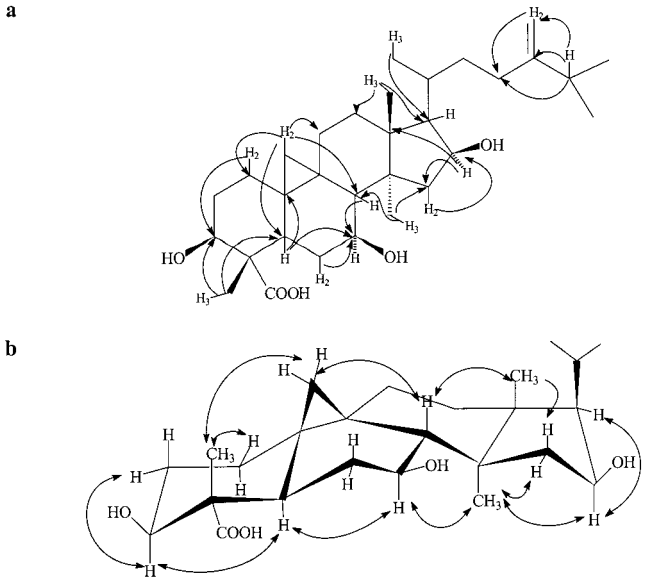

Figure 2. (a) Significant correlation observed in the HMBC spectrum of compound **3**, and (b) NOEs observed in the ROESY spectrum of compound **3**.

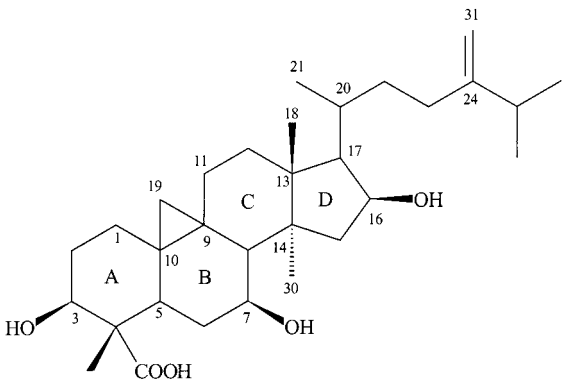

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Table 2. Antimicrobial Activity (μg/mL) of Compounds **1**−**3**

organism	compound			
	1	**2**	**3**	penicillin G
Staphylococcus aureus 375	32	32	16	0.06
S. aureus 310 (MR)[a]	64	128	>128	128
Enterococcus faecium 379 (VR)[b]	8	32	8	128
Escherichia coli imp 389	64	>128	>128	2
Bacillus subtilis PY79	32	64	16	8

[a] MR: Meticillin resistant. [b] VR: Vancomycin resistant.

Table 3. In Vivo Activity of Compound **3** (*Staphylococcus aureus* Smith Acute Lethal Infection Model in Mice)

compound	ED_{50} (mg/kg)
3	no protection up to 64 mg/kg
vancomycin	1.0
saccharomycin	0.6

Compound **3** is thus an isomer of 7β-hydroxy-23-deoxyjessic acid.[13] The difference between **3** and this compound is the presence of a hydroxyl group at C-16 and its absence at C-1 in **3**. This assignment was deduced from the long-range correlation observed in the HMBC spectrum between H-16 and C-13, C-14, and C-15 and between H-15 and C-16 (Figure 2a).

The configuration of the hydroxyl groups at C-3, C-7, and C-16 was concluded to be β on the basis of coupling constants of H-3 (dd, J = 10.8, 4.4 Hz), H-7 (ddd, J = 10.9, 8.5, 4.1 Hz), and H-16 (dt, J = 7.8, 6.0 Hz). The diaxial coupling of H-7 and H-6_{ax} (10.5 Hz) and H-8_{ax} (8.7 Hz) suggested the β configuration of the OH group at C-7. In the case of H-16, the diaxial coupling of H-16 and H-15_{ax} (7.8 Hz) and the axial−equatorial coupling of H-16 with both H-15_{eq} and H-17_{eq} indicated the β configuration of the OH group at this position. These observations were further confirmed by the correlation observed in the NOESY spectrum (Figure 2b).

Compounds **1**−**3** were tested for their antimicrobial activity against Gram-positive and -negative bacteria (Table 2). Compounds **1**−**3** exhibited moderate antimicrobial activity against Gram-positive bacteria. The MIC values for *E. faecium*, *S. aureus*, and *B. subtillis* were 8−32, 16−32, and 16−64 μg/mL, respectively. Interestingly, the three compounds showed better antimicrobial activity against vancomycin-resistant enterococci than penicillin G, which was used as control (MIC 128 μg/mL). In addition, compound **1** was also found active against methicillin-resistant staphylococci, having a MIC value of 64 μg/mL. Compound **2** and penicillin G were found to be equipotent against methicillin-resistant staphylococci (MIC of 128 μg/mL). In contrast, compounds **1**−**3** were essentially inactive against *E. coli*, with MIC values ranging between 64 and >128 μg/mL.

Compound **3** was also tested in an in vivo model. The results summarized in Table 3 reveal that it did not provide protection to mice infected with *S. aureus*.

Experimental Section

General Experimental Procedures. NMR spectra including DEPT 135, HMBC, HSQC, NOESY, and ROESY experiments were recorded on a Bruker DRX 600 spectrometer equipped with a triple resonance indirect detection probe (^{1}H {^{13}C, ^{15}N}). All the spectra were recorded in methanol-d_4, at 293 K operating at 600.133 MHz for ^{1}H and 150.903 for ^{13}C. Solvent signals δ_H 3.30 and δ_C 49.0 were used to reference the spectra. HR-FABMS spectra were recorded using a JEOL HX110 spectrometer with a resolution of 10 000, using a mixed matrix consisting of glycerol, thioglycerol, and m-NBA. Optical

rotations were performed on a JASCO P1020 polarimeter. IR spectra were obtained using KBr disks on a Buck Scientific 500 spectrometer. Melting points were measured in a Fisher-Johns apparatus and are uncorrected. TLCs were sprayed with 0.5% anisaldehyde in methanol and heated until colored spots appeared.

Plant Material. Aerial parts of *Acalypha communis* Müll. Arg. var. *saltensis* Pax et Hoffman were collected and identified by Renée H. Fortunato in November 1995, in the district Ojo del Agua, Santiago de Estero, Argentina. A specimen has been deposited in the herbarium of INTA, Castelar, Buenos Aires, Argentina (coll. no. RF5063). Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and INTA.

Extraction and Isolation. The air-dried aerial parts (1 kg) of *A. communis* were extracted by maceration using a mixture of CH_2Cl_2−MeOH (1:1) at room temperature. The organic extract was concentrated under reduced pressure to yield 40.5 g of residue. The resulting extract was evaluated for its antimicrobial activity on a panel of selected microorganisms.[21] The results indicated antimicrobial activity against *S. aureus* and *E. faecium* when tested using the agar diffusion method.

The active extract was then fractionated by column chromatography on silica gel (400.0 g) (Merck 63, 0.2 mm) and eluted with a step gradient of acetone in hexane starting with 100% hexane. Fractions were collected and pooled on the basis of their TLC profiles to yield 22 primary fractions (F001−F022). Fractions F015−F017, F018−F021, and F022 were found active against Gram-positive bacteria. Further column chromatography of the most active fraction F022 (30.0 g) on RP-18 (100 g), eluting with a step gradient of methanol in water starting with 100% water, afforded seven fractions (F0023−F029). The most polar fraction F029 (6.1 g) exhibited good activity against *S. aureus* and *E. faecium*. Reversed-phase HPLC of this fraction led to the isolation of the active compounds. RP-HPLC was carried out using a 10 × 250 mm Reliasil C_{18} column (Column Engineering), eluting with a gradient of 60% acetonitrile in aqueous 0.15% HCOOH to 100% acetonitrile in 20 min at a flow rate of 5.0 mL/min, and detection at 200 nm led to the isolation of the active compounds. In these chromatographic conditions, compounds **1**−**3** have retention times of 12.56, 13.10, and 15.28 min, respectively.

16α-Hydroxymollic acid (1): white crystals, mp 233−236 °C; $[\alpha]^{25}_D$ +43.7° (*c* 0.55, MeOH); IR (KBr) ν_{max} 3447, 3439, 3423, 2930, 2886, 1706, 1458, 1376, 1261, 1083 cm^{-1}; ^{13}C NMR, ^1H NMR data, see Table 1; FABMS *m/z* 488.3543 (calcd for $C_{31}H_{50}O_5$).

15α-Hydroxymollic acid (2): white crystals, mp 232−234 °C; $[\alpha]^{25}_D$ +67.22° (*c* 0.18, MeOH); IR (KBr) ν_{max} 3440, 3423, 2929, 1701, 1458, 1376, 1262; ^{13}C NMR, ^1H NMR data, see Table 1; FABMS *m/z* 488.3492 (calcd for $C_{31}H_{50}O_5$).

7β,16β-Dihydroxy-1,23-dideoxyjessic acid (3): white crystals, mp 229−231 °C; $[\alpha]^{25}_D$ +61.39° (*c* 1.02, MeOH); IR (KBr) ν_{max} 3446, 3430, 3355, 2958, 2942, 2870, 1706, 1458, 1376, 1255, 1098; ^{13}C NMR, ^1H NMR data, see Table 1; FABMS *m/z* [M − H] 502.03750 (calcd for $C_{31}H_{50}O_5$); FABMS *m/z* 503 $[M + 1]^+$, 485 $[M + 1 - H_2O]^+$, 467 $[M + 1 - 2H_2O]^+$, 449 $[M + 1 - 3H_2O]^+$.

Preparation of the Trimethylsilyl Derivatives. Compounds **1** (1.5 mg), **2** (1.0 mg), and **3** (2 mg) were dissolved in anhydrous methanol and incubated overnight with 100 μL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Aldrich). The resultant derivatives were analyzed using FABMS.

Antimicrobial Activity. Preliminary screening of the plant extract was performed by the agar diffusion method. The media used were Difco nutrient agar (pH 6.8) for *S. aureus* and LB (Luria-Bertani) agar for *E. faecium*. Assay plates (12 in. × 12 in. Sumilon) were prepared by pouring a 125 mL volume of agar medium (tempered at 50 °C) inoculated with an overnight broth culture of the test organisms (adjusted to approximately 10^6 cells per mL). Ten microliters volume of antibiotic solution diluted in DMSO was spotted onto the agar surface, and the plates were incubated at 37 °C for 18 h. The zone of growth inhibition was measured using a hand-held digital caliper. Minimum inhibitory concentrations (MICs) were determined by a standard broth microdilution method as described earlier.[21] Briefly, 5 μL of an overnight broth bacterial culture (adjusted to density of 1×10^7 to 5×10^7 CFU/ml) was added to 0.1 mL of broth medium in polystyrene plates containing the drug at 0.03−128 μg/mL concentration. The MIC was defined as the lowest concentration of antibiotic that prevented visual turbidity after 18−20 h of incubation at 37 °C. The bacterial strains used are laboratory cultures maintained in the Wyeth Research collection.

In Vivo Activity. In vivo activity was assessed in female strain CD-1 mice (Charles River Laboratories, Kingston NY), weighing 20 ± 2 g each, which were infected intraperitoneally with sufficient bacterial cells suspended in broth or with about 5% mucin to kill 95−100% of the untreated mice within 48 h. Antibiotic was administered in single doses 0.5 h after infection. Seven-day survival ratios from three or four separate tests, each with five dose levels and five animals per dose level, were pooled for the determination of the median effective dose (ED_{50}) by probit analysis.[21]

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