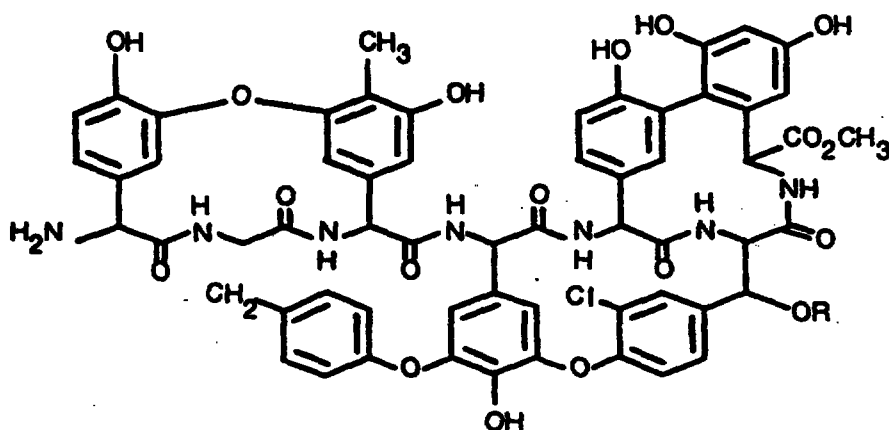


Description

Background of the Invention

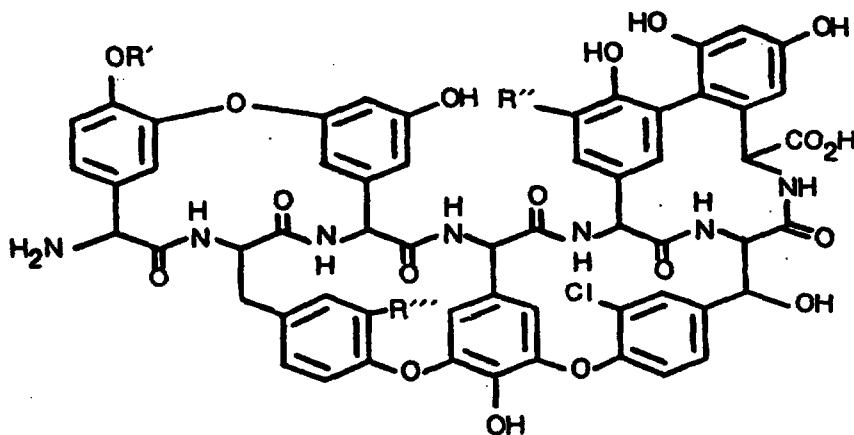
Novel AAD 216 antibiotics of the vancomycin-class are produced by cultivating the new micro-organism, *Kibdelosporangium aridum* Shearer, gen. nov., sp. nov. SK&F AAD 216 (ATCC 39323) in aqueous nutrient medium, containing assimilable sources of carbon and nitrogen, under submerged aerobic conditions until a substantial amount of the AAD 216 antibiotic complex is produced and optionally recovering the AAD 216 complex from the culture medium and isolating the individual major antibiotic factors, AAD 216A, AAD 216B, and AAD 216C. The AAD 216 antibiotics and the microorganism, *K. aridum*, are disclosed and claimed in a co-pending European Patent Application (EP-A-132118), Attorney Docket No. SKB 14168 (claiming priority from US Serial No. 513,513 filed July 13, 1983), filed concurrently herewith. A copy of this is enclosed with the documents of the present application and is to form part of the dossier.

European patent application 55069 describes an antibiotic A-4696 pseudo-aglycone, produced by mild acid hydrolysis of the actaplanin antibiotic A-4696 complex, and is of the formula:



wherein R is L-ristosamine.

European patent application 90578 describes A 41030 antibiotics of the formula:



wherein

- R' is H, R'' is Cl and R''' is Cl (Factor A);
- R' is H, R'' is H and R''' is Cl (Factor B);
- R' is Galactose, R'' is Cl and R''' is Cl (Factor C);
- R' is H, R'' is H and R''' is H (Factor E); or
- R' is Galactosylgalactose, R'' is Cl and R''' is Cl (Factor F).

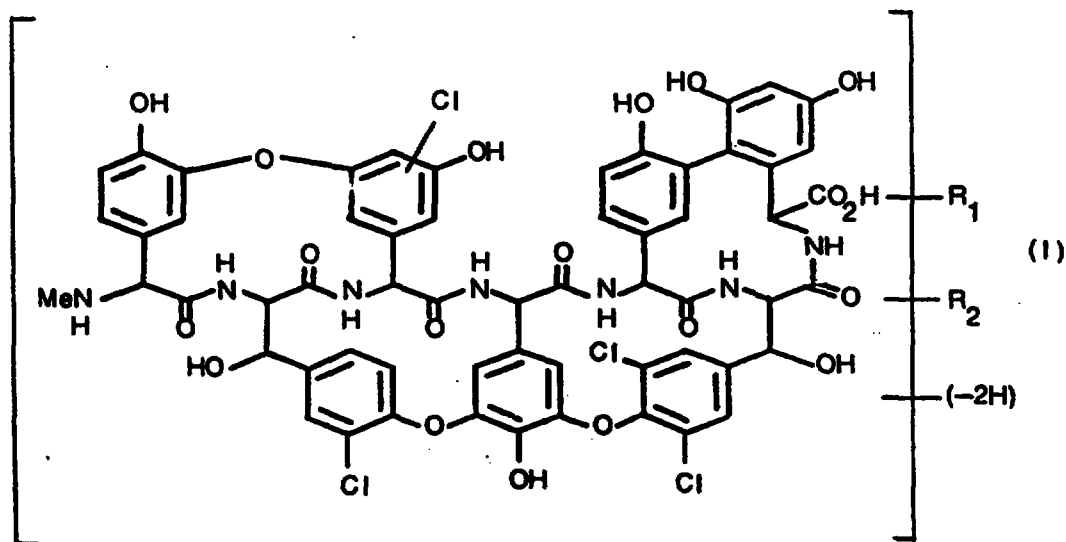
Summary of the Invention

This invention relates to the novel aglycone and pseudo-aglycones of the AAD 216 antibiotics which are prepared by the partial acid hydrolysis of the AAD 216 antibiotics complex or the individual antibiotic factors which comprise the AAD 216 complex followed by chromatographic isolation. The compounds of this invention exhibit antibacterial activity and are useful in animal health applications such as growth promotants and the treatment of bovine mastitis.

Detailed Description of the Invention

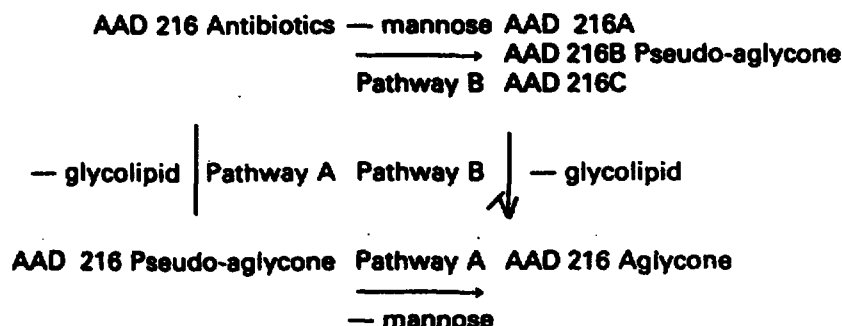
The aglycone and pseudo-aglycones of the instant invention are prepared by the partial acidic

hydrolysis of the AAD 216 antibiotics, which for the purpose of the present application is defined as the AAD 216 antibiotic complex and its major individual antibiotic factors, AAD 216A, AAD 216B and AD 216C. The aglycone and the pseudo-aglycones of the AAD 216 antibiotics are represented by the following general structural formula (I):



wherein R_1 is hydrogen or a mannosyl radical and R_2 is hydrogen or a glycolipid radical of unknown structure derived from the hydrolyzed AAD 216 antibiotic with the proviso that at least one of R_1 and R_2 is hydrogen. The AAD 216 antibiotic complex is the compound of formula (I) wherein R_1 is mannosyl and R_2 is a glycolipid radical.

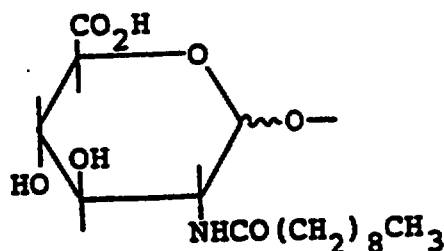
The hydrolysis of the AAD 216 antibiotics proceeds along two pathways to afford the AAD 216 aglycone of the formula (I) wherein R_1 and R_2 are hydrogen as follows:



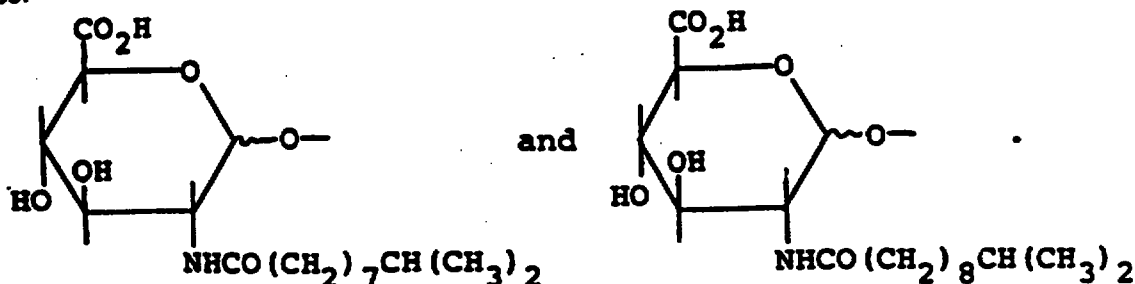
The initial step of the hydrolysis pathway A involves the loss of the glycolipid radicals from the AAD 216 antibiotics to yield the pseudo-aglycone of formula (I) wherein R_1 is mannosyl and R_2 is hydrogen. This pseudo-aglycone is denominated AAD 216 pseudo-aglycone since it is independent of which AAD 216 antibiotic is hydrolyzed. Subsequent hydrolysis of the AAD 216 pseudo-aglycone yields the AAD 216 aglycone upon the loss of the mannosyl radical.

The initial step of the hydrolysis pathway B involves the loss of the mannosyl radical from the AAD 216 antibiotics to give pseudo-aglycones of the formula (I) wherein R_1 is hydrogen and R_2 is a glycolipid radical of unknown structure which is derived from the AAD 216 antibiotic factor hydrolyzed. Accordingly, these pseudo-aglycones are denominated AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone or AAD 216C pseudo-aglycone depending on the AAD 216 antibiotic factor hydrolyzed. Where the AAD 216 complex is hydrolyzed, a mixture of these pseudo-aglycones will result.

The AAD 216A pseudo-aglycone contains a glycolipid radical of unknown structure with a molecular weight of 330 amu and an empirical formula of $C_{16}H_{28}NO_6$. The methanolysis of AAD 216A pseudo-aglycone produced two major products which were identified as the methylglycosidemethyl ester of an *n*-decanoyldeoxyaminoglycuronic acid and the methylglycoside of either *n*-decanoyldeoxyaminoglycofuran-3,6-lactone or *n*-decanoyldeoxyaminoglycopyran-3,6-lactose. The diacetate derivative of first major methanolysis product of AAD 216A pseudo-aglycone was compared with and found to be identical to the diacetate derivative of methyl-2-deoxy-2-[(1-oxodecyl)amino]- α -D-glycopyranosiduronic acid methyl ester, which was prepared in a simple two-step process from D-glucosamine. From the above evidence, the structure of the glycolipid radical of AAD 216A pseudo-aglycone is most probably as follows:



Since AAD 216A, AAD 216B and AAD 216C are homologs, the glycolipid radicals of AAD 216B pseudo-aglycone and AAD 216C pseudo-aglycone have molecular weights of 344 and 358, respectively, and empirical formulae of $C_{17}H_{30}NO_6$ and $C_{18}H_{32}NO_6$, respectively. Utilizing comparative studies of the ^{13}C nuclear magnetic resonance spectra of AAD 216A, AAD 216B and AAD 216C as well as a comparative studies of the fatty acid hydrolysis products of the parent antibiotic factors, the glycolipid radicals of AAD 216B pseudo-aglycone and AAD 216C pseudo-aglycone are tentatively assigned the following respective structures:



The AAD 216 pseudo-aglycone of the formula (I) wherein R_1 is mannosyl and R_2 is hydrogen has the following characteristics:

- (a) a pale white-yellow solid which decomposes at 300–350°C;
- (b) an empirical formula $C_{35}H_{55}N_7O_{24}Cl_4$;
- (c) an approximate elemental composition of 47.54 percent carbon, 4.00 percent hydrogen, 5.89 percent nitrogen and 8.63 percent chlorine when the water content was 12 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1230, 1180, 1150, 1120, 1060, 1010, 970 and 810;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1458 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acid conditions with an $E_{1\%} = 79.7$ and at 300 nm under basic conditions with an $E_{1\%} = 136$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 8.7 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 177.9, 174.6, 171.7, 170.9, 170.0, 169.2, 162.3, 158.8, 157.9, 155.2, 155.1, 153.9, 151.9, 149.7, 147.6, 147.2, 144.4, 141.0, 139.0, 138.8, 137.5, 136.1, 134.6, 130.7, 130.0, 129.8, 129.2, 128.9, 128.7, 127.5, 127.3, 126.9, 126.4, 126.0, 125.2, 122.7, 122.2, 121.1, 119.9, 119.7, 118.4, 116.6, 110.7, 110.4, 108.5, 104.4, 103.3, 100.5, 98.1, 74.0, 72.1, 71.6, 71.4, 70.8, 67.4, 65.8, 63.7, 62.2, 61.6, 60.2, 56.0, 55.9, 55.0 and 32.9; and
- (h) pK_a values in acetonitrile:water (3:7) as follows: 3.3, 7.1, 8.3, 9.1, 10.0 and 11.2 pK_a values above 11.2 not determined.

The AAD 216A pseudo-aglycone of the formula (I) wherein R_1 is hydrogen and R_2 is a glycolipid of unknown structure having an empirical formula $C_{16}H_{28}NO_6$ has the following characteristics:

- (a) a pale white-yellow solid which decomposes at 300–350°C;
- (b) an empirical formula $C_{75}H_{72}N_8O_{25}Cl_4$;
- (c) an approximate elemental composition of 49.76 percent carbon, 4.51 percent hydrogen, 5.99 percent nitrogen and 8.19 percent chlorine when the water content was 9.5 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1460, 1430, 1300, 1240, 1140, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1625 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 63.2$ and at 302 nm under basic conditions with an $E_{1\%} = 94$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.2, 178.0, 176.0, 175.6, 171.6, 170.9, 170.6, 170.5, 169.3, 164.6, 161.4, 159.3, 157.1, 156.5, 153.5, 152.5, 151.8, 151.1, 146.4, 144.9, 141.7, 138.6, 138.4, 136.9, 134.5, 134.4, 133.9, 130.8, 129.8, 129.7, 129.4, 128.6, 128.5, 128.3, 127.7, 127.3, 125.9, 125.7, 125.5, 122.5, 122.1, 120.1, 119.4, 118.0, 116.9, 109.6, 109.0, 108.7, 104.5, 104.4, 103.2, 98.9, 78.4, 74.3, 73.3, 72.1, 71.5, 66.2, 63.7, 61.9, 60.6, 56.9, 56.1, 55.8, 55.4, 37.2, 33.3, 32.0, 29.6, 29.4, 26.1, 22.9 and 14.4; and

(h) pK_a values in acetonitrile:water (3:7) as follows: 3.0, 4.3, 7.4, 8.5, 9.9 and 10.9 pK_a values above 10.9 not determined.

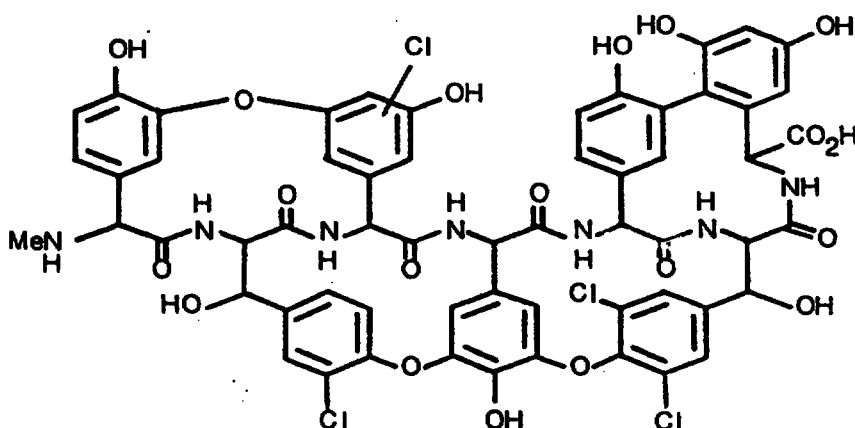
The AAD 216B pseudo-aglycone of the formula (I) wherein R_1 is hydrogen and R_2 is a glycolipid radical of unknown structure having an empirical formula $C_{17}H_{30}NO_6$ has the following characteristics:

- (a) a white solid which decomposes at 250–300°C;
- (b) a empirical formula $C_{76}H_{74}N_8O_{25}Cl_4$;
- (c) an approximate elemental composition of 48.98 percent carbon, 4.56 percent hydrogen, 5.64 percent nitrogen and 8.29 percent chlorine when the water content was 6.9 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1480, 1430, 1300, 1240, 1150, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1639 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 61.8$ and at 303 nm under basic conditions with an $E_{1\%} = 88$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.3, 177.6, 175.9, 175.6, 171.5, 171.0, 170.7, 170.3, 169.4, 164.0, 159.3, 158.9, 156.4, 152.5, 151.7, 151.1, 146.2, 144.7, 141.8, 138.7, 138.6, 136.8, 134.4, 133.8, 130.9, 129.8, 129.5, 128.6, 128.4, 127.9, 127.3, 126.0, 125.8, 122.5, 119.9, 119.3, 118.2, 116.9, 109.3, 108.8, 104.3, 104.2, 103.0, 99.4, 78.7, 74.4, 73.5, 72.1, 71.6, 65.8, 63.8, 62.5, 60.6, 57.1, 56.1, 55.9, 55.4, 39.7, 37.3, 33.1, 30.3, 29.9, 29.7, 28.5, 28.0, 26.3 and 23.4.

The AAD 216C pseudo-aglycone of the formula (I) wherein R_1 is hydrogen and R_2 is a glycolipid radical of unknown structure having an empirical formula $C_{18}H_{32}NO_6$ has the following characteristics:

- (a) a white solid which decomposes at 250–300°C;
- (b) a empirical formula $C_{77}H_{78}N_8O_{25}Cl_4$;
- (c) an approximate elemental compositions of 47.58 percent carbon, 4.51 percent hydrogen, 5.33 percent nitrogen and 8.08 percent chlorine when the water content was 7.8 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1430, 1300, 1240, 1140, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1653 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 60.0$ and at 303 nm under basic conditions with an $E_{1\%} = 83$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.1, 177.4, 175.5, 175.4, 171.4, 171.0, 170.7, 170.3, 169.3, 163.6, 159.1, 158.6, 156.5, 156.2, 152.7, 151.9, 151.8, 151.0, 146.2, 144.6, 142.0, 138.8, 138.6, 136.9, 134.8, 134.7, 134.0, 130.6, 129.9, 129.8, 129.4, 128.8, 128.3, 127.8, 127.5, 127.2, 126.4, 126.2, 125.8, 122.5, 122.0, 119.6, 119.1, 118.4, 116.9, 109.5, 109.2, 108.7, 104.2, 103.6, 99.8, 78.6, 74.6, 73.5, 72.1, 71.7, 66.0, 63.7, 62.1, 60.6, 56.9, 56.1, 55.9, 55.4, 39.6, 37.3, 33.2, 30.4, 30.0, 29.8, 28.5, 27.9, 26.3 and 23.1.

The AAD 216 aglycone, represented by the structural formula (II)



(II)

has the following characteristics:

- (a) a pale white-yellow solid which decomposes at 300–350°C;
- (b) an empirical formula $C_{59}H_{45}N_7O_{19}Cl_4$;
- (c) an approximate elemental composition of 47.92 percent carbon, 3.78 percent hydrogen, 6.58 percent nitrogen and 9.50 percent chlorine when the water content was 10.70 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1240, 1150, 1080, 1060 and 1010;

(e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1296 (major cluster);

(f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 83$ and at 300 nm under basic conditions with an $E_{1\%} = 140$;

(g) a carbon magnetic resonance spectrum at 90.56 MHz in $(CD_3)_2SO$ at a pH of 3.3 which exhibits the following chemical shifts in parts per million (ppm) using TMS as the internal standard: 172.6, 172.1, 170.0, 169.2, 168.4, 167.2, 167.0, 157.2, 156.4, 155.5, 155.0, 154.6, 151.4, 147.5, 145.8, 144.7, 142.8, 141.7, 139.0, 138.7, 136.2, 135.6, 134.5, 128.5, 128.2, 128.0, 127.9, 127.8, 127.4, 127.3, 126.3, 126.0, 125.3, 125.0, 121.1, 118.1, 117.7, 117.5, 116.6, 113.7, 108.7, 106.3, 105.9, 105.4, 103.7, 102.5, 71.3, 70.1, 65.3, 61.4, 60.1, 56.8, 54.8, 53.9, 53.6 and 33.4; and

(h) pK_a values in acetonitrile:water (3:7) as follows: 3.3, 7.1, 8.4, 9.2, 10.1 and 11.4 pK_a values above 11.4 not determined.

The compounds of the instant invention are conveniently prepared from the AAD 216 antibiotics along the following lines:

The AAD 216 pseudo-aglycone is prepared by hydrolyzing the AAD 216 antibiotics, exemplified by AAD 216A, in aqueous organic solvent and very dilute mineral acid at reflux until a substantial amount of the AAD 216 pseudo-aglycone is formed and subsequently isolating it from the reaction mixture. Illustrative of this hydrolysis process is the treatment of AAD 216A in 10 percent aqueous acetonitrile with 0.001 N hydrochloric acid at reflux for 48 hours and the isolation of the AAD 216 pseudo-aglycone by chromatographic means.

The AAD 216 aglycone, the AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone and AAD 216C pseudo-aglycone are prepared by the hydrolysis of the appropriate AAD 216A, AAD 216B and AAD 216C in a polar organic solvent and dilute mineral acid at elevated temperature until a substantial amount of the hydrolysis products are formed and subsequently isolating the individual desired products. Illustrative of this hydrolysis process is the treatment of AAD 216A in dimethylsulfoxide with 5 percent hydrochloric acid at 100°C for 15 minutes. The isolation of the individual AAD 216 aglycone and the AAD 216A pseudo-aglycone may be accomplished by chromatographic means.

Alternatively, the AAD 216 aglycone and the pseudo-aglycones may be prepared by the mild acid hydrolysis of the AAD 216 complex or the individual factors followed by a chromatographic isolation of the desired compounds.

Biological Activity Data

The *in vitro* minimum inhibitory concentrations (MIC) of the AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone and vancomycin were determined for a number of microorganisms using the standard microtiter assay procedures. The AAD 216 aglycone, the AAD 216 pseudo-aglycone, the AAD 216A pseudo-aglycone, the AAD 216B pseudo-aglycone and the AAD 216C pseudo-aglycone were neutralized with sodium bicarbonate prior to testing. The results are shown in the following Tables A—F.

EP 0 132 116 B1**Antimicrobial Spectrum****TABLE A**

Test Organism	AAD 216 Aglycone	MIC in µg/ml		Vancomycin
		AAD 216 Pseudo- Aglycone	AAD 216A Pseudo- Aglycone	
<i>Staph. aureus</i> HH127	0.4	1.6	0.8	1.6
<i>Staph. aureus</i> SK&F 910	0.4	1.6	0.4	1.6
<i>Strep. faecilis</i> HH34358	0.8	1.6	0.4	3.1
<i>Proteus mirabilis</i> SK&F 444	25	100	100	110
<i>E. coli</i> 12140 (SK&F 809)	100	>100	>100	100
<i>k. pneumoniae</i> 4200 (SK&F 798)	>100	>100	>100	>100
<i>Pseudomonas aeruginosa</i> HH63	>100	>100	>100	>100
<i>Serratia marcesens</i> ATCC 13880	>100	>100	>100	>100
<i>Proteus morgani</i> SK&F 179	>100	>100	>100	>100
<i>Providencia</i> SK&F 276	>100	>100	>100	>100
<i>Enterobacter cloacae</i> HH31254	>100	>100	>100	>100
<i>Salmonella gallinarum</i> SK&F BC595	25	25	~100	25
<i>Staph. epidermidis</i> SK&F 2479	0.8	~3.1	0.8	1.6
<i>Listeria monocytogenes</i> SK&F 2255	0.8	1.6	0.4	1.6
<i>Staph. epidermidis</i> SK&F 651	3.1	12.5	3.1	1.6

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TABLE B
(Methicillin Sensitive)

Test Organism	AAD 216 Aglycone	AAD 216 Pseudo- Aglycone	MIC in µg/ml	
			AAD 216A Pseudo- Aglycone	Vancomycin
<i>Staph. aureus</i> HH127	0.4	1.6	0.4	1.6
<i>Staph. aureus</i> SK&F 674	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 910	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 1761	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2666	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2677	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2678	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2680	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2682	0.8	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2736	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2743	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2776	3.1	3.1	1.6	1.6
<i>Staph. aureus</i> SK&F 2777	0.4	1.6	0.4	0.8
<i>Staph. aureus</i> SK&F 2613	0.4	1.6	0.4	1.6
<i>Staph. aureus</i> SK&F 2615	0.8	3.1	0.8	3.1

EP 0 132 116 B1**TABLE C**
(Methicillin Resistant)

Test Organism	AAD 216 Aglycone	AAD 216 Pseudo- Aglycone	MIC in µg/ml	
			AAD 216A Pseudo- Aglycone	Vancomycin
<i>Staph. aureus</i> SK&F 675	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2612	0.8	3.1	~0.8	1.6
<i>Staph. aureus</i> SK&F 2614	0.4	0.8	0.2	1.6
<i>Staph. aureus</i> SK&F 2616	0.4	1.6	0.2	0.8
<i>Staph. aureus</i> SK&F 2620	0.8	3.1	0.8	3.1
<i>Staph. aureus</i> SK&F 2621	0.4	1.6	0.4	1.6
<i>Staph. aureus</i> SK&F 2594	0.4	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2589	0.4	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2590	0.4	3.1	0.4	1.6
<i>Staph. aureus</i> SK&F 2593	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2591	0.8	3.1	0.8	1.6
<i>Staph. aureus</i> SK&F 2592	0.8	3.1	1.6	3.1
<i>Staph. aureus</i> SK&F 2595	0.8	3.1	1.6	1.6
<i>Staph. aureus</i> SK&F 2596	0.4	3.1	1.6	3.1
<i>Staph. aureus</i> SK&F 2597	0.8	3.1	1.6	3.1

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TABLE D
(Anaerobes)

Test Organism	MIC in µg/ml			Vancomycin
	AAD 216 Aglycone	AAD 216 Pseudo- Aglycone	AAD 216A Pseudo- Aglycone	
<i>Bacteroides fragilis</i> ATCC 25285	32	>32	16	32
<i>B. fragilis</i> H145	32	>32	8	32
<i>B. fragilis</i> SK&F 3060	32	>32	16	32
<i>B. loechis</i> SK&F 3087	16	32	8	32
<i>B. thetaotamicron</i> SK&F 3089	32	>32	16	32
<i>Fusobacterium nucleatum</i> ATCC 25586	32	>32	16	32
<i>Clostridium perfringens</i> MCP-1	≤0.016	≤0.016	≤0.016	0.5
<i>C. perfringens</i> MCP-2	≤0.016	0.031	≤0.016	0.5
<i>C. perfringens</i> ATCC 19408	0.5	0.05	0.125	1.0
<i>Clostridium difficile</i> SK&F 3062	2	2	0.125	2
<i>C. difficile</i> SK&F 3065	2	2	0.25	2
<i>C. difficile</i> SK&F 3091	≤0.016	≤0.016	0.125	2
<i>C. difficile</i> SK&F 3092	2	2	0.125	2
<i>C. difficile</i> SK&F 3096	1.0	2	0.25	2
<i>C. difficile</i> SK&F 3098	≤0.016	≤0.016	≤0.016	2

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TABLE E

Test Organism	MIC in µg/ml			Vancomycin
	AAD 216A Pseudo- Aglycone	AAD 216B Psuedo- Aglycone	AAD 216C Pseudo- Aglycone	
<i>Staph. aureus</i> HH127	0.4	0.4	0.2	0.2
<i>Staph. aureus</i> SK&F 910	0.4	0.2	0.8	1.6
<i>Staph. aureus</i> SK&F 209P	0.1	0.2	0.1	1.6
<i>Staph. aureus</i> SK&F 209P-mutant	100	50	50	100
<i>Staph. aureus</i> SK&F 674-P6-mutant	50	50	25	100
<i>Staph. aureus</i> SK&F 675	1.6	1.6	3.1	3.1
<i>Staph. epidermidis</i> SK&F 2479	3.1	6.3	6.3	3.1
<i>Staph. epidermidis</i> SK&F 2683	6.3	3.1	6.3	3.1
<i>Staph. epidermidis</i> SK&F 651	12.5	12.5	12.5	3.1
<i>Staph. epidermidis</i> SK&F 2265	12.5	6.3	12.5	3.1
<i>Staph. faecalis</i> SK&F 34358	0.2	0.1	0.1	3.1
<i>Staph. faecalis</i> SK&F 657	0.4	0.1	0.1	3.1
<i>Listeria monocytogenes</i> SK&F 2255	0.1	0.1	0.1	0.8
<i>E. coli</i> SK&F 12140	>100	>100	>100	>100
<i>Salmonella gallinarum</i> BC-595	>100	>100	>100	>100

TABLE F

Test Organism	MIC in µg/ml			
	AAD 216A Pseudo- Aglycone	AAD 216B Pseudo- Aglycone	AAD 216C Pseudo- Aglycone	Vancomycin
<i>Bacteroides Fragilis</i> ATCC	>32	>32	32	>32
<i>B. thetaiotomicron</i> H-145	>32	>32	>32	>32
<i>Fusobacterium nucleatur</i> ATCC 25586	>32	16	16	32
<i>C. perfringens</i> SK&F 2769	0.063	0.031	0.125	0.5
<i>C. perfringens</i> MCP-2	0.5	0.5	0.5	2.0
<i>C. difficile</i> SK&F 3062	0.125	0.125	0.125	0.5
<i>C. difficile</i> SK&F 3065	0.25	0.25	0.25	1.0
<i>C. difficile</i> SK&F 3092	0.25	0.25	0.5	0.5
<i>C. difficile</i> SK&F 3141	0.5	0.25	0.5	1.0

The *in vivo* activity of the AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone and vancomycin, measured as ED₅₀, was demonstrated against intraperitoneal infections with 46.8 LD₅₀'s of *Staph. aureus* HH 127 in mice by treatments with the antibiotics s.c., 1 and 5 hours post infection. The ED₅₀'s were as follows: AAD 216 aglycone, 7.6 mg/kg; AAD 216 pseudo-aglycone, 7.6 mg/kg; AAD 216A pseudo-aglycone, 5.0 mg/kg; vancomycin, 1.56 mg/kg. Similarly, the ED₅₀'s of AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone and vancomycin, determined by utilizing a similar protocol, were 12.5 mg/kg, 7.6 mg/kg, 10.8 mg/kg and 1.92 mg/kg, respectively.

The antibiotic compounds of the present invention including AAD 216 aglycone, AAD 216 pseudo-aglycone and AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone and mixtures thereof, exhibit antibacterial activity. The invention includes within its scope pharmaceutical compositions containing at least one of the above-mentioned antibiotic compounds and a pharmaceutically acceptable carrier. The compositions may also contain other active antibacterial agents. The compositions may be made up in any pharmaceutical form appropriate for the route of administration in question. Such compositions are exemplified by solid compositions for oral administration, such as tablets, capsules, pills, powders and granules; liquid compositions for oral administration such as solutions, suspensions, syrups and elixers; and preparations for parenteral administration such as sterile solutions, suspensions or emulsions.

For use as an antibacterial agent, the compositions are administered so that the concentration of the active ingredient is greater than the minimum inhibitory concentration for the particular organism treated.

The activity of the AAD 216 aglycone, AAD 216 pseudo-aglycone and AAD 216A pseudo-aglycone was demonstrated *in vitro* against a total of 58 bovine mastitis isolates using the conventional agar dilution method to determine minimum inhibitory concentrations (MICs). The MICs for AAD 216 aglycone, AAD 216 pseudo-aglycone and AAD 216 pseudo-aglycone ranged from 0.5 to >128 µg/ml, 0.25 to >128 µg/ml and 0.03 to >128 µg/ml, respectively. In comparison, vancomycin has MICs for the same microorganisms ranging from 0.25 to >128 µg/ml.

Growth Promotant Activity

The growth promotant activities of the AAD 216 aglycone, AAD 216 pseudo-aglycone, and AAD 216A pseudo-aglycone were determined in a swine *in vitro* model to predict utility in monogastric animals, such as swine and poultry; and a rumen *in vitro* model to predict utility in beef, dairy and sheep production.

Swine *in vitro* model

A Yorkshire barrow is surgically prepared either with an ileal cannula, which is placed 15 cm from the ileo-caecocolic junction, or a cecal cannula, which placed midway between the apex and origin of the cecum. The animal is fed 4 times daily to restrict intake to 4.5% of body weight in a 30 kg animal or 2.5% of body weight in a 100 kg animal. The swine grower ration is:

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	(% w/w)	(lbs/ton)
Medium ground shelled corn	70.60	1412
Soybean meal, 44%	22.00	440
Dehydrated alfalfa mea, 17%	4.50	90
Calcium propionate	0.15	3
Vitamin/mineral premix	2.75	55

Sampling of the material, via the cannula, begins 150—180 minutes following the first morning feeding and continues any time from 30—120 minutes thereafter, depending on the quantity of material needed. The sample is maintained in crushed ice, no cooler than 5°C, and is gassed continuously with carbon dioxide. The collected material is filtered. The filtrate is the inoculum used for incubations of the test and control samples. The gassed inoculum, 2.25 ml, is placed in each of 10 gassed test tubes, each containing 0.75 ml of a nutrient solution and 0.5 mg of each test compound. Four blank control tubes, along with the test compound tubes, are incubated 5 hours at 37°C with agitation. Four more killed tubes are included which are not incubated.

The tubes are each treated with 0.60 ml of a 25% solution of metaphosphoric acid, then, stored at -4°C until analysis. Samples are thawed and centrifuged for 25 minutes at 20,000 r.p.m. The supernatant liquid is decanted, sampled for gas chromatography and automatic analysis. The results are fed into a computer for finishing to give figures in which the blank control value is 100%; see the following tables. Virginiamycin and vancomycin or avoparcin are used as positive controls.

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I. Compound (ppm)	VFA* (% Control)	LYS* (% Control)	GLU* (% Control)	LAC* (% Control)
Virginiamycin				
(166.67)	93	163	197	81
(16.67)	130	127	191	76
(1.67)	248	82	182	70
Vancomycin				
(166.67)	250	48	190	64
(16.67)	280	42	189	59
(1.67)	92	83	100	99
AAD 216 Aglycone				
(166.67)	255	65	180	62
(16.67)	307	39	182	58
(1.67)	86	91	94	101
AAD 216 Pseudo-aglycone				
(166.67)	266	64	183	62
(16.67)	238	76	171	69
(1.67)	96	110	94	101
AAD 216A Pseudo-aglycone				
(166.67)	302	52	187	58
(16.67)	361	59	184	58
(1.67)	106	106	101	98

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II.	Compound (ppm)	VFA* (% Control)	LYS* (% Control)	GLU* (% Control)	LAC* (% Control)
	Virginiamycin				
	(1.67)	240	96	837	21
	Avoparcin				
	(1.67)	85	101	115	102
	AAD 216B Pseudo-aglycone				
	(16.67)	313	96	894	16
	(1.67)	118	102	169	95
	AAD 216C Pseudo-aglycone				
	(16.67)	252	91	895	16
	(1.67)	131	94	225	85

*VFA refers to the total of volatile fatty acids, namely acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. LYS is lysine, GLU is glucose and LAC is L-lactic acid.

Rumen *in vitro* model

The protocol for the rumen *in vitro* model is analogous to the protocol for the swine *in vitro* model with the following modifications:

- (1) A 400 kg steer is surgically prepared with a rumen cannula.

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(2) The animal is fed one time a day with the following ration:

<u>Finished Feed</u>	<u>% w/w</u>
Cottonseed hulls	44.0
Cracked corn	22.0
Alfalfa Hay 1"	20.0
Pellet Supplement*	10.0
Liquid Molasses	4.0
	<u>100.0</u>

<u>* Pellet Supplement</u>	<u>% w/w</u>
Soybean Oil Meal (50% protein)	50.0
Medium Ground Corn	32.5
D/Calcium Phosphate	6.50
Plain Salt	2.50
Ground limestone (Thomasville)	3.50
Urea	2.50
Vitamin A & D ₂ Premix **	2.50
	<u>100.00</u>

<u>**Vitamin A & D₂ Premix</u>	<u>% w/w</u>
Vitamin A (30,000 IU/gm)	5.87
Vitamin D ₂ (16,000,000 IU/lb)	0.50
Fine ground corn	93.63
	<u>100.00</u>

(3) Manipulation of VFA production is described as the production ratio of propionate as a percentage of total VFA produced.

(4) Sampling of the material, via the cannula, is at 120 minutes post feeding.

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III.	Compound (ppm)	VFA* (% Control)	LYS* (% Control)	GLU* (% Control)	Propionate* (% Control)
	Vancoymycin				
	(50.0)	107	97	189	127
	(5.0)	108	85	165	130
	(0.5)	97	83	17	105
	Avoparcin				
	(50.0)	113	97	141	132
	(5.0)	105	85	166	121
	(0.5)	103	96	116	106
	Monensin Sodium				
	(50.0)	109	147	0	156
	(5.0)	104	141	126	149
	(0.5)	94	109	11	119
	AAD 216 Aglycone				
	(50.0)	93	99	14	135
	(5.0)	110	101	22	131
	(0.5)	100	98	0	102
	AAD 216 Pseudo-aglycone				
	(50.0)	104	101	104	127
	(5.0)	95	96	0	15
	(0.5)	88	118	47	101
	AAD 216A Pseudo-aglycone				
	(50.0)	113	115	38	149
	(5.0)	110	97	30	139
	(0.5)	98	102	534	106

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Yellow corn meal	67.35%
Soybean oil meal	24.00%
Menhaden fish meal	6.00%
Steamed bone meal	1.00%
Ground limestone	1.00%
Iodized salt	0.34%
25% choline chloride	0.13%
Vitamin B ₁₂	0.10%
Manganese sulfate	0.02%
Vitamin mix	0.06%

Swine feed from weanling to fattening or finishing rations may be supplemented. Swine eat from about 2 lb of ration per day (for a 25 lb pig) to 9 lb per day (for a 150 lb pig). Most rations are comprised of a corn base supplemented with legume silage, wheat bran, oats, barley, molasses or a protein supplement.

Poultry feeds comprise starter rations, broiler rations and laying rations. The rations are usually based on ground corn, corn meal or soybean meal. The broiler rations, often, contain high energy supplements such as added fats, proteins and vitamins. Turkey rations are similar, but comprise only a starting ration and a growing ration. Chickens or pheasants eat from .03—0.3 lbs of feed per day, turkeys twice that much. Estimated intake of feed is dependent on the weight and age of the meat producing animal.

The active ingredients selected from the group consisting of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone or a mixture thereof are mixed uniformly with such feed rations to give supplemented rations which are, then fed as to custom, which is, most often, ad libitum. Conveniently, to do this, a premix of the supplemental growth promotant of this invention, optionally combined with or without other supplements known to this art such as an anthelmintic, a nitrogen source or an antibiotic, for example, virginiamycin or oxytetracycline is prepared by the manufacturer for sale to the formulators or feed lot operators. The concentration of the active ingredients selected from the group consisting of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone or a mixture thereof in the premix is usually from 5—75% by weight or a concentration 100—2000 times greater than that in the complete feed ration. The premix form may be liquid or solid. Premix vehicles are corn oil, cottonseed oil, molasses or distillers solubles to form a liquid premix preparation. Sucrose, lactose, corn meal, ground corn, flour, calcium carbonate or soybean meal are often used as bases for solid premix preparations. The premix composition is, then, mixed uniformly with whole ration which is fed to the target animal. Such premix compositions are included in the term "feed compositions" as used herein.

The concentration of the active ingredients selected from the group consisting of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone or a mixture thereof in the complete ration is a nontoxic but active quantity chosen, for example, from a range of about 1—1000 parts of active ingredient by weight per million parts of whole feed (ppm) or about 2—115 grams per ton. Advantageously, a nontoxic quantity of active ingredient is chosen from the range of 10—50 ppm.

The method of this invention comprises feeding to monogastric or ruminant, meat or milk producing animals, especially beef and dairy cattle, sheep, swine and poultry, an effective promoting but nontoxic quantity of an active ingredient selected from the group consisting of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, AAD 216C pseudo-aglycone or a mixture thereof. Other monogastric animals whose digestive tract also features fermentation in a cecum or cecum-like chamber are rabbits and horses.

The supplemented feed rations, described above, are presented to the animal by methods known to the art. Ad libitum feeding in the pasture, pen or growing shed is most convenient to increase the growth and milking rate of the animal and to increase the feed efficiency of the operation.

The following examples are illustrative of the production, isolation and purification of the antibiotics of the present invention and are not therefore to be considered in limiting the present invention as described in the claims appended hereto.

Example 1

Preparation of AAD 216 pseudo-aglycone

AAD 216A (1.2 g) was partially dissolved in 10 percent aqueous acetonitrile and 0.0001 N hydrochloric

acid (total volume 1500 ml) and heated to reflux for 48 hours. The reaction mixture was lyophilized and then chromatographed on reverse phase HPLC with a column of Whatman Partisil 40[®] (ODS-3) (25 × 500 mm) at a flow rate of 15 ml/minute. The column was eluted with 15 percent acetonitrile — 0.1 M pH 3.2 phosphate buffer (2000 ml) and 18 percent acetonitrile — buffer solution (1000 ml). The appropriate fractions were combined, lyophilized and desalted as described below to yield the desired AAD 216 pseudo-aglycone.

The desalting procedure involved a pooling of the appropriate fractions from the HPLC and removal of the acetonitrile at reduced pressure. The resulting aqueous samples were loaded onto an XAD-7 resin column and eluted with deionized water until the conductivity of the outflow was less than 1.5 µMHO. The column was then eluted with aqueous acetonitrile (50%) and the eluant lyophilized to afford the desired products.

Example 2

Preparation of AAD 216 aglycone and AAD 216A pseudo-aglycone

AAD 216 (0.74 g) was dissolved in 5 percent concentrated hydrochloric acid and dimethylsulfoxide (40 ml) and heated for 15 minutes at 100°C. The reaction mixture was diluted with 14 percent acetonitrile 0.1 M pH 3.2 phosphate buffer and chromatographed with the equipment described in Example 1. The column was eluted with 14 percent acetonitrile-buffer solution (1000 ml) followed by 18 percent (500 ml), 22 percent (500 ml), 26 percent (500 ml) and 30 percent (1000 ml) acetonitrile-buffer solution. The appropriate fractions containing AAD 216A pseudo-aglycone were combined and the appropriate fractions containing AAD 216 aglycone were combined. Both of the combined fractions were separately lyophilized and desalted using the procedure in Example 1 to afford the desired products.

Example 3

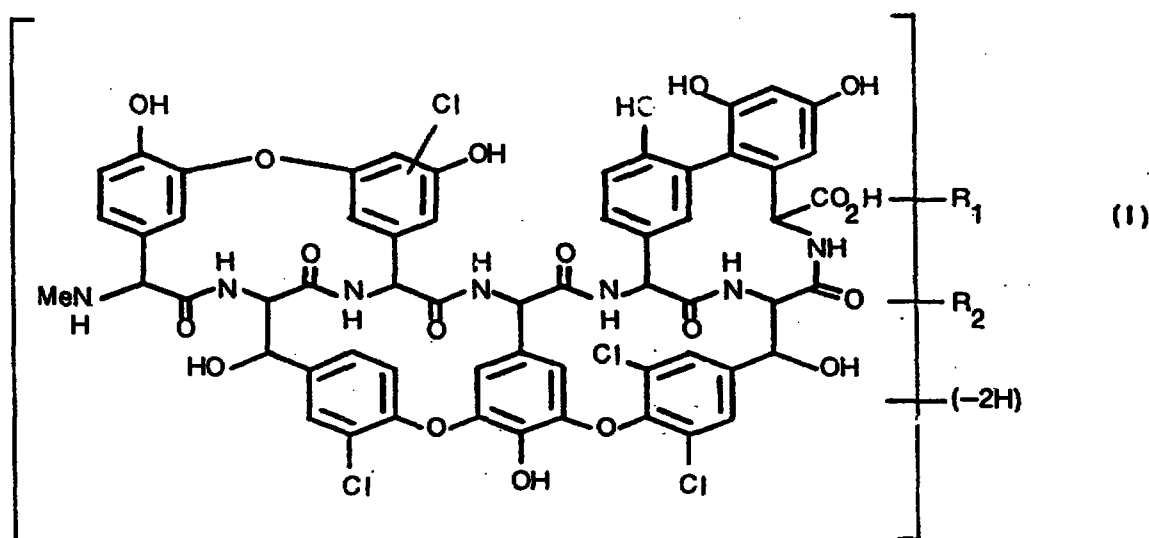
Preparation of AAD 216B Pseudo-Aglycone

AAD 216B (1.64 g) was dissolved in dimethylsulfoxide (40 ml) and concentrated hydrochloric acid (2 ml) was added. The reaction mixture was heated at 100°C for 15 minutes and then diluted with 31 percent acetonitrile 0.1 M pH 6.0 phosphate buffer (450 ml) which was loaded onto an affinity chromatography column (700 ml — Affigel 10 D-ala-D-ala). The column was washed with 0.1 M pH 6.0 phosphate buffer (100 ml), water (2000 ml) and 1 percent aqueous acetonitrile (1000 ml). The reaction products were eluted off the affinity chromatography column with 50 percent acetonitrile in 0.15 N ammonia (2000 ml). The eluant was lyophilized, dissolved in 10 percent acetonitrile in 0.1 M pH 6.0 phosphate buffer and chromatographed on the equipment described in Example 1. The column was eluted with 10 percent acetonitrile in 0.1 M pH 6.0 phosphate buffer (500 ml), followed by 15 percent (750 ml), 18 percent (750 ml), 22 percent (500 ml), 26 percent (50 ml), 30 percent (500 ml) and 35 percent (1000 ml) acetonitrile buffer solution. The appropriate fractions containing AAD 216B pseudo-aglycone were combined and the fractions containing AAD 216 aglycone were combined. Both combined fractions were separately lyophilized and desalted using the procedure in Example 1 to afford the desired products.

Utilizing the procedure of Example 3, AAD 216C (2.0 g) was hydrolyzed to yield AAD 216C pseudo-aglycone and AAD 216 aglycone. AAD 216A pseudo-aglycone was also prepared via the procedure of Example 3.

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

1. An antibiotic compound represented by the structural formula (I)





acid (total volume 1500 ml) and heated to reflux for 48 hours. The reaction mixture was lyophilized and then chromatographed on reverse phase HPLC with a column of Whatman Partisil 40® (ODS-3) (25 x 500 mm) at a flow rate of 15 ml/minute. The column was eluted with 15 percent acetonitrile — 0.1 M pH 3.2 phosphate buffer (2000 ml) and 18 percent acetonitrile — buffer solution (1000 ml). The appropriate fractions were combined, lyophilized and desalted as described below to yield the desired AAD 216 pseudo-aglycone.

The desalting procedure involved a pooling of the appropriate fractions from the HPLC and removal of the acetonitrile at reduced pressure. The resulting aqueous samples were loaded onto an XAD-7 resin column and eluted with deionized water until the conductivity of the outflow was less than 1.5 µMHO. The column was then eluted with aqueous acetonitrile (50%) and the eluant lyophilized to afford the desired products.

Example 2

Preparation of AAD 216 aglycone and AAD 216A pseudo-aglycone

AAD 216 (0.74 g) was dissolved in 5 percent concentrated hydrochloric acid and dimethylsulfoxide (40 ml) and heated for 15 minutes at 100°C. The reaction mixture was diluted with 14 percent acetonitrile 0.1 M pH 3.2 phosphate buffer and chromatographed with the equipment described in Example 1. The column was eluted with 14 percent acetonitrile-buffer solution (1000 ml) followed by 18 percent (500 ml), 22 percent (500 ml), 26 percent (500 ml) and 30 percent (1000 ml) acetonitrile-buffer solution. The appropriate fractions containing AAD 216A pseudo-aglycone were combined and the appropriate fractions containing AAD 216 aglycone were combined. Both of the combined fractions were separately lyophilized and desalted using the procedure in Example 1 to afford the desired products.

Example 3

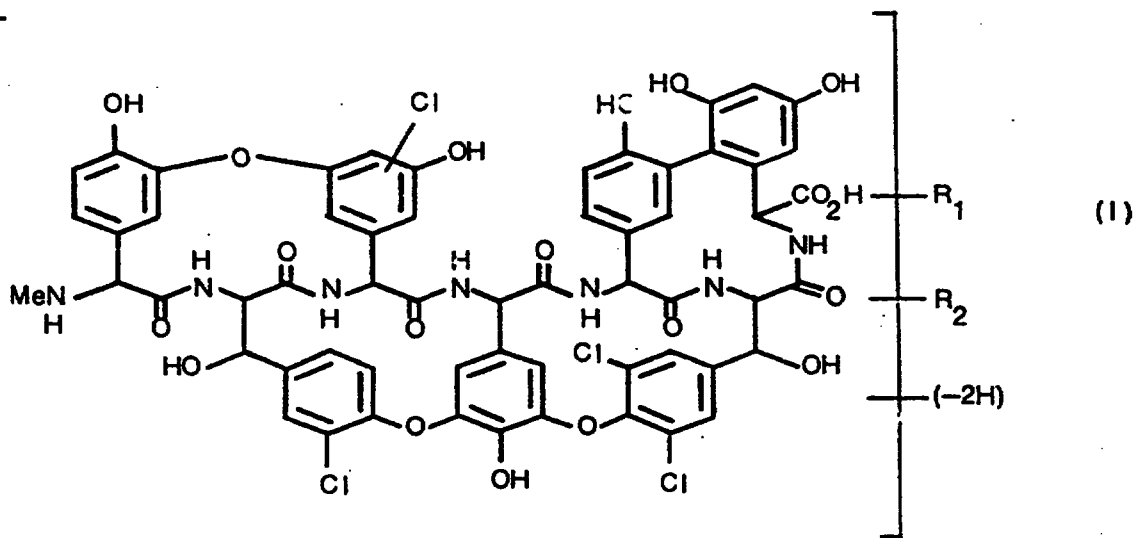
Preparation of AAD 216B Pseudo-Aglycone

AAD 216B (1.64 g) was dissolved in dimethylsulfoxide (40 ml) and concentrated hydrochloric acid (2 ml) was added. The reaction mixture was heated at 100°C for 15 minutes and then diluted with 31 percent acetonitrile 0.1 M pH 6.0 phosphate buffer (450 ml) which was loaded onto an affinity chromatography column (700 ml — Affigel 10 D-ala-D-ala). The column was washed with 0.1 M pH 6.0 phosphate buffer (100 ml), water (2000 ml) and 1 percent aqueous acetonitrile (1000 ml). The reaction products were eluted off the affinity chromatography column with 50 percent acetonitrile in 0.15 N ammonia (2000 ml). The eluant was lyophilized, dissolved in 10 percent acetonitrile in 0.1 M pH 6.0 phosphate buffer and chromatographed on the equipment described in Example 1. The column was eluted with 10 percent acetonitrile in 0.1 M pH 6.0 phosphate buffer (500 ml), followed by 15 percent (750 ml), 18 percent (750 ml), 22 percent (500 ml), 26 percent (50 ml), 30 percent (500 ml) and 35 percent (1000 ml) acetonitrile buffer solution. The appropriate fractions containing AAD 216B pseudo-aglycone were combined and the fractions containing AAD 216 aglycone were combined. Both combined fractions were separately lyophilized and desalted using the procedure in Example 1 to afford the desired products.

Utilizing the procedure of Example 3, AAD 216C (2.0 g) was hydrolyzed to yield AAD 216C pseudo-aglycone and AAD 216 aglycone. AAD 216A pseudo-aglycone was also prepared via the procedure of Example 3.

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

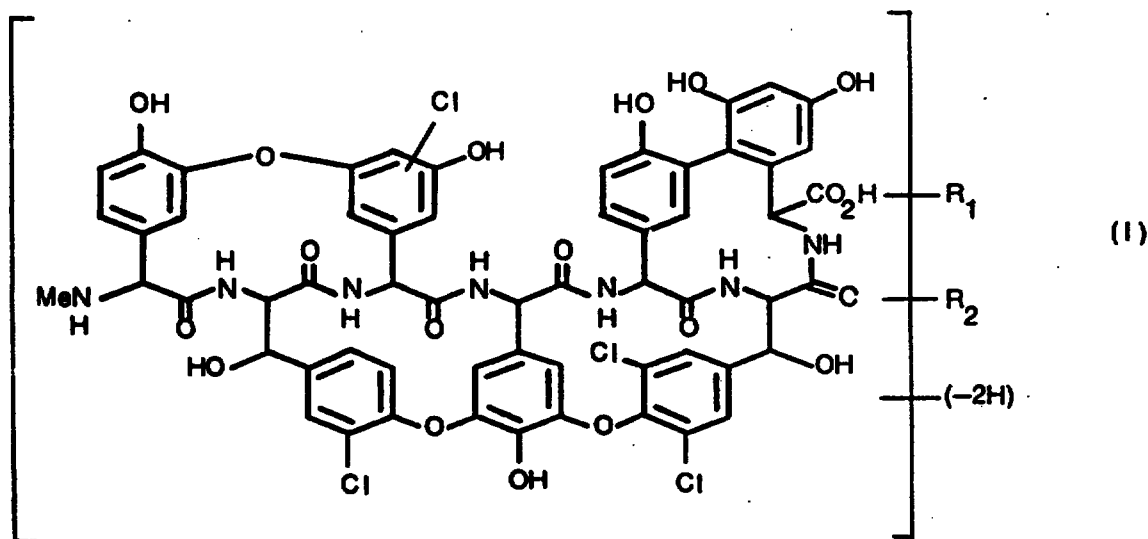
1. An antibiotic compound represented by the structural formula (I)





wherein R_1 is hydrogen or mannosyl and R_2 is hydrogen or a glycolipid radical of unknown structure with the proviso that at least one of R_1 and R_2 is hydrogen, prepared by the removal by acid hydrolysis of a glycolipid radical and/or a mannosyl radical from an antibiotic selected from the group consisting of AAD 216 complex, AAD 216A, AAD 216B and AAD 216C.

2. An antibiotic compound represented by the structural formula (I)



wherein R_1 is mannosyl and R_2 is hydrogen having the following characteristics:

(a) pale white-yellow solid which decomposes at 300–350°C;

(b) an empirical formula $C_{48}H_{56}N_7O_{24}Cl_4$;

(c) an approximate elemental composition of 47.54 percent carbon, 4.00 percent hydrogen, 5.89 percent nitrogen and 8.63 percent chlorine when the water content was 12 percent;

(d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1230, 1180, 1150, 1120, 1060, 1010, 970 and 801;

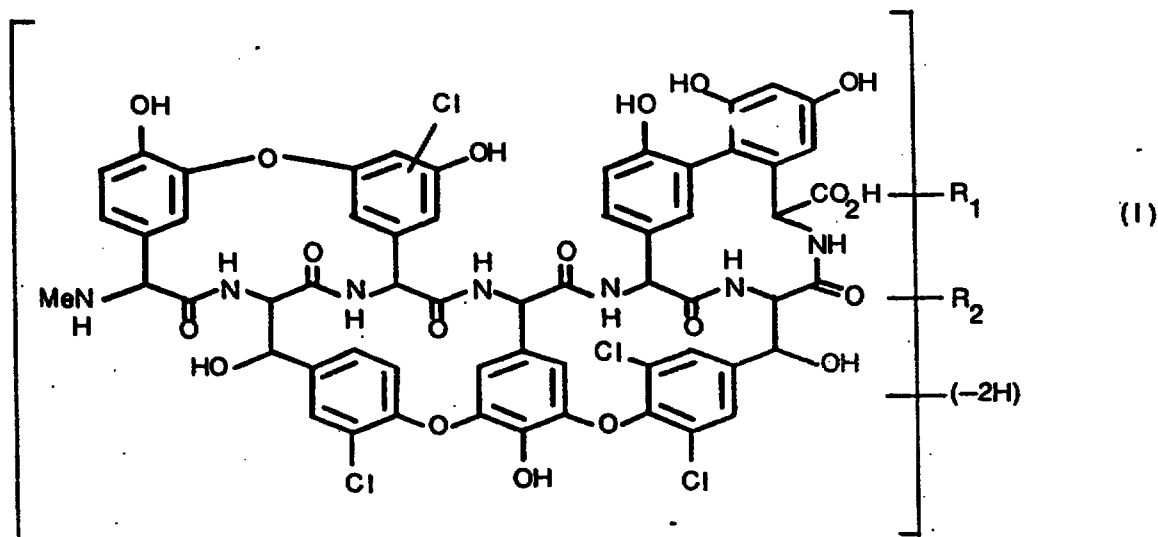
(e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1458 (major cluster);

(f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acid conditions with an $E_{1\%} = 79.7$ and at 300 nm under basic conditions with an $E_{1\%} = 136$;

(g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 8.7 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 177.9, 174.6, 171.7, 170.9, 170.0, 169.2, 162.3, 158.8, 157.9, 155.2, 155.1, 153.9, 151.9, 149.7, 147.6, 147.2, 144.4, 141.0, 139.0, 138.8, 137.5, 136.1, 134.6, 130.7, 130.1, 129.8, 129.2, 128.9, 128.7, 127.5, 127.3, 126.9, 126.4, 126.0, 125.2, 122.7, 122.2, 121.1, 119.9, 119.7, 118.4, 116.6, 110.7, 110.4, 108.5, 104.4, 103.3, 100.5, 98.1, 74.0, 72.1, 71.6, 71.4, 70.8, 67.4, 65.8, 63.7, 62.2, 61.6, 60.2, 56.0, 55.9, 55.0 and 32.9; and

(h) pK_a values in acetonitrile:water (3:7) as follows: 3.3, 7.1, 8.3, 9.1, 10.0 and 11.2 pK_a values above 11.2 not determined.

3. An antibiotic compound represented by the structural formula (I):

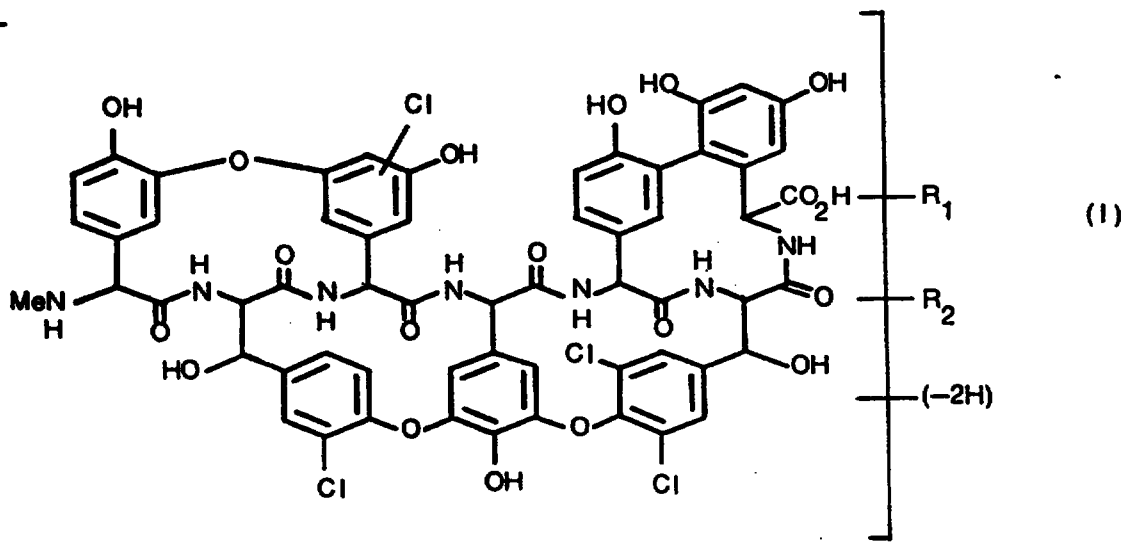




wherein R_1 is hydrogen and R_2 is a glycolipid of unknown structure having an empirical formula $C_{18}H_{28}NO_8$ and having the following characteristics:

- (a) pale white-yellow solid which decomposes at 300–350°C.
- (b) an empirical formula $C_{78}H_{72}N_8O_{25}Cl_4$;
- (c) an approximate elemental composition of 49.76 percent carbon, 4.51 percent hydrogen, 5.99 percent nitrogen and 8.19 percent chlorine when the water content was 9.5 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1460, 1430, 1300, 1240, 1140, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1625 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 63.2$ and at 302 nm under basic conditions with an $E_{1\%} = 94$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.2, 178.0, 176.0, 175.6, 171.6, 170.9, 170.6, 170.5, 169.3, 164.6, 161.4, 159.3, 157.1, 156.5, 153.5, 152.5, 151.8, 151.1, 146.4, 144.9, 141.7, 138.6, 138.4, 136.9, 134.5, 134.4, 133.9, 130.8, 129.8, 129.7, 129.4, 128.6, 128.5, 128.3, 127.7, 127.3, 125.9, 125.5, 122.5, 122.1, 120.1, 119.4, 118.0, 116.9, 109.6, 109.0, 108.7, 104.5, 104.4, 103.2, 98.9, 78.4, 74.3, 73.3, 72.1, 71.5, 66.2, 63.7, 61.9, 60.6, 56.9, 56.1, 55.8, 55.4, 37.2, 33.3, 32.0, 29.6, 29.4, 26.1, 22.9 and 14.4; and
- (h) pK_a values in acetonitrile:water (3:7) as follows: 3.0, 4.3, 7.4, 8.5, 9.9 and 10.9 pK_a values above 10.9 not determined.

4. An antibiotic compound represented by the structural formula (I)

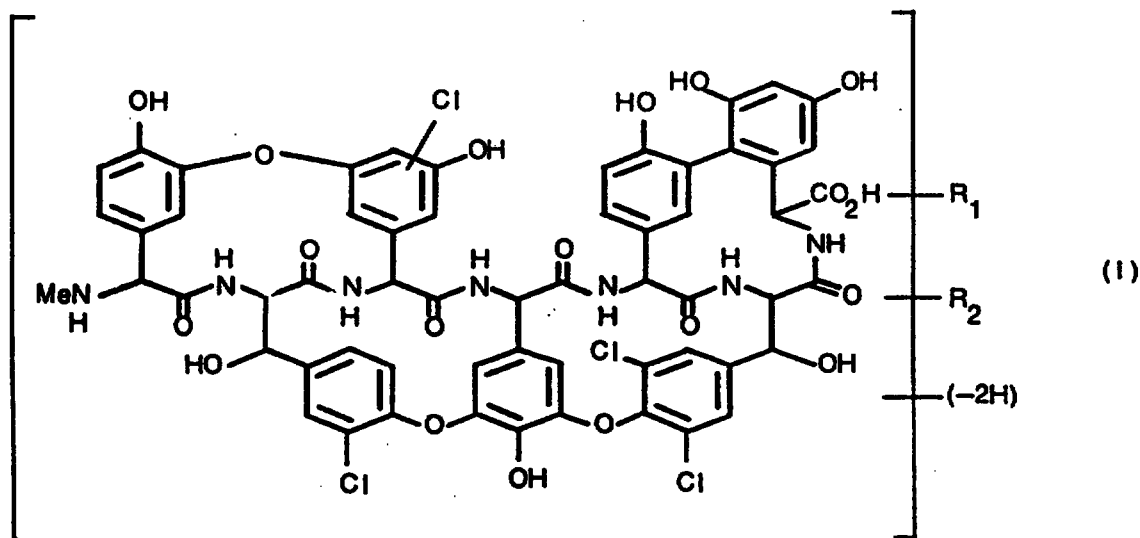


wherein R_1 is hydrogen and R_2 is a glycolipid radical of unknown structure having an empirical formula $C_{17}H_{30}NO_8$ has the following characteristics:

- (a) a white solid which decomposes at 250–300°C;
- (b) an empirical formula $C_{78}H_{74}N_8O_{25}Cl_4$;
- (c) an approximate elemental composition of 48.98 percent carbon, 4.56 percent hydrogen, 5.64 percent nitrogen and 8.29 percent chlorine when the water content was 6.9 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1480, 1430, 1300, 1240, 1150, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1639 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 61.8$ and at 303 nm under basic conditions with an $E_{1\%} = 88$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.3, 177.6, 175.9, 175.6, 171.5, 171.0, 170.7, 170.3, 169.4, 164.0, 159.3, 158.9, 156.4, 152.5, 151.7, 151.1, 146.2, 144.7, 141.8, 138.7, 138.6, 136.8, 134.4, 133.8, 130.9, 129.8, 129.5, 128.6, 128.4, 127.9, 127.3, 126.0, 125.8, 122.5, 119.9, 115.3, 118.2, 116.9, 109.3, 108.8, 104.3, 104.2, 103.0, 99.4, 78.7, 74.4, 73.5, 72.1, 71.6, 65.8, 63.8, 62.5, 60.6, 57.1, 56.1, 55.9, 55.4, 39.7, 33.1, 30.3, 29.9, 29.7, 28.5, 28.0, 26.3 and 23.4.



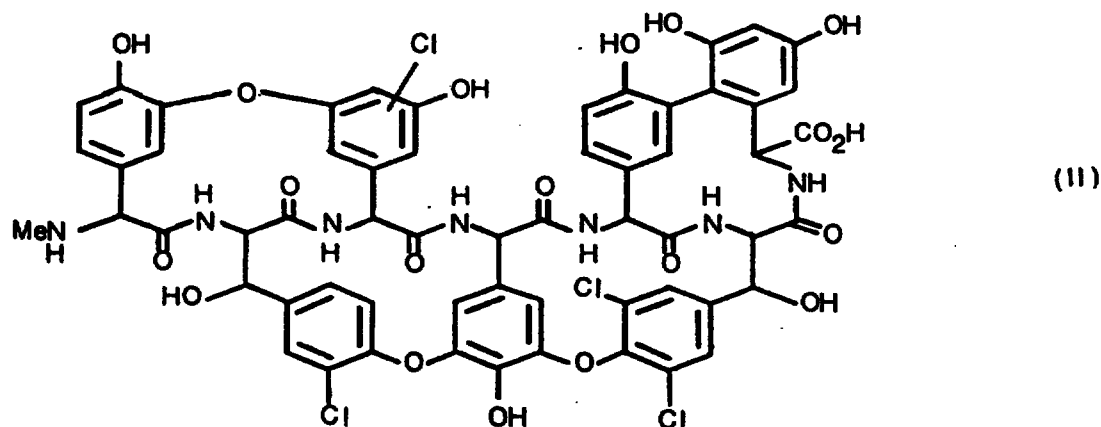
5. An antibiotic compound represented by the structural formula (I)



wherein R_1 is hydrogen and R_2 is a glycolipid radical of unknown structure having an empirical formula $C_{18}H_{32}NO_8$ has the following characteristics:

- (a) a white solid which decomposes at 250–300°C;
- (b) an empirical formula $C_{77}H_{78}N_8O_{25}Cl_4$;
- (c) an approximate elemental composition of 47.58 percent carbon, 4.51 percent hydrogen, 5.33 percent nitrogen and 8.08 percent chlorine when the water content was 7.8 percent;
- (d) an infrared absorption spectrum in potassium bromide which exhibits peaks at the following wave numbers in cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1430, 1300, 1240, 1140, 1080, 1060 and 1010;
- (e) a fast atom bombardment (FAB) mass spectrum with $M + H$ at 1653 (major cluster);
- (f) an ultraviolet spectrum in acetonitrile:water (1:1) which exhibits an absorption maximum at 281 nm under acidic conditions with an $E_{1\%} = 60.0$ and at 303 nm under basic conditions with an $E_{1\%} = 83$;
- (g) a carbon magnetic resonance spectrum at 90.56 MHz in $CD_3OD:D_2O$ (1:9) at a pH of 9.4 which exhibits the following chemical shifts in parts per million (ppm) relative to TMS as standard: 178.1, 177.4, 175.5, 175.4, 171.4, 171.0, 170.7, 170.3, 169.3, 163.6, 159.1, 158.6, 156.5, 156.2, 152.7, 151.9, 151.8, 151.0, 146.2, 144.6, 142.0, 138.8, 138.6, 136.9, 134.8, 134.7, 134.0, 130.6, 129.9, 129.8, 129.4, 128.8, 128.3, 127.8, 127.5, 127.2, 126.4, 126.2, 125.8, 122.5, 122.0, 119.6, 119.1, 118.4, 116.9, 109.5, 109.2, 108.7, 104.2, 103.6, 99.8, 78.6, 74.6, 73.5, 72.1, 71.7, 66.0, 63.7, 62.1, 60.6, 56.9, 56.1, 55.9, 55.4, 39.6, 37.3, 33.2, 30.4, 30.0, 29.8, 28.5, 27.9, 26.3 and 23.1.

6. An antibiotic compound represented by the structural formula (II)



7. A process for the preparation of an antibiotic compound of Claim 1 which comprises the removal by acid hydrolysis of a glycolipid radical and/or a mannosyl radical from an antibiotic selected from the group consisting of AAD 216 complex, AAD 216A, AAD 216B, and AAD 216C.

8. A process for the preparation of the antibiotic compound of Claim 2 which comprises the removal by acid hydrolysis of a glycolipid radical from an antibiotic selected from the group consisting of AAD 216 complex, AAD 216A, AAD 216B and AAD 216B.

9. A process according to Claim 8 wherein the antibiotic hydrolyzed is AAD 216A, AAD 216B or AAD 216C.

- ### Claims for the Contracting State: AT

- 24

3. The process according to Claim 1 for preparing AAD 216 pseudo-aglycone which comprises the removal by acid hydrolysis of a glycolipid radical from AAD 216 complex, AAD 216A, AAD 216B or AAD 216C.

5. The process according to Claim 1 for preparing AAD 216B pseudo-aglycone which comprises the removal by acid hydrolysis of a mannosyl radical from AAD 216B.

6. The process according to Claim 1 for preparing AAD 216C pseudo-aglycone which comprises the removal by acid hydrolysis of a mannosyl radical from AAD 216C.

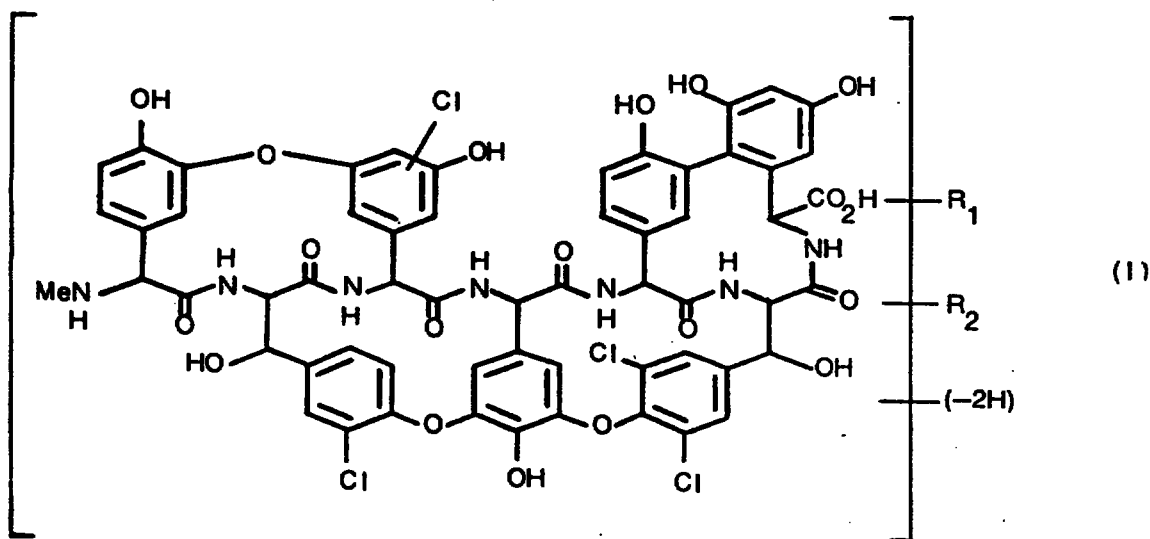
7. The process according to Claim 1 for preparing AAD 216 aglycone which comprises the removal by acid hydrolysis of a glycolipid radical and a mannosyl radical from AAD 216 complex, AAD 216A, AAD 216B or AAD 216C.

8. A process for preparing a composition comprising the admixing of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone, or AAD 216C pseudo-aglycone, or any mixture thereof, with a pharmaceutically acceptable carrier.

9. A process for preparing an animal feed composition comprising the admixing of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone or AAD 216C pseudo-aglycone, or any mixture thereof, with an animal feed.

10. A process for preparing an animal feed premix composition comprising the admixing of AAD 216 aglycone, AAD 216 pseudo-aglycone, AAD 216A pseudo-aglycone, AAD 216B pseudo-aglycone or AAD 216C pseudo-aglycone, or any mixture thereof with a premix vehicle.

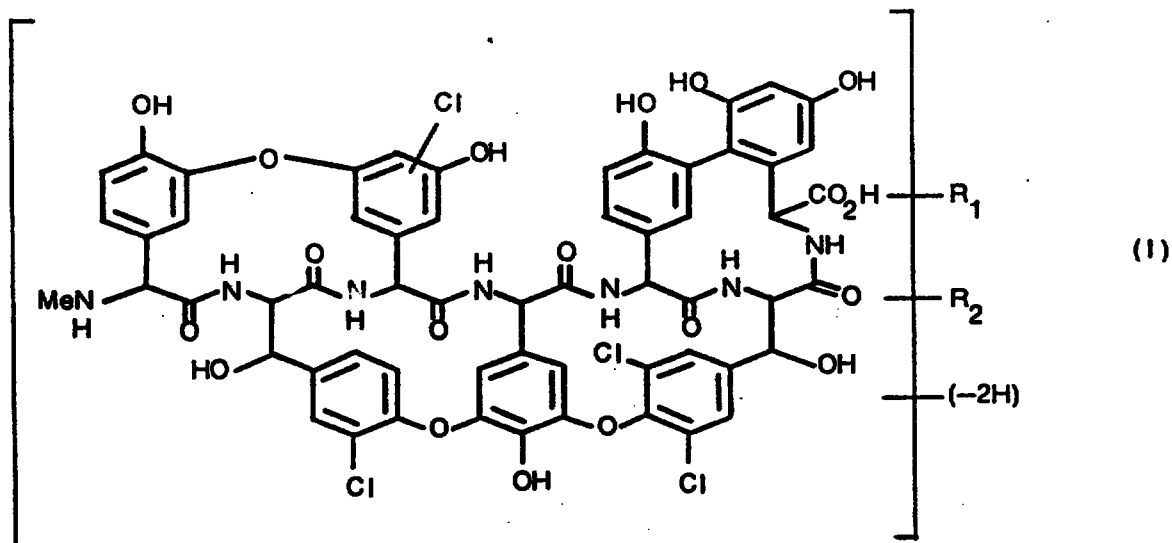
1. Antibiotikum der Strukturformel (I),



in der R₁ ein Wasserstoffatom oder ein Mannosylrest und R₂ ein Wasserstoffatom oder ein Glycolipidrest unbekannter Struktur ist, mit der Maßgabe, daß mindestens einer der beiden Reste R₁ und R₂ ein Wasserstoffatom ist, hergestellt durch saure hydrolytische Abspaltung eines Glycolipid- und/oder eines Mannosylrestes aus einem der Antibiotika AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C.



2. Antibiotikum der Strukturformel (I),



in der R_1 ein Mannosylrest und R_2 ein Wasserstoffatom ist, mit den folgenden Merkmalen:

(a) blaßweiß-gelber Feststoff, der sich bei 300 bis 350°C zersetzt,

(b) empirische Formel: $C_{66}H_{55}N_7O_{24}Cl_4$,

(c) ungefähre Elementarzusammensetzung: von 47,54% Kohlenstoff, 4,00% Wasserstoff, 5,89% Stickstoff und 8,63% Chlor bei einem Wassergehalt von 12%,

(d) Infrarot-Absorptionsspektrum in Kaliumbromid mit Peaks bei folgenden Wellenzahlen (in cm^{-1}): 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1230, 1180, 1150, 1120, 1060, 1010, 970 und 810;

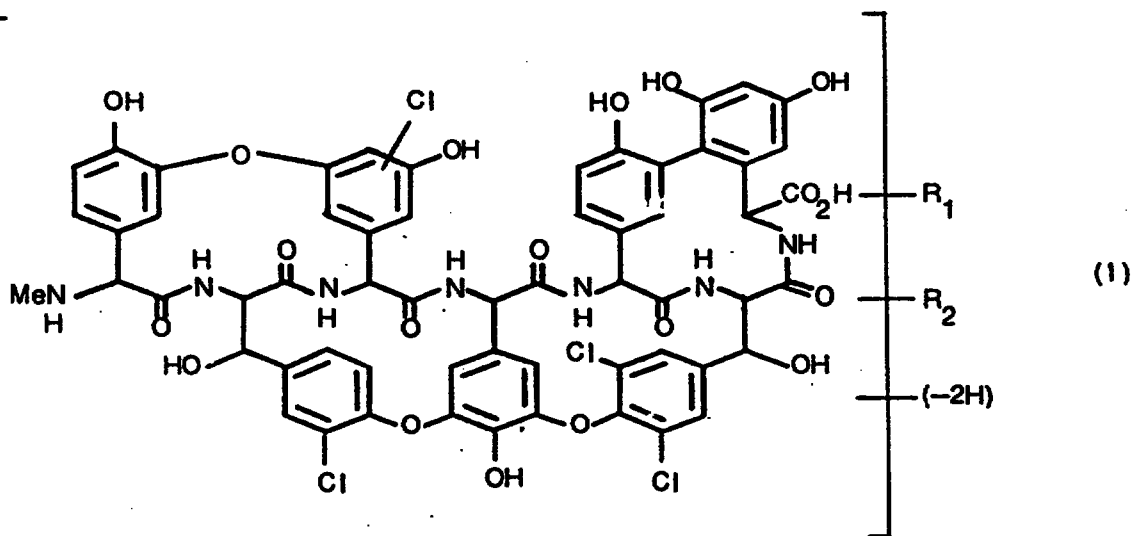
(e) FAB (fast atom bombardment)-Massenspektrum mit einem $M+H$ -Signal bei 1458 (Haupt-Cluster);

(f) Ultraviolett-Spektrum in Acetonitril:Wasser (1:1), mit einem Absorptionsmaximum bei 281 nm mit $E_{1\%} = 79,7$ unter sauren und einem Absorptionsmaximum bei 300 nm mit $E_{1\%} = 136$ unter basischen Bedingungen;

(g) 90,56 MHz Kohlenstoff-Kernmagnetisches Resonanzspektrum in $CD_3OD:D_2O$ (1:9) bei pH 8,7 mit folgenden chemischen Verschiebungen (in parts per million, ppm) relativ zum TMS-Standard: 177,9, 174,6, 171,7, 170,9, 170,0, 169,2, 162,3, 158,8, 157,9, 155,2, 155,1, 153,9, 151,9, 149,7, 147,6, 147,2, 144,4, 141,0, 139,0, 138,8, 137,5, 136,1, 134,6, 130,7, 130,0, 129,8, 129,2, 128,9, 128,7, 127,5, 127,3, 126,9, 126,4, 126,0, 125,2, 122,7, 122,2, 121,1, 119,9, 119,7, 118,4, 116,6, 110,7, 110,4, 108,5, 104,4, 103,3, 100,5, 98,1, 74,0, 72,1, 71,6, 71,4, 70,8, 67,4, 65,8, 63,7, 62,2, 61,6, 60,2, 56,0, 55,9, 55,0 und 32,9

(h) pK_a -Werte in Acetonitril:Wasser (3:7): 3,3, 7,1, 8,3, 9,1, 10,0 und 11,2 (pK_a -Werte über 11,2 wurden bestimmt).

3. Antibiotikum der Strukturformel (I),

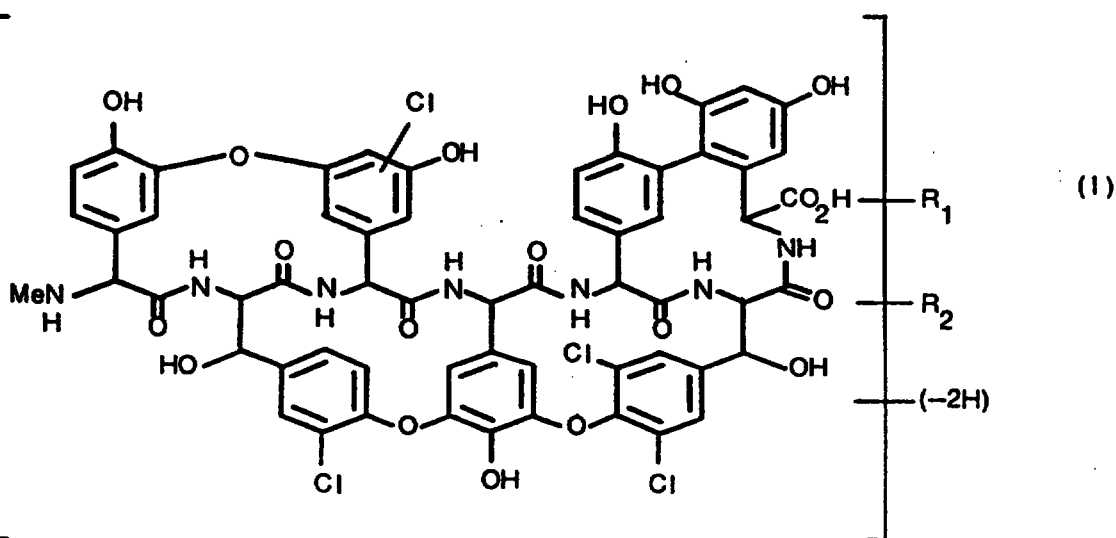


in der R_1 ein Wasserstoffatom und R_2 ein Glycolipidrest unbekannter Struktur mit der empirischen Formel $C_{18}H_{20}NO_8$ ist, das die folgenden Merkmale aufweist:



- (a) blaßweiß-gelber Feststoff, der sich bei 300 bis 350°C zersetzt;
 (b) empirische Formel: $C_{78}H_{72}N_8O_{25}Cl_4$;
 (c) ungefähre Elementarzusammensetzung: 49,76% Kohlenstoff, 4,51% Wasserstoff, 5,99% Stickstoff und 8,19% Chlor bei einem Wassergehalt von 9,4%;
 (d) Infrarot-Absorptionsspektrum in Kaliumbromid mit Peaks bei den folgenden Wellenzahlen (in cm^{-1}): 3400, 2920, 1660, 1590, 1500, 1460, 1430, 1300, 1240, 1140, 1080, 1060 und 1010;
 (e) FAB (fast atom bombardment)-Massenspektrum mit einem $M+H$ -Signal bei 1625 (Haupt-Cluster);
 (f) Ultraviolett-Spektrum in Acetonitril:Wasser (1:1), mit einem Absorptionsmaximum bei 281 nm mit $E_{1\%} = 63,2$ unter sauren und einem Absorptionsmaximum bei 302 nm mit $E_{1\%} = 94$ unter basischen Bedingungen;
 (g) 90,56 MHz Kohlenstoff-Kernmagnetisches Resonanzspektrum in $CD_3OD:D_2O$ (1:9) bei pH 9,4 mit folgenden chemischen Verschiebungen (in parts per million, ppm) relativ zum TMS-Standard: 178,2, 178,0, 176,0, 175,6, 171,6, 170,9, 170,6, 170,5, 169,3, 164,6, 161,4, 159,3, 157,1, 156,5, 153,5, 152,5, 151,8, 151,1, 146,4, 144,9, 141,7, 138,6, 138,4, 136,9, 134,5, 134,4, 133,9, 130,8, 129,8, 129,7, 129,4, 128,6, 128,5, 128,3, 127,7, 127,3, 125,9, 125,7, 125,5, 122,5, 122,1, 120,1, 119,4, 118,0, 116,9, 109,6, 109,0, 108,7, 104,5, 104,4, 103,2, 98,9, 78,4, 74,3, 73,3, 72,1, 71,5, 66,2, 63,7, 61,9, 60,6, 56,9, 56,1, 55,8, 55,4, 37,2, 33,3, 32,0, 29,6, 29,4, 26,1, 22,9 und 14,4; und
 (h) pK_a -Werte in Acetonitril:Wasser (3:7): 3,0, 4,3, 7,4, 8,5, 9,9 und 10,9 (pK_a -Werte über 10,9 wurden nicht bestimmt).

4. Antibiotikum der Strukturformel (I);

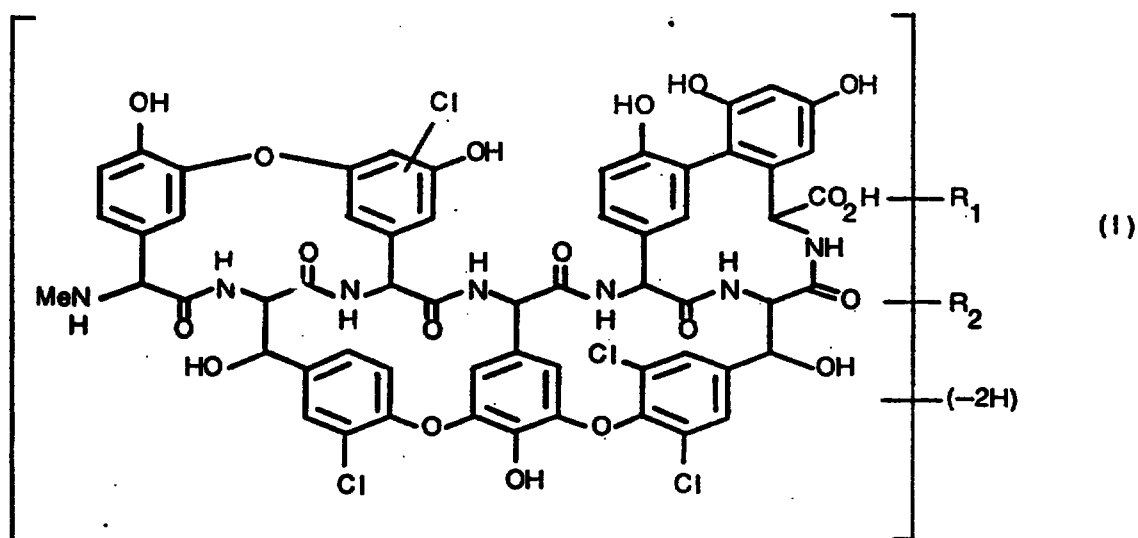


in der R_1 ein Wasserstoffatom und R_2 ein Glycolipidrest unbekannter Struktur mit der empirischen Formel $C_{17}H_{30}NO_6$ ist, das die folgenden Merkmale aufweist:

- (a) weißer Feststoff, der sich bei 250 bis 300°C zersetzt;
 (b) empirische Formel: $C_{78}H_{74}N_8O_{25}Cl_4$;
 (c) ungefähre Elementarzusammensetzung: 48,98% Kohlenstoff, 4,56% Wasserstoff, 5,64% Stickstoff und 8,29% Chlor bei einem Wassergehalt von 6,9%;
 (d) Infrarot-Absorptionsspektrum in Kaliumbromid mit Peaks bei folgenden Wellenzahlen (in cm^{-1}): 3400, 2920, 1660, 1590, 1500, 1480, 1430, 1300, 1240, 1150, 1080, 1060 und 1010;
 (e) FAB (fast atom bombardment)-Massenspektrum mit einem $M+H$ -Signal bei 1639 (Haupt-Cluster);
 (f) Ultraviolett-Spektrum in Acetonitril:Wasser (1:1) mit einem Absorptionsmaximum bei 281 nm mit $E_{1\%} = 61,8$ unter sauren und einem Absorptionsmaximum bei 303 nm mit $E_{1\%} = 88$ unter basischen Bedingungen;
 (g) 90,56 MHz Kohlenstoff-Kernmagnetisches Resonanzspektrum in $CD_3OD:D_2O$ (1:9) bei pH 9,4 mit folgenden chemischen Verschiebungen (in parts per million, ppm) relativ zum TMS-Standard: 178,3, 177,6, 175,9, 175,6, 171,5, 171,0, 170,7, 170,3, 169,4, 164,0, 159,3, 158,9, 156,4, 152,5, 151,7, 151,1, 146,2, 144,7, 141,8, 138,7, 138,6, 136,8, 134,4, 133,8, 130,9, 129,8, 129,5, 128,6, 128,4, 127,9, 127,3, 126,0, 125,8, 122,5, 119,9, 119,3, 118,2, 116,9, 109,3, 108,8, 104,3, 104,2, 103,0, 99,4, 78,7, 74,4, 73,5, 72,1, 71,6, 65,8, 63,8, 62,5, 60,6, 57,1, 56,1, 55,9, 55,4, 39,7, 37,3, 33,1, 30,3, 29,9, 29,7, 28,5, 28,0, 26,3 und 23,4.



5. Antibiotikum der Strukturformel (I),

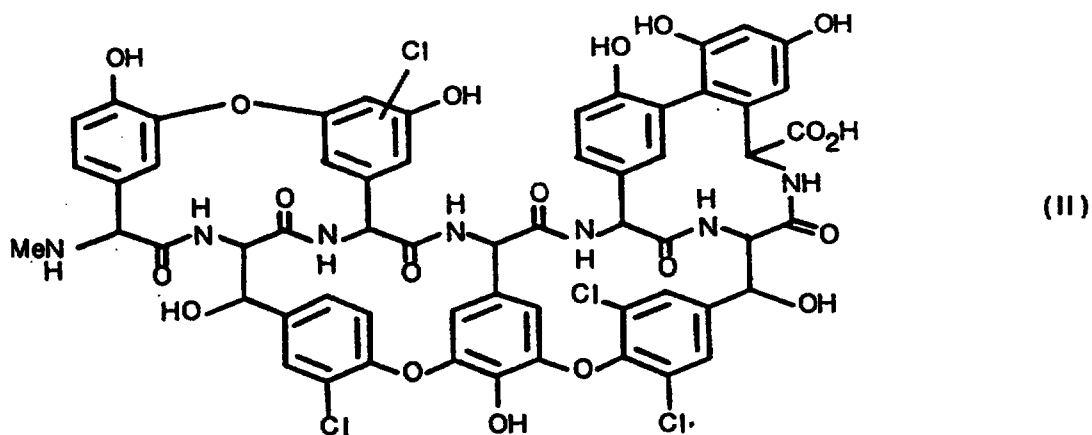


in der R_1 ein Wasserstoffatom und R_2 ein Glycolipidrest unbekannter Struktur mit der empirischen Formel $C_{18}H_{32}NO_8$ ist, das die folgenden Merkmale aufweist:

- (a) weißer Feststoff, der sich bei 250 bis 300°C zersetzt;
- (b) empirische Formel: $C_{77}H_{78}N_8O_{26}Cl_4$;
- (c) ungefähre Elementarzusammensetzung: 47,58% Kohlenstoff, 4,51% Wasserstoff, 5,33% Stickstoff und 8,08% Chlor bei einem Wassergehalt von 7,8%.
- (d) Infrarot-Absorptionsspektrum in Kaliumbromid mit Peaks bei den folgenden Wellenzahlen (in cm^{-1}): 3400, 2920, 1660, 1590, 1550, 1430, 1300, 1240, 1140, 1080, 1060 und 1010;
- (e) FAB (fast atom bombardment)-Massenspektrum mit einem $M+H$ -Signal bei 1653 (Haupt-Cluster);
- (f) Ultraviolett-Spektrum in Acetonitril:Wasser (1:1) mit einem Absorptionsmaximum bei 281 nm mit $E_{1\%} = 60,0$ unter sauren und einem Absorptionsmaximum bei 303 nm mit $E_{1\%} = 83$ unter basischen Bedingungen;

(g) 90,56 MHz Kohlenstoff-Kernmagnetisches Resonanzspektrum in $CD_3OD:D_2O$ (1:9) bei pH 9,4 mit folgenden chemischen Verschiebungen (in parts per million, ppm) relativ zum TMS-Standard: 178,1, 177,4, 175,5, 175,4, 171,4, 171,0, 170,7, 170,3, 169,3, 163,6, 159,1, 158,6, 156,5, 156,2, 152,7, 151,9, 151,8, 151,0, 146,2, 144,6, 142,0, 138,8, 138,6, 136,9, 134,8, 134,7, 134,0, 130,6, 129,9, 129,8, 129,4, 128,8, 128,3, 127,8, 127,5, 127,2, 126,4, 126,2, 125,8, 122,5, 122,0, 119,6, 119,1, 118,4, 116,9, 109,5, 109,2, 108,7, 104,2, 103,6, 99,8, 78,6, 74,6, 73,5, 72,1, 71,7, 66,0, 63,7, 62,1, 60,6, 56,9, 56,1, 55,9, 55,4, 39,6, 37,3, 33,2, 30,4, 30,0, 29,8, 28,5, 27,9, 26,3 und 23,1.

6. Antibiotikum der Formel (II),



7. Verfahren zur Herstellung eines Antibiotikums nach Anspruch 1, umfassend die Abspaltung durch saure Hydrolyse eines Glycolipid- und/oder eines Mannosylrestes von einem der Antibiotika AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C.

8. Verfahren zur Herstellung eines Antibiotikums nach Anspruch 2, umfassend die Abspaltung durch saure Hydrolyse eines Glycolipidrestes von einem der Antibiotika AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C.



9. Verfahren nach Anspruch 8, bei dem das hydrolysierte Antibiotikum AAD 216, AAD 216B oder AAD 216C ist.

10. Verfahren nach Anspruch 9, bei dem die Hydrolyse in einem wäßrigen organischen Lösungsmittel mit 0,001 N Salzsäure unter Rückfluß durchgeführt wird.

11. Verfahren zur Herstellung des Antibiotikums nach Anspruch 3, bei dem ein Mannosylrest von AAD 216A durch saure Hydrolyse abgespalten wird.

12. Verfahren zur Herstellung des Antibiotikums nach Anspruch 4, bei dem ein Mannosylrest von AAD 216B durch saure Hydrolyse abgespalten wird.

13. Verfahren zur Herstellung des Antibiotikums nach Anspruch 5, bei dem ein Mannosylrest von AAD 216C durch saure Hydrolyse abgespalten wird.

14. Verfahren zur Herstellung des Antibiotikums nach Anspruch 6, bei dem ein Glycolipid- und ein Mannosylrest von einem Antibiotikum AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C durch saure Hydrolyse abgespalten werden.

15. Verfahren Anspruch 14, bei dem das hydrolysierte Antibiotikum AAD 216A, AAD 216B oder AAD 216C ist.

16. Verfahren nach einem der Ansprüche 11, 12, 13 oder 15, bei dem die Hydrolyse in einem polaren organischen Lösungsmittel mit 5% Salzsäure bei erhöhter Temperatur durchgeführt wird.

17. Verfahren nach einem der Ansprüche 9, 11, 12, 13 oder 15, bei dem das Antibiotikum auf chromatographischem Wege isoliert wird.

18. Zusammensetzung, umfassend ein Produkt nach einem der Ansprüche 1 bis 6 oder ein Gemisch dieser Produkte und einen pharmazeutisch verträglichen Träger.

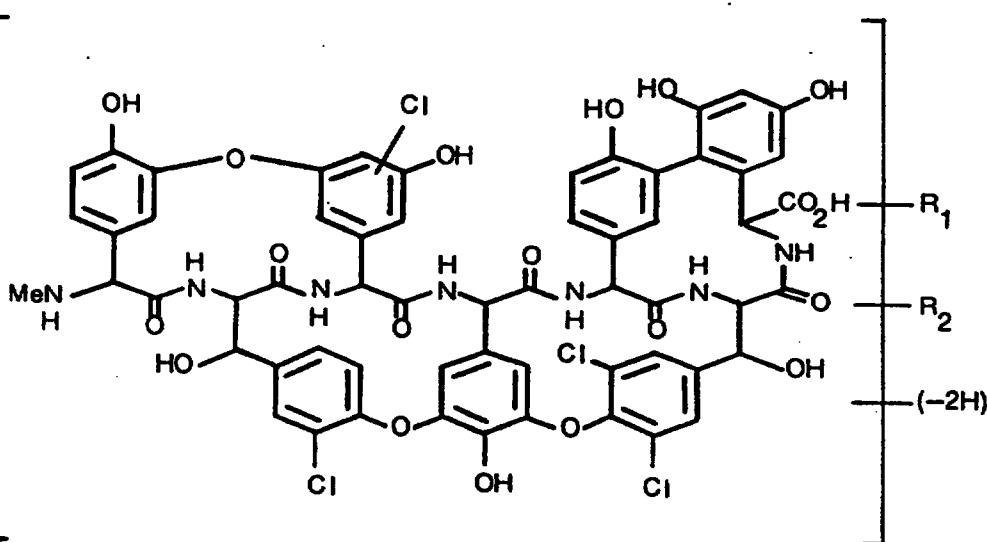
19. Tierfuttermittel, ergänzt mit einer nicht-toxischen Menge eines Produktes nach einem der Ansprüche 1 bis 6 oder mit einem Gemisch dieser Produkte.

20. Vorgemischtes Tierfuttermittel, umfassend vorgemischte Hilfsstoffe und ein Produkt nach einem der Ansprüche 1 bis 6 oder ein Gemisch dieser Produkte.

21. Verfahren zur Verbesserung der Wachstumsgeschwindigkeit und der Futterverwertung von Fleisch oder Milch produzierenden Tieren, umfassend die Verabreichung eines Produkts oder einer Zusammensetzung nach einem der Ansprüche 1 bis 6, 18, 19 oder 20.

Patentansprüche für den Vertragsstaat: AT

1. Verfahren zur Herstellung eines Antibiotikums der Formel (I)



(I)

in der R_1 ein Wasserstoffatom oder ein Mannosylrest und R_2 ein Wasserstoffatom oder ein Glycolipidrest unbekannter Struktur ist, mit der Maßgabe, daß mindestens einer der beiden Reste R_1 und R_2 ein Wasserstoffatom ist, umfassend:

i) für eine Verbindung, in der R_1 ein Wasserstoffatom und R_2 ein Glycolipidrest ist, die hydrolytische Demannosylierung des AAD 216-Komplexes, welcher die Verbindung der allgemeinen Formel I ist, in der R_1 ein Mannosyl- und R_2 ein Glycolipidrest ist,

ii) für eine Verbindung, in der R_1 ein Mannosylrest und R_2 ein Wasserstoffatom ist, die hydrolytische Abspaltung eines Glycolipidrestes aus dem AAD 216-Komplex, welcher die Verbindung der allgemeinen Formel I ist, in der R_1 ein Mannosyl- und R_2 ein Glycolipidrest ist,

und gegebenenfalls danach Erzeugung einer Verbindung, in der sowohl R_1 , als auch R_2 Wasserstoffatome sind, durch



i) hydrolytische Abspaltung eines Glycolipidrestes aus einer Verbindung, in der R_1 ein Wasserstoffatom und R_2 ein Glycolipidrest ist oder

ii) hydrolytische Demannosylierung einer Verbindung, in der R_1 ein Mannosylrest und R_2 ein Wasserstoffatom ist.

2. Verfahren zur Herstellung einer Verbindung nach Anspruch 1, umfassend die Abspaltung eines Glycolipid- und/oder eines Mannosylrest vom AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C durch saure Hydrolyse.

3. Verfahren nach Anspruch 1 zur Herstellung von AAD 216 Pseudoaglycon, umfassend die Abspaltung eines Glycolipidrestes vom AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C durch saure Hydrolyse.

4. Verfahren nach Anspruch 1 zur Herstellung von AAD 216A Pseudoaglycon, umfassend die Abspaltung eines Mannosylrestes von AAD 216A durch saure Hydrolyse.

5. Verfahren nach Anspruch 1 zur Herstellung von AAD 216B Pseudoaglycon, umfassend die Abspaltung eines Mannosylrestes von AAD 216B durch saure Hydrolyse.

6. Verfahren nach Anspruch 1 zur Herstellung von AAD 216C Pseudoaglycon, umfassend die Abspaltung eines Mannosylrestes von AAD 216C durch saure Hydrolyse.

7. Verfahren nach Anspruch 1 zur Herstellung von AAD 216 Aglycon, umfassend die Abspaltung eines Glycolipid- und eines Mannosylrestes vom AAD 216-Komplex, AAD 216A, AAD 216B oder AAD 216C durch saure Hydrolyse.

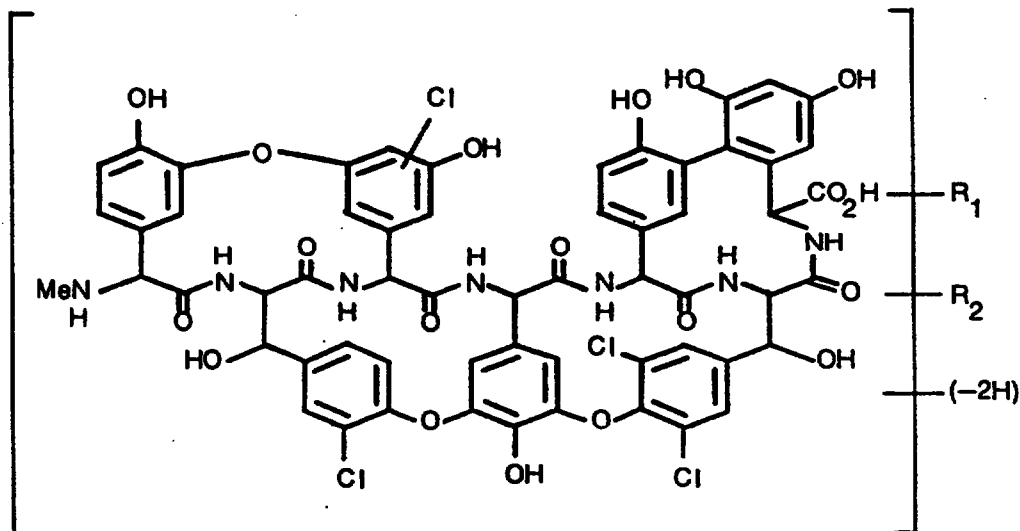
8. Verfahren zur Herstellung einer Zusammensetzung, umfassend das Vermischen von AAD 216 Aglycon, AAD 216 Pseudoaglycon, AAD 216A Pseudoaglycon, AAD 216B Pseudoaglycon oder AAD 216C Pseudoaglycon oder irgendeines Gemisches dieser Stoffe mit einem pharmazeutisch verträglichen Trägermaterial.

9. Verfahren zur Herstellung eines Tierfuttermittels, umfassend das Vermischen von AAD 216 Aglycon, AAD 216 Pseudoaglycon, AAD 216A Pseudoaglycon, AAD 216B Pseudoaglycon oder AAD 216C Pseudoaglycon oder irgendeines Gemisches dieser Stoffe mit einem Tierfutter.

10. Verfahren zur Herstellung eines vorgemischten Tierfuttermittels, umfassend das Vermischen von AAD 216 Aglycon, AAD 216 Pseudoaglycon, AAD 216A Pseudoaglycon, AAD 216B Pseudoaglycon oder AAD 216C Pseudoaglycon oder irgendeines Gemisches dieser Stoffe mit vorgemischten Hilfsstoffen.

Revendications pour les Etats contractants: BE CH DE FR GB IT LI LU NL SE

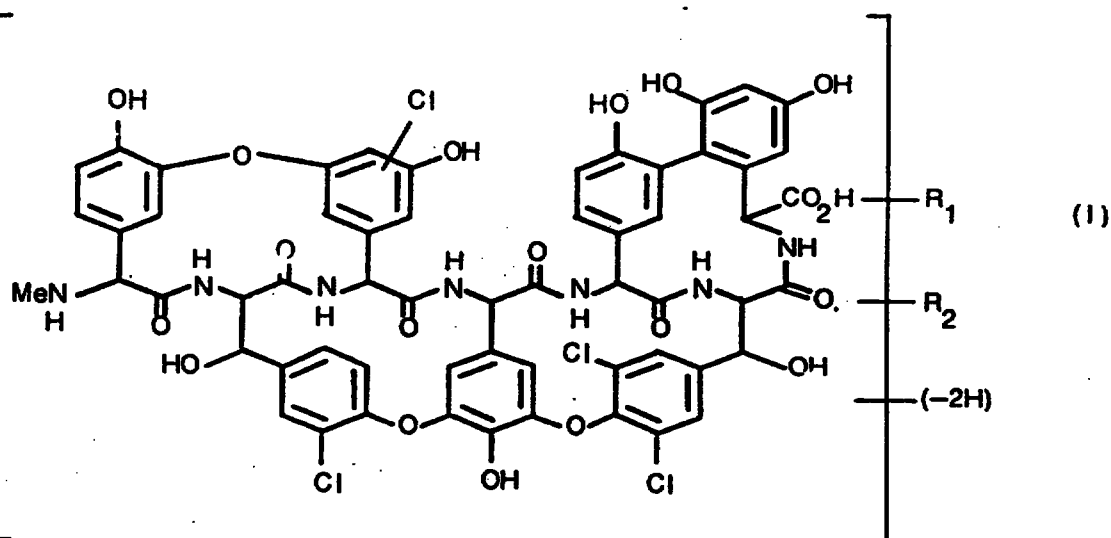
1. Composé antibiotique représenté par la formule de structure (I):



dans laquelle R_1 est de l'hydrogène ou mannosyle et R_2 est de l'hydrogène ou un radical glycolipidique de structure inconnue à la condition que au moins un des R_1 et R_2 soit de l'hydrogène, préparé par l'élimination par hydrolyse acide d'un radical glycolipidique et/ou d'un radical mannosyle d'un antibiotique choisi dans le groupe composé par le complexe AAD 216, l'AAD 216A, l'AAD 216B et l'AAD 216C.



2. Composé antibiotique représenté par la formule de structure (I):



dans laquelle R_1 est mannosyle et R_2 est de l'hydrogène ayant les caractéristiques suivantes:

(a) solide blanc-jaune pâle qui se décompose à 300–350°C;

(b) une formule empirique $C_{65}H_{55}N_7O_{24}Cl_4$;

(c) une composition élémentaire approximative de 47,54 pourcent de carbone, 4,00 pourcent d'hydrogène, 5,89 pourcent d'azote et 8,63 pourcent de chlore lorsque la teneur en eau est de 12 pourcent;

(d) un spectre d'absorption infrarouge dans le bromure de potassium qui présente des pics aux nombres d'ondes suivants en cm^{-1} : 3400, 1660, 1610, 1590, 1510, 1460, 1430, 1390, 1300, 1230, 1180, 1150, 1120, 1060, 1010, 970 et 810;

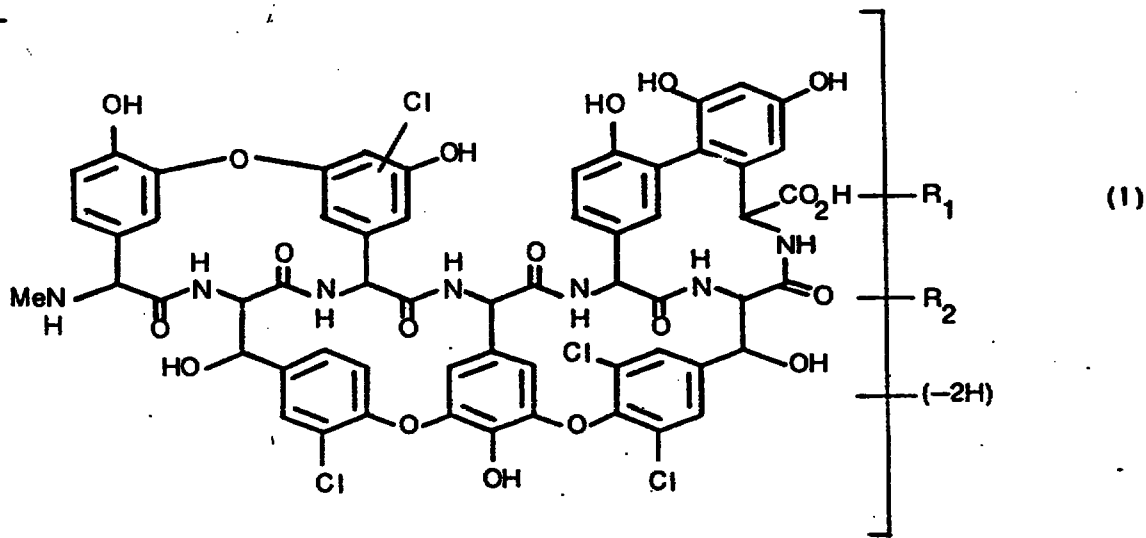
(e) un spectre de masse par bombardement atomique rapide (FAB) avec $M+H$ à 1458 (groupement principal);

(f) un spectre ultraviolet dans le mélange acétonitrile:eau (1:1) qui présente un maximum d'absorption à 281 nm dans des conditions acides avec un $E_{1\%} = 79,7$ et à 300 nm dans des conditions basiques avec un $E_{1\%} = 136$;

(g) un spectre de résonance magnétique du carbone à 90,56 MHz dans le mélange $CD_3OD:D_2O$ (1:9) à un pH de 8,7 qui présente les glissements chimiques suivants en partie par million (ppm) par rapport à TMS comme standard: 177,9, 174,6, 171,7, 170,9, 170,0, 169,2, 162,3, 158,8, 157,9, 155,2, 155,1, 153,9, 151,9, 149,7, 147,6, 147,2, 144,4, 141,0, 139,0, 138,8, 137,5, 136,1, 134,6, 130,7, 130,0, 129,8, 129,2, 128,9, 128,7, 127,5, 127,3, 126,9, 126,4, 126,0, 125,2, 122,7, 122,2, 121,1, 119,9, 119,7, 118,4, 116,6, 110,7, 110,4, 108,5, 104,4, 103,3, 100,5, 98,1, 74,0, 72,1, 71,6, 71,4, 70,8, 67,4, 65,8, 63,7, 62,2, 61,6, 60,2, 56,0, 55,9, 55,0 et 32,9 et

(h) des valeurs pK_a dans le mélange acétonitrile:eau (3:7) comme suit: 3,3, 7,1, 8,3, 9,1, 10,0 et 11,2, les valeurs pK_a au dessus de 11,2 n'étant pas déterminées.

3. Composé antibiotique représenté par la formule de structure (I):



dans laquelle R_1 est de l'hydrogène et R_2 est un glycolipide de structure inconnue de formule empirique $C_{16}H_{28}NO_6$ et ayant les caractéristiques suivantes:



(a) solide blanc-jaune pâle qui se décompose à 300—350°C;

(b) une formule empirique $C_{75}H_{72}N_8O_{25}Cl_4$;

(c) une composition élémentaire approximative de 49,76% de carbone, 4,51 pourcent d'hydrogène, 9,49 pourcent d'azote et 8,19 pourcent de chlore lorsque la teneur en eau est de 9,4 pourcent;

(d) un spectre d'absorption infrarouge dans le bromure de potassium qui présente des pics aux nombres d'ondes suivants en cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1460, 1430, 1300, 1240, 1140, 1080, 1060 et 1010;

(e) un spectre de masse par bombardement atomique rapide (FAB) avec $M+H$ à 1625 (groupement principal);

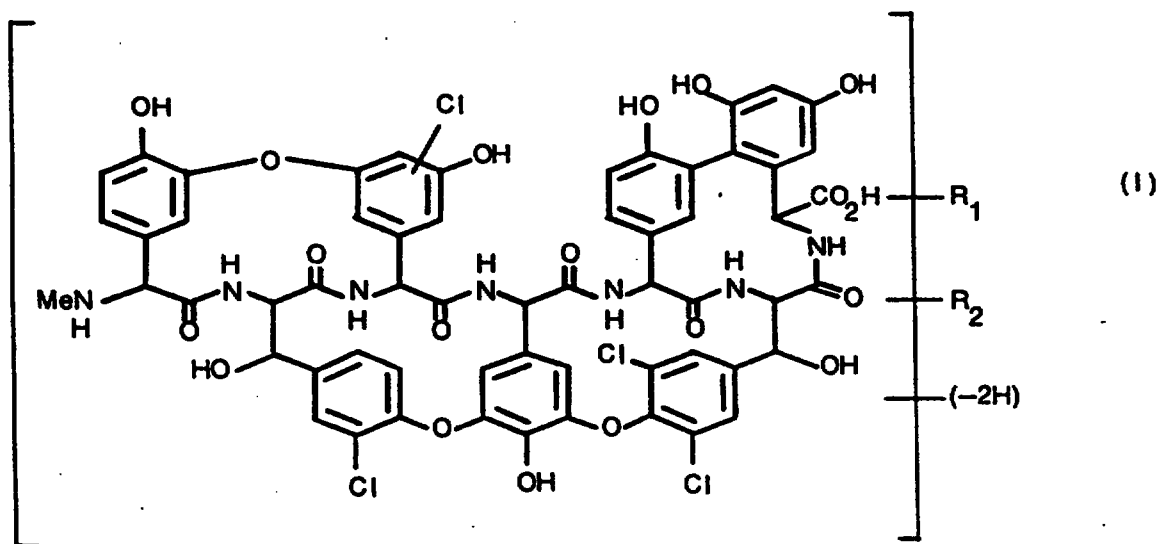
(f) un spectre ultraviolet dans le mélange acétonitrile:eau (1:1) qui présente un maximum d'absorption à 281 nm dans des conditions acides avec un $E_{1\%} = 63,2$ et à 302 nm dans des conditions basiques avec un $E_{1\%} = 94$;

(g) un spectre de résonance magnétique du carbone à 90,56 MHz dans le mélange $CD_3OD:D_2O$ (1:9) à un pH de 9,4 qui présente les glissements chimiques suivants en partie par million (ppm) par rapport à TMS comme standard:

178,2, 178,0, 176,0, 175,6, 171,6, 170,9, 170,6, 170,5, 169,3, 164,6, 161,4, 159,3, 157,1, 156,5, 153,5, 152,5, 151,8, 151,1, 146,4, 144,9, 141,7, 138,6, 138,4, 136,9, 134,5, 134,4, 133,9, 130,8, 129,8, 129,7, 129,4, 128,6, 128,5, 128,3, 127,7, 127,3, 125,9, 125,7, 125,5, 122,5, 122,1, 120,1, 119,4, 118,0, 116,9, 109,6, 109,0, 108,7, 104,5, 104,4, 103,2, 98,9, 78,4, 74,3, 73,3, 72,1, 71,5, 66,2, 63,7, 61,9, 60,6, 56,9, 56,1, 55,8, 55,4, 37,2, 33,3, 32,0, 29,6, 29,4, 26,1, 22,9 et 14,4 et

(h) des valeurs pK_a dans le mélange acétonitrile:eau (3:7) comme suit: 3,0, 4,3, 7,4, 8,5, 9,9 et 10,9, les valeurs pK_a au-dessus de 10,9 n'étant pas déterminées.

4. Composé antibiotique représenté par la formule de structure (I):



dans laquelle R_1 est de l'hydrogène et R_2 est un radical glycolipidique de structure inconnue de formule empirique $C_{17}H_{30}NO_8$ ayant les caractéristiques suivantes;

(a) solide blanc qui se décompose à 250—300°C;

(b) formule empirique $C_{78}H_{74}N_8O_{25}Cl_4$;

(c) une composition élémentaire approximative de 48,98 pourcent de carbone, 4,56 pourcent d'hydrogène, 5,64 pourcent d'azote et 8,29 pourcent de chlore lorsque la teneur en eau est de 6,9 pourcent;

(d) un spectre d'absorption infrarouge dans le bromure de potassium qui présente des pics aux nombres d'ondes suivants en cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1480, 1430, 1300, 1240, 1150, 1080, 1060 et 1010;

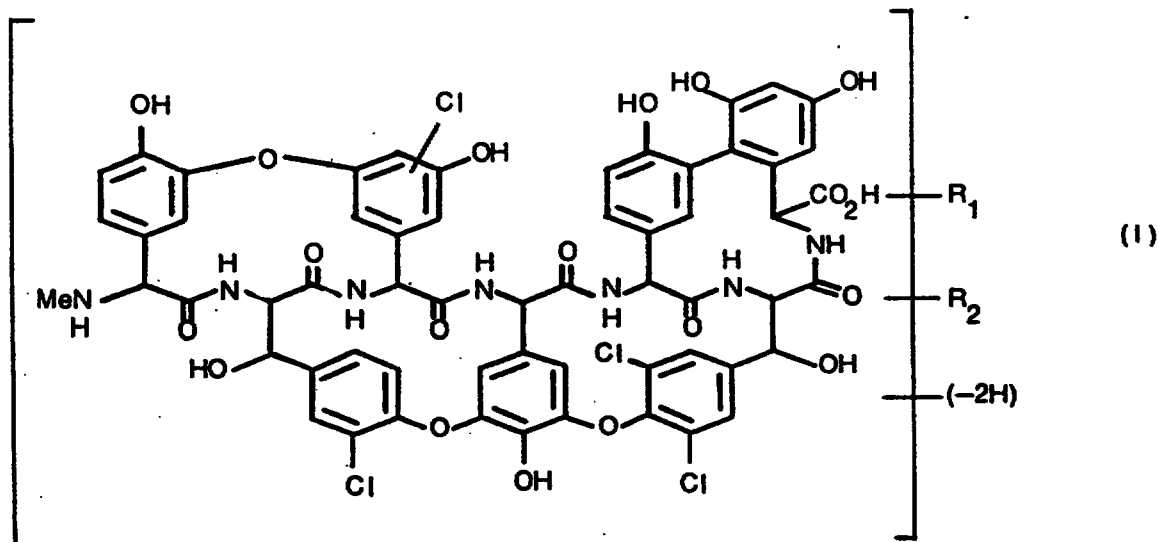
(e) un spectre de masse par bombardement atomique rapide (FAB) avec $M+H$ à 1639 (groupement principal);

(f) un spectre ultraviolet dans le mélange acétonitrile:eau (1:1) qui présente un maximum d'absorption à 281 nm dans des conditions acides avec un $E_{1\%} = 61,8$ et à 303 nm dans des conditions basiques avec un $E_{1\%} = 88$;

(g) un spectre de résonance magnétique du carbone à 90,56 MHz dans le mélange $CD_3OD:D_2O$ (1:9) à un pH de 9,4 qui présente les glissements chimiques suivants en parties par million (ppm) par rapport à TMS comme standard: 178,3, 177,6, 175,9, 175,6, 171,5, 171,0, 170,7, 170,3, 169,4, 164,0, 159,3, 158,9, 156,4, 152,5, 151,7, 146,2, 144,7, 141,8, 138,7, 138,6, 136,8, 134,4, 133,8, 130,9, 129,8, 129,5, 128,6, 128,4, 127,9, 127,3, 126,0, 125,8, 122,5, 119,9, 119,3, 118,2, 116,9, 109,3, 108,8, 104,3, 104,2, 103,0, 99,4, 78,7, 74,4, 73,5, 72,1, 71,6, 65,8, 63,8, 62,5, 60,6, 57,1, 56,1, 55,9, 55,4, 39,7, 37,3, 33,1, 30,3, 29,9, 29,7, 28,5, 28,0, 26,3 et 23,4.



5. Composé antibiotique représenté par la formule de structure (I):



dans laquelle R_1 est de l'hydrogène et R_2 est un radical glycolipidique de structure inconnue, de formule empirique $C_{18}H_{32}NO_8$ ayant les caractéristiques suivantes:

(a) solide blanc qui se décompose à 250–300°C;

(b) une formule empirique $C_{77}H_{78}N_8O_{26}Cl_4$;

(c) une composition élémentaire approximative de 47,58 pourcent de carbone, 4,51 pourcent d'hydrogène, 5,33 pourcent d'azote et 8,08 pourcent de chlore lorsque la teneur en eau est de 7,8 pourcent;

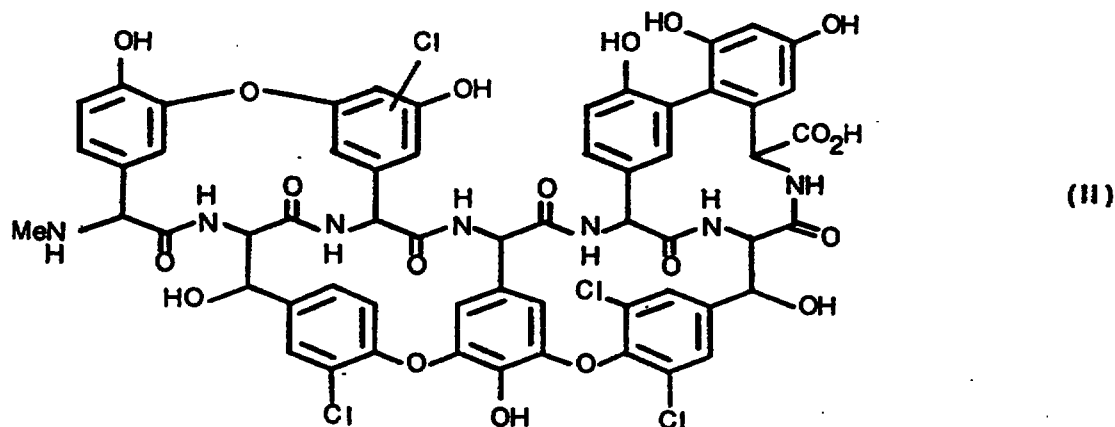
(d) un spectre d'absorption infrarouge dans le bromure de potassium qui présente des pics aux nombres d'ondes suivants en cm^{-1} : 3400, 2920, 1660, 1590, 1500, 1430, 1300, 1240, 1140, 1080, 1060 et 1010;

(e) un spectre de masse par bombardement atomique rapide (FAB) avec $M+H$ à 1653 (groupement principal);

(f) un spectre ultraviolet dans le mélange acétonitrile:eau (1:1) qui présente un maximum d'absorption à 281 nm dans des conditions acides avec un $E_{1\%} = 60,0$ et à 303 nm dans des conditions basiques avec un $E_{1\%} = 83$;

(g) un spectre de résonance magnétique du carbone à 90,56 MHz dans le mélange $CD_3OD:D_2O$ (1:9) à un pH de 9,4 qui présente les glissements chimiques suivants en parties par million (ppm) par rapport à TMS comme standard: 178,1, 177,4, 175,5, 175,4, 171,4, 171,0, 170,7, 170,3, 169,3, 163,6, 159,1, 158,6, 156,5, 156,2, 152,7, 151,9, 151,8, 151,0, 146,2, 144,6, 142,0, 138,8, 138,6, 136,9, 134,8, 134,7, 134,0, 130,6, 129,9, 129,8, 129,4, 128,8, 128,3, 127,8, 127,5, 127,2, 126,4, 126,2, 125,8, 122,5, 122,0, 119,6, 119,1, 118,4, 116,9, 109,5, 109,2, 108,7, 104,2, 103,6, 99,8, 78,6, 74,6, 73,5, 72,1, 71,7, 66,0, 63,7, 62,1, 60,6, 56,9, 56,1, 55,9, 55,4, 39,6, 37,3, 33,2, 30,4, 30,0, 29,8, 28,5, 27,9, 26,3 et 23,1.

6. Composé antibiotique représenté par la formule de structure (II):



7. Procédé pour la préparation d'un composé antibiotique de la Revendication 1 qui comprend l'enlèvement par hydrolyse acide d'un radical glycolipidique et/ou d'un radical mannosyle d'un antibiotique choisi dans le groupe composé du complexe AAD 216, de l'AAD 216A, de l'AAD 216B et de l'AAD 216C.



8. Procédé pour la préparation du composé antibiotique de la Revendication 2 qui comprend l'enlèvement par hydrolyse acide d'un radical glycolipidique d'un antibiotique choisi dans le groupe composé du complexe AAD 216, de l'AAD 216A, de l'AAD 216B et de l'AAD 216C.

9. Procédé suivant la Revendication 8 dans laquelle l'antibiotique hydrolysé est l'AAD 216A, l'AAD 216B ou l'AAD 216C.

10. Procédé suivant la Revendication 9 dans laquelle l'hydrolyse est effectuée dans un solvant organique aqueux avec de l'acide chlorhydrique 0,001 N au reflux.

11. Procédé pour la préparation du composé antibiotique de la Revendication 3 qui comprend l'enlèvement d'un radical mannosyle de l'AAD 216A, par hydrolyse acide.

12. Procédé pour la préparation du composé antibiotique de la Revendication 4 qui comprend l'enlèvement d'un radical mannosyle de l'AAD 216B, par hydrolyse acide.

13. Procédé pour la préparation du composé antibiotique de la Revendication 5 qui comprend l'enlèvement d'un radical mannosyle de l'AAD 216C, par hydrolyse acide.

14. Procédé pour la préparation du composé antibiotique de la Revendication 6 qui comprend l'enlèvement, par hydrolyse acide, d'un radical glycolipidique et d'un radical mannosyle d'un antibiotique choisi dans le groupe composé du complexe AAD 216, de l'AAD 216A, de l'AAD 216B et de l'AAD 216C.

15. Procédé suivant la Revendication 14 dans laquelle l'antibiotique hydrolysé est l'AAD 216A, l'AAD 216B ou l'AAD 216C.

16. Procédé suivant l'une quelconque des Revendications 11, 12, 13 ou 15 dans laquelle l'hydrolyse est effectuée dans un solvant organique polaire avec de l'acide chlorhydrique à 5% à température élevée.

17. Procédé suivant l'une quelconque des Revendications 9, 11, 12, 13, ou 15 dans laquelle le composé antibiotique est isolé par des moyens chromatographiques.

18. Composition comprenant un produit tel que défini dans l'une quelconque des Revendications 1, 2, 3, 4, 5 ou 6 n'importe lequel de leurs mélanges et un support pharmaceutiquement acceptable.

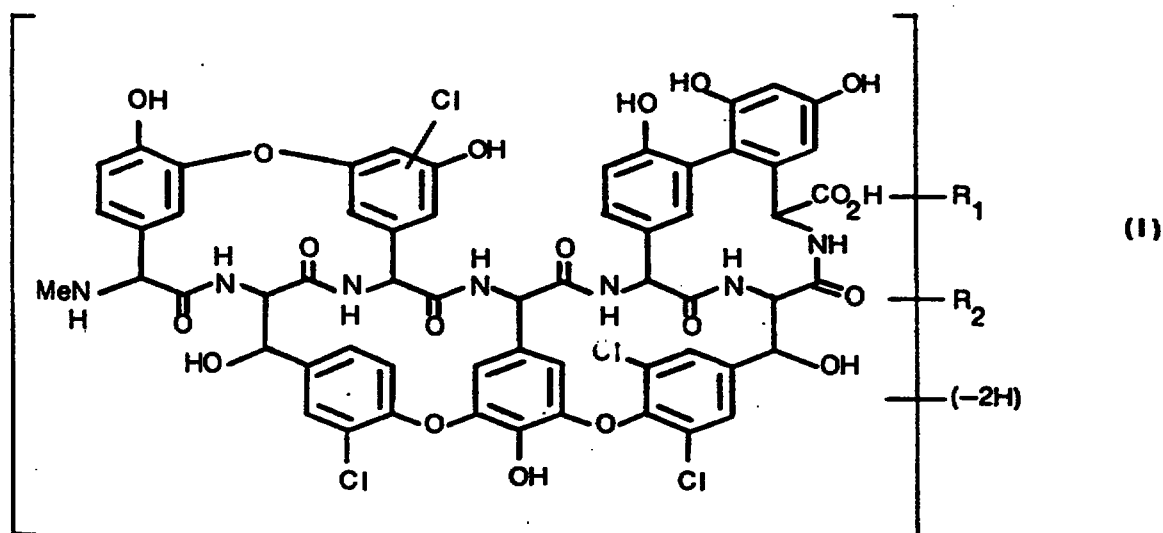
19. Composition alimentaire pour animaux additionnée d'une quantité non toxique d'un composé tel que défini dans l'une quelconque des Revendications 1, 2, 3, 4, 5 ou 6 ou de n'importe lequel de leurs mélanges.

20. Composition de prémélange pour aliment pour animaux comprenant un véhicule de prémélange et un produit tel que défini dans l'une quelconque des Revendications 1, 2, 3, 4, 5 ou 6 ou n'importe lequel de leurs mélanges.

21. Méthode pour améliorer la vitesse de croissance et l'efficacité alimentaire d'animaux producteurs de viande ou de lait qui comprend l'administration d'un produit ou d'une composition tels que définis dans l'une quelconque des revendications 1, 2, 3, 4, 5, 6, 18, 19 ou 20.

Revendications pour l'Etat contractant: AT

1. Procédé pour préparer un composé antibiotique de formule (I):



dans laquelle R_1 est de l'hydrogène ou mannosyle et R_2 est de l'hydrogène ou un radical glycolipidique de structure inconnue à la condition que au moins un des R_1 et R_2 soit de l'hydrogène, qui comprend:

i) pour un composé où R_1 est de l'hydrogène et R_2 est un radical glycolipidique, la dé-mannosylation par hydrolyse du complexe AAD 216 qui est le composé de formule (I) dans laquelle R_1 est mannosyle et R_2 est un radical glycolipidique;

ii) pour un composé où R_1 est mannosyle et R_2 est de l'hydrogène, l'enlèvement d'un radical glycolipidique par hydrolyse du complexe AAD 216, qui est le composé de formule (I) dans laquelle R_1 est mannosyle et R_2 est un radical glycolipidique;



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et, facultativement, la formation subséquente d'un composé où R_1 et R_2 sont tous deux de l'hydrogène par:
i) enlèvement d'un radical glycolipidique par hydrolyse d'un composé dans lequel R_1 est de l'hydrogène et R_2 est un radical glycolipidique; ou

ii) la dé-mannosylation par hydrolyse d'un composé dans lequel R_1 est mannosyle et R_2 est de l'hydrogène.

2. Procédé pour préparer un composé suivant la revendication 1 qui comprend l'enlèvement, par hydrolyse acide, d'un radical glycolipidique et/ou d'un radical mannosyle du complexe AAD 216, de l'AAD 216A, de l'AAD 216B ou de l'AAD 216C.

3. Procédé suivant la revendication 1 pour préparer la pseudo-aglycone d'AAD 216 qui comprend l'enlèvement, par hydrolyse acide, d'un radical glycolipidique du complexe AAD 216, de l'AAD 216A, de l'AAD 216B ou de l'AAD 216C.

4. Procédé suivant la revendication 1 pour préparer la pseudo-aglycone d'AAD 216A qui comprend l'enlèvement par hydrolyse acide, d'un radical mannosyle de l'AAD 216A.

5. Procédé suivant la revendication 1 pour préparer la pseudo-aglycone d'AAD 216B qui comprend l'enlèvement par hydrolyse acide d'un radical mannosyle de l'AAD 216B.

6. Procédé suivant la revendication 1 pour préparer la pseudo-aglycone d'AAD 216C qui comprend l'enlèvement, par hydrolyse acide, d'un radical mannosyle de l'AAD 216C.

7. Procédé suivant la revendication 1 pour préparer la pseudo-aglycone de l'AAD 216 qui comprend l'enlèvement, par hydrolyse acide, d'un radical glycolipidique et d'un radical mannosyle du complexe AAD 216, de l'AAD 216A, de l'AAD 216B ou de l'AAD 216C.

8. Procédé pour préparer une composition comprenant le mélange d'aglycone de l'AAD 216, de pseudo-aglycone d'AAD 216, de pseudo-aglycone d'AAD 216A, de pseudo-aglycone d'AAD 216B ou de pseudo-aglycone d'AAD 216C ou n'importe lequel de leurs mélanges avec un support pharmaceutiquement acceptable.

9. Procédé pour préparer une composition alimentaire pour animaux comprenant le mélange d'aglycone d'AAD 216, de pseudo-aglycone d'AAD 216, de pseudo-aglycone d'AAD 216A, de pseudo-aglycone d'AAD 216B ou de pseudo-aglycone d'AAD 216C ou de n'importe lequel de leurs mélanges, avec un aliment pour animaux.

10. Procédé pour préparer une composition de prémélange pour aliment pour animaux comprenant le mélange d'aglycone d'AAD 216, de pseudo-aglycone d'AAD 216, de pseudo-aglycone d'AAD 216A, de pseudo-aglycone d'AAD 216B ou de pseudo-aglycone d'AAD 216C ou n'importe lequel de leurs mélanges avec un support de prémélange.