ENHANCEMENT OF THE ACTIVITY OF THE ANTIBIOTIC LAIDLOMYCIN BY ACYLATION AND THE ^{13}C NMR SPECTRA OF LAIDLOMYCIN AND ITS ESTERS†

ROBIN D. CLARK*, GARY L. HEDDEN, ARTHUR F. KLUGE, MICHAEL L. MADDOX*

Institute of Organic Chemistry

HOWARD R. SPIRES

Institute of Agriscience, Syntex Research,
Palo Alto, California 94304, U.S.A.

and PETER F. LONG

Department of Poultry Science, University of Georgia,
Athens, Georgia, U.S.A.

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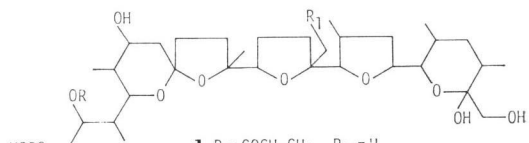
Selective monoacylation of the antibiotic laidlomycin at C-26 has led to derivatives that display enhanced activity toward favorably altering rumen fermentation and preventing avian coccidiosis. Diacylated derivatives (C-7, C-26) were found to be inactive. The structures of these compounds were determined by ^{13}C NMR spectroscopy.

The polyether ionophoric antibiotic laidlomycin (**1**)[1,2] obtained from a strain of *Streptomyces eurocidicus* var. *asterocidicus*, bears a close structural resemblance to the monensins (*e.g.* monensin **B**, **2B**).[3,4] The polyether ionophores have assumed considerable commercial importance as feed additives, enhancing feed efficiency in ruminants and acting as anticoccidial agents. Not surprisingly, laidlomycin also has activity that implies potential for both of these applications (*vide infra*). While investigating chemical modifications of laidlomycin we made the observation that acylation of the primary hydroxyl group (C-26) afforded compounds with a significantly enhanced profile in *in vitro* and *in vivo* activity relative to the parent compound in ruminal fermentation and anticoccidial screens. Acylation of both the C-7 and C-26 hydroxyls, however, destroyed activity. We now report these chemical and biological results as well as the first detailed ^{13}C NMR spectra of laidlomycin and its derivatives.



1 R = $COCH_2CH_3$, R_1 = H
2A R = CH_3, R_1 = CH_3
2B R = CH_3, R_1 = H

Chemistry

Acetylation of laidlomycin or sodium laidlomycin with excess acetyl chloride in pyridine at 5°C for 90 minutes gave selectively the monoacetate **3**. When the reaction was allowed to proceed for 24 hours the diacetate **4** was formed. However, with acyl halides or anhydrides of more that two carbons only monoacylation was observed utilizing either pyridine or methylene chloride - triethylamine as solvents at room temperature giving compounds **5**~**18**. Diacylation could be achieved under more

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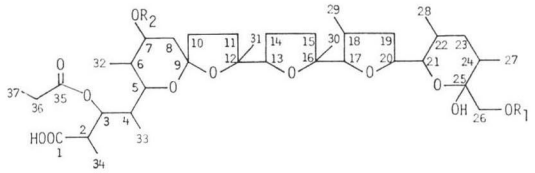
Table 1. Acylated laidlomycin derivatives.



Compound No.	R_1	R_2	Mp (°C)
3	CH_3CO	H	115~116
4	CH_3CO	CH_3CO	60~62
5	CH_3CH_2CO	H	190~192[a]
6	$CH_3(CH_2)_2CO$	H	77~80
7	$(CH_3)_2CHCO$	H	62~65
8	$(CH_2)_2CHCO$	H	85~87
9	$CH_3(CH_2)_3CO$	H	90~93
10	$(CH_2)_3CHCO$	H	68~70
11	$CH_3(CH_2)_4CO$	H	70~73, 80~82[a]
12	$(CH_2)_4CHCO$	H	32~34
13	$CH_3(CH_2)_5CO$	H	oil
14	$(CH_2)_5CHCO$	H	128~130
15	$CH_3(CH_2)_6CO$	H	60~62[a]
16	$CH_3(CH_2)_8CO$	H	54~56, 47~50[a]
17	$CH_3(CH_2)_{10}CO$	H	oil
18	$CH_3(CH_2)_{16}CO$	H	43~45
19	CH_3CH_2CO	CH_3CH_2CO	52~60[a]
20	$CH_3(CH_2)_2CO$	$CH_3(CH_2)_2CO$	48~53[a]
21	$CH_3(CH_2)_4CO$	$CH_3(CH_2)_4CO$	oil[a]
22	$CH_3(CH_2)_2CO$	CH_3CO	53~58[a]

[a] Denotes potassium salt; all other compounds are free carboxylic acid.

forcing conditions which involved use of excess anhydride in pyridine at 50°C to give **19**~**21** in low yield. Acetylation of butyrate **6** under these conditions gave **22**. The derivatives thus obtained are listed in Table 1.

These results indicate that acylation of the C-26 hydroxyl effectively retards subsequent acylation of the hydroxyl at C-7. This is reasonable in terms of the almost cyclic conformation of the molecule, which, as has been demonstrated by X-ray crystallography for monensin, places the C-26 and C-7 hydroxyls in close proximity with both oriented toward the interior of a hydrophilic cavity and coordinated to alkali metal ions when present.[5] Acylation of the more readily accessible C-26 hydroxyl may further bury the C-7 hydroxyl in the interior of the molecule thereby rendering it unreactive towards acylation.

^{13}C NMR Studies

The ^{13}C data for laidlomycin, **1**, its sodium (**1**-Na) and potassium (**1**-K) salts and the sodium salts of monensin A (**2A**) and B (**2B**) are listed in Table 2. The data for the potassium salts of the mono esters **3**, **5**, **6**, and **8**~**11** are contained in Table 3 while Table 4 contains the data for the potassium salts of the diesters **4**, and **19**~**22**. The assignments of the methyl and methine carbons are based on selective decoupling and confirmed by application of BIRDSALL's[6] off-resonance technique which also served

Table 2. ^{13}C NMR data for laidlomycin (**1**) and monensins A (**2A**) and B (**2B**)[a].

Carbon No.	**1**	**1**-Na	**1**-K	**2A**[b]	**2B**
1	176.46	179.98	181.70	181.40	181.33
2	40.67	43.54	43.07	45.12	45.03
3	73.86	75.70	75.29	83.16	83.03
4	39.69	40.38	39.69	37.58	37.47
5	67.20	68.29	68.40	68.46	68.31
6	34.72	35.25	35.57	34.92	34.91
7	70.81	70.58	70.51	70.61	70.55
8	32.87	33.34	34.07	33.61	33.58
9	108.00	106.99	107.80	107.15	106.96
10	38.49	39.25	39.33	39.34	39.34
11	34.10	33.16	33.68	33.35	33.22
12	85.21	85.23	85.60	85.37	85.32
13	82.67	81.52	82.05	82.64	81.59
14	27.80	27.39	26.98	27.31	27.42
15	32.28	30.33	30.92	29.97	30.40
16	84.62	83.88	84.33	86.02	83.91
17	86.44	86.46	85.95	85.04	86.47
18	33.81	34.54	34.95	34.46	34.60
19	32.38	33.02	31.99	33.35	33.05
20	77.53	76.60	77.60	76.53	76.68
21	73.76	74.54	74.64	74.58	74.61
22	32.86	31.76	32.44	31.92	31.85
23	36.74	35.63	36.83	35.76	35.76
24	34.95	36.45	37.03	36.61	36.51
25	97.27	98.14	98.11	98.44	98.27
26	67.81	64.86	66.90	65.02	64.91
27	15.51	16.04	16.38	16.12	16.05
28	17.55	16.77	17.33	16.77	16.76
29	15.31	14.09	14.73	14.63	14.14
30	24.71	23.92	23.67	30.69	23.92
31	27.89	27.60	28.09	27.50	27.65
32	10.11	10.18	10.05	10.53	10.51
33	10.96	11.06	11.61	11.05	11.01
34	16.45	16.61	17.03	16.77	16.68
35	173.41	173.51	173.83	58.00[c]	57.91[c]
36	28.19	27.86	27.93	8.26[d]	
37	9.27	9.33	9.36		

[a] δ Values in $CDCl_3$. [b] Numbered like laidlomycin for ease of comparison. [c] Methoxy group.
[d] Methyl attached to C-30.

to identify C-26 and C-36. The quaternary carbons were assigned on the basis of chemical shift (C-1, C-9, C-25 and C-35) and by comparison with closely related structures (C-12 and C-16). C-16 was also distinguished from C-12 upon comparison of the spectra of monensins A and B. In the spectrum of **2B**, C-16 is shifted 2.1 ppm upfield due to the removal of the methyl attached to C-30 in monensin A. The seven remaining methylenes were assigned by comparison with the spectra of mutalomycin[7] (C-19 and C-23), dianemycin[8] (C-8, C-10, and C-11), lenoremycin[8] (C-23) and lonomycin[7] (C-14 and C-15).

Table 3. ^{13}C NMR data for some 26-esters of laidlomycin[a].

Carbon No.	3	5	6	8	9	10	11
1	180.85	180.51	180.72	180.75	180.85	180.78	180.85
2	42.98	42.97	43.01	43.07	43.08	43.09	43.04
3	75.71	75.69	75.78	75.78	75.75	75.75	75.81
4	38.91	38.83	38.95	39.01	38.95	38.95	38.91
5	68.17	68.05	68.11	68.20	68.20	68.20	68.17
6	35.18	34.94	35.05	34.92	35.05	34.88	35.05
7	70.45	70.46	70.61	70.51	70.51	70.55	70.51
8	34.72	34.25	34.33	34.33	34.32	34.30	34.26
9	108.06	107.90	108.06	108.06	108.13	108.09	108.09
10	39.34	39.23	39.34	39.37	39.37	39.34	39.37
11	33.68	33.58	33.68	33.71	33.71	33.68	33.71
12	85.63	85.51	85.66	85.69	85.66	85.66	85.66
13	82.09	81.98	82.09	82.15	82.15	82.15	82.12
14	26.92	26.96	27.02	27.08	26.92	27.05	27.02
15	30.79	30.78	30.82	30.92	30.85	30.88	30.85
16	84.26	84.09	84.26	84.26	84.26	84.30	84.30
17	85.86	85.56	85.66	85.69	85.76	85.66	85.82
18	35.18	34.94	35.05	34.66	34.69	34.62	34.69
19	32.74	32.69	32.57	32.70	32.64	32.70	32.80
20	77.70	77.61	77.73	77.86	77.76	77.80	77.76
21	74.45	74.13	74.38	74.32	74.41	74.28	74.41
22	32.57	32.54	32.57	32.70	32.64	32.70	32.64
23	35.63	35.49	35.63	35.60	35.63	35.57	35.63
24	35.18	35.22	35.31	35.24	35.24	35.24	35.24
25	97.59	97.69	97.82	97.76	97.79	97.85	97.76
26	64.34	63.54	63.78	63.52	63.82	63.43	63.85
27	16.42	16.41	16.45	16.55	16.48	16.51	16.48
28	17.52	17.49	17.52	17.59	17.56	17.56	17.56
29	14.89	14.78	14.86	14.86	14.89	14.89	14.92
30	23.60	23.71	23.70	23.86	23.67	23.76	23.67
31	28.12	28.04	28.09	28.12	28.15	28.15	28.15
32	9.88	9.79	9.88	9.88	9.88	9.88	9.88
33	11.22	11.16	11.18	11.22	11.22	11.18	11.22
34	16.55	16.50	16.45	16.55	16.55	16.51	16.48
35	173.86	173.66	173.76	173.89	173.93	173.89	173.86
36	27.93	27.86	27.89	27.96	27.96	27.96	27.96
37	9.33	9.30	9.33	9.36	9.36	9.36	9.36
1′	171.03	174.11	173.47	174.93	173.93	175.29	173.70
2′	21.03	27.41	36.15	12.81	33.97	38.17	34.26
3′		8.93	18.21	8.42	27.96	24.67	24.51
4′			13.85	7.77	22.33	25.65	31.37
5′					13.81		22.43
6′							13.91

[a] δ Values in $CDCl_3$.

The previously reported chemical shift for C-11 in monensin A has been shown to be in error based on labelling experiments.[9] A recent partial assignment of monensin A by two-dimensional correlation

Table 4. ^{13}C NMR data for 7,26-diesters of laidlomycin[a].

Carbon No.	4-K[b]	19-K[c]	20-K[d]	21-K[e]	22-K[f]
1	181.40	176.20	175.97	176.20	175.58
2	43.79	39.40	39.43	39.47	39.40
3	75.71	76.04	75.91	76.01	75.88
4	39.89	38.88	39.01	38.95	39.24
5	69.77	68.56	68.63	68.69	68.59
6	32.15	32.54	32.31	32.44	32.05
7	73.86	72.58	72.46	72.56	72.46
8	34.23	33.12	33.16	33.25	33.00
9	108.32	107.15	107.18	107.15	107.48
10	38.91	39.17	39.21	39.21	39.24
11	34.36	34.07	34.26	33.87	34.62
12	85.69	86.35	86.28	86.31	86.35
13	80.92	81.34	81.37	81.31	81.47
14	25.71	26.20	26.11	26.20	25.84
15	29.55	31.31	31.11	31.31	30.43
16	84.46	83.97	83.91	83.97	83.87
17	85.69	85.92	85.89	85.95	85.63
18	34.88	35.04	34.92	34.95	32.44
19	32.90	32.35	32.31	32.57	32.05
20	77.83	76.95	76.92	76.95	76.75
21	74.61	73.89	73.86	73.86	73.99
22	32.37	32.83	32.90	32.90	32.90
23	35.63	36.86	36.86	36.87	36.80
24	35.88	35.40	35.40	35.40	35.37
25	97.20	96.59	96.62	96.62	96.65
26	65.31	67.62	67.49	67.59	67.46
27	16.32	15.47	15.47	15.44	15.51
28	17.26	17.20	17.13	17.20	17.13
29	14.66	14.43	14.56	14.43	15.18
30	23.20	23.43	23.37	23.40	22.17
31	26.53	25.13	25.52	25.26	26.11
32	10.83	10.21	10.31	10.34	10.14
33	10.96	11.57	11.57	11.64	11.22
34	16.64	16.19	16.12	16.16	16.12
35	173.86	173.53	173.56	173.57	173.54
36	27.96	27.76	27.80	27.80	27.83
37	9.36	9.20	9.23	9.27	9.27

[a] δ Values in $CDCl_3$.

Ester resonances: [b] 170.32, 170.77, 21.10, 21.94. [c] 174.54, 174.97, 27.67, 27.47, 9.10, 8.97. [d] 173.73, 174.25, 36.05, 35.92, 18.40, 18.17, 13.78, 13.69. [e] 173.93, 174.38, 2×34.17, 2×24.64, 31.53, 31.31, 22.40, 22.72, 13.95, 14.04. [f] 173.76, 36.05, 18.40, 13.69, 171.84, 21.88.

spectroscopy[10] is in complete agreement with these studies. Further multiple labelling and associated $^{13}C\{^{13}C\}$ decoupling experiments have completely established all the assignments for monensin A.[11]

The differences between the ^{13}C NMR spectra of **2A** and **2B** are exactly those expected for the removal of the methyl group at C-30, *i.e.* an upfield shift of C-30 and C-16 and downfield shifts for C-15 and

C-17.[12] The chemical shifts for the remainder of the molecule are virtually identical leading to the conclusion that the conformations of monensins A and B are essentially identical. Similarly replacement of the 3-methoxy group in **2** with a propionate ester to give **1** is expected to give rise only to upfield shifts of C-2, C-3, and C-4.[13] The expected shifts are observed for C-2 and C-3 but C-4 shifts downfield by 2.9 ppm. This downfield shift may be due to the anisotropy of the carbonyl group but is probably due to **1** adopting a conformation different than that of **2B**. The only other significantly different shift between **2B** and **1** is observed for C-32 and is probably also due to a slightly altered conformation.

The large differences observed between the spectra of **1**, **1**-Na, and **1**-K indicate substantial conformational changes which are dependent on the nature of the cation. Such differences have been observed in the crystal structure of monensin A.[5] Obviously, when comparing the ^{13}C spectra of ionophores of this type, care should be exercised to compare only similar salt or acid forms.

The shifts observed for the 26-acyl compounds are also somewhat unusual in that the expected downfield shift[13] of the carbinol carbon (C-26) is not observed. Instead, C-26 is approximately 2.6 ppm upfield in the C-26 esters as compared with the parent alcohol. WESTLEY *et al.*[14] have proposed that in the 26-urethanes of monensins A and B, the urethane carbonyl is coordinated to the cation instead of the 26-hydroxyl group.

Such a shift in coordination site and subsequent change in conformation could account for both the many differences in chemical shifts between **1** and its C-26-esters as well as the anomalous shift of C-26 upon acylation.

Acylation of the 7-hydroxyl group does lead to the expected shifts[13] of C-6, C-7, and C-8 as has been reported for monensin A.[9] Since the 7-OH group is one of the metal ligands in monensin A and therefor presumably in **1**, acylation of this group might be expected to influence the strength of coordination, the conformation of the molecule and its biological activity. Shift differences of 0.1 ppm or greater are observed for almost every carbon in the diesters as compared to the monoesters. These changes in chemical shift cannot be attributed only to acylation of the OH group but point to large conformational changes throughout the molecule.

Biological Testing

Two *in vitro* assays were used to determine the potential efficacy of laidlomycin esters as feed additives for ruminants.[15] For both assays, fluid digest obtained from a rumen-fistulated bovine was mixed with an appropriate buffer and after administration of test compound, the production of volatile fatty acids (VFA) or lactic acid was measured.

It has been established that the feed efficiency enhancing activity of monensin A is related to the enhancement of intraruminal propionic acid production.[16] Thus an *in vitro* fermentation model which simulated a normal rumen environment was used in which propionic acid production was measured at various concentrations of test compound. The results of this screen, represented as percentage increase in propionic acid relative to control, are presented in Table 5.

A second characteristic desirable for a feed additive for ruminants is related to the prevention of lactic acidosis.[17] This condition, which sometimes occurs when animals are introduced to a high concentrate diet in the feedlot, appears to be due to an initial rapid proliferation of the lactate producing rumen bacterium *Streptococcus bovis*.[18] Clinical signs of lactic acidosis may range from loss of appetite to death. A propionate enhancing antibiotic which would also inhibit the development of lactic aci-

Table 5. Activity of laidlomycin esters toward altering rumen fermentation *in vitro*.

Compound	Propionic acid production (% of control)[a]				Lactic acid production (% of control)[a]			
	Concentration (μg/ml)[b]				Concentration (μg/ml)[b]			
	0.31	0.62	1.25	5	0.31	0.62	1.25	5
Laidlomycin (**1**)	113	116	127	139	122	110	83	20
Monensin A (**2A**)	116	124	133	147	78	68	66	57
Laidlomycin esters:								
Acetate (**3**)	119	130	140	145	103	55	17	11
Propionate (**5**)	116	122	133	148	71	51	14	13
Butyrate (**6**)	125	128	141	154	38	32	15	14
Isobutyrate (**7**)	121	124	139	155	38	31	13	13
Cyclopropanecarboxyl (**8**)	132	128	137	141	38	5	6	8
Pentanoate (**9**)	127	133	135	138	60	29	12	10
Cyclobutanecarboxyl (**10**)	132	130	138	143	57	20	9	11
Hexanoate (**11**)	116	121	131	142	104	38	5	3
Cyclopentanecarboxyl (**12**)	130	133	144	142	61	17	9	11
Heptanoate (**13**)	117	128	130	136	104	38	5	3
Cyclohexanecarboxyl (**14**)	131	136	139	143	88	31	9	12
Octanoate (**15**)	120	124	131	136	114	26	3	3
Decanoate (**16**)	112	122	128	131	100	84	2	2
Dodecanoate (**17**)	118	123	128	132	95	103	30	3
Stearate (**18**)	103	104	111	124	125	130	120	114
Diacetate (**4**)	97	100	102	99				
Dipropionate (**19**)			105					
Dibutyrate (**20**)			106					
Dihexanoate (**21**)			102					
Acetate-butyrate (**22**)			107					

[a] Numbers are propionic acid or lactic acid production expressed as a percentage of control incubations containing no experimental compound. Therefore the amount of either acid produced in the control incubation would be expressed as 100%. Pooled standard errors obtained in any given experiment typically were less than 4% of control incubations.

[b] Represents the concentration of the test compound in the incubation media. See experimental section for the details of both incubations.

dosis could afford a demonstrable advantage over the feed additives currently available. Thus a second fermentation model designed to simulate the intraruminal environment under conditions conducive to lactic acidosis (low buffering capacity, high substrate availability) was used to test compounds for their potential in preventing lactic acid production. Results are presented in Table 5 as percentage of lactic acid produced relative to control.

Anticoccidial efficacy was determined *in vivo* with *Eimeria tenella* infected chicks. Compounds were evaluated based upon (1) prevention of mortality due to coccidiosis, (2) maintenance of body weight gain, and (3) intestinal tract lesions which were scored on a scale of 0 to 4 based upon their number and severity. Results are presented in Table 6.

Results

As shown in Table 5 monoacylation of laidlomycin generally led to compounds with enhanced ac-

Table 6. *In vivo* anticoccidial testing[a].

	Concentration (ppm)	No. of birds	No. of deaths from coccidiosis	Survivors 6th day	
				Mean weight gain (g) 0~6 days	Mean lesion score[b]
Laidlomycin (**1**)	62.5	23	3	109.65	2.57
	125	23	0	130.3	2.42
Monensin (**2**)	100	24	1	147.5	0.94
Laidlomycin propionate (**5**)	62.5	24	4	124.2	2.68
	125	24	0	148.6	0.92
Laidlomycin butyrate (**6**)	62.5	24	3	113.7	2.60
	125	24	5	156.7	1.24
Laidlomycin hexanoate (**11**)	62.5	24	4	124.2	2.64
	125	24	4	153.7	1.65
Laidlomycin octanoate (**15**)	62.5	24	0	108.5	2.58
	125	24	1	128.1	2.27
Laidlomycin decanoate (**16**)	62.5	24	2	124.8	2.08
	125	24	0	137.8	2.12
Infected controls	—	24	6	118.3	3.06
Non-infected controls	—	24	0	160.3	0

[a] Chickens were infected with oöcysts of *Eimeria tenella*.
[b] Intestinal tract lesions were scored on a scale of 0 to 4 based upon their number and severity.

tivity toward increasing propionic acid production and decreasing lactic acid production. Diacylation led to compounds which were inactive as propionate enhancers. Among the monoacyl derivatives, the four carbon esters **6** and **7** were the best propionate enhancers although probably not statistically significantly different from several of the other homologs. The same trend was present in the lactic acid inhibition rate which indicated that **6**, **7**, and **8** were the most active compounds. From this series, the butyrate (**6**) was selected for evaluation as a ruminant growth promoter. This compound at 1.25 μg/ml, a concentration that may roughly approximate that used for monensin *in vivo*, effectively increased propionic acid 40% over control levels while inhibiting lactic acid to 15% of control. To achieve comparable results, concentration of 5 μg/ml or greater of laidlomycin were required. The *in vitro* activity of **6**, especially in terms of lactic acid inhibition, was also superior to that of monensin A (Table 5).

Of the compounds tested in the anticoccidial screen, the propionate ester **5** at 125 ppm was superior to laidlomycin at the same dose and comparable to monensin at 100 ppm by all three of the evaluative criteria (Table 6).

The reason for the enhanced activity of the monoacylated laidlomycins is only a matter of conjecture at this time. It is attractive to speculate that the acylation of the C-26 hydroxyl may enhance the ionophoric properties of the molecule by covering the C-7 hydroxyl thus contributing to the hydrophobic exterior of the ionophore as it wraps around an ion. Diacylation may seriously perturb the chelative properties and/or conformation of the molecule thereby leading to inactive derivatives. However, the increased activity of the monoacyl compounds could also be due to metabolic or transport phenomena and further study would be required to clarify this point.

In conclusion, we have demonstrated that acylation of laidlomycin at C-26 leads to compounds with a superior profile of antibiotic activity relative to the parent compound in two *in vitro* rumen fer-

mentation screens and in an *in vivo* coccidiosis screen. Compound **6** has been evaluated *in vivo* as a feed additive for cattle and the results of this field trial indicate that **6** has potential as a feed efficiency enhancer. Details of this trial will be reported in due course.

Experimental

^1H NMR spectra were measured on a Bruker WM-300 in $CDCl_3$ solution containing tetramethylsilane as an internal standard. The pulse width was 4 microseconds (80° flip angle), the spectral width 3KHz, using a 32K data table giving a digital resolution of 0.2 Hz. Concentrations were 50~200 mg/ml. Four or eight transients were collected using quadrature detection. The proton chemical shifts were observed to be concentration dependent but no effort was made to determine the nature of this dependence. ^{13}C NMR spectra were determined using Bruker WM-300 (75.475 MHz) or WH-90 (22.62 MHz) spectrometers. The samples were dissolved in $CDCl_3$ containing tetramethylsilane and placed in 5 or 10 mm NMR tubes. The spectrometer parameters were as follows: WM-300/WH-90 spectral width 18/6 KHz, pulse width 6.5/5 microseconds, data table 32/16 K, digital resolution 1.1/0.7 Hz, repetition rate 0.44/0.7 sec., a 1.0 Hz line broadening function was applied to the ^{13}C FID before Fourier transformation. For the selective decoupling experiments, the decoupler frequencies were determined on the same solution by using either a dual frequency ^1H-^{13}C-5 mm probe or by using the decoupler coils of the 10 mm probe to observe the ^1H spectrum. 1,000 to 80,000 transients were collected for each spectrum. Monensin A was purchased from Calbiochem, LaJolla, CA. Monensin B were gifts of Prof. DAVID CANE, Brown University and Drs. DOUG DORMAN and ROBERT HAMILL of Eli Lilly and Co. Melting points were uncorrected; infrared spectra were recorded with a Perkin-Elmer 237b spectrometer. Silica gel for column chromatography was that of Merck (Darmstadt), 70~230 mesh. Microanalyses were performed by Syntex Analytical Research.

Laidlomycin Diacetate (4)

To a solution of 1.0 g sodium laidlomycin (**1**) in 10 ml of pyridine, cooled in an ice bath, was added 0.7 ml of acetyl chloride. The solution was stored for 24 hours at 5°C and then diluted with dichloromethane and washed twice with dilute aqueous hydrochloric acid. Evaporation of the solvent and chromatography of the residue on silica gel (elution with ethyl acetate) gave 0.85 g of **4**: mp 60~62°C; IR (KBr) 3600~3200, 2950, 1730, 1380, 1250 cm^{-1}.

Anal. Calcd. for $C_{41}H_{66}O_{14}$ (1/2 H_2O): C 62.18, H 8.53.
Found: C 62.07, H 8.55.

Laidlomycin Butyrate (6)

A solution of 0.15 g of laidlomycin (**1**) in 5 ml of pyridine was cooled in an ice bath and 0.3 ml of butyryl chloride was added. The solution was allowed to warm to room temperature over 3 hours and was partitioned between dichloromethane and dilute aqueous hydrochloric acid. The dichloromethane was evaporated to an oil which was filtered through silica gel with ethyl acetate elution to afford 0.13 g of **6**: mp 77~80°C; IR (KBr) 3600~3200, 2950, 1730, 1580, 1460, 1380, 1180 cm^{-1}.

Anal. Calcd. for $C_{41}H_{68}O_{13}$ (H_2O): C 62.57, H 8.96.
Found: C 62.52, H 8.81.

Ruminal Fluid Incubations

Rumen fluid obtained from a rumen-fistulated bovine was strained through cheesecloth, and the fluid was mixed with an equal volume of CO_2-saturated buffer. Buffer used in the lactic acid fermentation model had the following composition in grams per liter: glucose 27, Trypticase 1.50, Na_2HPO_4 4.26, NaH_2PO_4 8.28, NaCl 0.43, KCl 0.43, $MgSO_4 \cdot 7H_2O$ 0.12, K_2SO_4 0.15, $CaCl_2$ 0.05, urea 0.60. Buffer for the volatile fatty acid fermentation model had the following composition in grams per liter: glucose 1.08, cellobiose 4.10, maltose 2.39, soluble starch 5.38, amino acids 4.68, $NaHCO_3$ 9.80, Na_2HPO_4 3.69, NaCl 0.43, KCl 0.43, $MgSO_4 \cdot 7H_2O$ 0.12, $CaCl_2$ 0.05, K_2SO_4 0.15, urea 0.12.

Compounds for testing were transferred into 18×150 mm incubation tubes and appropriate rumen fluid-buffer was pipetted into each incubation tube to bring the volume to 10 ml. All tubes were fitted

with gas-release stoppers and were incubated in a shaking water bath at 39°C. Fermentation was terminated at 5.5 hours for the lactic acid fermentations and at 9 hours for the volatile fatty acid fermentations by addition of 2 ml of 25% metaphosphoric acid to the incubation tubes. Aliquots were centrifuged at 30,000 $\times g$ for 10 minutes, and the resulting supernate was analyzed for 1(+)-lactic acid or volatile fatty acids.

1(+)-Lactic acid was analyzed *via* a specific enzymatic assay. Volatile fatty acids were analyzed *via* gas chromatography. The instrument used was a Perkin-Elmer 900B equipped with a hydrogen flame ionization detector. The acids were separated on a stainless-steel column (0.32 × 182.9 cm) packed with 10% SP-1200/1% H_3PO_4 on Chromosorb-WAW, 80~100 mesh. The following additional chromatographic conditions were employed: column temperature, 145°C; injector temperature, 190°C: detector temperature, 190°C; carrier gas flow, 45 ml/minute; injection volume, 1 μl. Duplicate incubations for the lactic acid fermentation model and triplicate incubations for the volatile fatty acid fermentation model were conducted for each level of compound tested.

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