ANTICOCCIDIAL ACTIVITY OF SALINOMYCIN DERIVATIVES

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In the course of our studies concerning the derivatization of the polyether ionophore nigericin[1,2] we observed a dependence of the antibacterial activity on the counter ion at C-1,[3] which prompted us to investigate if related effects can also be found for other polyethers and indications. Salinomycin (**1**), for example is another important polyether[4,5] which is produced in combination with related analogs by fermentation of *Streptomyces albus*.[6] Compound **1** is commercially applied as feed additive for the prophylaxis against coccidiosis of broiler chicken. Furthermore, an antibacterial, antiviral activity and effect on food utilization in ruminants is noteworthy.[4,6,7] Its mode of action is probably related to the ionophoric properties.

Hitherto, only the antibacterial activities of salinomycin derivatives in their free acid form were intensively studied.[7,8] Corresponding investigation concerning the anticoccidial activities of derivatives were limited to degradation products.[9] Effects of different counter ions at C-1 were not described. This study was therefore designed to investigate the anticoccidial activity of certain derivatives *in vivo* modified mainly in the C-ring system whereas the influence of the counter ion at the carboxylic acid moiety at C-1 should be especially considered.

The C-20 acylated compounds **2**~**7** were prepared from **1** by reaction with the corresponding anhydrides in pyridine for 12 hours as described by MIYAZAKI.[7] The dicarboxylic acid derivatives **8** and **9** were prepared in an analogous procedure using succinic anhydride or phthalic anhydride as acylation reagents. Hydrogenation of **1** to **10** was performed as described.[7] The free acids **1**~**11** (Table 1) were accessible by treatment of an organic layer with 1 N hydrochloric acid. Dissolving the acids **1**~**11** in hexane and stirring with a 1 N solution of potassium hydroxide or sodium hydroxide in water gives rise to the formation of the corresponding salts which were isolated by evaporation of the organic layer. The salt structure could be easily proved by the characteristic carbonyl frequencies in the IR spectra. For example, in the free acid of **1** signals at $\gamma = 1710$ (C=O) and 1740 (COOH) cm^{-1} were observed, whereas for the potassium salt signals at $\gamma = 1710$ (C=O) and 1570 cm^{-1} (COO^-) cm^{-1} were detectable.

The anticoccidial action of the compounds was assessed in chickens infected with *Eimeria tenella* (5×10^4 sporulated oocysts per bird).[10] The compounds were mixed with the feed and given *ad lib.* For each concentration (ppm), eight chickens were used. The anticoccidial effect was determined by parameters, such as lesion scores,[10] morbidity due to *E. tenella* infection, average weight gain per group and oocyst reduction on D+6 and D+7 (D=day of infection) compared to untreated infected control.

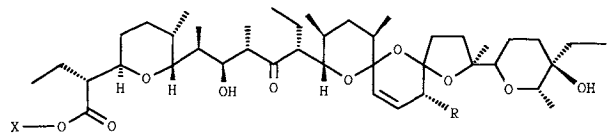
The results of the *in vivo* test are summarized in Table 1. At 60 ppm salinomycin (**1**) as sodium salt exhibited the best anticoccidial effect whereas potassium salt of **1** obviously produced weight depression due to drug toxicity. The free acid of **1** showed a distinct loss of activity. Based on these results it was of interest to investigate the activities of all free acid derivatives derived from standard work up, with a pronounced anticoccidial effect in Na^+ and K^+ form, too.

A decreased biological effect was observed for the dihydro derivative **10** as well as for the dicarboxylic acid compounds **8** and **9**. The less pronounced activities of **8** and **9** might be explained by the hydrophilicity which disturbs the transport through lipophilic membranes. Therefore, our interest was directed towards compounds with increased lipophilicity obtained by acylation of the hydroxyl moiety at C-20 in **1**. Among the unbranched acyl derivatives **2**~**6** in their free acid form a maximum activity was observed for the propanoyl compound **3** which was twice as active as **1**. The 3-methyl propanoyl ester (**7**) was about six times more active than **1**. The K^+ salt of **7** was distinctly less active than the Na^+ salt or H^+ form. In contrast, the K^+ salt of butanoyl derivative **4** proved to be superior to the Na^+ salt and the free acid form.

In general, the anticoccidial activities for the most active compounds was decreased in the order of **3** (H^+) > **7** (H^+, Na^+) > **3** (Na^+) = **11** (Na^+) > **1** (Na^+) = **2** (H^+). Obviously, the influence of the cation is not predictable.

We think that this work demonstrates the importance of evaluating counter ion effects on biological activities in the polyether field. The described cation effects cannot be explained yet.

Table 1. Anticoccidial activity of the salinomycin derivatives.



	R Effectivity of the salts	X	Concentration in ppm	Lesion score	Morbidity (%)	Average weight gain (%)	Reduction of oocyst output (%)
1	OH	H	60	1.9	12.5	74	71
	Na > K > H	Na	60	0.8	0	94	92
			30	3.0	50	61	12
		K	60[a]	0	0	37	84
			30	3.0	50	50	43
2	$OCOCH_3$	H	60	0.3	0	86	90
	H > K > Na	Na	60[a]	0.6	0	70	83
		K	60[a]	0.5	0	81	85
3	$OCOCH_2CH_3$	H	60	0	0	94	100
	H > Na > K		30	0	0	98	97
		Na	60	0	0	81	100
			30	0.9	0	90	82
		K	60	0	0	81	94
			30	1.9	0	79	66
4	$OCO(CH_2)_2CH_3$	H	60	1.3	0	71	76
	K > Na = H		30	2.5	0	73	66
		Na	60[a]	0.3	0	31	97
			30	3.0	0	81	63
		K	60[a]	0	0	25	98
			30	1.3	0	88	87
5	$OCO(CH_2)_3CH_3$	H	60[a]	0	0	68	99
	H > K > Na		30	1.6	0	70	37
		Na	60[a]	0.5	0	36	99
			30	2.8	0	56	65
		K	60[a]	1.0	0	55	70
			30	1.5	0	70	57
6	$OCO(CH_2)_4CH_3$	H	60	3.3	0	46	38
	Na = H ≫ K	Na	60	2.0	0	47	50
		K	60	2.5	25	69	13
7	$OCOCH(CH_3)_2$	H	60[a]	0	0	43	99
	Na > H ≫ K		30	0	0	88	89
		Na	30	0	0	88	96
		K	60[a]	0	0	42	99
			30	2.6	12.5	65	62
8	$OCOCH_2CH_2COOH$	H	60	3.5	50	52	26
9	$OCOC_6H_5$-*o*-COOH	H	60	3.5	75	57	0
	K > H	K	60	3.0	25	72	15
10	18,19-dihydro	H	60	3.5	75	50	5
11	O	H	60	3.2	25	42	40
	Na ≫ K ≫ H	Na	60	0.3	0	98	98
			30	0.9	0	89	90
		K	60	2.3	0	93	85
			30	2.3	0	78	70

[a] Toxic.

Table 2. Physico-chemical properties of the salinomycin derivatives.

No.	Formula	MW	Observed mass (MNa^+)	Calcd		Found	
				C	H	C	H
2	$C_{44}H_{72}O_{12}$	793.09	815	66.6	9.1	66.4	9.0
3	$C_{45}H_{74}O_{12}$	807.08	829	67.0	9.2	67.3	9.0
4	$C_{46}H_{76}O_{12}$	821.11	843	67.3	9.3	67.1	9.5
5	$C_{47}H_{78}O_{12}$	835.14	857	67.6	9.4	67.3	9.1
6	$C_{48}H_{80}O_{12}$	849.17	871	67.9	9.5	67.8	9.3
7	$C_{46}H_{76}O_{12}$	821.11	843	67.3	9.3	67.5	9.2
8	$C_{46}H_{74}O_{14}$	851.09	873	64.9	8.8	64.7	9.0
9	$C_{50}H_{74}O_{14}$	899.14	921	66.8	8.5	66.5	8.6
10	$C_{42}H_{72}O_{11}$	753.04	775	67.0	9.6	67.3	9.7
11	$C_{42}H_{68}O_{11}$	749.00	771	67.4	9.2	67.2	9.0

Therefore, a more detailed study upon transportation processes, biochemistry and pharmacology is required.

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