PREPARATION, PROPERTIES AND BIOLOGICAL ACTIVITY OF NATURAL AND SEMISYNTHETIC URETHANES OF MONENSIN

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Conversion of the monovalent polyether antibiotic monensin into a series of urethane derivatives substituted at C-26 causes a ten-fold increase in the cation transporting properties of the antibiotic as well as making the resulting semisynthetic urethanes divalent ionophores. These changes must in part account for the enhanced antimicrobial activities of the urethanes. The most active derivatives are the phenylurethanes which are ten times more active *in vitro* against Gram-positive bacteria and unlike monensin are active against *Candida albicans* and *Penicillium digitatum*. Another novel activity exhibited by four of the urethanes was against *Plasmodium berghei*, the causative agent for malaria.

The isolation of the novel antibiotics X-14667A and X-14667B from *Streptomyces cinnamonensis* subsp. *urethanefaciens* was recently described[1]. The confirmation of their structures as 2-phenethylurethanes of monensins B[2] and A[3] respectively by synthesis using 2-phenethylisocyanate suggested the synthesis of other monensin urethanes (**1**~**14**). In a similar study with the polyether antibiotic laidlomycin[4,5], monoacylation has led to derivatives that display enhanced activity towards favorably altering rumen fermentation and preventing avian coccidiosis[6]. In the study described here, many of the semisynthetic monensin urethanes displayed greater activity than monensin (**15**) in a variety of *in vitro* and *in vivo* screens. The derivatives were active *in vitro* against Gram-positive bacteria, *Penicillium digitatum, Candida albicans*, the anaerobes *Bacteroides fragilis* and *Clostridium histolyticum* and *C. septicum* and *Treponema hyodysenteriae*. In addition several of the urethanes were active against *Eimeria tenella* in chicks and *Plasmodium berghei* in mice.

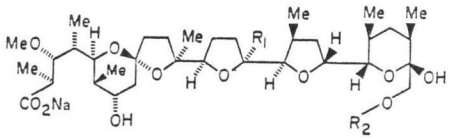
Chemistry

The preparation of antibiotics X-14667A (**2**) and X-14667B (**3**) from monensin B and A (**15**) has been described previously[1]. The other urethanes were made in the same way using monensin B, in the preparation of **4** and **5** and monensin A, to prepare the other urethanes. The reactions were carried out in benzene with an excess of the appropriate *iso*-cyanate and followed by silica gel TLC. At room temperature, the sodium salts were converted to their urethane analogs in one or two days whereas the free acid form of the antibiotics took about a week for complete reaction. Correct microanalyses were obtained for all fourteen analogs. The respective melting points of the sodium salts of the semisynthetic monensin urethanes are listed in Table 1.

Transport Properties

The U-tube system described by ASHTON and STEINRAUF[7] was employed to measure the monovalent and divalent cation transport properties of several monensin urethanes. A glass U-tube was filled with

Table 1. Structure and melting points of the monensin urethanes.

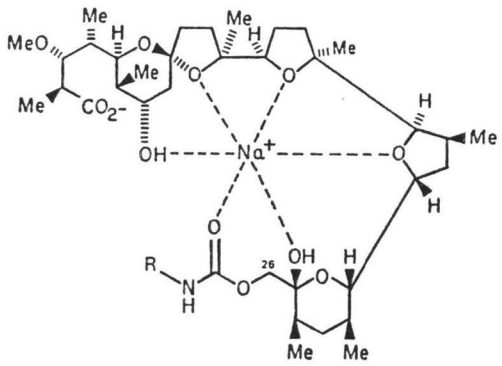


	R_1	R_2		Mp (°C)
Alkyl	Et	Me-NHCO	**1**	191~193
Phen-2-alkyl	Me	2-NHCO	**2**	70
	Et	2-NHCO	**3**	103
Phen-1-alkyl	Me	(*R*)-1-NHCO	**4**	89~93
	Me	(*S*)-1-NHCO	**5**	116~120
Cyclohexyl	Et	NHCO	**6**	110~123
4-Substituted phenyl	Et	4-O-	**7**	210~213
	Et	NHCO	**8**	199~210
	Et	4-Me-	**9**	220
	Et	4-F-	**10**	199~223
	Et	4-I-	**11**	203~206
	Et	4-Br-	**12**	201~203
	Et	4-Cl-	**13**	199~207
	Et	4-O_2N-	**14**	189~192
Monensin	Et	H	**15**	

Table 2. Cation transport of monensin urethanes as measured by the U-tube system of ASHTON and STEINRAUF.

	Monensin urethane	Rb^+ (%)	Ca^{2+} (%)
1	Methyl	42	8
2	2-Phenethyl (X-14667A)	31	2
3	2-Phenethyl (X-14667B)	26	1
4	(*R*)-1-Phenethyl	28	5
5	(*S*)-1-Phenethyl	29	7
6	Cyclohexyl	25	4
7	Phenoxyphenyl	34	10
8	Phenyl	30	12
10	Fluorophenyl	40	14
11	Iodophenyl	31	14
13	Chlorophenyl	27	17
14	Nitrophenyl	35	19
15	Monensin	3	0

Fig. 1. Proposed conformation of the sodium salt complex of the monensin urethanes.



5 ml of a chloroform solution of the monensin urethane (2×10^{-4} M). Two ml of an aqueous buffer (Tris-HCl, 20 mM, pH 9.5) containing 1 mM [^{45}Ca]calcium chloride or [^{86}Rb]rubidium chloride was placed in one arm and an equal volume of the same buffer solution with unlabelled calcium or rubidium chloride in the other arm. The experiment was started by the addition of the respective labelled metal chloride and the chloroform phase separating the two aqueous phases was then gently stirred with a magnetic stirrer overnight. The percentage of labelled cation transported overnight was determined by counting 50 μl samples taken from both aqueous phases with 10 ml Aquasol (New England Nuclear, Boston, Mass.) in an Intertechnique liquid scintillation spectrometer. The results are summarized in Table 2.

From the results in Table 2, there is a clear indication that the urethane moiety attached to the hydroxyl group at C-26 of monensin causes a ten-fold enhancement in the monovalent cation-transporting properties of the parent antibiotic. In addition, the urethanes have the ability to transport divalent cations which monensin does not. These divalent properties are particularly marked in the monensin phenylurethanes (**7**~**14**).

A possible explanation for the enhanced cation transporting properties of these semisynthetic deriva-

Table 3. *In vitro* minimum inhibitory concentrations (MIC) in μg/ml of monensin urethanes as Gram-positive antibacterial agents.

	Monensin urethane	*Staphylococcus aureus* ATCC 6538	*Micrococcus luteus* ATCC 9341	*Streptococcus faecium* ATCC 8043	*Bacillus* sp. E ATCC 27859	*Bacillus subtilis* NRRL 583	*Bacillus megaterium* ATCC 8011	*Bacillus* sp. TA ATCC 27860	*Mycobacterium phlei* ATCC 354	*Streptomyces cellulosae* ATCC 3313	*Paecilomyces varioti* ATCC 25820
1	Methyl	25	25	1.6	6.3	1.6	6.3	12.5	>25	>25	>25
2	2-Phenethyl (X-14667A)	6.3	3.1	0.8	1.6	3.1	3.1	3.1	25	12.5	>25
3	2-Phenethyl (X-14667B)	6.3	1.6	0.4	0.8	1.6	0.8	1.6	6.3	12.5	12.5
4	(*R*)-1-Phenethyl	6.3	3.1	0.8	0.4	1.6	1.6	0.8	12.5	3.1	3.1
5	(*S*)-1-Phenethyl	1.6	1.6	0.2	0.1	0.8	0.4	0.2	6.3	0.3	6.3
6	Cyclohexyl	1.6	0.8	0.1	0.2	0.8	0.4	0.8	3.1	3.1	3.1
7	Phenoxyphenyl	1.6	0.8	0.1	0.2	0.8	0.4	0.8	3.1	3.1	3.1
8	Phenyl	0.8	0.4	0.4	0.1	1.6	0.6	0.8	1.6	0.8	3.1
9	Methylphenyl	0.8	0.8	0.1	0.05	0.6	0.4	0.4	3.1	3.1	3.1
10	Fluorophenyl	0.4	0.4	0.2	0.05	0.4	0.2	0.2	1.6	0.8	1.6
11	Iodophenyl	0.6	0.2	0.1	0.02	0.4	0.2	0.2	1.6	0.4	1.6
12	Bromophenyl	0.8	0.2	0.1	0.05	0.4	0.1	0.2	1.6	0.8	1.6
13	Chlorophenyl	0.4	0.2	0.05	0.02	0.4	0.1	0.2	0.8	0.8	3.1
14	Nitrophenyl	0.4	0.6	0.1	0.4	0.6	0.4	0.2	1.6	0.8	0.8
15	Monensin	3.1	12.5	1.6	0.4	1.6	3.1	1.6	12.5	6.3	>25

Table 4. *In vitro* MIC's (μg/ml) of monensin urethanes against a mold, a yeast and a spirochete.

	Monensin urethanes	*P. digitatum*	*C. albicans*	*T. hyodysenteriae*
1	Methyl	>100	>100	50
2	2-Phenethyl (X-14667A)	>100	63	1.6
3	2-Phenethyl (X-14667B)	>100	25	0.4
4	(*R*)-1-Phenethyl	>100	25	0.6
5	(*S*)-1-Phenethyl			
6	Cyclohexyl	50	25	0.4
7	Phenoxyphenyl	100	100	0.4
8	Phenyl	50	6.3	0.08
9	Methylphenyl	25	25	0.08
10	Fluorophenyl	12.5	6.3	0.08
11	Iodophenyl	25	25	0.4
12	Bromophenyl	25	6.3	0.4
13	Chlorophenyl	6.3	0.08	
14	Nitrophenyl	25	25	0.4
15	Monensin	>100	>100	0.16

tives of monensin is the participation as a ligand of the urethane carbonyl oxygen (Fig. 1) as suggested earlier for antibiotics X-14667A and X-14667B[1] and also invoked by CLARK *et al.*[6] to explain the anomalous ^{13}C NMR upfield shift of the C-26 carbon in the acyl esters of laidlomycin.

Biological Properties

The *in vitro* activity of the fourteen semisynthetic monensin urethanes against ten Gram-positive bacteria are compared with that of monensin in Table 3. In the case of monensin (**15**) only one bacteria, *Bacillus* sp. E was sensitive at MIC levels less than 1 μg/ml. Apart from the monensin urethanes **1** and **2**, all the semisynthetic derivatives were active against three or more bacteria at levels less than 1 μg/ml with increasing activity down the Table to the phenyl derivatives, **8**~**14** which were active against between seven and nine of the ten Gram-positive microorganisms tested at levels less than 1 μg/ml. Against the most sensitive organism, *Bacillus* sp. E, five of the monensin phenylurethanes (**9**~**13**) were active at levels less than 0.1 μg/ml.

The monensin urethanes are also active *in vitro* against *P. digitatum, C. albicans* and *T. hyodysenteriae* (Table 4). Whereas monensin was inactive against both the mold, *P. digitatum* and the yeast, *C. albicans*, the cyclohexyl- (**6**) and phenylurethanes (**7**~**14**) were all active against both with monensin chlorophenylurethane (**13**) exhibiting activity against *C. albicans* at less than 0.1 μg/ml. In addition, four of the phenylurethanes (**7**, **9**, **11** and **14**) were compared with monensin (**15**) *in vitro* against three anaerobes, *Bacteroides fragilis, C. histolyticum* and *C. septicum*. The activities of the phenylurethanes were very similar to monensin against *B. fragilis* with MIC values between 25~100 μg/ml but were ten times more active against the two Clostridia with MIC values of 0.08~0.31 μg/ml compared to 3.1 μg/ml for monensin. In Table 5, the *in vivo* activities of the monensin urethanes are summarized. The 24 hour LD_{50} values and the antimalarial activities *vs. P. berghei* were determined in the mouse and the coccidiostat activities *vs. E. tenella* were studied in chicks.

The toxicities (i.p. and p.o.) of the alkylurethanes of monensin (**1**~**5**) were all less than the parent antibiotic (**15**), but the phenylurethanes were very similar. One exception was the bromophenylurethane (**12**) which was approximately three times less toxic than monensin and in addition was active against *P. berghei* in mice at 16 mg/kg p.o. whereas monensin was inactive at five times that level.

Table 5. Toxicity, antimalarial activity and coccidiostat activity of the monensin urethanes.

Monensin urethane	Mouse toxicity (mg/kg) 24 hours LD_{50}		Antimalarial activity *vs.* *P. berghei*		Coccidiostat activity *vs.* *E. tenella*		
	i.p.	p.o.	p.o. in mice	(mg/kg)	p.o. in chicks	(ppm)	ADI*
1	77	190			−	120	2.8
2	75	150	+	100			
3	27	245			−	50	3.4
4	77	250			+	70	1.1
5	35	120	−	10	−	120	3.0
6	14	140			+	120	0.8
7	9	120	−	8	−	120	2.1
8	22	77	+	4	+	120	0.5
9	15	150	−	5	+	70	1.1
10	24	45			+	70	0.8
11	24	55	−	5	+	120	0.1
12	50	140	+	16	+	70	0.5
13	9	22	+	2	+	70	0.9
14	12	45	−	5	+	70	0.5
Monensin (**15**)	17	44	−	80	+	120	0.8

* ADI is the average degree of infection and values of <1.5 indicate coccidiostat activity.

Phenylurethane (**8**) and chlorophenylurethane (**13**) derivatives were also active against *P. berghei* in mice, and at even lower levels than **12**, namely 4 and 2 mg/kg p.o. respectively.

In regard to the coccidiostat activity *vs. E. tenella* in chicks, the most active derivatives were the fluoro-, bromo-, chloro- and nitrophenylurethanes (**10**, **12**, **13** and **14**) followed by the (*R*)-1-phenethyl, cyclohexyl-, phenyl-, methylphenyl- and iodophenylurethanes (**4**, **6**, **8**, **9** and **11**) which were about on a par with the parent antibiotic, monensin (**15**).

Conclusions

The conversion of monensin to its C-26 hydroxyl urethane derivatives has a profound effect on the cation transporting properties of the antibiotic. The ability of monensin to transport Rb^+ is increased ten-fold and in addition these semi-synthetic urethanes can cause the transport of Ca^{2+} which monensin cannot.

This change in physical chemical properties accounts in part for the enhanced biological activity of some of the urethanes described in this report. The most active derivatives are the phenylurethanes which are approximately one order of magnitude more active *in vitro* against Gram-positive bacteria than monensin. In addition, the phenylurethanes, unlike monensin, are active against *P. digitatum* and *C. albicans*. When tested in mice, three of the phenylurethanes are active against *P. berghei* at levels ranging from $2\sim16$ mg/kg whereas monensin is inactive at 80 mg/kg. Finally, the phenylurethanes exhibit enhanced coccidiostat activity against *E. tenella* in chicks.

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