ISOLATION OF NOVEL ANTIBIOTICS X-14667A AND X-14667B FROM *STREPTOMYCES CINNAMONENSIS* SUBSP. *URETHANOFACIENS* AND THEIR CHARACTERIZATION AS 2-PHENETHYLURETHANES OF MONENSINS B AND A

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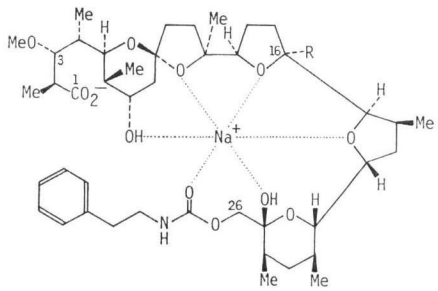
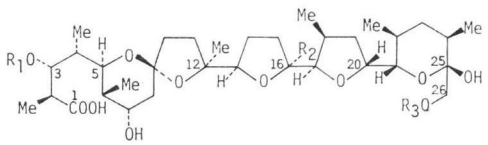
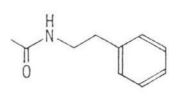
Antibiotics X-14667A (**1**) and X-14667B (**2**) are novel monovalent polyether antibiotics of the spiroketal type isolated from fermented cultures of *Streptomyces cinnamonensis* subsp. *urethanofaciens* together with monensin (**3**), its lower homolog, factor B (**4**) and 1,3-diphenethylurea (**6**). By a combination of microanalysis, mass spectrometry and ^{13}C nmr, antibiotics X-14667A and B have been shown to be natural 2-phenethylurethanes of monensin B and A respectively. Both structures have been confirmed by reacting the appropriate monensin with 2-phenethylisocyanate to yield semi-synthetic compounds that are identical to the natural products.

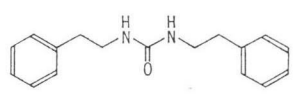
Antibiotics X-14667A (**1**) and X-14667B (**2**) are the latest additions to the more than sixty carboxylic acid ionophores[1] known as polyether antibiotics[2]. They are closely related to the first polyether antibiotic to be structurally defined[3], monensin (**3**), and a lower homolog of monensin called factor B[4], or monensin B (**4**). The first naturally occurring analog of monensin to be reported[5] was laidlomycin (**5**), which was also the first polyether claimed to exhibit antimycoplasmal activity. Laidlomycin differs from monensin B at carbon C-3 where the only methoxyl group present in the monensins is replaced by a propionyloxyl function. In contrast to this, the difference between antibiotic X-14667A and monensin B (and between X-14667B and monensin) is at the opposite end of the molecule, at C-26, where a primary alcohol is transformed into a 2-phenethylurethane moiety, a function not previously encountered amongst the polyether antibiotics. The availability of the alcohol at C-26 to both microbial and chemical transformation has been demonstrated in the glucosylation of monensin to antibiotic A27106 using

Sodium salt-complexes of antibiotics X-14667A (R = Me) and X-14667B (R = Et).





1 ; $R_1 = R_2 = Me$, $R_3 =$ 
2 ; $R_1 = Me$, $R_2 = Et$, $R_3 =$
3 ; $R_1 = Me$, $R_2 = Et$, $R_3 = H$
4 ; $R_1 = R_2 = Me$, $R_3 = H$
5 ; $R_1 = EtCO$, $R_2 = Me$, $R_3 = H$

6 

Streptomyces candidus[6] and by the chemical conversion of monensin to a C-(1→26) macrolide lactone by treatment with dipyridyl disulfide and triphenylphosphine[7]. With these reports in mind, the isolation of monensins A (**3**) and B (**4**) and 1,3-diphenethylurea (**6**) as co-metabolites of antibiotics X-14667A (**1**) and B (**2**), suggest the presence of an enzyme system in *Streptomyces cinnamonensis* subsp. *urethanofaciens* capable of converting the two monensins to their respective 2-phenethylurethanes, **1** and **2**. This enzyme is probably present in *Streptomyces* sp. AM-2498 which has also been reported[8] to produce 1,3-diphenethylurea.

As reported in the accompanying paper[9], antibiotics X-14667A and B, like the monensins and laidlomycin, complex alkaline but not alkaline earth cations. This makes them part of the monovalent class[2] of polyethers which already accounts for half of the carboxylic ionophores reported so far. As discussed recently in regard to another antibiotic[10], if the presence and nature of the spiroketal function is taken into account, the monovalent class of polyethers can be divided into three types:

(1) non-spiroketal (*e.g.*, alborixin[11] and X-206[12])
(2) spiroketal (the monensins and nigericin[13,14])
(3) dispiroketal (salinomycin[15] and the noboritomycins[16,17]).

This notation will be used in the present and any future communications from this laboratory concerning monovalent polyether antibiotics.

Specifically, this report describes the isolation and characterization of two novel monovalent polyethers of the spiroketal type, antibiotics X-14667A and X-14667B. The confirmation of the structures of these two novel antibiotics was accomplished by their synthesis from monensin and monensin B by reaction with 2-phenethylisocyanate, and this facile transformation suggested the production of other semisynthetic monensin urethanes. The variations of cation specificity and biological activity for a number of these compounds will be the subject of a future report.

Isolation of the Novel Antibiotics X-14667A and X-14667B and their Co-metabolites, Monensins A and B and 1,3-Diphenethylurea

The whole broth from a 254-gallon fermentation of *Streptomyces cinnamonensis* subsp. *urethanofaciens* was extracted at pH 7.6 with an equal volume of ethyl acetate. The extract was concentrated under reduced pressure and washed sequentially with equal volumes of 1 N HCl, water and saturated aqueous Na_2CO_3 and then dried over Na_2SO_4. Further concentration yielded 23 g of a crystalline mixture which was removed by filtration and subsequently shown to contain monensins A and B and 1,3-diphenethylurea. The mother liquor from the filtration was evaporated to yield 167 g of an oil which was dissolved in 2 liters of hexane and extracted twice with equal volumes of acetonitrile. The combined extracts were evaporated to an oil which, after trituration with *n*-hexane, yielded 32 g of an amorphous solid containing the two novel antibiotics, **1** and **2**. The crude solid was dissolved in diethyl ether, filtered and chromatographed on 500 g of silica gel. Gradient elution between 5 liters of CH_2Cl_2 and 5 liters of CH_2Cl_2 - ethanol (9: 1) gave 250×40 ml fractions. Fractions 120 to 180 were pooled and set aside for rechromatography. Fractions 185 to 235 were pooled, concentrated under reduced pressure and the residue dissolved in diethyl ether. The solution was washed with 1 N HCl, saturated aqueous Na_2CO_3 and water, and concentration of this ethereal solution gave 3.3 g antibiotic X-14667A (**1**), sodium salt, mp 70°C, $[\alpha]_D$ +48° (*c* 1, $CHCl_3$) and +44° (*c* 1, CH_3OH). Calcd. for $C_{44}H_{68}NO_{12}Na$ (826.02): C, 63.90; H, 8.41; N, 1.69; Na, 2.78. Found: C, 64.45; H, 8.68; N, 1.76; Na, 2.31.

The sodium salt of **1** was dissolved in ethyl acetate and washed with 1 N HCl. Concentration under

reduced pressure yielded the free acid form of X-14667A, mp 50°C, $[\alpha]_D$ +59.6° (*c* 1, $CHCl_3$) and +45.8° (*c* 1, CH_3OH). Calcd. for $C_{44}H_{69}NO_{12}$ (804.04): C, 65.65; H, 8.76; N, 1.74; O, 23.85. Found: C, 65.53; H, 8.16; N, 1.49; O, 24.16.

The pooled fractions 120 to 180 were concentrated and rechromatographed on a second silica gel column (700 g), which was eluted sequentially with ethyl acetate (4 liters), ethyl acetate - acetone (1: 1, 4 liters) and ethyl acetate - ethanol (95: 5, 4 liters) and 75 ml fractions were collected. Fractions 29 to 35 were pooled and concentrated to yield 1 g of 1,3-diphenethylurea (**6**), mp 136°C (literature value[8] 138~141°C), the structure (**6**) of which was established by microanalysis, mass spectrometry (M^+, *m/z* 268) and ^{13}C nmr in CD_2Cl_2 (see Table 1).

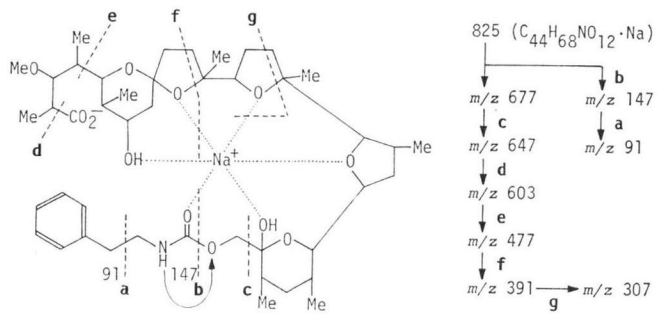
Pooled fractions 86~121 yielded a further 2 g of antibiotic X-14667A, and fractions 55~85 were concentrated under reduced pressure to 3.5 g of a mixture of **1** and **2** which was resolved on the Waters Prep LC-500™ using hexane - acetone (8: 2) as eluent to give a further 1.2 g of antibiotic X-14667A (**1**) and 1.8 g of antibiotic X-14667B (**2**), sodium salt, mp 103°C, $[\alpha]_D$ +50° (*c* 1, $CHCl_3$). Calcd. for $C_{45}H_{70}NO_{12}Na$ (840.04): C, 64,34; H, 8.40; N, 1.67; Na, 2.73. Found: C, 64.43; H, 8.29; N, 1.92; Na, 2.49.

The 23 grams of crystalline mixture isolated from the original extract (*vide supra*) was resolved by fractional crystallization into 6.7 g of 1,3-diphenethylurea (**6**), 6.5 g of monensin B (**4**) and 350 mg of monensin (**3**), which were all identified by methods described in the references[3,4,8] including microanalysis, TLC, mass spectrometry and ^{13}C nmr.

Characterization of Antibiotics X-14667A and 14667B

Field desorption mass spectrometry of antibiotic X-14667A sodium salt by Dr. CARTER COOK of the University of Illinois yielded a molecular ion that was in agreement with the molecular formula of $C_{44}H_{68}NO_{12}Na$ (826.02), calculated from microanalytical data. Conventional electron impact (EI) mass spectrometry did not yield a molecular ion but several fragment ions were noted, including those at *m/z* 677, 647, 603, 477, 391, 307, 147 and 91. The coincidence of finding the first six peaks in this list also in the EI mass spectrum of monensin B (**4**), isolated from *S. cinnamonensis* subsp. *urethanofaciens*, as well as the literature spectrum of monensin B[18], led us to the conclusion that antibiotic X-14667A must be a derivative of this lower monensin homolog, and a comparison of molecular formulae showed that the two molecules differed by C_9H_9NO. The presence in the fermentation of 1,3-diphenethylurea(**6**), in addition to monensin B (**4**), suggested that antibiotic X-14667A (**1**) is probably the 2-phenethylurethane of monensin B, as this would account for the difference in molecular formulae between **1** and **4**. Further support for this proposal is also to be found in the two mass spectral peaks which distinguish **1**

Fig. 1. Mass spectral fragmentation of antibiotic X-14667A, sodium salt.



from **4** at *m/z* 91 and 147. Both can be accounted for by cleavages **a** and **b** respectively in the mass spectral fragmentation scheme for antibiotic X-14667A illustrated in Fig. 1. The six fragment ions observed in both **1** and **4** are due to cleavages **b**~**g** (Fig. 1).

A comparison of the ^{13}C nmr spectra of the sodium salts of X-14667A (**1**) and monensin B (**4**) in CD_2Cl_2 revealed that there were forty-four carbons in **1**, which is nine carbons more than are present in **4**. This is in agreement with the molecular formula of $C_{44}H_{68}NO_{12}Na$ derived from microanalytical and mass spectral analysis. The presence of a 2-phenethylurethane was further supported by comparing the chemical shifts in the ^{13}C nmr spectra of **1** with that of its co-metabolite, diphenethylurea (**6**). There were peaks due to nine carbons that were very similar in **1** and **6** and did not appear in the spectrum of monensin B (Table 1).

Table 1. Comparison of ^{13}C nmr shifts of the nine 2-phenethylurethane carbons present in antibiotic X-14667A with those in the co-metabolite, 1,3-diphenethylurea as determined in CD_2Cl_2.

Functional group	δ-^{13}C shifts in ppm[a] of the 2-phenethylurethane	
	In antibiotic X-14667A	1,3-Diphenethylurea
$-CH_2-N$	42.7	41.5
$-CH_2-C=$	36.6	36.5
$=CH$	126.4	126.1
$=CH$	128.6 (×2)	128.3 (×2)
$=CH$	128.9 (×2)	128.6 (×2)
$=C-CH_2$	139.5	139.0
$C=O$	157.0	158.1

[a] Downfield from internal Me_4Si.

By analogous methods described for characterization of antibiotic X-14667A (**1**), antibiotic X-14667B was shown by microanalysis, mass spectrometry and ^{13}C nuclear magnetic resonance to be **2**, a higher homolog of X-14667A, the 2-phenethylurethane of monensin.

The final confirmation of the structures of antibiotics X-14667A and X-14667B as **1** and **2** was accomplished by synthesis from monensin B and A respectively.

Semi-synthetic Preparation of Antibiotic X-14667A

To a stirred solution of the sodium salt of monensin B (2.034 g, 3 mmole) in benzene was added 2-phenethylisocyanate (733 mg, 5 mmole). After reacting for 10 days at room temperature, the reaction mixture was washed in turn with aqueous Na_2CO_3 and water and then evaporated under reduced pressure to yield the 2-phenethylurethane of monensin B (2.11 g) which was identical by mp, $[\alpha]_D$, microanalysis, UV and IR spectra, NMR and mass spectrometry to the sodium salt of antibiotic X-14667A (**1**).

The analogous reaction using the sodium salt of monensin yielded the sodium salt of antibiotic X-14667B (**2**).

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