Communications to the Editor

ISOLATION AND CHARACTERIZATION OF ANTIBOTIC X-14931A, THE NATURALLY OCCURRING 19-DEOXYAGLYCONE OF DIANEMYCIN

Sir:

A number of polyether antibiotics containing two spiroketal functions in their molecular structure are known. They include dianemycin[1], lenoremycin[2] (A-130A)[3], and leuseramycin (TM-531A)[4]. In the most recent classification scheme proposed for the polyether antibiotics[5], these antibiotics constitute the 1b(2) type which, in addition to containing two spiroketals, are also β-glycosides due to the presence of the sugar-like moiety, trideoxy-4-*O*-methyl-D-erythro-hexapyranose (4-*O*-methylamicetose). In this report is described the first example of a polyether antibiotic containing two spiroketals but lacking the 4-*O*-methylamicetose or any other sugar moiety. This novel antibiotic, X-14931A (**1**), is formally the 19-deoxyaglycone of dianemycin (**2**).

Analysis of the sodium salt revealed the presence of two conformationally distinct molecules and preliminary efforts to obtain a structure from the data of this sodium salt failed. The structure of antibiotic X-14931A was subsequently determined by X-ray analysis of the silver salt hydrate (Fig. 1). The silver-oxygen and the hydrogen bond distances in the silver salt of antibiotic X-14931A are given in Table 1.

Table 1.

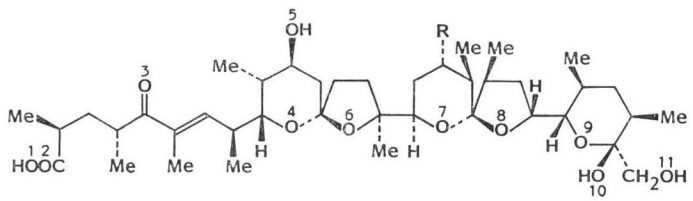
Silver-oxygen distances (Å) in the silver salt of X-14931A.

Oxygen	Ag ··· O
O (5)	2.44
O (6)	2.52
O (7)	2.40
O (8)	2.85
O (9)	2.63
O (10)	3.04
O (11)	2.48

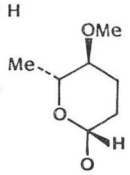
Hydrogen-bonding distances (Å) in the silver salt of X-14931A.

Hydrogen bond	H ··· O
O (5)–H ··· O (1)	2.79
O (10)–H ··· O (2)	2.67
O (11)–H ··· O (1)	2.65

Antibiotic X-14931A was isolated as part of a screen for novel ionophores by EtOAc extraction of whole fermentation broth (227 liters) from a culture of *Streptomyces* sp. X-14931. The crude extract was concentrated under reduced pressure to an oil which was dissolved in *n*-hexane and the solution extracted twice with equal volumes of CH_3CN, followed by three extractions with CH_3OH. The pooled CH_3CN and CH_3OH extracts were evaporated under reduced pressure. The residue was dissolved in EtOAc and washed sequentially with 1 N HCl, saturated Na_2CO_3 solution, then dried (Na_2SO_4) and concentrated under reduced pressure. The 55 g residue was chromatographed on 600 g silica gel. Gradient elution be-



1 X-14931A R = H



2 Dianemycin R =

Fig. 1. Stereoscopic diagram of the silver salt of antibiotic X-14931A, the naturally occurring 19-deoxy-aglycone of dianemycin.

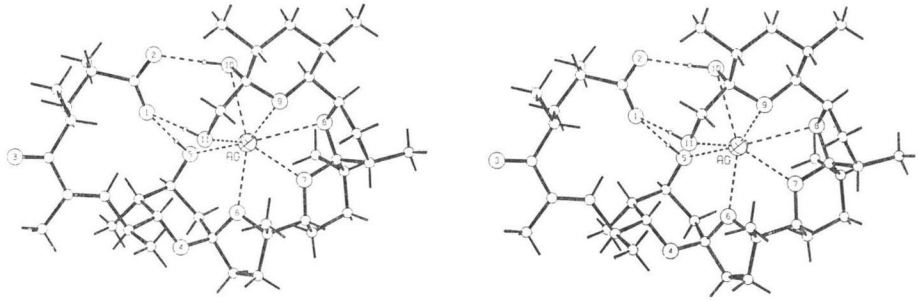


Table 2.

Organism	MIC (μg/ml)
Streptococcus faecium ATCC 8043	0.24
Staphylococcus aureus ATCC 6538P	0.98
Micrococcus luteus ATCC 9341	1.95
Bacillus megaterium ATCC 8011	0.98
Bacillus sp. E ATCC 27859	0.24
Bacillus subtilis NRRL 558	0.98
Bacillus sp. TA ATCC 27860	1.95
Mycobacterium phlei ATCC 355	7.8
Streptomyces cellulosae ATCC 3313	7.8
Paecilomyces varioti ATCC 26820	31.3
Penicillium digitatum ATCC 26821	62.5
Candida albicans NRRL 477	62.5
Saccharomyces cerevisiae ATCC 4226	62.5

tween CH_2Cl_2 - hexane (1: 1, 2 liters) and EtOAc - hexane (9: 1, 4 liters) gave 230×40 ml fractions. Fractions 134~230 were pooled, concentrated under reduced pressure and rechromatographed on 200 g silica gel. Gradient elution with EtOAc - hexane (1: 1, 2 liters) and EtOAc (3 liters) gave 130×40 ml fractions. Fractions 20~120 were pooled, concentrated under reduced pressure, dissolved in CH_2Cl_2, treated with charcoal, and were crystallized and recrystallized from CH_3CN - H_2O to give antibiotic X-14931A sodium salt, 7.2 g, mp 163~164°C, $[\alpha]_D^{25}$ +52.2° (*c* 1, $CHCl_3$). Calcd for $C_{40}H_{65}O_{11}Na \cdot H_2O$ (762.97): C 62.97, H 8.85, Na 3.01, H_2O 2.36. Found: C 63.05, H 8.75, Na 3.00, H_2O 2.88.

The sodium salt was dissolved in EtOAc and washed with 1 N HCl. Concentration under reduced pressure yielded the free acid form of antibiotic X-14931A, $[\alpha]_D^{25}$ +50.4° (*c* 1, $CHCl_3$). Calcd for $C_{40}H_{66}O_{11}$ (722.97): C 66.45, H 9.20. Found: C 66.59, H 9.30.

The silver salt of antibiotic X-14931A was prepared from the free acid by treating a diethyl ether solution first with saturated aqueous $Ba(OH)_2$ solution, followed by saturated aqueous Ag_2SO_4 solution. The salt was isolated by evaporation of the organic phase under reduced pressure and crystallization from diethyl ether - hexane to yield the silver salt which was recrystallized for X-ray analysis from CH_2Cl_2 - hexane to give the crystalline monohydrate, mp 152~155°C (dec), $[\alpha]_D^{25}$ +66.2° (*c* 1, $CHCl_3$). Calcd for $C_{40}H_{65}O_{11}Ag \cdot H_2O$ (847.85): C 56.67, H 7.97, Ag 12.72. Found: C 56.76, H 7.93, Ag 13.29.

Antibiotic X-14931A demonstrates *in vitro* bioactivity against Gram-positive microorganisms (cocci, bacilli, mycobacteria), molds and yeasts as shown in Table 2. Antibiotic X-14931A is active against mixed *Eimeria* infection in chickens at 50 μg/g in feed. The antibiotic also exhibits activity in the rumen growth promotant test.

After the conclusion of this study, a US patent appeared[6] for antibiotic 53,607 in which an identical structure to **1** is claimed although the microanalytical data were not in agreement with those found for antibiotic X-14931A and its salt.

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