TIRANDAMYCIN, A NEW ANTIBIOTIC ISOLATION AND CHARACTERIZATION

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This paper reports the isolation and characterization of tirandamycin, a new acidic antibiotic which has the molecular formula $C_{22}H_{27}NO_7$. This compound was isolated as the crystalline sodium salt and appears to be structurally related to streptolydigin.

This paper describes the isolation and physical and chemical properties of the new antibiotic tirandamycin. The taxonomy of the producing organism and the fermentation and biological properties of the antibiotic will be described elsewhere (DIETZ and SEBEK, in preparation).

Tirandamycin has a molecular formula of $C_{22}H_{27}NO_7$, deduced from its bromobenzene adduct, and is structurally related to streptolydigin (RINEHART, *et al.* in press). An outstanding characteristic of tirandamycin and its sodium salt is the tendency to form solvates, to the extent that the free compound has not been crystallized. The crystalline sodium salt has the following characteristics: U.V. max. 236 and 353 nm (0.1 N HCl in ethanol) and 253, 286 and 332 nm (0.1 N NaOH in ethanol); $[\alpha]_D^{25°}$ +51° (ethanol). It is soluble in most organic solvents but from some the solvate rapidly crystallizes.

Isolation and Purification

A filtered fermentation broth was extracted with methylene chloride at pH 2.

Fig. 1. The infrared absorption spectrum of tirandamycin.

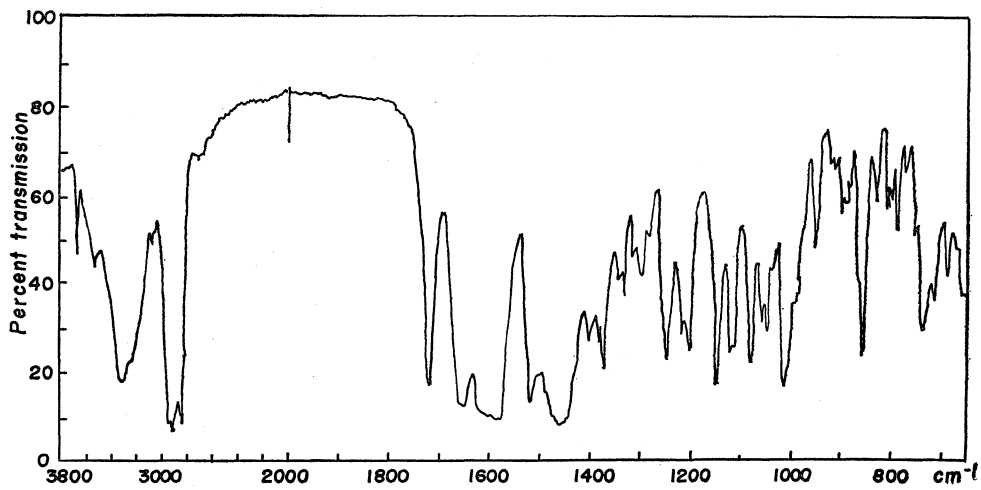
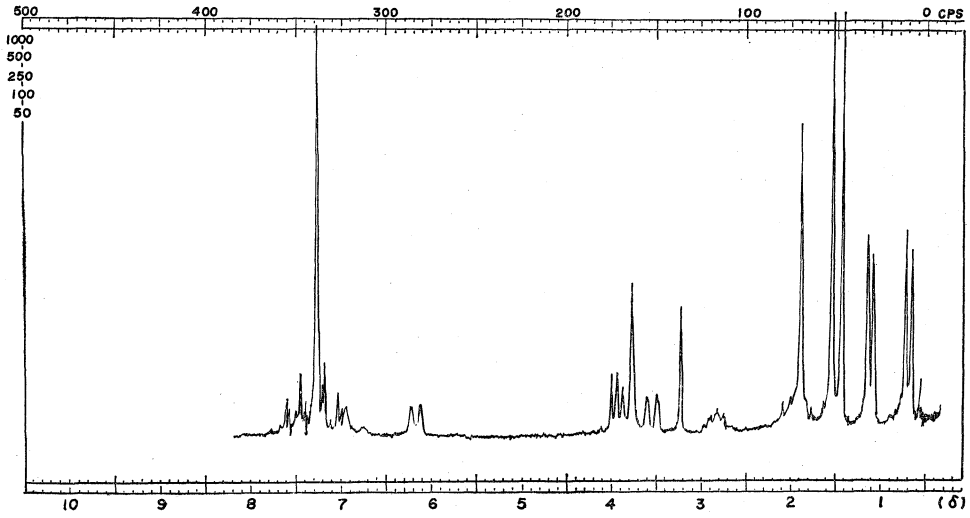


Fig. 2. The nuclear magnetic resonance spectrum of tirandamycin.



After concentration of the extract to a small volume, the antibiotic was transferred to water at pH 10 and again, as the sodium salt, into methylene chloride at pH 6.

The antibiotic was purified by chromatography on silica gel and recrystallized as the sodium salt.

Characterization

Sodium tirandamycin is a buff-colored micro-crystalline compound that is very soluble in methylene chloride. It is readily soluble in most of the usual organic solvents but the corresponding solvates rapidly crystallize therefrom. This is also true of the salt-free compound. Both the acid and salt forms are essentially insoluble in water.

Sodium tirandamycin has the following ultraviolet adsorption:

	max at nm	a
0.1 N HCl in ethanol:	236	19.3
	295 sl. sh.	16.8
	310 sl. sh.	24.7
	353	70.4
	365 sh.	64.0
	388 sl. sh.	27.4
0.1 N NaOH in ethanol:	253	29.5
	286	36.6
	332	40.0

The IR and NMR spectra of tirandamycin are shown respectively in Figs. 1 and 2.

Experimental

Isolation from Fermentation Broth

The whole broth (5,400 liters) was filtered at harvest pH 8.4 on a filter pre-coated with 250 lb (*ca.* 113 kg) of diatomaceous earth. The filtrate, adjusted to pH 2, was extracted with 200 liters of methylene chloride. The extract was concentrated to 6 liters, to which 10 liters of water was added and the pH raised to 10. Two more aqueous

extractions of 5 liters each were made. The combined aqueous phases were extracted with 20 liters of methylene chloride at pH 6, the extract dried with anhydrous sodium sulfate and the solvent removed.

Chromatography and Crystallization

Fourteen kg of silica gel (7734, E. Merck AG, Darmstadt) was added to a column 9 inches (22.86 cm) in diameter containing methylene chloride and allowed to settle to constant height. The charge, consisting of 223 g of crude powder dissolved in 1,260 ml of methylene chloride, was added and column developed with 168 liters of methylene chloride - methanol (19 : 1) followed by 350 liters of the same solvents (9 : 1, v/v). The effluent was monitored for absorbance at 352 nm and by TLC on silica plates developed with methylethyl ketone - acetone - water (186 : 52 : 20). The fractions which contained only material with an Rf value of 0.3 were combined and concentrated to dryness. The residue was crystallized from methylene chloride by the addition of 2.5 volumes of methanol. After drying *in vacuo*, the buff-colored crystals weighed 68.7 g. They were a methanol solvate of the sodium salt with variable composition, from which the methanol could not completely be removed. Analogous results were found with acetone, ethanol and benzene. Although satisfactory analyses were not obtainable with the latter compound, it was amenable to mass spectral analysis: m/e, found 417.1792, theory for $C_{22}H_{27}NO_7$ being 417.1786.

Tirandamycin · Bromobenzene Solvate

Sodium tirandamycin (1.0 g) was dissolved in 200 ml of methylene chloride and the pH adjusted to 2 with methanolic hydrogen chloride. After removing the sodium chloride with water, the solution was dried with anhydrous sodium sulfate and reduced to dryness, 800 mg of yellow powder being obtained. Of this, 600 mg were dissolved in 6 ml of bromobenzene. Light petroleum ether was added to incipient crystallization and the solution was refrigerated. The crystals were filtered off and rinsed repeatedly with petroleum ether but the odor of bromobenzene could not be removed. After drying in a stream of filtered air and *in vacuo*, they weighed 710 mg. Analyses were in agreement with a 1 : 1 solvate.

Analysis: $C_{22}H_{27}NO_7 \cdot C_6H_5Br$, Calculated: C 58.54, H 5.61, N 2.44, O 19.49, Br 13.91.
Found: C 58.68, H 5.44, N 2.35, O 19.92, Br 13.25.

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