THE ISOLATION AND CHARACTERIZATION OF RUBRADIRIN B

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Rubradirin B, $C_{40}H_{33}N_3O_{15}$, was separated from other components of the rubradirin complex by chromatographic and crystallization procedures. The spectrum of antibacterial activity is similar to that of rubradirin, but the antibiotic is less active.

Rubradirin was isolated in 1964 from a fermentation broth elaborated by *Streptomyces achromogenes* var. *rubradiris*[1,2]. The antibiotic inhibits susceptible bacteria by interfering with their synthesis of protein[3]. We report here the isolation, chemical characterization, and biological activity of rubradirin B, a second antibiotic produced by the above strain of *Streptomyces achromogenes*.

Materials and Methods

Mass spectroscopy.

The molecular weight was determined by field desorption on the Varian MAT CH5DF.

Nuclear magnetic resonance.

The ^{13}C NMR spectra were determined on the Varian CFT 20 and the ^{1}H NMR spectrum on the Varian XL-100-15 instruments, using d^6-DMSO as the solvent for rubradirin B and *d*-chloroform for rubradirin.

Infrared and ultraviolet spectra.

Infrared spectra were obtained on a Digilab FTS 14D spectrometer and ultraviolet spectra on a Cary model 15 spectrometer.

Chromatography.

Column chromatography employed buffered silica prepared as follows: A 1-kg quantity of silica gel 60, 70~230 mesh (E. M. Laboratories, Inc.) was mixed with 800 ml of 0.5 M KH_2PO_4 solution. This was then dried at 110°C for 20 hours. Aqueous suspensions of this silica had a pH of 5.8. Columns were prepared using a chloroform slurry of this silica.

Thin-layer chromatography was carried out in two systems. System A employed buffered silica on glass plates prepared as follows: A 60-g quantity of silica gel H.F. (E.M.) in 140 ml of a 0.4 M KH_2PO_4 solution was gauged onto glass plates which were then dried at 130°C. The development solvent was chloroform (stabilized with 0.75% ethanol) and methanol, (98 : 2, v/v). System B used silica gel GF on glass plates (Analtech) developed with ethyl acetate - acetone - water - methanol (8 : 5 : 10 : 6, v/v).

Assay.

The bioassay used was an agar dilution assay using a 12.7-mm disc and *Sarcina lutea* as the test organism. A biounit (B.U.) was defined as the concentration of active material required to give a 20-mm zone of inhibition.

In Vivo Testing.

The biological testing of rubradirin B was conducted in infected mice according to the procedures previously described[4].

Isolation of Rubradirin B.

A 1-g quantity of crude rubradirin mixture was chromatographed on 500 g of buffered silica in a 5-cm (diam.) column. The first 3.5 liters of chloroform eluant was discarded. The next 5.4 liters of chloroform eluant contained rubradirin by TLC analysis. The elution solvent was changed to chloroform and methanol (97 : 3, v/v) and 800 ml of this eluant was collected. TLC analysis showed it to contain a predominance of rubradirin B contaminated with traces of rubradirin. After concentration to 20 ml on the rotary evaporation followed by addition to 200 ml of Skellysolve B, 310 mg of red precipitate was collected.

A total of 660 mg of the above material (obtained by combining the foregoing 310 mg with product from a second column) was stirred in 30 ml of chloroform for 1 hour after which 160 mg of pure rubradirin B was collected as a crystalline solvate. These crystals deteriorated on drying at reduced pressure.

Anal. Calcd. for $C_{40}H_{33}N_5O_{15}$: C, 60.38; H, 4.18; N, 5.17; MW 795.
Found: C, 60.32; H, 4.45; N, 5.17; $M^{+\cdot}$ (by F.D.) 795.

Isolation and Characterization

Mixtures of rubradirin and rubradirin B, isolated from fermentation broths according to the procedure of MEYER[2], were chromatographed on silica gel buffered at pH 5.8 using combinations of chloroform and methanol as eluants. Pools of fractions containing the rubradirin B were reduced to a residue from which rubradirin B could be obtained in crystalline form by trituration with chloroform. These crystals became amorphous when dried under reduced pressure.

Rubradirin and rubradirin B have very similar ultraviolet spectra (Fig. 1). They have the same pH-dependency as to the colors of their solid powders and of their solutions, being red as free acids and green as basic salts. They show pKa' values of 6.2 (1 group) and 8.5 (2 groups)

Fig. 1. Ultraviolet spectra of rubradirin B.

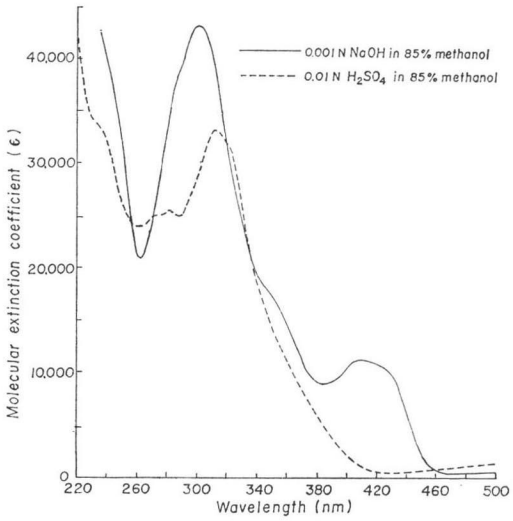
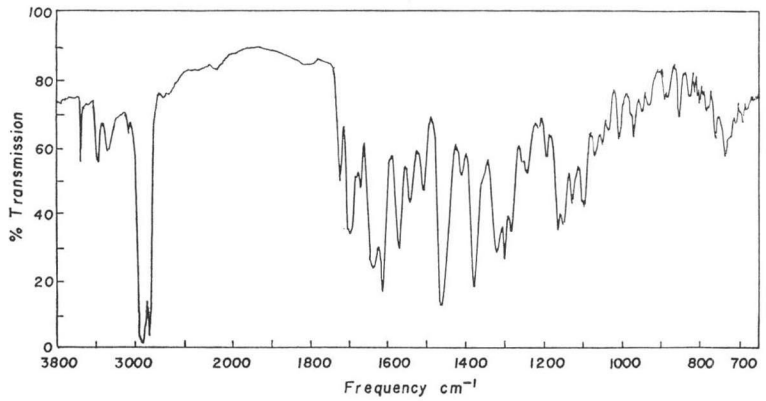


Fig. 2. Infrared spectrum of rubradirin B in nujol.



in 66% dimethyl sulfoxide. Rubradirin B has a molecular formula of $C_{40}H_{33}N_3O_{15}$ whereas the revised formula for rubradirin is $C_{48}H_{46}N_4O_{20}$. Carbonyl absorptions for rubradirin B in the infrared spectrum (Fig. 2) are very similar to those published for rubradirin although the band at 1550 cm^{-1} is much weaker for the former.

Signals corresponding to all 40 carbons are seen in the ^{13}C NMR spectrum (Fig. 3) for rubradirin

Fig. 3. ^{13}C NMR spectra.

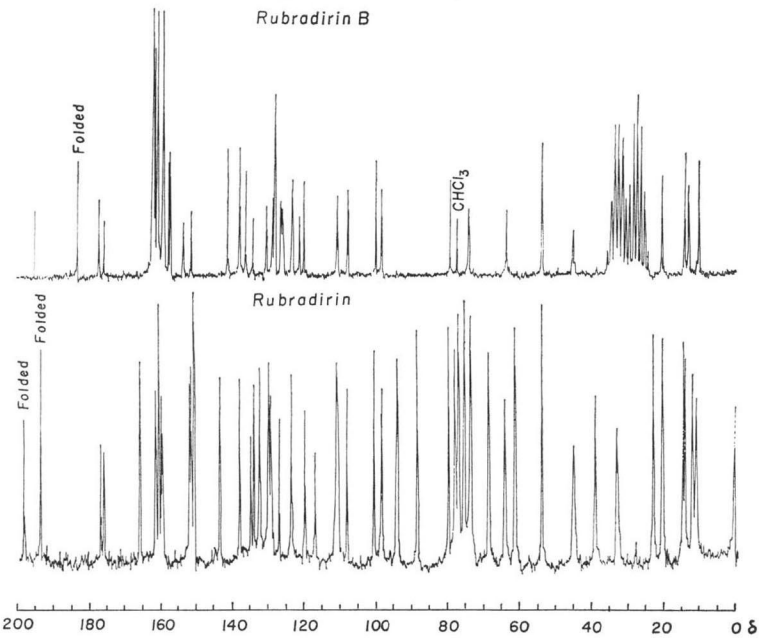
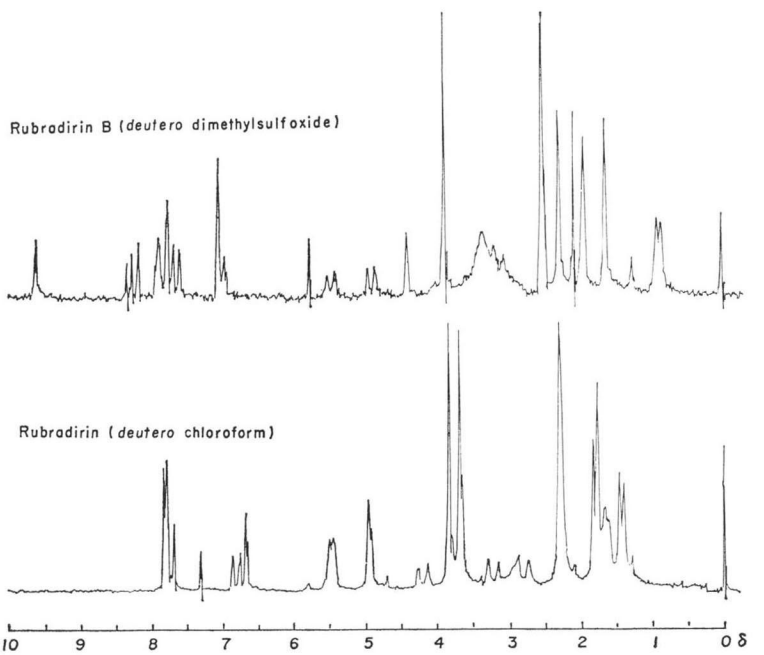


Fig. 4. ^{1}H NMR spectra.



B. These have their counterparts in the ^{13}C NMR spectrum of rubradirin, which also displays signals for 8 additional carbons. The decoupled spectra showed 3 quartets (δ 16.7, 24.8, 63.1), a triplet (δ 34.8), 3 doublets (δ 70.3, 79.5, 95.9) and a singlet (δ 90.3). A consideration of this 40-carbon similarity between the two antibiotics along with the other physical and analytical data suggests that rubradirin differs from rubradirin B by a discrete 8-carbon moiety, $C_8H_{13}NO_5$. The characterization of this moiety as a nitrosugar, stereoisomeric with evernitrose, has been described[4]. Other moieties present in both rubradirin and rubradirin B are a 3-amino-4-hydroxy-7-methoxy coumarin and a hydroxy dipicolinic acid[5]. About half of each antibiotic consists of an ansamycin moiety, containing the chromophore which accounts for the color of the two rubradirins[6].

Table 1. Antimicrobial spectrum of rubradirin B.

Organism		Minimum inhibitory concentration* (mcg/ml)	
		Rubradirin B	Rubradirin
S. aureus 284	UC 76	1.5	0.003
S. aureus	UC 570	3.1	0.012
S. aureus	UC 746	0.18	0.1
S. pyogenes	UC 152	6.2	0.20
St. faecalis	UC 694	>100	0.78
E. coli	UC 45	>100	>100
P. vulgaris	UC 93	>100	>100
K. pneumoniae	UC 58	>100	>100
S. schottmuelleri	UC 126	>100	>100
Ps. aeruginosa	UC 95	>100	>100
D. pneumoniae	UC 41	0.39	0.10

* Agar diffusion in brain heart-infusion medium.

Biological Characteristics

Agar diffusion assays in a brain-heart infusion agar (Table 1) show that rubradirin B is active at low concentrations against Gram-positive organisms. However, the activity is 4- to 500-fold less than that of rubradirin, depending on the test organism used. Mice infected with a strain of *Staphylococcus aureus* were protected by subcutaneously administered rubradirin B with a CD_{50} of 98 mg/kg. The corresponding dosage for rubradirin is 2.3 mg/kg.

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