A NEW ANTIBIOTIC, U-43,120 (NSC-163500)

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In the course of a screening program for antimetabolites[1] a new antibiotic (U-43,120) was isolated. The physical characteristics and composition of U-43,120 indicate that it is a member of the senfolomycins A and B[2] and proceomycin[3] family.

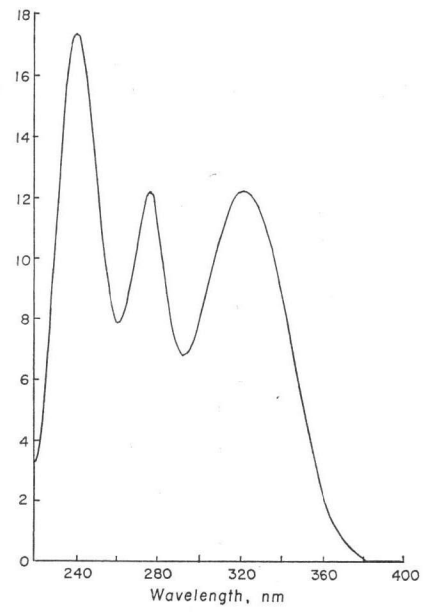
Isolation and Purification

The microorganism which produces U-43,120 is *Streptomyces paulus* DIETZ, sp.n. (UC-5231) isolated from soil. The fermentation conditions, microbiological assay and the taxonomy of the producing microorganism will be described by HANKA and DIETZ.[4] Seven hundred and twenty ml of fermented medium was mixed with 5% of diatomaceous earth (w/v), and the mixture was filtered washing the filter cake with 1/4 volume of water. The filtrate (pH 6.1) was adjusted to pH 2 with 6 N sulfuric acid and extracted with four 160-ml portions of methylene chloride. The combined extracts were evaporated *in vacuo* to give a black semi-solid residue weighing 125 mg and containing 60% of the original activity.

Five grams of material derived from an extraction similar but on a larger scale to that above was chromatographed on 300 g of silica gel using a solvent system consisting of ethyl acetate - ethanol - water (92: 5: 3) and collecting two hundred 20-ml fractions. Peak antibacterial activity occurred in fractions 57~95 which were combined and evaporated *in vacuo* to a dry residue weighing 500 mg. The activity yield was about 60%. Further purification was carried out by subjecting 50 g of chromatography product to countercurrent distribution in a 200-tube 50-ml per phase apparatus using cyclohexane - ethyl acetate - 95% ethanol - water (1: 1: 1: 1) as the solvent system and running 200 transfers. Fractions 116~175 were combined as a result of antibacterial assays and evaporated *in vacuo* at 35°C until the organic solvent was removed. The residue was extracted with three 100-ml portions of chloroform. Combination of the extracts and evaporation *in vacuo* at room temperature gave 2.9 g of residue representing an activity recovery of 30%.

Product from countercurrent distribution (14.4 g) was chromatographed on 1,080 g of silica using the same solvent system as previously. Six hundred 20-ml fractions were collected. On the basis of antibacterial activity fractions 204~395 were combined, and the solution was evaporated to dryness *in vacuo* at *ca.* 35°C. The yield of residue was 3.3 g with about 90% activity recovery. Crystallization of 2.7 g from chloroform gave 0.74 g of product which was nearly pure. Four more recrystallizations from the same solvent gave 149 mg of material which was judged to be pure on the basis of one spot, Rf 0.62, in tlc on silica gel

Fig. 1. UV Spectrum in EtOH



using methyl ethyl ketone - acetone - water (70: 20: 11) as the solvent system.

Physico-Chemical and Biological Properties

U-43,120 is a colorless crystalline solid melting at 119~122°C with prior softening. It is soluble in methanol, ethanol, chloroform, methylene chloride, acetone, ethyl acetate and ether but insoluble in water. It quickly loses its antibacterial activity in solution in lower alcohols. The optical rotation is $[\alpha]_D$+9.3° (*c* 1, $CHCl_3$), $[\alpha]_D$−34.9° (*c* 1, CH_3OH).

The ultraviolet spectrum (Fig. 1) showed maxima at 239 nm (a=17.3), 275 nm (a=12.2) and 321 nm (a=12.2) at a concentration of 50 μg/ml in ethanol. The infrared spectrum (Fig. 2) as a mull in Nujol had bands at 3560, 2070, 1730, 1690 sh, 1620, 1580, 1295, 1260, 1140, 1060, 1025, 993, 913, 820, 753 and 725 cm^{-1}. The NMR spectrum (Fig. 3) is a very complex one as would be expected from the large number of hydrogen atoms present. The few readily interpretable peaks are discussed in the next section.

Anal. Calcd. for $C_{34}H_{44}N_2SO_{18}$: C, 51.00; H, 5.53; N, 3.50; S, 4.01; O, 35.96.
Found: C, 50.64, 51.34; H, 5.88, 6.00; N, 3.60, 3.65; S, 4.07, 4.06; O, 34.41.

U-43,120 showed weak antibacterial activity against a few organisms (Table 1). It showed modest activity against L1210 and P388 cells

Fig. 2. Infrared spectrum (Nujol mull)

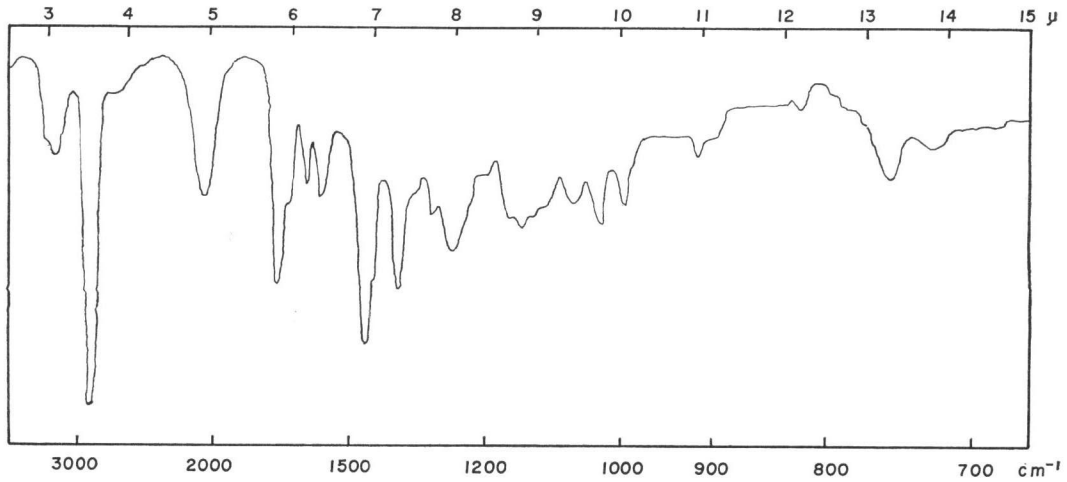
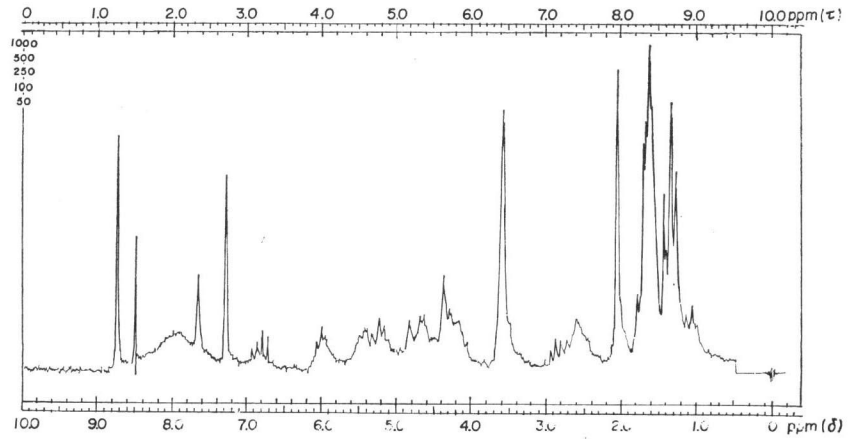


Fig. 3. NMR Spectrum in D_5C_5N (100 MHZ)



in vitro, $ID_{50} < 2.5$ μg/ml in both cases and it had a T/C of 1.50 at 25 mg/kg against a P388 tumor line in mice (G. L. NEIL, unpublished data, these laboratories).

Discussion

U-43,120 was differentiated from senfolomycin A by a direct tlc comparison using the already mentioned solvent system on silica. U-43,120 moved slightly faster than senfolomycin A and could be separated from it. Senfolomycin B was not available for direct comparison with U-43,120, and the meager data reported in the literature[2] for senfolomycin B made comparison difficult. However, the difference in optical rotation of senfolomycin B ($-60°$ in CH_3OH) and U-43,120 ($-34.9°$ in CH_3OH) indicate that they are not the same. Also the lower percentage of carbon and the higher percentage of sulfur and nitrogen as well as greater ultraviolet absorptivity of senfolomycin B suggest that it is a smaller molecule than U-43,120. The fact that proceomycin is colored differentiates it from U-43,120. In view of the close similarities in UV spectra of the four antibiotics and the presence of a band at about 2050 cm^{-1} in the infrared spectra of all four, it seems certain that they are chemically quite similar and make up a small family of antibiotics.

Very little has been reported concerning the structures of the senfolomycins and proceomycins. These antibiotics have been believed to contain an isothiocyanate group on the basis of the infrared spectra and the presence of sulfur. In the infrared spectrum of U-43,120 there is a band at 2070 cm^{-1} which would be consistent with the presence of an isothiocyanate group and sulfur is present. The infrared spectrum also shows the presence of a carbonyl (band at 1725 cm^{-1}). The molecule weight of the compound is not known as no satisfactory data were derived from mass spectra and molecular weight determinations were not done. However, the sulfur analysis is consistent with a molecular weight of about 800. The ultraviolet spectrum and analytical data are indicative of considerable unsaturation. The NMR spectrum shows the presence of seven methyl groups. Six of these are CH_3C and one is CH_3O (δ 3.56, s). A singlet at δ 2.04 is probably due to an acetyl group. The remaining signals due to methyl groups [δ 1.65 (d), δ 1.62 (s), δ 1.58 (d), δ 1.39 (d) and δ 1.30 (d)] are indicative of methyl groups β to a single bonded oxygen substituent.

Table 1. Antimicrobial activity of U-43,120

Test organism		MIC (μg/ml)
Staphylococcus aureus	UC80	15.6
Streptococcus hemolyticus	UC152	62.5
Streptococcus faecalis	UC3235	500
Escherichia coli	UC51	500
Proteus vulgaris	UC93	500
Klebsiella pneumoniae	UC57	500
Diplococcus pneumoniae	UC41	31.2

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