A NEW ANTITUMOR ANTIBIOTIC, KIDAMYCIN

III. PREPARATION AND PROPERTIES OF ACETYL KIDAMYCIN

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In attempts to reduce the toxicity and to enhance the activity of kidamycin many efforts have been made, and acetyl kidamycin was thus obtained in a pure crystalline state as one of the derivatives of kidamycin. It exsists as yellow needle crystals, and has three acetyl groups in the molecule. Acetyl kidamycin showed a lower toxicity than that of kidamycin, and its LD_{50} value was about 200 mg/kg intravenously.

Kidamycin[1], as reported by the present author, is an antitumor antibiotic of comparatively low toxicity, but not low enough to permit its use as an anticancer agent. Accordingly, in attempt to reduce the toxicity and to enhance the activity of kidamycin, various derivatives were prepared. Among them, the acetyl derivative of kidamycin obtained in a pure crystalline form exhibited a lower toxicity in mice.

Toxicology and antitumor activity on experimental animal tumors of acetyl kidamycin have been investigated. This paper deals with preparation and properties of acetyl kidamycin.

Preparation of Acetyl Kidamycin

An ordinary method can be applied for the acetylation of kidamycin. One gram of kidamycin was dissolved in the mixture of 4 ml acetic anhydride and 0.4 ml pyridine, and the reaction mixture was allowed to stand for 45 hours at 37°C in a sealed vessel, protected from light. After completion of reaction the resulting solution was poured onto pieces of ice and extracted with chloroform at below 5°C adjusting the pH at 4.5~5.0 with 5% NaOH with agitation. The separated chloroform layer was washed successively with 1% $NaHCO_3$ and water, dried with Na_2SO_4, and evaporated under vacuum to dryness. The residue obtained, after washing with petroleum ether and drying, was further purified by column chromatography, that is, the benzene solution of this residue was applied to the column of HCl-treated alumina slurried with benzene, and the column was eluted with ethyl acetate.

The yellow-colored effluent was collected and concentrated under vacuum to obtain yellow crystals. Crystals thus obtained were recrystallized from ethyl acetate, and 500 mg of pure acetyl kidamycin was obtained as bright yellow needle crystals.

Physical and Chemical Properties

Acetyl kidamycin forms bright yellow needle crystals, melting at 214.5~216.5°C with decomposition. Elemental analysis gives C 66.03%, H 6.56%, N 3.74%, no halogen and sulfur. The specific rotation is $[\alpha]_D^{20}+246\pm2°$ (c 1.5, chloroform). The ultraviolet spectrum in methanol was characterized by maxima at 238 mμ ($E_{1cm}^{1\%}$ 603), 270 mμ ($E_{1cm}^{1\%}$ 576) and 368~370 mμ ($E_{1cm}^{1\%}$ 160) as shown in Fig. 1. The infrared absorption spectrum in KBr shows characteristic bands at 1770, 1740, 1670, 1642, 1605, 1590, 1565, 1240, 1160,

Fig. 1. Ultraviolet absorption spectrum of acetylkidamycin (10 μg/ml methanol)

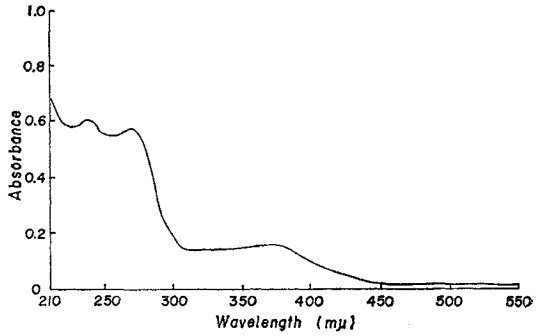


Fig. 2. Infrared absorption spectrum of acetylkidamycin (KBr)

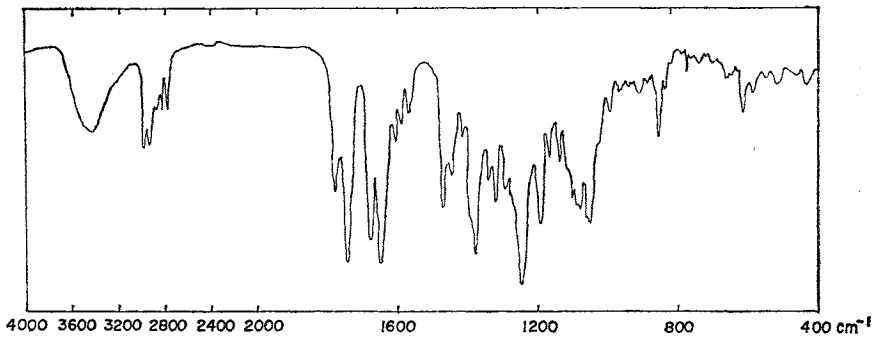
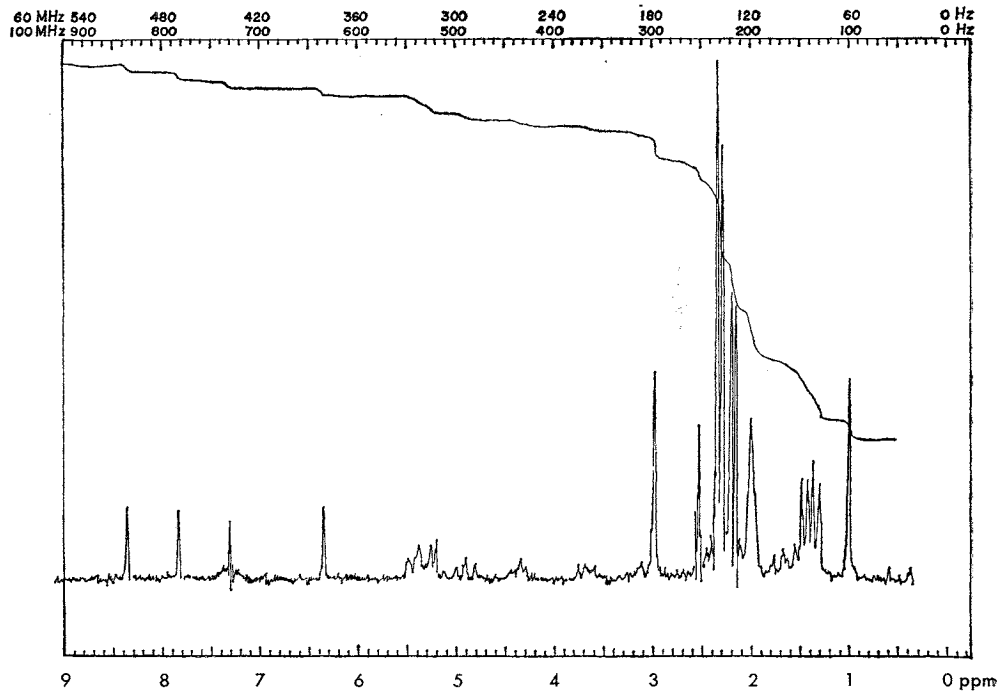


Fig. 3. NMR spectrum of acetyl kidamycin ($CDCl_3$, 100 MHz)



1140 and 850 cm^{-1} (Fig. 2.). The nuclear magnetic resonance spectrum of acetyl kidamycin in $CDCl_3$ indicates the presence of O-acetyl groups, N-methyl groups, and there is no exchangeable hydrogen as shown in Fig. 3. It contains three acetyl groups in the molecule. The molecular weight as determined by vapor pressure osmometry gives 800~820. The values calculated for $C_{44}H_{54}N_2O_{12}$ are C 65.84 %, H 6.73 % and N 3.49 %.

Acetyl kidamycin is easily soluble in chloroform, ethylene dichloride, carbon tetrachloride, benzene, pyridine, dioxane and aqueous acid; soluble in ethyl acetate, *n*-butyl acetate, acetone and *n*-butanol; slightly soluble in ethanol and methanol; insoluble in water, petroleum ether, ligroin and *n*-hexane.

Positive color reactions are as follows: reddish purple color with NaOH in MeOH and orange red color with conc. H_2SO_4. FEHLING, MOLISCH, ninhydrin, nickel acetate and magnesium acetate reactions are negative.

Rf values by thin-layer chromatography are as follows: 0.55 with ethanolpyridine (4 : 1, v/v) on silica gel (Merck Kieselgel G), and about 1.0 with water-saturated ethyl acetate on alumina oxide paper (Schleicher and Schüll No. 288).

Biological Properties

Though acetyl kidamycin is the product in which all hydroxyl groups of kidamycin are acetylated, it has a strong inhibitory activity upon a variety of microorganisms.

The antimicrobial activity of acetyl kidamycin on a variety of microorganisms were examined by the agar dilution method comparing with that of kidamycin[1] (Table 1.). Acetyl kidamycin is effective against many kinds of Gram-positive bacteria, but is not effective against Gram-negative bacteria and fungi similar to kidamycin free base.

Table 1. Antimicrobial spectrum of acetyl kidamycin and kidamycin

	Test organisms	Minimum inhibitory concentration (mcg/ml)	
		Kidamycin	Acetyl kidamycin
1	*Staphylococcus aureus* FDA 209 P	1.56	6.25
2	*Staphylococcus albus*	1.56	3.12
3	*Micrococcus flavus*	0.19	0.78
4	*Sarcina lutea* ATCC 9341	0.39	0.78
5	*Bacillus subtilis* PCI 219	1.56	6.25
6	*Bacillus cereus* ATCC 9634	0.78	3.12
7	*Corynebacterium sepedonicum*	0.78	6.25
8	*Escherichia coli* B	>100	>100
9	*Shigella flexneri* Komagome III	>100	>100
10	*Pseudomonas aeruginosa*	>100	>100
11	*Proteus vulgaris*	100	100
12	*Brucella melitensis*	1.56	1.56
13	*Salmonella enteritidis* No. 11	100	100
14	*Klebsiella pneumoniae*	100	100
15	*Serratia marcescens*	100	100
16	*Diplococcus pneumoniae* DP-1	0.39	1.56
17	*Streptococcus pyogenes* T-1	1.56	1.56
18	*Mycobacterium tuberculosis* 607	1.56	3.12
19	*Lactobacillus fermenti* 36	0.19	1.56
20	*Candida albicans* YU-1200	>100	>100
21	*Cryptococcus neoformans*	>100	>100
22	*Aspergillus niger*	>100	>100
23	*Trichophyton interdigitale*	>100	>100

The acute toxicities of acetyl kidamycin in mice in terms of LD_{50} are about 200 mg/kg intravenously, about 50 mg/kg intraperitoneally and about 600 mg/kg orally. It is rather interesting that the toxicity of acetyl kidamycin is apparently lower than that of kidamycin, however, the antimicrobial activity of acetyl kidamycin is similar to that of kidamycin.

As for antitumor activity, acetyl kidamycin exhibits

growth inhibition not only upon ascites tumors, such as EHRLICH ascites carcinoma, mouse leukemia SN-36 or mouse leukemia L 1210, but also upon solid type tumors such as Sarcoma-180 or EHRLICH carcinoma.

The results of antitumor experiment with acetyl kidamycin were partly reported at the 6 th International Congress of Chemotherapy[2] (Tokyo 1969), and will be reported in separate papers.

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