SIBIROMYCIN: ISOLATION AND CHARACTERIZATION

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(Received for publication July 31, 1972)

Sibiromycin, possessing strong antitumor activity in animal experiments, is produced by *Streptosporangium sibiricum*. Sibiromycin can be isolated from culture filtrate using ion-exchange or by solvent extraction. The antibiotic was obtained as a crystalline substance with ultraviolet absorption maxima at 230 and 310 nm, $[\alpha]_D$+525° (in dimethylformamide); the empirical formula-$C_{24}H_{31}N_3O_7$. In 1 N hydrochloric acid, sibiromycin is transformed into "the product of acidic inactivation" (PAI), $C_{24}H_{29}N_3O_6$. Hydrolysis of PAI with 6 N HCl affords "the product of acidic hydrolysis" (PAH), $C_{16}H_{14}N_2O_3$. Methanolysis of sibiromycin and PAI yields the methylglycoside of a new amino sugar sibirosamine, $C_8H_{16}NO_3$ (OCH_3). Aqueous alkaline hydrolysis of PAI and PAH affords the crystalline substance C_8H_9NO.

Sibiromycin is accumulaled in the culture liquid of *Streptosporangium sibiricum*[1] and can be isolated from culture filtrate by two methods: (1) by adsorbtion on ion-exchange resin and (2) by solvent extraction (Scheme 1).

Sibiromycin is an amphoteric substance soluble in diluetd acids and alkalies. The antibiotic can be adsorbed on sulfonic acid resins (NH_4^+-form) having high coefficient of swelling. We have found that the H^+-form of sulfonic acid resin inactivated sibiromycin. Sibiromycin is easily extracted from water solution by butanol and can be isolated from the culture filtrate with chloroform, ethyl acetate or methylene chloride. Extraction with the three last solvents depends on pH of the culture filtrate.

In alcoholic solution or as a dry powder sibiromycin is stable, but in water solution at pH lower than 3.0 and higher than 9.0, the antibiotic looses its biological activity. Crystalline sibiromycin has no sharp melting point and is decomposed above 120°C, $[\alpha]_D$ +230°→+106° (in 24 hours, *c* 0.5 in methanol) and +525° (*c* 0.35 in dimethylformamide). The molecular weight of sibiromycin as determined by ebullioscopy in chloroform, and the equivalent of neutralization, determined by titration in 75%

Scheme 1.

Culture filtrate

Adsorption on sulfonic acid resin SDV-3 (NH_4^+)	Extraction with CH_2Cl_2 (0.2 vol.) pH 7.6 in the presence of oleic acid (8 % to vol. CH_2Cl_2) used as a carrier.
Desorption with 0.25 N NH_4OH	Reextraction with citric-phosphate buffer pH 4.0, three times (0.1, 0.05, 0.05 vol.)
Extraction with $CHCl_3$	Extraction with $CHCl_3$, pH 7.8 (four times, 0.5 vol.)
Concentration	Concentration of solution to 150 mg/ml
Precipitation with petroleum ether	Precipitation with running of NH_3-gas through solution
Crystallization	Crystallization of sibiromycin from MeOH or $CHCl_3$

alcohol, both were estimated to be about 470. Sibiromycin has one basic group with a pKa 7.5. On the basis of elemental analysis and molecular weight determination, the molecular formula of sibiromycin is calculated to be $C_{24}H_{31}N_3O_7$ (M.W. 473). Sibiromycin decolorizes potassium permanganate solution and bromine solution, shows positive ninhydrin, $FeCl_3$, and diazo reactions. The last test can be used for detection of the antibiotic on chromatograms.

The ultraviolet spectrum of sibiromycin exhibits two maxima at 230 ($E^{1\%}_{1cm}$ 405) and 310 nm ($E^{1\%}_{1cm}$ 420) in methanol (Table 1, Fig. 1). In 0.1 N aqueous hydrochloric acid just after solution the spectrum is practically the same, but in several hours there is a perceptible and irreversible change in the character of absorption and the spectrum shows three maxima at 240, 405 and 445 nm[2,3].

In the NMR spectrum (in pyridine-d_5) of sibiromycin there are singnals of five methyl groups (δ 1.42 d, 1.72 s, 1.75 d, 2.4 s and 2.65 s) (Fig. 2). The infrared spectrum of sibiromycin is presented in Fig. 3. Physical and chemical properties of sibiromycin are

Table 1. Physical and chemical properties of sibiromycin

Melting point (°C)	decomp. above 120°C
Optical rotation $[\alpha]_D^{20°}$	+230°→+106° (in 24 hr., methanol) +525° (DMFA)
UV max. MeOH ($E^{1\%}_{1cm}$)	230, 310 nm 405, 420
Elementary analysis	Found : C 61.50, H 7.00, N 9.20 % Calc. for $C_{24}H_{31}N_3O_7$: C 60.87, H 6.85, N 8.88 %
Solubility	Soluble in DMFA, pyridine, acetone, sparingly soluble in alcohol, chloroform, ethyl acetate, insoluble in water (pH 7), petroleum ether
Color reactions	Positive ninhydrin, $FeCl_3$, diazo, decolorizes $KMnO_4$, Br_2-H_2O
TLC on silica gel (Rf)	0.5 ($CHCl_3$-MeOH 5 : 1) 0.45 (EtOAc-*n* BuOH, satur. with H_2O 1 : 1)

Fig. 1. Ultraviolet adsorption spectrum of sibiromycin in methanol.

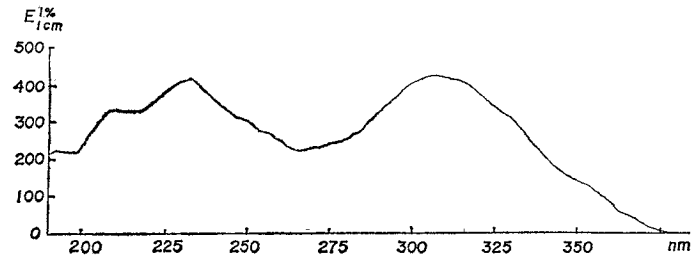


Fig. 2. NMR spectrum of sibiromycin in deuteropyridine (100 Mc, TMS as int, stand.)

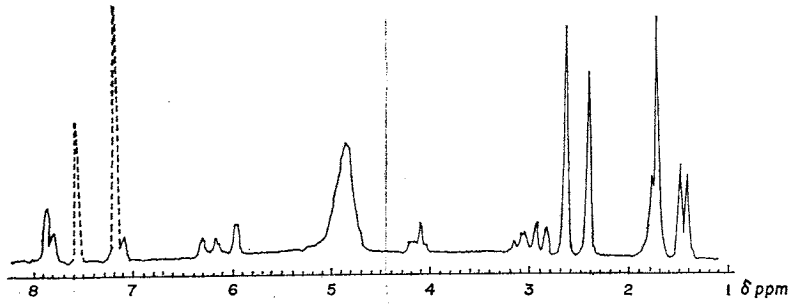


Fig. 3. Infrared spectrum of sibiromycin (in KBr)

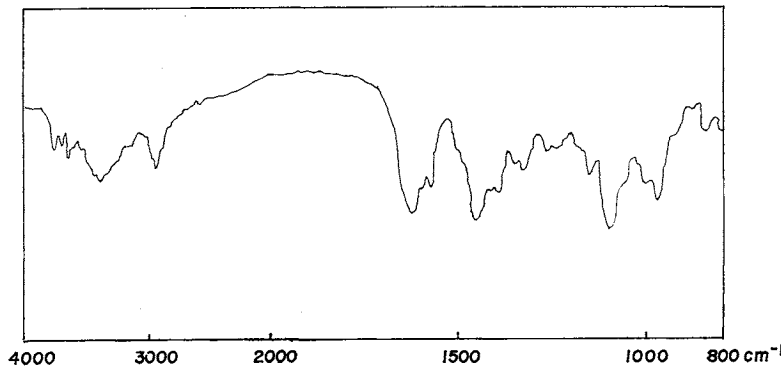
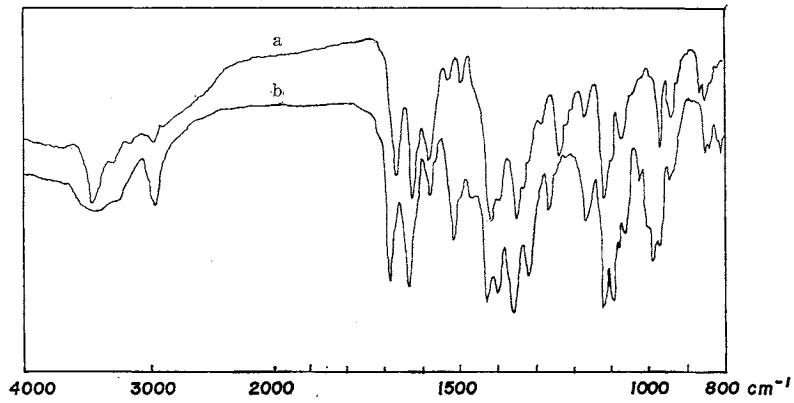


Fig. 4. Infrared spectra of "PAI" (a) and "PAH" (b) (in KBr)



tabulated in Table 1.

Interaction of sibiromycin with gaseous SO_2 in chloroform affords the crystalline derivative, $C_{24}H_{31}N_3O_7 \cdot SO_2$, possessing biological activity similar to that of sibiromycin. The sulfur-containing derivative of the antibiotic is crystallized from methanol and shows a UV spectrum practically identical with the spectrum of the parent sibiromycin. The same product yields on treatment of sibiromycin in chloroform solution with saturated aqueous solution of sodium hydrosulphite. The sulfur-containing derivative of sibiromycin is more stable to the action of acids[2].

As stated above sibiromycin is sensitive to the influence of acids. We have found that in 1 N alcoholic solution of hydrochloric acid, sibiromycin is transformed into a biologically inactive yellow crystalline substance named "the product of acidic inactivation" (PAI), $C_{24}H_{29}N_3O_6$. Some physical and chemical properties of PAI are presented in Table 2. Infrared spectrum is shown in Fig. 4 a.

On hydrogenation over palladium PAI consumes four moles of hydrogen and yields white crystalline substance, $C_{24}H_{37}N_3O_6$, with λ_{max} 229, 260 (shoulder) and 350 nm ($E^{1\%}_{1cm}$ 650, 190 and 75 respectively). It was shown that sibiromycin also consumes four moles of hydrogen during hydrogenation over palladium and affords the same product.

Methanolysis of sibiromycin and PAI, as well as methanolysis of their products

Table 2. Physical and chemical properties of degradation products of sibiromycin

	PAI	PAH	PBH	Methyl sbirosaminide horochloride
Melting point (°C)	203	270 (decomp.)	115~116	120~122
Molecular weight	455	282	135	205
Optical rotation	−170° (DMFA)	—	—	−50° (H_2O)
UV_{max} MeOH (nm)	277, 407, 435	290, 375, 410, 435	242, 250 (infl.), 325	—
$E^{1\%}_{1cm}$	880, 270, 250	1740, 350, 360, 280	1420, 1100, 720	—
Elementary analysis	Found : C 63.28, H 6.42, N 9.23 %	Found : C 68.25, H 5.22, N 9.75 %	Found : C 71.42, H 6.35, N 10.5	Found : C 44.96, H 8.52, N 5.55, Cl 14.4 %
	Calc. for $C_{24}H_{29}N_3O_6$: C 62.75, H 6.65, N 9.32 %	Calc. for $C_{16}H_{14}N_2O_3$: C 68.07, H 5.00, N 9.90	Calc. for C_8H_9NO: C 71.09, H 6.75, N 10.35,	Calc. for $C_9H_{19}NO_4 \cdot HCl$: C 44.71, H 8.34 N 5.79, Cl 14.68

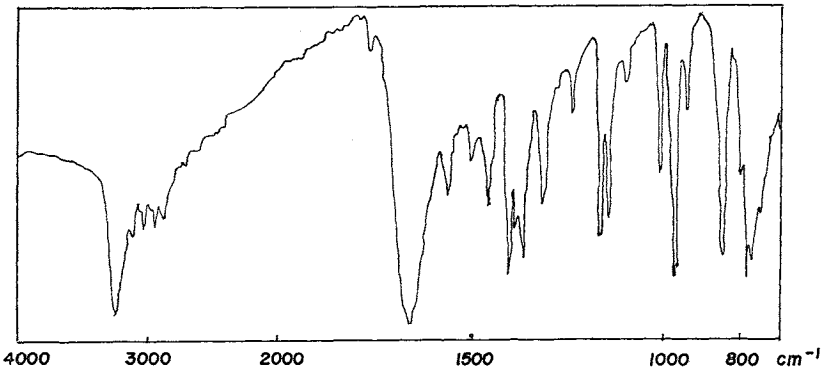
of hydrogenation (9 % HCl/MeOH 1 hour at 100°C) ield the meythylglycoside of a new amino sugar named sibirosamine. Methyl sibirosaminide was isolated using Dowex 50×8 (NH_4^+). Elution of the methylglycoside was carried out with 0.5 N NH_4OH. The ninhydrin-positive eluate was concentrated under reduced pressure and dried. Further purification of methyl sibirosaminide-base was made by column chromatography using silica gel and benzene - acetone (1 : 1) as the developing solvent. The pure methyl sibirosaminide base was converted into the hydrochloride and crystallized from anhydrous iso-propanol. On the basis of the elemental and functional analysis a molecular formula of methyl sibirosaminide hydrochloride was calculated to be $C_9H_{19}NO_4 \cdot HCl$, $[\alpha]_{20°}^{D}$ −50° (*c* 2.0, H_2O), m.p. 120~122°C (Table 2).

Methyl sibirosaminide is not hydrolyzed with 1 N aqueous HCl (3 hours, 100°C) and gives neither ammonia nor other volatile amines on treatment with hot alkali. Acetylation of the methyl glycoside with Ac_2O in pyridine yields the diacetate $C_{13}H_{23}NO_6$, $[\alpha]_D^{20°}$ −70° (*c* 0.4, methanol), m.p. 135~136°C. In the infrared spectrum of the diacetate there is absorption at 3450 cm^{-1} (OH). Additional acetylation of the diacetate with Ac_2O in triethylamine with 4-dimethylamino-pyridine[4] yielded the triacetate, $C_{15}H_{25}NO_7$, $[\alpha]_D^{20°}$ −25° (*c* 0.3, methanol), m.p. 127~128°C.

Hydrolysis of PAI with 6 N aqueous hydrochloric acid (45 min., 100°C) affords a bright yellow crystalline substance named "the product of acidic hydrolysis" (PAH) $C_{16}H_{14}N_2O_3$. PAH is soluble in pyridine, sparingly soluble in ether and acetone, slightly soluble in alcohol and insoluble in benzene, chloroform and water. Catalytic reduction (Pd-C) of PAH resulted in uptake of 4 equivalents of hydrogen and afforded the colourless octahydro-PAH, $C_{16}H_{22}N_2O_3$, the UV-spectrum shows the same maxima, as the UV-spectrum of octahydro-PAI, at 229, 260 (shoulder) and 350 nm. Infrared spectru m of PAH is shown in Fig. 4 b. PAH is very stable to the acids, but is easily hydrolized with 0.1 N aqueous NaOH (30 min., 100°C) yielding colorless crystalline substance, C_8H_9NO' named "the product of basic hydrolysis" (PBH).

PBH is very soluble in alcohols, ether, chloroform and benzene. In water and aqueous solutions of acids and alkalies PBH is insoluble. On hydrogenation over palladium on charcoal PBH consumes 2 moles of hydrogen. Infrared spectrum of

Fig. 5. Infrared spectrum of the product of alkaline hydrolysis (in KBr).

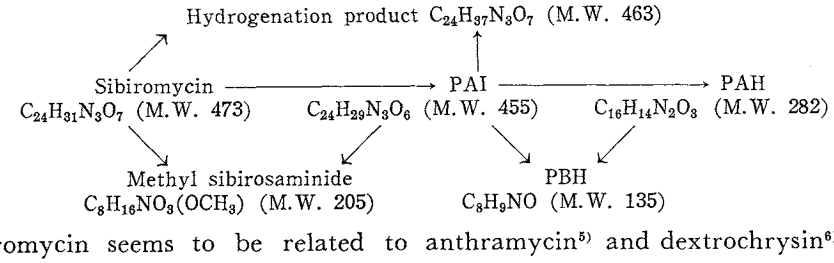


PBH in KBr tablet (Fig. 5) shows a strong C=O absorption band at 1670 cm^{-1}, with 2,4-dinitrophenyl hydrazine PBH affords the brown crystalline hydrazone. All these properties suggest that in the molecule of PBH there is carbonyl group.

We have found that PBH is formed also from PAI, but among the products of basic hydrolysis of parent sibiromycin PBH was not detected.

In the following scheme (Scheme 2) are summarized the results of studying chemical transformations of sibiromycin.

Scheme 2



Sibiromycin seems to be related to anthramycin[5] and dextrochrysin[6] in some physicochemical properties (UV-adsorption spectrum, extremely high dextrotation in dimethylformamide), but some distinct differences were found. The most important difference is that neither anthramycin nor dextrochrysin have an amino sugar moiety in their molecules. It was concluded that sibiromycin is a new aminoglycosidic antitumor antibiotic.

Sibiromycin inhibits growth of *Bacillus mycoides* and *Bacillus subtilis* (0.3 mcg/ml), *Staphylococcus aureus* (1 mcg/ml), and *Escherichia coli* (20 mcg/ml). The activity of sibiromycin is most pronounced in the treatment of mice with inoculated squamous praegastric cancer cells (strain OG-5). After two injections of sibiromycin in maximal tolerated doses the tumors disappeared completely. The development of lymphosarcoma (strain Lyo-1) was inhibited by sibiromycin in maximal tolerated dose by 90~97 %[7]. The LD_{50} of sibiromycin administered in a single dose to mice intravenously, intraperitoneally, subcutaneously and orally are 58, 32, 84 and 459 mcg/kg, respectively[8].

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