ISOLATION AND CHARACTERISTICS OF A NEW ANTIBIOTIC VARIAMYCIN*

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UDC 615.33

We have previously reported the isolation of an antibiotic from the culture fluid of actinomycete No. 6604-9 assigned to a new species Act. olivovariabilis sp. nov. Continuing a study of the metabolic products of this culture, we have obtained another new antibiotic, which we have called variamycin (No. 6604-9A).

Variamycin with the composition $C_{52}H_{76}O_{24}$, is a yellow substance readily soluble in the lower alcohols and in esters and moderately soluble in water. The antibiotic possesses a considerable activity against Gram-positive and acid-sensitive bacteria.† Below we give the antimicrobial spectrum of variamycin.

Name of the test organism	MBC, μg/ml
Staph aureus 209 P	0,06
Str. pyogenes	0,06
Str. faecalis	1,00
Bac. subtilis	1,00
Bac. anthracoides	0,06
Cor. diphtheriae	0,16
E. coli 675	50
Ps. aeruginosa	50
Proteus vulgaris	100
M. phlei	0,63
Candida albicans	50

The activity of the acetate of the antibiotic with respect to Gram-positive bacteria was approximately 100 times lower than that of the initial substance.

On comparing the properties (particularly the spectral characteristics)of variamycin and its acetate with the properties of substances having the same antibacterial activity described in the literature, a similarity was observed between our antibiotic and aureolic acid (mythramycin) [3, 4]. The two antibiotics were similar in chromatographic behavior in various solvent systems.‡

We compared the products of the acid degradation of variamycin and of aureolic acid. It is known [5] that the acid hydrolysis of this acid, depending on the conditions, forms the aglycone chromomycinone (I) and dideoxysugars [oliose (II), olivose (III), and D-mycarose (IV)] and also the tetraoside (V) (see Scheme).

From the products of the complete acid hydrolysis of variamycin we isolated the aglycone of the antibiotic, which was identified by direct comparison with an authentic sample of chromomycinone (I). We obtained the latter from aureolic acid by the method described by Bakhaeva et al. [5].

In a chromatographic study of the hydrolysates of variamycin, it was found that the molecule of the antibiotic contains, in addition to chromomycinone, the residues of three deoxy sugars, two of which, from

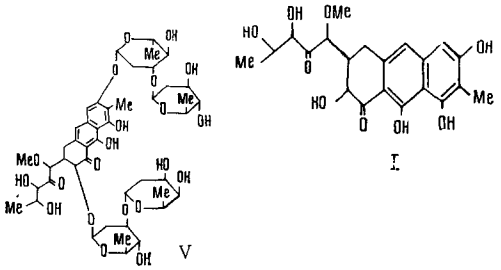
* For a preliminary communication, see [1].

† The determination of the antimicrobial spectrum of variamycin was performed by P. S. Braginskaya.

‡ Samples of mythramycin were kindly given to us by M. N. Kolosov, Corresponding Member of the Academy of Sciences of the USSR, and by Prof. S. M. Navashin.

All-Union Scientific-Research Institute of Antibiotics. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 646-649, September-October, 1971. Original article submitted May 6, 1971.

their chromatographic mobilities, are identical with oliose (II) and olivose (III), while the third differs from mycarose (IV); we have called it variose. The partial hydrolysis of variamycin forms a substance which was identical on direct comparison with the tetraoside (V).* This shows the presence in the molecule of the antibiotic of a fragment consisting of chromomycinone, one residue of oliose (II), and three residues of olivose (III). Since the difference between the molecular weight of variamycin (1084) and that of the tetraoside fragment (923) is comparable with the molecular weight of a single sugar, it may be assumed that the molecule of the antibiotic contains one variose residue.



I

V

As already mentioned, variose and mycarose have different chromatographic mobilities. Unlike mycarose [6], variose is not oxidized by sodium metaperiodate and, consequently, does not contain vicinal diol groupings. The presence in the structure of variamycin of a variose residue fundamentally distinguishes the antibiotic that we have isolated from the otherwise similar aureolic acid (mythramycin).

It follows from what has been said that variamycin is a new antibiotic of the aureolic acid group which has not been described previously.

EXPERIMENTAL

The substances were isolated and analyzed by column and thin-layer chromatography (in a nonfixed layer) on type hydrated silicic acid silica gel, using the following solvent systems: 1) chloroform–methanol (9 : 1), and 2) benzene–acetone (1 : 1).

The UV spectra were recorded on an SF-4A spectrophotometer (methanol), the IR spectra on a Zeiss UR-10 instrument (mulls with paraffin oil), and the NMR spectrum on a Varian HA-100 spectrometer (in $CDCl_3$). Substances were shown to be identical by comparing their melting points, specific rotations, and IR, UV, and NMR spectra. The analyses of all the compounds corresponded to the calculated figures.

Preparation of Variamycin. The deep fermentation of the antibiotic was performed by cultivating Act. olivovariabilis at 26-28°C for 130-140 h in an apparatus with a capacity of 100 liters on a medium with the composition (%): soya flour 2.0; starch 2.0; glucose 2.0; $CaCO_3$ 0.5; $(NH_4)_2SO_4$ 0.4; K_2HPO_4 0.04.

After the end of fermentation, the mycelia were filtered off, and the filtrate (~30 liters) was acidified with 10% HCl to pH 2-2.5. The active substance was extracted three times with ethyl acetate, and the combined extracts were re-extracted with 5% aqueous $NaHCO_3$ solution. The re-extract was acidified as described above, and the antibiotic was extracted with ethyl acetate. The extract was washed with saturated NaCl solution, dried over Na_2SO_4, and evaporated in vacuum to 0.1 of its original volume, and the concentrate was treated with a fivefold volume of petroleum ether (bp about 69°C). The residue (3.5 g) was dissolved in a small amount of mixture 1 and transferred to a column (55 × 500 mm). Elution was performed with the same mixture. The fractions collected (100 ml each) were analyzed by thin-layer chromatography in mixture 1. The fractions containing the variamycin were combined and evaporated to dryness in vacuum, and the residue was dissolved in a small amount of methanol and chromatographed in a thin layer in mixture 1 (plate dimensions 13 × 18 cm; amount deposited 30 mg).

The product was eluted from the zone with R_f 0.5-0.6 with methanol, the eluate was evaporated, and the residue was twice crystallized. The antibiotic obtained gave a single spot on chromatography in a thin layer in mixtures 1 and 2, and also on paper in the solvent mixture benzene–acetic acid–water (4 : 5 : 1) [7] (500 μg). The composition of variamycin is $C_{52}H_{76}O_{24}$, mp 162-165°C (ethyl acetate–hexane); $[\alpha]_D^{20}$ −42 ± 2° (c 0.5; ethanol). UV spectrum (in methanol), λ_{max}, nm: 230, 280, 317, 330 (shoulder), 412 (log ε 4.27, 4.61, 3.81, 3.74, 3.94). IR spectrum, cm^{-1}: 915, 1010, 1070, 1120, 1170, 1520, 1590, 1640, 1730,

*A sample of the tetraoside (V) was kindly given to us by I. V. Yartseva (Institute of the Chemistry of Natural Compounds).

3400-3450. The equivalent weight determined by potentiometric titration with 0.1 N methanolic NaOH solution in a mixture of benzene and methanol (1 : 1) was 1073 ± 50.

Acetylation of Variamycin. A mixture of 0.3 g of variamycin, 2 ml of dry pyridine, and 3 ml of acetic anhydride was kept at 20°C for 72 h, after which the solution was poured into cold water. The reaction product was extracted with chloroform, and the extract was washed with 5% solutions of $NaHCO_3$ and HCl, and with water. The residue after the evaporation of the dried extract was dissolved in a small amount of chloroform and chromatographed in a thin layer in benzene–acetone (3 : 1). The substance was eluted with methanol from the zone with R_f 0.6-0.7, and the eluate was evaporated. After crystallization, the acetate of the antibiotic was obtained, $C_{72}H_{96}O_{34}$, mp 145-148°C (benzene–hexane), $[\alpha]_D^{20}$ −43 ± 2° (c 0.5; ethanol). UV spectrum (in methanol), λ_{max}, nm: 225, 255, 267, 315, 325, 370 (log ε 4.57; 4.67; 4.87; 3.16; 4.1; 3.56). IR spectrum: 1745 cm^{-1} (C = O in esters).

Hydrolysis of Variamycin. A. A solution of 1 g of variamycin in 100 ml of 50% acetic acid was heated at 75°C for 3 h and evaporated to dryness in vacuum. A solution of the residue in 50 ml of ethyl acetate was washed with water, dried, and evaporated to dryness in vacuum. The residue was chromatographed in a thin layer in benzene–acetone (3 : 2). The substance was eluted with methanol from the zone with R_f 0.7. The residue from the evaporation of the eluate was recrystallized from glacial acetic acid, giving the solvate of chromomycinone $C_{21}H_{24}O_9 \cdot 2CH_3COOH$, mp 185-187°C. UV spectrum (in methanol), λ_{max}, nm: 232, 280, 325, 338, 415; IR spectrum, cm^{-1}: 1100, 1160, 1280, 1550, 1710, 1730, 3400; NMR spectrum: δ 2.09 ppm (s, 3H; $-CH_3$ in an aromatic ring).

B. A solution of 5 mg of variamycin in 5 ml of 50% acetic acid was heated at 75°C for 5 h and evaporated in vacuum to dryness; the residue was dissolved in ethanol and subjected to descending chromatography on paper (Leningrad type "S" ["medium"]) in butanol–ethanol–water (4 : 1 : 5). Solutions of triphenyltetrazolium chloride and $SbCl_3$ were used to reveal the spots [8]. Three spots were found with R_f 0.48, 0.54, and 0.70, respectively.

C. A solution of 10 mg of variamycin in 10 ml of 0.01 N HCl solution was heated at 75°C for 1.5 h and extracted with ethyl acetate. After washing with water and drying, the extract was evaporated to small volume, and the concentrate was chromatographed in a thin layer in mixture 1. The substance was extracted with methanol from the zone with R_f 0.2-0.3. The eluate was evaporated to dryness in vacuum, and the residue was dissolved in a small amount of ethyl acetate and precipitated with petroleum ether. This gave a yellow-orange amorphous powder of the tetraoside (V), $[\alpha]_D^{20}$ −82 ± 2° (c 0.5; ethanol). UV spectrum (in methanol), λ_{max}, nm: 230, 280, 315, 415 (log ε 4.69; 4.96; 4.24; 4.27); IR spectrum, cm^{-1}: 1070, 1170; 1520; 1590; 1635; 1720; 3400.

When the tetraoside V was hydrolyzed under the conditions described in paragraphs A and B, chromomycinone, oliose, and olivose were identified chromatographically.

D. A solution of 2 g of variamycin in 10 ml of 50% acetic acid was treated as described in paragraph A.

After evaporation in vacuum, the hydrolysate was dissolved in 50 ml of ethyl acetate, and the solution was extracted with water (5 × 15 ml). The combined extracts were evaporated in vacuum to dryness, and the residue was dissolved in methanol and chromatographed in a thin layer in solvent system 2 [the spots being revealed with H_2SO_4 in methanol (1 : 1)].

The substance was eluted with methanol from the zone with R_f 0.4-0.5, the eluate was concentrated to 1/4 of its original volume, and the concentrate was passed through a layer of Al_2O_3 to decolorize it, after which it was evaporated to dryness. A colorless syrup was formed which, according to chromatography, contained only variose $[\alpha]_D^{20}$ +54° (c 0.5; water).

SUMMARY

A new antibiotic, variamycin, belonging to the aureolic acid group and distinguished from the latter by the presence in the molecule of a residue of the deoxy sugar variose has been isolated.

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