BIOTRANSFORMATION OF ANTITUMOR AGENTS BY A STRAIN OF *WHETZELINIA SCLEROTIORUM*

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(Received for publication May 16, 1977)

Antitumor antibiotics of the olivomycin and chromomycin class were transformed when incubated with a culture of *Whetzelinia sclerotiorum*. The products, when purified by countercurrent distribution and column chromatography, were shown, by their physical properties, to be the deacylated analogues.

In the course of screening microorganisms for the ability to modify known antitumor agents, we identified a strain of *Whetzelinia sclerotiorum* which transformed several compounds related to aureolic acid[1]. As substrates we used compounds isolated in our laboratories: NSC–A649[2], an antibiotic which was subsequently characterized and named olivomycin by Russian workers[3,4] and NSC–135052, (D. E. NETTLETON, unpublished results) which we identified as a mixture of chromomycins A_2 and A_3[5]. The structures of these compounds had been elucidated by Russian[3,4] and Japanese[5] investigators.

The transformed substances were isolated from complex mixtures by methods similar to those employed for the starting materials. Their identities were established by comparison of TLC, IR, PMR and empirical formulas of the original and the transformed compounds and their acetates.

Experimental

Cultivation of the organism

Whetzelinia sclerotiorum ATCC–10939 was maintained on slants of BENNETT's medium[6] at 4°C. To prepare a seed culture, the growth of the slant was transferred aseptically to 25 ml of the following medium in a 125-ml Erlenmeyer flask and incubated for 48 hours at 27°C on a rotary shaker at 250 rpm: glucose 3%, lactose 1%, corn steep liquor 3% v/v, $(NH_4)_2SO_4$ 0.2%, KH_2PO_4 0.05%, $CaCO_3$ 0.25%. This seed culture was transferred to 100 ml of the same medium contained in 500-ml Erlenmeyer flasks for an additional 48 hours incubation under the same conditions. The growth from these flasks was used to inoculate 500-ml Erlenmeyer flasks containing 100 ml of the following medium: lactose 3.75%, Pharmamedia 2%, $CaCO_3$ 0.5%, $CaSO_4 \cdot 2H_2O$ 0.25%, Na_2SO_4 0.2%. After 48 hours of incubation at 27°C on a rotary shaker at 250 rpm, the compound to be transformed, dissolved in DMF, was added to give a final concentration of 1 mg/ml. The transformation was allowed to continue with further incubation on the shaker for 18~20 hours.

Preparation of substrates

Olivomycin A and chromomycin A_2 and A_3 were isolated and purified according to published procedures[3,4,5]. The major component from NSC–A649 gave IR and PMR spectra and elemental analysis, UV and visible spectra identical with those reported for olivomycin A. The optical rotation varied with the magnesium content of the samples and gave $[\alpha]_D^{22}$ +146° for a magnesium complex and −26° for the free acid (*c* 0.5, ethanol) compared to reported values of −18° to −21°.

Culture NSC–135052 yielded mostly chromomycin A_2, a small amount of A_3 and traces of A_1 and A_4[5]. The major components were identified by TLC, IR, UV, PMR spectra and elemental analysis. The optical rotation, as in the case of olivomycin, varied with the magnesium content. The free acid

chromomycin A_2 had $[\alpha]_D^{22}$ $-52.8°$ (*c* 0.5, ethanol) (reported: $-31°$ to $-61°$). A magnesium complex gave $[\alpha]_D^{22}$ $-52.8°$ under the same conditions.

Isolation of transformed products

The broths from the *Whetzelinia sclerotiorum* cultures were filtered and extracted twice with one-third volumes of methylene chloride. The extracts were concentrated to an oil, the residue washed with petroleum ether, and dissolved in a minimum amount of ethyl acetate. The solution was diluted with an equal volume of ether, and the crude solids precipitated by the addition of petroleum ether.

The crude material was subjected to counter-current distribution in the solvent mixtures water-saturated *n*-butanol - 5% ethyl acetate in ether (1: 1) or ethyl acetate - methanol - Skellysolve B - water (8: 5: 5: 5: 7). Purified compounds were chromatographed on silica gel (Grace-Davidson, Chemical Grade 62) activated at 110°C for 4 hours, using benzene-acetone gradient elution or a 1% solution of oxalic acid in ethyl acetate. No single method yielded pure compounds.

Fractions appearing homogeneous on TLC-bioautographs were crystallized from ethyl acetate-Skellysolve B. Thin-layer chromatograms were obtained on silica gel plates (EM Reagents, 60F 254 or Analtech Uniplates) with solvent mixtures methanol - chloroform - acetone - 17% ammonium hydroxide (3: 8: 8: 1) or chloroform - methanol - formic acid (85: 15: 1). The zones were localized by bioautographs with *Bacillus subtilis* ATCC 6633.

PMR spectra were obtained with Varian HA 100 and XL instruments. Magnesium was determined by energy X-ray analysis by Dr. D. L. JOHNSON, College of Environmental Science and Forestry, Syracuse, New York. Antimicrobial activity was assayed by two-fold tube dilution in nutrient broth of overnight cultures as indicated in Table 1.

Results and Discussion

Olivomycin A

The transformed material, m. p. 140~142°C, had $[\alpha]_D^{22}$ $-34.4°$ for the magnesium-free compound and $+40.5°$ for a magnesium complex (*c* 0.5, ethanol). The λ_{max} (log E) in ethanol were: 406, 318, 273, 225 nm (4.11, 3.69, 4.63, 4.31). The carbonyl absorption in the IR spectrum was less intense than that of olivomycin A. The PMR spectra in the region 1.1~1.4 ppm of the parent and transformed compounds indicated the loss of the isobutyryl group of olivomycin A, the presence of one acetyl at 2.15 ppm, two methoxyls at 3.3 and 3.5 ppm, and three aromatic protons at 6.8, 7.1 and 7.3 ppm.

Table 1. The antibacterial spectrum of olivomycin A, chromomycin A_2 and their derivatives

Organism	Dilution	MIC (mcg/ml)			
		Olivomycin A	Deisobutyryl olivomycin A	Chromomycin A_2	Deisobutyryl chromomycin A_2
Streptococcus pneumoniae A9585	10^{-3}	0.03	4	0.06	0.06
Streptococcus pyogenes A9604	10^{-3}	0.06	4	0.06	0.06
Staphylococcus aureus Smith A9537	10^{-4}	0.06	4	0.13	0.13
Staphylococcus aureus Smith +50% Human Serum A9537	10^{-4}	2	> 63	2	32
Staphylococcus aureus BX1633 A9606	10^{-3}	0.06	32	0.13	0.13
Staphylococcus aureus BX1633 A9606	10^{-2}	63	>125	16	>125
Staphylococcus aureus Methicillin resistant A15097	10^{-3}	0.03	16	0.13	0.13

Anal.Calcd. for $C_{54}H_{78}O_{25}$ (deisobutyrylolivomycin A): C, 57.54; H, 6.97
Found: C, 57.68; H, 6.77

Treatment of the transformed compound with acetic anhydride in pyridine in the dark for one week gave a colorless crystalline acetate, m.p. 172~174°C, $[\alpha]_D^{22}$ −162° (*c* 0.5, chloroform).

Anal. Calcd. for $C_{70}H_{94}O_{33}$: C, 57.30; H, 6.60
Found: C, 57.15; H, 6.37

The PMR spectrum showed seven methyl groups (1.1~1.45 ppm), nine acetyls (1.9~2.3 ppm), two methoxyls (3.3 and 3.5 ppm), and three aromatic protons (6.8, 7.1 and 7.3 ppm).

These data permit the formulation of the transformed product as deisobutyrylolivomycin A (b. Fig. 1).

A small amount of a second component of microbial action showed the loss of the isobutyryl and an acetyl group in the PMR spectrum.

Fig. 1. Structure of olivomycin, chromomycins and derivatives

a. Olivomycin A: R_1=H, R_2=CH_3CO, R_3=$COCH(CH_3)_2$
b. Deisobutyrylolivomycin A: R_1=H, R_2=CH_3CO, R_3=H
c. Chromomycin A_2: R_1=CH_3, R_2=CH_3CO, R_3=$COCH(CH_3)_2$
d. Chromomycin A_3: R_1=CH_3, R_2=R_3=CH_3CO
e. Deisobutyrylchromomycin A_2: R_1=CH_3, R_2=CH_3CO, R_3=H

Chromomycins A_2 and A_3

Chromomycins A_2 and A_3 gave identical mixtures of degradation products after incubation with *Whetzelinia sclerotiorum*. The major component from A_2 showed the loss of the isobutyryl group in the PMR spectrum (1.1~1.4 ppm) and the presence of one acetyl and one methyl group on an aromatic ring (2.1 ppm). An acetyl derivative prepared with acetic anhydride in pyridine showed nine acetyl groups (2.0~2.35 ppm).

The compound derived by microbial action and its acetate had the same TLC, PMR, m. p. and elemental analysis as mono-deacetylchromomycin A_3 or deisobutyrylchromomycin A_2 (e. Fig. 1), described by MIYAMOTO *et al.*[5], and prepared by alkaline hydrolysis of the antibiotics according to their procedures.

Biological Properties

The antimicrobial activities are reported in Table 1. The activity against Gram-positive bacteria of deisobutyrylolivomycin A was greatly reduced whereas deisobutyrylchromomycin A_2 was almost as active as the parent compound against most sensitive organisms. The activity of both deacyl derivatives was significantly reduced when tested in the presence of 50% pooled human serum.

Acknowledgements

We are indebted to Mr. R. GRULICH for the PMR spectra, to Miss ELIZABETH RAGAN for microanalysis, to Mr. K. DEN BLEYKER for antimicrobial assays, and to Mrs. JUDY VEITCH for preparations of the broth, and bioautographs of thin-layer chromatograms.

The work was performed under Public Health Service contract NOl-CM-43759 from the National Cancer Institute.

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