# OLIVOMYCIN AND RELATED ANTIBIOTICS

## XXIX. THE STRUCTURE OF AUREOLIC ACID

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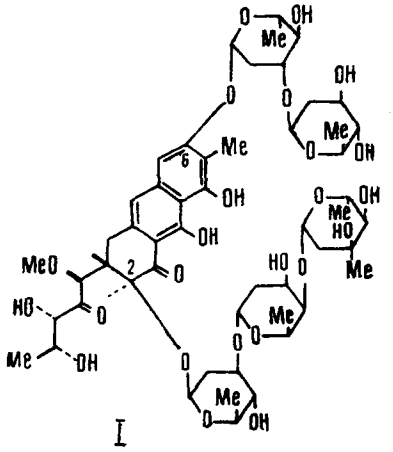
In a preceding communication [1] we have shown that aureolic acid is a glycoside of chromomycinone (X) containing D-olivose (II), D-oliose (III), and D-mycarose (IV) in a ratio of 3 : 1 : 1. As the result of further investigations, we have established that this antibiotic possesses the structure (I).



I

The action on aureolic acid of $NaIO_4$ oxidizes the single mycarose residue present in it and one of the olivose residues, while the three other monosaccharide residues are not affected. This shows that the antibiotic contains two terminal sugars (in the pyranose form) which is probable only if there is one branched or two unbranched carbohydrate chains. When the decabenzoate of aureolic acid* was subjected to acid hydrolysis, all the sugars were split off in the form of the benzoyl derivatives (V-IX); consequently, in each sugar residue of the antibiotic at least one hydroxyl is free. These results eliminate the possibility of the branching of the oligosaccharide part of the molecule; i.e., they show the presence in aureolic acid of two unbranched carbohydrate chains terminated by mycarose and olivose.

The positions of attachment of these chains to the aglycone were determined in the following way. Aureolic acid strongly and specifically lowers the pH of solutions of boric acid ($\Delta$pH 2.5), from which it follows (see [12]) that in the antibiotic the peri-dihydroxynaphthalene grouping is free. Furthermore, in

*Under the action of benzoyl chloride in pyridine, aureolic acid gives two benzoyl derivatives, one of which is the product of exhaustive benzoylation (undecabenzoate) while the second is a decabenzoate in which the tertiary hydroxyl of the mycarose residue is free ($\nu$ OH 3560 $cm^{-1}$). The hydrolysis of both benzoates (more accurately, methanolysis with subsequent hydrolysis) leads to mixtures of benzoylated monosaccharides containing the same derivatives of olivose and oliose and differing only by the presence of the mono- or the dibenzoate of mycarose.

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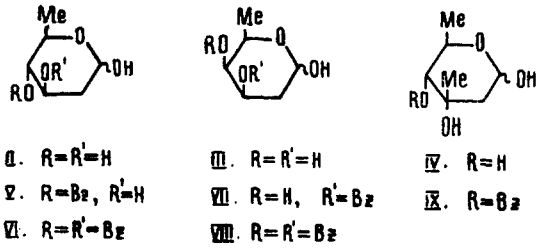
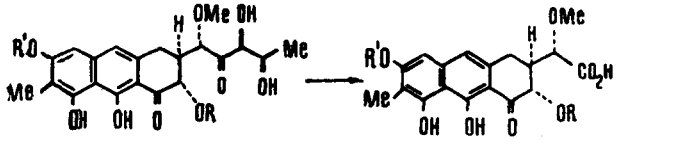
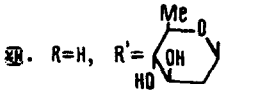
the periodate oxidation of aureolic acid mentioned above, in addition to the terminal sugar residues, the side chain of the aglycone is split off, one mole each of acetaldehyde and formic acid being liberated. The acid hydrolysis of the product of the periodate oxidation of the antibiotic (XV) forms chromomycinonic acid (XIV), which, furthermore, has also been obtained directly by the oxidation of chromomycinone (X). It follows from this that the hydroxyls of the side chain of the aglycone are also free, and therefore the two carbohydrate chains in aureolic acid are attached to the aglycones through 2-OH and 6-OH hydroxyls (XI).



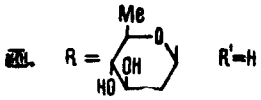
I. R=R'=H
II. R=Bz, R'=H
III. R=R'=Bz
IV. R=R'=H
V. R=H, R'=Bz
VI. R=R'=Bz
VII. R=H
VIII. R=Bz



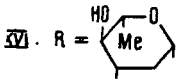
IX. R=R'=H
X. R and R' - carbohydrate chains
XIV. R=R'=H
XV. R and R' - oxidized carbohydrate chains



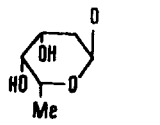
XII. R=H, R'=

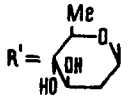


XIII. R=



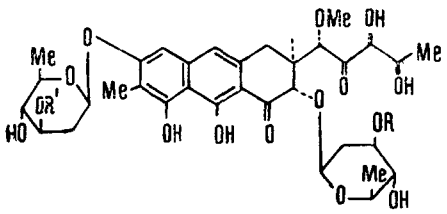
XVI. R=



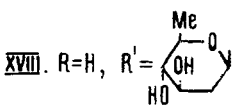


R'=

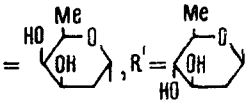
The structures of these chains were determined by the partial hydrolysis of the antibiotic. Thus, the action on aureolic acid of 0.1 N HCl yielded two isomeric chromomycinone monoolivosides in which the olivose residues were capable of being oxidized by periodate and, therefore, were present in the pyranose form. The UV spectrum of one of these olivosides, like the spectrum of unsubstituted chromomycinone (X), underwent a bathochromic shift on alkalization. This means that the 6-OH in it is free (see [3]) and, consequently, the structure of the glycoside will be (XIII) (for the configurations of the glycosidic bonds, see below, Table 1). The UV spectrum of the second olivoside did not change on alkalization, since in it the 6-OH phenolic hydroxyl is blocked by a sugar residue (XII). The formation of chromomycinone 2- and 6-olivosides shows that both carbohydrate chains of aureolic acid begin with olivose residues and, therefore, the antibiotic is based on the structure (XVII).



XVII. R=R'=H



XVIII. R=H, R'=



XIX. R=



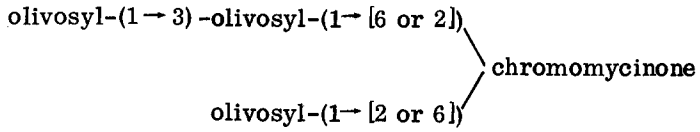
R'=

TABLE 1. Calculation of the Configurations of the Glycosidic Bonds of Aureolic Acid

| Glycoside | $[M]_D$ | Contribution of the sugar | $[M]_D$ of the anomeric methyl glycosides | Configuration of the glycosidic center |
|---|---|---|---|---|
| | deg | | | |
| Chromomycinone | +415 | | | |
| 6-Olivosylchromomycinone (XII) | −56 | −471 | α+212<br>β−138 | β |
| 2-Olivosylchromomycinone (XIII) | −168 | −583 | α+212<br>β−138 | β |
| Olivosylolivosylolivosylchromomycinone (XVIII) | −562 | 2×(−253) | α+212<br>β−138 | β β |
| Oliosylolivosylolivosylolivosylchromomycinone (XIX) | −385 | +177 | α+230*<br>β — | α |
| Mycarosyloliosylolivosylolivosylolivosylchromomycinone (aureolic acid) (I) | −552 | −167 | α+225<br>β−55 | β |

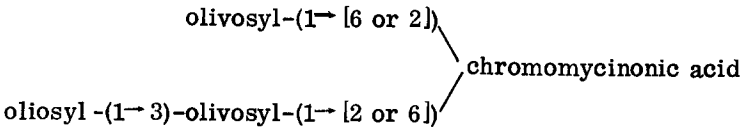
*According to [7] for the corresponding 3-O-acetate.

The hydrolysis of aureolic acid under milder conditions (0.01 N HCl) yielded a glycoside containing all three olivose residues of the initial antibiotic. The further hydrolysis of this trioside led to the 2-olivosylchromomycinone described above (XII) and its 6 isomer (XIII), and the hydrolysis of its benzoate formed, in addition to the dibenzoylolivose (VI), 4-benzoylolivose (V), which is stable to periodate. It follows from this that in the chromomycin triolivoside one of the hydroxyls of the aglycone (2-OH or 6-OH) is glycosylated with an olivose residue and the other with an olivosyl-(1→3)-olivosyl chain (XX):



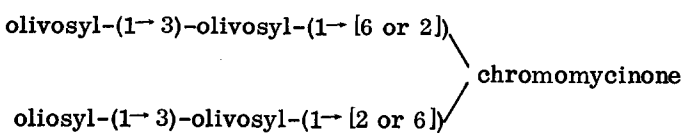

XX

The Smith degradation of aureolic acid (oxidation with $NaIO_4$ and then reduction with $NaBH_4$ and mild acid hydrolysis) shortened each of the carbohydrate chains of the antibiotic by one link (i.e., by mycarose and olivose), so that the substance obtained consisted of a trioside of chromomycinonic acid (XIV) and contained olivose and oliose in a ratio of 2 : 1. It was found that in this substance the oliose residue was oxidized by periodate, and on exhaustive benzoylation and subsequent hydrolysis the dibenzoates of olivose and oliose (VI) and (VIII) and also the 4-benzoate of olivose (V) were obtained. This shows that in the product of the Smith degradation of the antibiotic, the oliose residue and one of the olivose residues are terminal (both are present in the pyranose form); i.e., oliose glycosylates the 3-OH of the olivose residue directly attached to the aglycone (XXI):




XXI

From a comparison of the structures of the oliosyldiolivoside (XXI) and the triolivoside (XX) the arrangement of four of the five monosaccharide residues in the carbohydrate chains of the antibiotic is as follows:

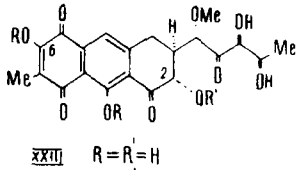



XXII

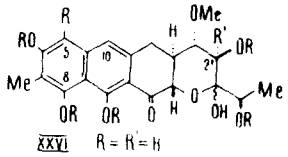
In actual fact, the hydrolysis of aureolic acid under conditions milder than those required to obtain the triolivoside (XX) yielded yet another product of the partial degradation of the antibiotic. This substance, like aureolic acid, contained olivose and oliose in a ratio of 3 : 1, but did not contain mycarose and therefore consisted of the tetroside represented by the partial formula (XXII). The formation of this tetroside, in which the oliose residue is capable of being oxidized by $NaIO_4$, shows that in the initial antibiotic the oliose is stable to periodate because it is blocked by the mycarose. Since, however, when the benzoate of aureolic acid was hydrolyzed the oliose was obtained in the form of the 3-benzoyl derivative (VII), it must be linked to the mycarose through the 4-OH hydroxyl. On the basis of what has been said, the arrangement of the monosaccharide residues in the carbohydrate chains of aureolic acid is as follows:

olivosyl-(1→3)-olivosyl-(1→[6 or 2])
chromomycinone
mycarosyl-(1→4)-oliosyl-(1→3)-olivosyl-(1→[2 or 6])

To determine which of these carbohydrate chains is attached to the chromomycinone through its 2-OH and which through its 6-OH, we performed a modification of the aromatic nucleus of the antibiotic that labilized the bond between the aglycone and one of the carbohydrate chains, thereby creating the possibility for its selective elimination. It was found that chromomycinone (X) is readily oxidized by potassium nitrosodisulfonate (Fremy's salt) [4] to a quinone, the structure of which was determined in the following way: on isomerization and deuteration under the action of NaOD in $D_2O$ solution, chromomycinone (X) forms 2',5-dideuteroisochromomycinone (XXVII), the positions of the deutrons in which are shown by the fact that the NMR spectrum of its tetraacetate (XXIX) lacks the signals of the $H_{2'}$ and $H_5$ protons (see the analogous conversion of olivin into 2',5-dideuteroolivin [5]). When the deuterated isochromomycinone (XXVII) was oxidized with potassium nitrosodisulfonate, the only aromatic proton of the initial compound ($H_{10}$) was retained, undergoing a paramagnetic shift as a consequence of descreening by the peri carbonyl. This shows that the terminal, resorcinol, ring was oxidized, i.e. the products of the oxidation of chromomycinone (X) and of isochromomycinone (XXVI) are, respectively, chromomycinone 5,8-quinone (XXIII) and isochromomycinone 5,8-quinone (XXX).

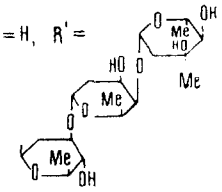


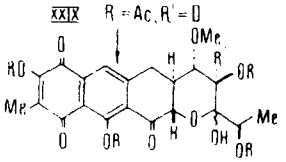
XXIII R = R' = H
XXIV R = R' = Ac



XXVI R = R' = H
XXVII R = H, R' = D
XXVIII R = Ac, R' = H
XXIX R = Ac, R' = D

XXV R = H, R' =





XXX R = R' = H
XXXI R = H, R' = D
XXXII R = Ac, R' = H
XXXIII R = Ac, R' = D

The oxidation of aureolic acid with potassium nitrosodisulfonate gave a quinone glycoside which, on acid hydrolysis, yielded the chromomycinone quinone (XXIII), olivose (II), oliose (III), and mycarose (IV) in equimolar amounts. Consequently, the oxidation of the aureolic acid to the corresponding quinone labilized the $C_6-O$ bond (one of the aryl glycoside bonds became the vinylog of an acyl glycoside bond) and caused the elimination of the olivosylolivosyl chain, forming the trioside (XXV). It follows from this that in aureolic acid the olivosylolivosyl chain is attached to the aglycone through 6-OH and the mycarosylolivosylolivosyl chain through 2-OH.

To confirm this conclusion, we investigated the analogous transformation of chromomycin $A_2$, the structure of which has been shown previously by an independent method (XI; R = olivomosylacetyloliosyl, R' = isobutyrylolivomycosylolivosylolivosyl) [6]. It was found that the oxidation of this antibiotic with

potassium nitrosodisulfonate formed a glycoside not containing olivomose and acetyloliose; i.e., the same selective elimination of the carbohydrate chain attached to the aglycone through the 6-OH took place as was observed in the case of aureolic acid.

Finally, the results of a comparison of the molecular rotations of aureolic acid and the products of its partial and complete hydrolysis showed, according to Klyne's rule, that of the five carbohydrate residues present in the antibiotic only the oliose is present in the $\alpha$ form, and all the other glycosidic bonds have the $\beta$ configuration (see Table 1). Thus, aureolic acid is 2-[$\beta$-mycarosyl-(1→4)-$\alpha$-oliosyl-(1→3)-$\beta$-olivosyl]-6-[$\beta$-olivosyl-(1→3)-$\beta$-olivosyl]chromomycinone (I), and the products of its partial hydrolysis possess the structures (XII, XIII, XVIII, and XIX).

## EXPERIMENTAL

Chromatography was performed in a thin nonfixed layer of silica gel of "aqueous silicic acid" type (smaller than 150 mesh, activity grade III-IV). The molecular weights were determined mass-spectrometrically, except where shown otherwise. The IR spectra were taken in mulls with paraffin oil, the UV spectra in 96% ethanol (i – inflection), and the NMR spectra in $CDCl_3$ at 100 MHz (s – singlet; d – doublet; t – triplet; m – multiplet). Chromatography was performed on Whatman No. 2 paper in the n-BuOH–EtOH–$H_2O$ (4 : 1 : 5) system.

1. Benzoylation of Aureolic Acid. A solution of 300 mg of the antibiotic and 1.2 ml of BzCl in 10 ml of pyridine was kept at 20°C for 72 h and was then heated at 80°C for 6 h; the pyridine was distilled off in vacuum, the residue was dissolved in chloroform, the solution was washed with water and evaporated, and the residue was chromatographed in the benzene–acetone (10 : 1) system. This gave 60 mg of undecabenzoate with $R_f$ 0.80 and 220 mg of the decabenzoate with $R_f$ 0.70; benzoylation at room temperature led to the formation of only the decabenzoate.

Undecabenzoate: mp 175°C (from ethanol), $[\alpha]_D^{18}$ –56° (c 0.2; chloroform); $\lambda_{max}$ 230, 262 i, 267, 325 nm (log ε 5.17, 4.87, 4.93, 4.14).

Decabenzoate: mp 173-176°C (from ethanol), $[\alpha]_D^{18}$ –54° (c 0.2; chloroform); $\lambda_{max}$ 231, 268, 327 nm (log ε 5.13, 4.87, 4.03).

2. Methanolysis of Aureolic Acid Decabenzoate. A solution of 200 mg of the decabenzoate in 10 ml of benzene and 10 ml of 1 N methanolic HCl was heated at 75°C for 3 h. After cooling, the mixture was neutralized with $Ag_2CO_3$, filtered, and evaporated. The residue was dissolved in ethyl acetate, the solution was washed with water and evaporated, and the purified residue was chromatographed in the benzene–acetone (10 : 1) system, giving zones with $R_f$ 0.70-0.77 (A), 0.59-0.65 (B), 0.47-0.54 (C), 0.42-0.47 (D), 0.35-0.42 (E), and 0.28-0.36 (F). The substances from zones A and B were rechromatographed in the same system on silica gel, and the substances from zones C-F on alumina (activity grade II) in the benzene–acetone (10 : 1 and 5 : 1) systems. The substances isolated were dissolved in 0.5 ml of 0.4 N ethanolic KOH, and after 4 h the solutions were neutralized with $CO_2$ and evaporated, and the saponification products were extracted with ethyl acetate. They were then hydrolyzed with 50% acetic acid (2 h at 80°C), and the sugars obtained were identified by paper chromatography. To determine the positions of the benzene residues in the monobenzoates of the methyl glycosides, the substances were hydrolyzed with 50% acetic acid (5-8 h at 85°C). The resulting sugar benzoates were treated with a 0.035 M solution of $NaIO_4$ (24-28 h at 20°C) and then with 0.4 N KOH, and the products were chromatographed on paper. As controls were used the corresponding methyl glycoside monobenzoates treated similarly but without the periodate oxidation. It was found that substance A (37 mg) consisted of the methyl glycoside of debenzoylolivose (VI) (no $\nu_{OH}$ in the IR spectrum), substance B (10.5 mg) the methyl glycoside of 4-benzoylmycarose (IX) [mp 47-48°C; mol. wt. 280 (for $C_{15}H_{20}O_5$, calculated 280)], substance D (14 mg) the methyl glycoside of 3-benzoyloliose (VII), and zones C, E, and F (33, 45, and 18 mg) contained the methyl glycosides of 4-benzoylolivose (V) [mp 60-61°C, $[\alpha]_D^{23}$ + 107° (c 1.5; chloroform), mol. wt. 266 (for $C_{14}H_{18}O_5$ calculated 266)] and 3-benzoyloliose (VII).

3. Periodate Oxidation of Aureolic Acid. This process was performed as described earlier for olivomycin A [8]. The distillate was found to contain 1.1 mole of formic acid (by the calomel method) and 1.0 mole of acetaldehyde (determined in the form of the dinitrophenylhydrazone). The nonvolatile oxidation product (X) was extracted from the acidified reaction mixture with ethyl acetate and was heated with 50% acetic acid (3 h at 75-80°C), after which the mixture was diluted with saturated NaCl solution and extracted with ethyl acetate. The aqueous solution was found by paper chromatography [9] to contain olivose (II) and

oliose (III) in a ratio of 1.9 : 1, but no mycarose (IV). Chromatography of the ethyl acetate extract in the benzene–acetone (3 : 1) system yielded chromomycinonic acid (XIV). Yield 48%, $R_f$ 0.34; the methyl ester was obtained by the action of $CH_2N_2$, mp 127-130°C (from ethanol).

Found: mol. wt. 404. $C_{20}H_{20}O_9$. Calculated: mol. wt. 404.

4. Acidity of the Borate Complex of Aureolic Acid. An 0.18 M solution of $H_3BO_3$ in 30% aqueous ethanol has pH 5.55; when aureolic acid was dissolved in it to a concentration of 0.009 M, the pH fell to 3.05.

5. Olivosyl-(1→2)-chromomycinone (XIII) and Olivosyl-(1→6)-chromomycinone (XII). A mixture of 150 mg of aureolic acid and 15 ml of 0.1 N $H_2SO_4$ in 50% methanol was heated for 1 h at 60°C, and after cooling, the mixture was concentrated to half its volume, diluted with water, and extracted with ethyl acetate. The extracted substance was chromatographed in the benzene–acetone (1 : 1) system. This gave 25 mg of chromomycinone (X), 17 mg of 2-olivosylchromomycinone (XIII), $[\alpha]_D^{20}$ −30° (c 0.4; ethanol), $R_f$ 0.52, $\lambda_{max}$ 232, 283, 324, 341, 420 nm (log ε 4.13, 4.30, 3.54, 3.57, 3.70), $\lambda_{max}^{0.05\ N\ KOH}$ 290, 420 nm (log ε 4.17, 3.75), and 13 mg of 6-olivosylchromomycinone (XII), $[\alpha]_D^{20}$ −10° (c 0.4; ethanol), $R_f$ 0.44, $\lambda_{max}$ 230, 279, 319, 331 i, 419 nm (log ε 4.27, 4.50, 3.70, 3.59, 3.78); $\lambda_{max}^{0.05\ N\ KOH}$ 278, 419 nm (log ε 4.28, 3.50).

Each of the monoolivosides (XII) and (XIII) was oxidized with 0.01 M $NaIO_4$ buffered with $NaHCO_3$ (pH 6.9; 2.5 h at 20°C). The oxidation products were extracted with ethyl acetate, hydrolyzed with 50% acetic acid (3 h at 70°C), and analyzed by paper chromatography. In neither case were monosaccharides detected.

6. The Olivosylolivosylolivosylchromomycinone (XVIII). A solution of 100 mg of aureolic acid in 5 ml of 0.01 N HCl was heated at 65°C for 1 h, and after cooling it was extracted with ethyl acetate and chromatographed in the benzene–acetone (2 : 3) system. The substance from the zone with $R_f$ 0.35-0.45 was separated from contamination by the initial antibiotic by crystallization from acetone. This gave 16 mg of the glycoside (XVIII) with mp 162-165°C, $[\alpha]_D^{20}$ −69.5° (c 0.3; ethanol); $\lambda_{max}$ 230, 279, 317, 334, 415 nm (log ε 4.38, 4.66, 3.80, 3.68, 4.10); $\lambda_{max}^{0.05\ N\ KOH\ in\ EtOH}$ 230, 279, 317 i, 334, 415 nm (log ε 4.30, 4.65, 3.58, 3.50; 4.00). After oxidation with 0.1 M $NaIO_4$ (3 h at 20°C) and heating with 50% acetic acid (3 h at 70°C), the amount of olivose (II) in the hydrolyzate was determined.

7. Benzoylation of the Olivosylolivosylolivosylchromomycinone (XVIII) and Methanolysis of its Benzoate. The triolivoside (XVIII) (37 mg) was benzoylated with 0.3 ml of BzCl in 1.2 ml of pyridine (24 h at 20°C). After the usual working up and chromatography in the benzene–acetone (20 : 1) system, the zone with $R_f$ 0.68-0.82 yielded 63 mg (80%) of benzoate with $[\alpha]_D^{20}$ + 2° (c 0.2; chloroform); $\lambda_{max}$ 230, 272, 280 i nm (log ε 5.16, 3.91, 3.77); $\nu_{OH}$ absent.

This benzoate was subjected to methanolysis and further treatment under the conditions of experiment 2. From 60 mg of the benzoate were obtained 17 mg of methyl glycosides of dibenzoylolivose (VI) and 13 mg of methyl glycosides of monobenzoylolivose (V). Acetic acid hydrolysis of the latter followed by periodate oxidation (0.03 M $NaIO_4$, 120 h at 20°C) with subsequent saponification under the conditions of experiment 2 led to the isolation of unchanged olivose (II).

8. The Oliosylolivosylolivosylchromomycinonic Acid (XVI) (Smith Degradation of Aureolic Acid). A solution of 200 mg of aureolic acid in 75 ml of 0.025 M $NaIO_4$ (pH 5) was kept at 20°C for 3 h and was then saturated with NaCl. The oxidation product was extracted with ethyl acetate and chromatographed in the benzene–acetone (1 : 1) system. The substance from the zone with $R_f$ 0.50-0.84 (70 mg) was dissolved in 1 ml of 0.18 N KOH, and then 9 ml of a 0.0095 M aqueous solution of $NaBH_4$ was added and the mixture was left for 20 min. After this, 50 ml of 0.1 N HCl was added, and after 4 h the mixture was extracted with ethyl acetate. On chromatography in the benzene–acetone (2 : 3) system, the zone with $R_f$ 0.26-0.51 yielded 23 mg of oliosylolivosylolivosylchromomycinonic acid (XVI) with $[\alpha]_D^{27}$ −54° (c 0.3; ethanol); $\lambda_{max}$ 280, 410 nm (log ε 4.57, 3.85). In the acetic acid hydrolyzate of this substance, chromomycinonic acid (XIV) was identified by comparison with an authentic sample, and the ratio of the amounts of olivose (II) and oliose (III) was found by paper chromatography to be 2.2 : 1. The periodate oxidation of the trioside (XVI) and subsequent acetic acid hydrolysis yielded only olivose (II).

9. Benzoylation of Oliosylolivosylolivosylchromomycinonic Acid (XVI) and Methanolysis of Its Benzoate. A solution of 25 mg of the trioside (XVI) and 0.1 ml of BzCl in 1.5 ml of pyridine was kept at 20°C for 12 h and was then heated at 85°C for 6 h. After the usual working up and chromatography in the benzene–acetone (20 : 1) system, the zones with $R_f$ 0.81-0.90 and 0.54-0.59 yielded, respectively, 12.5 and 8.5 mg of benzoates differing in the degree of benzoylation of the aglycone. The acid methanolysis of both

benzoates under the conditions of experiment 2 gave the same mixture of cleavage products, by analysis, as in experiment 2. The zones with $R_f$ 0.81-0.90 and 0.71-0.78 contained 14.8 mg of methyl glycosides of dibenzoylolivose (VI) and of dibenzoyloliose (VIII), and the zones with $R_f$ 0.43-0.48 and 0.38-0.43 contained 3.6 g of methyl glycosides of 4-benzoylolivose (V). The acid hydrolysis of the monobenzoylolivosides with subsequent periodate oxidation and alkaline saponification gave unchanged olivose (II).

10. Oliosylolivosylolivosylolivosylchromomycinone (XIX). A solution of 35 mg of aureolic acid in 1.5 ml of 0.01 N HCl was heated at 65°C for 30 min, and the product was extracted with ethyl acetate and chromatographed in the benzene–acetone (2 : 3) system. The zone with $R_f$ 0.35-0.45 yielded 13 mg of a mixture of the initial antibiotic and the triolivoside (XVIII) (see experiment 6), and the zone with $R_f$ 0.15-0.25 yielded 5 mg of the tetroside (XIX) (the same substance was obtained in lower yield on hydrolysis under the conditions of experiment 6) with $[\alpha]_D^{27}$ −41° (c 0.3; ethanol). By paper chromatography, an acetic acid hydrolyzate of this tetroside was found to contain olivose (II) and oliose (III) in a ratio of 3.2 : 1 (no mycarose was detected); on periodate oxidation and subsequent acetic acid hydrolysis only olivose (II) was found.

11. Chromomycinone 5,8-Quinone (XXIII) and Its Pentaacetate (XXIV). To a solution of 240 mg of potassium nitrosodisulfonate [10] in 10 ml of water and 2 ml of ethanol was added 100 mg of chromomycinone (X), and the mixture was kept at 20°C for 1.5 h and was extracted with ethyl acetate. The extract was washed with saturated NaCl solution until the wash waters were colorless, and then the wash waters were acidified to pH 3 and were again extracted with ethyl acetate. The substance isolated was recrystallized from isopropanol. The yield of chromomycinone quinone (XXIII) was 45 mg (44%), mp 190-192°C (decomp.); $\lambda_{max}$ 226, 238 i, 290, 395, 550 nm (log ε 4.39, 4.27, 4.47, 3.43, 3.22); $\nu_{max}$ 1600, 1660, 1693, 1721, 3440, 3560 $cm^{-1}$.

Found %: C 58.0; H 5.4. $C_{21}H_{22}O_{10}$. Calculated %: C 58.1; H 5.1.

The pentaacetate (XXIV) was obtained from the quinone (XXIII) by the action on it of $Ac_2O$ and a catalytic amount of $H_2SO_4$ (48 h at 20°C) and was purified by chromatography in the benzene–acetone (7 : 1) system (zone with $R_f$ 0.53-0.61). mp 194-196°C (from ethanol), $[\alpha]_D^{20}$ + 26° (c 1; chloroform); $\lambda_{max}$ 215, 263, 345 nm (log ε 4.51, 4.43, 3.43); $\nu_{max}$ 1600, 1645, 1675, 1720, 1750, 1770 $cm^{-1}$, δ 1.30 (3H, d, J 6.5; $3H_{5'}$), 2.00 (6H, s; $O_{4'}$-Ac and $C_7$-Me), 2.17 (3H, s; $O_{3'}$-Ac), 2.25 (3H, s; $O_2$-Ac), 2.34 (3H, s; $O_6$-Ac), 2.43 (3H, s; $O_9$-Ac), 3.33 (3H, s; $O_{1'}$-Me), 4.25 (1H, s; $H_{1'}$), 5.21 (1H, d, J 3; $H_{3'}$), 5.40 (2H, m; $H_2$ and $H_{4'}$), 7.84 (1H, s; $H_{10}$).

Found: mol. wt. 644. $C_{31}H_{32}O_{15}$. Calculated: mol. wt. 644.

12. Isochromomycinone (XXVI), 2',5-Dideuteroisochromomycinone (XXVII), and Their Pentaacetates (XXVIII) and (XXIX). A. A solution of 200 mg of chromomycinone (X) in 20 ml of 0.1 N NaOH was kept in an atmosphere of argon for 1 h and was then acidified with dilute HCl to pH 3 and extracted with ethyl acetate, and the extracted substance was chromatographed in the benzene–acetone (1 : 1) system. The zone with $R_f$ 0.54-0.62 yielded 105 mg (52%) of isochromomycinone (XXVI), mp 225°C (decomp.; from ethyl acetate), $\lambda_{max}$ 233, 280, 328, 341, 418 nm (log ε 4.37, 4.62, 3.84, 3.83, 4.05). (see [11]).

Found: mol. wt. 420. $C_{21}H_{24}O_9$. Calculated: mol. wt. 420.

Pentaacetylisochromomycinone (XXVIII) was obtained from (XXVI) by the action on it of $Ac_2O$ + Py (48 h at 20°C), followed by chromatography in the benzene–acetone (3 : 1) system (zone with $R_f$ 0.48-0.56) and reprecipitation from ethyl acetate with hexane, $[\alpha]_D^{20}$ + 36° (c 0.1; chloroform); $\lambda_{max}$ 219, 253 i, 259, 288 i, 300, 312, 365 nm (log ε 4.32, 4.71, 4.75, 3.69, 3.84, 3.61, 3.45); $\lambda_{max}$ 1610, 1630, 1710, 1750, 1780, 3500 $cm^{-1}$; δ 1.34 (3H, d, J 6.5; $3H_{5'}$), 2.01 (3H, s), 2.04 (3H, s), 2.07 (3H, s) ($O_{4'}$-Ac, $O_{2'}$-Ac and $C_7$-Me), 2.30 (3H, s, $O_6$-Ac), 2.36 (3H, s; $O_8$ - Ac), 2.44 (3H, s; $O_9$ - Ac), 3.46 (3H, s; $O_{1'}$-Me), 3.62 (1H, t, J 9.5; $H_{1'}$), 4.48 (1H, d, J 12; $H_2$), 5.00(1H, q, J 6.5; $H_{4'}$), 5.16 (1H, d, J 9.5; $H_{2'}$), 7.35 (1H, s; $H_5$, 7.46 (1H, s; $H_{10}$) (see [11]).

Found: mol. wt. 630. $C_{31}H_{34}O_{14}$. Calculated: mol. wt. 630.

B. 2',5-Dideuteroisochromomycinone (XXVII) was isolated by the action on 400 mg of chromomycinone (X) of a solution of 96 mg of Na in 4.5 ml of $D_2O$ under the conditions of experiment A. Yield 210 mg (52%); mp 223-227°C (decomp.; from acetone; $\lambda_{max}$ 233, 280, 328, 341, 418 nm (log ε 4.41, 4.61, 3.82, 3.81, 4.08).

The pentaacetate (XXIX) was obtained as in experiment 12A; $[\alpha]_D^{20}$ + 44° (c 0.2; chloroform); δ 1.33 (3H, d, J 6.5; $3H_{5'}$), 2.01 (3H, s), 2.03 (3H, s), 2.07 (3H, s)($O_{4'}$-Ac, $O_{2'}$ - Ac and $C_7$-Me), 2.28 (3H, s; $O_6$-Ac),

2.36 (3H, s; $O_8$-Ac), 2.44 (3H, s; $O_9$-Ac), 3.44 (3H, s; $O_{1'}$-Me), 3.60 (1H, d, J 9.5; $H_{1'}$), 4.43 (1H, d, J 12; $H_2$), 4.97 (1H, q, J 6.5; $H_4$), 7.44 (1H, s; $H_{10}$).

Found: mol. wt. 632. $C_{31}H_{32}D_2O_{14}$. Calculated: mol. wt. 632.

13. Isochromomycinone 5,8-Quinone (XXX), 2'-Deuteroisochromomycinone 5,8-Quinone (XXXI), and Their Tetraacetates (XXXII) and (XXXIII). A. To a solution of 100 mg of isochromomycinone (XXVI) in 4 ml of ethanol and 20 ml of water was added 270 mg of potassium nitrosodisulfonate; the mixture was stirred for 3 h and filtered, and ethyl acetate extracted from the residue 68 mg (65%) of the isochromomycinone quinone (XXX). After crystallization from ethyl acetate the mp of the substance was 145-152°C (decomp.); $\lambda_{max}$ 225, 238 i, 289, 395, 555 nm (log ε 4.27, 4.16, 4.34, 3.66, 3.39); $\nu_{max}$ 1615, 1655, 1675, 1720, 3500 $cm^{-1}$.

The tetraacetate (XXXII) was obtained by the action on (XXX) of $Ac_2O$ + Py (1 h at 20°C) with chromatography in the benzene–acetone (7 : 1) system, isolation of the material from the zone with $R_f$ 0.20-0.31, and reprecipitation from ethyl acetate with hexane; $[\alpha]_D^{20}$ + 85° (c 0.4; chloroform); $\lambda_{max}$ 234, 257 i, 284 i, 298 i, 341 nm (log ε 4.28, 4.09, 3.76, 3.71, 3.78); $\nu_{max}$ 1600, 1680, 1740, 1780, 3500 $cm^{-1}$; δ 1.38 (3H, d, J 6.5; $3H_{5'}$), 2.04 (3H, s), 2.06 (3H, s), 2.14 (3H, s) ($O_{4'}$-Ac, $O_{2'}$-Ac and $C_7$-Me), 2.37 (3H, s; $O_6$-Ac). 2.47 (3H, s; $O_9$-Ac), 3.50 (3H, s, $O_{1'}$-Me), 3.68 (1H, t, J 9.5; $H_{1'}$), 4.60 (1H, d, J 12; $H_2$) 5.02 (1H, q, J 6.5; $H_{4'}$), 5.17 (1H, d, J 9.5; $H_{2'}$), 7.92 (1H, s; $H_{10}$).

Found, %: C 57.7; H 5.4; $m/e_{max}$ 604 (M + 2) (see [12]). $C_{29}H_{30}O_{14}$. Calculated, %: C 57.9; H 5.0; mol. wt. 602.

B. 2',5-Dideuteroisochromomycinone (XXVII) (100 mg) was oxidized with potassium nitrosodisulfonate under the conditions of experiment A. This gave 68 mg (65%) of the 2'-deuteroisochromomycinone quinone (XXXI); mp 143-150°C (decomp., from ethyl acetate); $\lambda_{max}$ 226, 238i, 289, 395, 555 nm (log ε 4.32, 4.23, 4.34, 3.70, 3.35).

The tetraacetate (XXXIII): δ 1.38 (3H, d, J 6.5; $3H_5$), 2.04 (3H, s), 2.06 (3H, s), 2.12 (3H, s) ($O_{4'}$-Ac, $O_{2'}$-Ac and $C_7$-Me), 2.37 (3H, s; $O_6$-Ac), 2.47 (3H, s; $O_9$-Ac), 3.50 (3H, s; $O_{1'}$-Me), 3.68 (1H, d, J 9.5; $H_{1'}$), 4.60 (1H, d, J 12; $H_2$), 5.05 (1H, q, J 6.5; $H_{4'}$), 7.92 (1H, s; $H_{10}$).

Found: $m/e_{max}$ 605 (M + 2). $C_{29}H_{29}DO_{14}$. Calculated: mol. wt. 603.

14. The Mycarosyloliosylolivosylchromomycinone Quinone (XXV). To a solution of 500 mg of potassium nitrosodisulfonate in 30 ml of water and 5 ml of ethanol was added 200 mg of aureolic acid (I), and the mixture was kept at 20°C for 7 h, acidified with dilute HCl to pH 3, and extracted with ethyl acetate. The extracted substance (155 mg) was chromatographed in the benzene–acetone (2 : 3) system. The zone with $R_f$ 0.55-0.64 yielded 10 mg of the quinone trioside (XXV); $\lambda_{max}$ 227, 290, 400, 550 nm (log ε 4.41, 4.50, 3.65, 3.00); $\nu_{max}$ 1610, 1670, 1715, 3450 $cm^{-1}$.

The oxidation of 200 mg of aureolic acid with nitrosodisulfonate in 50% acetic acid gave 22 mg; in 0.03 N KOH 30 mg; and in 0.15 M $KH_2PO_4$, 23 mg of the trioside (XXV).

The acid hydrolysis of the quinone trioside (XXV) (0.05 N HCl, 2 h at 70°c) led to the formation of the chromomycinone quinone (XXIII), identical with respect to its $R_f$ values and UV and IR spectra with the product of the direct oxidation of chromomycinone (X) by Fremy's salt (experiment 11), and also mycarose (IV), olivose (II), and oliose (III) in a ratio of 1 : 1 : 0.9, 1 : 0.9 : 1 (for the determination of the sugars, see [1, 8]).

15. Oxidation of Chromomycin $A_2$. To a solution of 100 mg of chromomycin $A_2$ [6] in 7 ml of 50% AcOH was added 140 mg of potassium nitrosodisulfonate, and the mixture was stirred at 20°C for 1 h. Then it was diluted with a saturated solution of NaCl and extracted with ethyl acetate. The extracted substance was chromatographed in the benzene–acetone (3 : 2) system. The zone with $R_f$ 0.57-0.64 yielded 8 mg (11%) of isobutyrylolivomycosylolivosylolivosylchromomycinone quinone. The substance was hydrolyzed with 50% AcOH (4 h at 75°C). Isobutyrylolivomycose [13] and olivose (II) were found in the acetic acid hydrolyzed in a ratio of 1 : 2.1.

## SUMMARY

The results of the acid degradation of aureolic acid and of the products of its benzoylation and oxidation have shown that this antibiotic has the structure (I).

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