OLIVOMYCIN AND RELATED ANTIBIOTICS

# XVII. Partial Structure of the Carbohydrate Moiety of Olivomycin A*

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As a result of the investigations described in papers [3–7], we have established that olivomycin A is a glycoside of olivin (I) and contains five residues of 2, 6-dideoxy sugars—one residue each of isobutyrylolivomycose (III), of olivomose (VII), and of acetyloliose (XVI), and two residues of olivose (XI). The next stage of the establishment of the structure of the antibiotic was to answer the question of just how these monosaccharide residues are attached to one another and to the aglycone.

For this purpose we have investigated the acid degradation of two acyl derivatives of olivomycin A, namely its octabenzoate, in which all the hydroxyl groups are blocked, and the heptaacetate, in which the tertiary hydroxyl of the olivomycose residue remains free. The acetic acid hydrolysis of the heptaacetate of the antibiotic yielded olivin acetate and also isobutyrylolivomycose (III), acetylolivomose (VIII) [identified in the form of the methyl glycosides (IX)], acetyloliose (XVI), and acetylolivose (XII). The structure of the latter follows from its stability to periodate and its conversion into olivose (XI) on alkaline hydrolysis. In view of its lower solubility and greater resistance to the action of acetic acid, the octabenzoate of olivomycin A was subjected to alcoholysis by heating it with a methanol-benzene solution of HCl. In addition to olivin benzoate, we isolated benzoylisobutyrylolivomycosides (VI) [the structure of which was shown by hydrolysis to the free sugar IV, obtained independently by partial synthesis], benzoylolivomosides (X) (one of which was shown to be identical with the product of benzoylation of the $\alpha$-anomer of IX), benzoylolivosides (XIV) [the subsequent acid hydrolysis of which yielded a benzoylolivose (XIII), stable to $NaIO_4$ and saponified by $Ba(OH)_2$ to olivose (XI)], and acetyloliosides (XVIII) and the products of their deacetylation (XVII)**.

Since isobutyrylolivomycose (III) and olivomose (VII), each containing one free hydroxyl, were isolated in the form of the O-acyl derivatives IV, VIII, and X, it follows that these sugars consist of the terminal members either of one branched or of two unbranched carbohydrate chains. The only sugar containing two free hydroxyls (and therefore capable of being at the position of branching of the carbohydrate chain in olivomycin A) is olivose (XI). Since it, too, was isolated not as such but in the form of the 4-acyl derivatives XII and XIII, the possibility of branching is excluded and the presence in the initial molecule of two unbranched carbohydrate chains terminated by isobutyrylolivomycose (III) and by olivomose (VII) is demonstrated.

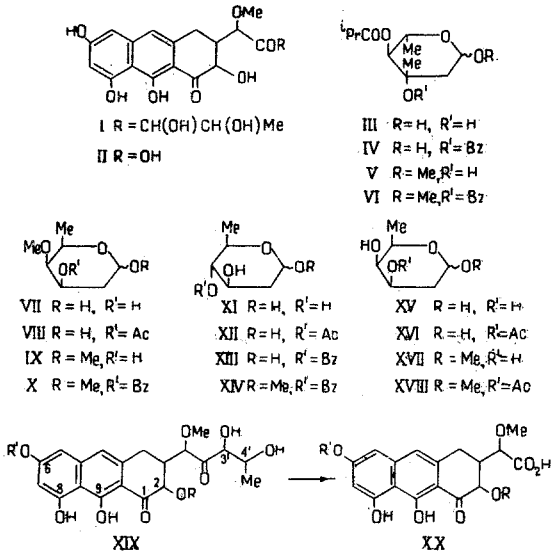
The position of attachment of the chains to the aglycone was established on the basis of the capacity of olivomycin A, like olivin (I), for giving an isopropylidene derivative under the action of acetone in the presence of $Cu_2SO_4$ and of forming a strongly acidic complex with boric acid. The first of these reactions shows that the antibiotic retains the 3', 4'-diol grouping of the aglycone, while the increase in the acidity of a solution of boric acid ($\Delta pH \sim 2.5$) caused by olivomycin A is characteristic in magnitude for peridihydroxynaphthalenes (see [3]) and shows that the phenolic 8-OH and 9-OH hydroxyls in the antibiotic are also free. Thus, the carbohydrate chains can be attached only to the 2-OH and 6-OH of the aglycone so that the typical formula XIX follows for olivomycin A.

An independent proof of formula XIX follows from the following properties of the antibiotic. We have previously found [7] that olivin (I) has two acidity constants, the first of which ($pK_a'$ 6.3) is due to a 1, 8, 9-oxodiol group and the

*For a preliminary communication, see [1], and for part XVI, see [2].

**In the benzoate of olivomycin A, the glycosidic bonds are more difficult to cleave than in the acetate of the antibiotic, in consequence of which fairly severe conditions are necessary for its degradation. At the same time, the O-acetyl group of acetyloliose is comparatively readily split off under the action of mineral acids [3]. Consequently, in the methanolysis of the benzoate of olivomycin A the acetyloliose is isolated mainly in the form of methyl oliosides (XVII) and only to a small extent (less than 10%) in the form of methyl actyloliosides (XVIII).

second ($pK_a$ 9.3) to the phenolic hydroxyl 6-OH, and that the ionization of this hydroxyl causes a considerable bathochromic shift of the strongest UV absorption maximum (277 → 286 mμ). It was found that, in contrast to olivin, olivomycin A has only one acid group ($pK_a$ 7.2) and its UV spectrum changes little when it is made alkaline. Consequently, the phenolic 6-OH hydroxyl in the antibiotic is blocked by a carbohydrate chain. Furthermore, on oxidation with an excess of periodate, olivomycin A reduces 2 moles of oxidizing agent and forms acetaldehyde, formic acid, and olivomycinic acid XX, the mild acid hydrolysis of which gives the olivinic acid II that we have described previously [9]. This conversion shows that in contrast to the dihydroxyketone side chain, the 1,2-ketol grouping in the antibiotic is protected from periodate oxidation, i.e., the second carbohydrate chain is attached to the aglycone through the 2-OH hydroxyl.




In conclusion, it must be mentioned that the product of the direct periodate oxidation of olivomycin A, olivomycinic acid (XX), on hydrolysis with 50% acetic acid, forms all four sugars—III, VII, IX, and XVI—in the same ratio as that in which they are present in the initial antibiotic. If, however, olivomycin A is first subjected to mild alkaline saponification and then to the action of periodate, oxidation of the olivomycose residue takes place, and on subsequent acetic acid hydrolysis only olivomose (VII), olivose (IX), and oliose (XV) are formed. This further confirms the fact that the terminal members of the two carbohydrate chains in the antibiotic are an acylated olivomycose and olivomose, which is stable to periodate.

## EXPERIMENTAL

General information on the experimental work has been given previously [3]. Chromatography was carried out in a thin layer of adsorbent in benzene–acetone (1:1) (system 1), 2:1 (system 2), 3:1 (system 3), 9:1 (system 4), and 20:1 (system 5).

1. **Heptaacetate of olivomycin A.** A solution of 1.2 g of olivomycin A in 60 ml of pyridine and 60 ml of acetic anhydride was kept at 20° C for 3 days and was then evaporated. The residue was dissolved in chloroform and the solution was washed with dil $H_2SO_4$, water, saturated $NaHCO_3$, and water again, and was dried and evaporated. The residue was chromatographed on silica gel in system 3. From the zone with $R_f$ 0.37–0.40 was isolated 600 mg (40%) of the heptaacetate of olivomycin A; mp 218–220° C (from absolute ethanol); $[\alpha]_D^{23}$ −17.5° (c 1; chloroform); $\lambda_{max}^{THF}$ mμ: 222, 248 (shoulder), 258 (shoulder), 266, 320, 351 (shoulder) (log ε: 4.57, 4.60, 4.74, 4.89, 4.04, 3.68); $\nu_{max}$, $cm^{-1}$: 1570, 1632, 1688, 1752, 1784 (shoulder), 3480 $cm^{-1}$.

Found, %: C 57.7; H 6.5. Calculated for $C_{72}H_{98}O_{33}$, %: C 58.0; H 6.6.

2. **Octabenzoate of olivomycin A.** To a solution of 2.4 g of olivomycin A in 30 ml of pyridine stirred at 0° C was added 4 ml of benzoyl chloride and the mixture was kept at 20° C for 2 days, after which another 2 ml of benzoyl chloride was added and it was heated at 75° C for 8 hr. After cooling, the mixture was poured into ice water and extracted with chloroform, the extract was treated in the preceding experiment and the substance obtained was

chromatographed on silica gel (column 300 × 30 mm) and was eluted with benzene. From the first 500 ml of eluate was isolated 3.25 g (80%) of the octabenzoate of olivomycin A; mp 172–175° C (from chloroform–hexane); $[\alpha]_D^{20}$ −15° (c 1; chloroform); $R_f$ 0.62 (on silica gel in system 4); $\lambda_{max}^{THF}$, mμ: 230, 258, (shoulder), 267, 320, 355 (shoulder); (log ε: 5.18, 4.83, 4.95, 4.08, 3.77); $\nu_{max}$, $cm^{-1}$: 706, 1590, 1604, 1632, 1694, 1733, 1793.

Found, %: C 66.9; H 5.9. Calculated for $C_{114}H_{116}O_{34}$, %: C 67.4; H 5.8.

From the next 200 ml of eluate was isolated 0.25 g (7%) of the hexabenzoate of olivomycin A; mp 179–182° C (from chloroform–hexane); $[\alpha]_D^{23}$ −31° (c 1; chloroform); $R_f$ 0.31 (on silica gel in system 4); $\lambda_{max}^{THF}$, mμ: 230, 258 (shoulder), 267, 322, 355 (shoulder) (log ε: 5.06, 4.74, 4.87, 3.98, 3.65); $\nu_{max}$, $cm^{-1}$: 710, 1573, 1590, 1608, 1634, 1698, 1735, 3440.

Found, %: C 65.5; H 5.8. Calculated for $C_{100}H_{108}O_{32}$, %: C 65.9; H 6.0.

3. **Hydrolysis of the heptaacetate of olivomycin A.** A solution of 1.45 g of the heptaacetate of olivomycin A in 150 ml of 50% acetic acid was heated at 80° C for 5 hr and was then evaporated, and the residue was dissolved in chloroform and washed with water. After the solvent had been evaporated off, the chloroform solution yielded 590 mg of a substance which was chromatographed on silica gel in system 2. From the zone with $R_f$ 0.60–0.65 was isolated 400 mg of olivin acetate; the eluates from the other zones were combined with the residue after the evaporation of the wash-waters (900 mg) and were rechromatographed on silica gel (in system 1). From the zone with $R_f$ 0.60–0.80 was isolated 350 mg of a mixture of 4-isobutyrylolivomycose (III) and 3-acetylolivomose (VIII), the zone with $R_f$ 0.30–0.50 yielded 380 mg of a mixture of 4-acetylolivose (XII) and 3-acetyloliose (XVI), and the zone with $R_f$ 0.20–0.30 yielded 100 mg (52%) of 3-acetyloliose (XVI), which was shown to be identical with an authentic sample [3]. The mixture of III and VIII obtained was chromatographed on $Al_2O_3$ in system 3; this gave 180 mg (77%) of 4-isobutyrylolivomycose (III), $R_f$ 0.28, and 150 mg (73%) of 3-acetylolivomose (VIII), $R_f$ 0.42; the two sugars were shown to be identical with those reported previously [4, 5]. The mixture of XII and XVI was rechromatographed on silica gel in system 1. The zone with $R_f$ 0.40–0.50 yielded 100 mg of 4-acetylolivose (XII), the zone with $R_f$ 0.35–0.40 180 mg of a mixture of 4-acetylolivose (XII) and 3-acetyloliose (XVI), and the zone with $R_f$ 0.30–0.35 30 mg of 3-acetyloliose (XVI). When the 4-acetylolivose (XII) was treated with a 0.02 N solution of $NaIO_4$ at 20° C for 48 hr, no consumption of oxidizing agent was observed. The hydrolysis of XII with a 0.3 N solution of $Ba(OH)_2$ (20° C, 3 hr) led to olivose (XI) with a yield of 95%, identified by comparison with an authentic sample [3].

4. **Methanolysis of the octabenzoate of olivomycin A.** A solution of 700 mg of the octabenzoate in 30 ml of benzene and 35 ml of 1 N methanolic HCl was heated at 75° C for 3 hr, and after cooling it was neutralized with $Ag_2CO_3$ and evaporated, and the residue was dissolved in ethyl acetate and washed with water. The substance obtained form the ethyl acetate solution (670 mg) was chromatographed on silica gel in system 4. This yielded 120 mg (95%) of methyl 3-benzoyl-4-isobutyrylolivomycosides (VI), 96 mg (98%) of methyl 3-benzoylolivomosides (X), 120 mg (65%) of methyl 4-benzoylolivosides (XIV), 30 mg (16%) of the individual α-anomer (α-XIV), 5 mg (7%) of methyl 3-acetyloliosides (XVIII), and 120 mg of olivin benzoate, which was not studied further. The residue after the evaporation of the wash-waters (55 mg) was chromatographed on $Al_2O_3$ in system 1; from the zone with $R_f$ 0.25–0.30 was isolated 40 mg (70%) of methyl oliosides (XVII).

5. **3-Benzoyl-4-isobutyrylolivomycose (IV).** A solution of 120 mg of the mixture of anomeric glycosides VI obtained in Experiment 4 in 15 ml of a 0.2 N solution of $H_2SO_4$ in 50% aqueous dioxane was heated at 80° C for 7 hr. after cooling, the reaction mixture was neutralized with $BaCO_3$, the precipitate was separated off by centrifuging and the solution was evaporated. The residue was chromatographed on silica gel in system 5. The zone with $R_f$ 0.74–0.78 yielded 14 mg (12%) of the initial glycosides (VI), and the zone with $R_f$ 0.36–0.45, 68 mg (60%) of benzoylisobutyrylolivomycose (IV); mp 115–120° C (from hexane); $[\alpha]_D^{22}$ +7.5° (c 0.7; chloroform).

Found, %: C 64.5; H 7.3. Calculated for $C_{18}H_{24}O_6$, %: C 64.3; H 7.2.

The same sugar was obtained by the benzoylation of the methyl 4-isobutyrylolivomycosides (VII) with benzoyl chloride in pyridine (7 hr at 75° C) with subsequent $H_2SO_4$ hydrolysis under the conditions described above. Yield 35%.

6. **Methyl α-3-benzoylolivomoside (α-X).** 96 mg of the mixture of anomeric glycosides X obtained in Experiment 4 were chromatographed on $Al_2O_3$ in system 3. This gave 70 mg (73%) of the methyl α-glycoside (α-X), $R_f$ 0.42; mp 93–94° C (from methanol); $[\alpha]_D^{22}$ +125° (c 0.9; chloroform).

The same glycoside was obtained by the benzoylation of methyl α-olivomoside (α-IX) [3] by BzCl + Py (48 hr at 20° C). Yield 89%.

7. **Methyl α-4-benzoylolivoside (α-XIV).** The substance isolated in Experiment 4 had mp 60–61° C (from hexane); $[\alpha]_D^{23}$ +139° (c 1; chloroform). $\nu_{max}$, $cm^{-1}$: 707, 1724, 3380.

Found, %: C 63.5; H 6.9. Calculated for $C_{14}H_{18}O_5$, %: C 63.2; H 6.8.

8. 4-Benzoylolivose (XIII). 87 mg of the mixture of glycosides XIV obtained in Experiment 4 was hydrolyzed with a 0.2 N aqueous dioxane solution of $H_2SO_4$ (4 hr at 75° C), treated as in Experiment 5, and chromatographed on silica gel in system 3. From the zone with $R_f$ 0.40–0.50 was isolated 70 mg (85%) of 4-benzoylolivose (XIII); $[\alpha]_D^{22}$ +34° (c 1; chloroform); $\nu_{max}$, $cm^{-1}$: 707, 1712, 3370. The substance is stable to the action of $NaIO_4$ at 20° C.

Found, %: C 61.0; H 6.8. Calculated for $C_{13}H_{16}O_5$, %: C 61.5; H 6.4.

9. **Hydrolysis of 4-benzoylolivose (XIII).** A solution of 50 mg of benzoylolivose (XIII) in 4 ml of a 0.4 N solution of NaOH in 75% ethanol was left at 20° C for 4 hr and was then neutralized with $CO_2$ and the ethanol was evaporated off. The residue was continuously extracted with ether (15 hr) and the substance extracted was chromatographed on silica gel in the chloroform–ethanol (3 : 1) system. The zone with $R_f$ 0.35–0.40 yielded 25 mg (84%) of olivose (XI), which was shown to be identical with a sample of the sugar obtained by the hydrolysis of olivomycin A [3].

10. **Acidity of the borate complex of olivomycin A.** A 0.18 M solution of $H_3BO_3$ in 30% aqueous ethanol has pH 5.55; when olivomycin A was dissolved in it to a concentration of 0.009 M, the pH fell to 3.05.

11. **3',4'-Isopropylideneolivomycin A.** A solution of 120 mg of olivomycin A in 6 ml of acetone was treated with 2.7 g of $CuSO_4$ and the mixture was stirred at 50° C for 14 hr. Then it was filtered, the filtrate was evaporated, and the residue was chromatographed on silica gel in system 3. This gave 15 mg (12%) of the isopropylidene derivative, $[\alpha]_D^{28}$ –13° (c 0.2; ethanol). The substance obtained was kept in a buffered 0.02 N aqueous ethanolic solution of $NaIO_4$ (pH 6.9) at 20° C for 10 hr, after which it was extracted with ethyl acetate and hydrolyzed with 50% acetic acid (5 hr at 75° C). The hydrolysate contained olivin ($R_f$ 0.70 on silica gel in system 1) and its carbohydrate composition was the same as that of olivomycin A.

12. **Periodate oxidation of olivomycin A.** At 0° C, 125 ml of 0.02 N $NaIO_4$ solution was added to a solution of 150 mg of olivomycin A in 20 ml of methanol, and the mixture was kept at 0° C in the dark for 40 min and was separated into three parts. One part was acidified with dil $H_3PO_4$ to pH 3 and was evaporated at 50° C/40 mm, and the formic acid in the distillate was determined by the calomel method [8]; found: 79% (in a control experiment, 70%). To the second part was added an excess of 0.1 N $Na_3AsO_3$, the solution was evaporated at 15° C/40 mm, and the acetaldehyde in the distillate was determined in the form of the 2,4-dinitrophenylhydrazone; found: 86% (in a control experiment, 87%). The third part was acidified with dil $H_2SO_4$ to pH 3 and extracted with ethyl acetate, and the extract was treated in the usual way and evaporated. The olivomycinic acid (XX) obtained was hydrolyzed by being heated with 50% acetic acid at 75° C for 3 hr. In the hydrolysate the 4-isobutylolivomycose (III), olivomose (VII), olivose (XI), and 3-acetyloliose (XVI) were determined by the method described previously [3] and were shown to be in a ratio of approximately 1 : 1 : 2 : 1. The hydrolysate was diluted with saturated NaCl solution and extracted with ethyl acetate. The solvent was distilled off and the residue was chromatographed in system 3. The zone with $R_f$ 0.23–0.30 yielded olivinic acid (II) (yield 56%), which was identical with a sample obtained by the periodate oxidation of olivin (I) [9].

## CONCLUSIONS

It has been shown that the five monosaccharide residues forming the carbohydrate moiety of the antibiotic olivomycin A form two unbranched chains attached to the hydroxyls in positions 2 and 6 of the aglycone and terminated by isobutyrylolivomycose and olivomose.

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