OLIVOMYCIN AND RELATED ANTIBIOTICS

XVIII. The Structure of Olivomycins A, B, C, and D*

Yu. A. Berlin, S. E. Esipov, and M. N. Kolosov

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In a preceding communication we showed that olivomycin A is a pentaoside of olivin in which residues of 4-isobutyrylolivomycose, olivomose, 3-acetyloliose, and two olivose residues form two unbranched chains attached to the hydroxyls in positions 2 and 6 of the aglycones and terminated by 4-isobutyrylolivomycose and olivomose [2]. To elucidate the sequence of individual members in the two chains it was necessary to have products of the partial hydrolysis of the antibiotics. However, the preparation of these compounds presented great difficulties since all the carbohydrate components of olivomycin A are 2,6-dideoxy sugars readily split out under the action of acids, in consequence of which the partial hydrolysis of the antibiotic always forms a complex mixture of substances differing little in chromatographic behavior. Nevertheless, by a careful selection of the conditions of acid degradation of olivomycin A (nature and concentration of the acid, solvent, temperature, time) we have succeeded in obtaining an olivin-containing mixture of hydrolysis products susceptible of chromatographic separation. The carbohydrate composition of the glycosides obtained as a result of this was determined by paper chromatography using a previously-described method [3] and the absence of contaminating free sugars was checked by analytical chromatography on alumina (on this adsorbent, the olivin-containing substances remain at the start in the form of chelates).

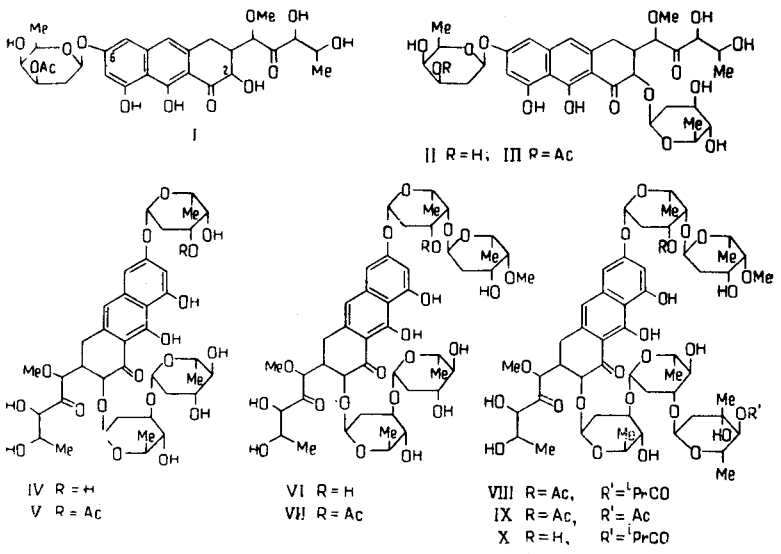
The simplest of the products of the partial hydrolysis of olivomycin A that we obtained was (acetyloliosyl)olivin. The isolation of this monosaccharide immediately showed that in olivomycin A the 3-acetyloliose residue is attached directly to the aglycone. Furthermore, the UV spectra of (acetyloliosyl)olivin in neutral and alkaline solutions proved to be almost identical, while in olivin a change to alkaline conditions causes a substantial bathochromic shift of the UV absorption [4]. This means that the 3-acetyloliose residue is attached to the chromophoric part of the aglycone and (acetyloliosyl)olivin must be ascribed structure I (on the configuration of the glycosidic center in this and other products of the partial hydrolysis of olivomycin A, see below).

Milder conditions of the degradation of olivomycin A led to olivosyl(acetyloliosyl) olivin. When this bioside was subjected to alkaline saponification and then to periodate oxidation, subsequent acid hydrolysis formed no sugars whatever. This shows, in the first place, that in the compounds studied the olivose residue is not attached to the 3-acetyloliose residue and, consequently, it is attached to C_2 of the aglycone and, in the second place, that both sugars exist in the pyranose form, i.e., the bioside has structure III.

The third product of the partial hydrolysis of olivomycin A, olivosylolivosyl(acetyloliosyl)olivin differs from the bioside III by the presence of another olivose residue. When this trioside was subjected to direct periodate oxidation, only one of the three sugar residues in it was degraded, the olivose residue, and on mild alkaline hydrolysis and subsequent treatment with $NaIO_4$ not only one olivose residue but also the oliose residue was oxidized. It follows from this that the second olivose residue is attached to the first and is also in the pyranose form. Furthermore, the formation of more than 1 mole of methyl 4-benzoylolivosides on the methanolysis of the octabenzoate of olivomycin A [2] shows that in the antibiotic the 4-OH hydroxyls of both olivose residues are free (consequently, both sugars are in fact in the pyranose form) and the 3-OH hydroxyls are glycosidated. Thus, the olivosylolivosyl(acetyloliosyl)olivin corresponds to formula V.

In the partial hydrolysis of olivomycin A we also isolated a tetraoside differing from the initial antibiotic only by the absence of a 4-isobutyrylolivomycose residue. It was found that the action of $NaIO_4$ on this substance oxidized only one of the two olivose fragments, and that periodate oxidation after previous alkaline hydrolysis takes place in a completely analogous manner (i.e., with the retention of the oliose residue). This shows that the olivomose is attached to the 3-acetylilose, protecting it from oxidative degradation after the elimination of the acetyl group, i. e., the tetraoside has structure VII. Hence, it follows, in its turn, that in olivomycin A the 4-isobutyrylolivomycose is

*For a preliminary communication, see [1].



attached to the 3-OH of the second olivose member and in this way the last question of the arrangement of the carbohydrate residues in the antibiotic is settled.

Having available the polarimetric characteristics of all the carbohydrate components and the products of partial hydrolysis of olivomycin A, we determined the configuration of the glycosidic centers of the molecule on the basis of calculations according to Klyne's rule (table). This permitted the conclusion that olivomycin A is 2-O-[O-α-4-isobutyrylolivomycosyl-(1 → 3)-O-β-olivosyl-(1 → 3)-β-olivosyl]-6-O-[O-α-olivomosyl-(1 → 4)-β-3- acetyl-oliosyl] olivin, i.e., it possesses the structure VIII.

So far as concerns the other olivomycins, in view of their closeness to olivomycin A in respect of biogenesis, antibiotic activity, physicochemical properties, and, particularly, monomeric composition [3], it may be assumed that all the antibiotics of this group are structurally related.

Thus, olivomycin B, in contrast to olivomycin A, contains a residue of the 4-acetate of olivomycose instead of its 4-isobutyrate [3]. In order to establish whether this difference is the only one, we subjected olivomycins A and B to mild alkaline hydrolysis with subsequent acetylation and established that the main product from both antibiotics as a result of these treatments was an acetate which is also obtained by the direct acetylation of olivomycin B. It follows from this that olivomycin B possesses the structure IX.

A study of the carbohydrate composition of olivomycin C showed that it contains the same sugars as olivomycin A but that oliose residue in its molecule is not acetylated [3]. Hence, the structure of desacetylolivomysin A (X) could be expected for olivomycin C. In actual fact, we demonstrated this formula by converting olivomycin C into the octaacetate, which was identical with the heptaacetate of olivomycin A, described previously [2].

Finally, olivomycin D, differing from olivomycin A by the absence of the 4-isobutyrylolivomycose residue [3] proved to be identical with one of the products of the partial hydrolysis of olivomycin A, namely (olivosylolivosyl)-[olivomosyl(acetyloliosyl)]olivin (VII).

Thus, the three most active components of native olivomycin, the antibiotics olivomycins A, B, and C, have a similar configuration of the glycosidic linkages and differ only in respect of the substituents in position 3 of the oliose residue and position 4 of the olivomycose residue. Olivomycin D, which lacks the olivomycose residue, forms, as it were, "incomplete" olivomycin A and can be regarded as a probable biogenetic precursor of this antibiotic.

EXPERIMENTAL

General information on the experimental work has been given previously [4]. Chromatography was carried out in a thin layer of silica gel in benzene–acetone (1 : 1) (system 1) or (3 : 1) (system 2).

1. **(3-Acetyloliosyl)olivin (I).** A solution of 560 mg of olivomycin A in 60 ml of a 0.1 N solution of H_2SO_4 in 50% methanol was boiled for 1.5 hr, concentrated to half volume, diluted with water, and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated, and the residue was triturated with ether. The precipitate was filtered off, and the addition of hexane to the filtrate precipitated an additional amount of substance (total of 180 mg). Chromatography in system 1 yielded 80 mg (40%) of olivin, R_f 0.67, and 80 mg (28%) of (acetyloliosyl)olivin (I), R_f 0.58; $[\alpha]_D^{20}$ − 18° (c 0.5; ethanol); λ_{max}, mμ: 227, 275, 307, 318, 332 (shoulder), 405 (log ε 4.39, 4.72, 3.84, 3.86, 3.67, 3.99); $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$, mμ: 227, 275, 307, 318, 332 (shoulder), 410 (log ε 4.37, 4.70, 3.81, 3.82, 3.64, 3.90); ν_{max}, cm^{-1}: 1510, 1582, 1640, 1720, 3370.

Found, %: C 56.5; H 6.0. Calculated for $C_{28}H_{34}O_{13} \cdot H_2O$, %: C 56.4; H 6.1.

Calculations for the Determination of the Configuration of the Glycosidic Centers of Olivomycin A

Glycoside	$[M]_D$	Contribution of the sugar	$[M]_D$ of the anomeric methyl glycosides	Configuration of the glycosidic center
	deg.			
Olivin	+240			
(Acetyloliosyl)-olivin	−110	−350	α+290 [5] β —	β
Olivosylolivosyl-(acetyloliosyl)-olivin	−320	−210	α+212 [3] β−138 [3]	β
Olivosylolivosyl-(acetyloliosyl)olivin	−486	−166	α+212 [3] β−138 [3]	β
Olivomosylolivosylolivosyl-(acetyloliosyl)olivin	−230	+256	α+284 [3] β − 62 [3]	α
(Isobutyrylolivomycosyl)-olivomosylolivosylolivosyl-(acetyloliosyl)olivin (olivomycin A).	−480	−250	α−282 [3] β + 67 [3]	α

2. **(3-Acetyloliosyl)olivosylolivin (III) and (3-acetyloliosyl)olivosylolivosylolivin (V).** A solution of 560 mg of olivomycin A in 60 ml of 50% acetic acid was heated at 60° C for 2 hr and evaporated, the residue was dissolved in ethyl acetate, and the solution was washed with water, concentrated to half-volume, and diluted with an equal amount of hexane. From the precipitate (200 mg) repeated chromatography in system 1 yielded 90 mg (44%) of olivin, R_f 0.67, 30 mg (8.5%) of (acetyloliosyl)olivosylolivin (III), R_f 0.57, and 50 mg (12%) of (acetyloliosyl)olivosylolivosylolivin (V), R_f 0.36.

(3-Acetyloliosyl)olivosylolivin (III): $[\alpha]_D^{20}$ − 44° (c 0.5; ethanol); λ_{max}, mμ: 228, 274, 318, 405 (log ε 4.46, 4.73 3.87, 4.10); ν_{max}, cm^{-1}: 1513, 1587, 1640, 1717, 3400.

Found, %: C 57.2; H 6.2. Calculated for $C_{34}H_{44}O_{16}$, %: C 57.6; H 6.3.

(3-Acetyloliosyl)olivosylolivosylolivin (V): $[\alpha]_D^{20}$ − 59° (c 0.6; ethanol); λ_{max}, mμ: 227, 277, 307, 318, 333 (shoulder), 408 (log ε 4.47, 4.74, 3.88, 3.89, 3.73, 4.16); ν_{max} 1510, 1582, 1638, 1725, 3360 cm^{-1}.

Found, %: C 55.8; H 6.9. Calculated for $C_{40}H_{54}O_{19} \cdot H_2O$, %: C 56.3; H 6.6.

3. Periodate oxidation of olivosylolivosylolivin (II). A solution of 14 mg of (acetyloliosyl)olivosylolivin (III) in 3 ml of 0.5 N KOH in methanol was left at 20° C for 2 hr and was neutralized with acetic acid and evaporated. The oliosylolivosylolivin (II) so obtained was dissolved in 15 ml of 0.04 N $NaIO_4$ solution buffered with $NaHCO_3$ to pH 6.5 and the solution was left in the dark at 20° C for 2 hr. The excess of periodate was reduced with Na_3AsO_3, the reaction

mixture was extracted with ethyl acetate, and the substance extracted (7 mg) was hydrolyzed with 50% acetic acid (4 hr at 75° C). When the hydrolysate was chromatographed on paper under the conditions described previously [3], no monosaccharides were detected.

4. Periodate oxidation of (3-acetyloliosyl)olivosylolivosylolivin (V). A solution of 8.5 mg of the trioside V in 9 ml of 0.04 N buffered $NaIO_4$ solution (pH 6.6–6.9) was kept at 20° C for 2 hr and was treated as in the oxidation of II. In the product (4 mg) 1 mole of 3-acetyloliose and 1 mole of olivose were found by the method described previously [3].

5. Periodate oxidation of oliosylolivosylolivosylolivin (IV). The acetyltrioside V was saponified with KOH to the trioside IV, and the latter was subjected to acetic-acid hydrolysis and also to periodate oxidation and then to hydrolysis by the method of Experiment 3. In the first hydrolysate 2 moles of olivose and 1 mole of oliose were found, and in the second only olivose.

6. Olivomosyl(3-acetyloliosyl)olivosylolivosylolivin (VII) (olivomycin D). A solution of 560 mg of olivomycin A in 20 ml of dioxane and 50 ml of 1 N acetic acid was heated at 70° C for 1.5 hr, concentrated in vacuum, and extracted with ethyl acetate. The extracted substance was chromatographed in system 1; the zone with R_f 0.70–0.75 yielded 100 mg (18%) of olivomycin A, and the zone with R_f 0.34–0.40 yielded 280 mg (50%) of the tetraoside VII; $[\alpha]_D^{20}$ – 23.5° (c 0.8; chloroform); λ_{max}, mμ: 227, 278, 307, 318, 332 (shoulder), 410 (log ε 4.34, 4.68, 3.77, 3.80, 3.58, 4.03); ν_{max}, cm^{-1}: 1510, 1582, 1638, 1727, 3360.

Found, %: C 56.4; H 6.8. Calculated for $C_{47}H_{66}O_{22} \cdot H_2O$, %: C 56.4; H 6.8.

7. Periodate oxidation of olivomosyloliosylolivosylolivosylolivin (VI). The acetyltetraoside VII (19.5 mg) was saponfied by KOH to form the tetraoside VI (yield 17 mg, 90%), and the latter was subjected to hydrolysis and oxidation-hydrolysis as in Experiment 5. Olivomose, oliose, and olivose in a ratio of 1 : 1 : 2 were found in the first hydrolysate, and the same sugars in a ratio of 1 : 1 : 1 in the second hydrolysate.

8. Periodate oxidation of the product of alkaline hydrolysis of olivomycin A. Olivomycin A was saponified with KOH, and the substance isolated was subjected to periodate oxidation and hydrolysis (cf. Experiments 3 and 5). It was shown chromatographically that the resulting hydrolysate contained oliose, olivose, and olivomose, but no olivomycose.

9. Acetylation of the product of the alkaline hydrolysis of olivomycin A. Olivomycin A (600 mg) was hydrolyzed with 10 ml of 0.5 N methanolic KOH (4 hr at 20° C), and the solution was neutralized with acetic acid and evaporated to dryness. The residue was acetylated with 20 ml of pyridine–acetic anhydride (1 : 1) (3 days at 20° C) and was worked up in the usual way. From the substances obtained, chromatography in system 2 yielded three individual acetates.

Acetate 1 (10 mg): R_f 0.62; mp 153–155° C (from chloroform–hexane); $[\alpha]_D^{22}$ – 21° (c 0.6; chloroform); λ_{max}^{THF}, mμ: 222, 248 (shoulder), 258 (shoulder), 266, 320, 351 (shoulder) (log ε 4.57, 4.57, 4.73, 4.89, 4.01, 3.69); ν_{max}, cm^{-1}: 1571, 1632, 1688, 1752, 1781.

Found, %: C 57.5; H 6.5. Calculated for $C_{71}H_{94}O_{34}$, %: C 57.1; H 6.3.

Acetate 2 (200 mg): R_f 0.38; mp 163–165° C (from chloroform–hexane); $[\alpha]_D^{22}$ – 34° (c 1; chloroform); λ_{max}^{THF}, mμ: 222, 249 (shoulder), 258 (shoulder), 266, 320 (log ε 4.58, 4.60, 4.75, 4.90, 4.04); ν_{max}, cm^{-1}: 1570, 1632, 1693, 1748, 1779, 3455.

Found, %: C 57.0; H 6.4. Calculated for $C_{69}H_{92}O_{33}$, %: C 57.0; H 6.4.

Acetate 3 (300 mg): R_f 0.15; mp 169–172° C (from chloroform–hexane); $[\alpha]_D^{29}$ – 42° (c 0.8; chloroform); on reacetylation, a mixture of acetates 1 and 2 was formed.

10. Acetylation of olivomycin B. Olivomycin B (117 mg) was acetylated in a similar manner to olivomycin A. Chromatography in system 2 yielded 4.5 mg of a substance with R_f 0.62, mp 153–155° C (from chloroform–hexane) and 88 mg of a substance with R_f 0.38, mp 164–166° C (from chloroform–hexane), which proved to be identical with acetates 1 and 2 obtained in the preceding experiment.

11. Acetylation of olivomycin C. The acetylation of olivomycin C with acetic anhydride in pyridine (3 days at 20° C) gave an acetate with mp 218–220° C (from absolute ethanol), $[\alpha]_D^{23}$ – 17.5° (c 1; chloroform) identical with the previously-described heptaacetate of olivomycin A [2].

CONCLUSIONS

By partial hydrolysis and correlation through the acetates, the complete structural formulas VIII, IX, X, and VII have been demonstrated for the antibiotics olivomycins A, B, C, and D.

REFERENCES

1. Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, and M. M. Shemyakin, Tetrah. Let., 1431, 1643, 1966.

2. Yu. A. Berlin, S. E. Esipov, M. N. Kolosov, and O. A. Chuprunova, KhPS [Chemistry of Natural Compounds], **5**, 1969 [in this issue].

3. Yu. A. Berlin, S. E. Esipov, O. A. Kiseleva, and M. N. Kolosov, KhPS [Chemistry of Natural Compounds], **3**, 331, 1967.

4. Yu. A. Berlin, I. V. Vasina, M. N. Kolosov, G. Yu. Pek, L. A. Piotrovich, and O. A. Chuprunova, KhPS [Chemistry of Natural Compounds], **5**, 304, 1969.

5. J. C. Brimacombe and D. Portsmouth, Chem. a. Ind., 468, 1965; Carbohydr. Res., **1**, 125, 1965.

6. M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara, and K. Nakanishi, Tetrah. Let., 545, 1966.

7. M. Miyamoto, Y. Kawamatsu, K. Kawashima, M. Shinohara, K. Tanaka, S. Tatsuoka, and K. Nakanishi, Tetrah., **23**, 421, 1967.

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Institute of the Chemistry of Natural Compounds, AS USSR