OLIVOMYCIN AND RELATED ANTIBIOTICS

XXXII. THE STRUCTURE OF CHROMOCYCLOMYCIN*

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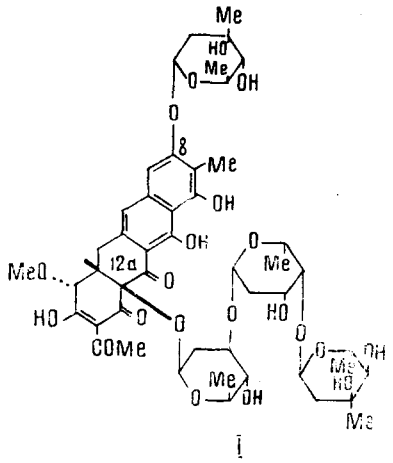
UDC 615.779.931+547.917

In studying the glycoside chromocyclomycin, isolated together with aureolic acid from the culture liquid of Streptomyces LA-7017, we have determined the structure and configuration of its aglycone, chromocyclin (VII) [2, 3]. The present paper gives information on the determination of the structure of the carbohydrate moiety of chromocyclomycin. It has been established that this glycoside has the structure (I).



I

The analysis of the mixture of monosaccharides formed in the complete acetic acid hydrolysis of chromocyclomycin showed that it contains the same sugars as aureolic acid, i.e., D-olivose (V), D-oliose (III), and D-mycarose (II), but in a ratio of 1 : 1 : 2. Under the action on chromocyclomycin of sodium periodate, the mycarose is oxidized completely, while the olivose and oliose remain unchanged. This shows that both mycarose residues in chromocyclomycin are terminal and are present in the pyranose form. As a result of mild acetic acid hydrolysis, we obtained from chromocyclomycin not only chromocyclin (VII) but also mycarosylchromocyclin (VIII), olivosylchromocyclin (IX), mycarosylolivosylchromocyclin (X), and mycarosylolivosyloliosylchromocyclin (XI) (for their structures, see below). The formation of mycarosylchromocyclin (VIII) means that one of the mycarose residues is directly attached to the aglycone; since the same sugar is terminal, it forms one of the carbohydrate chains.

The beginning of the other chain is formed by olivopyranose, as follows from the formation of olivosylchromocyclin (IX) in which the olivose residue is oxidized by periodate. In mycarosylolivosyloliosylchromocyclin (XI), the olivose is not affected by periodate, in contrast to the monoside (IX), while the oliose undergoes oxidation, from which it follows that the latter glycosylates the olivose and is also present in the pyranose form. Since in chromocyclomycin the oliose is resistant to periodate, it is substituted by a mycarose residue which, thus, completes the trisaccharide chain. The benzoylation of chromocyclomycin with subsequent methanolysis and hydrolysis yielded 4-benzoylolivose (VI) (stable to periodate) and 3-benzoyl-

* For a preliminary communication, see [1].

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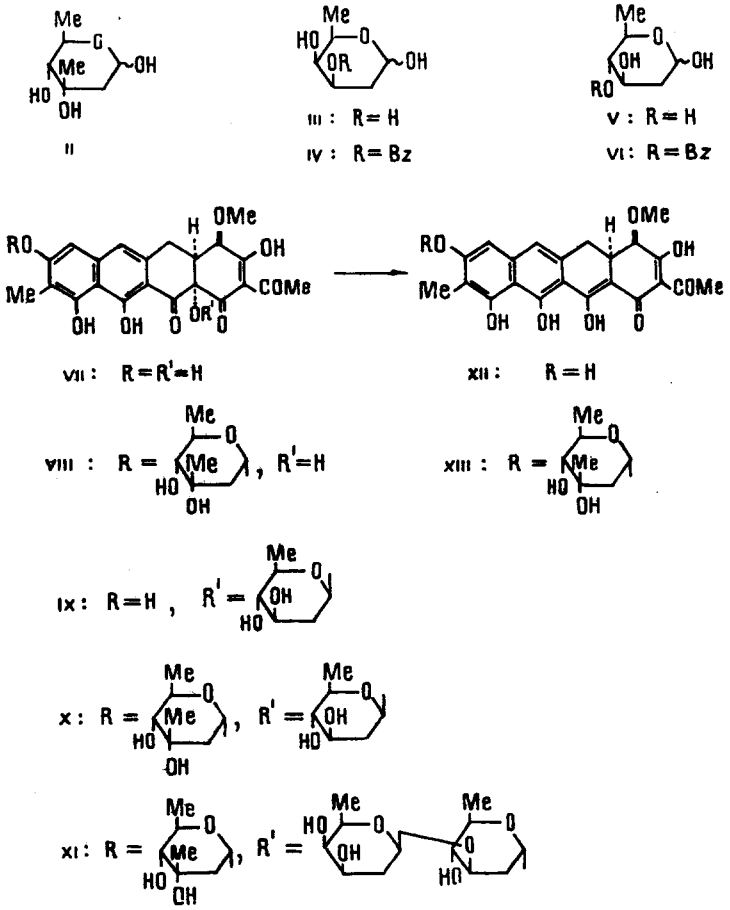
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 539-545, July-August, 1973. Original article submitted July 17, 1972.

TABLE 1. Details of the Calculation of the Configuration of the Glycosidic Centers of Chromocyclomycin

Glycoside	$[M]_D$	Contribution of the sugar	$[M]_D$ of anomeric methyl glycoside	Configuration of the glycosidic center
	degree			
Chromocyclin (VII)	−1860			
Olivosylchromocyclin (VIII)	−2020	−160	α: +212 β: −138	β
Mycarosylchromocyclin (IX)	−1270	+590	α: +225 β: −55	α
Olivosylmycarosylchromocyclin (X)	−1570	−300	α: +212 β: −138	β
Mycarosylolivosylchromocyclin (X)	−1570	+450	α: +215 β: −52	α
Oliosylolivosylmycarosylchromocyclin (XI)	−2160	−590	α: +230* β: —	β
Mycarosyloliosylolivosylmycarosylchromocyclin (chromocyclomycin) (I)	−1760	+400	α: +225 β: −55	α

* According to the literature [6] for methyl α-acetylolioside.

oliose (IV) (oxidized by periodate). This shows the position of the intercarbohydrate bonds and the mycarosyl-(1 → 4)-oliosyl-(1 → 3)-olivosyl structure for the second carbohydrate chain of chromocyclomycin.



Chromocyclomycin possesses approximately the same ionization constant as chromocyclin [2]. Consequently, the dihydroresorcinol and peri-dihydroxynaphthalene groupings in it are free and the carbohydrate chains are attached to the aglycone part of the molecule through the 8-OH and 12a-OH hydroxyls. In contrast to chromocyclomycin, the UV spectrum of which scarcely changes on the addition of alkalis, in chromocyclin in alkaline solution there is a bathochromic shift of the short-wave absorption band (280 → 290 nm) because of the ionization of the phenolic hydroxyl, 8-OH (compare the similar difference in the UV

spectra of the olivomycins and their aglycone, olivin [4]). The same shift in the UV spectrum takes place in the case of olivosylchromocyclin but not in the case of mycarosylchromocyclin [nor for oliosylolivosylmycarosylchromocyclin (XI), which confirms the structure that has been assigned to it of a product of the splitting off of mycarose from the trisaccharide chain and not from the aglycone]. It follows from this that the mycarose residue blocks the phenolic hydroxyl at C_8 of chromocyclin, and the mycarosyloliosylolivosyl chain is attached to the aglycone through the alcoholic 12a-OH group.

An independent proof of the arrangement of the carbohydrate chains in chromocyclomycin was obtained by its reductive cleavage at the $C_{12a}-O$ bond. It was found that the reduction of the chromocyclomycin ring in aqueous ammonia leads to the elimination of the mycarosyloliosylolivosyl chain and to the formation of mycarosyl-12a-deoxychromocyclin (XIII), the acid hydrolysis of which gave mycarose (II) and 12a-deoxychromocyclin (XII), which is also obtained by the direct reduction of chromocyclin (VII) [2]. This degradation unambiguously shows that the trisaccharide chain in chromocyclomycin is attached to the 12a-OH hydroxyl of the aglycone.

The configurations of the glycosidic bonds in chromocyclomycin were determined from calculations of molecular rotations in accordance with Klyne's rule using information on the optical activity of the products of partial and complete hydrolysis (Table 1). It was found that the olivose and oliose residues in chromocyclomycin have the β configuration and both mycarose residues the α configuration. So far as concerns the spatial structure of the aglycone part of the molecule, we have established its relative and absolute configuration previously [3]. However, in view of the ease of epimerization of chromocyclin at the C_4 asymmetric atom, the question of the native configuration of this atom in the initial glycoside deserves discussion. We have found that the direction of mutarotation of chromocyclomycin when it is dissolved in pyridine coincides with the direction of mutarotation of chromocyclin [3]; i.e., C_4-epimerization takes place in the same way in the two cases. This means that in the acetic acid hydrolysis of chromocyclomycin to chromocyclin no inversion of the C_4 asymmetric center takes place and, consequently, in the natural glycoside this center also has the R configuration.

On the basis of the information presented, structure (I) for chromocyclomycin has been proved.*

EXPERIMENTAL

For general information on the experimental part, see [2].

1. Complete Hydrolysis of Chromocyclomycin (I). A solution of 1 g of chromocyclomycin (I) [2] in 40 ml of 50% acetic acid was heated at 75°C for 3.5 h and, after cooling, it was diluted with water and extracted with ethyl acetate. The extract was carefully washed with water and evaporated. This gave 320 mg (76%) of chromocyclin (VII), which has been described in communication XXX [2].

The aqueous solutions were combined and evaporated, and the residue was chromatographed in the benzene–acetone (1:1) system. A zone with R_f 0.44-0.78 yielded 280 mg (85%) of D-mycarose (II), and a zone with R_f 0.13-0.39 yielded 190 mg of a mixture of D-oliose (III) and D-olivose (V), which were separated by chromatography three times in the benzene–acetone (1:2) system. This gave 60 mg (40%) of olivose (V) (R_f 0.48) and 50 mg (33%) of oliose (III) (R_f 0.30). All three sugars were identified by direct comparison with the products of the hydrolysis of aureolic acid [5]. In a quantitative determination of the sugars (paper and gas-liquid chromatographies [5]), their ratio was found to be II: (III + V) = 1 : 0.90, and (III): (V) = 1 : 1.10.

2. Periodate Oxidation of Chromocyclomycin. A solution of 50 mg of chromocyclomycin (I) in 3 ml of ethanol and 6 ml of 0.03 M $NaIO_4$ was kept at 20°C for 12 h and was then acidified to pH 3 and extracted with ethyl acetate; the extract was washed with water and evaporated and the residue was chromatographed in the benzene–acetone (3:2) system. This gave two glycosides with R_f 0.58 (12 mg) and 0.67 (10 mg), which, according to the results of acetic acid hydrolysis (see above) contained olivose (V) and oliose (III) but no mycarose (II).

3. Benzoylation of Chromocyclomycin and Methanolysis of Its Benzoate. A solution of 700 mg of chromocyclomycin (I) and 2.5 ml of BzCl in 12 ml of pyridine was left at 20°C for 24 h, and then another 1.5 ml of BzCl was added and the mixture was heated at 50°C for 14 h. After cooling, the reaction mixture was treated with water and extracted with chloroform. The extracted substance was chromatographed on a col-

* This structure is more accurate than that published previously [1], where a 1 → 3 bond between the mycarose and oliose residues was shown erroneously.

umn of silica gel (15×3.5 cm) with elution first by benzene (400 ml) and then with a mixture of benzene and acetone (9:1) (600 ml). The benzene–acetone eluate gave 1 g of a mixture of benzoates which was subjected to TLC in the benzene–acetone (4:1) system. A zone with R_f 0.53-0.62 yielded 210 mg of chromocyclin benzoate with $[\alpha]_D^{25}$ −30° (c 0.3; chloroform). A solution of this benzoate in 10 ml of benzene and 15 ml of 1 N methanolic HCl was boiled for 3 h, neutralized with Ag_2CO_3, filtered, and evaporated. The residue was extracted with ethyl acetate. The mixture of methyl glycosides (148 mg) was chromatographed in the benzene–acetone (9:1) system. The zones with the R_f values given below yielded the amounts of substances shown: 0.75-0.81 (1) 13.5 mg; 0.68-0.72 (2) 5.5 mg; 0.59-0.65 (3) 13 mg; 0.48-0.52 (4) 23 mg; 0.36-0.46 (5) 53 mg; 0.28-0.34 (6) 7 mg.

The substances isolated were saponified with 0.5 ml of a 0.4 N ethanolic solution of KOH (4 h at 20°C), neutralized with CO_2, and evaporated. The debenzoylation products were extracted with ethyl acetate and hydrolyzed by heating with 50% acetic acid (2 h at 80°C), the sugars obtained being identified by paper chromatography. In this way it was shown that zones 1-2 contained methyl glycosides of benzoylmycarose and zones 4-6 methyl glycosides of benzoylmycarose, benzoylolivose, and benzoyloliose.

A mixture of the substances from zone 5 was heated with 50% acetic acid (6 h at 85°C) and chromatographed in the benzene– acetone (5:1) system. A zone with R_f 0.13-0.37 yielded 44 mg of a mixture of benzoates of the free sugars, which was oxidized with 0.35 M $NaIO_4$ (48 h at 20°C) and was then hydrolyzed with 0.4 N ethanolic KOH (4 h), after which olivose (V) and oliose (III) were found in the hydrolyzate in a ratio of 1:0.1. If the mixture of substances from zone 5 was subjected to acid and alkaline hydrolysis with the omission of the stage of periodate oxidation, the ratio of olivose and oliose was 1:0.85.

4. Partial Hydrolysis of Chromocyclomycin. A solution of 300 mg of chromocyclomycin in 15 ml of 50% acetic acid was heated at 65°C for 1 h, diluted twofold with water, and extracted with ethyl acetate, and the substance isolated was chromatographed in the benzene–acetone (3:2) system.

A zone with R_f 0.83-0.89 yielded 29 mg of chromocyclin (VII), which was identified by comparison with the product of complete hydrolysis of chromocyclomycin [2]; a zone with R_f 0.71-0.81 yielded 17 mg of 8-mycarosylchromocyclin (VIII) with $[\alpha]_D^{25}$ −222° (c 0.4; ethanol); λ_{max} 230, 281, 420 nm (log ε 4.46; 4.69; 3.97); $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$ 232, 280, 420 nm (log ε 4.33; 4.68; 4.10); a zone with R_f 0.62-0.72 yielded 33 mg of 12a-olivosylchromocyclin (IX) with $[\alpha]_D^{25}$ −362° (c 0.7; ethanol); λ_{max} 231, 280, 420 nm (log ε 4.47; 4.66; 4.07); $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$ 290, 423 nm (log ε 4.59; 4.17); a zone with R_f 0.49-0.59 yielded 23 mg of 8-mycarosyl-12a-olivosylchromocyclin (X) with $[\alpha]_D^{25}$ −223° (c 0.6; ethanol); λ_{max} 230, 282, 432 nm (log ε 4.47; 4.71; 4.11); $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$ 232, 280, 423 nm (log ε 4.43; 4.71; 4.12); there were no monosaccharides in the hydrolyzate after periodate oxidation (16 h at 20°C); a zone with R_f 0.51-0.60 yielded 27 mg of 8-mycarosyl-12a-oliosylolivosylchromocyclin (XI) with $[\alpha]_D^{25}$ −260° (c 0.3; ethanol); λ_{max} 230, 282, 423 nm (log ε 4.50; 4.69; 4.10); $\lambda_{max}^{0.01\ N\ KOH\ in\ EtOH}$ 228, 282, 420 nm (log ε 4.42; 4.64; 4.15); the hydrolyzate after periodate oxidation contained only olivose (V).

5. Mycarosyl-12a-deoxychromocyclin (XIII). A solution of 100 mg of chromocyclomycin (I) in 8 ml of 12% aqueous NH_3 was stirred with 500 mg of activated Zn dust at 20°C for 72 h, filtered, acidified with dilute H_2SO_4 to pH 3.5, and extracted with ethyl acetate. The extracted substance was chromatographed in the benzene–acetone (3:2) system. A zone with R_f 0.79-0.87 yielded 33 mg of mycarosyl-12a-deoxychromocyclin (XIII) with $[\alpha]_D^{25}$ +382° (c 0.4; ethanol); λ_{max} 235, 258, 382, 448 nm (log ε 4.48; 4.42; 4.06; 4.43).

When this substance was hydrolyzed under the conditions of expt. 1, the products were mycarose (II) and 12a-deoxychromocyclin (XII) (yield 36%), identified by comparison with the product of the direct reduction of chromocyclin (VII) [2].

SUMMARY

The structure of the carbohydrate moiety of chromocyclomycin, a metabolite of Streptomyces LA-7017, has been established and it has been shown that this glycoside has the structure (I).

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