OLIVOMYCIN AND RELATED ANTIBIOTICS

XXV. STEREOCHEMISTRY OF THE SIDE CHAIN OF OLIVIN*

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The aglycone of the olivomycin antibiotics, olivin (I), contains five chiral centers separated by the ketonic carbonyl of the side chain into two asymmetric moieties: $C_2-C_3-C_{1'}$ and $C_{3'}-C_{4'}$. The stereochemistry of the first of them ($C_2-C_3-C_{1'}$) has been determined by us previously [4]. The present paper gives the results of a proof of the relative and absolute configurations of the second asymmetric moiety ($C_{3'}-C_{4'}$) and $C_{1'}$, i.e., all three chiral centers of the side chain, thanks to which the spatial structure of olivin (I) has been established.

To determine the configuration of the $C_{4'}$ center we blocked all the hydroxyls in olivin except the 3'-OH and cleaved the 2', 3'-ketol grouping oxidatively with the isolation of a $C_{3'}-C_{5'}$ fragment. With this aim we obtained from olivin a tetraacetate (II) [5], which was then converted through the 3'-benzyloxycarbonyl derivatives (III) and (IV) into pentaacetylolivin (V). When this was oxidized with periodic acid followed by potassium permanganate, O-acetyl-D-lactic acid was isolated, this being identified in the form of the p-bromophenacyl ester (VIII). These results permitted the ascription to olivin of the 4'R configuration.

Information on the relative configuration of the $C_{3'}-C_{4'}$ asymmetric moiety (and hence on the absolute configuration of the $C_{3'}$ center) was initially obtained from the NMR spectrum of the isopropylidene derivative of tetraacetylolivin (VI) (for its preparation, see [5]) by comparing it with the spectra of the model compounds (XI) and (XV) synthesized from the methyl esters of threo- and erythro-dihydroxybutyric acids (X) and (XIV). It was found that the signals of the geminal methyls of the isopropylidene group in the spectrum of the threo-acetonide (XI) almost coincided ($\Delta\delta$ 0.02 ppm) while in the case of the erythro isomer (XV) their chemical shifts differed substantially ($\Delta\delta$ 0.23 ppm) as a consequence of the cis location of the other two substituents in the dioxolane ring (see [6]). In the spectrum of the olivin derivative (VI), the signals of the geminal methyls were separated by only 0.06 ppm, which shows the threo configuration of the 3', 4'-diol grouping and, consequently, the S configuration of the $C_{3'}$ center.

We have shown the validity of this conclusion by cleaving olivin at the $C_{1'}-C_{2'}$ bond with the isolation of the $C_{2'}-C_{5'}$ four-carbon fragment, which was done by the Baeyer–Villiger oxidation of olivin hexaacetate (VII) with subsequent hydrolysis. The (–) acid obtained as a result of these transformations was identified by a direct comparison as D-threo-dihydroxybutyric acid (IX), the absolute configuration of which had been established previously [7, 8]. This showed unambiguously the 3'S, 4'R configuration of olivin.

So far as concerns the $C_{1'}$ center, to determine its configuration we used the spectropolarimetric approach, the olivin molecule being previously simplified so as to eliminate as far as possible effects connected with the other chiral centers and the influence of the chromophoric system. For this purpose, olivin (I) was converted by a published method [9] into the ester of formyltrimethylolivinic acid (XII), from which the 2-formyloxy group and the 1-oxo group were subsequently eliminated by hydrogenolysis, first under the action of zinc in formic acid and then by hydrogenation in the presence of palladium; the resulting

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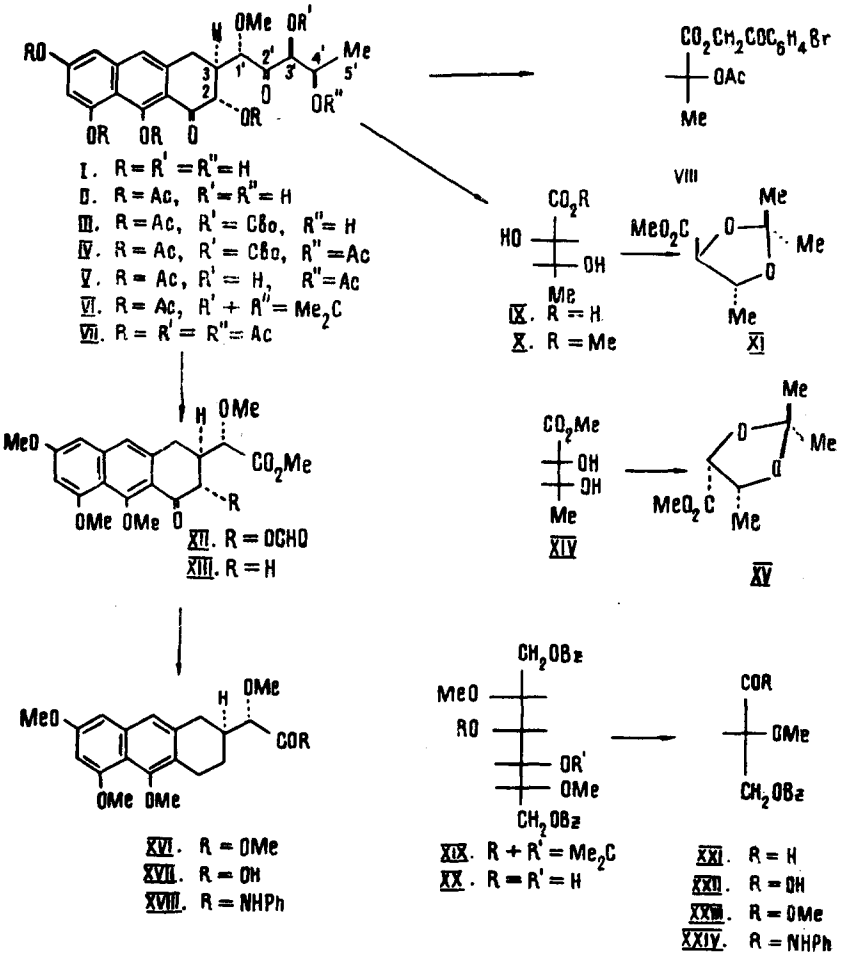

ester of trimethyldeoxodeoxyolivinic acid (XVI) was then saponified, and the free acid (XVII) was converted into the anilide (XVIII). In addition, the isopropylidene derivative of the substituted D-mannitol (XIX) [10] was hydrolyzed to the diol (XX), the oxidative cleavage of which with lead tetraacetate led to the aldehyde (XXI). The (benzoyl)(methyl)-D-glyceric acid (XXII) obtained by its permanganate oxidation was converted in the usual way into the ester (XXIII) and the anilide (XXIV). The results of a comparison of the ORD curves of compounds (XVI-XVIII) and of the model substances (XXII-XXIV) showed (Fig. 1) that they differed in the configuration of the asymmetric center $C-\overset{*}{C}H(OMe)-COR$ and, consequently, olivin (I) and the products of its degradation have the 1'S configuration.

Thus, the conversions described above have enabled the configurations of all three chiral centers of the side chain of olivin to be determined (1'S, 3'S, 4'R). In association with the stereochemistry of the C_2, C_3, and $C_{1'}$ centers (trans,threo configuration) that we established previously [4], this shows the absolute configuration 2S, 3R, 1'S, 3'S, 4'R for olivin (I).

EXPERIMENTAL

Chromatography was performed in a thin nonfixed layer of silica gel of "aqueous silicic acid" grade (less than 150 mesh, activity grade III-IV). Molecular weights were determined by mass spectrometry, unless otherwise stated. IR spectra were taken in mulls with paraffin oil, UV spectra in 96% ethanol, and NMR spectra in $CDCl_3$ at 100 MHz (s – singlet, d – doublet, t – triplet, q – quadruplet, m – multiplet). The elementary analyses of compounds (IV, V, VIII, XVIII, XX, and XXIV), of the bisphenylurethane of substance (X), and of the p-nitrophenylhydrazone of substance (XXI) corresponded to the calculated figures.

1. 2,6,8,9-Tetraacetyl-3'-benzyloxycarbonylolivin (III). Over 30 min, 2 ml of benzyl chloroformate was added to a solution of 574 mg of tetraacetylolivin (II) [5] in 3 ml of pyridine at −10°C with stirring. The mixture was stirred at 20°C for 45 min, and then 10 ml of methanol was added. It was left to stand for 30 min, and was evaporated at 30°C/0.01 mm. The residue was dissolved in 30 ml of chloroform, and

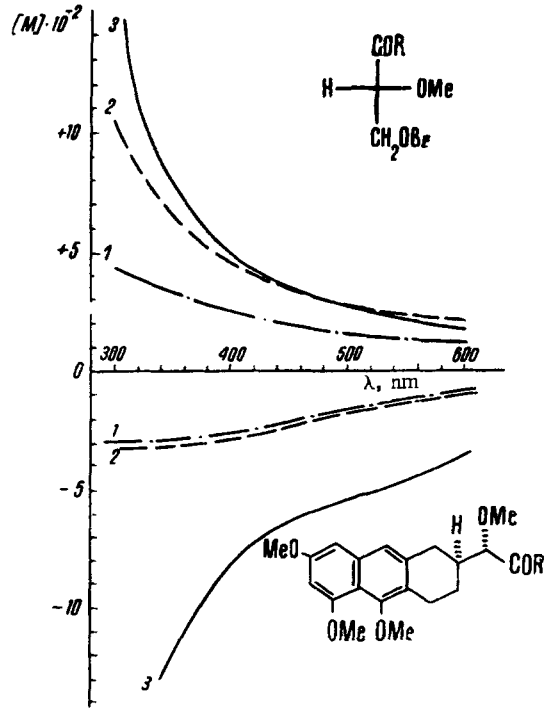

Fig. 1. ORD curves of trimethyldeoxodeoxyolivinic acid (XVII), its ester (XVI), and its anilide (XVIII) and of (benzoyl)(methyl)-D-glyceric acid (XXII) and its ester (XXIII) and anilide (XXIV) in 96% ethanol: 1) R = OH; 2) R = OMe; 3) R = NHPh.

the solution was washed with 1 N H_2SO_4, with 5% $NaHCO_3$, and with saturated NaCl solution, dried, and reevaporated. After chromatography in the benzene–acetone (5 : 1) system, 400 mg (57%) of the benzyloxycarbonyl derivative (III) with $[\alpha]_D^{23}$ +4° (c 1; (benzene); R_f 0.34, λ_{max} 259, 304, 359 nm (log ε 4.72; 3.86; 3.56); ν_{max} 1570, 1630, 1705, 1760, 1778, 3530 cm^{-1} was obtained.

Found: mol. wt. 708. $C_{36}H_{36}O_{15}$. Calculated: mol. wt. 708.

2. 2,4',6,8,9-Pentaacetyl-3'-benzyloxycarbonylolivin (IV). A solution of 180 mg of the tetraacetylbenzyloxycarbonylolivin (III) in 5 ml of pyridine was treated with 5 ml of Ac_2O, and the mixture was kept at 20°C for 24 h. The excess of reagent was evaporated off in vacuum, and the residue was worked up as in experiment 1. This gave 145 mg (77%) of the pentaacetate (IV) $C_{38}H_{38}O_{16}$ $[\alpha]_D^{23}$ +11.5° (c 1; benzene); R_f 0.65 [in the benzene – acetone (5 : 1) system], λ_{max} 259, 304, 359, nm (log ε 4.84; 3.89; 3.57); ν_{max} 1570, 1630, 1705, 1760, 1778 cm^{-1}.

3. 2,4',6,8,9-Pentaacetylolivin (V). A solution of 250 mg of the pentaacetylbenzyloxycarbonylolivin (IV) in 5 ml of glacial AcOH and 15 ml of ethanol was hydrogenated with Pd black (from 62 mg of $PdCl_2$) until 8.6 ml of hydrogen had been absorbed. After the usual working up and chromatography in the benzene–acetone system (5 : 1), 72 mg (29%) of the starting material (IV) and 67 mg (32%) of pentaacetylolivin (V) were obtained; the latter had $[\alpha]_D^{23}$ +33° (c 1; benzene), R_f 0.51, λ_{max} 259, 304, 359 nm (log ε 4.77; 3.86; 3.53) ν_{max} 1570, 1630, 1705, 1760, 1775, 3485 cm^{-1}.

Found: mol. wt. 616. $C_{30}H_{32}O_{14}$. Calculated: mol. wt. 616.

4. p-Bromophenacyl Esters of D-Acetyllactic Acid (VIII) and the L-Isomer. A. A mixture of 61 mg of pentaacetylolivin (V) in 2 ml of 2-methylpropan-2-ol and 3.5 ml of a 0.085 M solution of HIO_4 in the same solvent was kept at 20°C for 6 h, and then 1.75 ml of a 0.6% solution of $KMnO_4$ and 0.58 ml of 0.1 N H_2SO_4 were added. After 30 min, the IO_3^- and IO_4^- ions were precipitated with 60 mg of $Pb(NO_3)_2$ in 0.5 ml of water and, after filtration, the solution was neutralizaed with $NaHCO_3$ to pH 4.5 and was evaporated. The residue was extracted with ethanol (2 × 0.3 ml), and the solution was boiled for 1 h with 35 mg of p-bromophenacyl bromide. After chromatography in the benzene–acetone (10 : 1) system, 2.5 mg of the p-bromophenacyl ester of D-acetyllactic acid (VIII) was isolated with $[\alpha]_D^{25}$ +13.5° (c 0.3; benzene), R_f 0.78.

B. L-Lactic acid was alkylated with p-bromophenacyl bromide as in experiment A. The yield of the p-bromophenacyl ester $C_{11}H_{11}BrO_4$ was 53%, mp 106–107°C (from ethanol, $[\alpha]_D^{23}$ –1.2° (c 5; ethyl acetate).

The acetylation of this substance with Ac_2O + Py (24 h at 20°C) gave the p-bromophenacyl ester of L-acetyllactic acid, $C_{13}H_{13}BrO_5$, mp 84–85°C (from ethanol), $[\alpha]_D^{23}$ –49° (c 5; benzene), R_f 0.78 [in the benzene–acetone (10 : 1) system].

5. Acetonides of the Methyl Esters of Racemic threo- and erythro-Dihydroxybutyric Acids (XI and (XV). A. A solution of 630 mg of methyl threo-dihydroxybutyrate (X) [11] in 3 ml of acetone was treated with 430 mg of anhydrous $CuSO_4$ and 3 mg of conc. H_2SO_4, and the mixture was stirred for 24 h, filtered, and evaporated, and the residue was distilled. The yield of the acetonide (XI) was 260 mg (32%), bp 38°C (bath temperature)/0.3 mm, R_f 0.71 [benzene–acetone (20 : 1) system], V_{ret} 0.28 (relative to methyl stearate; polyethyleneglycol succinate on Chromosorb W, 160°C), δ 1.40 (3H, s) and 1.42 (3H, s) (Me_2C).

B. The methyl erythro-dihydroxybutyrate (XIV) [12] was converted into the acetonide (XV) under the conditions of experiment A (reaction time 3 h). Yield 40%, bp 30–32°C (bath temperature)/0.2 mm, R_f 0.63, V_{ret} 0.32, δ 1.36 (3H, s) and 1.59 (3H, s) (Me_2C).

6. D-threo-2,3-Dihydroxybutyric Acid (IX) and Its Derivatives. A. To a solution of 263 mg (0.4 mmole) of hexaacetylolivin (VII) [5] in 1 ml of methylene chloride was added 0.45 ml of a 6.25 M solution of trifluoroperacetic acid obtained from equimolar amounts of $(CF_3CO)_2O$ and anhydrous H_2O_2, and the mixture was left at 20°C for 72 h. Then 5 ml of water was added, the mixture was heated at 80°C for 4 h and evaporated; the residue was dissolved in water, and the solution was extracted with ethyl acetate. The aqueous solution was then evaporated to dryness, and the residue was chromatographed in ethyl acetate. This gave 22 mg of crude dihydroxybutyric acid (IX) with $[\alpha]_D^{24} - 4.5°$ (c 1; water), R_f 0.55 (on silica gel), R_f 0.37 [on paper in the n-BuOH–H_2O–HCOOH (10:5:2) system]. The acid was treated with an excess of diazomethane in ether–methanol. After chromatography in the benzene–acetone (2:1) system, 6 mg of the methyl ester (X) was isolated with R_f 0.37, V_{ret} 0.51 (relative to methyl acetate, 10% of PEGS, 179°C).

The bisphenylurethane of the ester (X) was obtained from 6 mg of (X) and 12 mg of PhNCO by boiling in 0.15 ml of tetrahydrofuran for 4 h. Yield 1.5 mg, mp 103–105°C (from benzene), $[\alpha]_D^{23} + 29°$ (c 0.13; ethanol), R_f 0.62 [benzene–ethyl acetate (5:1) system]; chromatographically and spectrally (IR, ORD), the substance was identical with the compound obtained in experiment B.

B. As described in experiment A, D-(–)-threo-dihydroxybutyric acid (IX) [13] was converted into the methyl ester (X) and then into the bisphenylurethane of the methyl ester, $C_{19}H_{20}N_2O_6$, mp 118–120°C (from benzene), $[\alpha]_D^{23} + 52°$ (c 1; ethanol).

7. Methyl 6,8,9-Trimethyl-2-deoxyolivinate (XIII). A solution of 100 mg of methyl formyltrimethylolivinate (XII) [9] in 4 ml of 85% formic acid was stirred at 0°C with 50 ml of Zn dust for 5 min and was then filtered, diluted with water, and extracted with ethyl acetate After chromatography in the hexane–ethyl acetate (2:1) system, the zone with R_f 0.23–0.35 yielded 72 mg (82%) of the reduction product (XIII), $[\alpha]_D^{25} - 8°$ (c 1.8; ethanol), λ_{max} 223, 270, 330 shoulder, 368 nm (log ε 4.40; 4.57; 3.76; 3.85); ν_{max} 1573, 1620, 1690, 1747 cm^{-1}.

Found: mol. wt. 388. $C_{21}H_{24}O_7$. Calculated: mol. wt. 388.

8. Methyl 6,8,9-Trimethyl-1-deoxo-2-deoxyolivinate (XVI). A solution of 30 mg of compound (XII) in 5 ml of ethanol was hydrogenated with 15 mg of Pd black (obtained from $PdCl_2$ by reduction with HCOOH) until 2 moles of hydrogen had been absorbed. After chromatography in the benzene–acetone (10:1) system, the zone with R_f 0.72–0.80 yielded 17 mg (61%) of the hydrogenolysis product (XVI), $[\alpha]_D^{25} - 33°$ (c 0.5; ethanol); λ_{max} 242, 290, 302, 340 nm (log ε 4.82; 3.74; 3.67; 3.39); ν_{max} 1578, 1607, 1627, 1747 cm^{-1}.

Found: mol. wt. 374. $C_{21}H_{26}O_6$. Calculated: mol. wt. 374.

9. 6,8,9-Trimethyl-1-deoxo-2-deoxyolivinic Acid (XVII) and Its Anilide (XVIII). The ester (XVI) (130 mg) was hydrolyzed with 0.6 N aqueous methanolic KOH (4 h at 20°C). After the usual working up, 86 mg (68%) of the acid (XVII) was obtained with mp 146–148°C (from ether), $[\alpha]_D^{25} - 31°$ (c 0.1; ethanol); λ_{max} 242, 290, 300, 340 nm (log ε 4.86; 3.82; 3.81; 3.53), ν_{max} 1579, 1602, 1635, 1750, 3280 cm^{-1}.

Found: mol. wt. 360. $C_{20}H_{24}O_6$. Calculated mol. wt. 360.

A solution of 45 mg of the acid (XVII), 31 mg of dicyclohexylcarbodiimide, and 30 mg of the anilide in 2.5 ml of benzene was kept at 20°C for 20 min and was then diluted with ethyl acetate, washed with dilute H_2SO_4 and with water, dried, and evaporated. The residue was chromatographed in the benzene–acetone (20:1) system, and the substance with R_f 0.45 was crystallized from ethanol. The yield of the anilide (XVIII), $C_{26}H_{29}O_5N \cdot C_2H_5OH$ was 21 mg (39%), mp 154–156°C, $[\alpha]_D^{25} - 87°$ (c 0.15; ethanol); λ_{max} 242, 290, 301, 340 nm (log ε 5.08; 3.90; 3.85; 3.58), ν_{max} 1518, 1580, 1610, 1633, 1665, 3280 cm^{-1}.

Found: mol. wt. 435. Calculated: mol. wt. (without ethanol) 435.

10. 3-Benzoyl-2-methyl-D-glyceric Acid (XXII), Its Methyl Ester (XXIII), and Its Anilide (XXIV). The 1,6-dibenzoyl-3,4-isopropylidene-2,5-dimethyl-D-mannitol (XIX) [10] was hydrolyzed by being heated with 50% acetic acid (5 h at 70°C). The yield of 1,6-dibenzoyl-2,5-dimethyl-D-mannitol (XX), $C_{22}H_{26}O_8$, was 78%, mp 101–102°C (from ethanol), $[\alpha]_D^{23} - 17°$ (c 1; chloroform).

A solution of 100 mg of the diol (XX) and 124 mg of 85% $Pb(OAc)_4$ in 6 ml of benzene was kept at 20°C for 1 h and was then filtered and evaporated. The resulting 3-benzoyl-2-methylglyceraldehyde (XXI) was converted into the p-nitrophenylhydrazone, $C_{17}H_{17}N_3O_5$. Yield 77 mg (93%), mp 119–121°C (from 50% aqueous ethanol), $[\alpha]_D^{24} + 43°$ (c 1; chloroform).

Solutions of 400 mg of the aldehyde (XXI) in 2 ml of 2-methylpropan-2-ol and of 200 mg of $KMnO_4$ in 20 ml of water were mixed. After 10 min the mixture was filtered, and the filtrate was extracted with

ethyl acetate, first at pH 8 and then at pH 2. The second extract yielded 183 mg (42%) of the acid (XXII), $[\alpha]_D^{25}$ +48° (c 3; ethanol), R_f 0.57 [benzene–acetone (5:1) system].

The methyl ester (XXIII) was obtained by methylating the acid (XXII) with an excess of diazomethane in ether. Yield 75%, $[\alpha]_D^{20}$ +83° (c 0.9; ethanol), R_f 0.70 [benzene–acetone (20:1) system].

The anilide (XXIV), $C_{17}H_{17}NO_4$, was obtained by the reaction of 120 mg of the acid (XXII), 62 mg of aniline, and 120 mg of N-cyclohexyl-N'-(γ-dimethylaminopropyl)carbodiimide in 0.5 ml of tetrahydrofuran under the conditions of experiment 9. Yield 50%, mp 98–99°C (from ethyl acetate), $[\alpha]_D^{24}$ +58° (c 0.9 in ethanol).

SUMMARY

The configurations of the $C_{3'}$ and $C_{4'}$ centers of olivin have been shown by the oxidative cleavage of olivin penta- and hexaacetates (V) and (VI) with the formation of D-acetyllactic and D-threo-dihydroxybutyric acids (VIII) and (IX). The stereochemistry of the $C_{1'}$ center has been determined by the spectropolarimetric correlation of the products of the degradation of olivin (XVI)–(XVIII) with the model substances (XXII)–(XXIV). As a result, the 2S, 3R, 1'S, 3'S, 4'R configuration has been established for olivin.

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